Compounds, Elderfield, R. C., Ed., New York, Wiley, p 207.
Schneider, W. C. (1948), *J. Biol. Chem. 176*, 259.
Slater, E. C., and Kemp, A., Jr. (1964), *Nature 204*, 1268.
Taurog, A. (1963), *Endocrinology 73*, 45.
Ter Welle, H. F., and Slater, E. C. (1964), *Biochim. Biophys. Acta 89*, 385.

Aliphatic Diether Analogs of Glyceride-Derived Lipids. III. Synthesis of Dialkenyl and Mixed Alkylalkenylglycerol Ethers*

B. Palameta† and M. Kates

ABSTRACT: L-2,3-Di-*O*-*cis*-9′-octadecenylglycerol (L-2,3-dioleylglycerol, III) was synthesized by alkylation of D-3-*O*-triphenylmethylglycerol with 1-bromo-*cis*-9-octadecene (oleyl bromide) and potassium hydroxide in boiling benzene, followed by acid hydrolysis of the triphenylmethyl group. L-2-*O*-Octadecyl-3-*O*-*cis*-9′-octadecenylglycerol (VII) was synthesized by blocking the 1 position of L-3-*O*-*cis*-9′-octadecenylglycerol (L-selachyl alcohol, IV) with the triphenylmethyl group, alkylation of the resulting triphenylmethyl derivative (V) with 1-bromooctadecane, and finally removing the blocking group by acid hydrolysis. The positional isomer of VII, namely, L-2-*O*-*cis*-9′-octadecenyl-3-*O*-octadecylglycerol (XI), was synthesized in an analogous way, starting with L-3-*O*-octadecylglycerol (L-batyl alcohol, VIII).

The oleyl bromide used was prepared by reduction of pure (>99%) oleic acid with lithium aluminum hydride to oleyl alcohol, formation of the *p*-toluenesulfonate, and conversion to the bromide by reaction with lithium bromide in boiling acetone. L-2-*O*-Hexadecanoyl-3-*O*-octadecylglycerol (XIII) was synthesized by acylation of L-3-*O*-octadecyl-1-*O*-triphenylmethylglycerol (IX) with palmitoyl chloride in pyridine followed by removal of the triphenylmethyl group by hydrogenolysis with palladium catalyst. The infrared spectra and physical properties of these compounds are described.

Previous communications in this series have dealt with the synthesis of 2,3-dialkyl ethers of glycerol (Kates *et al.*, 1963), and of the D and L isomers of 2,3-dihydrophytylglycerol (Kates *et al.*, 1965a). The latter compound was found to be identical with the glycerol diether isolated from hydrolysates of the lipids of *Halobacterium cutirubrum* (Kates *et al.*, 1965b).

These glycerol diethers have proved to be useful as starting materials for the synthesis of saturated dialkyl ether analogs of phosphatides, such as the diether analogs of phosphatidylcholine (Stanacev *et al.*, 1964) and of phosphonocephalin (Baer and Stanacev, 1965). In order to prepare diether phosphatides containing unsaturated hydrocarbon chains the corresponding unsaturated glycerol diethers were required. The latter were also required for metabolic studies and for identification of natural glycerol diethers. Synthesis of this class of diethers, however, required a modification of our previous procedure (Kates *et al.*, 1963) to avoid the use of catalytic hydrogenolysis for removal of the benzyl blocking group. This has been achieved by using the triphenylmethyl (trityl) group as a blocking agent, since it is readily removed by acid hydrolysis without affecting the double bond.

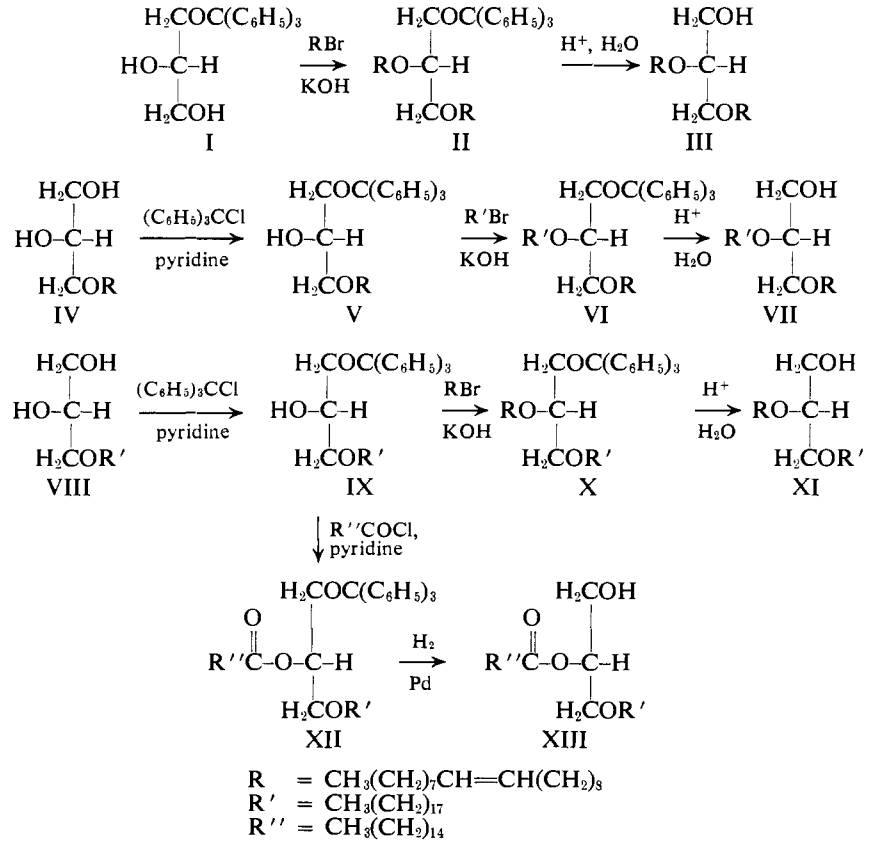
The present report describes the synthesis of a representative diether containing two unsaturated long-chain groups, namely, 2,3-di-*O*-*cis*-9′-octadecenylglycerol (dioleylglycerol), as the DL and L isomers, and two representative diethers containing one saturated and one unsaturated hydrocarbon group, namely, L-2-*O*-octadecyl-3-*O*-*cis*-9′-octadecenylglycerol and its positional isomer, L-2-*O*-*cis*-9′-octadecenyl-3-*O*-octadecylglycerol. The L stereoisomers were synthesized here since the only natural glycerol diether isolated to date proved to have the L configuration (Kates *et al.*, 1965a).

