Plant Response to Pesticides and Environment: Sicklepod (*Cassia tora* L.) Fatty Acid Separation by Gas Chromatography[1]

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Abstract

Fatty acid content of sicklepod (*Cassia tora* L.) leaves and stems was determined by GLC. Major constituents were identified as palmitate, stearate, oleate, linoleate and linolenate at 20.8%, 6.4%, 5.7%, 13.1% and 26.0%, respectively, of the total fatty acids present. The remainder of the fatty acids occurred in shorter or longer homologues and branched chain compounds. Chain lengths up to C_{34} were found.

Introduction

Analysis of the fatty acid composition of sicklepod (*Cassia tora* L.) vegetative structures was necessary to assist in an evaluation of the metabolic response of plants to herbicides. Although seed oil composition of several Indian *Cassia* species have been reported to include linoleic, oleic, palmitic, stearic and lignoceric acids (2,8,9) plus traces of myristic, arachidic and montanic acids, the vegetative fatty acid composition of sicklepod separate from seed oil composition has not been previously reported. Therefore, GLC analyses of the fatty acid content of sicklepod leaves and stems were undertaken.

Materials and Methods

Plant Growth

Three replicates of 100 scarified (conc. H_2SO_4–20 min) seed per replicate were planted in isolated flats in the field and harvested after 14 days.

Extraction

Immediately after harvesting, the monophasic chloroform-methanol-water total lipid extraction procedure described by Bligh and Dyer (1) was utilized on the total fresh weight of leaves and stems for each replicate. Solutions were allowed to stand until the final emulsion separated (usually 24 hr). Total and chloroform volumes were recorded, and a 50 ml chloroform aliquot was taken for analysis.

Esterification

The 50 ml aliquot was centrifuged at 1150 × *g* for 30 min to remove last traces of emulsion; evaporated to 5 ml under N_2 and esterified, separated and stored by the methods published by Jellum and Worthington (3) utilizing 25 ml absol. methanol, 5 ml benzene and 1 ml conc. H_2SO_4.

Thin-Layer Chromatography

Two hundred μl redist. Skellysolve F (pet. ether, bp 30–60 C) were injected into the vial, the sample material dissolved, and 5 aliquots of 20 μl each were applied to previously prepared TLC plates coated with 250 μ Silica Gel G and prewashed once in 2:1 chloroform-benzene solvent. Plate development utilized 2:1 chloroform-benzene and lipid fractions were made visible by the method of Jones et al. (4). Immediately after identification, the methyl ester spot was scraped from the plate and the esters eluted with Skellysolve F into serum vials and the solvent subsequently evaporated under N_2 at ambient temperature. Dry samples were stored at −10 C until analyzed by GLC.

Gas-Liquid Chromatography

A dual hydrogen flame ionization detector GLC (F&M, Model 5751A) equipped with a Moseley 7172 strip chart recorder and a digital integrator (Infotronics, Model CRS-100) was used. Calibration standards utilized for identification purposes were Hormel 2–5, 7–9 (Hormel Institute), Branched Chain Mixes L, 1–4 (Applied Science), and quantitative mixtures K-104, K-107, and K-108 (Applied Science). Columns used were ⅛ in. O.D., 6 ft stainless steel packed with Chromosorb W (AW) (DMCS) bearing

TABLE I
Composition of Sicklepod Vegetative Fatty Acids on DEGS Columns

C_n	Peak No.	Per cent of total fatty acids	Fresh wt. μg/gm
10	1	.4	7.1
11	2	+	.7
12	3	.1	2.8
12:1	4	.2	4.5
13	5	.4	7.3
I 14[a]	8	.5	9.2
14	9	2.4	45.9
14:1		+[c]	
A 15[b]		+	
15	10	.9	17.3
I 16		.2	4.3
16	11	20.8	397.4
16:1	12	.8	14.9
A 17		+	
17	13	1.0	
I 18		+	
18	14	6.4	122.9
18:1	15	5.7	109.2
18:2	17	13.1	250.3
18:3	18	26.0	495.4
19	16	.4	6.9
20		+	
20:1		+	
A 21		+	
21	19	.2	4.9
22	20	.9	18.1
22:1		.8	15.3
A 23		.6	12.1
23	21	.6	10.9
I 24		.3	5.7
24	22	.8	16.2
24:1	23	2.0	38.2
A 25		+	
25		.5	8.8
I 26		.1	1.6
26	24	.9	17.6
26:1	25	2.1	40.1
A 27		.6	10.9
27	26	1.0	18.4
I 28		+	
28	27	1.3	25.5
28:1		1.0	18.6
A 29		+	
29		1.0	19.0
I 30		+	
30		.6	12.4
A 31		3.4	65.6
31		+	
I 32		+	
32		+	
33		+	
34		+	

[a] I—iso.
[b] A—anteiso.
[c] + indicates concentration less than 0.1%.

