Purification and properties of two lectins from the latex of the euphorbiaceous plants *Hura crepitans* L. (sand-box tree) and *Euphorbia characias* L. (Mediterranean spurge)

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1. From the latex of two members of the plant family Euphorbiaceae, *Hura crepitans* L. (sand-box tree) and *Euphorbia characias* L. (Mediterranean spurge), two lectins were purified by affinity chromatography on acid-treated Sepharose 6B followed by elution with D-galactose. 2. The lectin from *E. characias* is a single molecular species with M_r 80 000, made up of two identical subunits with M_r 40 000, and is a glycoprotein containing 11% carbohydrate. 3. The lectin from *H. crepitans* appears as a mixture of three isolectins with M_r 140 000, consisting of four different subunits with M_r values 37 500, 35 500, 31 000, and 29 000. 4. Both lectins have haemagglutinating activity, with no specificity for human blood groups. The haemagglutinating activity is inhibited by D-galactose and by galactose-containing oligosaccharides. 5. The lectin from *H. crepitans* is mitogenic to human T-, but not to B-, lymphocytes. The latex of *E. characias* is mitogenic to T- and, to a lesser extent, to B-, lymphocytes, but the purified *E. characias* lectin has no mitogenic activity. 6. The lectin from *H. crepitans*, but not that from *E. characias*, inhibits protein synthesis by a rabbit reticulocyte lysate.

The latices of several members of the Euphorbiaceae are caustic and toxic to animals, and sometimes have been used in popular medicine. The latex of *Euphorbia characias* L. (Mediterranean spurge) is toxic and was used to remove warts. Richet (1909) reported that the latex of *Hura crepitans* L. (syn. *Hura brasiliensis* Willd.) (sand-box tree) has haemagglutinating properties and contains a toxic protein, crepitin, that he considered to be similar to ricin. Jaffé & Seidl (1969) purified from the latex of the same plant a protein, also called crepitin, that had haemagglutinating but not mitogenic activity. From the seeds of *H. crepitans* a mitogenic lectin was purified by McPherson & Hoover (1979), who called it 'hurin', and by Falasca *et al.* (1980), whereas three lectins were reported by Pere *et al.* (1981). A lectin was purified also from the latex of another member of the Euphorbiaceae, *Synadenium grantii* (Premaratna *et al.*, 1981).

We describe now the purification and some properties of two lectins from the latices of *H.*

crepitans and of *E. characias*. Both lectins have haemagglutinating activity without any specificity for human blood groups, and have the highest affinity for galactose and galactose derivatives. The lectin from the latex of *H. crepitans*, like that from the seeds of the same plant, is mitogenic to human T-lymphocytes, and inhibits protein synthesis in a cell-free system.

Experimental

Materials

The latex of *E. characias* was collected from plants grown in the Botanical Garden of the University of Bologna and from wild plants growing in the fields in the territory of Castro dei Volsci, Italy.

The latex of *H. crepitans* was collected from a plant grown in the Botanical Garden of the University of Bologna. Another sample was obtained from Dr. L. E. Newton, Kumasi, Ghana; this sample had been diluted with 10 vol. of 0.14 M-NaCl, and had been kept at 4°C for 5 days.

The lectin from *H. crepitans* seeds was prepared as described by Falasca *et al.* (1980).

Abbreviation used: SDS, sodium dodecyl sulphate.

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Human erythrocytes and blood samples used to separate human lymphocytes were obtained from the Centro Trasfusionale, Policlinico S. Orsola, Bologna, Italy. Rabbit erythrocytes were collected and washed as described by Falasca *et al.* (1979).

Standards for M_r determination were from Boehringer-Mannheim, Mannheim, Germany. [*methyl*- ^3H]Thymidine (specific radioactivity 20 Ci/nmol) and L-[^{14}C]leucine (340 Ci/mol) were from Amersham International, Amersham, Bucks., U.K. Acid-treated Sepharose 6B (Pharmacia Fine Chemicals, Uppsala, Sweden) was prepared as described by Ersson *et al.* (1973) with a 3 h acid hydrolysis. All other chemicals were from the same sources as in previous work (Falasca *et al.*, 1979, 1980).

