SYNTHESIS OF [20,21-$^{13}C_2$]-PREGNENOLONE

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SUMMARY

The synthesis of [20,21-$^{13}C_2$]-pregnenolone (**7**) from androst-5-en-3ß-ol-17-one (**1**) is described. Labelled carbons were introduced by two procedures, namely, condensation of **1** with K^{13}CN and Grignard reaction of nitrile derivative **5** with [^{13}C]-methylmagnesium iodide. Location of labels was confirmed by ^{13}C-NMR spectroscopy.

Key Words: [20,21-$^{13}C_2$]-pregnenolone; synthesis.

INTRODUCTION

It has been established by feeding experiments with labelled acetate and pregnenolone that the biological formation of the butenolide ring of cardenolides in plants of the genus *Digitalis* proceeds by the condensation of an acetate unit on the C-20 of a 20-keto-pregnane derivative (1). From the results of experiments conducted with labelled pregnane derivatives bearing hydroxyl groups at C-14ß and at C-21, Tschesche *et al.* proposed that the formation of the butenolide ring should occur after hydroxylations at C-14ß and at C-21 of the 20-keto-pregnane precursor (2). More recent results suggest that the condensation of the acetate unit on a 20-keto-pregnane to form the lactone ring of cardenolides could be produced before the hydroxylations at C-21 and at C-14ß of the pregnane intermediate (3,4). In order to get more information about the biosynthesis of the butenolide ring of cardenolides, pregnenolone labelled with C-13 at both carbons of the side chain was required.

RESULTS AND DISCUSSION

The synthesis of pregnenolone labelled at C-20 and C-21 was performed modifying the procedure previously used in our laboratory for the synthesis of [20,21-$^{13}C_2$]-progesterone (5). Condensation of androst-5-en-3ß-ol-17-one (**1**) with potassium ^{13}C-cyanide produced the

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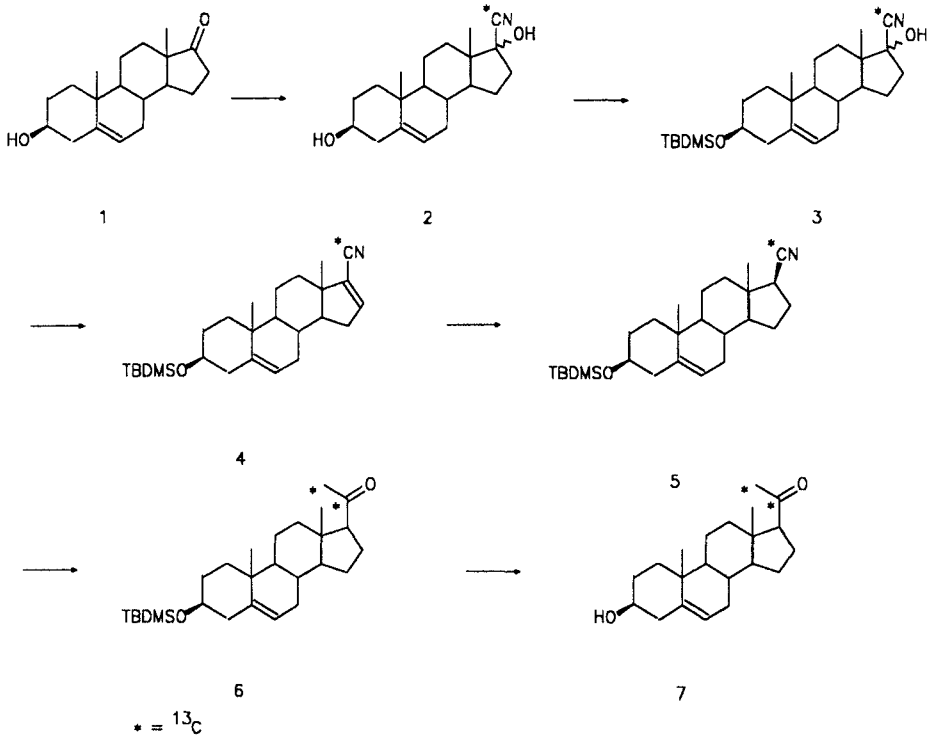
cyanohydrin (**2**) as a epimeric mixture in almost quantitative yield. Masking of the 3ß-hydroxy function in **2** as the correspondig t-butyldimethylsilylether was performed with t-butyldimethylsilyl chloride and imidazole in dry dimethylformamide (6) affording compound **3** in 100% yield. Compound **3** was dehydrated to the corresponding a,ß-unsaturated nitrile **4** by reaction with phosphorous oxychloride in pyridine at room temperature. Catalytic hydrogenation of **4** in the presence of palladium afforded compound **5** in 100% yield. In turn, compound **5** reacted with [^{13}C]-methylmagnesium iodide, prepared from labelled methyl iodide, to give



the 20-keto-pregnenesilylether **6** in good yield. Finally, removal of the protective group of compound **6** was achieved with tetra-n-butylammonium fluoride in dry tetrahydrofuran under mild conditions (7) affording the title compound **7** in excelent yield. The overall yield of the whole synthetic sequence from **1** was 30%. The broad band decoupled ^{13}C-NMR spectrum is shown in Fig. 1. Mass spectra of pregnenolone and [20,21-$^{13}C_2$]-pregnenolone are shown in Fig.2.

