SYNTHESES OF |24-^{14}C|-23-NORCHOLANOIC ACID DERIVATIVES

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SUMMARY

Syntheses of |24-^{14}C|3β,20ξ-dihydroxy-23-norchol-5-enoic acid, |24-^{14}C|3β,20ξ-dihydroxy-23-norcholanoic acid and |24-^{14}C|20ξ-hydroxy-3-oxo-23-norchol-4-enoic acid were accomplished by reaction of ethyl |1-^{14}C|bromoacetate, in Reformatsky conditions, with suitable 20-keto-pregnane derivatives. Dehydration of these products afforded |24-^{14}C|3β-hydroxy-23-norchola-5,20(22)E-dienoic acid, |24-^{14}C|3β-hydroxy-23-norchol-20(22)E-enoic acid and |24-^{14}C|3-oxo-23-norchola-4,20(22)E-dienoic acid respectively. The products were characterized by spectroscopic (IR, ^{1}H NMR, MS) methods.

Key Words: |24-^{14}C|-23-Norcholanic acid derivatives, Ethyl |1-^{14}C|bromoacetate, Reformatsky reaction, Synthesis.

In connection with our research on the biosynthesis of cardiotonic steroids (1) we needed 23-norcholanoic acid derivatives, labelled at the side-chain, to be tested as precursors of cardenolides in plants of the genus *Digitalis*. A preliminary assay with this type of compounds led to acceptable results (2). We wish to report here the preparation of six labelled compounds, namely, 3β,20ξ-dihydroxy-23-norchol-5-enoic acid (3), 3β,20ξ-dihydroxy-23-norcholanoic acid (8), 20ξ-hydroxy-3-oxo-23-norchol-4-enoic acid (17), 3β-hydroxy-23-norchola-5,20(22)E-dienoic acid (5), 3β-hydroxy-23-norchol-20(22)E-enoic acid (10) and 3-oxo-23-norchola-4,20(22)E-dienoic acid (19), all of them labelled with ^{14}C at carbon-24, by Reformatsky reaction of ethyl |1-^{14}C|bromoacetate with different 20-keto-pregnane derivatives.

RESULTS AND DISCUSSION

The starting materials and the labelled products obtained from them are presented in Scheme 1. The reaction of ethyl bromoacetate with 20-keto-

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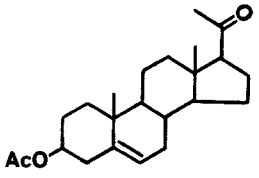

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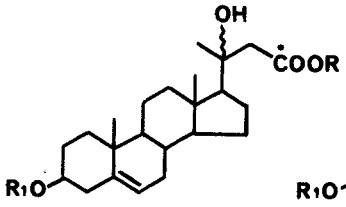

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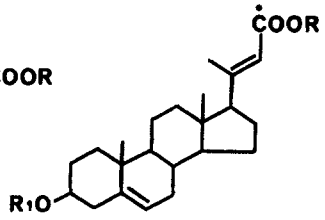

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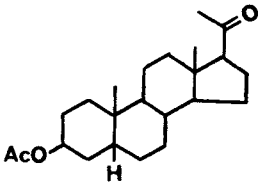

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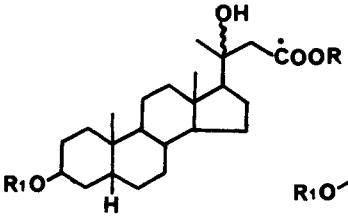

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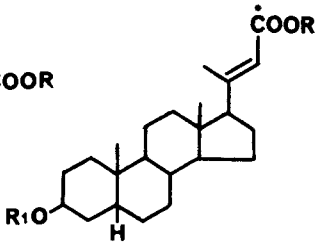

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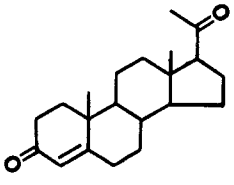

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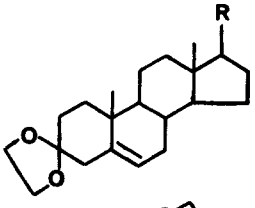

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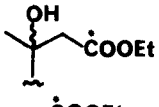
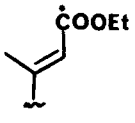
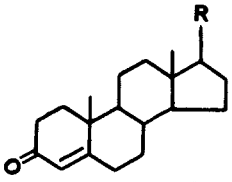
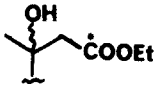
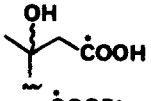
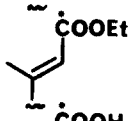
14 R = 