Purification of the lectins

Latices were diluted with 9–10 vol. of 0.14 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2, immediately after having been collected, and were cooled to 2–4°C, or were frozen at –25°C, if not used within the same day. All subsequent operations were performed at 2–4°C. The samples were centrifuged at 40 000 g at 2°C for 30 min, and the supernatants were dialysed against the same phosphate-buffered saline for at least 24 h. A milky supernatant was obtained from samples kept at 2°C or frozen for 24 h, whereas samples kept frozen for some days sometimes showed, on thawing, a sort of clot, and in these cases, on centrifuging, gave an almost clear supernatant. This difference did not affect the procedure, although a somewhat higher yield of lectin was obtained from supernatants which remained milky. The dialysed preparations were adjusted to 0.3 M with solid NaCl, and portions were applied to a column of acid-treated Sepharose 6B, previously equilibrated with 0.3 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2. The column was washed with the same solution until the A_{280} of the effluent was below 0.1, and the retained material was eluted with 0.2 M-galactose in the same medium. The fractions of the peaks were pooled, dialysed extensively against water, and freeze-dried.

Chemical determinations

The amino acid composition was determined in samples extensively dialysed against water as described by Falasca *et al.* (1980).

The neutral- and amino-sugar composition was analysed by g.l.c. as described by Dunstan *et al.* (1974), with D-mannitol as a standard.

M_r was determined by centrifugation in a sucrose density gradient (5–20%, w/v) as described by Martin & Ames (1961) with glutamate dehydrogenase (M_r , 336 000), lactate dehydrogenase (M_r , 140 000), malate dehydrogenase (M_r , 70 000) and ovalbumin (M_r , 43 000) as standards. Gradients

were centrifuged at 100 000 g for 16 h at 4°C in the SW39 rotor in a Beckman Spinco L50 centrifuge.

Determination of M_r by gel filtration was performed with a column (85 cm × 1.6 cm) of Bio-Gel P150 (Bio-Rad Laboratories) equilibrated with 0.3 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2, with lactate dehydrogenase, glucose 6-phosphatase (M_r , 104 000), bovine serum albumin (M_r , 67 000), ovalbumin and chymotrypsinogen (M_r , 25 000) as standards.

Isoelectric focusing was carried out on Ampholine/polyacrylamide-gel plates (LKB, Stockholm, Sweden) at a pH range 3.5–9.5, according to the instructions supplied by the manufacturer.

Electrophoresis under non-denaturing conditions was performed on acetate strips (Chemagel; Chemetron Chimica, Rozzano, Italy) with the following buffer solutions: 0.15 M-sodium phosphate, pH 7.2; 0.35 M- β -alanine/acetic acid, pH 4.5; 0.37 M-Tris/glycine, pH 9.5. SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970). Preparative polyacrylamide-gel electrophoresis was performed as described by Weber & Osborn (1969), but SDS was omitted.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma) as a standard, or spectrophotometrically (Warburg & Christian, 1941).

Haemagglutinating activity

Human blood was obtained from the Centro Trasfusionale, Policlinico S. Orsola, Bologna, Italy. Rabbit blood was collected from the ear vein, and was quickly mixed with 0.25 vol. of 0.38% sodium citrate. Erythrocytes were separated by centrifugation at 3000 g for 10 min at room temperature, were washed three times with 0.14 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2, and were resuspended in the same solution. Haemagglutinating activity was determined in Greiner microtitre plates as described by Falasca *et al.* (1980). Each well contained, in a final volume of 100 μl , 50 μl of 2% erythrocyte suspension and serial dilutions (by doubling, starting from 500 $\mu\text{g}/\text{ml}$) of the lectins. All solutions and suspensions were prepared in phosphate-buffered saline containing bovine serum albumin (15 $\mu\text{g}/\text{ml}$). The inhibitory activity of the sugars was tested in a similar system, with 50 μl of the lectins and 50 μl of erythrocyte suspension and with serial dilutions (by doubling) of the sugars, in a final volume of 150 μl .

Mitogenic activity

Human lymphocytes were separated by density-gradient centrifugation through Ficoll-Hypaque as described by Bøyum (1968). T- and B-lymphocytes were further purified from Ficoll-Hypaque-separated lymphocytes as previously described

(Franceschi *et al.*, 1981). The purity of T-lymphocytes identified by their capacity to form rosettes with sheep erythrocytes, and of B-lymphocytes, identified by the direct fluorescent-antibody technique with sheep anti-(human immunoglobulin) serum (Wellcome Research Laboratories, Beckenham, Kent, U.K.), was more than 90%.

