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

Gerardo M. Caballero and Eduardo G. Gros

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

SUMMARY

The synthesis of [20,21-$^{13}C_2$]-progesterone (9) from androst-4-ene-3,17-dione (1) is described. Labels were introduced by two procedures, namely, condensation of 1 with K^{13}CN and Grignard reaction of nitrile derivative 7 with [^{13}C]-methylmagnesium iodide. Location of labels was confirmed by ^{13}C-NMR spectroscopy.

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

It has been demonstrated that 20-keto-pregnane derivatives may act as biosynthetic precursors of plant cardenolides and bufadienolides. Among these, scillirosid, the main component of the bufadienolide fraction obtained from *Scilla maritima*, (1) is biologically derived from 20-keto-pregnane intermediates which provide 21-carbon atoms out of the 24-carbons of the bufadienolide (2). It is also known that oxalacetic acid or some related metabolite provides the 3-carbon atoms needed to complete the lactonic α-pyrone ring (3,4). In order to check the incorporation of an unmodified side chain of a 20-keto-pregnane derivative, progesterone labelled at both carbons of the side chain was required.

RESULTS AND DISCUSSION

The synthesis of progesterone labelled at C-20 and C-21 was performed modifying a procedure previously used for the synthesis of 20-keto-pregnanes (5,6). Condensation of androst-4-ene-3,17-dione (1) with potassium ^{13}C-cyanide produced the cyanohydrin 2 as a epimeric mixture; the main component (>95%) was the 17β-cyano-17α-hydroxy epimer (7). Compound 2 was dehydrated to the corresponding α,β-unsaturated nitrile 3 by reaction with phosphorous oxychloride in pyridine (8). Masking of the 3-keto-4-ene function in 3 as the corresponding acetal 4 was needed in order to isomerize the double bond from Δ^4 to Δ^5. This is important because Δ^5 double bond is not affected when submitted to hydrogenation in the presence of palladium catalysts (9,10); in this way, catalytic hydrogenation of 4 afforded compound 5 in 100% yield. When non-labelled 5 was submitted to the Grignard reaction with methylmagnesium

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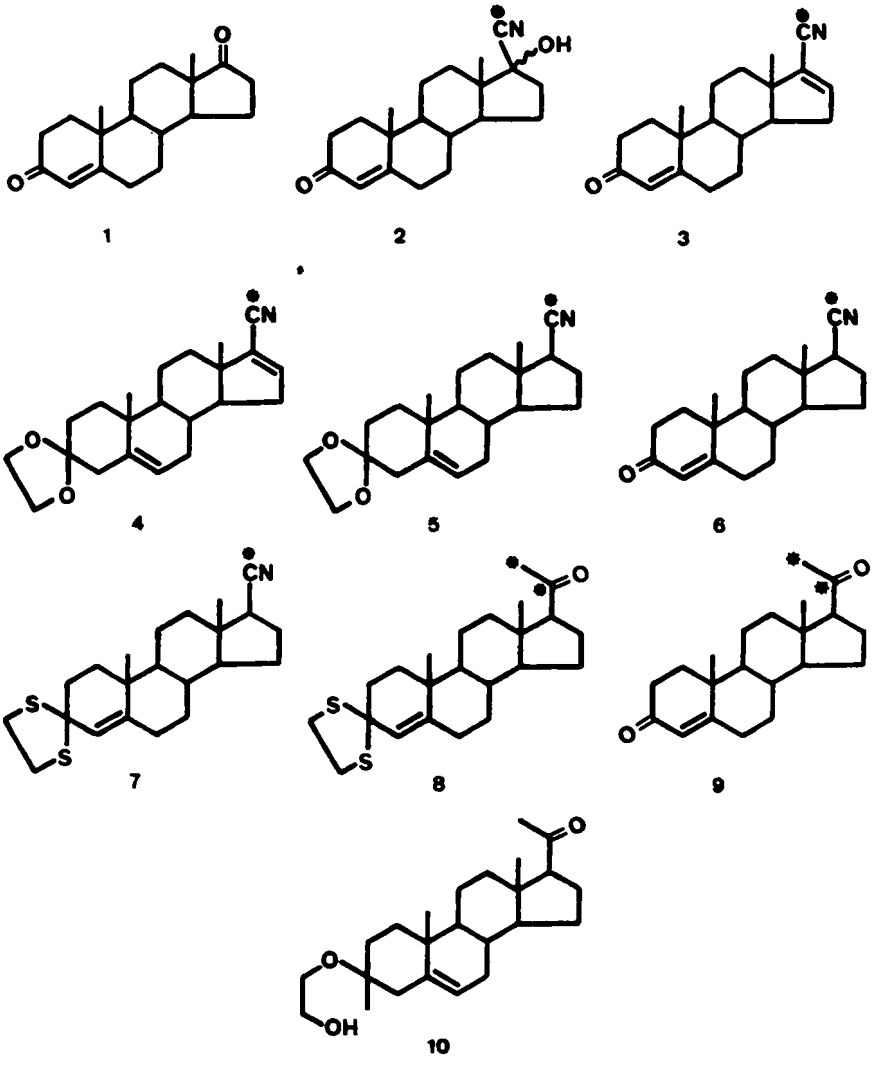
iodide, compound 10 was the unique product isolated. Formation of 10 may be due to the excess of Grignard reagent used in the reaction (20:1), and can be related to the preparation of N-substituted-amino ethers from aminoacetals and Grignard reagents described in the literature (11). Taking into account



