A method for rapid microsynthesis of radioactive cholesterol esters

KAROLY G. PINTER, JAMES G. HAMILTON, and JAMES E. MULDREY

Nutrition and Metabolism Laboratory of the Department of Medicine and the Department of Biochemistry, Tulane University School of Medicine, New Orleans, Louisiana

SUMMARY A simple method for synthesizing esters of labeled cholesterol of high specific activity is described. The formation of acyl chlorides takes 10 min and they are reacted with labeled cholesterol to form the cholesterol esters in another 5 min. Yields were 30–70% and radiopurities of 90–99.5% were achieved.

WITH THE growing interest in the metabolism of cholesterol esters, procedures have been developed which frequently make desirable the use of chemically pure compounds. Some of the radioactive cholesterol esters are either commercially unavailable or expensive when made to order. The high cost of the starting materials (cholesterol-4-C^{14}, pure fatty acids) makes it desirable to have a good yield of the end products. A rapid and efficient chemical synthesis of various labeled cholesterol esters is presented.

As early as 1930 Page and Rudy (1) prepared certain cholesterol esters; however, their synthetic method required large amounts of starting materials and the yield was not reported. An efficient preparation of acyl chlorides was accomplished by Wood et al. (2). Fatty acids and oxalyl chloride were refluxed for several hours. Further refluxing of acyl chlorides with cholesterol produced the desired esters. Mahadevan and Lundberg (3) prepared cholesterol esters of polyunsaturated fatty acids by the ester interchange method, in which cholesteryl acetate and the methyl ester of fatty acids are reacted in the presence of sodium ethoxide as catalyst. Purification was achieved by the combined use of repeated crystallizations and column chromatography. Recently Deykin and Goodman (4) reported a method of preparation of small quantities of radioactive cholesterol esters. However, these authors did not prepare cholesteryl linolenate or cholesteryl arachidonate.

The acyl chlorides of palmitic, stearic, oleic, linoleic, linolenic, and arachidonic acids were prepared as follows. The fatty acid (1 mmole) was placed in a small Florence flask with a side arm and oxalyl chloride then added in approximately 3-fold M excess. The flask was immediately closed with a ground glass stopper and the air was evacuated by a water aspirator (30 mm Hg) connected to the side arm. The flask was placed in a water bath at 65° and the mixture was allowed to boil for 5 min to drive off the excess oxalyl chloride. The conversion of fatty acids to acyl chlorides at this point is 30–45%. After addition of another 3-fold M excess of oxalyl chloride and repetition of the procedure the estimated yield of acyl chloride was raised to 75–85%. The yield of acyl chloride was estimated previously by preparing larger amounts of acyl chlorides and reacting these with equimolar amounts of nonradioactive cholesterol. Therefore the figures above represent a minimum yield of acyl chloride, since the reaction with cholesterol was assumed to be complete (100%).

TABLE 1 YIELD OF RADIOACTIVE CHOLESTEROL ESTERS AFTER ESTERIFICATION OF CHOLESTEROL-4-C^{14} AND RADIOPURITY OF THESE ESTERS AFTER SILICIC ACID COLUMN CHROMATOGRAPHY

Cholesterol Ester Prepared	Yield of Ester	Radiopurity after Column Chromatography
	%	%
Palmitate	57.1	99.5
Stearate	69.0	99.5
Oleate*	32.0–56.7	97.6–97.1
Linoleate	64.8	95.1
Linolenate	58.8	97.2
Arachidonate*	30.0–32.0	90.1–93.5

* Synthetic and purification procedures were carried out twice.

Cholesterol-4-C^{14} (5–50 μmoles), dissolved in 5 ml of dry diisopropyl ether, was added to the acyl chloride. The mixture was placed under vacuum at 60° and allowed to boil slowly until no visible amounts of the solvent remained. This step completes the esterification of the cholesterol-4-C^{14} with the fatty acid.

