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STUDIES OF MUSTARD OIL GLUCOSIDES

by

DENYS MEAKIN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

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UNIVERSITY OF ALBERTA AT CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Studies of Mustard Oil Glucosides", submitted by Denys Meakin in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The biosynthesis of glucotropaeolin in Tropaeolum majus L. has been studied by isotopic tracer techniques. It has been demonstrated that glucotropaeolin is derived directly from phenylalanine, essentially with retention of the nitrogen atom. Possible intermediates between phenylalanine and glucotropaeolin have also been explored; thioglucose has been shown to be a particularly good precursor of the thioglucosidic sulphur atom of glucotropaeolin.

Successful syntheses have been devised for three α-aminoacids, whose existence in nature has been postulated on the basis of the proven biosynthetic pathway for several mustard oil glucosides. These aminoacids are 7-thiomethyl-2-aminoheptanoic acid and its two oxidized derivatives, the sulphoxide and sulphone. The synthesis involved the condensation of dibromopentane with diethyl sodiophthalimidomalonate, followed by condensation with sodium methyl mercaptide. Hydrolysis of the product gave the thiomethylaminoacid, which was oxidized in two stages to the sulphoxide and sulphone.

Two new syntheses of mustard oil glucosides are described. The first, which was used to prepare glucolepidiin and glucoputranjivin, involved the addition

of tetraacetyl-thioglucose to a hydroxamoyl chloride, prepared by the chlorination of an aldoxime. In the second method the hydroxamoyl chloride was prepared by treating the sodium salt of a primary nitroalkane with concentrated hydrochloric acid. This route was used to prepare the desulphonated precursors of glucocapparin and glucolepidiin.

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"The Kingdom of Heaven is like to a grain of mustard seed, which a man took and sowed in his field:
Which indeed is the least of all seeds; but when it is grown, it is the greatest among herbs."

St. Matthew, XIII, 31-32.

SECTION 1

INTRODUCTION

GENERAL

It seems certain that primitive man must have been familiar with the pungent flavour of horse-radish and the black and white mustards, and probably used them as condiments, much as we do today. Certainly there are Biblical references to mustard, and in the Hippocratic writings and the Herbal of Dioscorides compiled more than twenty-five centuries ago there are references to the medicinal value of these plants.

In the case of the mustards and many related plants, the pungent principles are secondary plant products being formed by the breakdown of more complex compounds, the mustard oil glucosides. Because of the widespread use of these plants as foods, a considerable amount of attention has been paid to these various compounds.

STRUCTURE DETERMINATION OF THE MUSTARD OIL GLUCOSIDES

1. EARLY WORK

Chemical studies of mustard are comparatively recent. It appears that the formation of a volatile oil on distillation of mustard seeds with water was first noticed in 1608, but the preparation of oil of mustard and description of its properties did not appear until 1732. References to these reports which are contained in very obscure publications appear in an extensive historical account of the various mustard oil-producing plants by Gildemeister and Hofmann.(1)

The conclusion that the pungent principle of mustard is not present in the free state in the seeds was most probably reached by the early users of the plant, as they are likely to have noticed that grinding with water is necessary before the odour is produced. However, Gildemeister and Hofmann give the date of publication of this observation as 1825. The presence of sulphur in the oil had been demonstrated in 1819, and an elementary analysis was carried out in 1833.(2) The latter workers also showed that with ammonia, oil of mustard was converted to allylthiourea (I). Extraction of black mustard seeds with cold alcohol gave a solid substance(3)

$$CH_2 = CH.CH_2.NH.CS.NH_2.$$

I

which was named myrosin.[4] This was later shown to contain an enzyme, now known as myrosinase. Extraction of the residue with water yielded a crystalline substance which was shown to be the potassium salt of a complex acid. This "potassium myronate" was later given the name sinigrin by which it is known today. Treatment of the aqueous extract with myrosin liberated oil of mustard.

The first important advances into the nature of these compounds was when it was observed that "fermentation" of sinigrin with myrosin resulted in the formation of glucose, potassium hydrogen sulphate and allyl mustard oil.[5] Allyl mustard oil was synthesized by heating allyl iodide with potassium thiocyanate,[6][7] and the identity with the natural material confirmed by conversion to the thiourea.[7] This synthesis led to the erroneous conclusion that mustard oils were esters of thiocyanic acid, although their properties were different from other known esters. However, although allyl thiocyanate (II) is indeed the initial product of the reaction, it rapidly rearranges to allyl isothiocyanate (III). This facile rearrangement is typical only of allyl and t-butyl thiocyanates, others usually requiring prolonged heating.[8]

$$CH_2 = CH.CH_2I \xrightarrow{KSCN} CH_2 = CH.CH_2SCN$$

II

$$\longrightarrow CH_2 = CH.CH_2NCS$$

III

Hofmann first suggested that there were two isomeric series, the isothiocyanic esters (mustard oils) with linkage through the nitrogen, and the thiocyanates with linkage through the sulphur.(9) Studies of the reduction, oxidation and hydrolysis of the two isomeric types and of the interaction of the mustard oils with amines firmly established this view.(10)

2. THE GADAMER STRUCTURE

A contribution to the structure of the parent compound was made by the observation that allyl cyanide and free sulphur were formed in addition to allyl isothiocyanate, glucose and sulphate ion, on enzymatic decomposition of sinigrin.(11) Will and Körner also studied the cleavage of sinigrin with silver nitrate, which resulted in the precipitation of a glucose-free silver salt (V), which on decomposition with hydrogen sulphide yielded elemental sulphur and allyl cyanide. With this background, Gadamer(12) produced a structure (IV) for sinigrin, and thus by analogy for other mustard

oil glucosides, which remained virtually unchallenged for almost 60 years. The various reactions are shown in Figure 1.

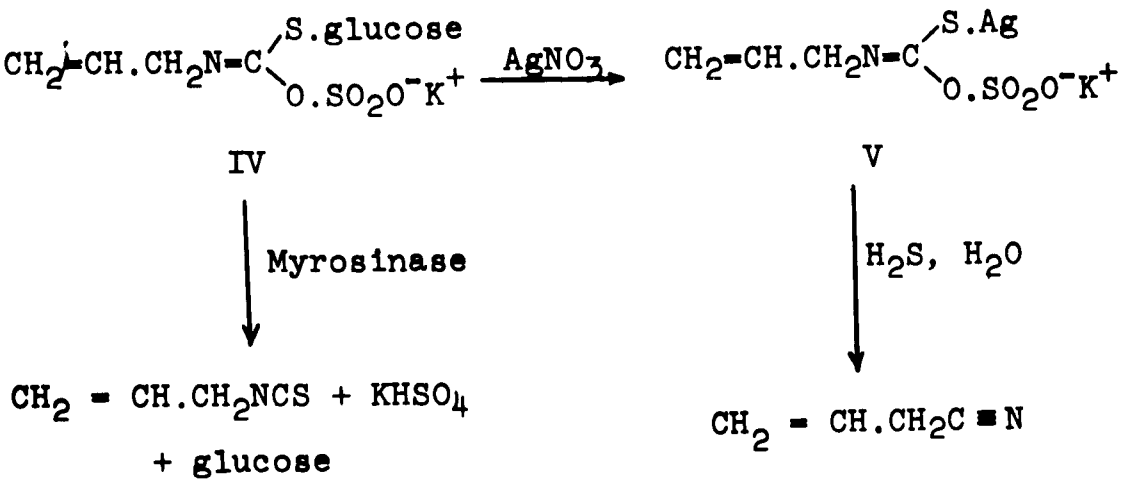


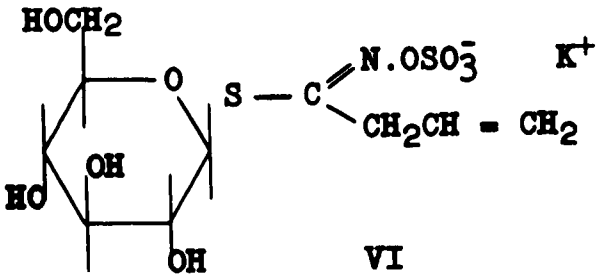
Figure 1

Gadamer's Structure for Sinigrin and Representation of its Reactions

The thioglucosidic character of the glucosides was suggested both by the removal of glucose by silver nitrate, and more positively by the isolation of the silver salt of 1-thio-D-glucose on treatment of the glucoside with potassium methoxide.[13] The small negative specific rotations of the glucoside suggested a β-linkage. It was demonstrated[14] that the silver induced cleavage was accompanied by Walden Inversion and yielded α-D-glucose, thus confirming the β-glucosidic linkage.

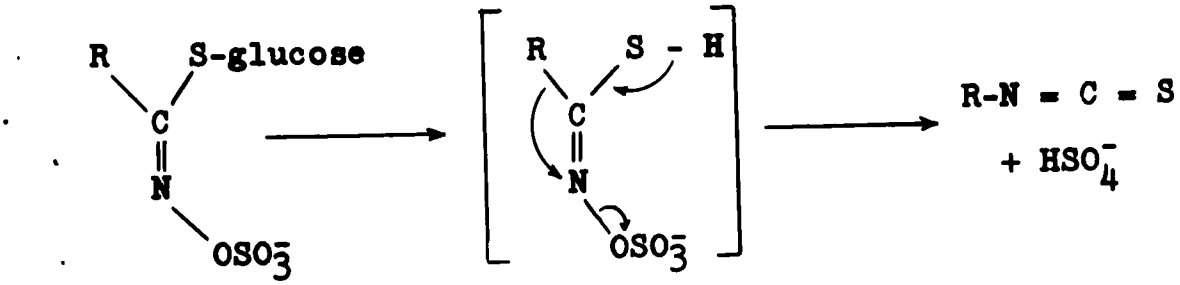
3. ETTLINGER AND LUNDEEN'S STRUCTURE FOR SINIGRIN AND OTHER MUSTARD OIL GLUCOSIDES

Gadamer's structure readily explains the enzymatic cleavage, but the production of nitriles is not easily reconcilable with this structure, and in 1956 Ettlinger and Lundeen[15] proposed a revised structure for sinigrin (VI) and the other mustard oil glucosides. This structure readily accounts for the formation of nitriles, but an

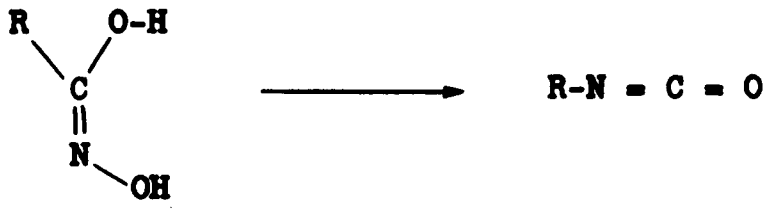


VI

intramolecular rearrangement related to the Lossen rearrangement is necessary to account for the formation of the isothiocyanates:



This type of reaction is known to occur <u>in vitro</u> with hydroxamic acids (VII).



VII

Additional evidence presented by Ettlinger and Lundeen for this structure was:

i) Raney nickel hydrogenolysis produces n-butylamine.

ii) Acid hydrolysis yields vinylacetic acid.

iii) Cold concentrated hydrochloric acid gives hydroxylamine.

A summary of the various degradations is given in Figure 2.

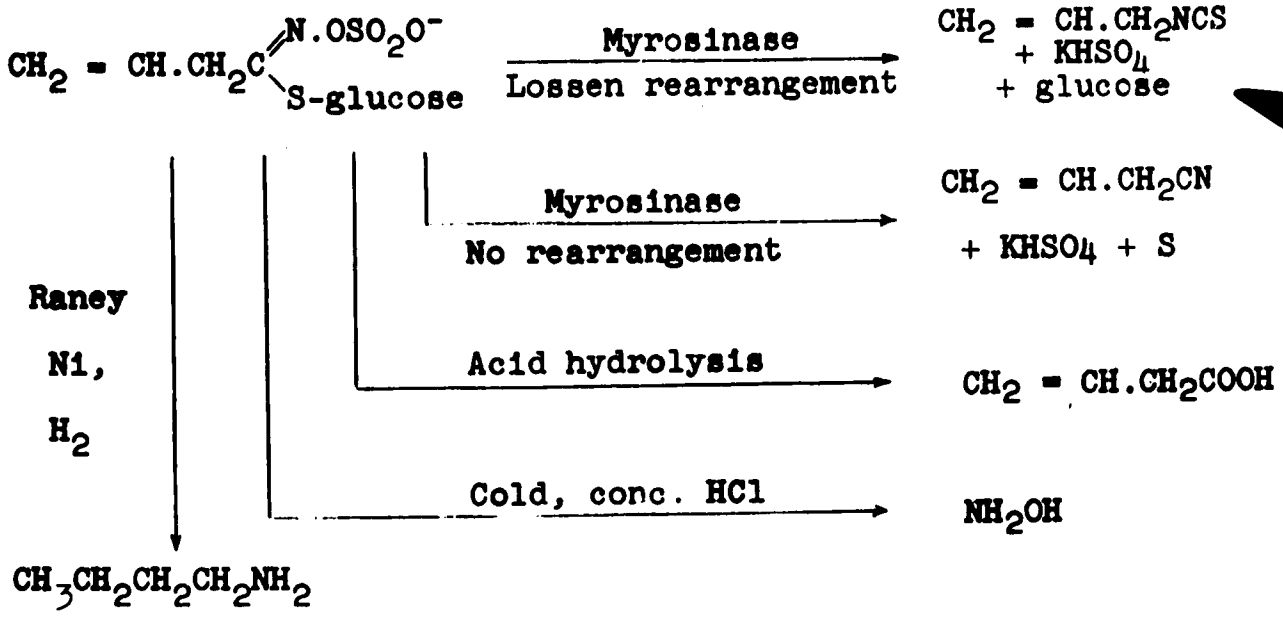
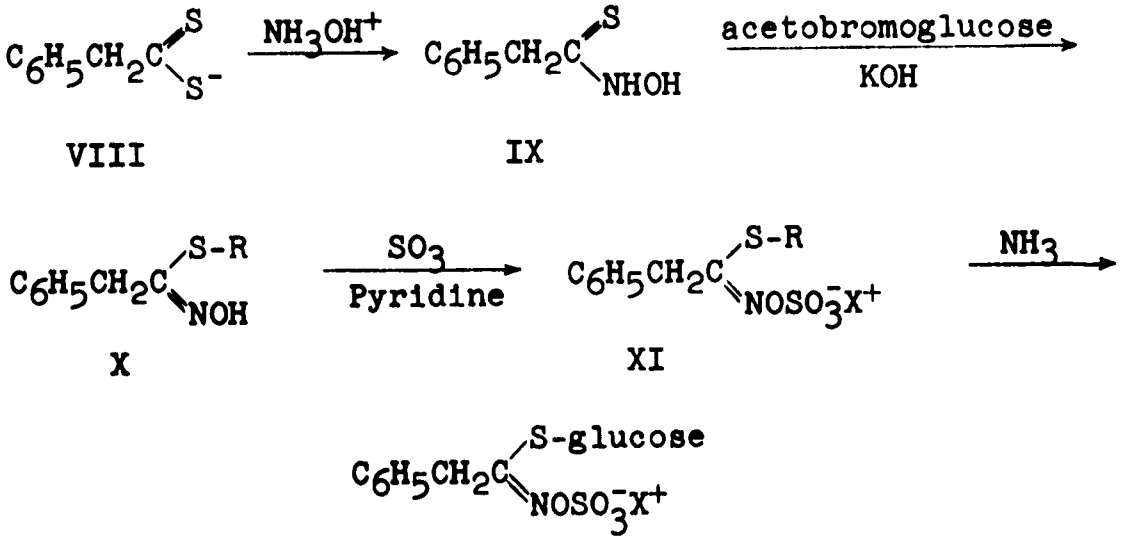


Figure 2. Evidence for Ettlinger and Lundeen Structure of Mustard Oil Glucosides

The structure was confirmed by the synthesis of glucotropaeolin (XII),[16] the mustard oil glucoside present in Tropaeolum majus L. (nasturtium) as shown in Figure 3.



R = Tetraacetyl-β-D-1-glucopyranosyl

$X^+ = K^+$, or $(CH_3)_4N^+$, which Ettlinger and Lundeen often found to be a superior cation for producing crystalline glucosides.

Figure 3. Synthesis of Glucotropaeolin

The only remaining structural detail is the stereochemistry around the oxime double bond. The likely mechanistic similarity of the implied rearrangement with that operating in the Hofmann-Curtius-Beckmann-Lossen types made it very likely that the sulphate residue and migrating R group were located anti to each other.

Very recently, an X-ray crystallographic analysis of sinigrin[17] has confirmed Ettlinger and Lundeen's structure for this mustard oil glucoside and it seems very reasonable to believe that the other mustard oil glucosides will have analogous structures.

NATURALLY DERIVED ISOTHIOCYANATES

Mustard oil glucosides are found in several plant families, but are particularly noticeable in plants of the family Cruciferae. Geographically, the glucoside-producing plants show no particular regional preference, being found in both the Old World and the New, the temperate zones and the tropics.

Considerable scanning of the botanical field for isothiocyanate-producing glucosides has been carried out by Kjaer.[18] Over 200 species of the family Cruciferae have been investigated, and hardly any have been devoid of thioglucosides. The family Capparidaceae is an equally constant source of thioglucosides, and the limited number of species of Resedaceae that have been studied all contained thioglucosides of the same general type. In addition to these three families, a number of sporadic appearances in species of other families have been noticed.

Taxonomically, the Capparidaceae, Cruciferae, Moringaceae and Resedaceae are usually grouped together with Papaveraceae in the order Rhoeadales.[19][20] However, assay of several Papaveraceae genera has failed to produce a single mustard oil glucoside-containing species,[21] leading to the conclusion that the Papaveraceae may be of different ancestry to the other four families and that they should

therefore be removed from the Rhoeadales. The linkage between Papaveraceae and the other four families has also been repeatedly questioned on classical taxonomic grounds. This evidence has recently been reviewed.[22] It has been suggested that the Papaveraceae may be derived directly from the Ranales.[23]

Some forty isothiocyanate-producing glucosides are now well authenticated. The side-chains on these comprise a wide range of structural types, both aliphatic, aromatic and heterocyclic. These are shown in Table I.

TABLE I

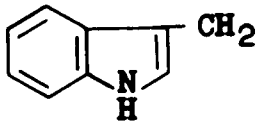
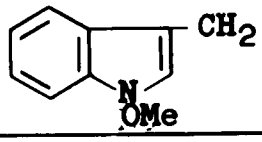
ISOTHIOCYANATE GLUCOSIDES WITH ESTABLISHED SIDE-CHAINS

Parent Glucoside	R of the Derived Isothiocyanate R-NCS	Reference
Glucocapparin	CH_3	24, 25
Glucolepidiin	CH_3CH_2	26
Glucoputranjivin	$CH_3CH(CH_3)$	27, 28
Glucococchlearin	$CH_3CH_2CH(CH_3)$	29, 30
Glucojiaputin	$CH_3CH_2CH(CH_3)CH_2$	31
Sinigrin	$CH_2 = CHCH_2$	11
Gluconapin	$CH_2 = CHCH_2CH_2$	32, 33
Glucobrassicanapin	$CH_2 = CHCH_2CH_2CH_2$	34

TABLE I (CONTINUED)

Parent Glucoside	R of the Derived Isothiocyanate R-NCS	Reference
Glucoiberverin	$CH_3S(CH_2)_3$	35
Glucoiberin	$CH_3SO(CH_2)_3$	36, 37
Glucocheirolin	$CH_3SO_2(CH_2)_3$	38
Glucoerucin	$CH_3S(CH_2)_4$	39
Glucoraphenin	$CH_3SOCH = CHCH_2CH_2$	40
Glucoraphanin	$CH_3SO(CH_2)_4$	41
Glucoerysolin	$CH_3SO_2(CH_2)_4$	42
Glucoberteroin	$CH_3S(CH_2)_5$	43
Glucoalyssin	$CH_3SO(CH_2)_5$	36, 37
Glucohesperin	$CH_3SO(CH_2)_6$	44
Glucohirsutin	$CH_3SO(CH_2)_8$	45
Glucoarabin	$CH_3SO(CH_2)_9$	46
Glucocamelinin	$CH_3SO(CH_2)_{10}$	47
Glucotropaeolin	$C_6H_5CH_2$	48, 49
Sinalbin	(p)-$HOC_6H_4CH_2$	50, 51
Glucoaubrietin	(p)-$CH_3OC_6H_4CH_2$	52
Glucolepigramin	(m)-$HOC_6H_4CH_2$	53
Glucolimnanthin	(m)-$CH_3OC_6H_4CH_2$	54
Gluconasturtiin	$C_6H_5CH_2CH_2$	49, 55
Glucoerypestrin	$CH_3OOC(CH_2)_3$	56
Glucomalcolmiin	$C_6H_5COO(CH_2)_3$	57, 58

TABLE I (CONTINUED)

Parent Glucoside	R of the Derived Isothiocyanate R-NCS	Reference
Glucoconringiin	$(CH_3)_2C(OH)CH_2$*	59, 60, 61
Progoitrin (Glucorapiferin)	$CH_2 = CHCHOHCH_2$*	60, 62
Glucobarbarin	$C_6H_5CHOHCH_2$*	63, 64
Glucosisymbrin	$HOCH_2CH(CH_3)$*	65
Glucobenzosisymbrin	$C_6H_5COOCH_2CH(CH_3)$	66
Glucosisaustricin	$HOCH_2CH(CH_2CH_3)$*	67
Glucobenzosisaustricin	$C_6H_5COOCH_2CH(CH_2CH_3)$	68
Glucocleomin	$CH_3CH_2C(CH_3)(OH)CH_2$*	31, 69, 70
Glucocapangulin	$CH_3CH_2CH_2CO(CH_2)_3$	71
Glucocappasalin	$CH_3CH_2CH_2CO(CH_2)_4$	72
Gluconorcappasalin	$CH_3CH_2CO(CH_2)_4$	73
Glucobrassicin		74
Neoglucobrassicin		75

*The isothiocyanate produced undergoes spontaneous ring closure to an oxazolidinethione (XI):

XI

The isothiocyanate side-chains can be divided into several prominent groups on a structural basis. These groups will be briefly discussed.

Aliphatic Side-Chains

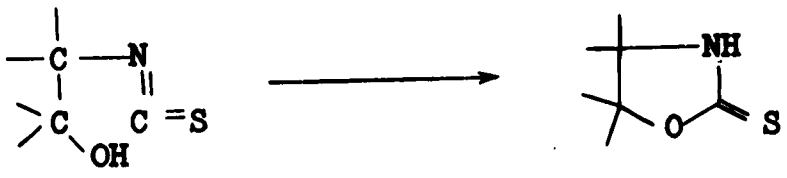
The simplest side-chain, that is a methyl group, has been repeatedly encountered in the thioglucoside glucocapparin,[25] an almost ubiquitous constituent of the Old World species of the family Capparidaceae, but has yet to be found with certainty in any cruciferous species. The ethyl side-chain, whose synthesis is described in this thesis, has been found only in a species of the genus Lepidium.[26] In contrast, the glucoside possessing an isopropyl grouping (gluco-putranjivin) is rather widely distributed, and often accompanied by the sec-butyl compound. A possible explanation for this will be presented later (page 22).

Three unsaturated aliphatic side-chains are known. The classical sinigrin with its allyl side-chain has recently been supplemented by two higher homologues, the 3-butenyl[32][33] and 4-pentenyl[34] glucosides, both occurring in the seeds of rape. It is noteworthy that sinigrin has not been found with its higher homologues in rape seed.

An interesting series of closely related glucosides has been established as a constituent of cruciferous species. These side-chains have a chain of methylenes terminated by a

thiomethyl group, usually with the sulphur in each of its three possible oxidation states, viz. the thioether, sulphoxide and sulphone. Glucosides with side-chains containing 3, 4, 5, 6, 8, 9 and 10 methylenes have been characterized. There is no apparent reason why the missing homologue with seven methylenes has not been located, and it is likely that it will eventually be discovered as a species of natural derivation. The series of sulphoxides all contain an asymmetric sulphur atom, and the plain, negative rotatory dispersion curves of the isothiocyanates and of several derivatives indicate that they all belong to the same stereochemical series.(76) This series of strictly homologous natural products poses a biogenetic problem of considerable interest.

Thioglucosides are also known with hydroxy groups in the side-chains, and it may be foreseen that when these occur in the β- or γ-positions spontaneous cyclization will occur on enzymatic hydrolysis, to five or six-numbered rings, as in fact does happen:



Extensive studies have been made of the absolute configurations of these hydroxylated compounds,(70) some of which also occur as the benzoate esters.

There are several other types of aliphatic side-chains containing ester and keto groups.

Aromatic Side-Chains

This group contains more of the classical mustard oil side-chains, for example sinalbin, glucotropaeolin and gluconasturtiin. The pair of *meta* derivatives (glucolepigramin and glucolimnanthin) is worthy of study in view of the comparatively rare occurrence of *meta* compounds in nature. The biosynthesis of glucotropaeolin will be further discussed in the body of this thesis. A somewhat related thioglucoside is glucobrassicin, first isolated from cabbage leaves,[74] and apart from neoglucobrassicin, its indole-N-methoxy derivative, the only thioglucoside yet known which contains a heterocyclic side-chain. On enzymatic hydrolysis, this compound follows either of two unorthodox alternative pathways, depending on pH. At pH values close to neutrality, the expected skatyl isothiocyanate is probably produced initially, but it immediately suffers further reactions, eventually producing di-indolyl-methane as the stable end product, together with the thiocyanate ion. In lower pH ranges, the enzymatic fission leads to indolylacetonitrile, a compound isolated several years ago from Brussels sprouts[77] and shown to have strong growth-promoting properties. From a biosynthetic viewpoint, it will be interesting to see whether other heterocyclic side-chains, particularly the one derived

from histidine, will be found.

The biogenetic implications of the side-chain patterns will be considered later in Part 3.

BIOLOGICAL PROPERTIES OF ISOTHIOCYANATES AND THEIR PARENT GLUCOSIDES

Several types of biological activity have been ascribed to mustard oils or their conversion products. An extensive literature exists on their antibacterial and fungiostatic properties (e.g.[78] which contains the results of a bacteriostatic survey of a large series of isothiocyanates).

Isothiocyanates originating from weeds have also been held as factors responsible for a "scorched" or "burnt" flavour of dairy products. The goitrogenic effect of glucobrassicin and of several oxazolidinethiones of plant origin has led to a series of studies to assess the possible importance of such compounds in animal feeding and in endemic goitre.[188][189] Certain other toxic effects observed occasionally in animals after feeding materials that contained excessive amounts of isothiocyanates have also called for much attention.

An interesting biological effect that has been attributed to the genuine glucosides rather than the free mustard oils is that of stimulating the feeding of certain insects. According to Thorsteinson,[79] two oligophagous insects, Plutella maculipennis (Curt) and Pieris brassicae (L), could be induced to feed on leaves which they ordinarily refuse by painting the leaves with sinigrin or

sinalbin solutions, but not with allyl isothiocyanate. The importance of such observations is obvious, considering the immense world-wide damages caused by host-specific insects to crops of important Cruciferae such as cabbage, turnip and rape. Curiously, β-phenylethyl isothiocyanate, produced from a glucoside present in watercress and turnip roots, among other plants, has been demonstrated to rank among the most potent insecticides.(80)

These examples characterize the isothiocyanate glucosides and their enzymatic fission products as natural products of considerable interest.

SECTION 2

OBJECTIVES

Three distinct, but biosynthetically related objectives form the basis of the research described in this thesis. These are:

1. An investigation of the biosynthesis of mustard oil glucosides.
2. The synthesis of some sulphur-containing α-aminoacids.
3. The synthesis of some mustard oil glucosides.

Each of these objectives is discussed in detail in the sections which follow.

SECTION 3

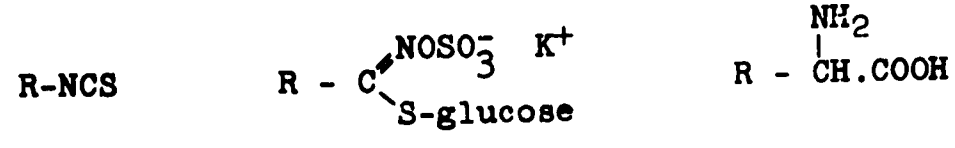
THE BIOSYNTHESIS OF MUSTARD OIL GLUCOSIDES

BACKGROUND

In connection with taxonomic studies of the distribution of isothiocyanates and their glucosidic progenitors in individual botanical species, the biogenesis of mustard oil glucosides is a challenging problem.