this result, compound 5 was submitted to hydrolysis under mild conditions (12) affording compound 6 in good yield. Compound 6 was treated with 1,2-ethanodi thiol giving the thioacetal 7 in almost quantitative yield (13). In turn, compound 7 reacted with [^{13}C]-methylmagnesium iodide, prepared from labelled methyl iodide, to give the 20-keto-pregnenethioacetal 8 (14). The final step

was performed by a procedure recently developed (15); thus, compound 8 in refluxing chloroform, in the presence of silica gel-supported copper (II) sulphate, afforded the title compound 9 in excellent yield. The overall yield of the whole synthetic sequence from 1 was 14%. The ^{1}H-NMR spectrum of 9 is shown in Fig. 1; normal broad band decoupled ^{13}C-NMR and the delayed decoupling ^{13}C-NMR spectra (16) are shown in Fig. 2.

EXPERIMENTAL

Melting points are uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ 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. Mass spectra were recorded at 70 eV (direct inlet) on a Varian-Mat CH7-A spectrometer coupled to a Varian-Mat Data System 166. The IR spectra were determined as nujol mulls.

[20-^{13}C]-17β-Cyano-17α-hydroxy-androst-4-en-3-one (2). To a suspension of 1 (1.5 g) and K^{13}CN (99 atom %, 1.44 g) in MeOH (15 ml), acetic acid (0.48 ml) was added dropwise during 20 min. The starting material was completely dissolved after 15 min; a few minutes later the product began to crystallize. The reaction mixture was kept at r.t. overnight, and acetic acid (1.5 ml) and water (30 ml) were added. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with 10% $NaCO_3H$ and water, and dried ($MgSO_4$). Evaporation of the solvent afforded crude 2 (1.64 g, 100%) as a mixture of C-17 epimers. The analytical sample of the main component, the 17β-cyano-17α-hydroxy epimer was obtained by HPLC separation (RP-18, 10 μm column, MeOH-H_2O 8:2).
IR: 3300 (OH), 2180 (CN), 1670 (C=O), 1580 (C=C) cm^{-1}.
^{1}H-NMR: δ 1.00 (s, 3H, 18-Me), 1.21 (s, 3H, 19-Me), 5.76 (m, 1H, H-4).
^{13}C-NMR: δ 120.7 (s, ^{13}C-20).
MS: m/z 314 [M+1]$^{+}$, 286 [(M+1) - H^{13}CN].

