THE SYNTHESIS OF POLYUNSATURATED GLYCERYL ALKYL ETHERS TRITIATED AT C-2

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SUMMARY

The synthesis of polyunsaturated trialkyl glyceryl ether tritiated at C-2 of the glycerol carbon chain having identical 1,3-alkyl groups is described.

Key Words: C-2-tritiated glyceryl trialkyl ethers, polyunsaturated glyceryl alkyl ethers.

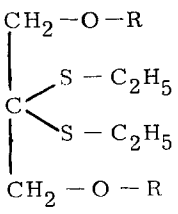
Long-chain alkenyl ethers of glycerol, being structurally related to the corresponding esters (1), may be used as non-degradable model substances for the study of the fate of lipoprotein triglycerides.

The present work was undertaken in order to prepare polyunsaturated glycerol ethers, since labeled unsaturated lipids can be efficiently introduced into lipoproteins (2, 3). In a method reported by Paltauf and Spener (1),[1-^{14}C] fatty acids were esterified, reduced and converted into the corresponding alkyl methane sulfonates, which were then reacted with 1,2-dialkyl glycerol to give the desired ethers. In this work, the starting material, dipropionoxy acetone diethyl mercaptal (4) was hydrolyzed under alkaline conditions and the dihydroxy compound was then etherified to di-*cis*-9-octadecenyloxy acetone diethyl mercaptal (1). The diethyl mercapto-group was removed to give di-*cis*-9-octadecenyloxy acetone (2), which was labeled with $NaB[^3H]_4$ at C-2. The resulting 1,3-di-*cis*-9'-octadecenyloxy-2-propanol-[2-^{3}H] (3) was converted to the trialkyl ethers (4, 5) with the respective alkyl methane sulfonates.

0362-4803/83/020269-07$01.00

Received July 15, 1982

R = cis-9-octadecenyl



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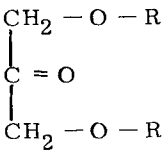

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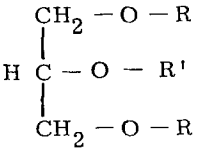

3 R' = H

4 R' = cis-9-octadecenyl

5 R' = cis,cis-9,11-octadecadienyl

The alkenyl ethers and their intermediates, being liquids at room temperature, were purified chromatographically rather than by crystallizations. The advantages of this method are as follows:

1. Inexpensive $NaB[^3H]_4$ is used for labeling.
2. Only the last reaction is conducted on a labeled intermediate (3), all the other steps are conventional chemical reactions, unhampered by the limitations involved in working with radioactive material.
3. As the 1,3-alkyl moieties are identical, only one symmetrical product is prepared, independent of the 2-alkyl chain chosen. The trialkyl ether, however, is still structurally similar to the natural triglycerides.

EXPERIMENTAL

Nmr spectra were recorded in a Jeol C-60-H high resolution spectrometer in $CDCl_3$ solution with tetramethylsilane as an internal standard. Mass spectra were taken through the direct inlet of LKB 2091 Gas Chromatograph Mass Spectrometer. The ultraviolet spectra were determined in a Varian Techtron spectrometer. Infrared spectra were measured (neat) in a Perkin Elmer Spectrophotometer, Model 337. TLC (on silica gel G) and silicic acid column chromatography were performed as described elsewhere (5). The method

for estimation of the radiochemical purity was previously reported (6). Aluminium oxide S (neutral) was purchased from Riedel-de Haën AG. The unsaturated alkyl methane sulfonates were obtained from Nu-Chek Prep. Inc. $NaB[^3H]_4$ (469 mCi/mmol) was purchased from the Radiochemical Centre, Amersham.

Cis-9-octadecenyl aldehyde was prepared as follows: 0.2 ml of cis-9-octadecenoyl chloride (Nu-Chek) in dry peroxide free ether (50 ml) was added dropwise into an etheral solution of excess diazomethane at ice bath temperature. A cooled solution of 7% $HClO_4$ (100 ml) was added dropwise with stirring until the yellow color disappeared. The ether phase was washed with water, a conc. solution of $NaHCO_3$, and again with water after which the solvent was removed. The residue was dissolved in 10 ml of isopropanol and $NaBH_4$ (0.25 g) was added. After 18 h, the product was extracted with ether and the solvent was removed. The residue was dissolved in ethanol (10 ml), and the following were added: $NaIO_4$ (0.3 g), CH_3COOH (0.5 ml) and water (2 ml). After two hours at room temperature the product was extracted with ether and the solvent was removed. The aldehyde exhibited a single spot on TLC in the system hexane : chloroform 7 :3 (R_f 0.35 visualization with iodine vapor); mass spectrum (m/e) 266 (M^+), 248 (M - H_2O, base peak). The 965 cm^{-1} ir band (trans $-CH=CH-$) (7) was not present in the spectra of the aldehyde and the other compounds studied.