To ensure that the final unsaturated diethers contained only the *cis*-9-octadecenyl group without contamination with the *trans* isomer it was found necessary to start with highly purified oleic acid uncontaminated with the *trans* isomer and to convert it to oleyl bromide by a procedure which did not produce any *cis–trans* isomerization. This was accomplished by reducing pure oleic acid with lithium aluminium hydride and converting the alcohol to the bromide by treating the *p*-toluenesulfonate with lithium bromide. In contrast to

* From The Division of Biosciences, National Research Council, Ottawa, Canada. *Received September 20, 1965.* Issued as N.R.C. No. 8875. Paper II in this series: Kates *et al.* (1965a).

† National Research Council Postdoctorate Fellow, 1963–1965.

SCHEME I



previous methods for preparation of oleyl bromide (*cf.* Loev and Dawson, 1956; Dankova *et al.*, 1945), this procedure was found to yield a product which contains no detectable *trans* isomer as shown by the absence of absorption in the infrared at 965 cm^{-1} (Figure 1A).

The L isomer of dioleylglycerol was synthesized by alkylation of D-3-*O*-tritylglycerol (I) with 1-bromo-*cis*-9-octadecene (oleyl bromide), and the resulting L-1-*O*-trityl-2,3-di-*O*-oleylglycerol (II) was converted to L-2,3-di-*O*-oleylglycerol (III) by acid-catalyzed hydrolysis (see Scheme I). The DL-2,3-dioleylglycerol was prepared in the same way, using the DL-3-*O*-tritylglycerol as starting material.

The L-2-*O*-stearyl-3-*O*-oleylglycerol (VII) was synthesized by tritylation of L-3-*O*-oleylglycerol (L-selachyl alcohol, IV), alkylation of the resulting L-1-*O*-trityl-3-*O*-oleylglycerol (V) with 1-bromooctadecane (stearyl bromide), and detritylation of the L-1-*O*-trityl-2-*O*-stearyl-3-*O*-oleylglycerol (VI) by acid hydrolysis. The L-2-*O*-oleyl-3-*O*-stearylglycerol (XI) was synthesized in an analogous manner, starting with L-3-*O*-stearylglycerol (L-batyl alcohol, VIII).

The above diethers were isolated by chromatography on silicic acid columns, and were shown to be homogeneous by thin layer chromatography. Their infrared spectra (Figure 1B and 1C) showed the expected absorption bands for OH, alcoholic C–O, ether C–O–C, CH_2, and CH_3 groups, and for *cis* double bonds; no absorption band for *trans* double bonds at 965 cm^{-1} was detected, showing that no *cis–trans* isomerization occurred during the alkylation or detritylation steps.

All of the unsaturated diethers had similar R_F values on thin layer chromatography, which did not differ significantly from the R_F values of the corresponding saturated diether, 2,3-distearylglycerol (Table I). The molecular rotations of the two alkylalkenyl diethers were very similar and equal to that of the corresponding saturated distearylglycerol (M_D +41°), but the dioleylglycerol had a significantly higher molecular rotation, due possibly to the presence of the two double bonds in the molecule.

For comparison with the diethers, we have also prepared a mixed glycerol monoether–monoester, L-2-*O*-hexadecanoyl-3-*O*-octadecylglycerol (XIII), by acylation of L-1-*O*-triphenylmethyl-3-*O*-octadecylglycerol (IX) with palmitoyl chloride in pyridine, and removal of the triphenylmethyl group by palladium-catalyzed hydrogenolysis (Stegerhoek and Verkade, 1956) (see Scheme I). The infrared spectrum of this compound showed strong ester absorption at 1725 and 1170 cm^{-1} as well as ether C–O–C absorption at 1110 cm^{-1} (Figure 1D). Its molecular rotation was surprisingly low (+2.5°), since it was expected to be intermediate between that for saturated diethers (+41°, Kates *et al.*, 1963) and for saturated diglycerides (+17°; Baer and Kates, 1950). The possibility of migration of the palmitoyl group to the 1 position during hydrogenolysis of the trityl group in XII is considered unlikely, since

