Lipid Constituents of Some Common Weed Seeds

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The fatty acid composition of the lipids extracted from weed seeds of 15 species which are members of the Compositae, Solanaceae, Malvaceae, Convolvulaceae, Gramineae, Amaranthaceae, and Polygonaceae families was determined by gas liquid chromatography. Linoleic acid was predominant in 14 species (36.5 to 76.5%), while all species contained oleic (10.3 to 42.9%), palmitic (6.0 to 22.1%), and stearic (1.2 to 6.3%) acids. Linolenic and palmitoleic acids were found in 13 species, arachidic acid in four species, and cyclopropenoic acids in the two species of Malvaceae. Sterols, sterol glycosides and -esters, α,α- and α,β-diglycerides and monoglycerides were found in each species. Lipid extracts from four species were separated into five lipid fractions by silicic acid chromatography. Triglycerides were predominant, followed by polar lipids in which phosphatidyl ethanolamine, -choline and -inositol, digalactosyl diglyceride, and monogalactosyl diglyceride were detected by thin-layer chromatography.

The seed from only approximately a dozen crops furnish the major portion of world needs for vegetable oils (Wolff, 1966). Of the large number of known higher plants, the oil of only about 900 species have been investigated for their fatty acid composition (Hilditch and Williams, 1964). Unfortunately, much of this work was done by methods which are no longer considered reliable. The introduction of gas and thin-layer chromatography has greatly improved the separation and quantitation of lipids.

Weeds would appear to have limited usefulness because of the considerable effort expended on their eradication (U.S. Dept. of Agriculture, 1968). These plants, however, possess many growth characteristics and adaptions that enable them to exploit their ecological niches successfully (National Academy of Sciences, 1968). Many weeds have a great capacity for reproduction and dispersal by means of very prolific seed productions (King, 1966; National Academy of Sciences, 1968). These growth and reproductive characteristics could be of value if we could develop an economic use for these plants.

Various plant constituents, including fatty acids, also have been used to establish chemotaxonomic relations for plants. In the experience of Shorland (1963), "seed fats (lipids) from the same species almost invariably contain the same type of fatty acids."

This is a study of the seed lipid composition of some of the common wild plants that are considered to be weeds in the United States and other regions.

MATERIALS AND METHODS

Plant Material. Mature seeds of 15 species were collected from the several locations indicated in Table I. The seeds were stored at −15° C until extraction. All seeds selected are serious weeds in several crops in certain areas of the United States (U.S. Dept. of Agriculture, 1968). Certain structures were removed from some seeds before extraction (Table I) in order to facilitate grinding and to eliminate interfering pigments. Dry weight was determined on seeds dried 24 hr at 100° C.

Extraction of Lipids. The seeds were ground in a ball mill. Lipids were extracted by homogenizing the powdered seeds in chloroform-methanol-water (Bligh and Dyer, 1959). The extracts were filtered and then evaporated to near dryness under vacuum. After the lipids had been redissolved in chloroform:methanol:water (86:14:1) and filtered, aliquots of the extracts were dried to constant weight for determination of lipid percentages. The extracts were stored in solution at −15° C under N_2 in darkness.

Halpen Test. Sida and velvetleaf extracts were tested for cyclopropenoid acids by the conventional Halpen color test (American Oil Chemists' Society, 1962).

Gas Liquid Chromatography. Fatty acid composition was determined with an F & M Model 400 gas chromatograph equipped with a flame ionization detector (Weber, 1969). Methyl esters of the fatty acids were prepared by transesterification of the lipids with methanol containing 5% sulfuric acid and were separated on a 1/4 in. x 6 ft glass column packed with 12% stabilized diethylene glycol succinate. Column temperature was 180° C and He flow was 40 ml per min. The column was standardized with equivalent NIH standards (Applied Science Laboratories, Inc.) as described by Horning *et al.* (1964). Fatty acid percentages were calculated by determining peak areas with the aid of a Disc integrator.

Column and Thin-Layer Chromatography. For four species the total lipid extracts were separated on silicic acid columns (Weber, 1969) into five fractions: (1) hydrocarbons and sterol esters, (2) triglycerides, (3) free fatty acids, (4) sterols, mono- and diglycerides, and (5) polar lipids. The fatty acid compositions of the triglyceride and polar lipid fractions were determined by gas chromatography.

