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STRUCTURAL AND SYNTHETIC STUDIES ON SOME FUNGAL METABOLITES

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

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STRUCTURAL AND SYNTHETIC STUDIES

ON SOME FUNGAL METABOLITES

submitted by ROBERT HUGH McCASKILL

in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

W.A. Ayer (Supervisor)

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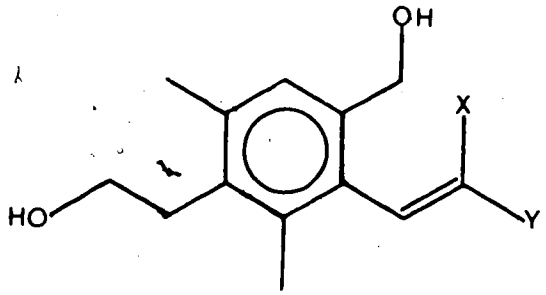
Date November 13, 1980

ABSTRACT

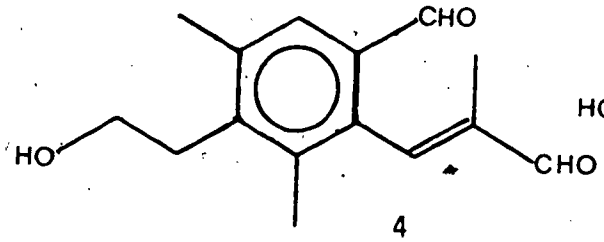
Part I of this thesis describes the isolation and structural elucidation of the metabolites produced in liquid culture by the bird's nest fungus *Cyathus bulleri* Brodie (strain 6680a). The structures of five new sesquiterpenoids cybrodol (1), isocybrodol (2), cybrodic acid (3), cybrodal (4), and trisnorcybrodolide (5) were established by physical and chemical methods. Compounds 1-5 are known collectively as cybrodins and



1 X = CH_3, Y = CH_2OH

2 X = CH_2OH, Y = CH_3

3 X = CH_3, Y = CO_2H



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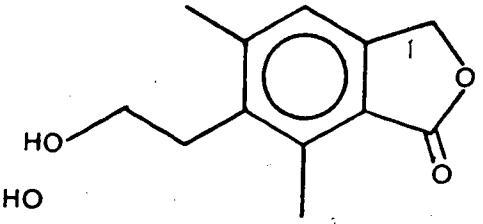

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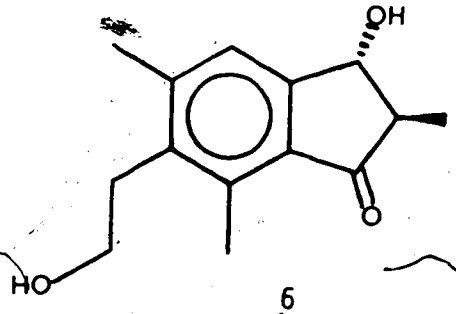
comprise a new class of sesquiterpenoids (*seco*-illudalanes). The known illudalane, pterosin C (6) is



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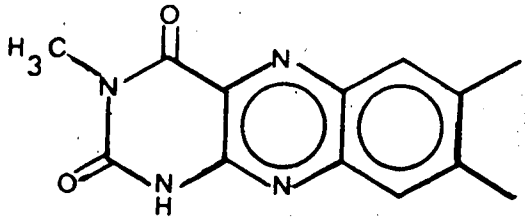

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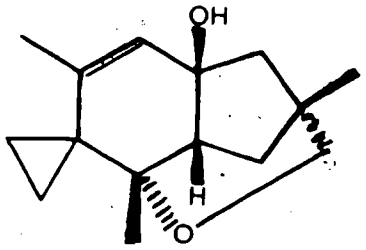
a major metabolite of *C. bulleri*. 3-Methyllumichrome (7), a compound not previously reported as a natural product is a minor metabolite, the structure of which was proven by total synthesis following literature precedents. Structures 8 and 9 are suggested for



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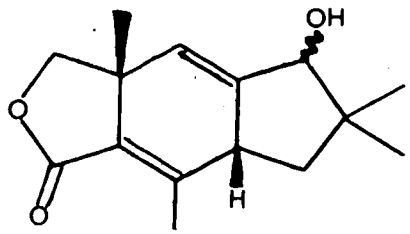

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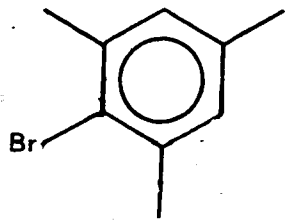
broderol and nidulol respectively, two minor metabolites.

Part II of this thesis describes the total synthesis of the cybrodins from 2-bromomesitylene (10). Salient features of the synthesis include: 1) regio-



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selective oxidation of the C-5 methyl group; 2) two carbon chain extension at C-2; and 3) ultimate elaboration of the fifth aromatic substituent required for the cybrodin skeleton.

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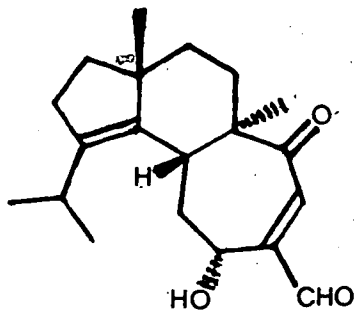
I: METABOLITES OF THE BIRD'S NEST FUNGUS *CYATHUS BULLERI*[1]

INTRODUCTION

The metabolites produced in liquid cultures by the gasteromycete fungi of the family Nidulariaceae have been investigated in these laboratories during the last decade[2]. In nature, members of this family produce small cup shaped fruiting bodies containing seed-like peridioles. The entire fruit body resembles a miniature bird's nest containing eggs. The family name is derived from the Latin word *nidula* meaning a little nest. The Nidulariaceae are commonly known as bird's nest fungi*.

Liquid cultures of bird's nest fungi elaborate a wide variety of interesting compounds. *Cyathus helenae* Brodie produces the diterpenoid antibiotic cyathin B_3 (1)[4]. More than one dozen compounds with the unique[5]



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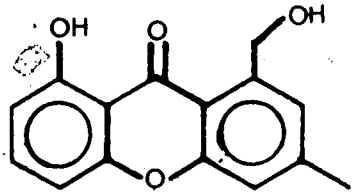
*For a thorough description of the members of this family see reference 3.

cyathane skeleton of cyathin B_3 (1) have been isolated from cultures of *C. helenae*[6], *C. africanus* Brodie[7] and *C. earlei* Lloyd[8]. The new xanthone 2 is produced by *C. intermedius* Tulasne[9].



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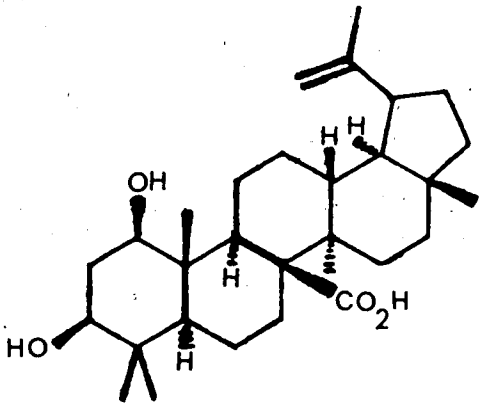
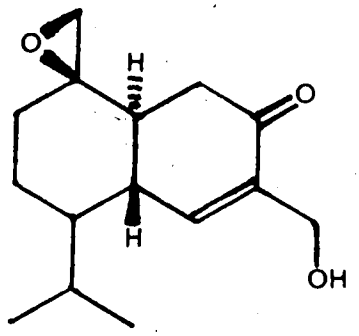
Structure 3 has been suggested for cyathic acid, a major



3

metabolite of *C. striatus* Hudson[10] and *C. pygmaeus* Lloyd[11]. Several cadinane-type[5] sesquiterpenoids, of which 8,15-β-epoxyschizandronol (4) is representative, were isolated from cultures of *C. striatus*[11]. *Mycocalia reticulata* Petch produces drimenin-type[5] sesquiter-



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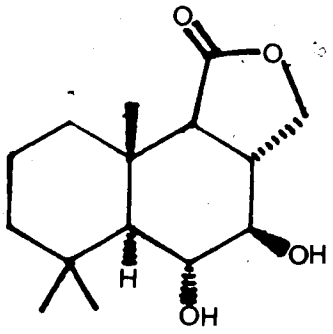
penoids such as 6α,7β-dihydroxydihydrodrimenin (5)[12].



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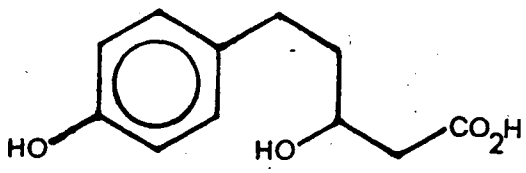
Nidula niveo-tomentosa Lloyd produces niduloic acid (6)



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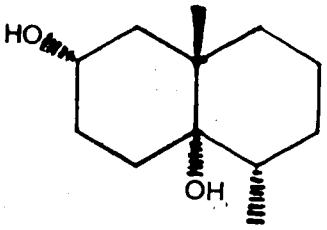
plus several similar phenolic compounds[13].

Cyathus bulleri Brodie is a species of bird's nest fungus native to the West Indies, Hawaii and Mexico[3]. Paice investigated the metabolites of *C. bulleri* (Brodie strain 6620, ATCC 38347)[14] and isolated the degraded eudesmane-type sesquiterpenoid cybullol (7).



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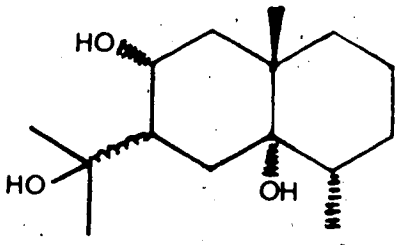

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Small quantities of a $C_{15}H_{22}O_3$ triol were also obtained. Structure 8 was proposed for this compound, however further quantities were required for a rigorous structural proof. Unfortunately, stock cultures of *C. bulleri* 6620 in our possession ceased production of cybullol (7) as well as the required triol. The loss of integrity of this strain was attributed to mutation. Replacement authentic cultures of *C. bulleri* 6620 were unavailable. Consequently, a second strain of *C. bulleri* (Brodie strain 6680a, ATCC 38351) was investigated. This strain does not produce cybullol (7) or the $C_{15}H_{22}O_3$ triol, however it does elaborate a number of interesting metabolites. Part 1 of this thesis describes

the isolation and structural elucidation of the metabolites of *C. bulleri* 6680a.

DISCUSSION AND RESULTS

Large scale cultures of *C. bulleri* 6680a were grown on Brodie's medium or yeast-malt extract medium (for compositions see Experimental Section) in a fermentation apparatus. After a growth period of approximately two weeks, the mycelial cells were removed by filtration. The culture broth was then extracted with ethyl acetate. The ethyl acetate soluble metabolites were divided into neutral and acidic components by extraction with saturated aqueous sodium bicarbonate.

The neutral and acidic extracts were separately chromatographed over LH-20 Sephadex. Constant volume (400 drop) fractions were collected. Sephadex chromatography provided a highly reproducible preliminary fractionation based on molecular size. In the case of the neutral extract this was necessary to remove the high molecular weight antifoaming agent, polypropylene glycol (mol. wt. > 2000) which contaminated the actual metabolites. Mixtures of carboxylic acids are usually not amenable to conventional silica gel or alumina chromatography, thus Sephadex chromatography was particularly useful for the fractionation of the acidic metabolites.

The major component of the neutral extract eluted in Sephadex fractions 40-41 and was named cybrodol to

reflect the fact that it is an alcohol. Cybrodol was obtained in pure form as a colourless oil when Sephadex fractions 38-41 were chromatographed over silica gel with chloroform-methanol, 20:1. Cybrodol is a rather polar compound having an R_f of 0.25 when subjected to thin layer chromatography (tlc) over silica gel with methylene chloride-methanol, 10:1. It is easily recognized by its characteristic colour reaction. When the developed plate is sprayed with 1% vanillin in sulfuric acid and then charred*, a reddish-brown colour appears on heating. Within ten minutes a bright green colour develops.

Cybrodol has a molecular formula of $C_{15}H_{22}O_3$ (mol. wt. 250) as determined by high resolution mass spectrometry (hrms)†. A molecular weight of 250 was confirmed by chemical ionization mass spectrometry $(NH_3)^{15}$. A strong peak at m/e 268 (M+18) corresponding to a collision complex of cybrodol plus an ammonium ion is observed. The infrared (ir) spectrum of cybrodol is dominated by strong hydroxyl absorption at 3300 cm^{-1}. Carbon-oxygen stretching bands (1030, 1010 cm^{-1}) are

---

*Unless specified, all references to tlc behaviour shall imply silica gel as the adsorbent and 1% vanillin in sulfuric acid followed by charring as the visualization method.

†The compositions of all parent and fragment ions reported in Part 1 of this thesis were determined by hrms. Because of the small quantities involved, elemental analyses were not performed.

also prominent. No strong bands are present in the carbonyl region, thus cybrodol must be an alcohol or an alcohol-ether.

The ultraviolet (uv) spectrum of cybrodol (λ_{max} (CH_3OH): 210 (ε 6200), 270 nm (ε ~320)) is typical of an alkyl substituted benzenoid compound. For example the uv spectrum of *m*-xylene (λ_{max} (CH_3OH): 212 (ε 7200), 264.5 nm (ε 300))[16] is very similar to that of cybrodol. A benzenoid structure is also compatible with the five sites of unsaturation implied by the molecular formula. The nuclear magnetic resonance (nmr) spectra of cybrodol are also consistent with a benzenoid nucleus. The ^{13}C nuclear magnetic resonance (^{13}Cmr) spectrum (CD_3OD) has eight signals in the region δ120-140* where sp^2 carbons not bonded to heteroatoms normally appear[17]. Off-resonance decoupling reveals that six of these signals are due to fully substituted carbons, the remaining two bear single hydrogen atoms. The ^1H nuclear magnetic resonance (^{1}Hmr) spectrum ($CDCl_3$) of cybrodol (Figure 1)† has one proton signals at δ7.10 and δ6.45 corresponding to aromatic and vinylic hydrogens respectively. A monocyclic molecule containing a penta-substituted benzene ring and a trisubstituted double bond would satisfy the requirement of five unsaturations

*All nmr shifts are relative to tetramethylsilane.
†Spectra are shown as figures in the appendix.

and accommodate the above nmr features. Bands at 1670(w) and 760 cm^{-1} in the ir are consistent with a trisubstituted olefin. A band at 890 cm^{-1} may be assigned to the out-of-plane deformation of a pentasubstituted benzene ring[18].

The ^{13}Cmr spectrum of cybrodol has three signals in the region δ60-70 typical of oxygenated sp^3 carbons[17]. Off-resonance decoupling shows that each of these signals is due to a methylene carbon. The ^{1}Hmr spectrum supports this observation. Three two proton signals (δ4.50, 4.23, 3.76) are observed in the region characteristic of hydrogens geminal to oxygen. Using the off-resonance ^{13}Cmr spectrum it is possible to count the number of carbon bound hydrogens in the molecule. Cybrodol has nineteen such hydrogens. It follows therefore, that each oxygenated methylene signal must represent a hydroxymethyl group. Three primary alcohols, accounting for the three oxygens present in the molecule, are consistent with the polar nature of cybrodol. Acetylation (acetic anhydride-pyridine-chloroform) confirmed the presence of three primary alcohols. Triacetylcybrodol (mol. wt. 376) lacks hydroxyl absorption and has a strong carbonyl band (1745 cm^{-1}) in the ir. The ^{1}Hmr spectrum ($CDCl_3$) of triacetylcybrodol has three acetyl methyl group signals. The three signals attributable to hydrogens geminal to oxygen all display

downfield acetylation shifts of about 0.45 ppm relative to cybrodol. Primary alcohols usually have downfield acetylation shifts of about 0.5 ppm, while the carbinol proton of a secondary alcohol is normally deshielded by at least 1 ppm on acetylation[19]. The ^{1}Hmr chemical shift ($CDCl_3$) of the methylene group of benzyl alcohol is δ4.58[20]. In view of cybrodol's apparent aromaticity, the signal at δ4.50 in the ^{1}Hmr of cybrodol is assigned to a benzyl alcohol function. Irradiation of the olefinic proton of cybrodol (δ6.45) sharpens the signal at δ4.23. Accordingly, this signal is assigned to the carbinol protons of an allylic alcohol function. The signal at δ3.76 is one half of an A_2X_2 system (J_{AX} = 7 Hz) whose second component appears at δ2.99†. The shifts of these signals are consistent with the presence of a β-phenethyl alcohol grouping. β-Phenethyl alcohol itself has ^{1}Hmr ($CDCl_3$) shifts of δ3.85 and δ2.82 for the α and β protons respectively[21]. Mass spectral evidence supports the presence of this moiety. The mass spectrum (ms) of cybrodol has a strong peak (95%)* at m/e 219 ($C_{14}H_{19}O_2$) corresponding to the loss of $\cdot CH_2OH$. Primary aliphatic alcohols commonly fragment in this manner[22]. This cleavage should be greatly facilitated in the case of cybrodol as the resulting

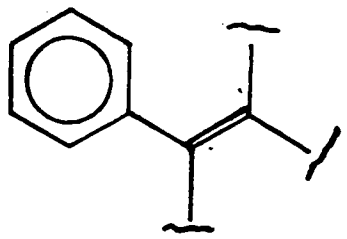
---

* Relative to the base peak as are all intensities quoted in this thesis.
† Verified by a decoupling experiment.

fragment ion would be a benzylic (or a tropylium) carbonium ion.

The ^{1}Hmr spectrum of cybrodol has three C-methyl group signals (δ2.36, .2.18, 1.44). Likewise the C-methyl group region of the ^{13}Cmr spectrum[17] displays three signals, all assigned to methyl groups by off-resonance decoupling. Given the benzenoid nucleus of cybrodol, the singlets at δ2.36 and δ2.18 must represent aromatic methyl groups. The signal at δ1.44 is a narrow doublet (J = 1 Hz) which collapses to a singlet upon irradiation of the vinyl proton (δ6.45). This methyl group must be vinylic. The sum total of all elements present in the structural fragments elucidated thus far exactly equals the molecular formula of cybrodol. Consequently the benzenoid and vinylic sections of the molecule must be directly linked. Partial structure 9 for cybrodol may now be formulated.

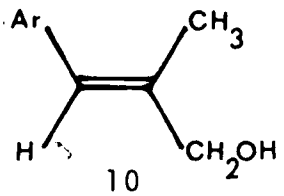
aromatic substituents: H, 2xCH_3, CH_2OH, CH_2CH_2OH



olefinic substituents: H, CH_3, CH_2OH

9

The chemical shifts of olefinic protons may be reliably predicted (± 0.3 ppm) using additive substituent coefficients[23]. The predicted shifts (CCl_4) for the six possible olefins (10-15) which can arise from part structure 9 are shown below.

δ6.32 

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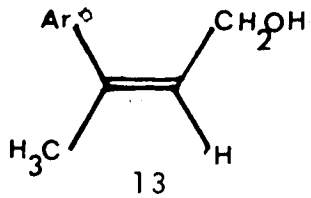
δ5.59 

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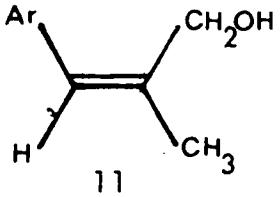
δ6.30 

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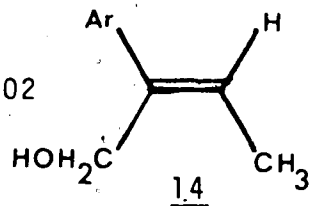
δ6.02 

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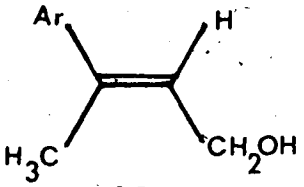
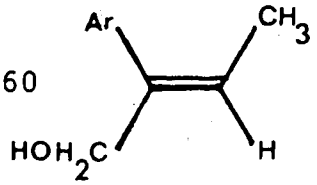
δ6.03 

12

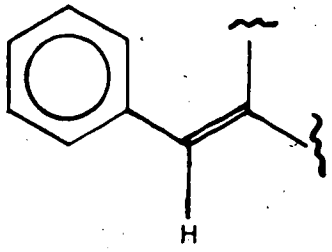
δ5.60 

15

Only structures 10 and 11, in which the vinyl hydrogen is geminal to the aromatic substituent, provide good agreement with the observed shift of δ6.45. Furthermore, in structures 14 and 15, the vinyl methyl group should exhibit a vicinal coupling (J ~ 7 Hz) to the vinyl hydrogen. Likewise, in structures 12 and 13 a vicinal coupling between the hydroxymethyl group and

the vinyl hydrogen should be seen. Only structures 10 and 11 are consistent with the observed (1 Hz) small allylic coupling between the vinyl methyl group and the vinyl hydrogen as well as the half-height widths (4 Hz, 2 Hz) of the vinyl hydrogen and hydroxymethyl group signals respectively. An expanded partial structure 16 can now be drawn on the basis of this analysis.

aromatic substituents: H, 2xCH_3, CH_2OH, CH_2CH_2OH



olefinic substituents: CH_3, CH_2OH

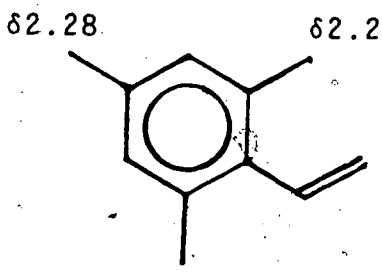
16

A careful comparison of the aryl methyl group signals of cybrodol reveals that the signal at δ2.18 is noticeably sharper than the one at δ2.36. Irradiation of the aromatic hydrogen (δ7.10) shows that both aryl methyl groups are weakly coupled to the aromatic proton. However, a nuclear Overhauser experiment demonstrated that the methyl group at δ2.36 is *ortho* to the aromatic proton. Irradiation of this methyl group

caused a five percent enhancement in the signal intensity of the aromatic proton. An *ortho* benzylic coupling (0.6-0.9 Hz) is usually larger than a *meta* (~0.4 Hz) or a *para* (0.5-0.6 Hz) coupling[24]. Thus the signal for the methyl group which is *ortho* to the aromatic hydrogen should be broader than the signal of the methyl group not so located. This feature allows one to indentify each methyl group in cybrodol derivatives.

The resolution of the cybrodol structural problem can now be divided into two independent pursuits: the elucidation of the benzene ring substitution pattern and the determination of the double bond geometry. The former objective will be addressed first.

Ozonolysis of the double bond in cybrodol followed by oxidative work-up should produce a benzoic acid derivative. The influence of the carbonyl group so generated upon substituents located *ortho* to it will be observable in the ^{1}Hmr spectrum of the acid derivative. The methyl group ^{1}Hmr shieldings ($CDCl_3$) of vinylmesitylene (17) and mesitoic acid (18) are shown[25].



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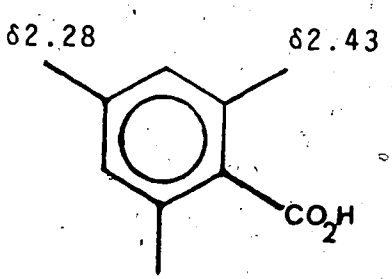


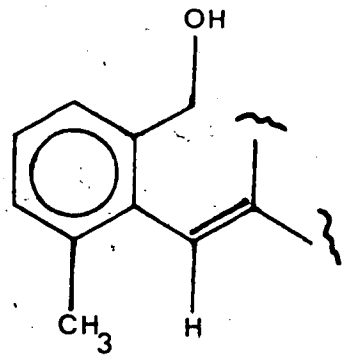
18

An examination of these figures reveals that a downfield shift of about 0.15 ppm on ozonolysis is anticipated for an alkyl group located *ortho* to the double bond in cybrodol. The effect on an *ortho* hydrogen is expected to be even more pronounced. The *ortho* protons of styrene and benzoic acid appear ($CDCl_3$) at $\delta 7.3$[26] and $\delta 8.2$[27] respectively. If either the β-phenethyl or benzyl alcohol moiety is located *ortho* to the acid function generated by the proposed degradation, lactonization would likely result. To prevent this and allow isolation of the benzoic acid derivative, triacetylcybrodol was chosen as the substrate for ozonolysis.

Triacetylcybrodol was ozonized in methanol solution at -78°C and then subjected to an oxidative work-up with hydrogen peroxide. The expected molecular weight of the product is 308, the highest mass peak in the ms is m/e 290 corresponding to dehydration of the molecular ion. Benzoic acids commonly fragment in this way if a hydrogen-bearing *ortho* group is available[28]. Comparison of the crude reaction product with triacetylcybrodol by ^{1}Hmr is in accord with expectations. One acetyl methyl group, the vinyl hydrogen, the allylic acetate methylene protons and the vinyl methyl group are not evident in the ^{1}Hmr spectrum ($CDCl_3$) of the product. The remaining features of the

starting material are all present. Only two of these groups are appreciably affected by the new carbonyl group. The benzylic acetate methylene group is deshielded by 0.17 ppm relative to the starting material. The methyl group which is not located *ortho* to the aromatic hydrogen is deshielded by 0.46 ppm, a somewhat larger effect than expected. With this information a refined partial structure 19 can be proposed for cybrodol.

aromatic substituents: H, CH_3, CH_2CH_2OH



olefinic substituents: CH_3, CH_2OH

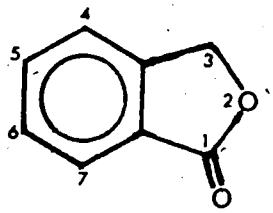
19

Treatment of the acid derivative with methanol in the presence of an acid catalyst should remove the acetyl groups by transesterification and cause lactonization to a phthalide derivative. The crude ozonolysis product, after oxidative work-up, was refluxed overnight in a benzene-methanol mixture in the presence of 10-camphorsulfonic acid. The product of this reaction was identical in all respects (tlc, ir, ^{1}Hmr, ms) with

natural trisnorcybrodolide, another component of the *C. bulleri* neutral extract.

Trisnorcybrodolide, so named because it is a lactone having three fewer carbons than cybrodol, eluted in Sephadex fractions 43-48 of the neutral extract. Silica gel chromatography of these fractions gave trisnorcybrodolide, mp 188-190°C. By tlc, this compound has a R_f of 0.58 when developed with methylene chloride-methanol, 10:1. It is best visualized under an ultraviolet lamp (254 nm).

Trisnorcybrodolide has a molecular formula of $C_{12}H_{14}O_3$ (mol. wt. 206). Chemical ionization mass spectrometry (NH_3) confirmed the molecular weight. A strong peak at m/e 224 (M + 18) is observed. The uv spectrum (λ_{max} (CH_3OH): 242 (ε 6400), 282 (ε 1100), 289 nm (ε 1300)) is very similar to that of phthalide (20, λ_{max} (C_2H_5OH): 227 (ε 9900), 273 (ε 1720), 280 nm (ε 1660))[29].



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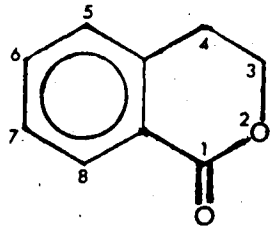
The ir ($CHCl_3$ cast) carbonyl frequency (1730 cm^{-1}) is in reasonable agreement with that of phthalide (ν_{max} (Nujol): 1745 cm^{-1})[30]. A comparison of the ^{1}Hmr spectra of trisnorcybrodolide ($CDCl_3$-CD_3OD, Figure 2) and cybrodol confirms that a five rather than a six-membered lactone was formed in the above interconversion. The ^{1}Hmr spectrum of trisnorcybrodolide has an A_2X_2 system (δ3.06, 3.79; J = 7 Hz) whose elements have virtually the same shifts as the β-phenethyl alcohol system in cybrodol. This suggests that trisnorcybrodolide does not possess nucleus 21.



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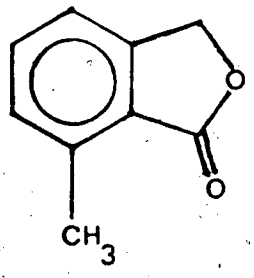
The C-3 protons in such a system should appear at *ca.* δ4.3 rather than δ3.79 as observed. The C-3 protons of phthalide (20) resonate at δ5.35 ($CDCl_3$)[31]. The ^{1}Hmr spectrum of trisnorcybrodolide has a two proton singlet at δ5.15, indicating the presence of nucleus 20. Finally, acetylation (acetic anhydride-pyridine) proved that trisnorcybrodolide has a free β-phenethyl alcohol system (ir: 3450 cm^{-1}). Acetyltrisnorcybrodolide (mol. wt. 248, mp 117-118°C) lacks hydroxyl

absorption in the ir and has a strong carbonyl band at 1741 cm^{-1}. In the ^{1}Hmr spectrum ($CDCl_3$) of this derivative, one acetyl methyl group signal is present and, most importantly, the elements of the β-phenethyl alcohol system display appropriate acetylation shifts[19] relative to trisnorcybrodolide. The two proton singlet (δ5.15) described above is not shifted on acetylation.

Having established that trisnorcybrodolide is a phthalide derivative, partial structure 22 for this metabolite can now be drawn. Placement of a methyl

aromatic substituents: H, CH_3, CH_2CH_2OH



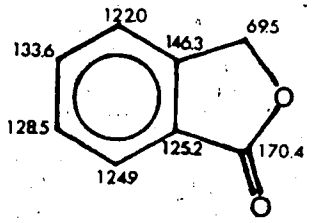
22

group at C-7 follows from the above discussion of the ^{1}Hmr spectrum of the acidic product of the ozonolysis of triacetylcybrodol. This assignment is reinforced by consideration of the nmr spectra of trisnorcybrodolide. The aromatic methyl groups appear at δ2.48 and δ2.73 in the ^{1}Hmr spectrum of this compound. The signal at δ2.48 is the broader of the two, indicating that

the aromatic hydrogen (δ7.11) is situated *ortho* to this methyl group. The same methyl group appeared at δ2.36 in the ^{1}Hmr spectrum of cybrodol, hence the degradation sequence resulted in a 0.12 ppm deshielding of this methyl group. The other methyl group however is shifted downfield by 0.55 ppm relative to its position in the ^{1}Hmr spectrum of cybrodol. These drastically different perturbations can only be rationalized if the methyl group resonating at δ2.73 in the ^{1}Hmr spectrum of trisnorcybrodolide is placed at C-7 where it lies in the deshielding region of the C-1 carbonyl group[32]. The ^{13}Cmr spectrum (DMSO-d_6) of trisnorcybrodolide is equally decisive in this connection. Methyl group resonances appear at δ12.7 and δ20.7. The methyl groups of *ortho*-xylene appear at δ19.6 (neat)[33], thus a shift of δ20.7 is not unusual for an aromatic methyl group with one alkyl group located *ortho* to it. A shift of δ12.7 is rather low for an aromatic methyl group flanked by two *ortho* alkyl substituents. For instance, the C-2 methyl group of 1,2,3-trimethylbenzene resonates at δ15.0 (neat)[33]. Placement of the highfield methyl group of trisnorcybrodolide (δ12.7) at C-7 allows it to experience a strong γ interaction with the C-1 carbonyl[33]. This interaction should repel electron density from the C-7 methyl hydrogens causing them to be deshielded in the ^{1}Hmr spectrum of trisnorcybrodolide.

The electron density released from the hydrogens enriches the electron population close to the C-7 methyl carbon causing it to be shielded in the ^{13}Cmr spectrum.

Further consideration of the ^{13}Cmr spectrum of trisnorcybrodolide allows a tentative assignment of the structure of this compound. The ^{13}Cmr ($CDCl_3$) spectrum of phthalide (20) has been assigned by MacLean. The shieldings (δ, ppm) are as follows[34].



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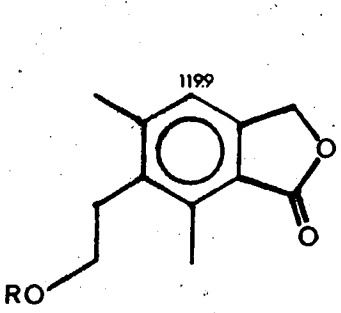
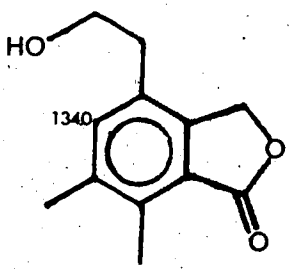
The carbonyl shielding agrees well with that observed for trisnorcybrodolide (δ171.1). The shift of C-3 allows assignment of the corresponding carbon (δ67.9) in trisnorcybrodolide. It therefore follows that the α and β carbons of the hydroxyethyl side chain of trisnorcybrodolide resonate at δ59.8 and δ32.1 respectively. The use of additivity parameters to estimate the ^{13}Cmr shieldings of aromatic carbons in cases where substituents are located *ortho* to one another is often subject to considerable error owing

to the unpredictable factor of steric interference between neighbouring groups[35]. Nevertheless, MacLean has successfully used additivity parameters to assign the ^{13}Cmr spectra of several phthalideisoquinoline alkaloids[34]. The empirical shielding parameters for an aromatic methyl group are: C-1 + 9.3 ppm, *ortho* + 0.8 ppm, *meta* 0 and *para* - 2.9 ppm[36]. No parameters are available for a hydroxyethyl group, however, for the purpose of crude estimation, the parameters for an ethyl group (C-1 + 15.6 ppm, *ortho* - 0.4 ppm, *meta* 0 and *para* + 2.9 ppm)[36] will suffice. The calculated shifts (δ_{ppm}) for the aromatic carbon bearing a hydrogen in each of the three possible structures (23a, 24, 25)* remaining for trisnorcybrodolide are as follows.



23a R = H

23b R = Ac



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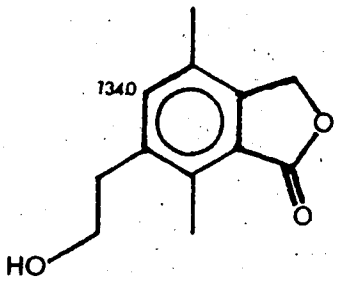

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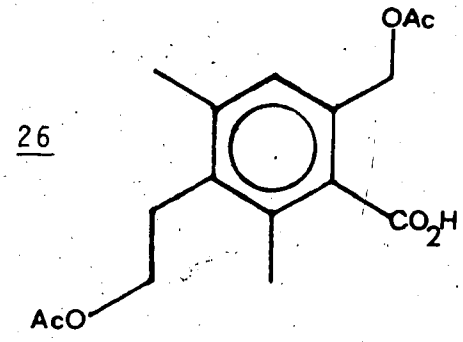
* Based on the constraint that only one methyl group may be located *ortho* to the aromatic hydrogen. Removal of this constraint admits three additional structural possibilities. Application of the above calculation to these compounds does not alter the conclusion reached below.

Since the calculations involve only the relatively small *ortho*, *meta* and *para* parameters, any discrepancies introduced by the substitution of an ethyl group for a hydroxyethyl group or by the neglect of steric and solvent factors will be minimal. The observed value for the carbon in question is δ121.3. The calculated value agrees with this figure only if the aromatic hydrogen is placed at C-4. Since a methyl group must be located *ortho* to this hydrogen, it follows that trisnorcybrodolide and acetyltrisnorcybrodolide have structures 23a and 23b respectively. The structures of the acid intermediate 26 from the ozonolysis of the triacetyl derivative (27b or 28b) of cybrodol (27a or 28a) may now be formalized.



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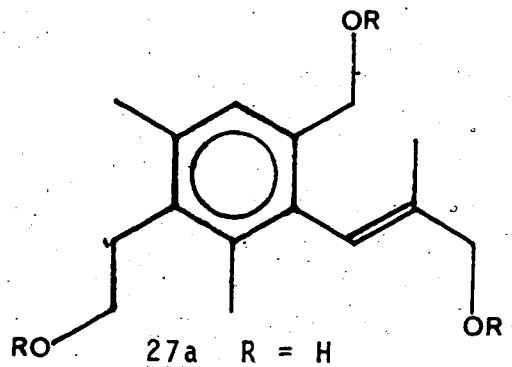
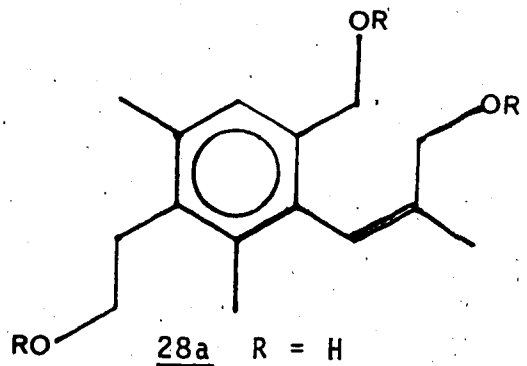


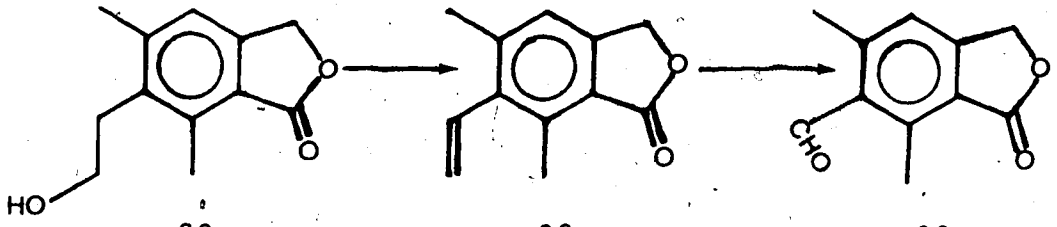
27a R = H
27b R = Ac



28a R = H
28b R = Ac

To buttress the above arguments which led to the tentative assignment of the ring substitution pattern of cybrodol (27a or 28a) and trisnorcybrodolide (23a), a second degradative sequence (Scheme 1) was undertaken.

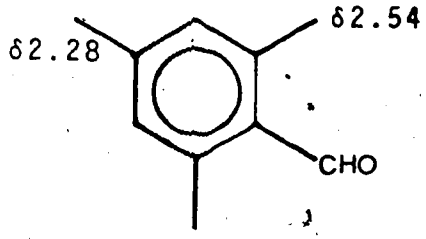
Scheme 1. Degradation of trisnorcybrodolide (23a).



23a 29 30

It was felt that conversion of the β-phenethyl alcohol system in 23a to a benzaldehyde derivative 30 via styrene 29 would provide confirmatory evidence for the proposed substitution pattern. Relative to 23a the formyl group should deshield both methyl groups in the ^{1}Hmr spectrum of 30[32]. The aromatic hydrogens in 30 and 23a should have approximately equal ^{1}Hmr shifts. In the case of compound 30 this hydrogen will not have a shift characteristic of an aromatic proton located *ortho* to a carbonyl group (δ7.6-8.0). Of the twelve possible arrangements of two aromatic methyl groups, one β-phenethyl alcohol function and one aromatic hydrogen on a phthalide nucleus, only

23a, when subjected to the degradation outlined in Scheme 1, will produce a derivative with the above properties. A benzaldehyde derivative was selected in preference to a methyl benzoate or a benzoic acid derivative after consideration of the tabulated ^{1}Hmr shifts ($CDCl_3$) of the methyl groups of three monosubstituted mesitylenes: 18, 31 and 32[25].



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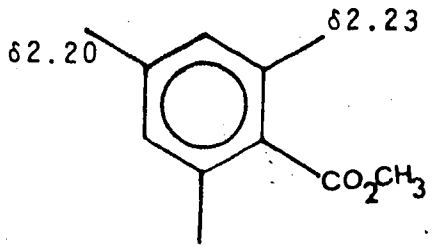
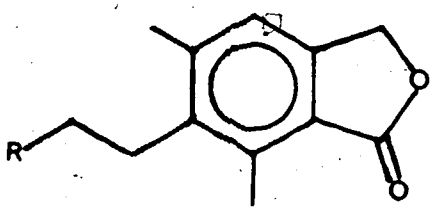


32

The ^{1}Hmr shift of an *ortho* methyl group is clearly most strongly influenced by a formyl group.

Three approaches to the preparation of 29 were investigated. Phosphorous oxychloride in pyridine has been used for the dehydration of alcohols[37]. This method was applied to the preparation of 29. Alcohol 23a was treated with phosphorous oxychloride in refluxing pyridine. The product, obtained in 64% yield, was chlorolactone 33 (mp 147-149°C). The formation of a chloride under these conditions is



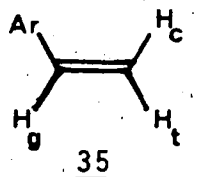
33 R = Cl

34 R = 4-NO_2-C_6H_4Se-

36 R = OMs

not unprecedented[38]. Attempted base induced dehydrochlorinations of 33 were unsuccessful. 1,5-Diazabicyclo[5.4.0]undec-5-ene (DBU) in refluxing toluene gave recovered 33 as did potassium t-butoxide in refluxing t-butyl alcohol.

Grieco has developed an effective primary alcohol dehydration method based on selenium chemistry[39]. In the presence of a phosphine condensing agent, primary alcohols and arylselenocyanates give selenides. These species are oxidized to selenoxides which fragment under mild conditions. The products are the dehydrated alcohol and an arylselenenic acid. Alcohol 23a was treated with 4-nitrophenylselenocyanate and tri-n-butylphosphine in tetrahydrofuran. A modest yield (43%) of selenide 34 was obtained after chromatography. Treatment of 34 with hydrogen peroxide in aqueous tetrahydrofuran gave a low (32%) yield of the required olefin 29 (mp 93-95°C) after preparative thin layer chromatography (ptlc). Spectral examination of this product readily confirms the formation of a vinyl group. In the ir spectrum bands at 1610, 1000 and 910 cm^{-1} are characteristic of this group[18]. A vinyl group 35 is clearly evident in the ^{1}Hmr spectrum. H_g appears



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as a doublet of doublets (J_{gc} = 18 Hz, J_{gt} = 11 Hz) centered at δ6.65. H_t is seen as a doublet of doublets (J_{gt} = 11 Hz, J_{ct} = 2 Hz) centered at δ5.65. A doublet of doublets (J_{ct} = 2 Hz, J_{gc} = 18 Hz) centered at δ5.24 is assigned to H_c.

In view of the rather low yields encountered in the above preparation of 29, an alternate route was explored. Treatment of alcohol 23a with methanesulfonyl chloride in pyridine gave 36 (mp 120-121°C) in 90% yield. Exposure of 36 to freshly distilled DBU in hot toluene gave, after ptlc purification, the elimination product 29 (21%) along with chlorolactone 33 (11%). Evidently 33 resulted from an S_N2 displacement of the mesyl group of 36 by chloride ion. The source of chloride ion is unclear. It could not have been introduced during the work-up sequence because tlc examination of the reaction mixture prior to work-up showed that 36 was absent and 33 was present. The DBU reagent was tested for the presence of chloride ion (perhaps in the form of DBU hydrochloride) as follows: an aqueous solution of DBU was acidified with nitric acid. Aqueous silver nitrate was added. No precipitate was observed.

Ozonolysis of 29 in methanol at -78°C was followed by reductive work-up (sodium iodide, acetic acid, methanol)[40]. Compound 30 (mp 159-160°C) was obtained

in 40% yield after ptlc purification. The formyl group is clearly evident in the ir (1683 cm^{-1}) and ^{1}Hmr (δ10.70) spectra of 30. The ^{1}Hmr shifts of the aromatic hydrogen (δ7.18) and aromatic methyl groups (δ2.69 (C-5 CH_3), δ2.98 (C-7 CH_3)) of 30 are in complete agreement with the above predictions. Relative to 23a, the aromatic hydrogen of 30 is only slightly deshielded (0.07 ppm) while the aromatic methyl groups are deshielded by roughly equal amounts (0.25 ppm (C-7 CH_3), 0.21 ppm (C-5 CH_3).

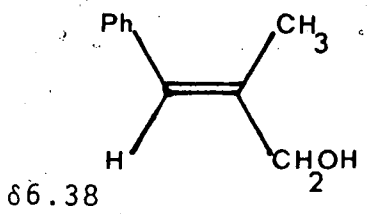
The determination of the double bond geometry of cybrodol (27a or 28a) was greatly facilitated by the fortuitous isolation of three closely related metabolites: isocybrodol, cybrodal and cybrodic acid. Moreover, spectral features of cybrodal and a cybrodic acid derivative supply further evidence for the aromatic substitution pattern determined for cybrodol (27a or 28a) and trisnorcybrodolide (23a).

Isocybrodol (mp 102-103°C), so named because it is an alcohol closely related to cybrodol (27a or 28a), eluted in Sephadex fractions 40-41 of the neutral extract. Isolation of pure isocybrodol required silica gel chromatography of Sephadex fractions 38-41, acetylation of the resultant crude isocybrodol, silica gel chromatography of the crude acetylation products, deacetylation and, finally, silica gel chromatography.

By tlc, isocybrodol has an R_f of 0.38 when developed with methylene chloride-methanol, 10:1. The colour reaction is very similar to that of cybrodol (27a or 28a), isocybrodol however eventually gives a yellow rather than a green spot.

Isocybrodol has a molecular formula of $C_{15}H_{22}O_3$ (mol. wt. 250). The ir spectrum of isocybrodol has strong hydroxyl (3290 cm^{-1}) and carbon-oxygen (1035, 1030, 1005 cm^{-1}) stretching bands. The uv spectrum of isocybrodol (λ_{max} (CH_3OH):210 (ϵ 6200), 270 nm (ϵ ~ 350)) is very similar to that of cybrodol (27a or 28a). Comparison of the ^{1}Hmr spectra of isocybrodol (Figure 3) and cybrodol (Figure 1) reveals that the two compounds share common aromatic nuclei . Isocybrodol has an aromatic hydrogen (δ7.05), aromatic methyl groups (δ2.16, 2.36) and a β-phenethyl alcohol moiety (δ2.97, 3.60) with chemical shifts practically identical with the same groups of cybrodol (27a or 28a). Whereas the benzyl alcohol methylene protons of cybrodol appeared as a broad singlet (δ 4.48), the corresponding protons of isocybrodol are seen as an AB quartet (J = 11 Hz) centered at δ4.44. Only the olefinic substituents of the two compounds have significantly different chemical shifts. The olefinic hydrogen (δ6.27) and allylic alcohol methylene group (AB quartet (J = 12 Hz), δ3.69) of isocybrodol are both

observed upfield relative to the analogous protons of cybrodol. The olefinic methyl group (δ2.02) of isocybrodol resonates at lower field than the corresponding group of cybrodol. These differences can be rationalized if the two compounds have opposite olefinic geometry. Consideration of the tabulated chemical shifts (CCl_4) of *E* (37)[41] and *Z* (38)[42]-2-methyl-3-phenyl-2-



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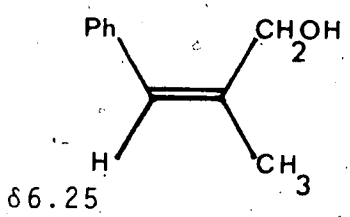
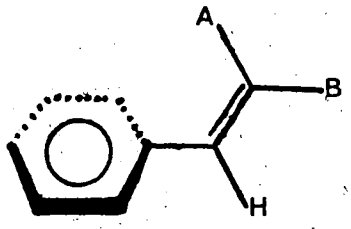


38

propenol allows tentative assignment of structure 27a to cybrodol and structure 28a to isocybrodol. Since the vinyl hydrogen appears further downfield in the case of cybrodol (27a), the *E* geometry in which the vinyl hydrogen is *cis* with respect to the hydroxymethyl group, is assigned to this metabolite.

Triacetylisocybrodol (28b, acetic anhydride-pyridine-methylene chloride) was subjected to the same sequence of ozonolysis and lactonization which was applied previously to triacetylcybrodol (27b). The product was identical in all respects (tlc, ir, ^{1}Hmr, ms) with 23a, proving the suggested *E*:*Z* relationship of cybrodol (27a) to isocybrodol (28a).

Comparison of the ^{1}Hmr shifts of the vinyl methyl groups and allylic alcohol methylene groups of cybrodol (27a) and isocybrodol (28a) indicates that the group located *trans* to the aromatic substituent is deshielded in both compounds while the group located *cis* to the aromatic substituent is shielded. Examination of space-filling models of 27a and 28a reveals the reason for this phenomenon. With two alkyl substituents located *ortho* to the olefinic group in these compounds it is impossible for the aromatic ring and the isobutenyl moiety to achieve coplanarity. Neither compound shows a styrene chromophore (λ_{max} (C_2H_5OH):248 (ε 14,000), 282 (ε 750), 291 nm (ε 500))[43] in the uv. This is consistent with a skewed relationship of the aromatic and olefinic systems in both compounds. Some degree of rotational restriction for the isobutenyl side chain also explains why in the case of isocybrodol (28a), the protons of the benzyl alcohol methylene group show non-equivalence*. This feature is precedented in biphenyl chemistry[44].

