SYNTHESIS OF |21-^{14}C|-3β-HYDROXY-5β-PREGNAN-20-ONE AND |21-^{14}C|-5β-CHOLESTAN-3β-OL

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SUMMARY

|21-^{14}C|-3β-Hydroxy-5β-pregnan-20-one was synthesised from 3β-acetoxy-5β-androstan-17β-carboxylic acid chloride and |$^{14}C_2$|dimethylcadmium. Reaction of the labelled product with isohexyl bromide yielded, after dehydration and catalytic hydrogenation, |21-^{14}C|-5β-cholestan-3β-ol (|21-^{14}C|-coprostanol).

Key Words: |21-^{14}C|-3β-Hydroxy-5β-pregnan-20-one; |21-^{14}C|-Coprostanol; Synthesis.

In connection with our studies on the biosynthesis of cardiotonic steroids in living organisms (1) we needed 5β-cholestan-3β-ol (coprostanol) labelled at the side chain. We have previously reported the synthesis of |20-^{14}C|-3β-hydroxy-5β-pregnan-20-one (2) which could have been used for the preparation of the corresponding labelled coprostanol but in the present case we choosed to have the label at C-21 for the sake of a simpler degradation reaction of the natural product resulting from the feeding experiment. Therefore, we report here the preparation of |21-^{14}C|-3β-hydroxy-5β-pregnan-20-one and its chemical transformation into |21-^{14}C|-5β-cholestan-3β-ol.

RESULTS AND DISCUSSION

3β-Hydroxy-5β-pregnan-20-one (1a) was acetylated to 1b which was transformed into 2a by oxidation with lead tetraacetate (3). In turn, compound 2a was deacetylated to 2b. Periodate oxidation afforded the etianic acid 3a which, after acetylation to 3b, was converted into the acid chloride 4 by treatment with oxalyl chloride in benzene (3). Treatment of compound 4 with |$^{14}C_2$|-dimethyl cadmium, prepared from |^{14}C|methylmagnesium iodide and cadmium chloride (3-5), afforded |21-^{14}C|-3β-acetoxy-5β-pregnan-20-one (5a) with identical properties to those of the unlabelled product.

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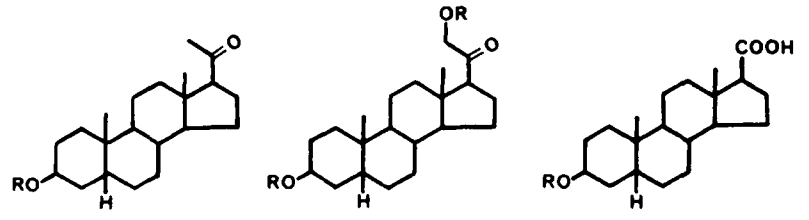

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1b R=Ac

2a R=Ac
2b R=H

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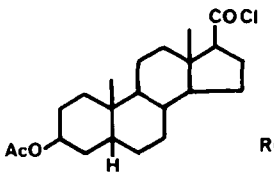
3b R=Ac



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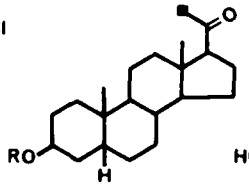

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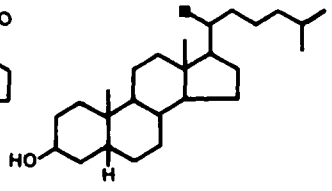
5b R=H



6 (■:^{14}C)

Reaction of compound 5a with isohexylmagnesium bromide under Grignard conditions (6) followed by acid-catalysed dehydration of the reaction product, yielded labelled 5β-cholest-20(22)-en-3β-ol which, without further purification, was hydrogenated over palladium/charcoal to |21-^{14}|-5β-cholestan-3β-ol (6) which resulted identical (IR,MS) to an authentic standard. The labelled coprostanol had an spec. act. of 1.43 mCi/mmol.

EXPERIMENTAL

Melting points were determined in a Fischer-Johns hot-plate and are uncorrected. IR spectra were measured using a Perkin-Elmer 421 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 100 and 25.2 MHz respectively in the FT mode with a Varian XL-100-15 spectrometer. Mass spectra were determined at 70 eV (direct inlet) with a Varian-MAT CH7-A spectrometer interfased to a Varian-MAT Data System 166 computer. Radioactivity was measured by liquid scintillation counting. |^{14}C|Methyl iodide was purchased from New England Nuclear Corp.

|^{14}C|-3β-*Acetoxy*-5β-*pregnan*-20-*one* (5a).

a. |$^{14}C_2$| *Dimethyl cadmium.* A small reaction flask containing metallic Mg (15 mg) was connected to the sealed tube containing the radioactive MeI (1 mCi, 58 mCi/mmol) and the system was evacuated. A solution of unlabelled MeI (20 µl) in anhydrous ethyl ether (1 ml) was introduced into the flask by injection through a silicone-rubber septum and the mixture was stirred for 30 min noting the consumption of some Mg and the formation of

a gray solution. The reaction flask was cooled in a liquid nitrogen bath and the breakseal of the tube having the radioactive MeI was broken. In these conditions the MeI glass container was gently warmed for 15 min. A second portion of unlabelled MeI (13 µl) was injected as before, the cooling bath was removed and, once at room temperature, the mixture was stirred for 30 min when all the Mg disappeared.The reaction flask was filled up with dry nitrogen gas and $CdCl_2$ (220 mg) was added and the mixture was stirred at room temperature for 2 hr.

