*Phytochemistry*, Vol. 25, No. 7, pp. 1553–1557, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

# LECTINS FROM LATICES OF *EUPHORBIA* AND *ELAEOPHORBIA* SPECIES*

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(*Revised received* 15 *November* 1986)

**Key Word Index**—*Euphorbia*; *Elaeophorbia*; Euphorbiaceae; latex; lectins;

**Abstract**—Crude latex sera from 17 members of the genus *Euphorbia* and from *Elaeophorbia drupifera* (Euphorbiaceae) contained a wide range of agglutinating abilities. Homogeneous lectins were isolated from latices of *Euphorbia coerulescens*, *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. lathyris*, *E. trigona* and *Elaeophorbia drupifera*. The $M_r$s of the lectins ranged from 60 to 67 000, and the unit weights from 27 to 38 000. pI measurements showed that each latex contained from five to 13 isolectins. The amino acid compositions of the seven lectins were determined: those from *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. trigona* and *Elaeophorbia drupifera* are related.

## INTRODUCTION

Lectins have been isolated from several genera of the Euphorbiaceae: from *Ricinus communis* [1], from *Hura crepitans* [2] where the seeds [3, 4] and latex [5] have yielded related but different agglutinins, and from *Synadenium grantii*, the latex of which yields another lectin [6].

Members of the genus *Euphorbia*, which are largely latex carrying plants, have not been extensively examined for lectins. Nsimba-Lubaki *et al.* [7] reported that while the seeds of both *Euphorbia helioscopia* (sun spurge) and *E. lathyris* (caper spurge) did not contain lectins, those of *E. heterophylla* were a source of an agglutinating agent. Barbieri *et al.* [5] have similarly isolated and characterized a lectin from the latex of *E. characias* (Mediterranean spurge).

During a study of some of the proteases from latices of various *Euphorbia* spp. [8–10] we have also isolated, and examined, the properties of lectins from the latices of *E. coerulescens*, *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. lathyris* and *E. trigona*, as well as from the related West African plant *Elaeophorbia drupifera*. As a preliminary to that work we surveyed the agglutinating properties of the sera from the latices of other members of the same genus, and shall report here the results of those investigations.

## RESULTS AND DISCUSSION

Sera of the latices of both the 'leafy' and the 'succulent' varieties of *Euphorbia* spp. were surveyed for the presence of lectins using sialidase-treated sheep erythrocytes and human A, B, AB and O types. The latex-sera of the plants were also assayed for protein content using bovine serum albumin as the reference material, and the procedure of Lowry *et al.* [11]. The results are collected in Table 1.

A significant value for the titer of the latex of *E. lathyris* was observed, though we, like Nsimba-Lubaki [7], did not find lectin in the seeds of that plant. Essentially constant haemagglutinating activity per unit weight of protein in the latex of *E. lathyris*, followed from 9 weeks after germination (when the latex could be conveniently collected without destruction of the plants) to 52 weeks from that time, was found. Further, lectin was detected in the latex of *E. helioscopia* with both sheep and human erythrocytes (Table 1), though none was found in the seeds of this species [7] (assayed with human blood). While our assay with sheep blood failed to detect agglutinating ability in crude latex from *E. characias*, such ability is clearly apparent, in Table 1, when measurement was made with human erythrocytes. The presence of lectin in this latex has been reported previously [5] using rabbit erythrocytes for detection.

In general, human cells of the four types examined are more sensitive to the lectins of the latex sera than are those of sheep, though there is considerable variability in the degree of enhancement observed in Table 1. Thus, the latex of *E. tirucalli* is equally effective with erythrocytes from either source tested, while that of *E. trigona* is about 80-fold more potent with blood of human than ovine origin. The data of Table 1 show, also, that the plant latex-sera tested have very limited ability to discriminate between the four human blood types used in this work. Only the latex-sera of *E. platyphylla* and *E. lactea cristata* had agglutinating activity significantly different for one of the four blood types employed.