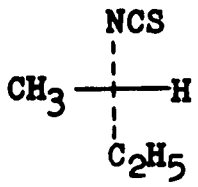
At the time the investigations described in this thesis were commenced, there were no reports of systematic experimental investigations of mustard oil glucoside biosynthesis. However, during the course of this work, several papers have appeared which contain the work of other groups on this problem, much of which parallels the work reported here. This activity reflects the interesting nature and importance of the problem.

In the course of his studies of naturally occurring mustard oils, Kjaer noted[81] that in several cases there was a striking similarity between the side-chains of the oils (XII) and therefore of the glucosidic precursors (XIII), and those of some commonly occurring α-aminoacids (XIV). Thus the series methyl, isopropyl and (+)-sec-

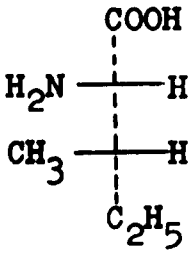


XII XIII XIV

butyl isothiocyanates, arising from the decomposition of glucocapparin, glucoputranjivin and glucocochlearin respectively, could be biogenetically derived from alanine, valine and isoleucine. In support of this, Kjaer demonstrated[30] that the absolute configuration of the naturally derived dextrorotatory sec-butyl isothiocyanate (XV) was the same as that about C-3 of the L-isoleucine (XVI).



XV



XVI

Perhaps very significantly, the glucosidic progenitor of ethyl isothiocyanate (glucolepidiin) occurs only rarely in nature[26] as does the corresponding α-aminoacid butyrine[82] (XIV, R = CH_3CH_2). Inspection of Table I (page 11) reveals further examples of isothiocyanates corresponding to naturally occurring α-aminoacids, although in many other cases the "corresponding" α-aminoacids are not known to occur naturally.

It appeared very reasonable to assume that many, if not all, of the mustard oil glucosides either share a

common biosynthetic pathway with α-aminoacids (e.g. both might be derived from the α-ketoacid), or else are derived directly from the corresponding α-aminoacids. The implications of this last possibility will be considered in more detail in the next section (see page 26).

DISCUSSION AND RESULTS

We elected to study the mechanism of the biogenesis of glucotropaeolin (XIII, R = $C_6H_5CH_2$) in Tropaeolum majus L. (nasturtium) plants. Also very briefly investigated was the biosynthesis of glucoputranjivin (XIII, R = $(CH_3)_2CH$) in Tropaeolum peregrinium (canary bird vine). Although the genus Tropaeolum does not belong to the family Cruciferae* which contains most of the mustard oil producing plants, Tropaeolum majus was convenient experimentally, as the plants are hardy, easy to grow and contain relatively large amounts of the mustard oil glucoside.

Isotopic "tracer" evidence was already at hand demonstrating that phenylalanine was incorporated, with loss of the carbon atom of the carboxyl group, into glucotropaeolin.(83)(84) However the alternative remained that some precursor of phenylalanine might have been a more direct precursor of glucotropaeolin, rather than phenylalanine itself, i.e. the glucotropaeolin might arise from phenylalanine by either of the alternative pathways 1 and 2 indicated in Figure 4.

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*Tropaeolum is the sole genus of the family Tropaeolaceae and contains some 65 species native mainly to the mountain regions of Mexico and South America.

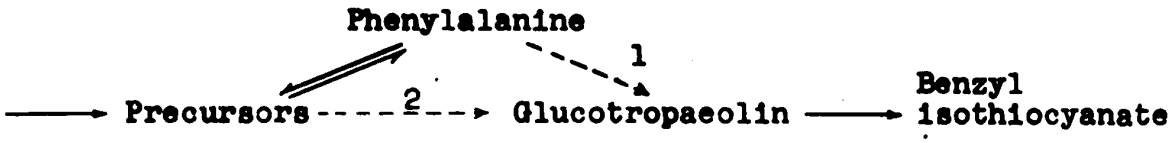

Figure 4. Possible Alternatives for the Biosynthesis of Glucotropaeolin

Gamborg and Neish demonstrated that phenylalanine (XX) is formed in plants by the same route as that established in bacteria.(85) Shikimic acid (XVII), derived from primary materials via glucose, is converted to prephenic acid (XVIII), which on decarboxylation gives phenylpyruvic acid (XIX), and hence, by transamination, phenylalanine.(86)(87) These transformations are outlined in Figure 5, together with the

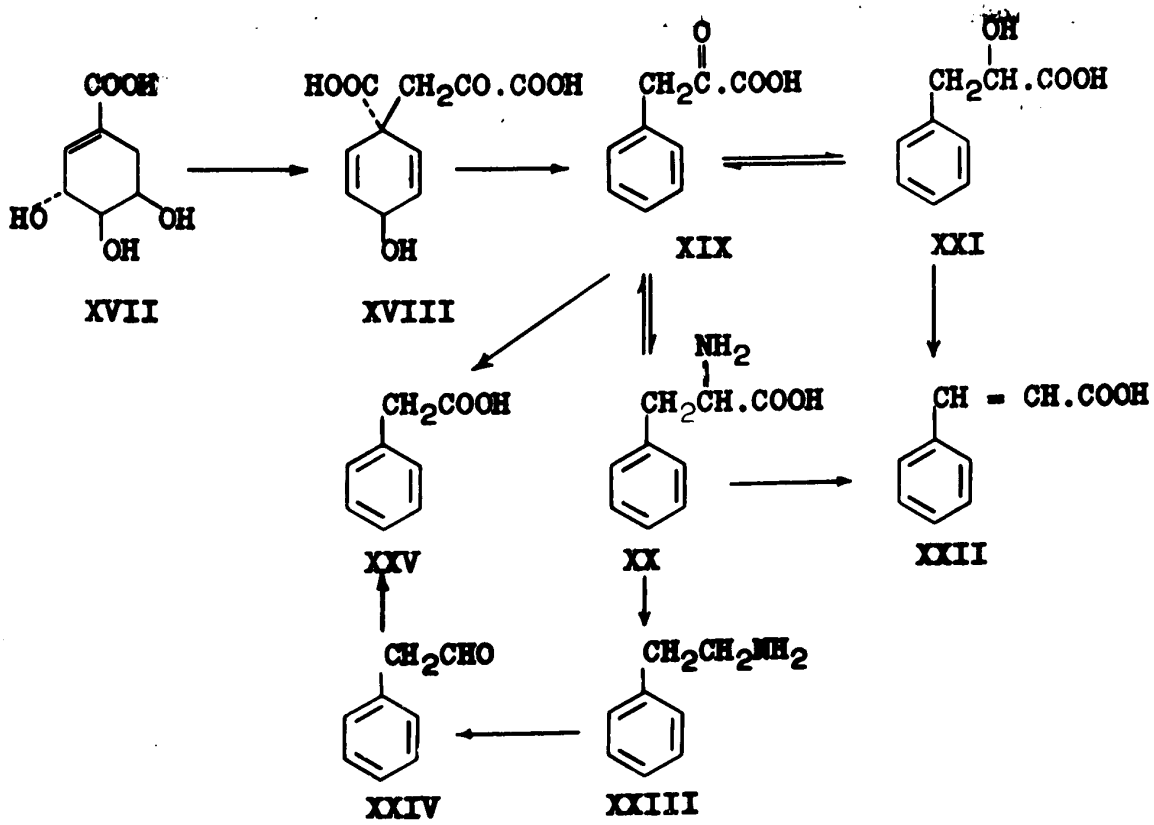

Figure 5. Metabolism of Phenylalanine

products which arise from phenylalanine by various metabolic processes. Thus deamination of phenylalanine by the enzyme deaminase gives rise to cinnamic acid (XXII),[88] a compound which may also arise by dehydration of phenyllactic acid (XXI).[85] Decarboxylation of phenylalanine gives β-phenylethylamine (XXIII),[89] which may undergo further transformation to phenylacetaldehyde (XXIV) and phenylacetic acid (XXV),[90] which is also obtainable by decarbonylation of phenylpyruvic acid.[91]

We thus have several possibilities for the biosynthesis of glucotropaeolin. Phenylalanine, phenylpyruvic acid and phenyllactic acid should form a pool and any of them should be readily converted to glucotropaeolin. Cinnamic acid should only be incorporated if it is specifically on the route from the pool. Whether or not phenylalanine itself is involved as a direct precursor can only really be tested by following the path of the nitrogen. These possibilities were tested by the administration of various carbon-14 and nitrogen-15 labelled compounds to the plants. The results are shown in Table II and Table III.

TABLE II

INCORPORATION OF CARBON-14 LABELLED PRECURSORS INTO GLUCOTROPAEOLIN

Compound administered			Benzylthiourea		% Incorp.
	Spec. Act. μc/mM	Act. μc	Specific Activity μc/mM	Activity μc	
Phenylalanine-3-^{14}C	40.5	31.4	3.23	0.75	2.4
Phenylpyruvic acid-2-^{14}C	31.7	16.4	1.81×10^{-2}	4.29×10^{-3}	2.6×10^{-2}
Cinnamic acid-2-^{14}C	144	55.9	2.90×10^{-3}	3.17×10^{-3}	5.67×10^{-3}
Phenylacetic acid-1-^{14}C	224	14.3	1.89×10^{-2}	3.65×10^{-3}	2.6×10^{-2}

Pathway 2 (Figure 4) was ruled out by the low incorporation of phenylpyruvic acid-2-^{14}C, which was synthesized from glycine-2-^{14}C by the route shown in Figure 6. This result was rather surprising, since the work

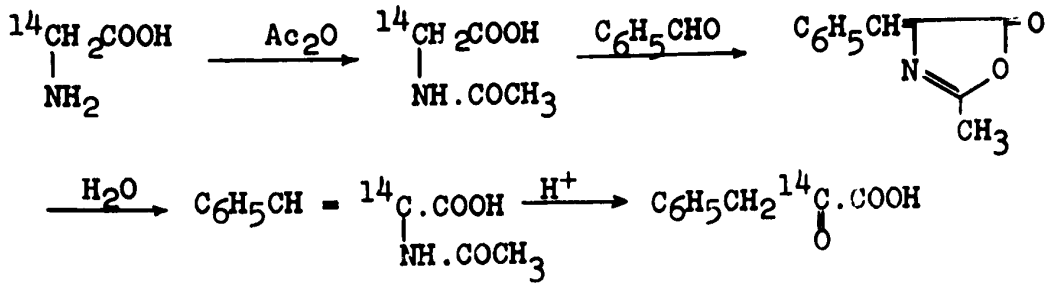

Figure 6. Synthesis of Phenylpyruvic Acid-2-^{14}C

of Gamborg and Neish[85] suggested that phenylpyruvic acid, phenylalanine and phenyllactic acid should all be readily interconvertible. Possibly the phenylpyruvic acid does not reach the site of biosynthesis before being destroyed, or is unable to become incorporated into some complex necessary for the conversion. Degradation of the radioactive benzylthiourea derived from phenylpyruvic acid did show that the activity appeared in the isothiocyanate carbon as predicted on the basis of conversion to phenylalanine.

Passing to known metabolic derivatives of phenylalanine (Figure 5), the incorporations of cinnamic acid and phenylacetic acid were measured. Cinnamic acid-2-^{14}C, prepared by the condensation of malonic acid-2-^{14}C with benzaldehyde (Knoevenagel reaction) as shown in Figure 7,

$$^{14}CH_2(COOH)_2 \xrightarrow[\text{Pyridine}]{C_6H_5CHO} C_6H_5.CH = {}^{14}C(COOH)_2$$

$$\longrightarrow C_6H_5.CH = {}^{14}CH.COOH$$

Figure 7. Synthesis of cinnamic acid-2-^{14}C

had the lowest incorporation of all the compounds fed, and

it is unlikely that this compound is on the biosynthetic route.* This result is in agreement with the results of Gamborg and Neish (discussed above) which demonstrated that cinnamic acid does not form part of the phenylpropanoid "pool". Underhill, Chisholm and Wetter have also found[84] that cinnamic acid is a very poor precursor of glucotropaeolin; however they also reported that phenyllactic acid was a reasonably efficient precursor of this glucoside, a result which would appear to require phenylpyruvic acid to be even better!

The decarboxylated derivatives of phenylalanine were also very poor precursors. Phenylacetic acid-1-^{14}C gave a very low incorporation, and Underhill and Chisholm[92] showed that β-phenylethylamine was no better.

It is therefore concluded that phenylalanine itself is converted to glucotropaeolin by a route involving intermediates other than those shown in Figure 5. The important question to be considered is whether there is retention or loss of the nitrogen atom. In order to

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*Experiments by Kindl indicate that p-coumaric acid (the hydroxylated derivative of cinnamic acid) is a better precursor of sinalbin than tyrosine, indicating a loss of nitrogen. This will be discussed later (page 34).

distinguish between these two possibilities, experiments were carried out with ^{15}N-labelled phenylalanine. This was prepared from ordinary DL-phenylalanine by replacing the amino group by bromine, followed by a Gabriel reaction with potassium phthalimide-^{15}N (see Figure 8). The phenylalanine-

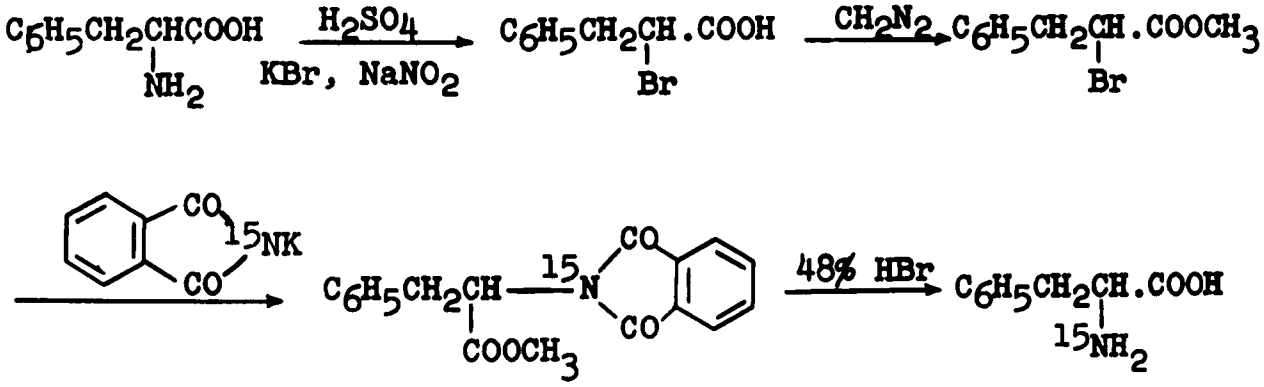


Figure 8. Synthesis of Phenylalanine-^{15}N

^{15}N was fed together with phenylalanine-3-^{14}C, and the results are shown in Table III. The relative dilutions of the ^{14}C and ^{15}N were used as a measure of the incorporation of the nitrogen-α-carbon as an intact unit. The atoms per cent nitrogen-15 content obtained from the benzylthiourea was corrected for the nitrogen atom derived from the ammonia.

It can be seen that the nitrogen-15 suffered a dilution roughly twice that of the carbon-14. We

TABLE III

INCORPORATION OF DL-PHENYLALANINE-^{14}C-^{15}N

INTO GLUCOTROPAEOLIN

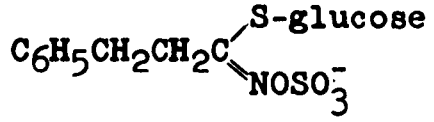
DL-Phenylalanine-3-^{14}C-^{15}N		Benzylthiourea		^{14}C	^{15}N
Specific activity $\mu c/mM$	Atoms % excess ^{15}N	Specific activity $\mu c/mM$	Atoms % excess ^{15}N	diln.	diln.
74.8	95.7	0.418	0.212	179	451
38.2	95.7	0.180	0.260	212	370

interpreted this as indicating that the biogenesis proceeds through the aminoacid (since much larger dilution would be anticipated if a reversion of phenylalanine to the phenylpropanoid pool was necessary), but that some loss of nitrogen occurs. This seems not unreasonable as transamination reactions are believed to be readily reversible. In fact, Gamborg and Wetter [93] have isolated a transaminase enzyme from plants and measured the kinetics of the conversion of phenylalanine to phenylpyruvic acid. By this mechanism D-phenylalanine can be converted to L-phenylalanine, since a transamination reaction was also observed with the D-isomer, albeit slower than the L-isomer. The L-phenylalanine then synthesized from the phenylpyruvic acid would still contain the

carbon-14, but would be much lower in nitrogen-15 so contributing to the dilution of nitrogen-15.

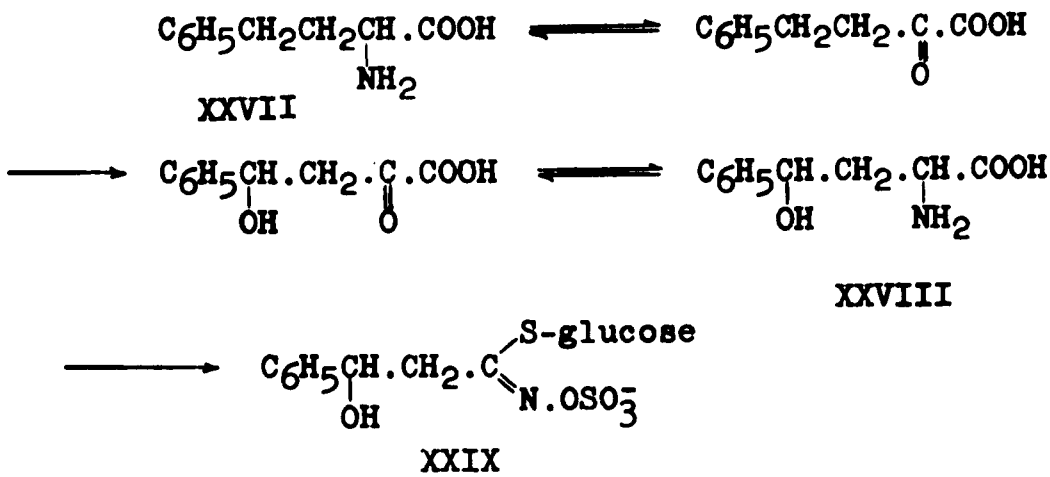
An identical experiment was carried out by Underhill and Chisholm[92] who however reported approximately identical dilutions for the carbon-14 and nitrogen-15 thus firmly establishing that the carbon and nitrogen of the glucoside are derived as a unit from phenylalanine and that phenylalanine itself is a direct precursor. We can only speculate on the reason why our results differ numerically from those obtained by these workers. One possible cause is a difference in feeding methods. We allowed the cut plants to stand in a relatively dilute solution of the tracer for 24 hours allowing a relatively slow uptake, whereas in general (although it is not specified in this particular paper) the other workers fed a concentrated solution initially, followed by water when the solution had been absorbed.[84][94] Thus if transamination is occurring, but the amount of enzyme is limited (Gamborg and Wetter suggest that this is often the case, and also that the amount is variable, depending on the stage of growth of the plants), then the rapid administration of the phenylalanine could "saturate" the transaminase enzyme, permitting less direction of nitrogen into the nitrogen "pool" and allowing more unchanged phenylalanine to partake in mustard oil glucoside synthesis.

Underhill has also demonstrated that gluconasturtiin (XXVI) is biosynthesized from L-γ-phenylbutyrine (XXVII) with



XXVI

retention of the α-aminoacid nitrogen atom.[94] However in the case of glucobarbarin (XXXIX),[95] for which γ-phenylbutyrine is also an efficient precursor, there is extensive loss of nitrogen. This is not entirely unexpected, since several steps are probably necessary for introduction of the hydroxyl group. Underhill has postulated the pathway shown in Figure 9,[95] and it is possible that administration of



XXVII

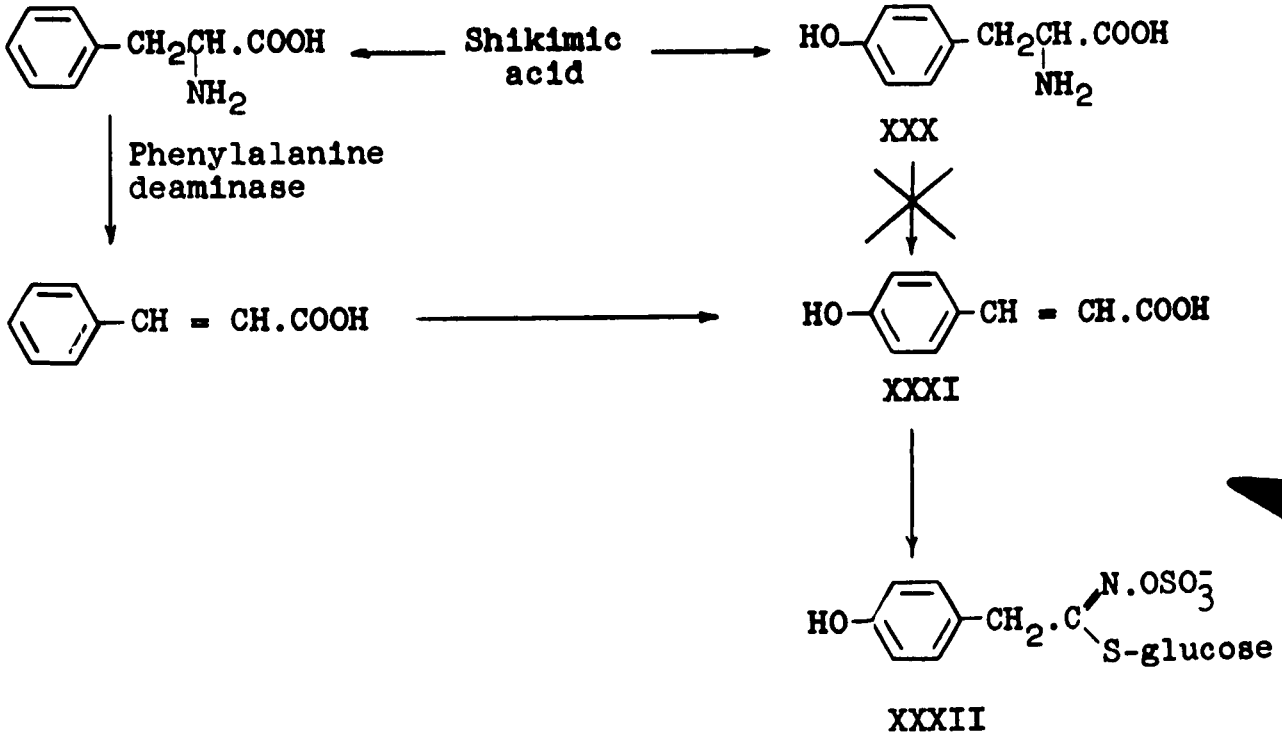
XXVIII

XXIX

Figure 9. Biosynthesis of Glucobarbarin as Postulated by Underhill

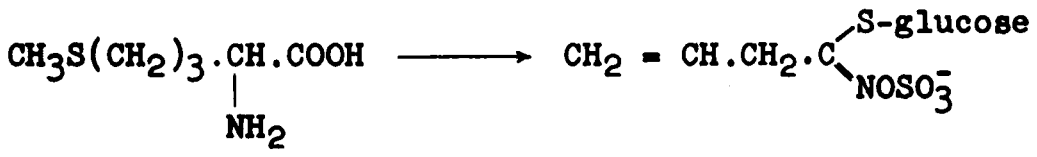
L-γ-hydroxy-γ-phenylbutyrine (XXVIII) will result in incorporation with retention of nitrogen. An alternative possibility, since the relative dilution of nitrogen is quite small, is that the nitrogen atom is being lost by reversible transamination as discussed on page 31.

Another apparently anomalous case is the biosynthesis of sinalbin (XXXII) in Sinapis alba L.[96] Here p-coumaric acid (XXXI) was found to be a better precursor than phenylalanine, which itself was better than tyrosine (XXX). Kindl suggested the following scheme for the biosynthesis of sinalbin:

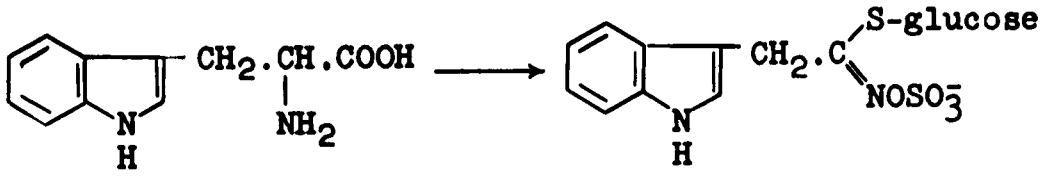


This scheme, in contrast to the ones previously presented, involves loss of nitrogen from the aminoacid. This may be a unique pathway caused by the deficiency of the enzyme or enzymes necessary to convert tyrosine directly to sinalbin.

Evidence for the involvement of aminoacids has also been presented for other systems. Thus sinigrin (XXXIV) is derived from homomethionine (XXXIII)[97] and glucobrassicin (XXXVI) from tryptophan (XXXV).[98]

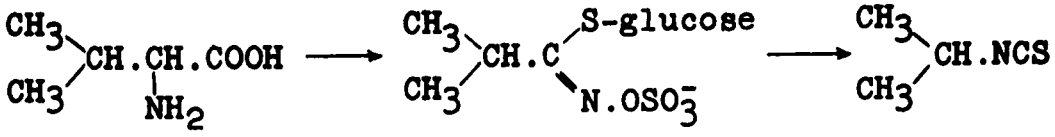


XXXIII XXXIV



XXXV XXXVI

In addition we have carried out a single preliminary experiment which indicated that valine (XXXVII) is almost certainly a precursor of glucoputranjivin (XXXVIII) in Tropaeolum peregrinum. The amount of isopropyl



XXXVII XXXVIII

isothiocyanate isolated after feeding Tropaeolum peregrinum plants DL-valine-4-^{14}C was too small to permit separation of the thiourea derivative from that of 2-methylbutyl

isothiocyanate with which it occurs. However, paper chromatography revealed that a spot identical with isopropylthiourea contained the bulk of the radioactivity.

If the α-carbon and the nitrogen of the phenylalanine are incorporated into the mustard oil glucoside as a unit, this also means that any intermediates must also be nitrogeneous. In the conversion of phenylalanine to glucotropaeolin, it is necessary for the nitrogen to be oxidized from its formal -3 state to a -1 state, a reversal of the situation believed to be operative in the formation of the aminoacid amino group from inorganic nitrate.(99) This oxidation is not unknown; the oxidation of ammonia to hydroxylamine has been demonstrated in Azotobacter chroococcum.(100) The α-oximinoacid (XL) thus appeared to be a possible intermediate on the route to glucotropaeolin. Yamafuji et al.(101) have demonstrated the natural occurrence of α-oximinopropanoic acid in silkworms, and an enzyme system capable of converting this to alanine. The reverse of this reaction would enable phenylalanine to be converted to α-oximino-β-phenylpropanoic acid. In addition, α-hydroxyaminoacids (XXXIX) have been shown to disproportionate, in vitro, into α-oximinoacids (XL) and α-aminoacids,(102) and so α-oximino-β-phenylpropanoic acid might arise by the pathway shown in Figure 10.

XXXIX XL

Figure 10. Disproportionation of α-hydroxyaminoacids

The test of this hypothesis involved feeding Tropaeolum majus L. plants with labelled α-oximino-β-phenylpropanoic acid, synthesized by the condensation of hydroxylamine with phenylpyruvic acid. The results are shown in Table IV.

TABLE IV

INCORPORATION OF α-OXIMINO-β-PHENYLPROPANOIC ACID-2-^{14}C INTO GLUCOTROPAEOLIN

Expt.	α-oximino-β-phenyl propanoic acid		Benzylthiourea		^{14}C incorp.	^{14}C diln.	^{15}N diln.
	^{14}C Spec. act. μc/mM	Atoms % excess ^{15}N	^{14}C Spec. act. μc/mM	Atoms % excess ^{15}N			
1	92.3	-	3.81×10^{-2}	-	0.21	2415	-
2	-	98	-	0.050	-	-	1960

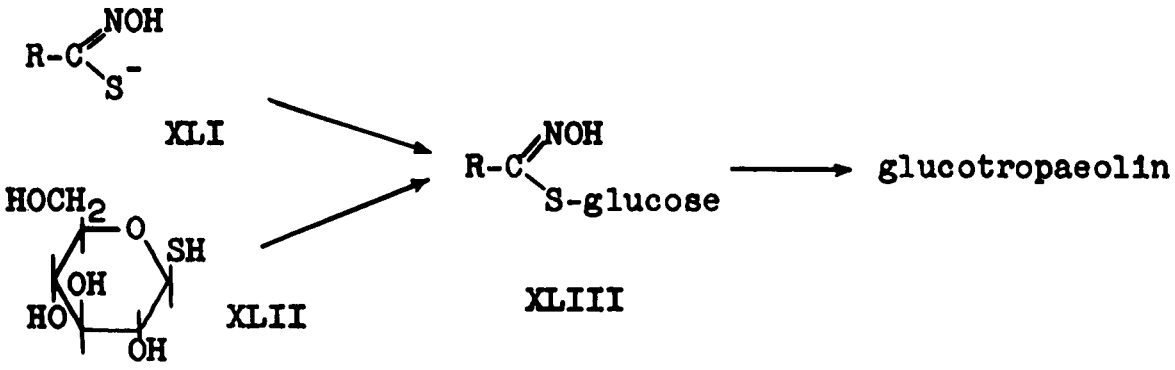
Comparison of the incorporation of α-oximino-β-phenylpropanoic acid-2-^{14}C with the values obtained for other compounds (Table II), shows that, with the exception of phenylalanine, this is the highest incorporation yet obtained. However, it is still considerably lower than would be expected if α-oximino-β-phenylpropanoic came after phenylalanine in the metabolic pathway. Whilst the carbon-14 and nitrogen-15 results are from separate experiments and therefore not really comparable, the dilutions are of the same order of magnitude in agreement with the general hypothesis. This should be checked with a simultaneous double-labelled experiment.

