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Synthesis of 16-Methyl-14,17-*cis*-5-dehydro- and -5α-Isobufadienolides[1]

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In order to examine the cardiotonic and anti-tumor activities, synthesis of 16-methyl-14,17-*cis*-5-dehydro- and 5α-isobufadienolides, in which C-17 is linked to 6-position of the 2-pyrone ring, has been undertaken. These cardiotonic steroid analogs have been prepared through the general route, namely condensation with ethyl orthoformate and then with malonic acid followed by cyclization to 2-pyrone, starting from the 16-methylpregnan-20-ones, which possess the desired configuration at C-14 and C-17. The synthesis of the corresponding 14α,17β-isobufadienolides is also described.

It has been generally accepted that there are certain molecular structures essential for the cardiotonic activity of cardenolides and bufadienolides: C/D-*cis* fusion, hydroxyl groups in positions 3β and 14β, and a five- or six-membered α,β-unsaturated lactone ring at 17β.[3] Of these structural features, the significance of the 14β-hydroxyl group has not fully been clarified, although an alteration of the stereochemistry at C-14 results in loss of the toxicity. As yet no cardiac-active steroids, either natural or synthetic have been found or prepared where ring C and D are arranged *cis* without a hydroxyl group being present at C-14.[4] Hence it seems to be quite attractive to explore whether or not the *cis* fusion of C/D-ring is mainly responsible for the physiological potency. In the modified steroidal hormones the introduction of substituent, such as halogen and methyl group, to steroid nucleus often enhances the biological activity. As to the cardiotonic steroids, however, the detailed examinations in these respects have not hitherto been made. In addition, considerable attentions have been drawn to the finding that some of the cardiac steroids exhibit the remarkable anti-tumor activity.[5] The interest in structure-activity relationship prompted the authors to explore the synthesis of the cardiotonic steroid analogs. The present paper describes the preparation of 16-methyl-14,17-*cis*-5-dehydro- and -5α-isobufadienolides, where the 2-pyrone ring is linked to C-17 with 6-position.

The desired compounds were prepared by the general route from the 16-methylpregnan-20-ones having the requisite configuration at C-14 and C-17. First, 3β-acetoxy-16-methylpregna-5,14,16-trien-20-one (I)[6] was chosen as the starting material. Hydrogenation over palladium-on-charcoal gave the partially reduced 3β-acetoxy-16α-methyl-14β,17β-pregn-5-

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[1] This paper constitutes Part II of the series entitled "Studies on Cardiotonic Steroid Analogs"; Part I: T. Nambara, S. Goya, J. Goto and K. Shimada, *Chem. Pharm. Bull.* (Tokyo), **16**, 2228 (1968).

[2] Location: *Kita-4-bancho, Sendai.*

[3] Ch. Tamm, "Proceedings of the 1st International Pharmacological Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p.11.

[4] During the preparation of this manuscript the authors received a personal communication from Dr. M. Okada, Tokyo Biochemical Research Institute, that they recently succeeded in synthesis of 3β-hydroxy-5α-card-20(22)-enolide.

[5] J.E. Pike, J.E. Grady, J.S. Evans and C.G. Smith, *J. Med. Chem.*, 7, 348 (1964); S.M. Kupchan, R.J. Hemingway and R.W. Doskotch, *ibid.*, 7, 803 (1964); S.M. Kupchan, J.R. Knox, J.E. Kelsey and J.A.S. Renauld, *Science*, **146**, 1685 (1964); R.B. Kelly, E.G. Daniels and L. B. Spaulding, *J. Med. Chem.*, **8**, 547 (1965); S.M. Kupchan, M. Mokotoff, R.S. Sandhu and L.E. Hokin, *ibid.*, **10**, 1025 (1967).

[6] R. Mickova and K. Syhora, *Collection Czech. Chem. Commun.*, 29, 2513 (1964).