The 'leafy' *Euphorbia* spp. examined in this work, namely *E. characias*, *E. cyparissias*, *E. esula*, *E. helioscopia*, *E. lathyris* and *E. platyphylla*, carry latices containing, in general, less agglutinating ability per unit weight of protein than do those of the 'succulents' (*E. coerulescens*, *E. cylindrifolia*, *E. globosa*, *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. mammilaris*, *E. splendens*, *E. stapelioides*, *E. tirucalli*, *E. trigona* and *Elaeophorbia drupifera*), though there are wide differences between members in the same group. Thus it will be seen, in Table 1, that the agglutinating properties of the serum from *E. lathyris* is greater than those of *E. aphylla*, *E. globosa* and *E. tirucalli*, but 50–100 fold lower than that of *E. trigona*. Latex-serum from the

*NRCC No. 25557.

Table 1. Measurement of agglutination of sialidase-stripped sheep and human A, B, AB and O erythrocytes using latex-sera from 17 *Euphorbia* spp. and from *Elaeophorbia drupifera*

| Source of latex | Protein (mg/ml) | Titers | | | | |
|---|---|---|---|---|---|---|
| | | Sheep | Human A | Human B | Human AB | Human O |
| *E. characias* | 18.6 | 0 | 1600 | 1600 | 1600 | 3200 |
| *E. cyparissias* | 59.7 | 128 | 800 | 800 | 800 | 800 |
| *E. esula* | 43.4 | 64 | 256 | 256 | 256 | 256 |
| *E. helioscopia* | 55.3 | 4 | 16 | 16 | 8 | 32 |
| *E. lathyris* | 49.4 | 800 | 3200 | 3200 | 1600 | 3200 |
| *E. platyphylla* | 36.8 | 16 | 256 | 256 | 256 | 2408 |
| *E. coerulescens* | 42.3 | 12800 | 128000 | 64000 | 64000 | 64000 |
| *E. cylindrifolia* | | | 128000 | 256000 | 128000 | 128000 |
| *E. globosa* | | 0 | 0 | 0 | 0 | 0 |
| *E. hermentiana* | 25.8 | 3200 | 102400 | 102400 | 102400 | 102400 |
| *E. lactea* | 19.1 | 1600 | 6400 | 6400 | 6400 | 6400 |
| *E. lactea cristata* | 9.7 | 10240 | 6400 | 12800 | 12800 | 51200 |
| *E. mammillaris* | 5.1 | | 0 | 0 | 0 | 0 |
| *E. splendens* | 34.7 | 700 | 6400 | 6400 | 6400 | 12800 |
| *E. stapelioides* | | | 0 | 0 | 0 | 0 |
| *E. tirucalli* | 4.3 | 16 | 64 | 32 | 32 | 64 |
| *E.trigona* | 13.0 | 12800 | 256000 | 256000 | 128000 | 256000 |
| *Elaeophorbia drupifera* | 24.5 | 25600 | 32000 | 32000 | 32000 | 32000 |

West African tree *Elaeophorbia drupifera* is also an excellent source of lactin.

We have purified to homogeneity, defined by exclusion chromatography on Biogel P150 and on TSK-G3000SW, lectins from the latex-sera of seven of the plants listed in Table 1, and compared some of the properties of the compounds isolated. The plants chosen for a more detailed examination of their lectins are: *E. lathyris*, the only member of the 'leafy' category from which we could obtain enough latex for the purification required: *E. coerulescens*, *E. lactea*, *E. lactea cristata*, *E. hermentiana*, *E. trigona* and *Elaeophorbia drupifera*.

In all elution profiles of the lectins isolated by HPLC on Spherogel TSK-G3000SW gel the coincidence of elution of protein and agglutinating titer was apparent. Similarly, single protein peaks coincident with titers for lectins were obtained for the seven lectins isolated on a Biogel P150 column. The $M_r$s, determined by that latter procedure using a column standardized with a range of proteins of known weights [12], and data from SDS-polyacrylamide disc gel electrophoresis, are presented in Table 2.

