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THE UNIVERSITY OF ALBERTA

Studies on the Agglutinins of *Artocarpus altilis* and *Ficus deltoidea*

by

John Bradley Hunter

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

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DATED July 11 1986

"All noble things are as difficult as they are rare"

Benedict de Spinoza

1632-1677

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Studies on the Agglutinins of *Artocarpus altilis* and *Ficus deltoidea* submitted by John Bradley Hunter in partial fulfilment of the requirements for the degree of Master of Science in Pharmaceutical Sciences.

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Supervisor

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Date..... 9th July 1986.....

DEDICATED

To my parents, John M.W. and Marion A. Hunter,

for their understanding and encouragement;

To the memory of R.A.M.,

for shaping my destiny.

ABSTRACT

A variety of plant seeds were screened for hemagglutinating activity, immunochemical relatedness and bacterial agglutination. Extracts from the seeds of breadfruit (*Artocarpus altilis*) and jackfruit (*Artocarpus integrifolia*) exhibited a high titer non-specific for human erythrocytes. They produced immunoprecipitin reactions of partial identity with antisera directed against Osage orange (*Maclura pomifera*) lectin suggesting a structural and evolutionary relationship of Moraceae lectins.

The breadfruit lectin (BFA) was purified by salt fractionation followed by affinity chromatography on immobilized β-D-gal(1→3)αgalNAc. Gel filtration using Bio-Gel P150 suggested a molecular weight around 44,500. Electrophoresis of the purified lectin in SDS polyacrylamide gels revealed two dissimilar subunits of approximately 19,000 and 22,000 daltons. Amino acid analysis and isoelectric focusing of BFA indicated that the lectin may be acidic in nature. This lectin did not agglutinate any of a variety of bacterial species and strains. In view of its binding affinity to the Thomsen-Friedenreich (T) antigen purified BFA may prove useful in tumor binding studies.

An extract from mistletoe fig (*Ficus deltoidea*) (FDA) seeds produced non-specific precipitin reactions with human serum thus affecting immunodiffusion studies. The crude samples were also observed to agglutinate human erythrocytes and some strains of bacteria including *Chlamydia trachomatis*, a clinically significant pathogen causing a sexually transmitted disease.

Fast protein liquid chromatography (FPLC) was utilized to partially purify the lectins from crude extracts of breadfruit and mistletoe fig seeds. Comparison of the elution profiles of the affinity purified and crude extracts of breadfruit showed gel filtration FPLC can offer advantages over the traditional affinity methods in that this rapid and simple one step procedure gives a considerable purification of the lectin from the crude extract. This technique

was also used to enrich a crude FDA extract, resolving it into nine major peaks. Although every fraction agglutinated red blood cells, only the fourth fraction was active against purified elementary bodies of the bacterium *Chlamydia*. FDA may be valuable as a probe for studying bacteria, particularly clinically important pathogens such as *Chlamydia*.

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LIST OF ABBREVIATIONS

BFA	–	breadfruit agglutinin
cm	–	centimetre
Con A	–	Concanavalin A
cpm	–	counts per minute
°C	–	degrees Celsius
DTT	–	dithiothreitol
EDTA	–	ethylenediaminetetraacetic acid
FDA	–	*Ficus deltoidea* agglutinin
FPLC	–	fast protein liquid chromatography
fuc	–	fucose
gal	–	galactose
galN	–	galactosamine
galNAc	–	N-acetyl-galactosamine
glc	–	glucose
glcN	–	glucosamine
glcNAc	–	N-acetyl-glucosamine
g	–	gram
x g	–	times gravity
h	–	hour(s)
IgA	–	immunoglobulin A
IgE	–	immunoglobulin E
IgM	–	immunoglobulin M
L	–	litre
lact	–	lactose
M	–	molar
man	–	mannose

manN	–	mannosamine
mg	–	milligram
min	–	minute
ml	–	millilitre
mm	–	millimetre
mM	–	millimolar
MPA	–	*Maclura pomifera* agglutinin
NA	–	neuraminidase
NeuNAc	–	N-acetyl-neuraminic acid
n.s.	–	neutral sugar
PAGE	–	polyacrylamide gel electrophoresis
PBS	–	phosphate buffered saline
pI	–	isoelectric point
PNA	–	peanut agglutinin
RIA	–	radioimmunoassay
SDS	–	sodium dodecyl sulfate
T antigen	–	Thomsen-Friedenreich antigen
TCA	–	trichloroacetic acid
TES	–	N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid
Tris	–	Tris(hydroxymethyl)aminomethane
uCi	–	microcurie(s)
ul	–	microlitre
um	–	micromole(s)
Ve	–	volume of elution

I. INTRODUCTION

A. Lectins

1) Definition and Classification

Stillmark's discovery of a lectin in *Ricinus communis* in 1888[1] initiated research into one of nature's most interesting and complex classes of proteins. By 1975, 20 lectins had been isolated in homogeneous form[2]. Ten years later the number of plant lectin structures and specificities elucidated has risen to more than 100. This increased interest has also been reflected in growing numbers of review papers[3-18].

Lectins are studied because they act as determinants of cellular recognition and many possess interesting structural features. In addition, they are useful for the investigation of protein-carbohydrate interactions, and as reagents for the characterization and separation of cells and glycoconjugates. However, their regulation and biological role still remain a mystery[19].

Early observations of the presence of red blood cell agglutinating material in plant seeds led to the term "phytohemagglutinin" or "phytoagglutinin" or "agglutinin". The word "lectin", derived from the Latin *lectus* (past participle *legere*) meaning to choose or select, was proposed in 1954[20]. Goldstein *et al*[21] clarified this term to define **"a sugar-binding protein or glycoprotein of non-immune origin which agglutinates cells and/or precipitates glycoconjugates."** Lectins have at least two sugar-binding sites and a specificity expressed as the monosaccharide or simple oligosaccharide responsible for inhibiting lectin-induced agglutination.

Others[22,23] prefer a definition based on physicochemical properties rather than biological activities. Choosing to emphasize the interaction with carbohydrates, Kocourek and Horejsi[22] define lectins as **"sugar-binding proteins or glycoproteins of non-immune origin which are devoid of enzymatic activity towards sugars to which they bind and do not require free**

glycosidic hydroxyl groups on these sugars for their binding." The Nomenclature Committee of the International Union of Biochemistry (NC-IUB) dismisses this definition as being too broad and the criterion of lack of enzymatic activity difficult to apply[24,25]. Consequently, the operational definition of Goldstein *et al* is now generally accepted.

More specific terms also appear in the literature. "Protectins" was used to describe a class of invertebrate agglutinins with a protective function as protease inhibitors[26]. "Electrolectins" refer to endogenous β-D-galactoside binding lectins found in vertebrates such as chick, eel and mouse[27,28]. "Isolectins" are chromatographically distinct forms of the agglutinin similar in molecular weight, with subtle differences in amino acid composition and isoelectric point. They are not uncommon in lectins characterized to date[29-42]. A clear distinction between "isolectins" and "multiple lectins" has not been made in the literature and the terms are often used interchangeably. Isolectins are analogous to isoenzymes and represent different molecular forms of the same lectin, whereas multiple lectins are distinct and unique in their properties despite being isolated from the same source. Lectins are sometimes included under the broader classification "affinitins", a collective name for combining sites containing proteins (or glycoproteins) such as lectins, antibodies and enzymes[43-45].

In some instances, an immunochemical relationship exists between a toxin and agglutinin isolated from the same species[46]. These toxins usually have two subunits, one toxic and one sugar-binding. While similar to lectins, toxins have only one binding site and do not agglutinate cells or precipitate glycoconjugates[21,47].

Lectins resemble antibodies in that they form precipitates through specific interactions with glycoproteins and polysaccharides which are concentration dependent and may be inhibited by low molecular weight "haptens", analagous to an antigen-antibody reaction[13]. Thus, many immunological tests and procedures are applicable to lectin studies[48]. Cold agglutinins, anti-carbohydrate antibodies that precipitate at low temperatures (4°C) and can exhibit agglutinating phenomena, have been described recently[49].

The differences between antibodies and lectins are more pronounced than their similarities. Immunoglobulins are restricted to vertebrates while lectins may be found in plants and microorganisms as well as animals. Unlike lectins (constitutive), antibodies require an antigenic stimulus for their formation (induced). The immune system is flexible, allowing a variety of antibody specificities whereas lectin binding characteristics are pre-determined and restricted to carbohydrates[13]. Other differences include the metal ion requirement of some lectins for their activity unlike antibodies and the existence of isolectins as opposed to classes and subclasses of antibodies[48].

Attempts have been made to classify lectins according to their carbohydrate-binding characteristics[17,50]. This may not be entirely satisfactory since lectins exhibiting a specificity not due to simple sugars or oligosaccharides (some bacterial lectins) may be excluded[21]. In addition, some rare lectins bind amino acids[52] instead of carbohydrates. Most lectins are cytosolic although some lectins from mammalian sources are membrane bound. Hemagglutination is a general phenomenon. Antibodies and certain enzymes involved in carbohydrate metabolism could also bring about agglutination due to their polymeric nature, but are not considered lectins. The nomenclature has not yet been standardized, hence both "lectin" and "agglutinin" appear in the the literature. This is reflected in Table I-1 where the literature nomenclature is used for the specific lectins listed.

2) Distribution in Nature

Early screening studies[53-55] demonstrated the wide distribution of lectins throughout the plant kingdom. Of 5156 species tested, 16% were positive for hemagglutinating activity. Agglutinating material was present in 30% of the 852 genera evaluated[56]. A recent screen of plants from the Indian subcontinent[57] found 50 out of the 543 species tested (9%) agglutinated erythrocytes. Lectins have also been detected in commonly consumed foods[58,77,78].

Lectin research has focused on plants and plant seeds because these are inexpensive and abundantly available. However, lectins are found in many diverse taxa from bacteria to

mammals, including vertebrates[60] and mammals[61,62], invertebrates[63,64], algae[65,66], slime molds[67,68,69], bacteria[69,70], fungi[71], lichens[72], earthworms[73], fish eggs[74], and molluscs, crustaceans and gastropods[75,76].

Plant seeds generally contain the highest concentration of lectins, in the range of 2 to 10% of the total protein in most leguminous seeds[11]. Lesser amounts may also be present in stems, leaves and roots. The agglutinin content accumulates and reaches a maximum as the seed matures. Upon germination there is a progressive decline in lectin content, in parallel with the decrease of reserve protein. In mature plants lectin activity is localized within the seeds[11]. Material that cross-reacts with antiserum against the seed lectin has been found in stems and leaves but not immature seeds[80-82]. Lectin synthesis has also been detected in the apical part of adventitious roots[83].

The agglutinating activity of lectins was speculated to be under genetic control[84]. A lectin gene mutation in *Phaseolus vulgaris* resulted in reduced levels of seed lectin[85]. The chromosomes responsible for lectin synthesis in *Triticum aestivum* and *Aegilops umbellulata* have also been investigated[86].

3) Function and Physiological Role in Plants

The exact function and physiological role of lectins in plants is not clear and is the subject of much speculation. The high amounts of lectins found in Leguminosae seeds and the homologous nature of lectins of different Leguminosae tribes suggest that lectins are important to the plant[87]. Many functions have been proposed[11] including antibody-like activity against soil bacteria[88] and viruses[15,89], transport and storage of sugars[56], control of seed germination and development, regulation of plant cell extension[89], binding of symbiotic nitrogen-fixing bacteria to legumes[11,18,56,90-93], protection against insect predators[94] and fungal phytopathogens[95,96], enzymes[18,97,98] and metabolism[99], and determinants of inter- and intracellular recognition[100-102].

If lectins are necessary to protect plants against microorganisms it is difficult to explain why some plants in which lectins have not been detected are able to flourish[11], or why lectins appear to act as receptors for some bacterial pathogens[28]. The presence of lectins in stems, leaves and roots create some problems for the theory of seed germination and development. The hypothesis of plant-symbiont attachment has generated conflicting data[18]. No conclusive evidence has been presented for any of the proposed physiological roles.

The properties of lectins in themselves may suggest some unique biological roles. For example, the specificity of binding sites implies the recognition of complementary saccharide receptors with which the lectin may interact. This interaction could involve the cross-linking of glycoproteins and glycolipids as part of a structural role, if more than one binding site was present. The binding of cells evidenced by agglutination activity may promote functions such as morphogenesis and phagocytosis. The abundance of lectins suggests a structural rather than enzymatic role[105]. While it is reasonable to expect the biological role of lectins to be determined by their known biochemical properties, it may be that the biological properties have no relation to their function in nature[18].

4) Physicochemical Properties

Lectins differ chemically and structurally and exhibit dissimilar biological and physicochemical properties within the same botanical family or genus[104]. Plant lectins have molecular weights ranging from 36,000 (wheat germ agglutinin) to 250,000 (lima bean lectin) with most around 100,000 daltons. The majority are composed of two or four identical subunits. However the existence of lectins with nonidentical subunits have been reported in soybean, horse gram, lentil and garden pea. Most lectins are themselves glycoproteins with a carbohydrate content of 4 to 10%. Examples of a few lectins lacking carbohydrates are concanavalin A and peanut lectin. The amino acid composition of lectins usually shows a high amount of serine, threonine and aspartic acid with significantly low sulfur-containing amino acids. Exceptions such as wheat germ, potato and pokeweed lectins rich in cysteine, are also

known. In some instances (Con A, pea, lima bean), biological activity is dependent on the presence of divalent metal ions[14]. The diversity of physicochemical properties among lectins is comprehensively summarized in Table 1-1.

Table I-1. Physicochemical properties of plant lectins characterized up to October, 1985.

Species[1]	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates[2]	Specificity Human blood group[3]	Specificity Sugar	Mitogenic	Ref.
Abrus precatorius (jequirity bean)	Leguminosae					None	D-gal		105-112
APA									
Abrus		134,900	4	32,200 33,800					
Abrin A		62,500	2		Yes (Abrin)			Yes (Abrin)	
Abrin C		65,600	2						
Adenia digitata (modecca flower)	Passifloraceae	63,000	2	28,000 38,000			terminal gal		113-118
Aegopodium podagraria (ground elder)	Apiaceae	480,000	8	55,000 60,000	11% n.s.	None	galNAc		119

[1]Nomenclature used (lectins or agglutinins) as given in the literature
[2]"Yes" indicates carbohydrate present, with amount and type undetermined
[3]"None" signifies non-specific agglutination of A,B,O and AB blood groups

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity Human blood group	Sugar	Mitogenic	Ref.
Agropyrum repens (couch grass)	Gramineae								
AREL (embryo)		36,000	2	18,000		None	glcNAc		120
ARLL (leaf)		39,000	2	19,500		A	galNAc		
Aloe arborescens (aloe)	Liliaceae								
P-2		18,000	2	7,500 10,500	>18%	None		Yes	121
S-1		24,000	2	12,000	>50%	(stronger than P-2)		No	
Amphicarpaea bracteata (hog peanut)	Leguminosae				2% n.s.	A_1, A, B	galNAc		122
Arachis hypogaea (peanut) PNA	Leguminosae	110,000	4	27,500	0	T, Tx (NA treated)	β-D-gal(1→3)D-galNAc D-gal	some sources	123-133
Artocarpus integrifolia (jackfruit)	Moraceae	62,000	4	13,000 18,000	3%	None	α-D gal galNAc	Yes	134-143
		39,500	4	10,000					

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity Human blood group	Specificity Sugar	Mitogenic	Ref.
Bauhinia purpurea (camels foot tree)	Leguminosae	195,000			7.7% n.s. 1.4% glcNAc	None M,N	galNAc D-gal		144-148
Brachypodium sylvaticum (false brome grass) BsyL	Gramineae	36,000	2	18,000		None	glcNAc		149
Butea frondosa (dhak or palas tree)	Leguminosae	141,000		36,000 65,000	4.2% n.s. 0.5% glcN	O>A=B	D-gal		150
Cannabis sativa (hemp, marijuana)	Cannabinaceae					None (papain treated)	glc and its derivatives		151-152
Canavalia ensiformis (jack bean) Con A	Leguminosae	55,000 110,000	2 (pH<5.6) 4 (pH 5.8-7.0) higher aggregates (pH>7.0)	25,500	0	None	α-D-man α-D-glc	Yes (dependent on aggregation)	153-166
Caragana arborescens (Siberian pea tree) CAA	Leguminosae	60,000	2	30,000	Yes	None	galNAc D-gal		167
Cerastium tomentosum (Mouse ear)	Caryophyllaceae					II	glcNAc sialon		168