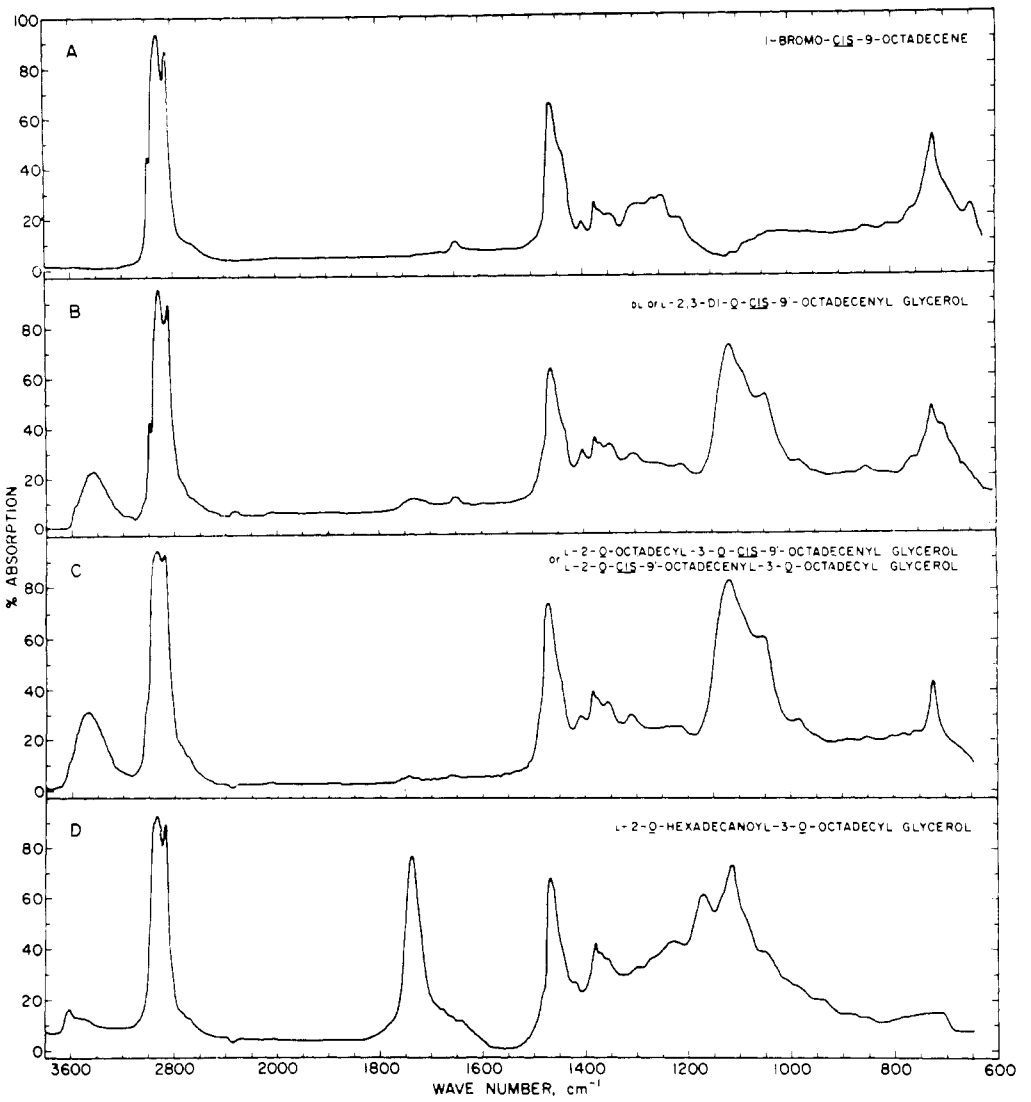


FIGURE 1: Infrared spectra of: A, oleyl bromide (oil); B, DL- or L-2,3-di-*O-cis*-9'-octadecenylglycerol (oil); C, L-2-*O*-octadecyl-3-*O-cis*-9'-octadecenylglycerol (oil) or L-2-*O-cis*-9'-octadecenyl-3-*O*-octadecylglycerol (oil); and D, L-2-*O*-hexadecanoyl-3-*O*-octadecylglycerol (in carbon tetrachloride).

Stegerhoek and Verkade (1956) have shown that acyl migration does not occur during this step. The R_F values of this compound on thin layer chromatography were, as expected, intermediate between those of the glycerol diethers and of diglycerides (Table I).

The physical properties of these synthetic diethers should prove useful in studies, now in progress, on the occurrence of glycerol diethers in various biological materials.

Experimental and Results

Gas–liquid partition chromatography, column chromatography, and thin layer preparative chromatography (silicic acid) were carried out as described elsewhere (Kates *et al.*, 1965a). For monitoring column chromatography and routine analysis, thin layer chromatography was done on microscope slides (25 × 75 mm) coated with silica gel TLC plain (Research Specialties Co. Richmond, Calif.) using screw-capped, wide-mouthed jars (7 cm in diameter, 9 cm in height), lined with filter paper. The plates were stored in an oven at 110°, and the solvents were freshly prepared before use. Spots were visualized by spraying the plates with 40% sulfuric acid and charring. All R_F values given are for microslides. Infrared spectra and melting points were measured as described elsewhere (Kates *et al.*, 1965a).

Starting Materials

D-2,3-*O*-Isopropylideneglycerol was synthesized according to the procedure of Baer (1952); $[\alpha]^{22}D$ +12.9° (pure liquid); lit. (Baer, 1952) $[\alpha]D$ +13.6°.

D- *or* DL-3-*O*-*Triphenylmethylglycerol* (*Tritylglycerol*,

TABLE I: Physical Properties of Dialkenyl-, Mixed Alkenylalkyl-, and Dialkylglycerol Ethers and Their Trityl Derivatives.

Substance	Mp (°C)	$[\alpha]_D$[a] (deg)	M_D[a] (deg)	Thin Layer Chromatography,[b] R_F in: Petroleum Ether–Benzene (1:3)	Benzene	Chloroform	Chloroform–Ether (9:1)
L-2,3-Di-*O*-*cis*-9′-octadecenyl-1-*O*-trityl-glycerol (II)	...	...	...	...	0.60	...	...
L-2,3-Di-*O*-*cis*-9′-octadecenylglycerol (III)	...	+7.4	+43.8	...	...	0.12	0.52
L-2-*O*-Octadecyl-3-*O*-*cis*-9′-octadecenyl-1-*O*-tritylglycerol (VI)	...	−4.3	−36.0	0.42	0.55	...	...
L-2-*O*-Octadecyl-3-*O*-*cis*-9′-octadecenyl-glycerol (VII)	...	+6.7	+39.9	...	...	0.12	0.45
L-2-*O*-*cis*-9′-Octadecenyl-3-*O*-octadecyl-1-*O*-tritylglycerol (X)	...	−4.2	−35.2	0.45	0.56	0.70	...
L-2-*O*-*cis*-9′-Octadecenyl-3-*O*-octadecyl-glycerol (XI)	...	+6.9	+41.0	...	...	...	0.47
L-2-*O*-Hexadecanoyl-3-*O*-octadecyl-1-*O*-tritylglycerol (XII)	35	−7.6	−62.7	...	0.38	0.49	...
L-2-*O*-Hexadecanoyl-3-*O*-octadecyl-glycerol (XIII)	67.5–68.5	+0.43	+2.5	...	...	...	0.38
D-2,3-Di-*O*-octadecylglycerol	53.5–54.5	−6.9[c]	−40.8[c]	...	...	0.12	0.47
D-2,3-Di-*O*-hexadecanoylglycerol	68–69	−2.9[d]	−16.5[d]	...	...	...	0.29
D-2,3-Di-*O*-octadecanoylglycerol	76–77	−2.8[d]	−17.5[d]	...	...	...	0.29