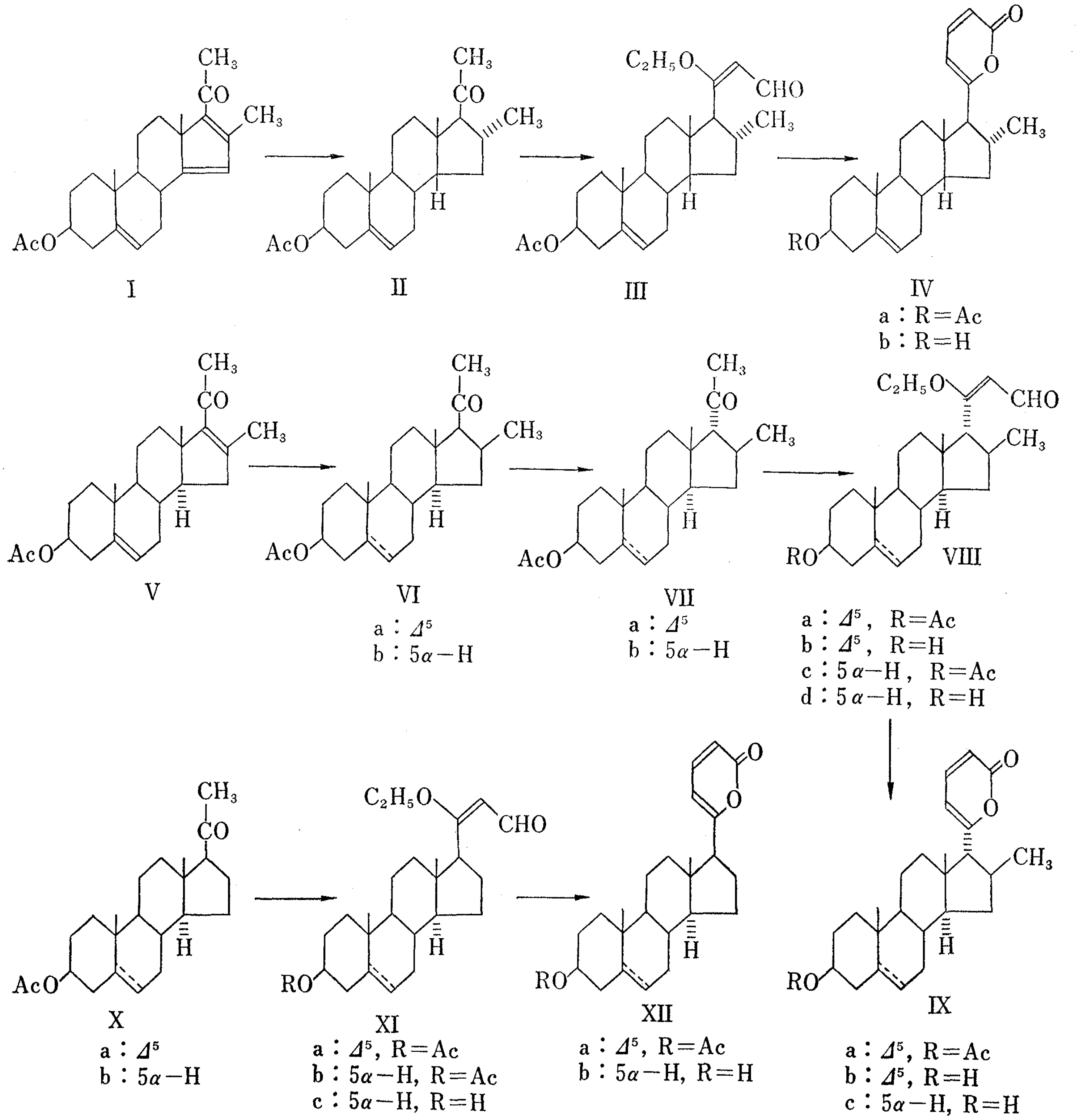

Chart 1

en-20-one (II) without disturbance of the 5,6-double bond.[7] The stereochemistry in ring D was verified on the basis of the following evidences. The optical rotatory dispersion curve exhibiting the positive Cotton effect indicated the formation of 17β-side chain. The nuclear magnetic resonance spectra showed a singlet peak at 2.74 ppm assigned to 17α-proton, which supported the *trans* arrangement of H16,17, that is, α-configuration of 16-methyl group. Furthermore the reduction product could be easily differentiated from the known 14α-epimer[9] by the usual criteria. Addition of hydrogen to the 16-methyl-$\Delta^{14,16}$-diene system would take place from the β-side in the usual way[10] and the resulting product would then be

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7) Mickova, *et al.* obtained 3β-acetoxy-16α-methyl-5α,14β,17β-pregnan-20-one from the same compound on catalytic hydrogenation over palladium-on-calcium carbonate.[8]

8) R. Mickova and K. Syhora, *Collection Czech. Chem. Commun.*, **30**, 2771 (1965).

