SYNTHESIS OF |24-^{14}C|-3β,14β,20ξ-TRIHYDROXY-23-NOR-5β-CHOLANOIC ACID

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SUMMARY

The synthesis of |24-^{14}C|-3β,14β,20ξ-trihydroxy-23-nor-5β-cholanoic acid was accomplished by Reformatsky reaction of ethyl |1-^{14}C|-bromo acetate with 3β-acetoxy-14β-hydroxy-5β-pregnan-20-one which was obtained from digitoxigenin. The final and intermediate products were characterized by spectroscopic (IR, ^{1}H and ^{13}C-NMR, MS) methods.

Key Words: |24-^{14}C|-3β,14β,20ξ-Trihydroxy-23-nor-5β-cholanoic acid, Synthesis, Reformatsky reaction.

In the course of our investigations on the biosynthesis of cardiotonic steroids (1) we have recently reported the syntheses of labelled 23-nor-cholanoic acids belonging to the series 3β-hydroxy-Δ^5- and 3β-hydroxy-5β-steroid derivatives (2). In order to have a closer intermediate to the final cardenolide structure, we needed a 23-nor-cholanoic acid derivative having a 14β-hydroxyl group and an isotopic label at the side chain. We wish to report the synthesis of |24-^{14}C|-3β,14β,20ξ-trihydroxy-23-nor-5β-cholanoic acid which was obtained from 3β-acetoxy-14β-hydroxy-5β-pregnan-20-one which was prepared, in turn, from digitoxigenin.

RESULTS AND DISCUSSION

The synthetic sequence shown in Fig 1 started with digitoxin (1) which, following a described procedure (3), was transformed into 3β-acetoxy-14β,21-dihydroxy-5β-pregnan-20-one (2). The transformation of a 21-hydroxy-20-keto-pregnane derivative such as compound 2 into a 20-keto-pregnane derivative as 4 has been performed through the corresponding etianic acid and condensation with a methyl-organometallic derivative (4); this approach has been used to introduce a label at C-21 (2). In the present case it resulted more convenient

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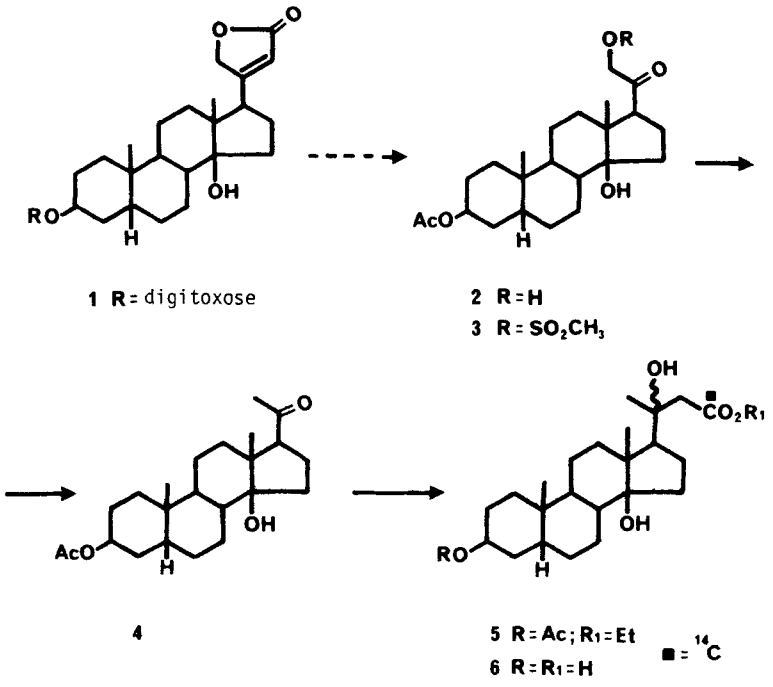
to eliminate the hydroxyl group at C-21 through the 21-O-mesyl derivative 3 and treatment of that with sodium iodide in acetic acid (5) to afford compound 4 in excellent yield. Reaction of 4 with ethyl |1-^{14}C|-bromoacetate in Reformatsky conditions yielded the hydroxy ester 5 which was hydrolyzed to the title compound 6. All compounds were fully characterized by spectroscopic (^{1}H and ^{13}C-NMR, MS) methods.



