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Ribosome-inactivating proteins from the seeds of *Saponaria officinalis* L. (soapwort), of *Agrostemma githago* L. (corn cockle) and of *Asparagus officinalis* L. (asparagus), and from the latex of *Hura crepitans* L. (sandbox tree)

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1. Ribosome-inactivating proteins, similar to those already known [Barbieri & Stirpe (1982) *Cancer Surveys* **1**, 489–520] were purified from the seeds of *Saponaria officinalis* (two proteins), of *Agrostemma githago* (three proteins), and of *Asparagus officinalis* (three proteins), and from the latex of *Hura crepitans* (one protein). The yield ranged from 8 to 400 mg/100 g of starting material. 2. All proteins have an M_r of approx. 30000 and an alkaline isoelectric point. Their sugar content varies from 0 (proteins from *S. officinalis*) to 40% (protein from *H. crepitans*). 3. The ribosome-inactivating proteins inhibit protein synthesis by rabbit reticulocyte lysate, the ID_{50} (concentration giving 50% inhibition) ranging from 1 ng/ml (a protein from *S. officinalis*) to 18 ng/ml (a protein from *A. githago*). Those which were tested (the proteins from *S. officinalis* and from *A. githago*) also inhibit polymerization of phenylalanine by isolated ribosomes, acting in an apparently catalytic manner. The protein from *H. crepitans* inhibited protein synthesis by HeLa cells, with an ID_{50} of 4 μg/ml, whereas the proteins from *S. officinalis* and from *A.* githago had an ID_{50} of more than 50–100 μg/ml. 4. The ribosome-inactivating proteins from *S. officinalis* and from *A. githago* reduced the number of local lesions by tobacco-mosaic virus in the leaves of *Nicotiana glutinosa*.

Extracts from various plant materials (mostly seeds) inhibit protein synthesis in a cell-free system (a rabbit reticulocyte lysate) (Gasperi-Campani *et al.*, 1977, 1980). The inhibitory activity of some extracts was very high, and from some of them the active principles were purified (Barbieri *et al.*, 1980, 1982; Stirpe *et al.*, 1980, 1981), identified as single-chain proteins similar to the 'pokeweed anti-viral protein' (Obrig *et al.*, 1973) and provisionally named 'ribosome-inactivating proteins' (RIPs) type 1 (review by Barbieri & Stirpe, 1982) [as opposed to the two-chain RIPs type 2, which include ricin and similar toxins (review by Olsnes & Pihl, 1982)]. The RIPs type 1 damage eukaryotic ribosomes in a similar manner to the A-chains of the toxins, reduce the number of local lesions induced by tobacco-mosaic virus (Stevens *et al.*, 1981; Stirpe *et al.*, 1981), and inhibit multiplication of animal viruses (Tomlinson *et al.*, 1974; Ussery *et al.*, 1977; Aron & Irvin, 1980; Barbieri *et al.*, 1982; Foà-Tomasi *et al.*, 1982).

We describe now the purification and partial characterization of some proteins from the seed extracts of two members of the Caryophyllaceae, *Saponaria officinalis* L. (soapwort) and *Agrostemma githago* L. (corn cockle) and a member of the Liliaceae, *Asparagus officinalis* L., and from the latex of a member of the Euphorbiaceae, *Hura crepitans* L. (sandbox tree). These proteins inhibit protein synthesis and have antiviral activity similar to that of the RIPs already described.

Abbreviations used: RIP(s), ribosome-inactivating protein(s); SDS, sodium dodecyl sulphate.

Experimental

Materials

Seeds were obtained as follows: *Saponaria officinalis*, from the Botanical Garden of the

University of Bologna and from the Botanical Officinal Garden of Casola Valsenio (Ravenna), Italy; *Agrostemma githago*, from the University of London Botanical Supply Unit, Egham, Surrey, U.K.; *Asparagus officinalis*, from a dealer in Bologna. The latex of *Hura crepitans* was obtained in part from a plant grown in the Botanical Garden of the University of Bologna, and was immediately diluted with buffered saline (0.14 M-NaCl in 5 mM-sodium phosphate buffer, pH 7.2) and frozen. Another sample was kindly provided by Dr. L. E. Newton, Kumasi, Ghana, and had been diluted with 0.14 M-NaCl and kept in a refrigerator for 5 days before freezing.

