Chemical Conversion of Corticosteroids to 3 α ,5 α -Tetrahydro Derivatives. Synthesis of 5 α -Cortol 3-Glucuronides and 5 α -Cortolone 3-Glucuronides

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The synthesis of the 3-glucuronides of 5 α -cortol-20 α , 5 α -cortolone-20 α and their 20 β -epimers is described. The 5 α -cortol 20,21-diacetates (12, 17) and 5 α -cortolone 20,21-diacetates (14, 19) were the key intermediates. Sodium borohydride reduction of the carbonyl group at C-20 in 5 α -tetrahydrocortisol 3-*tert*-butyldimethylsilyl ether 17,21-acetonide (8) gave the 20 α -hydroxy-acetonide (9). Selective removal of the acetonide ring was successful when the 20 α -acetoxy-17 α ,21-acetonide (10) was treated with 50% acetic acid. Subsequent acetylation with acetic anhydride in pyridine, followed by removal of the protecting group at C-3 in the silyl ether-acetate (11) gave the desired 20 α -intermediate (12). The 11-ketone (14) was prepared from 11 by oxidation with pyridinium chlorochromate, followed by desilylation. The 20 β -acetates (17, 19) were synthesized from 21-acetoxy-3 α ,11 β ,17 α -trihydroxy-5 α -pregnan-20-one 3-*tert*-butyldimethylsilyl ether (15). Introduction of the glucuronyl residue at C-3 was carried out by means of the Koenigs–Knorr reaction.

Keywords cortisol metabolite; 5 α -cortol; 5 α -cortolone; 5 α -cortol-20 α 3-glucuronide; 5 α -cortol-20 β 3-glucuronide; 5 α -cortolone-20 α 3-glucuronide; 5 α -cortolone-20 β 3-glucuronide; Koenigs–Knorr reaction

Cortisol metabolism in humans involves various transformations, for example, reduction of the Δ^4 -3-keto group to form 3 α -hydroxy products, oxidation at C-11, and reduction at C-20. Most of the metabolites, such as tetrahydrocortisol, tetrahydrocortisone and their 5 α -isomers, are excreted in the urine largely as conjugates with glucuronic acid. Measurement of the urinary 17-hydroxy-corticosteroids has long been an important index of adrenocortical activity. We have previously prepared the glucuronides of these metabolites for use in metabolic studies and immunoassays of corticosteroids.¹⁾ Mattox *et al.* have also synthesized the 5 β -steroid glucuronides.²⁾

The existence of the 3-monoglucuronides of 5 α -cortol-20 α ,³⁾ 5 α -cortolone-20 α and their 20 β -isomers, in addition to the corresponding 5 β -metabolites, in the urine⁴⁾ and plasma⁵⁾ has also been reported. The latter four 5 β -compounds have been synthesized earlier.⁶⁾ The recent development of high-performance liquid chromatography/mass spectrometry may enable us to characterize individually these polar metabolites or to perform a steroid profile analysis without the enzymatic or acid hydrolysis of the conjugates. For this purpose, it is essential to have authentic steroid samples; this prompted us to prepare possibly rather minor metabolites of cortisol in normal subjects for use as standards in analytical studies. This paper deals with the synthesis of 5 α -cortol-20 α 3-glucuronide (24), 5 α -cortolone-20 α 3-glucuronide (25) and their 20 β -isomers (26, 27).

In the present work, the 20,21-diacetates of 5 α -cortol and 5 α -cortolone were the key intermediates for introduction of the glucuronyl residue at C-3 and cortisol (1) was selected as a starting material. Transformation of 1 into the target compounds involves the stereoselective reduction of the Δ^4 -3-keto group and the carbonyl group at C-20. We previously developed a route *via* a 3,5-diene derivative, which yielded the desired 5 α -corticosteroid with a satisfactory stereoselectivity.⁷⁾ Concerning the carbonyl group at C-20, it has been shown that reduction of 17 α ,21-dihydroxy-20-ketones with sodium borohydride yields predominantly 20 β -alcohols.⁸⁾ On the other hand, when 17 α ,21-cyclic derivatives of corticosteroids are reduced with metal hydrides the 20 α -ol is practically the sole

product.⁹⁾ In previous work,^{1b)} the chemical conversion of 1 to various 5 α -tetrahydrocortisol derivatives was undertaken, and some of these were used as starting materials.