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity		Mitogenic	Ref.
						Human blood group	Sugar		
Chelidonium majus (greater celandine) CMA	Papaveraceae	23,000	2	9,500 11,500	0	None	glcNAc		169
Cicer arietinum (chick or gram pea)	Leguminosae	44,000	2	26,000	<0.5%	None (papain treated)	IgM, fetuin no simple sugars	No	170
Clerodendron trichotomum (glory vine) CTA	Verbenaceae	56,000	2	28,000	Yes	Cad(+)	galNAc D-gal		171
Crotalaria juncea (sunn hemp)	Leguminosae	120,000			5-9.8%	None	D-gal		172-173
Croton tiglium (purging nut)	Euphorbiaceae	120,000	2	55,000	0.75% n.s.	sheep, animals (hemolytic for rabbits)	no simple sugars		174
Cytisus scoparius (Scotch broom) CSI	Leguminosae								
CSIa		70,000		30,000			D-gal, galNAc		175
CSIb				30,000			galNAc		
CSII		75,000		30,000			galNAc		

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity: Human blood group	Specificity: Sugar	Mitogenic	Ref.
Cytisus sessilifolius (common cytisus) CSA	Leguminosae	110,000				O(II)	α-L-Fuc β-D-glcNAc(1→4)-β-D-glcNAc		176
Datura stramonium (Jimson weed, thorn apple)	Solanaceae	86,000	2	40,000 46,000	28-37% n.s. (mainly arabinose) 4.5% glcN, 6.3% hydroxproline residues	None	β(1→4) linked oligomers of glcNAc	Yes	177-184
Dolichos biflorus (horse gram) DBA	Leguminosae								
A		113,000	4	27,300 27,700	3.8%	A_1	α-D-galNAc		185-192
B		109,000	4	26,000					
Dolichos lablab (Indian or hyacinth bean)	Leguminosae	110,000		16,000 40,000	2% n.s.	None	glcNAc methyl α-D-mannopyranoside		193
Eranthis hyemalis (winter aconite) EHL	Ranunculaceae	62,000	2	30,000 32,000	5%	O	galNAc		194
Erythrina cristagalli (cockspur coral tree) ECA	Leguminosae	56,800	2	26,000 28,000	4.5% man, fuc, xylose, glcNAc	None	lacNAc, galNAc D-gal		195

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity Human blood group	Specificity Sugar	Mitogenic	Ref.
Erythrina edulis	Leguminosae	56,000	2	27,000	7.8% n.s.	None	D-lac p-nitrophenyl-β-D-galactoside		196
Erythrina indica (E. variegata) (Indian coral tree)	Leguminosae	68,200 66,200	2 2	30,000 33,000 30,000 34,000	5.6, 9-12% n.s. 0.46-1.5% glcN	O>B>A	D-gal		197-199
Euonymus europeus (prickwood) EEA	Celastraceae	127,000 166,000			4.8% D-gal 2.9% D-glc 2.8% glcNAc	B, O(H)			200-201
Euphorbia characias (Mediterranean spurge)	Euphorbiaceae	80,000	2	40,000	11.3%	None	D-gal	Yes (latex) No (purified lectin)	202
Euphorbia heterophylla (annual poinsettia) EHA	Euphorbiaceae	65,000	2	32,000		None	galNAc		203
Glycine max (Soja hispida) (soybean) SBA	Leguminosae	120,000	4	30,000	5.5-6%	None	galNAC, D-gal	Yes (polymeric forms)	204-218

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity Human blood group	Specificity Sugar	Mitogenic	Ref.
Griffonia simplicifolia (Bandeiraea simplicifolia)	Leguminosae				Yes (GSA-I)				
GSA						(GSA) B, Tk	α-D-gal		219-230
BS-I		114,000	4 (A,B)			(GS-I) B	D-glcNAc		
5 isolectins									
BSI-B$_4$						(GS-IA$_4$) A	-galNAc, α-D-gal		
BSI-AB$_4$						(GS-IB$_4$) B	α-D-gal, β-D-gal		
BSI-A$_1$B$_4$									
BSI-A$_1$B						(GS-II) Tk	glcNAc		
BSI-A$_4$									
BS-II		113,000	4	30,000					
Hordeum vulgare (barley)	Gramineae	31,000 36,000	2	18,000	0	None	glcN, galN, manN (but not with N-acetyl derivatives)		231-233
Hura crepitans (H. brasiliensis) (sand-box tree)	Euphorbiaceae	140,000		29,000 31,000 35,500 37,500	Yes	None		Yes	234-235
3 isolectins									

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity		Mitogenic	Ref.
						Human blood group	Sugar		
Iberis amara (candytuft) IAA	Cruciferae					M	galNAc		236-237
Laburnum alpinum (Scotch laburnum) LAA	Leguminosae					O(H)	glcNAc N,N'-diacetylchitobiose		238-240
Lactuca scariole (prickly lettuce) PLA	Compositae (Cichorium tribe)	84,000			Yes	mouse, rat	glycoproteins best inhibitors	Yes	241-242
Lathyrus odoratus (sweet pea) LOA	Leguminosae	37,000 42,800	2	19,000 4,400 17,000	0.5% n.s.	None	D-glc, D-man, their α-glycosides	Yes	243-245
Lathyrus sativus (grass pea, chickling vetch)	Leguminosae	42,900 49,000	2	21,000 4,400 19,000	2% n.s.	None	D-glc, D-man, their α-methyl-glucosides	Yes	246-247
Lens culinaris (*L. esculenta*) (lentil) LcH, LcA	Leguminosae				2%	None	α-D-man, α-D-glc		248-253

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity Human blood group	Specificity Sugar	Mitogenic	Ref.
LcH-A		60,000	2					Yes (LcH-A)	
LcH-B		42,000	2						
Lepidium sativum (garden cress)	Cruciferae	120,000	4	30,000	2.2%	None	NeuNAc, galNAc desialylated glycoproteins		254-255
Lotononis bainesii	Leguminosae								
Root		118,000		32,000 35,000	12% (root) hexose (fuc, man, xylose, glcN)		lac, lactulose, melibiose		256
Seed		160-200,000			50% (seed) hexose		gal, galactono-1,4-lactone		
Lycopersicon esculentum (tomato) LEA	Solanaceae	71,000			50% (arabinose 85%, gal 15%)	None	glcNAc oligomers	No	257-260
Maackia amurensis (Cladrastis amurensis)	Leguminosae	130,000							
MAH (strongly hemagglutinating)			4	33,000	9.5%		lac, NeuNAc		261
MAM (strongly mitogenic)			2	75,000	8.7%		weak inhibitory activity	Yes	

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity: Human blood group	Specificity: Sugar	Mitogenic	Ref.
Maclura pomifera (Osage orange) MPA	Moraceae	42,000		10,000 12,000	0	None	α D-gal galNAc	Yes	262-267
Momordica charantia (bitter pear melon)	Cucurbitaceae	49,000 120,000 129,000	2	26,000 28,000 30,000	2% glc, 8% man, 2.5% glcN	O	D-gal, methyl-α-gal		268-272
Momordica dioica	Cucurbitaceae			23,000 37,000	Yes	None	D-gal		273
Onobrychis viciifolia (sainfoin)	Leguminosae	53,000	2	26,000	2.6% n.s.	cat, rabbit	D-man, D-glc, α-glycosidic derivatives		274-276
Ononis hircina (restharrow)	Leguminosae	110,000	4	31,000	7.2% n.s.	O	galNAc, D-gal	No	277
Oryza sativa (rice) RBM, RGL	Gramineae	44,000 19,000 23,000	 2 2	 8,200 11,000 11,300 13,700	0.8%	None	glcNAc	Yes	278-280
Persea americana (avocado) PAA	Lauraceae					A>B=O	basic proteins basic polyamino acids	No	281

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity: Human blood group	Specificity: Sugar	Mitogenic	Ref.
Phaseolus limensis ***(P. lunatus, Lunatus limensis)*** (lima bean) LBA	Leguminosae	247,100 124,400		31,000	4%	A	α-D-galNAc	Yes	282-288
Phaseolus vulgaris (red kidney beans) PHA 5 isolectins $L_4, L_3E_1, L_2E_2, L_1E_3, E_4$	Leguminosae	115-140,000	4			None	galNAc,		289-304
PHA-E		143,000	4	37,000				Low	
PHA-L		114,000	4	30,000	7.3% man, 2.8% glcN			High	
Phlomis fruticosa (Jerusalem sage)	Labiatae					A,B,AB	galNAc, D-gal		305
Phytolacca americana (pokeweed, pigeon berry) PWA, PWM 5 isolectins, Pa-1 to Pa-5	Phytolaccaceae	20-30,000			1.8-12.5%	None	glcNAc oligomers		306-312
Pisum sativum (garden pea) PSA, PEA 2 isolectins	Leguminosae	53,000				None	D-man, D-glc, glcNAc	Yes	313-319
I		54,000		7,000	1.42% n.s.				
II		53,000		17,000	0.88% n.s.				

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity: Human blood group	Specificity: Sugar	Mitogenic	Ref.
Psophocarpus tetragonolobus (winged bean)	Leguminosae								
acidic I,II,III		54,000	2	27,000	7% (man, fuc, xylose, glcNAc)		galNAc, D-gal		320-323
basic B1,B2,B3		58,000	2	29,000	5% (man, fuc, xylose, glcNAc)	A,B (trypsinized)			
Ricinus communis (castor bean) RCA	Euphorbiaceae					None	D-gal, galNAc		324-330
RCA I		118- 120,000	4						
RCA II (toxin)		60- 65,000	2						
Robinia pseudoacacia (black locust, black acacia) RPA	Leguminosae	100-110,000		30,000 32,000	14-17%	None	galNAc		331-336
Salvia horminum (annual clary, common sage)	Labiatae					Tn, Cad			337-339
Salvia sclarea (clary) SSA	Labiatae					Tn			340-344

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity: Human blood group	Specificity: Sugar	Mitogenic	Ref.
Sambucus nigra (European elder) SNA	Caprifoliaceae	140,000	2 2	34,500 37,500	Yes	A>B>O	lac, galNAc		345
Secale cereale (rye germ)	Gramineae	36,000	2	18,000		None	glcNAc		346
Solanum tuberosum (potato) STA	Solanaceae	100,000	2	54,000	50% (92% arabinose)	None	glcNAc oligomers		347-354
Sophora japonica (Japanese pagoda tree)	Leguminosae	132,800	4	33,000	5.9% man, xylose	B>A	D-gal, galNAc	No	355-361
Sorghum bicolor	Gramineae					None	glcNAc, D-maltose		362
Tetragonolobus purpureas ***(Lotus tetragonolobus)*** (winged or asparagus pea) 3 isolectins Lotus A	Leguminosae	120,000 58,000 117,000		27,000		O(H)	α-L-fuc		363-369
Triticum vulgaris ***(T. aestivum)*** (wheat germ) several isolectins WGA	Gramineae	36,000	2	18,000	0	None	glcNAc oligomers	No	370-379

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity Human blood group	Specificity Sugar	Mitogenic	Ref.
Ulex europeus (gorse, furze, prickly broom)	Leguminosae								380-386
UEA									
UEA I		170,000			5.2% (1.6% man, 1.4% glcN)	O	α-L-fuc		
UEA II		23,000			21.7% (8.7% man, 6.3% gal)	O	N,N'-diacetylchitobiose		
Vicia cracca (tufted or cow vetch)	Leguminosae	125,000	4	33,000	5%	A	α-galNAc		387-393
		44,000	2	5,700		None	man, glc		
			2	17,500					
Vicia ervilia (bitter vetch)	Leguminosae	60,000	2	21,000	0	None	D-man, trehalose		394
			2	4,700					
Vicia faba (fava or broad bean)	Leguminosae	50,000	2	5,600	3% n.s.	None	D-man, D-glc	Yes	393-400
			2	20,000					
VFA		47,500	2	22,500					
Vicia graminea VGA	Leguminosae	105,000	4	25,000	7.3% man, glcNAc	N	galNAc	No	401-404
Vicia sativa (tare, spring vetch)	Leguminosae	70,000	2	20,000	9.4%	None	melezitose	Yes	405-407
				27,500					
VSL		40,000	2	6,000					
			2	14,000					

Species	Family	Molecular weight (daltons)	Sub-units	Subunit size (daltons)	Carbohydrates	Specificity: Human blood group	Specificity: Sugar	Mitogenic	Ref.
Vicia unijuga	Leguminosae					N			408
Vicia villosa ***(Visea villosa)*** (hairy or winter vetch) 3 isolectins B_4, A_2B_2, A_4 VVA	Leguminosae	120,000	4	33,600 35,900	4.3% n.s.		galNAc		409-411
					6.7-9.8% (B_4, A_4)	(B_4) Tn (A_4) A_1 (A_2B_2) Intermediate between A_1, B_4			
Vigna radiata (mung bean) MBA	Leguminosae	160,000	4	45,000		rabbit	α-D-galactosyl residues		412
Viscum album (European mistletoe) VAA	Loranthaceae				10.1-11%	None			413-419
I		115,000	2 2	29,000 34,000			D-gal		
II		60,000		27,000 32,000			D-gal, galNAc		
III		50,000		25,000 30,000			galNAc		
Wistaria floribunda (Japanese wistaria) WFA	Leguminosae	68,000	2	32,000	11.4% (5.8% man, 3.5% glcN)	None	galNAc	Yes	420-425

5) Biological Properties

Lectins display a variety of biological functions which enhance their usefulness. Properties such as the agglutination of erythrocytes and specific binding to carbohydrates are important features of a lectin.

The hemagglutinating activity of plant extracts that aroused early interest in lectins is well documented[18,424-436]. In some cases (*Dolichos biflorus*, *Arachis hypogaea*, *Griffonia simplicifolia* among others) this activity is blood group specific. In others, the agglutinin is specific for red blood cells of a particular species such as sheep (*Croton tiglium*), mouse or rat (*Lactuca scariole*) and cat or rabbit (*Onobrychis viciifolia*). Most lectins will agglutinate untreated erythrocytes but some require pretreatment of the red blood cells with neuraminidase (*Arachis hypogaea*), papain (*Cicer arietinum*) or trypsin (*Psophocarpus tetragonolobus*) for activity.

Lectins are also known for their sugar recognition specificities. They interact with the carbohydrate moieties of glycoproteins or glycolipids on cell surfaces[18,437] and induce cell-to-cell binding[438]. The ability to function as a lectin receptor is dependent upon the number and nature of sugar residues in the molecule[439]. Lectin binding may be affected by several factors including the presence of terminal sugar capping which may hinder binding[439], capacity of the adsorbent, temperature dependence, pH effects, ionic strength, concentration requirements for glycoside[440] and divalent metal ions[441].

This specific binding, as described above, produces a number of biological effects. Two of the most studied are the mitogenic stimulation of lymphocytes[8,9,15,16,18,446-449] and the agglutination of transformed cells[7-9,15,16,451-457]. Several other unique properties reported include:

- Insulin-like activity[442] and the stimulation of insulin release[16,443]
- Inhibition of interferon action[444], fungal growth[18,445] and epinephrine-stimulated lipolysis[270]

- Suppression of antibody response[430]
- Binding to sperm[431] and interactions with developing cells[432]
- Induction of the platelet release reaction[433,434]
- Inhibition of phagocytosis and protein synthesis[435,436].

6) Applications

In view of their unique biological properties, a number of clinical and research-oriented applications for lectins have emerged:

a) Clinical

- Routine and theoretical investigations of blood grouping and detection of "secretors"
- Chromosome analysis and fractionation of lymphocyte sub-populations
- Histopathological studies and comparative investigations on normal and transformed cells
- Immunohistochemical probes for metabolic and glycoprotein storage diseases
- Target-oriented therapy of malignant tumors
- Detection and identification of human pathogens

b) Research

- Isolation and purification of glycoproteins, glycopeptides and glycosylated nucleic acids by affinity chromatography
- Cell separations
- Identification and quantification of glycoproteins
- Structural studies on carbohydrates and glycoproteins using lectins and lectin-resistant cell surface variants of eukaryotic cells and selected cell lines with altered membrane carbohydrates

- Probes for the characterization of cell surfaces and the examination of membrane topography
- Eliciting monoclonal antibodies
- Retrograde and anterograde study of neural transport
- Plant chemotaxonomy

Lectins have been widely utilized as blood typing reagents in immunohematology[460]. Plant agglutinins have been used to determine the antigenic relations of blood group antigens[421] and the diagnosis of blood subgroups A and AB[462]. Secretors and non-secretors can be distinguished by *Ulex europeus* agglutinin[462].

The preparation of metaphase chromosomes from lymphoid cells is facilitated by mitogenic stimulation with lectins such as *Phaseolus vulgaris* agglutinin. Structural analysis of stained chromosome bands allows the detection of environmentally-induced and inherited genetic abnormalities, which manifest themselves in pathological conditions such as Klinefelter's, Turner's, Down's, Patau's and Edwards' syndromes. Lymphocytes have been fractionated using lectin binding techniques that capitalize on the surface differences between normal lymphocytes and those modified by lymphoproliferative diseases[463]. Selective agglutination is also the basis for separation of mouse lymphocyte classes[464-466].

