Synthesis of Cholesterol-20-^{14}C

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SUMMARY

Cholesterol-20-^{14}C was synthesized in four steps from pregnenolone-20-^{14}C. The labelled intermediates were proven to be cholest-5-ene-3β, 20α-diol acetate-3, cholesta-5, 20 (22)-diene-3β-ol acetate, and cholesterol acetate by ^{1}H-n.m.r. spectroscopical analysis. Cholesterol-20-^{14}C was obtained with a radiochemical purity exceeding 99 %.

In connection with our work on the biosynthesis of bufadienolides from animal and plant origin [1], it was required the preparation of cholesterol labelled with ^{14}C at any carbon-atom of the side chain but carbons 25, 26 and 27.

Since pregnenolone-20-^{14}C had been previously synthesized [2] we chose this labelled steroid as starting material for the preparation of cholesterol labelled with ^{14}C at C-20.

When pregnenolone-20-^{14}C acetate (I) was treated with the Grignard reagent prepared from isohexyl bromide [3], cholest-5-ene-3β, 20α-diol-20-^{14}C acetate-3 (II) was obtained in good yield. This compound was first synthesized by Petrow and Stuart-Webb [4] although at that time the authors did not disclosed the stereochemistry of the product. The same method was utilized later by Bergman *et al.* [5] without making any comments about the chirality at C-20. Recently, by analysis of n.m.r. spectra, Mijares *et al.* [6] presented evidence that the reaction between pregnenolone acetate and isohexyl magnesium bromide only produces the 20α epimer.

The labelled 20-hydroxy-cholesterol (II), without purification, was dehydrated by reaction with $POCl_3$ and pyridine in a sealed tube [7] to cholesta-5, 20 (22)-diene-3β-ol-20-^{14}C acetate (III). It seemed reasonable to suppose that

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dehydration of 20-hydroxy-cholesterol might produce a mixture of cholestadiene derivatives because of the three possible orientations of the elimination reaction [8]; however, analysis of the n.m.r. spectrum of III indicated that the predominant isomer (more than 90 %) had the second double bond between C-20 and C-22; the same structure had been correctly assumed by previous workers [5]. That some amount of isomeric products were present, could be inferred from the fact that the product could not be induced to crystallize.

Compound III was selectively hydrogenated over Pt in dioxane-acetic acid, under conditions which do not involve the saturation of the $\Delta^{5,6}$ double bond [9], to cholesterol-20-^{14}C acetate (IV) which, upon saponification, yielded cholesterol-20-^{14}C (V). The crude product was purified by chromatography on neutral alumina to pure V, whose i.r. and n.m.r. spectra were identical to the similar from authentic sample. The radio-thin-layer chromatogram of V indicated a radiochemical purity higher than 99 %.

EXPERIMENTAL.

I.r. spectra were recorded with a Perkin-Elmer Infracord spectrophotometer. N.m.r. spectra were measured with a Varian A-60 spectrometer in deuterochloroform; tetramethylsilane was used as an internal standard and to provide a lock signal. T.l. chromatography was conducted on silica-gel chromatoplates (Eastman, type K301R2) using chloroform:methanol mixtures. Radioactivity was assayed with a Packard Liquid Scintillation Spectrometer model 3305 in the usual scintillation solutions. Solvents were removed under diminished pressure below 50°.

Cholest-5-ene-3β, 20α-diol-20-^{14}C Acetate-3 (II).

Pregnenolone-20-^{14}C acetate (500 mg, 2.00×10^7 dpm/mmole) in benzene (30 ml) was added to a boiling solution of isohexylmagnesium bromide in ether (prepared from isohexyl bromide (2 ml), magnesium (300 mg) and ether (40 ml)). The mixture was boiled for 1 hr, the ether was distilled off, and the remained benzenic solution was heated under reflux for another 4 hr. The cool reaction mixture was poured into HCl-water and the product was extracted with benzene; the dried benzenic solution was evaporated, and the oily residue was treated with pyridine (10 ml) and acetic anhydride (10 ml). After 16 hr at room temperature the mixture was poured into ice-water, and the insoluble was extracted with chloroform; the chloroformic solution was washed with 2N HCl, saturated $NaHCO_3$ solution, water, and it was dried over $MgSO_4$. The oily residue obtained from removal of the solvent (550 mg) had an i.r. spectrum in agreement with the proposed structure; n.m.r. data : δ 0.83 (3-protons singlet, CH_3-18); δ 0.88 (6-protons doublet, CH_3-26 and CH_3-27); δ 1.03 (3-protons singlet, CH_3-19); δ 1.15 (3-protons

