Preparation of 3\(\beta\)-Acetoxy-5\(\beta\)-pregnan-20-one from 3\(\alpha\)-Acetoxy-5\(\beta\)-cholan-24-oic Acid Aimed at the Isotopic Labelling of the Pregnan Side Chain

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A mixture of 3\(\alpha\)-acetoxy-5\(\beta\)-cholan-24-oic acid (800 mg, 1.90 mmoles), cupric acetate (80 mg, 0.20 mmoles) and benzene (15 ml) was ozonised giving the ketone 2a. The unlabelled compound had been previously obtained from lithocholic acid which was ozonised giving the ketone 2a.

In connection with our research on the biosynthesis of cardiotonic steroids [1] it was required the preparation of 3\(\beta\)-hydroxy-5\(\beta\)-pregnan-20-one labelled at either C-20 or C-21. The unlabelled compound have been obtained by different methods such as reduction of pregnandione [2], or pregnenolone [3], and by degradation of smilagenin [4] but these procedures follow unappropriate routes for introducing a label at the side chain and produce the needed compound in very low yields.

We wish to report that using a low cost compound commercially available in large quantities as lithocholic acid, the title compound was prepared following a procedure that would allow the introduction of an isotopic carbon-atom at C-21.

Results and Discussion

As it is indicated in the Scheme, copper catalysed oxidative decarboxylation of 3\(\alpha\)-acetoxy-5\(\beta\)-cholan-24-oic acid (1) with lead tetraacetate in pyridine-containing benzene gave 3\(\alpha\)-acetoxy-24-nor-5\(\beta\)-chol-22-ene (2). Treatment of 2 with N-lithioethylenediamine afforded the A\(20,22\) olefin 3a as the sole reaction product [5] with no traces of the A\(17,20\) isomer, the 13C NMR spectrum of 3a indicated the presence of just the Z isomer. Acetylation of 3a afforded 3b which was ozonised giving the ketone 4; compound 4 had been previously obtained from lithocholic acid but following a different degradation approach [6]. Acetoxylation of compound 4 gave compound 5a [7] which after hydrolysis [8] afforded the \(\alpha\)-ketol 5b. Treatment of 5b with sodium periodate produced the cleavage of the side chain giving the etianic acid 6a which was isolated as its methyl ester.

Inversion of C-3 configuration was performed by displacement of a toslyoxy group on the etianic ester 6b. This classical method gave in this case better results than those obtained by the formic acid—ethyl azodicarboxylate procedure or by oxidation—reduction of the C-3 hydroxy group. Formylation of 6b yielded the 3\(\beta\)-formyloxy derivative 7a which was transformed into the acetyl derivative 7c by usual methods.

For construction of the pregnane side chain, the acid 7c was converted into the acyl chloride derivative 8 which was subjected to alkylation by an organometalic reagent. For this step, and considering the requirements of millimole scale that has to be used for the introduction of the labelled carbon, the usual procedures employing a large excess of a Grignard reagent were not suitable. The best results were obtained using dimethyl cadmium on the acyl chloride 8 adapting the original technique [9, 10] to microscale preparation.

Experimental

Melting points are uncorrected. IR spectra were determined as Nujol dispersions using a Perkin Elmer 421 spectrophotometer. 1H and 13C FT NMR spectra were recorded with a Varian XL-100-15, using TMS as internal standard and solvents indicated in each case. Mass spectra (MS) were performed at 70 eV (direct inlet) with a Varian-Mat CH7-A spectrometer interfaced to a Varian-Mat Data System 166 computer.

3\(\beta\)-Acetoxy-24-nor-5\(\beta\)-chol-22-ene (2)