First, 5 α -cortol-20 α 20,21-diacetate (12) and 5 α -cortolone-20 α 20,21-diacetate (14) were prepared. The route *via* 3,11 β ,17 α ,21-tetrahydroxy-3,5-pregnadien-20-one 3-methyl ether 17,21-acetonide (2) seemed attractive for this preparation in view of the combination of the 3,5-diene and cyclic side-chain systems. Therefore, this route was examined as follows: hydrogenation of the γ,δ -double bond in 2 over palladium, followed by reduction of the product (3) with sodium borohydride was carried out to give the 20 α -hydroxy compound (4). Subsequent acid hydrolysis, followed by acetylation with acetic anhydride in pyridine, gave the 20 α ,21-diacetoxy-3-ketone (5), which in turn was treated with potassium tri-*sec*-butylborohydride (K-Selectride) to give the desired 12. Preliminary experiments showed that this route was not necessarily practical owing to the instability of the intermediate 3 and to the difficulty in chromatographic purification of the product in each step. An alternative route was therefore explored. Silylation of 5 α -tetrahydrocortisol 17,21-acetonide (7) with *tert*-butyldimethylsilyl chloride and imidazole in pyridine–dimethylformamide afforded the 3-silyl ether-17 α ,21-acetonide (8). On reduction with sodium borohydride in methanol, 8 was converted to the 20 α -hydroxy-17 α ,21-acetonide (9). Selective removal of the acetonide ring in the presence of the silyl group at C-3 in 9 was not successful under the acidic conditions tested. However, when the 20 α -acetoxy-17 α ,21-acetonide (10) was treated with 50% acetic acid under mild conditions, selective cleavage of the side-chain ring took place, giving a 20 α -hydroxy-3-silyl ether¹⁰⁾ in good yield. Subsequent acetylation with acetic anhydride in pyridine, followed by removal of the protecting group at C-3 with 1% hydrofluoric acid in acetonitrile gave the desired 12 (58% yield from 7). Meanwhile, 11 was oxidized with pyridinium chlorochromate in methylene chloride to give the 11-ketone (13). Desilylation of 13 yielded the desired 14.

5 α -Cortol-20 β 20,21-diacetate (17) and 5 α -cortolone-20 β 20,21-diacetate (19) were then synthesized. Treatment of

Chart 1

Chart 2

diacetate (16). The formation of the 20α -isomer was less than 10%, as judged from the proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectrum. Desilylation of 16 with hydrofluoric acid in acetonitrile gave 17 in good yield. Compound 16 was oxidized with pyridinium chlorochromate in methylene chloride to give the 11-ketone (18), which, on removal of the protecting group at C-3 with hydrofluoric acid yielded the desired compound (19).

The stereochemistry at C-20 was confirmed on the basis of $^1\text{H-NMR}$ 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.^{6b,11} The C-18 protons of 12, 17, 14 and 19 resonate at δ 1.08, 0.95, 0.80 and 0.67, respectively, showing that the configuration at C-20 in 12 or 14 is α and in 17 or 19 is β .

Finally, the preparations of 5 α -cortol-20 α 3-glucuronide (24), 5 α -cortolone-20 α 3-glucuronide (25), 5 α -cortol-20 β 3-glucuronide (26), and 5 α -cortolone-20 β 3-glucuronide (27) were carried out. Introduction of the glucuronyl residue into 12, 14, 17 and 19 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, yielding the corresponding glucuronide acetate-methyl esters (20, 21, 22, 23). Subsequent removal of the protecting groups with methanolic potassium hydroxide provided the desired compounds (24–27). The very polar glucuronides were isolated by the solid-phase extraction method using Amberlite XAD-2 as an adsorbent.

In the $^1\text{H-NMR}$ spectra of 20, 21, 22 and 23, the anomeric proton of the sugar moiety resonates in the range of δ 4.54–4.56 as a doublet of $J = 7$ Hz, showing β -configuration of the anomeric center. In the case of the free glucuronides (24–27), the water-eliminated Fourier transform method

21-acetoxy-3 α ,11 β ,17 α -trihydroxy-5 α -pregnan-20-one 3-*tert*-butyldimethylsilyl ether (15) with sodium borohydride in methanol, followed by acetylation with acetic anhydride in pyridine gave the 5 α -cortol-20 β 3-silyl ether-20,21-

was employed on measurement of their $^1\text{H-NMR}$ spectra. The anomeric proton signal in each compound was observed at δ 4.33–4.35 as a doublet of $J=7$ Hz.