Di-cis-9-octadecenyloxy acetone diethyl mercaptal (1). A solution of KOH (3 g) in water (6 ml) was added to dipropionoxyacetone diethyl mercaptal (2 g) (4) in 50 ml methanol. The solution was kept at room temperature overnight. The product was extracted with chloroform (200 ml) and washed with water (200 ml, 3 times). The chloroform solution was dried (Na_2SO_4), evaporated and the residue (1 g) was chromatographed on alumina (40 g). The dihydroxyacetone diethyl mercaptal was eluted with 100 ml portions of increasing concentrations of chloroform in hexane. Two fractions of 20% and three of

25% chloroform respectively contained impurities. The product (364 mg) was eluted with 200 ml of 30% chloroform (m.p. 22-24°; R_f 0.1 in the TLC system, chloroform : methanol : water 90 : 10 : 1). The eluate was refluxed with cis-9-octadecenyl methane sulfonate (1718 mg) and KOH in benzene under N_2 as previously described (1). After extraction with peroxide free ether the product (1.8 g) was chromatographed on silicic acid (40 g). The column was eluted with increasing concentrations of benzene in hexane. The 5% and 7% benzene fractions (100 ml) contained impurities, the pure di-cis-9-octadecenyloxy acetone diethyl mercaptal (1) was eluted with 10% and 15% benzene in hexane (900 mg). R_f in TLC system hexane chloroform 7 : 3 0.3; NMR δ (ppm) 0.879 (6H, m, CH_3), 1.283 (broad s, CH_2-groups of alkyl moieties) (5), 2.614 (4H, m, CH_2-S), 3.462 and 3.684 (8H, m, $O-CH_2$), 5.40 (4H, m, $-CH=CH-$); IR 1475, 1030, 730 cm^{-1}; mass spectrum (m/e) 634 ($M - C_2H_5SH$). Anal. calcd. for $C_{43}H_{84}O_2S_2$; C, 74.14%; H, 12.07%; S, 9.20%; Found C, 74.40%; H, 11.88%; S, 8.82%.

Di-cis-9-octadecenyloxyacetone (2). 700 mg of 1 were subjected to reaction with $HgCl_2$ (25 g) in acetone solution as previously described (4) to give 550 mg of crude product. Chromatography on silicic acid (16 g) and elution with 30% benzene in hexane yielded 300 mg of 2 which crystallized in the refrigerator and melted at 23°C. R_f in hexane : chloroform 1 : 1, 0.57; NMR δ (ppm) 0.867 (6H, m, $17-CH_3$), 1.277 (broad s), 3.463 (4H, m, $O-CH_2-$), 4.191 (4H, s, $CO-CH_2-O$), 5.333 (4H, m, $-CH=CH-$); IR 1750 (C=O), 1475, 1025 cm^{-1}; mass spectrum (m/e) 592 (M^+). Anal. calcd. for $C_{39}H_{74}O_3$: C, 79.32%; H, 12.54%; Found: C, 79.61%; H, 12.26%.

1,3-Di-cis-9'-octadecenyloxy-2-propanol (3). $NaBH_4$ (40 mg) was added to a solution of 2 (145 mg) in isopropanol (5 ml). The solution was stirred overnight at room temperature, water (2 ml) was added and the stirring was

continued for 2 hr. The mixture was extracted with hexane (60 ml), the organic phase was washed with water (50 ml). The organic phase was then dried and evaporated under reduced pressure. The product which seemed almost pure (TLC, chloroform :ethyl acetate 95 :5; R_f 0.50) was further purified on silicic acid column (14 g). Elution with 50% benzene in hexane gave 125 mg of 3. A satisfactory chemical analysis was achieved after additional purification by preparative TLC. M.p. 17-18°C; NMR δ (ppm) 0.863, 1.277, 1.942 (m), 3.386 (8H, m, CH_2-O-), 4.370 (1H, m, OH), 5.33 (4H, m, $-CH=CH-$); IR 3520, 1765, 1425 cm^{-1}; mass spectrum (m/e) 592 (M^+). Anal. calcd. for $C_{39}H_{76}O_3$: C, 79.05%; H, 12.84%; Found: C, 79.30%; H, 12.98%.