A mixture of 3\(\beta\)-acetoxy-5\(\beta\)-cholan-24-oic acid (800 mg, 1.90 mmoles), cupric acetate (80 mg,
0.44 mmoles) and anhydrous pyridine (0.01 ml, 0.12 mmoles) in dry benzene (30 ml) was refluxed under nitrogen for 4 h while lead tetraacetate (2.4 g, 5.40 mmoles) was added in portions. The solids were then collected and the filtrate was evaporated to dryness. The residue was dissolved in methylene chloride, washed with 5% hydrochloric acid, 5% sodium hydrogen carbonate solution and water, dried over magnesium sulphate and evaporated. The crude extract was chromatographed on silica gel G, elution with toluene afforded compound 2 (450 mg, 63%). It was crystallized from methanol, m.p. 84–85 °C. IR (cm⁻¹) 1730 (C = O), 1630 (C = C). ¹H NMR (Cl₃CD)(δ): 0.68 (s, 3H, 18-CH₃), 0.94 (s, 3H, 19-CH₃), 1.03 (d, 3H, J = 6 Hz, 21-CH₃), 2.04 (s, 3H, CH₃CO), 4.75 (m, 1H, 3β-H), 4.90 (m, 2H, 23-CH₂), 5.70 (m, 1H, 22-CH). ¹³C NMR (Cl₃CD) (ppm): 35.10 (C-1), 26.39 (C-2), 74.39 (C-3), 32.31 (C-4), 41.94 (C-5), 35.85 (C-6), 40.51 (C-7), 35.10 (C-8), 35.10 (C-9), 35.10 (C-10), 20.90 (C-11), 40.10 (C-12), 42.71 (C-13), 56.55 (C-14 or C-17), 24.27 (C-15), 28.51 (C-16), 56.55 (C-17 or C-14), 12.28 (C-18), 23.40 (C-19), 41.22 (C-20), 20.15 (C-21), 145.14 (C-22), 111.43 (C-23), 21.54 (CH₃CO), 170.47 (CH₃CO). MS: m/e (%): 372 (M⁺, 2.4), 357 (M⁺-CH₃, 4.1), 317 (M⁺-side chain, 2.9), 312 (M⁺-CH₃COOH, 38.4), 297 (M⁺-CH₃COOH-CH₃, 12.9), 257 (M⁺-CH₃COOH-side chain, 100), 215 (28.6).

3α-Hydroxy-24-nor-5β-chol-20(22)-ene (3a) and 3α-acetoxy-24-nor-5β-chol-22(22)-ene (3b)

3α-Acetoxy-24-nor-5β-chol-22-ene (2) (3.5 g, 9.40 mmoles) was added in one lot to a stirred and heated solution (125–130 °C) of 7.8 equivalents of N-lithioethylene-diamine (234 mg Li added in portions to stirred dry ethylenediamine (7.9 ml) at 90–100 °C bath temperature, under nitrogen and heating for 2 h to complete the reaction) under nitrogen and the mixture refluxed for 15 min. After cooling, water was added and the solution extracted twice methylene chloride. The organic extracts were washed with water until neutral reaction, dried over magnesium sulphate and evaporated under reduced pressure to afford crude 3a which crystallized from diluted ethanol; m.p. 114–115 °C (2.8 g, 90%). IR (cm⁻¹) 3300 (broad, OH), 1650 (C=O). ¹H NMR
(Cl3CD) (δ): 0.52 (s, 3H, 18-CH3), 0.93 (s, 3H, 19-CH3), 1.58 (s, 3H, 23-CH3), 1.62 (s, 3H, 21-CH3), 3.65 (m, 1H, 3β-H), 5.25 (m, 1H, 22-CH). 13C NMR (Cl3CD) (ppm): 35.42 (C-1), 30.48 (C-2), 71.62 (C-3), 36.42 (C-4), 42.16 (C-5), 27.20 (C-6), 26.44 (C-7), 36.16 (C-8), 40.74 (C-9), 34.63 (C-10), 20.82 (C-11), 39.05 (C-12), 43.71 (C-13), 55.98 (C-14), 24.21 (C-15), 27.20 (C-16), 59.02 (C-17), 13.40 (C-18), 23.36 (C-19), 135.15 (C-20), 24.78 (C-21), 118.72 (C-22), 17.51 (C-23). MS: m/e (%): 316 (M+- H2O, 1.5), 302

which crystallized from ethanol; m.p. 104—105 °C.

6.36 (C-2), 74.34 (C-3), 32.30 (C-4), 41.95 (C-5), 56.04 (C-14), 24.25 (C-15), 26.25 (C-16), 59.10 (C-17), 34.69 (C-10), 20.85 (C-11), 39.05 (C-12), 43.79 (C-13), 55.98 (C-14), 24.21 (C-15), 27.20 (C-16), 59.02 (C-17), 13.40 (C-18), 23.36 (C-19), 135.15 (C-20), 24.78 (C-21), 118.72 (C-22), 17.51 (C-23). MS: m/e (%): 316 (M+- H2O, 1.5), 302

Acetylation with pyridine—acetic anhydride (1:1) at room temperature for 18 h afforded 3b as a solid

at room temperature for 4 h during which a white solid appeared. The mixture was poured into methanol and washed with water, dried over magnesium sulphate and evaporated. The residue was chromatographed on silica gel G. Elution with 95:5 afforded 5b (230 mg, 72%) of m.p. 130—131 °C (Lit. [8] m.p. 152.5—153.5 °C from acetone). IR (cm−1) 3300 (broad, OH), 1705 (C=O). 1H NMR (Cl3CD) (δ): 0.62 (s, 3H, 18-CH3), 0.93 (s, 3H, 19-CH3), 2.04 (s, 3H, 21-CH3), 4.18 (b.s., 2H, CH2). MS: m/e (%): 334 (M+, 0.9), 316 (M+-H2O, 0.8), 303 (M+-CH2OH, 9.4), 301 (M+-H2O—side chain, 100), 215 (5.8).