b. *3β-Acetoxy-5β-androstan-17β-carboxylic acid chloride* (4). 3β-Acetoxy-5β-androstan-17β-carboxylic acid (3b) obtained as described elsewhere (3) (100 mg) was dissolved in anhydrous benzene (2 ml) and treated with a solution of oxalyl chloride (1 ml) in benzene (2 ml). The reaction was maintained at room temperature for 2 hr. Evaporation of the solvent afforded compound 4 (105 mg); IR (film): 1800, 1730 cm^{-1}.

c. *|21-^{14}C|-3β-Acetoxy-5β-pregnan-20-one* (5a) *by reaction of compound 4 with |$^{14}C_2$|-dimethyl cadmium.*

Compound 4 (180 mg) in anhydrous benzene (2 ml) was added through the septum to the radioactive dimethyl cadmium solution and the mixture was stirred at room temperature for 18 hr and then heated at 50°C for 1 hr. The mixture was cooled to 0°C and conc. HCl (0.5 ml) was added dropwise followed by water (2 ml). It was extracted with CH_2Cl_2 (2 x 20 ml) and the organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent yielded 158 mg of a residue that was purified by column chromatography on silica gel G (CH_2Cl_2-MeOH, 98:2) giving pure 5a (68 mg) of m.p. 117-119°C (Lit. (7) m.p. 121°C) from EtOH and sp. act. 1.43 mCi/mmol. IR: 1735, 1695 cm^{-1}. ^{1}H NMR ($CDCl_3$-TMS): δ 0.62 (3H, s, Me-18), 0.98 (3H, s, Me-19), 2.06 (3H, s, CH_3CO), 2.12 (3H, s, Me-21), 5.10 (1H, m, H-3). ^{13}C NMR ($CDCl_3$-TMS): δ 13.28 (C-18), 20.94 (C-11), 21.30 (CH_3-CO), 22.73 (C-16), 23.63 (C-19), 24.25 (C-15), 24.83 (C-2), 25.99 (C-6), 26.26 (C-7), 30.42 (C-1 or C-4), 30.58 (C-1 or C-4), 31.31 (C-21), 34.73 (C-10), 35.48 (C-8), 37.11 (C-5), 39.06 (C-12), 39.70 (C-9), 44.11 (C-13) 56.55 (C-14), 63.61 (C-17), 70.31 (C-3), 170.14 (CH_3-CO), 208.80 (C-20). MS (m/z, %): 360 (M^+, 1.1), 342 (2.2), 300 (100), 285 (21.9), 257 (10.6), 215 (31.8), 43 (60.1).

|21-^{14}C|-3β-Hydroxy-5β-pregnan-20-one (5b). Compound 5a (5 mg) in EtOH (10 ml) was treated with conc. H_2SO_4 (2 drops) and refluxed for 24 hr. It was poured into water (30 ml) and extracted with CH_2Cl_2 (3 x 20 ml). The residue obtained by evaporation of the organic solvent was purified by preparative TLC (silica gel G) affording pure 5b of m.p. 140-143°C (Lit. (7) m.p. 149°C) and sp. act. 1.46 mCi/mmol. IR: 3500-3200, 1705 cm^{-1}. ^{1}H NMR ($CDCl_3$-TMS): δ 0.62 (3H, s, Me-18), 0.97 (3H, s, Me-19), 2.11 (3H, s, Me-21), 4.13 (1H, m, H-3). ^{13}C NMR

($CDCl_3$-TMS): δ 13.52 (C-18), 21.00 (C-11), 22.89 (C-16), 23.87 (C-19), 24.52 (C-15), 26.00 (C-6), 26.32 (C-7), 27.92 (C-2), 30.01 (C-1), 31,49 (C-21), 33.46 (C-4), 35.70 (C-10 and C-8), 36.36 (C-5), 39.29 (C-12), 39.82 (C-9), 44.36 (C-13), 56.78 (C-14), 63.99 (C-17), 66.86 (C-3), 209.62 (C-20). MS (m/z, %): 318 (M^+, 57.3), 303 (24.5), 300 (94.3), 285 (30.7), 257 (21.2), 233 (26.4), 215 (62.8), 43 (100).

|21-^{14}C|-5β-Cholestan-3β-ol (6). A solution of compound 5a (50 mg) in anhydrous benzene (1 ml) was added to a solution of isohexylmagnesium bromide prepared from isohexyl bromide (0.25 ml) and Mg (50 mg) in anhydrous ethyl ether (8 ml)(8).The mixture was refluxed for 12 hr, cooled to 0°C and the reaction was quenched by dropwise addition of conc. HCl (0.8 ml) and water (10 ml). The mixture was extracted with CH_2Cl_2 (3 x 20 ml) and the organic layer was washed with water and dried over $MgSO_4$. The residue obtained by evaporation of the solvent was treated with H_2SO_4 (0.2 ml) in EtOH (10 ml) and refluxed for 3 hr. It was poured into ice-water and extracted with CH_2Cl_2 (3 x 20 ml). The organic layer was washed with water and dried ($MgSO_4$). Evaporation of the solvent afforded a residue that was purified by preparative TLC (silica gel G, CH_2Cl_2-MeOH 98:2) yielding 27 mg of |21-^{14}C|-5β-cholest-20(22)-en-3β-ol of sp. act. 1.45 mCi/mmol which without further purification was dissolved in EtOAc (10 ml) and hydrogenated at atmospheric pressure and room temperature over 10% Pd/C for 5 hr. The catalyst was filtered off and the filtrate was evaporated yielding 27 mg of compound 6 of m.p. 95-98°C (EtOH) (Lit.(9) m.p. 101°C). The labelled 6 had IR and MS spectra identical to those from an authentic standard (10). Its sp. act. was 1.43 mCi/mmol.

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