The $M_r$s of the lectins described here fall in the narrow range of 60–67 000 (compared with 80 000 for the lectin isolated from the latex of *E. characias* [5]). The data reported in Table 2 for weight measurements with the SDS/PAGE technique also suggest that several of the agglutinins described here, like that from *E. characias* [5], exist in dimeric form. However, the lectins obtained from the latex sera of both *E. lactea* and *E. lactea cristata* must contain sub-units other than those composing dimers, a circumstance comparable with that found for the lectin of latex from *Hura crepitans* [5].

All of the lectins purified in this work were subjected to isoelectric focusing, and each was observed to be composed of a number of isolectins: 13, for example, in the agglutinating agent of the latex from *E. lactea cristata*. The results from these experiments are collected in Table 3. There it is evident that the isolectins of the 'succulent' group have pIs ranging in values between 4.7 and 6.9. The five isolectins obtained from the 'leafy' *E. lathyris* latex serum have a narrower range of pIs (4.7–5.9) which encompasses the pI (5.3) reported for the lectin from *E. characias* latex [5].

In Table 4 are collected the results of amino acid analyses for the lectins isolated in the work described here. To facilitate comparison of these with each other and with

Table 2. Molecular weights of lectins determined by gel-exclusion chromatography and by SDS-polyacrylamide disc gel electrophoresis

| Method | Source of latex serum | | | | | | |
|---|---|---|---|---|---|---|---|
| | *E. coerulescens* | *E. hermentiana* | *E. lactea* | *E. lactea cristata* | *E. lathyris* | *E. trigona* | *Elaeophorbia drupifera* |
| | | | $M_r \times 10^{-3}$ | | | | |
| P-150 | 60 | 65 | 66 | 60 | n.m.* | 60 | 67 |
| SDS/PAGE | 35 | 27 | 37, 32, 30 | 38, 31 | 30 | 32 | 30 |

*Not measured.

Table 3. Ranges of pIs and numbers of isolectin bands observed on isoelectric focusing with polyacrylamide disc gel electrophoresis

| Plant source | No. of isolectins | Range of pI |
|---|---|---|
| *E. coerulescens* | 8 | 4.8–6.9 |
| *E. hermentiana* | 10 | 4.8–6.9 |
| *E. lactea* | 7 | 5.0–6.8 |
| *E. lactea cristata* | 13 | 4.7–6.9 |
| *E. lathyris* | 5 | 4.7–5.9 |
| *E. trigona* | 10 | 4.8–6.9 |
| *Elaeophorbia drupifera* | 8 | 5.1–6.8 |

the results from analyses of the lectins from *E. characias* and *H. crepitans* we have also presented the data as percent weight compositions. It is clear from Table 4 that there are differences amongst the seven lectins purified in this work, but that the molecules appear to be similar to each other and to the lectins isolated from the latices of *E. characias* and *H. crepitans* [5].

To determine more precisely the relationship between these agglutinins, we have used the procedure of Cornish-Bowden [13] for measuring the relatedness of proteins from their amino acid compositions. It was then found that the lectins of *E. hermentiana*, *E. lactea*, *E. lactea cristata*, *E. trigona* and *Elaeophorbia drupifera* are apparently members of the same family of proteins by the more rigorous test possible [13]. The size of the lectin from *E. coerulescens* was such as to prevent a valid comparison of it with the other agglutinins described here, nor could the data for the lectins from *E. characias* and *H. crepitans* be included for the same reason [13]. The lectin from the latex serum of *E. lathyris* showed no relation with those from the other *Euphorbia* spp. (and *Elaeophorbia drupifera*) examined here, which is of interest as *E. lathyris* is the only non-succulent member of the genus studied in this work.

The purified lectins were assayed for comparative agglutinating ability with the sialidase-treated sheep erythrocytes, and the results are reported in Table 5. Included in that Table are values in nanograms, calculated from the titer and protein concentrations listed, of minimal weights of proteins which produce agglutination under the assay conditions described above. Those figures show that the purified lectin from *E. hermentiana* is much more effective in agglutination than the others examined. It was observed that the lectins of Table 5 did not discriminate, in agglutinating ability, between human A, B, AB and O blood types.

