Isolation and Partial Characterization of Virus Inhibitors From Plant Species Taxonomically Related to Phytolacca

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Accepted for publication 21 June 1977.

ABSTRACT

GRASSO, S., and R. J. SHEPHERD. 1978. Isolation and partial characterization of virus inhibitors from plant species taxonomically related to Phytolacca. Phytopathology 68:199-205.

A procedure was developed for obtaining a proteinaceous virus inhibitor from *Phytolacca americana* in an electrophoretically pure state. The final steps of the procedure involved adsorption to and elution from the cation exchange resin CM-Sephadex followed by passage through DEAE-Sephadex under nonadsorbing conditions. When the same procedure was applied to 14 other plant species reported to contain virus inhibitors, including several species of the order Centrospermae which is believed to be phylogenetically derived from the Phytolaccaceae, similar biologically active proteins were obtained in every case. However, in tests to evaluate further similarities only 2 of 8 species of the Centrospermae yielded substantial amounts of virus inhibitors that had the same molecular weight and were serologically related to the *Phytolacca* protein.

Substances that interfere with the mechanical transmission of plant viruses occur in many species of higher plants and pose a common problem in the bioassay of viruses by mechanical inoculation. Even so, only a few of the inhibitory substances have been isolated and characterized and fewer yet have been appraised for their mode of action during the transmission process.

Several inhibitors of infection have been isolated and have been found to be proteins. For example, the potent inhibitors from *Phytolacca americana* (6, 16) and *Dianthus caryophyllus* (11, 12), are proteins, and substances associated with the inhibitory activity exhibited by many other species have been found to behave like polypeptides when subjected to treatments and conditions which inactivate or precipitate proteins. Hence, inhibitors from many other species are probably proteins also.

Inhibitors of infection show some specificity of action. For example, they are not generally active in inhibiting infection of the species of their origin, or of closely related species (2), a factor suggesting that these substances act on the assay host instead of the virus itself. Moreover, some may be enzymes which act on the assay host to block the process of infection. As a notable example, the virus inhibitor from *P. americana* is a potent inactivator of almost all eukaryotic ribosomes except those of *Phytolacca* itself (9, 10). The mechanism of inhibition of protein synthesis was shown to be catalytic, hence enzymatic, since inhibition was found at levels as low as one molecule of inhibitor per 20 ribosomes. The implication of these observations is that virus replication is probably prevented as a result of interference with protein synthesis on host ribosomes. One can conjecture that viral RNA entering the cell during mechanical inoculation, in the presence of inhibitor, probably cannot function as messenger RNA to synthesize the early proteins needed for virus replication. As a result infection is aborted.

Species which have inhibitors of infection are not randomly distributed throughout the plant kingdom. Instead some taxonomic groups are notable for having virus inhibitors. For example, chenopodiaceous plants invariably have inhibitors. As pointed out by Smookler (14) the Chenopodiaceae, Amaranthaceae, and Basellaceae, three families of the order Chenopodiales (4), or more properly perhaps, the Centrospermae (1), all have inhibitors of infection. Tests with 29 species, selected as representatives of the families more or less at random, all contained inhibitors which would not pass through a dialysis membrane, thus suggesting a polypeptide nature. In further tests with two species each in the Chenopodiaceae and Amaranthaceae, the inhibitory substance was destroyed by emulsification with phenol, was precipitated by the various organic solvents, organic acids, and salts known to precipitate proteins, and was adsorbed as a basic material on carboxymethyl Sephadex (CM-Sephadex), a cation exchanger from which it was eluted by high-ionic-strength solutions (14). This behavior suggested the inhibitors were basic proteins.

The purpose of the present investigation was to extend the investigation of Smookler (14) to determine if selected species of the order Centrospermae contain proteinaceous inhibitors similar or identical to the basic polypeptide inhibitor of *P. americana* (16). The Centrospermae, as defined by Cronquist (1), consists of 11 families with some 10,000 species. It is believed to have its phylogenetic origin in the Phytolaccaceae. Species within the Centrospermae characteristically have betalains as pigments rather than anthocyanins (8) and share an assortment of other features indicating a common ancestry. It seemed reasonable that the

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production of virus inhibitors might be controlled by genes that conferred some special advantage, such as disease or insect resistance, which led to their retention during the course of evolution. It was the objective of this investigation to assess the presence, distribution, and biological activity of these particular proteins in the Centrospermae.

