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SHORT COMMUNICATION

HIGHLY OPTICALLY ACTIVE TRIGLYCERIDES OF *SEBASTIANA LIGUSTRINA* AND RELATED SPECIES

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Abstract—The highly optically active triglycerides (OAT) of the seed oil of *Sebastiana ligustrina* were isolated and characterized. As with the similar known triglycerides from *Sapium sebiferum*, the optically active 8-hydroxy-5,6-octadienoic acid is esterified to glycerol and to 2,4-decadienoic acid. The other two acyl moieties in the triglycerides of both these species are common fatty acids, principally 16:0, 18:1, 18:2 and 18:3 which occur in pairs ranging in degree of unsaturation from 16:0, 18:1 to 18:3, 18:3. Analyses of lipids of parts of the related species *Stillingia sylvatica* and *S. texana* revealed no allene or carbonyl conjugation, and thus by implication no OAT. A substance having IR absorptions characteristic of allenes and carbonyl conjugated dienes, but different from OAT, was found in the stems of *S. texana*. The seeds of S. sebiferum are known to contain OAT, but it was not found in fresh leaves.

INTRODUCTION

PREVIOUSLY, we elucidated the structure of the highly optically active triglycerides (OAT) of the seed oil of *Sapium sebiferum* (Euphorbiaceae), an import from China. These triglycerides contain 2,4-decadienoic acid esterified to the hydroxyl group of 8-hydroxy-5,6-octadienoic acid which is, in turn, esterified to a primary hydroxyl group of glycerol.[1] Recently, Christie[2] has shown that the allenic component is esterified at position 3 of glycerol. We now report a search for the same substance in three closely related American species, plus additional studies on the OAT from *S. sebiferum*.

RESULTS AND DISCUSSION

Sebastiana lingustrina

The total oil from the seeds of *S. ligustrina* had an optical rotation of $[\alpha]_D^{24}$ −4·7°, and the optically active component of the oil was separated by column chromatography on silica gel and found to have $[\alpha]_D^{24}$ −17·6°. Comparable values for the oil and the OAT of *S. sebiferum*[3,4] were −5 to −6° and −22°. To avoid loss of volatile methyl esters, OAT from *S. ligustrina* was interesterified in a sealed tube in 1 ml of 5% HCl in anhydrous methanol under N_2 at 100° for 1·5 hr. The methyl ester composition was found to be, in area per cent, <0·4% A (ECL 12·3), 19·5% 10:2 (ECL 13·0), 1·7% B (ECL 13·8), 0·4% C (ECL 14·4). 0·7% D (ECL 14·9), 2·2% E (ECL 15·3), 6·8% 16:0, 3·3% 18:0, 6·6% 18:1, 39·8% 18:2 and 18·8% 18:3. The IR spectrum of the OAT from *S. ligustrina* was the same as that of the OAT from *S. sebiferum* and showed an allenic structure to be present.[1]

[1] H. W. SPRECHER, R. MAIER, M. BARBER and R. T. HOLMAN, *Biochem.* **4**, 1856 (1965).
[2] W. W. CHRISTIE, *Biochim. Biophys. Acta* **187**, 1 (1969).
[3] R. MAIER and R. T. HOLMAN, *Biochem.* **3**, 270 (1964).
[4] P. T. HUANG, R. T. HOLMAN and W. M. POTTS, *J. Am. Oil Chem. Soc.* **26**, 405 (1949).

To isolate the allenic moiety, OAT of *S. ligustrina* was subjected to $LiAlH_4$ reduction followed by acetylation with Ac_2O. Separation on a silica gel column revealed the presence of a substance other than fatty alcohol acetates which had the same IR spectrum as 2,3-octadiene-1,8-diol diacetate derived from the OAT of *S. sebiferum*. The mass spectrum of this compound was identical to that of 2,3-octadiene-1,8-diol diacetate obtained from the oil of *S. sebiferum*. Hydrogenation yielded a substance which had the same IR spectrum and retention time on a GLC column as did synthetic octane-1,8-diol diacetate and the hydrogenated 2,3-octadiene-1,8-diol diacetate from *S. sebiferum*.[1] The 2,3-octadiene-1,8-diol diacetate derivatives of OAT from both *S. ligustrina* ($[\alpha]_D^{24}$ −29·0°) and *S. sebiferum* ($[\alpha]_D^{20}$ −38°) have strong optical activities. The preponderance of chemical and spectral evidence for identity suggests that partial racemization or isomerization to the acetylene is responsible for the discrepancy between their respective specific activities.