We feel that the low incorporation of α-oximino-β-phenylpropanoic acid should be viewed with some caution, and should not be held as a basis for rejection of the hypothesis that this is in fact closer to glucotropaeolin than phenylalanine without further evidence. Underhill and Chisholm[92] have since reported a similar low incorporation and suggested that a conformation effect may be involved. The configuration around the C = N in mustard oil glucosides has been shown[17] to be anti (referring to the side-chain, in this case benzyl, and the sulphate), whereas α-oximinoacids normally have these groups syn.[103] Thus the low incorporation may have been due to the use of the incorrect geometric isomer.

Another possibility is the involvement of a multi-enzyme complex, as postulated for fatty acid biosynthesis by Lynen and Tada.[104] Here the substrate remains bound to the enzyme system throughout a whole series of reactions (condensation, reduction, dehydration and reduction). In this way, the chain length of a fatty acid is lengthened by two carbon atoms without any of the intermediates having an independent existence. In such a situation, administration of one of the intermediates might well not give efficient incorporation.

An alternative system which has retention of the C-N system, that is phenylacetonitrile, is rejected on the basis of work by Underhill and Chisholm[92] who found it to be a very inefficient precursor.

The introduction of the thioglucoside moiety into the glucotropaeolin structure is an intriguing problem. We considered two alternatives: either the thiohydroximate aglucone (XLI) is a precursor and is glucosylated (the formal reverse of the thioglucosidase cleavage of the mustard oil glucosides) or thioglucose (XLII) is introduced as a unit.



Of these alternatives, the second appeared more likely in view of the instability of thiohydroxamic acids (see page 60). These possibilities were investigated by administration of sulphur-35 labelled phenylacetothiohydroxamate and thioglucoside, and also carbon-14 labelled thiohydroxamate to Tropaeolum majus L. These compounds were synthesized by the routes shown in Figure 11 and Figure 12.

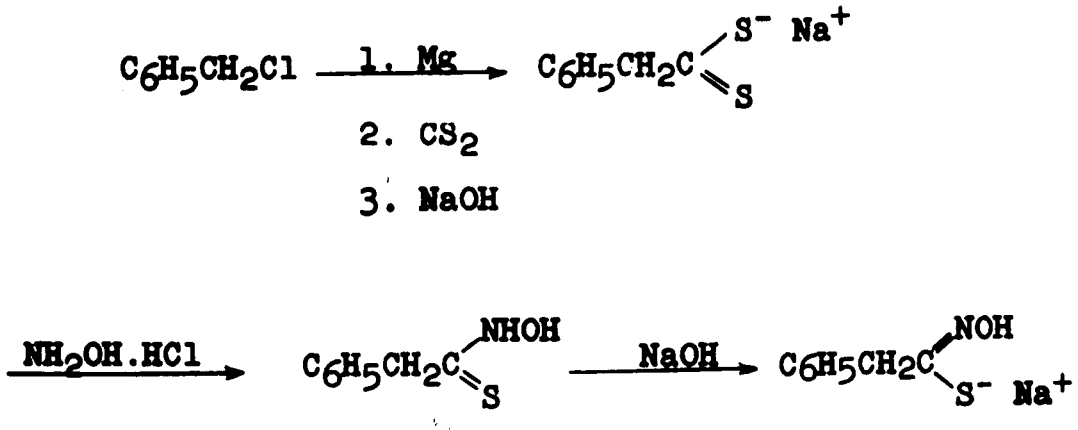


Figure 11. Synthesis of Sodium Phenylacetothiohydroxamate (^{35}S and ^{14}C-labelled)

($C_6H_5{}^{14}CH_2Cl$ and $C^{35}S_2$ were used as sources of the isotopic materials).

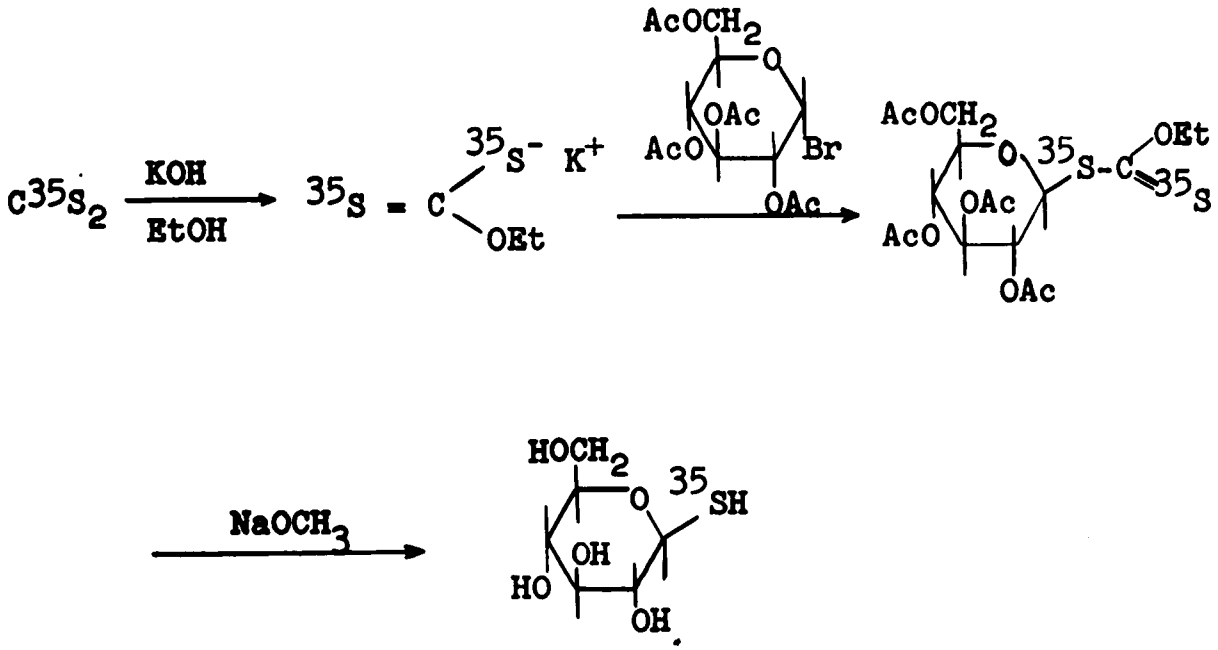


Figure 12. Synthesis of sodium-β-D-1-glucopyranosyl mercaptide-^{35}S (sodium thioglucoside)

The results of feeding (Table V) show that both of these compounds appear to be highly efficient precursors of glucotropaeolin. The apparent incorporation of the phenylacetothiohydroxamate-^{35}S into glucotropaeolin was unexpected. Our first supposition was that the thiohydroxamic acid had decomposed to simpler compounds which were then incorporated by the path Wetter had recently demonstrated for sinigrin.[105] However, the carbon-14 labelled thiohydroxamate was also incorporated. We now consider that the most likely explanation is that

TABLE V

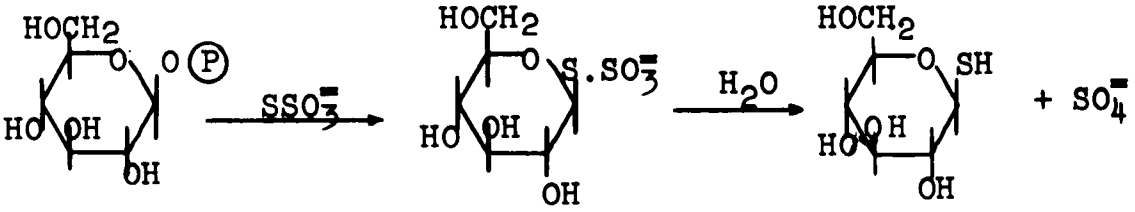
INCORPORATION OF SODIUM THIOGLUCOSIDE AND SODIUM PHENYLACETOTHIOHYDROXAMATE INTO GLUCOTROPAEOLIN

Expt.	Compound Administered	Spec. act. μc/mM	Wt. g	Benzyl-thiourea		% Incorp.	Diln. Ratio
				Spec. act. μc/mM	Wt. g		
1	Phenylaceto-thiohydroxamic acid-1-^{14}C	9.3	0.133	1.53	0.028	4.03	6.1
2	Phenylaceto-thiohydroxamic acid-^{35}S	18.8	0.096	6.21	0.012	4.75	3.0
3	Sodium thioglucoside-^{35}S	19.1	0.287	9.05	0.062	12.9	2.10

phenylacetothiohydroxamic acid decomposes to benzyl isothiocyanate which is still present at the time of extraction (or that this decomposition occurs during work-up). In other words, the sulphur-35 does not get into the glucoside. This could and should be tested by isolating glucotropaeolin from plants fed ^{35}S- or ^{14}C- labelled phenylacetothiohydroxamate.

Thioglucose might well arise from cysteine and glucose by trans-thiolation. An alternative possibility would be the formation of a Bunte salt (XLIV) from say glucosyl-1-phosphate or pyrophosphate and thiosulphate (which has been

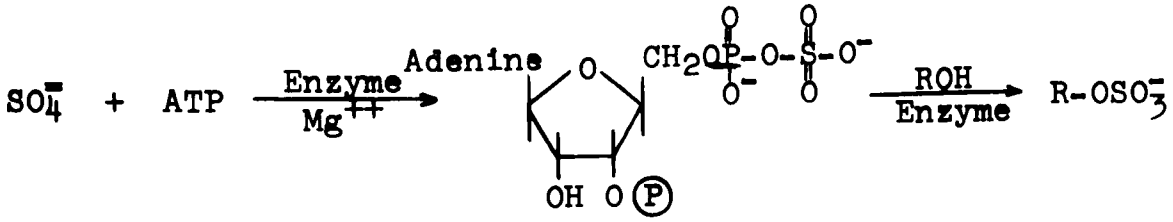
shown to be a decomposition product, *in vivo*, of cysteine[106], followed by hydrolysis:



XLIV

Wetter[105] has fed both "outer"- and "inner"-labelled sulphur-35 thiosulphate to *Armoracia lapathifolia* plants. In both cases, the bulk of the activity appeared in the sulphate portion, but of that appearing in the isothiocyanate sulphur, the "outer" sulphur atom of the thiosulphate is a significantly better contributor.

We consider that the final step in the biosynthesis of glucotropaeolin is probably the sulphonation of XLIII. The sulphonating agent is likely to be the "active sulphate" demonstrated for other systems:[151]



However, conceivably, sulphonation may occur earlier and perhaps lead to an intermediate of the type $R\text{-}C\equiv\overset{+}{N}\text{-}CSO_3^-$ (cf. page 45).

CONCLUSIONS

We believe that glucotropaeolin is biosynthesized directly from phenylalanine, rather than a precursor, with retention of its nitrogen atom. The sequence of reactions is probably close to that given in Figure 13.

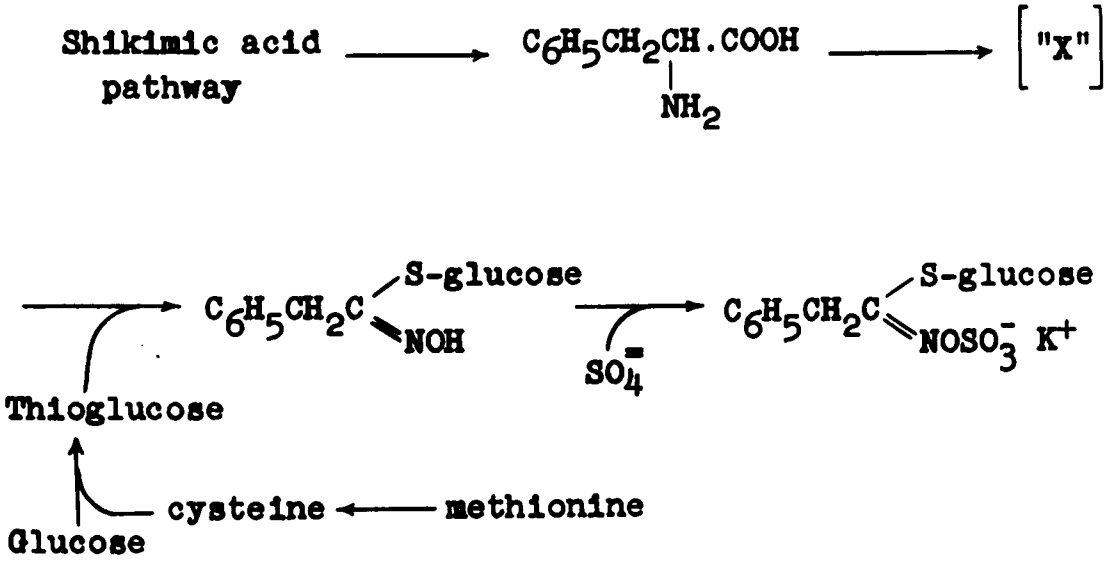
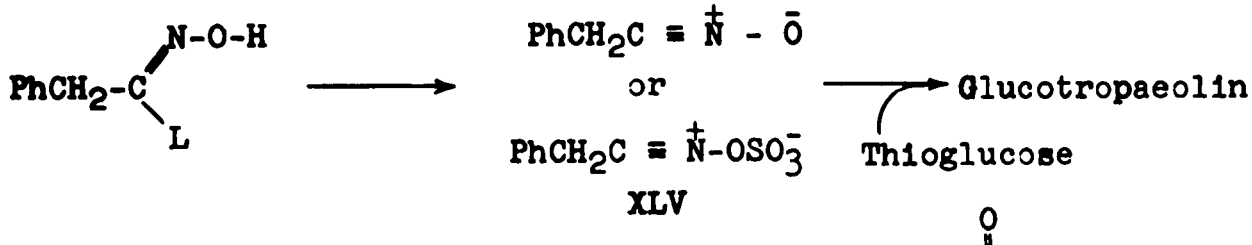


Figure 13. Probable Steps in Biosynthesis of Glucotropaeolin

On the basis of the high efficiency with which phenylalanine is converted to glucotropaeolin, the unidentified steps "X" in this pathway must be few in number and it is interesting to speculate on the possibilities. Benn originally considered that a nitrile oxide intermediate might be involved, i.e. (XLV), perhaps formed by a process similar to that indicated in Figure 14.

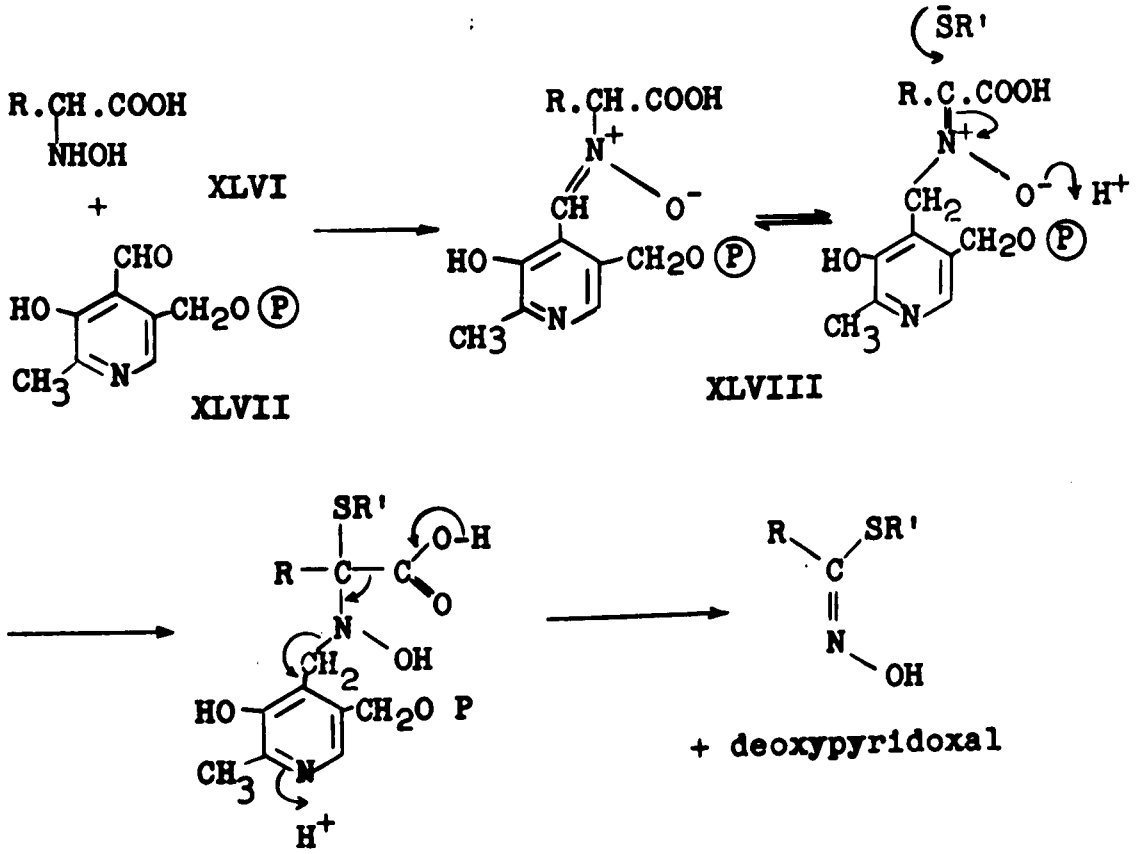


L = some leaving group (e.g. pyrophosphate or -C(=O).S.CoA)

Figure 14. Possible Conversion of Phenylpyruvic Acid Oxime to Glucotropaeolin

This induced him to attempt an *in vitro* synthesis of mustard oil glucosides patterned on this approach, as described elsewhere (page 61).

Another interesting intermediate would be the nitrone (XLVIII), derived from the N-hydroxyaminoacid (XLVI) and pyridoxal phosphate (XLVII):



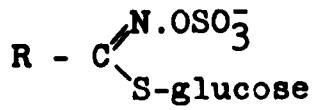
Nitrones are known[106] to undergo 1:3-additions with alcohols and many other compounds. This approach is being investigated further.

SECTION 4

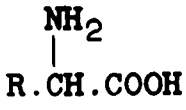
ω-THIOMETHYL-α-AMINOACIDS AND DERIVATIVES

INTRODUCTION

If the mustard oil glucosides (IL) are universally biosynthesized from α-aminoacids (L)*, a number of



IL



L

hitherto undescribed α-aminoacids must exist. Thus in Berteroa incana DC, a crucifer which has been shown to contain the mustard oil glucoside glucoberteroin (IL, R = $CH_3S(CH_2)_5$-),[43] one would predict the existence of the α-aminoacid (L, R = $CH_3S(CH_2)_5$-). However it is also quite possible that the concentration of such aminoacids might be extremely low, thus making their detection and isolation a matter of some difficulty. Accordingly it was decided to synthesize some of these α-aminoacids in order to have information about their chemical and physical properties (and in particular their chromatographic behaviour). It was also considered worthwhile to establish syntheses suitable for the preparation of ^{14}C-labelled α-aminoacids which could then be fed to plants in order to

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*cf. the discussion in Part 3, page 21.

check their role as biosynthetic precursors.

An obvious choice for study was one of the ω-thiomethyl-alkyl aminoacids. Since a homologous series of such glucosides has been isolated, the whole corresponding series of aminoacids (LI) would be of interest, and a

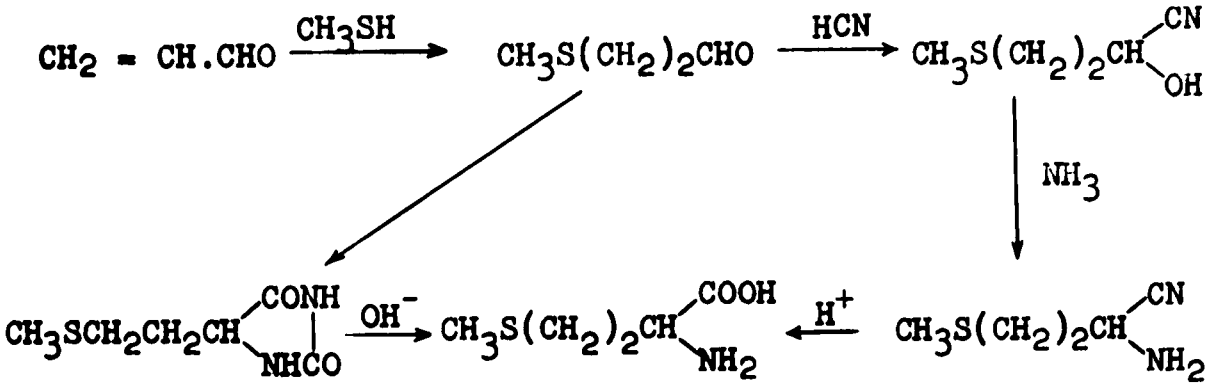
$$CH_3S(CH_2)_nCH(NH_2).COOH$$

LI

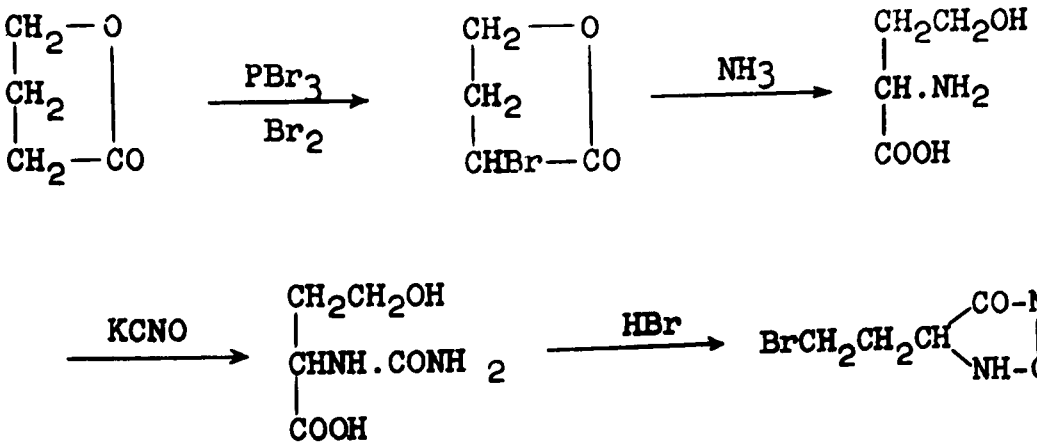
general synthetic route, applicable to any chain length, is desirable.

The particular acid synthesized was the one containing a chain of five methylene groups, that is 2-amino-7-thiomethylheptanoic acid, together with its oxidized derivatives, the sulphoxide and sulphone. These are the aminoacids corresponding to glucoberteroin, glucoalyssin and an as yet unknown mustard oil glucoside.

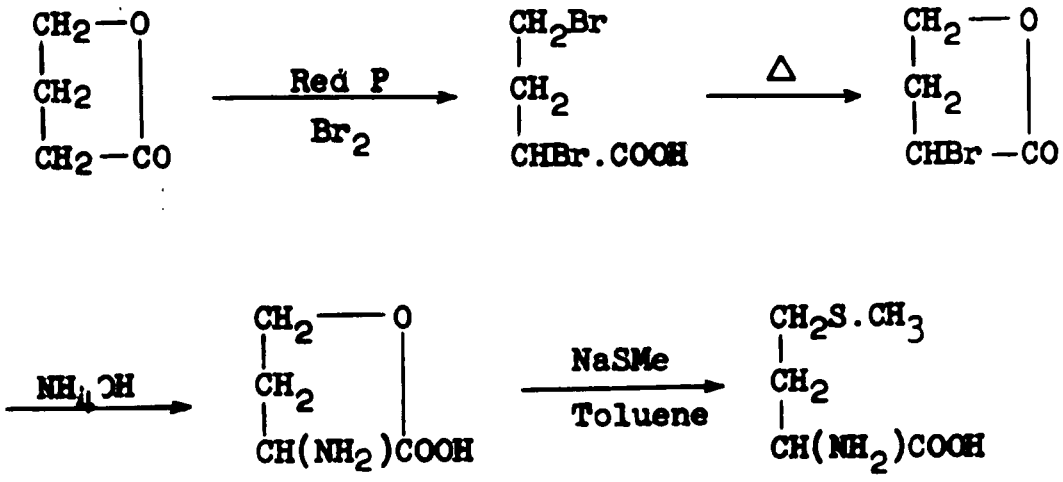
The synthesis of the lowest homologue of the series, methionine, has been accomplished by several different routes, but they are invariably specific for this particular compound, usually because of the lack of availability of starting materials for the higher homologues. Acrolein has been converted to methionine by several routes involving both the Strecker[108] and the Bucherer hydantoin[109] reactions:



γ-Butyrolactone has also served as a starting material, the synthesis proceeding via 5-(β-bromoethyl)hydantoin:(105)



Condensation with sodium methyl mercaptide gives 5-(β-methylmercapoethyl) hydantoin, which is then hydrolyzed to methionine. A simpler synthesis from γ-butyrolactone involved treatment of α-amino-γ-butyrolactone with sodium methyl mercaptide in toluene.(111)



Several routes have used 2-methylmercaptoethyl chloride, which can be prepared from the readily available ethylene chlorohydrin, as starting material. These have involved condensation with sodiomalonic ester,[112] diethyl sodiophthalimidomalonate[113] or diethyl sodioacetamidomalonate.[114]

The next higher homologue, 5-methylthionorvaline or homomethionine, has been synthesized from 1,3-dibromopropane,[115] γ-propylhydantoin[116] and allyl bromide.[117] Of these, only the 1,3-dibromopropane route appeared readily adaptable to higher homologues because of the difficulty of obtaining higher homologues of the other starting materials. This route however was involved, and designed primarily to produce the corresponding homologue of homocystine, known as pentahomocystine. After preliminary experiments, the best approach appeared to be a modification of the route

described by Birkofer and Morgenroth[118] for the synthesis of several sulphur containing aminoacids by the alkylation of acetaminomalonic ester with dihaloalkanes.

Oxidation of sulphides to sulphoxides and sulphones has been carried out by a number of methods. Oxidizing agents used have included hydrogen peroxide with hydrochloric acid[119] or with acetic acid,[120] potassium permanganate[121] and iodine.[119] The degree of oxidation obtained is controlled simply by the stoichiometry of the oxidizing agent used. Yields are generally high, often almost quantitative.

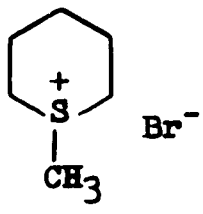
DISCUSSION

The ready condensation of 2-methylmercaptoethyl chloride with the sodium derivatives of substituted malonic esters as used in the synthesis of methionine led us to attempt a similar reaction sequence for the higher homologues. (Since the monothiomethylalkyl halide and the dithiomethylalkane could be readily separated by distillation, this sequence seemed preferable to an initial condensation of the dihaloalkane with the malonate). Accordingly, 6-thiomethyl-1-chlorohexane and 5-thiomethyl-1-chloropentane were prepared, and attempts made to condense them with both diethyl sodiophthalimidomalonate and diethyl sodioacetaminomalonate.

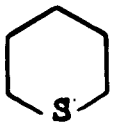
Varying reaction periods were tried, with a maximum of 18 hours in refluxing ethanol. The precipitate of sodium chloride was filtered off and the filtrate concentrated to remove ethanol. Only intractable brown oils were obtained, from which, with perseverance, some crystalline material was isolated. This was demonstrated to be unreacted phthalimido- or acetamino-ester by melting point, mixed melting point and infrared spectra. Hydrolysis of the oil produced only a small aminoacid fraction which was almost entirely glycine. A trace amount of another aminoacid (observed on paper chromatography, with an R_F

value of 0.69 in the solvent system described later, page 57) was presumed to be the desired 8-thiomethyl-2-amino-octanoic acid.