The components of the lipid classes were examined by comparing their mobilities against known standards by thin-layer chromatography (tlc). Neutral lipids were chromatographed on silica gel tlc plates developed in petroleum ether:diethyl ether:acetic acid (80:20:1) (Mangold, 1961). Sterols

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Table I. Identification, Description, and Some Analytical Data of the Experimental Material

Plant material[a]								
Common name	Scientific name	Family	State	Year	Part of seed removed prior to analysis	wt/100 seeds (mg)	lipid/100 seeds (mg)	lipid content (%)
Giant ragweed	*Ambrosia trifida* L.	Compositae	Illinois	1966	Involucre & testa removed	0.752	332	44.1
Common ragweed	*Ambrosia artemisiifolia* L.	Compositae	Illinois	1967	Involucre removed	0.494	103	20.8
Jimsonweed	*Datura stramonium* L.	Solanaceae	Illinois	1966	None	0.727	216	29.7
Prickly sida	*Sida spinosa* L.	Malvaceae	Illinois	1966	Carpel wall removed	0.194	30	15.7
Velvetleaf	*Abutilon theophrasti* Medic.	Malvaceae	Illinois	1964	None	0.811	106	13.1
Ivyleaf morning-glory	*Ipomoea hederacea* (L.) Jacq.	Convolvulaceae	Illinois	1966	None	3.070	387	12.6
Field bindweed	*Convolvulus arvensis* L.	Colvolvulaceae	California	1966	None	1.123	88	7.8
Yellow foxtail	*Setaria glauca* (L.) Beauv.	Gramineae	Wisconsin	1965	Bracts removed	0.172	25	14.6
Wild oat	*Avena fatua* L.	Gramineae	Illinois	1966	Bracts removed	1.036	100	9.6
Barnyard grass	*Echinochloa crusgalli* (L.) Beauv.	Gramineae	California	1966	Bracts removed	0.107	7	6.7
Giant foxtail	*Setaria faberii* Herrm.	Gramineae	Illinois	1965	Bracts removed	0.106	7	6.6
Johnson grass	*Sorghum halepense* (L.) Pers.	Gramineae	Arkansas	1965	Bracts removed	0.250	14	5.7
Redroot pigweed	*Amaranthus retroflexus* L.	Amaranthaceae	Illinois	1966	None	0.034	2	6.4
Wild buckwheat	*Polygonum convolvulus* L.	Polygonaceae	North Dakota	1965	None	0.348	10	2.9
Pennsylvania smartweed	*Polygonum pensylvanicum* L.	Polygonaceae	Illinois	1968	Perianth removed	0.580	16	2.7

[a] Botanical and common names according to the Weed Science Society of America (1966).

and partial glycerides were further checked by tlc in diethyl ether:benzene:ethanol:acetic acid (40:50:2:0.2) (Freeman and West, 1966) and benzene:diethyl ether:ethyl acetate:acetic acid (80:10:10:0.2) (Storry and Tuckley, 1967). Both solvent systems separated the α,α- from the α,β-diglycerides. Polar lipids were chromatographed both in one and two dimensions in chloroform:methanol:water:acetic acid (65:25:3:1) and chloroform:acetone:methanol:acetic acid:water (65:20:10:10:3) (Lepage, 1967).

Tlc sprays were used for identification of specific types of lipids. Amino phospholipids were detected by ninhydrin (Sambasivarao and McCluer, 1963). Aqueous 20% perchloric acid spray (Lepage, 1964) gave characteristic color reactions with the glycolipids. A molybdenum spray reagent (Dittmer and Lester, 1964) specific for phosphate esters was used to identify phospholipids.

RESULTS AND DISCUSSION

Seed Weight. The weights of the extracted seeds (Table I) indicate their relative size.

Lipid Content. Lipid contents of the seeds studied are shown in Table I. The lipid percentage ranged from 44.1 in giant ragweed to 2.7 in smartweed. The lipid content of nearly all the weed seeds was within the range of commercial oil-producing seeds, such as soybean with 20% oil (Rinne, 1969) and corn with 4.5% oil (Beadle *et al.*, 1965). In view of the fact that oil content varies only slightly due to environment and other factors (Shorland, 1963), our values are similar to those for the same species previously investigated (Earle *et al.*, 1960; Earle and Jones, 1962). We were unable to find reported values for the seed lipid content of sida, morningglory, giant foxtail, and common ragweed.

The total lipid yield per 100 seeds (Table I) was highest for morningglory and lowest for pigweed. Both seed size and lipid content are important characteristics in selecting plants for possible oil crops.