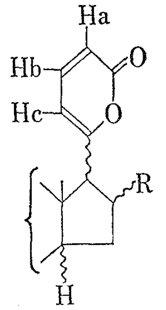
9) K. Heusler, J. Kebrle, C. Meystre, H. Ueberwasser, P. Wieland, G. Anner and A. Wettstein, *Helv. Chim. Acta*, **42**, 2043 (1959).

10) Pl. A. Plattner, H. Heusser and A. Segre, *Helv. Chim. Acta*, **31**, 249 (1948).

epimerized to the more stable 17β-pregnane derivative, in this case *trans* to the 16α-methyl group probably due to the steric hindrance.

Formation of the 6-substituted 2-pyrone ring was attempted according to the method developed by Knight, *et al.*[11] starting from II. Treatment with ethyl orthoformate in the presence of perchloric acid provided the 20-ethoxy-21-formylpregn-20-ene derivative (III). When the unsaturated aldehyde was treated with malonic acid in pyridine containing morpholine, condensation reaction and subsequent ring closure proceeded readily to give the 6-substituted 2-pyrone derivative (IVa). On prolonged contact with potassium bicarbonate the 3-acetate was hydrolyzed to result in the formation of the desired 6-(3β-hydroxy-16α-methyl-14β-androst-5-en-17β-yl)-2-pyrone (IVb).

It is of particular interest that the naturally occurring cardiotonic steroids have thermodynamically less stable 14,17-*cis* configuration. Accordingly the next project was directed to the preparation of the remaining 14α,17α-*cis*-isobufadienolide. 3β-Acetoxy-16β-methylpregn-5-en-20-one (VIa), obtained by partial hydrogenation of the 16-methyl-$\Delta^{5,16}$-diene (V), being refluxed with basic alumina in benzene and reacetylated, it was readily transformed into the epimeric 17α-pregnene derivative (VIIa). The occurrence of facile epimerization would also be attributable to the presence of the methyl group at 16β. Condensation of VIIa with ethyl orthoformate gave the α,β-unsaturated aldehyde 3-acetate (VIIIa), which in turn was led to the 3β-hydroxy derivative (VIIIb) upon hydrolysis with potassium carbonate. Reaction of VIIIb with malonic acid followed by cyclization to the lactone ring in the same manner gave the 3β-hydroxy-5-dehydro-14α,17α-isobufadienolide (IXb) in reasonable yield. Usual acetylation of IXb with acetic anhydride and pyridine afforded the 3-acetate (IXa), which proved to be identical with the substance derived from VIIIa by the ring closure with malonic acid.



R=CH_3 or H

Fig. 1

The corresponding 5,6-saturated 14α,17α-isobufadienolide (IXc) was prepared from 3β-acetoxy-16β-methyl-17α-pregnan-20-one (VIIb) employing the similar reaction sequence, *i.e.* condensation with ethyl orthoformate and then with malonic acid followed by cyclization to the six-membered lactone. In order to examine the structural significance of C/D-*cis* juncture in the physiological activities, the common C/D-*trans*-fused analogs (XIIa, b) were also synthesized starting from 3β-acetoxy-5α-pregnan-20-one (Xb) or its 5,6-unsaturated compound (Xa) through the 20,21-unsaturated aldehydes (XIa,b,c).

The physical data of all the isobufadienolides thus prepared were fairly consistent with their formulae. The ultraviolet spectra exhibited an absorption maximum at 305 mμ (ε 8100), characteristic to the unsaturated six-membered lactone. Three groups of signals in the nuclear magnetic resonance spectra, 6.00 (Ha or Hc, doublet, J_{ab}=6 cps), 7.25 (Hb, quartet, J_{ab}=6 cps, J_{bc}=9 cps) and 6.12 ppm (Hc or Ha, doublet, J_{bc}=9 cps), could be assigned to three ring protons on the 6-substituted 2-pyrone.

The results of the biological tests on these isobufadienolides will be reported elsewhere in near future.