1,3-Di-cis-9'-octadecenyloxy-2-propanol-[2-^{3}H]. Crystalline $NaB[^3H]_4$ (approx. 0.2 mg) was added to a solution of 2 (31 mg) in isopropanol (6 ml), which was then stirred as described above. Na_2CO_3 (5 mg) and water (0.5 ml) were added and the stirring was continued for another 2 hr. Extraction was carried out with toluene by which the organic phase and the washings contained 3.6×10^9 dpm and 4.1×10^9 dpm, respectively. The labeled residue from the organic phase was chromatographed on silicic acid (14 g) to give 0.74 mCi ($\sim 1.7 \times 10^9$ dpm) of 3 with radiochemical purity of 92.5%. Further purification of 160×10^6 dpm on preparative TLC yielded 140×10^6 dpm of 98% pure compound, however the crude labeled 3 was used for further reactions.

For estimation of the specific activity, a second part of the $NaB[^3H]_4$ was similarly reacted with excess of cis-9-octadecenylaldehyde. The resulting alcohol was purified by TLC (chloroform : ethyl acetate 95 : 5) to give the cis-9-octadecen-1-ol-[1-^{3}H] of >98% radiochemical purity and specific activity of approx. 118 mCi/mmol (1.7×10^9 dpm, 1.63 mg). The amount of the alcohol was estimated by GLC under the following conditions: column 3% OV-17; temp. 190°C; N_2, 40 psi; retention time 5.76 min; r.t. of internal standard (arachidyl alcohol) 11.42 min. The molar specific activity, being close to the calculated

value (i.e., nearly 1/4 that of the $NaB[^3H]_4$) was assumed correct for the labeled 3 as well.

Tri-cis-9'-octadecenyloxy-propane (4). 88 mg of 3 was etherified by cis-9-octadecenyl methane sulfonate (80 mg) as described for 1. The crude product was chromatographed on a silicic acid column with 100 ml fractions of 10%, 20%, 30%, 40% and 50% benzene in hexane. The product recovered from the 30% and 40% fractions (105 mg) was a pure compound as judged by TLC (hexane : chloroform 1 : 1, R_f 0.6). NMR δ (ppm) 0.892 (9H, m, 17'$-CH_3$), 1.264 (broad s), 1.949 (m), 3.476 (12H, $-CH_2-O$), 5.321 (6H, m, $-CH=CH-$); IR 2975, 1475, 1130 cm^{-1}; mass spectrum (m/e) 842 (M^+). The compound was identical with 4 prepared according to Paltauf and Spener (1).

Tri-cis-9'-octadecenyloxy-propane[2-^{3}H] was prepared from C-2 tritiated 3 (13×10^8 dpm) and cis-9-octadecenyloxy methane sulfonate (20 mg). Purification on silicic acid column gave 7.1×10^8 dpm of product being 98.4% pure.

1,3-Di-cis-9'-octadecenyloxy-2-cis,cis-9',11'-octadecadienyloxy-propane (5). 90 mg of 3 was etherified with 0.1 ml of 9,11-octadecadienyl methane sulfonate. The product was purified as described above to give 101 mg of 5. R_f 0.6; NMR δ (ppm) 0.887 (9H, m, 17'$-CH_3$), 1.265 (broad s), 2.016, 3.464 (12H, m, $-CH_2-O-$), 5.321 (8H, m, $-CH=CH-$); IR 2975, 1475, 1130 cm^{-1}. The uv spectrum did not exhibit the absorption near 230 nm associated with conjugated double bond system (7). Mass spectrum (m/e) 840 (M^+). The analytical batch was further purified by preparative TLC; calcd. for $C_{57}H_{108}O_3$: C, 81.43%; H, 12.86%; Found: C, 81.73%; H, 12.78%.

1,3-Di-cis-9'-octadecenyloxy-2-cis,cis-9',11'-octadecadienyloxy-propane-[2-^{3}H] was prepared from 3 (13×10^8 dpm) and cis,cis-9,11-octadecadienyl methane sulfonate (0.04 ml). Silicic acid column chromatography afforded 10.4×10^8 dpm of 5 (98% pure).

REFERENCES

1. Paltauf, F. and Spener, F. — Chem. Phys. Lipids. 2: 168 (1968).
2. Stein, Y. , Halperin, G., and Stein, O. — FEBS Letters 111: 104 (1979).
3. Stein, O., Halperin, G., and Stein, Y. — Biochim. Biophys. Acta 620: 247 (1980).
4. Barry, P. J. and Craig, B. M. — Can. J. Chem. 33: 716 (1955).
5. Halperin, G. and Gatt, S. — Steroids 35: 2541 (1980).
6. Stein, Y., Halperin, G., and Stein, O. — Biochim. Biophys. Acta 530: 420 (1978).
7. Bauman, W. J. and Mangold, H. K. — J. Org. Chem. 29: 3055 (1964).