Lectins are useful histochemical probes with potential as diagnostic tools. Tumors and metastases can be detected using radioiodinated lectins and monoclonal antibodies. Agglutinins conjugated to fluorochromes, peroxidase, ferritin or biotin, can localize glycosidic components of cells and trace their changes in the pathological state[467] of a variety of tissues[468]. While the emphasis has been on malignant cells[469,470], lectins have also been employed in other studies, such as the differentiation of normal and psoriatic epidermis[471].

The diagnostic use of lectins has been extended to the identification of glycoprotein storage diseases[472,473]. Lectin reagents are believed to be cheaper and simpler than conventional biochemical tests needed to identify specific sugars in undegraded stored

substances[472] or demonstrate a deficiency in enzyme activity.

Target-oriented therapy of malignant tumors using enzymes or drugs coupled to lectins may provide a new approach to chemotherapy[28]. Lectin derivatives of methotrexate and chlorambucil showed greater inhibitory effects on tumor cell DNA biosynthesis than equivalent doses of free drug and lectin[474]. Fluorescent-labelled lectin derivatives may also have value in the identification and detection of human fungal pathogens[475].

Agglutinins have many research applications in addition to their clinical potential. Immobilized lectins linked to inert supports have been used in the isolation and purification of glycoproteins, glycopeptides and glycosylated nucleic acids by affinity chromatography[8,16,32,34,39,440,476].

Affinity chromatography can also be used for cell separation, along with selective agglutination, mixed rosetting and flow microfluorimetry techniques. This application of lectins has been limited to the separation of mammalian cells, especially lymphocytes, but offers the advantage of high yields of viable cells since the binding of lectins to cells is reversible without damage to the cells[477].

Enzyme-linked lectin assays (ELLA), similar to the enzyme-linked immunosorbent assay (ELISA) but based on nonimmunologic reagents, have been developed as specific probes for end groups on immobilized glycoproteins[478,479]. Quantification can be achieved using rocket affinoelectrophoresis in a gel containing lectin[480,481]. The amount of glycoprotein applied and the rocket height correlate[481].

Structural studies on carbohydrates have been conducted using various combinations of immobilized lectin columns to fractionate sugar chains into various structural groups. Absolute configurations of sugars have been determined on the basis of inhibition of ^{125}I-labelled lectin binding[482]. Structural assessments of glycosylated macromolecules have also been achieved using lectin-resistant cell surface variants of eukaryotic cells and selected cell

lines with altered membrane carbohydrates.

Lectin probes have been employed in the characterization of cell surfaces and the examination of membrane topography[484]. The diversity of investigations ranges from cell surface glycoconjugates and membrane anomalies[485-488] to probing membrane asymmetry and compartmentalization[489] and the binding of glycoproteins from microsomal and Golgi membranes to lectins[490].

Agglutinins can be used to induce "capping", an effect characterized by the displacement of antigenic molecules to one pole of the cell. This phenomenon allows membrane fluidity to be studied.

Other research applications of lectins include the elicitation of monoclonal antibodies[491], retrograde and anterograde study of neural transport[492] and the chemotaxonomic classification of plants based on N-terminal sequence data[493].

B. Microorganisms and Lectins

A number of bacterial species have been shown to interact with lectins[493]. The binding of lectins to microbial cell walls involves a complex reaction[494]. Concanavalin A is known to agglutinate bacterial spheroplasts[495] and interact specifically with bacterial cell walls containing the appropriate glycosidic components on their teichoic acids[496]. Concanavalin A and wheat germ agglutinin both agglutinate *Streptococci*[497-501]. Lectins have been used to study the surface carbohydrates of various *Mycoplasma* membranes as well[502-505]. Lectins will disrupt the attachment and internalization of *Chlamydia*[506-508]. Other studies have demonstrated the interaction of lectins with *Salmonella*[509-510], *Staphylococcus*[511], *Mycobacterium*[512], *Bacillus*[513], *Legionella*[514], *Haemophilus*[515], *Escherichia*[516], *Campylobacter*[517] and *Pseudomonas*[518].

While lectins can agglutinate and interact with bacteria, the bacteria themselves also have lectins[519-525]. Fimbriae (pili) and some outer membrane proteins are known to contain bacterial surface lectins[526-528]. These lectins have been postulated to mediate the adherence of bacteria to epithelial cells[529-531] and may be involved in the initial stages of bacterial infection [532-535]. Bacterial lectins also bind lymphocytes[536-537] and may act as determinants of recognition in phagocytosis[538]. Bacterial-induced histamine release in intrinsic asthma has been attributed to bacterial lectins[539-540].

Lectin-microbe interactions are potentially useful for distinguishing bacterial species and identifying particular sugar residues on bacterial cell surfaces. In particular, the interaction of lectins with *Neisseria gonorrhoeae*[541-543] has lead to the epidemiological characterization of this bacterium[544] and rapid diagnostic tests for laboratory identification[545-547]. The differentiation of *Bacillus* species[548-549], *Brucella* strains[550], coagulase positive and negative *Staphylococci*[551] and detection of group C *Streptococci*[552] have also been achieved using lectins. Lectin-carbohydrate interactions have been used to quantify microbial cells[553] and investigate receptors for bacterial toxins[554].

Although limited studies have been reported on bacterial lectins and the use of lectins in microbiology, even less is known about agglutinins and viruses. A sialic acid binding hemagglutinin has been identified in influenza virus. Given the potential for antigenic shift and drift in this virus, lectins gain importance in clinical epidemiology particularly since the lectin part may change.

Lectins are reported to agglutinate cells following infection with nononcogenic viruses[356-360], and transformed avian RNA tumor viruses[361,362]. This property of lectins to induce viral agglutination has been used to purify oncornaviruses[363] and viral glycoproteins[555].

Lectins are also known to affect the growth and release of viruses[364,365], including myxovirus. The cytopathogenicity of Newcastle disease virus was modified by agglutinins[366,367]. Similar treatment with Con A rendered Sendai virus and herpes simplex virus non-infectious[368].

C. Botany of the Moraceae Family

The Urticales (nettle) order is comprised of approximately 125 genera and 3,100 species in the Ulmaceae (elm), Moraceae (mulberry), Cannabinaceae (hemp) and Urticaceae (nettle) families[569]. The Moraceae family, consisting of about 75 genera and 1850 species, is distributed mainly in tropical and subtropical regions[570]. Corner[571] describes six tribes (Ficeae, Dorstenieae, Brosimeae, Moreae, Olmedieae and Artocarpeae) whereas Hutchinson[570] lists three subfamilies and nine tribes (Moroideae; Fatoueae, Moreae, Broussonetieae, Strebleae, Dorstenieae; Artocarpoideae; Euartocarpeae, Olmedieae, Brosimeae, Ficeae; and Conocephaloideae). *Ficus* represents the largest genus with more than 800 species. *Dorstenia* contains about 170 species while *Brosimum* and *Artocarpus* each number around 50 species. Another 12 Moraceae genera contain ten to 25 species each, while the remaining genera have less than four species each.

Moraceae plants generally contain a milky latex. The leaves tend to be simple and alternate and the flowers small and apetalous. Many species possess multiple fruits that result from different flowers fusing together to form an aggregate fruit[569,572-574]. Economically the family is important for edible fruit (*Artocarpus*, *Ficus*, *Treculia*, *Morus*, *Chlorophora*), timber (*Ficus*, *Antiaris*, *Castilloa*, *Musanga*, *Brosimum*), latex products such as components in rubber, glue and caulking compounds and an arrow poison (*Ficus*, *Artocarpus*, *Castilloa*, *Cecropia*, *Antiaris*), ornamentals (*Maclura*, *Ficus*, *Dorstenia*, *Cudrania*, *Chlorophora*), fibres for bark cloth or paper (*Artocarpus*, *Broussonetia*) and leaves for feeding silkworms (*Morus*, *Maclura*[570,572]).

Artocarpus plants are monoecious, with both male and female flowers on the same plant, and contain a sticky, white latex[573]. The leaves alternate or form a spiral arrangement. The fruit is a fleshy, pulpy syncarp from eight to about 20 cm in diameter, containing many seeds which lack an endosperm[573,575]. The genus has been thoroughly described by Fosberg[576] and Jarrett[577,578]. Trees and shrubs of the genus *Ficus* are also monoecious flowers, have alternate leaves and produce latex[573,575].

D. Aims and Objectives of Current Investigation

Most previous investigations of lectin activity have focused primarily on lectins of the Leguminosae family, because of the economic importance of this taxa and abundance of agglutinating material in seeds of these plants. In the case of the Moraceae family, the lectins have not been studied or characterized extensively with the exception of *Maclura pomifera* (Osage orange) and *Artocarpus integrifolia* (jackfruit). One of the objectives of this study was to obtain more information on agglutinating substances in this botanical group.

Initial studies were directed towards the screening of lectins for serological cross-reactivity and relatedness to known lectins and the evaluation of crude extracts from new plant sources for potentially useful lectins. Based on this screening, the objective was to identify, purify and characterize new Moraceae lectins. The intent was to find new lectins which could have applications in the field of microbiology. Gram negative bacilli such as *Escherichia*, *Proteus* and *Pseudomonas* species, are part of the normal endogenous flora. However, these organisms can cause opportunistic infections upon leaving their normal habitat or after their introduction into debilitated patients. *Proteus* strains show a high degree of multiple drug resistance and *Pseudomonas* species are known to resist most antimicrobial agents. The cell wall of a bacterium exhibits many dynamic functions including rigidity and shape determination, protection and provision of a site of antigenic specificity. Its role as a permeability barrier may play a significant part in resistance to antibiotics. The identification and characterization of new lectins will increase the number of useful probes for studying the binding sites and moieties on the cell walls of drug resistant bacteria. This information should contribute to the design of better, more effective antimicrobial agents and the development of diagnostic kits.

A secondary objective involved the evaluation of different techniques and the application of new developments in protein chemistry to the purification and study of plant lectins.

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II. SCREENING OF CRUDE SEED EXTRACTS

A. Introduction

Since lectins have many applications and are potentially useful in a variety of fields, attempts were made to search for lectins from new plant sources. For this purpose, 23 species representing 10 botanical families including one gymnosperm (Table II-1, page 65) were screened using hemagglutination and Ouchterlony immunodiffusion assays.

The immunochemical analysis of lectins has proven valuable as a tool for defining phylogenetic relationships and chemotaxonomical classifications. Concanavalin A and lentil lectins are thought to have a common ancestor[1] and it appears from the sequence homologies determined to date that the genes coding for leguminous lectins in general share a common ancestry[2-4]. Ouchterlony immunodiffusion assays were conducted to determine if this type of relationship also extends to Moraceae agglutinins.

In addition to the above immunochemical/serological procedures, bacterial agglutinability of Moraceae extracts were carried out to identify potential applications, such as a means to quickly distinguish bacterial species.

B. Methods

1) Materials

Plant seeds were obtained from the various sources listed in the Appendix. Antisera directed against eight lectins (see page 65) were purchased from Sigma Chemical Co. Sugars used in the immunodiffusion medium were bought from Aldrich Chemical Co., except for lactose (Pfanstiehl Labs Inc.).

2) Preparation of crude seed extracts

One gram of seeds was ground using a mortar and pestle, mixed with 10 ml of 10 mM phosphate buffered saline (PBS) and tumbled overnight at 4°C. After centrifuging for 15 min.

at 320 *x g* in a Dynac table top centrifuge, the supernatant was collected and used as such or stored at -20°C.

3) Screening of crude seed extracts

Hemagglutination assays were conducted in plastic microtiter plates with samples added in 100 ul doubling dilutions and 75 ul of a 1.5% suspension of human erythrocytes in PBS. Fresh red blood cells provided by the Canadian Red Cross Blood Transfusion Service washed four times with PBS were used for this assay. *Dolichos biflorus*, an A_1 specific lectin, was used as an internal control. Ouchterlony immunodiffusion[3] was carried out in plastic Petri plates filled with 15 ml of 1.5% agar containing 0.05% sodium azide and 10 mg/ml concentrations of lactose, D-glucose, D-galactose, L-fucose, N-acetyl-α-D-glucosamine, D-mannose and D-maltose monohydrate. The sugars were added to eliminate any spurious precipitin lines due to lectin-glycoprotein interactions. Wells were filled with 20 ul of antisera or extract and incubated overnight in a humid chamber. Precipitin lines observed were washed for two days with 0.9% NaCl to remove non-involved proteins, dried using Whatman 1 filter paper, then stained for 18 min. with amido black and destained with 2% acetic acid.

4) Bacterial culturing and agglutination

The following bacteria were cultured on nutrient agar at 37°C: *Escherichia coli* 10418, *Proteus vulgaris* 13315, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* 13883, *Salmonella oranienburg* and *Staphylococcus aureus* 6538P (FDA 209P). *Bacillus cereus* and *Micrococcus luteus* 10240 were grown on the same media at 30°C, while *Serratia marcescens* was incubated at 26°C. *Staphylococcus aureus* (strains N2, a laboratory strain which is a producer of penicillinase, and 25923) was grown on blood agar plates at 37°C. *Salmonella typhimurium* 13311, *E. coli* (B strain 11303 and ESS, a mutant strain very sensitive to B-lactam antibiotics) were cultured on trypticase soy agar at 37°C. *Micrococcus luteus* 9341 and *Comamonas terrigena* 8461 were grown on the same media at 30°C.

A loop of bacteria was stirred in 100 μl of water on a glass slide, 100 μl extract added, rocked for a few minutes, and observed for agglutination reactions.

Chlamydia trachomatis serovar L_2 was harvested from infected monolayers cultivated in HeLa cells and elementary bodies were purified by layering over a discontinuous Renografin (Squibb) gradient and centrifugation at 43,000 *x g* for 60 min. at 4°C. Elementary bodies were collected from the 44-52% interface and diluted with three volumes of N-tris(hydroxymethyl)methyl 2-aminoethanesulfonic acid (Sigma Chemical Co.) buffer (TES). This preparation was centrifuged for 30 min. at 30,000 *x g* and 4°C. The purified material was resuspended in 10 mM TES in 0.85% NaCl and stored at -70°C. Agglutination tests were performed on glass slides and viewed under a microscope.

C. Results and Discussion

The extracts prepared from the two *Artocarpus* species and *Ficus deltoidea* displayed a high hemagglutination titer for A, B, O and AB human erythrocytes. *Mangifera indica* exhibited a slight preference for blood group O, despite its ability to agglutinate the other blood types as well. Moderate hemagglutination was observed with *F. vogelii* and *A. squamosa*. Weak agglutination activity was detected in *A. cherimolia*, *H. japonicus*, *B. papyrifera*, *D. contrajerva*, *P. mungo*, *P. aconitifolius*, *L. chinensis* and the two *Brassica* species. No activity was seen in the four Pinaceae species, *D. virginiana*, *P. radiatus*, *M. pumila* and *C. sativa*. One feature of the current investigation was to examine readily available Canadian seeds and taxa, including gymnosperms. These species produced low titers (*Brassica*) or failed to agglutinate human red blood cells (Pinaceae species) (Table II-2, page 66). A sample of *A. integrifolia* from Bangalore, India produced an exceptionally high titer (1/25,600) compared to a sample from Madras, India (1/64). This may be the result of using seeds which have been ground to a fine powder, as supplied by the Bangalore source. Grinding intact seeds from Madras using a mortar and pestle produced much lower titers suggesting this approach is not nearly as efficient or that large strain or variety differences may exist in this species.

The absence of agglutination or low titers in these results may not give an entirely accurate picture of lectin content, since no provision was made to remove oils, fats and other materials which could have been responsible for low lectin yields. The two *Brassica* species are particularly rich in oils and utilized commercially for canola oil. In addition, untreated blood was used in the assays and a minority of lectins are known to require pretreatment with neuraminidase, trypsin or papain for their agglutination activity. A third factor involves regional differences and variations in plants. It is also possible the lectin could be localized in a part of the plant other than the seeds. Agglutination could be prevented by the existence of glycoprotein-lectin complexes covering the active site of the lectin as well. Thus, while Hardman *et al*[6] also failed to detect a lectin in *C. sativa*, agglutination material may be present

as suggested by Tumosa[7-8]. As a technique, hemagglutination is simple and rapid but only semiquantitative at best. The results are subject to varied interpretation and provide few details as to specificity.

