SYNTHESIS OF [21-^{13}C]-CHOLESTEROL

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SUMMARY

The synthesis of [21-^{13}C]-cholesterol (**5**) from 3β-O-(t-butyldimethylsilyl)-17β-cyano-androst-5-ene (**1**) is described. Labelled carbon-atom was introduced by Grignard reaction of nitrile derivative with [^{13}C]-methylmagnesium iodide. Location of label was confirmed by ^{13}C-NMR spectroscopy.

Key Words: [21-^{13}C]-cholesterol; synthesis.

INTRODUCTION

It has been demonstrated that sterols such as cholesterol and coprostanol may act as biosynthetic precursors of bufadienolides in toads of the genus *Bufo* (1,2). Taking into account that the ^{14}C label is retained when [20-^{14}C]-cholesterol is converted to animal bufadienolides (1) but lost when [24-^{14}C]-cholesterol is the tested precursor (3), it has been postulated that to get into the bufadienolide-producing gland the steroid precursor should have a specific structural requirement. Results also point to the hypothesis that once inside the gland, cholesterol or a closely related compound, would be first converted into an intermediate with a 20-keto-pregnane structure which could be subsequently transformed into the bufadienolide (4). In order to verify this hypothesis, cholesterol labelled with carbon-13 at C-21 was required.

RESULTS AND DISCUSSION

The 3ß-O-(t-butyldimethylsilyl) derivative of 17ß-cyano-androst-5-ene (**1**) was prepared as previously described (5) in 58% yield. Compound **1** was submitted to the Grignard reaction with [^{13}C]-methylmagnesium iodide, prepared from labelled methyl iodide, affording the 20-keto-pregnenesilylether **2** in good yield. This compound was converted almost quantitatively into [21-^{13}C]-pregnenolone (**3**) by reaction with tetra-n-butylammonium fluoride in dry tetrahydrofuran under mild conditions (5). It is known that Wittig reactions between pregnenolone and bulky phosphoranes yield only the *E*-20(22)-dehydro-steroids isomers (6). It has also been informed that regio and stereoselective reductions of *E*-20(22)-Δ^5-dehydrosterols can be accomplished by catalytic hydrogenation over PtO_2 in dioxane-acetic acid at a slightly

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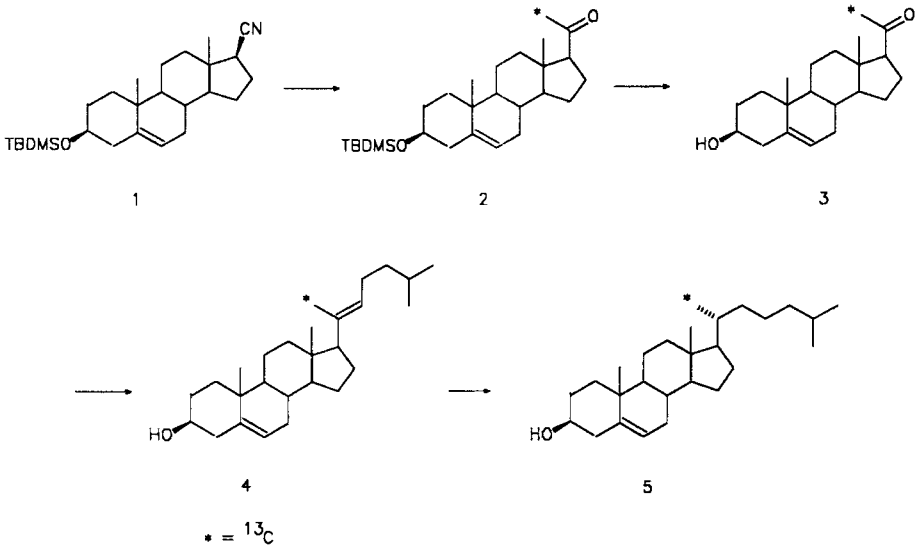

elevated pressure. The selectivity of natural to unnatural stereochemistry which is possible from such a reaction may approximate 70:30 in favor of the natural C-20(*R*) stereochemistry (7). Accordingly, compound **3** was treated with triphenyl-1-(4-methyl)-pentyl-phosphorane, prepared from the corresponding phosphonium salt and n-butyllithium, to give the *E*-cholestene (**4**) in 25% yield. In turn, catalytic hydrogenation of cholestene **4** in the presence of prereduced PtO_2 afforded [21-^{13}C]-cholesterol as a C-20 epimeric mixture. This was purified by HPLC giving the title compound **5a** (the 20(*R*) epimer) in 43% yield and the unnatural 20(*S*) epimer **5b** in 13% yield. The overall yield of the whole synthetic sequence starting from **1** was 7 %. The ^{1}H-NMR and normal broad band decoupled ^{13}C-NMR spectra of **5a** were in agreement with the proposed structure. Fig. 1 shows mass spectra of cholesterol and [21-^{13}C]-cholesterol.