[a] In chloroform (containing 0.75% ethanol). [b] On microscope slides; values are averages of at least three determinations, with standard deviations ±0.05. [c] From data of Kates *et al.* (1963). [d] From data of Baer and Kates (1950).

I). The D isomer was synthesized as described by Baer and Fischer (1945), starting with D-2,3-*O*-isopropylideneglycerol. The product had mp 98° and $[\alpha]_D$ −16.8° (*c* 8.0, anhydrous pyridine); lit. (Baer and Fischer, 1945) mp 97°, $[\alpha]_D$ −17.7°.

The DL isomer was prepared by treating an excess of glycerol with trityl chloride in pyridine, as described by de Freitas (1961) and by Jackson and Lundberg (1963); yield 87%, mp 109–110°; lit. (de Freitas, 1961) mp 109–110°.

1-Bromo-cis-9-octadecene (Oleyl Bromide). A solution of 50 g of oleic acid (>99% pure; iodine value, 89.9; diene conjugation, 0.03%; obtained from the Hormel Institute, University of Minnesota) in 150 ml of anhydrous ethyl ether was added dropwise to 8 g of lithium aluminium hydride suspended in 150 ml of ethyl ether, and the mixture was refluxed for 36 hr. After destroying the excess hydride by cautious addition of water, the oleyl alcohol was isolated in almost quantitative yield; it was free of *trans* isomer as shown by the absence of absorption near 965 cm^{-1}; R_F on thin layer chromatography: 0.17 (in chloroform), 0.46 (in chloroform–ether, 3:1).

The oleyl alcohol (48 g, 0.177 mole) was esterified with *p*-toluenesulfonyl chloride (42 g, 0.22 mole) in anhydrous pyridine (100 ml), initially at 0°, and then at 25° for 24 hr, under nitrogen. The reaction mixture was diluted with 2 N sulfuric acid and extracted with several portions of ethyl ether, and the combined ether extracts were washed with 2 N sulfuric acid until free from pyridine, and then with water, saturated sodium bicarbonate, and finally water. Concentration of the ether extract *in vacuo* yielded 60 g (80% based on oleic acid) of crude oleyl *p*-toluenesulfonate as a slightly yellow oil.

For analytical purposes, a small sample (112 mg) was purified on a column of silicic acid (4 g) to remove oleyl chloride which was probably formed by chlorination of oleyl alcohol by the tosyl chloride. Elution of the column with 150 ml of petroleum ether (bp 30–60°) yielded 20 mg of oleyl chloride (R_F on thin layer chromatography, 0.43 in petroleum ether; retention relative to 1-chlorooctadecane and 1-bromooctadecane, 1.08 and 0.72, respectively, on butanediol succinate polyester at 178°; its infrared spectrum was identical with that of oleyl bromide, Figure 1A). The pure oleyl *p*-toluenesulfonate was eluted with 25 ml of benzene; yield, 82 mg of colorless oil; R_F on thin layer chromatography: 0.23 (in petroleum ether–benzene, 1:1), 0.47 (in benzene). Its infrared spectrum showed no absorption for OH groups (3400–3600 cm^{-1}), and had strong absorption bands for the O–SO_2 group (1365, 1175–1185 (doublet) 950, 830 cm^{-1}), for the aryl group (3050, 1595, 1490, 815 cm^{-1}), and for the C=C group (2980, 1650 cm^{-1}).

The pure oleyl tosylate was converted to oleyl bromide by reaction with lithium bromide in acetone, as described below. It gave only a single peak on gas–liquid partition chromatography with retention relative to 1-bromooctadecane of 1.09 on butanediol succinate polyester at 178°; R_F on thin layer chromatography, 0.44 (in petroleum ether). The infrared spectrum of oleyl bromide (Figure 1A) showed the expected absorption bands for CH_2 and CH_3 groups (2905, 2840, 1465, 1380, and 720 cm^{-1}), *cis* double bond (2980, 1650, 695 (sh) cm^{-1}), and no absorption for OH groups (3400–3600 cm^{-1}) or for *trans* double bond (960–970 cm^{-1}).

In large-scale preparations, it was not found necessary to remove the oleyl chloride from the oleyl tosylate, since it reacted in subsequent ether condensation steps. The crude oleyl *p*-toluenesulfonate (59.3 g, 0.14 mole) was then treated with 48.8 g (0.56 mole) of anhydrous lithium bromide in 250 ml of anhydrous acetone under reflux for 12 hr. After removal of the solvent *in vacuo*, the residue was extracted with ethyl ether and the extract was washed with water and dried over sodium sulfate. Concentration of the extract *in vacuo* yielded 44 g (95% based on tosylate, 75% over-all yield) of oleyl bromide, which was shown to contain 20–25% of oleyl chloride by gas–liquid partition chromatography. This crude oleyl bromide was used in the subsequent reactions.

