[Chem. Pharm. Bull. 33(3)1049—1054(1985)]

Chemical Synthesis of 11-Deoxycortisol Metabolites[1)]

HIROSHI HOSODA, HIROMITSU YOKOHAMA, HIROAKI SHIOYA and TOSHIO NAMBARA*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

(Received July 9, 1984)

The synthesis of the 3-glucuronides of 11-deoxycortisol metabolites is described. 3α,17α,20α-Trihydroxy-5β-pregnan-21-oic acid 3-glucuronide (**13**) and its 20β-epimer (**14**) were prepared starting from 5β-dihydro-11-deoxycortisol (**1**). The 20-acetoxy-21-oic acid methyl esters (**7**, **8**) were the key intermediates. Oxidation of **1** with cupric acetate followed by the intramolecular Cannizzaro reaction gave the 20-epimeric 20-hydroxy-21-acids (**5**, **6**), which, on methylation with diazomethane followed by acetylation, were converted into the acetate-methyl esters (**3**, **4**). Reduction of the carbonyl group at C-3 in **3** and **4** with sodium borohydride gave the desired intermediates. Introduction of the glucuronyl residue at the C-3 position was carried out by means of the Koenigs–Knorr reaction. 20-Epimeric 5β-pregnane-3α,17α,20,21-tetrol 3-glucuronides (**26**, **27**) were also prepared.

Keywords——11-deoxycortisol metabolite; 3α,17α,20α-trihydroxy-5β-pregnan-21-oic acid 3-glucuronide; 3α,17α,20β-trihydroxy-5β-pregnan-21-oic acid 3-glucuronide; 5β-pregnane-3α,17α,20α,21-tetrol 3-glucuronide; 5β-pregnane-3α,17α,20β,21-tetrol 3-glucuronide; Koenigs–Knorr reaction

11-Deoxycortisol is an intermediate in cortisol biosynthesis in the human adrenal cortex. The metyrapone test for evaluation of pituitary-adrenal function is based on the inhibition of conversion of 11-deoxycortisol to cortisol.[2)] 11-Deoxycortisol is metabolized by the liver to tetrahydro-11-deoxycortisol. In addition, the metabolism of this steroid, like cortisol metabolism, must include reduction of the carbonyl group at C-20 and the transformation into 17-hydroxy-20-oic acids.[3)] The possible metabolites are 5β-pregnane-3α,17α,20α,21-tetrol, 3α,17α,20α-trihydroxy-5β-pregnan-21-oic acid and their 20β-epimers, and these may be excreted in the urine as conjugates with glucuronic acid. We have previously prepared the glucuronides of tetrahydro-11-deoxycortisol[4)] and various cortisol metabolites[5)] for use in metabolic studies and immunoassays of corticosteroids. This paper deals with the synthesis of the 3-glucuronides of the tetrahydroxy and acidic metabolites of 11-deoxycortisol. The preparation of related 17-hydroxy-21-oic acid derivatives was also carried out.

First, 20-epimeric 3α,17α,20-trihydroxy-5β-pregnan-21-oic acid 3-glucuronides (**13**, **14**) were prepared starting from 5β-dihydro-11-deoxycortisol (**1**).[4)] Treatment of **1** with cupric acetate in methanol, according to the method of Lewbart and Mattox,[6)] gave the 20-oxo-21-aldehyde (**2**). The intramolecular Cannizzaro reaction of **2** with sodium hydroxide in 10% methanol yielded a mixture of the 20-epimeric 20-hydroxy-21-acids (**5**, **6**). On treatment with diazomethane followed by acetylation with acetic anhydride in pyridine, these epimers were converted into the acetate-methyl esters (**3**, **4**) (40% yield from **1**). The epimeric mixture was separated into the 20α-acetate (**3**) and the 20β-epimer (**4**) in the ratio of *ca.* 2 : 1 by centrifugal liquid chromatography on silica gel. Saponification of these compounds with potassium hydroxide afforded 17α,20α-dihydroxy-3-oxo-5β-pregnan-21-oic acid (**5**) and its 20β-epimer (**6**), respectively.