Chemicals for chromatography and polyacrylamide-gel electrophoresis were obtained from the same sources as in a previous paper (Barbieri *et al.*, 1982). Protein markers for M_r determinations were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden (markers for gel filtration), or from Boehringer-Mannheim G.m.b.H., Mannheim, Germany (markers for polyacrylamide-gel electrophoresis). Trypsin (bovine, type XI), chymotrypsin (bovine, type II) and proteinase (subtilisin) were from Sigma Chemical Co., St. Louis, MO, U.S.A.

The preparation of a lysate of rabbit reticulocytes and of ribosomes, and the sources of chemicals for protein synthesis, were as described by Stirpe *et al.* (1981).

Tobacco-mosaic virus was purified by the method of Gooding & Herbert (1967). The final A_{266}/A_{280} ratio was 1.05 (uncorrected for light-scattering). Samples were diluted in distilled water to give countable numbers of local lesions.

Preparation of extracts and chromatography

Seeds were extracted by grinding in 0.14 M-NaCl in 5 mM-sodium phosphate buffer, pH 7.2 (8 ml per g). After overnight stirring at 4°C, extracts were strained through cheese-cloth and were centrifuged at 28000 *g* for 30 min. The supernatant was separated from the sediment and from floating fat, and is referred to as 'crude extract'.

The latex of *H. crepitans*, diluted with 9 vol. of, and dialysed against, the buffered saline described above for at least 24 h, was centrifuged as for the seed extracts. The supernatant was chromatographed through a column of acid-treated Sepharose 6B to remove a lectin present in the latex (Barbieri *et al.*, 1983). The unretained effluent was treated as for the seed extracts as described below.

Crude extracts were dialysed against 5 mM-sodium phosphate buffer, pH 6.5, centrifuged at 28000 *g* for 30 min and applied to a CM-cellulose column (CM 52; Whatman, Maidstone, Kent, U.K.), which, after washing, was eluted with a 0–0.3 M-NaCl gradient in the same buffer. Chromatography of the *A. officinalis* seed extract was performed at pH 6.0.

Protein synthesis

Protein synthesis by a lysate of rabbit reticulocyte or by HeLa cells was measured from the incorporation of L-[^{14}C]leucine as described by Barbieri *et al.* (1982). Poly(U)-directed polymerization of L-[^{14}C]phenylalanine by isolated rabbit reticulocyte ribosomes was assayed as described by Montanaro *et al.* (1978). Details are given in the legends to the appropriate Figures and Tables. The ID_{50} (concn. giving 50% inhibition) was calculated by linear-regression analysis. The radioactivity was measured as described by Gasperi-Campani *et al.* (1980).

Chemical determinations

M_r values were determined by the polyacrylamide-gel-electrophoresis method of Laemmli (1970), with the following markers: bovine serum albumin (M_r 68000), abrin A (29500) and B (35000) chains, modeccin A (28000) and B (35000) chains, and soya-bean trypsin inhibitor (21500). M_r values were also estimated by gel filtration through a column (100 cm × 2 cm) of Bio-Gel P-100, equilibrated with 0.3 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2, eluted at the rate of 14 ml/h, at room temperature (24°C). The column was calibrated with bovine serum albumin (68000), ovalbumin (43000), chymotrypsinogen (25000) and ribonuclease (13700).

Isoelectric points and the amino acid, amino sugar, and neutral sugar composition of the proteins were determined as described by Falasca *et al.* (1982).

Proteinase activity, with Azocoll (Calbiochem-Behring, San Diego, CA, U.S.A.) as a substrate, and ribonuclease activity, with yeast RNA (Type XI, Sigma) as a substrate, were determined as described by Nelson *et al.* (1981) and by Razzel (1963) respectively.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard, or spectrophotometrically (Kalb & Bernlohr, 1977).

Toxicity experiments

The toxicity of the purified proteins was evaluated in Swiss male mice weighing 35–40 g, fed *ad libitum*. The proteins, dissolved in 0.9% NaCl, were injected intraperitoneally at six different doses, ranging from 10 μg to 1 mg per 100 g of body wt. LD_{50} was evaluated from the deaths occurring within 6 days, by the method of Spearman-Kärber as described by Finney (1964).

Anti-viral activity

The effect of ribosome-inactivating proteins on the infectivity of tobacco-mosaic virus was determined by the number of lesions produced by the virus in

Nicotiana glutinosa as described previously (Stirpe *et al.*, 1981).