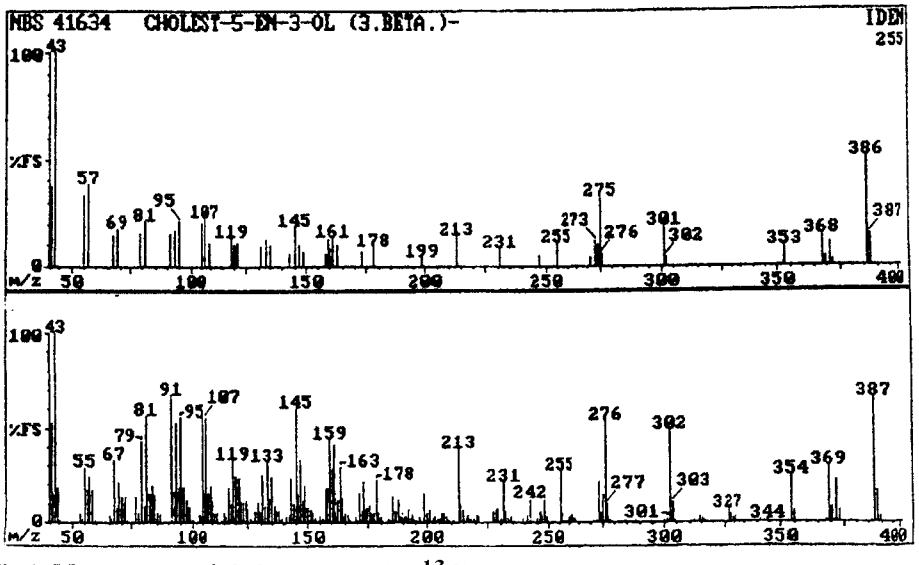

Fig 1. Mass spectra of cholesterol and [21-^{13}C] cholesterol.

EXPERIMENTAL

Melting points are uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ solutions (with the exception of compound **2** whose spectra were obtained in Py-d_5 solutions) using TMS as internal standard and were recorded on a Varian XL-100-15 spectrometer, at 100 and 25.2 MHz respectively, operating in the FT mode, and on a Bruker ACE-200 spectrometer at 200.13 and 50.33 MHz respectively. EI- mass spectra were recorded at 70 eV (direct inlet) on a Varian-Mat CH7-A spectrometer coupled to a Varian-Mat Data System 166, and on a Trio-2/2000 VG Masslab spectrometer.

[21-^{13}C]-3β-O-(t-Butyldimethylsilyl)-pregn-5-en-20-one (2). To a solution of [^{13}C]-methylmagnesium iodide, prepared from Mg (0.11 g) and [^{13}C]-methyl iodide (99 atom%, 0.3 ml, 4.9 mmol) in dry ether (1 ml), a solution of 3β-O-(t-butyldimethylsilyl)-17β-cyano-androst-5-ene (**1**) (0.20 g, 0.48 mmol) in benzene (8 ml) was added. The mixture was refluxed under a N_2 atmosphere for 40 hr. It was cooled to 0°C, treated with saturated solution of NH_4Cl, and maintained at r.t. for 2 hr. The organic layer was separated, and the aqueous layer was extracted with benzene; the combined organic extract was washed as usual and dried. Evaporation of the solvent gave a residue (0.20 g) that was chromatographed on a silica gel column eluted with hexane-EtOAc (90:10) affording pure **2** (0.14 g, 65%).
^{1}H-NMR: δ 0.06 (s, 6H, Me_2Si), 0.64 (s, 3H, 18-Me), 0.90 (s, 9H, Me_3C), 1.01 (s, 3H, 19-Me), 2.13 (d, 3H, J ^{13}C-H = 127.0 Hz, 21-Me), 3.55 (m, 1H, H-3), 5.35 (m, 1H, H-6).
^{13}C-NMR: δ 31.6 (s, ^{13}C-21).
EI-MS: m/z 431 [M+1], 416 [(M+1] - CH_3], 374 [(M+1) - Me_3C], 75 [Me_2SiOH], 44 [$CH_3{}^{13}CO$].

[21-^{13}C]-Pregnenolone (3). A solution of compound **2** (0.13 g, 0.3 mmol) and tetrabutylammonium fluoride (0.35 g) in anh. THF (2.5 ml) was kept at r.t. for 20 hr. EtOAc (20 ml) was added and the mixture was washed with water and dried ($MgSO_4$). Evaporation of the solvent gave pure **3** (0.09 g, 95%).
^{1}H-NMR: δ 0.64 (s, 3H, 18-Me), 1.01 (s, 3H, 19-Me), 2.13 (d, 3H, J ^{13}C-H = 127.0 Hz, 21-Me), 3.55 (m, 1H, H-3), 5.35 (m, 1H, H-6).
^{13}C-NMR: δ 31,6 (s, ^{13}C-21).
EI-MS: m/z 317 [M+1], 301 [(M+1) - $^{13}CH_3$], 299 [(M+1) - H_2O], 44 [$CH_3{}^{13}CO$].

