United States Patent Office

3,784,576
Patented Jan. 8, 1974

3,784,576

HALOGENATED CHOLESTEROL

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No Drawing. Filed Sept. 24, 1970, Ser. No. 75,269
Int. Cl. C07c *169/58*
U.S. Cl. 260—397.2 3 Claims

ABSTRACT OF THE DISCLOSURE

Disclosed herein is a halogenated cholesterol which can be utilized as an intermediate to prepare radioactive pharmaceuticals and to increase the fat content in animals.

BACKGROUND OF INVENTION

It has been known that cholesterol will localize in the adrenal. Because of this property various radioisotope derivatives of cholesterol have been prepared and tested. However, to be satisfactory the labeled compounds must meet several criteria among which is the ability of the compound to localize in an organ to a greater degree than the surrounding organs so a definitive test of the organ can be accomplished.

Various iodocholesterols have been tested, but for one reason or another have failed to localize and enable one to study the adrenal cortex satisfactorily. Among those prepared are those disclosed in the following articles:

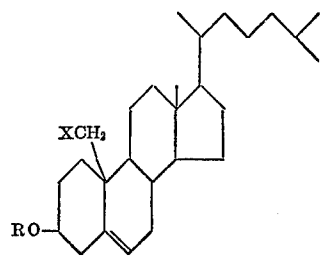
Nagai, T., Solis, B. A. and Koh, C. S., J. Nuclear Med., 9, 576 (1968); and

Kalvoda, J., Hensler, K., Ueberwasser, H., Anner., G., and Wettstein, A., Helv. Chim. Acta, 46, 1361 (1963).

THE INVENTION

This invention relates to 19-halocholesterols which can be utilized as fat producing agents and intermediates in the preparation of radioiodinated cholesterol which localizes in the adrenal cortex to provide a definitive scan of this organ. This end product when injected into a patient localizes in the adrenal cortex and allows a radiologist to obtain a scan of the organism as its uptake ratio here is greater than in the adrenal per se. Scans of the organ utilizing known techniques would then enable a practitioner ot visualize anatomical and functional differences in the adrenal gland, e.g., adrenocortical carcinomas, cushings syndrome. The amount of the iodocholesterol to be utilized in each patient is determined by known procedures as the amount of radioactivity will determine the volume amount to be injected into a patient. Therefore, from 0.1 mCi. to 10 mCi. per dose with from about 0.5 mCi. to about 5 mCi. is the preferred range.

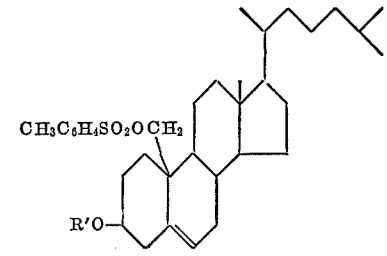
The 19-halocholesterols of this invention have the structure I:



(I)

wherein X is chloro, bromo, iodo or fluoro and R is hydrogen or acyl. The preferred acyl radicals are those of hydrocarbon carboxylic acids of less than twelve carbon atoms, as exemplified by the lower alkanoic acids (e.g., acetic, propionic butyric and pivalic acid), the lower alkanoic acids, the monocyclic aryl carboxylic acids (e.g., benzoic and toluic acids), the monocyclic aryl lower alkanoic acids (e.g., phenacetic and β-phenylpropionic acid), the cycloalkane carboxylic acids and the cycloalkene carboxylic acids.

The compounds of this invention are prepared by reacting cholest-5-ene-3,19-diol-3-acyl with a sulfonyl halide in an organic base such as pyridine. p-Toluene sulfonyl chloride is the preferred reactant, however, other sulfonyl halides such as methane sulfonyl bromide, ethoxy phenyl sulfonyl chloride and p-bromo phenyl sulfonyl chloride, and so forth, can be utilized. A tosylate of the structure II is thus formed:

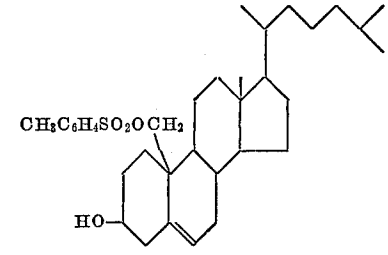


(II)

wherein R' is acyl.

The acyl group is then hydrolyzed by utilizing a basic solution of an alkali metal hydroxide such as sodium hydroxide, potassium hydroxide, and so forth; or other known means of hydrolyzing the 3-position of cholesterol as by utilizing an alkali metal carbonate or bicarbonate (e.g., sodium carbonate, potassium bicarbonate, etc.), an alkali metal (e.g., powdered sodium, potassium, etc.) or an alkali metal hydride (sodium hydride, potassium hydride, etc.).

The hydroxide thus formed has the structure III:



(III)

Treatment of Compound III with an alcoholic solution of an alkali metal halide such as sodium iodide, potassium bromide, and so forth, in propanol or butanol or other aliphatic alcohol will yield the corresponding 19-halocholesterol.