Attempts were made to prepare the previously unknown ω-thiomethyl pentyl and hexyl bromides with the object of using milder conditions in the condensation step, but these both proved to be unstable. Although 5-thiomethyl-1-bromopentane could be distilled under oil pump vacuum, the distillate rapidly precipitated the cyclic sulphonium salt (LII). 6-thiomethyl-1-bromohexane decomposed on attempted distillation giving a low melting, waxy solid, insoluble in water, ethanol or ether, but extremely soluble in chloroform. The structure is as yet unknown, but the NMR spectrum was almost identical with that of thiacyclohexane (LIII),[122] the difference being



LII



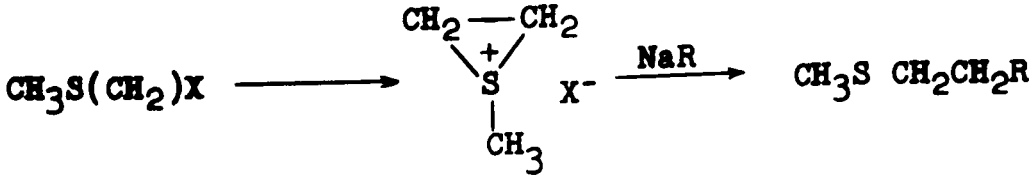
LIII

attributable to the extra methylene group. The infrared spectrum also was very similar to that of thiacyclo-heptane.[123] However the physical properties do not agree with those of thiacycloheptane, which is reported

as being a liquid, boiling point 170-171°.[124] The possibility of a polymeric material with this as a basic sub-unit would seem to be ruled out by a molecular weight determination which gave a value of approximately 135.

These results were rather unexpected in view of the reported smooth condensation of the lower homologous 2-thiomethylethyl chloride in the methionine synthesis, but are in line with the results of Kjaer[117] who reported a maximum yield of only 11% of alkylated acetaminocyanoacetate or formamidomalonate from the reaction of 3-thiomethylpropyl chloride or bromide with the appropriate sodium derivative. Large amounts of dark-coloured, oily, secondary products, possibly due to the formation of sulphonium type compounds, were also reported.[117]

Presumably, as Eliel has suggested,[125][126] the anomalous ease with which thiomethylethyl halides react with nucleophiles, compared to the higher homologues, is due to ready participation by sulphur, a cyclic three-membered sulphonium intermediate (LIV) being formed. The



LIV

higher homologues do not form cyclic sulphonium compounds as readily, as a result of increased steric repulsion and entropy factors, although once formed, these large ring compounds are more stable than (LIV). The rates of ring closure by compounds of the type $RS(CH_2)_nCl$ to cyclic sulphonium compounds are in the ratio of 5700:75:1 for five-, six- and seven-membered rings.[127][128]

Since ω-thiomethyl halides were unsatisfactory starting materials, we investigated the alternative approach outlined in Figure 15. In order to minimize replacement of

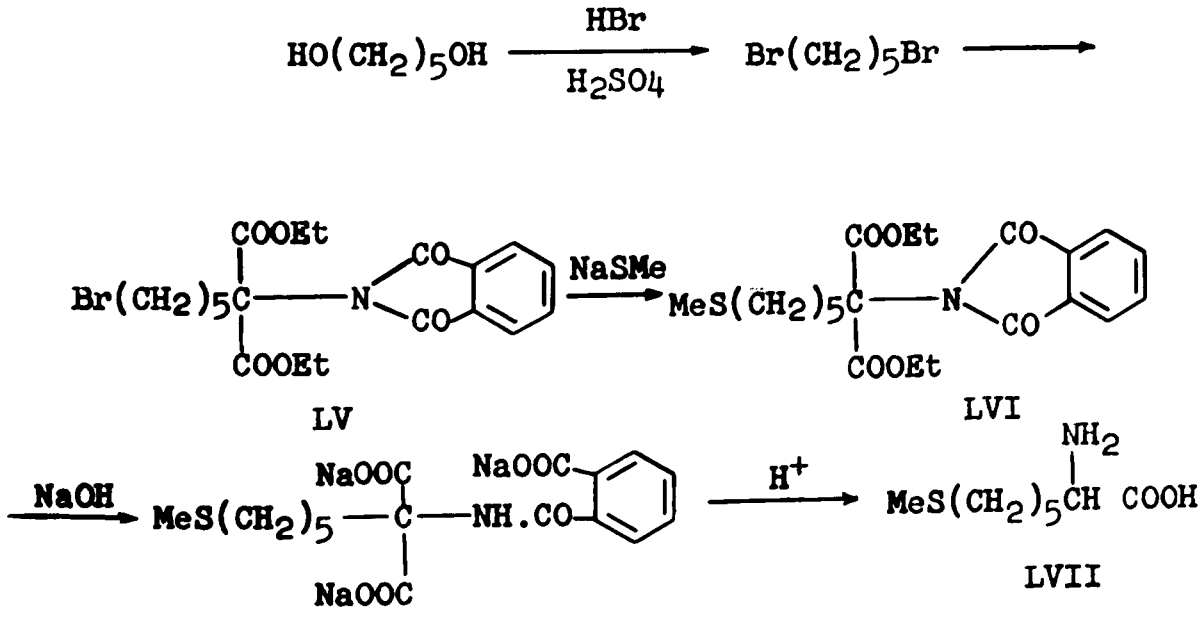
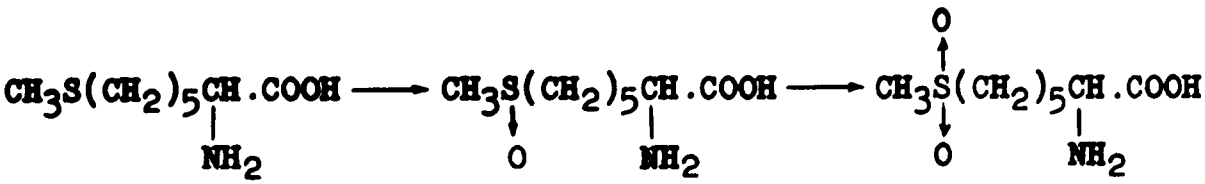


Figure 15. Synthesis of 2-amino-7-thiomethylheptanoic acid

both bromine atoms with phthalimidomalonate in the reaction of dibromoalkane with sodium phthalimidomalonate an almost four-fold excess of dibromopentane was used. (The excess was subsequently removed by distillation under high vacuum). The diethyl 2-(5'-bromopentyl)-2-phthalimidomalonate (LV) was then condensed with sodium thiomethylate, using ethanol as solvent. The reaction was almost instantaneous as evidenced by the immediate precipitation of sodium bromide. A two-stage hydrolysis of the phthalimido compound (LVI) was carried out, first with dilute sodium hydroxide solution, then with dilute hydrochloric acid. This allows much milder conditions than the often used single-stage hydrolysis, in which the phthalimido derivative is refluxed overnight with concentrated mineral acid, a treatment which would almost certainly cleave the thioether linkage. The product initially isolated contained a small amount of glycine (shown by paper chromatography) arising from the hydrolysis of diethyl phthalimidomalonate itself. Recrystallization of the crude product yielded pure 2-amino-7-thiomethyl-heptanoic acid (LVII).

Oxidation of (LVII) with the calculated amounts of hydrogen peroxide, using glacial acetic acid as solvent yielded the sulphoxide (LVIII) and sulphone (LIX).



LVII LVIII LIX

The infrared spectra of these three aminoacids are given in Appendix 2. Each shows the characteristic aminoacid bands at 2950, 2090, 1567 and 1406 cm^{-1}. The sulphoxide and the sulphone both showed bands characteristic of these functional groups at 1021 cm^{-1} (for the sulphoxide), 1126 and 1298 cm^{-1} (for the sulphone).

The paper chromatographic behaviour of these amino-acids, together with some other common α-aminoacids was studied in a 1-butanol/acetic acid/water (4:1:3) system. The results are given in Table VI. The value reported for homomethionine is 0.54.(117)

Whilst the overall yield was low (9.2%), the method would be suitable for preparing 2-^{14}C labelled acids using 2-^{14}C labelled malonate derivatives as starting materials. The method should also be generally applicable to the synthesis of any of the other ω-methylthioalkyl-α-amino-acids.

TABLE VI

R_F VALUES OF α-AMINOACIDS DETERMINED ON WHATMAN NO. 1 PAPER BY THE ASCENDING TECHNIQUE

Aminoacid	R_F value
Glycine	0.13
β-Phenylalanine	0.51
Methionine	0.49
2-Amino-7-thiomethylheptanoic acid	0.62
2-Amino-8-thiomethyloctanoic acid	0.69
2-Amino-7-methylsulphinylheptanoic acid	0.19
2-Amino-7-methylsulphonylheptanoic acid	0.21

Solvent system: 1-butanol/acetic acid/water (4:1:3).

Temperature: 21° (approx).

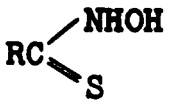
SECTION 5

THE SYNTHESIS OF MUSTARD OIL GLUCOSIDES

INTRODUCTION

The first successful synthesis of a mustard oil glucoside was that devised by Ettlinger and Lundeen[16] to confirm their structure for glucotropaeolin. The reaction sequence for this synthesis is shown in Figure 3, page 8. Dithiophenylacetate (VIII), prepared by the reaction of benzylmagnesium chloride with carbon disulphide, was treated with hydroxylamine hydrochloride to form phenylacetothiohydroxamic acid (IX). This, on treatment with acetobromoglucose in the presence of base, yielded S-(tetraacetyl-β-D-1-glucopyranosyl)-phenylacetothiohydroximic acid (X). Treatment of the thiohydroximic acid with a sulphur trioxide-pyridine complex, followed by deacetylation with methanolic ammonia gave glucotropaeolin (XII).

The success of this synthesis was dependent on a suitable route to the thiohydroximic acid. The Houben-Grignard method[129] used for the synthesis of dithiophenylacetate by Ettlinger and Lundeen is not generally applicable, particularly for alkyl dithio acids which are supposed to be formed only in low yields. In addition, several of the desired side-chains (e.g. those containing hydroxy or ester groups) react with Grignard reagents, precluding the use of this method. The thiohydroxamic acids (LX), when formed, are not particularly stable.



LX

Benzothiohydroxamic acid (LX, R = C_6H_5), first prepared from dithiobenzoic acid,[130] is an unstable oil which decomposes to benzonitrile, sulphur and water. More stable is anisothiohydroxamic acid (LX, R = p-$CH_3OC_6H_4$) which after two weeks at room temperature required heating to decompose it to anisonitrile.[131] Phenylacetothiohydroxamic acid (LX, R = $C_6H_5CH_2$) decomposes in a few days at room temperature. In contrast, an attempt to produce acetothiohydroxamic acid resulted in an unstable product which decomposed to acetonitrile and sulphur before it could be characterized.[132] However, it is reported that the sodium salts of these compounds are considerably more stable.[132]

In summary, the Ettlinger-Lundeen method, whilst being suitable for the synthesis of a number of aromatic mustard oil glucosides, did not appear attractive as a route to the alkyl compounds.

NEW ROUTES TO THE MUSTARD OIL GLUCOSIDES

1. FROM ALDEHYDES

An alternative route to the thiohydroximate system (LXV), the addition of a thiol to a nitrile oxide (LXIV, Figure 16), was first postulated during speculation on the biosynthesis of mustard oil glucosides (see page 44). In fact, Bachetti and Alemagna[133] had previously prepared some benzothiohydroximates by this method. Nitrile oxides are usually unstable compounds, best prepared *in situ* by treatment of a hydroxamoyl chloride (LXIII) with base.[134][135][136] The hydroxamoyl chloride is prepared by chlorination of the corresponding aldoxime (LXI).

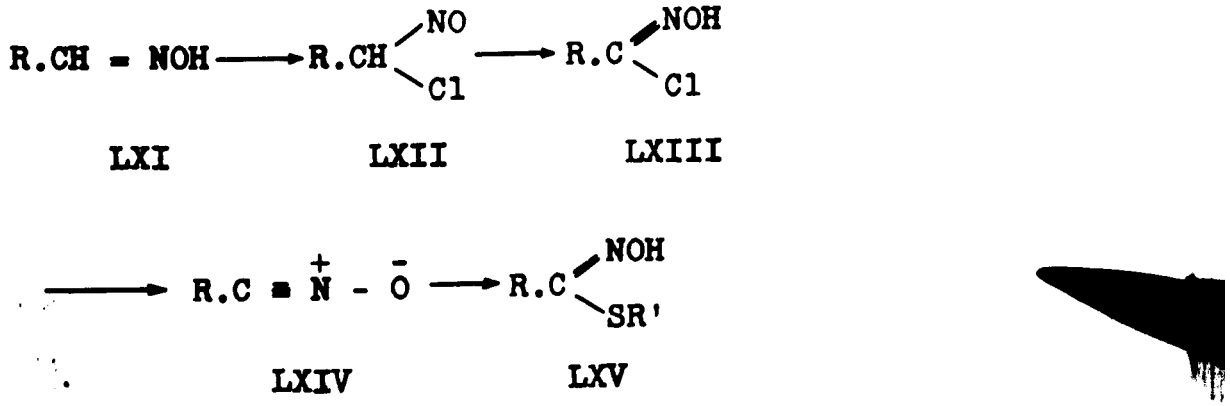


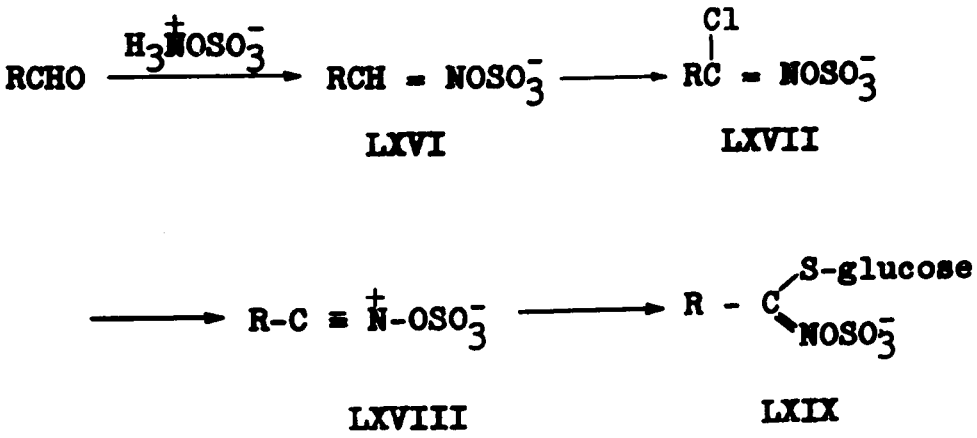
Figure 16. Synthesis of Thiohydroximates

This reaction sequence was further explored by Benn[137] who demonstrated its general applicability by synthesizing a wide range of thiohydroximates in good yield by condensing hydroxamoyl chloride with thiols in the presence

of triethylamine. Various combinations of aliphatic and aromatic for R and R' were represented. The reaction was also extended to a selenium analogue, Se-phenyl-benzoseleno-hydroximate. The investigation culminated in a successful synthesis of glucotropaeolin,[138] the remaining steps from the thiohydroximate being the same as in the original synthesis.

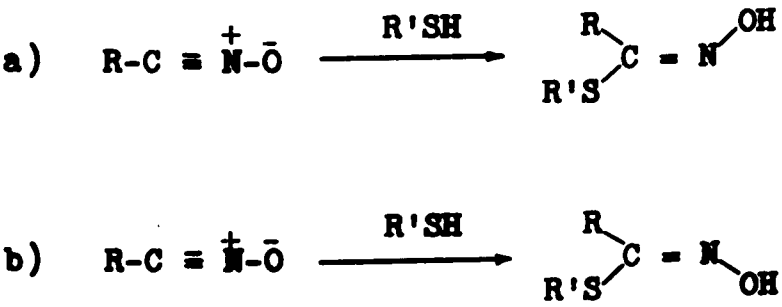
Evidence was produced[137] for the involvement of the nitrile oxide (XLIV) as an intermediate. Thus, thiols do not react with hydroxamoyl chlorides at an appreciable rate without the addition of triethylamine. However, when triethylamine was added to a cold ethereal solution of a hydroxamoyl chloride alone, immediate precipitation of triethylamine hydrochloride occurred, and on filtration, the solution exhibited the intense absorption at 2320 cm^{-1} characteristic of nitrile oxides.[135] This absorption rapidly disappeared on addition of a thiol, and evaporation of the solution yielded the thiohydroximate. On the other hand, Ettlinger and Dateo, who had independently developed a very similar route (unpublished, but reported in (139)), have shown that O-sulphonated oximes (LXVI) can be chlorinated, and the resulting O-sulphonated hydroxamoyl chlorides (LXVII) condensed with

sodium β-D-1-glucopyranosyl mercaptide to give mustard oil glucosides(LXIX) directly. In this case, formation of the nitrile oxide is blocked. There may be direct displacement of halogen by the mercaptide nucleophile, or alternatively, the reaction could presumably proceed through the intermediate (LXIII) which is analogous to a nitrile oxide. This is comparable to the intermediate (CVI, see page 76) proposed by Kornblum[140] to explain the conversion of nitro compounds to hydroxamoyl chlorides.



LXVI LXVII LXVIII LXIX

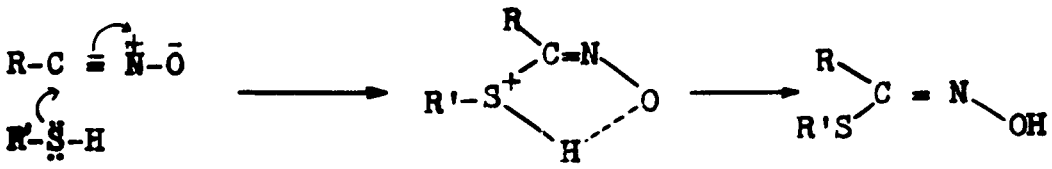
Stereochemically, two modes of addition to the nitrile oxide are possible:



a) syn

b) anti

X-ray crystallography[17] of sinigrin has shown it to have the anti configuration, and if by analogy this is extended to the other mustard oil glucosides, they too will have this configuration. Since the product of the reaction of nitrile oxides and glucosyl mercaptide has a configuration identical with the natural compounds, the reaction must proceed to give the anti thiohydroxamate as in (b). The reaction is taking place in basic solution and the mechanism appears to involve attack by the mercaptide anion on the nitrile oxide. It may be visualized as a trans addition in which the lone pair of electrons develops on the nitrogen trans to the attacking mercaptide anion. A possible alternative would involve the addition of the unionized mercaptan to the nitrile oxide, a reaction analogous to the 1,3-dipolar addition of nitrile oxides to olefins.[134][136]



The mustard oil glucosides selected for synthesis by this method were glucoputranjivin, glucolepidiin and the trio glucoiberverin, glucoiberin and glucocheirolin, which contain sulphur in the side-chain in various stages of oxidation.

The natural occurrence of glucoputranjivin (LXXVII) was first assumed when Puntambekar[27] isolated isopropyl isothiocyanate from the seeds of the Indian tree Putranjiva roxburghii Wall. Taxonomically, this is of great interest as this is the only well-established example of the occurrence of mustard oils in the family Euphorbiaceae*, a family which is normally regarded as taxonomically remote from the order Rhoedales which contains the families Cruciferae and Capparidaceae. However, Hutchinson[142] regards the Euphorbiaceae as a heterogeneous family, i.e. a collection of species, derived from several different stocks, including the Bixales, which he also regards as the ancestral stock from which the Capparidaceae were derived, so other examples of mustard oil producing plants may be encountered in this "family".

Isopropyl isothiocyanate has since been found to be fairly widely distributed, particularly within the Cruciferae[18] and in 1959 Kjaer[143] succeeded in isolating glucoputranjivin from the seeds of Lunaria annua L. (Lunaria biennis Moench.). In addition, its presence

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*Another example, the occurrence of glucotropaeolin in the latex of Latropha multifida L.[141] remains in doubt.

has also been demonstrated[144][145] in a species (Tropaeolum peregrinum, synonym T. canariense Hort.) of the family Tropaeolaceae, which is of the order Geraniales, again somewhat removed from the Rhoedales.

The synthesis of glucoputranjivin was carried out by the addition of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl mercaptan (LXXIV) to isobutyronitrile oxide (LXXIII), followed by sulphonation and deacetylation as shown in Figure 17.

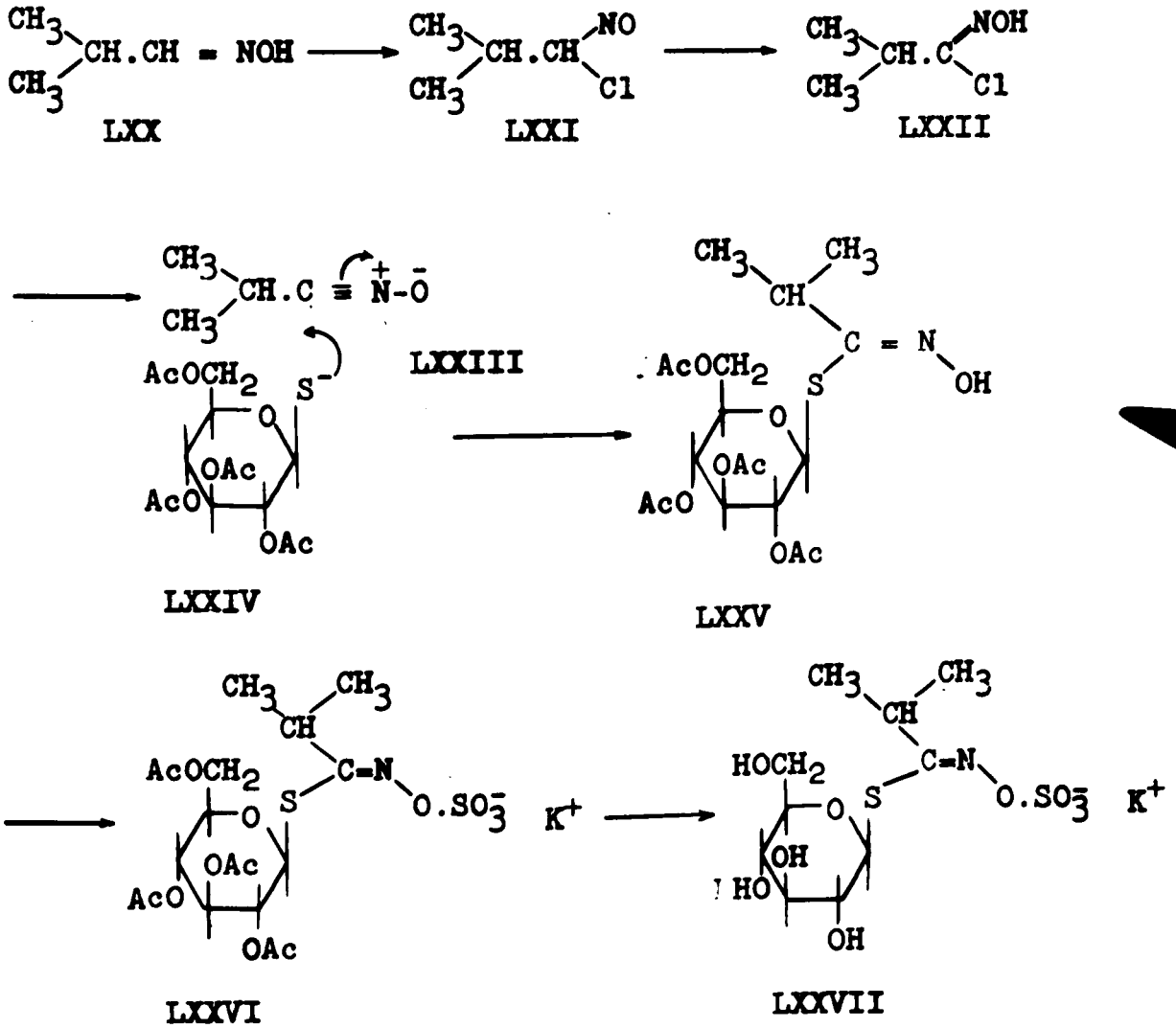


Figure 17. Synthesis of Glucoputranjivin

Isobutyraldoxime (LXX), dissolved in anhydrous ether was chlorinated to give initially the bright blue chloronitroso compound (LXXI), which spontaneously rearranges to the hydroxamoyl chloride (LXXII). Treatment of this with tetra-O-acetyl-β-D-glucopyranosyl mercaptan in the presence of excess base (triethylamine) gave the thiohydroximic acid (LXXV).

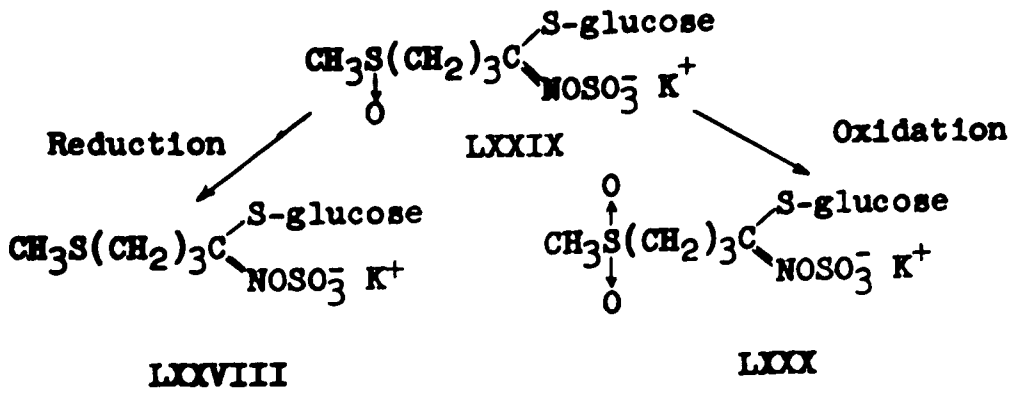
Sulphonation of the S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-isobutyrothiohydroximic acid (LXXV) with a pyridine-sulphur trioxide complex gave the tetra-O-acetylglucoputranjivin anion, isolated as the crystalline potassium salt (LXXVII), which was identical in all respects with an authentic specimen of natural origin.

Deacetylation of this material with an anhydrous solution of ammonia in methanol yielded glucoputranjivin. Although it was chromatographically homogeneous, it refused to crystallize. Since it has been demonstrated previously[15] that the tetramethylammonium salts of mustard oil glucosides often crystallize better than the potassium salts, the potassium cation was replaced by tetramethyl-ammonium ion by passing the salt through an ion exchange column in the tetramethylammonium form. However, the material obtained still refused to crystallize and it is quite possible that glucoputranjivin will prove to be a non-crystalline mustard oil glucoside.

Glucolepidiin is a comparatively rare mustard oil glucoside with an ethyl side-chain, the only known occurrence to date being in the North American crucifer Lepidium Menziesii DC.[26] as discussed in Part 3 (Biosynthesis, page 22). This concurs with the notable absence in nature of the α-aminoacid with the ethyl side-chain (α-aminobutyric acid).

The synthesis of glucolepidiin was carried out in the same manner as the glucoputranjivin synthesis. However, in this case, the glucoside finally obtained crystallized extremely readily in its potassium form.

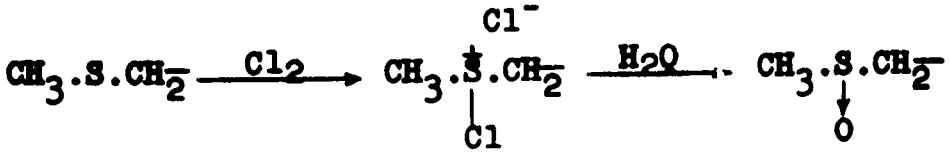
An attempt was also made to synthesize glucoiberin (LXXIX), which by reduction and oxidation would lead to glucoibervirin (LXXVIII) and glucocheirolin (LXXX) respectively.



Glucoibervirin, glucoiberin and glucocheiroline have been isolated from Iberis sempervirens L.,[35] Iberis amara L.,[37] and Cheiranthus cheiri L.[146] Glucocheirolin

has been known for a considerable length of time and the mustard oil derived from it was used as an analogy in the structural proof of methionine.(147)

In this case, a key intermediate was 4-methylthio-butyraldoxime. In view of the reported reaction of chlorine with thioethers, to give chlorosulphonium chlorides (LXXXI) which are readily hydrolyzed to sulphoxides (LXXXII), it was anticipated that chlorination of (XCVII) followed by condensation with tetraacetyl-glucosyl mercaptan would yield the thiohydroximic acid, desulphoiberin (C).



