Fatty Acid Composition of Extract from Pyrethrum Flowers *(Chrysanthemum cinerariaefolium)*

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The fatty acid fraction from an unrefined commercial pyrethrum extract containing 30% "pyrethrins" was isolated and the methyl esters were separated by column chromatography on silica gel into saturated or olefinic nonhydroxy acids and a hydroxy acid fraction. Combined fatty acids represented up to 40% of the pyrethrum extract, approximately 18% being nonhydroxy fatty acids. The latter were examined by gas chromatography and a series of normal saturated acids from C_{14} to C_{30} was identified. The major acids were palmitic, stearic, oleic, linoleic, and linolenic, which together constituted up to 90% of the nonhydroxy fatty acids. Pyrethrum flowers of clonal origin containing different proportions of the six insecticidal constituents had similar fatty acid composition.

Pyrethrum extract is normally manufactured by extracting the ground dry flower heads of the pyrethrum plant (*Chrysanthemum cinerariaefolium*) with a light petroleum solvent. Concentration of the miscella yields an oleoresin which contains about 30 weight % of the insecticidally active constituents, the "pyrethrins," the remaining 70% being largely of unknown composition. Detailed investigation of the noninsecticidal constituents has to date been confined to relatively insoluble by-products of the extraction process—e.g., the elucidation of the structure of pyrethrosin (Barton and de Mayo, 1957; Barton *et al.*, 1960) and, more recently, identification of the structure of "pyrethrol" with taraxasterol (Herz and Mirrington, 1966). This paper reports on the identification and quantitative estimation of the saturated and olefinic nonhydroxy fatty acids (henceforth termed fatty acids) present in pyrethrum extract and flowers.

The presence of fatty acids in pyrethrum extract has long been recognized. Ripert (1934) reported the presence of lauric, palmitic, oleic, and linoleic acids in both the free and combined state, and later Acree and La Forge (1937) reported pyrethrolone palmitate and linoleate in pyrethrum extracts. The Association of Official Agricultural Chemists (1955) method for analysis of pyrethrins depends on separation and estimation of chrysanthemum mono- and dicarboxylic acids derived from "pyrethrin I" (a mixture of cinerin I, jasmolin I, and pyrethrin I) and "pyrethrin II" (a mixture of cinerin II, jasmolin II, and pyrethrin II), respectively. The method recognizes possible interference by fatty acids and includes a step for their removal through the insolubility of their barium salts.

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SAMPLES AND SCOPE OF EXAMINATION

The fatty acid composition of a typical commercial pyrethrum extract was examined in detail, and the major acids were examined in four extracts manufactured at different times of the year. Four samples of pyrethrum flowers examined were of clonal origin—i.e., they were picked from plants obtained by vegetative propagation of a single plant. The clones selected contain different proportions of the six insecticidally active constituents which are characteristic of these clones (Head, 1967). The major fatty acids present in the *n*-hexane extracts of the flowers were determined, and a more detailed examination of their chloroform extracts was undertaken.

EXPERIMENTAL

Flower extracts were prepared by Soxhlet extraction for 6 hours with redistilled *n*-hexane as solvent, and the distribution of the pyrethrins was determined by gas-liquid chromatography (GLC), using electron capture for detection (Head, 1966). The pyrethrins in the commercial extracts were determined by the AOAC (1955) method.

Isolation of Acids. Pyrethrum oleoresin (1 gram) containing 30% pyrethrins was saponified by refluxing under nitrogen with alcoholic 0.5*N* KOH (40 ml.) for $1^1/_2$ hours. Water (300 ml.) was added and the mixture boiled until the volume was reduced to 150 ml. After cooling to room temperature, the solution was treated with 10% barium chloride solution (about 15 ml.), and the insoluble barium salts were filtered off through a bed of Celite. The filtrate and precipitate were separately acidified with HCl and the liberated acids extracted with ether. The acids were then separated from nonsaponifiable matter through their potassium salts and esterified with methanol-BF_3 reagent, using the sealed tube method of de Mann (1967). The yield of methyl esters from the acids having soluble barium

salts was 254 mg., 176 mg. of which, based on the AOAC analysis of pyrethrins, were the mixed esters of chrysanthemum mono- and dicarboxylic acids. The yield of esters from the acids with insoluble barium salts was 381 mg. GLC examination showed that the precipitation of barium salts under the conditions described was in the order of 98%. There was no marked differential solubility between the barium salts of the saturated and unsaturated fatty acids.