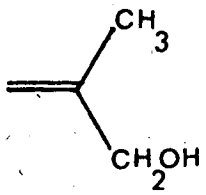


39

* Crystalline isocybrodol possessed no measurable rotation ($[\alpha]_D$ C 0.49 in CH_3OH).

Referring to diagram 39 it is clear that substituent A (*i.e.* the vinyl methyl group of 27a or the allylic alcohol methylene group of 28a) lies in the shielding region of the aromatic ring[45]. Substitutent B (*i.e.* the allylic alcohol methylene group of 27a or the vinyl methyl group of 28a) resides in the deshielding zone. If the tabulated ^{1}Hmr shieldings[46] ($CDCl_3$) of 2-methyl-2-propenol (40) are considered

40



δ1.71

δ3.92

as standards, then the aromatic ring shields the vinyl methyl group of 27a by 0.25 ppm and the hydroxymethyl group of 28a by 0.27 ppm. Likewise the hydroxymethyl group of 27a is deshielded by 0.31 ppm and the vinyl methyl group of 28a by 0.31 ppm.

A brief digression is in order at this point. Earlier, during the discussion of the ^{1}Hmr spectrum (Diagram 35) of compound 29, it was noted without comment that H_c, the proton located *cis* with respect to the aromatic substituent appears at higher field (δ5.24) than H_t (δ5.65), the proton located *trans* with respect to the aromatic substituent. The corresponding protons of styrene appear ($CDCl_3$) at δ5.70 and δ5.20 respectively[47]. One can apply the arguments implicit

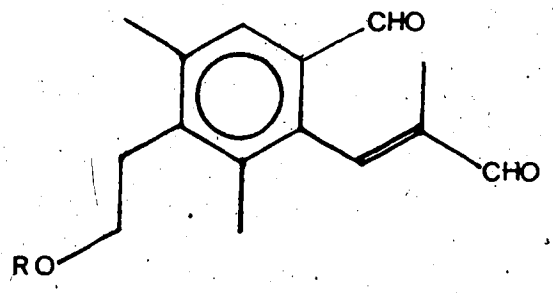
in diagram 39 to explain this anomaly. Thus H_t occupies position B and is deshielded relative to H_c which occupies position A. This phenomenon has been discussed by Stothers[47]. For example in the case of compound 17, H_t and H_c resonate ($CDCl_3$) at δ5.46 and δ5.20 respectively[47].

Cybrodal, an aldehyde related to cybrodol (27a), eluted in Sephadex fractions 40-41 of the neutral extract. Silica gel chromatography of Sephadex fractions 38-41 gave crude cybrodal which was again chromatographed over silica gel affording pure cybrodal as a yellow oil. By tlc, cybrodal has an R_f of 0.52 when developed with methylene chloride-methanol, 10:1. The colour reaction of cybrodal is characteristic: upon charring cybrodal gives a purple spot which slowly turns brown.

Cybrodal has a molecular formula of $C_{15}H_{18}O_3$ (mol. wt. 246). The molecular weight was confirmed by chemical ionization (NH_3) mass spectrometry which gave a peak at m/e 264 (M + 18). The ir spectrum of cybrodal shows hydroxyl (3450 cm^{-1}) and carbon-oxygen (1040 cm^{-1}) stretching bands. The presence of a single hydroxyl group was proven by the formation (acetic anhydride-pyridine-methylene chloride) of a monoacetyl derivative (mol. wt. 288) which lacks hydroxyl absorption in the ir. Bands at 2740 (w),

1688 and 1630 (w) cm^{-1} in the ir of cybrodal are indicative of the presence of an α,β-unsaturated aldehyde function.

Comparison of the ^{1}Hmr spectra of cybrodal ($CDCl_3$, Figure 4), cybrodol (27a) and isocybrodol (28a) allows tentative assignment of structure 41a to cybrodal. The



41a R = H

41b R = Ac

^{1}Hmr spectrum of cybrodal (41a) shows a β-hydroxyethyl function (δ3.10, 3.80), two aromatic methyl groups (δ2.26, 2.46), two aldehyde protons (δ9.75, 9.92) and a vinyl methyl group (δ1.57). Apparently the vinyl and aromatic protons have coincident chemical shifts. A broad two proton signal at δ7.61 is assigned to these hydrogens. Relative to cybrodol (27a) the vinyl and aromatic hydrogens are deshielded by 0.51 ppm and 1.16 ppm respectively. The former perturbation is consistent with an *ortho* relationship of the formyl group to the aromatic hydrogen in 41a. The latter deshielding reflects the β position of the vinyl hydrogen in an α,β-unsaturated system. Based on the relative chemical

shifts of the vinyl methyl groups of 27a (δ1.46) and 28a (δ2.02), the observed shift of δ1.57 for cybrodal (41a) suggests the *E* olefinic geometry.

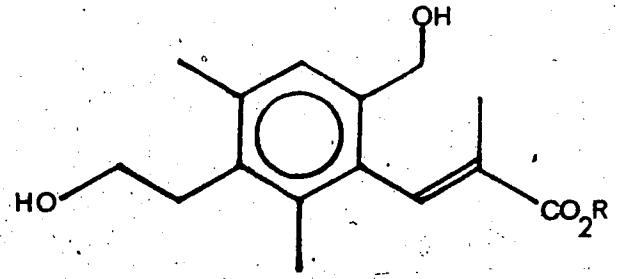
The proposed relationship of cybrodal (41a) to cybrodol (27a) was proven by two correlations. Manganese dioxide oxidation of 27a gave a product identical in all respects (tlc, ^{1}Hmr, ms) with cybrodal (41a). Acetylcybrodal (41b) was reduced with lithium aluminum hydride. The product was identical (tlc, nmr) with cybrodol (27a).

Cybrodic acid (mp 176-178°C), a carboxylic acid related to cybrodol (27a), eluted in Sephadex fractions 39-41 of the acidic extract. Ptlc of these fractions (toluene-acetone-acetic acid, 75:25:1) gave pure cybrodic acid, R_f 0.14. Cybrodic acid produces a characteristic red spot when charred.

Cybrodic acid has a molecular formula of $C_{15}H_{20}O_4$ (mol. wt. 264). The ir spectrum shows hydroxyl (3320 cm^{-1}) and carbon-oxygen (1040, 1030 cm^{-1}) stretching bands. Absorption at 2600 (broad), 1693 and 1640 cm^{-1} is indicative of the presence of an α,β-unsaturated carboxylic acid function. Treatment of cybrodic acid with diazomethane afforded a monomethyl ester, confirming the presence of a carboxylic acid function. Methyl cybrodate has a molecular formula of $C_{16}H_{22}O_4$ (mol. wt. 278). The α,β-unsaturated ester moiety is evident in

the ir spectrum (1715, 1640 cm^{-1}). Isolation of cybrodic acid by ptlc was an inefficient process, consequently the acid was normally obtained in the form of its methyl ester derivative. Thus Sephadex fractions 39-41 of the acidic extract were treated with diazomethane. Silica gel column chromatography gave methyl cybrodate as a yellow oil.

Comparison of the ^{1}Hmr spectra of cybrodic acid (CD_3OD, Figure 5), 27a and 41a leads to the assignment of structure 42a to cybrodic acid. Cybrodic acid (42a)

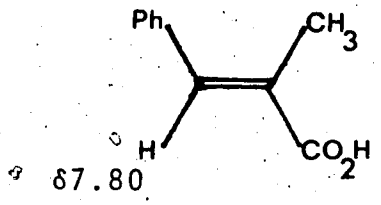


42a R = H

42b R = CH_3

and cybrodol (27a) clearly have common aromatic substituents. The chemical shifts of the two aromatic methyl groups (δ2.19, 2.38), the β-hydroxyethyl function (δ2.96, 3.61), the benzyl alcohol methylene group (δ4.38) and the aromatic hydrogen (δ7.15) of cybrodic acid (42a) do not differ significantly from the chemical shifts of the corresponding features of 27a. The allylic alcohol methylene group is not evident in the ^{1}Hmr spectrum of 42a. The vinyl hydrogen (δ7.65) and vinyl methyl group (δ1.60) have shifts comparable to the analogous groups of 41a. Irradiation of the

vinylic proton of cybrodic acid collapses the vinyl methyl group signal to a singlet, hence the identification of the vinylic proton signal is unambiguous. These facts are consistent with the presence of an α-methyl cinnamic acid moiety in cybrodic acid (42a). Since the vinyl methyl group ^{1}Hmr shifts of cybrodic acid (42a) and cybrodal (41a) are very similar, the *E* geometry is suggested for the acid. Consideration of the vinyl hydrogen ^{1}Hmr shifts ($CDCl_3$) of *E* (43) and *Z* (44) α-methylcinnamic acid[48] affords more compelling evidence



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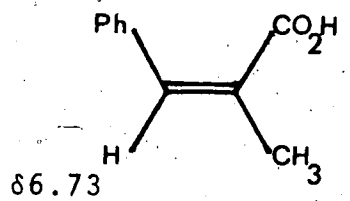
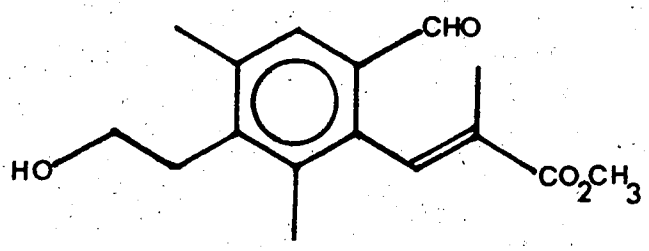


44

for the *E* geometry.

Methyl cybrodate (42b) was correlated with 27a. Lithium aluminum hydride reduction of 42b afforded material identical with 27a (tlc, ^{1}Hmr, ms).

Manganese dioxide oxidation of 42b gave the benzaldehyde derivative 45. The aromatic hydrogen (dis-

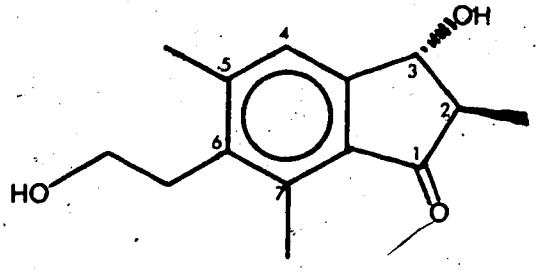


45

tinguished from the vinyl hydrogen (δ7.80) by a decoupling experiment) appears at δ7.60 in the ^{1}Hmr spectrum of 45. This proton appears 0.46 ppm downfield from the position of the same hydrogen of compound 42b (δ7.14) confirming that the free active alcohol function of 42b is benzylic and *ortho* to the aromatic hydrogen[32].

The uv spectrum of 42b (λ_{max} (CH_3OH):215 (ε 18,000), 258 nm (ε 4300)) reveals that the benzene and acrylate chromophores of 42b are twisted out of conjugation as are the benzene and vinyl chromophores of 27a and 28a. The uv spectrum of 42b is the superposition of an acrylate chromophore and a benzene chromophore and not that of a *trans*-cinnamate (λ_{max} (C_2H_5OH) 275 nm (ε~ 20,000))[29].

When Sephadex fractions 43-46 of the neutral extract were allowed to slowly evaporate, a metabolite (mol. formula $C_{14}H_{18}O_3$) identified as (2*R* , 3*R*)-pterosin C (46)[49,50] generally crystallized as clear needles (mp

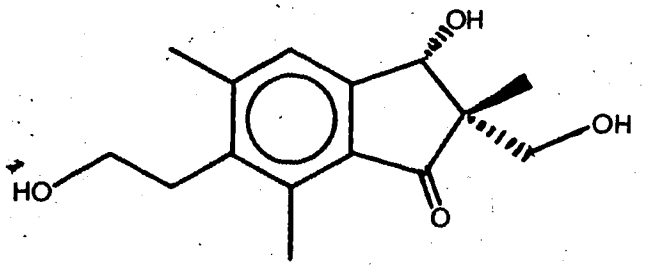


46

160-162°C). Roughly two dozen 1-indanone sesquiterpenes and norsesquiterpenes with the skeleton of 46 and collectively known as pterosins have been isolated by

Japanese workers[50,51] from extracts of the fern *Pteridium aquilinum* var. *latiusculum*. Reported[50] spectral (ir, ^{1}Hmr (CD_3OD)) and physical (mp 162-164°C) data of 46 closely matched that of the *C. bulleri* metabolite. The relative stereochemistry of the C-2 methyl group (δ1.30) and the C-3 hydroxyl group followed from the small (4 Hz) vicinal coupling constant between the C-2 (δ2.5) and C-3 (δ4.67) hydrogens. In the case of the (2*R* , 3*S*) isomer of 46, the reported[51] coupling constant is 6.8 Hz, while a value of 3.8 Hz is quoted[50] for 46. The reported chiroptical properties of 46[50,52] ($[\alpha]_D^{25}$ -65.3° (c 0.59, CH_3OH); cd (c 0.024, CH_3OH): $[\theta]_{325}$ -17,200) agree well with those observed ($[\alpha]_D^{25}$ -61° (c 0.36, CH_3OH); cd (c 0.02, CH_3OH): $[\theta]_{325}$ -15,000) for the *C. bulleri* metabolite.

Pterosin C (46) is a norsesquiterpene of the illudalane class[5,53]. (2*S* , 3*S*)-Pterosin L (47), the antipode of a sesquiterpene isolated[51] from the afore-



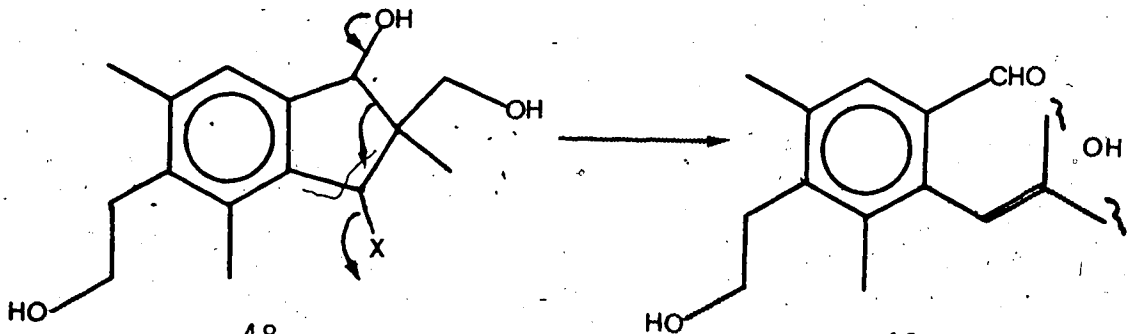
47

mentioned *Pteridium* fern, is a possible biogenetic

precursor to pterosin C* (46). *In vivo* oxidation of the C-2 hydroxymethyl group of 47 and decarboxylation could lead to 46.

The family of *C. bulleri* metabolites 23a, 27a, 28a, 41a and 42a are collectively known as cybrodins[1,53]. The fifteen carbon cybrodins (27a, 28a, 41a and 42a) bear an obvious familial resemblance to the pterosins. One can imagine production by the fungus of an intermediate (48) closely related to 47. Cleavage (Scheme 2) could

Scheme 2. Relationship between the pterosins and the cybrodins.



48 49

lead to the *seco* derivative 49. Adjustment of the oxidation levels would then give rise to the cybrodins. *In vivo* cleavage (*cf. in vitro* transformation of 27a and 28a to 23a) of the olefinic linkage presumably

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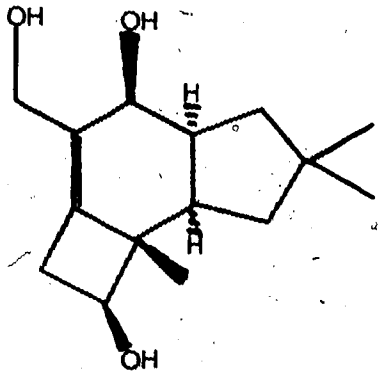
* The name pterosin C is applied to four different compounds arising from combinations of configurations at C-2 and C-3. All four compounds have been isolated from the *Pteridium* fern[50-52].

leads to trisnorcybrodolide (23a). The cybrodins can be classified as *seco*-illudalane sesquiterpenoids. Compounds of this skeletal class have not been reported previously.

The biogenesis* of the illudalane skeleton (50) from farnesyl pyrophosphate (51) is outlined in Scheme 3. Cyclization of 51 gives humulene (52), which cyclizes to the protoilludane cation (53). Illudol (54), elaborated by the fungus *Clitocybe illudens*[54] is repre-



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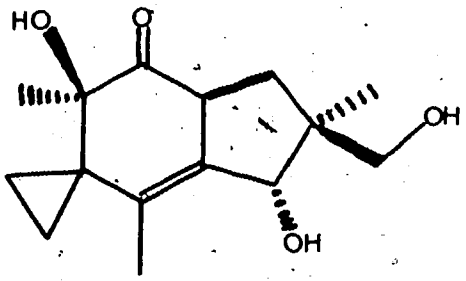

56

sentative of the protoilludane structural class. Bond migration (path a) leads to the illudane cation (55). Illudin S (56) obtained from the toadstool *Lampteromyces japonicus* is one member of the illudane family of sesquiterpenoids. Ring opening of 53 (path b) or 55 by nucleophile Z affords the illudalane skeleton (50).

The ^{13}Cmr spectrum of cybrodol (27a) was partially

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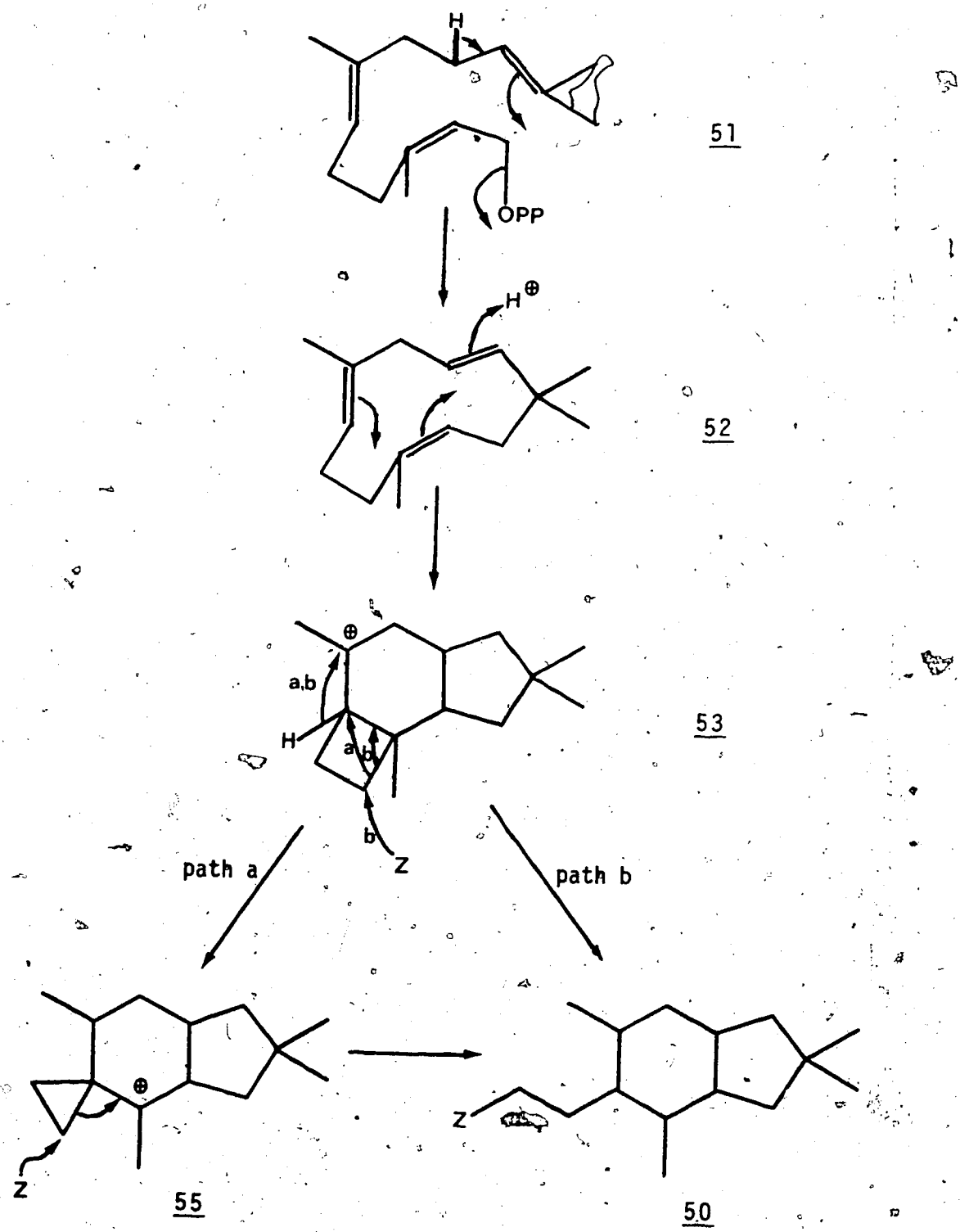
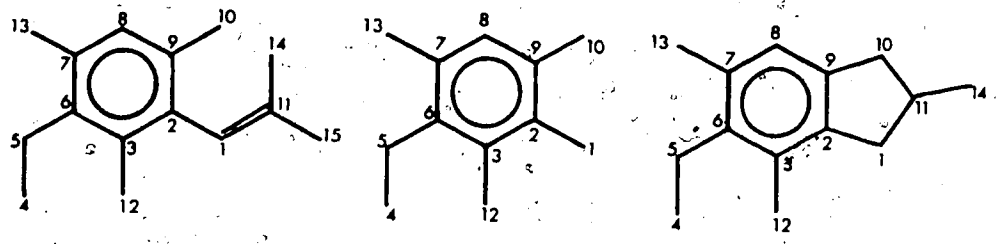
* For a complete discussion of the biogenesis of fungal sesquiterpenoids see reference 53.

Scheme 3. Illudoid biogenesis.



assigned by selective heteronuclear decoupling. Comparison with 27a allowed partial assignment of the ^{13}Cmr spectra of 28a, 42a, 42b, 45 and 46. In the interests of clarity, the numbering system of Nakanishi[55] (Scheme 4), which is based on illudoid biogenesis (Scheme 3)

Scheme 4. Numbering system for ^{13}Cmr assignments.



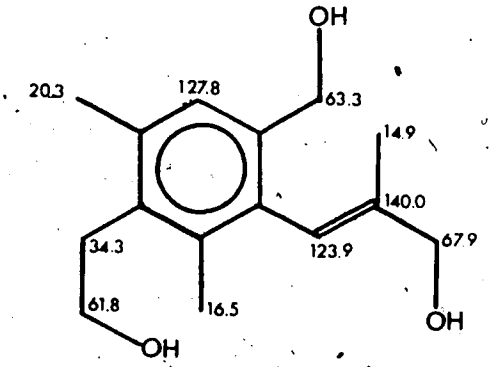
27a, 28a, 42a, 42b, 45 23a 46

will be adopted for the course of this discussion*. The partial ^{13}Cmr assignments for the above mentioned compounds as well as trisnorcybrodolide (23a) are shown in Scheme 5.

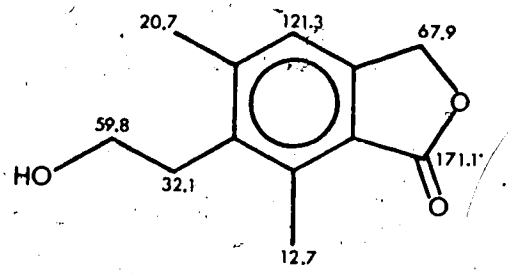
The assignable carbons of cybrodol (27a) fall into four groups. C-5 is the only non-oxygenated methylene group in the molecule, hence the peak at δ34.3 is assigned[17] to this carbon. The three oxygenated methylene group signals[17] (δ61.8, 63.3, 67.9) account for C-4, 10 and 15 respectively. Two selective ^{13}C-^{1}H decoupling

* This numbering scheme must not be confused with those applied previously to 23a and 46 or with the IUPAC numbering scheme used in the experimental section.

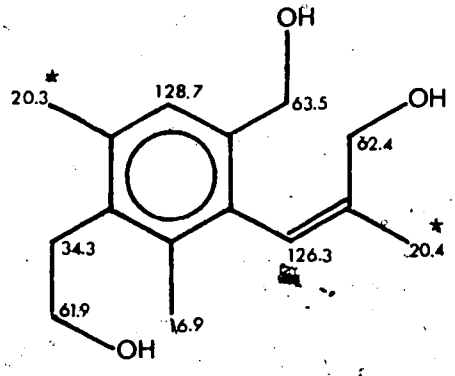
Scheme 5. Partial ^{13}Cmr (δ ppm) assignments (solvent in brackets).



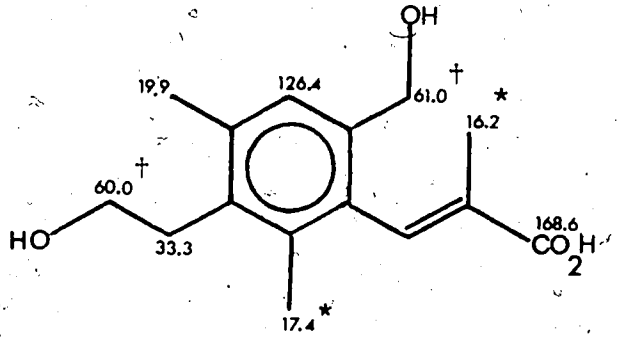
27a (CD_3OD)



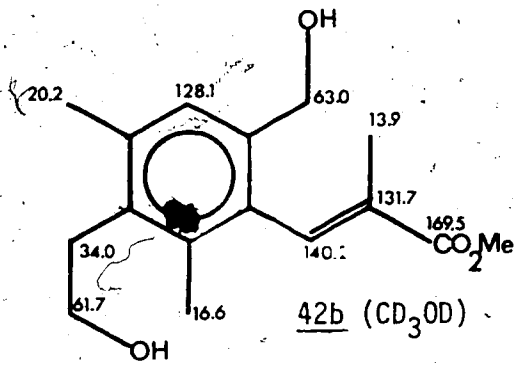
23a (DMSO-d_6)



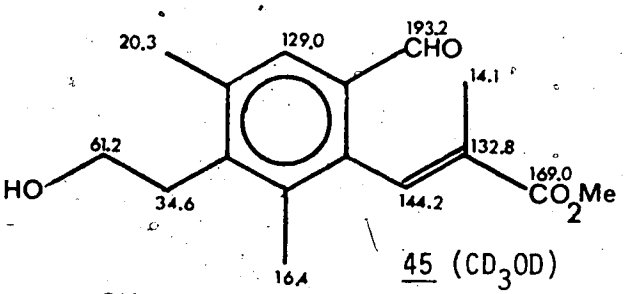
28a (CD_3OD)



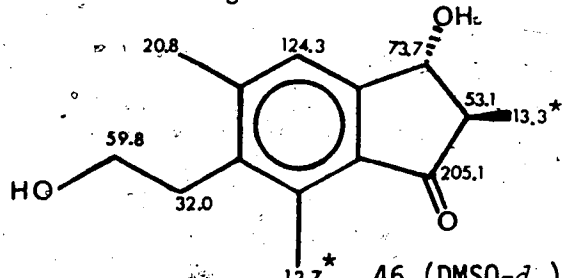
42a (DMSO-d_6)



42b (CD_3OD)



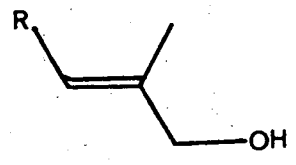
45 (CD_3OD)



46 (DMSO-d_6)

* † may be interchanged

experiments allowed assignment of these signals. Similarly, two selective decoupling experiments were sufficient to assign the three methyl group signals[17] (δ14.9, 16.5, 20.3) to C-14, 12 and 13 respectively. Finally, the two methine group signals (δ123.9, 127.8) were attributed to C-1 and 8 respectively by one decoupling experiment. The remaining six fully substituted carbon signals cannot be assigned with any degree of certainty although tentative assignment of C-11 (δ140.0) follows from consideration of the ^{13}Cmr spectrum of 42b (*vide infra*). In the case of isocybrodol (28a), comparison with 27a allows ready assignment of C-4, 5, 12, 13 and 15. Assignment of C-1, 8, 10 and 14 required two additional selective decoupling experiments. Unambiguous assignment of the ^{13}Cmr signals caused by the allylic alcohol methylene groups of 27a (C-15) and 28a (C-14) provides additional evidence for the proposed olefinic geometries of 27a and 28a. In a series of trisubstituted primary allylic alcohols, it is observed[56] that the α carbon of the *E* isomer (57) consistently



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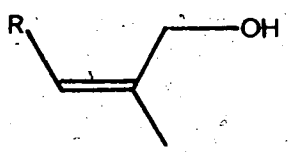


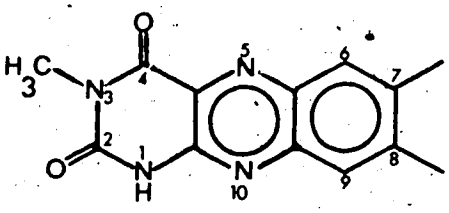
58

appears at lower field (*ca.* δ65-69 ppm) than the α carbon

of the Z isomer (58, *ca.* δ60-63 ppm). Assignment of the spectra of 42b and 45 follows readily from comparison with 27a. The transformation of an allylic alcohol to an α,β-unsaturated ester (27a → 42b) is expected to deshield C-1 by *ca.* 16 ppm while C-11 should be shielded by *ca.* 10 ppm[57]. In the ^{13}Cmr spectrum of 42b a methine group signal (δ140.2), attributable to C-1, is observed 16.3 ppm downfield from the position of C-1 in 27a. In the spectrum of 27a, there is a fully substituted carbon signal (δ140.0) which appears to be shifted upfield by 3 ppm to δ131.7 in the spectrum of 42b. These signals are tentatively assigned to C-11. The transformation of benzyl alcohol to benzaldehyde perturbs the ring carbons as follows: C-1 (-)3.3 ppm, *ortho* (+)2.6 ppm, *meta* (+)2.6 ppm and *para* (+)7.4 ppm[35]. On this basis, it was hoped that comparison of the ^{13}Cmr spectra of 42b and 45 would allow assignment of some of the ring carbons of these compounds, however no meaningful correlations could be ascertained. The ^{13}Cmr spectrum of 42a was assigned after comparison with 23a, 27a and 42b. In the case of pterosin C (46), C-4, 5, 8, 12 and 13 were assigned after comparison with 23a. The remaining four carbons (C-1, 10, 11 and 14) were assigned by inspection[17].

During the final chromatographic purification of trisnorcybrodolide (23a), a highly uv active (λ_{max}

(CH_3OH): 250, 337, 383 nm) substance* (mp > 300°C), with a molecular formula of $C_{13}H_{12}N_4O_2$ was isolated. This compound was identified as 3-methyllumichrome (59), a riboflavin (60) derivative not previously



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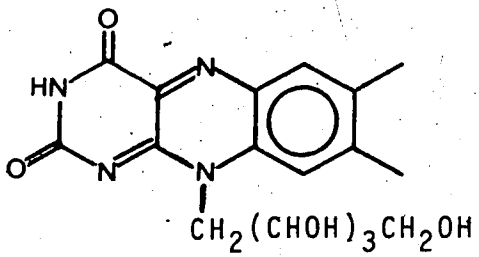
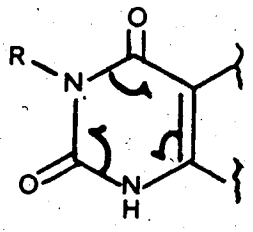


60

reported as a natural product. 3-Methyllumichrome (59) has been synthesized[58,59].

Literature spectral data[59] of 59 closely matched that of the *C. bulleri* metabolite. The only ambiguity was in the position of the N-methyl group. Compounds containing structural fragment 61 are known to undergo



61

a retro Diels-Alder mass spectral fragmentation as indicated. A peak at M-(R-N=C=O) is usually prominent in the ms of compounds of this type[60]. In the ms, the *C. bulleri* metabolite shows a strong loss of C_2H_3NO

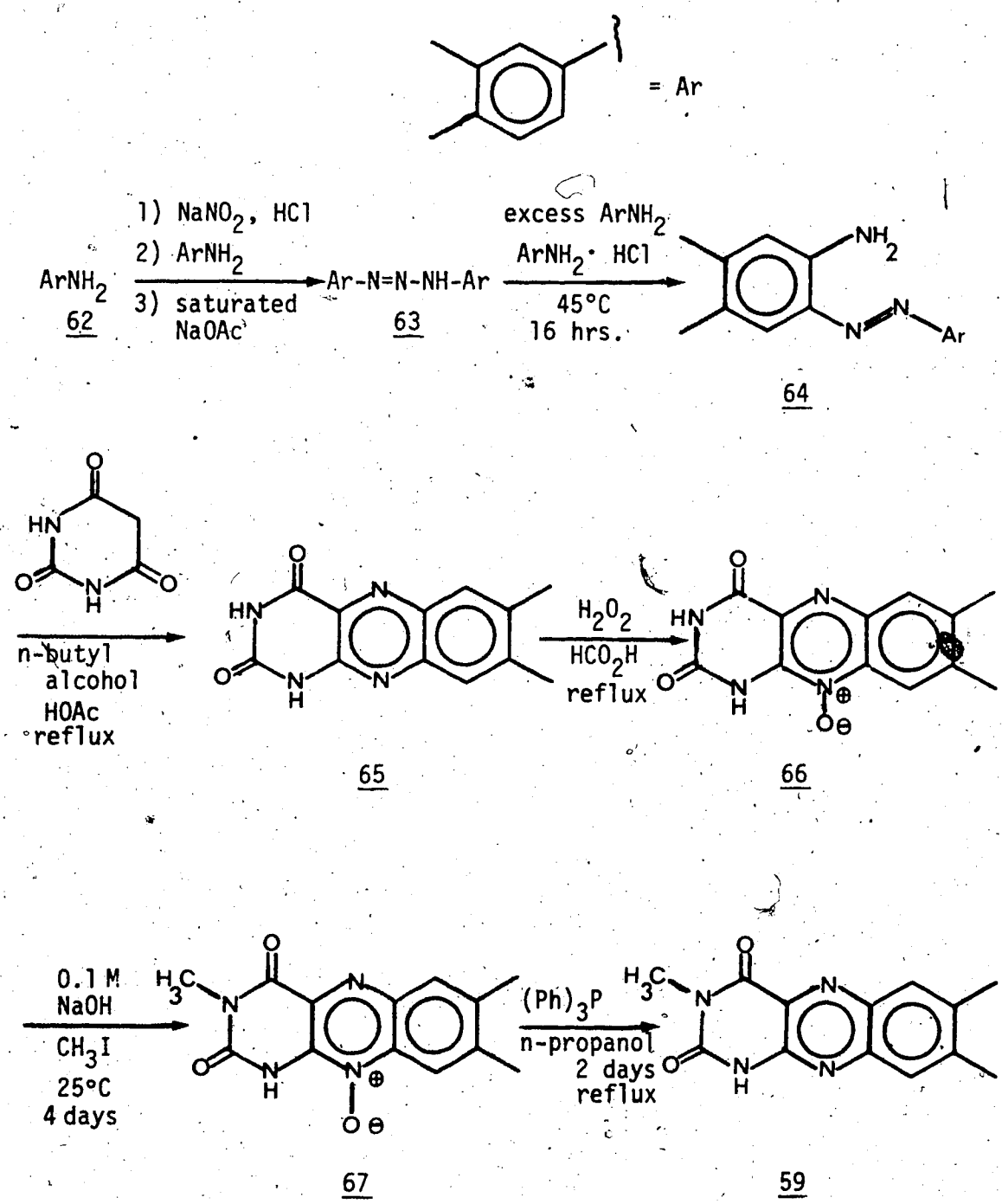
* This material was also produced by *C. bulleri* 6620 before this strain lost its viability.

(m/e 199) and C_2H_3NO + CO (m/e 171). This information serves to locate the methyl group at the 3 position.

As a final structural proof, 3-methyllumichrome (59) was synthesized. The route employed (Scheme 6) closely follows that of Berezovskii[61-64] who has synthesized a series of analogues of 3-methyllumichrome (59).

3,4-Xylidine (62) was added to the diazonium salt of the same amine in the presence of a sodium acetate buffer. The diazoamino compound (63) was formed in 91% yield. Compound 63 was isomerized in 64% yield to the phenylazoaniline derivative 64 by heating in the presence of a large excess of 62 plus a small amount of 3,4-xylidinium hydrochloride. Compound 64 readily condensed with barbituric acid affording a good (73%) yield of lumichrome (65). The first three reactions in this synthesis exactly duplicate the work of Berezovskii[61,62]. The Russian group performed the next two reactions in this sequence using the 8-desmethyl and 7,8-desmethyl analogues of 65 [63,64]. Lumichrome (65) was treated with hydrogen peroxide in boiling formic acid. Oxidation occurred preferentially[63] at the electron-rich 10 position giving 66 (83%). Methylation occurred at the less hindered 3 position affording a modest yield (41%) of 67. Berezovskii successfully reduced the N-oxide of the 8-desmethyl and 7,8-des-

Scheme 6. Synthesis of 3-methyllumichrome (59).



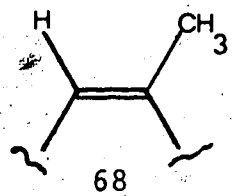
methyl analogues of 67 by treatment with alkaline sodium bisulfite at 50°C[64]. These results could not be reproduced in the case of 67. Instead, 59 was prepared in 80% yield by heating a n-propanol solution of 67 and triphenylphosphine for two days. The synthetic compound so produced was identical in all respects (tlc, ir, ^{1}Hmr, ms) with the natural material.

Broderol (mp 113-115°C), another minor component of the *C. bulleri* neutral extract, eluted in Sephadex fractions 42-43. Silica gel chromatography of these fractions gave pure broderol. This compound was observed in the extracts of two early cultures grown on Brodie's medium. It was absent from later extracts. By tlc, broderol has an R_f of 0.66 when developed with methylene chloride-methanol, 10:1. Broderol gives a purple spot when charred.

Broderol has a molecular formula of $C_{15}H_{22}O_2$ (mol. wt. 234). The ir spectrum displays hydroxyl (3400 cm^{-1}) and carbon-oxygen (1090, 1070, 1060, 1020 cm^{-1}) stretching bands. No strong bands are present in the carbonyl region, hence broderol must be a diol or an ether-alcohol.

The ^{13}Cmr spectrum ($CDCl_3$) of broderol shows two sp^2 carbons (δ127.5, 133.7)[17]. Off-resonance decoupling reveals that one of these carbons (δ127.5) bears a single hydrogen, the other sp^2 carbon is fully substi-

tuted. Broderol therefore contains a trisubstituted doublebond. This is supported by ir bands[18] at 1660 (w) and 800 cm^{-1}. The ^{1}Hmr spectrum ($CDCl_3$, Figure 6) is also in agreement with this deduction. One vinylic proton is observed at δ5.70 as a broad singlet. Irradiation of this proton collapses the three proton doublet (J = 1 Hz) at δ1.49 to a sharp singlet. This indicates that the vinylic proton is allylically coupled to a methyl group. Part structure 68 is implicated.



68

The ^{13}Cmr spectrum has three signals (δ74.0, 76.1, 79.4) in the sp^3 oxygenated carbon region[17]. One of these signals (δ74.0) represents a methylene carbon, the other two represent quaternary carbons. In the ^{1}Hmr spectrum, two protons geminal to oxygen are observed. A pair of double doublets at δ3.25 (J = 10.8, 2.5 Hz) and δ3.52 (J = 10.8, 1 Hz) indicate a geminate pair of hydrogens mutually coupled by 10.8 Hz. This coupling was proven by a double irradiation experiment. In the off-resonance decoupled ^{13}Cmr spectrum, twenty-one carbon-bound hydrogens can be accounted for. Consequently broderol has one active alcoholic hydrogen.

The alcohol function is either primary or tertiary.

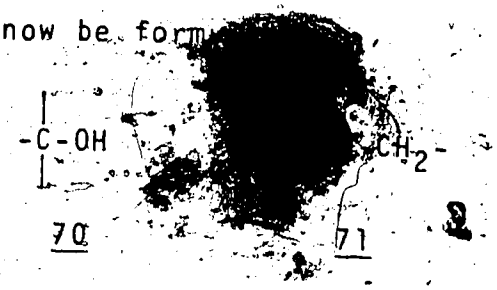
In an effort to determine the nature of the hydroxyl group of broderol, acetylation was attempted without success. Pyridine-acetic anhydride in boiling chloroform (overnight) gave recovered starting material as did acetic anhydride in the presence of 4-dimethylaminopyridine (25°, eleven days)[65]. Resistance to acetylation strongly suggests that the alcohol function is tertiary.

The molecule was successfully derivatized with trichloroacetyl isocyanate (TCAI). This reagent is known[66] to functionalize even highly hindered tertiary alcohols. TCAI was added to a deuteriochloroform solution of broderol in a nmr tube. A carbamate derivative (69) of broderol formed instantly. The

69

excess reagent gives no ^{1}Hmr signals. A single carbamate NH signal (1 H, δ8.25) is seen in the ^{1}Hmr spectrum of 69, proving that broderol has only one hydroxyl group. Furthermore, since the protons geminal to oxygen have virtually identical chemical shifts in the cases of broderol and its carbamate derivative (69), the alcohol function must be tertiary. The carbinol protons

of a primary alcohol are usually shifted downfield by 0.5-0.9 ppm after TCAI derivatization[66]. It follows that the methylene group bonded to oxygen must be part of a primary-tertiary ether moiety. Part structures 70 and 71 can now be formed.

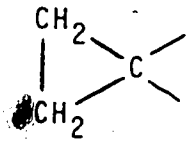


70 71

The most striking feature of the ^{13}Cmr spectrum of broderol is the presence of two methylene group signals at unusually highfield (δ6.3, 8.7). Only two types of carbon-bound methylene groups are known to resonate at such high field[17]. Methylene groups located α to a triple bond appear at high field. For example, C-2 of 3-heptyne appears at δ13.2 while C-2 of heptane appears at δ23.0[67]. Acetylenic carbons resonate in the range δ70-90[68]. The ^{13}Cmr of broderol displays three signals in this region. However, since one of the two fully substituted carbon signals in this region is accounted for in the tertiary alcohol function (70) known to be present in broderol, a triple bond can be excluded.

Cyclopropyl methylene carbon signals are usually observed in the region δ(-)10-(+)10[17,69]. If the two high field signals in the ^{13}Cmr spectrum of broderol

represent such carbons, then either part structure 72 or 73 is present. The ^{1}Hmr spectrum of broderol has



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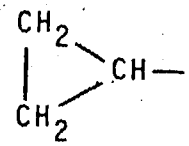
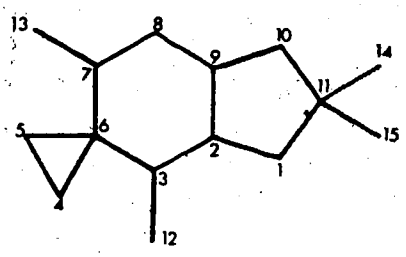


73

four complex one proton multiplets (δ0.54, 0.68, 0.82, 0.95) in the region where cyclopropyl protons commonly resonate. The methine carbon signal of 73 should appear in the region δ5-15 of the ^{13}Cmr spectrum[17,69], but no methine carbon signals are seen in this region. However, one quaternary carbon of broderol resonates at unusually high field (δ27.9)[17]. Fragment 72 is therefore indicated.

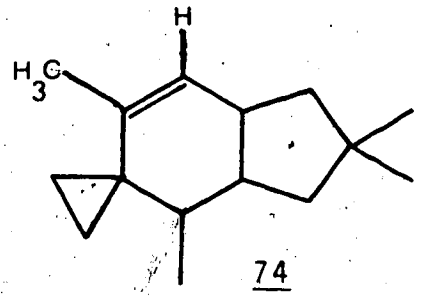
Given the demonstrated proclivity of *C. bulleri* to produce metabolites derived from the illudoid biogenetic pathway (Scheme 3), it is reasonable to assume that broderol possesses the illudane skeleton 55a. Indeed, this is the only known sesquiterpenoid skeleton



55a

capable of accommodating fragment 72[5,70]. Skeleton 55a will be used as a working hypothesis for the balance of this discussion.

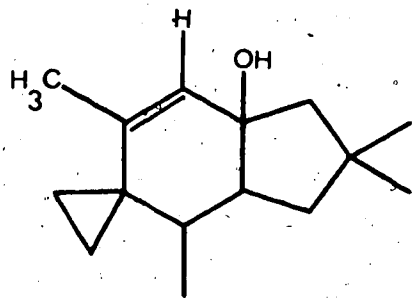
Fragment 68 can be uniquely located (C-7, C-8) giving rise to part structure 74 for broderol. The



74

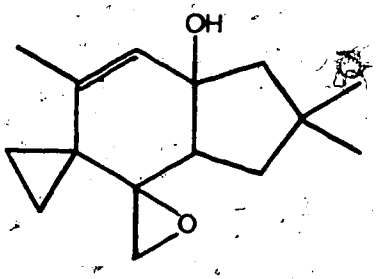
tertiary alcohol, fragment 70, can be located at C-2, 3 or 9. This function is placed at C-9 on the basis of the following considerations. The electronegative carbamate function of derivative 69 is expected to deshield nearby protons[66]. A comparison of the chemical shifts of the vinyl hydrogen of broderol (δ5.70) and derivative 69 (δ6.11) reveals that this hydrogen is substantially deshielded (0.41 ppm) by the carbamate group. The methyl groups are not appreciably deshielded. This suggests that the tertiary hydroxyl group (70) and the vinyl hydrogen of broderol are in close proximity. The pyridine induced chemical shift method has been used to locate hydroxyl groups. In general, hydrogens which are proximate to a hydroxyl function display Δ values ($\delta_{CDCl_3} - \delta_{C_5D_5N}$) in the range (-)0.15 - (-)0.40[71]. In the case of broderol, the Δ value for

the yinyl hydrogen is -0.32. This data strongly suggests that the hydroxyl group (70) is vicinal to the vinyl hydrogen. Partial structure 75 can now be drawn.



75

Fragment 71 may now be introduced. The tertiary oxygenated carbon of 71 must be either C-2 or 3. The primary oxygenated carbon can be C-12, 14 or 15. Application of these constraints allows structures 76 - 79 to be formulated. Each structure satisfies the



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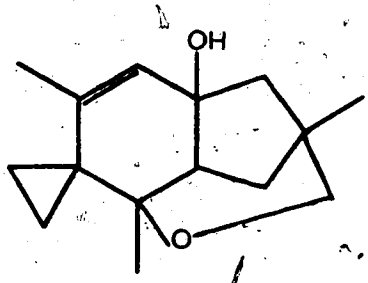

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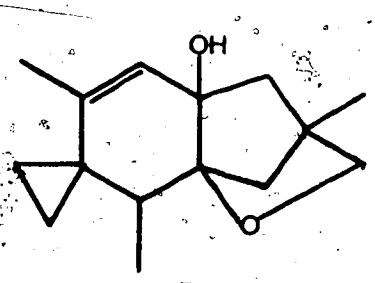

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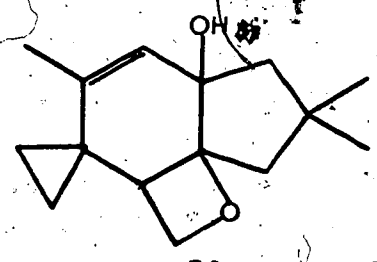

79

molecular formula of $C_{15}H_{22}O_2$. Structure 78 can be discounted immediately. The C-12 methyl group of 78 is secondary. With the exception of the vinyl methyl group, the methyl groups of broderol (^{1}Hmr ($CDCl_3$): δ0.88, 0.97) are tertiary.

Structure 76 contains an epoxide function. The mutual coupling constant for a pair of geminal epoxy protons is typically 4-6 Hz[72]. For example the epoxy protons of 4 display a geminal coupling constant of 4 Hz[11]. In the case of broderol, the geminal coupling constant of the methylene protons of fragment 71 is 10.8 Hz, considerably greater than the value anticipated for structure 76. On this basis 76 can be excluded.

