A simple method for the synthesis of cholesteryl ethers[1]

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Summary A novel method for the synthesis of cholesteryl ethers is described. The mesylates of fatty alcohols were treated with the sodium salt of cholesterol in toluene at 80°C in the presence of anhydrous dimethyl formamide. The hexyl, tetradecyl, and oleyl cholesteryl ethers were synthesized in yields varying between 55 and 70%. Tritiated cholesteryl oleyl ether was also synthesized in good chemical (45%) and radiochemical (45%) yields. — **Sripada, P. K.** A simple method for the synthesis of cholesteryl ethers. *J. Lipid Res.* 1986. **27:** 352–353.

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Cholesteryl ethers serve as non-metabolizable analogs of cholesteryl esters and are used to follow the metabolic pathway(s) of the latter and the particles in which they are incorporated, particularly plasma lipoproteins (1). Recently Halperin and Gatt (2) have synthesized a series of cholesteryl ethers and reported their chemical analysis, melting points as well as their infrared, proton NMR, and mass spectra. They prepared tosyl derivatives of cholesterol and tritiated cholesterol using a reaction that took 20 hr. In the second step the tosyl derivative was heated with the fatty alcohol at 110°C for 2.5 hr in a sealed tube, to yield, after purification, the cholesteryl ethers. In contrast, Hirth and Barner (3) reported the synthesis of glycerol ethers by reacting the tosyl derivatives of fatty alcohols with the glycerol derivative for 1 hr at 80°C in the presence of DMF. This simpler method has been used to synthesize unlabeled $C_{6:0}$, $C_{14:0}$, $C_{18:1}$ and tritium-labeled $C_{18:1}$ cholesteryl ethers.

MATERIALS AND METHODS

Anhydrous toluene (gold label), DMF (gold label) reagents and NaH in 60% oil dispersion were obtained from Aldrich Chemical Co. (Milwaukee, WI). Chloroform and methanol were of HPLC grade, obtained from EM Science (Gibbstown, NJ). Benzene (HPLC grade) was freshly distilled over calcium hydride before use. Unisil (activated silicic acid, 100–200 mesh) was from Clarkson Chemical Co., Inc. (Williamsport, PA). TLC plates, silica gel G 5 × 20 cm, 250 microns, were from Analtech (Newark, DE), and Rexyn I-300 was from Fisher Chemical Co. (Fairlawn, NJ).

Cholesterol and hexyl, tetradecyl, and oleyl mesylates were obtained from Nu-Chek-Prep, Inc. (Elysian, MN). Tritiated cholesterol was from New England Nuclear (Boston, MA) and Amersham (Chicago, IL).

The melting points were determined by a Sybron/Thermolyne (Dubuque, IA) apparatus. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory (Woodside, NY).

The reaction was carried out under anhydrous conditions in a dry argon atmosphere, which is preferred over dry nitrogen. TLC was carried out using silica gel G plates developed in two systems: *i*) hexane–benzene 2:3 and *ii*) hexane–diethyl ether 1:1. The cholesteryl ethers were made visible by exposing the plates to iodine in a chamber.

The ethers were taken up in heptane and applied to a silicic acid column and eluted with heptane containing 5%, 10%, and 20% benzene.

The HPLC analyses were carried out using a Varian 5000 liquid chromatograph equipped with a Varian variable wavelength detector UV 50 and Hewlett-Packard 3390A integrator. The conditions used were those of Carroll and Rudel (4), except that an Altex C-18 column was used. HPLC solvents consisted of CH_3CN-THF 65:35 (v/v) and double-distilled water. Cholesteryl ethers were eluted from the column using a linear water gradient. The water concentration in CH_3OH–THF was reduced from 3% to 0% over a period of 20 min, with a solvent flow rate 2.0 ml/min. Cholesteryl ethers eluting from the column were detected by their UV absorption at 212 nm.

^{1}H- and natural abundance proton-decoupled Fourier transform ^{13}C-NMR spectra were obtained at 47 KG (200 MHz for ^{1}H, 50.3 MHz for ^{13}C) with a Bruker WP-200 spectrometer equipped with an Aspect 2000 data system.