Hexane (10 ml) was added to the crude cholesterol ester. An aliquot (10–25 μl) of the solution of each labeled cholesterol ester was chromatographed on silica gel-impregnated glass fiber paper (5) in isooctane (2,2,4-trimethylpentane), together with the corresponding nonradioactive cholesterol esters as markers. The following R_F values were observed: cholesteryl palmitate 0.75, stearate 0.75, oleate 0.63, linoleate 0.55, linolenate 0.55, arachidonate 0.43, and cholesterol 0.10. After chromatography the glass paper sheets were air-dried and exposed for 5 sec to iodine vapors in a glass cylinder containing iodine crystals. The unreacted cholesterol and the synthesized cholesterol esters appeared briefly as two separate yellow spots against the white background of the paper. Both spots were quickly cut out, each was placed in a separate scintillation vial (6), and counted in the scintillation well. A third piece from a clear area of the same sheet of paper was counted simultaneously to determine background. The yield of the esterification products is shown in Table 1.

Downloaded from www.jlr.org by guest, on June 19, 2012

The remainder of the hexane solution of the reaction mixture containing cholesterol, cholesterol ester, and acyl chloride was transferred to a silicic acid column (7) and eluted with hexane–benzene 100:15. The column effluent was collected in 20-ml portions; an aliquot of each fraction was chromatographed on glass paper and the distribution of radioactivity was determined. The order of elution was the following: cholesterol ester, cholesterol and acyl chloride.

Fractions containing radioactivity only at the cholesterol ester spot were accepted as "pure." The impure fractions were pooled, concentrated under a stream of nitrogen, and rechromatographed on the silicic acid column until satisfactory (better than 90%) radiopurity was obtained (Table 1).

The quantities of radioactive cholesterol esters synthesized by this procedure are too small to be crystallized. The identification of the radioactive cholesterol esters by means of glass fiber paper chromatography and the use of labeled cholesterol esters is particularly helpful. Since throughout the entire synthetic process there is no dilution of radioactivity on a molar basis a final product of high specific activity (cpm/mg) is obtained by this method.

The longer heating times ordinarily used for the formation of acyl chlorides and for the reaction of the acyl chloride with cholesterol were found to be unnecessary. The cholesterol ester is easily separated from unreacted fatty acid, acyl chloride, and cholesterol. No other products of side reactions were observed. Although the ester interchange method (3) should work well for these small quantities of cholesterol esters it would be difficult to separate unreacted methyl ester and cholesteryl acetate from the cholesterol ester.

Any unreacted cholesterol-4-C^{14} can be readily recovered from the columns for future use. The cholesterol ester could also be readily purified by thin-layer chromatography. This method probably could also be adapted for the synthesis of cholesterol esters labeled in the fatty acids.

This work was supported by PHS Research Grant HE-04150 from the National Institutes of Health, U.S. Public Health Service, and formed part of a dissertation submitted by K. G. Pinter in partial fulfillment of the requirements for the Ph.D. Degree in Biochemistry, 1962, Tulane University.

Manuscript received September 9, 1963; accepted November 29, 1963.

References

1. Page, I. H., and H. Rudy. *Biochem. Z.* **220:** 304, 1930.
2. Wood, T. R., F. L. Jackson, A. R. Baldwin, and H. E. Longenecker. *J. Am. Chem. Soc.* **66:** 287, 1944.
3. Mahadevan, V., and W. O. Lundberg, *J. Lipid Res.* **3:** 106, 1962.
4. Deykin, D., and D. S. Goodman. *J. Biol. Chem.* **237:** 3649, 1962.
5. Hamilton, J. G., and J. E. Muldrey. *J. Am. Oil Chemists' Soc.* **38:** 582, 1961.
6. Pinter, K. G., J. G. Hamilton, and O. N. Miller. *Anal. Biochem.* **5:** 458, 1963.
7. Hirsch, J., and E. H. Ahrens, Jr. *J. Biol. Chem.* **233:** 311, 1958.

Downloaded from www.jlr.org by guest, on June 19, 2012