The ^{1}Hmr spectrum ($CDCl_3$) of broderol exhibits a complex five proton multiplet in the region δ1.5-2.0. The protons causing this pattern are insufficiently resolved (even at 400 MHz) to allow the elucidation of a coupling pattern. However, when the spectrum is recorded in pyridine-d_5, these signals are well resolved. The coupling pattern (Scheme 7) determined (at 400 MHz) for these protons identifies stereostructure 77a* as the correct† structure of broderol.

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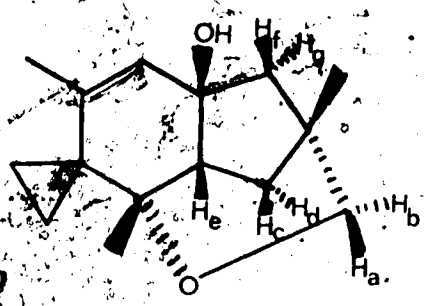
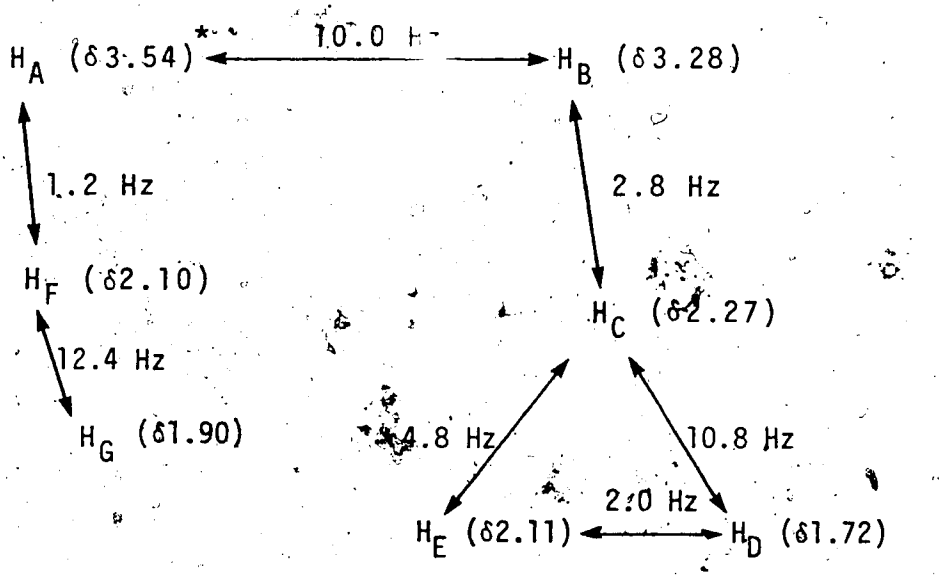
* Attempts to construct Dreiding molecular models of 77 will quickly convince one that 77a is the only possible relative stereostructure arising from 77.

† Contingent, of course, on the above biogenetic hypothesis.



77a

Scheme 7. Coupling pattern for broderol (77a).



* Recorded in C_5D_5N.

H_E (δ2.11), the ring junction proton has *cis* (4.8 Hz) and *trans* (2.0 Hz) couplings to H_C (δ2.27) and H_D (δ1.72) respectively. These protons are geminally coupled (10.8 Hz) and H_C has an additional four bond W coupling[73] (2.8 Hz) to H_B (δ3.28) which in turn is geminally coupled (10.0 Hz) to H_A (δ3.54). H_A has a four bond W coupling[73] (1.2 Hz) to H_F (δ2.10) which is geminally coupled (12.4 Hz) to H_G (δ1.90). H_F and H_C are both *cis* with respect to the hydroxyl group and are therefore deshielded relative to their geminal partners[71]. Structure 79 is inconsistent with Scheme 7. If 79 was the correct structure, H_A and H_B would both be coupled to a methine proton (H_E).

Tlc of the neutral extract of *C. bulleri* reveals a prominent blue coloured spot at R_f 0.50 (benzene-ether, 1:1). The same component is present in the extracts of *C. pygmaeus*[11]. Attempts to purify the compound responsible for this spot using extracts from either fungus by silica gel column chromatography led to the isolation of a mixture of two compounds. The so-called "blue compound" was always contaminated by a second component which by tlc exhibits a grey coloured spot at R_f 0.80 (benzene-ether, 1:1). The so-called "grey compound" was not present in the original extracts. The ms of this mixture indicated the presence of two compounds with molecular formulae $C_{15}H_{18}O_3$ (mol. wt.

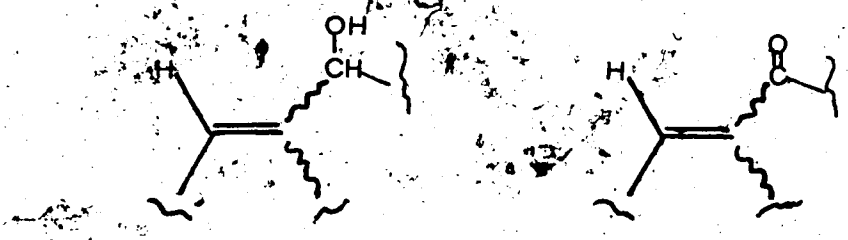
246) and $C_{15}H_{20}O_3$ (mol. wt. 248). Further purification by silica gel column chromatography invariably caused the disappearance of the "blue compound" and the isolation of the pure "grey compound" (yellow semi-solid), known henceforth as nidulone. Nidulone is a ketone (ir 1705 cm^{-1}) with a molecular formula of $C_{15}H_{18}O_3$. Dr. Reffstrup managed to obtain a small sample of the "blue compound" (slightly contaminated by nidulone) by ptlc. The "blue compound" hereafter called nidulol is an alcohol (ir 3400 cm^{-1}) with a molecular formula of $C_{15}H_{20}O_3$. Examination of the nidulol sample by tlc, ir and ^{1}Hmr several months after its isolation revealed a mixture of nidulol and nidulone (1:1)[11]. Apparently then, nidulone is an artifact resulting from oxidation of nidulol. This transformation is accelerated during chromatographic purification.

Nidulone has seven sites of unsaturation. The ^{13}Cmr spectrum ($CDCl_3$) of nidulone displays two signals in the carbonyl region (δ208.2, 168.6) attributable[17] to ketone and ester functions respectively. In addition to the aforementioned ketone carbonyl stretching band, the ir spectrum of nidulone shows an ester carbonyl peak (1744 cm^{-1}). The three oxygens present in the molecule are thereby accounted for.

The olefinic carbon region[17] of the ^{13}Cmr spectrum has four signals (δ130.2, 130.6, 140.2, 142.4). Off-

resonance decoupling reveals that the signal at δ130.6 is caused by a carbon bearing a single hydrogen, the remaining sp^2 carbons are fully substituted. Nidulone contains fully and trisubstituted double bonds. This is supported by ir bands[18] (1660, 780 cm^{-1}) and a single olefinic proton signal in the ^{1}Hmr spectrum (Figure 7; δ6.83, d (3.5 Hz)). With four sites of unsaturation accounted for, nidulone must be tricyclic.

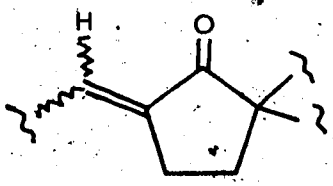
In the ^{1}Hmr spectrum ($CDCl_3$) of nidulol (Figure 8), a one proton signal at δ6.11 (dd; 3, 2.5 Hz) can be assigned to the vinylic proton. The transformation of nidulol to nidulone deshields this proton by 0.72 ppm. In the case of nidulol, this proton is coupled (2.5 Hz)* to a one proton doublet at δ4.14. This signal is assigned to the carbinol proton of nidulol, since it is absent in the ^{1}Hmr spectrum of nidulone. The deshielding of the vinylic proton of nidulol on oxidation plus the fact it is coupled to the carbinol proton suggest that partial structures 80 and 81 are present in nidulol and nidulone respectively. The olefinic proton is



80 81

*Verified by a decoupling experiment. All spectral data of nidulol was provided by Dr. Reffstrup[11].

placed in the β position of enone 81 for the following reason. If the proton is α to the carbonyl it should not be greatly deshielded by the conversion of 80 to 81. In contrast, if the proton is β with respect to the carbonyl a deshielding of as much as 1 ppm is anticipated[23]. Furthermore, since the carbinol proton of 80 is coupled only to the vinylic proton, the carbinol carbon of nidulol must be flanked on either side by fully substituted carbons. The α,β-unsaturated ketone stretching frequency of nidulone (1705 cm^{-1}) implies that the keto group is contained in a five membered ring[18]. Partial structures 82 or 83 follow for nidulone.



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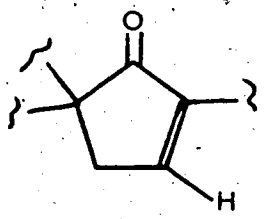
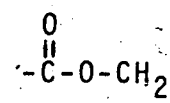


83

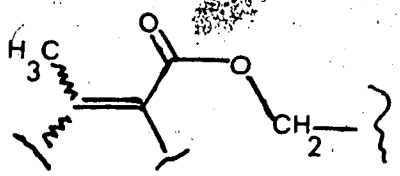
In the oxygenated carbon region[17] of the ^{13}Cmr spectrum of nidulone there is one signal (δ78.7) assigned to a methylene carbon. The ^{1}Hmr spectrum of nidulone displays a pair of one proton signals (δ4.05, 4.27) indicating two protons geminal to oxygen and mutually coupled by 9 Hz*. The ester function of nidulone can thus be formulated as in part structure 84.



84

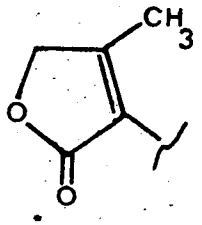
* Verified by a decoupling experiment.

The ^{1}Hmr spectra of nidulol and nidulone both display a singlet methyl group signal (nidulol δ2.26, nidulone δ2.33) at rather low field. To rationalize the chemical shift of this methyl group requires that it be located in the β position of an α,β-unsaturated carbonyl system[74]. Since this methyl group appears at low field in the spectra of both compounds it follows that both compounds contain an α,β-unsaturated ester system 85. The α,β-unsaturated ester carbonyl stretch-



85

ing frequencies of nidulol (1740 cm^{-1}) and nidulone (1744 cm^{-1}) require the presence in both compounds of an α,β-unsaturated γ-lactone (86 or 87) fragment[18].



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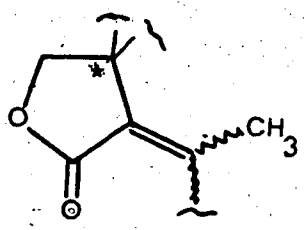


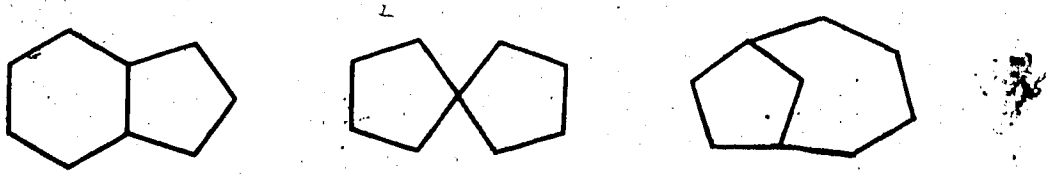
87

Part structure 86 can be discounted. One proton (δ4.05) of the methylene group bonded to oxygen is weakly coupled† to a methyl group (δ1.00) in the case of nidulone. Structure 86 cannot accommodate such a

† Demonstrated by a decoupling experiment.

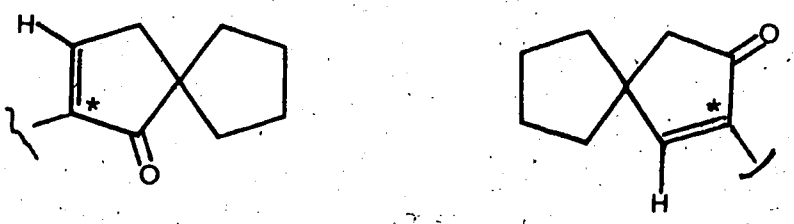
coupling, however placement of a methyl group at the asterisked position of 87 allows for a small four bond coupling[73] between the methylene and methyl group protons.

Since nidulone is tricyclic, the requirement of a lactone moiety implies the presence of two carbocyclic rings. In addition to the two methyl group signals mentioned above, the ^{1}Hmr spectrum of nidulone displays two tertiary methyl group signals (1.10, 1.22). This leaves nine carbons to form the carbocyclic skeleton of nidulone. One ring must be five membered, consequently three skeletons (88-90) are possible. Incorporation of



88 89 90

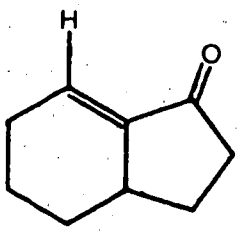
the enone moiety (82 or 83) into skeleton 90 violates Bredt's rule[75]. The enone function can be added to skeleton 89 giving part structures 91 and 92, however



91 92

in both cases a methyl group must be placed at the

asterisked positions. The vinyl hydrogen would be allylically coupled to this methyl group. No coupling is observed between the vinyl hydrogen and any of the methyl groups of nidulone. Nidulone must have skeleton 88. The enone system can be introduced in two ways (93 and 94). Part structure 94 can be eliminated



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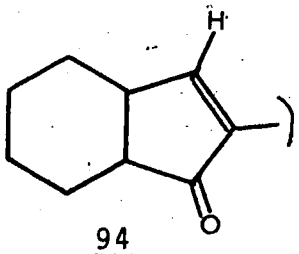

94

for the same reason 91 and 92 were rejected.

The vinyl hydrogen of nidulone is coupled (3.5 Hz) to a single hydrogen resonating at δ2.98. This hydrogen in turn displays couplings of 8 Hz and 11 Hz respectively to single hydrogens appearing at δ1.87 and δ1.72. These two hydrogens are also mutually coupled by 12.5 Hz*. The hydrogen appearing at δ2.98 is a methine hydrogen, the corresponding carbon resonates at δ40.9 in the ^{13}Cmr spectrum of nidulone. The signals at δ1.87 and 1.72 represent a geminate pair of hydrogens, the corresponding methylene carbon is seen at δ34.6 in the ^{13}Cmr spectrum. With this information isolated spin systems 95 and 96 can be constructed. In-

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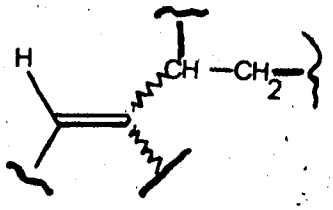
* These couplings were demonstrated by decoupling experiments. An analogous coupling scheme was established for nidulol[11].



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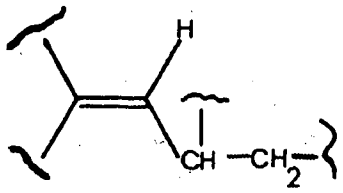
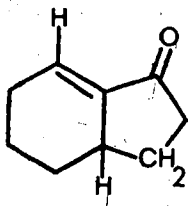
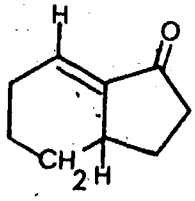


96

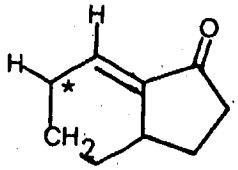
corporation of these spin systems into skeleton 93 leads to three possible improved structures: 93a, 93b and 93c. Structure 93c can be rejected. It is



93a

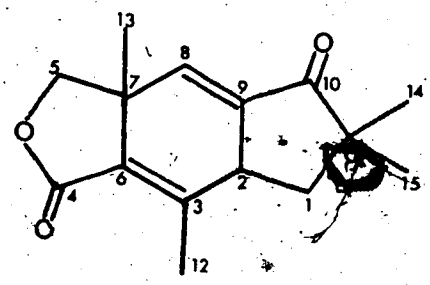


93b

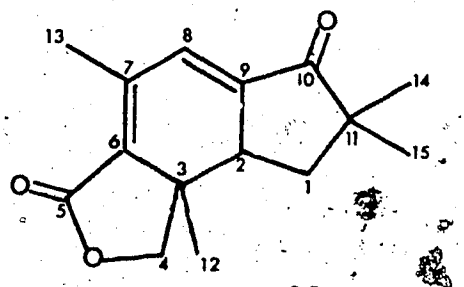


93c

impossible to substitute the asterisked position of 93c with a fully substituted carbon as required by the above coupling scheme. Addition of the lactone moiety 87 requires three contiguous ring carbons, eliminating 93b from contention. Two complete structures 97 and 98 (without stereochemistry) result from the addition of the remaining structural elements to 93a.

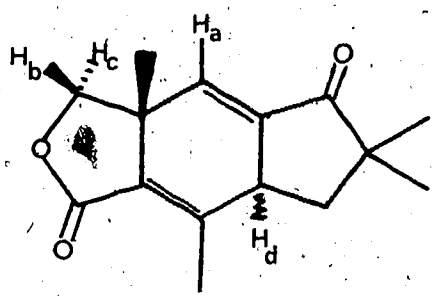


97



98

In order to distinguish between structures 97 and 98, a nuclear Overhauser experiment was conducted. The results of this experiment indicate that 97 is the correct constitution of nidulone. This experiment further suggests that nidulone has *syn* (97a) rather than *anti* (97b) relative stereochemistry. Dreiding molecular models of 97a and 97b indicate that in 97a,



97a β-H_d

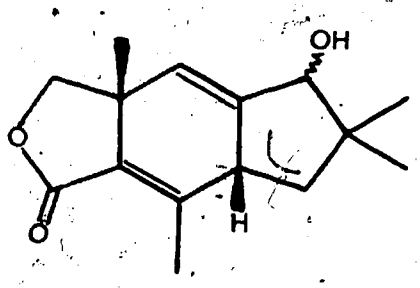
97b α-H_d

the six membered ring must exist in a boat-like conformation wherein the C-13 methyl group and the C-2 hydrogen (H_D) are in a bowsprit-flagpole relationship. In 97b, these groups are remote. The C-13 methyl group (δ1.00) is identified by a small coupling to one of the C-5 hydrogens (δ4.05). Irradiation of the C-13 methyl group in the nuclear Overhauser mode caused: 1) a 6.2% enhancement in the intensity of the proton signal at δ4.27 (H_B) confirming the vicinal relationship between the methyl group resonating at δ1.00 and the C-5 hydrogens. The C-13 methyl group hydrogens can adopt a distorted "W" relationship with H_C (δ4.05) explaining the small coupling observed

between these hydrogens[73]. On the other hand, the shielding effect of the C-13 methyl group should cause H_B to appear at higher field than H_C[76]; 2) a 2.5% enhancement in the intensity of the vinylic proton (H_A) signal, justifying the rejection of structure 98; 3) a small (2.8%) enhancement in the signal intensity of the proton (H_D) appearing at δ2.98, indicating that nidulone has the *syn* relative stereochemistry (97a).

The uv spectrum of nidulone (97a, λ_{max} (CH_3OH): 218 (ε 8600), 260 (ε 1100), 333 nm (ε 3900)) is not consistent with the structure as formulated (97a). The predicted[77] absorption maximum for 97a is *ca.* 240 nm. The uv spectrum seems more consistent with the extended chromophore present in 98[78].

Determination of the stereochemistry of nidulol (99) must await the isolation of additional material.



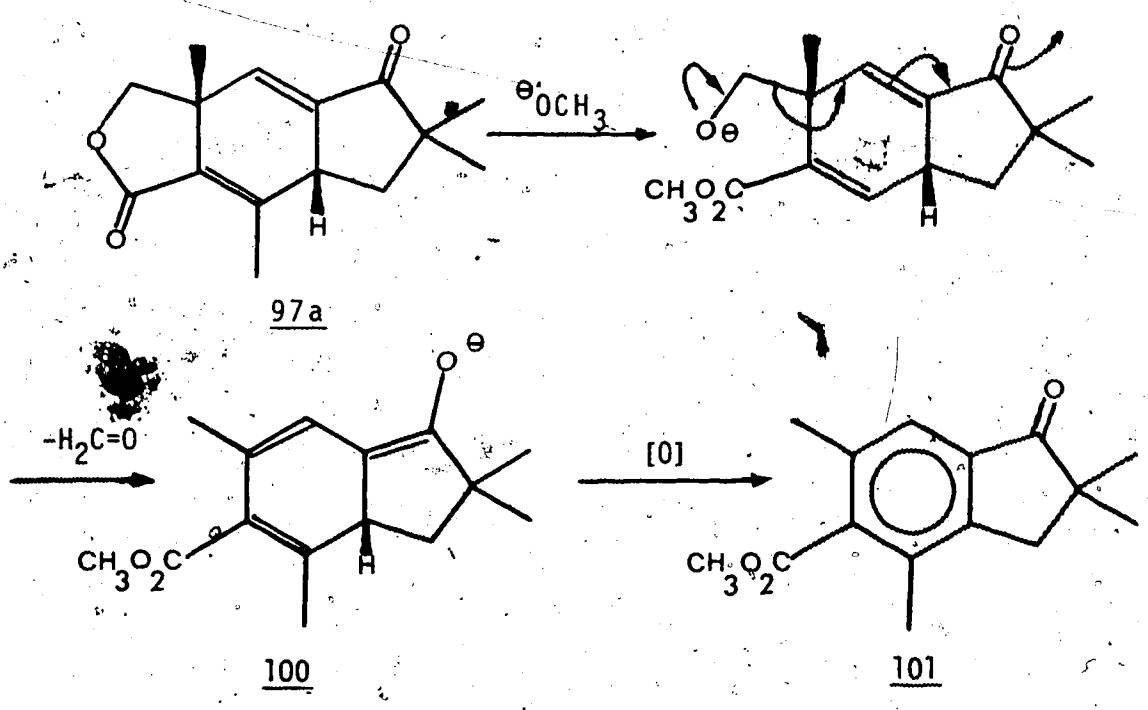
99

In addition an unambiguous correlation between nidulone (97a) and nidulol (99) must be established.

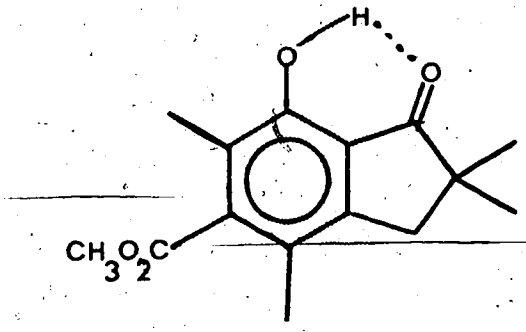
If 97a is the correct structure of nidulone, then treatment with base should open the lactone ring and

cause a retro-aldol fragmentation giving enolate 100 (Scheme 8). Aerial oxidation should then afford the

Scheme 8. Degradation of nidulone (97a).



1-indanone derivative 101*. Treatment of nidulone (97a) with sodium methoxide in methanol gave two major products identified as 101 and phenol 102.



102

* Compound 98 should fragment analogously giving 101 as well.

The uv spectrum of 101 (λ_{max} (CH_3OH): 213 (ε* ~ 20,000), 253 (ε~ 10,000), 306 nm (ε~ 1000)) is consistent with a 1-indanone nucleus. For example pterosin C (46)[51] has a very similar uv spectrum (λ_{max} (C_2H_5OH): 217 (ε 32,000), 259 (ε 16,000), 301 nm (ε 1700)). The ir spectrum of 101 shows ketone (1715 cm^{-1}) and carbomethoxyl (1733 cm^{-1}) stretching bands.

Phenol 102 could be formed from 100 by further aerial oxidation. The uv spectrum of 102 (λ_{max} (CH_3OH): 220 (ε~ 15,000), 262 (ε~ 11,000), 337 nm (ε~ 3000)) is consistent with a 7-hydroxy-1-indanone system[79]. In the presence of alkali the long wavelength absorption peak undergoes a bathochromic shift to 379 nm with an approximate doubling in intensity, this behaviour is characteristic of phenols[79]. The ^{1}Hmr spectrum of 102 shows an exchangeable one proton singlet at δ9.11 assigned to the intramolecular hydrogen bonded phenolic proton. The difference in the ketone carbonyl stretching frequencies of 101 (1715 cm^{-1}) and 102 (1688 cm^{-1}) reflects the hydrogen bonded system of 102[18].

Biogenetically, nidulone appears to be a novel *seco*-isolactarane (diagram 103, cleavage as indicated)[53] sesquiterpenoid. The fungal metabolite isolactarorufin (104) is an example of the rare isolactarane skeletal

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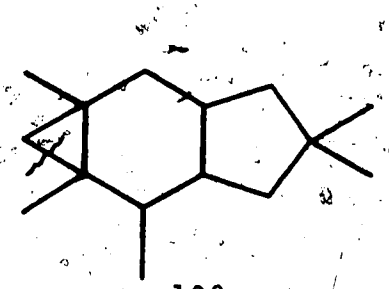
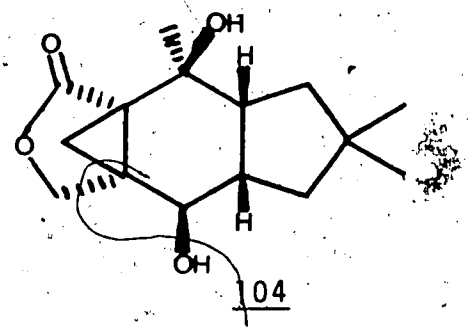
* ε Values are very approximate because of the small quantities employed.



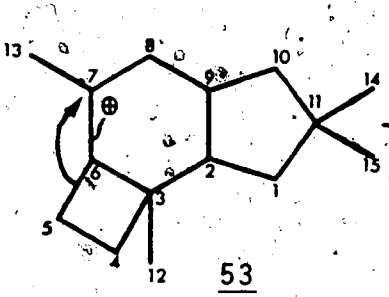
103



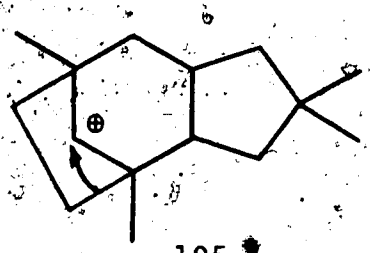
104

class[80]. Recent results[53] indicate that the isolactarane skeleton is closely related to the illudoids (Scheme 9) discussed earlier (Scheme 3).

Scheme 9. Isolactarane biogenesis.



53



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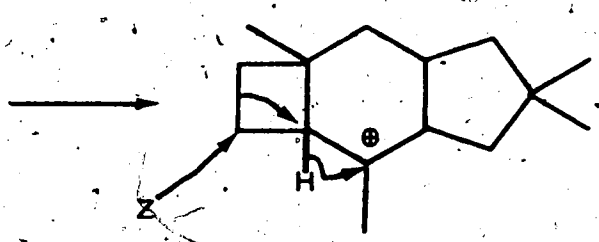

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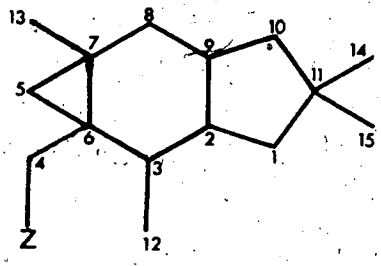

103a

The protoilludane* cation (53) rearranges to the secondary carbonium ion 105 which in turn rearranges

* Compound 98 is also biogenetically plausible as it is formally a *seco*-protoilludane.

to the sterpurane skeleton (106)*. Nucleophilic attack as indicated produces the isolactarane skeleton 103a. The numbering system adopted for nidulone (97a) is a consequence of the above biogenetic scheme.

Compound A, the final metabolite to be considered, eluted in Sephadex fractions 42-43 of the neutral extract. It was obtained in pure form as a yellow oil by silica gel column chromatography of these fractions. Compound A has an R_f of 0.75 (benzene-ether, 1:1) and gives a red coloured spot after visualization.

Compound A has a molecular formula of $C_{15}H_{18}O_3$ (mol. wt. 246). The solid phase ir ($CHCl_3$-cast) spectrum shows a sharp carbonyl band at 1752 cm^{-1} which is significantly broadened (but unshifted) in the solution phase ($CHCl_3$) ir spectrum. Hydroxyl absorption is absent in both spectra.

The ^{13}Cmr spectrum ($CDCl_3$) displays only one signal (δ214.4) in the carbonyl region[17]. Compound A is therefore a keto-diether. In the olefinic carbon region[17] of the spectrum, four signals (δ121.9, 132.9, 134.1, 144.9) are evident, indicating the presence of two double bonds in a tetracyclic molecule. Two of these signals (δ121.9, 132.9) correspond to methine carbons, the other two represent fully substituted carbons.

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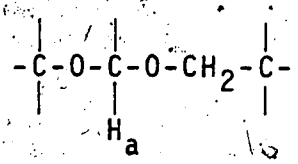
* One could argue that 97a is a *seco*-sterpurane sesquiterpenoid, however cleavage of the cyclopropyl ring of 103a seems more plausible than cleavage of the cyclobutane ring of 106.

The ^{1}Hmr spectrum ($CDCl_3$) of compound A (Figure 9) displays three one proton signals at low field (δ5.58 (d, 1.5 Hz), 5.62 (d, 1.5 Hz), 5.62 (s))*, two of which must be due to olefinic protons.

The oxygenated carbon region of the ^{13}Cmr spectrum shows two signals (δ77.6, 77.7) assignable to quaternary and methylene carbons respectively. The ^{1}Hmr spectrum reveals protons with chemical shifts of δ3.43 and δ3.84 mutually coupled† by 6 Hz. These protons must represent a geminate pair. The ^{13}Cmr spectrum shows a methine carbon (δ101.3) in the region where doubly oxygenated[17] carbons usually resonate. One of the three signals in the region δ5.58-5.62 must represent the proton attached to this carbon. Partial structure 107 is suggested by this data+. In an effort to demonstrate the



107

presence of an acetal linkage, acid catalyzed methanolysis was attempted. Boron trifluoride etherate in

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* When the ^{1}Hmr spectrum was recorded in benzene-d_6, these three protons were well resolved (δ5.14 (d, 1.5 Hz), 5.35 (d, 1.5 Hz), 5.69 (s)). A coupling (1.5 Hz) between the doublets in this set was verified by a double irradiation experiment.

† Demonstrated by a decoupling experiment.

\+ It would be presumptuous to assume that H_a corresponds to the singlet in the region δ5.58-5.62, therefore H_a may be coupled (allylically perhaps) to one olefinic proton.

methanol (two days, 25°C) gave only recovered starting material as did methanol saturated with hydrogen chloride (two days, 25°C). When the latter reaction mixture was heated at reflux overnight a complex mixture of very polar material resulted.

Compound A has three methyl groups (^{1}Hmr: δ1.16, 1.19, 1.50) bonded to fully substituted carbons. The ^{13}Cmr spectrum reveals two methylene group signals (δ33.5, 35.1). The corresponding protons appear in the ^{1}Hmr spectrum as a set of four single proton triple doublets (δ1.70, 1.82, 1.90, 2.11). These protons form an isolated spin system, irradiation of the other protons in the molecule does not perturb this region of the spectrum. The complexity of these signals is inconsistent with the presence of isolated methylene groups (which would give a pair of AB quartets). Isolated spin system 108 wherein each proton could potentially give

$$-CH_2-CH_2-$$

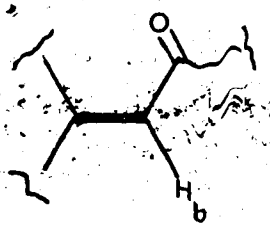
108

an eight line ^{1}Hmr signal is implicated.

Reduction of compound A (sodium borohydride-methanol) gave a complex reaction mixture from which the dihydro derivative (2H-A; mol. formula $C_{15}H_{20}O_3$, mol. wt. 248) was obtained in low yield (20%) by ptlc. When the method of Luche[81] (sodium borohydride, ceric chloride,

methanol) was employed a greatly superior (88%) yield of 2H-A was obtained. The ir spectrum of this derivative lacks carbonyl absorption, while a hydroxyl band (3420 cm^{-1}) is prominent. The carbinol proton and carbon are evident in the ^{1}Hmr (Figure 10, $CDCl_3$, δ3.26 (d, 1.5 Hz)) and ^{13}Cmr (methine, δ84.2) spectra respectively. Acetylation (acetic anhydride-pyridine, three days, 25°C) gave the acetyl derivative (Ac-2H-A; mol. formula $C_{17}H_{22}O_4$, mol. wt. 290) in which a characteristic secondary alcohol acetylation shift[19] of 1.50 ppm is observed for the carbinol proton (δ4.76) in the ^{1}Hmr spectrum.

The carbinol proton of 2H-A is coupled* (1.5 Hz) to a proton resonating at δ5.45 which in turn is coupled* (1.5 Hz) to a proton at δ5.73. This information suggests that 2H-A is an allylic alcohol, the carbinol proton being coupled to a vinyl proton. This would imply that compound A is an α,β-unsaturated ketone (part structure 109)†. The carbonyl stretching



109

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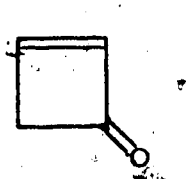
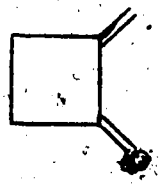
* Demonstrated by double irradiation experiments.
† The small shielding (0.15 ppm) of H_b on reduction of compound A requires that H_b be situated α to the carbonyl in 109[23].

frequency (1752 cm^{-1}) is too high for an α,β-unsaturated cyclopentanone (1710-1720 cm^{-1})[18] and somewhat low for an α,β-unsaturated cyclobutanone (110[82]: 1792 cm^{-1};



110



111

111[83]: 1765 cm^{-1}). The uv spectra of compound A (λ_{max} (CH_3OH): 215 (ε 2800), 250 (ε 3300), 342 nm (ε 600)) and its dihydro derivative (λ_{max} (CH_3OH): 246 nm (ε 3500)) are not entirely consistent with the presence of an enone moiety in compound A. Enones[84] exhibit two characteristic uv bands: an intense (ε> 10,000) π-π* band between 220 and 250 nm and a weak (ε< 100) n-π* band above 300 nm. In the spectrum of compound A the observed bands lack the proper intensities (both relative and absolute) expected of an enone. Comparison of the two spectra reveals a persistent absorption (A: 250 nm; 2H-A: 246 nm) which could be caused by a conjugated diene[85], perhaps the bands at 215 and 342 nm in the spectrum of compound A are caused by highly uv active impurities.

A conjugated diene moiety would explain the small (1.5 Hz) coupling existing between the protons resonating ($CDCl_3$) at δ5.58 and δ5.62. The vinyl protons of a

conjugated diene can exhibit four and/or five bond couplings of *ca.* 1 Hz[73]. An attempt to chemically investigate the olefinic system of compound A by ozonolysis (ozone in methylene chloride at -78°C; dimethyl sulfide work-up) produced only tar.

Based on the above information, it is not possible to propose a structure for compound A. Lack of material prevented further work on this compound.

EXPERIMENTAL

Fermentations were carried out in a New Brunswick Scientific MF-214 microferm laboratory fermentor. Water used at all stages of the fungal culturing process was distilled in an all glass apparatus.

All solvents were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum, bp 62-70°C. Anhydrous solvents were distilled from appropriate drying agents (in brackets): tetrahydrofuran (sodium), ether (sodium), t-butyl alcohol (sodium) and methanol (magnesium). A Hitachi CLC-3 centrifugal liquid chromatograph packed with Baker TLC Silica Gel 7 (<40 μm) was used for centrifugal liquid chromatography. Macherey Nagel Silica Gel 60 (<80 μm) was used for column chromatography. Fractions were collected with an Isco Model 1200 fraction collector. Whatman LPS-2 Chromedia (37-53 μm) was used for flash chromatography[86]. Analytical thin layer chromatography (tlc) was carried out on glass plates (75 x 25 or 75 x 50 mm) coated (~0.3 mm) with silica gel G (W. Merck, Darmstadt) containing 1% electronic phosphor (General Electric, Cleveland). Preparative thin layer chromatography (ptlc) was carried out on glass plates (20 x 20, 10 x 20 or 5 x 20 cm) coated (0.5 mm) with the same adsorbent. Materials were detected by visualization under an ultraviolet lamp (254 or 350 nm). The plate (only a

thin vertical band in the case of ptlc) was then sprayed with a solution of vanillin (1%) in concentrated sulfuric acid. Careful charring with a heat gun followed by a brief cooling period produced the colour reactions indicated in the text. Nitrogen was purified by passage through a column (4 x 45 cm) of Central Dynamics Corporation catalyst R3-11 followed by a column (4 x 50 cm) packed with potassium hydroxide and anhydrous calcium sulfate. Ozone was generated with a Welsbach Ozonator.

Mass spectra (MS) were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS 50 computer, or on an A.E.I. MS-9 mass spectrometer (chemical ionization). Data is reported as m/e (relative intensity). Unless diagnostically significant, peaks with intensities less than 20% of the base peak are omitted. Infrared (IR) spectra were recorded on a Nicolet 7199 FT interferometer, ultraviolet (UV) spectra on a Unicam SP 1700 ultraviolet spectrophotometer and optical rotations on a Perkin Elmer Model 141 polarimeter. Optical rotatory dispersion (ORD) and circular dichroism (CD) measurements were made with a Durrum Jasco ORD/UV-5 (SS-20 modification) recording spectropolarimeter. ^{1}H nuclear magnetic resonance (^{1}HMR) spectra were measured on a Bruker WP-60 spectrometer interfaced to a Nicolet 1080 computer, a Varian HA-100 spectrometer interfaced to

a Digilab FTS/NMR-3 data system, a Bruker WH-200 spectrometer or a Bruker WH-400 spectrometer. ^{13}C nuclear magnetic resonance (^{13}Cmr) spectra were measured on the aforementioned Bruker WP-60 instrument or a Bruker HFX-90 spectrometer interfaced to a Nicolet 1085 computer. All nuclear magnetic resonance measurements employed tetramethylsilane as an internal standard. Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected.

Growth of *Cyathus bulleri* cultures and extraction of metabolites

During the early phases of this work *C. bulleri* 6680a was grown on sterile Brodie's liquid medium. This aqueous medium consists of the following nutrients per litre: dextrose, 12 g; maltose, 5 g; yeast extract, 2 g; asparagine, 0.2 g; peptone, 0.2 g; $MgSO_4$, 0.25 g; KH_2PO_4, 0.5 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 g; $Fe_2(SO_4)_3$, trace amount. The medium was autoclaved at 120°C prior to use. The sterilization period varied from twenty minutes for volumes up to 500 mL, to one hour for ten litre batches.

Stock cultures of *C. bulleri* 6680a (ATCC 38351)* were maintained in slant tubes at 5°C on agar impregnated

* Obtained from Dr. J.H. Ginns, Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario.

with Brodie's medium. To initiate large scale cultures, small fragments of agar containing the mycelium were aseptically transferred to Erlenmeyer flasks (4 x 300 mL) containing sterile Brodie's medium (100 mL). The cultures were allowed to mature at room temperature on a rotary shaker. After three days incubation, thirty sterile glass beads (6 mm) were added and vigourous agitation was continued for a further seven days. The contents of these inoculation flasks were transferred to a fermentation apparatus charged with ten litres of sterile Brodie's medium and approximately 1 mL of the antifoaming agent polypropylene glycol. Fermentation proceeded under the following standard conditions: 25°C, 200 r.p.m. agitation rate, 3 L/min air flow. This culturing technique was plagued by bacteriological contamination of undetermined origin. On one occasion this method produced a total metabolite yield (*vide infra*) of 2.7 g after a seventeen day fermentation period.

A modified culturing technique, which resolved the problem of contamination, was used for the bulk of this work. Three or four petri dishes (9 cm x 1 cm) containing agar impregnated with Brodie's medium were inoculated from stock cultures. These growths were then allowed to mature for two weeks at which time the mycelial growth covered nearly the entire surface of

the agar plate. The agar discs were blended (Waring blender) along with sterile Brodie's medium (500 mL). This inoculum was gently agitated in an Erlenmeyer flask (2 L) for a two week period, the contents were then transferred to a fermentation apparatus as before. Initially, growths produced in this fashion gave total metabolite yields (*vide infra*) of 3.2 g after a twelve day fermentation period. Later cultures produced severely diminished metabolite yields (~0.6 g). This problem was solved, at least temporarily, by recourse to a simpler culture medium known as yeast-malt extract medium. This aqueous medium contains the following nutrients per litre: dextrose, 4 g; yeast extract, 4 g; malt extract, 10 g. Cultures were produced in exactly the same way as before with yeast-malt extract medium replacing Brodie's medium at each stage. This method produced a somewhat slower growth with a two month period elapsing between the initiation of the growth and the harvest of metabolites. Initial metabolite yields (*vide infra*) were in the 2-5 g range, one year later the yields had fallen to 1.0 g. It is likely that this tendency to lower metabolite production with the passage of time reflects a decreased viability of the original stock cultures.

Regardless of the culturing method or medium employed, metabolites were always isolated in the fol-

lowing fashion: The mycelium was removed by vacuum filtration through coarse filter paper (Grade 230). The culture broth was then extracted with ethyl acetate (4 x 1/3 volumes). The ethyl acetate extracts were heavily emulsified and filtration through Celite facilitated the separation of the organic and aqueous phases. The extracts were then concentrated *in vacuo* to 150 mL. Acidic components were removed by extraction with saturated aqueous sodium bicarbonate (3 x 50 mL). The bicarbonate extracts were then back-extracted with ethyl acetate (2 x [illegible] mL). The combined ethyl acetate solutions were washed with water (50 mL) and brine (50 mL). After drying over magnesium sulfate, filtration and concentration gave the neutral extract as a yellow oil. The bicarbonate extracts were acidified (~pH 1) with [illegible]trated hydrochloric acid and then extracted with [illegible] acetate (4 x 50 mL). These combined ethyl acetate extracts were washed with water (50 mL) and brine (50 mL) and then dried over magnesium sulfate. Filtration and concentration gave the acidic extract as a dark brown oil. From twenty litres of fermentation broth a typical yield was: neutral extract 1.6 g, acidic extract 0.6 g, total metabolite yield 2.2 g.

Preliminary fractionation of the crude metabolites

The neutral extract (1.6 g) was dissolved in

methanol (5 mL) and filtered through a cotton wool plug. The filtrate was applied to a column of LH-20 Sephadex (100 g, 65 cm x 2.8 cm). The column was eluted with methanol at a flow rate of 30 mL/hr. Fractions (400 drops, ~7 mL) were collected with the aid of an automatic fraction collector equipped with a drop counter. The antifoaming agent, polypropylene glycol (mol. wt. >2000) eluted in tubes 18-25 (480 mg). The metabolites eluted in tubes 30-60 (1.14 g). The acidic extract (0.6 g) was handled in exactly the same fashion. No antifoaming agent was present in this extract. The acidic metabolites also eluted in tubes 30-60.

The next step in the fractionation of the neutral extract involved silica gel chromatography of selected Sephadex fractions. During the early phases of this work, a Centrifugal Liquid Chromatograph (CLC) was used for this purpose, however ordinary column chromatography was found to be more reliable and convenient. Thus column chromatography (50 g of silica gel) of Sephadex fractions 38-41 (450 mg) gave some virtually pure metabolites as well as several semi-pure compounds. The preliminary column was eluted with the following solvent mixtures: Skellysolve B-chloroform (1:1, 500 mL), chloroform (500 mL), chloroform-methanol (100:1, 500 mL), chloroform-methanol (50:1, 500 mL), chloroform-methanol (20:1, 500 mL) and chloroform-methanol

(10:1, 500 mL).

Isolation of cybrodol (27a, 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenol)

Cybrodol (27a) eluted in Sephadex fractions 40-41 of the neutral extract. It was obtained in pure form as a colourless oil (62 mg) when the preliminary silica gel column of Sephadex fractions 38-41 was eluted with chloroform-methanol (20:1).

TLC: R_f 0.25 (methylene chloride-methanol, 10:1), green spot.

UV (CH_3OH) λ_{max}: 210 (ε 6200), 270 nm (sh, ε~ 320).

IR ($CHCl_3$ cast): 3300, 1670 (w), 1440, 1380, 1030, 1010, 890, 760 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.46 (3 H, d (1 Hz), vinyl CH_3), 1.8 (3 H, bs, 3xOH), 2.18 (3 H, s, C-2 CH_3), 2.36 (3 H, s, C-4 CH_3), 2.99 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.76 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.23 (2 H, bs, $W_{½}$ = 2 Hz, CH_2O), 4.50 (2 H, s, $Ar\underline{CH_2}O$), 6.45 (1 H, bs, $W_{½}$ = 4 Hz, vinyl H), 7.10 (1 H, s, ArH).

^{13}CMR (CD_3OD): δ14.9, 16.5, 20.3, (CH_3); 34.3, 61.8, 63.3, 67.9, (CH_2); 123.9, 127.8, (CH); 134.9, 135.0, 135.8, 136.1, 137.9, 140.0, (C).

MS: m/e calcd. for $C_{15}H_{22}O_3$ (M^+): 250.1569; found: 250.1562 (5), 232 (35), 219 (95), 217 (90), 201 (100),

199 (35), 191 (68), 187 (42), 183 (20), 173 (29), 171 (38), 159 (20), 157 (32), 156 (20), 143 (24), 128 (20). Chemical Ionization (NH_3) MS shows an M + 18 (m/e 268) peak.

Isolation of trisnorcybrodolide (23a, 6-(2-hydroxyethyl)-5,7-dimethylphthalide)

Trisnorcybrodolide (23a) eluted in Sephadex fractions 43-48 (66 mg) of the neutral extract. Silica gel chromatography (chloroform-methanol, 50:1; 20 g of silica gel) of these combined fractions gave pure trisnorcybrodolide (23a, 13 mg) as a tan powder. Crystallization (methanol) afforded white prisms, mp 188-190°C.

TLC: R_f 0.58 (methylene chloride-methanol, 10:1), visible at 254 nm only.

UV (CH_3OH) λ_{max}: 242 (ε 6400), 282 (ε 1100), 289 nm (ε 1300).

IR ($CHCl_3$ cast): 3450, 1730, 1610, 1590, 1460, 1380, 1350, 1260, 1040, 1030, 1010, 860, 800 cm^{-1}.

^{1}HMR ($CDCl_3/CD_3OD$)*: δ2.48 (3 H, s, C-5 CH_3), 2.59 (5 H, s (D_2O exchangeable), H_2O + OH), 2.73 (3 H, s, C-7 CH_3), 3.06 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.79 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 5.15 (2 H, s, CH_2O), 7.11 (1 H, s, ArH).

^{13}CMR (DMSO-d_6): δ12.7, 20.7, (CH_3); 32.1, 59.8, 67.9,

* CD_3OD was wet.

(CH_2); 121.3, (CH); 120.5, 137.1, 137.4, 143.9, 145.6, (C); 171.1, (C=O).

MS: m/e calcd. for $C_{12}H_{14}O_3$ (M^+): 206.0944; found: 206.0944 (78), 191 (66), 175 (100), 163 (16), 147 (43), 119 (20).

Chemical Ionization (NH_3) MS shows a peak at m/e 224 (M + 18).

Isolation of isocybrodol (28a, 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*Z*)-2-propenol)

Isocybrodol (28a) eluted in Sephadex fractions 40-41 of the neutral extract. It was obtained in very crude form (75 mg) when the preliminary silica gel column of Sephadex fractions 38-41 was eluted with chloroform-methanol (20:1). The crude fractions of isocybrodol (28a) along with pyridine (0.1 mL) and acetic anhydride (1 mL) were refluxed overnight in methylene chloride (10 mL). Evaporation to dryness *in vacuo* gave a brown oil (123 mg) which was chromatographed (chloroform-ether, 97:3; 10 g of silica gel) affording crude triacetylisocybrodol (28b, 65 mg). This material was stirred overnight in methanol (10 mL) along with potassium carbonate (1 g). The volatiles were removed *in vacuo* and the residue was partitioned between ethyl acetate (50 mL) and water (10 mL). The

organic solution was dried over magnesium sulfate, filtered and evaporated to dryness leaving crude isocybrodol (28a, 45 mg). Chromatography (chloroform-methanol, 50:1; 10 g of silica gel) gave pure isocybrodol (28a, 39 mg). Crystallization (chloroform-methanol) gave white prisms, mp 102-103°C.

TLC: R_f 0.38 (methylene chloride-methanol, 10:1), yellow spot.

UV (CH_3OH) λ_{max}: 210 (ε 6200), 270 nm (sh, ε~ 350).

IR ($CHCl_3$ cast): 3290, 1660 (w), 1440, 1380, 1035, 1030, 1005, 880, 750 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.02 (3 H, d (1 Hz), vinyl CH_3), 2.16 (3 H, s, C-2 CH_3), 2.36 (3 H, s, C-4 CH_3), 2.97 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.60 (1 H, d (12 Hz), CH_2O), 3.70 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 3.78 (1 H, d (12 Hz), CH_2O), 4.35 (1 H, d (11 Hz), $ArCH_2O$), 4.54 (1 H, d (11 Hz), $ArCH_2O$), 6.27 (1 H, bs, vinyl H), 7.05 (1 H, s, ArH).

