ACYL MIGRATION IN THE REFORMATSKY REACTION OF 21-ACYLOXY-5-PREGNEN-20-ONE DERIVATIVES WITH ETHYL BROMOACETATE

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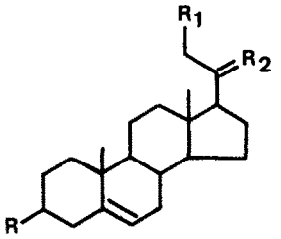
ABSTRACT

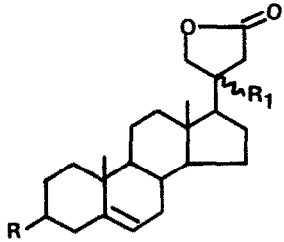
Reformatsky reaction of 3β,21-diacyloxy- and 3β-methoxy-21-acyloxy-5-pregnen-20-one with ethyl bromoacetate yields, through an intramolecular 1,2-acyl migration, 3β,20ξ-diacyloxy- and 3β-methoxy-20ξ-acyloxy-14α-card-5-enolide respectively. The 20ξ-acyloxy-14α-card-5-enolides were converted into the respective 20ξ-hydroxy-14α-card-5-enolides and the 14α-carda-5,20(22)-dienolides. Experimental support to the proposed intramolecular 1,2-acyl migration is provided by the use of labelled compounds.

We have recently reported (1) that the reaction of 3β,21-diacetoxy-5-pregnen-20-one (I) with ethyl bromoacetate in Reformatsky conditions gave a hitherto unknown main product that turned to be 3β,20ξ-diacetoxy-14α-card-5-enolide (II). We wish now to report that compound II is formed by an intramolecular 1,2-acyl migration from C-21 to C-20 before the closing of the lactone ring.

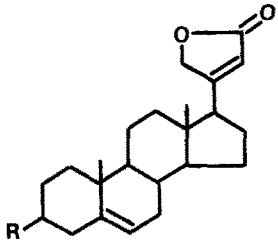
The facile migration of acyl groups in partially acylated polyhydroxylic compounds is well known and has been the subject of numerous reports in the literature (2-4) although to the best of our knowledge this rearrangement has not been previously described during a Reformatsky reaction. The fact that this migration is not privative of acetyl groups came from the Reformatsky reaction of 3β,21-dibenzoyloxy-5-pregnen-20-one (III), which was obtained by benzoylation of 3β,21-dihydroxy-5-pregnen-20-one (IV) (5), with ethyl bromoacetate in ether: benzene affording 3β,20ξ-dibenzoyloxy-14α-card-5-enolide (V) as the prioritary product. When the same reaction was performed in benzene, 3β-benzoyloxy-14α-carda-5,20(22)-dienolide (VI) was obtained as the major product. Successive treatment of V with alkali and acid gave a mixture of the known 3β,20ξ-dihydroxy-14α-card-5-enolide (VII) and 3β-hydroxy-14α-carda-5,20(22)-dienolide (VIII). This result ruled out any possible isomerization of the acylated ketol prior to condensation



I:R = R_1 = OAc; R_2 = O
III:R = R_1 = OCO-Ph; R_2 = O
IV:R = R_1 = OH; R_2 = O
IX:R = OMe; R_1 = H; R_2 = O
X:R = OMe; R_1 = OAc; R_2 = O
Xa:R = OMe; R_1 = OAc; R_2 = ^{18}O
XI:R = OMe; R_1 = OH; R_2 = O
XIa:R = OMe; R_1 = OH; R_2 = ^{18}O
XII:R = OMe; R_1 = OCO-Ph; R_2 = O
XIIa:R = OMe; R_1 = O^{14}CO-Ph; R_2 = O
XIIb:R = OMe; R_1 = OCO-Ph; R_2 = ^{18}O
XV:R = OMe; R_1 = H; R_2 = ^{18}O



II:R = R_1 = OAc
V:R = R_1 = OCO-Ph
VII:R = R_1 = OH
XIII:R = OMe; R_1 = OCO-Ph
XIIIa:R = OMe; R_1 = O^{14}CO-Ph
XIIIb:R = OMe; R_1 = ^{18}OCO-Ph
XIV:R = OMe; R_1 = OH
XIVa:R = OMe; R_1 = ^{18}OH