Antisera directed against *Maclura pomifera*, *Arachis hypogaea*, *Triticum vulgaris*, *Dolichos biflorus*, *Canavalia ensiformis*, *Limulus polyphemus*, *Tetragonolobus purpureas* and *Pisum sativum* agglutinins were used in the Ouchterlony immunodiffusion assays. The *F. deltoidea* extract showed a precipitin line with all of the above antisera. However, these precipitin reactions were eliminated when the crude FDA extract was mixed with normal rabbit sera, indicating the formation of a non-specific precipitin line with serum (see also chapter IV). Both *Artocarpus* extracts showed cross-reactivity with antisera directed against *M. pomifera* (see also chapter III). The remaining species did not produce precipitin lines with any of the eight antisera used. The spurs obtained with *Artocarpus* indicate a reaction of partial identity. Spur formation gives an indication of whether two proteins are antigenically similar or distinct. A single spur results when two different antigens share some common determinant(s)[9]. Three types of precipitation patterns are recognized (Figure II-1, page 68): A pattern of identity is represented by one continuous line indicating both antigens possess identical determinants with respect to the antiserum used. In a reaction of nonidentity, the lines form independently of each other without significant interaction meaning the antiserum does not contain antibodies directed against any determinant groups common to both antigens. In partial identity, a spur is generated by determinants found in one antigen but not the other. It is a case of one antigen reacting more fully with the antiserum than the second antigen. Thus the cross-reacting *Artocarpus* lectins are immunologically related, but not identical to the lectin from *Maclura pomifera*. Different proteins can be functionally similar while totally unrelated antigenically. But antigenically similar proteins possess a strong probability of both a functional and evolutionary relationship[10]. Cross-reactivity has been found among some lectins within the Solanaceae[11-12] and Leguminosae[10,13-14]. The present study suggests lectins within the Moraceae family may also be related. Cross-reactivity does seem to occur between

families as indicated in both literature and current investigations.

Precipitin lines were generated between some pairs of lectins in the outer wells of the agar plates, including *M. indica* and *P. mungo*, and *M. indica* and *A. squamosa*. This observation of reactivity indicating an apparent relationship between a member of Anacardiaceae and Leguminosae, and Anacardiaceae and Annonaceae, was further investigated to eliminate the possibility of 'lectin-lectin' interaction (where at least one should be a glycoprotein). No precipitin lines were obtained between the same pairs of seed extracts when immunodiffusion plates containing lactose, D-glucose, D-galactose, L-fucose, N-acetyl-α-D-glucosamine, D-mannose and D-maltose monohydrate were used. Analogous to these spurious lines, false reactivities could also be generated due to the presence of glycoproteins in serum. Such false positives were also eliminated by sugars in the media, leaving only true antigen/antibody reactions.

Preliminary experiments showed samples of FDA agglutinated trypticase soy broth, nutrient broth and trypticase, so bacteria were cultured on agar rather than in broth. Slight agglutination by the *F. deltoidea* extract was observed with *Staphylococcus aureus* and *Comamonas terrigena* and fair agglutination was seen using *Bacillus cereus*, *S. aureus* N2 and *Salmonella typhimurium*. *Chlamydia trachomatis* produced a strong agglutination. The other species and strains tested failed to agglutinate with the crude FDA. *Broussonetia papyrifera*, the only other Moraceae extract to interact with bacteria, slightly agglutinated *B. cereus* and *C. trachomatis* (Table II-3, page 67). *Chlamydia* causes the most prevalent sexually transmitted disease in Canada and is difficult to diagnose because of its asymptomatic nature. It can result in scarring of the fallopian tubes, infertility and pelvic inflammatory disease in women and epididymitis and prostitis in men. In addition, one-third of children born to infected mothers develop conjunctivitis, an eye infection. Due to its agglutination of this bacterium, FDA may be a clinically important lectin.

Table II-1. Species examined in the current studies.

CLASS	FAMILY	SPECIES	COMMON NAME
Gymnospermae	Pinaceae	*Larix sibirica* Ledeb	Siberian larch
		Picea glauca (Moench) Voss	white spruce
		Pinus contorta Loudon var. latifolia Engelm	lodgepole pine
		Pseudotsuga menziesii (mirb.) Franco	Douglas fir
Angiospermae	Ebenaceae	*Diospyros virginiana* Linn.	persimmon
	Sapindaceae	*Litchi chinensis* Sonner. (*Nephelium litchi* Linn.)	lychee
	Leguminosae	*Phaseolus mungo* Linn.	black manh, black bean
		Phaseolus aconitifolius Jacq.	moth
		Phaseolus radiatus (L.) Wilzeck	red chori
	Rosaceae	*Malus pumila* Mill. cultivar McIntosh	McIntosh apple
	Cruciferae	*Brassica campestris* Linn.	Polish rapeseed
		Brassica napus Linn.	Argentina rapeseed
	Moraceae	*Ficus vogelii* Miq.	African tropical fig
		Ficus deltoidea Jack. (*F. diversifolia* Blume)	mistletoe fig
		Dorstenia contrajerva Linn.	American tropical fig
		Artocarpus integrifolia Linn.	jackfruit
		Artocarpus altilis (Parkinson) Fosberg	breadfruit
		Broussonetia papyrifera Vent.	paper mulberry
	Cannabinaceae	*Cannabis sativa* Linn.	hemp
		Humulus japonicus Sieb. & Zucc.	Japanese hop
	Annonaceae	*Annona squamosa* L.	sitaphal, custard apple
		Annona cherimolia Mill.	cherimoya
	Anacardiaceae	*Mangifera indica* Linn.	mango

Table II-2. Hemagglutination and Ouchterlony immunodiffusion studies on extracts from a variety of plant seeds.[1]

SPECIES	SOURCE	HEMAGGLUTINATION TITER[2]				OUCHTERLONY ASSAY
		Human Blood Groups				*Maclura pomifera* antisera
		A	B	O	AB	
Larix sibirica	Local	0	0	0	0	–
Picea glauca	Local	0	0	0	0	–
Pinus contorta	Local	0	0	0	0	–
Pseudotsuga menziesii	Local	0	0	0	0	–
Diospyros virginiana	Local	0	0	0	0	–
Litchi chinensis	Local	4	4	4	4	–
Phaseolus mungo	Local	64	16	64	64	–
Phaseolus acontitifolius	Local	1	1	0	0	–
Phaseolus radiatus	Local	0	0	0	0	–
Malus pumila	Local	0	0	0	0	–
Brassica campestris	Local	1	1	1	1	–
Brassica napus	Local	1	1	1	1	–
Ficus vogelii	Redwood City, Ca.	128	128	128	128	–
Ficus deltoidea	Local	6400	6400	6400	6400	+[3]
Dorstenia contrajerva	Local	32	16	16	16	–
Artocarpus integrifolia[4]	Madras, India	64	64	64	64	+
Artocarpus altilis	Local	2048	1024	1024	1024	+
Broussonetia papyrifera	Sandwich, Mass	2	2	2	2	–
Cannabis sativa	Ottawa, Ont	0	0	0	0	–
Humulus japonicus	Redwood City, Ca	8	8	8	4	–
Annona squamosa	Local	64	128	128	128	–
Annona cherimolia	Local	40	40	40	40	–
Mangifera indica	Local	1024	2048	4096	2048	–

[1] *Dolichos biflorus* agglutinin, an A_1 specific lectin, was used as a reference in hemagglutination studies.
[2] Titer refers to the reciprocal of the last doubling dilution showing agglutination.
[3] Positive results were also obtained with normal sera and therefore represent non-specific precipitin reactions.
[4] Powdered seeds from Bangalore, India source showed a surprisingly high titer of 25,600.

Table II-3. Bacterial agglutination tests with extracts from Moraceae species.

BACTERIUM	DESCRIPTION	F. DEBTOIDEA[1]	B. PAPYRIFERA[1]
Staphylococcus aureus	Gram + cocci	+	-
Staphylococcus aureus N2		+ +	-
Micrococcus luteus	Gram + cocci	-	-
Bacillus cereus	Gram + endospore-forming rods	+ +	+
Chlamydia trachomatis	Gram - spheroidal	+ + +	+
Klebsiella pneumoniae	Gram - facultatively anaerobic rods	-	-
Salmonella oranienburg	Gram - facultatively anaerobic rods	-	-
Salmonella typhimurium		+ +	-
Serratia marcescens	Gram - facultatively anaerobic rods	-	-
Pseudomonas aeruginosa	Gram - aerobic rods	-	-
Proteus vulgaris	Gram - facultatively anaerobic rods	-	-
Escherichia coli	Gram - facultatively anaerobic rods	-	-
Escherichia coli B		-	-
Escherichia coli ESS		-	-
Comamonas terrigena	Gram - facultatively anaerobic curved rods	+	-

Note: Extracts prepared from *F. vogelii*, *D. contrajerva*, *A. intergrifolia* and *A. altilis* did not agglutinate any of the bacterial species tested.
[1]Agglutination was assessed visually as weak (+), medium (+ +), strong (+ + +) or absent (-).

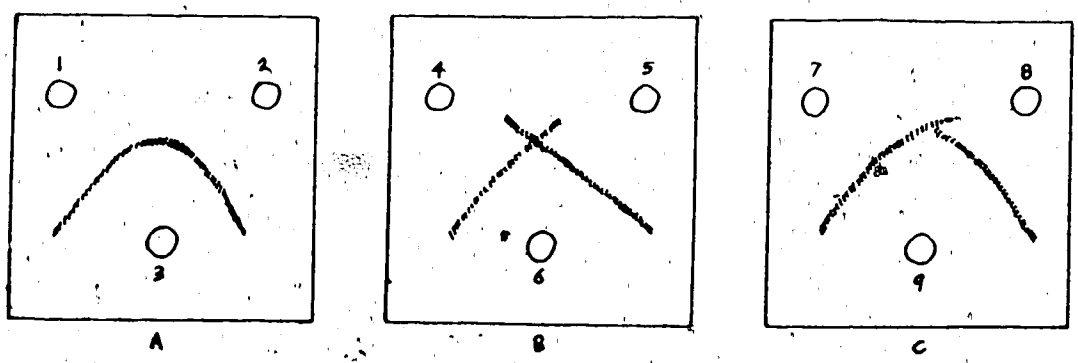

Figure II-1. Illustrations of Ouchterlony immunodiffusion precipitin reactions in agar. In the reaction of identity (A), wells 1 and 2 contain the same antigen with the antisera in well 3. A reaction of nonindentity (B) was obtained by placing different antigens in wells 4 and 5 and antisera to both in well 6. Partial identity (C) was achieved by using an antigen and its antiserum in wells 7 and 9, respectively, and a crossreacting antigen in well 8. (Adapted from Davis, B.D. *et al*, 1980).

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III. STUDIES ON A LECTIN FROM *ARTOCARPUS ALTILIS* SEEDS *

A. Introduction

Artocarpus altilis[1] (*A. communis*, *A. incisa*) or breadfruit is cultivated throughout the tropics but is indigenous to New Guinea, the Moluccas, Micronesia and western Melanesia regions of the South Pacific[2,3]. It was introduced to the West Indies by Capt. William Bligh in 1793[4]. The breadfruit has remained a tropical/subtropical plant since it requires hot, moist climates with temperatures of 25-30°C, 150-250 cm annual rainfall and well-drained soils[5].

The tree ranges from 40 to 60 feet (12-18 metres) in height, with many large, glossy green leaves arranged spirally on the branches. The plant is monoecious with male and female flowers in separate groups on the same tree. Ripe fruits measure 4-8 inches (10-20 cm) in diameter and are characterized by a thick, green to brownish-yellow rind enclosing a pale white, starchy, fibrous pulp[6,7] (Plates III-1,2; Figure III-1, pages 92 and 89).

The considerable amounts of starch and sugars present make breadfruit a staple food and it can be cooked and prepared in a number of ways[8]. In addition to being a good source of vitamin B, it is also a fair source of vitamins A and C[9]. Its potential for commercial processing has also been studied[10]. While breadfruit is mainly a food staple, the plant has been utilized in a variety of ways. The wood has been used to make canoes and furniture, the latex for caulking material and glue; the fibrous inner bark transformed into cloth and dried leaves used for mats or polishing bowls[11,12].

Detailed scientific studies of the breadfruit are lacking. Phytochemical investigations have detected sterols and triterpenes in the fruit and bark[13-15]. One other study[16] analyzed ground breadfruit seeds and found dried samples contained 3.4% ash, 12.8% fat, 16.0% soluble carbohydrates, 20.0% total proteins and 3.9% crude fiber. The content of globular proteins was twice that of non-globular proteins.

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As alluded to in the previous chapter, cross-reactivity was observed between a breadfruit extract and antisera directed against *M. pomifera*. As a result it was decided to purify the agglutinating material. This chapter deals with the isolation and characterization of a lectin from breadfruit seeds.

B. Material and Methods

1) Materials

Breadfruit imported from Fiji was obtained from a local store. The Synsorb™, carbohydrate-coated latex beads and synthetic carbohydrate haptens were provided by Chembiomed Ltd. (Edmonton). All chemicals and reagents used were of analytical grade.

2) Purification of breadfruit agglutinin

Preliminary studies on the distribution indicated the lectin was primarily localized in the seeds (Table III-1, page 82). The average breadfruit yielded 10 seeds weighing a total of about 40 g (fresh).

In a typical purification, the seed coats were removed and 78 g of breadfruit seeds along with 780 ml of PBS (1:10 W/V) ground in a blender for 1 min. The mixture was sonicated on ice with three 30 sec bursts (0.2 relative output, Fisher Sonic Dismembrator Model 300) and stirred overnight at 4°C. The crude breadfruit extract was centrifuged for 20 min, at 2100 *x g* and 4°C. The supernatant was filtered through glass wool. 1M $MnCl_2$ (5.8 ml) was added dropwise with stirring to remove nucleoproteins from the filtrate. The mixture was centrifuged as before and the pellet discarded. The supernatant was brought to 30% saturation of $(NH_4)_2SO_4$ and stirred on ice for 30 min.[17] The extract was centrifuged and the pellet discarded. The supernatant was subsequently brought to 75% saturation of $(NH_4)_2SO_4$ and stirred on ice for 30 min. After centrifugation the pellet was resuspended in 150 ml PBS and dialyzed for 2 days at 4°C against 2L of PBS with two changes. Following a preliminary characterization using sugar-bound latexes (see later section), the dialyzed extract was added to T-Synsorb™ (immobilized β-D-gal(1→3)αgalNAc with an incorporation of 1.89 um of T

hapten/g of Synsorb™) and tumbled for 4 h at room temperature. The Synsorb™ was then washed with 500 ml of PBS and poured into a column. Washings were continued until the absorption at 280 nm registered a baseline. Elution was carried out using a pH gradient prepared from 10 mM Tris-HCl - 10% sucrose (added as a protein stabilizer) - 0.9% NaCl - 0.1% NaN_3 (pH 7) and 10 mM acetic acid - 10% sucrose - 0.9% NaCl (pH 2), followed by an additional 100 ml of the same solution. Ten ml fractions were collected and pooled aliquots containing the eluted peak neutralized with 0.5M Na_2HPO_4. This material was concentrated to 25 ml in an Amicon Ultrafitration cell model 402 with a PM-10 membrane under 25 psi nitrogen (Table III-2, page 83). The 78 g of breadfruit seeds yielded approximately 15 mg of purified lectin (by weight after lyophilization). All of the fractions from the different purification steps were tested for hemagglutination as described in the previous chapter. The units of activity were determined by multiplying the titratable endpoint of a given fraction using human red blood cells and doubling dilution of the fraction by the volume. Specific activity represents the units of activity /g protein.