[21-^{13}C]-(20E)-Cholesta-5,20(22)-dien-3β-ol (4). To a suspension of triphenyl-(4-methyl)-1-pentyl-phosphonium bromide (0.89 g, 2.07 mmol) in dioxane (2 ml) kept in a centrifuge tube at r.t., a solution 1.5 M of n-butyllithium (1.1 ml, 1.65 mmol) was added dropwise under a N_2 atmosphere. The mixture was centrifugated and the supernatant taken with a syringe. The deep red solution thus obtained was added to a solution of compound **3** (85 mg, 0.27 mmol) in dioxane (2 ml) and the mixture was heated under reflux for 20 hr. It was cooled to r.t., treated with NH_4Cl (ss), and extracted with CH_2Cl_2. The extract was washed with water and dried ($MgSO_4$). Evaporation of the solvent gave a residue that was chromatographed on a silica gel column eluted with hexane-EtOAc (75:25) affording pure **4** (26 mg, 25%).

^{1}H-NMR: δ 0.55 (s, 3H, 18-Me), 0.89 (d, 6H, J = 6.5 Hz, 26- and 27-Me), 1.63 (d, 3H, J ^{13}C-H = 124.0 Hz, 21-Me), 3.54 (m, 1H, H-3), 5.18 (m, 1H, H-22), 5.36 (m, 1H, H-6).
^{13}C-NMR: δ 17,8 (s, ^{13}C-21).
EI-MS: m/z 385 [M+1], 370 ([M+1] - CH_3), 367 ([M+1] - H_2O).

*[21-^{13}C]-Cholesterol (**5a,b**).* A solution of **4** (25 mg, 0.06 mmol) in dioxane:AcOH (50:1) was hydrogenated at atmospheric pressure over PtO_2 (35 mg) at r.t. for 4 hr. Excess PtO_2 was added (35 mg) and the hydrogenation was continued for 6 hr. The catalyst was filtered off and the solvent was evaporated affording crude **5a,b** (21 mg, 90%) as a mixture of C-20 epimers. The mixture was purified by HPLC (ODS-2, 5 μm, MeOH) giving pure **5a** (10 mg, 43%) and pure **5b** (3 mg, 13%).

5a

^{1}H-NMR: δ 0.68 (s, 3H, 18-Me), 0.87 (d, 6H, J = 6.6 Hz, 26-and 27-Me),
0.93 (dd, 3H, J ^{13}C-H = 124.3 Hz, J ^{1}H-^{1}H = 6.4 Hz, 21-Me), 1.00 (s, 3H, 19-Me),
3.50 (m, 1H, H-3), 5.35 (m, 1H, H-6).
^{13}C-NMR: δ 18.7 (s, ^{13}C-21).
EI-MS: m/z 387 [M+1], 372 ([M+1] - CH_3), 369 ([M+1] - H_2O).

5b

^{1}H-NMR: δ 0.68 (s, 3H, 18-Me), 0.82 (dd, 3H, J ^{13}C-^{1}H = 124.3 Hz, J ^{1}H-^{1}H = 6.4 Hz,
21-Me), 0.87 (d, 6H, J = 6.6 Hz, 26- and 27-Me), 1.01 (s, 3H, 19-Me), 3.52 (m, 1H, H-3),
5.35 (m, 1H, H-6).
^{13}C-NMR: δ 18.7 (s, ^{13}C-21).
EI-MS: m/z 387 [M+1], 372 ([M+1] - CH_3), 369 ([M+1] - H_2O).

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REFERENCES

1. Porto, A.M. and Gros, E.G. - *Experientia*, **27**: 506 (1971).
2. Garraffo, H.M. and Gros, E.G. - *Steroids*, **48**: 251 (1986), and previous papers cited therein.
3. Garraffo, H.M., Burton, G., Porto, A.M. and Gros, E.G. - *An.Asoc. Quim. Argent.* **70**: 743 (1982).
4. Gros E.G. - *Anal. Acad. Nac. Cs. Ex. Fis. Nat.* **38**: 17 (1986).
5. Corey, E.J. and Venkateswarlu, A. - *J. Am. Chem. Soc.* **94**:6190 (1972).
6. Schow, S.R. and McMorris, T.C. - *J. Org. Chem.*, **44**: 3760 (1979).
7. DuBois, G.E. - *J. Org. Chem.* **47**: 5035 (1982).