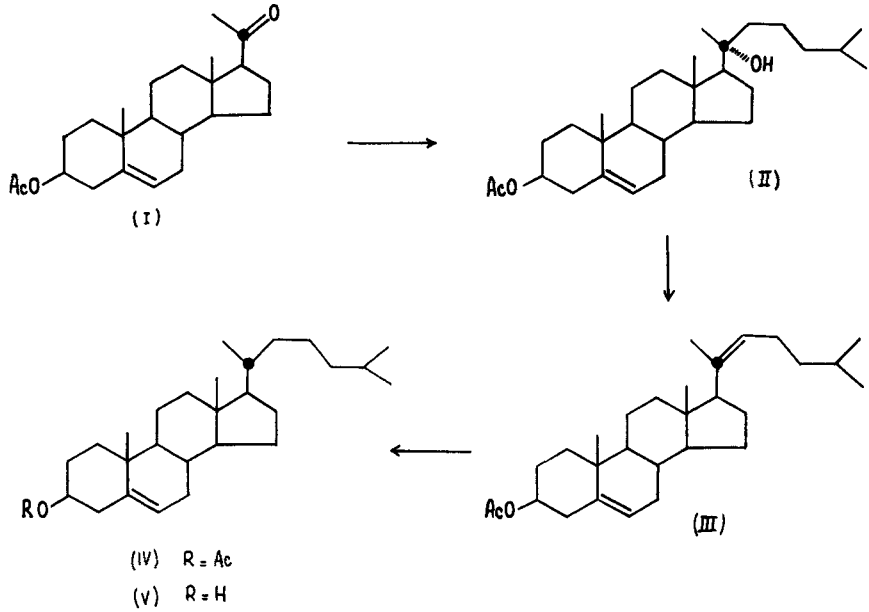
singlet, CH_3-21); δ 2.03 (3-protons singlet, CH_3-acetyl); δ 4.63 (1 proton broad signal, H-3); δ 5.38 (1-proton, H-6); the product had spec. act. of 1.97 × 10^7 dpm/mmole.

Cholesta-5, 20 (22)-dien-3β-ol-20-^{14}C Acetate (III).

Compound II (530 mg), pyridine (8 ml), and $POCl_3$ (1.2 ml) were heated in a sealed tube at 140° for 6 hr. Once at room temperature the mixture was poured into ice-HCl, and the insoluble was extracted with ether. The extract was washed with saturated $NaHCO_3$ solution, with water, and dried over $MgSO_4$. The non-crystalline residue obtained for evaporation of the solvent (440 mg) showed to be expected product; the i.r. spectrum showed no absorption in the hydroxyl region; n.m.r. data : δ 0.83 (3-protons singlet, CH_3-18); δ 0.88 (6-protons doublet, CH_3-26 and CH_3-27); δ 1.03 (3-protons singlet, CH_3-19); δ 2.01 (6-protons singlet, CH_3-21 and CH_3-acetyl); δ 4.66 (1-proton broad signal, H-3); δ 5.16 (1-proton, H-22); δ 5.38 (1-proton, H-6); the spec. act. of the product was 1.99 × 10^7 dpm/mmole.

Cholesterol-20-^{14}C Acetate (IV).

Compound III (420 mg) was dissolved in dioxane (25 ml) and acetic acid (0.5 ml). The solution was hydrogenated for 24 hr at room temperature.



Synthesis of cholesterol-20-^{14}C from pregnenolone-20-^{14}C. Labelled carbon-atoms are indicated with heavy dots.

and atmospheric pressure over Pt (from 100 mg of PtO_2). The catalyst was filtered off, and the filtrate was evaporated. The residue (380 mg) showed by n.m.r. analysis to be the expected product : δ 0.68 (3-protons singlet, CH_3-18); δ 0.87 (6-protons doublet, CH_3-26 and CH_3-27); δ 0.90 (3-protons doublet, CH_3-21); δ 1.02 (3-protons singlet, CH_3-19); δ 2.01 (3-protons singlet, CH_3-acetyl); δ 4.88 (1-proton broad signal, H-3); δ 5.35 (1-proton, H-6); spec. act. 2.00×10^7 dpm/mmole.

Cholesterol-20-^{14}C (V).

Without purification, compound IV (370 mg) was dissolved in ethanol (10 ml), the solution was treated with NaOH (0.8 g) in water (1.6 ml), and the mixture was heated under reflux for 3 hr. When cool, the heterogeneous mixture was extracted with chloroform. The extract was washed with 2N HCl, saturated $NaHCO_3$ solution, and water. The oily residue (215 mg) obtained for evaporation of the dried extract, was taken in benzene and chromatographied on aluminium oxide, (Woelm neutral, activity I) (20 g) eluting with the same solvent. Fractions were monitored by t.l.c., and those containing cholesterol were combined and evaporated to dryness. The crystalline residue was recrystallized once from ethanol yielding 145 mg of pure compound; its i.r. and n.m.r. spectra were identical to those from an authentic sample; spec. act. 2.00×10^7 dpm/mmole; the radio-thin-layer chromatogram of the product indicated a radiochemical purity higher than 99 %.

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