3) Characterization of breadfruit agglutinin

a. Latex agglutination assay

The partially purified (after salt precipitation and dialysis) breadfruit seed extract was tested for agglutination against a series of carbohydrate-bound latexes (developed by Chembiomed Ltd. and described in reference 18) in order to quickly identify the sugar specificity. Based on the results obtained (Table III-3, page 84), a 1:10 dilution of BFA extract in 3% BSA was adsorbed overnight on 50 mg of three different Synsorbs™ (βgal(1→3)αgalNAc, αgal, βgal(1→4)βglcNAc) and a control. After centrifugation in an Eppendorf microfuge for 4 min., 100 ul of the supernatants were diluted serially in PBS. One hundred ul of 2% red blood cells were added and the tubes allowed to sit at room temperature for 1 h before checking for agglutination. Weak agglutinating activity would be indicative of BFA binding to the specific Synsorb™. The results suggested the T-Synsorb™ (βgal(1→3)-αgalNAc) was the most suitable adsorbent to use in an affinity chromatography column.

b. Radioligand inhibition studies

i) Preparation of RIA tubes

A solution of purified BFA containing 50 ug protein/ml in dilution buffer (50mM NaH_2PO_4/Na_2HPO_4, 5 mM $MgCl_2$, 15mM NaN_3 pH 7.5) was prepared and 100 ul used to coat RIA tubes[19,20]. After an overnight incubation at room temperature, the coating solution was aspirated and 200 ul of 5% BSA in PBS added to prevent non-specific binding. Following a 4 h incubation at room temperature the BSA solution was removed and replaced by 1.9 ml of PBS for 10 min. The RIA tubes were aspirated three times to thoroughly remove the PBS and air dried for 1 h at room temperature. The coated tubes were stored at 4°C in a sealed plastic bag.

ii) ^{125}I-labeling of T-BSA

One mg of iodogen (1,3,4,6-tetrachloro-3α, 6α-diphenyl-glycouril) was dissolved in 100 ml of chloroform. One hundred ul of this solution was dispensed into a tube and dried under a gentle nitrogen stream until ready to use. A 1 mg/ml solution of T-hapten-BSA (β-D-gal(1→3)αgalNAc-BSA) (Chembiomed Ltd.) to be labeled was prepared in iodination buffer (0.5M NaH_2PO_4/Na_2HPO_4 pH 7.1). Twenty ul of iodination buffer was placed in another tube, 10 ul of $Na^{125}I$ (4000 MBq/ml in 0.1M NaOH) added and mixed thoroughly. Following the addition and mixing of 10 ul of the T-BSA solution, the contents were transferred to the iodogen-plated tube, stoppered and incubated for 45 min. with occasional gentle agitation. The sample was removed from the iodogen tube to a fresh tube and 20 ul of 1M NaI and 50 ul of 1% BSA/PBS added. This was incubated for 40 min. to allow non-radioactive I^- to exchange with any non-specifically bound $^{125}I^-$.

iii) Gel filtration chromatography of ^{125}I-labeled T-BSA

Gel filtration chromatography is required to separate the labeled T-BSA from radioactivity which did not bind (usually in form of I^-). A 1 by 10 cm Bio-Gel P6DG column was washed with 1 ml of 1% BSA/PBS followed by 20 ml of PBS. Column chromatography was initiated by washing the labeled sample in with small applications of PBS, connected to a PBS reservoir. At approximately 1.5 ml prior to the void volume, 8-drop fractions were collected in tubes previously exposed to a Zerostat™ gun to remove static. After collecting the last fraction the column was washed with 20 ml PBS. The five or six fractions containing the bulk of the radioactivity were pooled and a 1/100 dilution of radioactive stock prepared. A working solution of about 9.65 uCi (250,000 cpm)/ 50 ul was derived from the radioactive stock and 1% BSA/PBS added to bring the volume to 40 ml.

iv) Inhibition of ^{125}I-T-BSA binding

Two mg/ml concentration in PBS of 13 free haptens (Table III-4, page 85), were screened in triplicate for inhibition of ^{125}I-T-BSA binding to BFA coated RIA tubes. Three coated (as described above) and three uncoated tubes contained 100 ul 1% BSA in PBS, while the remaining coated tubes received 100 ul of hapten. Fifty ul of ^{125}I-labeled T-BSA solution was added to all the tubes. The following day, tubes were aspirated, washed three times with PBS and measured for radioactivity in a Beckman Gamma 400 spectrometer. To determine the 50% inhibition value of the T-hapten, serial dilutions of hapten from 2-500 ug/ml in 1% BSA in PBS were prepared and the procedure described above repeated.

c. Gel filtration

Five g of Bio-Gel P150 (100-200 mesh) were hydrated in PBS for 4 hr, then degassed under vacuum and poured into a column. Bed height measured 48 cm and column diameter, 1.5 cm. Uniform packing of the column and void volume were checked using 2 ml of blue dextran (10 mg/ml). A flow rate of 12 ml/hr was established (LKB Bromma 2132 Microperpex peristaltic pump) and absorbance monitored at 280 nm. A 1 ml sample

containing 1 mg of each of the following molecular weight markers (Sigma Chemical Co.) were used to calibrate the column: Cytochrome C from horse heart (12,400), trypsinogen from bovine pancreas (24,000), ovalbumin (45,000), albumin from bovine serum (66,000), alcohol dehydrogenase from yeast (150,000) and β-amylase from sweet potato (200,000). Gel filtration[21] was also conducted using a Pharmacia FPLC system with a Pharmacia HR 10/30 Superose 12™ high performance gel filtration column (chapter IV). After calibration, 1 ml of purified BFA was chromatographed and its Ve determined.

d. SDS polyacrylamide gel electrophoresis

Electrophoresis was performed in 20% (w/v) polyacrylamide slab gels of 1.5 mm thickness, in the presence of 0.1% SDS, employing the discontinuous buffer system of Laemmli[21]. The gels were run at 30 mAmps per slab using a Bio-Rad Protean 16CM unit. Reduced gels were obtained with 10 mM dithiothreitol (DTT). Gels were stained on a shaking bath with 0.15% (w/v) Coomassie Brilliant Blue R-250 in 50% ethanol/10% acetic acid and destained in 20% ethanol/10% acetic acid.

e. Isoelectric focusing in polyacrylamide gels

Isoelectric focusing gels were cast in glass tubes of 2.5 mm internal diameter and 15 cm length. The gel composition was: 4% acrylamide, 0.24% N,N'-methylene bis-acrylamide, 9M urea, 0.66% NP40 and 2% pH 3.5-10 ampholines (LKB Chemical). Samples were dissolved in freshly prepared buffer containing 9M urea and 1 mM DTT, centrifuged to remove insoluble material and then loaded onto gels and subjected to nonequilibrium pH gradient electrophoresis[23]. The extracts were run for 16 h at 250 volts, making a total of 4000 Vhrs. Gels were stained with Coomassie Brilliant Blue R-250, after pretreatment with 15% trichloroacetic acid (TCA) (three changes in three h) and 20% ethanol/10% acetic acid (four changes in two h), to remove ampholines. Approximate isoelectric points were determined by sectioning the gels, immediately after electrophoresis, into 1 cm segments and eluting the ampholines into 1 ml of degassed water over a period of 10 min. and measuring the pH. The

gradient obtained for the major central region of the gel was found to be linear with a ΔpH value of 0.04/mm gel.

f. Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was performed by running an isoelectric focusing gel in the first dimension followed by SDS PAGE in the second dimension[24-26].

g. Amino acid analysis

Timed acid hydrolysis of 24, 48, 72, and 96 h was conducted on 200 ug samples of purified BFA which had been mixed with 1 ml 6N HCl in sealed glass tubes, evacuated and hydrolyzed at 110°C. After removing the HCl by evaporating to dryness and dissolving the residue in sodium citrate buffer pH 2.2, 0.5 ml aliquots were analyzed on a Durrum amino acid analyzer[27].

h. Carbohydrate determination

The phenol-sulfuric acid method[28] was employed to determine the sugar content of the purified lectin. Galactose standards containing 0, 10, 25, 50, 100 or 200 ug/ml dH2O were

dialyzed samples[10].

j. Densitometry

BFA protein bands on the SDS PAGE gels were analyzed using a Joyce Loebl Chromoscan 3 densitometer, scanning from the bottom of the gel to the top.

k. Preparation of antisera in rabbits

One ml of purified BFA (0.6 mg) was mixed with 1 ml complete Freunds' adjuvant and vortexed thoroughly to form an emulsion. One-half ml was injected intramuscularly into four sites of female New Zealand white rabbits weighing about 4 kg each. The animals received a booster injection of BFA after five weeks and were bled from the marginal vein of the ear one week later. The blood was placed in a water bath at 37°C for 30-35 min., then refrigerated at 4°C overnight. The serum was collected and centrifuged for 15 min. at 450 *x g* in a Dynac centrifuge. The supernatant was frozen at -20°C following the addition of 0.1M sodium azide.

C. Results and Discussion

The extracts prepared from the two *Artocarpus* species displayed a high hemagglutination titer for A, B, O and AB human erythrocytes (chapter II). Both showed cross-reactivity with antisera directed against *Maclura pomifera* agglutinin, as evidenced by the presence of spurs (Plates III 3 i. and ii. and 4, page 93). The jackfruit precipitin line (Plate III-4, page 94) was clearer in other cases, but gave the same appearance. The spurs generated indicate a reaction of partial identity, suggesting lectins within the Moraceae family are immunochemically related.

The breadfruit agglutinin (BFA) was purified to homogeneity using an affinity chromatography procedure employing T-Synsorb™ in view of its apparent binding specificity towards the disaccharide based on latex agglutinations and preliminary screening. $MnCl_2$ treatment to remove nucleoproteins increased the specific activity marginally, compared to the 67-fold enrichment achieved by $(NH_4)_2SO_4$ salt fractionation (Table III-2, page 83). The affinity chromatography step produced a 147-fold enrichment (determined by hemagglutination assays) resulting in a homogeneous product. The apparent increases in yields observed in the $(NH_4)_2SO_4$ fractionation steps is perhaps indicative of the removal of a soluble inhibitor. It is relevant to draw attention to the fact that substantial amounts of sugars are co-purified along with the lectin (see Table III-5, page 86). It is likely that the high salt content could dissociate some of the saccharides bound to the lectin active site and thus enhance toxic activity. Homogeneity of the protein was shown by the following biochemical techniques: A single peak on gel filtration (Figure III-2, page 90) and a single precipitin line in Ouchterlony immunodiffusion analysis (Plates III-3 i. and ii., page 93). Further support to the purity of the lectin preparation is indicated by SDS polyacrylamide gel electrophoresis (Plate III-5 lanes 2 and 3, page 95) wherein the seed proteins are essentially absent in the purified sample. The two-dimensional electrophorectic gels also indicate the extent of purification (Plate III-7, page 97).

No difference in the titer was observed following dialysis of BFA against 100 mM EDTA. This suggests divalent ions are not required for activity or are too tightly bound to be removed by the method employed. Galbraith and Goldstein[10] were able to completely inhibit the precipitation reaction caused by lima bean lectin using 0.1 mM EDTA, a 1000-fold lower concentration.

The carbohydrate determination indicated that BFA is a glycoprotein containing 4.3% carbohydrate (Table III-5, page 86). However, a sample not run through the gel filtration column with 0.1M NH_4OH apparently showed 210% carbohydrate implying that other glycans are copurified with the lectin. Thus, the use of NH_4OH to dissociate complexes appears to be well founded and could have influenced the final yield of lectin from the crude extract. By comparison, the jackfruit lectin is reported to have 3% carbohydrate[31].

Gel filtration of BFA through a column of Bio-Gel ,P150 produced a single symmetrical peak around 44,500 in molecular weight (Figure III-2, page 90). This value is subject to some error in view of the fact BFA is a glycoprotein[32]. The figure estimated may be high owing to the copurification of other glycans. Two dissimilar components of 19,000 and 22,000 daltons were seen on the SDS reduced electrophoretic gel (Plate III-5, page 95). The non-reduced gel gave a similar pattern. The pattern was not altered in the absence of reducing agents such as DTT which suggests that subunit interactions are not through a disulfide bond. Care was taken to prepare fresh samples since aliquots frozen with reducing agents such as DTT showed altered solubility characteristics and electrophoretic patterns[33]. The disparity in molecular weight between the subunits and native protein can be attributed to the carbohydrate moieties which affect the electrophoretic mobility of glycoproteins in SDS polyacrylamide gels[34,35]. Densitometric analysis of the purified BFA resolved on the gel (Figure III-4, page 91) indicates the protein was essentially pure (93% integration value). The antisera raised against the purified lectin gave a single precipitin line (Plate III-3 ii, page 93), evidence that the purified material contained a single lectin.

BFA is similar to the other two Moraceae lectins characterized to date (Table III-7, page 88). *Maclura pomifera* agglutinin with a molecular weight of 42,000 is a tetramer composed of subunits of 10,000 and 12,000[34]. *Artocarpus integrifolia* lectin has dissimilar subunits of 13,000 and 18,000 and an overall molecular weight of 62,000[33] although a molecular weight of 39,500 with one subunit of 10,000 has also been proposed[34]. An earlier study[35] reported subunits of 11,250 and 15,000 for the jackfruit.

Isoelectric focusing revealed that the 19,000 dalton subunit of BFA (determined by two-dimensional electrophoresis) exhibits an isolectric point of 5.2 (Plate III-6, page 96). When the isoelectric focusing gel was transferred onto a SDS polyacrylamide gel and the proteins electrophoresed in a second dimension (Plate III-7, page 97), the 22,000 subunit was located at a position corresponding to the origin of the isolectric focusing gel. The absence of the second band suggests this component possesses a sufficiently acidic pI to prevent it from entering the isoelectric focusing gel or that it forms aggregates, even in the presence of 9M urea, which are too large to penetrate the gel matrix. However, the aggregates are dissociated in SDS PAGE gels, as is evident by the presence of two bands.

The total amino acid composition of BFA was characterized by a high glycine (14.2%) and serine (11.5%) content as well as a moderately high number of glutamyl (9.3%) and aspartyl (9.0%) residues. In comparison, the levels of histidine (1.2%), arginine (2.0%) and methionine (0.7%) were relatively low (Table III-6, page 87). It is interesting to note that jackfruit lectin is high in glycine and serine and low in histidine, arginine and methionine[33] as well.

The acidic nature of BFA was also suggested in the isoelectric focusing studies described above. Despite its similarity to peanut agglutinin (PNA) in terms of T-binding specificity, BFA's amino acid composition is considerably different from that of PNA.

Among the various synthetic carbohydrate haptens used to determine the sugar binding specificity, β-D-gal(1→3)αgalNAc produced the highest inhibitory activity of the

disaccharides tested (Table III-4, page 85). This specificity corresponds to the anti-T lectin from peanut[40] which has been thoroughly investigated. BFA binds the α-anomer (β-D-gal(1→3)αgalNAc) better than the β-anomer (β-D-gal(1→3)βgalNAc) and this is the inverse of that observed with peanut agglutinin[41]. However, the affinity of BFA to T-hapten gave a 50% inhibition value of 0.15 mM (81 ug/ml). BFA is paradoxical in that it has an affinity for binding the T-antigen, but will hemagglutinate erythrocytes without prior treatment with neuraminidase. From limited data (Table III-4, page 85), it appears that the lectin binds a subterminal T as well (αfuc(1→2)βgal(1→3)αgalNAc) since this compound also inhibits in this assay. The inhibition by galNAc in the free form suggests binding perhaps also involves the N-acetyl portion. In addition to BFA and PNA, *Bauhinia purpurea*, *Maclura pomifera* and *Sophora japonica* are also specific for D-galβ(1→3)DgalNAc residues[42]. The jackfruit lectin is specific for α-D-galactose[31] and N-acetyl-galactosamine[37]. An anti-T lectin in jackfruit has been described as well[43]. In view of the T-antigen binding characteristic, BFA may be potentially useful in T-antigen expressing tumor binding and biodistribution studies.

The jackfruit lectin has a number of interesting properties including the ability to agglutinate sperm[37], mitogenicity[44,45], IgA-binding[46] and enhancement of IgE synthesis through multiple oral dosing[47]. Given the taxonomic and botanical relatedness of jackfruit and breadfruit, along with the physicochemical similarities of the lectins from these species, BFA may also share some of these interesting and unique immunological properties.

Table III-1. Distribution of breadfruit agglutinin in the various parts of the plant as determined by immunodifusion analysis.

Part	Anti-MPA[1]
Fruit	-
Stalk	-
Seed	+ + +
Seed coat	+
Latex	+ +

[1]No precipitin line (-), faint (+), moderate (+ +) or intense (+ + +)

Table III-2. Purification protocol of breadfruit agglutinin[1].

Purification step	Total Protein (mg)	Vol (ml)	Reciprocal endpoint	Units of activity[2]	Specific activity[3]	Yield	Fold purification
crude extract	1247	2710	1024	2.8×10^7	22.3	–	–
$MnCl_2$ supernatant	840	2710	6144	1.7×10^7	19.8	60	0.9
30% supernatant	486	2860	2048	5.9×10^7	120.5	211	5.4
30-75% dialyzed	189	215	131,072	2.8×10^8	1491.0	1016	67
affinity purified	10	25	131,072	3.3×10^7	3276.8	118	147

[2]A unit of agglutination activity represents the titratable endpoint of a given fraction using human red blood cells and doubling dilutions of the fraction. Thus for the sample showing an endpoint of 1/1024 units and volume of 2710 ml, the total units will be 2710 ml x 10 (since 100 μl used in hemagglutination assay) x 1024 = 2.8×10^7 units of activity.
[3]Specific activity is defined as the units of activity/g of protein.

[1]Flow diagram of purification shown below.