[20-^{13}C]-17-Cyano-androsta-4,16-dien-3-one (3). Crude 2 (1.6 g) dissolved in anh. pyridine (35 ml), treated with phosphorous oxychloride (2.6 ml) in a a screw capped tube, was heated at 150°C for 10 hr. The mixture was carefully poured into water-HCl (30:20 ml) and extracted with CH_2Cl_2. The extract was washed with water and dried. Evaporation of the solvent afforded a residue (1.5 g) that was purified by column chromatography (silica gel) eluted with hexane-EtOAc (65:35) giving pure 3 (0.71 g, 45%) of m.p. 144-146°C.
IR: 2170 (CN), 1670 (C=O), 1580 (C=C) cm^{-1}.
^{1}H-NMR: δ 0.98 (s, 3H, 18-Me), 1.22 (s, 3H, 19-Me), 5.76 (m, 1H, H-4), 6.64 (m, 1H, H-16).
^{13}C-NMR: δ 115.4 (s, ^{13}C-20).
MS: m/z 296 [M+1]$^{+}$, 254 [(M+1) - CH_2CO].

[20-^{13}C]-17-Cyano-3-cycloethylenedioxy-androsta-5,16-diene (4). Compound 3 (0.71 g) was suspended in ethyl orthoformate (2.5 ml) and ethylene glycol (8 ml)