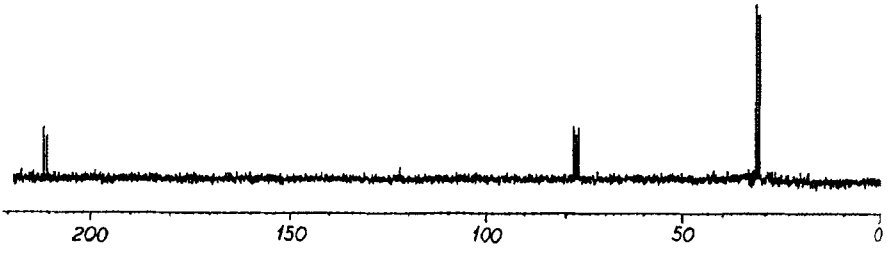


Fig. 1. ^{13}C-NMR spectrum of [20,21-$^{13}C_2$]-pregnenolone.

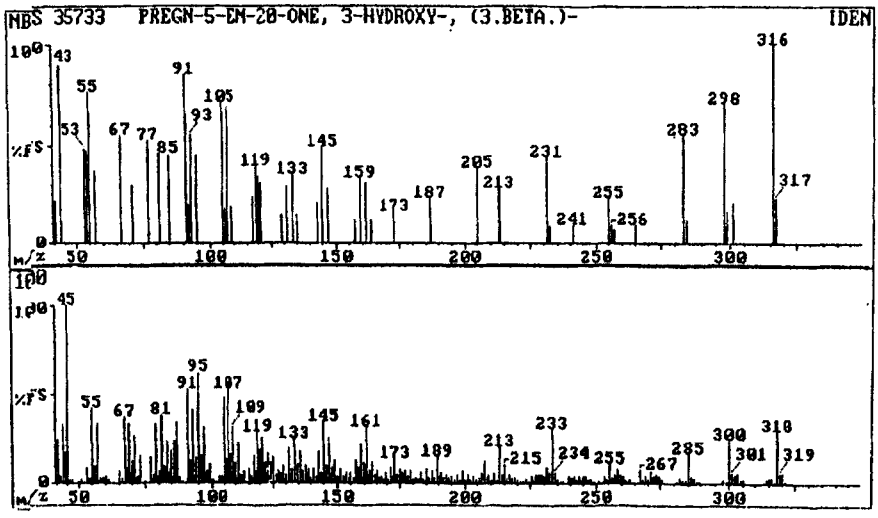


Fig. 2. Mass spectra of pregnenolone and [20,21-$^{13}C_2$]-pregnenolone.

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. 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.

[20-^{13}C]-17ξ-Cyano-17ξ-hydroxy-androst-5-en-3β-ol (2). To a suspension of **1** (1.04 g) and K^{13}CN (99 atom %, 1 g) in EtOH (15 ml), acetic acid (0.70 ml) was added dropwise during 1 h. The reaction mixture was kept at r.t. overnight, and acetic acid (0.7 ml) and water (50 ml) were added. The precipitate thus formed was filtered, washed with water and dried under vacuum giving crude **2** (1.13 g, 99%) of m.p. 140-141°C.

^{1}H-NMR: d 0.97 (s, 3H, 18-Me), 0.99 (s, 3H, 19-Me), 3.70 (m, 1H, H-3), 5.30 (m, 1H, H-6).

^{13}C-NMR: d 122.3 (s, ^{13}C-20).

MS: m/z 316 [M+1]+, 298 [(M+1) - H_2O], 288[(M+1) - H^{13}CN].

[20-^{13}C]-3β-O-(t-Butyldimethylsilyl)-17ξ-cyano-17ξ-hydroxy-androst-5-ene (3). A solution of crude **2** (1.1 g), t-butyldimethyl silyl chloride (1.76 g) and imidazole (2.2 g) in anh. DMF (30 ml) was maintained at r.t. for 2 hr. The mixture was diluted with CH_2Cl_2, washed with brine (5x100 ml) and dried ($MgSO_4$). Evaporation of the solvent afforded pure **3** (1.49 g, 100%) of m.p. 187-189°C.

^{1}H-NMR: d 0.06 (s, 6H, **Me**$_2$Si), 0.90 (s, 9H, **Me**$_3$CSi), 0.96 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me), 3.50 (m, 1H, H-3), 5.32 (m, 1H, H-6).