3α-Hydroxy-5β-androstan-17β-carboxylic acid methyl ester (6a) and 3α-tosyloxy-5β-androstan-17β-carboxylic acid methyl ester (6b)

To a solution of 3α,21-dihydroxy-5β-pregnan-20-one (5b) (1.0 g, 2.99 mmole) in methanol (50 ml) there was added the precipitate formed was filtered off, washed with water and dried. The solid was dissolved in methanol and treated with diazomethane. Evaporation of the solvent afforded 3α-hydroxy-5β-androstan-17β-carboxylic acid methyl ester (6a) (913 mg, 91.3%) which crystallized from methanol; m.p. 142—146 °C (Lit. [11] m.p. 142—146 °C from ether—petroleum ether). IR (cm−1) 3300 (broad, OH), 1740 (C=O). 1H NMR (Cl3CD) (δ): 0.65 (s, 3H, 18-CH3), 0.93 (s, 3H, 19-CH3), 3.60 (m, 1H, 3β-H), 3.68 (s, 3H, COOCH3). MS: m/e (%): 316 (M+-H2O, 1.5), 302
(M⁺→CH₃OH, 100), 287 (M⁺→CH₃OH→CH₃, 70), 275 (M⁺→side chain, 4.1), 257 (M⁺→side chain→H₂O, 3.6), 230 (21.1), 215 (59.3).

To a solution of 6a (140 mg, 0.42 mmoles) in dry pyridine (5 ml) a solution of p-toluenesulphonyl chloride (280 mg, 1.47 mmoles) in dry pyridine (5 ml) was added. The mixture was maintained at room temperature for 20 h and poured into diluted hydrochloric acid–ice. It was extracted twice with methylene chloride and the combined organic extracts were washed with water, dried over magnesium sulphate and the solvents evaporated under reduced pressure. Crystallization from methanol afforded 3α-tosyloxy-5β-androstan-17β-carboxylic acid methyl ester (6b) (194 mg, 95%) of m.p. 120–121 °C. IR (cm⁻¹) 1735 (C=O), 1300, 1170 and 1000–750 (tosyloxy group). ¹H NMR (CDCl₃) (δ): 0.64 (s, 3H, 18-CH₃), 0.91 (s, 3H, 19-CH₂), 2.46 (s, 3H, H₂C=CH₂SO₂), 3.68 (s, 3H, COOCH₃), 4.45 (m, 1H, 3/β-H). ¹³C NMR (CDCl₃): 31.6 (M⁺→CH₃CC₆H₄SO₂H, 100), 301 (M⁺→CH₃CC₆H₄SO₂H→CH₂OH, 87.6), 284 (M⁺→CH₃CC₆H₄SO₂H→CH₃OH, 4.1), 257 (M⁺→CH₃CC₆H₄SO₂H→side chain, 37.1), 230 (11.6), 215 (52.5), 172 (88.0).

3α-Formyloxy-5β-androstan-17β-carboxylic acid methyl ester (7a), 3β-hydroxy-5β-androstan-17β-carboxylic acid (7b) and 3β-acetoxy-5β-androstan-17β-carboxylic acid (7c)

A solution of 3α-tosyloxy-5β-androstan-17β-carboxylic acid methyl ester (6b) (100 mg, 0.20 mmoles) in dry N,N-dimethylformamide (4.0 ml, 52.00 mmoles) was stirred under nitrogen at 80 °C for 50 h. It was then poured onto ice-water and the mixture extracted twice with methylene chloride. The combined organic extracts were washed with water several times, dried over magnesium sulphate and evaporated under reduced pressure. The crude extract was chromatographed on silica gel G. Elution with toluene afforded 5β-androst-3-en-17β-carboxylic acid methyl ester (22 mg, 34%). Further elution with toluene–ethyl acetate 95:5 afforded 3β-formyloxy-5β-androstan-17β-carboxylic acid methyl ester (7a) (45 mg, 61%) which crystallized from ethanol; m.p. 95–96 °C. ¹H NMR (CDCl₃) (δ): 0.66 (s, 3H, 18-CH₃), 0.99 (s, 3H, 19-CH₂), 3.68 (s, 3H, COOCH₃), 5.20 (m, 1H, 3a-H), 8.08 (s, 1H, HCOO). MS: m/e (%): 316 (M⁺→HCOOH, 100), 303 (M⁺→side chain, 4.1), 301 (M⁺→HCOOH→CH₃, 40.5), 257 (M⁺→HCOOH→side chain, 12.3), 215 (19.9).