VI:R = OCO-Ph
VIII:R = OH
XVI:R = OMe

as it has been described for a related system (6). In order to provide further support to the postulated intramolecular 1,2-benzoyl migration and to avoid any interference from the benzoyl group at C-3, the synthesis of 3β-methoxy-21-benzoyloxy-5-pregnen-20-one (XII) was performed starting with 3β-methoxy-5-pregnen-20-one (IX) which was converted by reaction with lead tetraacetate into 3β-methoxy-21-acetoxy-5-pregnen-20-one (X) which, in turn, was saponified to 3β-methoxy-21-hydroxy-5-pregnen-20-one (XI); benzoylation of XI afforded XII in excellent yield. Reformatsky reaction on compound XII gave 3β-methoxy-20ξ-benzoyloxy-14α-card-5-enolide (XIII) which could be transformed into

3β-methoxy-20ξ-hydroxy-14α-card-5-enolide (XIV) by saponification. When compound XI was benzoylated with benzoyl-(carbonyl-^{14}C) chloride radioactive 3β-methoxy-21-benzoyloxy-(carbonyl-^{14}C)-5-pregnen-20-one (XIIa) was obtained which was submitted to the Reformatsky reaction affording 3β-methoxy-20ξ-benzoyloxy-(carbonyl-^{14}C)-14α-card-5-enolide (XIIIa). Saponification of XIIIa gave compound XIV and radioactive benzoic acid which carried all the radioactivity showing that the label had been exclusively located at the benzoyl group. These results can be interpreted as strong evidence that the rearrangement reaction path is entirely intramolecular through a sequence involving a transient cyclic and essentially strain-free intermediate as presented in Fig. 1.

The proposed mechanism was confirmed by Reformatsky reaction on compound XII labelled with ^{18}O at the C-20 carbonyl group (XIIb). Compound XIIb was prepared from 3β-methoxy-5-pregnen-20-one(^{18}O) (XV) through a reaction sequence involving compounds Xa and XIa by application of the same procedures used for the unlabelled compounds. The 3β-methoxy-21-benzoyloxy-5-pregnen-20-one(^{18}O) (XIIb) when submitted to the Reformatsky reaction in benzene afforded non-labelled 3β-methoxy-14α-carda-5,20(22)-dienolide (XVI) while using benzene:ether as solvent it gave 3β-methoxy-20ξ-benzoyloxy(^{18}O)-14α-card-5-enolide (XIIIb); upon saponification compound XIIIb was converted into 3β-methoxy-20ξ-hydroxy (^{18}O)-14α-card-5-enolide (XIVa). The position of the labelled oxygen-atom was determined by mass spectrometry analysis of the reaction products (7). The mass spectra of the ^{18}O-labelled compounds resulted identical to those of the unlabelled products except for the two mass units shift of the fragments carrying the labelled oxygen-atom.

It is interesting to note the importance of the reaction temperature in the formation of the rearranged product which is the sole product when the Reformatsky reaction is conducted in a mixture of solvents of low boiling points. When benzene or tetrahydrofuran are used, the 20ξ-hydroxy- or/and the unsaturated lactone are obtained (8,9).

EXPERIMENTAL

Melting points are uncorrected. Solvents were evaporated *in vacuo* at temperature below 50°. The ^{1}H and fully decoupled ^{13}C-NMR spectra were recorded with a Varian XL-100-15 spectrometer interfaced with a 620L VFT computer at 100.1 and 25.2 MHz respectively. Pulsed FT carbon spectra were obtained in 12 mm tubes using approximately 3.5 ml of 0.1 M

solutions in the indicated solvent containing 1% tetramethylsilane (TMS) Typically, spectra were run using 45° pulses, spectral width of 5438 Hz, 8K data table, 0.754 pulse repetition rate, and *ca.* 0.7 Hz of line broadening due to exponential weighting of the free induction decay (FID). Chemical shifts are reported as δ values referred to TMS internal standard; asterisk indicates interchangeable values. IR spectra were obtained as Nujol dispersions using a Perkin-Elmer 421 spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter using a 1 dm cell. Mass spectra were obtained at 70 eV with a Varian-Mat CH-7A mass spectrometer coupled to a computer Varian-Mat Data System 166. Radioactive samples were measured by scintillation counting using a Packard Tri-Carb spectrometer. $H_2{}^{18}O$ was purchased from Merck, Sharp & Dohme (Canada) and $C_6H_5{}^{14}COCl$ from the Comisión Nacional de Energía Atómica, Argentina.