An incomplete interesterification was carried out on a 500 mg sample of OAT in 180 ml of 5% HCl in anhydrous methanol under N_2 at room temp. The reaction mixture was periodically monitored by TLC. At 4 hr, a thin-layer plate of the lipids showed five prominent spots. Column chromatography of the lipid soluble extract separated the five components. The IR spectrum of Fraction I (fastest migration) revealed no allene. IR and mass spectra and TLC suggested it to be the methyl esters of the ordinary fatty acids. The IR and mass spectra of Fraction II were almost identical to those of the methyl ester of the ten carbon acid esterified to the eight carbon hydroxy acid obtained from *S. sebiferum* ($[\alpha]_D^{2.0}$ −46°). Fraction II had a specific rotation of $[\alpha]_D^{24}$ −28·6°. Interesterification and acetylation yielded a very polar compound whose IR and mass spectra were identical to those of the methyl 8-acetoxy-5,6-octadienoate derived from the OAT from *S. sebiferum*. Fraction III had an IR spectrum, rate of migration in TLC analyses and specific rotation identical to those for the OAT itself. Fraction IV was quite polar and contained no allene grouping detectable by IR spectroscopy, but it had a specific rotation of $[\alpha]_D^{24}$ +14·3° and an IR absorption in the OH region. Its mass spectrum showed fragments at *m/e* 267, 265, 263 and 261, corresponding to acyl fragments from glycerides of the fatty acids, 18:0, 18:1, 18:2 and 18:3. TLC, IR and mass spectral data suggest Fraction IV to be diglycerides of common acids but its optical rotation is of unexpected sign and magnitude.[5] Fraction V was almost identical to that of OAT of *S. ligustrina* except for an hydroxyl absorption at 2·9 μ, and the fraction had a specific rotation of $[\alpha]_D^{24}$ −2·3°. Thus, Fraction V probably was a diglyceride which contained the eight carbon hydroxy acid esterified to decadienoic acid.

These observations show that 2,4-decadienoic acid is esterified to the hydroxyl group of 8-hydroxy-5,6-octadienoic acid which is, in turn, esterified to glycerol, was as found in the OAT of *S. sebiferum*.

TLC on plates impregnated with $AgNO_3$ separated OAT from *S. ligustrina* into 9 components made visible by spraying with 2,7-dichlorofluorescein or Rhodamine G and viewing in UV light. Fatty acid compositions of these were determined by GLC analysis. The principal components had the following combinations of common fatty acids: (1) the farthest migrating spot on TLC, 16:0, 18:1, (2) 16:0, 18:2, (3) 18:1, 18:2; (4) 18:2, 18:2; (5) 16:0, 18:2, 18:3; (6) 18:2, 18:3; (7) 18:3, 18:3; (8) 16:0, 18:2; (9) 16:0, 18:3. Fraction 5 probably was contaminated by neighboring fractions, and fractions 8 and 9 perhaps represent polar decomposition products formed during the analysis. GLC analyses of all cell fractions revealed the presence of five minor components A–E and 10:2. Fully hydrogenated OAT recrystallized twice from acetone had a m.p. range of 38–44°.

[5] J. C. SOWDEN and H. O. L. FISCHER, *J. Am. Chem. Soc.* **63**, 3244 (1941).

To identify the minor unknown fatty acid components, A–E, the composite methyl esters from OAT were examined with a gas chromatograph directly coupled to a mass spectrometer. The column was 120 cm × 3 mm packed with 20% EGS and was programmed between 100 and 205°. The mass spectrum of the 10:2 was identical to that published previously.[6] Component A, migrating ahead of 10:2, was identified as 8:3 which could have been derived from methyl hydroxyoctadienoate by loss of water or from methyl methoxyoctadienoate by loss of methanol. Component B, following 10:2, was an isomer of 10:2, (perhaps t,t–10:2) having a mass spectrum which differed only in relative intensities from that of the dominant *trans*-2, *cis*-4-decadienoate isomer.[7] Components C and D had molecular ions of 188, but their structures could not be deduced. The mass spectrum of component E exhibited no molecular ion, but had prominent ions at *m/e* 152 and 153, suggesting the loss of methanol and of OCH_3 from a molecular ion of 184. The fragmentation pattern suggests the structure CH_3—O—CH_2—CH=C=$CH(CH_2)_3$—$COOCH_3$, which could have been produced in the methylation. These minor components are probably derived from the C_8 and C_{10} moieties of OAT. No evidence could be found for 12:2 which has been previously reported to be present in the seed oil of *S. ligustrina*.[8]

Sapium sebiferum (Stillingia sebiferum *previously considered*)

Individual triglycerides were isolated by $AgNO_3$ TLC from the OAT of *S. sebiferum*. Fatty acid analysis revealed, in addition to 10:2 and minor components A–E, the following fatty acid combinations: (1 and 2) 16:0, 18:1; (3) 16:0, 18:2; (4) 18:1, 18:2, (5) 18:2, 18:2; (6) 18:1, 18:2, 18:3 (contamination?); (7) 18:2, 18:3. Gas chromatography and mass spectrometry of the methyl esters of OAT revealed that the minor components A–E were the same as those found in OAT from *S. ligustrina*. Fully hydrogenated OAT recrystallized twice from acetone had a m.p. range of 38–47°.

The similarities of the products of methanolysis and of other derivatives of the OAT preparation from *S. ligustrina* and *S. sebiferum* show that the structures are alike, and suggest that the strong optical activity in OAT from *S. ligustrina* is probably due to the allenic structure also. OAT preparations from the two species differ principally in the proportions of the individual triglycerides, all of which bear 2,4-decadienoic acid esterified to 8-hydroxy-5,6-octadienoic acid which is in turn esterified to glycerol.