The following sugars tested failed to inhibit the agglutination caused by the purified lectins described here: L-fucose, 1-*O*-methyl α-D-glucopyranoside, methyl α-D-mannopyranoside and *N*-acetyl-D-glucosamine. In Table 6 are listed the lowest concentrations of D-galactose and lactose at which inhibition of agglutination occurs. It is apparent that the lactose is a more effective inhibitor of all the lectins discussed, except that from *Elaeophorbia drupifera*, which is not affected by either sugar.

## EXPERIMENTAL

*Reagents.* Unless otherwise noted, these were of analytical grade. Bio-gel P150, all reagents for SDS-PAGE and for polyacrylamide gel isoelectric focusing were obtained from BioRad. The Folin–Ciocalteau reagent was supplied by Fisher Scientific, Ottawa. Sigma, supplied the L-fucose, D-galactose, *N*-acetyl-D-glucosamine, methyl α-D-mannopyranoside and 1-*O*-methyl α-D-glucopyranoside. Lactose was obtained from Anachemia Chemicals, Montreal, Quebec, and sialidase (*Vibrio cholerae*) from Calbiochem. The sheep erythrocytes employed in

Table 4. Amino acid compositions as residues/molecule and, in parentheses, weight percent for lectins from *E. coerulescens* (*E. coer.*), *E. hermentiana* (*E. herm.*), *E. lactea* (*E. lact.*), *E. lactea cristata* (*E. lact. crist.*), *E. lathyris* (*E. lath.*), *E. trigona* (*E. trig.*) and *Elaeophorbia drupifera* (*El. drup.*) [data for *E. characias* (*E. char.*) and *Hura crepitans* (*H. crep.*) [5] are included for comparison]

| Residue | *E. coer.* | *E. herm.* | *E. lact.* | *E. lact. crist.* | *E. lath.* | *E. trig.* | *El. drup.* | *E. char.* | *H. crep.* |
|---|---|---|---|---|---|---|---|---|---|
| Cys | 15 (2.6) | 20 (3.2) | 20 (3.2) | 17 (3.0) | 17 (3.0) | 15 (2.6) | 17 (2.7) | (3.1) | (3.4) |
| Asx | 87 (17.0) | 88 (15.9) | 91 (16.2) | 76 (14.9) | 80 (15.7) | 76 (14.9) | 88 (15.5) | (16.9) | (12.7) |
| Thr | 51 (8.8) | 46 (7.3) | 53 (8.3) | 41 (7.0) | 48 (8.3) | 46 (7.9) | 51 (7.9) | (6.9) | (7.2) |
| Ser | 38 (5.7) | 36 (5.0) | 37 (5.0) | 26 (3.9) | 39 (5.9) | 33 (5.0) | 39 (5.2) | (12.1) | (6.0) |
| Glx | 53 (11.6) | 49 (9.9) | 54 (10.8) | 42 (9.2) | 52 (11.5) | 51 (11.2) | 49 (9.7) | (9.4) | (11.7) |
| Pro | 47 (7.7) | 42 (6.4) | 42 (6.3) | 31 (5.1) | 16 (2.6) | 38 (6.3) | 51 (7.6) | (2.6) | (4.9) |
| Gly | 48 (4.6) | 49 (4.4) | 50 (4.4) | 41 (4.0) | 38 (3.7) | 44 (4.3) | 51 (4.4) | (4.2) | (3.6) |
| Ala | 13 (1.6) | 21 (2.3) | 30 (3.3) | 24 (2.9) | 20 (2.4) | 17 (2.1) | 22 (2.4) | (1.4) | (4.1) |
| Val | 31 (5.2) | 35 (5.4) | 23 (3.5) | 27 (4.6) | 18 (3.0) | 26 (4.4) | 26 (3.9) | (5.8) | (8.7) |
| Met | 6 (1.3) | 2 (0.4) | 1 (0.2) | 2 (0.4) | 2 (0.4) | 0 (0) | 4 (0.8) | (2.3) | (1.0) |
| Ile | 28 (5.4) | 37 (6.6) | 27 (4.7) | 31 (6.0) | 33 (6.4) | 28 (5.4) | 30 (5.2) | (4.7) | (6.1) |
| Leu | 41 (7.9) | 45 (8.0) | 50 (8.7) | 42 (8.1) | 45 (8.7) | 39 (7.5) | 47 (8.1) | (7.0) | (9.9) |
| Tyr | 18 (5.0) | 24 (6.2) | 30 (7.6) | 26 (7.2) | 21 (5.8) | 21 (5.8) | 26 (6.5) | (4.8) | (7.9) |
| Phe | 15 (3.8) | 20 (4.6) | 14 (3.2) | 12 (3.0) | 11 (2.8) | 15 (3.8) | 16 (3.6) | (6.3) | (1.2) |
| His | 3 (0.7) | 3 (0.6) | 6 (1.3) | 6 (1.4) | 6 (1.4) | 5 (1.2) | 3 (0.6) | (1.5) | (0.9) |
| Lys | 18 (3.9) | 21 (4.2) | 28 (5.5) | 23 (5.0) | 7 (1.5) | 20 (4.4) | 22 (4.3) | (5.0) | (5.0) |
| Arg | 18 (4.8) | 21 (5.2) | 20 (4.8) | 20 (5.3) | 28 (7.5) | 21 (5.6) | 25 (6.0) | (6.0) | (5.6) |
| Trp | 0 (0) | 0 (0) | 0 (0) | 16 (5.1) | 10 (3.2) | 10 (3.2) | 7 (2.0) | — | — |
| GlcN | 9 (2.5) | 16 (4.0) | 12 (3.0) | 14 (3.8) | 22 (6.0) | 16 (4.4) | 14 (3.5) | — | — |