Although a limited number of species within a family were investigated, our data (Table I) suggest that lipid content may be relatively similar within a particular family. Other workers (Earle *et al.*, 1960; Earle and Jones, 1962; Genest and Sahasrabudhe, 1966; Lambertsen *et al.*, 1966; Sahasrabudhe and Genest, 1965) have determined the lipid content of species in these families. The data which they obtained closely resembles ours and this range of seed lipid content therefore seems to be a characteristic which could be ascribed to the particular families investigated. When our values are listed according to decreasing lipid content by family (Table I), the order closely follows that listed by Earle and Jones (1962).

Fatty Acid Composition. Linoleic acid (18:2) constituted from 36.5 to 76.5% of the total fatty acids in the extracts (Table II), and was the predominant acid in all species except wild oat. Oleic acid (18:1) composed from 10.3 to 42.9% of the acids, and was the second most abundant component in all samples except sida, velvetleaf, morningglory, and wild oat. From 6.0 to 22.1% of the acids in the seeds was made up of palmitic (16:0); it was third in abundance in all species except the same four listed above. Small quantities (1.2 to 6.3%) of stearic acid (18:0) were present in all of the species. Linolenic acid (18:3) constituted from 1.2 to 5.3% of the acids in 11 species; it was not detected in ragweed and only a trace was found in sida and jimsonweed. Levels of palmitoleic acid (16:1) above trace amounts were found only in four species. Arachidic acid (20:0) was detected in only four species. The C_{18} unsaturated acids comprised from 70.0 to 92.5% of the acids in the seeds (Table II). From 7.5 to 28.4% of the total were saturated C_{16} and C_{18} acids.

The fatty acid compositions of the oils of giant ragweed, jimsonweed, velvetleaf, morningglory, and pigweed reported by Hilditch and Williams (1964) differ markedly from ours. Their results were, however, not obtained by gas chromatography and the disparities probably result from dif-

Table II. Percentage Distribution of Fatty Acids in the Extracts of the Weed Seeds and Two Edible Oils

Species	Fatty acids[a]							
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Giant ragweed	...	8.0	...	3.6	17.8	70.6	...	...
Common ragweed	...	7.6	T[b]	3.7	12.2	76.5	...	...
Jimsonweed	T	12.3	T	2.7	29.2	55.8	T	T
Prickly sida	T	17.7	1.5	3.2	10.3	67.3	T	...
Velvetleaf	...	15.8	T	3.4	11.7	66.9	2.2	...
Ivyleaf morningglory	T	22.1	T	6.3	16.6	49.5	3.9	1.6
Field bindweed	T	16.4	T	5.2	24.3	52.9	1.2	...
Yellow foxtail	...	6.0	T	1.5	15.9	74.4	2.2	T
Wild oat	T	15.6	...	2.0	42.9	36.5	3.0	...
Barnyardgrass	...	7.7	T	1.2	20.3	68.1	2.7	...
Giant foxtail	T	6.8	T	1.2	20.0	68.8	3.2	T
Johnsongrass	...	14.5	1.7	2.0	37.7	42.5	1.6	...
Redroot pigweed	...	11.8	2.0	3.4	22.7	58.3	1.8	...
Wild buckwheat	...	11.9	T	2.4	38.2	42.4	5.1	...
Pennsylvania smartweed	...	9.7	7.6	3.0	28.3	46.1	5.3	...
Corn[c]	...	11.5	...	2.2	26.6	58.7	0.8	0.2
Soybean[d]	...	11.3	...	4.1	26.7	51.4	6.5	...

[a] In abbreviations of fatty acids the first number refers to the number of carbons in the molecule and the second number to the total number of double bonds, *e.g.*, 14:0 myristic acid. [b] T = trace. [c] Data from Beadle *et al.* (1965). [d] Data from Rinne (1969).

ferences in the extraction and analytical procedures employed. No recent work has been done on the species we investigated, but when gas chromatography was used to analyze seed lipids of other members of the same genus, the fatty acid patterns were similar. The fatty acid distribution for seven members of the Ipomoea genus (Genest and Sahasrabudhe, 1966; Sahasrabudhe and Genest, 1965) corresponded to our values for morningglory (Table II). Similarities in patterns for bindweed (Table II) and *Convolvulus tricolor* (Genest and Sahasrabudhe, 1966; Sahasrabudhe and Genest, 1965) were also noted. In addition, our distribution pattern for wild oat shows good agreement to that of *Avena sativa* reported by Lambertsen *et al.* (1966).