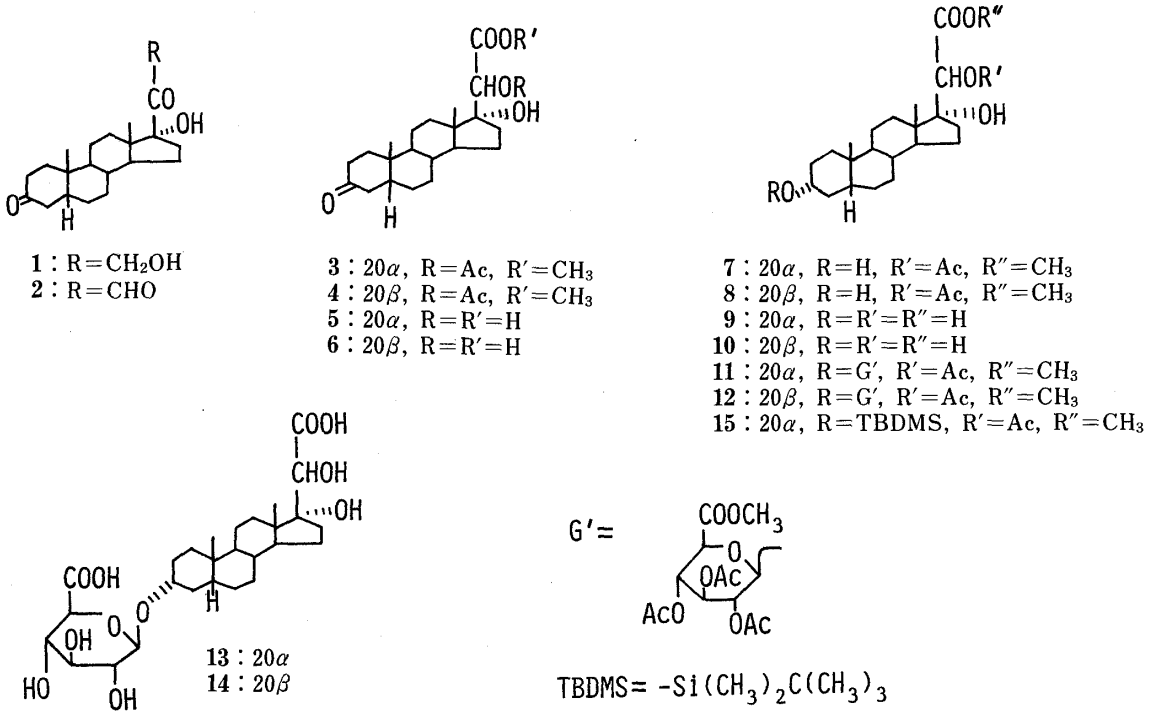
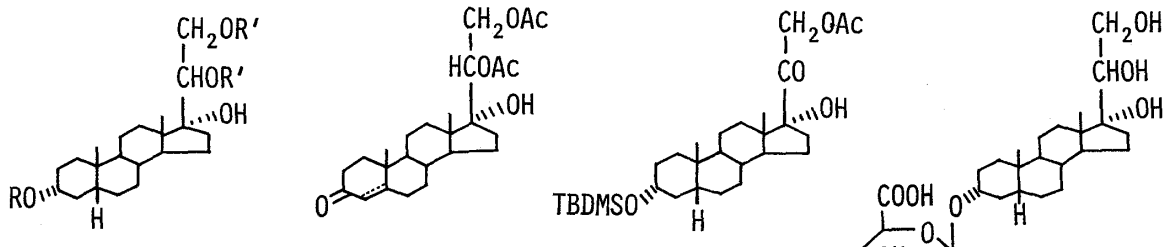


Chart 1

The stereochemistry at C-20 was determined on the basis of the proton nuclear magnetic resonance (^{1}H-NMR) spectral data. It has been reported that the C-18 proton signal of a 20α-acetate appears at lower field than that of the corresponding 20β-epimer.[3] The C-18 protons of **3** and **4** resonate at 0.92 and 0.76 ppm, respectively, showing that the configuration at C-20 in **3** is α and that in **4** is β. This was confirmed by the chemical method described below for the preparation of the 20,21-diacetate (**18**).

Reduction of **3** with sodium borohydride under mild conditions gave the desired 3α-alcohol (**7**) and its 3β-epimer, in 59 and 16% yields, respectively. In the ^{1}H-NMR spectra, the C-3 proton signal of **7** was observed at 3.60 ppm as a multiplet with the half-band width of *ca.* 20 Hz, showing the axial nature of this proton, whereas the 3β-epimer exhibited a signal of $W_{1/2}$ = *ca.* 10 Hz at 4.10 ppm. In a similar manner, the 20β-compound (**8**) was prepared from **4** by sodium borohydride reduction. Saponification of **7** and **8** with potassium hydroxide gave 20-epimeric 3α,17α,20-trihydroxy-5β-pregnan-21-oic acids (**9** and **10**, respectively). Introduction of the glucuronyl residue at the C-3 position of the 3α-hydroxy-20α-compound (**7**) was achieved by using the Koenigs–Knorr reaction with methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate in toluene in the presence of silver carbonate, giving the glucuronide acetate-methyl ester (**11**) in 29% yield. Subsequent removal of the protecting groups with methanolic potassium hydroxide provided the desired 20α-hydroxy-21-oic acid 3-glucuronide (**13**). The very polar product was isolated by the solid-phase extraction method using Amberlite XAD-2 as an adsorbent. Similarly, the 20β-hydroxy-21-oic acid 3-glucuronide (**14**) was prepared through the sequence of reactions **8**→**12**→**14**.