Chromatographic Separation of Fatty Acids. The methyl esters of the acids with insoluble barium salts were separated by chromatography on a silica gel column (Mold *et al.*, 1966). The first fraction, which was eluted with 120 ml. of a solvent mixture of ether in light petroleum (15 to 85), yielded 194 mg. of fatty acid methyl esters. Two further fractions, eluted with acetone (100 ml.) and methanol (100 ml.), contained 63 and 126 mg. of material, respectively. The latter had infrared spectra characteristic of hydroxy acids. Complete separation of the nonhydroxy acid methyl esters from the hydroxy acid methyl esters was confirmed by thin-layer chromatography on silica gel. The author further established that on gas-liquid chromatography the methyl esters of chrysanthemum mono- and dicarboxylic acids were eluted before the major fatty acid components and that the presence of the suspected hydroxy acids did not give rise to serious interference. Accordingly, investigations on other commercial extracts and pyrethrum flowers were carried out on the methyl esters of the total acid fraction.

Separation and Identification of Acids. The methyl esters of the fatty acids were analyzed by GLC on an Aerograph 204 gas chromatograph using dual 5-foot × $^1/_8$-inch diameter stainless steel columns and flame ionization for detection. Two types of column packing were employed: polyethylene glycol adipate on acid-washed Chromosorb W 80- to 100-mesh (10 to 90) and Apiezon L on acid-washed Chromosorb W 80- to 100-mesh (5 to 95).

Separation on both polar and nonpolar columns was achieved by temperature programming at 2° per minute with a nitrogen carrier gas flow rate of 35 ml. per minute. The polar column was programmed from 30° to 200° C., which allowed separations up to methyl behenate (n-C_{22}) and the nonpolar column was programmed from 50° to 300° C., allowing separations up to methyl triacontanate (n-C_{30}).

Identification of the components was based on determination on polar and nonpolar columns of retention times of the methyl esters of normal even-numbered acids between C_8 and C_{18}; a graph of retention times against chain lengths of these acids, the line being extrapolated for the higher members found in pyrethrum extract; and position and relative intensity of peaks of samples before and after hydrogenation.

Quantitative measurements were based on the response of pure samples of methyl palmitate and methyl stearate, assuming, as an approximation, an equal weight-response factor for all fatty acid esters identified.

RESULTS AND DISCUSSION

Commercial Pyrethrum Extracts. Results of the detailed investigation of commercial pyrethrum extract are given in Table I. The total fatty acid content of 20.3 weight % found by GLC may be compared with the 18.4% calculated from the weight of acids isolated. The major acids are palmitic, stearic, oleic, linoleic, and linolenic, a composition typical of many seed oils (Hilditch and Williams, 1964). The series of normal acids ranges from C_{14} to C_{30}. The GLC trace of the methyl esters on the nonpolar column before and after hydrogenation is given in Figure 1,*A* and *B*. Identification of unsaturated components between C_{15} and C_{20}, inclusive, should be considered as only tentative, since satisfactory confirmation of minor compounds of this type can be achieved only by sample collection and rechromatography on a polar column before and after hydrogenation (Iverson *et al.*, 1965). The minor peaks preceding some of the saturated esters are attributed to branched acids and are clearly visible in the GLC trace of the hydrogenated sample (Figure 1,*B*).

Table I. Fatty Acid Composition of a Commercial Pyrethrum Extract Containing 30% Pyrethrins