LXXXI LXXXII

The preparation of the starting material (4-thiomethyl-butanal) itself involved a lengthy series of reactions. The first attempt used a sequence devised for the synthesis of 3-thiomethylpropanal.(148) 4-Thiomethylbutyronitrile (LXXXIV), prepared from 1,3-bromochloropropane (LXXXIII), was converted through the iminoester hydrochloride (LXXXV) to the orthoester (LXXXVI) which was reduced (lithium aluminum hydride) to the acetal (LXXXVII) and hydrolyzed. All stages proceeded in adequate yields, apart from the final hydrolysis. The conditions for this, as given by

$$Cl(CH_2)_3Br \xrightarrow{KCN} Cl(CH_3)_3CN \xrightarrow{CH_3SNa} CH_3S(CH_2)_3CN$$

LXXXIII LXXXIV

$$\xrightarrow[EtOH]{HCl} CH_3S(CH_2)_3C(OEt)=NH.HCl \xrightarrow{EtOH} CH_3S(CH_2)_3C(OEt)_3$$

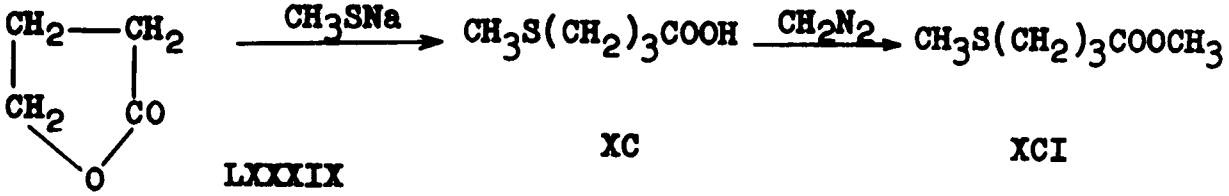
LXXXV LXXXVI

$$\xrightarrow{LiAlH_4} CH_3S(CH_2)_3CH(OEt)_2 \xrightarrow[H_2O]{H^+} CH_3S(CH_2)_3CHO$$

LXXXVII

Barger and Coyne[147] (boil the acetal for 30 minutes with 2 volumes of water containing 1-2 ml of dilute hydrochloric acid) proved far too mild, and the acetal was recovered unchanged. Refluxing for a longer period (3 hours) with stronger (6N) hydrochloric acid also had no effect. An attempted hydrolysis with sulphuric acid (6N) in the presence of dioxan to increase the solubility of the acetal resulted only in complete decomposition, tars being the only isolable product.

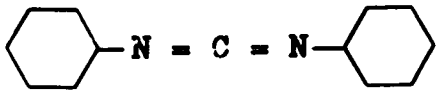
An alternate route through the corresponding alcohol (XCII) was somewhat more successful. γ-butyrolactone (LXXXIX) was cleaved with sodium thiomethylate and the resulting acid (XC) converted to the ester (XCI) with diazomethane. Reduction of the ester with lithium



LXXXIX XC XCI

XCII XCIII

aluminum hydride gave the alcohol (XCII). Oxidation of the alcohol to the aldehyde (XCIII) by simple oxidative procedures failed because the lack of volatility of the aldehyde prevented its removal from the reaction mixture, resulting in over-oxidation. Also many of the common oxidizing agents are capable of oxidizing the sulphur atom. As a result, we used the Moffat-Pfitzner reagent (dicyclohexylcarbodiimide, DCCD, XCIV, in dimethyl sulphoxide with anhydrous phosphoric acid as catalyst), recently reported[149] as a mild and highly specific reagent for the oxidation of



XCIV

alcohols to aldehydes. The reaction was carried out at room temperature and in our case, a mixture of four products was obtained, from which the aldehyde (XCIII) was isolated in very low yield. One possible explanation is that the

thiomethyl group was oxidized by dimethyl sulphoxide, volatile dimethyl sulphide being produced, so helping to displace the equilibrium to the right:

$$CH_3S(CH_2)_3CHO + CH_3\underset{\underset{O}{\downarrow}}{S}CH_3 \rightleftharpoons CH_3\underset{\underset{O}{\downarrow}}{S}(CH_2)_3CHO + CH_3SCH_3\uparrow$$

XCVI

The sulphoxide aldehyde (XCVI) was sufficiently water soluble to resist attempts to extract it from the reaction mixture.

The aldehyde was converted to the oxime (XCVII) and chlorinated in an aqueous medium, rather than the usual anhydrous ether or chloroform. It was known that chlorine reacts with sulphides and the conditions chosen were such as to cause hydrolysis of the initially produced sulphonium chloride (XCVIII) to the sulphoxide (IC). Extraction of the

$$CH_3S(CH_2)_3CH = NOH \xrightarrow{Cl_2} CH_3\overset{Cl^-}{\overset{+}{\underset{\underset{Cl}{|}}{S}}}(CH_2)_3\underset{\underset{Cl}{|}}{C} = NOH$$

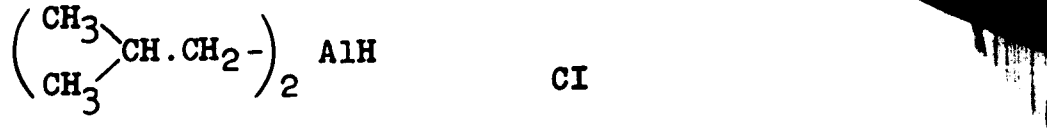
XCVII XCVIII

$$\xrightarrow{H_2O} CH_3\underset{\underset{O}{\downarrow}}{S}(CH_2)_3\underset{\underset{Cl}{|}}{C} = NOH \xrightarrow{RSH} CH_3\underset{\underset{O}{\downarrow}}{S}(CH_2)_3C\begin{matrix}NOH\\ \diagdown\\ SR\end{matrix}$$

IC C

aqueous solution with ether gave the blue colour (due to the chloronitroso compound) always observed in solutions of hydroxamoyl chlorides, but attempts to condense the hydroxamoyl chloride with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl mercaptan in the usual way gave octa-O-acetyl-diglucosyl disulphide as the only crystalline product. This also happened when the chlorination was carried out in chloroform.

No further work was carried out on this synthesis, due to lack of time. It seems likely that the best route to the aldehyde (XCIII) would be to reduce the ester (XCI) with diisobutyl aluminum hydride (CI), and Mrs. F. Willis of this Department is now exploring this approach, as well as attempting to prepare glucoiberin <u>via</u> 4-methylthio-1-nitrobutane (see next section).



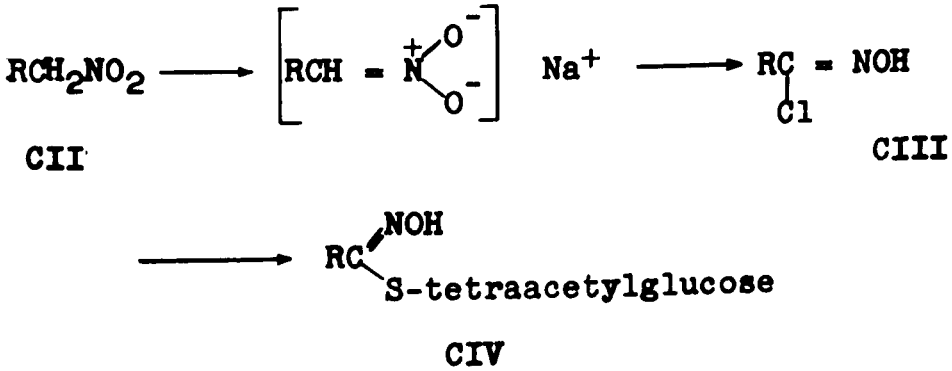
CI

2. FROM NITRO COMPOUNDS

Very recently during an investigation of the mechanism of the Nef reaction, Kornblum and Brown[140] noted that when the sodium salt of phenylnitromethane (CII, R = C_6H_5) was added to concentrated hydrochloric acid, phenylaceto-hydroxamoyl chloride (CIII, R = C_6H_5) was produced. This observation prompted us to investigate the behaviour of other sodium salts of nitrocompounds to see whether the reaction was general.

Nitroalkanes are attractive starting materials as they are often readily prepared by the displacement of bromide with sodium nitrite using dimethyl sulphoxide as solvent.[150] In contrast aldehydes, particularly non-volatile compounds, are not easily prepared especially when unsaturation (when rearrangement may occur) or when oxidizable groups (e.g. sulphur-containing groups) are present in the molecule. Furthermore a number of the oximes are sensitive to chlorination.

This route to the hydroxamic acid chloride is therefore an alternative to the chlorination of an aldoxime in the synthesis of mustard oil glucosides. The precursors, S-β-D-1-(tetraacetylglucopyranosyl)-acetohydroximic acid (CIII, R = CH_3) and S-β-D-1-(tetraacetylglucopyranosyl)-propiohydroximic acid (CIV, R = C_2H_5), of the mustard oil glucosides glucocapparin and glucolepidiin were synthesized from the nitroparaffins by this route:



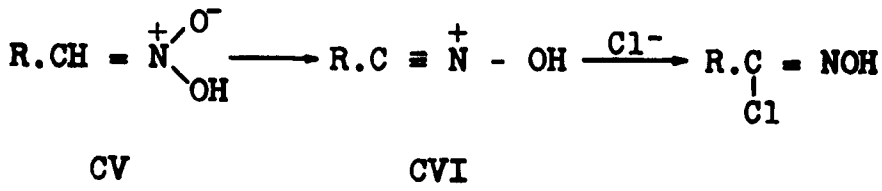
CII CIII CIV

Glucocapparin, the simplest member of the mustard oil glucoside series has been found many times in plants of the family Capparidaceae,[25] but has not been definitely identified in plants of the Cruciferae family. Glucolepidiin, which was also synthesized by the oxime route, is discussed in the preceding section (page 68).

The starting materials for these compounds were the commercially available nitroethane and nitropropane. The sodium salt of the nitroparaffin was formed in good yield by treatment with sodium ethoxide in absolute ethanol. Addition of this salt to concentrated hydrochloric acid containing 20% of anhydrous lithium chloride at 0° produced the hydroxamic acid chloride (CIII), isolated by pouring the reaction mixture into a large excess of water and extracting with ether. The ether solution, after drying, was condensed directly with tetraacetyl-β-D-glucopyranosyl mercaptan in the presence of triethylamine, to give the thiohydroximic acid (CIV). The melting points and infrared spectra of both compounds prepared (CIV, R = CH_3 and R = C_2H_5) were identical with those of specimens prepared by the chlorination of the oximes of acetaldehyde and propionaldehyde.[186]

The reaction is believed to proceed through the aci-form (CV) of the nitroparaffin, and an intermediate (CVI) was postulated by Kornblum,[140] but the existence of this

intermediate has not yet been verified.



CV CVI

This route to mustard oil glucosides from compounds is somewhat simpler than the route from aldehydes and is subject to less limitations in the starting materials. It appears to be the most versatile of the various syntheses so far devised for mustard oil glucosides.

SECTION 6

EXPERIMENTAL

GENERAL

1. SOLVENTS

Anhydrous solvents are specified in a number of the experimental procedures. These were prepared by the following methods.

Ether. Commercial anhydrous ether (Mallinckrodt or Shawinigan Chemicals) was used without further treatment.

Ethanol. Commercial absolute ethanol was dried by refluxing it with magnesium turnings, followed by fractional distillation from the magnesium ethylate produced.[152]

Methanol. Commercial anhydrous methanol was dried by the same method used for ethanol.

Dimethyl sulphoxide. Commercial material (Matheson, Coleman and Bell) was refluxed over calcium oxide overnight and distilled under reduced pressure.

Pyridine. Commercial material (B.D.H. Analar) was stored over potassium hydroxide pellets.

2. EQUIPMENT

Gas-liquid chromatographic analyses were performed on a Wilkens Instrument Company Aerograph Hy-Fi instrument using a 5' x 1/8" stainless steel column packed with 5%

SE30 (silicone oil) on 60/80 Chromosorb W, with nitrogen as carrier gas, and a hydrogen flame detector. Melting points are uncorrected on a Zeiss hot-stage. Infrared spectra were run on a Perkin-Elmer model 137B Infracord, or a Perkin-Elmer model 337B (grating) Spectrometer.

Microanalyses were performed by the Schwarzkopf Microanalytical Laboratory.

SYNTHESIS OF LABELLED COMPOUNDS

Before attempting the synthesis of any isotopically labelled compound, the proposed synthesis was carried out at least once using inactive material. Only when the procedure was suitably modified for a small scale, and the final product shown to have the correct structure and desired purity was the synthesis carried out on labelled material.

1. CARBON-14 LABELLED COMPOUNDS

Phenylacetic acid-1-^{14}C

The preparation was carried out in an evacuated manifold system.[153] Carbon-14 labelled barium carbonate (0.719 g, 0.00383 moles, 0.98 mc) was treated with excess concentrated sulphuric acid (5.0 ml), and the carbon dioxide produced condensed out in a gas storage bulb. In another reaction flask was placed magnesium turnings (0.5 g) and ether (30 ml). Benzyl chloride (2.0 ml) was distilled into it at liquid nitrogen temperature. The mixture, which was magnetically stirred, was gradually warmed until reaction took place and the ether refluxed (solid carbon dioxide condenser). When formation of the Grignard reagent was

complete, the carbon dioxide from the gas storage bulb was allowed to evaporate into the reaction flask which was cooled to -20° in an ice-salt bath. When all the carbon dioxide had been absorbed, the flask was removed from the manifold and treated with ice and dilute hydrochloric acid. The mixture was extracted with ether (3 x 10 ml), the combined ether solutions extracted with 10% sodium hydroxide (3 x 10 ml) and the combined alkaline extracts acidified. The white crystalline precipitate of phenylacetic acid was collected and washed with water and dried. Yield 0.503 g (almost theoretical), m.p. 76-77° (lit.[154] m.p. 76°). Specific activity 224 μc/mmole.

Phenylpyruvic acid-2-^{14}C

i) Acetylglycine-2-^{14}C[155]

Glycine-2-^{14}C (0.150 g, 0.002 mole, activity 100 μc) was dissolved in water (1 ml) and stirred with acetic anhydride (0.7 g) for 20 minutes. The mixture was left in the refrigerator overnight to crystallize and then filtered. The filtrate was concentrated to half its volume, cooled and refiltered. The two crops of crystals were combined to give 0.157 g (0.00137 mole, 69%).

ii) 2-methyl-4-benzylidene oxazolone-3-^{14}C (Azlactone)[156]

Acetylglycine-2^{14}C (0.157 g, 0.00137 mole) was refluxed for one hour with freshly distilled benzaldehyde (0.24 g,

0.0026 mole), anhydrous sodium acetate (0.085 g) and acetic anhydride (0.36 g). After leaving the mixture in the refrigerator for two days, water (0.5 ml) was added and the crystals of the azlactone were filtered off and used directly without further purification.

iii) Phenylpyruvic acid-2-^{14}C[157]

The azlactone crystals were treated with acetone (1.25 ml) and water (0.5 ml) and heated under reflux for four hours. At the conclusion of this period the condenser was removed to allow the acetone to distil off. More water (1 ml) was added and the mixture placed in the refrigerator overnight to crystallize. After filtration, hydrolysis was completed by refluxing a solution of the crystals in 1N hydrochloric acid (1 ml) for three hours. On cooling, pale yellow crystals of phenylpyruvic acid separated from the solution. The yield was 0.167 g (51%), m.p. 147-152° (lit.[158] m.p. 152-154°). Specific activity 31.7 μc/mmole.

<u>α-oximino-β-phenylpropanoic acid-2-^{14}C</u>

The above preparation of phenylpyruvic acid-2-^{14}C was repeated, using 0.2 mc glycine-2-^{14}C (0.160 g) as starting material. The yield of phenylpyruvic acid was 0.115 g. This was dissolved in ethanolic sodium hydroxide solution to which a few drops of water were added and then treated at 0° with a slight excess of hydroxylamine hydrochloride (0.06 g)

dissolved in water (5 ml). The mixture was left at room temperature for 40 minutes, then acidified with concentrated hydrochloric acid and extracted with ether (3 x 10 ml). The combined ethereal extracts were dried ($MgSO_4$) and evaporated to dryness at room temperature under reduced pressure. The residual solid was purified by sublimation under high-vacuum (130°, 5 x 10^{-4} mm pressure). The yield of fine white crystals was 0.090 g. (72%), m.p. 165-167° (decomp.) Lit. m.p. 159-160° (decomp.)[159], 166-167° (decomp.)[103]. Specific activity 92.3 μc/mmole.

Cinnamic acid-2-^{14}C[160]

Malonic acid-2-^{14}C (0.070 g, 0.1 mc) was treated with freshly distilled benzaldehyde (0.25 g), pyridine (0.25 g), sodium sulphate (0.1 g) and piperidine (2 drops), and the mixture heated first at 100° for two hours, and then at 120° for two hours, after which it was cooled and poured into ice water (10 ml). Concentrated hydrochloric acid was added to precipitate the cinnamic acid which was extracted with ether (3 x 10 ml), the combined, dried ($MgSO_4$) extracts evaporated to dryness and the residue recrystallized from water. Yield 0.082 g (69%), m.p. 131° (lit.[161] m.p. 133°). Specific activity 144 μc/mmole.

Sodium phenylacetothiohydroxamate-2-^{14}C

i) Sodium dithiophenylacetate-2-^{14}C

Benzyl chloride-7-^{14}C (1.32 g, 0.1 mc) was dissolved in anhydrous ether (20 ml) and allowed to react with magnesium (0.24 g) in vacuum. The Grignard solution was removed by means of a syringe, through a serum cap, and added dropwise to carbon disulphide (0.75 g), at 0°, under an atmosphere of nitrogen. After 20 minutes, the solution was acidified with 6N hydrochloric acid, extracted with ether (3 x 10 ml) and the combined ether extracts washed with 2% sodium hydroxide solution (25 ml total) to give an aqueous alkaline solution of sodium dithiophenylacetate.

ii) Sodium phenylacetothiohydroxamate-2-^{14}C

A solution of hydroxylamine hydrochloride (0.7 g) in water (25 ml), neutralized with potassium carbonate (0.7 g), was added dropwise at 0° to the solution of sodium dithiophenylacetate prepared above. After standing at room temperature for one hour, the solution was acidified with 6N hydrochloric acid and extracted with ether (3 x 10 ml). The combined ether extracts were dried ($MgSO_4$) and evaporated to dryness, under vacuum, at room temperature. The residue was dissolved in ethanol and the solution made just alkaline to phenolphthalein with ethanolic sodium hydroxide. The solution was evaporated to dryness under reduced pressure and the residue recrystallized from

ethanol-ether. Yield 0.263 g (13.4% overall). Specific activity 9.3 μc/mmole.

2. NITROGEN-15 LABELLED COMPOUNDS

DL-Phenylalanine-^{15}N (162)

i) Preparation of methyl 2-bromo-3-phenylpropanoate.

Potassium bromide (40.0 g) was added to an ice-cold, stirred solution of DL-phenylalanine (16.5 g, 0.1 mole) in 3N sulphuric acid (165 ml). A saturated aqueous solution of sodium nitrite (9.0 g) was then added dropwise over three hours, the internal temperature being maintained at -10°. The resulting mixture was diluted with water (50 ml) and extracted with ether (100 ml and 2 x 50 ml). The combined ether extracts were dried over magnesium sulphate and treated with an ethereal solution of diazomethane (158) until no further effervescence was observed. On evaporation of the ether, the yield of methyl 2-bromo-3-phenylpropanoate was 20.5 g (84.5%). For the next step, a portion was purified by distillation. The fraction b.p. 101-102° at 0.24 mm (lit. (162) b.p. 90° at 0.05 mm) was shown to be at least 99% pure by gas-liquid chromatography.

ii) Preparation of DL-phenylalanine-^{15}N

Potassium phthalimide-^{15}N (0.93 g, 0.005 moles, 96% ^{15}N)

was added to methyl 2-bromo-3-phenylpropanoate (1.35 g, 0.0055 moles) dissolved in redistilled dimethylformamide (3.0 ml). The solution was heated on a steam bath for three hours, then cooled to room temperature. The precipitated material was filtered off, washed with dimethylformamide (1 ml), and the combined filtrate and washings evaporated to dryness under reduced pressure. The residual solid was triturated with chloroform (4 ml) and left at 0^{o} overnight. The insoluble material was removed by filtration and washed with more chloroform (1 ml). The combined solids were washed with water to remove potassium bromide and yielded 0.316 g of uncondensed phthalimide. The chloroform solution was evaporated under reduced pressure to give the phthaloylaminoester-^{15}N (1.65 g) which was hydrolyzed without further purification.

The phthaloylaminoester was hydrolyzed by refluxing it with glacial acetic acid (2.7 ml) and 40% hydrobromic acid (2.7 ml) for 18 hours. The reaction mixture was cooled, and then diluted with an equal volume of water to precipitate phthalic acid which was filtered off and washed with water (1.5 ml). The filtrate plus washings were evaporated to dryness under reduced pressure. The solid residue of phenylalanine hydrobromide was dissolved in water (5 ml) and the free aminoacid was precipitated by addition of ammonia until the solution was neutral to bromocresol

purple. After the solution had stocd at 0° for six hours, the phenylalanine-^{15}N was filtered off and washed with alcohol until free of bromide ion. Yield 0.60 g (94%, allowing for recovered phthalimide).

α-oximino-β-phenylpropanoic acid-^{15}N

Phenylpyruvic acid (1.3 g. 0.0079 mole) was dissolved in ethanolic sodium hydroxide solution to which a few drops of water were added. An ice-cold solution of hydroxylamine-^{15}N hydrochloride (0.5 g, 98.4% ^{15}N) was added to this chilled solution. The mixture was left for 40 minutes at room temperature, acidified with concentrated hydrochloric acid and extracted with ether (3 x 10 ml). The combined ethereal extracts were evaporated to dryness at room temperature under reduced pressure and the residue purified by sublimation under vacuum (130°, 5 x 10^{-4} mm). Yield 0.96 g (75%) m.p. 165-166° (decomp.). Lit. m.p. 159-160° (decomp.)[159], 166-167° (decomp.).[103]

3. SULPHUR-35 LABELLED COMPOUNDS

Due to the comparatively short half-life of sulphur-35 (87 days), little was to be gained by assaying and reporting the activities of the materials at the time of preparation.

The activity was measured at the same time as the activity of the benzylthiourea (prepared from the isothiocyanate from the plant after administration of the labelled compound). In this way, correction of activities for decay was unnecessary. The source of the sulphur-35 for these two syntheses was carbon disulphide-^{35}S. Total activity used (at the time of the syntheses) was approximately 4 mc.

Sodium phenylacetothiohydroxamate-^{35}S

1) Sodium dithiophenylacetate-^{35}S

A stock solution of benzyl magnesium chloride solution was prepared from benzyl chloride (12.7 g) and magnesium (2.43 g), in ether, and standardized by titration immediately before use. The concentration was found to be 0.000718 moles/ml. Carbon disulphide-^{35}S (1.0 ml) was transferred from its break-seal container to a freshly dried flask, under vacuum with liquid nitrogen cooling. The reaction flask, still evacuated, was transferred to an ice-bath and the Grignard solution (25 ml) was added by syringe over a period of one hour with continuous magnetic stirring. After a further 20 minutes, the solution was acidified with dilute hydrochloric acid, extracted with ether (3 x 10 ml), and the combined ether extracts washed

with sodium hydroxide solution (a total of 25 ml containing 0.7 g of sodium hydroxide) to give an alkaline solution of sodium dithiophenylacetate-^{35}S.

ii) Sodium phenylacetothiohydroxamate-^{35}S

An aqueous solution (15 ml) containing hydroxylamine hydrochloride (1.1 g) neutralized with potassium carbonate (1.1 g) was added dropwise, with stirring, to an ice-cold solution of sodium dithiophenylacetate (prepared above). The solution was allowed to stand at room temperature for one hour, then acidified with dilute hydrochloric acid, and extracted with ether (3 x 10 ml). The ether extracts were combined, dried ($MgSO_4$) and evaporated to dryness at room temperature under vacuum. The residue was recrystallized from ether-petroleum ether to yield the free thiohydroxamic acid (0.286 g), m.p. 71-73° (lit.[131] 74-75°).

The sodium salt was prepared by dissolving the acid in ethanol and treating the solution with ethanolic sodium hydroxide until it was just alkaline to phenolphthalein. The solution was then evaporated to dryness and the residue recrystallized from ethanol/ether. Yield 0.290 g (11.7% overall).

β-D-1-glucopyranosyl mercaptan-^{35}S (sodium salt)

1) Potassium O-ethyl xanthate-^{35}S(163)

Potassium hydroxide (0.924 g) was heated under reflux with dry ethanol (7.5 ml) for one hour, by which time the solid had dissolved. After cooling, carbon disulphide-^{35}S (1.0 ml) was distilled in under vacuum at liquid nitrogen temperature. The mixture was stirred magnetically as it was allowed to warm to 0°. The almost solid mass was filtered on a sintered glass funnel and washed with anhydrous ether (3 x 2 ml). Yield of pale yellow crystals 1.975 g (77%). This was used directly in the next step.

ii) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl O-ethyl-xanthate(164)

Potassium ethyl xanthate-^{35}S (1.97 g, 0.0126 mole) was dissolved in ethanol (25 ml), and acetobromoglucose (5.27 g, 0.0128 mole, see page 107) was added. The reaction mixture was heated to boiling on a steam bath for five minutes, during which time potassium bromide precipitated. Cold water was added to the hot solution, first to dissolve the potassium bromide, and then until the solution just became turbid. The solution was cooled and stirred until crystallization commenced and left in a refrigerator until ice-cold. The crystals were filtered off, yield 4.9 g (86%). Recrystallization from ethanol gave 4.2 g, m.p. 88-89°. Lit.(164) m.p. 88-89°.

iii) β-D-1-Glucopyranosyl mercaptan-^{35}S (sodium salt)

Acetylglucosyl xanthate-^{35}S (4.2 g, 0.0094 mole) was dissolved in chloroform (10 ml), the solution cooled to -20° and then treated with a solution of sodium (0.43 g, 0.019 mole) in methanol (14.5 ml). The solution was allowed to warm until a gel formed, and was then replaced in the ice-bath for a further 5-8 minutes. Water (10 ml) was added, followed by acetic acid until the pH of the reaction mixture was 8. The aqueous layer was separated and evaporated to dryness under reduced pressure (bath temperature less than 40°). The residual oil was taken up in a small volume of methanol, a few drops of aqueous ethanol added, and the solution stirred until crystallization occurred. The crystals of the dihydrate of sodium glucosyl mercaptide were filtered off, washed with methanol and dried. Yield 2.19 g (almost theoretical), m.p. 177-178° (decomp.). Lit.[139] m.p. 175° (decomp.).

The overall yield based on carbon disulphide was 57%.

ISOTOPIC TRACER FEEDING EXPERIMENTS

1. LABELLED COMPOUNDS

All labelled compounds were synthesized (see previous section) apart from DL-phenylalanine-3-^{14}C and DL-valine-4-^{14}C, which were obtained from commercial sources.

2. ADMINISTRATION OF LABELLED COMPOUNDS

The labelled compound was dissolved in water (100 ml) and the pH adjusted, when necessary, to between 6 and 8. The leafy part of the plants was removed from the roots by a diagonal cut with a sharp knife just above soil level. A further cut was made when the stems were immersed in the feeding solution in order to avoid any air blockages in the stems. The plants were allowed to take up the solution for a period of 24-96 hours, after which they were worked up to isolate the isothiocyanate. The solution was assayed for material not absorbed.

3. ISOLATION OF THE ISOTHIOCYANATE

The plants were washed free of any adhering radioactive solution and macerated in a Waring Blendor. The homogenate was allowed to stand for one hour to allow enzymatic hydrolysis of the glucoside to take place. A few drops of concentrated hydrochloric acid were then added and the

mixture steam-distilled, the distillate being collected in a large excess of aqueous-ethanolic ammonia. The distillate was left at room temperature overnight to complete conversion of the isothiocyanate to thiourea, and then evaporated to dryness. The residue was recrystallized twice from aqueous ethanol.

4. DEGRADATION OF BENZYLTHIOUREA

Benzylthiourea was intimately mixed with five times its weight of anhydrous sodium carbonate and placed in the end of a sealed tube. A further 1-2" of sodium carbonate was packed into the tube. The mixture was heated strongly with a Bunsen burner and the benzylamine produced was condensed in a liquid nitrogen-cooled U-bend in the tube. The condensate was taken up in ether (5 ml) and extracted with 6N hydrochloric acid (2 x 5 ml). The aqueous, acidic solution was neutralized with sodium hydroxide solution and extracted with ether (2 x 5 ml). The combined ether extracts were re-extracted with 6N hydrochloric acid (2 x 5 ml). The aqueous solution was evaporated to dryness to yield benzylamine hydrochloride.