Stimulation of lymphocytes with lectins, culture conditions, and the [^3H]thymidine pulse were performed as previously described (Falasca *et al.*, 1979). After incubation, cultures were frozen at -20°C and, after thawing, were automatically collected on glass-fibre filters with a cell-culture harvester (Skatron, Oslo, Norway). Filters were transferred to counting vials and processed as described previously (Franceschi *et al.*, 1981). Radioactivity was determined in a scintillation spectrometer.

Assay of protein synthesis

The effect of lectins on protein synthesis was evaluated with a rabbit-reticulocyte-lysate system as described by Stirpe *et al.* (1981); the details are given in the legend to Table 6 (below).

Results

Preliminary experiments showed that diluted latices of *H. crepitans* and of *E. characias* agglutinated rabbit erythrocytes and that agglutination was prevented by galactose. This prompted us to purify the lectins by affinity chromatography on acid-treated Sepharose 6B, as practised with the galactose-specific lectin from *H. crepitans* seeds (Falasca *et al.*, 1980). Lectins were eluted as single, sharp peaks with galactose; recoveries are given in Table 1.

Chemical properties of the purified lectins

Both *H. crepitans* and *E. characias* lectins showed a single band on centrifugation on a sucrose density gradient, with sedimentation rates corresponding to M_r 140 000 for the *H. crepitans* and 80 000 for

the *E. characias* lectin. The *H. crepitans* lectin gave three partially resolved peaks when it was eluted from an acid-treated Sepharose 6B column with a 0–0.2 M-galactose gradient (results not shown). When applied to a Bio-Gel P-150 column, the lectin from *H. crepitans* was eluted in a position corresponding to M_r 112 000, and that from *E. characias* in a position corresponding to M_r 80 000.

On cellulose acetate electrophoresis, performed under non-denaturing conditions at pH 4.5, 7.2 and 9.5, the *E. characias* lectin consistently showed a single band, whereas the *H. crepitans* lectin showed three bands at the same three pH values. Three bands were also obtained from *H. crepitans* lectin on preparative gel electrophoresis. These bands were eluted from the gel, and all of them had haemagglutinating activity towards rabbit erythrocytes and were mitogenic to human lymphocytes (Fig. 1).

On SDS/polyacrylamide-gel electrophoresis in the presence of 2-mercaptoethanol, the lectin from *E. characias* showed a single subunit with mobility corresponding to M_r 40 000, and the *H. crepitans* lectin showed four subunits, with M_r values respectively 37 500, 35 500, 31 000 and 29 000 (mean values from four determinations). The two heaviest subunits seemed more abundant with respect to the smaller ones, as judged from the intensity of the coloured bands. If treatment with 2-mercaptoethanol was omitted, the lectin from *H. crepitans* showed three bands only.

On isoelectrofocusing, the lectin from *E. characias* showed a single molecular species with pI 5.3, whereas the lectin from *H. crepitans* showed again three bands, with pI values respectively, 7.3, 7.0 and 6.7. A summary of the physical properties of the *H. crepitans* and *E. characias* lectins is given in Table 2.

The amino acid composition of the lectins is given in Table 3. In this respect the lectin from *H. crepitans* latex is similar to, but not identical with,

Table 1. *Purification of the lectins from the latices of Hura crepitans and of Euphorbia characias*
Experimental conditions are described in the text. Results refer to 1 ml of fresh latex.

Preparation	Agglutinating activity			Recovery (%)
	Protein (mg)	Specific activity (units*/mg of protein)	Total activity (units)	
<i>Hura crepitans</i>				
Dialysed latex	101.2	58.8	5993	
Galactose-eluted peak (lectin)	4.65	1111	5166	87
<i>Euphorbia characias</i>				
Dialysed latex	37.8	31.2	1181	
Galactose-eluted peak (lectin)	0.8†	285	228	19

* One unit is defined as the minimal amount giving visible agglutination of human erythrocytes group O, Rh $^+$.

† This was the lowest yield obtained. The mean value from five preparations was 2.5 mg/ml of fresh latex.