MATERIALS AND METHODS

Inhibitor purification.—Leaves and stems of the following species of plants grown in the greenhouse or in the field were used as sources of inhibitor: *Gomphrena globosa* L., *Dianthus caryophyllus* L., *Stellaria media* Cyr., *Chenopodium album* L., *C. amaranticolor* Coste & Reyn., *C. quinoa* Will., *Phytolacca americana* L., *Montia* sp., *Rumex* sp., *Alyssum* sp., *Brassica chinensis* L., *B. nigra* L., *Datura stramonium* L., and *Nicotiana tabacum* L. 'Turkish'. Plants were harvested and stored in polyethylene bags at −20 C until used.

Initial fractionation of the tissue for isolation of inhibitor was done by the same procedure used for purification of the inhibitor from *Phytolacca* (6). Frozen tissue was homogenized in a Waring Blendor, using 500 ml of water and 1.0 ml of mercaptoethanol per kilogram of tissue. The homogenate then was filtered through cheesecloth and fractionated exactly as described by Wyatt and Shepherd (16).

The final steps of purification of the inhibitors were carried out on a column of carboxymethyl Sephadex (CM-Sephadex) prepared as follows: 3 g of dry CM-Sephadex were suspended in 200 ml of 0.05 M sodium phosphate buffer, pH 6.0. After hydrating for a few hours at room temperature the material was then heated to 100 C for a few minutes, cooled, and a few milligrams of sodium azide were added before aging the solution in a cold room for 1-2 wk before use. These steps, to obtain full swelling of the CM-Sephadex, were found to be necessary to obtain reproducible behavior of columns during the final chromatographic isolation of the inhibitors. The fully swelled CM-Sephadex was poured into a 2-cm diameter glass column and allowed to settle under gravity.

After dialysis against water and low-speed centrifugation, one-tenth volume of 0.5 M phosphate buffer, pH 6.0, was added to the inhibitor solution before it was applied to the column. After application of the inhibitor solution to the CM-Sephadex column, 1,000 ml of 0.05 M phosphate, pH 6.0, was passed through the column to remove nonadsorbing proteins. All chromatography steps were done in a cold room at 4 C. In order to avoid packing of the Sephadex, which caused poor flow rates, only low pressure (i.e., about 10 cm of hydrostatic head) was used during all column elution steps.

A system of stepwise elution developed for the inhibitor from *Phytolacca* was tested for purification of the inhibitors from several other species and found to be useful. It consisted of an initial elution of largely inactive proteins from the CM-Sephadex column by applying 100 ml of 0.05 M sodium chloride in 0.05 M phosphate, pH 6.0. Subsequently, two active fractions were collected by further elution with buffered salt solutions. The first of these, of low purity and designated E_3, was obtained by elution of the CM-Sephadex column with an additional 150 ml of 0.05 M sodium chloride, 0.05 M phosphate, pH 6.0. After elution of E_3, the second active fraction which was of better purity and designated E_4, was obtained by applying an additional 250 ml of 0.1 M sodium chloride, 0.05 M phosphate, pH 6.0, to the column. The E_3 and E_4 fractions were dialyzed separately against 0.02 M Tris, pH 8.5, centrifuged at low speed to remove insoluble matter, and final purification achieved by passing these fractions through a column of DEAE-Sephadex equilibrated with 0.02 M Tris, pH 8.5. At this pH and salt concentration the inhibitors did not adsorb to the DEAE-Sephadex as did some contaminating proteins. The final E_3 and E_4 fractions were dialyzed against water (four to six 1-liter changes over a period of 2 days) and lyophilized. These purified fractions were stored dry at −20 C until used.

Biological activity.—The inhibitory effect of the purified E_3 and E_4 fractions was tested using partially purified preparations of the Mexican strain (3) of southern bean mosaic virus (SBMV) at 10 μg/ml for half-leaf comparisons on primary leaves of *Phaseolus vulgaris* L. 'Sutter Pink'. In these assays the inhibitor solution, resuspended at a known dry weight per unit volume, was mixed with an equal volume of the virus solution; the control half-leaf was inoculated with a mixture of the same virus suspension and an equal volume of water. Some additional bioassays were done to determine if the isolated inhibitors from other species were active on *Phytolacca*. In this case, tobacco mosaic virus was used as the lesion-inciting agent. Mechanical inoculations were performed by rubbing the leaves dusted with 22-μm (600-mesh) corundum with a finger dipped into the inoculum. Each test solution was inoculated to 10 half-leaves per bioassay. The inhibitory activity of each test solution, based on the local lesions induced, was estimated according to the following formula:

$$\% \text{ inhibition} = \frac{\left(\begin{array}{c}\text{No. lesions}\\ \text{without}\\ \text{inhibitor}\end{array}\right) - \left(\begin{array}{c}\text{No. lesions}\\ \text{with}\\ \text{inhibitor}\end{array}\right)}{\left(\begin{array}{c}\text{No. lesions}\\ \text{without}\\ \text{inhibitor}\end{array}\right)} \times 100$$

Electrophoresis on polyacrylamide gels.—The homogeneity of proteins purified from different plants was assessed by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS). Fifty μliters of each protein solution (3 mg/ml) were denatured at 100 C for 5 min after being mixed with an equal volume of 10 M urea, 7% SDS, and 10 μliters of 2-mercaptoethanol. Approximately 30-μliter samples of each protein were applied to each column (0.7 × 9 cm) of 10% polyacrylamide gel. The Tris-borate buffer system of Lane (7) was used. Electrophoresis was performed at 2 ma/tube for the first 15 min and then at 5 ma/tube for an additional 1.5 hr. The gels were stained with 0.2% Coomassie Blue for 2 hr and destained with 7% acetic acid in 23% methanol. In addition to the SDS gel system, a cationic detergent system using cetyltrimethylammonium bromide as denaturing agent was done as described by

Rice (13).

The molecular weights of the isolated inhibitor proteins were determined from their mobilities in SDS-polyacrylamide gels in comparison with marker proteins of known molecular weights (15).

Serology.—Antisera to the inhibitor from *Phytolacca* were prepared in rabbits by subcutaneous injections of purified inhibitor emulsified with an equal volume of Freund's incomplete adjuvant. In first attempts to prepare antiserum, in which several mg of *Phytolacca americana* peptide (PAP) were injected, the animals died because of the high mammalian toxicity of the protein. Eventually two antisera were prepared, using electrophoretically pure PAP (fraction E_4), by injecting very small amounts of material at first, followed by larger amounts later. Initially three injections of 10 μg each were made at weekly intervals followed by 100 μg on the 4th wk, and 1 mg on the 5th wk. Test bleedings were made on alternate days starting 10 days after the final injection. The serum had a titer of about 1:250 in microprecipitin tests beneath mineral oil. Tests for relationships among the proteins from various species were done by agar gel diffusion tests.

RESULTS

Isolation and biological activity of proteins from species of the Centrospermae.—The procedure described in materials and methods was developed for the inhibitor from *Phytolacca* and is believed to give a highly purified product. Figure 1 shows the results of an experiment in which the E_3 and E_4 fractions from several different purified preparations of *Phytolacca* were electrophoresed into polyacrylamide gels with either cationic (cetyltrimethylammonium bromide) (13) or anionic (sodium dodecyl sulfate) detergents as the denaturation and charge swamping reagent. The results show that both the E_3 and E_4 fractions consist largely of a single protein with only minor amounts of impurities present in some preparations. When contaminating proteins were present they occurred generally in the E_3 fraction. In fact, the E_3 fraction frequently contained considerable amount of other proteins. For this reason the final CM-Sephadex column eluate was collected as two fractions rather than a single one, as mentioned previously. However, it should be pointed out that even the E_4 fraction could not be assumed to have good purity without actually assessing its quality by gel electrophoresis experiments.

The same procedure was tested for other species of the Centrospermae under the assumption that if these contained similar or identical proteins that these would behave in the same manner during fractionation. Tests for inhibitory potency with the isolated products could then be used to establish biological similarity and hence support the probable identity of the final products. Table 1 shows the results of tests with the E_4 fraction from 14 species of plants, eight representatives of the Centrospermae plus six other species which have been reported to contain inhibitors from families outside the Centrospermae. All of these plants were found to contain proteins that can be isolated by this procedure and which inhibit the transmission of southern bean mosaic virus. However, the proteins from the various species of Centrospermae are, in the main, much more potent than