Next, the preparation of 20-epimeric 5β-pregnane-3α,17α,20,21-tetrol 3-glucuronides (**26**, **27**) was carried out. The 20-epimeric 20,21-diacetates (**18**, **23**) were the key intermediates. The 3α-hydroxy-20α-compound (**7**) was treated with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide–pyridine to give the 3-silyl ether (**15**). On treatment with lithium aluminum hydride in ether, **15** was transformed into the tetrol 3-silyl ether (**16**) in good yield. Acetylation of **16** with acetic anhydride in pyridine, followed by removal of the silyl group with sulfuric acid in acetone, afforded the desired 20α-intermediate (**18**). This compound was



16 : 20α, R=TBDMS, R′=H
17 : 20α, R=TBDMS, R′=Ac
18 : 20α, R=H, R′=Ac
22 : 20β, R=TBDMS, R′=Ac
23 : 20β, R=H, R′=Ac
24 : 20α, R=G′, R′=Ac
25 : 20β, R=G′, R′=Ac

19 : Δ^4
20 : 5β-H

21

26 : 20α
27 : 20β

Chart 2

also derived from the known 17α,20α,21-trihydroxy-4-pregnen-3-one 20,21-diacetate (**19**).[7] Hydrogenation of **19** with palladium-on-calcium carbonate in pyridine[8] gave the 5β-3-ketone (**20**), which, on reduction with sodium borohydride, was converted into **18**. Thus, the stereochemistry at C-20 in **3** and **4** was confirmed.

For the preparation of the 20β-compound (**23**), the route *via* direct reduction of the 20-oxo group rather than that *via* the 21-oic acid derivative (**8**) was advantageous in terms of the overall yield. Reduction of 21-acetoxy-3α,17α-dihydroxy-5β-pregnan-20-one 3-*tert*-butyldimethylsilyl ether (**21**)[4] with sodium borohydride in methanol, followed by acetylation with acetic anhydride in pyridine, gave the 20β-derivative (**22**). Subsequent removal of the silyl group at C-3 in **22** with sulfuric acid in acetone gave **23** (71% yield from **21**). The formation ratio of the 20β-alcohol to the 20α-alcohol in the sodium borohydride reduction was 91 : 9, as determined by ^{1}H-NMR spectroscopy; a similar result (89 : 11) was obtained in a reduction with lithium aluminum hydride in ether. The 3-glucuronides (**26**, **27**) were prepared by the Koenigs–Knorr reaction of **18** and **23**, followed by removal of the protecting groups with methanolic potassium hydroxide in satisfactory yields.

In the ^{1}H-NMR spectra of **11**, **12**, **24** and **25**, the anomeric proton of the sugar moiety resonates as a doublet of $J=7$ Hz in the range of 4.62—4.66 ppm, showing β-configuration of the anomeric center. In the case of the free glucuronides (**13**, **14**, **26**, **27**), the anomeric proton signal in each compound was observed at 4.43 ppm as a doublet of $J=7$ Hz.

The glucuronides and related compounds obtained here should be useful in metabolic studies and immunoassays of corticosteroids. Synthesis of 5α-corticosteroid derivatives, which also exist in human plasma and urine, is being conducted in these laboratories.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were determined in $CHCl_3$ unless otherwise specified. ^{1}H-NMR spectra were measured with a JEOL FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard.