L-3-*O*-*cis*-9′-*Octadecenylglycerol* (L-*selachyl alcohol, IV*) was synthesized by a modification of the procedure of Baer *et al.* (1944): a mixture of 3.25 g (25 mmoles) of D-2,3-*O*-isopropylideneglycerol, 8.3 g (25 mmoles) of oleyl bromide, and 4 g of finely powdered potassium hydroxide in 60 ml of anhydrous benzene was heated under reflux with stirring under nitrogen, for 10 hr. The reaction mixture was diluted with ice–water and neutralized with 10% sulfuric acid; the benzene phase was removed, and the aqueous phase was extracted with several portions of ethyl ether. The combined benzene and ether extracts were washed with water and concentrated *in vacuo*. The residual crude D-2,3-*O*-isopropylidene-1-*O*-*cis*-9′-octadecenylglycerol was hydrolyzed in a mixture of 100 ml of ethyl ether, 40 ml of methanol, and 6 ml of concentrated HCl, saturated with gaseous HCl, under reflux for 16 hr. The mixture was diluted with water and extracted with ether; the ether extract was washed with water and dried over sodium sulfate. The crude product obtained after removal of the solvent *in vacuo* was purified by chromatography on a column of silicic acid (80 g), eluted successively with the following solvents: petroleum ether (bp 30–60°, 200 ml), chloroform (100 ml), and chloroform–ethyl ether (1:1, 150 ml). The desired L-selachyl alcohol appeared in the chloroform–ether eluate which yielded 7.4 g (87% over-all yield) of chromatographically pure product (oil); $[\alpha]^{22}D$ +4.7°; lit. (Baer and Fischer, 1947) for D-selachyl alcohol, $[\alpha]D$ −4.5°; R_F on thin layer chromatography, 0.31 in ethyl ether, 0.51 in chloroform–methanol–water (90:10:1). The infrared spectrum showed the expected bands for OH, CH_2 and CH_3, ether C–O–C, and *cis* double bond; no *trans* double bond absorption was detected.

L-3-*O*-*Octadecylglycerol* (L-*batyl alcohol, VIII*) was synthesized by a modification of the procedure of Baes and Fischer (1941). A mixture of 6.5 g (50 mmoles) of D-2,3-*O*-isopropylideneglycerol, 16.5 g (50 mmoles) of 1-bromooctadecane, and 8 g of potassium hydroxide in 150 ml of anhydrous benzene was heated under reflux for 10 hr. The D-2,3-*O*-isopropylidene-1-*O*-octadecylglycerol was isolated as described for the corresponding oleyl derivative and was hydrolyzed in 150 ml of 50% acetic acid at 80° for 2 hr. The hydrolysate was poured onto an excess of ice–water, and the resulting precipitate was collected by filtration and dissolved in ethyl ether. The ether solution was washed with water, saturated sodium bicarbonate solution, and finally water, dried over sodium sulfate, and concentrated *in vacuo* to dryness. The residue was crystallized from acetone, yielding 10.2 g of pure L-3-*O*-octadecylglycerol, mp 71–72°; lit. (Baer and Fischer, 1941) mp 71–72°. A second crop of crystals (mp 70–71°) weighing 2.6 g was obtained from the filtrate; over-all yield 12.8 g (75%); R_F values on thin layer chromatography identical with those of selachyl alcohol.

Since the optical rotation of L-batyl alcohol in solution is very small, the diacetyl derivative was prepared as described by Baer and Fischer (1941), and found to have $[\alpha]^{22}D$ +7.1° (*c* 10.8, anhydrous alcohol-free chloroform); lit. (Baer and Fischer, 1941) $[\alpha]D$ +7.6° (*c* 11.2, chloroform); R_F value in chloroform 0.18; in chloroform–ether (9:1), 0.53.

Synthesis of Glycerol Diethers

L-2,3-*Di-O-cis*-9′-*octadecenylglycerol* (*III*). A mixture of 3.3 g (0.01 mole) of D-3-*O*-triphenylmethylglycerol (I), 6.6 g (0.02 mole) of 1-bromo-*cis*-9-octadecene, and 2 g of powdered potassium hydroxide in 150 ml of anhydrous benzene was heated under reflux with stirring for 16 hr, using a phase-separating head for removal of water. Another 2-g portion of potassium hydroxide was added, and the mixture was refluxed for 24 hr. A further 3.3 g of oleyl bromide and 3 g of potassium hydroxide were added and refluxing was continued for another 24 hr. Finally, another 2-g portion of potassium hydroxide was added and the mixture refluxed for 24 hr to ensure complete alkylation of the tritylglycerol. The reaction mixture was acidified with 10% sulfuric acid and extracted with ethyl ether, and the organic layer was washed with several portions of water and concentrated *in vacuo* to dryness. A solution of the residual crude trityl ether in 100 ml of ether and 40 ml of methanol was acidified with 6 ml of concentrated HCl and saturated with HCl gas, and the mixture was refluxed with stirring for 15 hr. The solvent was evaporated *in vacuo* and the residue was extracted with ether; the ether extract was washed with several portions of water and dried over sodium sulfate, and the solvent was removed *in vacuo*.

The residual oil (11.4 g) was dissolved in petroleum ether (bp 30–60°), freed from crystals of triphenylmethanol by centrifugation, and fractionated on a

column (3.3 cm × 25 cm) of silicic acid (125 g; Bio-Rad Laboratories, Richmond, Calif.). The column was eluted in succession with petroleum ether (500 ml, fraction 1), petroleum ether–benzene (1:1, 1100 ml, fraction 2), benzene (450 ml, 50-ml portions collected, fractions 3–11), benzene–ether (10:1, 400 ml, 50-ml portions collected, fractions 12–19), and finally benzene–ether (3:1, 150 ml, 50-ml portions, fractions 20–22). Fraction 1 contained mostly *cis*-1,9-octadecadiene (characterized by absorption for the vinyl group in the infrared at 3060, 1638, 1420, 990, and 907 cm^{-1}; R_F on thin layer chromatography in petroleum ether, 0.66; retention relative to *n*-octadecane on butanediol succinate at 184°, 1.17), together with a smaller amount of oleyl halide. Fraction 2 was mainly triphenylmethanol. The desired product appeared in the benzene eluates, fractions 4–11, and in the benzene–ether (10:1) eluates, fractions 12–17, together with decreasing amounts of triphenylmethanol. Fractions 18–22 contained a small amount of L-3-*O*-*cis*-9′-octadecenylglycerol, having R_F values on thin layer chromatography and an infrared spectrum identical with those of the authentic compound (see above). After purification by thin layer chromatography this product had $[\alpha]^{22}D$ −1.1° (*c* 3.8, chloroform); lit. (Baer *et al.*, 1944) $[\alpha]D$ +1.2° (*c* 5.1, chloroform) for the D isomer.