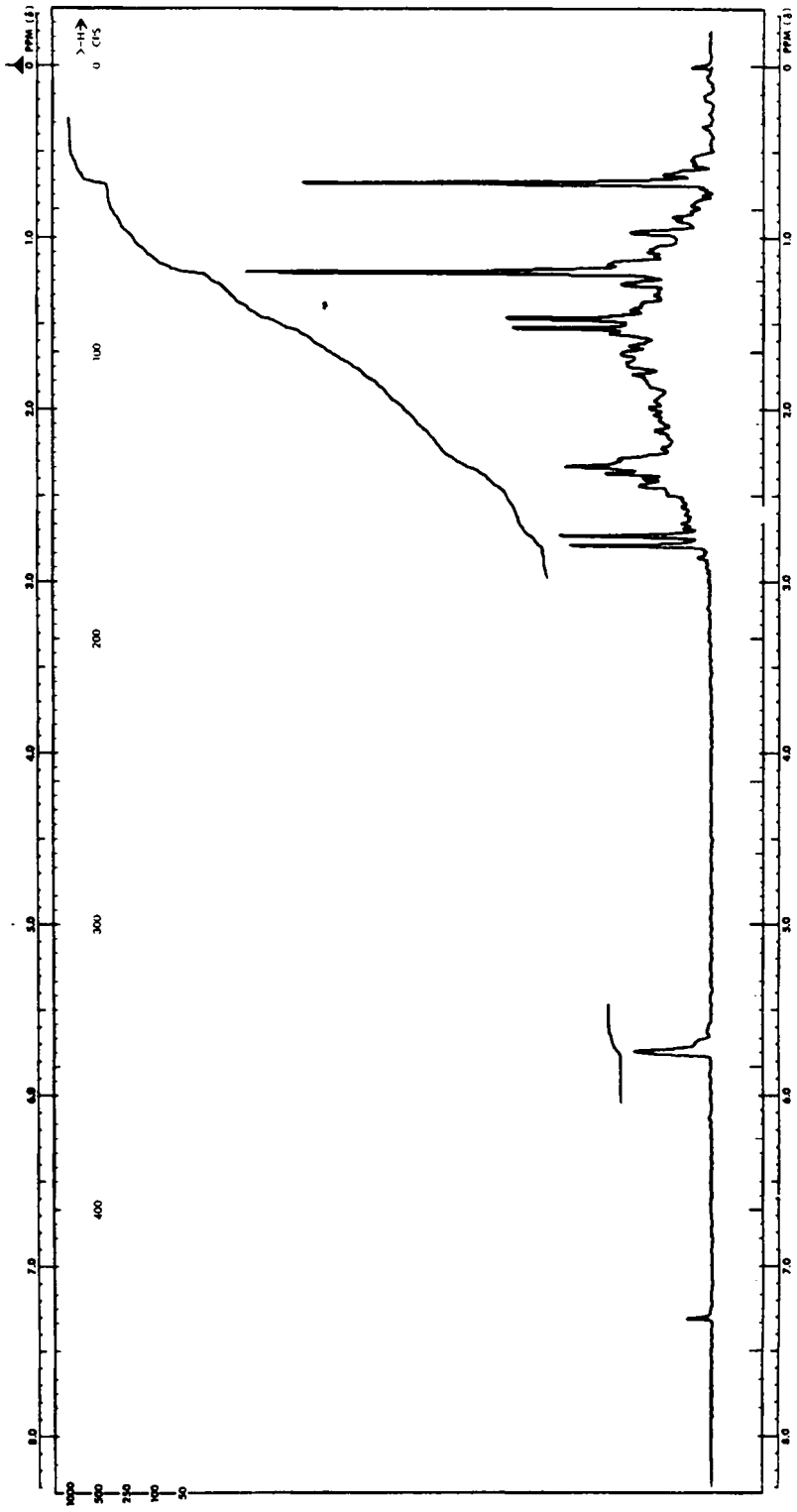


Fig. 1. ^{1}H-NMR spectrum of [20,21-$^{13}C_2$]-progesterone.

and treated with a catalytic amount (ca. 5 mg) of p-toluenesulphonic acid. The mixture was maintained at 40°C for 22 hr with vigorous stirring, and then 5% $NaCO_3H$ sol (50 ml) was added, and the mixture extracted with CH_2Cl_2. After the usual work-up, evaporation of the solvent gave crude 4 (0.74 g, 90%)