Methyl 20α-Acetoxy-17α-hydroxy-3-oxo-5β-pregnan-21-oate (3) and the 20β-Epimer (4)——A solution of **1**[4] (2.95 g) and $Cu(OAc)_2 \cdot H_2O$ (424 mg) in MeOH (300 ml) was stirred at room temperature for 2 h while air was bubbled through it. After addition of ethylenediaminetetraacetic acid (EDTA)·2Na (800 mg) in H_2O (100 ml) followed by removal of the MeOH, the mixture was extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O, then dried over anhydrous Na_2SO_4, and evaporated down. A stirred suspension of the product (**2**) in MeOH (20 ml)–H_2O (200 ml) was treated with 1 N NaOH (20 ml) under a nitrogen atmosphere. After 10 h, the reaction mixture was extracted with ether. The aqueous layer was acidified with conc. HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. A solution of the acidic product in MeOH (3 ml) was treated with diazomethane. The crude product obtained was chromatographed

on silica gel (60 g) with hexane–AcOEt (3 : 2) as an eluent, yielding a mixture of methyl 17α,20α-dihydroxy-3-oxo-5β-pregnan-21-oate and its 20β-epimer. This was treated with acetic anhydride (2.5 ml) in pyridine (5 ml) overnight at room temperature. After addition of H_2O, the mixture was extracted with AcOEt. The organic layer was washed with 5% HCl, 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Purification of the crude product by centrifugal liquid chromatography on silica gel with benzene–AcOEt (5 : 2) as an eluent gave the 20α-acetate (**3**) (930 mg) and 20β-acetate (**4**) (480 mg).

3: Colorless semi-crystals. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.15 (3H, s, –$OCOCH_3$), 3.76 (3H, s, –$COOCH_3$), 5.07 (1H, s, 20β-H).

4: Colorless leaflets from MeOH. mp 153—154 °C. $[\alpha]_D^{15}$ +14° (*c*=1.0). *Anal.* Calcd for $C_{24}H_{36}O_6 \cdot 3/4H_2O$: C, 66.41; H, 8.71. Found: C, 66.58; H, 8.99. ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 2.14 (3H, s, –$OCOCH_3$), 3.76 (3H, s, –$COOCH_3$), 5.12 (1H, s, 20α-H).

17α,20α-Dihydroxy-3-oxo-5β-pregnan-21-oic Acid (5)——A solution of **3** (150 mg) and 10% KOH (0.5 ml) in MeOH (2 ml) was stirred overnight at room temperature. After addition of H_2O, the resulting solution was acidified with conc. HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from benzene–CH_2Cl_2 gave **5** (90 mg) as colorless leaflets. mp 107—111 °C. $[\alpha]_D^{18}$ +6° (*c*=0.3). *Anal.* Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.06; H, 9.11. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 4.20 (1H, s, 20β-H).

17α,20β-Dihydroxy-3-oxo-5β-pregnan-21-oic Acid (6)——Saponification of **4** (155 mg) with KOH was carried out in the manner described for **5**. The crude product obtained was recrystallized from benzene–CH_2Cl_2 to give **6** (100 mg) as colorless leaflets. mp 115—120 °C. $[\alpha]_D^{18}$ −4° (*c*=0.2). *Anal.* Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 68.96; H, 9.00. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 4.37 (1H, s, 20α-H).

Methyl 20α-Acetoxy-3α,17α-dihydroxy-5β-pregnan-21-oate (7)——A solution of **3** (365 mg) and $NaBH_4$ (40 mg) in MeOH (3 ml) was stirred at 0 °C for 20 min. After addition of AcOH to decompose the excess reagent, the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue was subjected to column chromatography on silica gel (25 g) with benzene–AcOEt (1 : 1) as an eluent, yielding **7** (215 mg) and its 3β-epimer (60 mg).

7: Colorless prisms from hexane–AcOEt. mp 192—193 °C. $[\alpha]_D^{13}$ +18° (*c*=1.0). *Anal.* Calcd for $C_{24}H_{38}O_6$: C, 68.22; H, 9.06. Found: C, 68.01; H, 9.12. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 2.14 (3H, s, –$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.76 (3H, s, –$COOCH_3$), 5.05 (1H, s, 20β-H).

The 3β-Epimer: ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 2.14 (3H, s, –$OCOCH_3$), 3.74 (3H, s, –$COOCH_3$), 4.10 (1H, m, 3α-H), 5.06 (1H, s, 20β-H).

Methyl 20β-Acetoxy-3α,17α-dihydroxy-5β-pregnan-21-oate (8)——Reduction of **4** (270 mg) with $NaBH_4$ in MeOH and purification by chromatography were carried out in the manner described for **7**, yielding **8** (160 mg) and its 3β-epimer (55 mg).

8: Colorless needles from hexane–AcOEt. mp 174—176 °C. $[\alpha]_D^{13}$ +13° (*c*=1.0). *Anal.* Calcd for $C_{24}H_{38}O_6 \cdot 1/2H_2O$: C, 66.79; H, 9.11. Found: C, 67.06; H, 8.96. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 2.14 (3H, s, –$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.76 (3H, s, –$COOCH_3$), 5.10 (1H, s, 20α-H).