5. DETERMINATION OF ISOTOPES

Carbon-14

The samples were converted to carbon dioxide by the

Van Slyke-Folch method[165] in the apparatus shown in Figure 18. A weighed amount of solid sample or a measured aliquot of solution was mixed with a solid combustion reagent, and after evacuation of the apparatus, the liquid combustion reagent was added. On strong heating, carbon dioxide was evolved and swept into the ionization chamber by a stream of inactive, dry carbon dioxide. A condenser and a stannous chloride scrubber removed impurities. The chamber was filled with inactive carbon dioxide to a standard pressure and the carbon-14 content assayed on the Nuclear-Chicago Dynacon Model 6000 Vibrating Reed Electrometer by the rate of charge method.

Preparation of Combustion Reagents.

Dry reagent. Reagent grade potassium iodate (2 parts) was ground with reagent grade potassium dichromate (1 part) to give an intimate mixture.

Liquid reagent. Phosphoric acid (85% H_3PO_4, 33 ml) was cautiously mixed with fuming sulphuric acid (30% free SO_3, 67 ml). Potassium iodate (1 g) was added and the mixture heated until it dissolved. The solution was cooled and stored.

Standardization of Equipment.

The equipment was standardized using standard barium carbonate (Chemtrac 1-19A) which had an activity of 1304 d.p.m./mg. The results appear in Table VII and

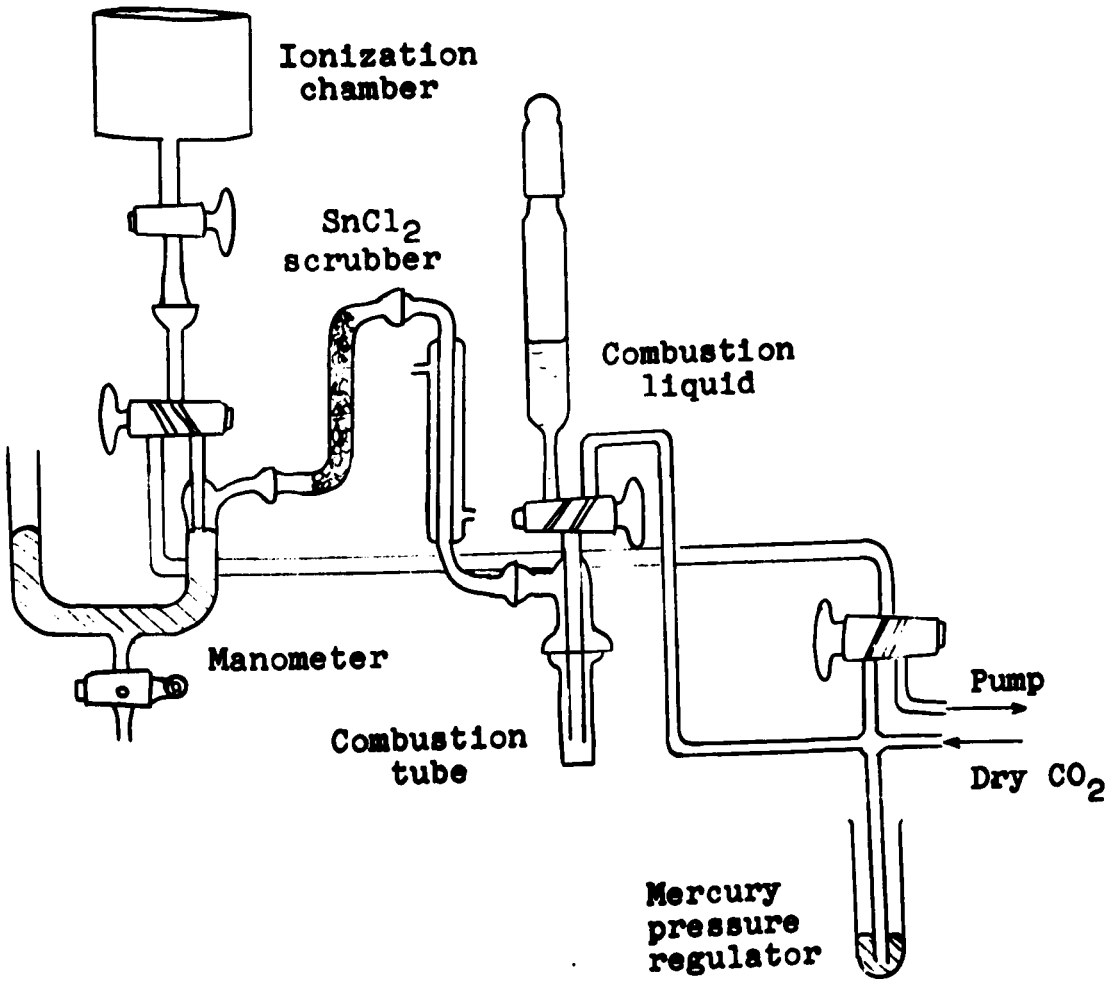


Figure 18

Apparatus for Combustion of Samples to Carbon Dioxide for Carbon-14 Determination

Table VII Calibration of Carbon-14 Counting Equipment

Sample Number	Wt. of $BaCO_3$ mg	d.p.m.	Measured current amps	Chamber Background current amps	Net current amps
1	16.4	21,390	4.56×10^{-14}	1.584×10^{-16}	4.54×10^{-14}
2	38.9	50,370	9.94×10^{-14}	1.584×10^{-16}	9.92×10^{-14}
3	63.9	83,400	1.670×10^{-13}	1.584×10^{-16}	1.668×10^{-13}
4	81.5	106,200	2.158×10^{-13}	1.691×10^{-16}	2.156×10^{-13}
5	109.1	142,300	3.025×10^{-13}	1.691×10^{-16}	3.023×10^{-13}

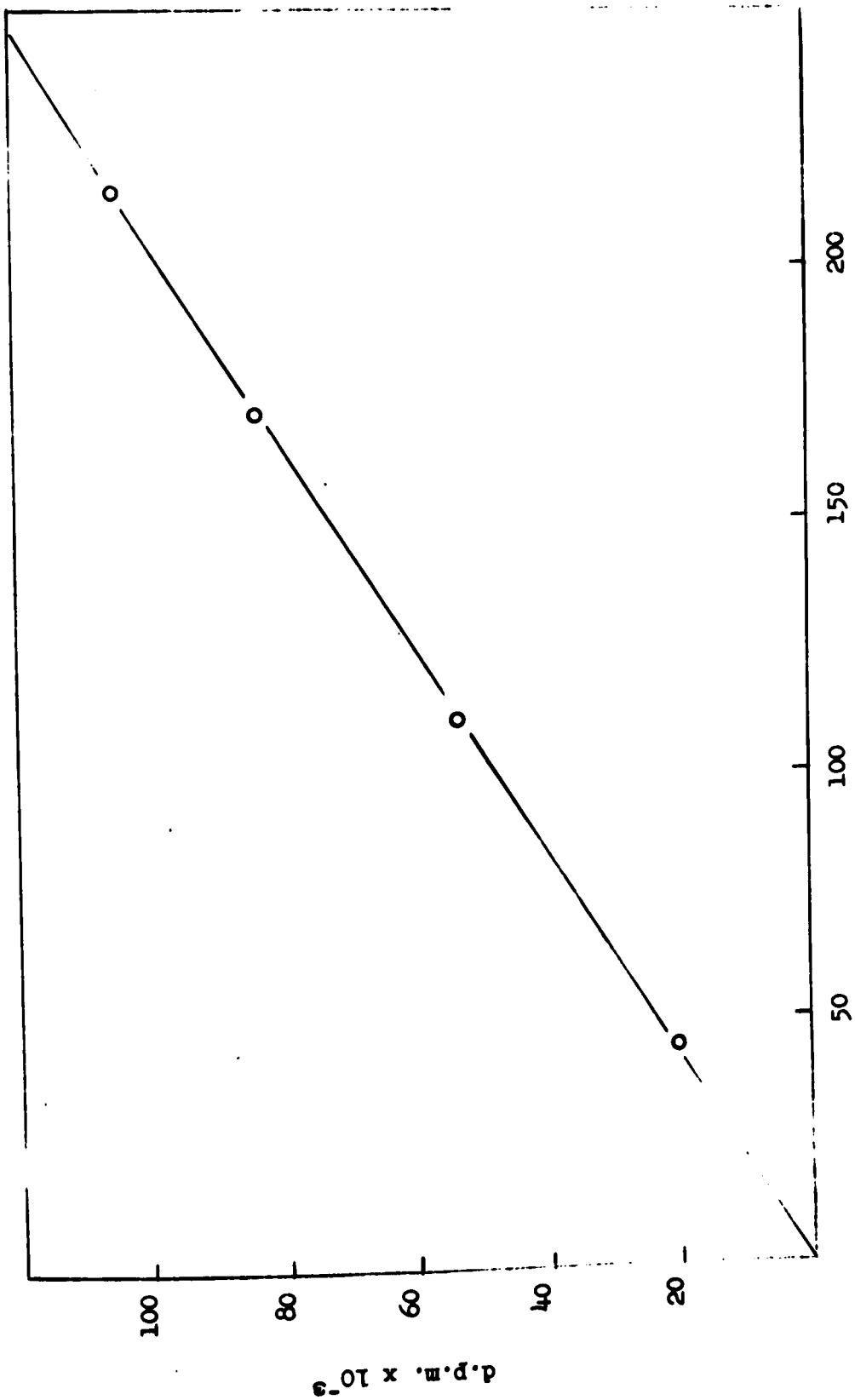


Figure 19 Calibration Graph for Dynacon Electrometer

Figure 19. From the graph, 1×10^{-15} amps is equivalent to 494 d.p.m. or 2.226×10^{-4} μc.

Nitrogen-15

The samples were converted to nitrogen gas by the method of Rittenberg(166)(167) and assayed with a mass spectrometer.

The sample (corresponding to about 2 mg of nitrogen) was subjected to a Kjeldahl digestion with concentrated sulphuric acid in the presence of selenium and mercuric sulphate. After distillation of the ammonia into 0.10 N sulphuric acid (5 ml), the ammonium sulphate solution was oxidized to nitrogen with sodium hypobromite in the apparatus shown in Figure 20.

$$2NH_4^+ + 2OH^- + 3NaOBr \longrightarrow N_2 + 3NaBr + 5H_2O$$

Preparation of sodium hypobromite reagent.

Bromine (50 ml) was added slowly with vigorous stirring to a 40% (by weight) solution (150 ml) of reagent grade sodium hydroxide held at 0°. Since hypobromite is more stable in strongly alkaline solution, a further 150 ml of 40% sodium hydroxide solution was added after the addition of bromine was complete. This solution could be kept for several months in the refrigerator without decomposition.

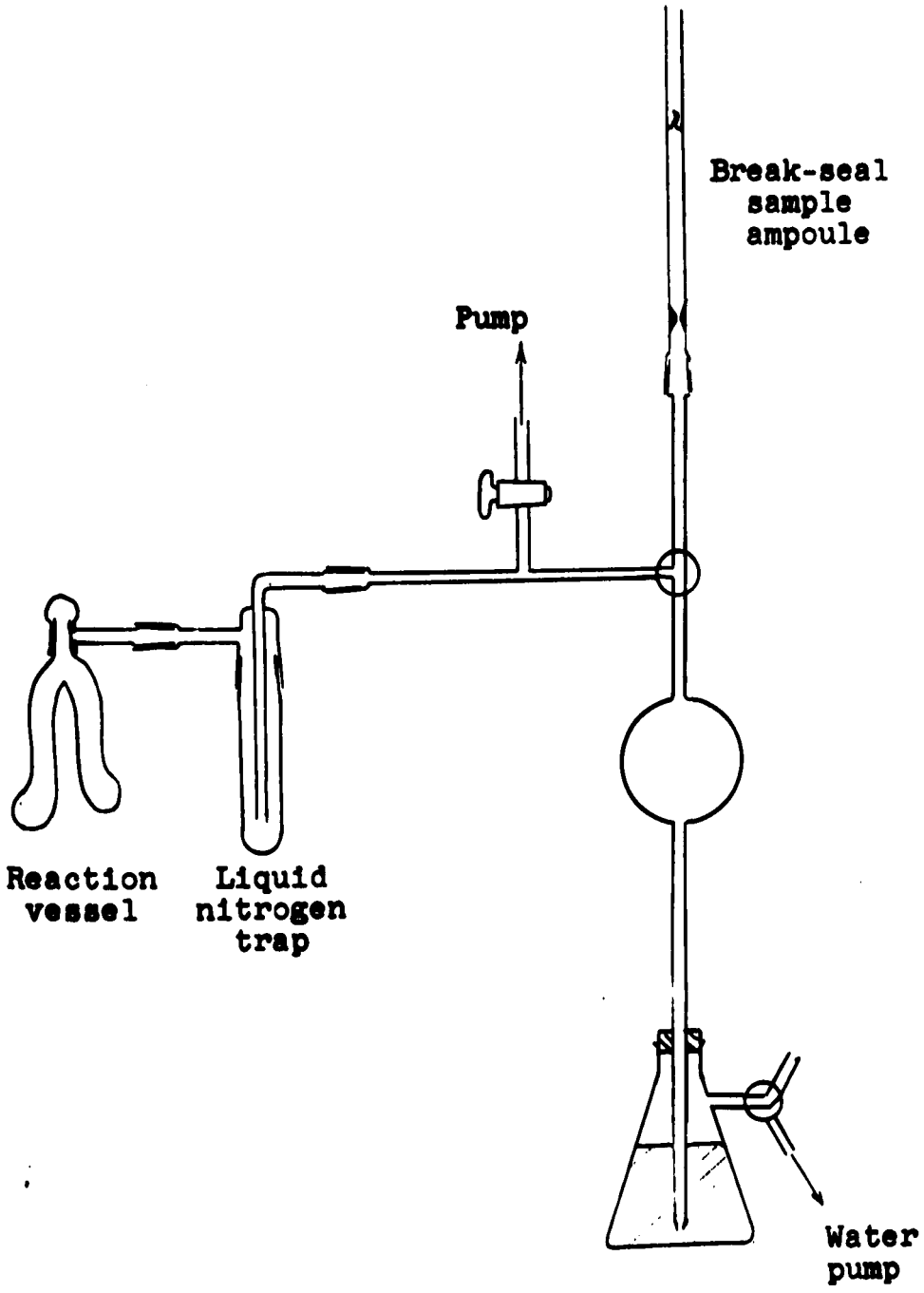


Figure 20

Apparatus for Preparation of Nitrogen Samples for Mass Spectrometric Analysis

The freshly prepared solution tended to liberate oxygen and precipitate sodium bromide, but this effect stopped after a few days and the clear stable solution was decanted off. 1 ml of this solution, diluted with an equal volume of water immediately before use, was sufficient to oxidize 10 to 12 mg of ammonia.

Sulphur 35

The activity of sulphur-35 was determined with a Nuclear Chicago Model 725 Liquid Scintillation spectrometer. A weighed amount of sample was dissolved in a suitable solvent, and a standard amount of the fluors (PPO, 2,5-diphenyloxazole and POPOP, 2-p-phenylene-bis-(5-phenyl-oxazole)) in toluene solution was added. Corrections to counting efficiency were made by reference to standard samples.

SYNTHESIS OF ω-THIOMETHYL-α-AMINOACIDS AND DERIVATIVES

5-Thiomethylhexyl chloride

A solution of sodium thiomethylate (prepared from 3.7 g of sodium in 50 ml of ethanol and excess methyl mercaptan) was added dropwise to ice-cold 1,6-dichlorohexane (25.0 g). When addition was complete, the ice-bath was removed and the internal temperature rose rapidly with precipitation of sodium chloride. After a further one hour, the reaction mixture was filtered and the filtrate concentrated. Fractional distillation of the crude product gave 6-thiomethylhexyl chloride (14.9 g, 56%, b.p. 104-108°/8 mm.). Some dichlorohexane and dithiomethylhexane were also obtained.

5-Thiomethylpentyl chloride

This was prepared in 63% yield in an identical manner to the 6-thiomethylhexyl chloride. The boiling point was 84-86°/8 mm. (lit.[127] b.p. 94°/15 mm.).

Condensation of Chlorides with Diethyl Sodiophthalimidomalonate and Diethyl Sodioacetamidomalonate

Equimolar amounts of the thiomethylalkyl chloride and the sodium salt of the malonate derivative were refluxed in

ethanol solution for periods of from 3 to 18 hours. Water was added to the cooled solution which was then extracted with chloroform. The chloroform solution was dried ($MgSO_4$) and the chloroform evaporated, leaving a viscous, dark brown oil. Attempted crystallization yielded some pale yellow crystals from benzene-petrol which were shown by melting point (93-95°), mixed melting point and infrared spectrum to be unreacted malonate derivative.

The crude oil was hydrolyzed by boiling with 10% sodium hydroxide solution, followed by acidification and boiling for a further 30 minutes. The aqueous layer was washed with ether and evaporated to dryness under reduced pressure. Examination of the crystalline residue by paper chromatography (ascending, Whatman No. 1 paper, 1-butanol: acetic acid:water, 4:1:3, solvent system) showed it to contain glycine, and in one case another aminoacid. This had an R_F value of 0.69 and was presumed to be 8-thiomethyl-2-aminooctanoic acid. There was insufficient present to isolate.

6-Thiomethylhexylbromide

A solution of sodium thiomethylate (prepared from 4.6 g sodium in 150 ml of ethanol and an excess of methyl mercaptan was added dropwise to 1,6-dibromohexane (49 g). After one hour, the precipitated sodium bromide was filtered

off and the ethanol evaporated from the filtrate under reduced pressure. An attempt to distil the product under vacuum gave a small amount of cloudy distillate, with the remainder solidifying in the still-pot.

The solid residue was insoluble in water, ether and ethanol, but very soluble in carbon tetrachloride. On recrystallization from the latter, a white waxy solid was obtained which gave a positive sulphur test, but was negative for halogen. Its molecular weight in benzene (by osmometer) was approximately 136. Its infrared spectrum had no distinguishing features but was very similar to that of thiacycloheptane.[123] The NMR spectrum with two multiplets at 1.35τ and 2.40τ was also very similar to that of thiacyclohexane.[122]

5-Thiomethylpentyl bromide

This was prepared from 1,5-dibromopentane by the method used for 6-thiomethylhexyl bromide. The distillation was carried out under high vacuum, but the clear distillate rapidly deposited crystals of methyl thiacyclohexyl sulphonium bromide (identified by elemental analysis, solubility, infrared and NMR). The liquid eventually became completely solid.

Dibromopentane

1,5-pentanediol (175 g) was added to a mixture of 48% hydrobromic acid (600 ml) and concentrated sulphuric acid (205 ml) and the mixture heated under reflux for two hours. The mixture was then left overnight at room temperature, by which time the mixture had separated into two phases. The lower dibromide layer was separated, washed successively with water, sodium carbonate solution and water, dried ($MgSO_4$) and fractionally distilled under reduced pressure, collecting the fraction b.p. 99-100°/ 13 mm. Yield of dibromopentane 269 g (70%), shown by VPC to be homogeneous.

Diethyl 2-(5-thiomethylpentyl)-2-phthalimidomalonate

Sodium (4.3 g, 0.184 mole) was dissolved in dry ethanol (100 ml) and diethyl phthalomidomalonate (56 g, 0.184 mole) added to give a deep yellow solution. Dibromopentane (154.8 g, 0.675 mole) was added and the mixture allowed to reflux overnight. The ethanol was distilled off, and distillation continued under reduced pressure to remove the excess dibromopentane (121 g). The residue was taken up in ethanol and filtered to remove sodium bromide (16 g). The crude material was treated with sodium thiomethylate in ethanol until no further precipitation of sodium bromide occurred. The

precipitate (12 g) was filtered off and the ethanol removed under reduced pressure. No further purification was carried out.

2-Amino-7-thiomethylheptanoic acid

The substituted malonate was hydrolyzed by refluxing for one hour with 10% aqueous sodium hydroxide solution (125 ml), acidifying with concentrated hydrochloric acid, and refluxing for a further 30 minutes. Some organic phase remained which was taken up in ether and separated from the aqueous layer, which was then evaporated under reduced pressure. Four crops of crystals were removed as the solution was concentrated, the last two of which (total weight 6.5 g) gave positive nitrogen and sulphur tests. Recrystallization from water gave 2-amino-7-thiomethyl-heptanoic acid, which was shown by paper chromatography to contain a trace of glycine. A further recrystallization from ethanol removed this and gave 3.2 g (9.2% based on diethyl phthalimidomalonate) of thin white plates, m.p. 227-229° (decomp.).

Analysis $C_8H_{17}O_2NS$

Calculated	C, 50.24%	H, 8.90%	N, 7.32%	S, 16.76%
Found	C, 50.66%	H, 8.90%	N, 7.20%	S, 16.76%

Paper chromatography on Whatman No. 1 paper using the ascending technique with a 1-butanol:acetic acid:water

(4:1:3) solvent system gave a single spot, R_F value = 0.62. The infrared spectrum is given in Appendix 2.

2-Amino-7-methylsulphinylheptanoic acid

2-Amino-7-thiomethylheptanoic acid (0.191 g, 1.0 mmole) was dissolved in glacial acetic acid (1 ml) and 30% hydrogen peroxide (0.13 ml, 1.1 mmole) added. The solution was allowed to stand overnight at room temperature, cooled in ice, and ethanol (2 ml) and ether (1 ml) were added. The slurry was left in the refrigerator overnight to complete precipitation, the white crystals were filtered off, and recrystallized from water-ethanol. Yield of fine, white needles was 0.143 g (69%), m.p. 216-219° (decomp.).

Analysis $C_8H_{17}O_3NS$

Calculated	C, 46.35%	H, 8.27%	N, 6.76%	S, 15.47%
Found	C, 46.29%	H, 7.85%	N, 6.80%	S, 15.82%

The infrared spectrum (potassium bromide pellet), reproduced in Appendix 2, shows the characteristic sulphoxide peak at 1021 cm^{-1}. Paper chromatography (ascending) on Whatman No. 1 paper with 1-butanol: acetic acid:water (4:1:3) as the solvent system gave a single spot, R_F 0.19.

2-Amino-7-methylsulphonylheptanoic acid

2-Amino-7-thiomethylheptanoic acid (0.191 g, 0.001 mole) was dissolved in glacial acetic acid (1 ml) and 30% hydrogen peroxide (0.5 ml, 0.004 moles) added. The mixture was left overnight at room temperature, cooled in ice, and the aminoacid precipitated by the addition of ethanol (2 ml) and ether (2 ml). Precipitation was completed by leaving overnight in the refrigerator. After filtration, the crystals were recrystallized from aqueous ethanol to give thin, white plates (0.177 g, 80%), m.p. 226-228° (decomp.).

Analysis $C_8H_{17}O_4NS$

Calculated	C, 43.03%	H, 7.68%	N, 6.27%	S, 14.36%
Found	C, 42.91%	H, 7.76%	N, 6.07%	S, 14.41%

The infrared spectrum (potassium bromide pellet), reproduced in Appendix 2, shows the characteristic sulphone peaks at 1126 and 1298 cm^{-1}. Paper chromatography (ascending) on Whatman No. 1 paper with 1-butanol:acetic acid:water (4:1:3) as the solvent system gave a single spot R_F 0.21.

SYNTHESIS OF MUSTARD OIL GLUCOSIDES

1. GENERAL REAGENTS

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl mercaptan

Potassium O-ethyl xanthate

Potassium O-ethyl xanthate was prepared by the method of Vogel,[163] using 14.0 g (0.25 mole) of potassium hydroxide and 19.0 g (0.25 mole) of carbon disulphide. Yield of pale yellow, fluffy xanthate was 27.7 g (71%).

2,3,4,6-Tetra-O-acetyl-β-D-glucosyl bromide (acetobromo-glucose.

The method of Bárczai-Martos and Korosy[168] was used. Glucose (100 g) was added to acetic anhydride (400 ml) containing perchloric acid (2.5 ml) keeping the temperature between 30 and 40°. Then red phosphorus (30 g), bromine (180 g) and water (36 ml) were added sequentially, with the temperature kept below 20°. On work-up, 151 g (66%) of recrystallized acetobromoglucose, m.p. 85-86° (lit.[168] 87°) was obtained. It was stored in a desiccator until use.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl O-ethyl xanthate

Potassium ethyl xanthate (9 g) in hot ethanol was treated with acetobromoglucose (23 g).[164] Yield of pale yellow glucosyl xanthate was 19.8 g (79%), m.p. 88-89°

(lit.[164] m.p. 88-89°).

Sodium β-D-1-glucopyranosyl mercaptide[164]

Treatment of a chloroform solution of the glucosyl-xanthate (12.5 g) with a solution of sodium (1.2 g) in absolute methanol gave a theoretical yield of the dihydrate of the sodium salt of glucosyl mercaptan (6.1 g), m.p. 198° (decomp.). Lit.[139] 175° (decomp.).

Octa-O-acetyl-di-β-D-glucopyranosyldisulphide

The sodium salt of glucosyl mercaptan (6.0 g) was oxidized with iodine (3.5 g) using the procedure of Schneider and Bansa.[169] This was followed by acetylation with an acetic anhydride/pyridine mixture. Recrystallization from ethanol gave a 71% yield (7.2 g) of white crystalline material, m.p. 143-144° (lit.[169] 144°).

Preparation of Active Amalgam[170]

Aluminum foil (4 g), cut into small pieces, was covered with 0.2 N sodium hydroxide solution (100 ml). Hydrogen evolution was brisk. After 30 minutes, a 0.5% solution of mercuric chloride in water (50 ml) was added with stirring, followed by the dropwise addition of a 1% solution of sodium cyanide in water until the mercuric oxide just redissolved. The amalgam was drained, rinsed with water

(2 x 100 ml) and ethanol (2 x 100 ml) and used immediately. (The material was extremely reactive and rapidly became hot after the final decantation of ethanol).

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl mercaptan

Octaacetyldiglucosyldisulphide (4.0 g) was reduced with aluminum amalgam/acetic acid as described by Schneider and Bansa.[169] Total yield of mercaptan was 3.38 g (84%), m.p. 116-117°, (lit. 112-113°,[168] 75°[171]).

Pyridine-sulphur trioxide complex[172]

A solution of dry pyridine (31 g) in chloroform (175 ml) was placed in a three-necked flask cooled in an ice-salt mixture. Chlorosulphonic acid (reagent grade from a freshly opened bottle, 20 g) was added dropwise with stirring at such a rate as to maintain the temperature (internal thermometer) at 0° ± 3°. When addition was complete (1½ hours), the reaction mixture was filtered, the finely divided white precipitate washed with ice-cold chloroform (3 x 30 ml) and then dried over concentrated sulphuric acid in a vacuum desiccator. Yield 25.6 g, m.p. 171-172° (lit.[173] m.p. 175°).

This material undergoes fairly rapid decomposition, the life-time when stored in a desiccator being about 1-2

months. On decomposition it becomes freely soluble in water.

2. GLUCOPUTRANJIVIN

Isobutyraldoxime

Pyridine (2 ml) and hydroxylamine hydrochloride (5.0 g) were added to a solution of isobutyraldehyde (5.0 g) in ethanol (50 ml) and the mixture refluxed for one hour on the steam bath. The ethanol was evaporated under reduced pressure, water (20 ml) added, and the reaction mixture extracted with ether (3 x 25 ml). The combined, dried ($MgSO_4$) ether extracts were evaporated and the residual oil fractionally distilled, the fraction b.p. 137-141° being collected. Yield 4.4 g (72%). Lit.[174] b.p. 140°.

Isobutyrohydroxamoyl chloride

Chlorine was bubbled through a sulphuric acid drying bottle into a solution of the oxime (2.0 g) in anhydrous ether (50 ml), cooled in an ice-salt mixture, for 30 minutes. The solution passed through varying shades of deep blue and finally became green. The ether was evaporated under reduced pressure at room temperature, at the same time removing excess chlorine, and the residual

oil used immediately in the next step.

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-isobutyro-thiohydroximic acid (tetraacetylproglucoputranjivin)

The partially solidified, oily hydroxamoyl chloride dissolved in ice-cold anhydrous ether (50 ml) was added to a solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl mercaptan (1.0 g) dissolved in cold, dry ether, followed by triethylamine (2.8 ml) dissolved in dry ether (10 ml). A flocculent precipitate of triethylamine hydrochloride was rapidly formed and the blue colour of the solution was discharged. After 30 minutes the reaction mixture was washed with 1N sulphuric acid (50 ml) to remove triethylamine and triethylamine hydrochloride. The ether phase was separated, dried (magnesium sulphate) and evaporated to yield a colourless oil which crystallized from the concentrated ethereal solution on addition of petroleum ether. Recrystallization from ether-petroleum ether gave colourless needles of tetraacetylprogluco-putranjivin, m.p. 93-95° (0.75 g, 60%). Further recrystallization raised the m.p. to 98-100°.