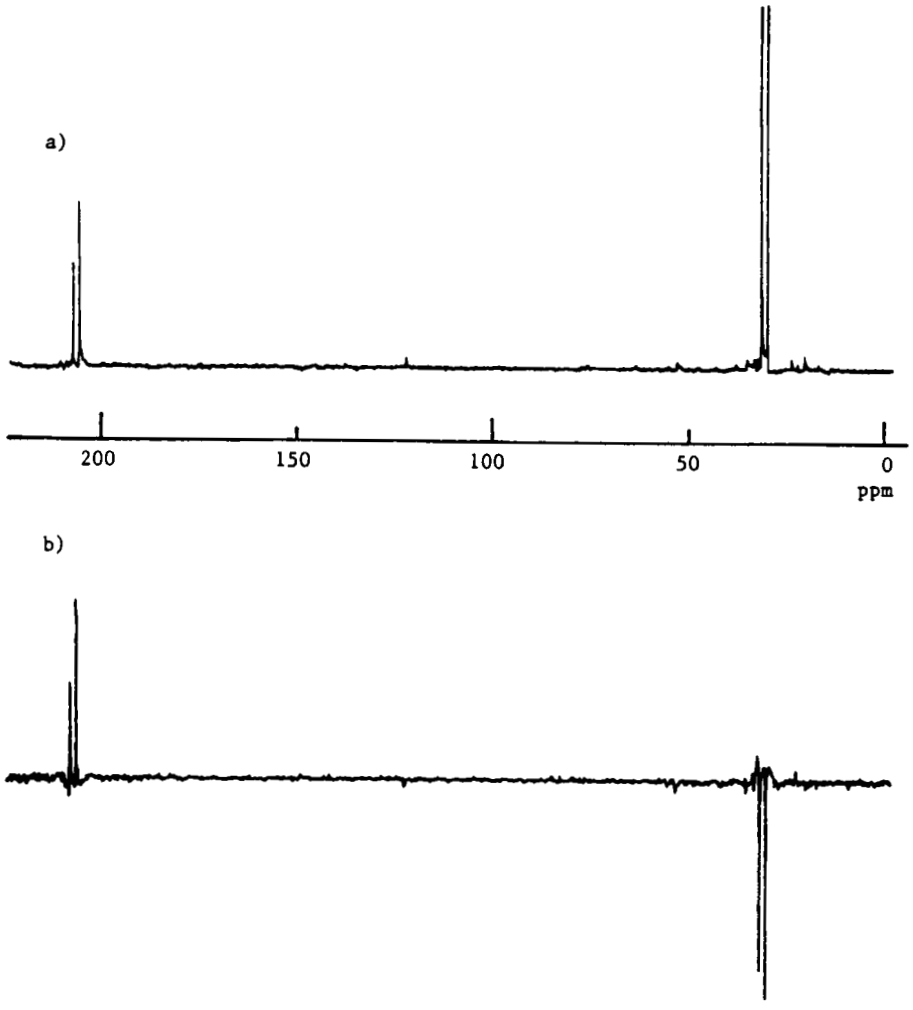


Fig. 2. a) ^{13}C-NMR spectrum of [20,21-$^{13}C_2$]-progesterone.

b) delayed decoupling ^{13}C-NMR spectrum of [20,21-$^{13}C_2$]-progesterone.

Recrystallization from MeOH-water afforded pure product of m.p. 192-195°C.
IR: 2170 (CN), 1098 (C-O-C) cm^{-1}.
^{1}H-NMR: δ 0.96 (s, 3H, 18-Me), 1.06 (s, 3H, 19-Me), 3.95 (m, 4H, O-C$\underline{H}_2$-), 5.36 (m, 1H, H-5), 6.64 (m, 1H, H-16).
^{13}C-NMR: δ 115.6 (s, ^{13}C-20).
MS: m/z 340 $[M+1]^+$, 99 $[C_5H_7O_2]$.

[20-^{13}C]-17β-Cyano-3-cycloethylenedioxy-androst-5-ene (5). A solution of 4 (0.73 g, 2.17 mmol) in EtOAc (50 ml) was hydrogenated at atmospheric pressure and room temperature over 10% Pd/C (100 mg/mmol) for 20 hr. The catalyst was filtered off and washed with EtOAc. Evaporation of the solvent gave pure 5 (0.74 g, 100%) which after recrystallization from MeOH-water had m.p. 84-86°C.
IR: 2180 (CN), 1098 (C-O-C) cm^{-1}.
^{1}H-NMR: δ 0.94 (s, 3H, 18-Me), 1.05 (s, 3H, 19-Me`, 3.95 (m, 4H, O-C$\underline{H}_2$-), 5.36 (m, 1H, H-5).
^{13}C-NMR: δ 121.1 (^{13}C-20).
MS: m/z 342 $[M+1]^+$, 99 $[C_5H_7O_2]$.

[20-^{13}C]-17β-Cyano-androst-4-en-3-one (6). To a solution of 5 (0.73 g) in THF (15 ml), 10% HCl (8 ml) was added, and the mixture was kept at r.t. for 8 hr. The organic solvent was removed, sat. solution of $NaCO_3H$ (50 ml) was added, and the suspension was extracted with CH_2Cl_2. After the usual work-up the solvent was evaporated giving 6 (0.57 g, 90%) which was recrystallized from EtOH to m.p. 174-175°C.
IR: 2180 (CN), 1670 (C=O), 1580 (C=C) cm^{-1}.
^{1}H-NMR: δ 0.98 (s, 3H, 18-Me), 1.21 (s, 3H, 19-Me), 5.75 (m, 1H, H-4).
^{13}C-NMR: δ 120.8 (s, ^{13}C-20).
MS: m/z 298 $[M+1]^+$, 256 $[(M+1) - CH_2CO]$.

[20-^{13}C]-17β-Cyano-3-cycloethylenedithio-androst-4-ene (7). Compound 6 (0.57 g) was dissolved in MeOH (20 ml) and ethanedithiol (0.24 ml) and boron trifluoride etherate (0.3 ml) were added. After stirring at r.t. for 1 hr, the solvent was removed and the residue was taken in EtOAc, washed with 5% $NaCO_3H$ sol. and water, and dried ($MgSO_4$). Evaporation of the solvent afforded pure 7 (0.68 g, 95%).
IR: 2180 (CN) cm^{-1}.
^{1}H-NMR: δ 0.95 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me), 3.36 (m, 4H, S-C$\underline{H}_2$-), 5.50 (m, 1H, H-4).
^{13}C-NMR: δ 121.0 (^{13}C-20).
MS: m/z 374 $[M+1]^+$, 314 $[(M+1) - C_2H_4S]$.