The 3β-Epimer: ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 2.15 (3H, s, –$OCOCH_3$), 3.75 (3H, s, –$COOCH_3$), 4.10 (1H, m, 3α-H), 5.09 (1H, s, 20α-H).

3α,17α,20α-Trihydroxy-5β-pregnan-21-oic Acid (9)——Saponification of **7** (185 mg) with KOH was carried out in the manner described for **5**. The crude product obtained was recrystallized from acetone–AcOEt to give **9** (95 mg) as colorless needles. mp 176—178 °C. $[\alpha]_D^{15}$ +6° (*c*=0.5, MeOH). *Anal.* Calcd for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.48; H, 9.57. ^{1}H-NMR (CD_3OD) δ: 0.86 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.50 (1H, m, 3β-H), 4.09 (1H, s, 20β-H).

3α,17α,20β-Trihydroxy-5β-pregnan-21-oic Acid (10)——Saponification of **8** (120 mg) with KOH was carried out in the manner described for **5**. The crude product obtained was recrystallized from aqueous MeOH to give **10** (40 mg) as colorless leaflets. mp 213—216 °C. $[\alpha]_D^{11}$ +4° (*c*=0.4, MeOH). *Anal.* Calcd for $C_{21}H_{34}O_5 \cdot 1/4H_2O$: C, 67.98; H, 9.37. Found: C, 68.24; H, 9.60. ^{1}H-NMR (CD_3OD) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.50 (1H, m, 3β-H), 4.27 (1H, s, 20α-H).

Methyl 20α-Acetoxy-3α,17α-dihydroxy-5β-pregnan-21-oate 3-(2′,3′,4′-Tri-*O*-acetyl-β-D-glucopyranosid)uronic Acid Methyl Ester (11)——Freshly prepared Ag_2CO_3 (690 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (1 g) were added to a solution of **7** (210 mg) in toluene (12 ml), and the suspension was stirred at room temperature for 20 h. After addition of AcOEt, the resulting solution was passed through Florisil (5 g) on a sintered-glass funnel, and the filtrate was evaporated down. The crude product obtained was chromatographed on silica gel (20 g) with benzene–AcOEt (2 : 1) as an eluent, yielding **11** as semi-crystals (105 mg). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 2.00, 2.03 and 2.13 (12H, –$OCOCH_3$), 3.6 (1H, m, 3β-H), 3.74 (6H, s, –$COOCH_3$), 4.00 (1H, m, 5′-H), 4.62 (1H, d, *J*=7 Hz, 1′-H), 4.8—5.4 (3H, 2′-, 3′-, 4′-H), 5.05 (1H, s, 20β-H).

Methyl 20β-Acetoxy-3α,17α-dihydroxy-5β-pregnan-21-oate 3-(2′,3′,4′-Tri-*O*-acetyl-β-D-glucopyranosid)uronic Acid Methyl Ester (12)——The Koenigs–Knorr reaction of **8** (70 mg) was carried out in the manner described for **11**. Purification of the crude product obtained by column chromatography on silica gel with benzene–AcOEt (2 : 1) as an

eluent, followed by recrystallization from acetone–hexane, gave **12** (52 mg) as colorless prisms. mp 244—246 °C. $[\alpha]_D^{12}$ −9° (c=0.3). *Anal.* Calcd for $C_{37}H_{54}O_{15}$: C, 60.15; H, 7.37. Found: C, 59.89; H, 7.50. ^{1}H-NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 2.01, 2.04 and 2.14 (12H, –$OCOCH_3$), 3.6 (1H, m, 3β-H), 3.74 (6H, s, –$COOCH_3$), 4.00 (1H, m, 5′-H), 4.64 (1H, d, J=7 Hz, 1′-H), 4.8—5.3 (3H, 2′-, 3′-, 4′-H), 5.12 (1H, s, 20α-H).

3α,17α,20α-Trihydroxy-5β-pregnan-21-oic Acid 3-Glucuronide (13)——Saponification of **11** (105 mg) with KOH was carried out in the manner described for **5**. The reaction mixture was neutralized with AcOH. After removal of the MeOH followed by addition of H_2O, the mixture was subjected to column chromatography on Amberlite XAD-2. Elution with MeOH and removal of the solvent gave **13** (60 mg) as colorless hygroscopic crystals. ^{1}H-NMR (CD_3OD) δ: 0.85 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 4.08 (1H, s, 20β-H), 4.43 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250 °C. $[\alpha]_D^{16}$ −15° (c=0.3, AcOH–MeOH (1 : 3)). *Anal.* Calcd for $C_{27}H_{40}BaO_{11}\cdot 2H_2O$: C, 45.41; H, 6.21. Found: C, 45.08; H, 6.42.