Results

Purification

The crude extracts, prepared as described above, were subjected to a single-step chromatography on CM-cellulose. Several peaks absorbing at 280nm were obtained from each extract and were numbered in the order of elution (Fig. 1). The effect of the material from each peak on protein synthesis by a lysate of rabbit reticulocytes was tested (Table 1). Most of the inhibitory activity was recovered in peaks 6, 8 and 9 of *S. officinalis*, in peaks 2, 5 and 6 of *A. githago*, and in peaks 2, 3 and 5 of *A. officinalis* seed extracts, and in peak 5 of *H. crepitans* latex. In all cases but peak 8 of *S. officinalis* (not studied any further) the inhibitory activity was destroyed by heating for 20min in a boiling-water bath. Active fractions were pooled, dialysed extensively against distilled water, and freeze-dried. Yields are given in Table 1. It should be noted that the total activity of *S. officinalis* extract increased after dialysis at pH6.5, as has been observed with other RIPs (Barbieri *et al.*, 1980, 1982; Stirpe *et al.*, 1981; Kishida *et al.*, 1983).

Physico-chemical characteristics

The material from the peaks showing high inhibitory activity towards protein synthesis gave a single protein band on SDS/polyacrylamide-gel electrophoresis (Fig. 2).

M_r values, determined from the mobility on SDS/polyacrylamide gels and in some cases by gel filtration, are reported in Table 2, and all are near 30000.

On isoelectric focusing the components from the peaks examined all had a pI in the alkaline region (Table 2).

All proteins but those from *S. officinalis* are glycoproteins, and their sugar contents and compositions are reported in Table 3.

The amino acid composition of the proteins obtained from *S. officinalis* and from *H. crepitans* is shown in Table 4. These proteins all contain a relatively high amount of lysine, and those from *S. officinalis* have a very low content of cystine and a higher content of phenylalanine, as compared with the protein from *H. crepitans* and with other RIPs

Table 1. *Purification of ribosome-inactivating proteins*

The purification procedure is described in the text. Peak numbers refer to Fig. 1. Reaction mixtures contained, in a final volume of 125μl: 10mM-Tris/HCl buffer, pH7.4, 100mM-ammonium acetate, 2mM-magnesium acetate, 1mM-ATP, 0.2mM-GTP, 15mM-phosphocreatine, 6μg of creatine kinase, 0.05mM-amino acids (minus leucine). 0.19μCi of L-[^{14}C]leucine and 50μl of a rabbit reticulocyte lysate. Incubation was at 27°C for 5min, and the radioactivity incorporated into protein was measured in 25μl samples as described by Gasperi-Campani *et al.* (1980). A unit of activity is defined as the amount giving 50% inhibition of protein synthesis in the reticulocyte-lysate system.

Preparation	Total protein (mg)	Specific activity (units/mg of protein)	10^{-3} × Total activity (units)	Yield (%)	
Saponaria officinalis seeds (25g)					
Crude extract	1417	113636	161079	100	
Dialysed pH6.5	1054	444444	468666	290	
Peak 4	6.9	591715	4083	2.5	
Peak 5	15.7	806452	12661	7.9	
Peak 6	103.5	884955	91593	57	84
Peak 7	2.7	245700	663	0.4	
Peak 8	9.6	164474	1579	1.0	
Peak 9	28.8	869565	25043	15.5	
Agrostemma githago seeds (50g)					
Crude extract	3882	1590	6182	100	
Dialysed pH6.5	1682	2220	3727	60	
Peak 2	4.2	55000	233	3.8	
Peak 5	17.1	71400	1214	19.6	32
Peak 6	9.2	58500	540	8.7	
Hura crepitans latex (8ml)					
Dialysed pH7.2	810	8600	6966	100	
Dialysed pH6.5	750	6700	5025	72	
Peak 5	11.7	293000	3788	54	
Asparagus officinalis seeds (45g)					
Crude extract	765	3448	2638	100	
Peak 2	10.7	76923	823	31	
Peak 3	3.5	90909	318	12	104
Peak 5	8.1	200000	1610	61	