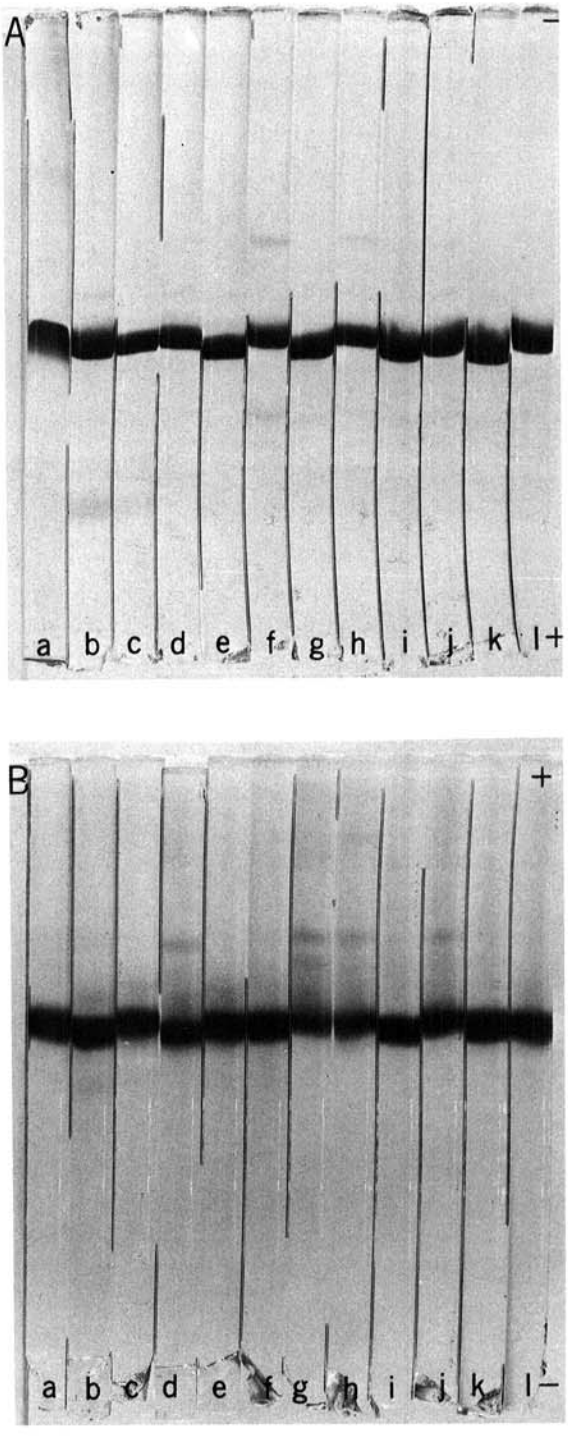

Fig. 1-(A,B). Results of polyacrylamide gel electrophoresis tests with several preparations (E_4 fractions except for E_3 fractions on gels d, f, h, and j) of *Phytolacca americana* inhibitor. **A**) a through l show the results of separations in a sodium dodecyl sulfate containing system. **B**) a through l, show the same preparations after electrophoresis in a system containing cetyltrimethylammonium bromide. Forty-five μg of protein was applied to each gel.

similar proteins from other species, although some variation exists among different members of the Centrospermae with some lacking the potency exhibited by species such as *Phytolacca americana*, *Dianthus* caryophyllus, or *Chenopodium amaranticolor*. In general though, proteins from the Centrospermae are 10- to 100-fold more potent than those from other taxonomic groups. None of the species tested equal *Phytolacca* in yield of inhibitor (Table 1). Similar results were obtained with the E_3 fractions, but the activity was usually somewhat lower than that of the E_4 fractions.

Behavior of proteins in polyacrylamide gel electrophoresis.—The occurrence, in various plants, of proteins with the same behavior during column chromatography suggested considerable homology between the various polypeptides, hence, further trials were done to evaluate these relationships. When the proteins were subjected to SDS-polyacrylamide gel electrophoresis, only two of the proteins migrated with the same mobility as the protein from *Phytolacca* and showed a great degree of homogeniety. Interestingly, these were the same proteins which showed the most potency as virus inhibitors; i.e., those from *C. amaranticolor*, and *D. caryophyllus* (Fig. 2). The proteins isolated from other species, both from the Centrospermae and other taxonomic groups, gave a variety of components of different mobilities none of which coincided exactly with the position of the major components of these three species. In most cases the gel bands of other species which approximated this position were much less intense in spite of the fact that the same amount of the lyophylized column product (40 μg) was applied to each gel. The material from *Datura* gave an intense double band of greater mobility than the most