3α,17α,20β-Trihydroxy-5β-pregnan-21-oic Acid 3-Glucuronide (14)——Saponification of **12** (40 mg) with KOH and Amberlite XAD-2 chromatography were carried out in the manner described above, yielding **14** (25 mg) as colorless hygroscopic crystals. ^{1}H-NMR (CD_3OD) δ: 0.86 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 4.28 (1H, s, 20α-H), 4.43 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250 °C. $[\alpha]_D^{16}$ −20° (c=0.4, AcOH–MeOH (1 : 3)). *Anal.* Calcd for $C_{27}H_{40}BaO_{11}\cdot 2H_2O$: C, 45.41; H, 6.21. Found: C, 45.72; H, 6.49.

Methyl 20α-Acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-5β-pregnan-21-oate (15)——A solution of **7** (60 mg), imidazole (140 mg), and *tert*-butyldimethylsilyl chloride (100 mg) in pyridine (0.2 ml)–dimethylformamide (0.4 ml) was stirred at room temperature for 1 h. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from aqueous MeOH gave **15** (72 mg) as colorless needles. mp 147—148 °C. $[\alpha]_D^{20}$ +34° (c=0.5). *Anal.* Calcd for $C_{30}H_{52}O_6Si$: C, 67.12; H, 9.76. Found: C, 66.82; H, 9.81. ^{1}H-NMR ($CDCl_3$) δ: 0.04 (6H, s, 3-OSi(CH_3)$_2$), 0.88 (15H, s, 18-CH_3, 19-CH_3 and 3-OSi-*tert*-Bu), 2.14 (3H, s, –$OCOCH_3$), 3.56 (1H, m, 3β-H), 3.74 (3H, s, –$COOCH_3$), 5.06 (1H, s, 20β-H).

5β-Pregnane-3α,17α,20α,21-tetrol 3-*tert*-Butyldimethylsilyl Ether (16)——A mixture of **15** (60 mg) and $LiAlH_4$ (50 mg) in dry ether (2 ml) was stirred at room temperature for 1 h. After careful addition of H_2O to decompose the excess reagent, the mixture was extracted with AcOEt. The organic layer was washed with 10% Rochelle salt and H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from acetone gave **16** (35 mg) as colorless needles. mp 208—209 °C. $[\alpha]_D^{20}$ +16° (c=0.3). *Anal.* Calcd for $C_{27}H_{50}O_4Si$: C, 69.48; H, 10.80. Found: C, 69.35; H, 11.01. ^{1}H-NMR ($CDCl_3$) δ: 0.05 (6H, s, 3-OSi(CH_3)$_2$), 0.73 (3H, s, 18-CH_3), 0.88 (9H, s, 3-OSi-*tert*-Bu), 0.89 (3H, s, 19-CH_3), 3.4—3.9 (4H, 3β-, 20β-, 21-H).

5β-Pregnane-3α,17α,20α,21-tetrol 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (17)——A solution of **16** (100 mg) and acetic anhydride (0.5 ml) in pyridine (1 ml) was allowed to stand overnight at room temperature. After addition of H_2O, the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from MeOH gave **17** (110 mg) as colorless needles. mp 195—197 °C. $[\alpha]_D^{20}$ −14° (c=0.4). *Anal.* Calcd for $C_{31}H_{54}O_6Si$: C, 67.59; H, 9.88. Found: C, 67.36; H, 9.96. ^{1}H-NMR ($CDCl_3$) δ: 0.05 (6H, s, 3-OSi(CH_3)$_2$), 0.84 (3H, s, 18-CH_3), 0.88 (12H, s, 19-CH_3 and 3-OSi-*tert*-Bu), 2.02 and 2.11 (each 3H, s, –$OCOCH_3$), 3.58 (1H, m, 3β-H), 4.03 (1H, dd, J=9 and 12 Hz, one of 21-H), 4.52 (1H, dd, J=3 and 12 Hz, one of 21-H), 5.30 (1H, dd, J=3 and 9 Hz, 20β-H).