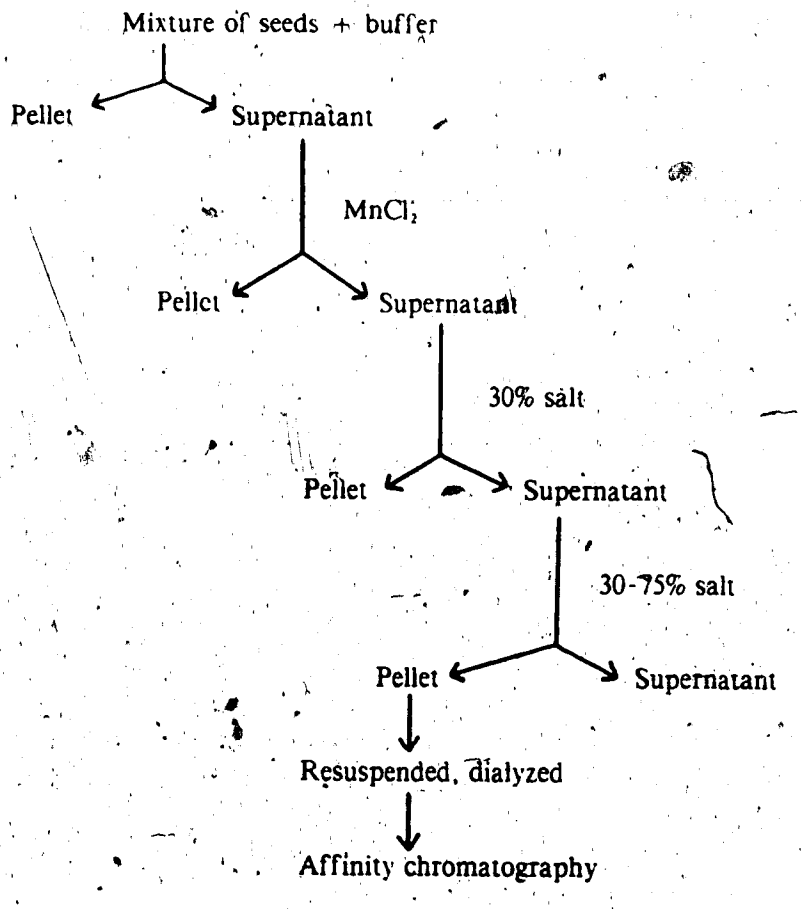

Table III-3. Agglutination of carbohydrate coated latex beads by BFA extract.

Carbohydrate structure	Strength of agglutination
αgal(1→3)βgal(1→2)αfuc-R	+
αgal(1→3)βgal-R	+
βgal(1→4)βglc-R	+
βgal(1→3)βglcNAc-R	+
βgal-R	+
αgal(1→4)βgal-R	+
βgal(1→3)βgalNAc-R	+
βgal(1→4)βglcNAc(1→3)βgal(1→4)βglcNAc-R	+
βgal(1→4)βglcNAc-R	+ +
βgal(1→3)βgal-R	+ +
βgal(1→2)αman-R	+ +
αgal-R	+ + +
βgal(1→3)αgalNAc-R	+ + +

R = $(CH_2)_8$-$COOCH_3$

Table III-4. Inhibition of binding of T-BSA to breadfruit lectin coated tubes by carbohydrates.

Structure	Conc. (mM)	% Inhibition
αgalNAc-R	5.1	92.2
αgal-R	5.7	88.6
βgal(1→3)αgalNAc-R	3.6	94.1
βgal(1→3)βgalNAc-R	3.6	33.2
αgalNAc(1→4)βgal-R	3.6	32.1
αman(1→2)αman-R	3.9	27.7
βglcNAc(1→6)αgalNAc-R	3.6	25.7
αgalNAc(1→3)βgal-R	3.6	25.6
αgal(1→3)βgal-R	3.9	14.5
αfuc(1→2)βgal(1→3)αgalNAc-R	2.9	57.7
αgalNAc(1→3)βgal(1→4)βglcNAc-R	2.8	21.2
αgalNAc(1→3)βgal(1→2)αfuc-R	2.9	18.8
βgalNAc(1→3)αgal(1→4)βgal (1→4)βglc-R	2.3	19.9

R = $(CH_2)_1$-$COOCH_3$

Table III-5. Carbohydrate analysis of purified breadfruit lectin using the phenol-sulfuric acid method.

BFA	ug/galactose equivalent	Percentage of carbohydrate
Affinity purified BFA		
60 ug	193.61	209.7
150 ug	316.94	
300 ug	315.83	
Affinity purified BFA rechromatographed[1]		
60 ug	3.61	4.26
150 ug	5.56	
300 ug	10.00	

[1]On Bio-Gel P150 using 0.1M NH_4OH as the elution buffer

Table III-6. Amino acid composition of the breadfruit agglutinin.

Amino acid	Breadfruit[1] Residues/ mole protein[2]	Breadfruit[1] Percentage	Jackfruit[3] Percentage	Peanut[4] Percentage
Asx	34	8.97	9.47	18.35
Thr[5]	30	7.16	7.94	9.21
Ser[5]	56	11.51	9.93	9.41
Glx	31	9.34	7.12	4.43
Pro	19	4.33	5.16	2.11
Gly	106	14.18	12.16	8.37
Ala	27	4.44	3.37	5.56
Val	32	7.44	8.16	12.44
Met	2	0.71	1.19	
Ile	23	6.13	6.39	6.59
Leu	22	5.79	6.02	4.83
Tyr	14	5.31	7.35	2.26
Phe	17	5.81	7.07	6.63
His	4	1.22	0.75	1.07
Lys	19	5.80	6.51	4.20
Arg	6	2.02	1.42	2.10

[1]Includes both subunits.
[2]Calculated on basis of molecular weight determined from gel filtration.
[3]From reference 37.
[4]Recalculated from reference 40.
[5]Extrapolated to zero time of hydrolysis.

Table III-7. Comparative properties of breadfruit (BFA), jackfruit (JFA), Osage orange (MPA) and peanut (PNA) lectins.

Property	BFA	JFA	MPA	PNA
Molecular weight (daltons)	44,500	62,000 (39,500)[1]	42,000	110,000
Subunits	2	4	4	4
Subunit size (daltons)	19,000 22,000	13,000 18,000 (10,000)[1]	30,000 12,000	27,500
Carbohydrates	4.3%	3%	0	0
Blood group specificity	None	None	None	T, Tk
Sugar specificity	β-D-gal(1→3)αgalNAc	α-D-gal galNAc	α-D-gal galNAc	β-D-gal(1→3)D-galNAc D-gal
Mitogenic		Yes	Yes	Some sources

[1]Values reported by a different set of authors.

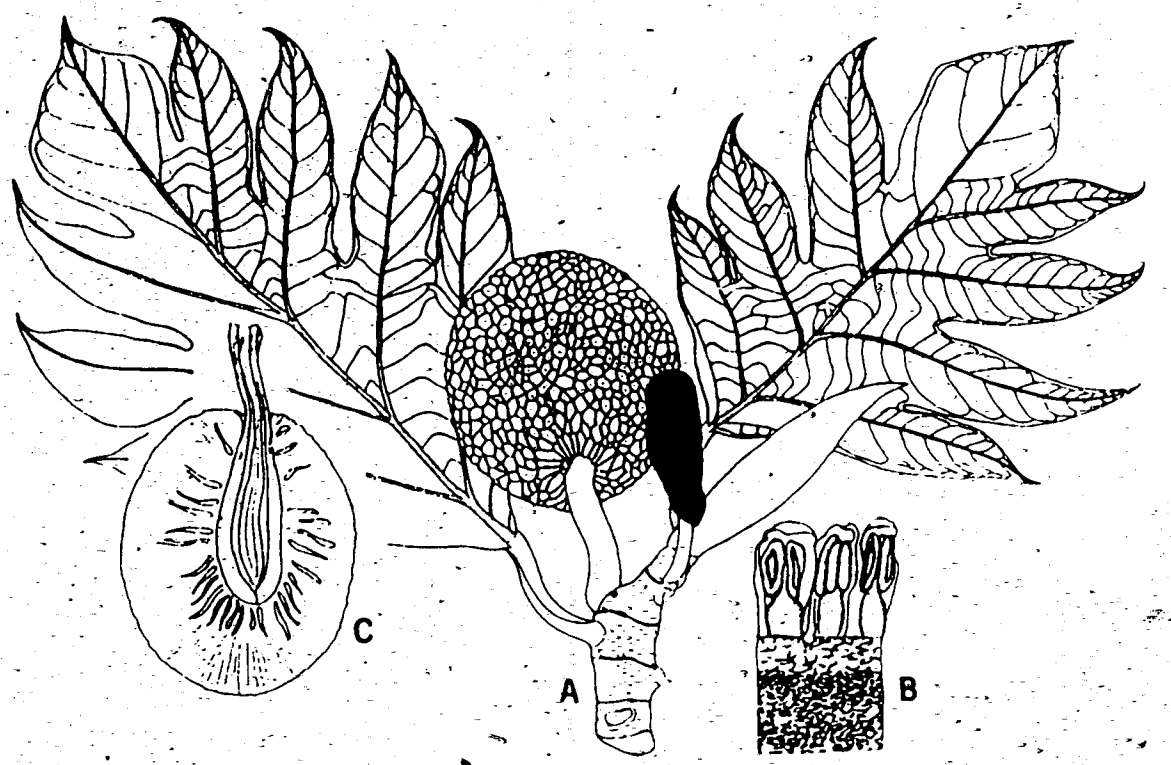

Figure III-1. Illustration of the breadfruit. A, shoot with male inflorescence and fruit; B, individual male flowers; C, fruit in longitudinal section. (Taken from Purseglove, J.W., 1968).

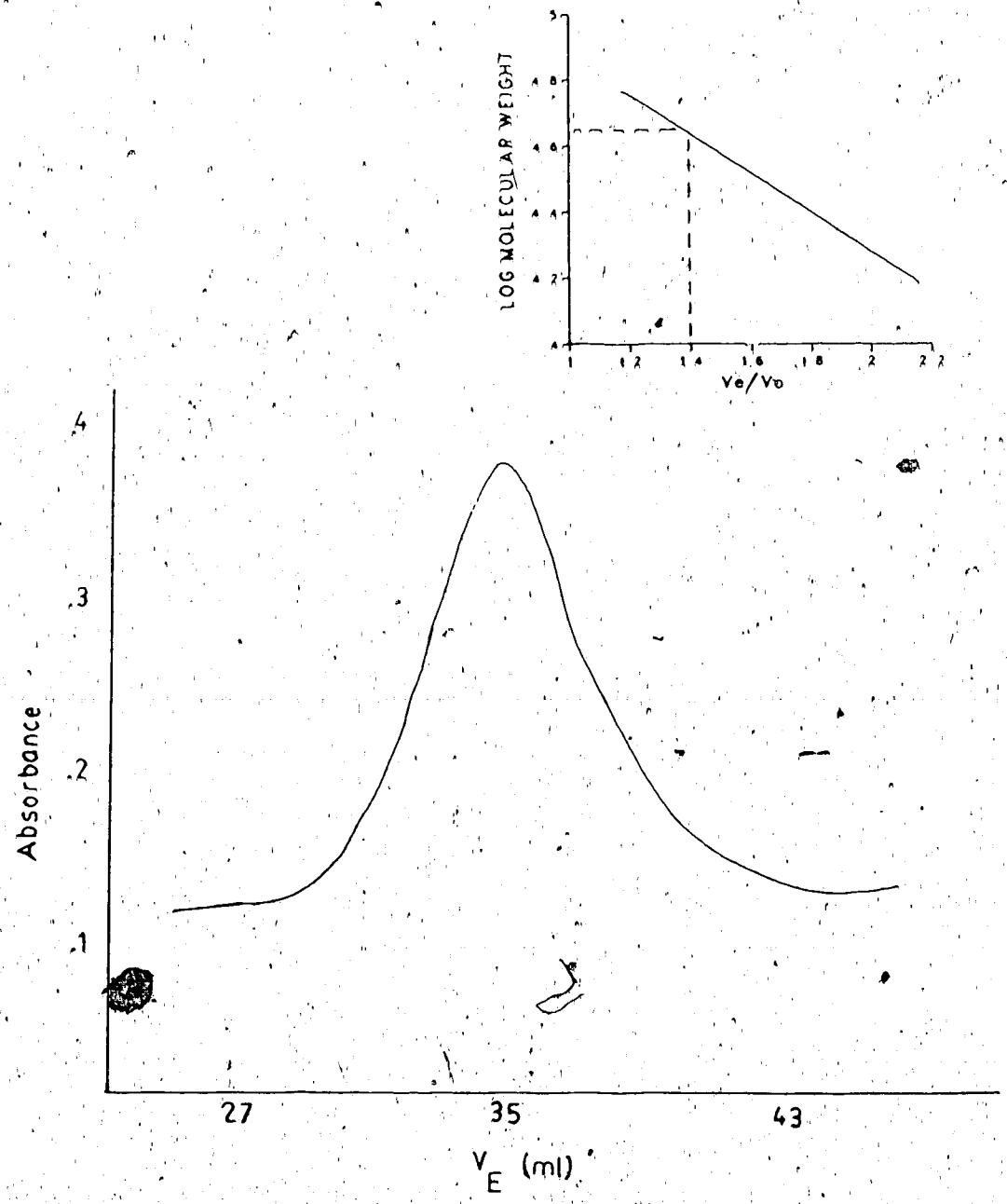

Figure III-2. Gel filtration profile of breadfruit agglutinin on Bio-Gel P150. Inset illustrates standard curve used to estimate molecular weight of the native protein.

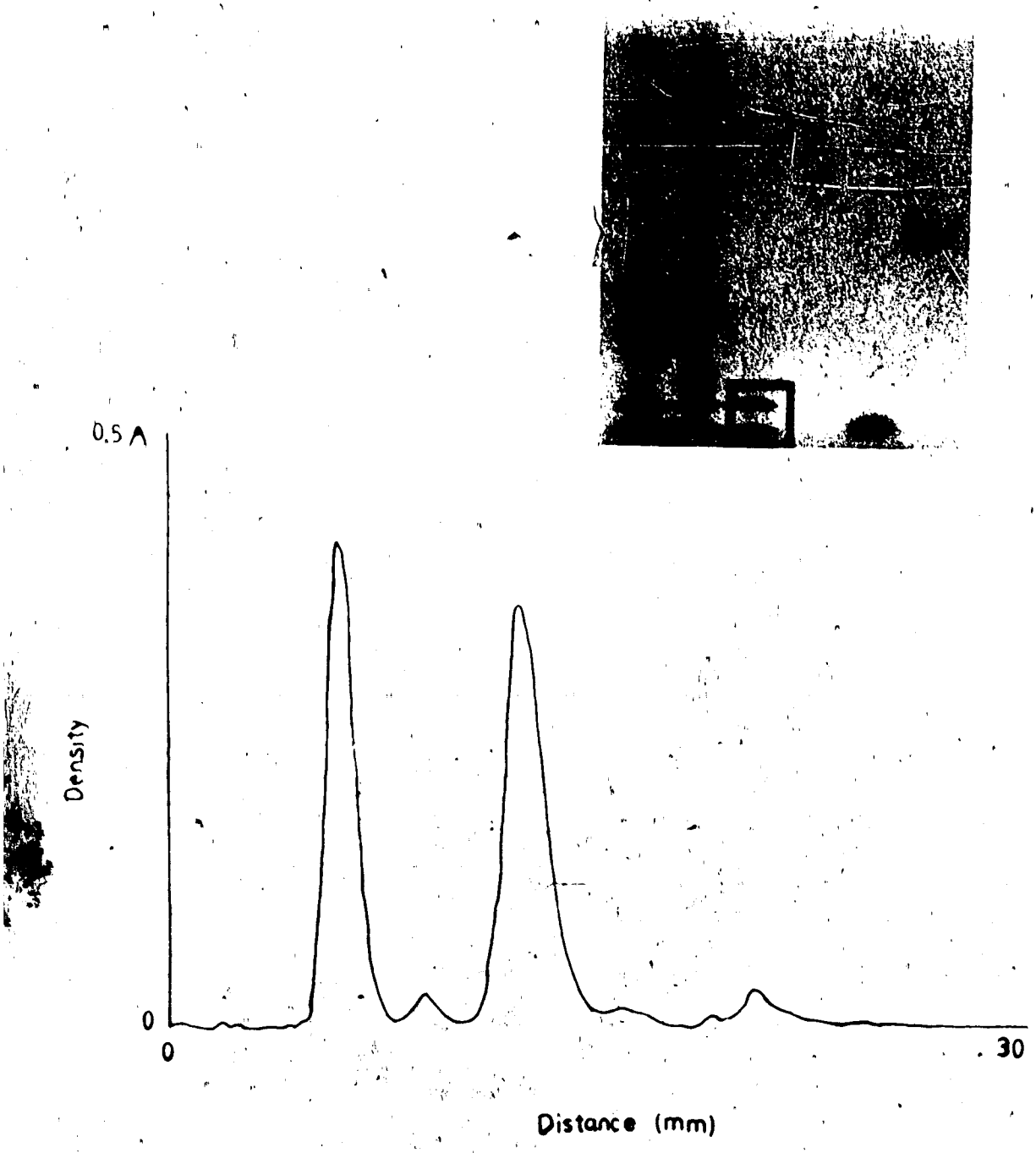

Figure III-4. Densitometric scan of purified BFA on reducing gel (boxed lane of inset).