$[\alpha]_D^{25}$ -29° (c, 3 in chloroform)

Analysis. $C_{18}H_{27}NO_{10}S$

Calculated	C, 48.10%;	H, 6.06%;	N, 3.12%
Found	C, 48.31%;	H, 6.09%;	N, 3.29%

2,3,4,6-Tetra-O-acetylglucoputranjivin

Freshly prepared sulphur trioxide-pyridine complex (0.42 g) was added to a solution of tetraacetylprogluco-putranjivin (0.37 g) in dry pyridine (5 ml) and the reaction mixture stirred at room temperature overnight with exclusion of moisture. The reaction mixture was poured into water (about 20 ml), containing potassium bicarbonate (0.75 g). After effervescence ceased, the aqueous solution was washed with ether (2 x 20 ml), the combined ether extracts washed with water (2 x 20 ml), and the combined aqueous solution freeze-dried. The residual solids were extracted with boiling 95% (v/v) ethanol and filtered hot. On cooling, the filtrate deposited fine colourless needles (0.22 g, 47%, m.p. 180.5-182°, $[\alpha]_D^{25}$-18.3° (c, 1.6, H_2O). Lit.[175] $[\alpha]_D^{23}$-15.4° (c, 1.2, H_2O). The infrared spectrum in a KBr disc, shown in the appendix, was superimposable on that of an authentic specimen of natural origin.

Glucoputranjivin

Tetraacetylglucoputranjivin (0.95 g) was dissolved in anhydrous methanol previously saturated with ammonia, and

kept at room temperature overnight. The solution was evaporated to dryness under reduced pressure to give a gum which refused to crystallize. The gum was dissolved in water and passed through a Dowex 50 ion-exchange column (in the tetramethylammonium form). The aqueous eluates on freeze-drying yielded a gummy residue of tetramethylammonium glucoputranjivin which once again refused to crystallize. Paper chromatography (ascending, Whatman No. 1 paper), using the upper phase of 1-butanol:acetic acid:water (4:1:5) as eluant, yielded a single yellow spot (R_F 0.094) on an orange background on development. (The development was carried out by spraying with 0.02N silver nitrate solution, drying at 100°, and respraying with 0.02N potassium dichromate solution.[176][177])

3. GLUCOLEPIDIIN

Propionaldoxime

Propionaldehyde (10 g) dissolved in ethanol (50 ml) was treated with hydroxylamine hydrochloride (10 g) and pyridine (5 ml) and the solution refluxed for one hour. The ethanol was evaporated under reduced pressure, water (20 ml) added, and the mixture extracted with ether (3 x 25 ml). The residual oil obtained on evaporation of the dried ($MgSO_4$) extracts was fractionally distilled, the

fraction b.p. 128-132° being collected. Yield 8.4 g (67%). Lit.[178] b.p. 131.5°.

Propionohydroxamoyl chloride

Propionaldoxime (2.0 g) in anhydrous ether (50 ml) was treated with dry chlorine for 30 minutes at 0°. The colour changed through deep blue to green. The ether was removed under reduced pressure at room temperature to give a colourless solid used directly in the next step.

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-propionothiohydroximic acid (tetraacetylproglucolepidiin)

The hydroxamoyl chloride was taken up in ether (20 ml), cooled to 0°, and treated with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl mercaptan (1.0 g) dissolved in dry ether (200 ml). On addition of triethylamine (2.8 ml), rapid precipitation of triethylamine hydrochloride occurred. After 30 minutes, the reaction mixture was poured into 1N sulphuric acid (50 ml), extracted with ether (2 x 25 ml), and the ether extracts dried over magnesium sulphate. On evaporation of the ether, crystals of tetraacetylproglucolepidiin separated. The residue was taken up in boiling ether and reprecipitated by the addition of petroleum ether. Yield 1.03 g (86%), m.p. 155-156°.

Analysis $C_{17}H_{25}NO_{10}S$

Calculated	C, 46.89%;	H, 5.79%;	N, 3.22%;	S, 7.36%
Found	C, 47.07%;	H, 6.04%;	N, 3.25%;	S, 7.27%

2,3,4,6-Tetra-O-acetylglucolepidiin

Tetraacetylproglucolepidiin (0.209 g) was treated with pyridine-sulphur trioxide complex (0.23 g) in dry pyridine (3 ml) and left overnight at room temperature. A solution of potassium bicarbonate (0.5 g) in water (15 ml) was added, and after effervescence ceased, the aqueous solution was washed with ether (2 x 10 ml), the combined ether extracts washed with water (5 ml) and the combined aqueous extracts freeze-dried. The residue was recrystallized from 95% ethanol to yield fine white needles (0.19 g, 72%), m.p. 200-201°.

Analysis $C_{17}H_{24}NO_{13}S_2K$

Calculated	C, 36.88%;	H, 4.37%;	N, 2.53%;	S, 11.58%;	K, 7.07%
Found	C, 37.45%;	H, 4.30%;	N, 2.86%;	S, 11.66%;	K, 7.30%

Glucolepidiin

Tetraacetylglucolepediin (0.156 g) was dissolved in dry methanol (20 ml) saturated with ammonia and left overnight at room temperature. The solution was evaporated under reduced pressure to dryness and yielded an oil which was recrystallized from ethanol. Yield 0.077 g (71%),

m.p 180-182°. The infrared spectrum is given in Appendix 2.

Paper chromatography, as described under glucoputranjivin (page113) gave a single spot, R_F = 0.13.

4. ATTEMPTED SYNTHESIS OF GLUCOIBERIN

3-Chloropropanol(179)

1,3-Propanediol (about 25 g) was placed in a 100 ml round-bottomed flask, equipped for distillation and heated by means of an oil bath maintained at 150-170°. A rapid stream of dry hydrogen chloride was passed in and a yellow distillate passed over. As the diol in the flask was used up, more was added. When a total of 100 g of diol had been used, the distillate was heated on a steam bath for one hour to drive out most of the hydrogen chloride and then fractionated under reduced pressure. The material boiling at 60-65°/10 mm. was collected. The yield was 57 g (46%). Lit.(179) b.p. 60-64°/10 mm.

1-Bromo-3-chloropropane

3-chloropropanol (57 g) was treated with 48% hydrobromic acid (200 ml) and concentrated sulphuric acid (75 ml) and left overnight at room temperature. The reaction mixture was then heated on a steam bath for two hours, the

organic layer separated and the aqueous phase washed with ether (2 x 50 ml). After drying ($MgSO_4$), the combined organic layers were concentrated and fractionally distilled collecting the fraction b.p. 135-137°/660 mm. (lit.[180] 143°). The yield was 75 g (79%).

4-Chlorobutyronitrile[181]

Potassium cyanide (37 g, 0.55 mole) and water (75 ml) were warmed and stirred until the cyanide was completely dissolved. To this solution was added ethanol (250 ml), followed by 1-chloro-3-bromopropane (75 g, 0.47 mole) and the mixture heated on a steam bath for 1½ hours. The solution was cooled, diluted with water (400 ml) and extracted with chloroform (2 x 50 ml). The chloroform solution was washed with 20% calcium chloride solution (50 ml), water (50 ml) and dried over calcium chloride. The dried solution was fractionally distilled, collecting the fraction b.p. 80-82°/13 mm. (lit.[181] 93-96°/ 26 mm.). The yield was 24.8 g (52%).

4-Thiomethylbutyronitrile[182]

4-Chlorobutyronitrile (24.8 g, 0.24 mole) was treated with an ice-cold solution of sodium thiomethylate (from 6 g sodium and 12 g methyl mercaptan) in absolute ethanol (150 ml). After 0.5 hour, the mixture was heated on a

steam bath for 1.5 hour. After filtering to remove sodium chloride, the ethanol was removed under reduced pressure and the residual oil fractionally distilled, collecting the fraction b.p. 93-95° at 13 mm. (Lit.[182] 218° at atmospheric pressure). The yield was 24.1 g (87%).

Ethyl 4-thiomethyliminobutyrate hydrochloride

Dry hydrogen chloride was bubbled through an ice-cold solution of 4-thiomethylbutyronitrile (24.1 g, 0.21 mole) in absolute ethanol (19.3 g, 0.42 mole) until 9.1 g (0.25 mole) had been absorbed. The resulting solution was allowed to stand for three days in the refrigerator after which dry ether (200 ml) was added. After a further 18 hours at 0°, the solution was cooled to -30° and filtered. The pale-yellow crystals were dried in a vacuum desiccator over potassium hydroxide and phosphorus pentoxide. The solid was then triturated with dry ether at -40° and again filtered and dried. Yield 34.8 g (84%).

Ethyl 4-thiomethylorthobutyrate (1,1,1-triethoxy-4-thiomethylbutane)

The iminoester hydrochloride (34.8 g, 0.176 mole) was stirred in absolute ethanol (156 g, 2.0 mole) until solution was complete. Anhydrous ether (400 ml) was then added and the resulting solution heated under reflux for

18 hours. The reaction mixture was cooled to 0° and the precipitated ammonium chloride filtered off. The filtrate was washed with an equal volume of 10% sodium carbonate solution and dried over potassium carbonate. The ether was evaporated under reduced pressure and the residual oil fractionated. The fraction, b.p. 68-72° at 0.7 mm. was collected and shown by VPC to be homogeneous. Yield, 25.3 g (61%).

4-Thiomethylbutanal diethylacetal (1,1-diethoxy-4-thiomethylbutane)

Lithium aluminum hydride (3.1 g, 0.08 mole) suspended in dry ether (100 ml) was added to a stirred solution of the orthoester (25.3 g, 0.107 mole) in sodium-dried benzene (100 ml) at such a rate as to maintain steady boiling. After addition was complete, the viscous mixture was refluxed for a further two hours. The complex was then cautiously decomposed with a 30% aqueous solution of Rochelle salt (150 ml). The benzene layer was separated and the aqueous phase extracted with ether (100 ml). The combined organic solutions were dried ($MgSO_4$), the solvent evaporated under reduced pressure and the residual oil fractionally distilled, collecting the fraction b.p. 48-50° at 0.1 mm. Lit.[183] 88° at 1.5 mm. Yield, 14.6 g (71%).

Hydrolysis of 4-thiomethylbutanal diethylacetal

4-Thiomethylbutanal diethylacetal (14.6 g) was refluxed, with vigorous stirring, for 30 minutes with 0.5N hydrochloric acid (30 ml). The reaction mixture was extracted with ether (2 x 25 ml), and the ether extracts dried ($MgSO_4$) and concentrated. Examination of the concentrated solution by VPC showed that no hydrolysis had occurred. The procedure was repeated using 6N hydrochloric acid (30 ml) and refluxing for 3 hours. Once again, no hydrolysis occurred. The acetal was then treated with 6N sulphuric acid (30 ml), with the addition of dioxan (10 ml) until the mixture was homogeneous. This solution was refluxed for one hour, and worked up as before. Only very high boiling material remained after evaporation of the ether.

Methyl 4-thiomethylbutyrate[184]

Methyl mercaptan (10 g, 0.21 mole) was added to an ice-cold solution of sodium (4.4 g, 0.19 mole) in anhydrous methanol (100 ml). The methanol was removed by distillation and toluene was added to maintain the volume. The distillation was continued until the boiling point rose to that of toluene (111°). The suspension was then cooled to 40° and butyrolactone (17.2 g, 0.20 mole) was added with stirring. On heating to boiling, the reaction mixture solidified to a gel. Heating was continued for

one hour, the solid mass being broken occasionally. The reaction mixture was filtered, the filter-cake washed with ether and then taken up in 2N hydrochloric acid (200 ml). The oil which separated was collected and the acidic, aqueous solution was extracted with ether (2 x 50 ml). The ether extracts were combined with the oil, dried ($MgSO_4$) and filtered. To this solution was added an ethereal solution of diazomethane[158] until esterification was complete (termination of effervescence and development of a yellow colour by the ether solution), a few drops of acetic acid were added to destroy the excess diazomethane and the ether evaporated under reduced pressure. The residual oil was distilled, collecting the fraction b.p. 85-87° at 13 mm. The yield of ester was 20.2 g (68%), and the product was homogeneous on VPC analyses.

4-Thiomethylbutanol

A solution of methyl 4-thiomethylbutyrate (20.2 g, 0.14 mole) in anhydrous ether (100 ml) was added dropwise to a stirred suspension of lithium aluminum hydride (3.1 g, 0.08 mole) in anhydrous ether (100 ml) at a rate sufficient to maintain steady boiling. After addition was complete, the reaction mixture was refluxed one hour, after which ethyl acetate was added to decompose the excess lithium aluminum hydride. This was

followed by ice-cold 6N sulphuric acid until all the solid was dissolved (approximately 50 ml required). The ether layer was separated, and the aqueous layer extracted with ether (3 x 25 ml). The combined ether extracts were dried ($MgSO_4$), concentrated, and fractionally distilled under high vacuum. The fraction b.p. 73-75° at 0.3 mm was collected. The yield was 15.8 g (94%) with n_D^{27} 1.4816. Lit.[185] b.p. 81-85° at 3 mm., n_D^{20} 1.4856.

Oxidation of 4-thiomethylbutanol with dicyclohexylcarbodiimide

Dicyclohexylcarbodiimide (6.18 g, 0.03 mole) was added to a solution of 4-thiomethylbutanol (2.4 g, 0.02 mole) and anhydrous phosphoric acid (0.49 g, 0.005 mole) in anhydrous dimethyl sulphoxide (30 ml). A rapid exothermic reaction took place with formation of a copious precipitate of dicyclohexylurea. The mixture was left at room temperature for 18 hours and then filtered. The filter cake was washed with dimethyl sulphoxide (10 ml) and ether (10 ml). The combined filtrates were poured into water (100 ml) and extracted with ether (3 x 25 ml). The ether extracts were dried ($MgSO_4$) and concentrated. VPC analysis on this crude product showed four components, with unreacted alcohol being present in the largest amount, and two peaks either of which could be the desired

4-thiomethylbutanol, present only in minor amounts. Reaction of the crude material with 2,4-dinitrophenylhydrazine gave a small amount of 2,4-dinitrophenylhydrazone derivative. This on recrystallization from aqueous ethanol had a m.p. 94.5-95°.

Analysis:

Calculated for 4-thiomethylbutanal 2,4-dinitrophenylhydrazone, $C_{11}H_{14}O_4N_4S$ C, 44.3% H, 4.70% N, 18.78%

Found C, 44.57% H, 5.23% N, 19.11%

Fractional distillation of the mixture was attempted, but with only slight concentration of the lower boiling components.

The reaction was repeated on a larger scale, with a greater proportion of dicyclohexylcarbodiimide, and the addition of benzene as diluent. Dicyclohexylcarbodiimide (26.0 g, 0.125 mole) was added to an ice-cold solution of 4-thiomethylbutanol (8.0 g, 0.068 mole) and anhydrous phosphoric acid (1.5 g, 0.015 mole) in anhydrous dimethyl sulphoxide (40 ml) and benzene (80 ml). The reaction mixture was cooled in ice during the initial exothermic period, and then kept at room temperature for three hours. After work-up as before, the crude product was fractionally distilled under reduced pressure. The yield of aldehyde (b.p. 80-82° at 19 mm.) was 0.9 g (11%).

4-Thiomethylbutanal oxime

The aldehyde (0.9 g, 0.0077 mole) was added to a solution of hydroxylamine hydrochloride (1.0 g, 0.0145 mole) and pyridine (3 ml) in ethanol (20 ml). The mixture was left overnight at room temperature, poured into water (50 ml) and extracted with ether (3 x 10 ml). The dried ($MgSO_4$) extracts were concentrated and fractionally distilled under reduced pressure. The yield of oxime (b.p. 114°/12 mm.) was 0.5 g (49%). $C_6H_{11}NOS$ requires N, 10.52%. Found 10.39%.

4-Methylsulphinylbutyrohydroxamoyl chloride

4-Thiomethylbutanal oxime (0.5 g) in 6N hydrochloric acid (20 ml) was treated with chlorine for 30 minutes at 0°. The solution was diluted with water to 100 ml and extracted with ether (3 x 25 ml). The ether solution was concentrated to half-volume (also to remove dissolved chlorine) and dried ($MgSO_4$).

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-methylsulphinylbutyrothiohydroximic acid (tetraacetylproglucoiberin)

The pale blue, ethereal solution of 4-methylsulphinylbutyrohydroxamoyl chloride was treated with a solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl mercaptan (0.2 g)

in ether (100 ml). On addition of triethylamine (2 ml) rapid precipitation of triethylamine hydrochloride occurred. After 30 minutes, the reaction mixture was poured into 1N sulphuric acid (50 ml), extracted with ether (2 x 25 ml), and the ether extracts dried ($MgSO_4$). Evaporation of the ether gave an oil which crystallized from an ether-petrol mixture, giving white prisms (0.13 g). The melting point (143°) and infrared spectrum of this material were identical with those of octa-O-acetyl-diglucosyldisulphide. None of the desired product was isolated.

5. THIOHYDROXIMATES FROM ALIPHATIC NITROCOMPOUNDS

S-(2,3,4,6-Tetra-O-acetyl-β-D-1-glucopyranosyl)-acetothiohydroximic acid (tetraacetylproglucocapparin)

Nitroethane (8 g, 0.106 mole) was treated with sodium ethoxide (0.1 mole) in ethanol (100 ml) and then diluted to a total volume of 600 ml with anhydrous ether. The slurry was filtered and washed with anhydrous ether (200 ml). The pale yellow sodium salt was dried overnight in vacuum. Yield, quantitative.

The nitrosalt (1.0 g, 0.010 mole) was added over a two minute period to a well stirred, ice-cold, solution of lithium chloride (4.0 g) in 37% hydrochloric acid (20 ml). After 10 minutes of stirring, the mixture was poured into

ice-water (150 ml) and extracted with ether (2 x 35 ml). The dried ($MgSO_4$) ether solution, cooled in ice, was treated with 2,3,4,6-tetra-O-acetyl-β-D-1-glucopyranosyl mercaptan (0.5 g, 0.0015 mole), dissolved in ether (150 ml), followed by triethylamine (5 ml). After 30 minutes, the reaction mixture was washed with 0.5N sulphuric acid (100 ml) to remove triethylamine and triethylamine hydrochloride. The dried ($MgSO_4$) ethereal solution was concentrated under reduced pressure and the thiohydroximic acid precipitated by the addition of petroleum ether. Yield of tetraacetylproglucocapparin (fine white needles) was 0.41 g (72%), m.p. 170-171°. The infrared spectrum was identical to that of material produced by chlorination of acetaldoxime.(186)

S-(2,3,4,6-Tetra-O-acetyl-β-D-1-glucopyranosyl)-propionothiohydroximic acid (tetraacetylproglucolepidiin)

This was prepared exactly as the tetraacetylproglucocapparin above, starting from the nitropropane. The product (0.43 g, 70%) crystallized as white leaflets, m.p. 155-156°, and had an infrared spectrum identical to that of material prepared by chlorination of propanaldoxime (see page 114).

BIBLIOGRAPHY

1. E. Gildemeister and F. Hofmann, "Die Atherischen Ole", 3rd Ed. Vol. I, 145, Schimmel and Co., Leipzig (1927).
2. J.B.A. Dumas and J. Pelouze, Ann.Chim.Phys., 53, II, 181 (1833).
3. A.F. Boutron and E. Fremy, Ann., 34, 230 (1840).
4. A. Bussy, ibid., 34, 233 (1840).
5. E. Ludwig and W. Lange, Zeit.chem.Pharm., 3, 430, 577 (1860).
6. N. Zinin, J.prakt.Chem., 64, 504 (1855).
7. M. Bertholet and S. de Luca, Compt.rend., 41, 21 (1855).
8. A. Iliceto, A. Fava and U. Mazzucato, Tetrahedron Letters, No. 11, 27 (1960).
9. A.W. von Hofmann, Ber., 1, 28 (1868).
10. V. Mayer and P. Jacobson, "Lehrbuch der Organischen Chemie", Vol. I, pt. 2, 1313, von Veit and Co., Leipzig (1913).
11. H. Will and W. Körner, Ann., 125, 257 (1863).
12. J. Gadamer, Arch.Pharmaz., 235, 44 (1897); Ber., 30, 2322 (1897).
13. W. Schneider and F. Wrede, Ber., 47, 2225 (1914).
14. W. Schneider, H. Fischer and W. Specht, ibid., 63, 2787 (1930).
15. M.G. Ettlinger and A.J. Lundeen, J.Am.Chem.Soc., 78, 4172 (1956).
16. Idem, ibid., 79, 1764 (1957).

17. J. Waser and W.H. Watson, Nature, 198, 1297 (1963).

18. A. Kjaer, Fortschr.Chem.Org.Naturstoffe, 18, 122 (1960).

19. R. Wettstein, "Handbuch der Systematischen Botanik", 4th Ed., Vol. II, 716, F. Deuticke, Leipzig (1935).

20. H. Harms in "Die natürlichen Pflanzenfamilien", Vol. 17b, 1, Duncker and Humblot, Berlin (1936).

21. A. Kjaer in "Chemical Plant Taxonomy", 468, Pergamon Press, Oxford (1963).

22. A. Takhtajan, "Die Evolution der Angiospermin", G. Fischer, Jena (1959).

23. R. Hegnauer, Planta Medica, 9, 37 (1961).

24. A. Kjaer, R. Gmelin and I. Larsen, Acta Chem.Scand., 9, 857 (1955).

25. A. Kjaer and R. Gmelin, ibid., 10, 335 (1956).

26. A. Kjaer and I. Larsen, ibid., 8, 699 (1954).

27. S.V. Puntambekar, Proc.Indian Acad.Sci., 32A, 114 (1950).

28. A. Kjaer and J. Conti, Acta Chem.Scand., 7, 1011 (1953).

29. J. Gadamer, Arch.Pharmaz., 237, 92 (1899).

30. A. Kjaer and S.E. Hansen, Acta Chem.Scand., 11, 898 (1957).

31. A. Kjaer and P. Friis, ibid., 16, 936 (1962).

32. A. Kjaer, J. Conti and K.A. Jensen, ibid., 7, 1271 (1953).

33. M.G. Ettlinger and J.E. Hodgkins, J.Am.Chem.Soc., 77, 1831 (1955).

34. A. Kjaer and R.B. Jensen, Acta Chem.Scand., 10, 1365 (1956).

35. A. Kjaer, R. Gmelin and I. Larsen, ibid., 9, 1143 (1955).

36. O.E. Schultz and R. Gmelin, Arch.Pharmaz., 287/59, 404 (1954).

37. A. Kjaer and R. Gmelin, Acta Chem.Scand., 10, 1100 (1956).

38. W. Schneider, Ann., 375, 207 (1910).

39. A. Kjaer and R. Gmelin, Acta Chem.Scand., 9, 542 (1955).

40. H. Schmidt and P. Karrer, Helv.Chim.Acta, 31, 1017 (1948).

41. Ž.Procházka, Coll.Czech.Chem.Comm., 24, 2429 (1959).

42. W. Schneider and H. Kaufmann, Ann., 392, 1 (1912).

43. A. Kjaer, I. Larsen and R. Gmelin, Acta Chem.Scand., 9, 1311 (1955).

44. A. Kjaer and B.W. Christensen, ibid., 17, 846 (1963).

45. Idem, ibid., 12, 833 (1958).

46. A. Kjaer and R. Gmelin, ibid., 10, 1358 (1956).

47. A. Kjaer, R. Gmelin and R.B. Jensen, ibid., 10, 1614 (1956).

48. J. Gadamer, Arch.Pharmaz., 237, 111 (1899).

50. H. Salkowski, Ber., 22, 2137 (1889).

51. A. Kjaer and K. Rubenstein, Acta Chem.Scand., 8, 598 (1954).

52. A. Kjaer, R.B. Jensen and R. Gmelin, ibid., 10, 26, (1956).

53. A. Kjaer and P. Friis, ibid., 17, 1515 (1963).

54. M.G. Ettlinger and A.J. Lundeen, J.Am.Chem.Soc., 78, 1952 (1956).

55. J. Gadamer, Ber., 32, 2335 (1899).

56. A. Kjaer and R. Gmelin, Acta Chem.Scand., 11, 577 (1957).

57. Idem, ibid., 10, 1193 (1956).

58. A. Kjaer and R.B. Jensen, ibid., 12, 1746 (1958).

59. A. Kjaer, R. Gmelin and R.B. Jensen, ibid., 10, 432 (1956).

60. O.E.Schultz and W. Wagner, Arch.Pharmaz., 289/61, 597 (1956).

61. R. Gmelin and A.I. Virtanen, Acta Chem.Scand., 13, 1474 (1959).

62. M.A. Greer, J.Am.Chem.Soc., 78, 1260 (1956).

63. A. Kjaer and R. Gmelin, Acta Chem.Scand., 11, 906 (1957).

64. Idem, ibid., 12, 1693 (1958).

65. A. Kjaer and B.W. Christensen, ibid., 13, 1575 (1959).

66. Idem, ibid., 15, 1477 (1961).

67. Idem, ibid., 16, 71 (1962).

68. Idem, ibid., 16, 83 (1962).

69. A. Kjaer and H. Thomsen, ibid., 16, 591 (1962).

70. A. Kjaer and B.W. Christensen, ibid., 17, 279 (1962).

71. A. Kjaer, H. Thomsen and S.E. Hansen, ibid., 14, 1226 (1960).

72. A. Kjaer and H. Thomsen, ibid., 16, 2065 (1962).

73. Idem, ibid., 17, 561 (1963).

74. R. Gmelin and A.I. Virtanen, Ann.Acad.Sci.Fennicae, AII, 107 (1961).

75. Idem, Acta Chem.Scand., 16, 1378 (1962).

76. W. Klyne, J. Day and A. Kjaer, ibid., 14, 215 (1960).

77. E.R. Henbest, E.R.H.Jones and G.F. Smith, J.Chem.Soc., 3796 (1953).

78. A.F. Mackay et al., J.Am.Chem.Soc., 81, 4328 (1959).

79. A.J. Thorenstein, Canad.J.Zool., 31, 52 (1953).

80. E.P. Lichtenstein, F.M. Strong and D.G. Morgan, J.Agr.Food Chem., 10, 30 (1962).

81. A. Kjaer, Acta.Chem.Scand., 8, 1110 (1954).

82. J.P. Greenstein and M. Winitz, "Chemistry of the Aminoacids", Vol. I, 35, John Wiley and Sons, New York (1961).

83. M.H. Benn, Chemistry and Industry, 1907 (1962).

84. E.W. Underhill, M.D. Chisholm and L.R. Wetter, Can.J. Biochem.Physiol., 40, 1505 (1962).

85. O.L. Gamborg and A.C. Neish, ibid., 37, 1277 (1959).

86. B.D. Davies, Adv.in Enzymol., 16, 247 (1955).

87. Idem, Arch.Biochem.Biophys., 78, 497 (1956).

88. J. Koukol and E.E. Conn, J.Biol.Chem., 236, 2692 (1961).

89. S. Udenfriend and J.R. Cooper, ibid., 203, 953 (1953).

90. R.H. Kenten and P.J.G. Mann, Biochem.J., 50, 360 (1952).

91. N. Kunita, Med.J.Osaka Univ., 7, 203 (1956).

92. E.W. Underhill and M.D. Chisholm, Biochem.Biophys. Res.Commun., 14, 425 (1964).

93. O.L. Gamborg and L.R. Wetter, Can.J.Biochem.Physiol., 41, 1733 (1963).

94. E.W. Underhill, Can.J.Biochem., 43, 179 (1965).

95. Idem, ibid., 43, 189 (1965).

96. H. Kindl, Monatsh., 95/2, 439 (1964).

97. M.D. Chisholm and L.R. Wetter, Can.J.Biochem., 42, 1033 (1964).

98. M. Kutáček, Ž.Prochúzka and K. Vereš, Nature, 194, 393 (1962).

99. A.I. Virtanen, Ann.Rev.Plant Physiol., 12, 1 (1961).

100. N.E. Saris and A.I. Virtanen, Acta Chem.Scand., 11, 1438 (1957).

101. K. Yamafuji, T. Kawakami and K. Shinohara, Enzymologia, 15, 199 (1951).

102. A. Ahmad and I.D. Spenser, Proc.Chem.Soc., 375 (1961).

103. Idem, Can.J.Chem., 39, 1340 (1961).

104. F. Lynen and M. Tada, Angew.Chem., 73, 513 (1961).

105. L.R. Wetter, Phytochemistry, 3, 57 (1964).

106. C. Fromageot and P. Desmuelle, Compt.Rend., 214, 647 (1947).

107. J. Hamer and A. Macaluso, Chem.Rev., 64, 473 (1964).

108. J.R. Catch et al., Nature, 159, 578 (1947); J.Chem. Soc., 1609 (1947)

109. E. Pierson, M. Giella and M. Tishler, J.Am.Chem.Soc., 70, 1450 (1948).

110. J.E. Livak et al., J.Am.Chem.Soc., 67, 2218 (1945).

111. H. Plieninger, Ber., 83, 265 (1950).

112. W. Windus and C.S. Marvel, J.Am.Chem.Soc., 52, 2575 (1930).

113. G. Barger and T.E. Weichselbaum, Biochem.J., 25, 997 (1931).

114. D. Goldsmith and M. Tishler, J.Am.Chem.Soc., 68, 144 (1946).

115. V. du Vigneaud et al., J.Biol.Chem., 106, 401 (1934).

116. R. Gaudry et al., Rev.Can.Biol., 11, 32 (1952).

117. A. Kjaer and S. Wagner, Acta Chem.Scand., 9, 721 (1955).

118. L. Birkhofer and K. Morgenroth, Ber., 96, 1909 (1963).

119. T.F. Lavine, J.Biol.Chem., 169, 477 (1947).

120. A. Lepp and M.S. Dunn, Biochemical Preparations, 4, 80 (1955).

121. W. Schneider, Ann., 375, 207 (1910).

122. Catalogue of NMR Spectra, 118, Varian Associates, Palo Alto (1962).

123. Infrared Spectral Data, No. 1237, American Petroleum Institute (1951).

124. A. Cerniani, G. Modena and P.A. Todesco, Gazz. chim.Ital., 90, 382 (1960).

125. E.L. Eliel in "Steric Effects in Organic Chemistry", M.S. Newman, Ed., 116, John Wiley and Sons, Inc., New York (1956).

