

## Seed Extracts Inhibiting Protein Synthesis *in vitro*

Anna GASPERI-CAMPANI, Luigi BARBIERI, Patrizia MORELLI and Fiorenzo STIRPE  
*Istituto di Patologia generale dell'Università di Bologna, I-40126 Bologna, Italy*

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Of 33 seed extracts examined, 12 inhibited protein synthesis in a rabbit reticulocyte lysate. This activity seems to be due to a protein, since (i) it was recovered with the  $(\text{NH}_4)_2\text{SO}_4$  precipitate, (ii) it was retained by dialysis membranes, and (iii) in all cases but one was destroyed by boiling. Only the extracts from the seeds of *Adenia digitata* and, to a lower extent, of *Euonymus europaeus* inhibited protein synthesis in intact cells.

Proteins which inhibit protein synthesis in eukaryotic systems have been found in seeds or other parts of several plants. Among these proteins, ricin and abrin (Olsnes & Pihl, 1977) and modeccin (Refsnes *et al.*, 1977; Stirpe *et al.*, 1978; Olsnes *et al.*, 1978; Gasperi-Campani *et al.*, 1978) inhibit protein synthesis in cells as well as in cell-free systems, and are highly toxic to animals, whereas others are effective on cell-free systems only, and are scarcely or non-toxic to animals, presumably because they do not penetrate into cells (Obrig *et al.*, 1973; Stirpe *et al.*, 1976; Gasperi-Campani *et al.*, 1977).

The present experiments were performed to investigate further the distribution of these inhibitors among plants, to search for more powerful substances, and to ascertain whether some of them could be found in seeds easily available in quantities that could be used for further purification.

Of over 30 seed extracts examined, approximately one-third inhibited protein synthesis in a lysate of rabbit reticulocytes, in some cases at very low concentrations. Only the extracts from the seeds of *Adenia digitata* and from the arils of *Euonymus europaeus* inhibited protein synthesis also in cells, and the former was also toxic to rats.

### Experimental

#### Materials

The sources of seeds are indicated in the legend to Table 1. All chemicals were from the same sources as in previous work (Gasperi-Campani *et al.*, 1977).

#### Preparation of seed extracts

Extracts were prepared as described previously (Gasperi-Campani *et al.*, 1977). Briefly, seeds were shelled, when feasible, and were ground several times

with diethyl ether in a blender or with an Ultra-Turax apparatus. The resulting powder was extracted overnight with 10 vol. of cold 0.2 M-NaCl containing 0.005 M-sodium phosphate buffer, pH 7.2. After centrifugation, the supernatant was fractionated with  $(\text{NH}_4)_2\text{SO}_4$  at 0–40%, 40–60% and 60–100% saturation, and the precipitates obtained were redissolved in phosphate-buffered saline and dialysed against the same solution.

#### Biochemical determinations

Protein synthesis was determined as described previously (Gasperi-Campani *et al.*, 1977) with a lysate of rabbit reticulocytes prepared as described by Allen & Schweet (1962) and with Ehrlich or Yoshida AH-130 ascites cells.

Ribonuclease activity was determined as described by Razzel (1963) with yeast RNA (type XI; Sigma Chemical Co., St Louis, MO, U.S.A.) as substrate; proteinase activity was determined by a phenol colour method (Greenberg, 1955) with bovine serum albumin (Sigma) as substrate.

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

### Results

The activity of the extracts inhibiting protein synthesis and a list of the inactive extracts are given in Table 1. In all cases inhibition occurred immediately after addition of the extracts and was the same whether they were added before or after the reaction was started (results not shown; cf. Fig. 1 of Gasperi-Campani *et al.*, 1977). None of the extracts had proteinase activity, and only the extract from *Datura stramonium* seeds had ribonuclease activity. The inhibitory activity of all extracts was destroyed