EXPERIMENTAL

Synthesis of cholesteryl tetradecyl ether

Cholesterol (445 mg) was converted into the sodium salt (using NaH in 60% oil dispersion). Na-cholesterol salt was heated to 80°C with tetradecyl mesylate in dry toluene in the presence of anhydrous DMF for 1 hr. The reaction mixture was filtered, evaporated, and dissolved in an organic phase (CH_2Cl_2–diethyl ether 1:3), washed with water, and passed over Rexyn-I-300 to remove

Abbreviations: DMF, dimethyl formamide; THF, tetrahydrofuran; TLC, thin-layer chromatography; HPLC, high pressure liquid chromatography; NMR, nuclear magnetic resonance.

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residual DMF. The product was further purified by silicic acid chromatography. Cholesteryl tetradecyl ether was eluted in hexane containing 20% benzene and crystallized from acetone at 0°C. The yield was 62%. Analysis: $C_{41}H_{74}O$ requires C = 84.4, H = 12.79; Found C = 84.21, H = 12.91.

The tetradecyl ether melted sharply at 51–52°C (lit. value 47–49°C). The elution of cholesteryl tetradecyl ether was compared with that of an authentic sample in two TLC systems and found to have the same R_f value. In HPLC, under the conditions of Carroll and Rudel (4), the ether was eluted in 22 min as a single peak.

The ether linkage in the cholesteryl tetradecyl ether was identified by the ^1H as well as ^{13}C NMR spectra in $CDCl_3$. The proton resonance of the methylene protons adjacent to the ether oxygen is characterized by a well-resolved, narrow triplet centered at 3.44 ppm. In the ^{13}C spectrum, a peak at 68.2 ppm is characteristic of a methylene carbon adjacent to the ether oxygen. The detailed assignments of the ^{13}C spectra of the hexyl, tetradecyl, and cis-octadecenyl ethers will be reported separately (D. Croll and J. Hamilton, unpublished observations).

Synthesis of hexyl and oleyl cholesteryl ethers

These were prepared as above in yields of 68% and 65% and had melting points of 69–70°C and 39–40°C, respectively.

Synthesis of [1,2-^{3}H]cholesteryl oleyl ether

One mCi of tritiated cholesterol in toluene was evaporated under anhydrous conditions under argon at 25°C. Unlabeled cholesterol (40 mg) and anhydrous toluene were added, followed by NaH in 60% oil dispersion, and heated at 80°C. Oleyl mesylate (60 mg) and anhydrous DMF (0.3 ml) were added after 1 hr and heating was continued for another hour. Cholesteryl oleyl ether was separated from the reaction mixture as described above for the tetradecyl ether. The cholesteryl oleyl ether fractions were pooled and kept at 0°C. The radiochemical yield was 42–44% and the radiopurity was >99% in four different experiments.

DISCUSSION

The success of the method described here for the synthesis of cholesteryl ethers using DMF is due to the fact that highly polar DMF enhances the nucleophilic reaction of the cholesterol anion with the mesylates of the alcohols. In addition, the mesylate on the primary hydroxyl is a better leaving group than on the secondary hydroxyl of cholesterol. The advantages of this synthesis over the recently described synthesis (2) are threefold: *1*) lower reaction temperature (80°C), *2*) unsealed reaction flask at atmospheric pressure, and *3*) shorter reaction time.

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REFERENCES

1. Stein, Y., Y. Kleinman, G. Halperin, and O. Stein. 1983. Hepatic retention and elimination of cholesteryl linoleyl ether after injection of labeled acetylated LDL or chylomicrons. *Biochim. Biophys. Acta.* **750:** 300–305.
2. Halperin, G., and S. Gatt. 1980. The synthesis of cholesteryl alkyl ethers. *Steroids.* **35:** 39–42.
3. Hirth, G., and R. Barner. 1982. Synthese von Glyceryl-ätherphosphatiden. Herstellung von 1-*O*-Octadecyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphorylcholin ('Platelet Activating Factor'), des Enantiomeren sowie einiger analoger Verbindungen. *Helv. Chim. Acta.* **65:** 1059–1084.
4. Carroll, R. M., and L. L. Rudel. 1981. Evaluation of a high-performance liquid chromatography method for isolation and quantitation of cholesterol and cholesteryl esters. *J. Lipid Res.* **22:** 359–363.