3β,21-Dibenzoyloxy-5-pregnen-20-one (III). 3β,21-Dihydroxy-5-pregnen-20-one (IV) (5) (3.8 g, 11.6 mmoles) was treated with benzoyl chloride-pyridine (1:1; 10 ml) at room temperature for 20 hr. The reaction mixture was poured onto ice-water and the precipitate was filtered off and dried *in vacuo* over KOH pellets. The product (5.8 g, 10.9 mmoles, 94%) was recrystallized from methanol giving pure III, mp 177-180° and $|\alpha|_D^{25}$ +76.2° (c 0.6, $CHCl_3$); IR 1730 and 1720 (C=O), 1600 and 1580 (Ar) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.73 (3H, s, 18-Me), 1.15 (3H, s, 19-Me), 4.85 (2H, q_{AB}, J 16Hz, 21-CH_2), 5.40 (1H, b.s., 6-H), 7.60-8.00 (10H, m, 3-C_6H_5 and 21-C_6H_5); ^{13}C-NMR ($CDCl_3$): δ 13.26 (18-C), 19.33 (19-C), 21.04 (11-C), 22.86 (16-C), 24.60 (15-C), 27.81 (2-C), 31.80 (7-C and 8-C), 36.66 (10-C), 37.01 (4-C), 38.13 (1-C), 38.52 (12-C), 44.71 (13-C), 49.79 (9-C), 56.94 (14-C), 59.21 (17-C), 69.48 (21-C), 74.29 (13-C), 122.18 (6-C), 128.08, 128.21, 129.24, 129.33, 129.69, 130.60, 132.55 and 133.08 (C_6H_5CO), 139.50 (5-C), 165.72 (C_6H_5CO), 203.30 (20-C); MS m/z 418 (M - $PhCOOH^+$), 283 (M - PhCOOH - 21-C), 255 (M - PhCOOH - side chain), 105 ($PhCO^+$, 100%).
Anal. Calcd. for $C_{35}H_{40}O_5$: C, 77.78; H, 7.41.
Found: C, 77.89; H, 7.70%.

3β,20ξ-Dibenzoyloxy-14α-card-5-enolide (V). 3β,21-Dibenzoyloxy-5-pregnen-20-one (III) (4.5 g, 8.3 mmoles) was dissolved in a mixture of benzene-ethyl ether (1:1, 85 ml) and the solution was treated with activated zinc powder (10) (2.5 g) and a few crystals of iodine. To the stirred and heated (50°) solution ethyl bromoacetate (7.0 g) was added dropwise maintaining a gentle reflux. When the addition was over, the

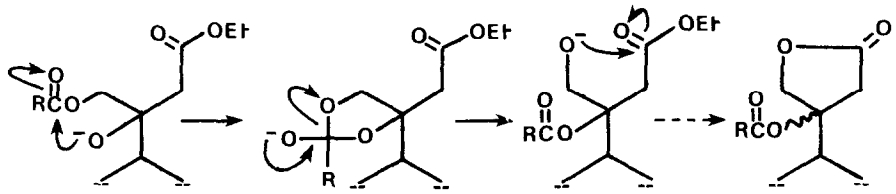


FIGURE 1
Possible mechanism for the formation of 20ξ-acyloxy-cardenolides

mixture was refluxed for 1 hr and it was poured onto ice-1 N hydrochloric acid (1:1, 100 ml). The organic layer was washed with saturated sodium bicarbonate solution (3 x 100 ml), water (2 x 100 ml), dried over magnesium sulphate and evaporated. The product (4.05 g, 6.9 mmoles, 84%) was crystallized from a mixture of ethanol-benzene (1:1) affording pure compound V, mp 231-232° and $|\alpha|_D^{25}$ +16.2° (c 0.6, $CHCl_3$); IR 1790 (lactone C=O), 1720 (Ph-C=O), 1600 and 1580 (Ar) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.90 (3H, s, 18-Me), 1.05 (3H, s, 19-Me), 3.00 (2H, b.s., 22-CH_2), 4.80 (2H, q_{AB}, J 12Hz, 21-CH_2), 5.40 (1H, m, 6-H), 7.00-8.00 (10H, m, 3-C_6H_5 and 20-C_6H_5); ^{13}C-NMR ($CDCl_3$): δ 14.16 (18-C), 19.31 (19-C), 20.03 (15-C), 20.63 (11-C), 24.43 (16-C), 27.76 (2-C), 31.28* (8-C), 31.64* (7-C), 36.54 (10-C), 36.90 (4-C), 38.09 (1-C), 38.50 (12-C and 22-C), 43.39 (13-C), 49.65 (9-C), 53.05 (17-C), 55.71 (14-C), 74.23 (3-C), 75.59 (21-C), 88.08 (20-C), 122.11 (6-C), 139.50 (5-C), 128.07, 129.47, 129.86 and 132.54 (C_6H_5CO), 165.68 (C_6H_5CO); MS m/z 460 (M - PhCOOH), 338 (M - 2 PhCOOH, 100%), 105 ($PhCO^+$).
Anal. Calcd. for $C_{37}H_{42}O_6$: C, 76.29; H, 7.22.
Found: C, 76.48; H, 7.42%.