TABLE 1. Yield and biological activity of proteins obtained from 14 different species of plants reported to have virus inhibitors[a]

Plant source of inhibitors Order, Family, Species	Yield (mg/kg of tissue)	Inhibition at indicated concentration[b]			
		1,000 μg/ml (%)	100 μg/ml (%)	10 μg/ml (%)	1 μg/ml (%)
Species of the Centrospermae:					
Amaranthaceae					
Gomphrena globosa	56	96	67	45	0
Caryophyllaceae					
Dianthus caryophyllus	14	100	99	98	75
Stellaria media	11	98	65	49	33
Chenopodiaceae					
Chenopodium album	18	92	84	25	22
C. amaranticolor	21	100	99	89	86
C. quinoa	19	87	58	28	0
Phytolaccaceae					
Phytolacca americana	65-129	100	96	84	76
Portulacaceae					
Montia sp.	6	95	64	45	29
Species not in the Centrospermae:					
Polygonaceae					
Rumex sp.	10	59	40	0	0
Cruciferae					
Alyssum sp.	12	58	24	0	0
Brassica chinensis	16	74	48	14	0
B. nigra	7	90	77	25	15
Solanaceae					
Datura stramonium	21	89	24	0	0
Nicotiana tabacum 'Turkish'	34	77	26	0	0

[a]Proteins purified by elution from a CM-Sephadex column and passage through DEAE-Sephadex. The E_4 fraction was tested in all cases.

[b]Purified inhibitor was mixed, at the indicated concentrations, with 10 μg/ml of southern bean mosaic virus. The inoculum was applied to halves of primary leaves of *Phaseolus vulgaris* 'Sutter Pink'. The opposite half-leaf was inoculated with a mixture of the virus and water. Inhibitory activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{No. of lesions without inhibitor}) - (\text{No. of lesions with inhibitor})}{\text{No. of lesions without inhibitor}} \times 100$$

homogenous and potent inhibitors named above (Fig. 2).

Some of the proteins were subjected to more extensive SDS gel electrophoretic experiments with marker proteins in order to estimate molecular weights (15). The following values were obtained: inhibitor from *Phytolacca*, 27,800; inhibitor from *Dianthus*, 28,500; inhibitor from *C. amaranticolor*, 29,000; and the inhibitor from *Datura*, 17,000.

Biological effect of selected inhibitors on Phytolacca.—The gel electrophoresis results suggested that the proteins from *C. amaranticolor* and *D. caryophyllus* might be identical to the inhibitor from *Phytolacca*. If so, these proteins should show no biological activity in inhibiting the mechanical transmission of viruses to *Phytolacca* since inhibitors of infection are not active on the species of their origin (2). This was confirmed when PAP containing extracts on *Phytolacca* showed no inhibition of virus infection. With this in mind, tests were done to see if the *C. amaranticolor* and *D. caryophyllus* proteins would interfere with the transmission of tobacco mosaic virus to *Phytolacca*. This virus induces local necrotic lesions on the latter and hence provides a good biological system for the evaluation. In tests with isolated E_4 fraction proteins from these two species at 100 μg/ml and 10 μg/ml, 60% and 42% inhibition, respectively, was obtained with the *Dianthus* protein and 95% and 84%, respectively, was obtained with the *Chenopodium* protein. From comparison of these data with those in Table 1 for inhibition of southern bean mosaic on bean, one can see that even though the *Dianthus* protein shows less potency in tests on *Phytolacca* it is still highly active. However, the *Chenopodium* protein is virtually as active in the test on *Phytolacca* hence one can conclude that, although the proteins are similar in many ways, they are not identical.

Serological tests.—Reactions in agar gel diffusion tests with PAP and its antiserum showed that the protein was immunologically heterogenous. In several tests, most purified preparations of the protein produced two precipitin bands, a major band and a minor band that was very weak, or even absent, in some preparations [Fig. 3-(A to C)]. Sometimes when agar gel plates were allowed to incubate for 36-48 hr, evidence for still a third component was evident. In this case a spur extension of the main precipitin line was evident when less-pure *Phytolacca* preparations were reacted in wells adjoining pure preparations (Fig. 3-A). Immunological heterogeniety was not expected since a high degree of purity had been indicated by gel electrophoresis experiments and only material with the highest degree of purity had been injected for preparation of the antisera. The presence of the second precipitin band was not always related to the presence of minor components detected in the gel electrophoresis experiments.