^{13}CMR ($CDCl_3$): δ16.9, 20.3, 20.4, (CH_3); 34.3, 61.9, 62.4, 63.5, (CH_2); 126.3, 128.7, (CH); 134.3, 135.2, 136.0, 136.5, 138.0, 139.9, (C).

MS: m/e calcd. for $C_{15}H_{22}O_3$ (M^+): 250.1569; found: 250.1578 (3), 232 (52), 219 (92), 217 (85), 201 (100), 199 (37), 191 (62), 187 (46), 183 (21), 173 (34), 171 (34), 159 (22), 158 (22), 157 (38), 156 (21), 143 (27), 142 (22), 141 (21), 129 (21), 128 (23).

Isolation of cybrodal (41a, 3-((2-formyl-5-(2-hydroxyethyl)-4,6-dimethyl)phenyl)-2-methyl-(*E*)-2-propenal)

Cybrodal (41a) eluted in Sephadex fractions 40-41 of the neutral extract. It was obtained in crude form (26 mg) when the preliminary silica gel column of Sephadex fractions 38-41 was eluted with chloroform-methanol, 100:1. The crude material was chromatographed (Skellysolve B-ether, 3:1; 10 g of silica gel) affording pure cybrodal (41a) as a yellow oil (12 mg).

TLC: R_f 0.52 (methylene chloride-methanol, 10:1), brown spot.

IR ($CHCl_3$ cast): 3450, 2740, 1688, 1630, 1040, 1010, 890, 860, 830 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.57 (3 H, d (1 Hz), vinyl CH_3), 2.26 (3 H, s, C-6 CH_3), 2.46 (3 H, s, C-4 CH_3), 3.10 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.80 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 7.61 (2 H, bs, ArH + vinyl H), 9.75 (1 H, s, CHO), 9.92 (1 H, s, CHO).

MS: m/e calcd. for $C_{15}H_{18}O_3$ (M^+): 246.1256; found: 246.1254 (1), 217 (100), 186 (24).

Chemical Ionization (NH_3) MS shows a peak at m/e 264 (M + 18).

Isolation of cybrodic acid (42a, 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenoic acid)

Cybrodic acid (42a) eluted in Sephadex fractions 39-41 (96 mg) of the acidic extract. In early work, fractions similar to these (40 mg) were subjected to ptlc (toluene-acetone-acetic acid, 75:25:1) affording pure acid 42a as a yellow solid (5 mg). Crystallization (ethyl acetate-Skellysolve B) gave white plates, mp 176-178°C.

TLC: R_f 0.14 (toluene-acetone-acetic acid, 75:25:1), red spot.

IR (CH_3OH cast): 3320, 2600, 1693, 1640, 1440, 1400, 1380, 1340, 1260, 1130, 1040, 1030, 980, 870, 810, 750 cm^{-1}.

^{1}HMR (CD_3OD): δ1.60 (3H, d (1 Hz), vinyl CH_3), 2.19 (3 H, s, C-2 CH_3), 2.38 (3 H, s, C-4 CH_3), 2.96 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.61 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.38 (2 H, bs, CH_2O), 7.15 (1 H, s, ArH), 7.65 (1 H, bs, vinyl H).

^{13}CMR (DMSO-d_6): δ16.2, 17.4, 19.9, (CH_3); 33.3, 60.0, 61.0, (CH_2); 126.4, 134.1, (CH); 130.8, 131.5, 133.0, 135.3, 136.6, 137.9, (C); 168.6, (C=O).

MS: m/e calcd. for $C_{15}H_{20}O_4$ (M^+): 264.1361; found: 264.1365 (4), 246 (47), 233 (64), 215 (100), 201 (24), 187 (48), 173 (32), 171 (54), 159 (23), 129 (21), 128 (23).

Isolation of methyl cybrodate (42b, methyl 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenoate)

The natural acid 42a was more conveniently isolated as its methyl ester derivative, methyl cybrodate (42b). Thus Sephadex fractions 39-41 (96 mg, *vide supra*) in methanol (5 mL) were treated with an excess of diazomethane in methylene chloride at 0°C. Evaporation to dryness gave crude methyl cybrodate (42b, 116 mg). Silica gel chromatography (chloroform-methanol, 50:1; 20 g of silica gel) gave pure methyl cybrodate (42b) as a yellow oil (75 mg).

TLC: R_f 0.54 (methylene chloride-methanol, 10:1), red spot.

UV (CH_3OH) λ_{max}: 215 (ε 18,000), 258 nm (ε 4300).

IR ($CHCl_3$ cast): 3400, 1715, 1640, 1430, 1380, 1340, 1260, 1210, 1190, 1120, 1080, 1040, 1000, 750 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.65 (3 H, d (1 Hz), vinyl CH_3), 2.18 (3 H, s, C-2 CH_3), 2.39 (3 H, s, C-4 CH_3), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.75 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 3.82 (3 H, s, CO_2CH_3), 4.48 (2 H, bs, CH_2O), 7.14 (1 H, s, ArH), 7.66 (1 H, bs, vinyl H).

^{13}CMR (CD_3OD): δ13.9, 16.6, 20.2, 52.4, (CH_3); 34.0, 61.7, 63.0, (CH_2); 128.1, 140.2, (CH); 131.7, 133.1, 134.6, 135.4, 137.0, 137.3, (C); 169.5, (C=O).

MS: m/e calcd. for $C_{16}H_{22}O_4$ (M^+): 278.1518; found:

278.1522 (4), 260 (38), 247 (42), 233 (36), 229 (100), 218 (24), 203 (21), 201 (41), 188 (20), 187 (52), 173 (31), 171 (41), 170 (22), 128 (20).

O,O,O-Triacetylcybrodol (27b, 3-((3-(2-acetoxyethyl)-6-acetoxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenyl acetate)

A mixture of cybrodol (27a, 109 mg, 0.44 mmol), pyridine (0.1 mL), acetic anhydride (1 mL) and chloroform (10 mL) was refluxed for six hours and then evaporated to dryness *in vacuo*. This gave triacetylcybrodol (27b, 156 mg, 95%) as a yellow oil.

TLC: R_f 0.44 (Skellysolve B-acetone, 7:3), green spot.

IR ($CHCl_3$ cast): 1745 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.44 (3 H, d (1 Hz), vinyl CH_3), 2.05 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.12 (3 H, s, OAc), 2.20 (3 H, s, C-2 CH_3), 2.37 (3 H, s, C-4 CH_3), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.14 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.65 (2 H, bs, CH_2O), 4.91 (2 H, s, $ArCH_2O$), 6.39 (1 H, bs, vinyl H), 7.05 (1 H, s, ArH).

MS: m/e calcd. for $C_{21}H_{28}O_6$ (M^+): 376.1886; found: 376.1890 (1), 196 (100).

Ozonolysis of triacetylcybrodol (27b)

A stirred solution of triacetylcybrodol (27b, 78 mg, 0.21 mmol) in methanol was cooled to -78°C. Ozone

was bubbled through the solution for forty-five minutes (0.03 mL/min). After a further forty-five minutes at -78°C, the solution was purged with nitrogen and then warmed to room-temperature. Aqueous hydrogen peroxide (30%, 1 mL) was added and the mixture was refluxed for one hour. Most of the volatiles were removed *in vacuo*, the residue was taken up in ethyl acetate (100 mL) and then washed with water (10 mL) and brine (10 mL). After drying over magnesium sulfate, filtration and concentration gave crude 3-(2-acetoxyethyl)-6-(acetoxymethyl)-2,4-dimethylbenzoic acid (26, 73 mg).

^{1}HMR ($CDCl_3$): δ1.98 (6 H, s, 2X OAc), 2.42 (3 H, s, C-4 CH_3), 2.66 (3 H, s, C-2 CH_3), 3.01 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.10 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 5.08 (2 H, s, CH_2O), 7.04 (1 H, s, ArH).

MS: m/e calcd. for $C_{16}H_{18}O_5$ (P-H_2O, parent ion not seen): 290.1154; found: 290.1154 (5), 248 (26), 188 (100), 187 (54), 175 (26), 160 (23), 159 (21), 147 (24), 115 (20), 91 (26).

The crude acid (26, 73 mg) and 10-camphorsulfonic acid (100 mg) in benzene-methanol (10:1, 30 mL) were refluxed for one day. Evaporation to dryness and column chromatography (methylene chloride-methanol, 50:1; 10 g of silica gel) of the residue afforded a white powder (29 mg, 69%) judged to be identical with natural trisnor-

cybrodolide (23a) by comparison of tlc, ir, ^{1}Hmr and ms data.

O-Acetyltrisnorcybrodolide (23b, 6-(2-acetoxyethyl)-5,7-dimethylphthalide)

A mixture of trisnorcybrodolide (23a, 20 mg, 0.097 mmol), pyridine (10 ml) and acetic anhydride (1 mL) was stirred overnight at room temperature. Evaporation to dryness *in vacuo* gave acetyltrisnorcybrodolide (23b, 23 mg, 96%) as a white powder. Crystallization (acetone) gave long clear needles, mp 117-118°C.

TLC: 0.62 (methylene chloride-methanol, 20:1), visible at 254 nm only.

IR ($CHCl_3$ cast): 1741 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.04 (3 H, s, OAc), 2.48 (3 H, s, C-5 CH_3), 2.73 (3 H, s, C-7 CH_3), 3.08 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.17 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 5.14 (2 H, s, CH_2O), 7.10 (1 H, s, ArH).

MS: m/e calcd. for $C_{14}H_{16}O_4$ (M^+): 248.1048; found: 248.1050 (14), 188 (100), 176 (20), 159 (28), 147 (36), 129 (24).

6-(2-Chloroethyl)-5,7-dimethylphthalide (33)

Trisnorcybrodolide (23a, 17 mg, 0.083 mmol) and phosphorous oxychloride (100 µL, 168 mg, 1.1 mmol) were heated at reflux in pyridine (1 mL) for one day. After

cooling, water (10 mL) and concentrated hydrochloric acid (1 mL) were carefully added. The mixture was extracted with methylene chloride (4 x 10 mL). The combined extracts were washed with saturated aqueous sodium carbonate (10 mL), water (10 mL) and brine (10 mL). After drying over magnesium sulfate, filtration and concentration gave chlorolactone 33 (12 mg, 64%). Recrystallization (methylene chloride-Skellysolve B) gave white plates, mp 147-149°C.

TLC: R_f 0.43 (methylene chloride-methanol, 50:1), purple spot visible only at 254 nm.

IR ($CHCl_3$ cast): 1741 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.49 (3 H, s, C-5 CH_3), 2.73 (3 H, s, C-7 CH_3), 3.23 (2 H, t (7 Hz), $ArCH_2$-CH_2), 3.60 (2 H, t (7 Hz), $ArCH_2CH_2$), 5.17 (2 H, s, CH_2O), 7.13 (1 H, s, ArH).

MS: m/e calcd. for $C_{12}H_{13}O_2{}^{37}Cl$ (M + 2): 226.0575; found: 226.0585 (12); calcd. for $C_{12}H_{13}O_2{}^{35}Cl$ (M^+): 224.0604; found: 224.0607 (40), 189 (100), 175 (48).

6-(2-(4-Nitrophenylseleno)ethyl)-5,7-dimethylphthalide (34)

Freshly distilled tri-n-butylphosphine (45 μL, 36.5 mg, 0.18 mmol) and 4-nitrophenylselenocyanate (40 mg, 0.18 mmol)* in dry tetrahydrofuran (500 μL) were added to a stirred solution of trisnorcybrodolide (23a, 16.7 mg, 0.081 mmol) in dry tetrahydrofuran (500 μL)

* Kindly supplied by Prof. D. Clive, University of Alberta.

under a nitrogen atmosphere. The deep-red mixture was stirred for thirty minutes at room temperature and then evaporated to dryness *in vacuo*. The residue was chromatographed (10 g of silica gel), non-polar side products eluted with Skellysolve B and then chloroform eluted selenide 34 (15 mg, 43%) as a yellow foam. Continued elution with chloroform gave recovered starting material 23a (6 mg).

TLC: R_f 0.42 (Skellysolve B-acetone, 7:3), visibly yellow spot.

^{1}HMR ($CDCl_3$): δ2.23 (3 H, s, C-5 CH_3), 2.70 (3 H, s, C-7 CH_3), 3.10 (2 H, t (7 Hz), $ArCH_2CH_2$), 4.28 (2 H, t (7 Hz, $ArCH_2CH_2$), 7.11 (1 H, s, ArH), 7.59 (2 H, d (9 Hz), 2xArH), 8.10 (2H, d (9 Hz), 2xArH).

MS: m/e calcd. for $C_{18}H_{17}NO_4Se$ (M^+): 391.0323; found: 391.0318 (5), 189 (100).

6-Vinyl-5,7-dimethylphthalide (29)

Aqueous hydrogen peroxide (30%, 0.1 mL) was added to a solution of selenide 34 (15 mg, 0.038 mmol) in tetrahydrofuran (2 mL). The mixture was stirred at room temperature for one day and then evaporated to dryness *in vacuo*. The residue was dissolved in ether (50 mL) and washed with saturated aqueous sodium bicarbonate (10 mL), water (10 mL) and brine (10 mL). After drying over magnesium sulfate, filtration and

concentration gave crude 29 (7.4 mg) as a yellow oil. Ptlc purification (Skellysolve B-ether, 3:1, triple elution) gave pure 29 (2.3 mg, 32%) as a white powder. Crystallization (methylene chloride) gave clear needles, mp 93-95°C.

TLC: R_f 0.72 (Skellysolve B-ether, 3:1, triple elution); purple spot visible only at 254 nm.

IR ($CHCl_3$ cast): 1740, 1610, 1000, 910 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.38 (3 H, s, C-5 CH_3), 2.65 (3 H, s, C-7 CH_3), 5.16 (2 H, s, CH_2O), 5.24 (1 H, dd (2, 18 Hz), vinyl H), 5.65 (1 H, dd (2, 11 Hz), vinyl H), 6.65 (1 H, dd (11, 18 Hz), vinyl H), 7.10 (1 H, s, ArH).

MS: m/e calcd. for $C_{12}H_{12}O_2$ (M^+): 188.0837; found: 188.0836 (100), 187 (22), 159 (26), 143 (38), 129 (43), 128 (25), 115 (20).

6-(2-Methanesulfonyloxyethyl)-5,7-dimethylphthalide (36)

Trisnorcybrodolide (23a, 50 mg, 0.24 mmol) and methanesulfonyl chloride (0.5 mL, 0.74 g, 6.5 mmol) in pyridine (5 mL) were stirred overnight at room temperature. The volatiles were removed *in vacuo* and the residue was partitioned between water (20 mL) and ethyl acetate (20 mL). The aqueous phase was extracted with additional ethyl acetate (2 x 20 mL); the combined organic phases were then washed with saturated aqueous sodium bicarbonate (2 x 10 mL) and brine (10 mL). After

drying over magnesium sulfate, filtration and concentration gave crude 36 (106 mg) as a brown oil. Column chromatography (methylene chloride-methanol, 50:1; 20 g of silica gel) gave pure 36 (62 mg, 90%) as a tan solid. Crystallization (methylene chloride) gave colourless needles, mp 120-121°C.

TLC: R_f 0.56 (methylene chloride-methanol, 50:1), purple spot visible only at 254 nm.

IR ($CHCl_3$ cast): 1750, 1340, 1170 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.47 (3 H, s, C-5 CH_3), 2.72 (3 H, s, C-7 CH_3), 2.94 (3 H, s, CH_3SO_2), 3.23 (2 H, t (7 Hz), $ArCH_2CH_2$), 4.28 (2 H, t (7 Hz), $ArCH_2CH_2$), 5.14 (2 H, s, CH_2O), 7.12 (1 H, s, ArH).

MS: m/e calcd. for $C_{13}H_{16}O_5S$ (M^+): 284.0719; found: 284.0724 (1), 189 (100), 130 (31).

Compound 29 by DBU treatment of 36

Compound 36 (40 mg, 0.14 mmol) and freshly distilled DBU (1 mL) were stirred overnight in toluene (5 mL) at 90°C. The cooled reaction mixture was diluted with ether (20 mL) and washed with 5% aqueous hydrochloric acid (5 mL), saturated aqueous sodium bicarbonate (5 mL), water (5 mL) and brine (5 mL). After drying over magnesium sulfate, filtration and concentration gave the crude product (12 mg). Ptlc purification (Skellysolve B-ether, 3:1; triple elution) gave two

fractions: R_f 0.72 (5.5 mg, 21%) and R_f 0.56 (3.7 mg, 11%). The R_f 0.72 fraction was identical with compound 29 by tlc and ^{1}Hmr comparison. The R_f 0.56 component was identical with compound 33 by the same criteria.

6-Formyl-5,7-dimethylphthalide 30

Ozone was bubbled (0.03 mL/min) through a stirred solution of olefin 29 (4 mg, 0.021 mmol) in methanol (5 mL) at -78°C for thirty minutes. The ozone was then purged with nitrogen and the solution was warmed to room temperature. Sodium iodide (150 mg, 1 mmol) and acetic acid (75 µL) were added and the solution was stirred for two days at room temperature. The volatiles were removed *in vacuo*, the residue was dissolved in ether (50 mL) and washed with water (10 mL), 10% aqueous sodium thiosulfate (10 mL), saturated aqueous sodium bicarbonate (10 mL), water (10 mL) and brine (10 mL). After drying over sodium sulfate, filtration and concentration gave crude aldehyde 30 (3 mg) which was purified by ptlc (benzene-ether, 1:1) affording pure 30 (1.6 mg, 40%) as a white powder. Crystallization (methylene chloride) gave white prisms, mp 159-160°C.

TLC: R_f 0.59 (benzene-ether, 1:1), purple spot visible only at 254 nm.

IR ($CHCl_3$ cast): 1746, 1683 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.69 (3 H, s, C-5 CH_3), 2.98 (3 H, s,

C-7 CH_3), 5.21 (2 H, s, CH_2O), 7.18 (1 H, s, ArH), 10.70 (1 H, s, CHO).

MS: m/e calcd. for $C_{11}H_{10}O_3$ (M^+): 190.0629; found: 190.0629 (100), 162 (37), 161 (27), 149 (23), 133 (31), 103 (21).

O,O,O-Triacetylisocybrodol (28b, 3-((3-(2-acetoxyethyl)-6-acetoxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*Z*)-2-propenyl acetate)

A mixture of isocybrodol (28a, 40 mg, 0.16 mmol), pyridine (0.1 mL), acetic anhydride (0.5 mL) and methylene chloride (10 mL) was refluxed for six hours and then evaporated to dryness *in vacuo*. This gave triacetylisocybrodol (28b, 59 mg, 98%) as a yellow oil.

TLC: R_f 0.44 (Skellysolve B-acetone, 7:3), green spot.

IR ($CHCl_3$ cast): 1745 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.93 (3 H, d (1 Hz), vinyl CH_3), 1.98 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.08 (3 H, s, OAc), 2.21 (3 H, s, C-2 CH_3), 2.36 (3 H, s, C-4 CH_3), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.13 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.26 (2 H, bs, CH_2O), 4.84 (1 H, d (12 Hz), $ArCH_2O$) 4.98 (1 H, d (12 Hz), $ArCH_2O$), 6.30 (1 H, bs, vinyl H), 7.02 (1 H, s, ArH).

MS: m/e calcd. for $C_{21}H_{28}O_6$ (M^+): 376.1886; found: 376.1903 (3), 256 (24), 248 (28), 196 (100), 183 (20).

Ozonolysis of triacetylisocybrodol (28b)

Ozone was bubbled (0.03 mL/min) through a stirred solution of triacetylisocybrodol (28b, 14.6 mg) in methanol (5 mL) at -78°C for forty-five minutes. The solution was stirred at -78°C for a further forty-five minutes, purged with nitrogen and then warmed to room temperature. Aqueous hydrogen peroxide (30%, 0.2 mL) was added and the mixture was refluxed for one hour. Benzene-methanol (10:1, 30 mL) and 10-camphorsulfonic acid (100 mg) were added and reflux was continued overnight. Evaporation to dryness and column chromatography (methylene chloride-methanol, 50:1; 10 g of silica gel) gave a white powder (7 mg, 70%) judged to be identical with natural trisnorcybrodolide (23a) by comparison of tlc, ir, ^{1}Hmr and ms data.

O-Acetylcybrodal (41b, 3-((2-formyl-5-(2-acetoxyethyl)-4,6-dimethyl)phenyl)-2-methyl-(*E*)-2-propenal)

A mixture of cybrodal (41a, 6 mg, 0.024 mmol), pyridine (0.5 mL), acetic anhydride (1 mL) and methylene chloride (8 mL) was refluxed overnight and then evaporated to dryness. Ptlc purification (Skellysolve B-acetone, 7:3) gave pure acetylcybrodal (41b, 5 mg, 71%) as a clear oil.

R_f: 0.45 (Skellysolve B-acetone, 7:3), brown spot.

IR ($CHCl_3$ cast): 2730, 1740, 1689, 1630 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.58 (3 H, d (1 Hz), vinyl CH_3), 2.07 (3 H, s, OAc), 2.27 (3 H, s, C-2 CH_3), 2.47 (3 H, s, C-4 CH_3), 3.12 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.19 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 7.63 (2 H, bs, vinyl H + ArH), 9.77 (1 H, s, CHO), 9.95 (1 H, s, CHO).

MS: m/e calcd. for $C_{16}H_{19}O_3$ (M-CHO, parent peak not seen): 259.1334; found: 259.1337 (100), 199 (73). Chemical Ionization (NH_3) MS shows a peak at m/e 306 (M + 18).

Manganese dioxide oxidation of cybrodol (27a)

Cybrodol (27a, 9.7 mg, 0.039 mmol) and activated manganese dioxide[87] (150 mg, 1.7 mmol) in benzene (2 mL) were stirred for one day at room temperature. The mixture was filtered through Celite, the filter cake was washed with chloroform (5 x 10 mL) and the combined washings were concentrated to a syrup (8 mg). Ptlc (methylene chloride-methanol, 10:1) purification gave a clear oil (6.7 mg, 69%) with physical properties (tlc, ^{1}Hmr, ms) identical with those of natural cybrodal (41a).

Lithium aluminum hydride reduction of acetylcybrodal (41b)

Lithium aluminum hydride (20 mg, 0.53 mmol) was added to a stirred solution of acetylcybrodal (41b, 5 mg, 0.017 mmol) in dry ether (2 mL). After two and one-half hours at room temperature, water (10 mL) was added

and the mixture was extracted with ether (5 x 10 mL). The combined ether extracts were washed with brine (10 mL) and dried over magnesium sulfate. Filtration and concentration followed by ptlc purification (methylene chloride-methanol, 10:1) gave a clear oil (3 mg, 70%) deemed to be identical with natural cybrodol (27a) by comparison of tlc and ^{1}Hmr data.

Lithium aluminum hydride reduction of methyl cybrodate (42b)

Lithium aluminum hydride (15 mg, 0.39 mmol) was carefully added to a stirred solution of methyl cybrodate (42b, 5 mg, 0.018 mmol) in dry tetrahydrofuran (5 mL). The mixture was stirred for six hours at room temperature. Water (15 mL) was added and the solution was extracted with chloroform (3 x 30 mL). The chloroform extracts were washed with water (10 mL) and brine (10 mL). After drying over magnesium sulfate, filtration and concentration gave a light yellow oil (4 mg, 89%) judged to be identical with natural cybrodol (27a) by comparison of tlc, ^{1}Hmr and ms data.

Methyl 3-((2-formyl-5-(2-hydroxyethyl)-4,6-dimethyl)-phenyl)-2-methyl-(*E*)-2-propenoate (45)

Activated manganese dioxide (150 mg, 1.7 mmol) was added to a solution of methyl cybrodate (42b, 6 mg,

0.022 mmol) in benzene (1 mL). The slurry was stirred at room temperature for one hour and then filtered through Celite. The filter cake was washed with chloroform (5 x 10 mL), the filtrates were concentrated leaving 45 as a yellow oil (5 mg, 83%).

TLC: R_f 0.50 (methylene chloride-methanol, 10:1), brown spot.

IR ($CHCl_3$ cast): 3420, 1714, 1689 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.65 (3 H, d (1 Hz), vinyl CH_3), 2.24 (3 H, s, C-6 CH_3), 2.42 (3 H, s, C-4 CH_3), 3.04 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.78 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 3.83 (3 H, s, CO_2CH_3), 7.60 (1 H, s, ArH), 7.80 (1 H, bs, vinyl H), 9.94 (1 H, s, CHO).

^{13}CMR (CD_3OD): δ14.1, 16.4, 20.3, 52.6, (CH_3); 34.6, 61.2, (CH_2); 129.0, 144.2, (CH); 132.8, 133.2, 136.7, 137.7, 138.2, 138.5, (C); 169.0, 193.2, (C=O).

MS: m/e calcd. for $C_{16}H_{20}O_4$ (M^+): 276.1362; found: 276.1359 (2), 217 (100).

Isolation of (2*R*,3*R*)-pterosin C (46)

(2*R*,3*R*)-Pterosin C (46) eluted in Sephadex fractions 43-46 (50 mg) of the neutral extract. Slow evaporation of these fractions usually caused this compound to crystallize as clear needles. Alternatively, it could be isolated by silica gel column chromatography (20 g of silica gel). Compound 46 (18 mg) eluted

with chloroform-methanol, 50:1. Crystallization (ethyl acetate-Skellysolve B) afforded clear needles, mp 160-162°C (lit.[50] 162-164°C), $[\alpha]_D^{25}$ -61° (c 0.36, CH_3OH); cd/ord (c 0.02, CH_3OH) 25°C: $[\theta]_{325}$ -15,000; $[\phi]_{310}$ +19° (peak), $[\phi]_{262}$ 0° (intersects), $[\phi]_{250}$ -152°(trough).

TLC: R_f 0.40 (methylene chloride-methanol, 10:1), brown spot.

IR ($CHCl_3$ cast): 3360, 1681, 1600, 1070, 1040, 1020, 1010 cm^{-1}.

^{1}HMR (CD_3OD): δ1.30 (3 H, d (7 Hz), C-2 CH_3), 2.48 (3 H, s, C-5 CH_3), 2.5 (1 H, dq (J_d = 4 Hz, J_q = 7 Hz), C-2 H), 2.65 (3 H, s, C-7 CH_3), 2.99 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.62 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.67 (1 H, d (4 Hz), C-3 H), 7.34 (1 H, s, ArH).

^{13}CMR (DMSO-d_6): δ12.7, 13.3, 20.8, (CH_3); 32.0, 59.8, (CH_2); 53.1, 73.7, 124.3, (CH); 130.7, 135.5, 136.9, 144.0, 153.6, (C); 205.1, (C=O).

MS: m/e calcd. for $C_{14}H_{18}O_3$ (M^+): 234.1256; found: 234.1254 (55), 203 (100), 185 (21).

Chemical Ionization (NH_3) MS shows peaks at m/e 235 (M + 1) and 252 (M + 18).

Isolation of 3-methyllumichrome (59)

3-Methyllumichrome (59) eluted in Sephadex fractions 43-46 (50 mg). Silica gel column chromatog-

raphy (chloroform-methanol, 50:1; 20 g of silica gel) of fractions 43-48 (66 mg) gave 3-methyllumichrome (59, 8 mg) as a yellow powder. This compound eluted prior to trisnorcybrodolide (23a). Crystallization (acetone-Skellysolve B) gave fine yellow needles, mp > 300°C (lit.[59] > 300°C).

TLC: R_f 0.60 (methylene chloride-methanol, 10:1), blue spot at 350 nm.

UV(CH_3OH) λ_{max}: 250 (ε 9000), 337 (ε 2400), 383 nm (ε 2400).

IR ($CHCl_3$ cast): 3170, 3060, 1680, 757, 678 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.49 (3 H, s, $ArCH_3$), 2.52 (3 H, s, $ArCH_3$), 3.56 (3 H, s, CH_3N), 7.76 (1 H, bs, ArH), 8.06 (1 H, bs, ArH), 8.7 (1 H, bs, NH).

MS: m/e calcd. for $C_{13}H_{12}N_4O_2$ (M^+): 256.0959; found: 256.0959 (100), 199 (33), 171 (50), 156 (28).

Chemical Ionization (NH_3) MS shows peaks at m/e 530 (2M + 18) and 274 (M + 18).

Preparation of 3-methyllumichrome (59)

3-Methyllumichrome-10-N-oxide ((67), 100 mg, 0.37 mmol) and triphenylphosphine (200 mg, 0.76 mmol) in 1-propanol (100 mL) were heated at reflux for two days. The solvent was removed *in vacuo* and the residue was chromatographed over silica gel (chloroform, 130 g of silica gel) affording 3-methyllumichrome (59, 75 mg, 80%). Crystallization (acetone-Skellysolve B) gave

fine yellow needles, mp > 300°C, the physical properties of which were identical with those of the natural product (tlc, ir, ^{1}Hmr, ms).

Isolation of broderol (77a)

Broderol (77a) eluted in Sephadex fractions 42 and 43 (195 mg) of the neutral extract. It was obtained in pure form as white flakes (17 mg) when these fractions were subjected to column chromatography (chloroform, 10 g of silica gel). This compound eluted after compound A (*vide infra*). Broderol (77a) was only observed in two early growths using Brodie's medium. The first isolation afforded 17 mg of 77a from ten litres of broth, the next harvest gave 2.5 mg of 77a from twenty litres of broth. Broderol (77a) was crystallized (carbon tetrachloride) affording white plates mp 113-115°C; $[\alpha]_D^{25}$ -99 (c 0.09, CH_3OH).

TLC: R_f 0.66 (methylene chloride-methanol, 10:1), purple spot.

IR ($CHCl_3$ cast): 3400, 3360, 1740 (w, impurity?), 1660 (w), 1460, 1400, 1380, 1090, 1070, 1060, 1020, 800 cm^{-1}.

^{1}HMR ($CDCl_3$): δ0.54 (1 H, m), 0.68 (1 H, m), 0.82 (1 H, m), 0.88 (3 H, s), 0.95 (1 H, m), 0.97 (3 H, s), 1.49 (3 H, d (1 Hz)), 1.5-2.0 (5 H, m), 3.25 (1 H, dd (10.8, 2.5 Hz)), 3.52 (1 H, dd (10.8, 1 Hz)), 5.70 (1 H, bs).

^{1}HMR (C_5D_5N): δ0.40 (1 H, ddd (4.8, 6.4, 9.2 Hz)), 0.58 (1 H, ddd (4.8, 6.4, 9.2 Hz)), 0.84 (3 H, s), 0.92 (3 H, s), 0.9 (2 H, m), 1.40 (3 H, d (1 Hz)), 1.72 (1 H, dd (2.0, 10.8 Hz)), 1.90 (1 H, d (12.4 Hz)), 2.10 (1 H, dd (1.2, 12.4 Hz)), 2.11 (1 H, dd (2.0, 4.8 Hz)), 2.27 (1 H, ddd (2.8, 4.8, 10.8 Hz)), 3.28 (1 H, dd (2.8, 10.0 Hz)), 3.54 (1 H, dd (1.2, 10.0 Hz)), 6.02 (1 H, q (1 Hz)).

^{13}CMR ($CDCl_3$): δ14.5, 18.8, 21.3, (CH_3); 6.3, 8.7, 36.8, 50.6, 74.0, (CH_2); 53.5, 127.5, (CH); 27.9, 41.2, 76.1, 79.4, 133.7, (C).

MS: m/e calcd. for $C_{15}H_{22}O_2$ (M^+): 234.1620; found: 234.1615 (48), 205 (22), 161 (23), 159 (27), 151 (70), 149 (36), 137 (100), 135 (48), 134 (20), 124 (29), 123 (50), 122 (37), 121 (20), 119 (34), 109 (46), 107 (28), 105 (38), 97 (49), 96 (20), 93 (31), 91 (41), 79 (26), 77 (31), 69 (24), 55 (35).

Derivatization of broderol (77a) with trichloroacetyl isocyanate

A few drops of trichloroacetyl isocyanate were added to a chloroform-*d* solution of broderol (77a, 1 mg) in an nmr tube.

^{1}HMR ($CDCl_3$): δ0.90 (3 H, s), 0.98 (3 H, s), 1.49 (3 H, d (1 Hz)), 3.26 (1 H, d (10.8 Hz)), 3.55 (1 H, d (10.8 Hz)), 6.11 (1 H, bs), 8.25 (1 H, s).

Isolation of nidulone (97a)

The tlc (benzene-ether, 1:1) of the neutral extract has a prominent blue spot at R_f 0.50. The compound responsible for this spot elutes in Sephadex fractions 40-42 (310 mg). Chromatography (chloroform-methanol, 100:1; 20 g of silica gel) of these fractions led to the isolation of semi-purified material (20 mg) whose tlc showed mainly two compounds: the blue spot plus a new grey spot (R_f 0.80) representing a co-eluting compound. The grey spot was not detected in the original extract. This semi-purified material was chromatographed (chloroform-ether, 10:1; 10 g of silica gel) affording nidulone (97a), the so-called "grey compound", as a yellow semi-solid* (8 mg). Negligible amounts of the impure original "blue compound" were found in subsequent fractions. Nidulone (97a, $[\alpha]_D^{25}$ -29°, (c 0.03, CH_3OH)), has the following properties.

TLC: R_f 0.80 (benzene-ether, 1:1), grey spot.

UV (CH_3OH) λ_{max}: 218 (ε 8600), 260 (sh, $\varepsilon \approx$ 1100), 333 nm (ε 3900).

IR ($CHCl_3$ cast): 1744, 1705, 1660, 1460, 1380, 1240, 1040, 780 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.00 (3 H, s), 1.10 (3 H, s), 1.22

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* Repeated attempts to crystallize nidulone (97a) by slow evaporation from a wide variety of solvent systems eventually caused extensive decomposition as indicated by ^{1}Hmr and tlc.

(3 H, s), 1.72 (1 H, dd (11, 12.5 Hz), 1.87 (1 H, dd (8, 12.5 Hz)), 2.33 (3 H, s), 2.98 (1 H; ddd (3.5, 8, 11 Hz)), 4.05 (1 H, d (9 Hz)), 4.27 (1 H, d (9 Hz)), 6.83 (1 H, d (3.5 Hz)).

^{13}CMR ($CDCl_3$): δ16.3, 16.4, 23.4, 24.8, (CH_3); 34.6, 78.7, (CH_2); 40.9, 130.6, (CH); 40.4, 47.2, 130.2, 140.2, 142.4, (C); 168.6, 208.2, (C=O).

MS: m/e calcd. for $C_{15}H_{18}O_3$ (M^+): 246.1256; found: 246.1248 (100), 231 (53), 218 (18), 215 (23), 203 (82), 187 (30), 177 (51), 161 (68).

The "blue compound", nidulol (99) has the following physical properties (This compound was isolated by Dr. Reffstrup from extracts of *C. pygmaeus*[11]).

TLC: R_f 0.50 (benzene-ether, 1:1), blue spot.

IR ($CHCl_3$ cast): 3400, 1740, 1705 (w), 1655, 1600, 1450, 1380, 1260, 1200, 1180, 1140, 1090, 1070, 1040, 1020, 780 cm^{-1}.

^{1}HMR ($CDCl_3$): δ0.90 (6 H, s), 1.18 (3 H, s), 1.31 (1 H, dd (12.5, 12.5 Hz)), 1.57 (1 H, dd (8, 12.5 Hz)), 2.26 (3 H, s), 2.73 (1 H, ddd (3, 8, 12.5 Hz)), 3.92 (1 H, d (8 Hz)), 4.14 (1 H, d (8 Hz)), 4.14 (1 H, d (2.5 Hz)), 6.11 (1 H, dd (3, 2.5 Hz).

MS: m/e calcd. for $C_{15}H_{20}O_3$ (M^+): 248.1412; found: 248.1405 (100), 233 (22), 230 (18), 217 (20), 215 (34), 203 (72), 201 (31), 189 (17), 187 (70), 161 (73), 146 (21), 137 (22), 133 (22), 125 (25), 122 (30), 119 (30),

115 (22), 105 (49).

Treatment of nidulone (97a) with sodium methoxide

A solution of nidulone (97a, 13.4 mg, 0.054 mmol) in dry methanol (0.6 mL) was added to a solution of sodium (24 mg, 1 mmol) in dry methanol (0.4 mL). A dark red colour appeared on mixing. The solution was stirred at room temperature overnight. No attempt was made to exclude oxygen. Acetic acid (0.1 mL) and acetone (10 mL) were added and the mixture was dried over magnesium sulfate, filtered and concentrated. The resultant powder was triturated with methylene chloride (4 x 2 mL). The combined, concentrated supernatant liquids were purified by flash chromatography (1 cm column) eluting successively with Skellysolve B, benzene, methylene chloride and methanol. Two major components were isolated. 5-Carbomethoxy-7-hydroxy-2,2,4,6-tetramethyl-1-indanone (102, 1.9 mg, 13%) eluted with Skellysolve B-benzene (1:3) while 5-carbomethoxy-2,2,4,6-tetramethyl-1-indanone (101, 1.0 mg, 7%) eluted with benzene. Compound 102 (white powder) has the following physical properties.

TLC: R_f 0.75 (benzene-ether, 3:1), turquoise spot at 350 nm.

UV (CH_3OH) λ_{max}: 220 (ϵ~ 15,000), 262 (ϵ~ 11,000), 337 nm (ϵ~ 3000).

UV (3% KOH in CH_3OH) λ_{max}: 223, 237 (sh), 267 (sh), 379 nm.

IR ($CHCl_3$ cast): 3340, 1725, 1688 cm^{-1}.

^{1}HMR ($CDCl_3$): 1.26 (6 H, s, 2XC-2 CH_3), 2.14 (3 H, s, $ArCH_3$), 2.17 (3 H, s, $ArCH_3$), 2.85 (2 H, s, $ArCH_2$), 3.94 (3 H, s, CO_2CH_3), 9.11 (1 H (D_2O exchangeable), s, OH).

MS: m/e calcd. for $C_{15}H_{18}O_4$ (M^+): 262.1205; found: 262.1207 (100), 247 (60), 231 (26), 230 (22), 202 (29).

Chemical Ionization (NH_3) MS shows peaks at m/e 263 (M + 1) and 280 (M + 18).

Compound 101 (clear oil) has the following physical properties.

TLC: R_f 0.70 (benzene-ether, 3:1), purple spot visible at 254 nm only.

UV (CH_3OH) λ_{max}: 217 (ε~ 20,000), 253 (ε~ 10,000), 306 nm (ε~ 1000).

IR ($CHCl_3$ cast): 1733, 1715 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.23 (6 H, s, 2XC-2 CH_3), 2.25 (3 H, s, $ArCH_3$), 2.44 (3 H, s, $ArCH_3$), 2.86 (2 H, s, $ArCH_2$), 3.94 (3 H, s, CO_2CH_3), 7.43 (1 H, s, ArH).

MS: m/e calcd. for $C_{15}H_{18}O_3$ (M^+): 246.1256; found: 246.1257 (49), 231 (100), 215 (22).

Chemical Ionization (NH_3) MS shows peaks at m/e 247 (M + 1) and 264 (M + 18).

Isolation of compound A

Compound A eluted in Sephadex fractions 42 and 43 (195 mg) of the neutral extract. It was obtained in pure form as a yellow oil (18 mg) when these fractions were subjected to silica gel column chromatography (Skellysolve B-chloroform, 1:1; 10 g of silica gel).

TLC: R_f 0.75 (benzene-ether, 1:1), red spot.

UV (CH_3OH) λ_{max}: 215 (ε 2800), 250 (ε 3300), 342 nm (ε 600).

IR ($CHCl_3$ cast): 1752, 1710 (w, impurity?), 1645, 1605, 1450, 1375, 1340, 1315, 1295, 1235, 1195, 1090, 1080, 1020, 1000, 990, 940, 910, 840 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.16 (3 H, s), 1.19 (3 H, s), 1.50 (3 H, s), 1.70 (1 H, ddd (6.8, 10.8, 12.2 Hz)), 1.82 (1 H, ddd (3.6, 10.8, 12.2 Hz)), 1.90 (1 H, ddd (6.8, 10.8, 12.8 Hz)), 2.11 (1 H, ddd (3.6, 10.8, 12.6 Hz)), 3.43 (1 H, d (6 Hz)), 3.84 (1 H, d (6 Hz)), 5.58 (1 H, d (1.5 Hz)), 5.62 (1 H, d (1.5 Hz)), 5.62 (1 H, s).

^{1}HMR (C_6D_6): δ0.99 (3 H, s), 1.16 (6 H, s), 1.3-1.7 (4 H, m), 3.29 (1 H, d (6 Hz)), 3.60 (1 H, d (6 Hz)), 5.14 (1 H, d (1.5 Hz)), 5.35 (1 H, d (1.5 Hz)), 5.69 (1 H, s).

^{13}CMR ($CDCl_3$): δ15.2, 17.2, 20.0, (CH_3); 33.5, 35.1, 77.7, (CH_2); 101.3, 121.9, 132.9, (CH); 47.2, 49.6, 77.6, 134.1, 144.9, (C); 214.4, (C=O).

MS: m/e calcd. for $C_{15}H_{18}O_3$ (M^+): 246.1255; found: 246.1245 (49), 231 (16), 218 (20), 216 (34), 203 (24), 201 (20), 188 (100), 173 (25), 160 (28), 145 (22), 128 (21). Chemical Ionization (NH_3) MS shows peaks at m/e 247 (M + 1) and 264 (M + 18).

Sodium borohydride reduction of compound A

A mixture of compound A (13.6 mg, 0.055 mmol) and sodium borohydride (50 mg, 1.3 mmol) in methanol (10 mL) was stirred at room temperature for one hour. Saturated aqueous ammonium chloride (20 mL) was added and most of the methanol was removed *in vacuo*. The residue was extracted with methylene chloride (5 x 10 mL). The extract was washed with brine (10 mL), dried over magnesium sulfate, filtered and concentrated. The crude product was purified by ptlc (methylene chloride-methanol, 10:1) affording 2H-A (2.7 mg, 20%).

TLC: R_f 0.56 (methylene chloride-methanol, 10:1), purple spot.

UV (CH_3OH) λ_{max}: 246 nm (ϵ 3500).

IR ($CHCl_3$ cast): 3420, 1650, 1610, 1080 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.17 (3 H, s), 1.21 (3 H, s), 1.52 (3 H, s), 1.5-1.8 (4 H, m), 3.26 (1 H, d (1.5 Hz)), 3.46 (1 H, d (6 Hz)), 3.86 (1 H, d (6 Hz)), 5.45 (1 H, dd (1.5, 1.5 Hz)), 5.68 (1 H, s), 5.73 (1 H, d (1.5 Hz)).

^{1}HMR (C_5D_5N): δ1.22 (3 H, s), 1.37 (3 H, s), 1.50 (3 H,

s), 3.48 (1 H, d (1.5 Hz)), 3.50 (1 H, d (6 Hz)), 3.96 (1 H, d (6 Hz)), 5.50 (1 H, dd (1.5, 1.5 Hz)), 5.85 (1 H, d (1.5 Hz)), 6.02 (1 H, s).

^{13}CMR ($CDCl_3$): δ19.8, 20.6, 21.9, (CH_3); 34.2, 36.2, 77.3, (CH_2); 84.7, 102.0, 125.1, 130.3, (CH); 44.4, 45.3, 78.1, 135.5, 140.3, (C).

MS: m/e calcd. for $C_{15}H_{20}O_3$ (M^+): 248.1413; found: 248.1416 (2), 190 (20), 175 (100), 173 (89).

Sodium borohydride reduction of compound A in the presence of ceric chloride

Sodium borohydride (5 mg, 0.13 mmol) was added to a stirred solution of compound A (25 mg, 0.10 mmol) and ceric chloride hexahydrate (353 mg, 1 mmol) in methanol at room temperature. The mixture was stirred for five minutes, diluted with water (25 mL), and extracted with methylene chloride (5 x 10 mL). These extracts were washed with brine (10 mL), dried over magnesium sulfate, filtered and concentrated leaving crude 2H-A as a yellow oil. Column chromatography (chloroform, 8g of silica gel) gave pure material (22 mg, 88%).

Acetylation of 2H-A

A mixture of 2H-A (2.7 mg, 0.011 mmol) and acetic anhydride-pyridine (10:1, 1 mL) was stirred at 25°C for three days. Evaporation to dryness gave Ac-2H-A

(2.8 mg) as a yellow oil.

TLC: R_f 0.80 (benzene-ether, 1:1), purple spot.

IR ($CHCl_3$ cast): 1742 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.09 (3 H, s), 1.14 (3 H, s), 1.50 (3 H, s), 1.5-1.8 (4 H, m), 2.00 (3 H, s), 3.47 (1 H, d (6 Hz)), 3.83 (1 H, d (6 Hz)), 4.76 (1 H, d (1.5 Hz)), 5.44 (1 H, dd (1.5, 1.5 Hz)), 5.61 (1 H, d (1.5 Hz)), 5.68 (1 H, s).

MS: m/e calcd. for $C_{17}H_{22}O_4$ (M^+): 290.1518; found: 290.1531 (4), 260 (35), 173 (100).

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APPENDIX

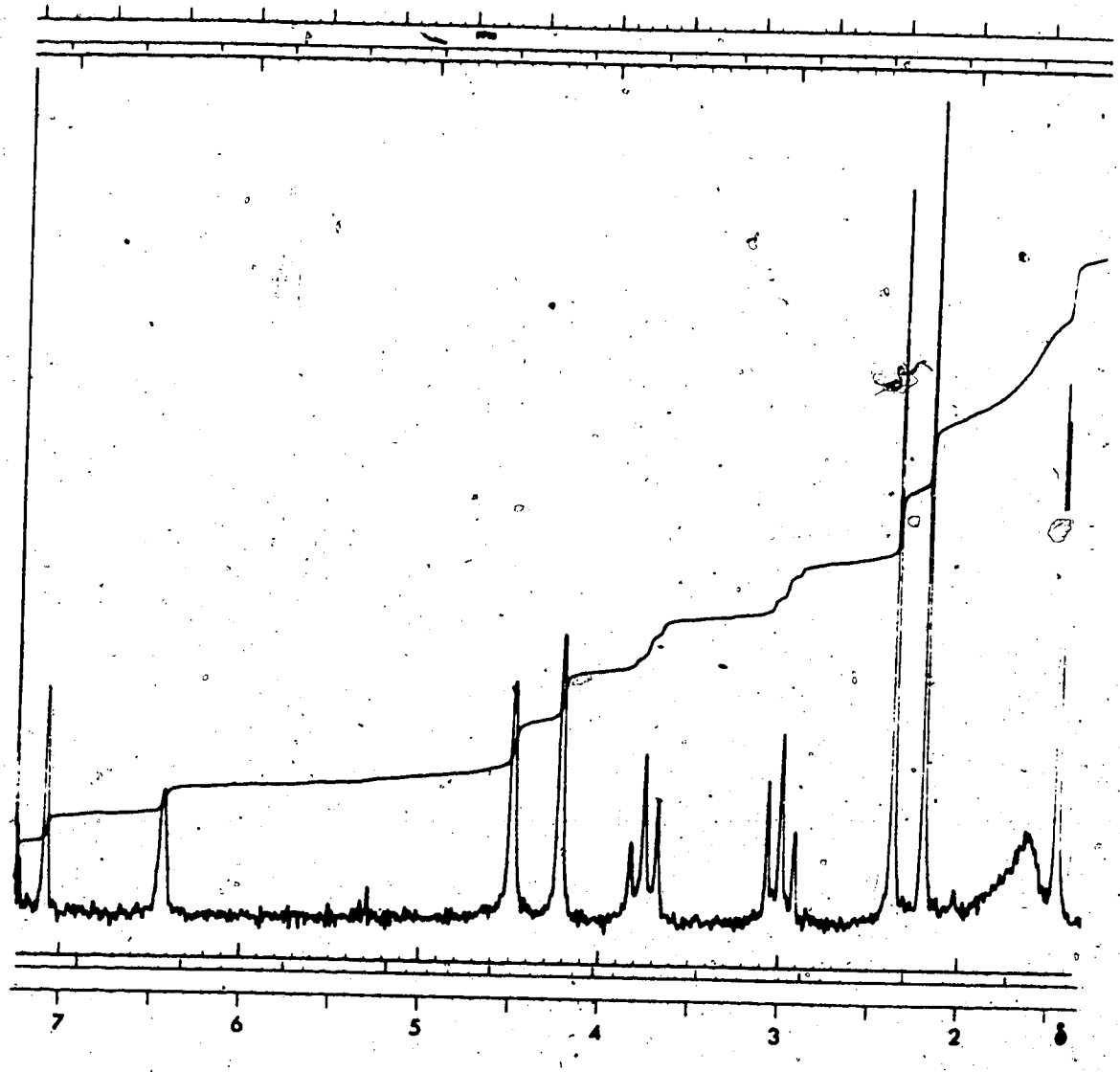


Figure 1: 100 MHz ^{1}Hmr spectrum ($CDCl_3$) of cybrodol (27a).