EXPERIMENTAL

Melting points were determined in a Fischer-Johns hot-plate and are uncorrected . ^{1}H and ^{13}C-NMR were recorded at 100 and 25.2 MHz respectively 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 interfaced to a Varian-Mat Data System 166 computer. Radioactivity was measured by liquid scintillation counting. Ethyl |1-^{14}C|-bromoacetate was purchased from the Comisiõn de Energía Atómica (Argentina).

3β-Acetoxy-14β-hydroxy-21-methansulfonyloxy-5β-pregnan-20-one (3). 3β-Acetoxy 14β,21-dihydroxy-5β-pregnan-20-one (2) (855 mg) was dissolved in pyridine (10 ml), treated with methansulfonyl chloride (1 ml) and the reaction mixture was shaked for 15 min. Ice-water was added and extracted with CH_2Cl_2 (3 x 30 ml).

The organic extract was washed with water (30 ml), with diluted HCl (30 ml) and water again (2 x 30 ml). The residue obtained by evaporation of the solvent (950 mg) was chromatographed on a silica gel G column which was eluted with CH_2Cl_2 and CH_2Cl_2-MeOH (99.5:0.5 and 99:1) affording compound 3 (813 mg, 80%) with m.p. 164-165°C (ethyl ether). IR (Nujol): 1710, 1370, 1170 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS): δ 0.98 (6H, s, Me-18 and Me-19), 2.06 (3H, s, $C\underline{H}_3CO_2$), 3.23 (3H, s, $C\underline{H}_3SO_2$), 3.40 (1H, bs, OH), 4.88 (2H, s, $C\underline{H}_2OSO_2CH_3$), 5.08 (1H, bs, H-3). ^{13}C-NMR ($CDCl_3$-TMS): δ 15.4 (C-18), 20.8 (C-11), 21.4 (C-16 and $C\underline{H}_3CO_2$), 23.7 (C-19), 24.7 (C-15), 25.0 (C-2), 26.3 (C-6), 30.5 (C-1 and C-4), 33.9 (C-7), 35.2 (C-8 and C-10), 36.9 (C-5), 39.1 (C-9 and C-12), 40.1 ($C\underline{H}_3SO_2$), 50.1 (C-13), 57.5 (C-17), 70.3 (C-3), 72.6 (C-21), 84.9 (C-14), 170.4 ($CH_3\underline{C}O_2$), 209.9 (C-20). MS (m/z, %): 470 (M^+, 1.5), 452 (1.5), 392 (11), 343 (10), 296 (32), 283 (54), 255 (64), 214 (14), 106 (100).

3β-Acetoxy-14β-hydroxy-5β-pregnan-20-one (4). Compound 3 (513 mg) was treated with NaI (940 mg) in acetic acid (7.5 ml) for 40 min with continous stirring. It was poured into 0.2 N aqueous $NaSO_3H$ (50 ml) and extracted with CH_2Cl_2 (4 x 20 ml). The organic extract was washed with water, dried over $MgSO_4$ and evaporated. The residue (360 mg) was chromatographed on a silica gel H column which was eluted with CH_2Cl_2 giving compound 4 (288 mg, 70%) of m.p. 141-142°C (MeOH); lit.(4) 150-151°.IR (Nujol): 3400, 1740, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS): δ 0.97 (6H, s, Me-18 and Me-19), 2.06 (3H, s, $C\underline{H}_3CO_2$), 2.24 (3H, s, Me-21), 4.38 (1H, bs, OH), 5.06 (1H, bs, H-3). ^{13}C-NMR ($CDCl_3$-TMS): δ 15.4 (C-18), 20.9 (C-11), 21.5 ($C\underline{H}_3CO_2$), 23.8 (C-19), 24.9 (C-15), 25.1 (C-2), 26.4 (C-6), 30.6 (C-1 and C-4), 33.3 (C-21), 34.0 (C-7), 35.2 (C-8 and C-10), 37.0 (C-5), 39.3 (C-9), 40.0 (C-12), 49.3 (C-13), 62.4 (C-17), 70.5 (C-3), 84.8 (C-14), 170.4 ($CH_3\underline{C}O_2$), 217.4 (C-20). MS (m/z, %): 376 (M^+, 1.5), 358 (2), 348 (8), 298 (3), 290 (44), 273 (8), 255 (10), 97 (100).