^{13}C-NMR: δ 120.7 (s, ^{13}C-20).
MS: m/z 402 [(M+1) - H^{13}CN], 373 [(M+1) - Me$_3$C], 345 [(M+1) -H^{13}CN - Me$_3$C].

[20-^{13}C]-3β-O-(t-Butyldimethylsilyl)-17-cyano-androsta-5,16-diene (4). Compound **3** (1 g) dissolved in anh. pyridine (30 ml) was treated with phosporous oxychloride (5.0 ml) and kept at r.t. for 72 hr. The mixture was carefully poured into HCl (30%) and extracted with CH_2Cl_2. The extract was washed with NaHCO3 (5%) and water, and dried (MgSO4). Evaporation of the solvent supplied a residue (0.60 g) that was purified by column cromatography (silica gel) eluted with hexane-EtOAc (90:10) giving pure **4** (0.43 g, 45%) of m.p. 142-145°C.
^{1}H-NMR: δ 0.06 (s, 6H, **Me**$_2$Si), 0.90 (s, 9H, **Me**$_3$CSi), 0.95 (s, 3H, 18-Me), 1.04 (s, 3H, 19-Me), 3.48 (m, 1H, H-3), 5.32 (m, 1H, H-6), 6.64 (m, 1H, H-16).
^{13}C-NMR: δ 115.9 (s, ^{13}C-20).
MS: m/z 397 [(M+1) - CH$_3$], 355 [(M+1) - Me$_3$C], 75 [Me$_2$SiOH].

[20-^{13}C]-3β-O-(t-Butyldimethylsilyl)-17β-cyano-androst-5-ene (5). A solution of **4** (0.40 g) in EtOAc (25 ml) was hydrogenated under atmospheric pressure at r.t. over Pd/C (10%) (0.11 g) for 7 hr. The catalyst was filtered off and evaporation of the solvent gave pure **5** (0.40 g, 100%) of m.p. 146-148°C.
^{1}H-NMR: δ 0.06 (s, 6H, Me$_2$Si), 0.90 (s, 9H, Me$_3$CSi), 0.93 (s, 3H, 18-Me),
1.02 (s, 3H, 19-Me), 3.48 (m, 1H, H-3), 5.32 (m, 1H, H-6).
^{13}C-NMR: δ 121.4 (s, ^{13}C-20).
MS: m/z 357 [(M+1) - Me$_3$C], 75 [Me$_2$SiOH].

[20,21-^{13}C2]-3β-O-(t-Butyldimethylsilyl)pregn-5-en-20-one (6).
To a solution of [^{13}C]-methylmagnesium iodide, prepared from Mg (0.23 g), [^{13}C]-methyl iodide (99 atom%, 0.6 ml, 9.8 mmol) in dry ether (2 ml), a solution of compound **5** (0.39 g, 0.94 mmol) in benzene (15 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 (0.36 g) that was cromatographed on a silica gel column eluted with hexane-EtOAc (90:10) affording pure **6** (0.29 g, 70%) of m.p. 164-165°C.
^{1}H-NMR: δ 0.06 (s, 6H, **Me**$_2$Si), 0.63 (s, 3H, 18-Me), 0.90 (s, 9H, **Me**$_3$CSi), 1.01 (s, 3H, 19-Me), 2.12 (dd, 3H, J ^{13}C-H =127 Hz, J ^{13}C-^{13}C-**H** = 6 Hz, 21-Me), 3.55 (m, 1H, H-3), 5.31 (m, 1H, H-6).
^{13}C-NMR: d 31.5 (d, J^{13}C-^{13}C = 39.4 Hz, ^{13}C-21), 209.6 (d, J^{13}C-^{13}C = 39.4 Hz, ^{13}C-20).
MS: m/z 375 [(M+2) - Me$_3$C], 75 [Me$_2$SiOH], 45 [$^{13}C_2H_3O$].

[20,21-$^{13}C_2$]-Pregnenolone (7). A solution of compound **6** (0.27 g, 0.63 mmol) and tetrabutylammonium fluoride (0.70 g) in anh. THF (5 ml) was kept at r.t. for 20 hr. EtOAc (50 ml) was added to the mixture and it was washed with water and dried ($MgSO_4$). Evaporation of the solvent gave pure **7** (0.2 g, 95%).

^{1}H-NMR: δ 0.63 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me), 2.12 (dd, 3H, J ^{13}C-H =127 Hz, J ^{13}C-^{13}C-**H** = 6 Hz, 21-Me), 3.53 (m, 1H, H-3), 5.35 (m, 1H, H-6).
^{13}C-NMR: δ 31,5 (d, J ^{13}C-^{13}C = 39.4 Hz, ^{13}C-21), 209.6 (d, J ^{13}C-^{13}C = 39.4 Hz, ^{13}C-20).
MS: m/z 318 [M+2], 302 [(M+2) - $^{13}CH_3$], 300 [(M+2) - H_2O], 45 [$^{13}C_2H_3O$].

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