Results of agar gel diffusion tests with antiserum to the inhibitor from *Phytolacca* and the purified proteins from other species is given in Table 2 and Fig. 3. All but 4 of the 14 species tested gave positive reactions consisting of a single precipitin line [Fig. 3-(A to C)] indicating a serological relationship between these proteins and the inhibitor from *Phytolacca*. The heterologous reactions always were much weaker than the reaction of the antiserum with its homologous antigen [Fig. 3-(A to C)] even though all the proteins were used at the same dry weight concentrations. Four of the species which gave positive reactions were from species not allied to the Centrospermae. Hence not only can similar proteins be

Fig. 2. Polyacrylamide gel electrophoresis of proteins from *Phytolacca americana* (a), *Dianthus caryophyllus* (b), *Chenopodium amaranticolor* (c), and *Datura stramonium* (d) in a sodium dodecyl sulfate containing system. Forty-five μg of protein was applied to each gel.

Fig. 3. Agar gel serological tests with proteins from *Phytolacca*, *Dianthus*, *Chenopodium*, and *Datura* reacted with antiserum to purified inhibitor from *Phytolacca* (center wells). Peripheral wells 1 and 2 in A, B, and C contained inhibitor from *Phytolacca* of 6 August 74. Wells 3 and 4 in A, B, and C contained *Phytolacca* inhibitor 6-25-74. Wells 5 and 6 of A contained *Phytolacca* inhibitor of 7-29-75. Inhibitor from *Dianthus* was in wells 7 and 8 of A and in wells 5 and 6 of B. Chenopodium inhibitor was in wells 7 and 8 of B and in wells 5 and 6 of C. Datura inhibitor was in wells 7 and 8 of C. All proteins were used at a concentration of 3 mg/ml.

isolated from species outside the Centrospermae but these proteins are also serologically related to those from the Centrospermae. Moreover, proteins isolated from two species of the Centrospermae (*Montia perfoliata* and *Gomphrena globosa*) did not give positive serological tests, suggesting that some species of the group do not contain proteins serologically related to the inhibitor from *Phytolacca*. As one can see in Fig. 3-B, the reactions of heterologous proteins cross the precipitin line of the minor component of *Phytolacca* peptide, indicating the serological relationship is to the major constituent of the *Phytolacca* preparations.

DISCUSSION

The column-chromatographic method for purification of the inhibitor from *Phytolacca*, using adsorption and elution from a cation exchanger followed by passage through an anion exchanger under nonadsorbing conditions, gave a much purer product than methods used formerly, as judged by gel electrophoresis of the final product in both anionic and cationic detergent systems. With this method electrophoretically pure material can be prepared consistently, although every preparation could not be relied upon to have high purity. Paradoxically, the material which showed a high degree of electrophoretic purity appeared to consist of two components immunologically, although some preparations had a scarcely detactable amount of the second component. The reason for the discrepancy between the two methods is unknown.

A similar method for purification of the inhibitor from *Phytolacca* has been reported recently by Irwin (5). Adsorption and elution from columns of DEAE cellulose and phosphocellulose were used. The product was found to be electrophoretically pure in SDS polyacrylamide gel experiments in which it exhibited a mobility equivalent to a molecular weight of 27,000, a value similar to that reported herein. Both values represent slightly more than twice the minimum molecular weight of about 13,000 determined by chemical methods by Wyatt and Shepherd (16). Hence there are probably two lysine residues per molecule instead of one residue assumed in the latter investigation.

The isolation of proteins from the various species by the same procedure used for isolation of the inhibitor from *Phytolacca* resulted in the yield of appreciable amounts of material in each case. This was true regardless of taxonomic position of the species. Moreover, the yield bore no relationship to taxonomic position, with about the same wide range of variation being shown by members of the Centrospermae and those not taxonomically allied to them. In addition, the isolated products showed biological activity in inhibiting mechanical transmission of southern bean mosaic virus in every case. In comparisons among species, those in the Centrospermae were more potent biologically than those belonging to other families (Table 1). For example, the proteins from *Chenopodium amaranticolor* and *D. caryophyllus* were fully as active as the inhibitor from *Phytolacca*. The proteins isolated from *C. album*, *C. quinoa*, *Stellaria*, *Montia*, and *Gomphrena*, also belonging to the Centrospermae, were significantly less potent.