Fig. 1. Preparative electrophoresis of *Hura crepitans* lectin

Electrophoresis was performed as described in the text, with 1 mg of lectin on a slab (9 cm × 14 cm × 0.3 cm) of 7.5% (w/v) polyacrylamide gel. After a run of 4 h at 70 mA, two small strips were cut at each side of the slab and stained with Coomassie Blue. The rest of the slab was cut into horizontal strips (3 mm each), which were eluted by homogenization in a Potter homogenizer with 1 ml of 0.14 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2, and 15 µg of bovine serum albumin/ml. The eluates were assayed for protein content (Warburg & Christian, 1941) (O), and for haemagglutinating (●) and mitogenic (■) activity [the latter being expressed as 'stimulation index' (ratio between incorporation of [³H]thymidine in the presence and in the absence of the lectin)].

that from the seeds of the same plant (Falasca *et al.*, 1980), having a lower content of arginine and a higher content of proline, alanine, valine, leucine and tyrosine. The abundance of dicarboxylic amino acids in *E. characias* lectin, consistent with the pI of this protein, and of sulphur amino acids, is noteworthy. The same lectin contains 11% carbohydrate, and the sugar composition is reported in Table 3.

Haemagglutinating activity

The lectin from *H. crepitans* agglutinated to a comparable extent human erythrocytes of all blood groups at concentrations of 24–48 ng/ml.

The *E. characias* lectin agglutinated human erythrocytes at concentrations ranging from 0.19 (group O) to 1.56 µg/ml (group A₂B). It should be noted that the crude latex of *E. characias* did not agglutinate human erythrocytes, although it ag-

Table 2. *M_r* and isoelectric point of the lectins from *Hura crepitans* and *Euphorbia characias* latices. Experimental conditions are described in the text.

Lectin ...	<i>Hura crepitans</i>	<i>Euphorbia characias</i>
<i>M_r</i> of lectin		
By sucrose density gradient	140 000	80 000
By gel filtration	112 000	80 000
<i>M_r</i> of subunits		
By SDS/polyacrylamide-gel electrophoresis	37 500 35 500 31 000 29 000	40 000
pI of native lectin	7.3 7.0 6.7	5.3

Table 3. (a) Amino acid and (b) sugar composition of *Hura crepitans* and *Euphorbia characias* lectins

Experimental conditions are described in the text. Results are the averages of duplicate determinations after hydrolysis for 24, 48 and 72 h. *M_r* values of 140 000 and 80 000 respectively were assumed for the *H. crepitans* and *E. characias* lectins.

			(a) Amino acid content (residues/molecule)	
Amino acid	Lectin	...	<i>H. crepitans</i>	<i>E. characias</i>
Lys			47.0	32.3
His			8.0	7.7
Arg			43.0	21.8
Asx			133.1	100.8
Thr*			86.1	47.1
Ser*			82.4	94.4
Glx			108.9	49.8
Pro			61.2	18.4
Gly			76.0	51.1
Ala			68.6	13.7
$\frac{1}{2}$ -Cys			40.1	20.7
Val			105.0	40.1
Met			8.3	11.8
Ile			65.3	28.4
Leu			105.6	42.7
Tyr*			58.3	20.0
Phe			9.9	29.3
Trp			Not determined	
			(b) Sugar content (residues/molecule) of <i>E. characias</i> lectin	
Sugar				
Fuc			12.2	
Gal			8.3	
Glc			0.5	
Man			12.5	
GlcNAc			12.5	
Xyl			2.9	
Total sugar content: 11.3%.				

* Values obtained from the hydrolysis for 24, 48 and 72 h were extrapolated to zero time.

Table 4. *Inhibition of haemagglutination by sugars*

Haemagglutination was determined, as described in the Experimental section, with human erythrocytes group O, Rh⁺. Lectins were added at twice the lowest concentration giving agglutination. The following sugars did not affect haemagglutination, at concentrations up to 50 mM: D-glucose, D-gulose, L-glucose, D-mannose, L-mannose, D-arabinose, D-ribose, L-fucose, D-xylose, 2-deoxy-2-amino-D-glucose, 2-deoxy-2-amino-D-galactose, neuraminic acid, methyl α -D-glucopyranoside, methyl β -D-glucopyranoside, 3-O-methyl α -D-glucopyranoside, *p*-nitrophenyl α -D-glucopyranoside, methyl α -D-mannopyranoside, 2-deoxy-2-acetamido- α -D-glucose, 2-deoxy-2-acetamido- α -D-mannose, D-maltose, cellobiose, sophorose, gentiobiose, threulose, sucrose and D-stachyose.