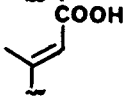
15 R = 



16 R = 

17 R = 

18 R = 

19 R = 

pregnanes has been used for the syntheses of cardenolides and 3β-hydroxy-cholanoic acid derivatives (3-8). In the present case the reaction of acetyl pregnenolone (1) and 3β-acetoxy-5β-pregnan-20-one (6) with ethyl bromoacetate under Reformatsky conditions produced, after acetylation, the expected products 2 and 7 respectively which were easily hydrolysed to the hydroxy acids 3 and 8. On the other hand, compounds 2 and 7 were submitted in separate experiments to dehydration reaction affording the α,β-unsaturated esters 4 and 9 respectively which were hydrolysed as before to the corresponding acids 5 and 10. The conformation of the double bond 20(22) was established as E by analyses of their respective ^{1}H-NMR spectra.

When the starting material was progesterone (11) a selective protection of the 3-keto group was needed. For this purpose progesterone was converted into its diethyleneketal derivative 12 (9) which was selectively hydrolysed to the monoethyleneketal derivative 13. Reformatsky reaction on compound 13 yielded the hydroxy ester 14 (9).

On one hand, compound 14 was converted into 16 which was, in turn, hydrolysed to the hydroxy acid 17. On the other hand, dehydration of 14 afforded the α,β-unsaturated ester 15 which was stepwise hydrolysed to 18 and then to the acid 19. The products presented IR, ^{1}H-NMR and mass spectra in accordance with the proposed structures.

When the same reaction sequences were performed using ethyl |1-^{14}C|bromo acetate the respective labelled compounds were obtained in good yields and resulted identical in every respect to the unlabelled authentic products.

EXPERIMENTAL

Melting points were determined in a Fischer-Johns hot-plate and are uncorrected. ^{1}H-NMR spectra were recorded at 100 MHz in the FT mode with a Varian XL-100-15 NMR spectrometer; solvents are indicated in each case. 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. Radiactivity was measured by liquid scintillation counting (6). Ethyl |1-^{14}C|bromoacetate was purchased from the Comisión Nacional de Energía Atómica (Argentina).

|24-^{14}C| *Ethyl 3β-acetoxy-20ξ-hydroxy-23-norchol-5-en-24-oate* (2). Pregnenolone acetate (1) (40.8 mg) was treated with ethyl |1-^{14}C|bromoacetate of sp.act. 1.08 mCi/mmol as described elsewhere (4). Compound 2 (48.0 mg, 94%) had IR and ^{1}H-NMR spectra identical to those previously reported (4) and sp.act. of 1.10 mCi/mmol.

|24-^{14}C| *3β,20ξ-Dihydroxy-23-norchol-5-enoic acid* (3). A solution of compound 2 (46.0 mg) in MeOH (0.6 ml) was treated with a 50% aqueous NaOH solution (0.1 ml) and heated under reflux for 3 hr. The MeOH was removed, water (2 ml) was added and extracted with ethyl ether which was discarded. The remaining aqueous solution was acidified with 2N HCl (0.5 ml) and extracted with ethyl ether (3 x 5 ml). The organic extract was washed with water and dried over $MgSO_4$. Evaporation of the solvent afforded a residue that was recrystallized from acetone yielding compound 3 (23.9 mg, 62%) of m.p. 202-203°; lit (10) 204-206°. IR (Nujol): 3500-2500, 1700 cm^{-1}. ^{1}H-NMR (pyridine-d_5:TMS) δ 1.06 (3H, s, Me-18), 1.12 (3H, s, Me-19), 1.60 (3H, s, Me-21 from isomer 20R), 1.78 (3H, s, Me-21 from isomer 20S), 2.86 (2H, s, CH_2-COOH), 3.84 (1H, bs, H-3), 5.44 (1H, m, H-6). MS (m/z, %): 376 (M^+, 10), 358 (78), 343 (28), 340 (57), 325 (52), 316 (5), 298 (20), 273 (8), 255 (8), 231 (38), 213 (84), 103 (100). Sp. act. 1.17 mCi/mmol.

|24-^{14}C| *Ethyl 3β-acetoxy-20ξ-hydroxy-23-norchola-5,20(22)E-dien-24-oate* (4). Compound 2 (150 mg) in dry pyridine (1.8 ml) was dehydrated by reaction with phosphorous oxychloride as already reported (4). Compound 4 (38.5 mg, 27%) had physical properties identical to those described (4) and sp. act. 1.02 mCi/mmol.