3β-Benzoyloxy-14α-carda-5,20(22)-dienolide (VI). Reformatsky reaction on compound III (1.61 g, 2.9 mmoles) was carried out in the same conditions as indicated for the preparation of V but using benzene as solvent. The product (1.24 g, 2.7 mmoles, 90%) was crystallized from isopropanol affording pure compound VI, mp 226-227° and $|\alpha|_D^{25}$ -16.6° (c 1.0, $CHCl_3$); IR 1790 and 1760 (unsaturated lactone C=O), 1720 (Ph-C=O) 1630 (C=C) and 1600 (Ar) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.65 (3H, s, 18-Me), 1.10 (3H, s, 19-Me), 4.80 (2H, b.s., 21-CH_2), 5.40 (1H, m, 6-H), 5.90 (1H, b.s., 22-CH), 7.00-8.00 (5H, m, C_6H_5); ^{13}C-NMR ($CDCl_3$): δ 13.10 (18-C), 19.38 (19-C), 20.82 (11-C), 24.38 (15-C), 25.90 (16-C), 27.78 (2-C), 31.66* (8-C), 32.09* (7-C), 36.66 (10-C), 36.98 (4-C), 37.94 (1-C), 38.09 (12-C), 44.25 (13-C), 49.88 (9-C), 50.69 (17-C), 56.39 (14-C), 73.39 (21-C), 74.23 (3-C), 115.85 (22-C), 122.10 (6-C), 128.07, 129.33, 130.55 and 132.55 (C_6H_5), 139.54 (5-C), 165.74 (Ph-CO), 170.90 (20-C); MS m/z 338 (M - $PhCOOH^+$, 100%), 323 (M - CH_3 - PhCOOH), 255 (M - PhCOOH - side chain), 105 ($PhCO^+$).
Anal. Calcd. for $C_{30}H_{36}O_4$: C, 78.26; H, 7.82.
Found: C, 78.10; H, 8.01%.

3β,20ξ-Dihydroxy-14α-card-5-enolide (VII). To a stirred solution of compound V (0.174 g, 0.30 mmoles) in chloroform (25 ml), 0.5N sodium methoxide (25 ml) was added and the mixture was maintained at 0° for 2 hr. Water (50 ml) was added and the solution was acidified (pH 3) by addition of 2N hydrochloric acid (7.5 ml) and heated to reflux temperature for 2 hr. The reaction mixture was extracted with dichloromethane (3 x 25 ml) and the combined extracts were washed with saturated sodium bicarbonate solution (3 x 25 ml), water (2 x 25 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was crystallized from isopropanol giving pure VII (0.11 g, 0.29 mmoles, 95%), mp 261-263° and $|\alpha|_D^{25}$ -15.2° (c 0.8, tetrahydrofuran) (Lit. (1) mp 260-263° and $|\alpha|_D^{25}$ -15.8°(c 0.9, tetrahydrofuran)); ^{13}C-NMR ($CDCl_3$-CD_3OD, 8:2) δ 13.38 (18-C), 19.17 (19-C), 20.59 (11-C), 23.39 (16-C), 23.98 (15-C), 31.03* (7-C), 31.13* (8-C), 31.54 (2-C), 36.34 (10-C), 37.08 (1-C), 39.24 (12-C), 41.67 (4-C), 41.93 (22-C),

42.87 (13-C), 49.62 (9-C), 56.05 (17-C), 56.21 (14-C), 71.06 (3-C), 78.87 (21-C), 79.02 (20-C), 120.91 (6-C) and 140.61 (5-C).

3β-Hydroxy-14α-carda-5,20(22)-dienolide (VIII). Saponification of compound VI (0.230 g, 0.5 mmoles) was performed as already indicated for compound V. Crystallization from dichloromethane-methanol (1:1) afforded compound VIII (0.156 g, 0.43 mmoles, 88%), mp 235-238° (Lit. mp (8) 260-262°, (11) 240-245° and (1) 235-240°), with spectral properties identical to those previously described (1).

