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A Preliminary Survey of Some Midwestern Plants for Substances Inhibiting Human Plasma Cholinesterase *in Vitro*¹

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Abstract. Substances which inhibit the action of human plasma cholinesterase *in vitro* have been found in aqueous extracts of various members of the plant family Solanaceae. A preliminary survey of some weedy and cultivated midwestern representatives of this family revealed eleven active species in six genera: *Datura*, *Lycopersicon*, *Nicotiana*, *Petunia*, *Physalis*, and *Solanum*. A member each of the genera *Capsicum* and *Lycium* were inactive. Inhibitory substances were not found in thirty-one species representing nineteen plant families other than Solanaceae.

Substances which inhibit the action of the enzyme cholinesterase *in vitro* have been found in aqueous extracts of potato (*Solanum tuberosum* L.) foliage (Orgell, 1958a). Substances with a similar effect were subsequently found in tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melongena* L.) extracts. These observations resulted in a preliminary examination of representatives of several plant families available in the vicinity of Ames, Iowa.

MATERIALS AND METHODS

The methods of collection, extraction, and assay were similar to those described previously (Orgell, 1958b). Plants were collected from the field and extracted within two hours after harvest. Ten grams of fresh tissue were homogenized in a Waring Blendor with 40 ml. of distilled water for 3 minutes at room temperature. The homogenate was filtered through a cotton plug, and the volume of filtrate measured. The filtrate was brought to pH 7.35 with 0.1 N sodium hydroxide, and diluted to 1.1 times its initial volume. Where semi-quantitative comparisons of many tissues were desired, 10 ml. aliquots of each filtrate (extract) were removed for assay. When quantitative determinations on single tissues were made, logarithmic series of five aliquots ranging from 0.01 to 3.16 ml., were taken for assay.

The various aliquots of aqueous plant extracts were each mixed with 5 ml. of standardized human blood plasma and made up to a total volume of 50 ml. with distilled water. The solutions were

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Table 1
Inhibition of Plasma Cholinesterase by Aqueous Extracts of Various Plant Tissues

Scientific name	Common name	Tissue	% I
Amaranthaceae			
<i>Amaranthus retroflexus</i> L.	Pigweed	Leaves	0
		Roots	0
Asclepiadaceae			
<i>Asclepias syrica</i> L.	Milkweed	Leaves	0
Chenopodiaceae			
<i>Chenopodium album</i> L.	Lamb's quarters	Leaves	0
Compositae			
<i>Arctium minus</i> Benth.	Burdock	Leaves	0
<i>Helianthus annuus</i> L.	Sunflower	Leaves + flowers	0
<i>Taraxacum officinale</i> Weber	Dandelion	Tops	0
Cruciferae			
<i>Brassica oleracea</i> L.			
var. <i>botrytis</i>	Broccoli	Head	2
<i>Brassica oleracea</i> L.			
var. <i>capitata</i>	Cabbage	Head	0
		Roots	2
<i>Brassica napobrassica</i> Mill.	Rutabaga	Roots	0
Cucurbitaceae			
<i>Cucurbita pepo</i> L.			
var. <i>melopepo</i>	Squash	Leaves	0
		Fruits	0
		Roots	0
<i>Cucurbita pepo</i> L.	Conn. field pumpkin	Fruit	0
		Foliage	2
		Roots	0
Convolvulaceae			
<i>Convolvulus arvensis</i> L.	Morning glory	Leaves	0
Equisetaceae			
<i>Equisetum arvense</i> L.	Horsetail	Shoots	0
Euphorbiaceae			
<i>Ricinus communis</i> L.	Castor bean	Leaves	0
Gramineae			
<i>Zea mays</i> L.	Corn	Foliage	0
Iridaceae			
<i>Gladiolus</i> sp.	Gladiolus	Corm	0
<i>Iris</i> sp.	Wild iris	Leaves	0
Labiatae			
<i>Nepeta cataria</i> L.	Catnip	Leaves	0
Leguminosae			
<i>Phaseolus limensis</i> Macf.	Lima bean	Foliage	0
		Roots	0
		Pods	0
<i>Trifolium repens</i> L.	White Dutch clover	Tops	46
<i>Medicago sativa</i> L.	Alfalfa	Tops	0
Liliaceae			
<i>Allium</i> sp.	Japanese onion	Leaves	0
Malvaceae			
<i>Hibiscus trionum</i> L.	Flower-of-an-hour	Leaves	0
<i>Abutilon theophrasti</i> Medic.	Velvet weed	Leaves	0
Polygonaceae			
<i>Polygonum pennsylvanicum</i> L.	Smartweed	Leaves	0
<i>Rumex crispus</i> L.	Sourdock	Leaves	0
Portulacaceae			
<i>Portulaca oleracea</i> L.	Purslane	Leaves	0
		Roots	0