|24-^{14}C| *3β-Hydroxy-23-norchola-5,20(22)E-dienoic acid* (5). Compound 4 (30.5 mg) dissolved in EtOH (0.9 ml) was treated with 50% aqueous NaOH solution (0.15 ml) and refluxed for 3 hr. Application of a procedure similar to that described for compound 3 produced compound 5 (23.0 mg, 90%) of m.p. 263-265° from EtOH; lit (10) m.p. 262-265°. IR (Nujol) 3300, 3200-2300, 1670, 1620, 1050 cm^{-1}. ^{1}H-NMR (pyridine-d_5:TMS) δ 0.62 (3H, s, Me-18), 1.03 (3H, s, Me-19), 2.42 (3H, d, J=1 Hz, Me-21), 3.83 (1H, bs, H-3), 5.40 (1H, m, H-6), 6.15 (1H, m, H-22). MS (m/z, %): 358 (M^+, 45), 343 (7), 340 (63), 313 (6), 308 (22), 273 (4), 255 (2), 231 (5), 213 (100). Sp. act. 1.03 mCi/mmol.

|24-^{14}C| *Ethyl 3β-acetoxy-20ξ-hydroxy-23-nor-5β-cholan-24-oate* (7). To a solution of 3β-acetoxy-5β-pregnan-20-one (6) (80.0 mg) in dry benzene (1.6 ml) activated zinc powder (120 mg) and a few crystals of iodine were added. To the stirred mixture, kept under a nitrogen atmosphere, ethyl |1-^{14}C|bromoacetate (0.21 ml) was added dropwise maintaining a gentle reflux. When the addition was over, the mixture was stirred and refluxed for 30 min and it was then poured onto ice-2N HCl. The reaction mixture was extracted with ethyl ether (3 x 10 ml) and the organic layer was washed with water, with saturated $NaCO_3H$ solution, with water again, and dried over $MgSO_4$. The residue obtained for evaporation of the solvent was treated with acetic anhydride-pyridine (1:1, 0.5 ml) and let aside at room temp for 20 hr. The mixture was poured onto ice-2N HCl and

extracted with ethyl ether (3 x 10 ml). The organic phase was washed with water, concentrated $NaCO_3H$ solution, water again and dried over $MgSO_4$. The residue obtained after evaporation of the solvent (162 mg) was chromatographed on a silica-gel G column (6 g) eluting with toluene and toluene-ethyl acetate (98:2) affording compound 7 (85.2 mg, 85%) which was recrystallized from EtOH. It had m.p. 130-131° and sp. act. 1.04 mCi/mmol. IR (Nujol): 3550-3400, 1750 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS) δ 0.84 (3H, s, Me-18), 0.98 (3H, s, Me-19), 1.24 (3H, s, Me-21 from isomer 20R), 1.28 (3H, t, J=7 Hz, $\underline{CH_3}$-CH_2), 1.35 (3H, s, Me-21 from isomer 20S), 2.04 (3H, s, $\underline{CH_3}$-CO), 3.37 (1H, bs, OH), 4.18 (2H, q, J=7 Hz, CH_3-$\underline{CH_2}$), 5.08 (1H, bs, H-3). MS (m/z, %): 373 (M^+-AcOH-Me, 1), 355 (2), 257 (1), 229 (4), 215 (5), 131 (100).

|24-^{14}C| *3β,20ξ-Dihydroxy-23-nor-5β-cholanoic acid* (8). Compound 7 (80.2 mg) in MeOH (1.2 ml) was treated with 50% aqueous NaOH solution (0.17 ml) and the solution was refluxed for 3 hr. After evaporation of the MeOH, water (5 ml) was added and the solution was extracted with ethyl ether (2 x 5 ml) which was discarded. The remaining solution was acidified with 2N HCl (1 ml) and extrac ted with ethyl ether (3 x 5 ml). The organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave a crystalline residue (57.1 mg, 84%) which was recrystallized from acetone. Compound 8 had m.p. 202-204° and sp. act. 1.01 mCi/mmol. IR (Nujol): 3600-3050, 1730 cm^{-1}. ^{1}H-NMR (pyridine-d_5-TMS) δ 1.02 (6H, s, Me-18 and Me-19), 1.56 (3H, s, Me-21 from isomer 20R), 1.72 (3H, s, Me-21 from isomer 20S), 2.80 (2H, s, $\underline{CH_2}$-COOH), 4.34 (1H, bs, H-3). MS (m/z, %): 360 (M^+-H_2O, 2), 345 (3), 342 (2), 300 (21), 215 (37), 43 (100).