If the acyl derivative is desired, hydrolysis of Compound II is not performed as indicated above and the desired product is recovered.

The 19-iodo compounds of this invention can be labeled by isotope exchange of the compounds as by reaction with alkali metal halide, sodium iodide-125, potassium iodide-131, and so forth. By similar procedures isotopes-^{123}I and ^{130}I may also be placed in the 19-halo cholesterol of this invention.

It has further been found that this position is resistant to dehalogenation in the adrenal and does not markedly alter the structure of the cholesterol molecule. It is to be understood that this compound is to be further utilized as a diagnostic tool in humans.

The 19-iodocholesterol has the utility set forth above. The remaining 19-halocholesterol compounds of this instant invention have been found to increase the fat content in animals. Thus, from about 1.0 mg. to 10 mg. per kg. can be injected into a farm animal (e.g., sheep, cattle, pigs, and so forth) and a substantial increase in fat content noted. The injection can be administered as deemed necessary but for best results injection of a steroid of this invention should be within the first 6 months after birth.

The following examples are illustrative of the invention, all temperatures are in degrees centigrade, unless otherwise stated:

EXAMPLE 1

Cholest-5-ene-3β,19-diol 3-acetate 19-toluene-p-sulfonate

To a solution containing cholest-5-ene-3β,19-diol 3-acetate (300 mg.) in pyridine is added p-toluene sulfonyl chloride (300 mg.). The desired product is then recovered. The physical characteristics were identical with those reported by Akhtar and Barton, J. Amer. Chem. Soc., 86, 1528 (1964).

EXAMPLE 2

Cholest-5-ene-3β,19-diol 19-toluene-p-sulfonate

A solution of cholest-5-ene-3β,19-diol 3-acetate 19- toluene-p-sulfonate (200 mg.) in dioxane (7 ml.) is added dropwise to a solution of NaOH (100 mg.) in aqueous methanol (10 ml.). The solution is stirred at room temperature for 2 hours and then poured into ice-water. The resulting mixture is extracted with ether and the extract washed with water and dried over anhydrous sodium sulfate. Removal of the solvent and crystallization of the residue from acetone-water affords cholest-5-ene-3β,19-diol 19-toluene-p-sulfonate (120 mg.): M.P. 121–123°; NMR δ 0.58 (s, 3, C_{18}-protons), 2.53 (s, 3, $CH_3C_6H_4$—), 3.95 and 4.09 (d, 2, J_{gem}=10 c.p.s., C_{19}-protons), 5.50 (s, 1, vinylic proton), 7.31 and 7.73 (dd, 4, J_o=8 c.p.s., aromatic proton).

Analysis.—Calc'd for $C_{34}H_{52}SO_4$ (percent): C, 73.33; H, 9.41. Found (percent): C, 73.77; H, 9.55.

EXAMPLE 3

19-iodocholest-5-en-3β-ol

A solution of cholest-5-ene-3β,19-diol 19-toluene-p- sulfonate (200 mg.) and sodium iodide (100 mg.) in isopropanol (15 ml.) is gently refluxed under N_2 for 4 hours. The solution is concentrated to about 5 ml. in vacuo and poured into ice water. Extraction with ether and work up as in Example 2 furnishes an oily residue which solidifies upon trituration with petroleum ether (B.P. 30–40°). Recrystallization from methanol gives pure 19-iodocholest-5-en-3β-ol: M.P. 106–109°; NMR δ 0.69 (s, 3, C_{18}-protons), 3.24 and 3.51 (dd, 2, J_{gem}=11 c.p.s., C_{19}-protons), and 5.53 (s, 1, vinylic proton).

Analysis.—Calc'd for $C_{27}H_{45}IO$ (percent): C, 63.27; H, 8.85. Found (percent): C, 63.45; H, 8.91.

EXAMPLE 4

19-iodocholest-5-en-3β-ol acetate

A solution of cholest-5-ene-3β,19-diol 3-acetate 19-toluene-p-sulfonate (300 mg.) and sodium iodide (200 mg.) in isopropanol (20 ml.) is treated as above. Recrystallization of the crude product from acetone-methanol affords 19-iodocholest-5-en-3β-ol acetate (110 mg., 42%): M.P. 91–93° (reported [1] M.P. 97°); NMR δ 0.76 (s, 3, C_{18} protons), 2.0 (s, 3, CH_3COO—), 3.30 and 3.54 (dd, 2, J_{gem}=11 c.p.s., C_{19}-protons), and 5.61 (s, 1, vinylic proton).

[1] M. Akhtar and C. J. Gibbons, J. Chem. Soc., 5964 (1965).

Analysis.—Calc'd for $C_{29}H_{47}IO_2$ (percent): C, 62.79; H, 8.54. Found (percent): C, 62.78; H, 8.57.