Similarity in patterns of fatty acid distribution can be noted for the species within each of the Compositae, Malvaceae, Convolvulaceae, and Polygonaceae families (Table II), suggesting some taxonomic significance. In the Gramineae, there is similarity between wild oat and johnsongrass, and among barnyard grass, giant foxtail, and yellow foxtail. In the former two, the patterns of distribution resemble those of three other members of Gramineae, analyzed or reviewed by Lambertsen *et al.* (1966); for the latter three, there is similarity between them and nine other members of Gramineae (Lambertsen *et al.*, 1966).

Positive Halpen tests were obtained with both velvetleaf and sida, indicating the presence of cyclopropenoid acids. These acids, especially malvalic acid, are characteristic components in seeds of the Malvaceae (Shorland, 1963). The cyclopropenoid acids were not quantitated. Methyl malvalate and methylinoleate are not separated by gas chromatography on polar columns (Wolff and Miwa, 1965). Some of the acid attributed to linoleic in these seeds was probably malvalic.

No other unusual fatty acids in more than trace quantities were detected in the seeds. The fatty acid patterns of the species investigated were similar to widely used edible oils such as corn (Beadle *et al.*, 1965) and soybean (Rinne, 1969) oils (Table II).

Lipid Classes. We were not able to find reports on the classification of lipids in these species. Column separation of the lipid extracts revealed that the triglycerides constituted from 55.3 to 87.2% of lipids present in the plants studied (Table III). Polar lipids made up from 6.0 to 32.6% and were the second most abundant class. For each species, there was less lipid present in the remaining three classes than in the triglyceride or polar classes.

The fatty acid compositions of the triglyceride and polar lipids fractions are shown in Table IV. The fatty acid distribution pattern for the triglyceride fractions of each species is similar to the pattern for the total lipid extracts (Table II) because of the high proportion of triglycerides present. The polar lipids have a higher percentage of saturated fatty acids than the triglycerides; they are higher in palmitic and stearic acids. In wheat, each polar lipid had a characteristic fatty acid pattern (McKillican, 1964). Therefore, the fatty acid pattern of the polar lipid class probably reflects variations in the relative abundance of individual lipids in that class. Thin-layer chromatography revealed the presence of phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol, digalactosyl diglyceride, monogalactosyl diglyceride, sterol glycosides, and sterolglycoside esters in the polar lipids of each of the four species.

The fractions designated as sterols, mono- and diglycerides contained sterols, α,α-, and α,β-diglycerides, monoglycerides, and other unknowns.

We present these data as a basis for other researchers because we do not intend further research along these lines. Our data may be useful in the areas of new oil crop development, chemical plant taxonomy, and herbicide utilization.

For commercial use of these seed oils the possible presence of toxic factors should be considered. However, the toxins in

Table III. Percentage Distribution of Lipids in Five Classes Separated by Silicic Acid Chromatography

Species	Hydrocarbons and sterol esters	Triglycerides	Free fatty acids	Sterols, mono- and diglycerides	Polar lipids
Jimsonweed	1.0	85.7	0.3	3.0	10.0
Morningglory	1.0	55.3	0.3	10.8	32.6
Yellow foxtail	1.0	87.2	1.8	4.0	6.0
Giant foxtail	1.0	75.1	4.6	9.2	10.1

Table IV. Percentage Distribution of Fatty Acids in the Triglyceride and Polar Lipid Fractions of Four Species

Species and fraction	Fatty acid							
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Morningglory								
Triglyceride	T	23.1	T	5.9	16.8	49.1	3.7	1.4
Polar lipid	T	24.8	...	5.5	12.7	54.7	2.3	...
Jimsonweed								
Triglyceride	T	12.6	T	2.2	28.3	56.9	T	...
Polar lipid	T	20.3	T	5.1	33.5	41.1	...	T
Giant foxtail								
Triglyceride	...	5.9	...	1.0	18.9	72.2	2.0	...
Polar lipid	T	17.6	T	4.1	17.0	57.2	4.1	T
Yellow foxtail								
Triglyceride	...	5.0	...	1.4	15.7	76.3	1.6	...
Polar lipid	T	17.5	T	2.6	13.3	63.8	2.8	...

seeds such as castor bean and cotton have not prohibited the commercial use of their oils.

The relationship of our work to herbicide utilization has not been thoroughly researched, but it is known that herbicides affect lipolysis in plants. Research on this subject has been reported by Stevens *et al.* (1962) and Penner and Ashton (1967).

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