Sugar	Lectin ... Source ...	Relative inhibitory activity*		
		<i>H. crepitans</i>		<i>E. characias</i>
		Latex	Seeds	Latex
L-Arabinose		0.12	0.25	0.12
D-Talose, D-fucose		0.50	0.50	0.50
D-Galactose		1	1	1
Methyl α -D-galactopyranoside		2.5	1	1.25
Methyl β -D-galactopyranoside		5	2	2.5
<i>p</i> -Nitrophenyl α -D-galactopyranoside		5	2.5	2.5
<i>p</i> -Nitrophenyl β -D-galactopyranoside		20	5	10
2-Acetamido-2-deoxy- β -D-galactose		33	8	65
<i>p</i> -Nitrophenyl 2-acetamido-2-deoxy- β -D-galactopyranoside		1600	50	1600
<i>p</i> -Nitrophenyl β -D-fucopyranoside		10	5	5
Melibiose		1	0.5	2
Lactose		8	4	4

* Calculated by taking as 1 the minimal inhibitory concentration of D-galactose, which was 6.25 mM for *H. crepitans* latex lectin, 25 mM for *H. crepitans* seed lectin and 3.12 mM for *E. characias* lectin.

glutinated rabbit erythrocytes (results not shown).

The effect of simple sugars on haemagglutination is reported in Table 4. Among the non-derivatized monosaccharides tested, D-galactose appears to be the most efficient inhibitor.

No sugars with the arrangement at C-3 and C-4 different from that of D-galactose (i.e. D-gulose, D-glucose, D-mannose) have any inhibitory activity. Considerable modification at C-5 and C-6, on the other hand, seems to be tolerated, since D-fucose and L-arabinose are still inhibitory.

A substitution at C-2 can abolish (2-deoxy-2-amino-D-galactose) or greatly increase (2-deoxy-2-acetamido-D-galactose) the inhibitory potency of the monosaccharide, but has no effect if the hydroxy groups at C-3 and C-4 are not in the D-galactose configuration (2-deoxy-2-acetamido-D-glucose, 2-deoxy-2-acetamido-D-mannose). Both lectins react more strongly with β -substituted D-mono-saccharides [methyl- β -D- or *p*-nitrophenyl- β -D-pyranosides or β -D-linked disaccharides (lactose)]. The presence of a *p*-nitrophenyl group at C-1 gives a very strong enhancement of the inhibitory potency of D-galactose-related sugars, but does not have any effect if the hydroxy group at C-4 is not in the D-galactose configuration (*p*-nitrophenyl α -D-glucopyranoside).

The lectin purified from the seeds of *H. crepitans*, whose ability to agglutinate human erythrocytes in the presence of monosaccharides was evaluated at

the same time, showed behaviour identical with that of the two other lectins, the results being somewhat different from those reported previously (Falasca *et al.* (1980) with rabbit erythrocytes.

Mitogenic activity

The lectin from *H. crepitans*, when tested on separated human B- and T-lymphocytes, was mitogenic for the latter cells only, the lowest concentration stimulating [³H]thymidine incorporation being 0.71 nM. Maximal stimulation was approx. 66% of that given by phytohaemagglutinin at optimal concentration, and was obtained with *H. crepitans* lectin at 10 μ g/ml. Higher concentrations were less stimulatory.

The purified lectin from *E. characias*, at concentrations up to 100 μ g/ml, did not affect [³H]-thymidine incorporation by lymphocytes (results not shown). However, the dialysed latex from *E. characias* was mitogenic for both B- and T-cells (Table 5). This mitogenic activity was somewhat decreased if the latex was partially clarified by centrifugation, and was not modified after boiling for 2 h in a water bath.

Effect on protein synthesis

Some galactose-binding lectins inhibit protein synthesis in a cell-free system (Barbieri *et al.*, 1979). This prompted us to examine the effect on protein synthesis of the lectins from *E. characias* latex and

from *H. crepitans* latex and seeds. The *E. characias* lectin had little effect, whereas both the *H. crepitans* lectins significantly inhibited protein synthesis by a rabbit reticulocyte lysate (Table 6), although at a concentration (100 µg/ml) higher than those required to agglutinate erythrocytes or to stimulate lymphocytes. The inhibitory effect was not significantly modified in the presence of *N*-acetyl-D-galactosamine, was decreased if the lectins were preincubated at 37°C for 2 h, and was completely abolished by preincubation at 37°C in the presence of 2-mercaptoethanol.

