Occurrence of *Cis*-6-Hexadecenoic Acid as the Major Component of *Thunbergia alata* Seed Oil[1]

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ABSTRACT

An unusual series of monoenoic fatty acids constitutes about 85% of the total acids in seed oil from *Thunbergia alata*. The major component in the oil, *cis*-6-hexadecenoic acid (82%), is accompanied by the homologous 4-tetradecenoic (ca. 0.2%) and 8-octadecenoic (1.8%) acids. Another homologous series is represented by 5-tetradecenoic (ca. 0.2%), 7-hexadecenoic (1.8%) and the familiar 9-octadecenoic (4.4%) acids. Traces (<0.1%) of three other acids, 6-tetradecenoic and 10- and 11-octadecenoic, are also present along with palmitic (5.8%), stearic (0.6%) and linoleic (2.2%) acids. Some of the monoenoic acids have not previously been known to occur in seed oils.

INTRODUCTION

Members of the genus *Thunbergia* (family Acanthaceae) are mostly tall perennial climbers widely grown in greenhouses or in the open air in warm climates. *Thunbergia alata* Boj. ex Sims (Black-eyed Susan, but not to be confused with the nonviny *Rudbeckia* spp. also called black-eyed Susan) is often grown as an annual garden plant which flowers in the late summer (1). Gas liquid chromatography (GLC) of *T. alata* seed oil and the methyl esters prepared from it indicated that almost 90% of the acids in the oil contained 16 carbon atoms. Since these acids rarely constitute such a large proportion of a seed oil, the further characterization studies reported here were undertaken.

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TABLE I

Analytical Data on *Thunbergia alata* Seed and Oil[a,b]

Component	% by GLC
14:0	0.1
14:1[4], 14:1[5], 14:1[6]	0.4
15:1	0.1
16:0	5.8
16:1[6]	82.2
16:1[7]	1.8
17:1	0.2
18:0	0.6
18:1[8]	1.8
18:1[9]	4.4
18:2[9,12]	2.2
18:3	0.1
20:0	0.1
20:1	Trace
22:0	0.1

[a]Seed weight, 25 g/1000 g; per cent of oil, 20 db; per cent of protein, 24 db (N x 6.25); HBr equivalent as epoxyoleic acid, 0.7%; refractive index, 1.4625 n_D^{40}; iodine value, 88.7 Wijs, 88.8 calculated.

[b]Fatty acid composition based on area percentages of methyl esters; percentages of positional isomers based on peak areas of ozonolysis fragments (5).

EXPERIMENTAL PROCEDURES

Oil was extracted from ground seed with petroleum ether (bp 30-60 C) and was analyzed by direct GLC in a manner similar to that of Litchfield et al. (2). Methyl esters were prepared from the oil (3) and their equivalent chain lengths were determined by GLC (4) on a Packard 7401 gas chromatograph. Esters were fractionated by chain length by preparative GLC on an Autoprep A-700 gas chromatograph equipped with a 6 ft x 1/4 in. stainless steel column packed with 20% Apiezon L on 60/80 mesh Celite 545. In the column held at 225 C, helium served as the carrier gas at a rate of 180 ml/min. To minimize losses, the collection device on the chromatograph was modified by replacing the collector tip with a 1/4 in. brass, male, Swagelock fitting silver-soldered to the outlet. Fractions were collected in 8 in. x 1/4 in. O.D. glass tubes packed with Adsorbosil CAB and attached to the fitting with 1/4 in. Teflon ferrules and brass nuts.

Preparative thin layer chromatography (TLC) was performed on plates spread with a 0.25 mm layer of Silica Gel G containing 20% $AgNO_3$. The developing solvent was a 50:50 mixture of C_6H_6 and $CHCl_3$. After development the plates were sprayed with an alcoholic solution of 2',7'-dichlorofluorescein so that the bands could be observed under ultraviolet (UV) light. The bands were scraped from the plates and the esters were recovered from the adsorbent with ether.

Combinations of ozonolysis and GLC (5) and of GLC and mass spectrometry (MS) were used to locate double bonds. In the GLC-MS combination, methoxy derivatives were pre-

pared (6) from the mixed esters. These derivatives were introduced into a CEC 21-492-1 mass spectrometer by a GC inlet system (jet sample enricher) from a Packard 7401 gas chromatograph equipped with a 6 ft glass column packed with 3% OV-101 on Gas Chrom Q. The column oven was held at 200 C and the source of the mass spectrometer at 210 C. Infrared (IR) spectra of the samples were recorded on a Perkin-Elmer Model 137 spectrophotometer either from liquid films (sodium chloride disks) or from carbon disulfide solutions (1 mm sodium chloride cells).