3β-Methoxy-21-acetoxy-5-pregnen-20-one (X). To a solution of 3β-methoxy-5-pregnen-20-one (IX) (12) (9.9 g, 30 mmoles) and lead tetraacetate (17 g) in benzene (400 ml), a solution of boron trifluoride etherate (58 ml) in methanol (20 ml) was added. The mixture was stirred at room temperature for 4 hr. and poured onto water (500 ml). The organic layer was washed with water (3 x 200 ml), dried over magnesium sulphate and evaporated. The residue was crystallized from methanol giving pure X (8.9 g, 22.9 mmoles, 77%), mp 144-149° (Lit. (13) mp 146.5-147°); IR 1755 (acetyl C=O), 1735 (C=O) and 1070 (CH_3O) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.67 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 2.18 (3H, s, Me-CO), 3.37 (3H, s, MeO), 4.66 (2H, q_{AB}, J 17Hz, 21-CH_2), 5.38 (1H, m, 6-H); ^{13}C-NMR ($CDCl_3$): δ 13.06 (18-C), 19.33 (19-C), 20.44 (CH_3CO), 21.01 (11-C), 22.81 (16-C), 24.55 (15-C), 27.91 (2-C), 31.80 (7-C and 8-C), 36.84 (10-C), 37.13 (1-C), 38.55 (4-C and 12-C), 44.62 (13-C), 49.93 (9-C). 55.52 (CH_3O), 57.00 (14-C), 59.21 (17-C), 69.06 (21-C), 80.09 (3-C), 120.96 (6-C), 140.68 (5-C), 169.96 (MeCO), 203.37 (20-C); MS m/z 388 (M^+), 373 (M - Me), 356 (M - MeOH), 341 (M - MeOH - Me), 315 (M - CH_2OAc), 287 (M - side chain), 283 (M - MeOH - CH_2OAc), 255 (M - MeOH - side chain).

3β-Methoxy-21-hydroxy-5-pregnen-20-one (XI). A solution of compound X (8.0 g, 20.6 mmoles) in ethanol (400 ml) containing sulphuric acid (2 ml) was heated to reflux temperature for 30 hr. Water (500 ml) was added and on cooling a solid was obtained which was filtered off and dried *in vacuo* over KOH pellets. Crystallization from ethanol gave pure compound XI (6.95 g, 20.1 mmoles, 98%), mp 146-148° and $[\alpha]_D^{25}$ + 0.5° (c 6.9, $CHCl_3$); IR 3420 (hydroxyl), 1700 (C=O), 1060 (MeO) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.68 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 3.38 (3H, s, MeO), 4.22 (2H, b.s., 21-CH_2), 5.38 (1H, m, 6-H); ^{13}C-NMR ($CDCl_3$): δ 13.32 (18-C), 19.33 (19-C), 20.96 (11-C), 22.91 (16-C), 24.55 (15-C), 27.90 (2-C), 31.80 (7-C and 8-C), 36.83 (10-C), 37.12 (1-C), 38.55 (4-C and 12-C), 44.67 (13-C), 49.92 (9-C), 55.52 (CH_3O), 56.89 (14-C), 59.16 (17-C), 69.32 (21-C), 80.07 (3-C), 120.91 (6-C), 140.71 (5-C), 209.89 (20-C); MS m/z 346 (M^+), 331 (M - Me), 315 (M - CH_2OH), 314 (M - MeOH), 300 (M - CH_2OH - Me), 299 (M - MeOH - Me), 287 (M - side chain), 283 (M - CH_2OH - MeOH), 255 (M - side chain - MeOH, 100%).
Anal. Calcd. for $C_{22}H_{34}O_3$: C, 76.30; H, 9.83.
Found: C, 76.31; H, 9.88%.

3β-Methoxy-21-benzoyloxy-5-pregnen-20-one (XII). Compound XI (5.0 g, 14.4 mmoles) was treated with benzoyl chloride-pyridine (1:1) (6 ml) at room temperature for 18 hr. The reaction mixture was poured onto ice-water and the precipitate was filtered off and dried *in vacuo* over KOH