[24-^{14}C]Ethyl 3β-acetoxy-14β,20ξ-dihydroxy-23-nor-5β-cholan-24-oate (5). To a heated solution of compound 4 (70 mg) in anhydrous benzene containing activated Zn (99.5 mg) and few crystals of iodine and maintained under a nitrogen atmosphere, ethyl [1-^{14}C]bromoacetate (0.176 ml, 1 mCi/mmol) was added dropwise and the reaction was refluxed for 30 min. It was poured into ice-2 N HCl and extracted with ethyl ether (3 x 10 ml). The organic extract was washed with water, with saturated $NaCO_3H$ solution, water again and dried over $MgSO_4$. The residue obtained by evaporation of the solvent (89 mg) was chromatographed on a silica gel H column eluting with CH_2Cl_2 and CH_2Cl_2-MeOH (99:1). Compound 5 (40 mg, 45%) and unreacted compound 4 (24 mg) were thus obtained. The radioactive product was recrystallized from MeOH to constant spec. act. of 1.08 mCi/mmol. It had m.p. 160-161°C. IR (Nujol): 3550-3300, 1720, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$-TMS): δ 0.98 (6H, s, Me-18 and Me-19), 1.27 (3H, s, Me-21), 1.28 (3H, t, J=7 Hz, $-CO_2CH_2$

$\underline{CH}_3$), 2.05 (3H, s, $\underline{CH}_3CO_2$), 4.06 (1H, bs, OH), 4.20 (2H, q, J=7 Hz, $CO_2\underline{CH}_2CH_3$), 5.08 (1H, bs, H-3). ^{13}C-NMR ($CDCl_3$-TMS): δ 14.1 ($CO_2CH_2\underline{CH}_3$), 18.0 (C -18), 20.5 (C-11), 21.5 ($\underline{CH}_3CO_2$ and C-16), 23.8 (C-19 and C-15), 25.1 (C-2), 26.5 (C-21 of isomer 20S), 27.0 (C-21 of isomer 20R), 30.5 (C-1 and C-4), 31.8 (C-7), 35.2 (C-8 and C-10), 37.0 (C-5), 39.6 (C-9), 42.3 (C-12), 45.4 (C-13), 48.7 (C-22), 58.8 ($CO_2\underline{CH}_2CH_3$), 60.7 (C-17), 70.5 (C-3), 73.6 (C-20), 84.1 (C-14), 170.5 ($CH_3\underline{CO}_2$), 173.4 ($\underline{CO}_2CH_2CH_3$). MS (m/z, %): 446 (M^+, 3), 428 (3), 386 (2), 273 (2), 255 (2), 131 (38), 43 (100).

[24-^{14}C] 3β,14β,20ξ-Trihydroxy-23-nor-5β-cholanoic acid (6). Labelled compound 5 (37 mg) was dissolved in MeOH (1 ml), treated with 50% aqueous KOH (0.07 ml) and the solution was refluxed for 3 hr. The MeOH was removed and the residue was acidified with 2 N HCl (3 ml). The solid was filtered affording 22.3 mg of compound 6 which was recrystallized from acetone to constant spec. act. of 0.99 mCi/mmol. The product had m.p. 186-187°C. IR (Nujol): 3500-3000, 1720 cm^{-1}. ^{1}H-NMR (pyridine-d_5-TMS): δ 1.02 (6H, s, Me-18 and Me-19), 1.42 (3H, s, Me-21 of isomer 20R), 1.80 (3H, s, Me-21 of isomer 20S), 2.94 (2H, s, $\underline{CH}_2CO_2H$), 4.34 (1H, bs, H-3). ^{13}C-NMR (pyridine-d_5-TMS): δ 19.5 (C-18), 22.2 (C-11), 23.9 (C-16), 24.5 (C-15), 26.9 (C-2), 27.3 (C-21 of isomer 20S), 28.6 (C-21 of isomer 20R), 28.7 (C-6), 30.3 (C-1), 31.9 (C-7), 34.2 (C-4), 35.0 (C-8), 35.6 (C-10), 36.9 (C-5), 39.8 (C-9), 43.5 (C-12), 48.1 (C-22), 48.3 (C-13), 61.8 (C-17), 66.0 (C-3), 72.8 (C-20), 175.2 ($\underline{CO}_2H$). MS (m/z, %): 358 (M^+ - 36, 100), 343 (5), 316 (13), 273 (10), 255 (20), 103 (26), 43 (64).

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