[20,21-$^{13}C_2$]-3-Cycloethylenedithio-pregn-4-en-20-one (8). To a solution of [^{13}C]-methylmagnesium iodide, prepared from Mg (0.53 g), [^{13}C]-methyl iodide (99 atom %, 1.35 ml, 21.6 mmol) in dry ether (5 ml), a solution of compound 7 0.40 g, 1.07 mmol) in benzene (20 ml) was added. The mixture was refluxed under a nitrogen 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 (0.41 g) that was chromatographed on a silica gel column eluted with hexane-EtOAc (98:2) affording pure 8 (0.22 g, 51%) of m.p. 174-176°C.

IR: 1706 (^{12}C=O, weak), 1650 (^{13}C=O, strong) cm^{-1}.

^{1}H-NMR: δ 0.63 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me), 2.12 (dd, 3H, $J_{^{13}C-H}$ = 126.7 $J_{^{13}\underline{C}-^{13}C-\underline{H}}$ = 5.7 Hz, 21-Me), 3.36 (m, 4H, S-C$\underline{H}_2$-), 5.50 (m, 1H, H-4).

^{13}C-NMR: δ 31.3 (d, $J_{^{13}C-^{13}C}$ = 39.2 Hz, ^{13}C-21), 208.9 (d, $J_{^{13}C-^{13}C}$ = 39.2 Hz, ^{13}C-20).

MS: m/z 392 $[M+2]^+$, 332 [(M+2) - C_2H_4S], 45 ($^{13}C_2H_3O$].

[20,21-$^{13}C_2$]-Progesterone (9). Compound 8 (0.21 g, 0.54 mmol) was dissolved in $CHCl_3$ (20 ml) and silica gel-supported copper(II) sulphate (1.08 g) was added. The mixture was refluxed for 5 hr. The residue obtained for evaporation of the filtered solvent was chromatographed on silica gel eluting with hexane-EtOAc (80:20) to give pure 9 (0.14 g, 80%).

^{1}H-NMR: δ 0.67 (s, 3H, 18-Me), 1.19 (s, 3H, 19-Me), 2.13 (dd, 3H, $J_{^{13}C-H}$ = 126.9 , $J_{^{13}\underline{C}-^{13}C-\underline{H}}$ = 5.6 Hz, 21-Me), 5.74 (m, 1H, H-4).

^{13}C-NMR: δ 31.3 (d, $J_{^{13}C-^{13}C}$ = 39.2 Hz, ^{13}C-21), 208.7 (d, $J_{^{13}C-^{13}C}$ = 39.2 Hz, ^{13}C-20).

MS: m/z 316 $[M+2]^+$, 45 [$^{13}C_2H_3O$]

3-(2-Hydroxy-ethoxy)-3-methyl-pregn-5-en-20-one (10). Unlabelled compound 5 (50 mg, 0.15 mmol) was treated with unlabelled methylmagnesium iodide as described for the preparation of 8. After chromatographic purification, pure compound 10 (32 mg, 60 %) was obtained.

^{1}H-NMR: δ 0.63 (s, 3H, 18-Me), 1.01 (s, 3H, 19-Me), 1.09 (s, 3H, 3-Me), 2.12 (s, 3H, 21-Me), 3.62 (m, 4H, HO-CH_2-CH_2-O-), 5.35 (m, 1H, H-5).

^{13}C-NMR: δ 13.2 (C-18), 19.3 (C-19), 20.9 (Me at C-3), 31.8 (C-21), 61.7 and 62.2 (HO-CH_2-CH_2-O-), 63.6 (C-17), 76.2 (C-3), 121.6 (C-6), 140.7 (C-5), 209.2 (C-20).

MS: m/z 374 $[M]^+$, 312 [M - ($HOCH_2CH_2OH$)], 43 [CH_3CO].

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