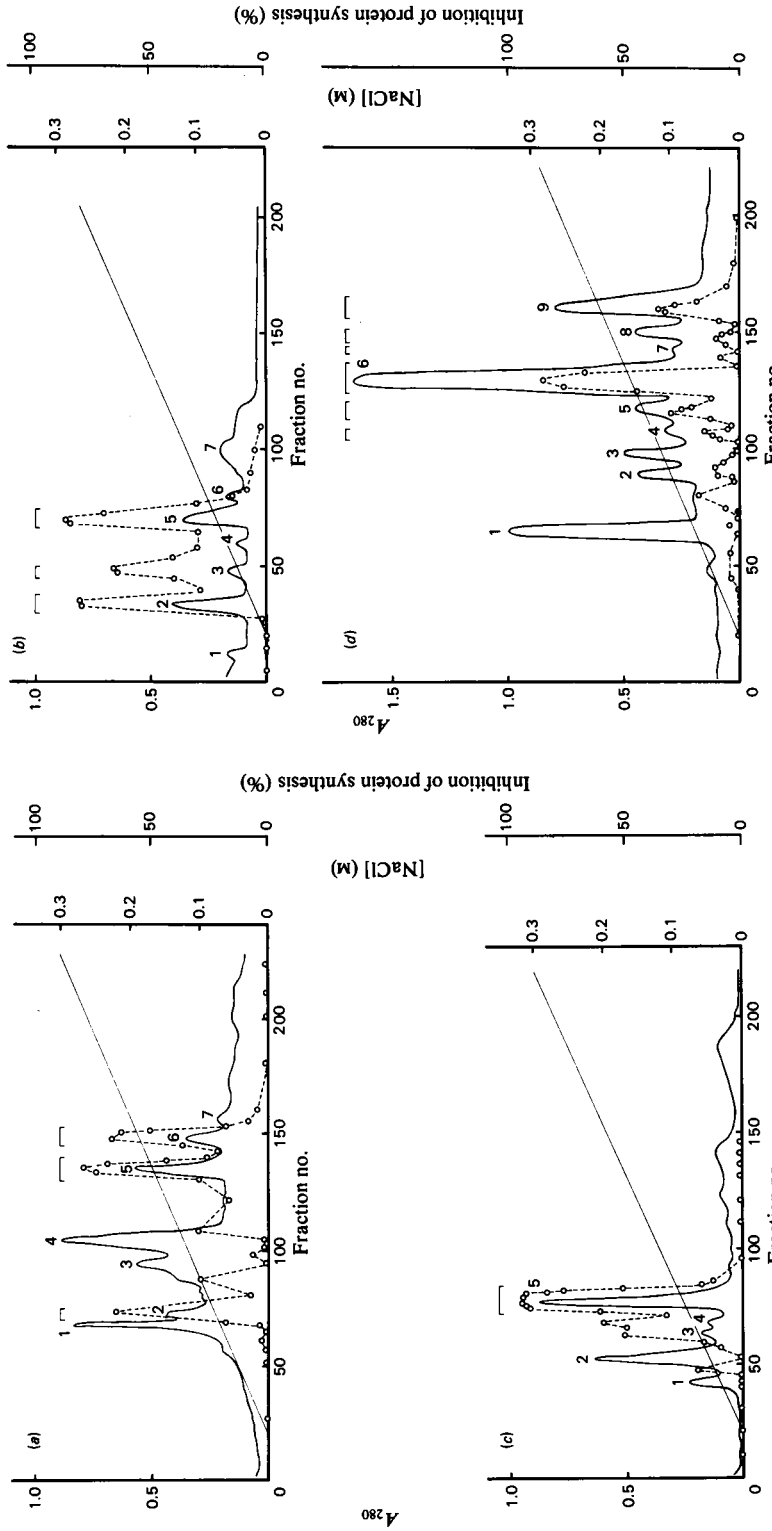

Fig. 1. *Purification of ribosome-inactivating proteins*

The dialysed extracts (details in Table 1) were chromatographed on a column of CM-cellulose (CM 52) as described in the text. In the case of *S. officinalis* the column was 20 cm × 2 cm, was eluted with a 1-litre gradient (0–0.3 M-NaCl), and 4 ml fractions were collected; in all other cases the column was 20 cm × 1.6 cm, the gradient was 500 ml, and fractions were 2 ml. Fractions were eluted with a 0–0.3 M-NaCl gradient (thin line), their A_{280} was recorded (thick line), and the inhibitory activity on protein synthesis (O) was assayed in the lysate system as described in the text, with 10 μl of a suitable dilution. (*a*) *Agrostemma githago* seed extract; (*b*) *Asparagus officinalis* seed extract; (*c*) *Hura crepitans* dialysed latex; (*d*) *Saponaria officinalis* seed extract.