Fractions 4–17 were combined, and the solvent was removed *in vacuo*, yielding 4.1 g (69% over-all yield) of L-2,3-dioleylglycerol (III) as a colorless oil. The contaminating triphenylmethanol was largely removed by dissolving the oil in petroleum ether, cooling to 0°, and removing the crystals of triphenylmethanol by centrifugation. At this stage the dioleyl ether was estimated to contain less than 1% of triphenylmethanol.

For analytical purposes, a small sample (200 mg) of the diether was purified by thin layer chromatography using chloroform–ether (3:1) as solvent. The diether recovered from the plate was chromatographically pure and had $[\alpha]^{23}D$ +7.4° (*c* 4.0 chloroform); MD +43.8°.

Anal. Calcd for $C_{39}H_{76}O_3$ (593.0): C, 78.99; H, 12.92. Found: C, 79.39; H, 13.20.

The infrared spectrum of the diether (Figure 1B) showed the expected absorption bands for OH (3420 cm^{-1}), CH_2 and CH_3 (2910, 2840, 1465, 1380, 720 cm^{-1}), ether C–O–C (1115 cm^{-1}), and *cis* double bond (2980, 1650, 700 cm^{-1}); no *trans* double bond absorption (965 cm^{-1}) was present.

DL-2,3-*Di*-*O*-*cis*-9′-*octadecenylglycerol* was prepared by the same procedure described for the L isomer, except that DL-3-*O*-triphenylmethylglycerol was used as starting material; yield, 69% based on tritylglycerol. The product obtained was identical in all respects with the L isomer except that it had no optical activity.

Anal. Calcd for $C_{39}H_{76}O_3$ (593.0): C, 78.99; H, 12.92. Found: C, 78.30; H, 12.58.

L-3-*O*-*cis*-9′-*Octadecenyl*-1-*O*-*triphenylmethylglycerol* (*V*). L-3-*O*-*cis*-9′-Octadecenylglycerol (IV) was converted to the triphenylmethyl derivative essentially as described by Stegerhoek and Verkade (1956). A solution of 3.4 g (0.01 mole) of IV and 5.6 g (0.02 mole) of triphenylchloromethane in 20 ml of anhydrous pyridine was stirred overnight at 25°. The reaction mixture was added to an excess of ice and water and extracted with ethyl ether. The ether extract was washed with 1 N sulfuric acid, water, saturated sodium bicarbonate solution, and finally water, dried over sodium sulfate, and concentrated *in vacuo*. The residual oil (6 g) was fractionated on a column of 60 g of silicic acid, which was eluted with the following solvents, 50-ml fractions being collected: petroleum ether (fractions 1–3), petroleum ether–benzene (1:1, fractions 4–7), benzene (fractions 8–9), chloroform (fractions 10–13), and ethyl ether (fraction 14). The desired compound (V) was obtained in almost quantitative yield in fractions 5–13, but was contaminated with a small amount (less than 1%) of triphenylmethanol. For analytical purposes, the purest fraction (fraction 10) was further purified by thin layer chromatography with chloroform as solvent (R_F 0.15); the product was obtained as a colorless oil with $[\alpha]^{22}D$ −2.8° (*c* 8.3, chloroform).

Anal. Calcd for $C_{40}H_{56}O_3$ (584.84): C, 82.14, H, 9.65. Found: C, 82.18; H, 9.56.

The infrared spectrum of V (as oil) showed the expected absorption bands for OH (3600, 3480 cm^{-1}), CH_2 and CH_3 (2940, 2870, 1465, 1380 cm^{-1}), triphenylmethyl (3050, 1600, 1495, 1450, 770, 760, 743, 695 cm^{-1}), and ether C–O–C (1115, 1075 (doublet) cm^{-1}). Absorption due to the *cis* double bond was masked by the strong aryl bands.

L-2-*O*-*Octadecyl*-3-*O*-*cis*-9′-*octadecenyl*-1-*O*-*triphenylmethylglycerol* (*VI*). The crude compound V obtained above (5.7 g, 0.01 mole) was alkylated with 3.3 g (0.01 mole) of 1-bromooctadecane and 5 g of powdered potassium hydroxide in 75 ml of anhydrous benzene heated under reflux for 6 hr. Another 3.3-g portion of 1-bromooctadecane and a 5-g portion of potassium hydroxide were added and refluxing again continued for 6 hr. The crude product (brown oil) was isolated from the reaction mixture as described for compound II and was partially purified by chromatography on a column of silicic acid (150 g), eluted with the following solvents (50-ml fractions): petroleum ether (fractions 1–3, containing 1-octadecene and 1-bromooctadecane); petroleum ether–benzene (3:1, fractions 4–6); petroleum ether–benzene (1:1, fractions 7–8). The desired product (VI) was eluted in fractions 5–7, together with small amounts of fast-moving material. A small sample was finally purified by thin layer chromatography with petroleum ether–benzene (1:3) as solvent. The pure compound VI was recovered from the plate as a colorless oil with $[\alpha]^{22}D$ −4.3° (*c* 9.2, chloroform).

Anal. Calcd for $C_{58}H_{92}O_3$ (837.3): C, 83.19; H, 11.07. Found: C, 83.27; H, 10.34.

