TRANS-2-HEXADECENOIC ACID IN *ASTER SCABER* SEED OIL

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ABSTRACT.—Methyl *trans*-2-hexadecenoate has been isolated from *Aster scaber* seed oil after transesterification with boron trifluoride in methanol. Column and high-performance liquid chromatography were used for its isolation; chromatography and infrared, nuclear magnetic resonance and mass spectrometry were used for its characterization.

During an examination of the fatty acid composition of seed oils of the Compositae, 1% or less of an unknown component was noted in many of the species containing 5–10% *trans*-3 acids. This component had gas chromatographic retention times suggestive of a heptadecenoic acid. However, it exhibited an apparent molecular ion of *m/e* 268 instead of 282 when analyzed by mass spectrometry. This component was isolated from *Aster scaber* (Thunb.) seed oil and characterized as *trans*-2-hexadecenoic acid. *trans*-2 Acids are not commonly found in nature, although *trans*-2 fatty esters have been isolated from pear volatiles (1) and *trans*-2 monoenoic acids and esters have been reported in bean pod exudate (2). To our knowledge, this is the first finding of a *trans*-2 acyl group in seed oils.

EXPERIMENTAL

Ground seed was extracted with hexane in a Soxhlet apparatus for 24 hr. Acyl groups were converted to methyl esters with 10% BF_3 in methanol and subsequently analyzed by gas chromatography (gc) on a Hewlett-Packard 7610 gas chromatograph equipped with a dual flame ionization detector and two glass columns, each run independently. One column was packed with 5% Apiezon L, the other with 5% LAC-2-R 446 (3, 4). Methyl esters were initially separated by the degree of unsaturation on a dry 42 cm x 1.5 ID column of 45 g of 20% $AgNO_3$ on silica and eluted with 400 ml of isooctane. Fractions (5 ml) were collected, and progress of the chromatography was monitored by gc. The unknown ester was then isolated from fractions containing saturates and *trans*-3 monoenes on a Partisil M-9 10/50 ODS-2 column (Whatman Inc., Clifton, N.J.) in a Waters ALC high performance liquid chromatograph equipped with a differential refractometer with acetonitrile at 2.5 ml/min as eluting solvent. Individual peaks were collected and analyzed by gc.

The gas chromatography-mass spectrometry (gc-ms) and computerized data acquisition system (5) included a 3′ x ⅛″ glass column packed with 3% OV1 on Gas Chrom Q operated isothermally at 200°.

The infrared (ir) spectrum was obtained in CS_2 solution in a 1-mm NaCl cell. The proton magnetic resonance spectrum (pmr) was taken in $CDCl_3$ with a Varian XL-100 spectrometer. Hydrogenation of the isolated material was accomplished by bubbling H_2 through an ethanol solution of the isolate for 15 min with 10% palladium-on-powdered charcoal as catalyst.

RESULTS AND DISCUSSION

A. scaber seeds weighed about 1.5 mg each and contained 30.3% oil (dry basis). Gc of methyl esters prepared from *A. scaber* oil indicated the following composition (area percent): 16:0, 3.9%; 16:1^{3t}, (16:1^{3t} = 16 carbon monoenoic fatty acid with *trans* unsaturation at the 3 position) 10.2%; 17:0, 0.1%; 18:0, 1.7%; 18:1 (unless otherwise specified, unsaturated acids are those commonly found in seed oils) 12.6%; 18:1^{3t}, 1.9%; 18:2, 50.6%; 18:$2^{3t,9c}$, (6) 3.9%; 18:3, 0.3%; 18:$3^{3t,9c,12c}$, 10.7%; 20:0, 0.3%; 20:1, 0.1%; 20:1^{3t}, 1.4%; 21:0, 0.7%; unknown component

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with ECL (3) values of 16.6 on Apiezon L. and 17.5 on LAC-2-R 446, 1.1%; other, 0.5%.

Saturates and *trans*-3-monoenes were present in the $AgNO_3$ column fractions which contained the unknown ester. Dienoic, trienoic, and *cis*-monoenoic esters were retained on the column.

The unknown ester was isolated from the saturated and *trans*-3 esters by hplc, eluted between methyl *trans*-3-hexadecenoate and methyl palmitate. The retention volumes relative to methyl palmitate were as follows: $16:1^{3t}$, 0.705; $16:1^{2t}$, 0.918; 16:0, 1.000; $18:1^{3t}$, 1.046; 18:0, 1.448. Prior separation with the $AgNO_3$ column was necessary because methyl oleate (relative retention volume 0.888)could not be completely resolved from $16:1^{2t}$ by hplc.

Gc analysis of the esters prepared from the isolated triglycerides revealed the same relative abundance of $16:1^{2t}$ as found in the whole oil, indicating that the $16:1^{2t}$ is indeed a triglyceride constituent.

The mass spectrum of the isolated ester was analogous to that of synthetic $18:1^{2t}$ (7) with ions at *m/e* 43, 55, 74, 87, 113, 127, and 141. Ions were found at *m/e* 152, 194, and 236 that correspond to loss of 116, 74, and 32 from the molecular ion, *m/e* 268 (7). The McLafferty rearrangement ion, *m/e* 74, is usually more intense than *m/e* 87 in long-chain monoenoic methyl esters (8). However, in the spectrum of the isolated $16:1^{2t}$, *m/e* 87 was considerably stronger than *m/e* 74. This relationship appears to be characteristic of *trans*-2 esters (7 9).

The pmr spectrum included signals commonly found in methyl esters of unbranched long chain fatty acids: a singlet at δ 3.73 from CH_3–O, a triplet at δ 0.88 from C–CH_3 and a broad single line at δ 1.26 from C–(CH_2)–C. A complex multiplet was seen at δ 2.19 from the protons on the carbon alpha to the double bond. Doublets of triplets were present at δ 5.82 from $C=CH-CO_2CH_3$ and δ 6.98 from C–CH=C. The *J* values for the triplets were 1.5 and 7, respectively. Upon irradiation of the δ 2.19 signal, the δ 5.82 and δ 6.98 signals became doublets with *J* values of 16 Hz, indicative of a *trans* double bond. The chemical shifts agreed with those published for a synthetic methyl *trans*-2-octadecenoate (10), with the exception of a 0.10 to 0.15 upfield shift of the 6.98 and 5.82 signals and an equivalent downfield shift of the 1.26 signal probably resulting from solvent effects ($CHCl_3$ vs. CCl_4). The pmr spectrum of the *trans*-2 ester differs from the *cis*-2 in that the alkenyl coupling constant is larger, and the C–CH=C signal is shifted downfield 0.7 ppm (10).

The infrared (ir) spectrum of the $16:1^{2t}$ showed *trans* absorbance at 980 cm^{-1} and matched that of synthetic methyl *trans*-2-hexadecenoate (11). The product obtained from hydrogenation of the isolated $16:1^{2t}$ had ECL values of 16.0 by gas chromatography on both Apiezon L. and LAC-2-R 446 columns. The mass spectrum of the hydrogenated ester was identical to that of authentic methyl palmitate. The chromatographic and spectroscopic data, when taken as a whole, conclusively identify the unusual component of *A. scaber* seed oil as *trans*-2-hexadecenoic acid.

ACKNOWLEDGMENTS

L. W. Tjarks provided the pmr spectrum.

Received 30 June 1980

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