Table 2. *M_r values and isoelectric points of ribosome-inactivating proteins*
Experimental conditions are described in the text. Peak numbers refer to Fig. 1.

Ribosome-inactivating protein	M_r value determined by: Polyacrylamide-gel electrophoresis	Gel filtration	pI
Saponaria officinalis			
Peak 6	29500	29000	≥9.5
Peak 9	29500	27000	≥9.5
Agrostemma githago			
Peak 2	30600	29200	7.7
Peak 5	29500	25500	8.7
Peak 6	29600	27000	8.75
Asparagus officinalis			
Peak 2	32500	Not determined	≥9.5
Peak 3	32500	Not determined	≥9.5
Peak 5	32500	Not determined	≥9.5
Hura crepitans			
Peak 5	28000	Not determined	≥9.5

(Irvin *et al.*, 1980; Barbieri *et al.*, 1982; Falasca *et al.*, 1982).

Biological properties

Inhibition of protein synthesis. Cell-free systems. The purified proteins inhibited protein synthesis in a rabbit reticulocyte lysate with specific activities (Table 1) up to 45-fold higher than those of the respective crude extracts.

This inhibitory activity is heat-labile (see above). The protein from peak 6 of *S. officinalis* was subjected to a variety of treatments (Table 5): the activity was not affected by treatment with proteolytic enzymes, urea or *N*-ethylmaleimide, and was destroyed by overnight incubation at 37°C with 1% SDS, or 0.1M-NaOH, or 0.1M-HCl; the latter treatment had no effect at room temperature.

The proteins of *S. officinalis* and of *A. githago* (the only ones tested) also inhibited poly(U)-directed polyphenylalanine synthesis by purified reticulocyte ribosomes, either when the proteins were present in the reaction mixture, or when ribosomes were preincubated with the proteins and washed before the assay (Table 6). In the latter experiment, the protein from peak 6 of *S. officinalis* was also effective at a concentration less-than-equimolar with ribosomes.

The purified proteins showed no unspecific proteinase or RNAase activity, when assayed at concentrations ten times higher than those inhibiting protein synthesis (results not shown).

Cells. The proteins from *S. officinalis*, from *A. officinalis* and from *A. githago* had a moderate inhibitory activity towards protein synthesis by intact HeLa cells, with ID_{50} values of the same order of magnitude as those observed with other RIPs (Barbieri & Stirpe, 1982), whereas the protein from *H. crepitans* was somewhat more inhibitory (Table 7).

Toxicity

The RIPs of peak 6 and 9 of *S. officinalis* killed mice, with an LD_{50} at 6 days of 4mg/kg body wt. (95% confidence limits 3.3–4.8) and 1.7mg/kg body wt. (1.4–2.0) respectively. The proteins from *A. githago* did not cause any apparent harm when injected into mice at doses up to 1mg/kg body wt.

Anti-viral activity

The RIPs purified from *S. officinalis* and *A. githago* seeds, mixed with tobacco-mosaic virus before infection, greatly reduced the number of local lesions in the leaves of *Nicotiana glutinosa* (Table 8). A dose–response curve constructed with the RIPs from *S. officinalis* gave 50% inhibition of local lesions at a concentration of 50ng/ml or lower.

Table 3. *Sugar content of ribosome-inactivating proteins*
Experimental conditions are described in the text. Peak numbers refer to Fig. 1.

	Content (residues/molecule)						
	Agrostemma githago			*Asparagus officinalis*			*Hura crepitans*
Peak no. ...	2	5	6	2	3	5	5
Total neutral sugar (%) ...	6.68	6.87	7.17	1.42	1.20	1.32	39.9
Fucose	8.6	8.8	8.6	Traces	0	0	6.1
Galactose	0	0	0	0.3	Traces	0.3	6.4
Glucose	1.0	1.0	0.9	2.1	2.1	2.1	4.7
Mannose	1.9	2.0	2.0	0.4	0.3	0.3	48.4
Xylose	0.7	0.8	0.7	0	0	0	3.2
N-Acetylglucosamine							Traces

Table 4. *Amino acid composition of ribosome-inactivating proteins from Saponaria officinalis and from Hura crepitans*

Experimental conditions are described in the text. Peak numbers refer to Fig. 1.