Common Name	GLC Peak Ref.	Code	Acid, Wt. %	Distribution, %
Myristic	1	14:0	0.25	1.2
Pentadecanoic	2	15 (unsat)	0.02	0.1
	3	15 (br)	<0.01	
	4	15:0	0.05	0.25
Palmitic	5	16 (unsat)	0.04	0.2
	6	16 (br)	0.01	0.05
	7	16:0	4.64	22.8
Heptadecanoic	8	17 (unsat)	0.12	0.6
	9	17 (br)	0.02	0.1
	10	17:0	0.13	0.65
Stearic	11	18 (br)	0.04	0.2
	15	18:0	1.71	8.4
Oleic	12	18:1	2.09	10.3
Linoleic	13	18:2	6.62	32.5
Linolenic	14	18:3	2.94	14.5
Nonadecanoic	16	19 (unsat)	0.05	0.25
	17	19 (br)	0.01	0.05
	18	19:0	0.03	0.15
Arachidic	19	20 (unsat)	0.07	0.35
	20	20:0	0.34	1.7
Heneicosanoic	21	21:0	0.04	0.2
Behenic	22	22:0	0.37	1.8
Tricosanoic	23	23:0	0.07	0.35
Lignoceric	24	24:0	0.36	1.8
Pentacosanoic	25	25:0	0.05	0.25
Hexacosanoic	26	26:0	0.15	0.7
Heptacosanoic	27	27:0	0.02	0.1
Octacosanoic	28	28:0	0.07	0.35
Nonacosanoic	29	29:0	<0.01	
Triacontanoic	30	30:0	0.02	0.1
		Total	20.34	

Distribution of the major fatty acids in this extract and in four other commercial extracts is listed in Table II. While there is variation in total fatty acid content, the relative proportions of the major acids are similar.

Pyrethrum Flowers. Distribution of major fatty acids in the n-hexane extracts of flowers from the four pyrethrum clones is given in Table III. The relative proportions of the acids are again similar, though here the proportion of stearic to oleic acid present tends to be greater than that found in commercial pyrethrum extract. This is presumably the result of solubility differences between the

Table II. Fatty Acid Composition of Commercial Pyrethrum Extracts

Date of Production	Pyrethrins Analysis (AOAC) % Py. I	% Py. II	Total	Total Fatty Acids, %[a]	Distribution of Major Fatty Acids, % Palmitic	Stearic	Oleic	Linoleic	Linolenic
Unknown[b]	15.0	15.4	30.4	18.0	25.8	9.5	11.6	36.8	16.3
April 1967 (A)	15.4	14.5	29.9	16.3	28.3	8.6	12.1	39.7	11.3
December 1966 (B)	15.5	15.7	31.2	14.8	28.6	8.9	12.6	36.9	13.0
October 1966 (C)	13.7	15.3	29.0	17.8	26.5	8.3	10.9	38.6	15.7
July 1966 (D)	15.4	16.2	31.6	14.6	27.5	8.3	11.3	38.4	14.5
			Av.	16.3	27.3	8.7	11.7	38.1	14.2

[a] Major components only.
[b] Detailed composition given in Table I.

Table III. Fatty Acid Composition of *n*-Hexane Extract from Four Pyrethrum Clones Having Different Proportions of Insecticidally Active Constituents

Clonal Reference	Pyrethrins, Wt. %, in Dry Flowers[a] Cin. I	Jas. I	Py. I	Cin. II	Jas. II	Py. II	Total	Total Fatty Acids in Dry Flowers, %[b]	Distribution of Major Fatty Acids, % Palmitic	Stearic	Oleic	Linoleic	Linolenic
1708	0.12	0.05	0.36	0.25	0.10	0.46	1.34	0.73	25.3	10.7	9.7	37.9	16.4
4729	0.16	0.09	0.53	0.23	0.13	0.50	1.64	0.89	28.7	9.4	10.1	37.5	14.3
4331	0.10	0.13	0.85	0.05	0.05	0.23	1.41	0.79	27.2	10.6	9.8	35.5	16.9
194	0.16	0.08	0.47	0.31	0.13	0.56	1.71	0.80	24.5	12.1	11.6	37.0	14.8
								Av.	26.4	10.7	10.3	37.0	15.6

[a] Containing approximately 10% moisture.
[b] Major fatty acids only.