|24-^{14}C| *Ethyl 3β-acetoxy-23-nor-5β-chol-20(22)E-en-24-oate* (9). To a solution of compound 7 (110 mg) in pyridine (1.4 ml) phosphorous oxychloride (0.24 ml) was slowly added and the reaction mixture was kept at room temperature for 22 hr. It was poured onto ice-2N HCl and extracted with CH_2Cl_2 (3 x 20 ml). The organic layer was washed with water, aqueous $NaCO_3H$ solution, water again and dried over $MgSO_4$. Evaporation of the solvent yielded a residue (100 mg) that was purified by column chromatography (neutral alumina, 5 g). Elution with toluene (150 ml) afforded an isomeric mixture (5) which was recrystallized from EtOH to pure compound 9 (21 mg, 20%) of m.p. 108-109° and sp. act. 0.99 mCi/mmol. IR (Nujol): 1720, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS) δ 0.66 (3H, s, Me-18) 0.94 (3H, s, Me-19), 1.26 (3H, t, J=7 Hz, $\underline{CH_3}$-CH_2), 2.01 (3H, s, $\underline{CH_3}$-CO), 4.14 (2H, q, J=7 Hz, CH_3-$\underline{CH_2}$), 5.06 (1H, bs, H-3), 5.68 (1H, bs, H-22). MS (m/z, %): 430 (M^+, 1), 370 (100), 355 (32), 215 (20).

|24-^{14}C| *3β-Hydroxy-23-nor-5β-chol-20(22)E-enoic acid* (10). Compound 9 (40 mg) was treated as described above for the preparation of 3. In the present case compound 10 (32.5 mg, 86%) was recrystallized from EtOH to m.p. 132-133° and sp. sct. 0.98 mCi/mmol. IR (Nujol): 3500-2500, 1670, 1630 cm^{-1}. ^{1}H-NMR (pyridine-d_5-TMS) δ 0.62 (3H, s, Me-18), 1.00 (3H, s, Me-19), 2.44 (3H, s, Me-21), 3.42 (1H, s, OH), 6.20 (1H, s, H-22). MS (m/z, %): 360 (M^+, 5), 342 (30), 327 (25), 215 (100).

|24-^{14}C| *Ethyl 3-ethylenedioxy-20ξ-hydroxy-23-norchol-5-en-24-oate* (14). A solution of compound 13, obtained as described elsewhere (9), (260 mg) in dry benzene (2 ml) was treated with activated zinc (391 mg), iodine and ethyl |1-^{14}C| bromoacetate (0.67 ml) as already described for the preparation of 7. The crude product (650 mg) was purified by column chromatography (silica gel G, 30 g) eluting with toluene and toluene-ethyl acetate (98:2 and 95:5) yielding 255 mg (70%) of pure 14 of m.p. 165-166° and sp. act. 1.00 mCi/mmol after recrystallization from MeOH. IR (Nujol): 3610, 1730, 1098 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS) δ 0.86 (3H, s, Me-18), 1.02 (3H, s, Me-19), 1.20 (3H, s, Me-21 from isomer 20R), 1.26 (3H, t, J=7 Hz, CH_3-CH_2), 1.34 (3H, s, Me-21 from isomer 20S), 3.34 (1H, s, OH), 3.92 (4H, s, O-CH_2-CH_2-O), 4.20 (2H, q, J=7 Hz, CH_3-CH_2), 5.30 (1H, bs, H-6). MS (m/z, %): 446 (M^+, 1), 431 (1), 401 (1), 131 (3), 99 (100).

|24-^{14}C| *Ethyl 3-keto-20ξ-hydroxy-23-norchol-4-en-24-oate* (16). Compound 14 (99 mg) was dissolved in acetone and the solution was treated with *p*-toluene sulphonic acid (22.5 mg) at room temperature for 48 hr. After evaporation of the solvent the residue was suspended in saturated $NaCO_3H$ solution (6.8 ml) and extracted with ethyl acetate (3 x 15 ml). The organic extract was washed with water (2 x 10 ml) and dried. The residue obtained by evaporation of the solvent (90 mg) was chromatographed on a silica gel G column eluting with toluene and toluene-ethyl acetate (98:2) affording 77.5 mg (87%) of compound 16 which was recrystallized from EtOH to m.p. 127-128° and sp. act. 1.02 mCi/mmol. IR (Nujol): 3500-3100, 1750, 1690, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS) δ 0.90 (3H, s, Me-18), 1.20 (6H, s, Me-19 and Me-21 from isomer 20R), 1.28 (3H, t, J=7 Hz, CH_3-CH_2), 1.36 (3H, s, Me-21 from isomer 20S), 3.46 (1H, s, OH), 4.18 (2H, q, J=7 Hz, CH_3-CH_2), 5.74 (1H, bs, H-4). MS (m/z, %): 402 (M^+, 1), 387 (1), 384 (1), 272 (16), 131 (100), 124 (37), 85 (26).