A solution of 7a (100 mg, 0.28 mmoles) in 2% sodium hydroxide hydroalcoholic solution (10 ml) was refluxed for 2 h. The mixture was poured onto ice–water, acidified with hydrochloric acid and extracted twice with ethyl acetate. The combined organic extracts were washed with water until neutral reaction, dried over magnesium sulphate and evaporated under reduced pressure. Crystallization from ethanol afforded 3β-hydroxy-5β-androstan-17β-carboxylic acid (7b) (82 mg, 93%) of m.p. 239–241 °C (Lit. [12] m.p. 226–228 °C). IR (cm⁻¹) 3350 (broad, OH), 3500–2700 (broad, COOH), 1700 (C=O). ¹H NMR (CDCl₃—CD₃OD 1:1) (δ): 0.88 (s, 3H, 18-CH₃), 1.02 (s, 3H, 19-CH₂), 4.30 (m, 1H, 3a-H). MS: m/e (%): 302 (M⁺→H₂O, 4.7), 287 (M⁺→H₂O→CH₃, 2.7), 215 (1.0), 45 (100).

Acetylation of 7b (100 mg, 0.31 mmoles) with glacial acetic acid at reflux for 2 d afforded 7c as a solid which was purified by column chromatography on silica gel G using methylene chloride as eluent and crystallized from ethanol; m.p. 170–172 °C. ¹H NMR (CDCl₃) (δ): 0.73 (s, 3H, 18-CH₃), 0.95 (s, 3H, 19-CH₂), 2.07 (s, 3H, CH₃CO), 5.10 (m, 1H, 3α-H).

3β-Acetoxy-5β-pregnan-20-one (9)

a) Preparation of 3β-acetoxy-5β-androstan-17β-carboxylic acid chloride (8): To a solution of 3β-acetoxy-5β-androstan-17β-carboxylic acid (7c) (100 mg, 0.28 mmoles) in dry benzene (1 ml), oxalyl chloride (1 ml) was added. The mixture was stirred and maintained at room temperature for 2 h after which the solvents were evaporated at reduced pressure to afford a crude product which was identified as 3β-acetoxy-5β-androstan-17β-carboxylic acid chloride (8) and used without further purification. IR (cm⁻¹) 1800 (CIC=O), 1730 (C=O, acetoxy group).

b) Preparation of dimethyl cadmium and reaction with acid chloride 8: To magnesium turnings (8 mg) contained in an evacuated tube, dry ether (1 ml) and methyl iodide (0.018 ml) dried over phosphorous pentoxide were added. The mixture was stirred at room temperature until most of the magnesium disappeared (1 h). The then cadmium chloride (122 mg) was added under nitrogen atmosphere. The mixture was stirred at room temperature for 2 h followed by the addition of the solution of acid chloride 8 (105 mg) in benzene (2 ml). After 18 h in the same conditions the mixture was warmed to 50 °C and further stirred for 1 h. The reaction was quenched by addition of 1N hydrochloric acid, decanted and extracted twice with methylene chloride. The combined organic extracts were washed with water, dried over magnesium sulphate and the solvents evaporated under reduced pressure. Chromatography on
silica gel G using as solvent methylene chloride–methanol 98:2 afforded 3β-acetoxy-5β-pregnan-20-one (9) (37 mg, 38%) m.p. 120–121 °C (Lit. [2] m.p. 121 °C). IR (cm⁻¹) 1740 (C=O, acetoxyl group), 1700 (C=O). "H NMR (CDCl₃) (δ): 0.62 (s, 3H, 18-CH₃), 0.98 (s, 3H, 19-CH₃), 2.06 (s, 3H, CH₃CO), 2.12 (s, 3H, 21-CH₃), 5.10 (m, 1H, 3α-H). MS: m/e (%): 360 (M⁺, 1.1), 300 (M⁺−CH₃COOH, 100), 285 (M⁺−CH₃COOH−CH₃, 21.9), 257 (M⁺−side chain−CH₃COOH, 10.6), 215 (31.8).