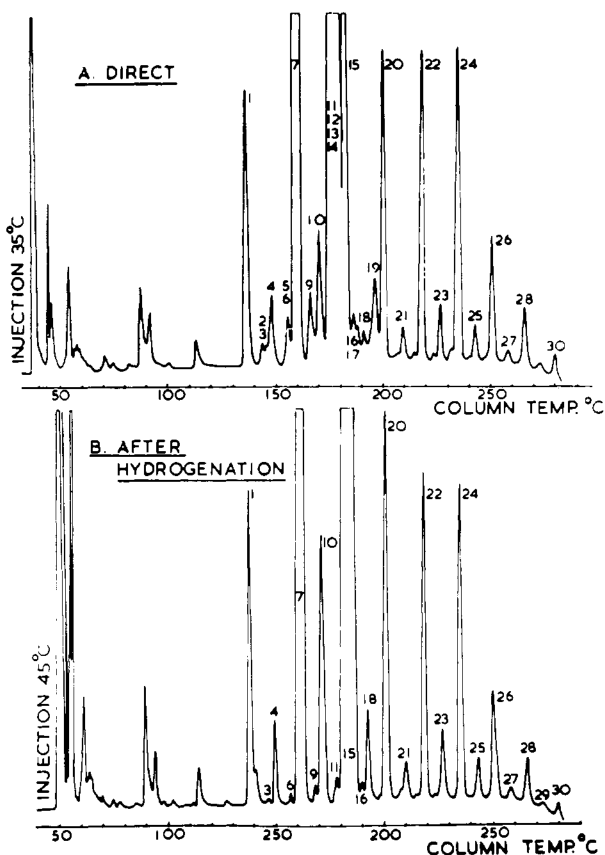

Figure 1. GLC chromatograms of fatty acid methyl esters on Apiezon L before and after hydrogenation

See Table I for peak identification

stearic and oleic esters present in pyrethrum flowers under conditions of laboratory Soxhlet and commercial factory scale extraction. A more complete analysis of the fatty acid composition of flowers from each of these four clones, obtained by examining their chloroform extracts, is shown in Table IV. Again, the relative proportions in

Table IV. Distribution of Fatty Acids in Chloroform Extracts of Four Pyrethrum Clones

Common Name	Code	Clonal Reference Number, Fatty Acids, % 4729	4331	194	1708
Myristic	14:0	1.8	1.4	2.0	1.5
Pentadecanoic	15:0	0.5	1.9	1.0	1.0
Palmitic	16:0	23.3	20.1	19.8	20.0
Heptadecanoic	17:0	0.9	1.1	1.6	2.1
Stearic	18:0	7.3	8.6	9.2	7.8
Oleic	18:1	7.9	6.8	8.0	7.0
Linoleic	18:2	33.0	29.6	28.7	31.0
Linolenic	18:3	13.7	14.2	12.8	13.7
Nonadecanoic	19:0	0.2	0.5	0.4	0.4
Arachidic	20:0	1.6	2.7	2.1	2.4
Heneicosanoic	21:0	0.6	0.8	1.0	0.8
Behenic	22:0	2.4	3.7	3.1	2.7
Tricosanoic	23:0	0.7	0.9	1.1	1.0
Lignoceric	24:0	2.6	3.9	3.8	3.6
Pentacosanoic	25:0	0.4	0.5	0.7	0.7
Hexacosanoic	26:0	1.7	1.7	2.2	2.0
Heptacosanoic	27:0	0.2	0.3	0.4	0.4
Octacosanoic	28:0	0.8	0.8	1.2	1.0
Nonacosanoic	29:0	Trace	Trace	0.3	0.3
Triacontanoic	30:0	0.4	0.5	0.6	0.6
Total fatty acids, wt. %[a]		1.16	1.13	1.04	0.94

[a] Based on dry flowers containing approximately 10% moisture.

which the acids are present are similar although the total fatty acid content varies. These results indicate that knowledge of the fatty acid composition of flowers from a clone does not aid clonal identification and that selection of clonal material for a specific insecticide composition is unlikely to alter the fatty acid composition of the oleoresin.

A comparison of the amount of acids of known constitution present in commercial pyrethrum extract, in relation to the total acid recovery, shows that unknown acids are present to an extent roughly equal to the fatty acid content. Evidence obtained during isolation of the purified fatty acids from pyrethrum extract indicates that these are mainly hydroxy acids, and their further investigation would greatly increase our knowledge of the composition of pyrethrum extract.

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Received for review March 5, 1968. Accepted June 10, 1968. Division of Agricultural and Food Chemistry, 155th Meeting, ACS, San Francisco, Calif., April 1968. Work forms part of a thesis for a higher degree of the University of East Africa.