	Composition (mol/mol of protein)		
	Saponaria officinalis		*Hura crepitans*
Amino acid	Peak 6	Peak 9	Peak 5
Lys	22.6	26.8	18.0
His	2.4	5.3	4.3
Arg	12.7	10.8	7.8
Asx	34.5	33.1	21.4
Thr*	17.3	16.1	16.0
Ser*	13.6	13.5	16.2
Glx	26.0	23.3	18.3
Pro	7.3	7.9	8.7
Gly	12.2	11.4	12.2
Ala	19.6	18.9	15.0
½-Cys†	0.6	0.4	4.1
Val	19.3	19.1	20.6
Met†	9.3	6.3	5.6
Ile	14.8	13.8	10.6
Leu	23.7	24.9	20.5
Tyr*	8.8	8.6	8.6
Phe	14	17.3	9.2
Trp‡	1.0	1.0	Not determined

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

† Determined as cysteic acid and methionine sulphone.

‡ Hydrolysis with *p*-toluenesulphonic acid.

Table 5. *Effect of various treatments on the protein-synthetic-inhibitory activity of a ribosome-inactivating protein from Saponaria officinalis*

Treatments of *S. officinalis* peak 6 protein were as follows. Expt. 1: the protein (200μg/ml) was incubated in 50mM-sodium phosphate buffer, pH7.4, with or without trypsin or chymotrypsin (2μg/ml). Expt. 2: the protein (480μg/ml) was incubated in 20mM-sodium phosphate buffer, pH7.4, with or without the indicated additions. Expt. 3: the protein (100μg/ml) was incubated in water (no additions) or in the solutions indicated. The inhibitory effect on protein synthesis was assayed with the lysate system described in the legend to Table 1, with the *S. officinalis* protein at the concentration of 10ng/ml.

		Protein synthesis	
Expt. no.	Additions and treatments	(d.p.m. incorporated)	(% of control)
1	Controls	2740	100
	S. officinalis peak 6, kept at 37°C overnight with:		
	No additions	868	32
	Trypsin	799	29
	Chymotrypsin	956	35
	Proteinase (subtilisin)	981	36
2	Controls	2830	100
	S. officinalis peak 6, kept at 37°C for 30min:		
	With no additions	66	2
	In 8M-urea	208	7
	In 1.7mM-*N*-ethylmaleimide	201	7
	In 8M-urea and 1.7mM-*N*-ethylmaleimide	232	8.2
3	Controls	2755	100
	S. officinalis peak 6, kept at 37°C overnight:		
	With no additions	50	2
	In 1% SDS	2745	100
	In 0.1M-NaOH	2805	102
	In 0.1M-HCl	2517	91
	S. officinalis peak 6, kept at 21°C overnight:		
	With no additions	95	3
	In 1% SDS	2486	90
	In 0.1M-NaOH	2615	95
	In 0.1M-HCl	135	5

Discussion

The proteins purified from the seeds of *S. officinalis*, of *A. githago* and of *A. officinalis*, and from the latex of *H. crepitans* have all the characteristics of previously described RIPs type 1 (Barbieri & Stirpe, 1982). Indeed, they are single-chain proteins of approx. M_r 30000, all are basic proteins, and inhibit protein synthesis in cell-free systems, with less effect on intact cells. As far as studied, they act by inactivating ribosomes in an apparently catalytic manner, in the absence of any cofactor, and make them unable to perform one (or more) step(s) of protein synthesis, subsequent to the formation of aminoacyl-tRNA.

The extracts examined contained more than one active protein, as observed with pokeweed (*Phytolacca americana* L.) (Irvin *et al.*, 1980; Barbieri *et al.*, 1982) and carnation (*Dianthus caryophyllus* L.) (Stirpe *et al.*, 1981). The proteins from the same extract had sometimes a markedly different specific activity. On the other hand, those examined in this respect showed immunological cross-reactivity (dianthins; Falasca *et al.*, 1982) and similar amino acid composition (*S. officinalis* proteins). It remains to be ascertained whether the active proteins from the same source have the same mechanism of action, thus being like isoenzymes or deriving from each other, or are different proteins.