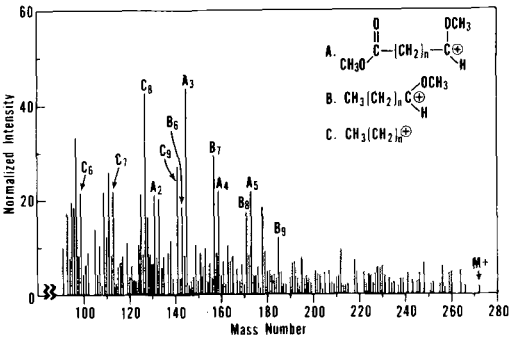

FIG. 1. Mass spectrum of methyl methoxytetradecanoates from *Thunbergia alata* esters by gas liquid chromatography-mass spectrometry. Fragment type is denoted by letter with subscript indicating the number of carbon atoms. Peaks below mass 90 are not essential to the identification of the esters and are not shown.

RESULTS AND DISCUSSION

GLC of *T. alata* oil indicated a range of triglycerides from C_{46} to C_{56} with the major peak at C_{48}. Over 98 (area) per cent of the material eluted from the column was included in the triglyceride region of the curve. IR and UV spectra of the oil were consistent with those of normal seed oils. GLC analysis of the methyl esters prepared from the oil indicated that almost 90% of the fatty acids were 16 carbon acids; small amounts of acids containing 14, 15, 17, 18, 20 and 22 carbon atoms were also in evidence.

Identification of Fatty Acid Esters

Preparative GLC yielded three fractions: C_{14}, C_{16} and C_{18} esters. These fractions were analyzed by GLC to determine the esters present. C_{16} and C_{18} fractions were essentially free of contamination by esters of other chain lengths. The C_{14} fraction was small and contained 20% of other esters carried over during collection and about 20% of BHT that had been concentrated from the ether used to recover the esters (7). IR analysis of the C_{16} and C_{18} fractions showed them to be free of *trans* unsaturation.

The C_{18} fraction was further separated according to degrees of unsaturation by preparative TLC on silver nitrate-impregnated plates. The saturated, monoenoic and dienoic esters were collected, and their purity was again established by GLC. The trace of trienoic ester (Table I) was not recovered.

Since the C_{14} and C_{16} fractions contained essentially no polyunsaturated esters by GLC, these fractions were subjected to ozonolysis-GLC without further fractionation. Of the aldehyde-ester (AE) and aldehyde (A) fragments produced from the C_{16} fraction, C_6 AE and C_{10} A made up 98%. Trace amounts of C_7 AE and C_9 A were also found. This evidence established that the major hexadecenoate had *cis*-6-unsaturation and that there was a small amount of 7-hexadecenoate. The C_{18} monoene fraction gave essentially four peaks totaling 96 area per cent from ozonolysis-GLC. These were C_9 AE, C_9 A, C_8 AE and C_{10} A. The fragments define the parent structures as $18{:}1^9$ and $18{:}1^8$. Traces of fragments which could have arisen from $18{:}1^{10}$ and $18{:}1^{11}$ were also found. The only component found in the C_{18} diene fraction was methyl linoleate.

Ozonolysis of the C_{14} fraction did not provide a clear-cut definition of the parent esters because the large amounts of impurities contributed fragments which were indistinguishable from those from the C_{14} esters. Combinations of the fragments indicated that a $14{:}1^4$, a $14{:}1^5$ and a $14{:}1^6$ could be present. Some of the AE and A formed from these esters also could have come from the 18:1 and 16:1 known to be present in this fraction. Nevertheless, the identities of the C_{14} esters were established by MS of their methoxy derivatives (6). Combined GLC-MS, applied to *T. alata* esters, showed strong peaks for the 4-, 5-, 6- and 7-methoxytetradecanoates (Fig. 1). Although these derivatives could come from only a $14{:}1^4$ and $14{:}1^6$, the relative intensities of the peaks together with ozonolysis results strongly suggest the presence also of a $14{:}1^5$. Reliable quantitative data for the C_{14} monoenes were not obtained. Indications were, however, that the $14{:}1^4$ was the most abundant followed by $14{:}1^5$ and then $14{:}1^6$. Spectra from the methoxyhexadecanoates and methoxyoctadecanoates were also consistent with ozonolysis-GLC results.

Total fatty acid composition and some characteristics of *T. alata* oil are given in Table I. Excellent agreement between the iodine value

derived by chemical means (Wijs) and that calculated from the fatty acid composition indicates that the amounts of unsaturated acids given are substantially correct.

According to the latest review of unusual fatty acids in plants (8), *cis*-6-hexadecenoic acid was not known to exist in seed oils. Since this compilation it has been found as a minor constituent of *Picramnia sellowii* (9) and *Beauprea balansae* (10) seed oils and now as a major constituent in *T. alata* oil. Although the unusual tetradecenoic acids have not heretofore been found in seed oils (8), *cis*-8-octadecenoic acid was recently discovered in many species of the Proteaceae (10).

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