pellets. The product was crystallized from ethanol giving pure XII (6.25 g, 13.8 mmoles, 97%), mp 142-144° and $|\alpha|_D^{25}$ +56.0° (c 2.5, $CHCl_3$); IR 1750 (PhC=O), 1735 (C=O), 1600 (Ar), 1070 (MeO) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.75 (3H, s, 18-Me), 1.03 (3H, s, 19-Me), 3.40 (3H, s, CH_3-O), 4.93 (2H, q_{AB}, J 18Hz, 21-CH_2), 5.42 (1H, m, 6-H), 7.50-8.30 (5H, m, C_6H_5); ^{13}C-NMR ($CDCl_3$): δ 13.11 (18-C), 19.28 (19-C), 21.05 (11-C), 22.85 (16-C), 24.55 (15-C), 27.92 (2-C), 31.78* (8-C), 31.85* (7-C), 36.87 (10-C), 37.14 (1-C), 38.60 (4-C and 12-C), 44.61 (13-C), 49.98 (9-C), 55.41 (CH_3O), 56.99 (14-C), 59.25 (17-C), 69.38 (21-C), 80.11 (3-C), 120.90 (6-C), 128.15, 129.28, 129.64 and 132.96 (C_6H_5CO), 140.71 (5-C), 165.58 (C_6H_5CO), 203.94 (20-C); MS m/z 450 (M^+), 435 (M - Me), 418 (M - MeOH), 403 (M - MeOH - Me), 315 (M - CH_2OCOPh), 287 (M - side chain), 283 (M - CH_2OCOPh - MeOH), 255 (M - side chain - MeOH), 105 ($PhCO^+$, 100%).

Anal. Calcd. for $C_{29}H_{38}O_4$: C, 77.33; H, 8.44.
Found: C, 77.49; H, 8.60%.

3β-Methoxy-20ξ-benzoyloxy-14α-card-5-enolide (XIII). Treatment of compound XII (3.0 g, 6.6 mmoles) in the same conditions as those indicated for the preparation of compound V gave compound XIII (2.56 g, 5.2 mmoles, 79%) which after recrystallization from ethanol had mp 183-184° and $|\alpha|_D^{25}$ -5.3° (c 0.6, $CHCl_3$); IR 1800 (lactone C=O), 1720 (PhC=O), 1600 (Ar), 1070 (MeO) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.88 (3H, s, 18-Me), 0.96 (3H, s, 19-Me), 3.01 (2H, q_{AB}, J 16Hz, 22-CH_2), 3.34 (3H, s, MeO), 4.58 (1H, b.s., 3-H), 4.75 (2H, q_{AB}, J 11Hz, 21-CH_2), 5.33 (1H, m, 6-H), 7.32-8.16 (5H, m, C_6H_5); ^{13}C-NMR ($CDCl_3$): δ 14.18 (18-C), 19.27 (19-C), 20.64 (11-C), 24.02 (15-C), 24.44 (16-C), 27.86 (2-C), 31.27* (8-C), 31.65* (7-C), 36.77 (10-C), 37.03 (1-C), 38.52 (4-C, 12-C and 22-C), 43.36 (13-C), 49.78 (9-C), 53.03 (17-C), 55.52 (CH_3O), 55.78 (14-C), 75.59 (21-C), 80.06 (3-C), 88.13 (20-C), 120.91 (6-C), 128.37, 129.46, 129.85 and 133.23 (C_6H_5-CO), 140.71 (5-C), 165.69 (C_6H_5CO); MS m/z 492 (M^+), 460 (M - MeOH), 370 (M - PhCOOH), 355 (M - PhCOOH - Me), 338 (M - PhCOOH - MeOH), 255 (M - lactone ring - MeOH), 105 ($PhCO^+$, 100%).

Anal. Calcd. for $C_{31}H_{40}O_5$: C, 75.60; H, 8.13.
Found: C, 75.43; H, 8.37%.

3β-Methoxy-20ξ-hydroxy-14α-card-5-enolide (XIV). Saponification of compound XIII (2.0 g, 4.07 mmoles) was carried out as described for the preparation of compound VII. Crystallization from ethanol afforded pure compound XIV (1.26 g, 3.24 mmoles, 80%), mp 216-218° and $|\alpha|_D^{25}$ -19.5° (c 0.85, $CHCl_3$); IR 3440 (hydroxyl), 1820 (lactone C=O), 1070 (MeO) cm^{-1} ^{1}H-NMR ($CDCl_3$): δ 0.84 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 2.55 (2H, q_{AB}, J 18Hz, 22-CH_2), 3.32 (3H, s, MeO), 4.20 (1H, b.s., 3-H), 4.23 (2H, s, 21-CH_2), 5.34 (1H, m, 6-H); ^{13}C-NMR ($CDCl_3$): δ 13.90 (18-C), 19.36 (19-C), 20.74 (11-C), 23.49 (16-C), 24.07 (15-C), 27.92 (2-C), 31.27* (8-C), 31.69* (7-C), 36.81 (10-C), 37.16 (1-C), 38.60 (4-C), 39.29 (12-C), 42.35 (22-C), 42.43 (13-C), 49.93 (9-C), 55.51 (CH_3O), 56.09 (17-C), 56.46 (14-C), 78.18 (21-C), 78.74 (20-C), 80.07 (3-C), 120.90 (6-C), 140.71 (5-C); MS m/z 388 (M^+), 373 (M - Me), 370 (M - H_2O) 356 (M - MeOH), 341 (M - MeOH - Me), 338 (M - MeOH - H_2O), 255 (M - lactone ring - MeOH).