|24-^{14}C| *3-Keto-20ξ-hydroxy-23-norchol-4-enoic acid* (17). A solution of 16 (77.5 mg) in MeOH (2 ml) was treated with 50% aqueous NaOH solution (0.14 ml) as previously described for the preparation of compound 3. In this case the crude product (63.5 mg) was purified by chromatography on a silica gel G column.

Elution with CH_2Cl_2 and CH_2Cl_2-MeOH (99:1) afforded pure 17 (43.3 mg, 60%) which was recrystallized from EtOH to m.p. 192-194° and sp. act. 1.02 mCi/mmol. IR (Nujol): 3600-3300, 1750, 1660 cm^{-1}. ^{1}H-NMR (pyridine-d_5-TMS) δ 1.04 (3H, s, Me-18), 1.12 (3H, s, Me-19), 1.62 (3H, s, Me-21 from isomer 20R), 1.78 (3H, s, Me-21 from isomer 20S), 2.88 (2H, m, CH_2-COOH), 5.88 (1H, bs, H-4). MS (m/z, %) 359 (M^+-Me, 2), 341 (2), 314 (19), 149 (13), 124 (37), 103 (100).

|24-^{14}C| *Ethyl 3-ethylenedioxy-23-norchola-5,20(22)E-dien-24-oate* (15). Compound 14 (126 mg) in anhydrous pyridine (1.6 ml) was treated with phosphorous oxychloride (0.27 ml) as already described for the preparation of compound 9. In this case, compound 15 (37.8 mg, 31%) was obtained by recrystallization from EtOH, m.p. 164-166° and sp. act. 1.00 mCi/mmol. IR (Nujol): 1700, 1630, 1098 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS) δ 0.60 (3H, s, Me-18), 1.04 (3H, s, Me-19), 1.28 (3H, t, J=7 Hz, CH_3-CH_2), 2.20 (3H, d, J=1 Hz, Me-21), 3.96 (4H, s, O-CH_2-CH_2-O), 4.16 (2H, q, J=7 Hz, CH_3-CH_2), 5.36 (1H, m, H-6), 5.70 (1H, bs, H-22). MS (m/z, %): 428 (M^+, 30), 413 (2), 367 (3), 113 (7), 100 (78), 99 (100).

|24-^{14}C| *Ethyl 3-Keto-23-norchola-4,20(22)E-dien-24-oate* (18). Compound 15 was hydrolysed as described for the preparation of compound 16. The reaction with 81 mg of 15 yielded 64 mg (92%) of compound 18 which was recrystallized from EtOH to m.p. 112-113° and sp. act. 0.99 mCi/mmol. IR (Nujol): 1700, 1660, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS) δ 0.64 (3H, s, Me-18), 1.18 (3H, s, Me-19), 1.28 (3H, t, J=7 Hz, CH_3-CH_2), 2.18 (3H, s, Me-21), 4.16 (2H, q, J=7 Hz, CH_3-CH_2), 5.72 (2H, m, H-4 and H-22). MS (m/z, %): 384 (M^+, 100), 396 (8), 338 (37), 230 (37), 147 (12), 124 (15).

|24-^{14}C| *3-Keto-23-norchola-4,20(22)E-dienoic acid* (19). Compound 18 (64 mg) in EtOH (1.8 ml) with the addition of 50% aqueous NaOH solution (0.13 ml) was refluxed for 3 hr. The solution was acidified with conc. HCl (0.35 ml) and poured onto water. The precipitate was filtered (64 mg) and chromatographed on a silica gel G column which was eluted with CH_2Cl_2 and CH_2Cl_2-MeOH (99:1) yielding compound 19 (42.2 mg, 71%) of m.p. 220-221° and sp. act. 0.99 mCi/mmol. IR (Nujol): 3500-2500, 1700, 1660, 1620 cm^{-1}. ^{1}H-NMR (pyridine-d_5-TMS) δ 0.60 (3H, s, Me-18), 1.00 (3H, s, Me-19), 2.42 (3H, d, J=1 Hz, Me-21), 5.85 (1H, s, H-4), 6.20 (1H, bs, H-22). MS (m/z, %): 356 (M^+, 100), 341 (15), 324 (10), 244 (20), 124 (15).

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