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On the Preparation of Cholesterol Labelled with Tritium at Carbon Atoms 24 and 25 (Cholesterol-24,25-T_2)

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Cholesterol-24,25-T_2 has been prepared by electrolysis of 3β-hydroxychol-5-enic acid and *iso*valeric acid-2,3-T_2 obtained by reduction of 3,3-dimethylacrylic acid with tritium gas.

In order to study the metabolism of cholesterol we have synthesized cholesterol labelled with tritium mainly at carbon atoms 24 and 25.

Cholesterol has been obtained by anodic (Kolbe) synthesis from 3β-hydroxychol-5-enic acid and *iso*valeric acid. With a 25-fold excess of *iso*valeric acid a 17 % yield of cholesterol calculated on the amount of bile acid used has been obtained (*cf.* Ref.[1]).

Adsorption chromatography on alumina followed by reversed phase partition chromatography had to be used to obtain a pure product from the reaction mixture.

Tritium labelled *iso*valeric acid was obtained by reducing 3,3-dimethylacrylic acid with tritium gas in dioxan solution in presence of platinum catalyst. The isotope is presumably located at carbon atoms 2 and 3 of the *iso*valeric acid but according to the experience of reduction with tritium gas in other cases, some isotope might also be located in the terminal methyl groups.

EXPERIMENTAL

All melting points were taken on an electrically heated aluminum block and are corrected. The radioactivity was estimated in a Tracerlab gas flow counter in an "infinitely thin" layer.

Δ^5-3β-Hydroxycholenic acid. An impure sample of the acid was purified by chromatography of its methyl ester on alumina. We are indebted to Ciba Ltd., Basel, for a generous sample of this acid. Electrolysis was carried out in wide test tubes (i.d. 35—38 mm)

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in which 3 platinum electrodes (20 × 26 mm) were fixed parallel to and at distances of 2 mm from each other. The middle one served as anode and the two outer ones as cathode. The current used was 2.2 A.

262 mg (0.7 mmole) of 3β-hydroxychol-5-enic acid, 2 ml (1.80 mmole) of isomer-free *iso*valeric acid and 42 mg (1.87 mmole) of sodium was dissolved in a mixture of 26 ml methanol and 9 ml dry pyridine. (The cholenic acid did not dissolve in methanol alone.)

The vessel was cooled in an ice-salt mixture and the solution was also cooled by dipping into it a glass tube through which cold water was circulated. The temperature of the reaction mixture remained between 35° and 45° during the electrolysis that was continued until pH 7.5 was reached. The solution was then taken to dryness *in vacuo*. 20 ml of 2 N hydrochloric acid was added and extracted with four 50 ml portions of ether. The ethereal solution was washed with 2 N hydrochloric acid, water, 10 % sodium hydroxide solution and water, and then taken to dryness *in vacuo*. The residue weighed 230 mg.

Chromatography. The residue was dissolved in benzene and brought onto a column (i.d. of tube 15 mm) of 20 g aluminum oxide (alkali free [2]), prepared with benzene. The column was eluted as shown in Table 1, fractions of 40 ml being collected.

Table 1.

Fraction	Eluent	Eluate		
1	Benzene	—		
2,3	Benzene/ether 19:1 v/v	11 mg oil		
4—13	»	small amount of oil in each		
14	Benzene/ether 17:3 v/v	5.9 mg, oily		
15	»	8.0 mg, crystalline		
16	»	10.1 mg	»	
17	»	12.6 mg	»	m.p. 95—105°
18	»	14.2 mg	»	m.p. 119—30°
19	»	21.6 mg	»	m.p. 126—32°
20	»	28.3 mg	»	m.p. 130—135°
21	»	27.7 mg	»	m.p. 128—34°
22	»	12.1 mg	»	m.p. 127—30°
23	»	4 mg	»	
24	»	2 mg	»	

Similar chromatograms were also obtained in the preparation of active cholesterol.

Fractions 19, 20 and 21 after one crystallization from methanol yielded 71 mg of crystals of m.p. 133—37°. 23.6 mg of this was mixed with 1 mg of cholesterol-4-^{14}C and

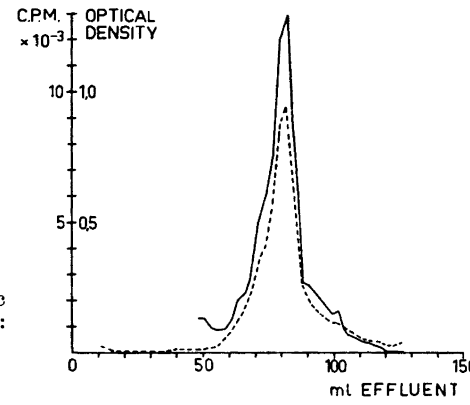

Fig. 1. Partition chromatogram of synthetic cholesterol. Full line: c.p.m. Broken line: cholesterol color reaction [4].

subjected to reversed phase partition chromatography at 23° on hydrophobic Supercel (4.5 g) with 55 % *iso*propanol in water as the mobile phase and chloroform/heptane (1:4) (4 ml) as the stationary phase as described by Danielsson [3].

Fractions of about 2 ml were collected with a fraction-collector. Aliquot parts of the fractions were taken and the radioactivity estimated by plating and counting. The total cholesterol was determined with the Tschugaeff's reaction [4]. The results are shown in Fig. 1.

The substance (17 mg) was collected from the fractions between 66 and 90 ml effluent. One crystallization from methanol gave 14.0 mg of crystals, m.p. 147–48°. A mixed melting point determination with authentic cholesterol gave no depression.

3,3-Dimethylacrylic acid was prepared by oxidation of mesityl oxide [5].

Isovaleric acid-2,3 T. The hydrogenation was carried out in a high vacuum apparatus similar to that described by Glascock [6]. The reaction vessel had a volume of 2.9 ml and was connected to the vacuum line with a balljoint. We have also found it convenient to introduce a 15 mm long gold-plated iron rod as a magnetic stirrer. By this technique the long reaction time described by Glascock can be much reduced.

2 mg of platinum oxide suspended in 0.1 ml dry dioxan was reduced with inactive hydrogen in the reaction vessel. 5 mg of dimethylacrylic acid in 0.15 ml of dioxan was then added. The apparatus was evacuated with the reaction vessel cooled in liquid air and 0.208 ml of tritium gas (0.5 C) pumped into the reaction space with the Toepler pump. After 1 h 95 % of the tritium gas had been taken up. 0.5 ml of inactive hydrogen was then pumped into the vessel. This gas was consumed in 2 h and the reduction then brought to completion with a further portion of inactive hydrogen.

Cholesterol-24,25-T. The labelled *iso*valeric acid was dissolved in 5 ml dioxan and added to the same mixture as described above and was then electrolysed and worked up as before. 230 mg of residue was obtained.

Chromatography on alumina followed by partition chromatography yielded in all 46 mg cholesterol (m.p. 147–48°), *i.e.* a 17 % yield calculated on the amount of 3β-hydroxychol-5-enic acid used.

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