The RIPs described above differ greatly in their sugar contents. The proteins from *S. officinalis* contain no sugar, and in this respect are similar to the pokeweed anti-viral protein (Barbieri *et al.*, 1982). At the other extreme, the RIP from *H.*

Table 6. *Effect of ribosome-inactivating proteins on poly(U)-directed phenylalanine polymerization*

Rabbit reticulocyte ribosomes (200 pmol in Expt. 1, 315 pmol in Expt. 2) in 1 ml of 80 mM-Tris/HCl buffer, pH 7.4, containing 120 mM-KCl, 7 mM-magnesium acetate and 2 mM-dithiothreitol, were preincubated at 24°C for 30 min, in the presence or in the absence of the ribosome-inactivating proteins. Ribosomes were washed by centrifugation at 105000 *g* for 3 h through 1.5 ml of 5% sucrose in the same medium. Assays were done with duplicate samples containing, in a final volume of 0.25 ml: 80 mM-Tris/HCl buffer, pH 7.4, 120 mM-KCl, 7 mM-magnesium acetate, 2 mM-GTP, 200 μg of poly(U), 250 μg (as protein) of 'pH 5 supernatant', 25 pmol of [^{14}C]phenylalanyl-tRNA and 20 pmol [calculated as described by Montanaro *et al.* (1978)] of ribosomes. After incubation at 24°C for 30 min, 0.25 ml of 10% (w/v) trichloroacetic acid was added, and the acid insoluble radioactivity was determined as described by Montanaro *et al.* (1978).

Expt. 1 *Saponaria officinalis* proteins		Polyphenylalanine polymerized (d.p.m. incorporated)	Percentage of control value
During preincubation	During assay		
None	None	6724	—
Peak 6 (500 pmol)	None	2881	43
Peak 6 (50 pmol)	None	2572	38
Peak 9	None	2273	34
None	Peak 6 (100 pmol)	1925	29
None	Peak 9 (100 pmol)	1897	28
Expt. 2 *Agrostemma githago* proteins			
During preincubation	During assay		
None	None	1706	—
Peak 2 (500 pmol)	None	332	19
Peak 5 (500 pmol)	None	484	28
Peak 6 (500 pmol)	None	431	25
None	Peak 2 (100 pmol)	383	22
None	Peak 5 (100 pmol)	490	29
None	Peak 6 (100 pmol)	407	24

Table 7. *Effect of ribosome-inactivating proteins on protein synthesis by HeLa cells*

Tests were performed in triplicate, with 10^5 HeLa cells grown in 1 ml of RPMI medium containing 10% human blood-group-AB serum in each well of 16 mm multiwell trays. Cells were incubated for 18 h at 37°C in a humidified atmosphere of air/CO_2 (19:1) in the RPMI medium without serum and containing the appropriate amount of RIPs. The medium was then removed and replaced with 1 ml of RPMI medium minus leucine but containing L-[^{14}C]leucine (0.25 μCi/well). After incubation for 2 h the medium was removed and the acid-insoluble radioactivity was measured as described by Barbieri *et al.* (1982) or as described by Sandvig & Olsnes (1982).

Ribosome-inactivating protein	ID_{50} (μg/ml)
Saponaria officinalis	
Peak 6	69
Peak 9	158
Agrostemma githago	
Peak 2	>100
Peak 5	>100
Peak 6	>100
Hura crepitans	
Peak 5	4
Asparagus officinalis	
Peak 2	>100
Peak 3	>100
Peak 5	>100

crepitans has a high content (40%) of sugars, among which mannose is predominant. These results confirm that sugars are not essential for the inhibitory activity of RIPs (Falasca *et al.*, 1982). On the other hand, it is known that sugars are important for the recognition and uptake of glycoproteins by cells (Ashwell & Morell, 1974; Stahl & Schlesinger, 1980), and therefore the high sugar content may account for the higher cytotoxicity of *H. crepitans* RIP.

The seeds of *S. officinalis* contain a surprisingly high amount of RIPs, one alone (peak 6) accounting for approx. 0.4% of the whole seed weight, or 7% of the total seed protein. Thus these seeds appear the richest source, and hence one of the most convenient, for the easy preparation of large quantities of a RIP.