EXAMPLE 5

Isotope exchange

A solution of $Na^{125}I$ (5 mc.) is placed in a 25 mg. round bottom flask and the water removed by azeotropic distillation with benzene. A solution of 19-iodocholest-5-en-3β-ol (100 mg.) in acetone (7 ml.) is added and the mixture refluxed under an atmosphere of nitrogen for 4 hours. The solution is allowed to cool and poured into cold water. The resulting mixture is extracted with ether and the ether extract washed with water and dried over anhydrous sodium sulfate. The ether is evaporated and the residue chromatographed over alumina (activity III). Elution with petroleum ether (B.P. 30–40°) ether (1:1) gives 19-iodocholesterol-^{125}I (80 mg.) with a specific activity of 28.25 mc./mg. (52% exchange). Thin layer chromatography using chloroform-ethanol (1:1) gives a single spot (R_f=0.66) coincident with the single radioactive peak appearing on the radiochromatogram.

EXAMPLE 6

Isotope exchange

Water from $Na^{125}I$ solution (3 mc.) is removed as described above. A solution of 19-iodocholest-5-en-3β-ol acetate (100 mg.) in acetone (5 ml.) is added and the clear solution gently refluxed with stirring for 4 hours under nitrogen. The solution is concentrated to about ½ the original volume and poured into ice water. The precipitate is collected, washed well with water, and recrystallized from methanolacetone. This gives 19-iodocholesterol acetate-^{125}I with a specific activity of 8.87 mc./mg. (68% exchange). T.L.C. using benzene:hexane (1:2) shows a single spot (R_f=0.74) coincident with the radioactive area shown by a radiochromatogram.

EXAMPLE 7

Twenty-six mongrel dogs weighing 7 to 13 kilograms are injected intravenously with 19-^{125}I-chloesterol. Ten to 90 μCi./kg. weight of the compound were administered to 13 dogs pretreated with 40 units of adrenal corticaltropic hormone gel daily for 2 to 4 days and continued to termination of the study period. The various daily intervals up to 8 days after the tracer dose scans were performed on the dogs and the results are as indicated in Table I.

TABLE I

Ratios of c.p.m./mg. adrenal cortex to liver [19-^{125} I-iodocholesterol (26 dogs)]

	ACTH			Non-ACTH		
Interval (hrs.)	No. at interval	Mean ratio	Range	No. at interval	Mean ratio	Range
24	1	5	---	1	7	---
48	2	19	10–27	3	31	29–35
72	3	94	74–102	3	22	6–32
96	2	85	78–92	2	33	29–36
120	1	100	---			
144	2	168	165–170	2	68	62–74
192	2	109	102–116	2	158	96–220

The scans were performed with the dogs under intravenous thiamylal and pentobarbital anesthesia in the prone position with a 5-inch crystal photoscanner using a coarse, 3-inch focus collimator. This example illustrates that 19-^{125}I-cholesterol will concentrate in the adrenal cortex at a sufficiently high level to enable the practitioner to satisfactorily visualize any malfunction therein. It also illustrates that ACTH accelerates the rate of uptake of the radioactive compound.

EXAMPLE 8

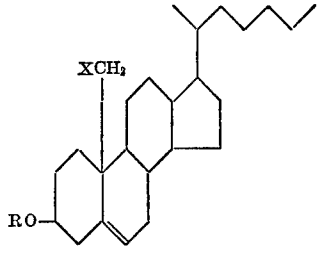
Following the procedure of Example 4 but utilizing the benzoate in lieu of the acetate, the corresponding 19-halocholesterol benzoate is recovered.

EXAMPLE 9

Following the procedure of Example 4 but utilizing the propionate cholesterol in lieu of the acetate cholesterol, the corresponding propionate is recovered.

What is claimed is:

1. A compound having the formula



wherein R is selected from the group consisting of hydrogen and acyl and X is selected from the group consisting of iodine-123, iodine-125 and iodine-131.

2. The compound of claim 1 wherein R is hydrogen.

3. The compound of claim 1 wherein R is acetyl.

References Cited

Akhtar, et al.: Jour. Chem. Soc. (London), November 1965, pp. 5964–5968 relied on.

ELBERT L. ROBERTS, Primary Examiner

U.S. Cl. X.R.

424—1

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,784,576 Dated January 8, 1974

Inventor(s) Raymond E. Counsell

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, line 45, "ot" should be -- to --;

Column 2, lines 5-6, "lower alkanoic" should be -- lower alkenoic --; and

Column 4, line 40, "chloesterol" should be -- cholesterol --.

Signed and sealed this 3rd day of September 1974.

(SEAL)
Attest:

McCOY M. GIBSON, JR.
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents

FORM PO-1050 (10-69)

USCOMM-DC 60376-P69
* U.S. GOVERNMENT PRINTING OFFICE : 1969 O—366-334

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