Only certain species of the Centrospermae contain significant amounts of basic, biologically active protein related to the polypeptide inhibitor in *Phytolacca*. Of the eight species of the Centrospermae investigated, *C. amaranticolor* and *D. caryophyllus* contained proteins of the same molecular weight and serologically related to the *Phytolacca* protein. However, in the serological tests the reactions were of the partial identity type. Moreover, in inoculations of TMV to *Phytolacca*, the purified proteins from *C. amaranticolor* and *D. caryophyllus* inhibited infection to a significant degree thus showing they are not identical to the *Phytolacca* protein. Consequently, one can conclude that, although some species of the Centrospermae contain proteins analogous to the *Phytolacca* protein, most species do not. If these proteins represent the conservation of certain genes during the course of evolution, many species have not found retention of these genes essential for their survival.

TABLE 2. Results of agar-gel serological tests with isolated proteins from various plant species and antiserum to the inhibitor from *Phytolacca*

Species and taxonomic position[a]	Serological reaction[b]
Species of the Centrospermae:	
Chenopodium album	+
Chenopodium amaranticolor	+
Chenopodium quinoa	+
Dianthus caryophyllus	++
Gomphrena globosa	−
Montia perfoliata	−
Phytolacca americana	+++
Stellaria media	+
Species not in the Centrospermae:	
Alyssum sp.	+
Brassica campestris	−
Brassica nigra	−
Datura stramonium	+
Nicotiana tabacum	+
Rumex crispus	+

[a]All species listed have been reported to have virus inhibitors.
[b]The degree of the reaction is indicated by:− no reaction, + faint reaction, ++ strong reaction,+++ very strong reaction.

LITERATURE CITED

1. CRONQUIST, A. 1968. The evolution and classification of flowering plants. Houghton Mifflin, Boston. 298 p.
2. GLENDRON, Y., and B. KASSANIS. 1954. The importance of the host species in determining the action of virus inhibitors. Ann. Appl. Biol. 41:183-188.
3. GROGAN, R. G., and K. A. KIMBLE. 1964. The relationship of severe bean mosaic virus from Mexico to southern bean mosaic and its related strain in cowpea. Phytopathology 54:75-78.
4. HUTCHISON, J. 1948. British flowering plants. Gawthorne Ltd., London. 468 p.
5. IRWIN, J. D. 1975. Purification and partial characterization of the antiviral protein from Phytolacca americana which inhibits eukaryotic protein synthesis. Arch. Biochem. Biophys. 169:522-528.

6. KASSANIS, B., and A. KLECZKOWSKI. 1948. The isolation and some properties of a virus-inhibiting protein from Phytolacca esculenta. J. Gen. Microbiol. 2:143-153.
7. LANE, L. C. 1974. The components of barley stripe mosaic and related viruses. Virology 58:323-333.
8. MABRY, T. J., and A. S. DREIDING. 1968. The betalains. *in* Recent Adv. Phytochem. 1:145-160.
9. OBRIG, T. G., J. D. IRWIN, and B. HARDESTY. 1973. The effect of an antiviral peptide on the ribosomal elongation enzymes, EF-1 and EF-11. Arch. Biochem. Biophys. 155:278-289.
10. OWENS, R. A., G. BRUENING, and R. J. SHEPHERD. 1973. A possible mechanism for the inhibition of plant viruses by a peptide from Phytolacca americana. Virology 56:390-393.
11. RAGETLI, H. W. J., and M. WEINTRAUB. 1962. Purification and characteristics of a virus inhibitor from Dianthus caryophyllus L. I. Purification and activity. Virology 18:232-240.
12. RAGETLI, H. W. J., and M. WEINTRAUB. 1962. Purification and characterization of a virus inhibitor from Dianthus caryophyllus L. II. Characterization and mode of action. Virology 18:241-248.
13. RICE, R. H. 1974. Minor components in cowpea chlorotic mottle virus and satellite of tobacco mosaic virus. Virology 61:249-255.
14. SMOOKLER, M. M. 1971. Properties of inhibitors of plant virus infection occuring in the leaves of species in the Chenopodiales. Ann. Appl. Biol. 69:157-168.
15. WEBER, K., and M. OSBORN. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.
16. WYATT, S. P., and R. J. SHEPHERD. 1969. Isolation and characterization of a virus inhibitor from Phytolacca americana. Phytopathology 59:1787-1794.