The infrared spectrum of compound VI showed the expected bands for CH_2 and CH_3 (2920, 2850, 1465, 1380, 715 (sh) cm^{-1}), triphenylmethyl (3050, 1600, 1493, 1450, 770, 760, 743, 700 cm^{-1}), and ether C–O–C (1115, 1075 (doublet) cm^{-1}); OH absorption was absent.

L-2-*O*-*Octadecyl*-3-*O*-*cis*-9′-*octadecenylglycerol* (*VII*). The partially purified compound VI (*ca.* 7 g) was

detritylated in a mixture of 120 ml of ether, 40 ml of methanol, and 6 ml of concentrated HCl, saturated with gaseous HCl under reflux with stirring for 18 hr. The crude product (VII) was isolated from the hydrolysate as described for III. The oil was dissolved in petroleum ether, freed of triphenylmethanol (1.4 g) by centrifugation, and fractionated on a column of silicic acid (80 g); elution was carried out with petroleum ether (fraction 1, 400 ml; containing 1-bromooctadecane and 1-octadecene); petroleum ether–benzene (1:1, fraction 2, 200 ml; containing compound VII, some fast-moving material, and triphenylmethanol); benzene (fraction 3, 400 ml; containing VII plus traces of triphenylmethanol); chloroform (fraction 4, 400 ml; containing only VII); and ethyl ether (fraction 5, 400 ml; containing VII and some slow-moving material). Fractions 2–5 were combined and evaporated to dryness, and the residue was dissolved in petroleum ether and freed from triphenylmethanol by centrifugation; total yield 3.8 g (64% over-all yield, based on L-selachyl alcohol). For analytical purposes, a small sample was finally purified by thin layer chromatography with chloroform–ether (9:1) as solvent. Compound VII was recovered from the plate as a colorless oil having $[\alpha]^{22}D$ +6.7° (*c* 7.7, chloroform); MD +39.9°.

Anal. Calcd for $C_{39}H_{78}O_3$ (595.0): C, 78.72; H, 13.21. Found: C, 79.10; H, 12.66.

The infrared spectrum of compound VII (Figure 1C) was very similar to that of dioleylglycerol (III).

L-3-*O*-*Octadecyl*-1-*O*-*triphenylmethylglycerol* (*IX*) was prepared by reaction of 10.2 g (0.03 mole) of L-3-*O*-octadecylglycerol (VIII) with 16.8 g (0.06 mole) of triphenylchloromethane in 60 ml of anhydrous pyridine at 25° for 18 hr. The reaction mixture was added to an excess of ice–water, and the precipitate was filtered by suction. It was then dissolved in ether, and the solution was washed with 1 N sulfuric acid, water, saturated sodium bicarbonate solution, and water, and dried over sodium sulfate. Removal of the solvent *in vacuo* gave a crude product which was dissolved in a small volume of petroleum ether and freed from triphenylmethanol by centrifugation; this procedure was repeated several times. The petroleum ether solution was finally concentrated *in vacuo*, and the residue was crystallized twice from acetone; yield of pure IX, 14.4 g (82%); mp 57–57.5°; $[\alpha]D$ −2.8 ± 0.3° (*c* 5.5, chloroform).

Anal. Calcd for $C_{40}H_{58}O_3$: C, 81.86; H, 9.96. Found: C, 82.08; H, 10.19.

Its infrared spectrum was identical with that of compound V.

L-2-*O*-*cis*-9′-*Octadecenyl*-3-*O*-*octadecyl*-1-*O*-*triphenylmethylglycerol* (*X*). Compound IX (11.7 g, 0.02 mole) was alkylated with 1-bromo-*cis*-9-octadecene in the same manner as V, and the product was isolated and partially purified as described for VI. After purification of a small sample on preparative thin layer chromatography with benzene–petroleum ether (2:1), the product (oil) had $[\alpha]^{22}D$ −4.2° (*c* 5.4, chloroform).

Anal. Calcd for $C_{58}H_{92}O_3$ (837.3): C, 83.19; H, 11.07. Found: C, 82.44; H, 10.81.

The infrared spectrum of X was identical with that of VI.

L-2-*O*-*cis*-9′-*Octadecenyl*-3-*O*-*octadecylglycerol* (*XI*). Detritylation of X was carried out as described for VI and the product was isolated and purified as described for VII; yield of purified XI, 6.8 g (57% over-all yield, based on VIII). After final purification by preparative thin layer chromatography with chloroform–ether (20:1), the material had $[\alpha]^{20}D$ +6.9° (*c* 5.9, chloroform); MD +41.0°.

Anal. Calcd for $C_{39}H_{78}O_3$ (595.0); C, 78.72; H, 13.21. Found: C, 78.64; H, 12.83.

The infrared spectrum XI was identical with that of its positional isomer VII (Figure 1C).

L-2-*O*-*Hexadecanoyl*-3-*O*-*octadecyl*-1-*O*-*triphenylmethylglycerol* (*XII*). L-3-*O*-octadecyl-1-*O*-triphenylmethylglycerol (compound IX, 2.9 g, 5 mmoles) was esterified with hexadecanoyl chloride (2.8 g, 10 mmoles) in anhydrous pyridine (20 ml) at 25° for 24 hr. Ice and water were added to the reaction mixture with stirring, the solid material was filtered with suction while cold and dissolved in ether, and the ether solution was washed with 1 N sulfuric acid, water, saturated bicarbonate solution, and water. The sodium sulfate dried ether solution was concentrated *in vacuo*, the residue was dissolved in petroleum ether, and the solution was filtered to remove insoluble material and evaporated to dryness. The residual crude XII solidified on cooling; yield, 4 g. For analytical purposes, a small sample was further purified by thin layer chromatography with chloroform as solvent; mp near 35°; $[\alpha]^{22}D$ −7.6° (*c* 11.1, chloroform).

Anal. Calcd for $C_{56}H_{88}O_4$ (825.3); C, 81.50; H, 10.75. Found: C, 80.92; H, 10.72.