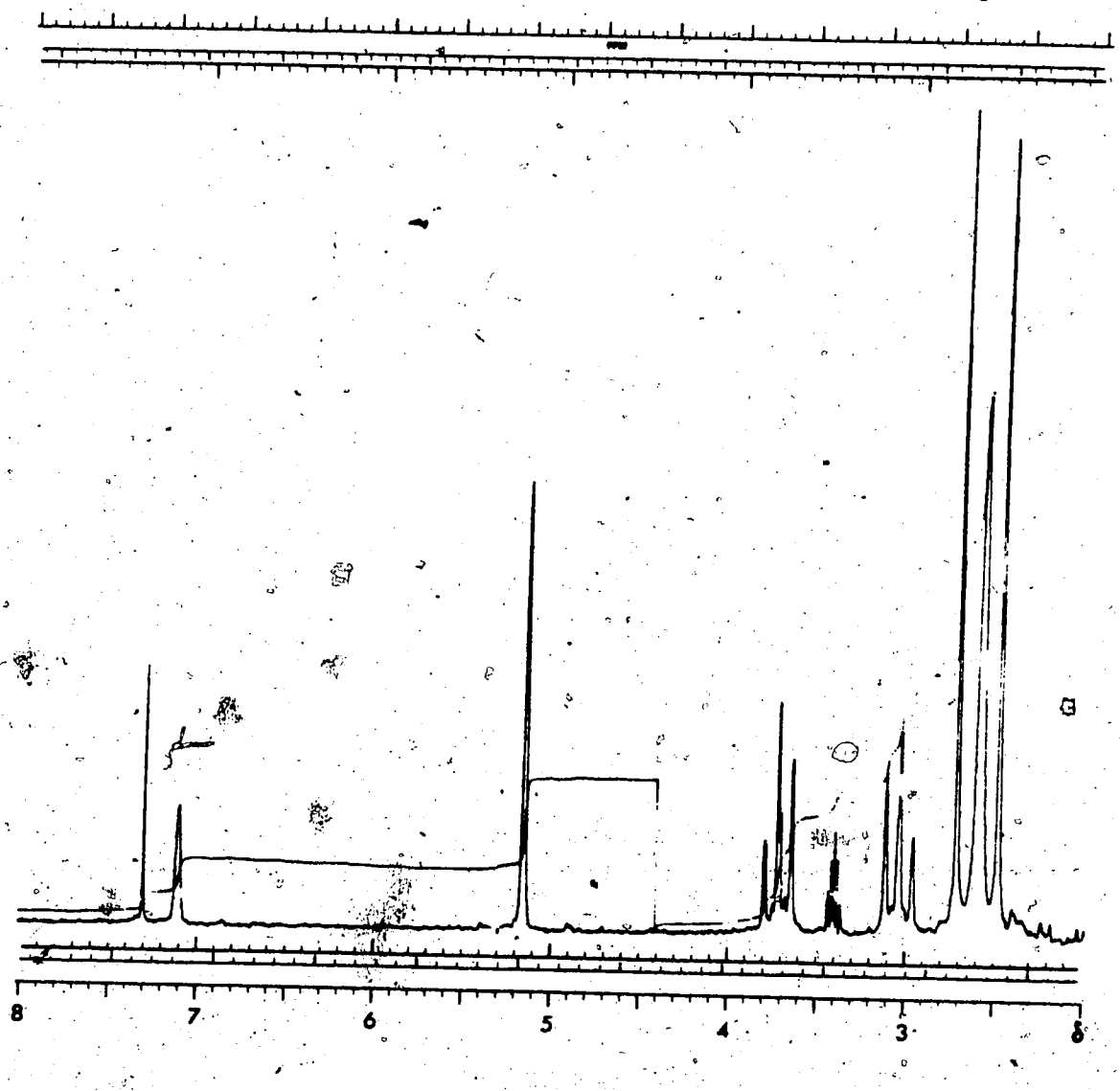


Figure 2: 100 MHz ^{1}Hmr spectrum ($CDCl_3$-CD_3OD) of tris-norcybrodolide (23a).

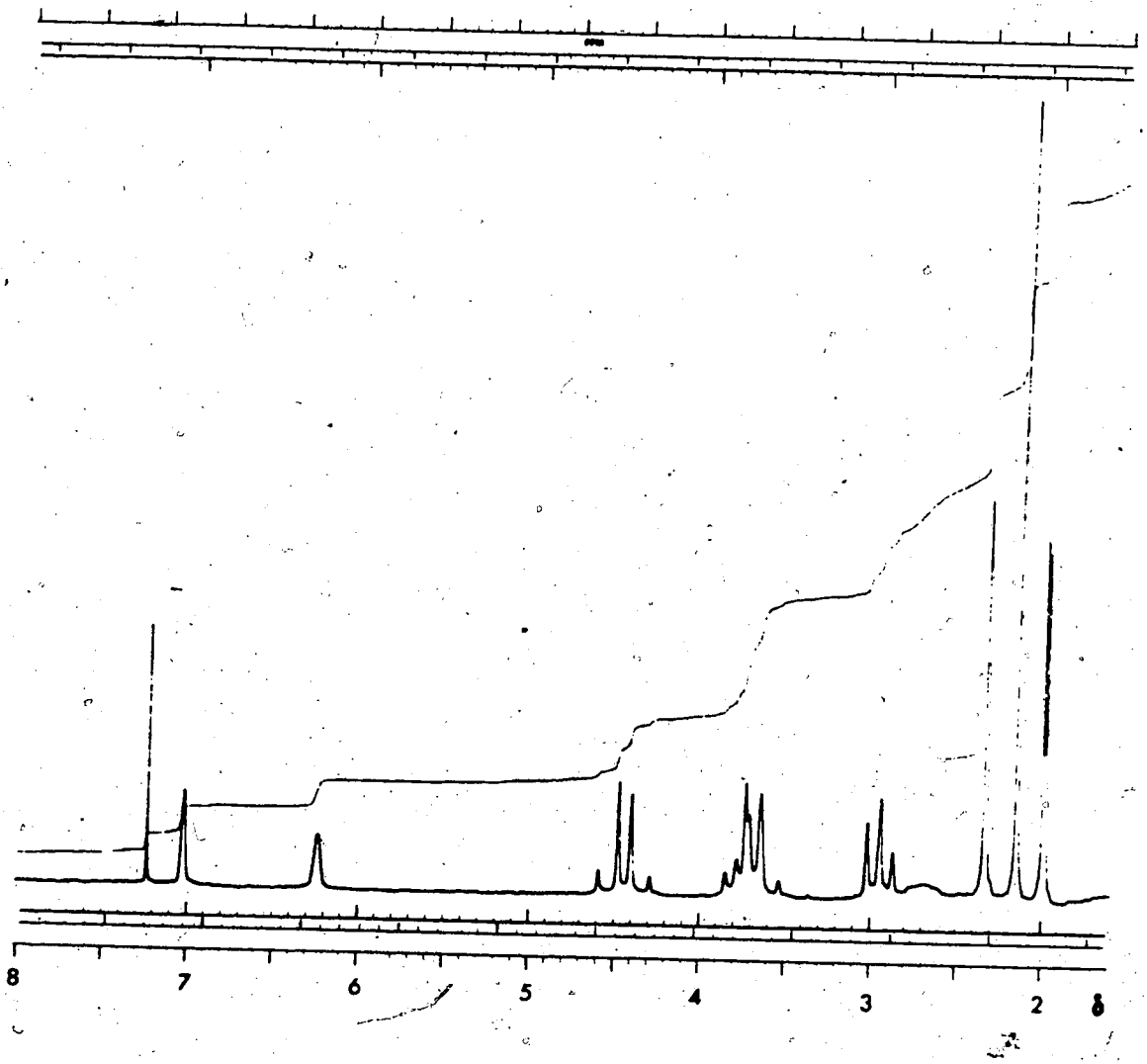


Figure 3: 100 MHz ^{1}Hmr spectrum ($CDCl_3$) of isocybrodol (23a).

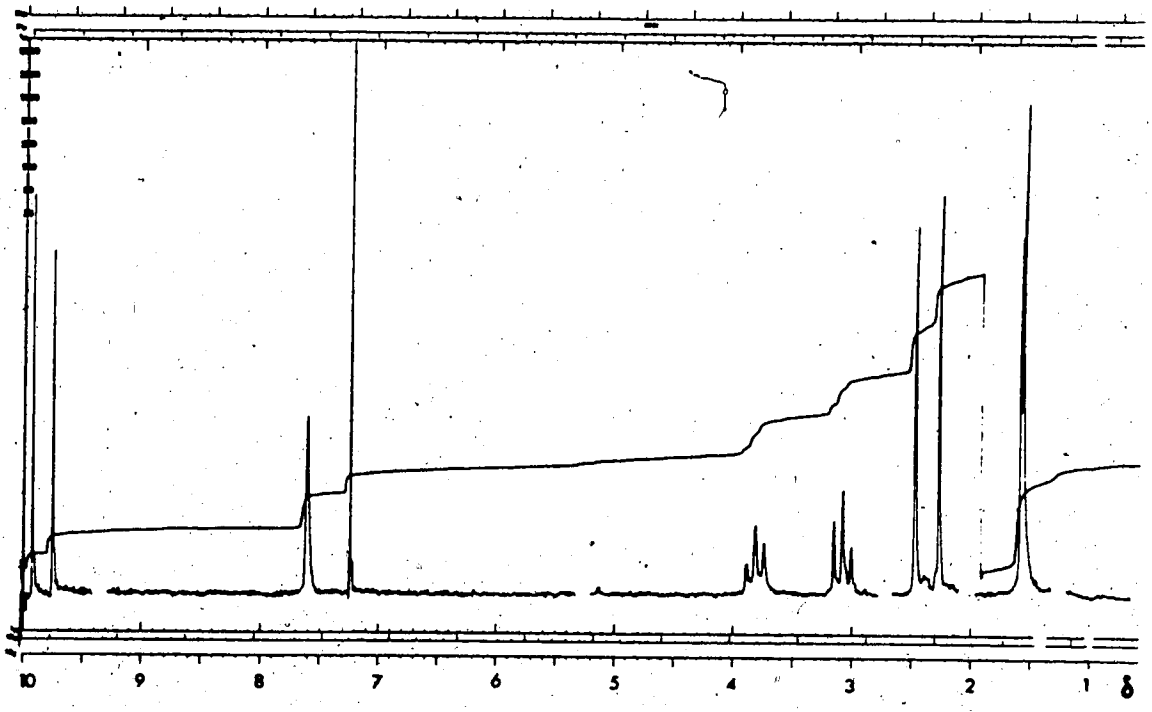


Figure 4: 100 MHz ^{1}Hmr spectrum ($CDCl_3$) of cybrodal (41a).

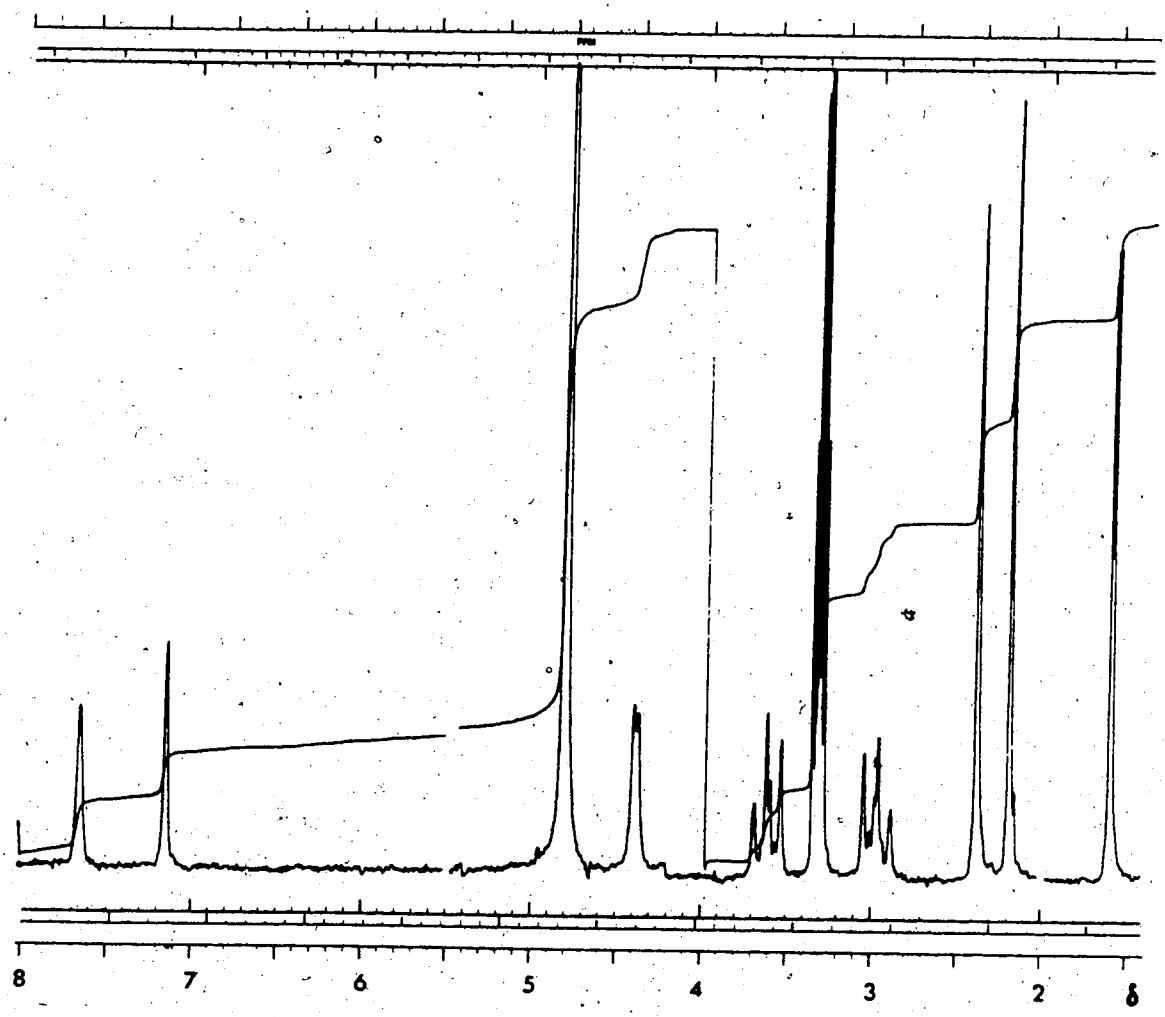


Figure 5: 100 MHz ^{1}Hmr spectrum (CD_3OD) of cybrodic acid (42a).

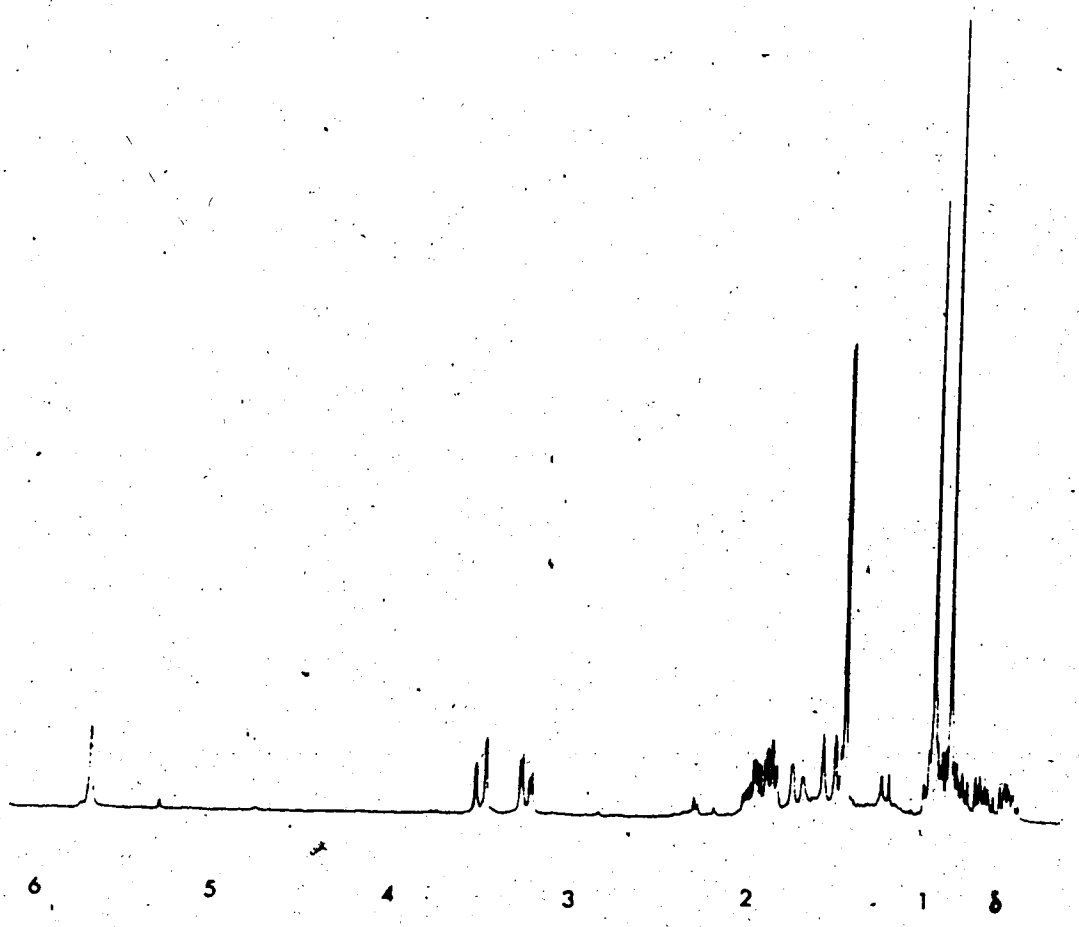


Figure 6: 200 MHz ^{1}Hmr spectrum ($CDCl_3$) of broderol (77a).

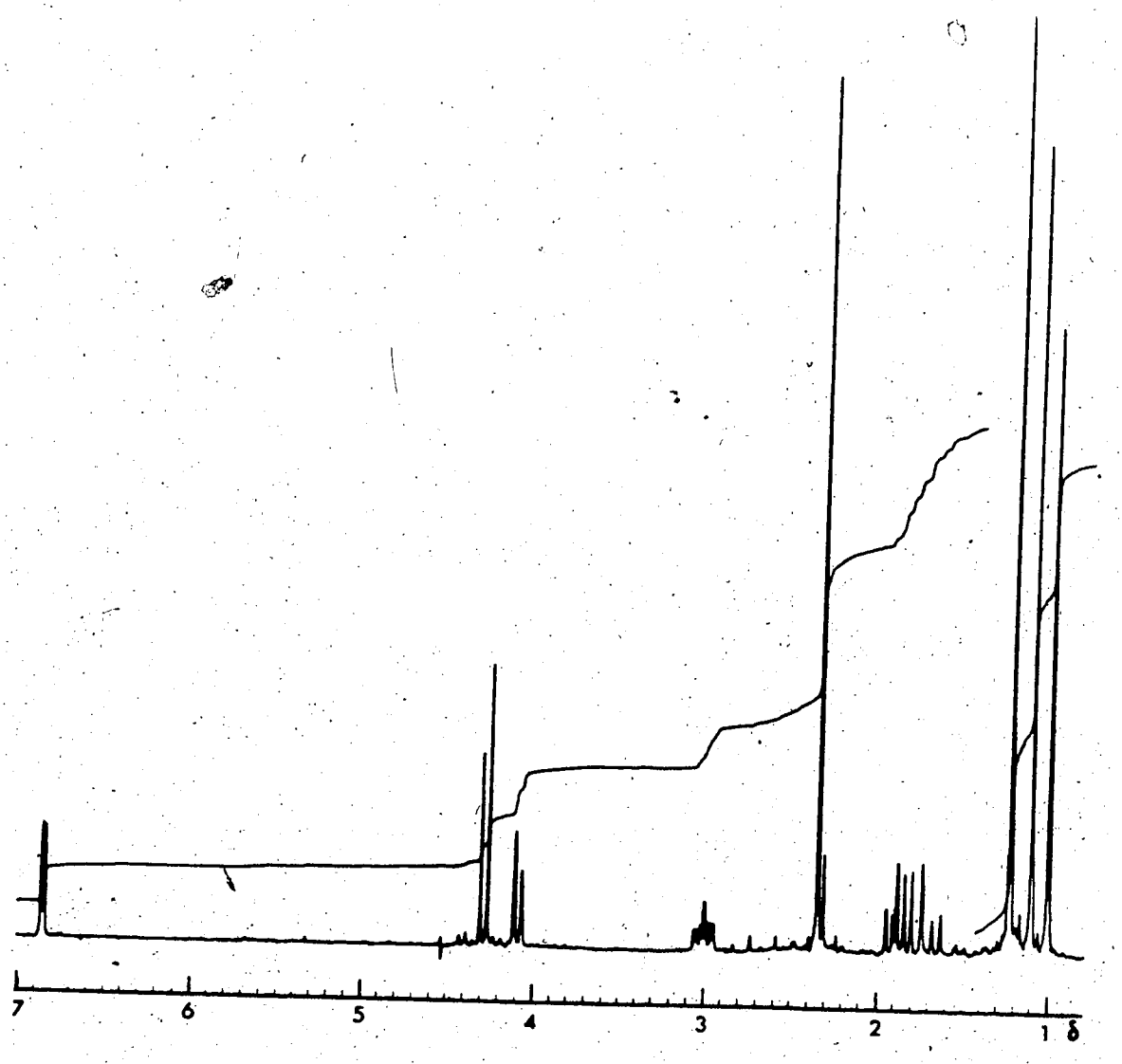


Figure 7: 200 MHz ^{1}Hmr spectrum ($CDCl_3$) of nidulone (97a).

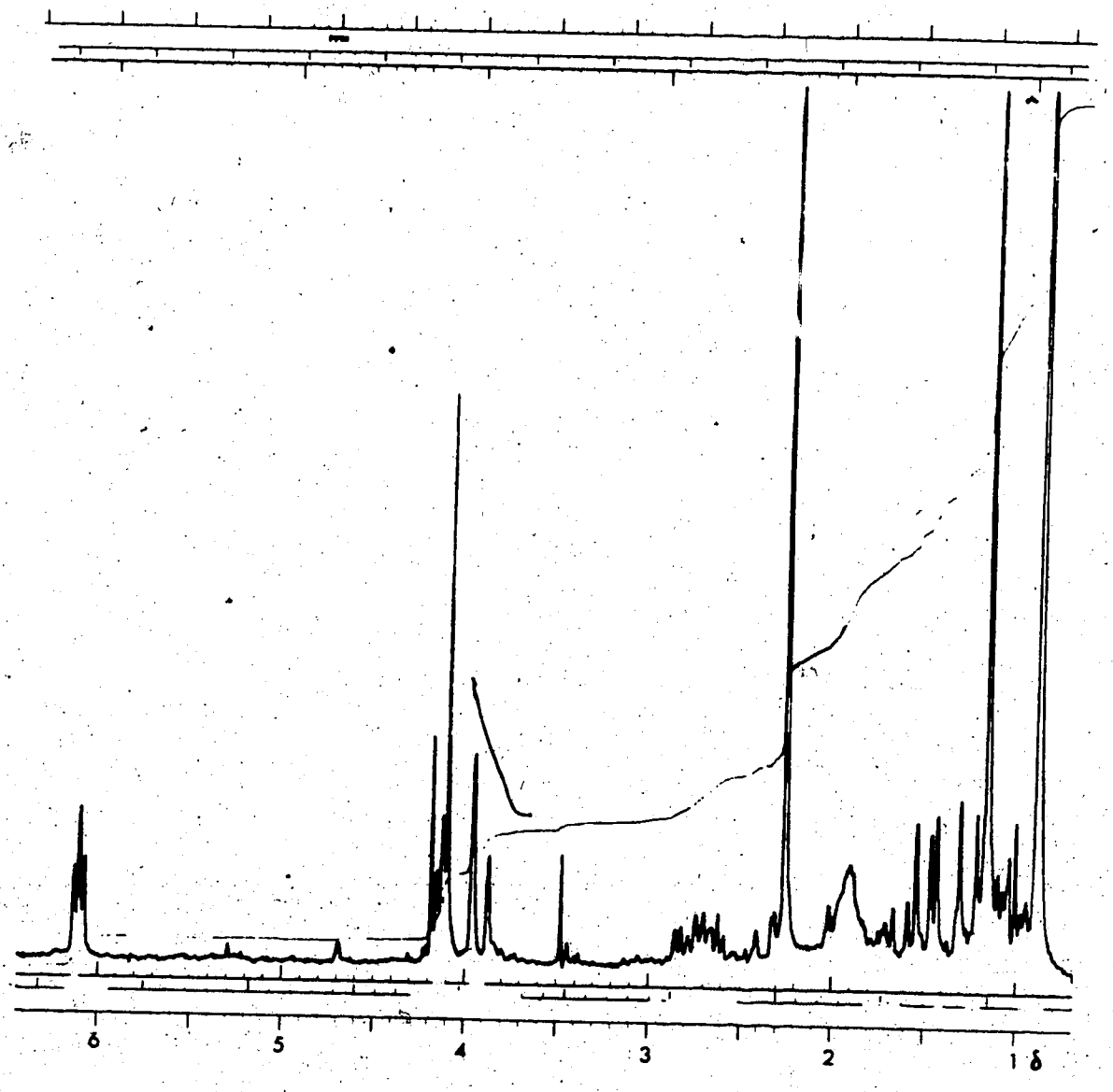


Figure 8: 100 MHz ^{1}Hmr spectrum ($CDCl_3$) of nidulol (99).

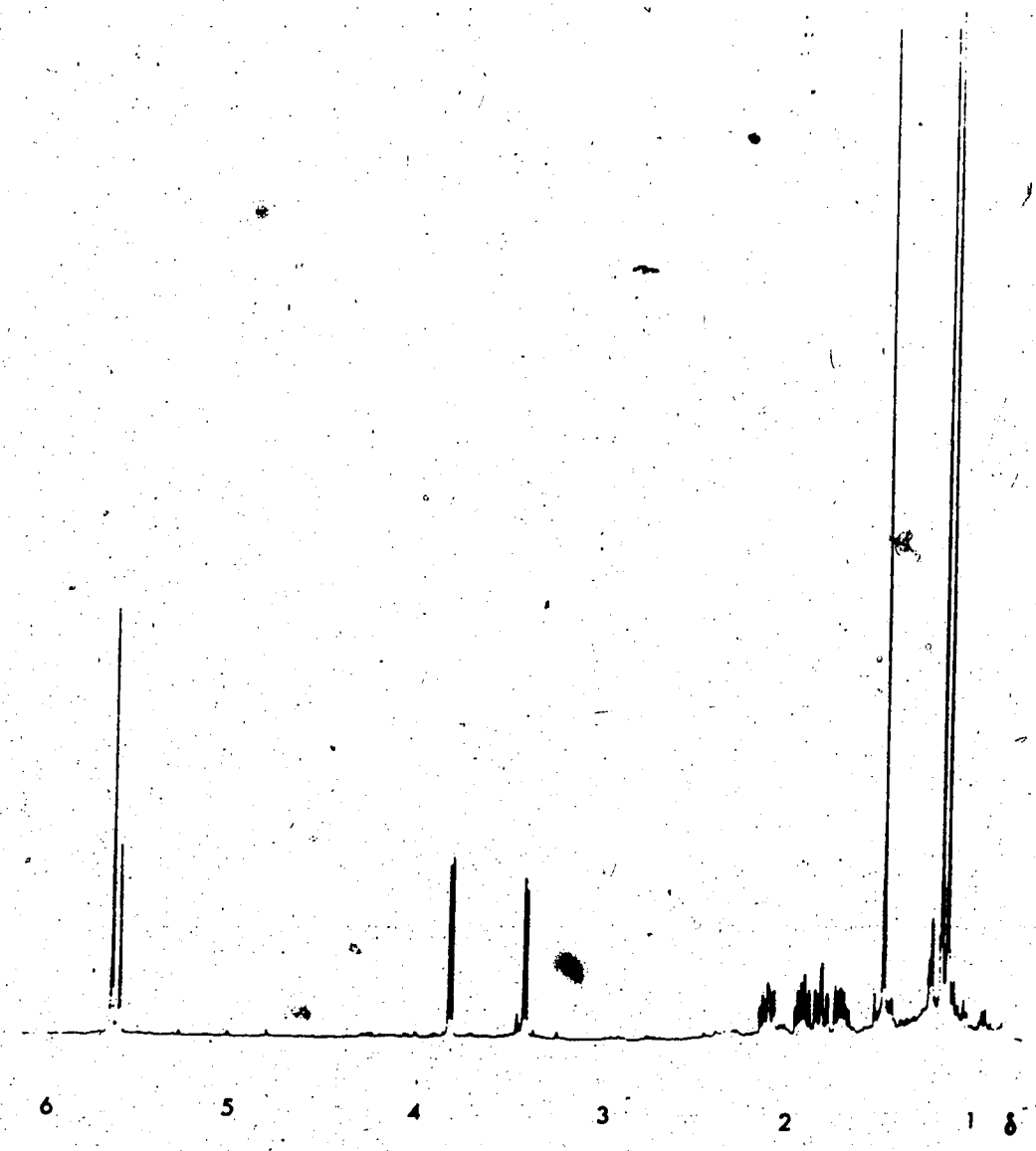


Figure 9: 400 MHz ^{1}Hmr spectrum ($CDCl_3$) of compound A.

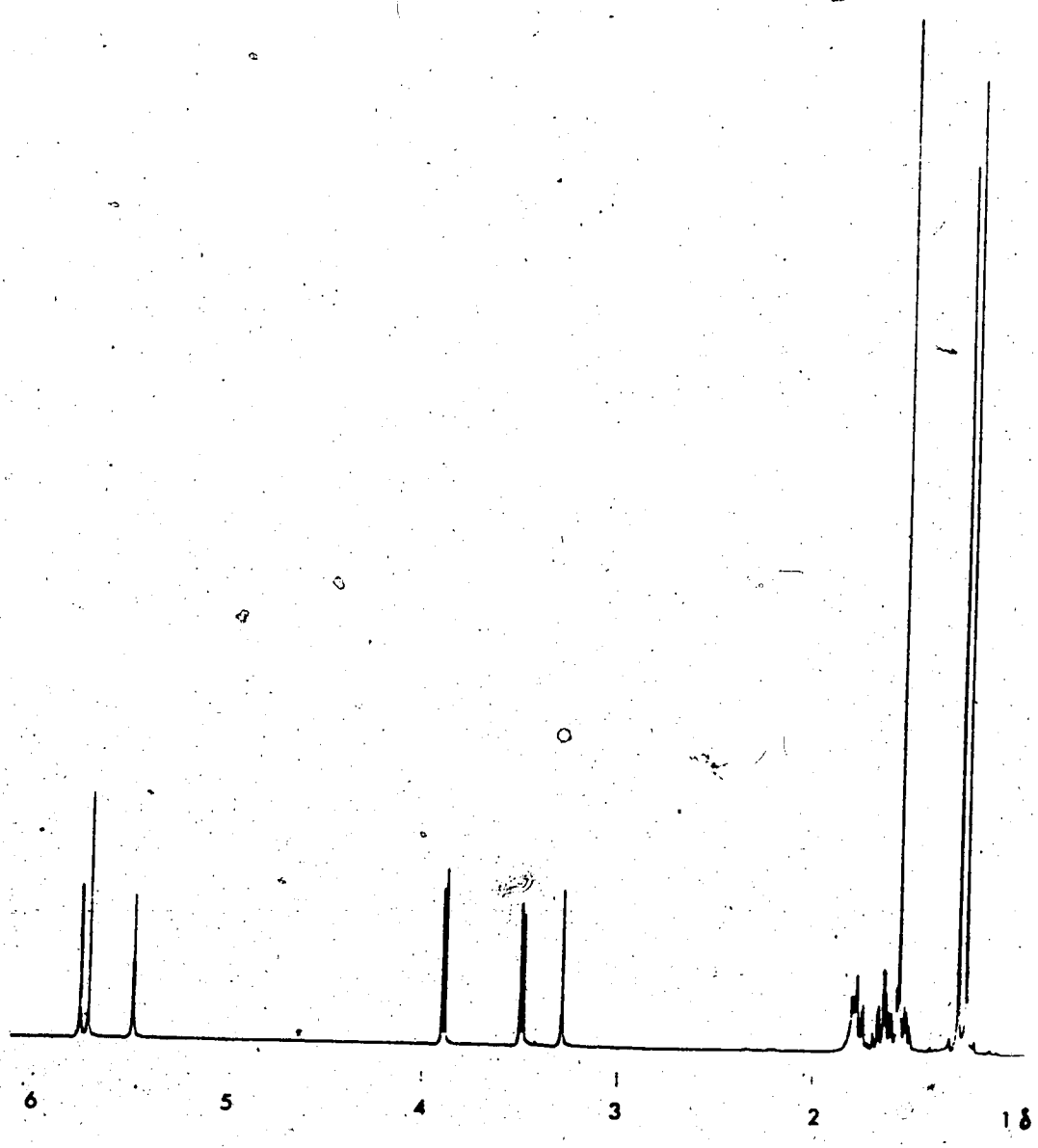
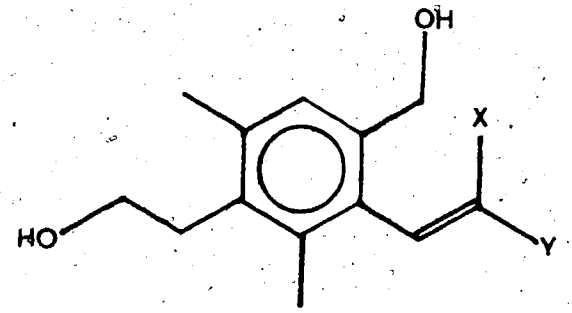


Figure 10: 400 MHz ^{1}Hmr spectrum ($CDCl_3$) of compound 2H-A.

II: THE TOTAL SYNTHESIS OF THE CYBRODINS[1]

INTRODUCTION

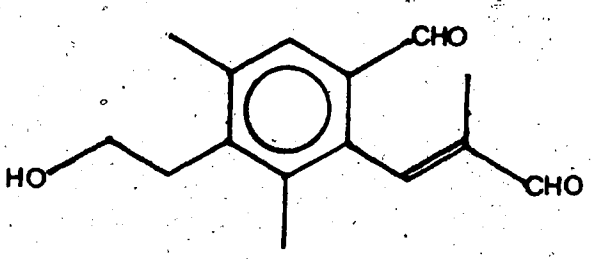
There are only three absolutely unequivocal methods for determining the structure of a natural product: 1) X-ray crystallographic solution, 2) unambiguous total synthesis, and 3) unambiguous correlation with a compound whose structure has been previously established by method 1 or 2. We decided therefore to undertake a rational total synthesis of the cybrodins[2], so that the structures proposed for these new *seco*-illudalane sesquiterpenoids produced by the bird's nest fungus *Cyathus bulleri* would not be subject to question. Five cybrodins, cybrodol (1), isocybrodol (2), cybrodic acid (3), cybrodal (4) and trisnorcybrodolide (5) were isolated from cultures of this fungus.



1 X = CH_3 Y = CH_2OH

2 X = CH_2OH Y = CH_3

3 X = CH_3 Y = CO_2H



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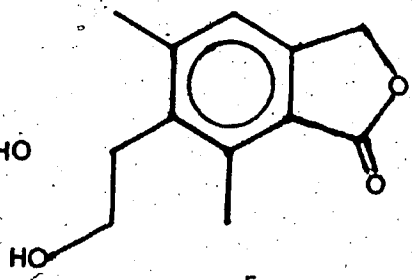
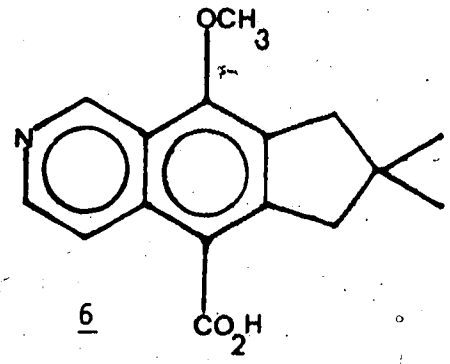


5

Since the cybrodins represent a new class of sesquiterpenoid[3], no synthetic efforts towards this skeleton have appeared*. Syntheses of the illudalanes illudinine (6), illudalic acid (7) and illudacetalic acid (8) have been described[5]. These compounds were



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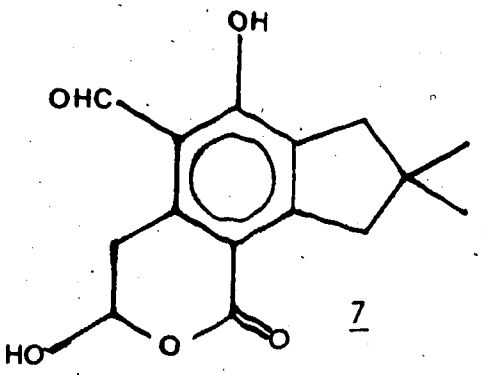

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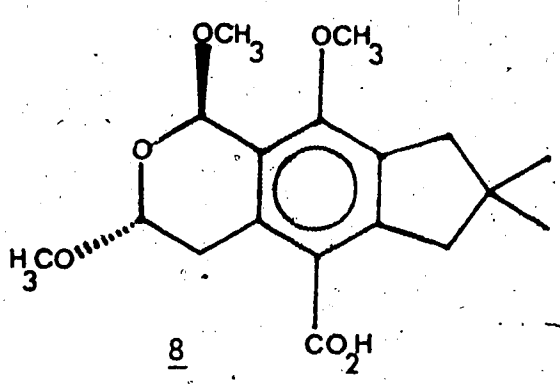

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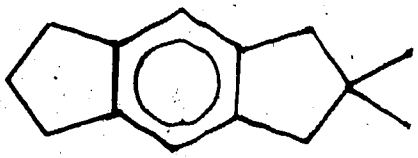

9

prepared in elegant fashion from hydrocarbon 9. The illudalane synthesis most germane to the problem of the cybrodin synthesis is that of Rao (Scheme 1)[6] who prepared pterosin E (10).

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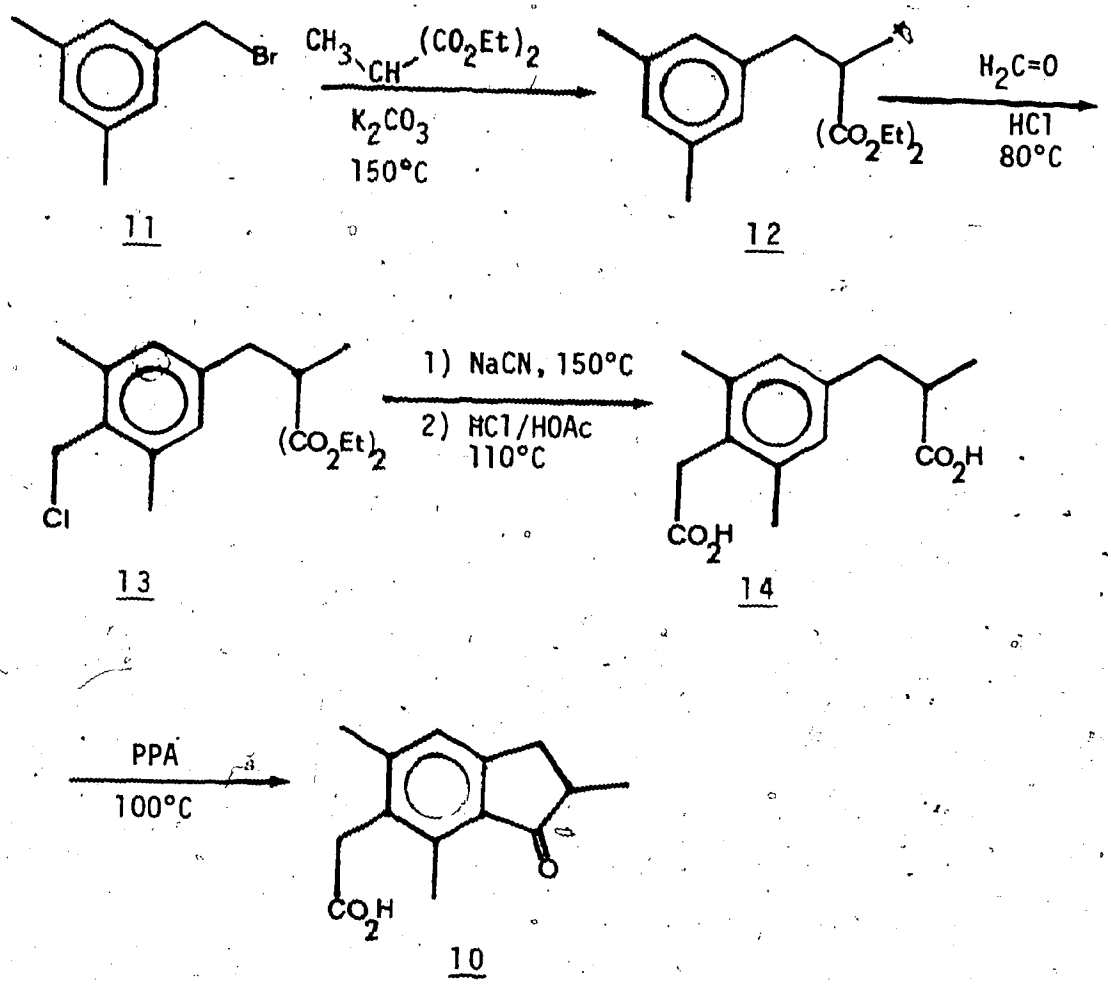
* For exhaustive accounts of sesquiterpenoid chemistry including total syntheses arranged by skeletal class see reference 4.

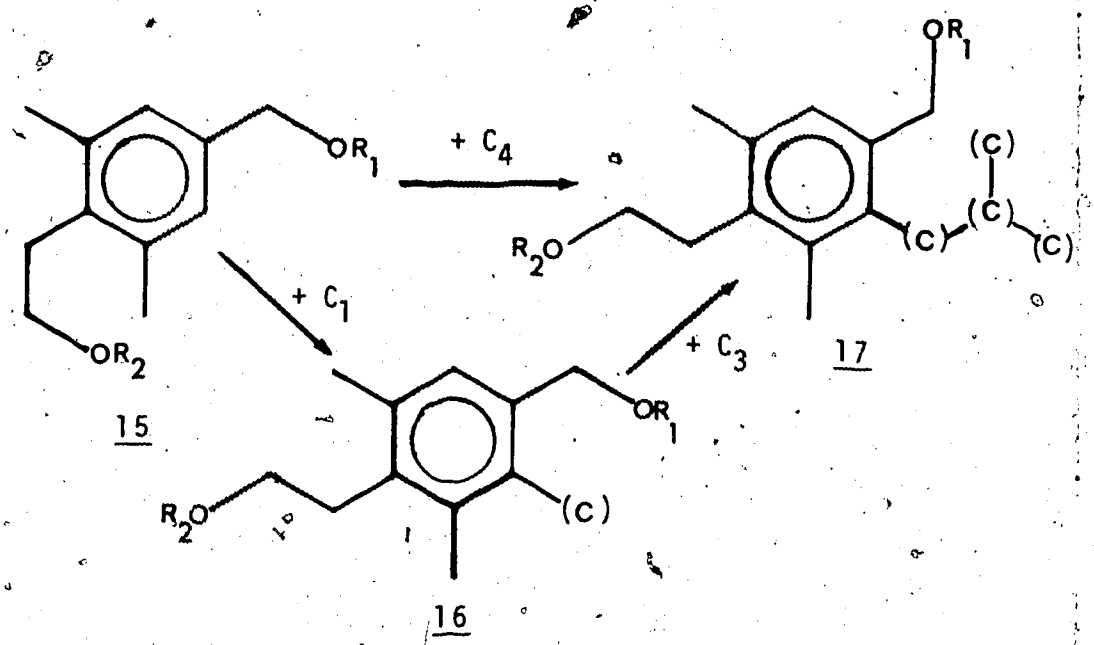
Scheme 1. Rao's synthesis of pterosin E (10).



A key aspect of this approach is the regioselective chloromethylation of intermediate 12 followed by chain extension with sodium cyanide. This serves to introduce the two carbon side chain. The bulky diester appendage forces chloromethylation to occur at the desired position. The introduction of the final aromatic substituent is achieved by polyphosphoric acid mediated cyclization of diacid 14.

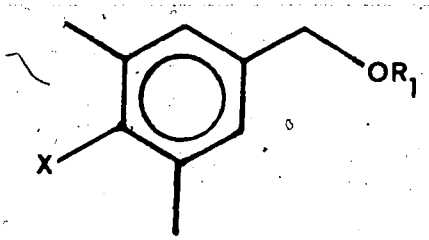
Our approach to the synthesis of the cybrodins (Scheme 2) involves elaboration of the symmetrical aromatic diether 15. We envisioned addition of a one carbon unit at either of the equivalent unsubstituted

Scheme 2. General strategy for the cybrodin synthesis.



aromatic centers. This should provide reasonably facile access to trisnorcybrodolide (5) through intermediate 16. Addition of an appropriate three carbon unit to 16 would afford the fifteen carbon cybrodin skeleton (17). The possibility of adding a four carbon fragment to 15 giving 17 directly would also be explored.

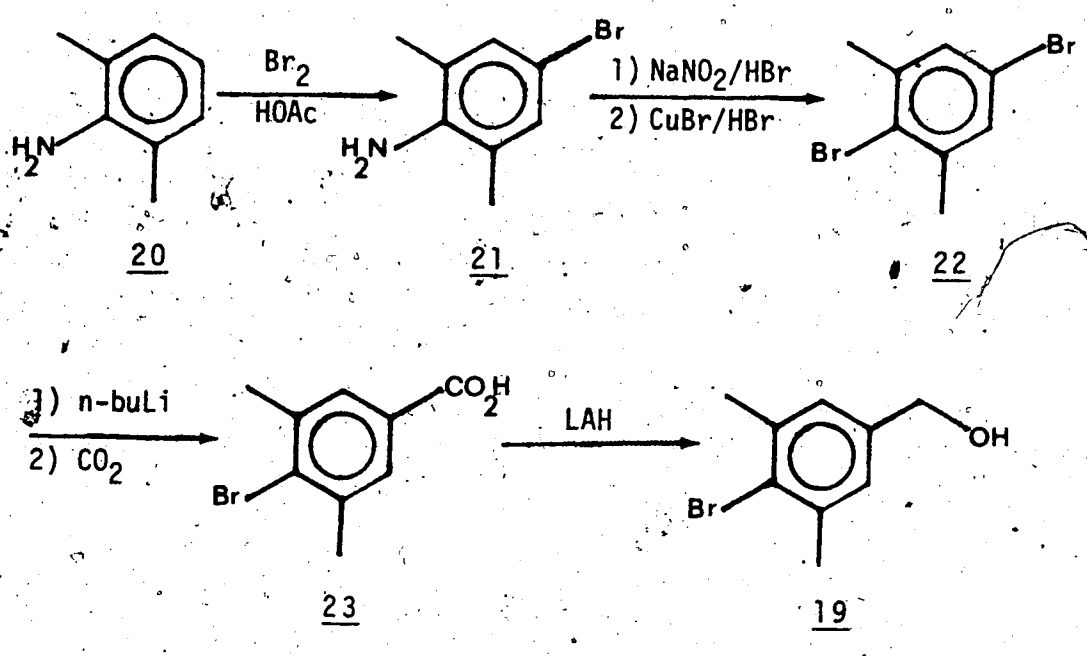
Simplification of intermediate 15 leads to general structure 18.



18

Compounds of this type have been reported. Grisdale has prepared alcohol 19[7] by methods outlined in Scheme 8.

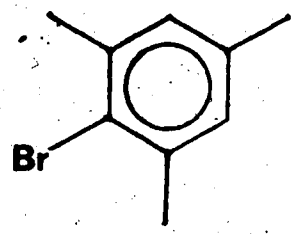
Scheme 3. Grisdale's preparation of alcohol 19.