Fresh leaves from *S. sebiferum* were extracted with $CHCl_3$–MeOH, and the total lipids were separated by silic acid column chromatography into 13 recognizable fractions. Several of these substances had R_fs similar to OAT on TLC and exhibited UV absorption, but their IR spectra revealed no allene absorption. Thus, no evidence was found that the complex mixture of lipids in the leaves contained OAT.

Stillingia sylvatica

TLC analysis of lipids from seeds and shucks revealed no components with R_fs similar to OAT. GLC analysis of the methyl esters of fatty acids from total lipids revealed no 2,4-decadienoic acid.

Stillingia texana

Analysis of the fatty acids of the total lipids of either roots or leaves by GLC–mass

[6] R. T. HOLMAN and J. J. RAHM, in *Progress in the Chemistry of Fats and Other Lipids*, Vol. IX, p. 13, Pergamon Press, Oxford (1971).
[7] L. CROMBIE, *J. Chem. Soc.* 1007 (1955).
[8] R. T. HOLMAN and D. P. HANKS, *J. Am. Oil Chem. Soc.* **32**, 356 (1955).

spectrometry revealed no 2,4-decadienoic acid, whereas this acid was detected in the lipids of the stems. The total lipids of the stems were fractionated by preparative TLC into nine fractions which were subjected to IR spectral analysis. One fraction exhibited the absorptions characteristic of allenes and of carboxyl conjugated dienes. The mass spectrum of this fraction was quite unlike that of OAT and could not be interpreted. Hydrogenation of this fraction produced a substances whose mass spectrum contained peaks at *m/e* 155 and 171 characteristic of decanoic acid, and at *m/e* 267, 341 and 395 characteristic of stearic acid in glycerides.[9,10] A prominent unidentified peak occurred at *m/e* 467. Prominent peaks at *m/e* 607 and 637 also occur in the mass spectrum of hydrogenated OAT from *S. sebiferum*.[1] In the latter spectrum these correspond to the ions $(M-C_9H_{19}COOC_7H_{14}COO]^+$ and $[M-C_{17}H_{35}COO]^+$, respectively, whose elemental compositions had been determined by high resolution measurements. The general correspondence of the two spectra is taken as evidence that a substance having some relationship to the structure of OAT occurs in the stem lipids of *S. texana*.

These studies show that of the four species analysed from three closely related genera, only *S. sebiferum* and *S. ligustrina* have OAT in the seed oil. In this biochemical respect, *S. sylvatica* and *S. texana* are not, therefore, closely related to *S. ligustrina* and *Sapium* (*Stillingia*) *sebiferum*.

EXPERIMENTAL

Seeds from *Sebastiana ligustrina* were collected in the Big Thicket of east Texas. Leaves of *Sapium sebiferum* (Chinese tallow tree) were collected and preserved in chloroform–methanol at College Station, Texas. Seeds and shucks of *Stillingia sylvatica* (Queen's Delight) were collected in Brazos County, Texas. Whole plants of *Stillingia texana* were collected in the vicinity of Fort Hood, Texas. Lipids were extracted repeatedly from the ground materials with light petroleum (b.p. 30–60°) or with $CHCl_3$–MeOH (2:1). The lipids from each sample were separated using TLC and column chromatography as described previously.[3]

IR spectra were recorded with a Beckman IR-8 grating instrument on CCl_4 solutions, or with a Perkin–Elmer Model 21 on films or on CCl_4 and CS_2 solutions. Mass spectra were recorded using a Hitachi–Perkin–Elmer RMU6D single focusing instrument equipped with a direct insertion sample inlet system and with a directly coupled gas chromatograph. Optical rotations were measured with a Bellingham–Stanley polarimeter at the sodium line in CH_2Cl_2 solution in a 5 cm cell.

GLC was performed using either an F&M 810 or a Beckman GC2A chromatograph equipped with hydrogen flame detectors. The former instrument was fitted with a 180 cm × 4 mm o.d. column packed with 20% EGS plus 2% H_3PO_4 on 100–200 mesh Gas Chrom P, and the latter with a 180 cm × 6 mm o.d. column packed with 20% EGS on 100–110 mesh Anachrome ABS. Identification of components was made by comparison with authentic standards and by equivalent chain length (ECL). Thin-layer plates were spread with a slurry of Silica Gel G or H, or with Silica Gel G plus aqueous $AgNO_3$.[11]

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[9] W. M. LAUER, A. J. AASEN, G. GRAFF and R. T. HOLMAN, *Lipids* **5**, 861 (1970).
[10] A. J. AASEN, W. M. LAUER and R. T. HOLMAN, *Lipids* **5**, 869 (1970).
[11] C. B. BARRET, M. S. J. DALLAS and F. B. PADLEY, *Chem. & Ind.* 1050 (1962).

Key Word Index—*Sebastiana lingustria*; *Stillingia*; Euphorbiaceae; lipids; optically active triglycerides; 8-hydroxy-5,6-octadienoic acid; 2,4-decadienoic acid.