Table 5. Measurements of agglutinations of sheep-erythrocytes (sialidase-stripped) with the purified lectins from *Euphorbia* latices

| Lectin source | Protein concn (mg/ml) | Titer | Nanograms of lectin required to produce agglutination |
|---|---|---|---|
| *E. coerulescens* | 1.76 | 800 | 55 |
| *E. hermentiana* | 2.2 | 3200 | 17 |
| *E. lactea* | 11.9 | 800 | 370 |
| *E. lactea cristata* | 17.3 | 6400 | 68 |
| *E. lathyris* | 0.2 | 64 | 78 |
| *E. trigona* | 20.7 | 3200 | 162 |
| *Elaeophorbia drupifera* | 27.6 | 1280 | 539 |

Table 6. Inhibition by D-galactose and lactose of agglutination of sheep erythrocytes by purified lectins from latex-sera of several *Euphorbia* spp.

| Lectin source | Lowest inhibiting concentration (mM) D-galactose | lactose |
|---|---|---|
| *E. coerulescens* | 12.5 | 3.13 |
| *E. hermentiana* | 12.5 | 0.78 |
| *E. lactea* | 25.0 | 6.25 |
| *E. lactea cristata* | 25.0 | 6.25 |
| *E. lathyris* | 1.56 | 0.78 |
| *E. trigona* | 25.0 | 6.25 |
| *Elaeophorbia drupifera* | no inhibition | |

the agglutination tests were purchased from Qualicum Scientific, Ottawa. Human cells of types A, B, AB and O were from the Canadian Red Cross, Ottawa. Affigel-10-β-D-lactoside was a gift from Dr. N. M. Young [14].