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.22; H, 9.27
Found: C, 74.10; H, 9.22%.

3β-Methoxy-21-benzoyloxy(carbonyl-^{14}C)-5-pregnen-20-one (XIIa). Compound XI (250 mg, 0.72 mmoles) was treated in the same conditions described for obtaining compound XII but using benzoyl(carbonyl-^{14}C) chloride (spec. act. 4.4 μCi/mmol). Crystallization from methanol gave pure compound XIIa (308 mg, 0.68 mmoles, 95%), mp 142-143° and spec. act. 4.17 μCi/mmol. IR, ^{1}H-NMR and MS resulted identical to those described for the unlabelled product.

3β-Methoxy-20ξ-benzoyloxy(carbonyl-^{14}C)-14α-card-5-enolide (XIIIa). Treatment of compound XIIa (300 mg, 0.66 mmoles, spec. act. 4.17 μCi/mmol) in the same conditions as those indicated for obtaining compound XIII gave compound XIIIa (262 mg, 0.53 mmoles, 80%) with mp, IR and ^{1}H-NMR identical to those described for the unlabelled compound and spec. act. 4.44 μCi/mmol.

3β-Methoxy-20ξ-hydroxy-14α-card-5-enolide (XIV) *(from compound* XIIIa) Saponification of compound XIIIa (250 mg, 0.50 mmoles, spec. act. 4.44 μCi/mmol) was performed as already described for compound XIII. After the acidic treatment, the reaction mixture was extracted with dichloromethane (3 x 30 ml) and the combined extracts were dried over magnesium sulphate and evaporated. The syrup obtained was sublimated and benzoic acid (51.2 mg, 0.42 mmoles, spec. act. 4.4 μCi/mmol) was isolated and fully characterized. The residue was crystallized from ethanol affording unlabelled XIV with mp and spectral properties identical to those previously described.

3β-Methoxy-5-pregnen-20-one(^{18}O) (XV). 3β-Methoxy-5-pregnen-20-one (IX) (500 mg, 1.16 mmoles) in tetrahydrofuran (15 ml) was treated with a mixture of $H_2{}^{18}O$ (0.2 ml, 11.1 mmoles, 97 atom % ^{18}O) and dry hydrogen chloride (0.1 ml) and heated (70°) in a sealed tube for 24 hr. The mixture was poured onto ice-water and extracted with chloroform (3 x 20 ml), washed with sodium bicarbonate solution (3 x 15 ml), water (3 x 15 ml) and dried over magnesium sulphate. Recrystallization from methanol afforded pure compound XV (482 mg, 1.12 mmoles, 97%) with mp and IR spectrum identical to those of an unlabelled standard. Mass spectrometry showed the product to be 73.1 atom% ^{18}O in the carbonyl position. MS m/z 332 (M^+), 300 (M - MeOH), 285 (M - MeOH - Me), 255 (M - MeOH - $MeC^{18}O$), 45 ($CH_3C^{18}O$).

3β-Methoxy-21-acetoxy-5-pregnen-20-one(^{18}O) (Xa). Treatment of compound XV (470 mg, 1.42 mmoles) in the same conditions as those indicated for obtaining compound X gave Xa (535 mg, 1.38 mmoles, 97%) with mp and IR spectrum according with that of an unlabelled sample. Mass spectrometry showed the product to be 50.2 atom % ^{18}O in the carbonyl position. MS m/z 390 (M^+), 358 (M - MeOH), 343 (M - MeOH - Me), 317 (M - CH_2OCOCH_3), 285 (M - MeOH - CH_2OCOCH_3), 255 (M - MeOH - $C^{18}OCH_2OCOCH_3$).