Table I (Continued)

Scientific name	Common name	Tissue	% I
Rosaceae			
<i>Malus sylvestris</i> Mill.	Jonathan apple	Fruits	0
<i>Fragaria chiloensis</i> var. <i>ananassa</i> Duchesne	Strawberry	Fruits	1
Solanaceae			
<i>Solanum rostratum</i> Dunal	Buffalo bur	Stem, seed pods dry leaves	8
<i>Solanum tuberosum</i> L.	White Russet potato	Tuber flesh	40
		Tuber peels	58
		Peels + flesh	51
		Peels + eyes	53
		Roots	47
		Leaves	58
		Flowers	55
		Berries	53
	Irish cobbler	Tuber peels + flesh	56
	Cherokee potato	Tuber peels + flesh	49
	Red Lasoda potato	Tuber peels + flesh	54
	Red Pontiac potato	Tuber peels + flesh	40
<i>Solanum melongena</i> L.	Egg plant	Fruit	19
<i>Petunia hybrida</i> Vilm.	Petunia	Leaves + flowers	42
		Flowers	55
<i>Nicotiana tabacum</i> L.	Tobacco	Leaves	5
<i>Nicotiana glauca</i>		Leaves	43
<i>Nicotiana glutinosa</i>		Leaves	31
<i>Nicotiana bigloveii</i>		Leaves	54
<i>Datura stramonium</i> L.	Jimson weed	Leaves	20
		Seed pods	0
		Roots	0
<i>Physalis</i> sp.	Ground cherry	Leaves + flowers	9
		Leaves	0
		Berries	35
<i>Solanum carolinense</i> L.	Horse nettle	Leaves + flowers	3
		Leaves	0
		Berries	44
<i>Capsicum frutescens</i> L.	Pepper	Leaves	0
		Fruits	0
		Roots	0
<i>Solanum americanum</i> Mill.	Nightshade	Leaves	30
		Berries	27
<i>Solanum dulcamara</i> L.	Nightshade bittersweet	Leaves	30
		Berries	0
<i>Lycium halimifolium</i> Mill.	Matrimony vine	Leaves	0
		Berries	0
<i>Lycopersicon esculentum</i> Mill.	Tomato	Ripe fruit	0
		Green fruit	0
		Leaves	9
		Roots	21
Umbelliferae			
<i>Daucus carota</i> L.	Carrot	Leaves	0
		Roots	0
Xanthaceae			
<i>Xanthium pennsylvanicum</i> L.	Cocklebur	Leaves	0

stirred for 40 minutes at room temperature, during which time inhibition of the enzyme occurred. One ml. aliquots of the partially inhibited enzyme solutions were then removed for assay by the electrometric method of Curry (1956). Results were expressed either as the per cent inhibition resulting from 10 ml. of plant extract, or as the quantity of extract giving 50 per cent inhibition of the cholinesterase present in 5 ml. of plasma (I-50). Individual per cent inhibition values were corrected for an effect of the buffer capacity of the plant extract upon the electrometric assay, whereas the I-50 values were not corrected and are relative only to each other.

RESULTS

Assay of 44 plant species representing 21 families revealed cholinesterase-inhibitory substances in individuals from two of the families, the Leguminosae and the Solanaceae (Table 1). Cholinesterase inhibitors have been previously reported in two legumes. The calabar bean (*Physostigma venenosum* Balf.) is native to the west coast of Africa and is the source of the pharmacologically active alkaloid physostigmine (eserine). Ladino clover (*Trifolium repens* L.) contains an inhibitor which is reported to be soluble in chloroform (Heath, 1953). We found a similar inhibitor in extracts of White Dutch clover.