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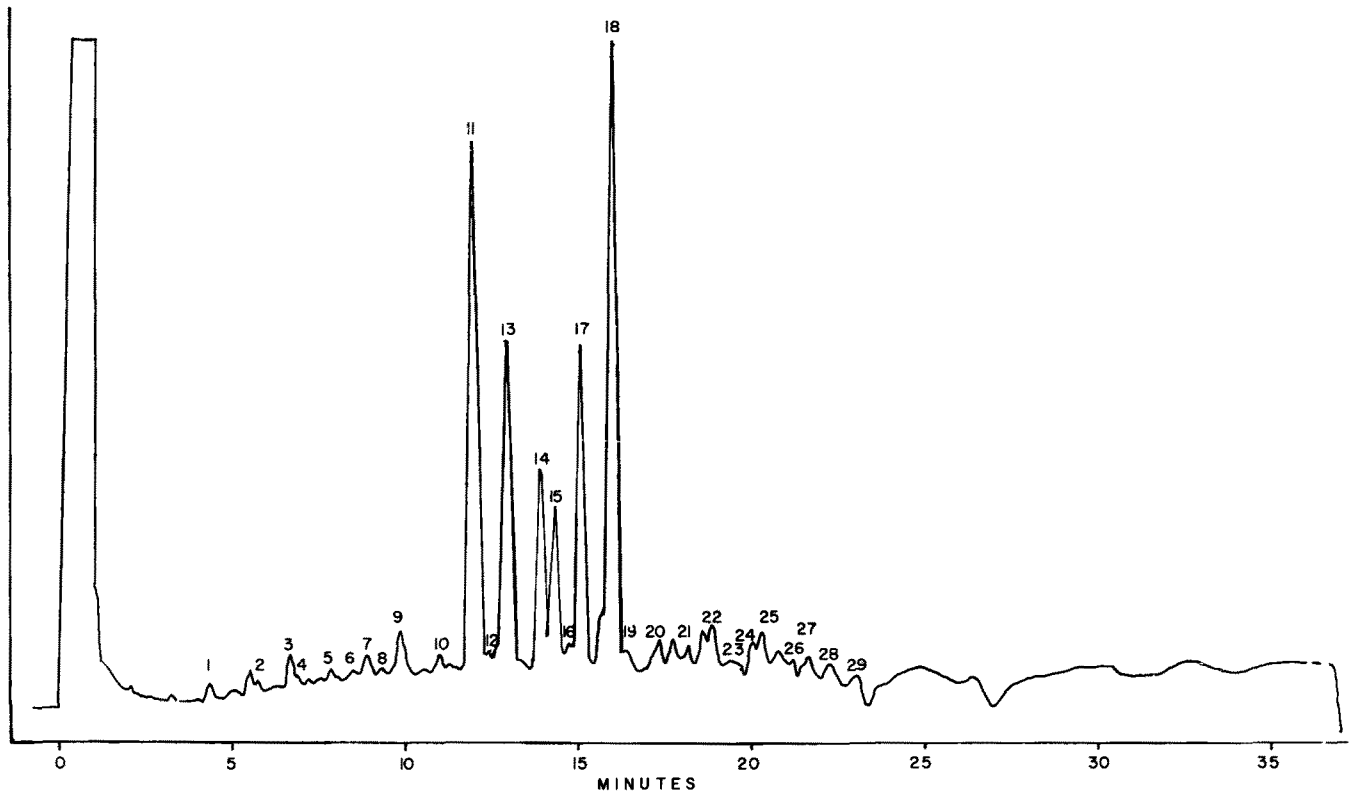

FIG. 1. Vegetative sicklepod fatty acid composition separated on 10% DEGS. Peak identification in Table I. Conditions as listed in text plus: 1) He −75 ml/min; 2) terminal temp. −234 C.

either 5% OV-1 or 10% DEGS (diethylene glycol succinate). Temperature was programmed at 8 C/min from 71 C to 234 C when DEGS was the liquid phase or 330 C when OV-1 was used. An upper limit hold of 15 min was utilized. Chart speed was ½ in./min and attenuation selected for optimum peak responses. Injected aliquots were 2 μl taken from N_2 dried TLC separated fatty acid methyl esters to which 100 μl redist. Skellysolve F had been added. Quantification was attained by addition of 1 mg heptadecanoic acid to the mixture prior to esterification. Values reported are the averages of three replications. Minor unidentified compounds were excluded from quantitative analyses.

Quantitative results with GL 0013 standard (Analabs) agreed with stated composition with an average (triplicate runs) of a relative error less than 3% for major components (> 4% of total mixture) but less than 4% for minor components (< 4% of total mixture).

Results and Discussion

A representative chromatogram of extracted fatty acid methyl esters is shown in Figure 1. Average μg quantities and percentages of the total fatty acids eluted are shown in Table I. On a percentage basis, 78.6% of the fatty acid found was within the series of myristoleate to erucate with major constituents being palmitate (20.8%), stearate (6.4%), oleate (5.7%), linoleate (13.1%) and linolenate (26.0%). Previous analyses[2] indicated that heptadecanoic acid was normally present at ca. 1% of the total fatty acids present.

Several iso- and anteiso-compounds were found. Identification, percentage, composition and concentrations are listed in Table I. Composition and concentration patterns follow those presented by Shorland (7) wherein the concentrations were generally below 1% with a maximum being anteiso C_{31} at 2.2%. The remainder of the fatty acids found were low in concentration and scattered throughout the series. Identifiable components of concentration less that 0.1% are indicated by a + in Table I. Peaks from fatty acids of greater molecular weight than C_{34} have been routinely observed but identification and quantification has not been possible due to a lack of standards.

[2] Wilkinson, R. E., unpublished data.

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