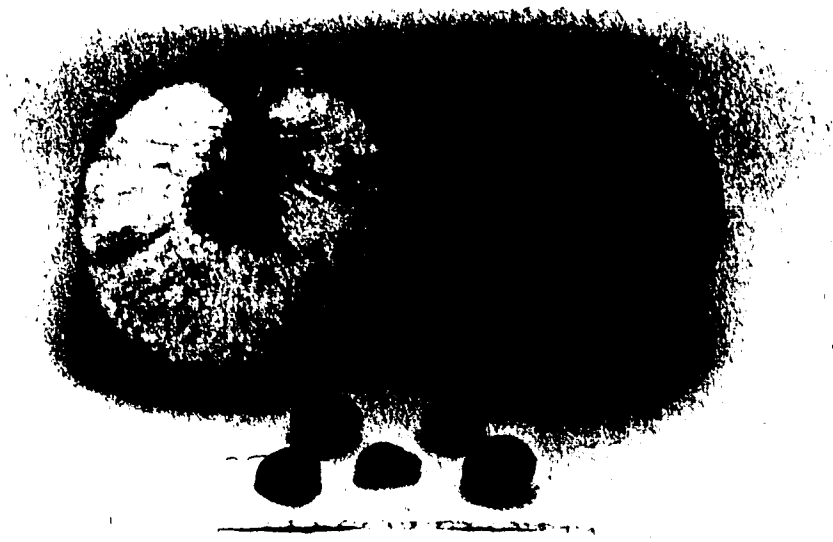

Plate III-1. An intact breadfruit (right) and a cross-section revealing the core (left). Seeds are in the foreground.

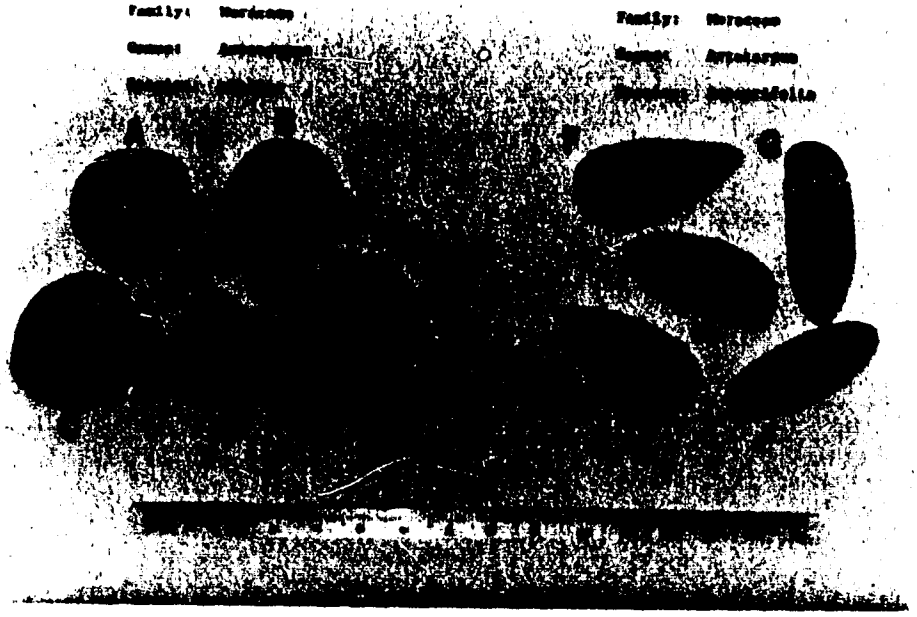

Plate III-2. Breadfruit (left) and jackfruit (right) seeds. A,E,F,G and I depict the intact seed. B and C show a cross-section of the seed. D,H and J illustrate the seed without its seed coat.

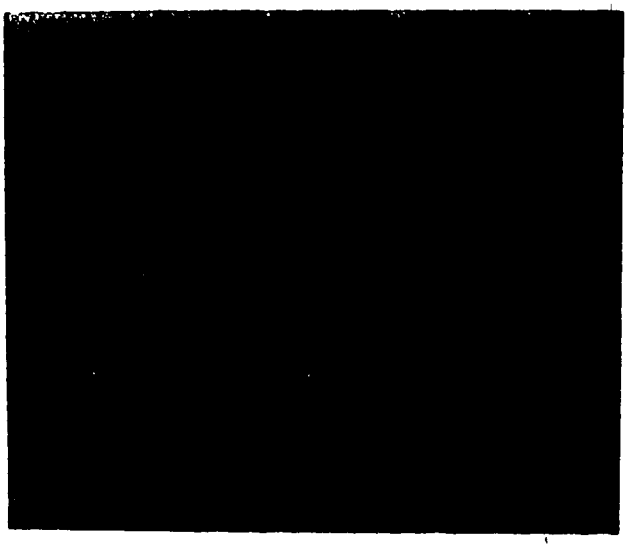

Plate III-3. i. Ouchterlony precipitin reactions of breadfruit lectin vs antisera against *Maclura pomifera* lectin. Antisera against *Maclura pomifera* (A) is surrounded by breadfruit agglutinin (1,3,5) and *Maclura pomifera* agglutinin (2,4). Note the spurs.

Plate III-3. ii. Ouchterlony precipitin reactions of *Maclura pomifera* lectin vs antisera against breadfruit lectin. Antisera against breadfruit agglutinin (B) is surrounded by preimmune sera (1), breadfruit agglutinin (2) and *Maclura pomifera* agglutinin (3).

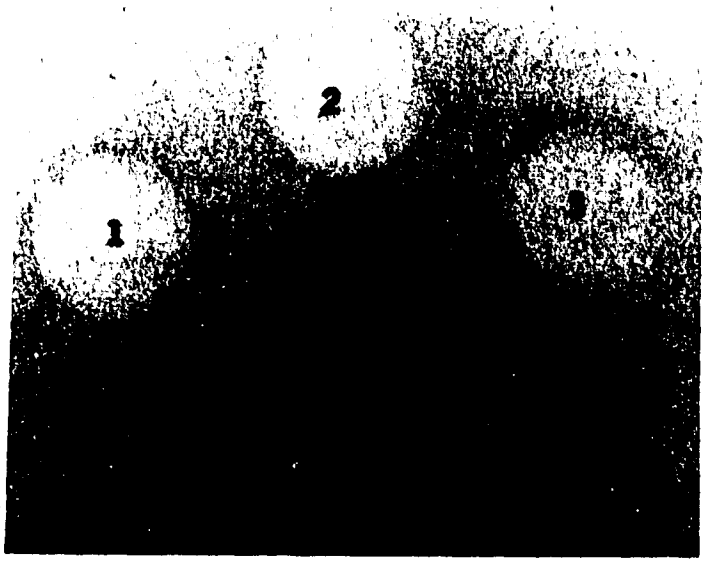

Plate III-4. Ouchterlony precipitin reaction of jackfruit extract vs antisera against *Maclura pomifera* lectin. Antisera against *Maclura pomifera* (A) is surrounded by jackfruit extract (2) and *Maclura pomifera* agglutinin (1,3).

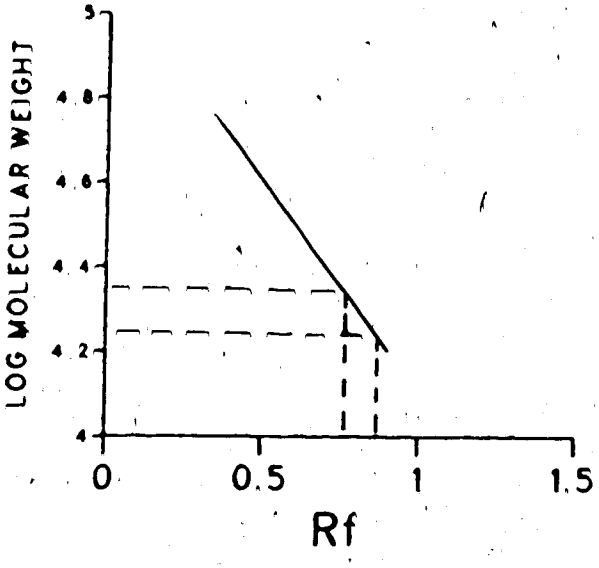

Figure III-3. The standard curve used for determining subunit molecular weights of breadfruit agglutinin.

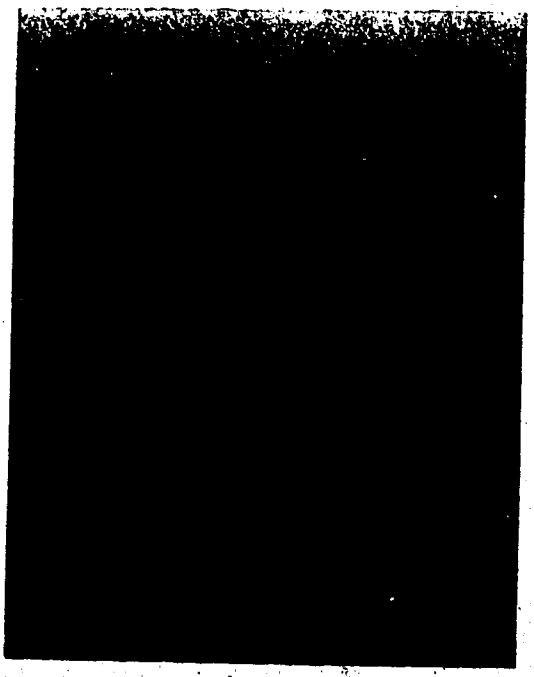

Plate III-5. SDS polyacrylamide gel electrophoresis of breadfruit lectin on a 12% reducing gel. Lane 1, crude jackfruit; lane 2, crude breadfruit; lane 3, purified BFA; lane 4, *Maclura pomifera* agglutinin; lane 5, peanut agglutinin.

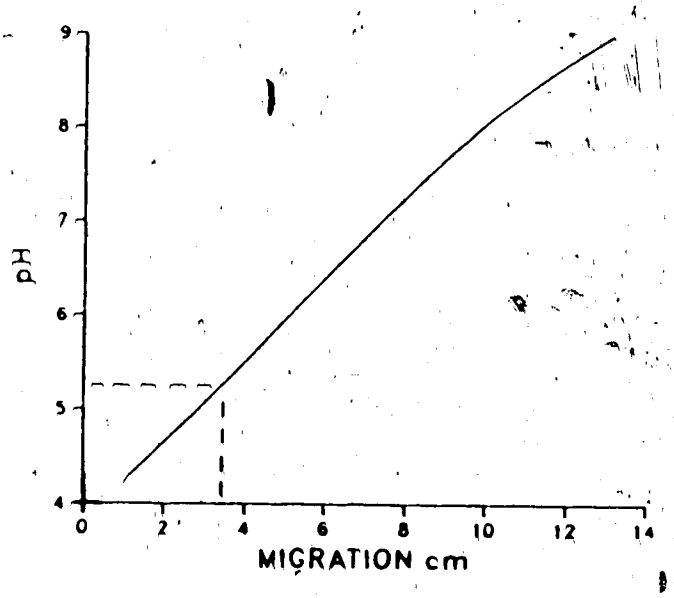

Figure III-5. The standard curve used to determine the isoelectric point of breadfruit aggulutinin.

Plate III-6. Polyacrylamide gel isoelectric focusing of breadfruit lectin.

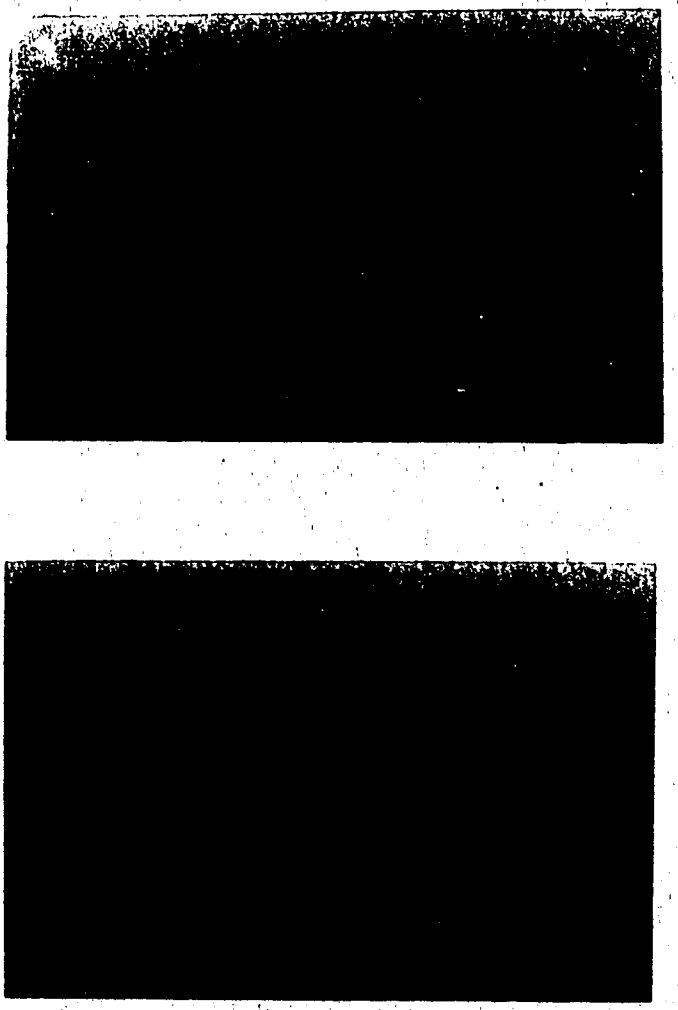

Plate III-7. Two-dimensional electrophoretic gels of crude breadfruit (top) and purified BFA (bottom). Isoelectric focusing was performed in the first dimension, followed by SDS PAGE in the second dimension.

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IV. PRELIMINARY INVESTIGATION OF AN AGGLUTININ FROM *FICUS DELTOIDEA* SEEDS *

A. Introduction

Traditionally, lectins have been purified by affinity chromatography with biospecific adsorbents[1-4]. This approach necessitates a preliminary study to determine the best adsorbent. In addition, unwanted starches and sugars may also bind, making further purification necessary. Fast protein liquid chromatography (FPLC)[5] offers a fast, reproducible method for protein purification. Developed by Pharmacia, the FPLC system is a modular high performance liquid chromatography apparatus designed to purify proteins, polypeptides and polynucleotides by ion exchange chromatography, chromatofocusing, gel filtration and reverse phase chromatography. The basis of the system is a series of prepacked high resolution columns and media. FPLC has been used to purify a number of proteins such as haemoglobin derivatives[6], snake venom[7], enzymes[8] and plasma proteins[9]. The suitability of using FPLC for lectin purification is described in this chapter. Affinity purified and crude samples of breadfruit agglutinin discussed in the previous chapter were analyzed on FPLC to test the efficacy of this approach. Subsequently, *Ficus deltoidea* Jack (*F. diversifolia* Blume) or mistletoe fig[10] was chosen for FPLC purification in view of the abundance of agglutinating material and bacterial agglutinations observed in preliminary screening experiments.

B. Materials and Methods

1) Materials

F. deltoidea seeds were obtained from plants growing in the Department of Plant Science, University of Alberta. Chembiomed Ltd. (Edmonton) provided carbohydrate-bound latexes. The FPLC equipment and column were bought from Pharmacia Fine Chemicals (Uppsala, Sweden).

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2) Preparation of crude seed extracts

Since the seeds are very small, the entire fruit and seeds were used. One gram of fruit and seeds (Plates IV-1, 2, page 106) was ground using mortar and pestle, mixed with 10 ml 0.01M PBS and tumbled overnight at 4°C. After centrifuging for 15 min. at 320 *x g* in a Dynac table top centrifuge, the supernatants were collected and centrifuged again under the same parameters. Extracts were stored at -20°C and then freeze-dried.

3) Latex screening for affinity purification

The extract was screened against a series of carbohydrate-bound latexes (developed by Chembiomed Ltd. and described in reference 11) to give an indication of the sugar specificity.

4) Fast protein liquid chromatographic purification of FDA and BFA

Crude seed extracts of *F.* deltoidea and *A. altilis* and the affinity purified BFA were chromatographed on a Pharmacia HR 10/30 Superose 12™ high performance gel filtration column, an agarose-based matrix in a prepacked glass column[12-13] with a fractionation range of 1,000-300,000 daltons. The Pharmacia FPLC system equipped with the Liquid Chromatography Controller (LCC), 280 nm UV monitor, P-500 high precision pump and Frac-100 fraction collector was utilized. Samples (200 ul of a 1.5 mg/0.5 ml solution) were loaded on the column and sodium acetate buffer (0.05M, pH 5.2) containing 0.1M NaCl was used as an eluant with a flow rate of 0.3 ml/min. The peak fractions were collected automatically in 5 ml tubes using the peak collection mode, dialyzed against 2L of distilled water overnight and tested for hemagglutination activity.

5) Screening assays

Hemagglutination and Ouchterlony assays and bacterial agglutinations were performed as described in chapters II and III.