Discussion

The lectins purified from the latices of *H. crepitans* and *E. characias* agglutinate human erythrocytes, the lectin from *H. crepitans* being the most potent. In the case of the former lectin, no significant differences were observed among various blood groups, whereas the haemagglutinating activity of the *E. characias* lectin was approx. 10-fold higher towards

erythrocytes of group O than towards those of group AB.

The inhibition of agglutination with simple sugars suggests an identical primary interaction site for both lectins. *N*-Acetyl-D-galactosamine and D-galactose are the most inhibitory monosaccharides, and the comparison with the effects of other sugars indicates that the D-galactose configuration at C-3 and C-4 is essential for the interaction with both lectins.

In spite of the identical sugar-binding properties, the two lectins have a completely different effect on human lymphocytes in that only the *H. crepitans* lectin is mitogenic to T-lymphocytes. Thus the mitogenic activity seems unrelated to the sugar specificity, which suggests one of the following alternatives: either (i) *E. characias* lectin binds to cell receptors different from those for *H. crepitans* lectin, or (ii) should the two lectins share the same receptors, binding *per se* is not sufficient to trigger blastization.

The lack of mitogenic activity of *E. characias* lectin is not inconsistent with the mitogenic effect of the crude latex of the same plant. The decreased activity of the centrifuged latex suggests that this effect, which resists prolonged boiling, is due to the presence of the latex particles, which could act either *per se* or as carriers of non-proteinaceous mitogenic substance(s).

The lectin from *H. crepitans*, like some other galactose-binding lectins (Barbieri *et al.*, 1979), inhibits protein synthesis in a cell-free system. This effect is exerted at concentrations much higher than those causing haemagglutination, and is independent of the lectin property, since it is not modified in the presence of *N*-acetyl-D-galactosamine. Treatment with 2-mercaptoethanol abolished the effect on protein synthesis; this is at variance with what has been observed with ricin and related toxins (reviewed by Olsnes & Pihl, 1982), and suggests that the inhibitory effect of *H. crepitans* lectin is exerted either by the whole lectin molecule rather than by

Table 5. *Effect of Euphorbia characias* latex on [³H]-thymidine incorporation by B- and T-lymphocytes

Experimental conditions are described in the text.

Latex added and dilution	[methyl- ³ H]Thymidine incorporated (c.p.m.)	
	B-cells	T-cells
None	1076 ± 150	3007 ± 1738
Non-centrifuged latex		
1:10000	397 ± 36	7079 ± 1092
1:1000	3231 ± 162	23323 ± 1675
1:100	7149 ± 551	26318 ± 1322
1:10	136 ± 51	5502 ± 354
Latex centrifuged for 30 min at 28000 g		
1:10000	954 ± 100	2359 ± 439
1:1000	294 ± 31	4100 ± 576
1:100	4793 ± 218	26759 ± 428
1:10	82 ± 15	19097 ± 160

Table 6. *Effect of Hura crepitans* and *Euphorbia characias* lectins on cell-free protein synthesis

Reaction mixtures contained, in a final volume of 125 µl: 10 mM-Tris/HCl buffer, pH 7.4, 100 mM-ammonium acetate, 2 mM-magnesium acetate, 1 mM-ATP, 0.2 mM-GTP, 15 mM-phosphocreatine, 6 µg of creatine kinase, 0.05 mM-amino acids (minus leucine), 0.19 µCi of L-[¹⁴C]leucine and 50 µl of a lysate of rabbit reticulocytes. Incubation was at 27°C for 5 min and the radioactivity incorporated into protein was measured in 25 µl samples.