126. E.L. Eliel, B.E. Novak and R.A. Daignault, J.Org. Chem., 30, 2448 (1965).

127. G.M. Bennett, F. Heathcoat and A.N. Mosses, J.Chem. Soc., 2567 (1929).

128. G.M. Bennett and E.G. Turner, J.Chem.Soc., 813 (1938).

129. J. Houben and H. Pohl, Ber., 40, 1303, 1725 (1907).

130. L. Cambi, Akti reale Accad.Lincei, Rend.Classe sci. fis.mat.nat., 5, 18, I, 687 (1909).

131. T. Bacchetti and A. Alemagna, Rend.ist.Lombardo sci., I, 91, 30, 574 (1957).

132. Idem, Atti Accad.naz.Lincei, Classe sci.fis.mat.nat., 8, 24, 161 (1958).

133. Idem, Rend.ist.Lombardo sci., I, 91, 30, 481 (1957).

134. A. Quilico, Gazz.chim.Ital., 67, 589 (1937).

135. R.H. Wiley and B.J. Wakefield, J.Org.Chem., 25, 546 (1960).

136. R. Huisgen and W. Mack, Tetrahedron Letters, No. 17, 583 (1961).

137. M.H. Benn, Can.J.Chem., 42, 2393 (1964).

138. Idem, ibid., 41, 2836 (1963). See also idem, ibid., 43, 1 (1965).

139. M.G. Ettlinger and G.P. Dateo, Jr., "Studies of Mustard Oil Glucosides", Final Report, Contract DA 19-129-QM-1059, Rice University, Houston (1961).

140. N. Kornblum and R.A. Brown, J.Am.Chem.Soc., 87, 1742 (1965).

141. F.W. Freise, Perfumery Essent.Oil Record. 26, 219 (1935).

142. J. Hutchinson, "The Families of Flowering Plants", 2nd Ed., Vol.I, Clarendon Press, Oxford (1959).

143. A. Kjaer, Acta Chem.Scand., 13, 851 (1959).

144. A. Kjaer, J.Conti and I. Larsen, ibid., 7, 1276 (1953).

145. P.G. Delaveau and A. Kjaer, ibid., 17, 2562 (1963).

146. W. Schneider and L. Schütz, Ber., 46, 2634 (1913).

147. G. Barger and F.P. Coyne, Biochem.J. 22, 1417 (1928).

148. C.J. Claus and J.L. Morgenthau, J.Am.Chem.Soc., 73, 5005 (1951).

149. K.E. Pfitzner and J.G. Moffat, ibid., 85, 3027 (1963).

150. N. Kornblum et al., ibid., 78, 1497 (1956).

151. G.C. Webster and J.E. Varner, ibid., 67, 733 (1954).

152. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., 167, Longmans Green and Co., London (1957).

153. R.P. Linstead, J.A. Elvidge and M. Whalley, "Modern Techniques in Organic Chemistry", 80, Butterworth, London (1955).

154. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., 778, Longmans Green and Co., London (1957).

155. Organic Syntheses, II, 11, John Wiley and Sons Inc., New York (1943).

156. Ibid., 1.

157. Ibid., 519.

158. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., 969, Longmans Green and Co., London (1957).

159. E. Erlenmeyer, Ann., 271, 137 (1892).

160. J.C. Bevington, M.G. Guzman and H.W. Melville, Proc. Roy.Soc. (London), 221A, 453 (1954).

161. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., 779, Longmans Green and Co., London (1957).

162. V.I. Maimind, K.M. Ermalaev and M.M. Shemyakin, Zhur. Obs.Khim., 26, 2313 (1956).

163. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., 499, Longmans Green and Co., London (1957).

164. W. Schneider, R. Gille and K. Eisenfeld, Ber., 61, 1244 (1928).

165. D.D. Van Slyke, T. Plazin and J.R. Weisiger, J.Biol. Chem., 191, 299 (1951).

166. D. Rittenberg et al., ibid., 125, 291 (1938).

167. D.B. Sprinson and D. Rittenberg, ibid., 180, 707 (1949).

168. M. Bárczai-Martos and F. Korosy, Nature, 165, 369 (1950).

169. W. Schneider and A. Bansa, Ber., 64, 1321 (1931).

170. F.L. Hahn and R. Schleipen, Z.anorg.allgem.Chemie, 153, 98 (1926).

171. F. Wrede, Z.physiol.Chem., 119, 46 (1922).

172. Inorganic Syntheses, II, 173, McGraw Hill, New York (1946).

173. L.F. Fieser, "Experiments in Organic Chemistry", 3rd Ed., 337, Heath and Co., Boston (1957).

174. Beilstein's Handbuch der Organischen Chemie, 4th Ed., HI, 674, Julius Springer, Berlin.

175. A. Kjaer, Acta Chem.Scand., 13, 851 (1959).

176. O.E. Schultz and R. Gmelin, Z.Naturforsch., 7b, 500 (1952).

177. O.E. Schultz and W. Wagner, ibid., 11b, 73 (1956).

178. Beilstein's Handbuch der Organischen Chemie, 4th Ed., HI, 631, Julius Springer, Berlin.

179. Organic Syntheses, I, 533, John Wiley and Sons, Inc., New York (1932).

180. A.I. Vogel, "Practical Organic Chemistry", 3rd Ed., 296, Longmans Green and Co., London (1957).

181. Organic Syntheses, I, 156, John Wiley and Sons, Inc., New York (1932).

182. W. Schneider and H. Kaufman, Ann. 392, 1 (1912).

183. Japanese Patent 2213 (1959). Chem.Abs., 54, 9772 (1960).

184. W. Reppe et al., Ann., 596, 80 (1955).

185. U.S. Patent 2,929,406 (1960). Chem.Abs., 55, 11921 (1961).

186. M.H. Benn, Can.J.Chem., 42, 163 (1964).

187. Organic Syntheses, I, 165, John Wiley and Sons, Inc., New York (1932).

188. E.B. Astwood, M.A. Greer and M.G. Ettlinger, J. Biol. Chem., 181, 121 (1949).

189. J.M. Bell, Can.J.Agr.Sci., 35, 242 (1955).

APPENDIX 1

THE SYNTHESIS OF ISOTHIOCYANATES AND THIOUREAS

In order to provide a set of standards, particularly for gas and paper chromatography, a number of isothiocyanates were prepared, and converted to the corresponding thiourea by the usual method of treating them with a large excess of concentrated ammonia solution, with addition of ethanol to increase the solubility of the isothiocyanate. The synthesis of the isothiocyanates involved treating an amine with thiophosgene, and it was found that yields were considerably higher when the reaction was carried out in the presence of chloroform and sodium bicarbonate,[58] rather than the Organic Syntheses method[187] using water alone.

$$RNH_2 + CSCl_2 + 2NaHCO_3 \xrightarrow{CHCl_3} RNCS + 2NaCl + 2H_2O + 2CO_2$$

$$RNCS + NH_3 \longrightarrow RNH.CS.NH_2$$

The yields obtained together with physical data on these compounds are shown in Table VIII.

GENERAL PROCEDURES FOR THE SYNTHESIS OF ISOTHIOCYANATES AND THIOUREAS

1. ISOTHIOCYANATES

An amine (0.04 mole) dissolved in saturated aqueous sodium bicarbonate solution, was treated with an equimolar quantity of thiophosgene dissolved in chloroform (100 ml). The mixture was stirred vigorously until the red colour of the thiophosgene in the chloroform layer was discharged (approximately 90 minutes). The chloroform layer was separated, washed with dilute hydrochloric acid and water, dried over magnesium sulphate and evaporated to yield an oil, which was fractionally distilled through a short column. The compounds prepared, with the yields and physical data are shown in Table VIII (page 141).

2. THIOUREAS

The isothiocyanate was added to a large excess of concentrated ammonia (specific gravity 0.88) mixed with an equal volume of ethanol. The mixture was allowed to stand at room temperature overnight and evaporated to dryness. The residue of mono-N-substituted thiourea was recrystallized from aqueous ethanol. The thioureas so prepared and their melting points are given in Table VIII (page 141).

Table VIII Synthesis of Isothiocyanates

Compound	Yield °/o	bp	n_D^{22}	IR NCS peak (in $CHCl_3$)	Thiourea mp
n-propyl	69	140-1° / 660 mm	1.5077	Triplet 4.50, 4.61, 4.69 μ	110°
isopropyl	49	124-6° / 660 mm	-	Triplet 4.57, 4.70, 4.92 μ	156°
n-butyl	79	155-164° /660 mm	1.5041	Triplet 4.52, 4.61, 4.68 μ	80°
sec-butyl	63	141-2° / 660 mm	1.4958	Doublet 4.55, 4.65 μ	136°
t-butyl	37	126-8° / 660 mm	1.4816	Doublet 4.65, 5.0 μ	165°
isobutyl	69	150-1° / 660 mm	1.4980	Doublet 4.50, 4.70 μ	93°
n-octyl	60	120-5° / 13 mm	1.4805	Doublet 4.50, 4.67 μ	-
benzyl	74	120-2° / 13 mm	1.6036	Doublet 4.50, 4.70 μ	165°

APPENDIX 2

INFRARED SPECTRA

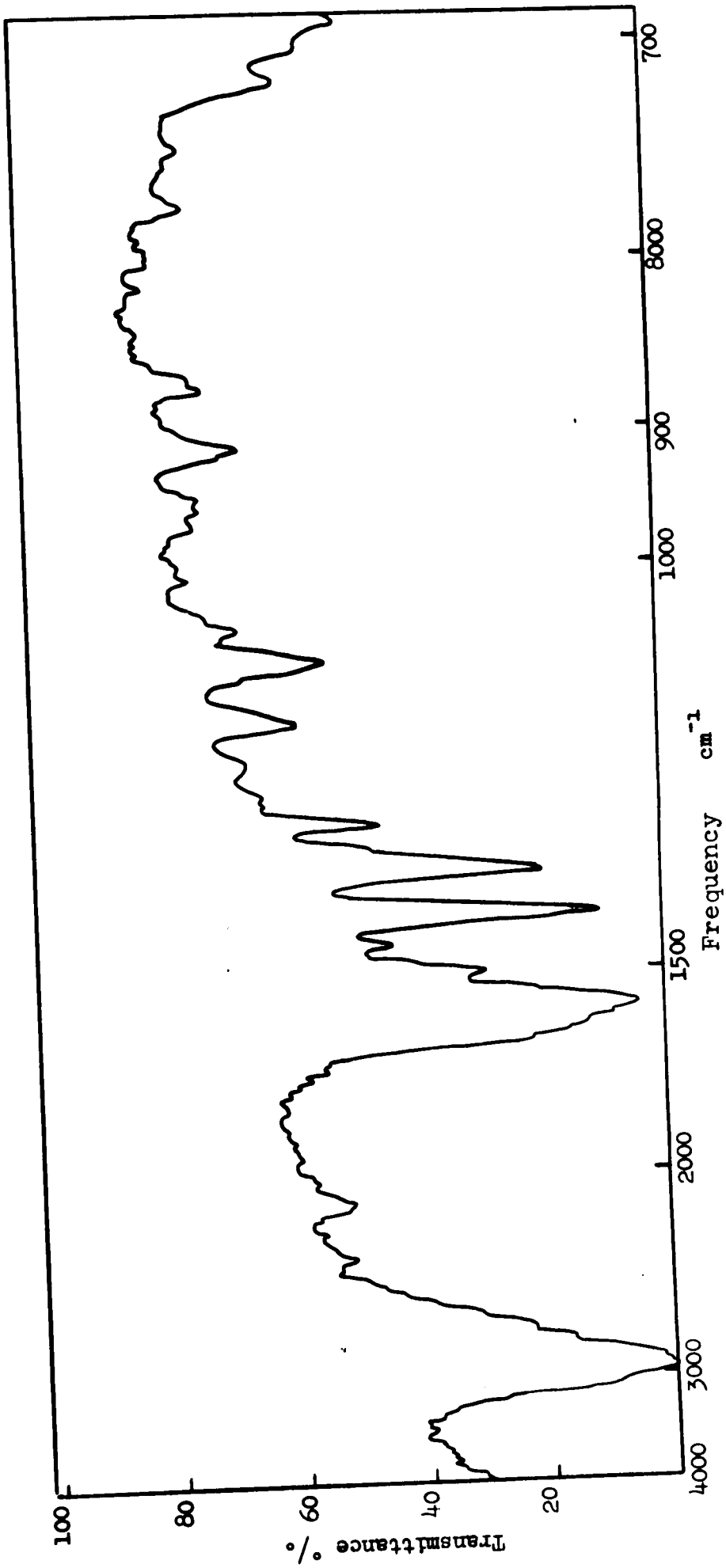


Figure 21 Infrared Spectrum of 2-amino-7-thiomethylheptanoic acid

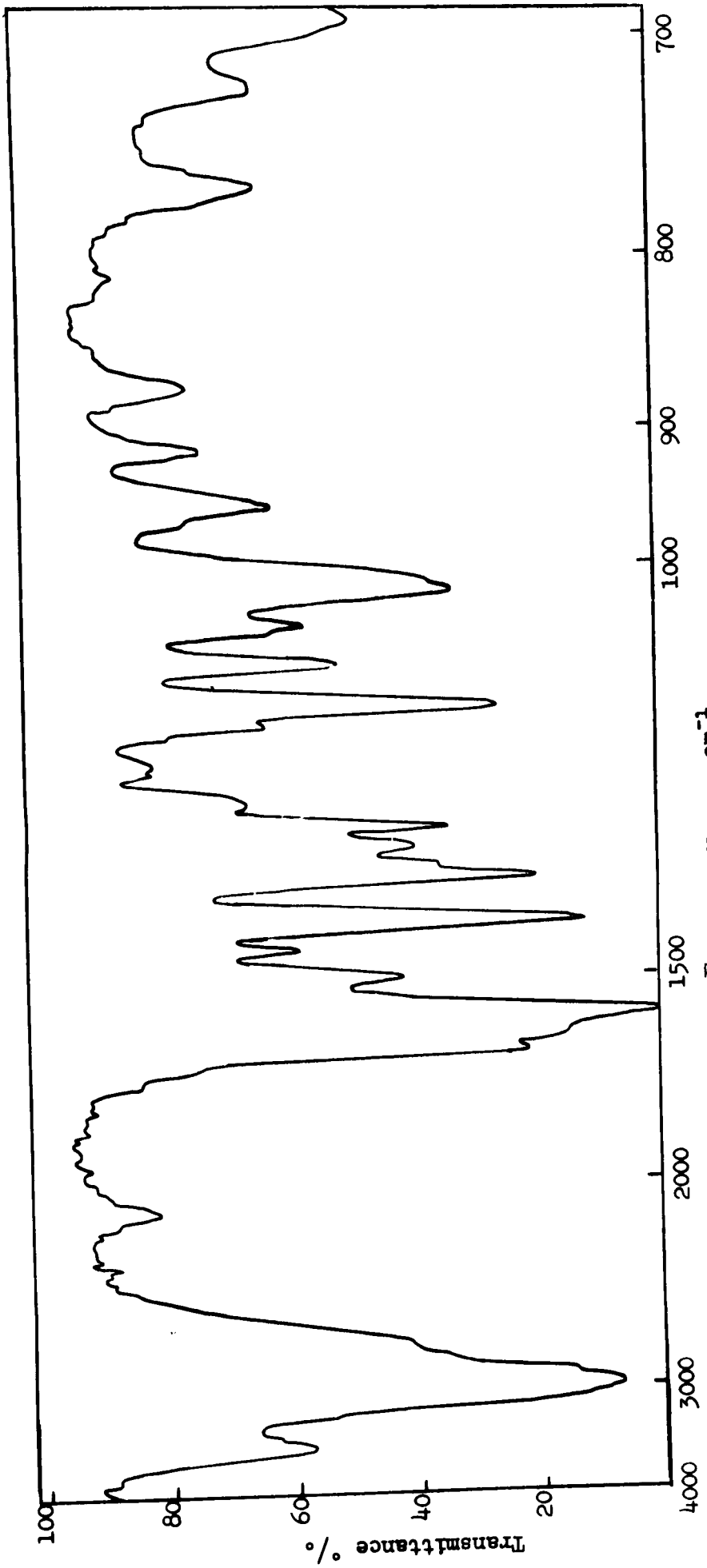


Figure 22 Infrared Spectrum of 2-amino-7-methylsulphinylheptanoic acid

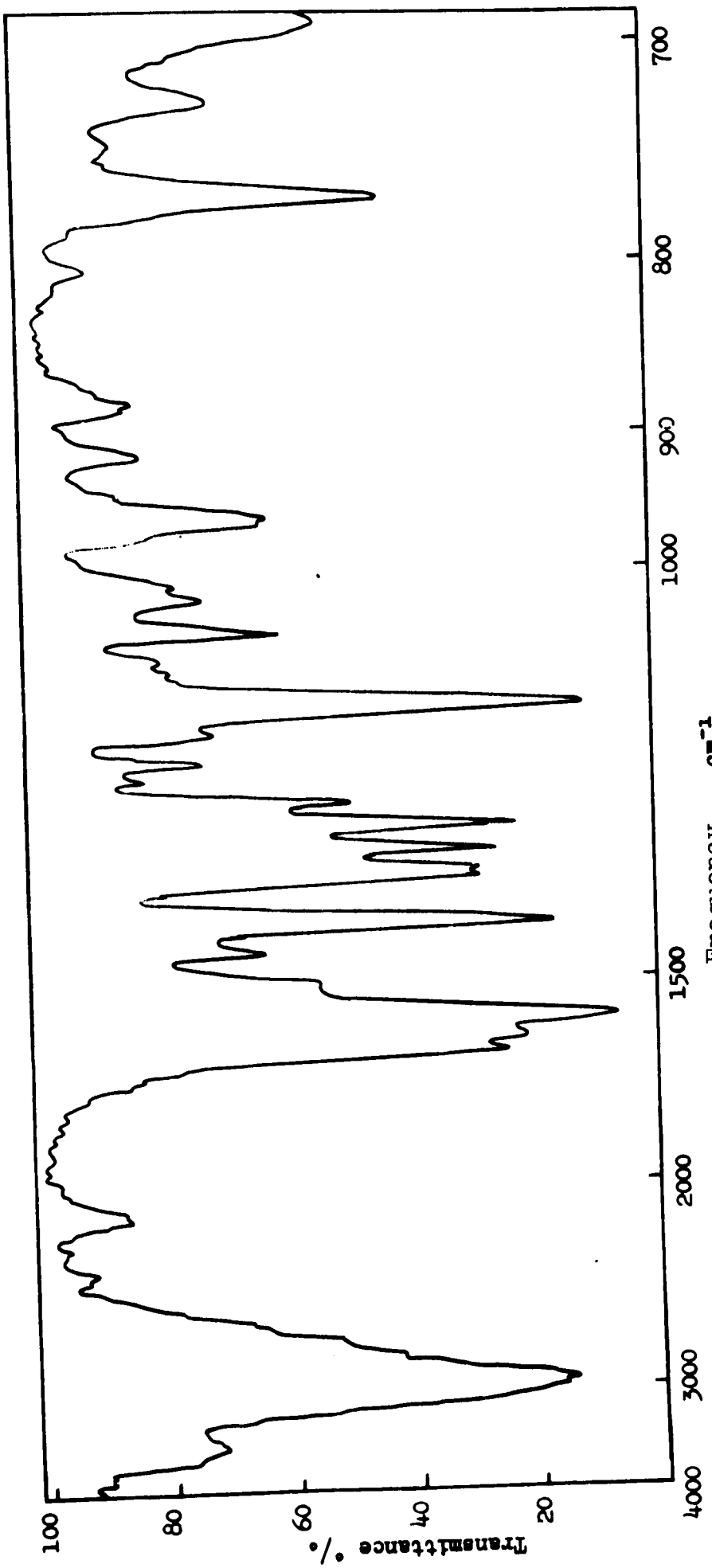


Figure 23 Infrared Spectrum of 2-amino-7-methylsulphonylheptanoic acid

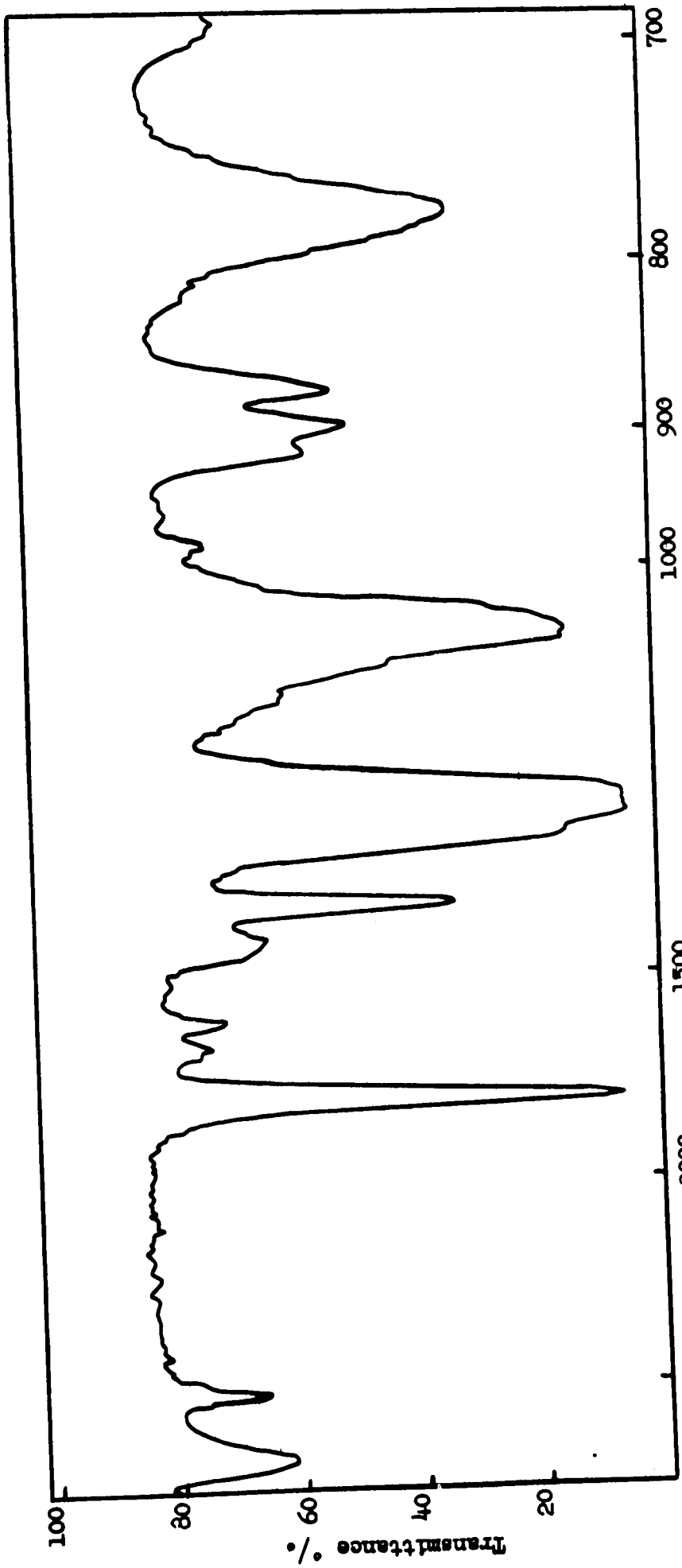


Figure 24 Infrared Spectrum of Tetraacetylglucoputranjivin

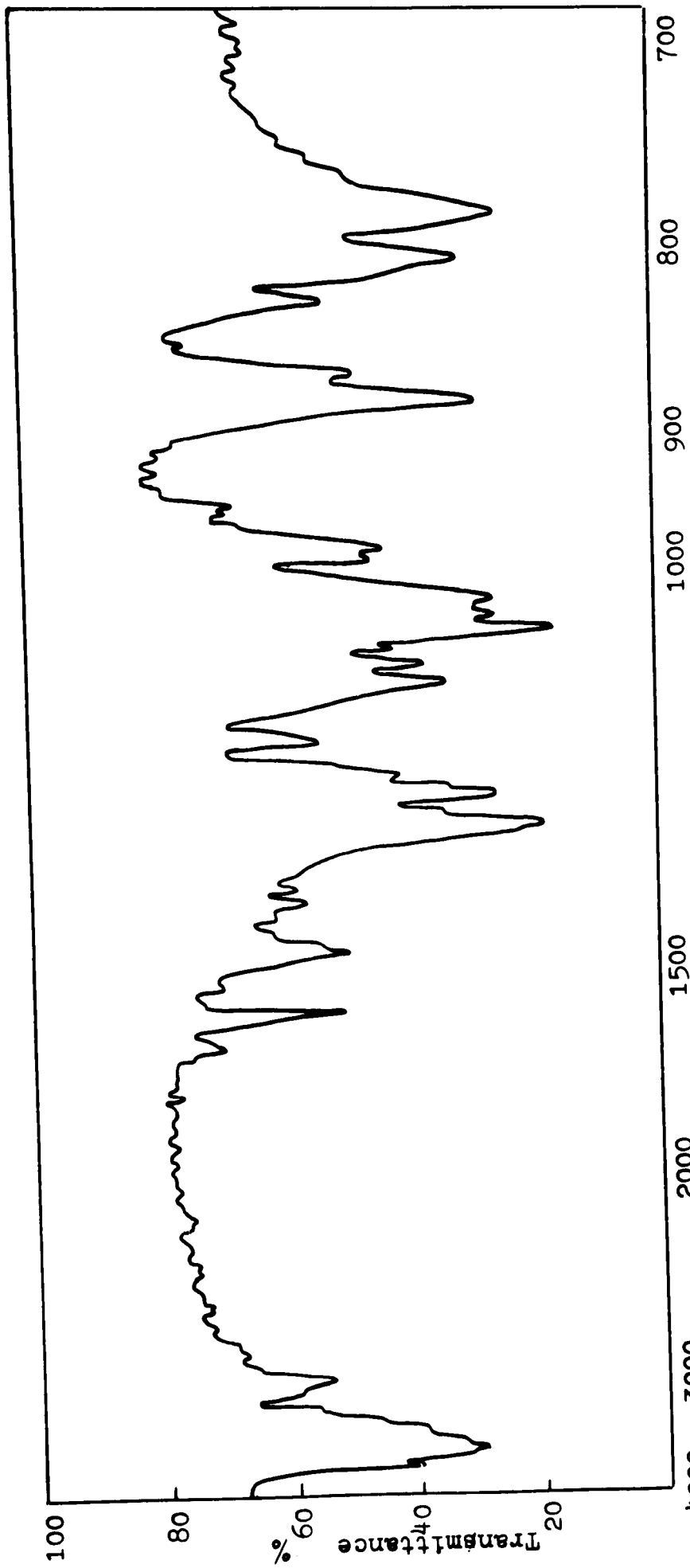


Figure 25 Infrared Spectrum of Glucolepidin