20 21 22 23 19

The first two reactions in this sequence date back to the turn of the century. Thus 2,6-xylidene (20) was brominated[8] and the product (21) was brominated by Sandmeyer methodology[9]. Grisdale selectively lithiated dibromide 22 at the less hindered centre. Carbonation

and reduction afforded alcohol 19 in about 30% overall yield*. It was felt that this rather circuitous route to our projected starting material 18 could be improved upon if we could, with reasonable efficiency, regioselectively functionalize the C-5 methyl group of 2-bromomesitylene (24).

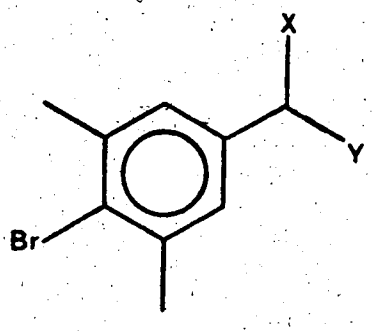


24

* Assuming that Sandmeyer bromination proceeds in 70% yield, the yield of this process is not quoted.

DISCUSSION AND RESULTS

Two classical methods have found much utility in the oxidation of aromatic methyl groups to useful functionality. The Étard reaction (chromyl chloride) is commonly used for this purpose, however the reaction is often difficult or even dangerous to carry out[10]. The results of Wheeler suggest that no regioselectivity can be expected in the Étard oxidation of 24. Under identical conditions, 4-bromotoluene gave an 85% yield (92% conversion) of 4-bromobenzaldehyde while 2-bromotoluene gave a 60% yield (95% conversion) of 2-bromobenzaldehyde[11]. The Thiele-Winter (chromyl acetate) oxidation of toluenes conveniently gives benzylidene diacetates which can be hydrolyzed to benzaldehydes[12]. Consideration of the procedures for the chromyl acetate oxidation of 2 and 4-nitrotoluene gave us some hope that oxidation of 24 under similar conditions would lead to 25 as the major product. Chromyl acetate



25 X,Y = OAc

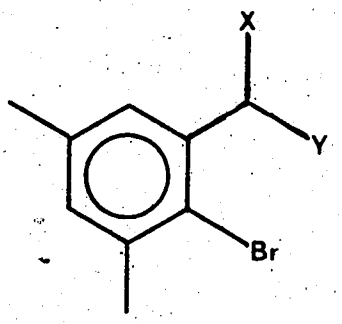
19 X = H, Y = OH

28 X = H, Y = OCH_3

30 X = H, Y = Br

oxidation of 4-nitrotoluene for two hours at 5-10°C gives 4-nitrobenzylidene diacetate (65%) while 2-nitrotoluene gives the corresponding product (36%) after three hours at 5-10°C[13].

2-Bromomesitylene (24) was subjected to chromyl acetate oxidation under the conditions recommended by Lieberman and Connor[14]. Under strict temperature control (5-10°C), oxidation for ninety minutes afforded a 41% (recrystallized) yield of 25 (mp 94-95°C) when the reaction was conducted on a modest (7 g of 24) scale. The isomeric product 26 was not isolated from



26 X,Y = OAc

29 X = H, Y = OCH_3

31 X = H, Y = Br

the product mixture, however subsequent results implied its presence. The ratio of 25 to 26 was apparently (*vide infra*) 89:11.

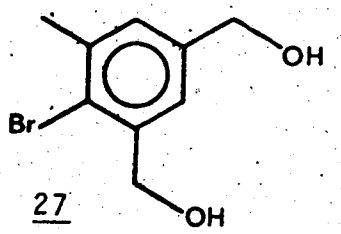
Nuclear magnetic resonance (nmr) spectra of product 25 clearly demonstrate that oxidation occurred *para* to the bromine. The ^1H nuclear magnetic resonance (^1Hmr) spectrum ($CDCl_3$) shows one signal (δ2.43)* for the aromatic methyl groups as well as one signal (δ7.20) for

* All nmr shifts quoted in Part II of this thesis are relative to tetramethylsilane.

the aromatic protons. The ^{13}C nuclear magnetic resonance (^{13}Cmr) spectrum ($CDCl_3$) shows only four signals (δ126.3*, 129.0, 134.0, 138.8*) assignable[15] to aromatic carbons.

When this reaction was performed on a synthetically practical scale (100 g of 24), a crystalline product could not be obtained although ^{1}Hmr evidence indicated that 25 was the major component of the product mixture. A cold ethanol solution of the crude reaction product seeded with crystalline 25 could not be induced to crystallize. Consequently, the unpurified product of chromyl acetate oxidation was used in the next step of the synthesis.

Crude 25 was reduced with an excess of lithium aluminum hydride in ether. We had hoped that pure alcohol 19 could be obtained by crystallization of the product mixture. Indeed, a cold chloroform solution of the crude reduction product did give some crystalline material (mp 124-125°C). The melting point of this compound did not agree with that reported for alcohol 19 (53-54°C)[7]. The crystalline material was identified as diol 27 resulting from over oxidation of 24. Alcohol



27

* Double intensity signals.

19 could not be induced to crystallize from the mother liquor. Small scale chromatography of the mother liquor gave pure 19 (mp 52-54°C). Large scale purification by this method was not attempted, instead the crude reduction product was used in the next step without purification.

Crude alcohol 19 was methylated by the method of Brown[16]. Methylation was deemed the most appropriate mode of alcohol protection[17] for several reasons. Two carbon chain extension (18 → 15) would require that the protecting group be stable under strongly basic conditions. Addition of the fifth aromatic substituent (15 → 16 or 17) would necessitate the use of a small protecting group and, depending on the method of introducing this substituent, stability to acid or base might be needed. A methyl ether seemed the most secure device for storing the hydroxymethyl group.

Polar impurities were removed from the methylated product by rapid passage through an alumina column. Distillation then gave an 89:11 mixture* of methyl ethers 28 and 29. The combined overall yield was 44% in three steps from 24. Pure samples of 28 and 29 were obtained by chromatography, however large scale separation of isomers 28 and 29 by this method was not practical.

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* As judged by ^{1}Hmr.

Nmr examination of the separated isomers clearly demonstrates that the major product of this sequence was the symmetrical bromoether (28). One signal is seen for the aromatic methyl groups of 28 in the ^{1}Hmr (δ2.42) and the ^{13}Cmr (δ23.7*) spectra ($CDCl_3$). The ^{13}Cmr spectrum of 28 has four signals attributable to aromatic carbons (δ126.4, 127.4*, 136.9, 138.1*)[15]. In contrast, the aromatic methyl groups of 29 are non-equivalent (^{1}Hmr: δ2.26, 2.35; ^{13}Cmr: δ20.8, 23.2) and six distinct aromatic carbon signals (δ121.8, 127.0, 130.6, 136.6, 137.6, 137.9) are seen in the ^{13}Cmr spectrum of this minor product.

Bromoethers 28 and 29 were also prepared by two additional methods. Free radical bromination of 24 (N-bromosuccinimide, benzoyl peroxide) gave a mixture of dibromides 30 and 31 which was not characterized. Treatment with sodium methoxide gave a 62:38 mixture of 28 and 29 in 72% combined overall yield from 24. Photobromination of 24 with bromotrichloromethane[18] gave, after the same sodium methoxide treatment, a 60:40 mixture of 28 and 29 in 86% combined overall yield from 24.

With the C-5 methyl group of 24 securely functionalized, we now turned our attention to the task of adding the two carbon β-hydroxyethyl side chain (18 → 15).

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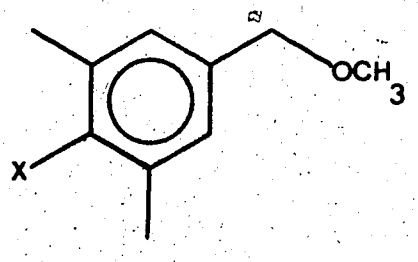
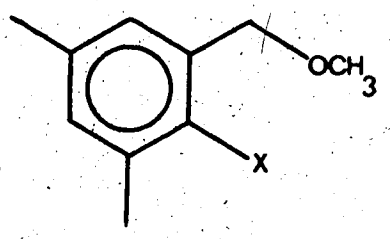
* Double intensity signals.

A Grignard reaction was attempted on a mixture of 28 and 29 (89:11). Ethylene oxide was added to a mixture of Grignard reagents 32 and 33 prepared by refluxing a tetrahydrofuran solution of bromides 28 and 29 in the

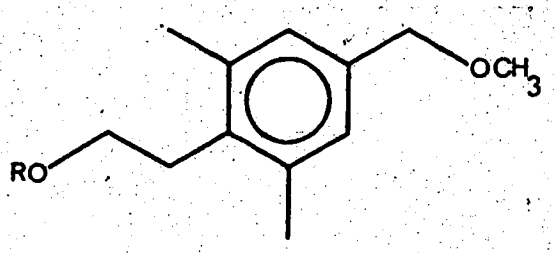




32 X = MgBr
35 X = H
36 X = OH
38 X = Li

33 X = MgBr
35 X = H
39 X = Li

presence of magnesium and 1,2-dibromoethane. Examination of the reaction products by thin layer chromatography (tlc) revealed that the desired alcohol 34

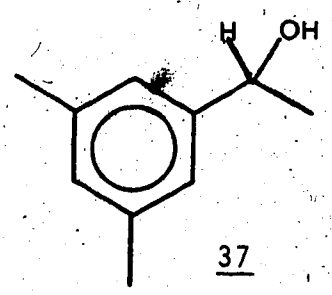


34 R = H
42 R = Ac
50 R = THP

(*vide infra*) was not formed in this reaction. The major products isolated by chromatography were methyl ether 35 (59%) and phenol 36 (6%). Compound 35 resulted from protonolysis of the Grignard reagents 32 and 33. Whether or not this occurred during work-up or during

the reaction (*i.e.* wet solvent, reagents and/or apparatus) is not clear. Phenol 36 likely resulted from oxidation of 32, suggesting the incursion of atmospheric oxygen.

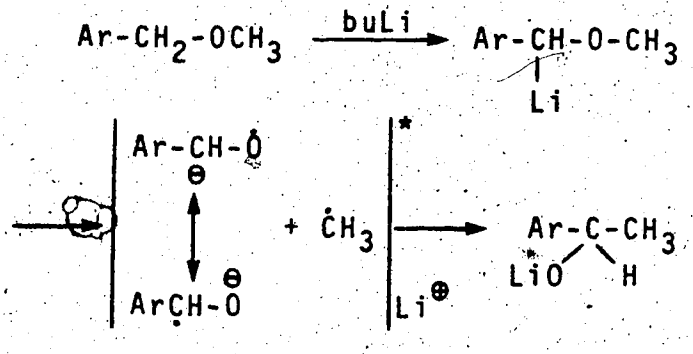
A mixture of bromides 28 and 29 (89:11) was lithiated (n-butyllithium) in tetrahydrofuran-hexane at room temperature. Ethylene oxide was added to the purple solution. Once again, tlc examination revealed the absence of alcohol 34 (*vide infra*). The major product, after chromatography of the complex product mixture, was alcohol 37 (37%). This compound likely resulted



37

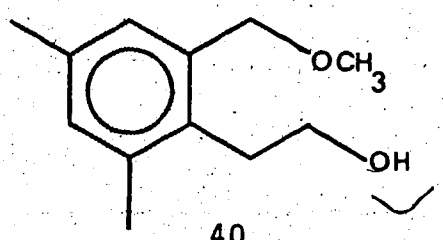
from Wittig rearrangement (Scheme 4)[19,20] of intermediate 38 or 39.

Scheme 4. Formation of 37.



* Caged radicals.

Low temperature (-78°C) lithiation (1.1 equivalents of n-butyllithium) of a tetrahydrofuran-hexane solution of bromides 28 and 29 (89:11) followed by addition of ethylene oxide (-78°C, five hours) gave a satisfactory result. On a small scale (5 g of 28 and 29), a 51% yield of alcohol 34 was obtained after chromatography. The purification of this alcohol on a larger scale (25 g of 28 and 29) was greatly facilitated by low temperature (-70°C) calcium chloride complexation[21] which removed the desired compound (34) from a rather complex product mixture. Distillation then gave alcohol 34 in 66% yield, isomerically pure. The isomeric alcohol (40, *vide infra*) did not contaminate the distilled product, nor was it observed on tlc examination of the reaction products prior to calcium chloride complexation.



40

An authentic sample of 40 was prepared by lithiation (n-butyllithium, tetrahydrofuran-hexane, -78°C) of pure 29 and treatment with ethylene oxide (-78°C, five hours; -5°C, overnight). Aryllithium 39 reacted slowly and inefficiently with ethylene oxide affording

a poor (19%) yield of 40. Methyl ether 35 (66%) was the major product obtained after chromatography. The sluggish reactivity of 39 is attributed to intramolecular complexation with the ethereal oxygen (41)[19].

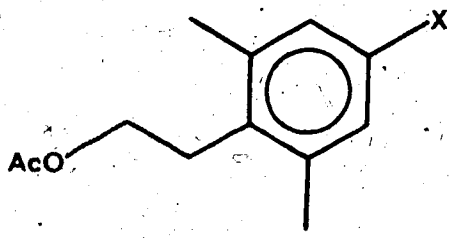
41

Comparison of the nmr spectra ($CDCl_3$) of 34 and 40 leaves no question as to the regiochemistry of the alcohol (34) used for subsequent synthetic transformations. The ^{1}Hmr spectrum of 34 shows one aromatic methyl group peak (6 H, δ2.33) and one sharp aromatic proton peak (2 H, δ6.99). The ^{1}Hmr spectrum of 40 has two aromatic methyl group signals (δ2.26, 2.29) and a broad aromatic hydrogen peak (2 H, δ6.95). The ^{13}Cmr spectrum of 34 has only four peaks assignable[15] to aromatic carbons (δ127.9*, 134.3, 136.0, 137.1*) while the ^{13}Cmr spectrum of 40 shows six such peaks (δ128.8, 131.6, 133.2, 135.6, 136.1, 137.3).

We now addressed the problem of adding the fifth substituent to the aromatic ring (15 → 16). Friedel-Crafts formylation was considered first, since this approach offered the most direct route to 16. Treat-

* Double intensity peaks.

ment of an aromatic compound with α,α-dichloromethyl methyl ether in the presence of either titanium tetrachloride[22] or stannic chloride[23] is a common formylation method*. For example, mesitylene can be formylated in 81-89% yield using this technique[22]. The acetyl derivative (42, acetic anhydride-pyridine-methylene chloride) of alcohol 34 was selected as the substrate for formylation experiments. Lewin's improvement[24] of the Rieche method was employed. The arene (42) was slowly added to a methylene chloride solution of the preformed complex (1.7 equivalents) of the Lewis acid and α,α-dichloromethyl methyl ether. When stannic chloride was used, 42 was consumed and an intractable mixture of highly polar products was formed. With titanium tetrachloride, a single product, identified as the benzylic chloride 43 was formed in good yield.



43 X = CH_2Cl

44 X = CHO

Compound 43 may be formed through the steps indicated in Scheme 5. Two other widely used formylation methods are the Gattermann[25] and the Gattermann-Koch[26] reactions. Both methods employ concentrated protic

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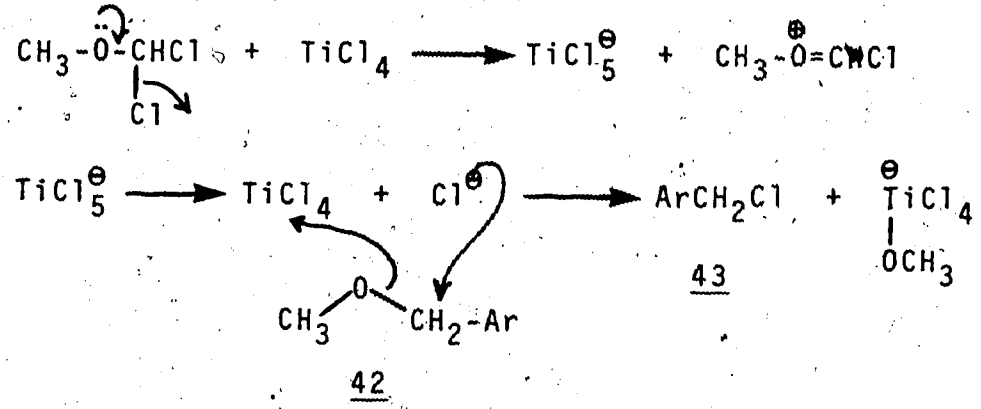
* Often called Rieche formylation.

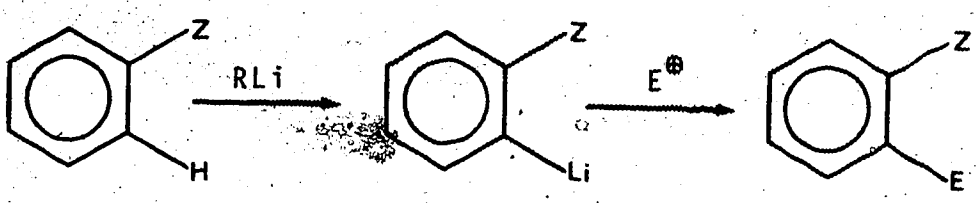
Scheme 5. Formation of 43.



(usually hydrochloric) acid at elevated temperatures. In view of the known[27] lability of methylbenzyl ether under strongly acidic conditions, these approaches were not investigated. The applicability of most other common formylation methods is usually restricted to reactive substrates (*i.e.* indoles, anisoles, *etc.*)[28].

Direct *ortho* metalation of aromatic compounds (Scheme 6) is an increasingly popular synthetic technique[29] which has recently been reviewed[30]. A wide

Scheme 6. *Ortho* metalation.

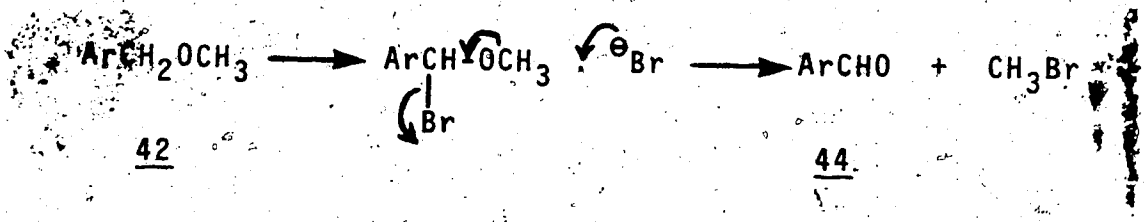


variety of functional groups (Z) are capable of pro-

moting efficient *ortho* metalation[29,30], however this methodology has not been extended to methylbenzyl ether derivatives. It is known[19,30] that attempted direct *ortho* metalation of methylbenzyl ether causes benzylic deprotonation and Wittig rearrangement (Scheme 4). Seebach has effected *ortho* metalation of benzyl alcohol[31], however application of this process to our synthesis would require suitable protection of the hydroxyl group of 34 followed by demethylation. An efficient synthetic approach to the cybrodins using *ortho* metalation would require the incorporation of the directing group Z (*eg.* N,N-diethylamido)[29] at the beginning of the synthesis. Under these circumstances Z could complicate the process of introduction of the β-hydroxyethyl group. We therefore chose to adopt the more classic approach of bromination followed by metal exchange.

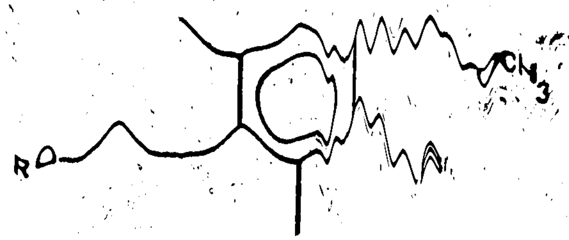
Compound 42 was used to establish favourable conditions for bromination. Treatment of 42 with bromine (1.2 equivalents) in carbon tetrachloride at 0°C gave aldehyde 44. Free radical bromination likely occurred at the benzylic position, elimination of methyl bromide would then give 44 (Scheme 7).

Scheme 7. Formation of 44.



42 44

Conversion of m[illegible] ether [illegible] with bromine is a know[illegible]ess[32]. Wh[illegible] (1.2 equivalents [illegible]mine) of 4[illegible] out in nitromethane (0[illegible] the desired r[illegible] species 45 was [illegible]. Br[illegible] 1.5 equivalents of bromine [illegible] alcohol 34 un[illegible] conditions



[illegible] R = A[illegible]
[illegible] R = H
[illegible] R = THP

gave bromoalcohol [illegible] 104-106°C [illegible]. Only very small samples [illegible] this oily pr[illegible] crystallized, at[illegible] bulb to pu[illegible] caused extensive d[illegible]sition. M[illegible] required that the h[illegible] group of [illegible] tetrahydropyranyl [illegible] was deemed [illegible] appropriate protection method. [illegible] treatment of [illegible] dihydropyran (2.8 equivalents) [illegible] methylene chlo[illegible] presence of a catalytic am[illegible] pyridinium [illegible] gave the oily tetrahyd[illegible] (THP) et[illegible] yield after chromatography.

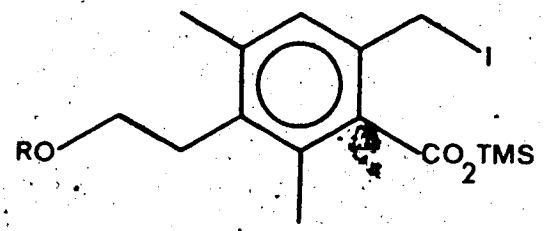
47 X = Br [illegible] X = CO_2H
48 X = MgBr [illegible] X = CO_2CH_3
49 X = Li [illegible] X = CHO
50 X = H [illegible] X = CH_2OH

Metalation of 47 was a facile process, likely made so by the presence of the methoxymethyl group (*cf.* Diagram 41)[19,29,30]. The Grignard reagent 48 was formed by refluxing a tetrahydrofuran solution of 47 and 1,2-dibromoethane in the presence of magnesium powder. Aryllithium 49 was formed by addition of 47 to a tetrahydrofuran-hexane solution of n-butyllithium (1.1 equivalents) at -78°C.

The metalated species 48 and 49 were treated with a variety of one carbon electrophiles (15→16). Gaseous carbon dioxide failed to react with either organometallic (49: two hours at 0°C), high yields of the protonolysis product 50 were isolated. Negligible acidic products (*i.e.* compound 51) were obtained. Treatment of an organometallic with trimethyl orthoformate followed by acid hydrolysis is occasionally used as an aldehyde synthesis[34]. Intermediates 48 and 49 failed to react with excess trimethyl orthoformate (48: two hours at reflux, 49: five hours at -78°C). Compound 50 was the sole product in each case. Likewise when 48 was treated with excess methyl chloroformate (overnight at reflux), 50 was the only product. However, treatment of 49 with methyl chloroformate (7.5 equivalents, overnight at 0°C) gave a respectable yield (62%) of ester 52 as a clear oil after chromatography.

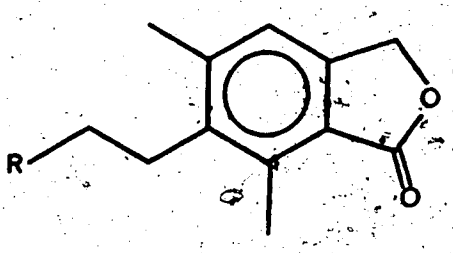
The preparation of trisnorcybrodolide (5) could

now be dealt with. Olah has reported that iodotrimethylsilane, generated *in situ* from chlorotrimethylsilane and sodium iodide in acetonitrile, will convert methyl esters to silyl esters, cleave methyl ethers to alcohols and transform benzylic alcohols to iodides[35]. We reasoned that exposure of 52 to this reagent might afford intermediate 53, a species which should be well



53

suited to lactonization. The fate of the THP ether protecting group was unclear from Olah's results. Treatment of 52 with an excess of the Olah reagent in refluxing acetonitrile gave a mixture of products from which material (11%) identical in all respects (tlc, ir, ms, ^{1}Hmr) with natural trisnorcybrodolide (5)[2] could be isolated by preparative thin layer chromatography (ptlc). The major product (60%) was the primary iodide 54 (mp 182-184°C), likely formed from 5 by the

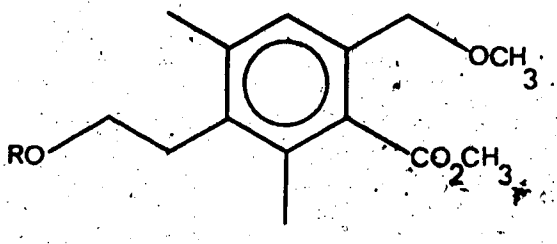


5 R = OH

54 R = I

57 R = OAc

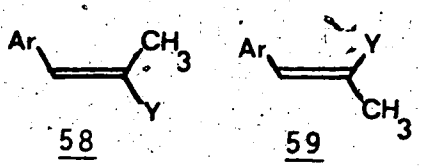
action of excess reagent. In order to avoid this side reaction, the THP protecting group was removed by methanolysis (pyridinium tosylate)[33]. Alcohol 55 was



52 R = THP
55 R = H
56 R = Ac

obtained as a clear oil (97%). Acetylation (acetic anhydride-pyridine) gave the acetyl derivative 56 in 98% yield. Exposure of 56 to an excess of Olah's reagent in refluxing acetonitrile gave a product identified as acetyltrisnorcybrodolide (57) by tlc comparison with authentic material[2]. Without purification this intermediate was treated with potassium carbonate in methanol affording synthetic trisnorcybrodolide (5, mp 189-191°C) in 75% overall yield from 52.

Addition of the final three carbons of the fifteen carbon cybrodin skeleton (16 → 17) was now considered. Since cybrodins with *E* (1, 3, 4) and *Z* (2) olefinic geometries were synthetic targets, we desired methodology which would produce roughly equal proportions of olefins 58 and 59*.



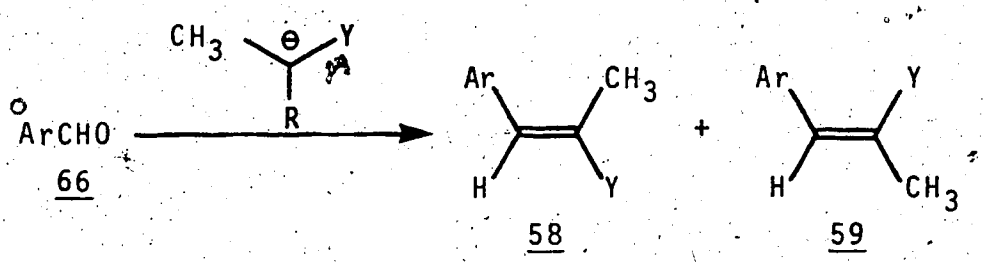
58 59

* Where Y is some functionality transformable to CH_2OH, CHO or CO_2H.

Our experience from the isolation of cybrodol (1) and isocybrodol (2)[2] gave us confidence that synthetic intermediates 58 and 59 would be separable, consequently stereoselective synthesis of either isomer 58 or 59 was considered counter-productive.

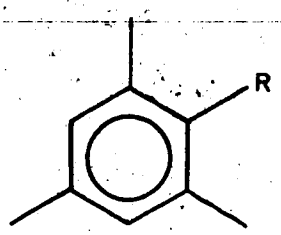
Three approaches received attention during our preliminary deliberations. A Wittig approach (Scheme 8) offered a direct solution to the problem. Addition of a suitable three carbon unit (60-65) to aldehyde 66

Scheme 8. Wittig approach to the cybrodins.



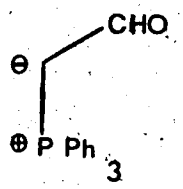
R	$P(C_6H_5)_3^{\oplus}$	$P(C_6H_5)_3^{\oplus}$	$P(C_6H_5)_3^{\oplus}$	$P(OC_2H_5)_2$ (O)	$P(OC_2H_5)_2$ (O)	$P(OC_2H_5)_2$ (O)
Y	$CO_2C_2H_5$	CN	CHO	$CO_2C_2H_5$	CN	CHO
	60	61	62	63	64	65

would give the complete fifteen carbon cybrodin skeleton. Since aldehyde 66 is *ortho* disubstituted, steric hindrance may prevent the success of this scheme. Condensations of ylides 60-62 with mesitaldehyde (67) have not been reported. Ylide 62 has not been reported,



67 R = CHO

74 R = CO_2CH_3

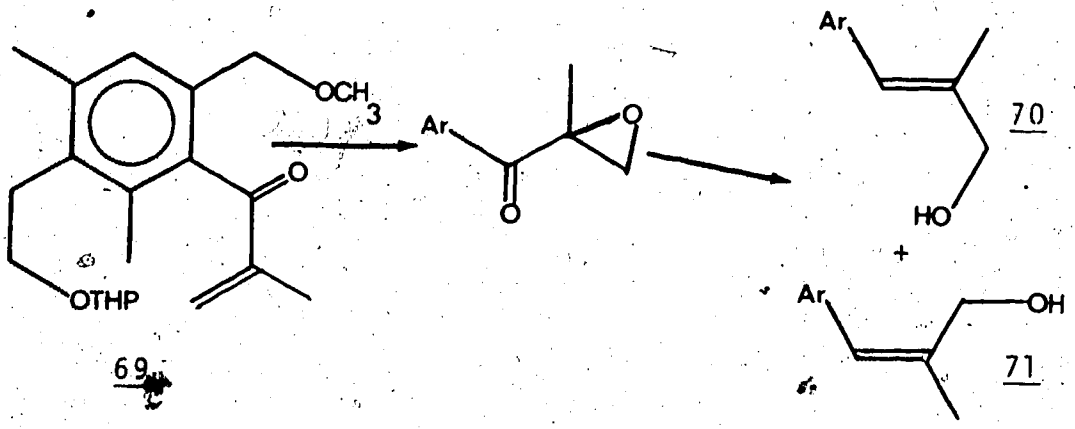


68

although analogue 68 is known to condense with benzaldehydes giving good yields of *E* cinnamaldehydes[36]. Likewise, ylides 60 and 61 give predominantly the *E* isomer on condensation with aldehydes[37]. The more nucleophilic phosphonate carbanions 63 and 64 offer a better prospect of a successful Wittig (Horner-Emmons modification) reaction with aldehyde 66[38]. Kinstle has condensed 63 with aldehyde 67 giving exclusively the *E* olefin. No yield was reported although the yields for a large series of aldehydes (aliphatic and aromatic) were in the range 65-95%[39]. The results of French workers using carbanion 64 and aromatic aldehydes (not *ortho* disubstituted however) suggest that the *E*/*Z* ratio using carbanion 64 can be dramatically influenced by experimental conditions[40]. Use of carbanion 65 or its analogues has not been reported.

An approach based on Wharton's epoxyketone reaction[41] (Scheme 9) also received consideration. Epoxidation of enone 69, hydrazine treatment and thermolysis would give the properly functionalized

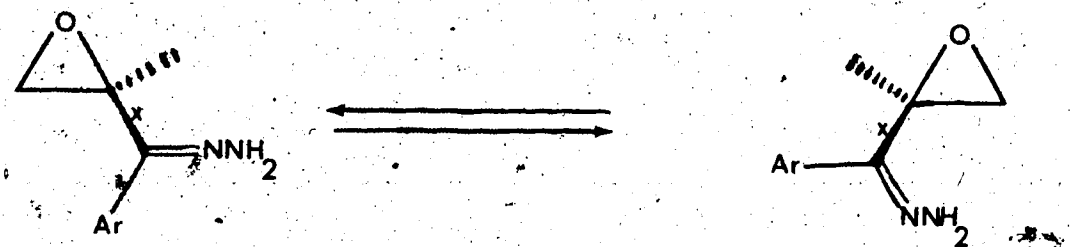
Scheme 9. Wharton approach to the cybrodins.



69

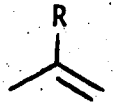
fifteen carbon cybrodin skeleton (70, 71). The *E*/*Z* ratio in this scheme likely would depend upon the rotomeric distribution about bond x of intermediate 72a,b (Scheme 10). Consideration of Dreiding models

Scheme 10. Geometry of the Wharton reaction.



72a 72b

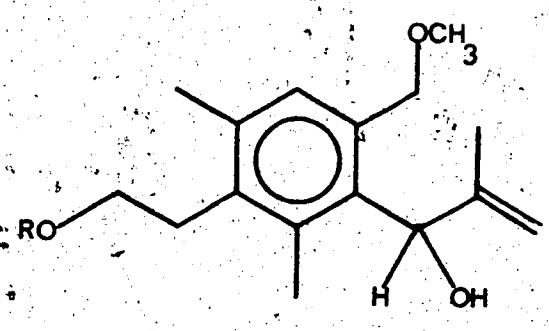
reveals little apparent predictable preference for either conformer. Enone 69 should be available via addition of isopropenyllithium (73) to acid 51.



73 R = Li

76 R = MgBr

Obtaining acid 51 would likely not be a trivial process. Methyl esters such as 52 (*cf.* methyl mesitoate (74)) are notoriously resistant to base hydrolysis[42]. Hydrolysis is usually achieved by recourse to a reagent capable of attacking the methyl carbon rather than the carbonyl carbon (*eg.* iodotrimethylsilane). Under these circumstances, concomitant methyl ether cleavage might greatly complicate the preparation of 51. Alternatively, oxidation of alcohol 75, prepared by addition of 73 or 76 to aldehyde 66, could provide ketone 69.

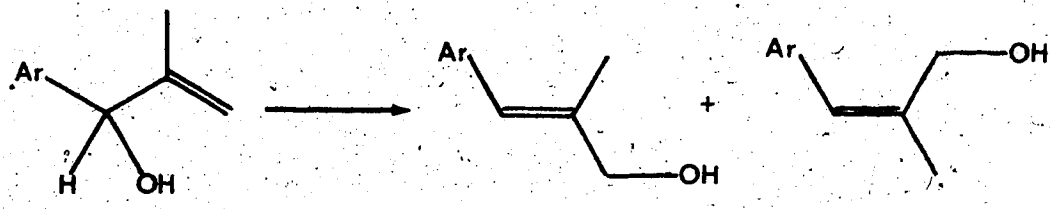


75 R = THP

81 R = H

Allylic rearrangement (Scheme 11) of alcohol 75 was the third approach contemplated. We deemed this

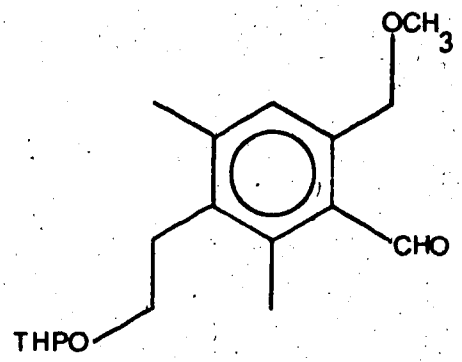
Scheme 11. Allylic rearrangement approach to the cybrodins.



75

route most worthy of priority consideration since it

involved addition of a very reactive nucleophile (73 or 76 rather than 60-65) to a receptive electrophile (66 rather than 51). We therefore required aldehyde 66.



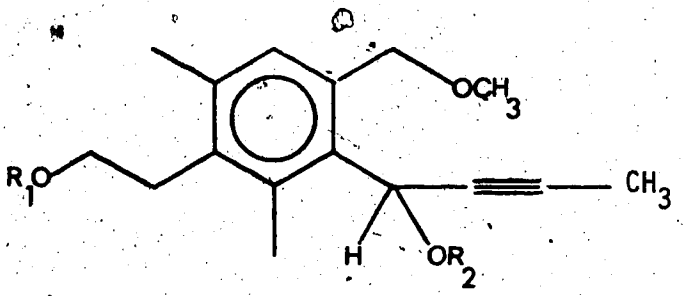
66

Controlled reduction of esters with diisobutylaluminum hydride is a well established aldehyde preparation method[43]. Exposure of ester 52 to one equivalent of this reagent in toluene at -78°C for two hours and then at room temperature for two hours gave only recovered 52. Ester 52 was easily reduced to alcohol 77 with lithium aluminum hydride in ether. The pure alcohol was obtained in 77% yield after chromatography.

Aldehyde 66 was prepared in 98% yield by oxidation of alcohol 77 with pyridinium chlorochromate in buffered methylene chloride. Sodium acetate buffering is recommended for pyridinium chlorochromate oxidations

when acid sensitive THP ether groups are present[44].

The aldehyde (66) was treated with excess isopropenyllithium (73) prepared from 2-bromopropene by the method of Braude[45]. The product, isolated in low (15%) yield after chromatography was propargylic alcohol 78 and not the expected allylic alcohol 75.

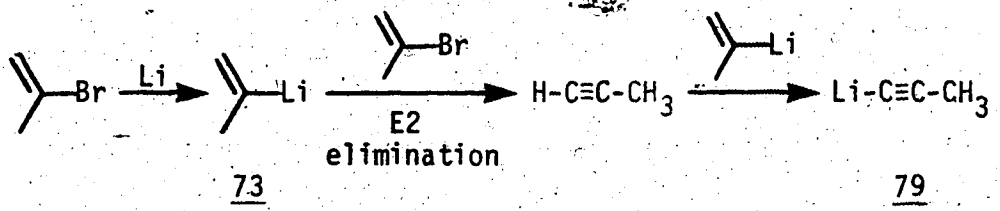


78 R_1 = THP, R_2 = H

80 R_1 = H, R_2 = CH_3

This result is rationalized in Scheme 12. The attack-

Scheme 12. Formation of 78.



73 79

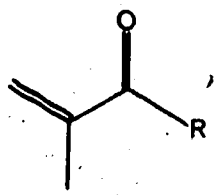
ing species was 1-lithiopropyne (79) and not isopropenyllithium (73). The alkyne moiety was evident on examination of nmr spectra of the deprotected (methanol-pyridinium tosylate)[33] species. Alkyne carbons generally resonate in the region δ70-90[46], the ^{13}Cmr spectrum of 80 shows fully substituted carbons at δ83.0 and δ1[illegible]8.4.

Methyl groups located α to a triple bond resonate at rather highfield, for instance the ^{13}Cmr shift of the C-1 carbon of 2-hexyne is δ2.9[46]. Compound 80 shows a methyl group signal at δ3.8 in the ^{13}Cmr spectrum. The alkyne methyl group of 80 is seen in the ^{1}Hmr spectrum (δ1.83) as a doublet (J = 3 Hz) coupled to the carbinol proton (δ5.38, q (3 Hz)). This is typical of acetylenic systems where protons on the α and α' carbons usually couple by ~3 Hz[47]. The failure to observe acetylenic carbon-carbon stretching bands (2260-2190 cm^{-1}) in the infrared (ir) spectra of 78 or 80 is not surprising, this band is often not observed in the ir spectrum of an internal alkyne[48]. The reactive propargylic alcohol 78 was solvolyzed to the methyl ether (80) under mildly acidic (pyridinium tosylate)[33] catalysis.

Recourse to the Grignard reagent 76, formed by addition of 2-bromopropene and 1,2-dibromoethane to a tetrahydrofuran suspension of magnesium powder, produced the desired result. Addition of aldehyde 66 to Grignard reagent 76 (five equivalents) gave carbinol 75 in 80% yield after chromatography. The deprotected version (81, pyridinium tosylate - aqueous tetrahydrofuran)[33] of 75 was fully characterized.

The possibility of direct addition of a four carbon unit to organometallic 48 or 49 (15 → 17) was now explored. Treatment of 48 or 49 with methacrylyl

chloride (82)[49] could potentially give ketone 69 and

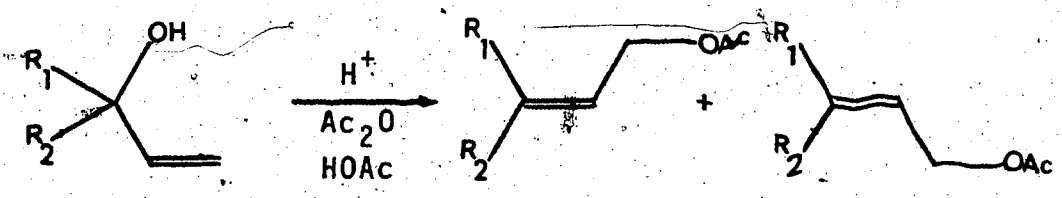


82 R = Cl
83 R = H

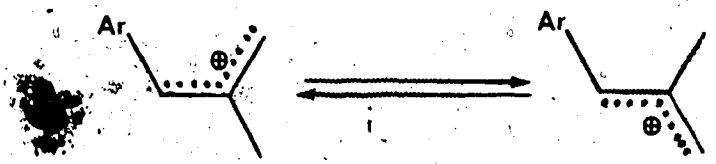
thereby afford entry to either the Wharton (Scheme 9) or allylic rearrangement (Scheme 11) approach to the fifteen carbon cybrodin skeleton. In both cases however (48, thirty equivalents of 82, one hour at reflux; 49, forty equivalents of 82, four hours at 0°C) compound 50 was the only product obtained. Treatment of 49 with methacrolein (83, five equivalents) gave a 25% yield of carbinol 75 after chromatography. While the yield of this reaction was unimpressive, it did provide a useful expedient as two steps (52 → 77 → 66) could be by-passed.

Babler has developed a trisubstituted olefin synthesis[50] based on the facile acid catalyzed isomerization of tertiary allylic alcohols (or acetates) to primary allylic acetates (Scheme 13). The rearrange-

Scheme 13. Babler's rearrangement scheme.

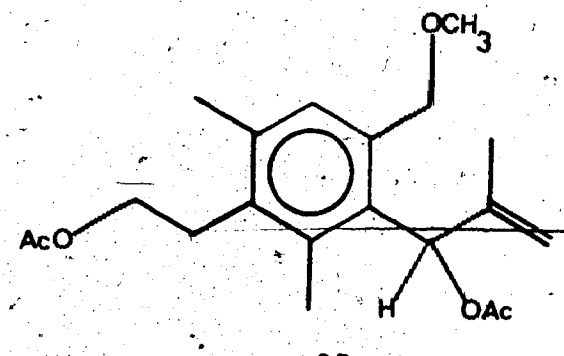


ment is carried out in acetic acid-acetic anhydride and is catalyzed by *p*-toluenesulfonic acid. The method has not been extended to include isomerization of secondary allylic alcohols, however we felt that this scheme might be applicable to our problem since the secondary alcohol function of compound 81 is both allylic and benzylic. This factor should promote the formation of carbonium ions 84a,b. However, the fact that the four



84a 84b

carbon side chain of alcohol 81 is flanked by two *ortho* substituents will likely prevent ideal overlap between the allylic and aromatic π-systems of 84a,b . Exposure of alcohol 81 to Babler's conditions gave the unrearranged compound 85.

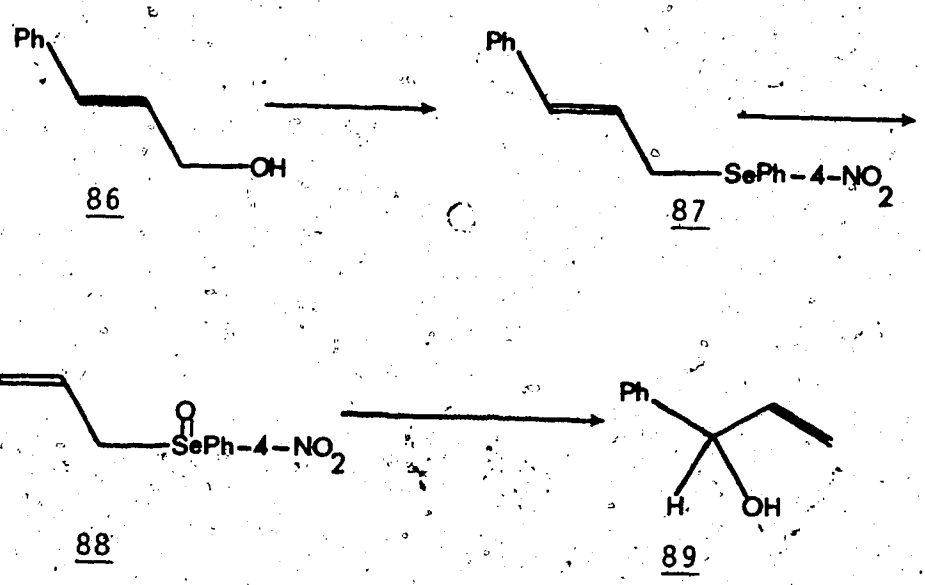


85

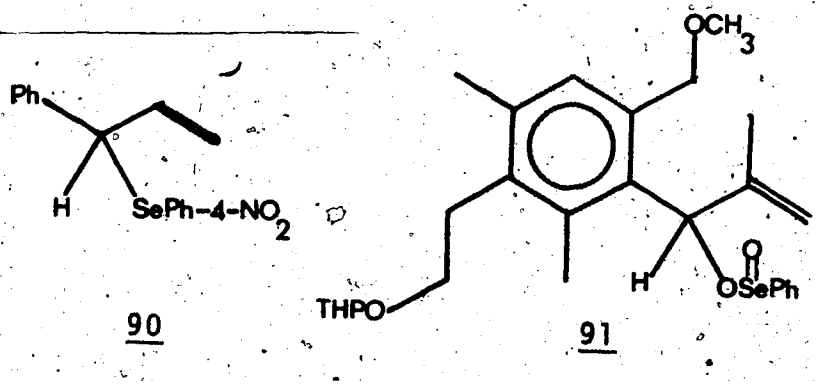
Clive has used selenium chemistry to effect 1,3

alcohol transposition[51] (Scheme 14). Alcohol 86 was

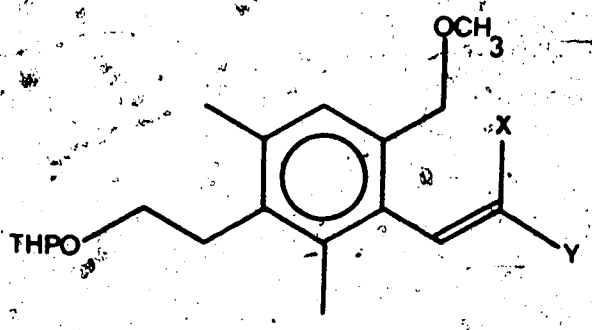
Scheme 14. 1,3 alcohol transposition using selenium chemistry.



converted to selenide 87, oxidation then afforded intermediate 88 which underwent [2,3] sigmatropic rearrangement under mild conditions affording, after work-up, alcohol 89. The methodology has not been applied to the reverse transformation (89 → 86), because of 1,3-seleno-allylic rearrangement of selenide 90 to 87[52,53]. It was suggested[52] that selenoester 91 might undergo



[3,3] sigmatropic rearrangement affording 92a and 92b.



92a X = CH_3, Y = CH_2-OSePh(=O)

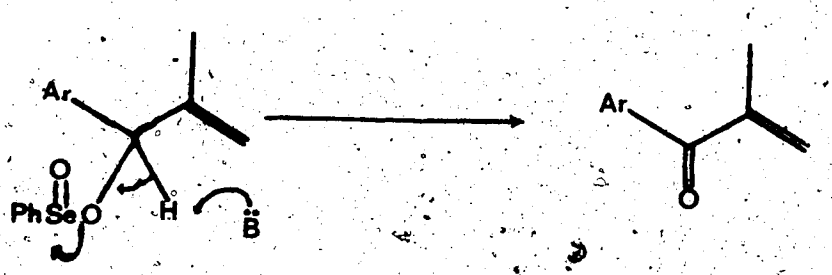
92b X = CH_2-OSePh(=O), Y = CH_3

Treatment of alcohol 75 with benzeneselenenic anhydride (93, 1.2 equivalents) and pyridine (1.2 equivalents) in

93

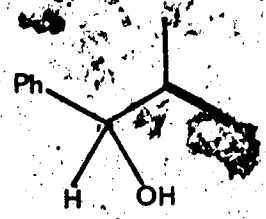
methylene chloride (overnight at room temperature) gave recovered 75 (20%) as well as a new product (49%) identified as ketone 69, likely formed by base (*eg* pyridine) induced fragmentation of intermediate 91 (Scheme 15).

Scheme 15. Formation of 69.