*Euphorbia lathyris* L., *E. helioscopia* L., *E. characias* L. and *E. platyphylla* L. (broad-leafed spurge) were grown from seeds in greenhouses, using a 50% sand–soil mixture with a daylight cycle of 18 hr, at 23°. Those seeds were from the Botanic Gardens of Lyon, France (*E. lathyris* and *E. characias*) and Bordeaux, France (*E. helioscopia* and *E. platyphylla*). The latex of *E. esula* L. (leafy spurge) was obtained from plants grown at Agriculture Canada, Ottawa, and that from *E. cyparissias* L. (cypress spurge) collected from plants growing wild at Arnprior, Ont. Latex from *E. mammilaris* L. and *E. stapelioides* Boiss. were taken from plants at the greenhouse, Carleton University, Ottawa. Cuttings of *E. globosa* (Haw.) Sims, *E. cylindrifolia* Marn-Lapostc and Rauh and of *Elaeophorbia drupifera* Thonn ex Schum were obtained from Dr. G. L. Webster, and cuttings of *E. coerulescens* Haw. and *E. tirucalli* L. from Dr. D. S. Verity. These were grown as described above. *Euphorbia splendens* Bojer., *E. lactea* Haw., *E. lactea* Haw. cv. '*cristata*', *E. hermentiana* Lem. and *E. trigona* Haw. were purchased from commercial suppliers in Ottawa.

Latex was collected from incisions in the stems of mature plants throughout their life times and stored at −20° until required. This was then thawed and centrifuged at 25 000 *g* for 1 hr, when clear, watery serum containing the protein material of the latex was separated.

*Purification of lectins.* Crude latex-serum (10–20 ml) was applied to a column (1.5 × 15 cm) of Affigel-10-β-D-lactoside at 4°. Elution of the lectin was effected with 10 mM NaPi/150 mM NaCl, pH 7.3 containing 100 mM lactose. After dialysis against 200 mM KPi buffer, pH 7, and concn on a Diaflo UM-10 membrane, the lectins were subjected to isocratic HPLC on a column of Spherogel TSK-G3000SW (60 cm; Altex, CA). A Beckman model 110A metering pump was used in this system with a Varian model 2050 detector system, and the column maintained at 23°. By this procedure the lectins isolated were each shown to have a unique $M_r$.

*Agglutination tests.* The same method was used in the cell preparation of the sheep erythrocytes commonly employed in this work, as with those of human A, B, AB and O blood types. The citrated cells obtained were washed × 3 with the phosphate-buffered saline described above, and diluted to 2% in that saline. Cell quantitation was performed by lysing 1 ml of cells in 14 ml of 0.1% $NaHCO_3$ and measuring $A_{541}$. $A_{541} = 0.70$ was accepted as the measure of 5% cell concn [15]. Blood cells were prepared freshly for the agglutination tests by treatment of 20 ml of 2% cells with 0.2 I.U. sialidase, incubation at 37° for 2 hr, and washing with the phosphate-buffered saline described above.

*Measurement of agglutination.* This was done on micro-titer plates where, to a series of 1:2 dilutions of lectin soln, 25 μl per well, were added 25 μl of a 2% suspension of the sialidase-stripped cells. After 1 hr at 23° the degree of agglutination was scored visually as 'titer', which is the reciprocal of the maximum dilution giving agglutination [16]. The inhibitory activity of various sugars was measured in the same way, using 25 μl of serially diluted inhibitor with 25 μl of stripped cells and 25 μl of (2 × titer) concn of each lectin.

*Protein determination.* The Folin procedure [11] was used, as well as $A_{280}$ measurements.

$M_r$ *determinations.* A column (1.5 × 100 cm) of Bio-gel P150 in 50 mM NaOAc, pH 6 at 4° was equilibrated with aldolase, bovine serum albumin, haemoglobin, ovalbumin, ribuonuclease, carbonic anhydrase and soybean trypsin inhibitor, and $M_r$s of the lectins determined following ref. [12].

Sub-unit weights of the lectins were measured in calibrated SDS-polyacrylamide gel electrophoresis following the procedure of ref. [17].

*Isoelectric focusing.* Biolyte (BioRad) ampholytes were employed following the procedure of ref. [18]. Staining was with Coomassie Brilliant Blue.