The eleven active species of the Solanaceae occurred in six genera: *Datura*, *Lycopersicon*, *Nicotiana*, *Petunia*, *Physalis*, and *Solanum*. All six species representing the genus *Solanum* were active. Garden pepper (*Capsicum frutescens* L.) and matrimony vine (*Lycium halimifolium* Mill.) were inactive. There appears to be no correlation between the poisonous character of some of these plants and their content of cholinesterase-inhibitor.

Table 2
Relative Concentration of Cholinesterase-Inhibitory Substances Present
In Extracts of Solanaceous Plants

Plant	Variety or Cross	Tissue	I-50(ml)
Petunia	—	foliage	0.03
Potato	AI-5561-8	foliage	0.08
Potato	I-5569	foliage	0.16
Potato	AI-5561-13	foliage	0.23
Potato	Irish Cobbler	tuber sprouts	0.53
Potato	Irish Cobbler	foliage	0.56
Potato	Irish Cobbler	tuber peel	0.63
Potato	Plymouth	foliage	0.60
Potato	Huron	foliage	0.69
Horse nettle	—	berries	2.8
Common nightshade	—	berries	3.3
Ground cherry	—	berries	7.0
Potato	White Russet	tuber flesh	32.1

The most potent source of inhibitory substances examined was the foliage of petunia (Table 2). As little as six milligrams of fresh petunia leaf resulted in 50 per cent inhibition of the cholinesterase present in 5 ml. of plasma. Foliage of certain experimental selections of potato were next in order of activity. Leaves, tuber skins, and tuber sprouts of commercial potato varieties contained lesser concentrations of inhibitor. Berries of horse nettle, common nightshade, and ground cherry were quite low in activity. Inhibitory substances of the potato plant were present in highest concentration in the foliage, flowers, berries, tuber sprouts, and skins. Lower levels were found in the stems and roots. The innermost flesh of potato tubers was practically devoid of inhibitors.

The distribution of cholinesterase-inhibitory substances within the plant varied from species to species within the Solanaceae. Inhibitory material was found primarily in the roots of garden tomato, with only low concentrations in the leaves and no activity in the fruits. The leaves of nightshade bittersweet contained inhibitor whereas none was present in the berries. The converse was true for the ground cherry. Leaves taken from different parts of the same field grown potato plant contained nearly equivalent levels of inhibitory substances.

Comparisons of the level of inhibitor in the foliage of various commercial varieties and in selections from a breeding program revealed considerable differences. In general, the common commercial varieties contained the lowest, and certain of the experimental crosses contained the highest levels of inhibitor. The AI-5561 series, which are partially derived from *Solanum chacoense*, are particularly rich in these substances.

Little is known about the effects of various environmental and climatic factors upon the concentration of inhibitory substances in potatoes. Plants grown in the greenhouse during the winter appear to have only one-half to one-fourth as much inhibitory material as those grown in the spring and summer in the field (Table 3). One experiment has indicated that the "greening" of potato tubers under artificial light results in an increased level of inhibitor in the tuber.

Table 3

Relative Concentrations of Inhibitory Substances Present in Foliage of Potato Selections Grown Under Field and Greenhouse Conditions

Potato Selection	Field (I-50)	Greenhouse (I-50)	Field
			Greenhouse
Plymouth	0.60	2.17	3.6
AI-5561-4	0.09	0.29	3.2
White Rose	1.16	2.58	2.2

DISCUSSION

Cholinesterase-inhibitory substances have been reported by others to be present in various plants, including raspberry (Beckett, 1954), boxwood (Vincent, 1945), and gelsemium (Vincent, 1951). Hence the presence of cholinesterase-inhibitory compounds is not unusual among higher plants. As yet, insufficient information is available to allow generalization with respect to the taxonomic distribution of these substances within the various plant families. The physical and chemical properties of these inhibitors suggest that they are alkaloids or alkaloidal glycosides, and that the substances from different families are also different chemically. The physiological and ecological role of these natural enzyme-inhibitors is at present obscure. It is perhaps possible that they play a part in the interactions of these plants with their predators and parasites.

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