3β-Methoxy-21-hydroxy-5-pregnen-20-one(^{18}O) (XIa). Hydrolysis of compound Xa (500 mg, 1.28 mmoles) in the same conditions as those used for the preparation of compound XI, gave compound XIa (415 mg, 1.20 mmoles, 93%) of mp and IR spectrum identical to those described for the unlabelled product. Mass spectrometry showed an isotopic enrichment

of 45.3 atom % ^{18}O at the carbonyl group. MS m/z 348 (M^+), 330 (M - $H_2{}^{18}O$), 317 (M - CH_2OH), 316 (M - MeOH), 301 (M - MeOH - Me), 285 (M - CH_2OH - MeOH), 255 (M - MeOH - $HOCH_2C^{18}O$).

3β-Methoxy-21-benzoyloxy-5-pregnen-20-one(^{18}O) (XIIb). Benzoylation of compound XIa (400 mg, 1.15 mmoles) was performed as indicated for the preparation of compound XII. Crystallization from ethanol afforded XIIb (472 mg, 1.05 mmoles, 91%) of mp and IR identical to those of the unlabelled product. Mass spectrometry showed an isotopic enrichment of 43.0 atom % ^{18}O at the C-20 carbonyl group. MS m/z 452 (M^+), 420 (M - MeOH), 317 (M - CH_2OCOPh), 285 (M - MeOH - CH_2OCOPh), 255 (M - $PhCOOCH_2C^{18}O$ - MeOH).

3β-Methoxy-20ξ-benzoyloxy(^{18}O)-14α-card-5-enolide (XIIIb). Reformatsky reaction on compound XIIb (200 mg, 0.44 mmoles) as indicated for the preparation of XIII afforded compound XIIIb (137 mg, 0.28 mmoles 65%) of mp and IR identical to those described for the unlabelled product. By mass spectrometry compound XIIIb had an isotopic enrichment of 43.0 atom % ^{18}O at the oxygen atom attached to C-20. MS m/z 494 (M^+), 462 (M - MeOH), 370 (M - $PhCO^{18}OH$), 355 (M - $PhCO^{18}OH$ - Me), 338 (M - $PhCO^{18}OH$ - MeOH), 255 (M - lactone ring - MeOH), 124 ($PhCO^{18}OH$), 105 ($PhCO^+$, 100%).

3β-Methoxy-20ξ-hydroxy(^{18}O)-14α-card-5-enolide (XIVa). Saponification of compound XIIIb (100 mg, 0.20 mmoles) to yield compound XIVa was performed in the conditions indicated for the preparation of compound XIV. Crystallization from ethanol afforded compound XIVa (56.6 mg, 0.14 mmoles, 73%) of mp and IR identical to those reported for the unlabelled product. By mass spectrometry compound XIVa had an enrichment of 40.0 atom % ^{18}O at the hydroxyl group. MS m/z 390 (M^+), 370 (M - $H_2{}^{18}O$), 358 (M - $Me^{18}OH$), 343 (M - Me - MeOH), 338 (M - MeOH - $H_2{}^{18}O$), 323 (M - MeOH-Me - $H_2{}^{18}O$), 255 (M - lactone ring - MeOH).

3β-Methoxy-14α-carda-5,20(22)-dienolide (XVI) *(from 3β-methoxy-21-benzoyloxy-5-pregnen-20-one* (XII)*)* Reformatsky reaction on compound XII (2.5 g, 5.5 mmoles) was performed in the conditions indicated for the preparation of XIII but using benzene as solvent. The product was crystallized from ethanol affording pure compound XVI (1.55 g, 4.2 mmoles, 78%) of mp 212-217°; IR 1785 and 1750 (unsaturated lactone C=O), 1630 (C=C) and 1070 (MeO) cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.63 (3H, s, 18-Me), 1.05 (3H, s, 19-Me), 3.50 (3H, s, CH_3O), 4.72 (2H, b.s., 21-CH_2), 5.38 (1H, m, 6-H), 5.85 (1H, b.s., 22-CH); MS m/z 370 (M^+), 338 (M - MeOH), 255 (M - MeOH - lactone ring, 100%).
Anal. Calcd. for $C_{24}H_{34}O_3$: C, 77.83; H, 9.18.
Found: C, 77.62; H, 9.38%.

3β-Methoxy-14α-carda-5,20(22)-dienolide (XVI) *(from 3β-methoxy-21-benzoyloxy-5-pregnen-20-one(^{18}O)* (XIIb)*)*. Reformatsky reaction on compound XIIb (200 mg, 0.44 mmoles) was carried out in the same conditions as indicated for the unlabelled compound. The product was crystallized from ethanol affording pure XVI (107 mg, 0.29 mmoles, 68%) whose mass spectrum showed no ^{18}O enrichment.

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