Table 1. *Effect of seed extracts on protein synthesis by a reticulocyte lysate*

The reaction mixture contained, in a final volume of 0.25 ml: 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, 12  $\mu$ g of creatine kinase, 0.05 mM amino acids (minus leucine), 0.75  $\mu$ Ci of L-[ $^{14}$ C]leucine, the appropriate amount of extract and 0.1 ml of lysate. Incubation was at 27°C for 5 min. Radioactivity incorporated into protein was determined on 25  $\mu$ l samples. The most active preparation is identified by the saturation of  $(\text{NH}_4)_2\text{SO}_4$  giving the most active precipitate. The  $\text{ID}_{50}$  (concentration giving 50% inhibition) was calculated by the linear regression method. Extracts inactive or with an  $\text{ID}_{50} < 100 \mu\text{g/ml}$  were as follows. Araceae: *Arum italicum* (1); Asclepiadaceae: *Arauja sericifera* (1); Bixaceae: *Bixa orellana* (3); Euphorbiaceae: *Euphorbia cyparissas* (1); *Euphorbia dracunculoides* (4), *Euphorbia helioscopia* (2), *Mallotus japonicus* (2), *Sapium sebiferum* (4), *Sarcococca saligna* (4), *Tragia involucrata* (4); Leguminosae: *Caesalpinia gillesii* (3), *Cassia occidentalis* (4), *Cercis siliquastrum* (1), *Crotalaria juncea* (6), *Gleditsia triachanthos* (1), *Gymnocladus canadensis* (1), *Ulex europaeus* (7); Papaveraceae: *Argemone mexicana* (3); Passifloraceae: *Passiflora caerulea* (1), *Passiflora foetida* (4); Rosaceae: *Cotoneaster* sp. (1). Sources of seeds: (1) fields or private gardens; (2) Botanical Garden of the University of Bologna; (3) Simes S.p.A., Milan, Italy; (4) Mr. F. G. Celo, Zweibrücken, West Germany; (5) Department of Botany, University of Pretoria, Pretoria, South Africa; (6) Dr. B. Ersson, Uppsala, Sweden; (7) Florsilva, Bologna, Italy.

Extract	Most active preparation	$\text{ID}_{50}$ ( $\mu\text{g/ml}$ )	Remarks
Celastraceae			
<i>Euonymus europaeus</i> (1)			
Arils	40–60%	7.1	Partially heat-resistant
Seeds	40–60%	100	
Cucurbitaceae			
<i>Bryonia alba</i> (2)			
<i>Momordica balsamina</i> (3)	60–100%	0.26	
<i>Momordica charantia</i> (4)	40–60%	0.09	
	30–60%	0.01	
Euphorbiaceae			
<i>Acalypha indica</i> (4)			
<i>Gelonium multiflorum</i> (4)	60–100%	43.5	
<i>Jatropha gossypifolia</i> (4)	60–100%	0.05	
<i>Jatropha podagrica</i> (2)	60–100%	25.5	
<i>Manihot palmata</i> (2)	0–100%	1.4	
	0–100%	0.06	
Passifloraceae			
<i>Adenia digitata</i> (5)			
	40–60%	9.6	
Sapindaceae			
<i>Koelerinteria paniculata</i> (2)			
	60–100%	14.7	
Solanaceae			
<i>Datura stramonium</i> (2)			
	60–100%	1.3	Contains ribonuclease

by boiling for 30 min, except for the extract from *Euonymus europaeus* arils, the activity of which was decreased by 50%.

The extract from *Adenia digitata* seeds and, to a lesser extent, that from *Euonymus europaeus* arils inhibited protein synthesis by Ehrlich or Yoshida ascites cells (Fig. 1). All other extracts were inactive on rats at a concentration of 100  $\mu\text{g}$  of protein/ml.

All animals injected with the *Adenia digitata* extract (1  $\mu\text{g}$  of extract protein/100 g body wt.) died within 24 h with lesions similar to those brought about by modeccin, the toxin of *Adenia digitata* (Sperti *et al.*, 1979). The extracts from *Momordica charantia* and from *Jatropha podagrica* were lethal to rats at a dose of 5 mg of protein/100 g body wt., given intraperitoneally, but not at a dose of 1 mg/100 g body wt. All other extracts did not cause any apparent harm to rats when injected intraperitoneally at a dose of 1 mg of protein/100 g body wt.