*Amino acid analysis.* Conventional hydrolysates were prepared in 6 M HCl, under vacuum at 110° for 22 hr. Tryptophan was determined after hydrolysis in 4 M methane sulphonic acid [19]. Amino sugars were measured after hydrolysis in 4 M HCl for 6 hr under vacuum at 110°, and the cysteine content assayed after oxidation and hydrolysis as described in ref. [20]. Amino acid and amino sugar analyses were performed on an automatic amino acid analyser.

*Acknowledgements*—We wish to thank for seeds, cuttings and access to *Euphorbia* spp.: The Directors of the Botanical Gardens of Bordeaux and of Lyon, France; Dr. G. L. Webster, Department of Botany, University of California at Davis, CA; Dr. D. S. Verity, Herbarium, Mildred E. Mathias Botanical Gardens, Los Angeles, CA, Drs. C. Crompton and A. Stahevitch, Agriculture Canada, Ottawa and Mr. H. Datema of Carleton University, Ottawa, who also grew the seeds and cuttings listed in the text. We wish also to thank: Dr. R. Labow of the Canadian Red Cross for supplies of human blood; Dr. M. Yaguchi who performed the amino acid and amino sugar analyses; Mrs. L. Bramall for the comparisons of the amino acid compositions, as well as Dr. N. M. Young who provided aid and advice during this work, and Dr. W. I. Illman, Biology Department, Carleton University, who generously assisted in the taxonomic verifications.

## REFERENCES


1. Lis, H. and Sharon, N. (1981) in *The Biochemistry of Plants* (Marcus, A., ed.) p. 371. Academic Press, New York.
2. Jaffé, W. G. and Seidl, D. (1969) *Experientia* **25**, 891.
3. McPherson, A. and Hoover, S. (1979) *Biochem. Biophys. Res. Commun.* **89**, 713.
4. Falasca, A., Franceschi, C., Rossi, C. A. and Stirpe, F. (1980) *Biochim. Biophys. Acta* **632**, 95.
5. Barbieri, L., Falasca, A., Franceschi, C., Licastro, F., Rossi, C. A. and Stirpe, F. (1983) *Biochem. J.* **215**, 433.
6. Premaratna, A., Shadaksharaswamy, M. and Nanjappa, S. (1981) *Indian J. Biochem. Biophys.* **18**, 32.
7. Nsimba-Lubaki, M., Peumans, W. J. and Carlier, A. R. (1983) *Biochem. J.* **215**, 141.
8. Lynn, K. R. and Clevette-Radford, N. A. (1983) *Biochim. Biophys. Acta* **18**, 154.
9. Lynn, K. R. and Clevette-Radford, N. A. (1984) *Phytochemistry* **23**, 682.
10. Lynn, K. R. and Clevette-Radford, N. A. (1985) *Phytochemistry* **24**, 925.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
12. Andrews, P. (1970) *Methods Biochem. Anal.* **18**, 1.
13. Cornish-Bowden, A. (1983) *Methods Enzymol.* **91**, 60.
14. Neurohr, K. J., Young, N. M. and Mantsch, H. H. (1980) *J. Biol. Chem.* **255**, 9205.
15. Kabat, E. A. and Mayer, M. M. (1967) in *Experimental Immunochemistry*, 2nd edn, p. 150. C. C. Thomas, Springfield, IL.
16. Goldstein, I. J. and Hayes, C. E. (1978) in *Advances in Carbohydrate Chemistry and Biochemistry* (Tipson, R. S. and Horton, D., eds) p. 127. Academic Press, New York.
17. Weber, K., Pringle, J. R. and Osborn, M. (1971) *Methods Enzymol.* **26**, 3.
18. Righetti, P. L. and Drysdale, J. W. (1974) *J. Chromatogr.* **98**, 271.
19. Simpson, R. J., Neuberger, M. R. and Lui, T. Y. (1976) *J. Biol. Chem.* **251**, 1936.
20. Hirs, C. H. W. (1967) *Methods Enzymol.* **19**, 197.