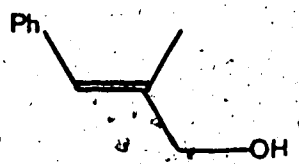


91 69

Braude[45] reports that alcohol 94 undergoes acid mediated allylic rearrangement in aqueous acetone affording primary alcohol 95 in 50% yield. Braude states



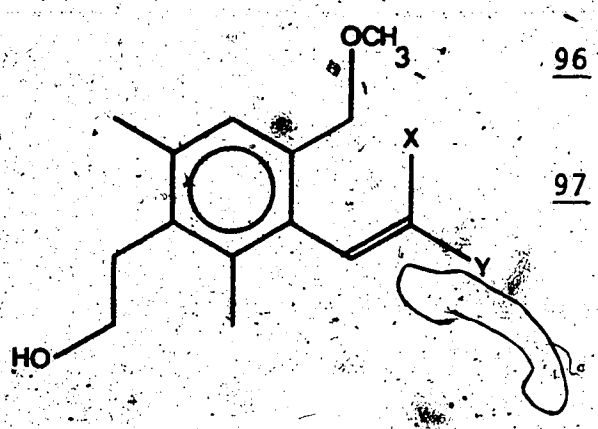
94



95

that the product likely possesses the *E* geometry although no spectral evidence was available to support his contention.

Alcohol 75 was exposed to dilute (0.12 M) sulfuric acid in refluxing 60% aqueous acetone. The THP ether function was cleaved in short order as tlc examination revealed that diol 81 was formed within a few minutes. Over a period of several hours, tlc monitoring indicated the disappearance of diol 81 while two new products, assigned structures 96 and 97, were formed. The combined yield of 96 and 97 was maximal (67%) after a



96 X = CH_3
Y = CH_2OH

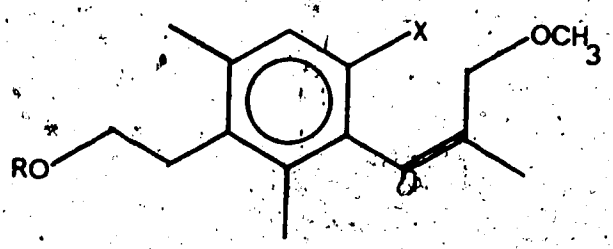
97 X = CH_2OH
Y = CH_3

reaction period of six to seven hours. The products were formed in roughly equal amounts and were separable by careful chromatography.

Assignment of structure 96 followed readily from comparison of the ^{1}Hmr spectrum ($CDCl_3$) of one product with that of cybrodol (1)[2]. Except for the presence of an O-methyl group signal (δ3.34) in the spectrum of the synthetic compound, the spectra are practically superimposable. The methylene group protons of a primary methyl ether normally appear 0.2-0.3 ppm upfield from the position of the carbinol protons of the corresponding primary alcohol[54]. Thus one would expect that the benzyl ether methylene group protons of the synthetic product should appear ~0.2-0.3 ppm upfield from the position of the benzyl alcohol methylene group protons (δ4.50) of cybrodol (1)[2]. In the ^{1}Hmr spectrum of the synthetic compound, the methylene protons assigned to the allylic alcohol function and the benzyl ether function appear as a broad singlet (4 H, δ4.3) in agreement with prediction. This synthetic compound is therefore cybrodol methyl ether (96).

Structure 97 was naively assigned to the other rearrangement product. The ^{1}Hmr spectrum ($CDCl_3$) of this product and that of isocybrodol (2)[2] are practically identical except for the presence of an O-methyl group signal (δ3.21) in the spectrum of the former.

However, close examination of these spectra led us to conclude that structure 97 was untenable. Instead, structure 98 was assigned to this rearrangement product



98 R = H, X = CH_2OH

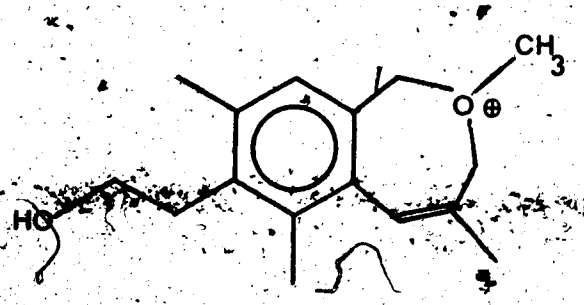
99 R = Ac, X = CH_2OAc

100 R = H, X = CHO

(isocybrodol methyl ether) on the basis of the following considerations. The benzyl alcohol methylene group protons of isocybrodol (2) appear as an AB quartet (J = 14 Hz) centered at δ4.40[2], the spectrum of isocybrodol methyl ether has a very similar pattern centered at δ4.45. The allylic alcohol methylene group protons of isocybrodol (2) appear as an AB quartet (J = 12 Hz) centered at δ3.69[2], the ^{1}Hmr spectrum of the synthetic compound has an AB quartet (J = 11 Hz) centered at δ3.58, suggesting that the synthetic compound has a free benzyl alcohol group rather than a free allylic alcohol group[54]. This suggestion was verified by two chemical transformations. Acetylation (acetic anhydride-pyridine) gave compound 99 (98%). The AB quartet assigned to the benzyl alcohol function (δ4.45) of isocybrodol methyl ether (98) is shifted downfield to δ4.85 in the ^{1}Hmr spectrum of 99. The methylene signal assigned to the allyl methyl ether

function of 98 is not greatly shifted on acetylation. The acetylation shift[55] is consistent with the presence of a free benzylic alcohol and a methylated allylic alcohol in 98. Activated manganese dioxide oxidation of 98 gave benzaldehyde derivative 100 (93%). The chemical shifts ($CDCl_3$) of the aromatic protons* of 98 and 100 are decisive. The aromatic proton of 98 appears at δ7.11, the aromatic proton of 100 appears at δ7.60. This 0.49 ppm deshielding on oxidation requires that the oxidation product be a benzaldehyde rather than a cinnamaldehyde derivative[56]. The ultraviolet spectrum (λ_{max} (CH_3OH): 208, 266, 310 nm) of 100 is similar to that of mesitaldehyde (67, λ_{max} (hexane): 264, 300 nm)[57].

The formation of the anomalous product 98 can be rationalized by invoking bicyclic cation 101, cleavage to a benzylic carbonium ion with attack by water would give the observed product.



101

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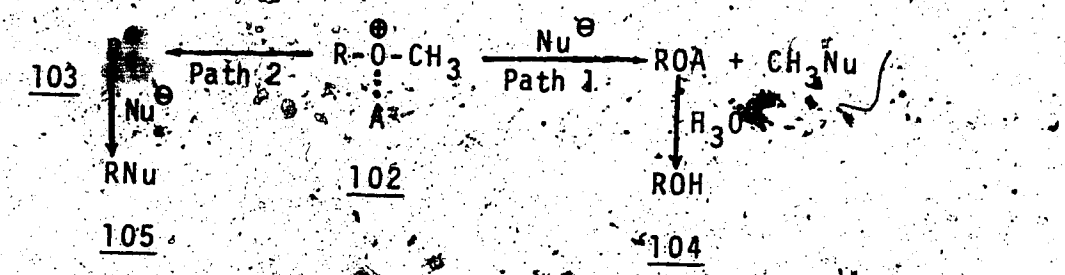
* Distinguished from the vinylic protons by decoupling experiments. The vinyl hydrogen is coupled (J = 1 Hz) to the vinyl methyl group in each case.

When the allylic rearrangement reaction (75 → 81 → 96 + 98) was allowed to proceed for extended periods (one day or more), the reaction product mixture became very complex*. Compound 98 disappeared from the reaction mixture within twenty-four hours. Cybrodol (1), likely from solvolysis of 96 appeared in low concentrations after two days, however this was not an efficient preparation of cybrodol (1) since the reaction product mixture had become very complex by this time. Compound 96 never completely disappeared from the product mixture. At no time was isocybrodol (2) evident in the product mixture.

Attention was now focussed on the problem of deprotecting the benzylic hydroxyl group of cybrodol methyl ether (96) and the allylic hydroxyl group of isocybrodol methyl ether (98). While methylation offers excellent protection for a hydroxyl group, the stability of a methyl ether often makes deprotection difficult[17]. Several demethylation procedures, (Scheme 16)

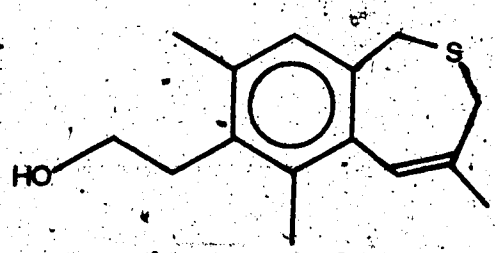
Scheme 16. Cleavage of methyl ethers.



* Experiments were conducted on a small scale (2-5 mg of 75) with tlc monitoring only, hence only qualitative conclusions can be drawn.

relying on nucleophilic attack (path 1) on the methyl carbon of a complex (102) of the methyl ether with a Lewis acid, have been reported[35,58-62]. In the present case, however, a competing mode of cleavage (path 2) may complicate the situation. If, as in this case, a stable carbonium ion 103 can be formed by fragmentation of complex 102, the product, after hydrolysis, will not be alcohol 104. Instead the product will be 105 f[illegible]ucleophilic attack on cation 103. The po[illegible]ty that cation 103 might rearrange also exists.

Isocybrodol methyl ether (98) was exposed to Fujita's[58] demethylation conditions (excess boron trifluoride etherate in ethanedithiol) at room temperature for four days. A single major (63%) product was isolated and assigned structure 106. This assignment



106

followed after comparison of the ^{1}Hmr spectra ($CDCl_3$) of the reaction product (mol. formula $C_{15}H_{20}OS$*) with that of isocybrodol (2)[2]. The formation of 106 is rationalized in Scheme 17.

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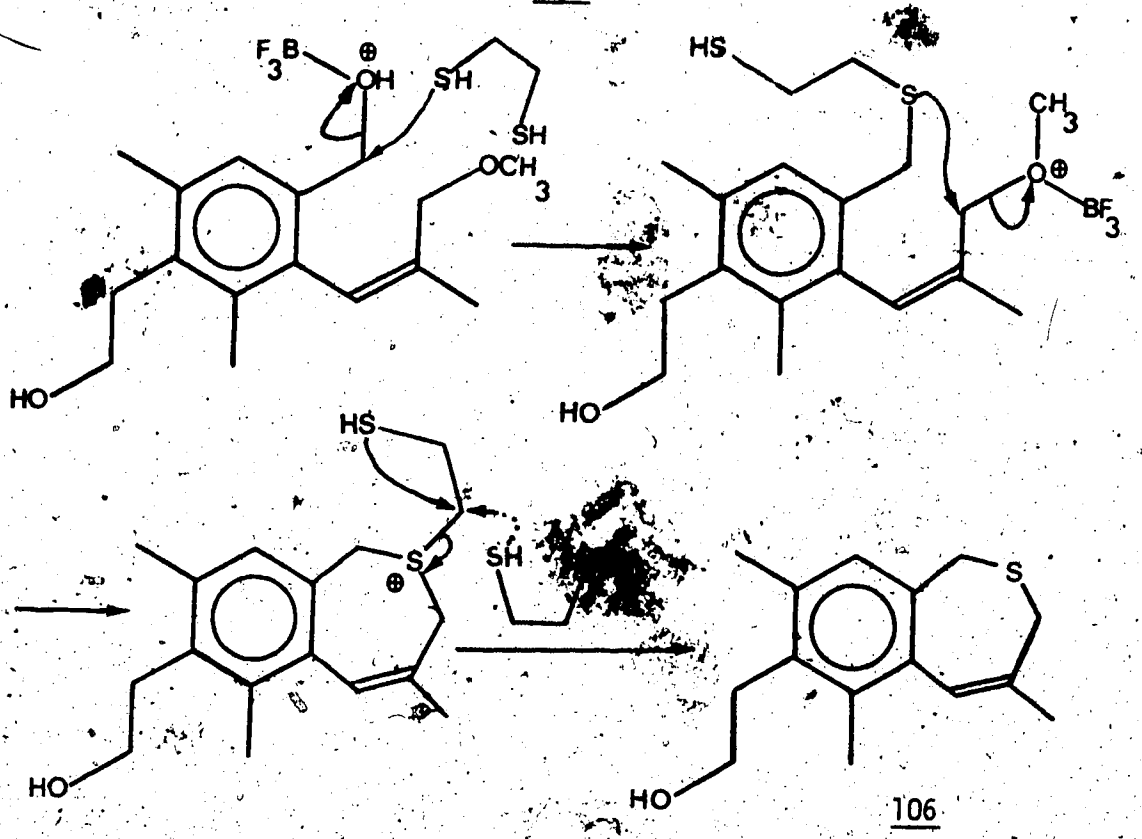
* Established by high resolution mass spectrometry (hrms) and chemical ionization (NH_3) mass spectrometry.

Scheme 17. Formation of 106.

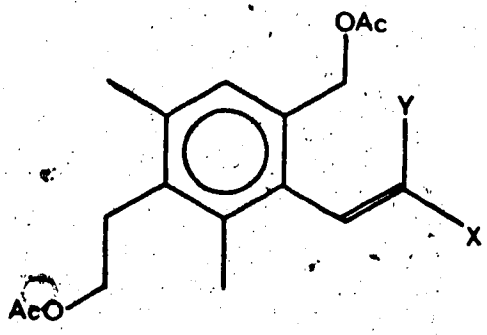


106

Pyridine hydrochloride in refluxing acetic anhydride has been used to cleave methyl ethers[59]. An acetic anhydride solution of isocybrodol methyl ether (98) and pyridine hydrochloride was refluxed for four hours. The sole product was diacetylisocybrodol methyl ether (99).

Ganem has reported the cleavage of methyl ethers by ferric chloride in acetic anhydride[60]. An acetic anhydride solution of 98 and ferric chloride (0.8 equivalents) was stirred overnight at room temperature. The product, isolated in 78% yield after chromatography

was a 78:22 mixture of triacetylcybrodol (107) and triacetylisocybrodol (108) as judged by ^{1}Hmr analysis[2].



99 X = CH_3, Y = CH_2OCH_3

107 X = CH_2OAc, Y = CH_3

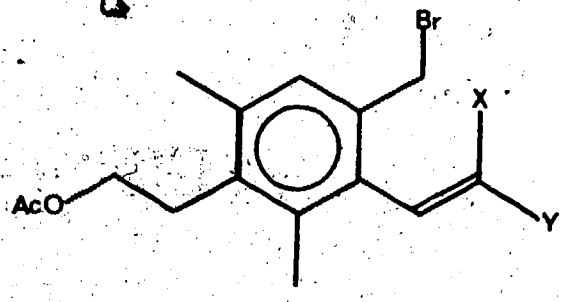
108 X = CH_3, Y = CH_2OAc

When cybrodol methyl ether (96) was treated in like fashion, the same product mixture resulted. Compounds 107 and 108 were chromatographically inseparable, hence the crude product mixture was stirred overnight in methanol with potassium carbonate. This treatment produced cybrodol (1) and isocybrodol (2) (78:22 ratio) which were then separated by chromatography. The combined overall yield of (1) and (2) was 75% in two steps from 96 or 98. Synthetic cybrodol (1, mp 109-110°C) and synthetic isocybrodol (2, mp 101-102°C) were identical with the natural products[2] by the following criteria: tlc, ir, ^{1}Hmr and ms. Interestingly, natural cybrodol (1) is a clear oil which resisted all attempts at crystallization while the synthetic compound could be crystallized from chloroform-methanol. A small sample of natural cybrodol (1) in chloroform-methanol (50:1) seeded with a crystal of synthetic 1 did crystallize, however.

The results of the ferric chloride-acetic anhydride cleavage experiment implied the intermediacy of carbonium ions (*cf.* 84a,b) similar to those involved in the sulfuric acid mediated rearrangement of 80. In this case however, the bulky acetyl groups appear to bias the product distribution in favour of the *E* olefinic geometry. Based on these results, we felt that a more direct route to cybrodol (1) in particular might be available if we were to use ferric chloride in acetic anhydride to promote the allylic rearrangement of 81. Treatment of diol 81 with ferric chloride (0.8 equivalents) in acetic anhydride (overnight at room temperature) gave, after deacetylation as before, a 51% combined overall yield of 1 and 2 (69:31 ratio of 1 to 2).

Since isocybrodol (2) was a minor product of the above deprotection sequence, we desired a superior synthesis of 2 which would avoid the substantial isomerization to the *E* geometry which occurred when isocybrodol methyl ether (98) was exposed to Ganem's conditions. Boron tribromide, has been utilized for methyl ether cleavage[64], therefore we explored the applicability of this reagent to our synthesis. Methyl ether 99 was treated with boron tribromide (2.6 equivalents) at 0°C in methylene chloride. Two products, separable by careful ptlc, were formed.

The less polar product (mol. formula $C_{17}H_{22}O_2Br_2$*) was assigned structure 109 after comparison of the ^{1}Hmr spectrum ($CDCl_3$) with that of isocybrodol (2)[2]. The



109 X = CH_2Br
Y = CH_3

110 X = CH_3
Y = CH_2Br

more polar component (mol. formula $C_{17}H_{22}O_2Br_2$) was assigned structure 110 after analogous comparison with cybrodol (1)[2]. The ratio of 109 to 110 prior to ptlc was 75:25 as determined by ^{1}Hmr. When the reaction was carried out at -20°C, the product mixture (86% total yield) consisted of 109 and 110 in approximately a 95:5 ratio as judged by ^{1}Hmr.

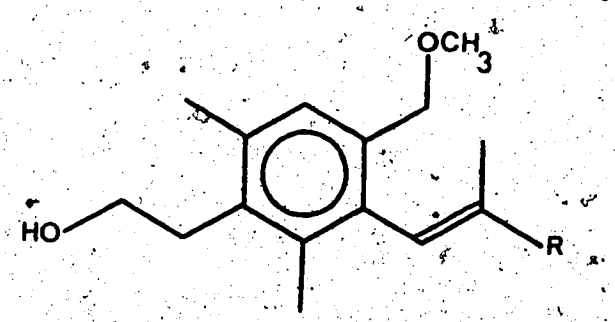
We anticipated that treatment of 109 with hydroxide would result in the direct production of 2. A vigourously stirred mixture of 109 (2 mg) in methylene chloride and tetra-n-butylammonium bromide in 40% aqueous sodium hydroxide was stirred at room temperature for eight hours. Tlc examination of the complex reaction product mixture showed that 109 had been completely consumed, however 2 had not been formed. In view of this rather unpromising result, this idea was not pursued further.

---

* As determined by hrms and chemical ionization (NH_3) mass spectrometry.

It is known that benzylic halides, when exposed to tetraethylammonium acetate in boiling acetone, afford benzylic acetates by a S_N2 displacement mechanism[63]. Treatment of 109 with this reagent should produce 108. Carbonium ion formation, which might result in formation of 107, should be minimal. Indeed, treatment of 109 with a ten-fold excess of tetraethylammonium acetate in refluxing acetone for one hour gave 108* in excellent yield (97%).

Finally, cybrodic acid (3) was prepared from cybrodol methyl ether (96) in the following manner. Activated manganese dioxide oxidation of 96 overnight in methylene chloride gave aldehyde 111 quantitatively.



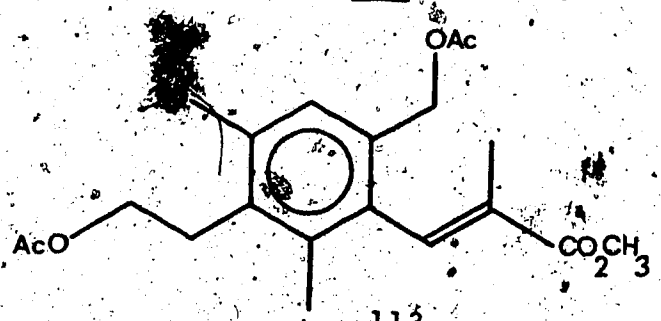
111 R = CHO

112 R = CO_2CH_3

Corey has developed an efficient process[64] for converting an α,β-unsaturated aldehyde to an α,β-unsaturated ester. The cyanohydrin of the aldehyde is oxidized with activated manganese dioxide to the acyl cyanide which, in the presence of the alcoholic solvent, gives the ester. A similar process[64] using argentic oxide as the oxidant gives α,β-unsaturated carboxylic acids

* Contaminated (~5%) with 107, the starting material 109 was also contaminated (~5%) with 110 (*vide supra*).

directly. We preferred an ester as an intermediate synthetic target so as to facilitate chromatographic purification. Treatment of aldehyde 111 with sodium cyanide (2.7 equivalents), acetic acid (2.4 equivalents) and activated manganese dioxide in methanol gave, after a two day reaction period, a 95% yield of methyl ester 112. The methyl ether protection was removed with Ganem's reagent. Treatment of 112 with ferric chloride (1.8 equivalents) in acetic anhydride (two hours at room temperature) gave 113 in 77% yield after chromatog-



113

Saponification of 113 (potassium hydroxide, re- aqueous methanol) gave synthetic cybrodic acid (3, 97%, mp 179-180°C) identical in all respects (mp, tlc, ir, ms, ^{1}Hmr) with the natural product[2].

EXPERIMENTAL

Unless specified all solvents with the exception of nitromethane and ether were distilled prior to use. Technical grade ether (U.S.P. quality) was used for extractions. For most other applications ACS quality nitromethane and anhydrous ether were used without purification. Skellysolve B refers to Skelly Oil Company light petroleum, bp 62-70°C. Anhydrous solvents and reagents were distilled from appropriate drying agents (in brackets): dimethoxyethane (sodium), tetrahydrofuran (sodium), dihydropyran (sodium), acetonitrile (calcium hydride) and methylene chloride (phosphorous pentoxide). Whatman LPS-2 Chromedia (37-53 μm) or Merck Silica Gel 60 (40-63 μm) were used for flash chromatography[65]. Merck Silica Gel 60 (70-230 mesh) was used for column chromatography. Fractions were collected with an Isco Model 1200 fraction collector. A Hitachi CLC-3 centrifugal liquid chromatograph packed with Baker TLC Silica Gel 7 (<40 μm) was used for centrifugal liquid chromatography. Analytical thin layer chromatography (tlc) was carried out on glass plates (75 x 25 or 75 x 50 mm) coated (~0.3 mm) with silica gel G (W. Merck, Darmstadt) containing 1% electronic phosphor (General Electric, Cleveland). Preparative thin layer chromatography (ptlc) was carried out on glass plates (20 x 20 cm) coated (0.5 mm) with the same

adsorbent. Materials were detected by visualization under an ultraviolet lamp (254 or 350 nm). The plate (only a thin vertical band in the case of ptlc) was then sprayed with a solution of vanillin (1%) in concentrated sulfuric acid. Careful charring with a heat gun followed by a brief cooling period produced the colour reactions indicated in the text. Gas chromatography was carried out on a Hewlett-Packard 5700 A gas chromatograph equipped with a flame ionization detector. Nitrogen was purified by passage through a column (4 x 45 cm) of Central Dynamics Corporation catalyst R3-11 followed by a column (4 x 50 cm) packed with potassium hydroxide and anhydrous calcium sulfate.

Mass spectra (MS) were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS 50 computer, or an A.E.I. MS-9 mass spectrometer (chemical ionization). Data is reported as m/e (relative intensity). Unless diagnostically significant, peaks with intensities less than 20% of the base peak are omitted. Infrared (IR) spectra were recorded on a Nicolet 7199 interferometer or a Perkin Elmer 297 infrared spectrometer. Ultraviolet (UV) spectra were recorded on a Unicam SP 1700 ultraviolet spectrophotometer. ^{1}H nuclear magnetic resonance (^{1}HMR) spectra were measured with a Varian A-60D spectrometer, a Varian HA-100 spectrometer, or a Varian HA-100 spectrometer interfaced to a Digilab

FTS/NMR-3 data system. ^{13}C nuclear magnetic resonance (^{13}CMR) spectra were measured on a Bruker WP-60 spectrometer interfaced to a Nicolet 1080 computer or a Bruker HFX-90 spectrometer interfaced to a Nicolet 1085 computer. All nuclear magnetic resonance measurements employed tetramethylsilane as an internal standard. Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were carried out by the microanalytical laboratory of this department or by Schwarzkopf Microanalytical Laboratory, New York.

4-Bromo-3,5-dimethylbenzylidene diacetate (25)

2-Bromomesitylene (24, 99.5 g, 0.5 mol)[66], acetic acid* (770 mL) and acetic anhydride* (770 mL) were cooled to 0°C in an ice-salt bath. Concentrated sulfuric acid (115 mL) was added dropwise over a ten minute period to the mechanically stirred solution. After the solution temperature was allowed to fall to 5°C, chromium trioxide (135 g, 1.35 mol) was added in small portions over a period of one hour. During the addition period the solution temperature was maintained between 5 and 10°C. The mixture was stirred at 5°C for a further thirty minutes and then carefully poured onto crushed ice (3 L). Water was added to bring the

* Reagent grade, undistilled solvents, were used in this case.

total volume to six litres. When the reaction was conducted on a smaller scale (7.16 g of 24) a crude, semi-crystalline product (5.20 g) could be filtered off at this point. This material was used to obtain spectral data. On a larger scale the product was quite oily and could not be effectively isolated by filtration. Instead, the product was isolated by ether extraction (3 x 2 L). The extract was taken to dryness leaving a green semi-solid which was dissolved in ether (500 mL). This solution was washed with water (100 mL) and brine (100 mL). After drying over magnesium sulfate, filtration and evaporation to dryness gave a yellow semi-solid (147 g) which was used without purification in the next step.

The crude product from 7.16 g of 24 was recrystallized (95% ethanol) affording pure 25 (4.65 g, 41%) as white prisms, mp 91-93°C. Sublimation of this material (80°C, 1 Torr) gave an analytically pure sample, mp 94-95°C (clear prisms).

TLC: R_f 0.57 (methylene chloride), red spot.

IR ($CHCl_3$ cast): 1751, 1240, 1205 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.10 (6 H, s, 2xOAc), 2.43 (6 H, s, 2x$ArCH_3$), 7.20 (2 H, s, 2xArH), 7.55 (1 H, s, CH).

^{13}CMR ($CDCl_3$): δ20.8 (2 C), 23.9 (2 C), (CH_3); 89.4 126.3 (2 C), (CH); 129.0, 134.0, 138.8 (2 C), (C); 168.7 (2 C), (C=O).

MS: m/e calcd. for $C_{13}H_{15}O_4{}^{81}Br$ (M + 2): 316.0133; found: 316.0140 (60); calcd. for $C_{13}H_{15}O_4{}^{79}Br$ (M^+): 314.0154; found: 314.0152 (64), 106 (100), 105 (48), 104 (77), 103 (56), 91 (28), 78 (33), 77 (65), 51 (21). ANALYSIS: calcd. for $C_{13}H_{15}O_4Br$: C 49.54, H 4.80, Br 25.35; found: C 49.52, H 4.78, Br 25.57.

4-Bromo-3,5-dimethylbenzyl alcohol (19)

A solution of crude 25 (147 g) in anhydrous ether (750 mL) was added over a two hour period to a mechanically stirred slurry of lithium aluminum hydride (31 g, 0.82 mol) in anhydrous ether (1.2 L). The mixture was stirred for a further ninety minutes and then quenched by sequential dropwise addition of water (31 mL), 15% aqueous sodium hydroxide (31 mL) and water (93 mL). The solids, after removal by filtration, were washed with anhydrous ether (10 x 50 mL). The combined ether solutions were concentrated *in vacuo* leaving crude 19 (100 g) as a semi-solid which was used without purification in the next step.

In a parallel run, a small sample of the crude product (4.70 g) in chloroform (10 mL) deposited a crystalline compound (702 mg) after refrigeration for several days. This material was recrystallized (95% ethanol) giving long clear needles (mp 124-125°C) and was identified as 2,4-dihydroxymethyl-6-methylbromo-

benzene (27). Flash chromatography (Skellysolve B - ethyl acetate, 3:1; 3 cm column) of the chloroform mother liquor gave pure 19 (3.76 g) as well as additional 27 (65 mg). Recrystallization (heptane) of 19 obtained in this fashion gave white plates mp 52-54°C (lit.[7] mp 53-54°C).

Compound 27 has the following physical properties.

TLC: R_f 0.17 (Skellysolve B - ethyl acetate, 1:1), red spot.

IR ($CHCl_3$ cast): 3300 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.65 (1 H, t (6 Hz), OH), 2.01 (1 H, t (6 Hz), OH), 2.43 (3 H, s, $ArCH_3$), 4.64 (2 H, d (6 Hz), CH_2O), 4.75 (2 H, d (6 Hz), CH_2O), 7.19 (1 H, bs, ArH), 7.29 (1 H, bs, ArH).

MS: m/e calcd. for $C_8H_8O^{81}Br$ (M-CH_3O, parent ion not seen): 200.9738; found: 200.9746 (27); calcd. for $C_8H_8O^{79}Br$ (M-CH_3O): 198.9759; found: 198.9749 (28), 185 (20), 183 (22), 151 (46), 133 (21), 105 (100), 93 (50), 92 (59), 91 (68), 77 (37).

Chemical Ionization (NH_3) MS shows peaks at m/e 248/250 (M + 18).

Compound 19 has the following physical properties.

TLC: R_f 0.53 (Skellysolve B - ethyl acetate, 1:1), red spot.

IR ($CHCl_3$): 3600 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.42 (6 H, s, 2x$ArCH_3$), 4.55 (2 H, s,

CH_2O), 7.03 (2 H, s, 2xArH).

MS: m/e calcd. for $C_9H_{11}O^{81}Br$ ($M^+ + 2$): 215.9973; found: 215.9973 (73); calcd. for $C_9H_{11}O^{79}Br$ (M^+): 213.9993; found: 213.9988 (77), 135 (73), 107 (100), 106 (60), 105 (32).

4-Methoxymethyl-2,6-dimethylbromobenzene (28, Chromyl acetate route)

To a magnetically stirred solution of crude 19 (100 g) and methyl iodide (100 g, 0.70 mol) in dry dimethoxyethane (200 mL), sodium hydride (57% in oil, washed with Skellysolve B; 17 g, 0.71 mol) was added in small portions over a thirty minute period. Addition of the sodium hydride caused vigourous boiling. The mixture was stirred for a further two hours at room temperature. The solution volume was reduced to 50 mL by distillation at atmospheric pressure, ether (200 mL) was added and the salts were removed by filtration. The filter cake was extracted with ether (5 x 50 mL) and the combined ethereal solutions were concentrated leaving a brown oil (62.5 g). Polar impurities were removed by rapid passage of a Skellysolve B solution of this material through a short column of acidic alumina (200 g). The washings were concentrated to a yellow oil (57.3 g) which on distillation (101-105°C, 0.8 Torr) gave an 89:11 mixture (as judged by ^{1}Hmr) of

4-methoxymethyl-2,6-dimethylbromobenzene (28) and 2-methoxymethyl-4,6-dimethylbromobenzene (29, 50.5 g, 44% from 2-bromomesitylene (24)). These isomers could be separated by flash chromatography (Skellysolve B-ether, 9:1; 1 g of sample/5 cm column), however for synthetic purposes the mixture was used as such in the next step. A small sample of pure 28 was evaporatively distilled (80°C, 0.03 Torr) for microanalysis.

Compound 28 has the following physical properties.
TLC: R_f 0.58 (Skellysolve B-ether, 3:1), red spot.
IR ($CHCl_3$ cast): 1105, 1030, 860 cm^{-1}.
^{1}HMR ($CDCl_3$): δ2.42 (6 H, s, 2xArCH_3), 3.38 (3 H, s, CH_3O), 4.35 (2 H, s, CH_2O), 7.04 (2 H, s, 2xArH).
^{13}CMR ($CDCl_3$): δ23.7 (2 C), 58.0, (CH_3); 73.9, (CH_2); 127.4 (2 C), (CH); 126.4, 136.9, 138.1 (2 C), (C).
MS: m/e calcd. for $C_{10}H_{13}O^{81}Br$ (M + 2): 230.0129; found: 230.0113 (40); calcd. for $C_{10}H_{13}O^{79}Br$ (M^+): 228.0150; found: 228.0143 (40), 149 (100), 119 (24).
ANALYSIS: calcd. for $C_{10}H_{13}OBr$: C 52.42, H 5.72, Br 34.88; found: C 52.40, H 5.67, Br 34.61.

Compound 29 has the following physical properties.
TLC: R_f 0.52 (Skellysolve B-ether, 3:1), orange spot.
IR (film): 1100 cm^{-1}.
^{1}HMR ($CDCl_3$): δ2.26 (3 H, s, ArCH_3), 2.35 (3 H, s, ArCH_3), 3.46 (3 H, s, CH_3O), 4.48 (2 H, s, CH_2O), 6.97 (1 H, s, ArH), 7.08 (1 H, s, ArH).

^{13}CMR ($CDCl_3$): δ20.8, 23.2, 58.5, (CH_3); 74.5, (CH_2); 127.0, 130.6, (CH), 121.8, 136.6, 137.6, 137.9, (C). MS: m/e calcd. for $C_{10}H_{13}O^{81}Br$ (M + 2): 230.0129; found: 230.0132 (30); calcd. for $C_{10}H_{13}O^{79}Br$ (M^+): 228.0150; found: 228.0156 (29), 149 (100), 119 (21), 104 (22).

Compound 28 by the N-Bromosuccinimide route

A mixture of 2-bromomesitylene (24, 1.56 g, 7.84 mol), N-bromosuccinimide (1.42 g, 7.98 mol) and benzoyl peroxide (100 mg, 0.41 mol) in carbon tetrachloride (40 mL) was refluxed for six hours. After cooling, the solids were removed by filtration and washed with carbon tetrachloride (2 x 50 mL). The combined carbon tetrachloride solutions were evaporated to dryness, methanol (100 mL) and sodium (5 g, 0.22 mol) were added and the mixture was refluxed for four hours. Most of the volatiles were removed *in vacuo* and water (100 mL) was added to the residue. The products were isolated by ether extraction (4 x 50 mL). After drying over sodium sulfate, filtration and concentration gave a crude mixture of ethers 28 and 29 (1.42 g). Flash chromatography (as above, 3 cm column) gave pure 28 (801 mg) and pure 29 (489 mg, 72% combined overall yield, ratio of 28 to 29 was 62:38).

Compound 28 by the Bromotrichloromethane route

A stirred mixture of 2-bromomesitylene (24, 39 g, 0.196 mol) and bromotrichloromethane* (49.6 g, 0.250 mol) was irradiated (General Electric 275 watt Sunlamp) for two days in a round bottom Pyrex flask. The reaction was monitored by gas chromatography (5 ft. x 1/8 in. glass column packed with 5% SE-30 on Chromosorb W). The volatiles were removed *in vacuo* and the resultant thick brown oil was dissolved in methanol (200 mL). Sodium (15 g, 0.65 mol) was added and the mixture was stirred overnight at room temperature. Water (1 L) was added and the products were isolated by ether extraction (5 x 100 mL). After drying over sodium sulfate, filtration and concentration gave crude material (48 g) which was fractionally distilled (70-90°C, 0.2 Torr) affording a mixture of bromoethers 28 and 29 (38.5 g, 86%). ^{1}Hmr examination of the distillate indicated that the ratio of 28 to 29 was 60:40.

Attempted preparation of 34 by a Grignard reaction

A solution of bromides 28 and 29 (2.29 g, 10 mmol, from the chromyl acetate route) plus 1,2-dibromoethane (0.86 mL, 1.87 g, 10 mmol) in dry tetrahydrofuran (40 mL) was added over a period of ninety minutes to a

* Undistilled.

stirred suspension of magnesium turnings (0.5 g, 21 mmol) in dry tetrahydrofuran (10 mL) under reflux. The mixture was refluxed for a further two hours under a nitrogen atmosphere and then cooled to -10°C. Ethylene oxide (5 g, 0.11 mol) was distilled through a drying tube and condensed into the reaction mixture by means of a dry-ice condenser. The mixture was stirred for one hour at room temperature and then saturated aqueous ammonium chloride (100 mL) was added. The products were isolated by ether extraction (5 x 30 mL), the extracts were dried over magnesium sulfate, filtered and concentrated to a thick oil (1.68 g). Tlc examination of the products revealed that compound 34 (*vide infra*) was not formed in this reaction. The product mixture was subjected to centrifugal liquid chromatography (5 cm spacer; 100 g silica gel; Skellysolve B-ether, 5:1). Two major components were isolated. 1-Methoxymethyl-3,5-dimethylbenzene (35) was obtained as a yellow oil (967 mg, 59%).

TLC: R_f 0.44 (methylene chloride), orange spot.

IR (film): 1100 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.20 (6 H, s, 2x$ArCH_3$), 3.23 (3 H, s, CH_3O), 4.28 (2 H, s, CH_2O), 6.85 (1 H, s, ArH), 6.90 (2 H, s, 2xArH).

MS: m/e calcd. for $C_{10}H_{14}O$ (M^+): 150.1045; found: 150.1020 (23), 149 (100), 134 (24), 133 (81), 105 (40).

4-Methoxymethyl-2,6-dimethylphenol (36) was obtained as a yellow oil (107 mg, 6%).

TLC: R_f 0.58 (benzene-ether, 3:1), red spot.

IR (film): 3350 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.18 (6 H, s, 2x$ArCH_3$), 3.30 (3 H, s, CH_3O), 4.28 (2 H, s, CH_2O), 5.7 (1 H, bs, OH), 6.85 (2 H, s, 2xArH).

MS: m/e calcd. for $C_{10}H_{14}O_2$ (M^+): 166.0994; found: 166.0994 (76), 165 (30), 151 (34), 135 (100).

Attempted preparation of 34 using n-butyllithium at room temperature

n-Butyllithium (8.8 M in hexane, 2.5 mL, 22 mmol) was added to a mixture of bromides 28 and 29 (4.35 g, 19 mmol, from the chromyl acetate route) in dry tetrahydrofuran (20 mL) at room temperature under a nitrogen atmosphere. The mixture was stirred for one hour during which time a purple colour developed. Ethylene oxide (5 g, 0.11 mol) was distilled through a drying tube and condensed into the reaction vessel by means of a dry-ice condenser. The mixture was stirred at room temperature for one hour and then saturated aqueous ammonium chloride (10 mL) was added. The products were isolated by ether extraction (3 x 10 mL). The combined ether extracts were dried over magnesium sulfate, filtered and concentrated to a brown oil (3.73 g). Tlc

examination of the reaction mixture revealed that compound 34 (*vide infra*) was not present. The major product, isolated as a clear oil by column chromatography (chloroform, 150 g of silica gel) was 1-((3,5-dimethyl)phenyl)ethanol (37, 1.06 g, 37%).

TLC: R_f 0.47 (methylene chloride-methanol, 20:1), purple spot.

IR (film): 3450 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.49 (3 H, d (7 Hz), $\underline{CH_3}CH$), 2.32 (6 H, s, $2xArCH_3$), 4.82 (1 H, q (7 Hz), $\underline{CH}CH_3$), 6.90 (1 H, s, ArH), 6.97 (2 H, s, 2xArH).

MS: m/e calcd. for $C_{10}H_{14}O$ (M^+): 150.1045; found: 150.1047 (55), 135 (53), 107 (100), 91 (28).

2-((4-Methoxymethyl-2,6-dimethyl)phenyl)ethanol (34)

A magnetically stirred solution of n-butyllithium (1.6 M in hexane, 75 mL, 0.12 mol) in dry tetrahydrofuran (80 mL) was cooled to -78°C under a nitrogen atmosphere. A mixture of bromides 28 and 29 (from the chromyl acetate route, 25.0 g, 0.109 mol)* in dry tetrahydrofuran (30 mL) was added over a period of fifteen minutes causing a creamy white precipitate to form. The solution was stirred at -78°C for a further

* When the mixture of bromides 28 and 29 from the bromotrichloromethane route was used, the yield of 34 was proportionately lower and the product was contaminated (*ca.* 10%) by alcohol 40 (*vide infra*).

hour. Ethylene oxide (25 g, 0.57 mol), purified by passage through a drying tube (2 x 30 cm) containing potassium hydroxide and anhydrous calcium sulfate, was condensed in the reaction flask by means of a dry-ice condenser. Introduction of the ethylene oxide required one hour. The reaction mixture was stirred for an additional four hours. Saturated aqueous ammonium chloride (50 mL) was added and the mixture was allowed to warm to room temperature. Water (200 mL) was added to dissolve the salts and the organic layer was removed. The aqueous phase was extracted with ether (5 x 100 mL), the combined organic extracts were dried over magnesium sulfate, filtered and concentrated. On a smaller scale (5 g of starting material) purification was conveniently achieved by column chromatography (chloroform, 100 g of silica gel) which afforded pure 34 (2.16 g, 51%). On the present scale however, the above crude product (24.6 g) was dissolved in Skellysolve B (250 mL). Finely powdered anhydrous (dried overnight at 120°C) calcium chloride (50 g) was added and the mixture was cooled to -70°C for two hours. The solids were filtered off and washed with cold (0°C) Skellysolve B (3 x 30 mL). The filtrate was set aside and water (200 mL) was added to the filter cake. After thirty minutes, the aqueous solution was extracted with ether (3 x 200 mL). The ether extracts were dried

over magnesium sulfate, filtered and evaporated to dryness leaving crude alcohol 34 (15.2 g) as a viscous yellow oil. Distillation (120-130°C, 0.12 Torr) gave pure 34 (14.0 g, 66%).

TLC: R_f 0.30 (methylene chloride-methanol, 20:1), orange spot.

IR ($CHCl_3$ cast): 3400 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.33 (6 H, s, 2xArCH_3), 2.91 (2 H, t (7 Hz), CH_2CH_2O), 3.37 (3 H, s, CH_3O), 3.67 (2 H, t (7 Hz), CH_2CH_2O), 4.35 (2 H, s, CH_2OCH_3), 6.99 (2 H, s, 2xArH).

^{13}CMR ($CDCl_3$): δ20.0 (2 C), 58.2, (CH_3); 32.8, 61.6, 74.7, (CH_2); 127.9 (2 C), (CH); 134.3, 136.0, 137.1 (2 C), (C).

MS: m/e calcd. for $C_{12}H_{18}O_2$ (M^+): 194.1307; found: 194.1310 (44), 163 (100), 132 (24).

ANALYSIS: calcd. for $C_{12}H_{18}O_2$: C 74.19, H 9.34; found: C 74.03, H 9.36.

2-((2-Methoxymethyl-4,6-dimethyl)phenyl)ethanol (40)

n-Butyllithium (1.6 M in hexane, 0.63 mL, 1 mmol) was added to a solution of bromide 29 (200 mg, 0.87 mmol) in dry tetrahydrofuran (1 mL) at -78°C under a nitrogen atmosphere. The mixture was stirred at -78°C for one hour. Ethylene oxide (2 mL, 40 mmol) was added (cooled syringe) in one portion. The mixture was

stirred at -78°C for five hours and then left overnight at -5°C.. The reaction mixture was diluted with water (20 mL) and extracted with ether (4 x 20 mL). The combined ether extracts were dried over sodium sulfate, filtered and concentrated. Flash chromatography (methylene chloride-methanol, 100:1; 2 cm column) gave 40 as a clear oil (33 mg, 19%) as well as 35 (86 mg, 66%). The isomeric alcohols 34 and 40 could be distinguished by tlc (Skellysolve B-ethyl acetate, 1:1). TLC: R_f 0.30 (Skellysolve B-ethyl acetate, 1:1), dark orange spot.

IR (film): 3400 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.26 (3 H, s, $ArCH_3$), 2.29 (3 H, s, $ArCH_3$), 2.92 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.38 (3 H, s, CH_3O), 3.76 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.40 (2 H, s, $\underline{CH_2}O$), 6.95 (2 H, s, 2xArH).

^{13}CMR ($CDCl_3$): δ19.8, 20.7, 58.0, (CH_3); 32.1, 61.7, 73.7, (CH_2); 128.8, 131.6, (CH); 133.2, 135.6, 136.1, 137.3, (C).

MS: m/e calcd. for $C_{12}H_{18}O_2$ (M^+): 194.1306; found: 194.1313 (17), 149 (34), 133 (68), 132 (100).

2-((4-Methoxymethyl-2,6-dimethyl)phenyl)ethyl acetate (42)

Alcohol 34 (750 mg, 3.87 mmol), acetic anhydride (1 mL) and pyridine (1 mL) in methylene chloride (10 mL) were stirred overnight at room temperature.

Evaporation to dryness gave 42 as a clear oil (863 mg, 95%).

TLC: R_f 0.52 (Skellysolve B-acetone, 7:3), orange spot.

IR (film): 1740 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.05 (3 H, s, OAc), 2.36 (6 H, s, 2x$ArCH_3$), 2.98 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.37 (3 H, s, CH_3O), 4.15 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.35 (2 H, s, $\underline{CH_2}O$), 6.98 (2 H, s, 2xArH).

MS: m/e calcd. for $C_{14}H_{20}O_3$ (M^+): 236.1412; found: 236.1420 (35), 176 (100), 163 (43), 161 (59), 149 (20), 145 (36), 144 (21), 131 (20).

Attempted Rieche formylation of 42

A solution of titanium tetrachloride (56 μL, 0.5 mmol) and α,α-dichloromethyl methyl ether (44 μL, 0.5 mmol) in dry methylene chloride (10 mL) was stirred at 0°C for thirty minutes. Arene 42 (70 mg, 0.3 mmol) in dry methylene chloride (10 mL) was added over a ninety minute period. The solution was then refluxed for two hours during which time a dark green colour developed. The mixture was poured into ice-water (100 mL) and the products were extracted into methylene chloride (4 x 30 mL). The combined extracts were washed with 3 M aqueous hydrochloric acid (40 mL) and water (40 mL). After drying over magnesium sulfate, filtration and concentration gave a clear oil which was sub-

jected to ptlc (methylene chloride). In this fashion 2-((4-chloromethyl-2,6-dimethyl)phenyl)ethyl acetate (43) was isolated as a clear oil (55 mg, 76%).

TLC: R_f 0.74 (methylene chloride), red spot.

IR (film): 1740 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.05 (3 H, s, OAc), 2.37 (6 H, s, 2xArCH$_3$), 2.95 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.12 (2 H, t, (7 Hz), $CH_2\underline{CH_2}O$), 4.47 (2 H, s, CH_2Cl), 7.02 (2 H, s, 2xArH).

MS: m/e 242 (M + 2, 4), 240 (M, 12), 182 (28), 180 (87), 169 (17), 167 (54), 145 (100), 132 (37).

Attempted bromination of 42 with bromine in carbon tetrachloride

Bromine (28 µL, 87 mg, 0.54 mmol) in carbon tetrachloride (10 mL) was added to a solution of arene 42 (105 mg, 0.45 mmol) in carbon tetrachloride (10 mL) at 0°C. The mixture was stirred at 0°C for three hours and then washed with water (5 mL), 20% aqueous sodium hydroxide (2 x 5 mL) and water (5 mL). After drying over magnesium sulfate, filtration and concentration gave a crude product which was purified by ptlc (Skellysolve B-acetone, 10:1). In this way 4-(2-acetoxyethyl)-3,5-dimethylbenzaldehyde (44, 57 mg, 58%) was isolated as a yellow foam.

TLC: R_f 0.28 (Skellysolve B-acetone, 10:1), red spot.

IR (film): 1740, 1690 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.03 (3 H, s, OAc), 2.42 (6 H, s, 2x$ArCH_3$), 3.03 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.16 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 7.47 (2 H, s, 2xArH), 9.85 (1 H, s, CHO).

MS: m/e calcd. for $C_{13}H_{16}O_3$ (M^+): 220.1099; found: 220.1099 (4), 160 (100), 159 (48), 148 (25), 131 (24).

2-((3-Bromo-4-methoxymethyl-2,6-dimethyl)phenyl)ethyl acetate (45)

Bromine (13 μL, 40 mg, 0.25 mmol) in nitromethane (10 mL) was added to a solution of arene 42 (50 mg, 0.21 mmol) in nitromethane (10 mL) at 0°C. The mixture was stirred at 0°C for two hours and then partitioned between saturated aqueous sodium carbonate (50 mL) and ether (50 mL). The ether layer was dried over sodium sulfate, filtered and concentrated. Bromide 45 (37 mg, 56%) was isolated by ptlc (Skellysolve B-acetone, 10:1) as a clear oil.

TLC: R_f 0.38 (Skellysolve B-acetone, 10:1), red spot.

IR ($CHCl_3$ cast): 1740 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.03 (3 H, s, OAc), 2.35 (3 H, s, $ArCH_3$), 2.47 (3 H, s, $ArCH_3$), 3.03 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.45 (3 H, s, CH_3O), 4.12 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.47 (2 H, s, CH_2O), 7.11 (1 H, s, ArH).

MS: m/e calcd. for $C_{14}H_{19}O_3{}^{81}Br$ (M + 2): 316.0497;

found: 316.0496 (11); calcd. for $C_{14}H_{19}O_3{}^{79}Br$ (M^+): 314.0518; found: 314.0522 (11), 175 (100).