Experimental[12]

3β-Acetoxy-16α-methyl-14β-pregn-5-en-20-one (II)——A solution of 3β-acetoxy-16-methylpregna-5,14,16-trien-20-one (I)[6] (130 mg) in EtOH (4 ml) was shaken with 5% Pd/C (70 mg) under a stream of H_2

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[11] J.C. Knight, G.R. Pettit and C.L. Herald, *Chem. Commun.*, **1967**, 445.

[12] All melting points were taken on a micro hot-stage apparatus and uncorrected. Optical rotations were measured in $CHCl_3$ solution unless otherwise stated. The rotatory dispersion curves were obtained on Nihon-Bunko Model ORD/UV-5 optical rotatory dispersion recorder. The nuclear magnetic resonance spectra were obtained on Hitachi Model H-60 spectrometer at 60 Mc in $CDCl_3$ employing $(CH_3)_4$-Si as an internal standard. The thin-layer chromatography (TLC) plates were prepared according to the Stahl's procedure using silica gel HF (E. Merck, Darmstadt) as adsorbent.

at room temperature for 16 hr. After removal of catalyst by filtration, the filtrate was concentrated to give the crystalline product. Recrystallization from MeOH gave II (80 mg) as colorless needles. mp 127—128.5°. $[\alpha]_D^{12}$ $-19.1°$ ($c=0.38$). *Anal.* Calcd. for $C_{24}H_{36}O_3$: C, 77.37; H, 9.74. Found: C, 77.08; H, 9.73. ORD ($c=0.19$, MeOH), $[\alpha]^{23}$: $+53°$ (350), $+289°$ (308) (peak), 0° (290), $-500°$ (260) (trough), $-473°$ (250). NMR (4% solution in $CDCl_3$) δ:0.97 (3H, singlet, 19–CH_3), 1.02 (3H, doublet, $J=6$ cps, 16-CH_3), 1.07 (3H, singlet, 18-CH_3), 2.00 (3H, singlet, 3β-$OOCCH_3$), 2.08 (3H, singlet, 21-CH_3), 2.74 (1H, singlet, 17α-H), 4.60 (1H, multiplet, 3α-H), 5.40 (1H, multiplet, 6-H).

3β-Acetoxy-16α-methyl-20-ethoxy-21-formyl-14β-pregna-5,20-diene (III)——To a solution of II (300 mg) in ethyl orthoformate (5 ml) was added a few drops of $HClO_4$ dropwise under ice–cooling over the period of 20 min. After addition of several drops of pyridine to decompose the perchlorate, the resulting solution was extracted with ether. The organic layer was washed with cold 5% HCl, 5% $NaHCO_3$ and H_2O successively, and dried over anhydrous Na_2SO_4. On usual work-up the yellow oily product was obtained. This product was not crystallized and therefore submitted to further elaboration without purification. NMR (4% solution in $CDCl_3$) δ:3.79 (2H, quartet, $J=6.5$ cps, -OCH_2Me), 4.55 (1H, multiplet, 3α-H), 5.35 (1H, multiplet, 6-H), 5.45 $\left(1H,\ \text{doublet},\ J=8\ \text{cps},\ \rangle C{=}C\langle^{CHO}_{H}\right)$, 9.75 (1H, doublet, $J=8$ cps, –CHO).

6-(3β-Acetoxy-16α-methyl-14β-androst-5-en-17β-yl)-2-pyrone (IVa)——To a solution of III (300 mg) in pyridine (5 ml) were added a trace of morpholine and malonic acid (80 mg), and the resulting solution was allowed to stand at 65—70° for 16 hr. The reaction mixture was poured into ice-cooled 10% HCl and stirred for 2 hr. The solution was extracted with ether, washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using hexane-AcOEt (4:1) as developer. The adsorbent corresponding to the zone (*Rf* value 0.46) was eluted with AcOEt and recrystallization of the eluate from MeOH using active charcoal gave IVa (40 mg) as colorless plates. mp 174—175.5°. $[\alpha]_D^{7}$ $-92.9°$ ($c=0.25$). *Anal.* Calcd. for $C_{27}H_{36}O_4$: C, 76.38; H, 8.55. Found: C, 76.52; H, 8.45.

