SHORT COMMUNICATIONS

γ-Linolenic Acid in *Acer* Seed Oils

ABSTRACT

The octadecatrienoic acids in *Acer negundo* L. (maple family) seed oil include both 9,12,15- (1%) and 6.9,12- (7%) isomers. The chief monoenoic acids identified were 9-octadecenoic (21%), 11-eicosenoic (7%), 13-docosenoic (15%), and 15-tetracosenoic (7%). Also present is a considerable amount of 9,12-octadecadienoic acid. Investigation of ten other Aceraceae revealed their seed oils to have a similar fatty acid composition.

INTRODUCTION

Hopkins and coworkers (1) reported fatty acid composition and positions of unsaturation in many acids from a series of Aceraceae seed oils. They indicated that these seed oils resemble those from Cruciferae species because they contain large amounts of docosenoic and tetracosenoic acids. However, the publication (1) does not mention the presence of C_{22} and C_{24} saturated acids or C_{20} and C_{22} dienoic acids; neither does it note the positions of unsaturation in the octadecatrienoic acids in the Aceraceae seed oils.

EXPERIMENTAL PROCEDURES

Seed oils were extracted and analyzed as previously described (2,3). Methyl esters of the fatty acids were prepared (4) and analyzed by both gas liquid chromatography (GLC) and thin layer chromatography (TLC) (2,5). Methyl esters of *Acer negundo* L. were separated according to degree of unsaturation by preparative TLC on plates coated with a 1 mm thick layer of Silica Gel G containing 20% silver nitrate. The plates were developed with benzene, which separated the esters into five fractions (I-V). Each fraction was subsequently analyzed by GLC. Olefinic bond positions were located either by GLC-ozonolysis (6) procedures or by combined GC-MS (mass spectrometry) of methoxy derivatives (7). Infrared (IR) and ultraviolet (UV) absorption of the oils were measured as previously described (8). Nuclear magnetic resonance (NMR) data of the trienoic esters from *A. negundo* were obtained with a Varian HA-100 spectrometer from a deuteriochloroform solution containing 1% tetramethylsilane as internal standard.

RESULTS AND DISCUSSION

Fraction I from $AgNO_3$-TLC of *A. negundo* methyl esters contained mostly even chain saturated compounds ranging from C_{14} to C_{20}. The monoenes in fraction II ranged from C_{18} to C_{24}, and fraction III was 98% C_{18} dienes. Fractions IV and V were essentially all trienes, fraction IV being rich in 9,12,15-18:3 and fraction V in 6,9,12-18:3. The NMR spectrum of fraction IV exhibited a well defined symmetrical triplet at δ 1.0 defining the terminal methyl protons as β to an olefinic bond, whereas fraction V showed an unsymmetrical triplet representing the terminal methyl protons at δ 0.9 and indicating those protons were remote to olefinic unsaturation. Mass spectra of the trienoic isomers were consistent with NMR data. Mass spectra of fractions IV and V were characteristic of 9,12,15-18:3 and 6,9,12-18:3, respectively, as compared to those reported by Holman et al. (9).

Positions of unsaturation in the trienoic isomers were confirmed by GLC of the reduced ozonides (6). The major ozonolysis product from fraction IV (9,12,15-:18:3) was the C_9 aldehyde-ester (9AE); C_3 aldehyde (3A) fragments expected from this isomer are not observed under these conditions (6). Fraction V yielded 6AE and 6A in a ratio of 1:1. These ozonolysis products firmly establish fraction V to be 6,9,12-18:3.

Double bond positions of the components in fractions II and III were identified by GC-MS of the methoxy derivatives (7). Mass spectra of the methoxylated monoenoic esters in fraction II all exhibit major ions at m/e 157 and at m/e 171 resulting from the fragment

$$^{+}\text{CH}(\text{OCH}_3)-(\text{CH}_2)_n-\text{CH}_3$$

(n = 7,8), respectively, indicative of $\omega 9$ unsaturation. In addition, the ions representing the corresponding fragments containing the ester moiety

$$\left(\text{O}=\text{C}(\text{OCH}_3)-(\text{CH}_2)_x-\overset{\oplus}{\text{C}}\text{H}(\text{OCH}_3)\right)$$