2-((3-Bromo-4-methoxymethyl-2,6-dimethyl)phenyl)ethanol (46)

A stirred solution of arene 34 (10.0 g, 51.5 mmol) in nitromethane (50 mL) was cooled to 0°C. Bromine (4.0 mL, 12.4 g, 78 mmol) in nitromethane (50 mL) was added dropwise over a thirty minute period. The reaction mixture was stirred at 0°C for a further thirty minutes and then saturated aqueous sodium carbonate (50 mL) was added. Most of the nitromethane was removed *in vacuo* and the residue was extracted with ether (5 x 50 mL). The combined ether extracts were washed with water (50 mL) and brine (50 mL). After drying over magnesium sulfate, filtration and concentration gave bromide 46 (13.3 g, 94%) as a viscous orange oil. Attempted bulb to bulb distillation (150°C, 0.1 Torr) caused extensive decomposition. A small sample of crude 46 was crystallized from Skellysolve B-methylene chloride (1:1) affording buff coloured crystals, mp 101-106°C, which were sublimed (100°C, 0.023 Torr) giving analytically pure 46 (mp 104-106°C).

TLC: R_f 0.63 (Skellysolve B-acetone, 7:3), red spot.

IR ($CHCl_3$ cast): 3250 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.29 (3 H, s, $ArCH_3$), 2.42 (3 H, s,

$ArCH_3$), 2.95 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.42 (3 H, s, CH_3O), 3.65 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.44 (2 H, s, $\underline{CH_2}OCH_3$), 7.09 (1 H, s, ArH).

MS: m/e calcd. for $C_{12}H_{17}O_2{}^{81}Br$ (M + 2): 274.0392; found: 274.0395 (1); calcd. for $C_{12}H_{17}O_2{}^{79}Br$ (M^+): 272.0411; found: 272.0403 (1), 243 (100), 241 (94), 213 (44), 211 (45), 132 (35), 131 (21), 115 (24), 91 (21).

ANALYSIS: calcd. for $C_{12}H_{17}O_2Br$: C 52.76, H 6.27, Br 29.25; found: C 52.87, H 6.12, Br 29.13.

1-Bromo-3-(2-(tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxymethyl-2,4-dimethylbenzene (47)

A solution of alcohol 46 (18.05 g, 66.1 mmol), dihydropyran (17 mL, 15.7 g, 0.186 mol) and pyridinium tosylate (700 mg, 2.8 mmol)[33] in methylene chloride (70 mL) was stirred at room temperature for one hour. The solution was washed with water (10 mL) and dried over magnesium sulfate. Filtration and evaporation to dryness gave crude ether 47 as a brown oil. Flash chromatography (Skellysolve B-ethyl acetate, 20:1; 5 cm column; 4 runs) gave pure 47 (22.5 g, 95%) as a light yellow oil.

TLC: R_f 0.60 (benzene-ether, 3:1), red spot.

IR ($CHCl_3$ cast): 1120, 1030 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 2.32 (3 H, s,

$ArCH_3$), 2.45 (3 H, s, $ArCH_3$), 3.00 (2 H, t (7 Hz), $ArCH_2CH_2$), 3.42 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.70 (2 H, t (7 Hz), $ArCH_2CH_2$), 4.44 (2 H, s, CH_2OCH_3), 4.56 (1 H, bs, CH), 7.09 (1 H, s, ArH).

MS: m/e calcd. for $C_{17}H_{25}O_3{}^{81}Br$ (M + 2): 358.0965; found: 358.0968 (2); calcd. for $C_{17}H_{25}O_3{}^{79}Br$ (M^+): 356.0985; found: 356.0988 (2), 274 (1), 272 (1), 85 (100).

Attempted preparation of acid 51 by a Grignard reaction

A solution of bromide 47 (270 mg, 0.76 mmol) and 1,2-dibromoethane (0.2 mL, 0.44 g, 2.3 mmol) in dry tetrahydrofuran (10 mL) was added dropwise to a suspension of magnesium powder (200 mg, 8.2 mmol) in gently refluxing tetrahydrofuran (5 mL) under nitrogen. The mixture was refluxed for three hours and then cooled to 0°C. Carbon dioxide (Matheson, "Bone Dry") was bubbled through the stirred solution for fifteen minutes. Saturated aqueous ammonium chloride (20 mL) was added and the products were extracted into ether (2 x 50 mL). The combined ether solutions were extracted with saturated aqueous sodium carbonate (3 x 10 mL), water (10 mL) and brine (10 mL) and then dried over magnesium sulfate. Filtration and concentration gave the neutral product 1-(2-(tetrahydro-2H-pyran-2-yl)oxyethyl)-4-methoxymethyl-2,4-dimethylbenzene (50) as a yellow oil (171 mg, 81%).

TLC: R_f 0.63 (Skellysolve B-ether, 1:1), orange spot.

IR (film): 1030 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 2.35 (6 H, s, 2x$ArCH_3$), 2.97 (2 H, t (7 Hz), $Ar\underline{CH_2}CH_2$), 3.37 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.76 (2 H, t (7 Hz), $ArCH_2\underline{CH_2}O$), 4.35 (2 H, s, $\underline{CH_2}OCH_3$), 4.58 (1 H, bs, CH), 6.96 (2 H, s, 2xArH).

MS: m/e calcd. for $C_{17}H_{26}O_3$ (M^+): 278.1882; found: 278.1863 (2), 143 (33), 85 (100).

The combined sodium carbonate extracts were acidified (~pH 1) and extracted with ether (2 x 50 mL). These ether extracts were dried over magnesium sulfate, filtered and concentrated leaving negligible (< 1 mg) acidic products.

Methyl 3-(2-(tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxymethyl-2,4-dimethylbenzoate (52)

A solution of n-butyllithium (1.6 M in hexane, 44 mL, 70 mmol) in dry tetrahydrofuran (100 mL) was cooled to -78°C under a nitrogen atmosphere. Bromide 47 (22.5 g, 63.0 mmol) in dry tetrahydrofuran (20 mL) was added over a ten minute period, the mixture was then stirred at -78°C for a further fifty minutes. Freshly distilled methyl chloroformate (36 mL, 44 g, 0.47 mol) was rapidly injected (syringe) into the reaction pot. The solution was warmed to 0°C and kept

at this temperature for sixteen hours. The reaction was quenched by addition of saturated aqueous sodium carbonate (50 mL) followed by water (100 mL). The organic phase was removed and the aqueous residue extracted with ether (3 x 50 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Flash chromatography (Skellysolve B-ethyl acetate, 3:1; 5 cm column; 5 runs) provided pure ester 52 (13.2 g, 62%) as a clear oil.

TLC: R_f 0.42 (benzene-ether, 10:1), brown spot.

IR ($CHCl_3$ cast): 1730 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 2.31 (3 H, s, $ArCH_3$), 2.37 (3 H, s, $ArCH_3$), 2.98 (2 H, t (7 Hz), $Ar\underline{CH_2}CH_2$), 3.30 (3 H, s, $\underline{CH_3}OCH_2$), 3.5 (2 H, m, CH_2O), 3.75 (2 H, t (7 Hz), $ArCH_2\underline{CH_2}$), 3.87 (3 H, s, CO_2CH_3), 4.38 (2 H, s, $\underline{CH_2}OCH_3$), 4.56 (1 H, bs, CH), 7.00 (1 H, s, ArH).

MS: m/e calcd. for $C_{19}H_{28}O_5$ (M^+): 336.1936; found: 336.1939 (1), 305 (6), 190 (28), 85 (100).

Trisnorcybrodolide (5, 6-(2-hydroxyethyl)-5,7-dimethylphthalide, direct route from 52)

Chlorotrimethylsilane (140 µL, 120 mg, 1.1 mmol) was added to a solution of ester 52 (52 mg, 0.15 mmol) and sodium iodide (165 mg, 1.1 mmol) in dry acetonitrile (5 mL). The mixture was refluxed for one day

under nitrogen, extra chlorotrimethylsilane (140 μL) was added and reflux was continued for a further twenty-four hours. The reaction mixture was cooled and then diluted with ether (100 mL). The ether solution was washed with water (3 x 10 mL), 10% aqueous sodium thiosulfate (10 mL) and saturated aqueous sodium bicarbonate (3 x 10 mL). After drying over sodium sulfate, filtration and concentration gave a crude product mixture (53 mg) which was subjected to ptlc (Skellysolve B-ethyl acetate, 3:1) affording two major components: R_f 0.33 (28 mg, 60%) and R_f 0.09 (3.4 mg, 11%).

The R_f 0.33 component (mp 182-184°C, Skellysolve B) was identified as 6-(2-iodoethyl)-5,7-dimethylphthalide (54) on the basis of the following spectral properties.

IR ($CHCl_3$ cast): 1750 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.43 (3 H, s, $ArCH_3$), 2.67 (3 H, s, $ArCH_3$), 3.2 (4 H, m (A_2B_2), CH_2CH_2), 5.11 (2 H, s, CH_2O), 7.08 (1 H, s, ArH).

MS: m/e calcd. for $C_{12}H_{13}O_2I$ (M^+): 315.9960; found: 315.9949 (3), 189 (100).

The R_f 0.09 component was identical (tlc, ir, ^{1}Hmr, ms) with natural trisnorcybrodolide (5)[2].

Methyl 3-(2-hydroxyethyl)-6-methoxymethyl-2,4-dimethylbenzoate (55)

A solution of tetrahydropyranyl ether 52 (303 mg,

0.902 mmol) and pyridinium tosylate (100 mg, 0.4 mmol) in methanol (5 mL) was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was taken up in ether (100 mL) and then washed with water (10 mL) and brine (10 mL). After drying over sodium sulfate, filtration and concentration gave compound 55 (220 mg, 97%), a clear oil sufficiently pure for further work (*vide infra*). For the purpose of characterization, a small sample (500 mg) from a parallel run was chromatographed (Skellysolve B-ethyl acetate, 1:1; 20 g silica gel) affording pure 55 (450 mg). Evaporative distillation (110°C, 0.017 Torr) of a portion of this material provided an analytically pure sample.

TLC: R_f 0.33 (Skellysolve B-acetone, 7:3), brown spot.

IR ($CHCl_3$ cast): 3440, 1727 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.26 (3 H, s, $ArCH_3$), 2.32 (3 H, s, $ArCH_3$), 2.50 (1 H, s, OH), 2.90 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.28 (3 H, s, $\underline{CH_3}OCH_2$), 3.60 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 3.85 (3 H, s, CO_2CH_3), 4.36 (2 H, s, $\underline{CH_2}OCH_3$), 7.00 (1 H, s, ArH).

MS: m/e calcd. for $C_{14}H_{20}O_4$ (M^+): 252.1362; found: 252.1363 (29), 221 (25), 207 (21), 205 (100), 189 (32).

ANALYSIS: calcd. for $C_{14}H_{20}O_4$: C 66.65, H 7.99; found: C 66.75, H 7.87.

Methyl 3-(2-acetoxyethyl)-6-methoxymethyl-2,4-dimethylbenzoate (56)

A solution of crude alcohol 55 (*vide supra*, 220 mg) in acetic anhydride (5 mL) and pyridine (10 mL) was stirred at room temperature for three hours. Evaporation to dryness under high vacuum gave crude 56 (241 mg) as a brown oil, sufficiently pure for further work (*vide infra*). In a parallel experiment, pure alcohol 55 (330 mg) treated in the same fashion gave the pure acetyl derivative 56 (378 mg, 98%) as a clear oil.

TLC: R_f 0.66 (Skellysolve B-ethyl acetate, 1:1), brown spot.

IR (film): 1750, 1745 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.01 (3 H, s, OAc), 2.29 (3 H, s, $ArCH_3$), 2.35 (3 H, s, $ArCH_3$), 2.98 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.30 (3 H, s, $\underline{CH_3}OCH_2$), 3.85 (3 H, s, $CO_2\underline{CH_3}$), 4.10 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.36 (2 H, s, $\underline{CH_2}OCH_3$), 7.02 (1 H, s, ArH).

MS: m/e calcd. for $C_{16}H_{22}O_5$ (M^+): 294.1468; found: 294.1472 (7), 263 (42), 262 (92), 247 (20), 234 (38), 219 (100), 207 (72), 203 (21), 189 (21), 187 (55).

Trisnorcybrodolide (5) via compounds 56 and 57

A mixture of crude 56 (241 mg, *vide supra*), sodium iodide (1.2 g, 8 mmol) and chlorotrimethylsilane

(1.0 mL, 856 mg, 7.9 mmol) in dry acetonitrile (20 mL) was refluxed under nitrogen for twenty-four hours. Water (100 mL) was added and the product was extracted into ether (5 x 20 mL). The combined ether extracts were washed successively with water (20 mL), 10% aqueous sodium thiosulfate (20 mL), water (20 mL) and brine (20 mL). After drying over magnesium sulfate, filtration and concentration gave a brown powder. The major component of this crude product mixture was shown to be identical with the acetyl derivative (57) of natural trisnorcybrodolide (5)[2] by tlc examination. Crude 57 and potassium carbonate (1.0 g) were taken up in methanol (10 mL) and stirred overnight at room temperature. The methanol was removed *in vacuo* and the residue was partitioned between ethyl acetate (100 mL) and water (10 mL). The organic layer was washed with brine (20 mL), dried over magnesium sulfate, filtered and concentrated. Non-polar impurities were removed from the residue by trituration with methylene chloride (3 x 1 mL) leaving virtually pure trisnorcybrodolide (5, 139 mg, 75% overall from 52). Recrystallization (methanol) gave colourless prisms mp 189-191°C which were sublimed (120°C, 0.015 Torr) providing an analytical sample. ANALYSIS: calcd. for $C_{12}H_{14}O_3$: C 69.89, H 6.84; found: C 69.87, H 7.05.

3-(2-(Tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxymethyl-2,4-dimethylbenzyl alcohol (77)

Methyl ester 52 (7.07 g, 21.0 mmol) in dry ether (50 mL) was added over a thirty minute period to a stirred slurry of lithium aluminum hydride (1.00 g, 26.3 mmol) in dry ether (100 mL). The mixture was stirred at room temperature for two hours and then quenched by dropwise addition of water (1 mL), 15% aqueous sodium hydroxide (1 mL) and water (3 mL). The granular precipitate was filtered off and washed with ether (5 x 50 mL). The combined ether solutions were concentrated leaving crude alcohol 77 (6.5 g). Flash chromatography (Skellysolve B-ethyl acetate, 1:1; 5 cm column) afforded pure 77 (5.00 g, 77%) as a clear oil.

TLC: R_f 0.29 (Skellysolve B-ethyl acetate, 1:1), black spot.

IR ($CHCl_3$ cast): 3440 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 2.36 (3 H, s, ArCH_3), 2.46 (3 H, s, ArCH_3), 2.70 (1 H, t (7 Hz), OH), 3.03 (2 H, t (7 Hz), Ar$\underline{CH_2}CH_2$), 3.42 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.75 (2 H, t (7 Hz), Ar$CH_2\underline{CH_2}$), 4.48 (2 H, s, $\underline{CH_2}OCH_3$), 4.58 (1 H, bs, CH), 4.66 (2 H, d (7 Hz), $\underline{CH_2}OH$), 6.95 (1 H, s, ArH).

MS: m/e calcd. for $C_{18}H_{28}O_4$ (M^+): 308.1988; found: 308.1984 (1), 174 (21), 162 (34), 146 (32), 85 (100).

3-(2-(Tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxymethyl-2,4-dimethylbenzaldehyde (66)

A solution of alcohol 77 (5.00 g, 16.2 mmol) in methylene chloride (10 mL) was added in one portion to a solution of pyridinium chlorochromate (5.40 g, 25 mmol) and sodium acetate (400 mg, 4.88 mmol) in methylene chloride (20 mL). The mixture was stirred at room temperature for one hour, diluted with ether (100 mL) and then filtered through Celite. The filter cake was washed with additional ether (5 x 30 mL). The combined filtrates were rapidly passed through a Florosil column (50 g). An extra portion of ether (200 mL) completely eluted the product. Concentration provided aldehyde 66 (4.85 g, 98%) sufficiently pure for further work.

TLC: R_f 0.58 (Skellysolve B-ethyl acetate, 1:1), red spot.

IR ($CHCl_3$ cast): 1690 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 2.43 (3 H, s, ArCH_3), 2.61 (3 H, s, ArCH_3), 3.04 (2 H, t (7 Hz), Ar$\underline{CH_2}CH_2$), 3.42 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.77 (2 H, t (7 Hz), Ar$CH_2\underline{CH_2}$), 4.57 (1 H, bs, CH), 4.69 (2 H, s, $\underline{CH_2}OCH_3$), 7.21 (1 H, s, ArH), 10.54 (1 H, s, CHO).

MS: m/e calcd. for $C_{18}H_{26}O_4$ (M^+): 306.1831; found: 306.1832 (15), 204 (37), 189 (24), 85 (100).

1-((3-(2-(Tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxy-methyl-2,4-dimethyl)phenyl)-2-butyn-1-ol (78)

2-Bromopropene (3.0 mL, 4.1 g, 34 mmol) was added to a suspension of finely divided lithium (1% sodium, 270 mg, 38.9 mmol) in dry ether (50 mL)*. The mixture was refluxed for three hours under nitrogen at which time little reaction (as indicated by dissolution of lithium) was evident. Extra lithium (200 mg, 28.8 mmol) was added and the mixture was stirred overnight. Aldehyde 66 (2.28 g, 7.45 mmol) in dry ether (10 mL)* was added in one portion and the mixture was stirred for one hour. Saturated ammonium chloride (50 mL) was added and the products were extracted into ether (2 x 100 mL). After drying over magnesium sulfate, filtration and concentration, the crude product was chromatographed (Skellysolve B-ether, 1:1; 150 g of silica gel) affording recovered starting material 66 (210 mg) and product 78 (391 mg, 15%) as a yellow oil.

TLC: R_f 0.66 (benzene-ether, 1:3), green spot.

IR ($CHCl_3$ cast): 3400 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 1.84 (3 H, d (3 Hz), $CH_3C{\equiv}C$), 2.34 (3 H, s, $ArCH_3$), 2.48 (3 H, s, $ArCH_3$), 3.02 (2 H, t (7 Hz), $ArCH_2CH_2$), 3.40 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.77 (2 H, t (7 Hz),

* Distilled from sodium.

$ArCH_2\underline{CH_2}$), 4.32 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 4.60 (1 H, bs, CH), 5.26 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 5.85 (1 H, bs, $\underline{CH}OH$), 6.98 (1 H, s, ArH).

MS: m/e calcd. for $C_{21}H_{30}O_4$ (M^+): 346.2144; found: 346.2142 (1), 85 (100).

1-((3-(2-Hydroxyethyl)-6-methoxymethyl-2,4-dimethyl)-phenyl)-1-methoxy-2-butyne (80)

A solution of compound 78 (106 mg, 0.306 mmol) and pyridinium tosylate (50 mg, 0.199 mmol) in methanol (5 mL) was stirred overnight at room temperature. Methanol was removed *in vacuo*, the residue was dissolved in chloroform (100 mL) and washed with water (10 mL). After drying over magnesium sulfate, filtration and concentration gave 80 (82 mg, 97%) as a yellow oil.

TLC: R_f 0.56 (benzene-ether, 1:3), green spot.

IR (film): 3400 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.83 (3 H, d (3 Hz), $CH_3C\equiv C$), 2.32 (3 H, s, $ArCH_3$), 2.50 (3 H, s, $ArCH_3$), 2.95 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.33 (3 H, s, CH_3O), 3.36 (3 H, s, CH_3O), 3.67 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.44 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 4.60 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 5.38 (1 H, q (3 Hz), HCO), 7.00 (1 H, s, ArH).

^{13}CMR ($CDCl_3$): δ3.8, 16.2, 20.3, 56.2, 57.9, (CH_3); 33.2, 61.3, 73.1, (CH_2); 69.2, 129.5, (CH), 83.0,

108.4, 134.1, 134.2, 136.0, 136.7, 136.9, (C).
MS: m/e calcd. for $C_{17}H_{24}O_3$ (M^+): 276.1725; found: 276.1734 (2), 229 (100), 214 (32), 213 (22), 201 (33), 183 (21).

1-((3-(2-(Tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxy-methyl-2,4-dimethyl)phenyl)-2-methyl-2-propen-1-ol (75)

A solution of freshly distilled 2-bromopropene (7.1 mL, 9.67 g, 79.9 mmol) and 1,2-dibromoethane (0.9 mL, 1.96 g, 10.4 mmol) in dry tetrahydrofuran (30 mL) was added dropwise over a thirty minute period to a magnetically stirred suspension of magnesium powder (2.20 g, 90.5 mmol) in dry tetrahydrofuran (10 mL) under a nitrogen atmosphere. The addition of the bromide solution caused vigourous boiling. Reflux was maintained for two hours after the addition was complete. The mixture was cooled to 0°C and then a solution of aldehyde 66 (5.00 g, 16.4 mmol) in dry tetrahydrofuran (30 mL) was added in one portion. The mixture was warmed to room temperature and then refluxed for two hours. The mixture was carefully poured over crushed ice (~500 g), the products were isolated by ether extraction (5 x 100 mL). After drying over magnesium sulfate, filtration and concentration gave a brown oil (7.6 g). Flash chromatography (Skellysolve B-ethyl acetate, 5:1; 5 cm column; 3 runs) afforded pure

carbinol 75 (4.56 g, 80%) as a clear oil.

TLC: R_f 0.53 (Skellysolve B-ethyl acetate, 1:1), green spot.

IR ($CHCl_3$ cast): 3420, 1650, 900 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 1.56 (3 H, bs, vinyl CH_3), 2.30 (3 H, s, ArCH_3), 2.36 (3 H, s, ArCH_3), 2.98 (2 H, t (7 Hz), Ar$\underline{CH_2}CH_2$), 3.26 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.72 (2 H, t (7 Hz), Ar$CH_2\underline{CH_2}$), 4.11 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 4.56 (1 H, bs, CH), 4.75 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 4.93 (1 H, bs, vinyl H), 5.08 (1 H, bs, vinyl H), 5.47 (1 H, bs, $\underline{H}COH$), 6.91 (1 H, s, ArH).

MS: m/e calcd. for $C_{21}H_{34}O_4$ (M^+): 348.2301; found: 348.2295 (2), 214 (46), 199 (57), 85 (100).

1-((3-(2-hydroxyethyl)-6-methoxymethyl-2,4-dimethyl)-phenyl)-2-methyl-2-propen-1-ol (81)

A solution of tetrahydropyranyl ether 75 (890 mg, 2.56 mmol) and pyridinium tosylate (500 mg, 2 mmol) in water-tetrahydrofuran (1:2, 30 mL) was refluxed overnight. Most of the tetrahydrofuran was removed *in vacuo*, water (15 mL) was added and the product was isolated by chloroform extraction (5 x 20 mL). After drying over magnesium sulfate, filtration and concentration gave diol 81 (657 mg, 97%). Evaporative distillation (100-120°C, 0.017 Torr) of a small sample

(80 mg) afforded analytically pure material.

TLC: R_f 0.46 (methylene chloride-methanol, 10:1), green spot.

IR($CHCl_3$ cast): 3380, 1650, 900 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.56 (3 H, bs, vinyl CH_3), 2.26 (3 H, s, $ArCH_3$), 2.32 (3 H, s, $ArCH_3$), 2.90 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.29 (3 H, s, CH_3O), 3.63 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.12 (1 H, d (11 Hz), CH_2O), 4.73 (1 H, d (11 Hz), CH_2O), 4.93 (1 H, bs, vinyl H), 5.08 (1 H, bs, vinyl H), 5.46 (1 H, bs, $\underline{H}COH$), 6.91 (1 H, s, ArH).

MS: m/e calcd. for $C_{16}H_{24}O_3$ (M^+): 264.1725; found: 264.1735 (4), 232 (100), 217 (85), 201 (67), 199 (25), 191 (66).

ANALYSIS: calcd. for $C_{16}H_{24}O_3$: C 72.69, H 9.15; found: C 72.75, H 8.97.

Direct conversion of bromide 47 to alcohol 75

A solution of n-butyllithium (1.6 M in hexane, 10.6 mL, 17 mmol) in dry tetrahydrofuran (20 mL) was cooled to -78°C under nitrogen. A solution of bromide 47 (5.71 g, 16 mmol) in dry tetrahydrofuran (20 mL) was added and the mixture was stirred at -78°C for one hour. Freshly distilled methacrolein (83, 6.6 mL, 5.6 g, 80 mmol) was added in one portion and the cooling bath was removed. The mixture was stirred at room temperature for five hours whereupon water (100 mL)

was added. The organic layer was removed and the aqueous phase was extracted with ether (5 x 40 mL). The combined organic solutions were washed with brine (20 mL) and dried over sodium sulfate. Filtration and concentration gave a yellow oil (8.8 g) which was purified by flash chromatography (Skellysolve B-ethyl acetate, 5:1; 4 cm column), affording alcohol 75 (1.38 g, 25%).

Attempted allylic rearrangement of 81 using Babler's conditions

A mixture of alcohol 81 (6.8 mg, 0.026 mmol), *p*-toluenesulfonic acid (6 mg), acetic anhydride (0.1 mL) and acetic acid (0.5 mL) was stirred at room temperature for twenty minutes. The solution was diluted with water (10 mL) and extracted with methylene chloride (4 x 7 mL). The combined methylene chloride extracts were washed with saturated aqueous sodium carbonate (5 mL), water (5 mL) and brine (5 mL). After drying over magnesium sulfate, filtration and concentration gave 1-((3-(2-acetoxyethyl)-6-methoxymethyl-2,4-dimethyl)phenyl)-2-methyl-2-propenyl acetate (85) as a yellow oil (7.1 mg, 79%).

TLC: R_f 0.50 (Skellysolve B-ethyl acetate, 1:1), green spot.

IR ($CHCl_3$ cast): 1740 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.71 (3 H, bs, vinyl CH_3), 2.05 (3 H,

s, OAc), 2.07 (3 H, s, OAc), 2.35 (3 H, s, $ArCH_3$), 2.37 (3 H, s, $ArCH_3$), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.36 (3 H, s, CH_3O), 4.12 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.35 (1 H, d (12 Hz), CH_2O), 4.70 (1 H, bs, vinyl H), 4.71 (1 H, d (12 Hz), CH_2O), 4.93 (1 H, bs, vinyl H), 6.65 (1 H, bs, HCO), 7.08 (1 H, s, ArH).

MS: m/e calcd. for $C_{18}H_{26}O_4$ (M-CH_2C=O, parent ion not seen): 306.1831; found: 306.1821 (14), 288 (81), 274 (94), 215 (28), 213 (53), 201 (28), 199 (100), 197 (77), 196 (30), 185 (26), 183 (21), 171 (20).

Chemical Ionization (NH_3) MS shows a peak at m/e 366 (M + 18).

Attempted allylic rearrangement of 75 using benzeneselenenic anhydride (93)

A slurry of benzeneselenenic anhydride (93, 86 mg, 0.24 mmol)* in dry methylene chloride (5 mL) containing a small amount of pyridine (19 µL, 19 mg, 0.24 mmol) was added to a stirred solution of alcohol 75 (69 mg, 0.20 mmol) in dry methylene chloride (5 mL). The mixture was stirred under nitrogen for eighteen hours and then washed with saturated aqueous sodium carbonate (10 mL), water (10 mL) and brine (10 mL). After drying over sodium sulfate, filtration and concentration gave an oil which was subjected to ptlc

* Prepared by the t-butylhydroperoxide procedure, see reference 67.

(benzene-ether, 3:1). In this fashion, starting material 75 (14 mg, 20%) was recovered along with 1-((3-(2-(tetrahydro-2H-pyran-2-yl)oxyethyl)-6-methoxymethyl-2,4-dimethyl)phenyl)-2-methyl-2-propen-1-one (69) as a yellow oil (34 mg, 49%).

TLC: R_f 0.79 (benzene-ether, 3:1), red spot.

IR (film): 1660 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.4-1.8 (6 H, m, 3xCH_2), 2.02 (3 H, bs, vinyl CH_3), 2.13 (3 H, s, ArCH_3), 2.36 (3 H, s, ArCH_3), 2.97 (2 H, t (7 Hz), Ar$\underline{CH_2}CH_2$), 3.23 (3 H, s, CH_3O), 3.5 (2 H, m, CH_2O), 3.75 (2 H, t (7 Hz), Ar$CH_2\underline{CH_2}$), 4.21 (2 H, s, $\underline{CH_2}OCH_3$), 4.56 (1 H, bs, CH), 5.51 (1 H, bs, vinyl H), 5.87 (1 H, bs, vinyl H), 7.03 (1 H, s, ArH).

MS: m/e calcd. for $C_{21}H_{30}O_4$ (M^+): 346.2144; found: 346.2147 (6), 200 (33), 85 (100).

Cybrodol methyl ether (96, 3-((3-(2-hydroxyethyl)-6-methoxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propen-1-ol) and isocybrodol methyl ether (98, 4-(2-hydroxyethyl)-2-(3-methoxy-2-methyl-(*Z*)-1-propenyl)-3,5-dimethylbenzyl alcohol)

A mixture of alcohol 75 (610 mg, 1.75 mmol), 0.3 M aqueous sulfuric acid (16 mL) and acetone (24 mL) was heated at reflux for seven hours and then diluted with saturated aqueous sodium carbonate (50 mL). The products were extracted into methylene chloride (5 x 25 mL), the

organic extracts were dried over sodium sulfate, filtered and evaporated to dryness. Flash chromatography of the residue (methylene chloride-methanol, 50:1; 3 cm column) gave isocybrodol methyl ether (98, 152 mg) and cybrodol methyl ether (96, 158 mg) as yellow oils (67% combined yield). An analytically pure sample of 96 was obtained by evaporative distillation (130-140°C, 0.027 Torr). Compound 98 has the following physical properties.

TLC: R_f 0.43 (methylene chloride-methanol, 10:1), yellowish green spot.

IR ($CHCl_3$ cast): 3350, 1680 (w), 880 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.00 (3 H, d (1 Hz), vinyl CH_3), 2.19 (3 H, s, $ArCH_3$), 2.38 (3 H, s, $ArCH_3$), 2.99 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.21 (3 H, s, CH_3O), 3.48 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 3.68 (1 H, d (11 Hz), $\underline{CH_2}OCH_3$), 3.76 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.34 (1 H, d (12 Hz), $Ar\underline{CH_2}OH$); 4.56 (1 H, d (12 Hz), $Ar\underline{CH_2}OH$), 6.37 (1 H, bs, vinyl H), 7.11 (1 H, s, ArH).

MS: m/e calcd. for $C_{16}H_{24}O_3$ (M^+): 264.1725; found: 264.1723 (5), 232 (26), 219 (100), 217 (42), 202 (21), 201 (53), 197 (30), 191 (35), 187 (28), 183 (21), 171 (27), 157 (23).

Compound 96 has the following physical properties.

TLC: R_f 0.40 (methylene chloride-methanol, 10:1), green spot.

IR ($CHCl_3$ cast): 3350, 1680 (w), 870 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.44 (3 H, d (1 Hz), vinyl CH_3), 2.20 (3 H, s, $ArCH_3$), 2.37 (3 H, s, $ArCH_3$), 2.99 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.34 (3 H, s, CH_3O), 3.74 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.3 (4 H, bs, $2xCH_2O$), 6.38 (1 H, bs, vinyl H), 7.08 (1 H, s, ArH).

MS: m/e calcd. for $C_{16}H_{24}O_3$ (M^+): 264.1725; found: 264.1730 (5), 233 (33), 232 (74), 217 (60), 201 (100), 199 (29), 187 (36), 183 (22), 171 (35), 157 (27).

ANALYSIS: calcd. for $C_{16}H_{24}O_3$: C 72.69, H 9.15; found: C 72.35, H 9.39.

4-(2-Acetoxyethyl)-2-(3-methoxy-2-methyl-(Z)-1-propenyl)-3,5-dimethylbenzyl acetate (99)

Alcohol 98 (222 mg, 0.84 mmol), acetic anhydride (10 mL) and pyridine (10 mL) were stirred overnight at room temperature. Concentration under high vacuum gave compound 99 (287 mg, 98%) as a yellow oil.

TLC: R_f 0.64 (Skellysolve B-ethyl acetate, 1:1), green spot.

IR ($CHCl_3$ cast): 1740 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.95 (3 H, d (1 Hz), vinyl CH_3), 2.06 (3 H, s, OAc), 2.08 (3 H, s, OAc), 2.21 (3 H, s, $ArCH_3$), 2.37 (3 H, s, $ArCH_3$), 3.01 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.15 (3 H, s, CH_3O), 3.59 (2 H, s, CH_2O), 4.14 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.85 (1 H, d (12 Hz), $ArCH_2O$), 5.01

(1 H, d (12 Hz), $ArCH_2O$), 6.25 (1 H, bs, vinyl H), 7.02 (1 H, s, ArH).

MS: m/e calcd. for $C_{20}H_{28}O_5$ (M^+): 348.1937; found: 348.1944 (1), 288 (49), 273 (23), 213 (41), 201 (22), 199 (22), 197 (100), 196 (87), 185 (21), 183 (43), 181 (21).

4-(2-Hydroxyethyl)-2-(3-methoxy-2-methyl-(z)-1-propenyl)-3,5-dimethylbenzaldehyde (100)

Isocybrodol methyl ether (98, 27 mg, 0.10 mmol) and activated manganese dioxide (500 mg, 5.7 mmol)[68] in methylene chloride (10 mL) were stirred for two hours at room temperature. The mixture was filtered through Celite and the filter cake was washed with ether (5 x 50 mL). The combined filtrates were concentrated and the residue was purified by flash chromatography (methylene chloride-methanol, 100:1; 1 cm column) affording 100 as a clear oil (25 mg, 93%).

TLC: R_f 0.50 (methylene chloride-methanol, 10:1), brown spot.

UV (CH_3OH) λ_{max}: 208, 266, 310 nm.

IR ($CHCl_3$ cast): 3440, 1684 cm^{-1}.

^1HMR ($CDCl_3$): δ2.01 (3 H, d (1 Hz), vinyl CH_3), 2.25 (3 H, s, $ArCH_3$), 2.40 (3 H, s, $ArCH_3$), 3.04 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.10 (3 H, s, CH_3O), 3.58 (2 H, bs, $\underline{CH_2}OCH_3$), 3.76 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 6.47 (1 H, bs,

vinyl H), 7.60 (1 H, s, ArH), 9.38 (1 H, s, CHO).
MS: m/e calcd. for $C_{16}H_{22}O_3$ (M^+): 262.1569; found: 262.1568 (3), 217 (100).

Attempted demethylation of 98 with ethanedithiol

Isocybrodol methyl ether (98, 16.8 mg, 0.064 mmol) and boron trifluoride etherate (30 µL, 34 mg, 0.24 mmol) in ethanedithiol (0.5 mL)* were stirred at room temperature for four days. The solution was diluted with brine (10 mL) and extracted with methylene chloride (3 x 10 mL). The combined organic solutions were washed with 20% aqueous sodium hydroxide (2 x 10 mL), brine (10 mL) and dried over sodium sulfate. Filtration and concentration gave a foul smelling oil which was subjected to ptlc (benzene-ether, 1:3) affording 5-(2-hydroxyethyl)-4,6,10-trimethyl-2H-benzothiapin (106) as a clear oil (10 mg, 63%).
TLC: R_f 0.53 (benzene-ether, 1:3), purple spot.
IR ($CHCl_3$ cast): 3360 cm^{-1}.
^{1}HMR ($CDCl_3$): δ2.06 (3 H, d (1 Hz), vinyl CH_3), 2.20 (3 H, s, $ArCH_3$), 2.34 (3 H, s, $ArCH_3$), 2.81 (2 H, s, CH_2S), 2.96 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.43 (2 H, s, $ArCH_2S$), 3.74 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 6.34 (1 H, s, vinyl H), 6.93 (1 H, s, ArH).
MS: m/e calcd. for $C_{15}H_{20}OS$ (M^+): 248.1235; found:

* Undistilled.

248.1232 (78), 233 (22), 217 (13), 207 (100). Chemical Ionization (NH_3) MS shows a peak at m/e 266 (M + 18).

Cybrodol (1, 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propen-1-ol) and isocybrodol (2, 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*Z*)-2-propen-1-ol)

A solution of alcohol 98* (201 mg, 0.76 mmol) and ferric chloride (100 mg, 0.62 mmol) in acetic anhydride (5 mL) was stirred for eighteen hours at room temperature. The mixture was diluted with water (50 mL) and then extracted with methylene chloride (5 x 20 mL). In a preliminary experiment (88 mg of 96) the methylene chloride extracts were dried over sodium sulfate, filtered and concentrated. Flash chromatography (Skelly-solve B-acetone, 8:1; 1 cm column) gave an inseparable mixture of triacetylcybrodol (107) and triacetylisocybrodol (108, 98 mg total, 78%)[2]. The relative proportions were 78:22 in favour of the *E* isomer 107 as determined by ^{1}Hmr. Normally however, the wet methylene chloride extracts were taken to dryness under high vacuum and then dissolved in methanol (10 mL). Potassium carbonate (1 g) was added and the mixture was stirred at room temperature overnight. The volatiles

* Compound 96 or mixtures of compounds 96 and 98 can be used with the same result.

were removed *in vacuo* and the residue was partitioned between ethyl acetate (50 mL) and water (10 mL). The organic phase was dried over sodium sulfate, filtered and concentrated. Flash chromatography (methylene chloride-methanol, 20:1; 2 cm column) gave isocybrodol (2, 32 mg) and cybrodol (1, 111 mg, 75% combined overall yield) as yellow oils. These materials were identical in all respects (tlc, ir, ^{1}Hmr, ms) with the natural products[2]. Synthetic cybrodol (1) was crystallized (chloroform-methanol, 50:1) affording white prisms, mp 109-110°C. Synthetic isocybrodol (2) was crystallized (acetone-Skellysolve B) affording white prisms, mp 101-102°C. Analytically pure samples of both compounds were prepared by evaporative distillation (140-160°C, 0.025 Torr).

Cybrodol (1) analysis: calcd. for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found: C 72.27, H 9.08.

Isocybrodol (2) analysis: calcd. for $C_{15}H_{22}O_3$: C 71.97, H 8.86; found: C 72.21, H 9.09.

Allylic rearrangement of 81 with ferric chloride in acetic anhydride

A solution of alcohol 81 (103 mg, 0.39 mmol) and ferric chloride (50 mg, 0.31 mmol) in acetic anhydride (5 mL) was stirred for eighteen hours at room temperature. This reaction mixture was treated in exactly

the same fashion as the previous one. In this instance, after deacetylation and flash chromatography, isocybrodol (2, 15.3 mg) and cybrodol (1, 34.3 mg, combined overall yield of 51%) were obtained as yellow oils.

3-((3-(2-acetoxyethyl)-6-bromomethyl-2,4-dimethyl)-phenyl)-2-methyl-(Z)-2-propenyl bromide (109) and 3-((3-(2-acetoxyethyl)-6-bromomethyl-2,4-dimethyl)phenyl)-2-methyl-(E)-2-propenyl bromide (110)

A solution of boron tribromide (200 mg, 0.80 mmol) in dry methylene chloride (1 mL) was added to a solution of methyl ether 99 (105 mg, 0.30 mmol) in dry methylene chloride (12 mL) at -20°C. The mixture was stirred under nitrogen at -20°C for one hour and then diluted with saturated aqueous sodium carbonate (20 mL). The organic layer was removed and the aqueous residue was extracted with methylene chloride (3 x 20 mL). The combined extracts were dried over sodium sulfate, filtered and concentrated leaving dibromide 109* as an orange oil (114 mg, 86%). In a preliminary experiment, the reaction was conducted at 0°C resulting in the production of 109 and 110 in a 3:1 ratio as determined by ^{1}Hmr. Compounds 109 and 110 have practically identical tlc characteristics, however careful ptlc (Skellysolve B-acetone, 20:1; double elution) of this 3:1 mixture

* This material contained a small amount of isomer 110 (~5%) as judged by ^{1}Hmr.

did allow isolation of sufficient quantities for spectral analysis of each compound (brown oils). Compound 109 has the following physical properties.

TLC: R_f 0.70 (Skellysolve B-acetone, 20:1; double elution), blue spot.

IR ($CHCl_3$ cast): 1740, 1240, 605 cm^{-1}.

^{1}HMR ($CDCl_3$): δ2.05 (3 H, s, OAc), 2.10 (3 H, d (1 Hz), vinyl CH_3), 2.23 (3 H, s, $ArCH_3$), 2.35 (3 H, s, $ArCH_3$), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.70 (2 H, s, CH_2Br), 4.14 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.29 (1 H, d (11 Hz), $ArCH_2Br$), 4.46 (1 H, d (11 Hz), $ArCH_2Br$), 6.35 (1 H, bs, vinyl H), 7.07 (1 H, s, ArH).

MS: m/e calcd. for $C_{17}H_{22}O_2{}^{81}Br$ (M-Br, parent ion not seen): 339.0783; found: 339.0802 (1); calcd. for $C_{17}H_{22}O_2{}^{79}Br$ (M-Br): 337.0803; found: 337.0799 (1), 217 (100).

Chemical Ionization (NH_3) MS shows peaks at m/e 434, 436, 438, 440 (M + 18).

Compound 110 has the following physical properties.

TLC: R_f 0.66 (Skellysolve B-acetone, 20:1; double elution), blue spot.

IR ($CHCl_3$ cast): 1740, 1240, 605 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.60 (3 H, d (1 Hz), vinyl CH_3), 2.05 (3 H, s, OAc), 2.20 (3 H, s, $ArCH_3$), 2.36 (3 H, s, $ArCH_3$), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 4.14 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.17 (2 H, s, CH_2Br), 4.35 (2 H, s,

$ArCH_2Br$), 6.60 (1 H, bs, vinyl H), 7.07 (1 H, s, ArH). MS: m/e calcd. for $C_{17}H_{22}O_2{}^{81}Br$ (M-Br, parent ion not seen): 339.0783; found: 339.0786 (77); calcd. for $C_{17}H_{22}O_2{}^{79}Br$ (M-Br): 337.0803; found: 337.0806 (79), 279 (99), 277 (100), 257 (38), 215 (27), 198 (92), 197 (76), 185 (63), 184 (42), 183 (53), 171 (47), 170 (34), 169 (38), 156 (31), 155 (45), 153 (20), 141 (23), 128 (20), 87 (30).

Chemical Ionization (NH_3) MS shows peaks at m/e 434, 436, 438, 440 (M + 18).

Conversion of dibromide 109 to triacetylisocybrodol (108)

A solution of dibromide 109 (109 mg, 0.25 mmol) and tetraethylammonium acetate (500 mg, 2.6 mmol)[63], in acetone (10 mL) was refluxed for one hour and then taken to dryness. The residue was dissolved in ether (50 mL), washed with water (10 mL) and brine (10 mL) and then dried over magnesium sulfate. Filtration and concentration gave mainly (*vide infra*) triacetylisocybrodol (108, 91 mg, 97%). This material was deacetylated in the usual fashion (see previous deacetylation) and the product was purified by flash chromatography. In this way, isocybrodol (2, 55 mg 73% from 99) plus a small amount of cybrodol (1, 3 mg) were obtained.

3-((3-(2-hydroxyethyl)-6-methoxymethyl-2,4-dimethyl)-phenyl)-2-methyl-(*E*)-2-propenal (111)

Cybrodol methyl ether (96, 104 mg, 0.39 mmol) and activated manganese dioxide (622 mg, 7.1 mmol) in methylene chloride (10 mL) were stirred overnight at room temperature. The mixture was filtered through Celite, the filter cake was washed with methylene chloride (5 x 10 mL) and the combined filtrates were concentrated leaving aldehyde 111 (103 mg, 100%) as a clear oil.

TLC: R_f 0.39 (benzene-ether, 1:3), purple spot.

IR ($CHCl_3$ cast): 3460, 2850, 1687, 1640 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.57 (3 H, d (1 Hz), vinyl CH_3), 2.18 (3 H, s, $ArCH_3$), 2.38 (3 H, s, $ArCH_3$), 3.00 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.33 (3 H, s, CH_3O), 3.74 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.20 (2 H, s, CH_2O), 7.12 (1 H, s, ArH), 7.46 (1 H, bs, vinyl H), 9.79 (1 H, s, CHO).

MS: m/e calcd. for $C_{16}H_{22}O_3$ (M^+): 262.1571; found: 262.1597 (0.3), 217 (100).

Methyl 3-((3-(2-hydroxyethyl)-6-methoxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenoate (112)

A mixture of aldehyde 111 (98 mg, 0.37 mmol), acetic acid (50 μL, 52 mg, 0.87 mmol), sodium cyanide (50 mg, 1 mmol) and activated manganese dioxide (1.4 g,

16 mmol) in methanol (10 mL) was stirred at room temperature for two days. The mixture was filtered through Celite and the filter cake was washed with ether (5 x 50 mL). The combined filtrates were washed with saturated aqueous sodium carbonate (20 mL), water (20 mL) and brine (20 mL). After drying over sodium sulfate, filtration and concentration gave methyl ester 112 (103 mg, 95%) as a clear oil.

TLC: R_f 0.48 (benzene-ether, 1:3), red spot.

IR ($CHCl_3$ cast): 3400, 1720, 1640 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.65 (3 H, d (1 Hz), vinyl CH_3), 2.18 (3 H, s, $ArCH_3$), 2.38 (3 H, s, $ArCH_3$), 2.98 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.35 (3 H, s, CH_3O), 3.74 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 3.82 (3 H, s, CO_2CH_3), 4.22 (2 H, s, CH_2O), 7.11 (1 H, s, ArH), 7.64 (1 H, bs, vinyl H).

MS: m/e calcd. for $C_{17}H_{24}O_4$ (M^+): 292.1674; found: 292.1670 (1), 260 (56), 247 (32), 229 (100), 201 (29), 171 (25).

Methyl 3-((3-(2-acetoxyethyl)-6-acetoxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenoate (113)

A solution of ester 112 (50 mg, 0.17 mmol) and ferric chloride (50 mg, 0.31 mmol) in acetic anhydride (5 mL) was stirred for two hours at room temperature. The reaction mixture was diluted with water (20 mL) and the product was extracted into methylene chloride

(5 x 10 mL). The methylene chloride extracts were washed with saturated aqueous sodium carbonate (10 mL), water (10 mL) and brine (10 mL). After the solution was dried over sodium sulfate, filtration and concentration gave crude 113 (65 mg) as a brown oil. Flash chromatography (Skellysolve B-ethyl acetate, 3:1; 1 cm column) gave pure 113 as a clear oil (48 mg, 77%).

TLC: R_f 0.53 (Skellysolve B-ethyl acetate, 1:1), red spot.

IR ($CHCl_3$ cast): 1740, 1720 cm^{-1}.

^{1}HMR ($CDCl_3$): δ1.65 (3 H, d (1 Hz), vinyl CH_3), 2.05 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.20 (3 H, s, $ArCH_3$), 2.39 (3 H, s, $ArCH_3$), 3.03 (2 H, t (7 Hz), $\underline{CH_2}CH_2O$), 3.82 (3 H, s, CO_2CH_3), 4.15 (2 H, t (7 Hz), $CH_2\underline{CH_2}O$), 4.90 (2 H, bs, CH_2O), 7.07 (1 H, s, ArH), 7.63 (1 H, bs, vinyl H).

MS: m/e calcd. for $C_{20}H_{26}O_6$ (M^+): 362.1729; found: 362.1719 (13), 302 (48), 289 (37), 288 (23), 259 (26), 243 (38), 242 (61), 229 (43), 228 (36), 227 (22), 215 (26), 201 (23), 200 (23), 199 (62), 183 (100), 171 (22), 151 (23).

Cybrodic acid (3, 3-((3-(2-hydroxyethyl)-6-hydroxymethyl-2,4-dimethyl)phenyl)-2-methyl-(*E*)-2-propenoic acid)

A mixture of ester 113 (88 mg, 0.24 mmol), potassium hydroxide (1.2 g, 21 mmol), water (4 mL) and methanol

(8 mL) was heated at reflux for ninety minutes. The solution was cooled to 0°C and acidified (~pH 1) with concentrated sulfuric acid. Brine (100 mL) was added and the product was isolated by ethyl acetate extraction (5 x 30 mL). The combined extracts were dried over sodium sulfate, filtered and concentrated leaving crude acid 3 (63 mg, 97%) as a brown oil which on prolonged refrigeration in acetone (2 mL) gave crystalline cybrodic acid (3, mp 179-180°C). This material was identical with the natural acid[2] by the following criteria: mp, tlc, ir, ^{1}Hmr and ms. Sublimation (160°C, 0.025 Torr) of the crystalline synthetic acid afforded an analytically pure sample.

ANALYSIS: calcd. for $C_{15}H_{20}O_4$: C 68.16, H 7.63; found: C 68.18, H 7.62.

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