5β-Pregnane-3α,17α,20α,21-tetrol 20,21-Diacetate (18)——i) A solution of **17** (80 mg) and 30% H_2SO_4 (0.1 ml) in acetone (4 ml) was stirred at room temperature for 20 min. The reaction mixture was neutralized with 5% $NaHCO_3$. Upon addition of H_2O a precipitate was formed; this was collected by filtration and dried. Recrystallization of the crude product from MeOH gave **18** (50 mg) as colorless needles. mp 227—229 °C. $[\alpha]_D^{20}$ −32° (c=0.3). *Anal.* Calcd for $C_{25}H_{40}O_6$: C, 68.77; H, 9.24. Found: C, 68.64; H, 9.24. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 2.01 and 2.10 (each 3H, s, –$OCOCH_3$), 3.63 (1H, m, 3β-H), 4.08 (1H, dd, J=9 and 12 Hz, one of 21-H), 4.52 (1H, dd, J=3 and 12 Hz, one of 21-H), 5.32 (1H, dd, J=3 and 9 Hz, 20β-H).

ii) A solution of **20** (90 mg) and $NaBH_4$ (30 mg) in 90% tetrahydrofuran (2 ml) was stirred at 0 °C for 2.5 h. After usual work-up, the crude product obtained was chromatographed on silica gel (8 g) with benzene–ether (1 : 5) as an eluent, yielding **18** (60 mg). The infrared spectra of the two samples obtained in i) and ii) were identical.

17α,20α,21-Trihydroxy-5β-pregnan-3-one 20,21-Diacetate (20)——A solution of 17α,20α,21-trihydroxy-4-pregnen-3-one 20,21-diacetate (**19**) (120 mg), prepared according to the method of Gardi *et al.*,[7] in pyridine (5 ml) was stirred under a hydrogen gas stream for 12 h at atmospheric pressure in the presence of palladium-on-calcium carbonate (600 mg). After addition of AcOEt followed by removal of the catalyst by filtration, the filtrate was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Purification of the crude product by column chromatography on silica gel with benzene–ether (1 : 1) as an eluent, followed by recrystallization from acetone–hexane, gave **20** (100 mg) as colorless needles. mp 208—209 °C. $[\alpha]_D^{17}$ −27° (c=0.3). *Anal.* Calcd for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 68.83; H, 8.85. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.03 and 2.12 (each 3H, s, –$OCOCH_3$), 4.04 (1H, dd, J=9 and 12 Hz, one of 21-H), 4.55 (1H, dd, J=3 and 12 Hz, one of 21-H), 5.34 (1H, dd, J=3 and 9 Hz, 20β-H).

5β-Pregnane-3α,17α,20β,21-tetrol 20,21-Diacetate (23)——Sodium borohydride reduction of 21-acetoxy-3α,17α-dihydroxy-5β-pregnan-20-one 3-*tert*-butyldimethylsilyl ether (**21**)[4] (245 mg) was carried out in the manner

described for **7**. The product obtained was treated with acetic anhydride (1 ml) in pyridine (2 ml) to give **22**. Desilylation of **22** with H_2SO_4 was carried out in the manner described for **18**. Purification of the product by column chromatography on silica gel with hexane–AcOEt (1 : 3) as an eluent, followed by recrystallization from acetone–hexane, gave **23** (150 mg) as colorless prisms. mp 163—164 °C. $[\alpha]_D^{18}$ +58° (c=0.5). *Anal.* Calcd for $C_{25}H_{40}O_6$: C, 68.77; H, 9.24. Found: C, 68.52; H, 9.23. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 2.02 and 2.08 (each 3H, s, –$OCOCH_3$), 3.60 (1H, m, 3β-H), 4.16 (1H, dd, J=8 and 12 Hz, one of 21-H), 4.46 (1H, dd, J=3 and 12 Hz, one of 21-H), 5.34 (1H, dd, J=3 and 8 Hz, 20α-H).

Methyl (20α,21-Diacetoxy-17α-hydroxy-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (24)——The Koenigs–Knorr reaction of **18** (420 mg) was carried out in the manner described for **11**. Purification of the product by column chromatography on silica gel with hexane–AcOEt (1 : 3) as an eluent, followed by recrystallization from ether, gave **24** (400 mg) as colorless leaflets. mp 201—202 °C. $[\alpha]_D^{24}$ −35° (c=0.4). *Anal.* Calcd for $C_{38}H_{56}O_{15}\cdot 1/2H_2O$: C, 59.91; H, 7.54. Found: C, 59.75; H, 7.39. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 2.02, 2.07 and 2.12 (15H, –$OCOCH_3$), 3.6 (1H, m, 3β-H), 3.76 (3H, s, –$COOCH_3$), 3.9—4.6 (3H, 21- and 5′-H), 4.66 (1H, d, J=7 Hz, 1′-H), 4.8—5.5 (4H, 20β-, 2′-, 3′-, 4′-H).