6-(3β-Hydroxy-16α-methyl-14β-androst-5-en-17β-yl)-2-pyrone (IVb)——To a solution of IVa (50 mg) in MeOH (9.5 ml) was added 5% $KHCO_3$ (1.5 ml) and the resulting solution was allowed to stand at room temperature for 2 weeks. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using benzene-AcOEt (9:1) as developer. The adsorbent corresponding to the zone (*Rf* value 0.10) was eluted with AcOEt. Recrystallization of the eluate from MeOH gave IVb (10 mg) as colorless plates. mp 188—190°. $[\alpha]_D^{13}$ $-139.3°$ ($c=0.10$). *Anal.* Calcd. for $C_{25}H_{34}O_3$: C, 78.49; H, 8.96. Found: C, 78.26; H, 8.98.

3β-Acetoxy-16β-methylpregn-5-en-20-one (VIa)——A solution of 3β-acetoxy-16-methylpregna-5,16-dien-20-one (V)6) (350 mg) in AcOEt (15 ml) was shaken with 5% Pd/C (200 mg) under a stream of H_2 at room temperature for 16 hr. After removal of catalyst by filtration, the filtrate was concentrated to give the crystalline product. Recrystallization from MeOH gave VIa (200 mg) as colorless needles. mp 145—146°. $[\alpha]_D^{21}$ $-28.6°$ ($c=0.38$). *Anal.* Calcd. for $C_{24}H_{36}O_3$: C, 77.37; H, 9.74. Found: C, 77.57; H, 9.75. ORD ($c=0.10$, MeOH) $[\alpha]^{23}$: $-20°$ (400), 0° (350), $+20°$ (334) (peak), 0° (324), $-450°$ (275) (trough), $-300°$ (250). Romo, *et al.* prepared the same compound by the different method and reported it mp 150—153°.13)

3β-Acetoxy-16β-methyl-17α-pregn-5-en-20-one (VIIa)——A solution of VIa (500 mg) in benzene (20 ml) was refluxed for 10 hr with basic Al_2O_3 (15 g), which was previously treated with 2% methanolic KOH and activated at 160° for 2 hr. After removal of Al_2O_3 by filtration, the filtrate was concentrated. The residue obtained was reacetylated by treatment with pyridine and Ac_2O at room temperature overnight. Recrystallization from MeOH gave VIIa (450 mg) as colorless needles. mp 169—172°. $[\alpha]_D^{20}$ $-122.0°$ ($c=0.24$). *Anal.* Calcd. for $C_{24}H_{36}O_3$: C, 77.37; H, 9.74. Found: C, 77.51; H, 9.70. ORD ($c=0.15$, MeOH) $[\alpha]^{4}$: $-586°$ (350), $-1866°$ (307) (trough), 0° (282), $+933°$ (265) (peak), $+533°$ (250). Romo, *et al.* prepared the same compound by the different method and reported it mp 178—180°.13)

3β-Acetoxy-16β-methyl-20-ethoxy-21-formyl-17α-pregna-5,20-diene (VIIIa)——VIIa (300 mg) was treated with ethyl orthoformate (5 ml) and $HClO_4$ in the same manner as described in III. The crude product was submitted to further elaboration without purification.

3β-Hydroxy-16β-methyl-20-ethoxy-21-formyl-17α-pregna-5,20-diene (VIIIb)—— To a solution of VIIIa (480 mg) in MeOH (10 ml) was added 5% K_2CO_3 (2 ml) and the resulting solution was refluxed for 1 hr. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crude product was obtained. Recrystallization from AcOEt gave VIIIb (270 mg) as colorless needles. mp 199—201°. $[\alpha]_D^{15}$ $+69.8°$ ($c=0.14$). *Anal.* Calcd. for $C_{25}H_{38}O_3$: C, 77.67; H, 9.91. Found: C, 77.97; H, 9.76. UV λ_{max}^{EtOH} mμ (ε): 261 (21100). NMR (4% solution in $CDCl_3$) δ:2.87 (1H, doublet, $J=5$ cps, 17β-H), 3.50 (1H, multiplet, 3α-H), 3.78 (2H, quartet,

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13) J. Romo, J. Lepe and M. Romero, *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.*, **4**, 125 (1952) [*C.A.*, **48**, 9399*h* (1954)].