The RIPs from *S. officinalis* and from *A. githago*, the only ones tested, inhibit local lesions by tobacco-mosaic virus, consistent with the anti-viral activity of seed extracts from several members of the Caryophyllaceae (Barakat & Stevens, 1980) and of other RIPs (see the introduction). Thus the anti-viral activity may be a common property of all RIPs, which presumably act through the same mechanism, i.e. by entering into virus-infected cells and damaging their ribosomes (Owens *et al.*, 1973). RIPs inhibit also the multiplication of animal viruses (see the introduction). Since these proteins resist proteolytic digestion (Stirpe *et al.*, 1980; the present work), the possibility should be considered that RIPs

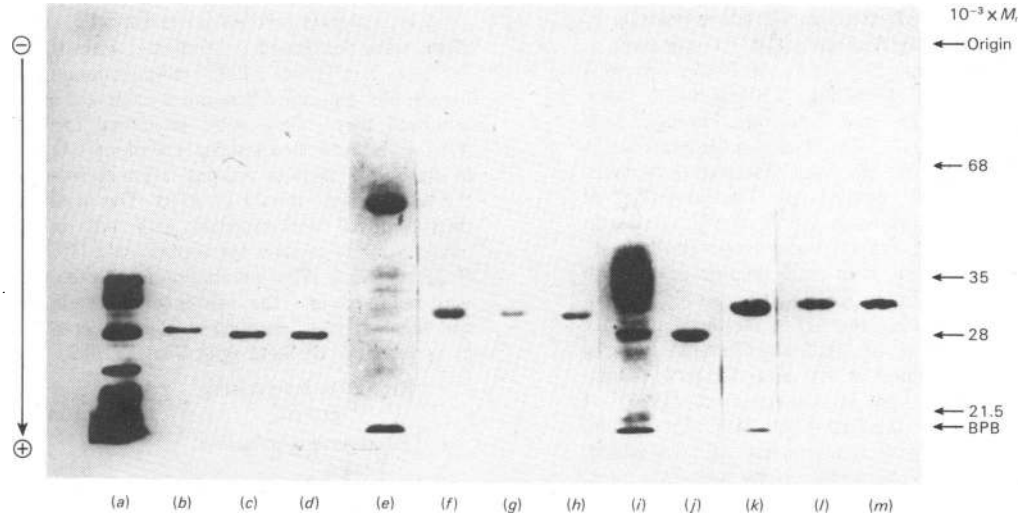

Fig. 2. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of ribosome-inactivating proteins*
Electrophoresis was performed as described in the text. Migration was from top to bottom, and the Bromophenol Blue front is indicated by 'BPB'. *Agrostemma githago*: (*a*) crude seed extract; (*b*) peak 2; (*c*) peak 5; (*d*) peak 6. *Asparagus officinalis*: (*e*) crude seed extract; (*f*) peak 2; (*g*) peak 3; (*h*) peak 5. *Hura crepitans*: (*i*) dialysed latex; (*j*) peak 5. *Saponaria officinalis*: (*k*) crude seed extract; (*l*) peak 6; (*m*) peak 9.

Table 8. *Effect of ribosome-inactivating proteins from Saponaria officinalis and from Agrostemma githago seeds on the infectivity of tobacco-mosaic virus*
Experimental conditions are described in the text.

Protein added (μg/ml)	Lesions (mean no.)	Inhibition (%)	Lesions (mean no.)	Inhibition (%)	Lesions (mean no.)	Inhibition (%)
Saponaria officinalis						
	Peak 6		Peak 9			
None	151	–	151	–		
0.5	62	59	75	50		
1.0	9	94	21	86		
5.0	3	98	10	93		
10.0	3	98	12	92		
50.0	0	100	0	100		
Agrostemma githago						
	Peak 2		Peak 5		Peak 6	
None	17	–	17	–	17	–
50.0	7.2	58	0.1	99	0.5	97

contained in vegetables eaten raw may contribute to control viral infections in the intestine of animals and man.

The pokeweed anti-viral protein is toxic to tobacco cells in culture (Grasso *et al.*, 1980), and it is possible that other RIPs are toxic to plant cells. This, and the fact that RIPs may be present at high concentrations in saps (e.g. *H. crepitans*), suggests that they may be one of the factors preventing the taking of heterografts amongst plants. Indeed, if a graft was attempted on a stock, the sap of which contained a RIP toxic to the cells of the scion, these would be killed.

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