Methyl (20β,21-Diacetoxy-17α-hydroxy-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (25)——The Koenigs–Knorr reaction of **23** (100 mg) was carried out in the manner described for **11**. The crude product obtained was chromatographed on silica gel (20 g) with benzene–AcOEt (1 : 1) as an eluent, yielding a mixture of **25** and a sugar derivative. Separation of these products was achieved after acetylation of the latter compound. Purification by chromatography on silica gel with benzene–ether (1 : 1) as an eluent, followed by recrystallization from ether–hexane, gave **25** (75 mg) as colorless prisms. mp 171—172 °C. $[\alpha]_D^{18}$ +22° (c=0.5). *Anal.* Calcd for $C_{38}H_{56}O_{15}\cdot 1/2H_2O$: C, 59.90; H, 7.54. Found: C, 59.75; H, 7.21. ^{1}H-NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.91 (3H, s, 19-CH_3), 2.02, 2.05 and 2.08 (15H, –$OCOCH_3$), 3.6 (1H, m, 3β-H), 3.74 (3H, s, –$COOCH_3$), 3.9—4.6 (3H, 21- and 5′-H), 4.64 (1H, d, J=7 Hz, 1′-H), 4.8—5.5 (4H, 20α-, 2′-, 3′-, 4′-H).

5β-Pregnane-3α,17α,20α,21-tetrol 3-Glucuronide (26)——Saponification of **24** (530 mg) with KOH and Amberlite XAD-2 chromatography were carried out in the manner described for **13**. The product obtained was purified by column chromatography on silica gel (30 g) with $CHCl_3$–MeOH–H_2O–AcOH (50 : 50 : 2 : 0.1) as an eluent, and then on Amberlite XAD-2 to give **26** (220 mg), which was recrystallized from MeOH–AcOEt. mp 190 °C (dec.). $[\alpha]_D^{20}$ −19° (c=0.4, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.76 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 4.43 (1H, d, J=7 Hz, 1′-H). The barium salt: *Anal.* Calcd for $C_{27}H_{43}Ba_{1/2}O_{10}$: C, 54.38; H, 7.27. Found: C, 54.72; H, 7.45.

5β-Pregnane-3α,17α,20β,21-tetrol 3-Glucuronide (27)——Saponification of **25** (400 mg) with KOH and purification of the product by chromatography were carried out in the manner described for **26**. Recrystallization of the product obtained from MeOH–AcOEt gave **27** (170 mg) as a colorless amorphous substance. mp 182—184 °C. $[\alpha]_D^{19}$ −7° (c=0.5, MeOH). *Anal.* Calcd for $C_{27}H_{44}O_{10}\cdot 5/4H_2O$: C, 58.84; H, 8.50. Found: C, 58.77; H, 8.30. ^{1}H-NMR (CD_3OD) δ: 0.82 (3H, s, 18-CH_3), 0.94 (3H, s, 19-CH_3), 4.43 (1H, d, J=7 Hz, 1′-H).

Acknowledgement The authors are indebted to the staff of the central analytical laboratory of this Institute for elemental analyses and spectral measurements.

References and Notes

1) Part CCIX of "Studies on Steroids" by T. Nambara; Part CCVIII: K. Shimada, K. Ohishi and T. Nambara, *J. Natur. Prod.*, in press.
2) G. W. Liddle, H. L. Estep, J. W. Kendall, Jr., W. C. Williams, Jr., and A. W. Townes, *J. Clin. Endocrinol. Metab.*, **19**, 875 (1959).
3) H. L. Bradlow, B. Zumoff, C. Monder, H. J. Lee, and L. Hellman, *J. Clin. Endocrinol. Metab.*, **37**, 811 (1973).
4) H. Hosoda, H. Yokohama, K. Ishii, Y. Ito, and T. Nambara, *Chem. Pharm. Bull.*, **31**, 4001 (1983).
5) H. Hosoda, K. Saito, Y. Ito, H. Yokohama, K. Ishii, and T. Nambara, *Chem. Pharm. Bull.*, **30**, 2110 (1982); H. Hosoda, H. Yokohama, and T. Nambara, *ibid.*, **32**, 1359 (1984).
6) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 1773, 1779 (1963).
7) R. Gardi, R. Vitali, A. Ercoli, and W. Klyne, *Tetrahedron*, **21**, 179 (1965).
8) M. G. Combe, H. B. Henbest, and W. R. Jackson, *J. Chem. Soc.*, (*C*), **1967**, 2467.