$J=6.5$ cps, -OCH$_2$Me), 5.32 (1H, multiplet, 6-H), 5.42 $\left(\text{1H, doublet, } J=8 \text{ cps, } \rangle C{=}C\langle^{CHO}_{H}\right)$, 9.75 (1H, doublet, $J=8$ cps, -CHO).

6-(3*β*-Acetoxy-16*β*-methylandrost-5-en-17*α*-yl)-2-pyrone (IXa)——i) VIIIa (300 mg) was treated with malonic acid (80 mg), pyridine (5 ml) and a trace of morpholine in the same manner as described in IVa. The crude product was submitted to the preparative TLC using benzene–AcOEt (10:1) as developer. The adsorbent corresponding to the zone (*Rf* value 0.39) was eluted with AcOEt. Recrystallization of the eluate from acetone gave IXa (10 mg) as colorless prisms. mp 257.5—259°. $[\alpha]^{13}_{D}$ +63.1° ($c=0.06$). *Anal.* Calcd. for $C_{27}H_{36}O_4$: C, 76.38; H, 8.55. Found: C, 76.58; H, 8.41.

ii) Usual acetylation of IXb with pyridine and Ac$_2$O followed by recrystallization from acetone gave IXa in quantitative yield. mp 256.5—258.5°. Mixed melting point on admixture with the sample obtained in i) showed no depression.

6-(3*β*-Hydroxy-16*β*-methylandrost-5-en-17*α*-yl)-2-pyrone (IXb)——To a solution of VIIIb (120 mg) in pyridine (5 ml) were added a trace of morpholine and malonic acid (60 mg), and the resulting solution was refluxed for 4 hr. The reaction mixture was diluted with CH_2Cl_2, washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the residue obtained was submitted to the preparative TLC using $CHCl_3$–AcOEt (10:1) as developer. The adsorbent corresponding to the zone (*Rf* value 0.20) was eluted with $CHCl_3$–AcOEt and recrystallization of the eluate from MeOH gave IXb (29 mg) as colorless prisms. mp 233—235°. $[\alpha]^{13}_{D}$ +92.7° ($c=0.44$). *Anal.* Calcd. for $C_{25}H_{34}O_3$: C, 78.49; H, 8.96. Found: C, 78.73; H, 8.99.

3*β*-Acetoxy-16*β*-methylpregnan-20-one (VIb)——A solution of V (1.2 g) in AcOH (12 ml) was shaken with PtO_2 (100 mg) under a stream of H_2 at room temperature for 6 hr. After removal of catalyst by filtration, 2% CrO_3–AcOH solution was added to the filtrate under cooling and allowed to stand at room temperature for 3 hr. To the resulting solution was added MeOH dropwise to decompose the excess CrO_3. The reaction mixture was extracted with ether, washed with 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. On usual work-up the crude product obtained was recrystallized from MeOH to give VIb (700 mg) as colorless prisms. mp 143—146.5°. Michova, *et al.* prepared the same compound by the different method and reported it mp 147—148° or 135—137° (polymorphism).[8]

3*β*-Acetoxy-16*β*-methyl-17*α*-pregnan-20-one (VIIb)——VIb (700 mg) was treated with basic Al_2O_3 (21 g) and acetylated with pyridine and Ac$_2$O in the same manner as described in VIIa. Recrystallization from MeOH gave VIIb (350 mg) as colorless needles. mp 125.5—127°. ORD ($c=0.18$, MeOH) $[\alpha]^{10}$: −366° (350), −1417° (309) (trough), 0° (288), +1500° (264) (peak), +1250° (250). Michova, *et al.* prepared the same compound by the different method and reported it mp 130—131°.[8]

3*β*-Acetoxy-16*β*-methyl-20-ethoxy-21-formyl-17*α*-pregn-20-ene (VIIIc)——VIIb (400 mg) was treated with ethyl orthoformate (5 ml) and $HClO_4$ in the same manner as described in III. The crude product was submitted to further elaboration without purification.