Lectin (100 µg/ml) added	Protein synthesis (% of controls)		
	Lectin (100 (3550 d.p.m.))	Lectin + 10 mM-β-D-GalNAc 100 (3582 d.p.m.)	Reduced lectin* 100 (1925 d.p.m.)
None (control)			
<i>H. crepitans</i>			
Latex	28	24	102
Seeds	10	3	113
<i>E. characias</i>	79	84	126

* Treated with 1% 2-mercaptoethanol for 2 h at 37°C; an equal amount of 1% 2-mercaptoethanol was added to controls.

one of its subunits, or by an unstable subunit that could be inactivated once it is separated from the other subunits. It is noteworthy that the latex of *H. crepitans* also contains a protein that inhibits protein synthesis with the characteristics of the ribosome-inactivating proteins present in many plants (reviewed by Barbieri & Stirpe, 1982). The inhibitory effect on protein synthesis of the *H. crepitans* lectin, however, does not seem to be due to contamination by traces of the ribosome-inactivating protein, since (i) it is also exerted by the lectin from *H. crepitans* seeds, which do not contain the other protein, and (ii) is abolished by treatment with 2-mercaptoethanol, which does not affect the activity of ribosome-inactivating proteins (Stirpe *et al.*, 1980).

The lectin purified from the latex of *H. crepitans* is similar to that obtained from the seeds of the same plant (Falasca *et al.*, 1980) in several aspects (the sugar specificity, the lack of specificity for blood groups, the effect on protein synthesis). However, the two lectins are not identical, as shown (i) by the presence of isolectins in the latex, (ii) by the subunit composition (four identical subunits in the lectin from seeds, four different subunits in the lectin from latex), and (iii) by the amino acid composition.

Lectins have been obtained mostly from seeds. Present results extend previous observations on the presence of lectins in the latices or saps of members of the Euphorbiaceae (Premaratna *et al.*, 1981), of the Cucurbitaceae (Sabnis & Hart, 1978) and other plants (Gietl *et al.*, 1979), and indicate that these materials can be useful sources for the purification of lectins.

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References

- Barbieri, L. & Stirpe, F. (1982) *Cancer Surveys* **1**, 489–520
- Barbieri, L., Lorenzoni, E. & Stirpe, F. (1979) *Biochem. J.* **182**, 633–635
- Bøyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, Suppl. **97**, 77–79
- Dunstan, D. R., Grant, A. M. S., Marshall, R. D. & Neuberger, A. (1974) *Proc. R. Soc. London Ser. E* **186**, 297–316
- Ersson, B., Aspberg, K. & Porath, J. (1973) *Biochim. Biophys. Acta* **310**, 446–452
- Falasca, A., Franceschi, C., Rossi, C. A. & Stirpe, F. (1979) *Biochim. Biophys. Acta* **577**, 71–81
- Falasca, A., Franceschi, C., Rossi, C. A. & Stirpe, F. (1980) *Biochim. Biophys. Acta* **632**, 95–105
- Franceschi, C., Licastro, F., Chiricolo, M., Bonetti, F., Zannotti, M., Fabris, N., Mocchegiani, E., Fantini, M. P., Paolucci, P. & Masi, M. (1981) *J. Immunol.* **126**, 2161–2164
- Gietl, C., Kauss, H. & Ziegler, H. (1979) *Planta* **144**, 367–371
- Jaffé, W. G. & Seidl, D. (1969) *Experientia* **25**, 891–892
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **263**, 1372–1379
- McPherson, A. & Hoover, S. (1979) *Biochem. Biophys. Res. Commun.* **89**, 713–720
- Olsnes, S. & Pihl, A. (1982) in *Molecular Action of Toxins and Viruses* (Cohen, P. & Van Heyningen, S., eds.), pp. 51–105, Elsevier Biomedical Press, Amsterdam
- Pere, M., Pere, D. & Rouge, P. (1981) *Planta Med.* **41**, 344–350
- Premaratna, F. A., Shadaksharaswamy, M. & Nanjappa, S. (1981) *Indian J. Biochem. Biophys.* **18**, 32–35
- Richet, C. (1909) *Ann. Inst. Pasteur, Paris* **23**, 745–800
- Sabnis, D. D. & Hart, J. W. (1978) *Planta* **142**, 97–101
- Stirpe, F., Olsnes, S. & Pihl, A. (1980) *J. Biol. Chem.* **255**, 6947–6953
- Stirpe, F., Williams, D. G., Onyon, L. J., Legg, R. F. & Stevens, W. A. (1981) *Biochem. J.* **195**, 399–405
- Warburg, O. & Christian, W. (1941) *Biochem. Z.* **310**, 384–421
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412