The infrared spectrum of compound XII showed the expected bands for ester C=O, ester C–O–, ether C–O–C, trityl group, and CH_2 and CH_3 groups; no OH band was present.

L-2-*O*-*Hexadecanoyl*-3-*O*-*Octadecylglycerol* (*XIII*). The crude XII (4 g) was detritylated by hydrogenolysis in absolute ethanol at 25° and atmospheric pressure with palladium-on-charcoal catalyst as described by Stegerhoek and Verkade (1956). The catalyst was removed by centrifugation, the alcohol solution was evaporated *in vacuo*, and the residue was crystallized from acetone; yield of XIII, 2.5 g (85% yield based on IX); mp 66–67°. After recrystallization from acetone, the product had mp 67.5–68.5°; $[\alpha]^{22}D$ +0.43°; MD +2.5°.

Anal. Calcd for $C_{37}H_{74}O_4$ (583.0): C, 76.22; H, 12.80. Found: C, 75.82; H, 13.19.

The infrared spectrum of XIII (Figure 1D) showed the expected bands for OH, CH_2 and CH_3, ester C=O, ester C–O–, ether C–O–C, and alcohol C–O.

Acknowledgment

The authors are grateful to Mr. D. Murray, Labatt's Brewing Co., London, Ontario, for assistance with the synthesis of the DL-dioleylglycerol. The technical as-

sistance of Mr. T. Shier (National Research Council summer assistant) is gratefully acknowledged.

References

Baer, E. (1952), *Biochem. Prepn. 2*, 31.
Baer, E., and Fischer, H. O. L. (1941), *J. Biol. Chem. 140*, 397.
Baer, E., and Fischer, H. O. L. (1945), *J. Am. Chem. Soc. 67*, 944.
Baer, E., and Fischer, H. O. L. (1947), *J. Biol. Chem. 170*, 337.
Baer, E., and Kates, M. (1950), *J. Am. Chem. Soc. 72*, 942.
Baer, E., Rubin, L. J., and Fischer, H. O. L. (1944), *J. Biol. Chem. 155*, 447.
Baer, E., and Stanacev, N. Z. (1965), *J. Biol. Chem. 240*, 44.
Dankova, T. F., Genkin, E. I., and Preobrazhenskii, N. A. (1945), *Zh. Obshch. Khim. 15*, 189.
de Freitas, A. S. W. (1961), Ph.D. Thesis, University of Minnesota.
Jackson, J. E., and Lundberg, W. O. (1963), *J. Am. Oil Chemists' Soc. 40*, 502.
Kates, M., Chan, T. H., and Stanacev, N. Z. (1963), *Biochemistry 2*, 394.
Kates, M., Palameta, B., and Yengoyan, L. S. (1965a), *Biochemistry, 4*, 1595.
Kates, M., Yengoyan, L. S., and Sastry, P. S. (1965b), *Biochim. Biophys. Acta 98*, 252.
Loev, B., and Dawson, C. R. (1956), *J. Am. Chem. Soc. 78*, 1180.
Stanacev, N. Z., Baer, E., and Kates, M. (1964), *J. Biol. Chem. 239*, 410.
Stegerhoek, L. J., and Verkade, P. E. (1956), *Rec. Trav. Chim. 75*, 143.

New Acetylenic Fatty Acids from *Acanthosyris spinescens* Seed Oil*

R. G. Powell and C. R. Smith, Jr.

ABSTRACT: The seed oil of *Acanthosyris spinescens* contains a number of previously unknown acetylenic fatty acids. These include 17-octadecen-9-ynoic acid, 18%; *trans*-10,16-heptadecadien-8-ynoic acid, 10%; and *trans*-11,17-octadecadien-9-ynoic acid, 4%. One other nonoxygenated C_{17} acid (9%) is also present but was not fully characterized.

A. spinescens is the first of the higher plants found to contain straight-chain C_{17} acids in more than very small amounts.

Investigations of the seed oil of *A. spinescens* (Mart. et Eich.) Griseb., family *Santalaceae*, by procedures conventionally applied to seed oils revealed that a number of unusual fatty acids were present. Infrared and ultraviolet spectra of the oil indicated the presence of hydroxyl, acetylene, terminal methylene, and conjugated enyne groupings. Gas–liquid partition chromatographic (glpc)[1] analyses of the fatty acid methyl esters revealed the presence of a number of unfamiliar components.

Previously, fatty acids containing terminal double bonds had been found only in the seed oil of *Onguekoa gore* (family *Olacaceae*), commonly referred to as isano oil, and in *Santalum acuminatum* (Bu'Lock and Smith, 1963). Conjugated enynoid fatty acids had been found in oils of a few genera in the *Santalaceae* and *Olacaceae* (Sørenson, 1963; Gunstone and Sealy, 1963) and in one member of the *Compositae*, *Helichrysum bracteatum*. 9-Hydroxy-*trans*-10-octadecen-12-ynoic acid has been characterized as a constituent of the seed oil of the latter (Powell *et al.*, 1965).

It appeared to us that investigation of *Acanthosyris* oil would yield results that might be significant biogenetically. Isolation and characterization of the nonhydroxylated acetylenic fatty acids are discussed in this paper. The structure of one of the hydroxyacetylenic acids has been indicated in a preliminary communication (Powell and Smith, 1965). The entire group of hydroxyacetylenic acids from *A. spinescens* oil will be the subject of a forthcoming paper from this laboratory.

Results

Separation of Nonoxygenated and Hydroxy Acids. Separation of *Acanthosyris* free fatty acids into hydroxy and nonhydroxy fractions was accomplished by using modifications of a procedure described by Frankel

* From the Northern Regional Research Laboratory, Peoria, Illinois. *Received June 28, 1965; revised November 15, 1965.* This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[1] Abbreviations used in this work: glpc, gas–liquid partition chromatographic; tlc, thin layer chromatography; nmr, nuclear magnetic resonance.