3*β*-Hydroxy-16*β*-methyl-20-ethoxy-21-formyl-17*α*-pregn-20-ene (VIIId)——VIIIc (350 mg) was treated with K_2CO_3 in aq. MeOH in the same manner as described in VIIIb. Recrystallization from acetone gave VIIId (270 mg) as colorless prisms. mp 198.5—200°. $[\alpha]^{14}_{D}$ +108.3° ($c=0.14$). *Anal.* Calcd. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.52; H, 10.22. UV λ^{EtOH}_{max} mμ (ε): 260 (20700). NMR (4% solution in CDCl$_3$) δ:2.87 (1H, doublet, $J=4.5$ cps, 17β–H), 3.67 (1H, multiplet, 3α–H), 3.81 (2H, quartet, $J=6.5$ cps, -OCH$_2$Me), 5.42 $\left(\text{1H, doublet, } J=8 \text{ cps, } \rangle C{=}C\langle^{CHO}_{H}\right)$, 9.80 (1H, doublet, $J=8$ cps, -CHO).

6-(3*β*-Hydroxy-16*β*-methylandrostan-17*α*-yl)-2-pyrone (IXc)——VIIId (130 mg) was treated with malonic acid (70 mg), pyridine (5 ml) and a trace of morpholine in the same manner as described in IXb. The crude product obtained was submitted to the preparative TLC using $CHCl_3$–AcOEt (10:1) as developer. The adsorbent corresponding to the zone (*Rf* value 0.20) was eluted with $CHCl_3$–AcOEt. Recrystallization of the eluate from MeOH gave IXc (41 mg) as colorless plates. mp 253—255°. $[\alpha]^{15}_{D}$ +141.7° ($c=0.20$). *Anal.* Calcd. for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 78.18; H, 9.43.

3*β*-Acetoxy-20-ethoxy-21-formylpregna-5,20-diene (XIa)——Prepared from 3*β*-acetoxypregn-5-en-20-one (Xa) according to the method of Dusza, *et al.* mp 174—177° (Reported mp 175—178°).[14]

6-(3*β*-Acetoxyandrost-5-en-17*β*-yl)-2-pyrone (XIIa)——Prepared from XIa according to the method of Knight, *et al.* mp 213—216° (Reported mp 213—216°).[11]

3*β*-Acetoxy-20-ethoxy-21-formylpregn-20-ene (XIb)——Prepared from 3*β*-acetoxypregnan-20-one (Xb) according to the method of Dusza, *et al.* mp 124—127° (Reported mp 126—127°).[14]

3*β*-Hydroxy-20-ethoxy-21-formylpregn-20-ene (XIc)——XIb (150 mg) was treated with K_2CO_3 in aq. MeOH in the same manner as described in VIIIb. Recrystallization from acetone gave XIc (130 mg) as colorless needles. mp 162—164°. $[\alpha]^{16}_{D}$ −105.3° ($c=0.08$). *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 77.18; H, 10.22. UV λ^{EtOH}_{max} mμ (ε): 261 (18700). NMR (4% solution in CDCl$_3$) δ:3.05 (1H, multip-

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14) J.P. Dusza, J.P. Joseph and S. Bernstein, *J. Am. Chem. Soc.*, **86**, 3908 (1964); *Idem*, U.S. Patent 3201425 (1965) [*C.A.*, 63, 18231*e* (1965)].

let, 17α-H), 3.67 (1H, multiplet, 3α-H), 3.79 (2H, quartet, J=6.5 cps, $-OCH_2Me$), 5.42 $\left(\text{1H, doublet, } J=8 \text{ cps, } >\mathrm{C{=}C}<^{\mathrm{CHO}}_{\mathrm{H}}\right)$, 9.80 (1H, doublet, J=8 cps, -CHO).

6-(3β-Hydroxyandrostan-17β-yl)-2-pyrone (XIIb)——XIc (130 mg) was treated with malonic acid (60 mg), pyridine (5 ml) and a trace of morpholine in the same manner as described in IXb. The crude product obtained was submitted to the preparative TLC using $CHCl_3$–AcOEt (10:1) as developer. The adsorbent corresponding to the zone (*Rf* value 0.15) was eluted with $CHCl_3$–AcOEt. Recrystallization of the eluate from MeOH gave XIIb (34 mg) as colorless needles. mp 212—214°. $[\alpha]_D^{13}$ $-17.0°$ (c=0.18). *Anal.* Calcd. for $C_{24}H_{34}O_3$: C, 77.80; H, 9.25. Found: C, 78.00; H, 9.29.

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