TABLE I

Composition of *Acer* Seed Oils

Species	Oil (% by wt)	Fatty acid (area percent by gas liquid chromatography)													HBr reactive[a] acids (%)
		16:0	18:0	18:1	18:2	18:3	18:3[b]	20:0	20:1	20:2	22:0	22:1	22:2	24:0	24:1	
A. buergerianum Miq.[c]	15.0	5.0	3.0	27.0	34.0	0.3	1.0	0.3	5.1	tr[d]	2.6	12.7	0.7	2.5	5.2	1.2
A. ginnala Maxim.	12.5	4.0	2.3	23.9	37.4	1.0	3.5	0.2	5.8	tr	0.7	14.0	---	0.3	6.0	2.2
A. heldreichii Orph. & Boiss	13.2	6.0	2.0	26.0	34.6	2.3	2.3	0.2	5.5	0.2	0.6	11.5	tr	0.2	4.4	3.9
A. hyrcanum Fisch. & Mey.[e]	16.0	7.0	2.0	26.0	35.0	0.2	1.5	0.1	5.6	tr	0.6	12.0	0.7	0.6	6.0	1.3
A. monspessulanum L.	34.7	5.0	3.0	29.0	34.0	0.5	1.5	0.2	7.0	0.2	0.7	13.0	---	0.5	5.3	1.4
A. negundo L.	9.0	4.0	1.0	21.0	34.0	1.0	7.0	0.3	7.0	0.2	0.9	15.0	tr	0.3	6.8	0.9
A. platanoides L.[c,e]	8.1	10.0	2.0	25.0	35.0	0.8	1.6	0.2	5.0	0.2	1.0	11.0	0.5	1.0	4.7	1.4
A. pseudoplantinus L.[e]	17.4	10.0	2.0	28.0	30.0	1.0	1.4	0.2	4.0	0.2	0.7	12.0	tr	0.6	6.4	3.6
A. saccharum Marsh[c,e]	17.0	6.4	3.2	28.2	36.6	0.5	1.8	0.6	6.6	tr	0.7	10.3	0.3	tr	3.8	2.0
A. tataricum L.	21.3	3.0	2.0	18.0	35.0	0.8	6.0	0.2	4.6	0.2	0.8	18.0	0.5	0.6	10.3	1.1
A. truncatum Bunge	18.0	5.0	3.0	27.0	38.0	0.4	1.0	0.2	7.5	0.2	0.9	11.8	0.6	0.8	3.6	0.5

[a]Material reacting to hydrogen bromide and calculated as epoxy oleic acid.

[b]γ-Linolenic.

[c]Also found 0.5% hexadecenoic.

[d]tr = Trace, <0.1.

[e]Also found 0.1-0.3% heptadecanoic.

from each monoene were observed. The spectrum of the methoxylated diene in fraction III was dominated by intense ions at m/e 129, 215, 115, and 201. These ions represent the methoxy derivatives of methyl 9,12-octadecadienoate.

The fatty acid composition of the 10 other Aceraceae seed oils is given in Table I. The percentages are based on GLC of the mixed esters. Although not analyzed as rigorously as *A. negundo*, all these oils appear to contain γ-linolenic acid as indicated by equivalent chain lengths (5) of 17.2 (Apiezon L. column) and 19.3 (Silar 5CP column).

In addition to the usual long chain fatty acids, the seed oils contain small quantities of material reacting to hydrogen bromide (Table I). They vary from 0.5 to 3.9% when calculated as epoxyoleic acid (2,3).

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Effect of Transplanted Human Ovarian Cancer Tissue on Liver Lipid Metabolism of Nude Mice

ABSTRACT

The changes in the lipids of liver tissues of nude mice with and without transplanted human cancerous tissues were studied to clarify the effect of transplanted human tumor tissues on host liver lipid metabolism. The total lipid was extracted and separated into phospholipid, triglyceride, and other fractions by thin layer chromatography. The amounts of methyl esters of fatty acids of each lipid fraction were measured by quantitative gas liquid chromatography after each lipid fraction had been subjected to methanolysis by 5% HCl-methanol. The phospholipid content of liver tissues of six tumor bearing nude mice was increased and the triglyceride content decreased in comparison with these fractions in three control nude mice. The ratio of the phospholipid fatty acid content to the triglyceride fatty acid content (phospholipid:triglyceride[PL:TG]) of six tumor bearing nude mice was distributed between 7.6 and 33.5, whereas PL:TG ratios of three control nude mice were distributed between 1.7 and 3.8. This result was similar to that reported for human liver tissues of patients with malignant neoplastic disease, indicating that nude mice with transplanted human cancer may be useful for clarifying the mechanisms of the lipid-chemical changes of liver tissues of patients with malignancies.

INTRODUCTION

In 1971, lipid-chemical changes of biopsied human lvier tissues, which might be useful for the diagnosis of gastroenterological and other cancers, including early cases of gastric cancer, were reported by Nakazawa and Yamagata (1).

Recently, reports by Flanagan (2) and Pantelouris (3) indicated that human malignant tumors are transplantable into the nude mouse.