C. Results and Discussion

Titers ranging from 1/6400 to 1/8200 found in hemagglutination screening studies (chapter II) indicated an abundance of agglutinating material present in the crude fig preparation. Antisera directed against *M. pomifera*, *A. hypogaea*, *T. vulgaris*, *D. biflorus*, *C. ensiformis*, *L. polyphemus*, *T. purpureas* and *P. sativum* agglutinins showed a precipitin line with the *F. deltoidea* extract using Ouchterlony immunodiffusion assays (Plate IV-3, page 107), despite the presence of various sugars in the agar to eliminate spurious interactions. It is difficult to justify a lectin cross-reacting with antisera of so many phylogenetically different and varied lectins. The lectins screened above belong to such diverse taxa as Moraceae, Leguminosae, Gramineae and even an invertebrate. Further, as already alluded to in chapter II, the fig extract also agglutinated various broths. These apparently interesting features were pursued to determine the nature of the active principle. When the crude FDA extract was mixed with normal rabbit sera, the precipitin reactions obtained with various antisera were eliminated suggesting the lectin is interacting with a component in sera to form a precipitin line. It is pertinent to note at this juncture, that extracts of certain bacteria such as *Staphylococcus aureus* possess or exhibit immunoglobulin binding properties. This protein (protein A) has been purified and extensively characterized and is routinely used as an important probe in immunology. Among all the sugars tested, it should be emphasized that free sialic acid and latex-bound sialic acid were omitted due to lack of availability. Sialic acid binding lectins are rare but known to exist, an example being the lectin from the invertebrate *Limulus polyphemus*.

FDA is also of interest for its ability to agglutinate some strains of bacteria, including *S. aureus*, *B. cereus* and *Chlamydia trachomatis*, the latter being a clinically significant pathogen responsible for a venereal disease. Chlamydial attachment to mouse fibroblasts (L cells) has been blocked using wheat germ agglutinin[14]. Other studies using this lectin as a probe[15-16] suggest the attachment of *C. trachomatis* is related to β1→4-linked oligomers of D-glcNAc and NeuNAc residues.

No agglutination was observed between different latexes and the FDA extract, making the affinity separation of the extract more difficult since a suitable adsorbent could not be readily identified. Attempts were made to purify the agglutinin from FDA using a novel approach. In order to validate the FPLC system, crude and affinity purified samples of BFA described in the previous chapter were chromatographed to determine the feasibility for lectin purification. Application of crude BFA to a Superose 12™ gel filtration column produced several peaks. Erythrocyte agglutination activity was exclusively associated to the peak which eluted at 20.15 min. Gel filtration of the affinity purified BFA produced a peak at a similar elution volume as compared to the active fraction of the crude extract (Figure IV-1 A and B, page 105). This demonstrates that gel filtration FPLC is very efficient and can eliminate the lengthy procedure of latex screening and affinity chromatography, rendering an enriched product in a very short time. Gel filtration of the crude FDA extract resulted in nine major peaks (Figure IV-2, page 105). When tested, all of the peaks agglutinated erythrocytes but only the fourth peak was active against the bacterium *Chlamydia*. Thus, by the use of gel filtration FPLC, the crude FDA was separated into nine components in less than 20 min, and only one fraction agglutinated *Chlamydia*. Evidently, gel filtration FPLC may be used in the purification of lectins from different sources and is an alternative to the traditional affinity chromatograpy methods because it is simple, fast and reproducible.

FDA has proved to be an interesting lectin since it can agglutinate *Chlamydia* as well as precipitate certain serum components as noted earlier. The same principle or two different ones may be involved. These properties may be useful in developing a rapid means of *Chlamydia* identification or as a probe for membrane studies of the bacterium.

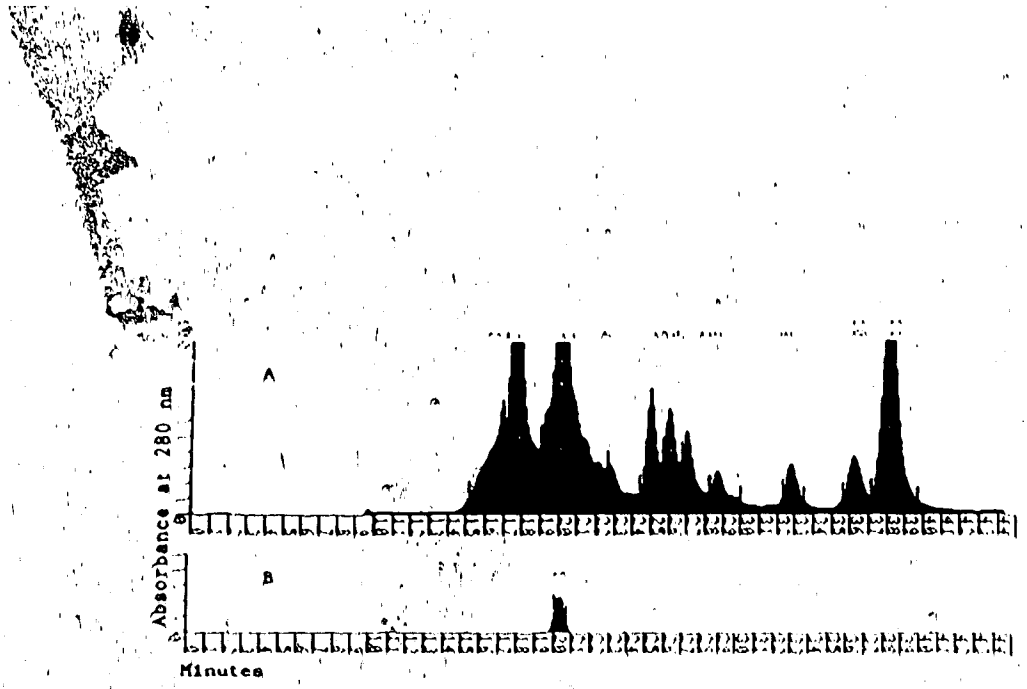

Figure IV-1. Gel filtration profile of crude (A) and affinity purified (B) BFA extracts on Superose 12™. Peaks 3 in A and 1 in B represent the active fractions.

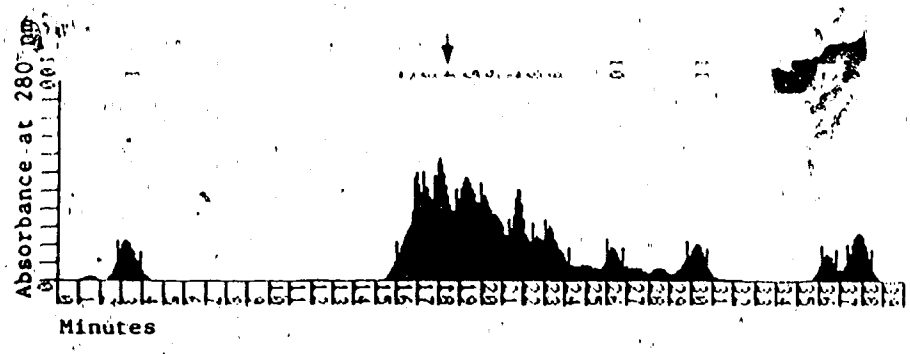

Figure IV-2. Gel filtration profile of FDA crude extract on Superose 12™. Peak 4 represents the active fraction.

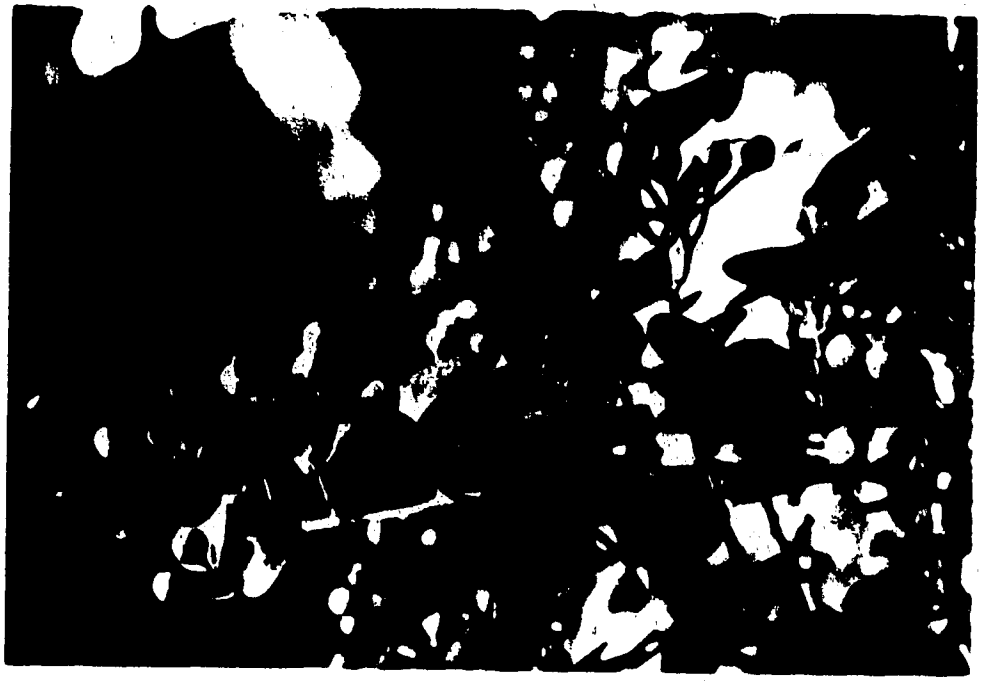

Plate IV-1. A branch of *Ficus deltoidea* bush showing leaves and fruit.

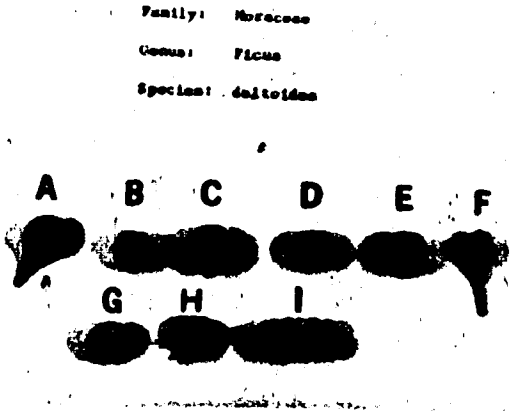

Plate IV-2. Fruit and seeds of *Ficus deltoidea*. A to F illustrate stages of ripening. G to I depict a cross-section of the fruit revealing the seeds.

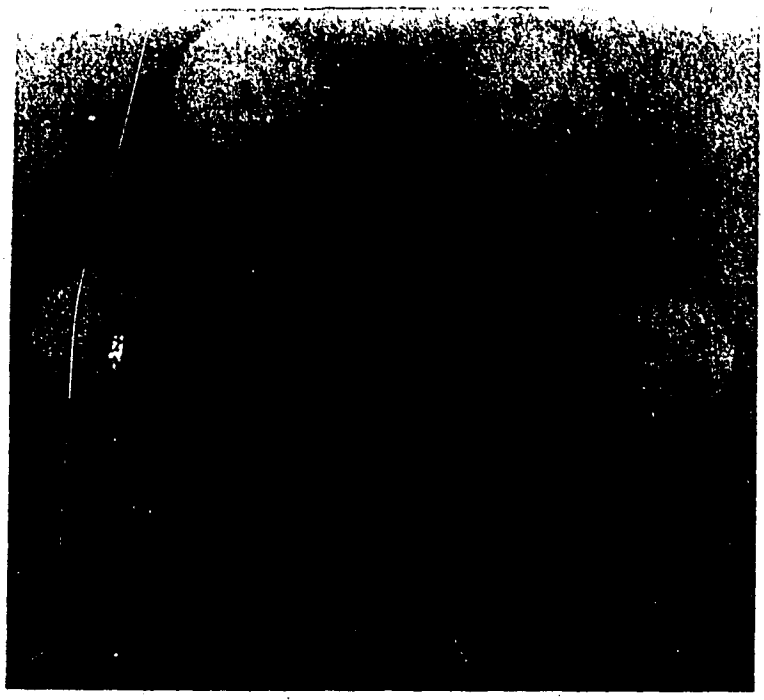

Plate IV-3. Ouchterlony immunodiffusion assay of *F. deltoidea* extract (A) surrounded by antisera directed against *T. purpureas* (1), *D. biflorus* (2), *A. hypogaea* (3), *M. pomifera* (4), *C. ensiformis* (5) and *L. polyphemus* (6) agglutinins. Similar reactions were observed with *P. sativum* and *T. vulgaris*.

D. References

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V. GENERAL SUMMARY AND CONCLUSIONS

The screening of 23 plant species using hemagglutination and Ouchterlony immunodiffusion assays revealed high titers of agglutinating material in six species, low titers in nine and no agglutination in eight. The agglutination was observed with A, B, O and AB classes of human erythrocytes in all cases. The two *Artocarpus* species (*A. integrifolia*, *A. altilis*) showed reactions of partial identity with antisera directed against *Maclura pomifera* agglutinin, another member of the Moraceae family. The cross-reactivity between the *Artocarpus* and *Maclura* lectins suggests a phylogenetic relationship between lectins of the Moraceae since all three belong to this taxon. Moraceae lectins thus appear to be related and exhibit considerable conserved domains in the protein structure.

An extract from *Ficus deltoidea* reacted with all eight antisera tested. These precipitin reactions were eliminated by mixing normal rabbit sera with the crude *F. deltoidea* extract, suggesting the active principle is precipitating some component in the serum. It would be interesting to pursue this further. In testing Moraceae species for bacterial agglutination, *F. deltoidea* proved interesting in agglutinating some bacteria, including *Chlamydia*. *Broussonetia papyrifera* lectin slightly agglutinated *Bacillus cereus* as well as *Chlamydia*. The remaining Moraceae extracts tested failed to agglutinate the different species of bacteria used in this study.

On the basis of the aforementioned results, *Artocarpus altilis* (breadfruit) and *Ficus deltoidea* (mistletoe fig) were selected for further investigation. The breadfruit agglutinin was purified by steps involving salt fractionation and affinity chromatography on immobilized β-D-gal(1→3)αgalNAc. Characterization of the purified material revealed a molecular weight of 44,500 for the native protein, consisting of two dissimilar subunits of approximately 19,000 and 22,000 daltons. The carbohydrate content was estimated to be 4.3%. The amino acid composition was noteworthy for a high content of glycine and serine and moderate amounts of glutamyl and aspartyl residues. The levels of histidine, arginine and methionine were

relatively low. β-gal(1→3)αgalNAc produced the highest inhibitory activity of the disaccharides tested. In view of this T antigen binding characteristic of the breadfruit agglutinin, tumor binding studies are proposed as an extension of this work.

The traditional approach to lectin purification involving affinity chromatography with biospecific adsorbents requires much ground work. Consequently, a novel approach to the purification of the lectins was undertaken. The viability of utilizing fast protein liquid chromatography was initially demonstrated with crude extracts as well as affinity purified samples of breadfruit agglutinin, which was purified by the conventional method noted above. FPLC offers the advantages of being fast, simple and highly reproducible. This procedure resolved the *F. deltoidea* extract into nine major peaks, all of which agglutinated human erythrocytes. Purified elementary bodies of the bacterium *Chlamydia* were only agglutinated by material in the fourth fraction. Thus, purification may be accomplished by means other than the conventional affinity chromatography. The serum binding characteristic of *F. deltoidea* agglutinin also merits further investigation. Given the importance of *Chlamydia* as a clinically significant pathogen, FDA may have value as a probe for studying and identifying this pathogen.

VI. APPENDIX

Seed Sources

Hemp

Bureau of Dangerous Drugs,
Health Protection Branch,
Department of National Health and Welfare,
3rd floor, Jackson Building,
122 Bank St.,
Ottawa, Ont. K1A 1B9

Douglas fir, white spruce, lodgepole pine, Siberian larch

Alberta Agriculture,
Alberta Tree Nursery and Horticulture Centre,
R.R. 6,
Edmonton, Alta. T5B 4K3

Polish rapeseed, Argentina rapeseed

United Grain Growers,
Seed Division,
7410 - 120 Ave.,
Edmonton, Alta.

Black manh, moth, red chori

Asia Import House (1980) Ltd.,
7912 - 104 St.,
Edmonton, Alta.

Paper mulberry, Osage orange

F.W. Schumacher Inc.,
Sandwich, Mass.
02563-1023
U.S.A.

Breadfruit, custard apple

JB Q-Mart,
8929 - 82 Ave.,
Edmonton, Alta.

Common fig, Japanese hop

J.L. Hudson, Seedsman,
P.O. Box 1058,
Redwood City, California
94064
U.S.A.

Jackfruit

Dr. A. Srinivasan,
Director, Protein Research Unit,
Department of Chemistry,
Loyola College,
Madras,
South India

Dr. C.K. Rao
Department of Botany,
Bangalore University,
Jnanabharathi,
Bangalore 560056
India

Lychee, persimmon, McIntosh apple, cherimoya, mango

Safeway Stores Ltd.,
Edmonton, Alta.

Mistletoe fig, American tropical fig

Mr. R. Kroon,
Greenhouse supervisor,
Department of Botany,
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Hunter, J.B. *et al*. Isolation and Characterization of a Lectin from Breadfruit (*Artocarpus altilis*) Seeds. *Biochemical Archives*, 1986 (in press).

Hunter, J.B. *et al*. Physicochemical Properties of Lectins - A Review. In preparation.