## Discussion

Approximately one-third of the seed extracts examined in the present experiments inhibit protein synthesis by a lysate of rabbit reticulocytes. In all cases the active principle(s) do not pass through dialysis membranes, are thermolabile (except for that from *Euonymus europaeus*), and are precipitated with  $(\text{NH}_4)_2\text{SO}_4$ , all this indicating that they may be proteins.

Only the extracts from *Adenia digitata* seeds and from *Euonymus europaeus* arils are active on protein synthesis in whole cells, and the former extract is also toxic to rats. Taking into account also the lesions observed in these animals, it seems very likely that the seeds of *Adenia digitata* contain modeccin, the toxin found in the roots of the same plant (Refsnes *et al.*, 1977; Stirpe *et al.*, 1978). All other extracts are very slightly toxic or non-toxic to

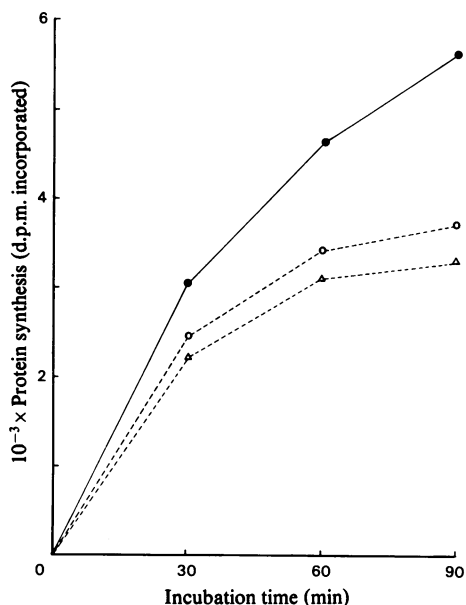


Fig. 1. Inhibition of protein synthesis in Yoshida cells by the extract from *Euonymus europaeus*

Yoshida AH-130 ascites cells ( $8 \times 10^6$ ) were incubated at  $37^\circ\text{C}$  in 1 ml of medium E 2a (Puck *et al.*, 1957), containing 5% calf serum and  $1 \mu\text{Ci}$  of L- $^{14}\text{C}$ leucine, in the absence (—) or in the presence (---) of the extract from *Euonymus europaeus* arils at the concentrations shown ( $\mu\text{g}$  of protein/ml). Radioactivity incorporated into protein was measured on  $25 \mu\text{l}$  samples.

rats, nor do they affect protein synthesis by cells, thus resembling other inhibitors of protein synthesis of plant origin (Obrig *et al.*, 1973; Stirpe *et al.*, 1976; Gasperi-Campani *et al.*, 1977).

Some of the extracts act at very low concentrations. Taking into consideration that these are crude preparations, the active principles can be considered among the most powerful inhibitors of protein synthesis hitherto described. This suggests that they may act catalytically, i.e. enzymically, as was demonstrated or postulated for other inhibitors of protein synthesis found in plant materials (Irvin, 1975; Sperti *et al.*, 1976; Gasperi-Campani *et al.*, 1977) and for the two active proteins purified from *Momordica charantia* seeds (Barbieri *et al.*, 1980). All these inhibitors but one (Stewart *et al.*, 1977) act by damaging ribosomes, and this suggests that many of them may have a similar mechanism of action. Thus the different activity of the various extracts could be due to different concentrations of their active principles.

The distribution of the inhibitors is quite irregular, although they seem to be particularly frequent among the Cucurbitaceae, of which the seven species examined all contained active inhibitors

(Gasperi-Campani *et al.*, 1977; the present work). However, it should be noticed that the extract of *Crotalaria juncea* seeds was inactive, although it contains a haemagglutinating lectin (Ersson *et al.*, 1973) inhibiting protein synthesis (Barbieri *et al.*, 1979). This apparent inconsistency may be due to a low concentration of the lectin in the crude extracts, or to an enhancement of the inhibitory activity of the lectin during the purification procedure. This observation suggests also that inhibitors may be more frequent among plants than it appears.

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