We have previously prepared the glucuronides of related cortisol^{1,6b} and 11-deoxycortisol^{7,12} metabolites. The glucuronides obtained here should also be useful as standard samples in the direct analysis of plasma and urinary corticosteroids by immunoassay or chromatography.

Experimental

All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 in CHCl_3 unless otherwise specified. $^1\text{H-NMR}$ spectra were taken with a JEOL JNM-FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard.

5 α -Tetrahydrocortisol 3-*tert*-Butyldimethylsilyl Ether 17,21-Acetonide (8) A solution of 5 α -tetrahydrocortisol (6) (1.13 g, 3.1 mmol), prepared from its 21-tetrahydropyranyl ether^{1b} by acid hydrolysis, 2,2-dimethoxypropane (1.7 ml) and *p*-toluenesulfonic acid (50 mg) in dimethylformamide (6 ml) was stirred at 80 °C for 4 h. The resulting solution was diluted with AcOEt, washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. The crude product obtained was chromatographed on silica gel (30 g) with hexane–AcOEt (2:1) as an eluent, yielding the acetonide (7) as semi-crystals. A solution of 7 (670 mg, 1.6 mmol), *tert*-butyldimethylsilyl chloride (615 mg, 4.1 mmol) and imidazole (560 mg, 8.2 mmol) in pyridine (1.3 ml)–dimethylformamide (2.6 ml) was allowed to stand at room temperature for 6 h. The resulting solution was diluted with AcOEt, washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. The residue obtained was chromatographed on silica gel (30 g). Elution with hexane–AcOEt (10:1) and recrystallization of the product from CHCl_3 gave 8 (870 mg, 54% from 6) as colorless needles. mp 156–158 °C. $[\alpha]_D^{25} + 40^\circ$ ($c=1.0$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.82 (3H, s, 18-CH₃), 0.88 (9H, 3-OSi-*tert*-Bu), 0.99 (3H, s, 19-CH₃), 1.38, 1.48 (each 3H, s, isopropyl CH₃), 3.9–4.2 (3H, 3 β -, 21-H), 4.40 (1H, m, 11 α -H). *Anal.* Calcd for C₃₃H₅₂O₅Si: C, 69.18; H, 10.06. Found: C, 69.00; H, 10.10.

20 α -Acetoxy-5 α -pregnane-3 α ,11 β ,17 α ,21-tetrol 3-*tert*-Butyldimethylsilyl Ether 17,21-Acetonide (10) A solution of 8 (870 mg, 1.7 mmol) and NaBH_4 (150 mg, 4.0 mmol) in MeOH (10 ml) was stirred at room temperature for 1 h. After careful addition of AcOH, the mixture was extracted with AcOEt. The organic layer was washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. A solution of the product (9) (840 mg, 1.6 mmol) and acetic anhydride (4 ml) in pyridine (8 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. The residue obtained was chromatographed on silica gel (30 g). Elution with hexane–AcOEt (4:1) as an eluent gave 10 (770 mg, 70% from 8) as colorless semi-crystals. $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.84 (3H, s, 18-CH₃), 0.89 (9H, 3-OSi-*tert*-Bu), 0.99 (3H, s, 19-CH₃), 1.33, 1.50 (each 3H, s, isopropyl CH₃), 2.04 (3H, s, 20-OCOCH₃), 3.54 (1H, dd, $J=6$, 12 Hz, one of 21-H), 3.8–4.0 (2H, 3 β -H, one of 21-H), 4.40 (1H, m, 11 α -H), 4.95 (1H, t, $J=6$ Hz, 20 β -H).

5 α -Cortol-20 α 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (11) A solution of 10 (770 mg, 1.4 mmol) in 50% acetic acid (6 ml) was allowed to stand at room temperature for 1 h. The resulting solution was diluted with AcOEt, washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. The product obtained was treated with acetic anhydride (2 ml) in pyridine (4 ml). Purification by chromatography on silica gel (30 g) using hexane–AcOEt (3:1) and recrystallization of the product from MeOH gave 11 (660 mg, 85% from 10) as colorless leaflets. mp 179–181 °C. $[\alpha]_D^{25} - 5^\circ$ ($c=1.1$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.88 (9H, s, 3-OSi-*tert*-Bu), 1.00 (3H, s, 19-CH₃), 1.08 (3H, s, 18-CH₃), 2.03, 2.11 (each 3H, s, -OCOCH₃), 3.9–4.7 (4H, 3 β -, 11 α -, 21-H), 5.34 (1H, dd, $J=3$, 9 Hz, 20 β -H). *Anal.* Calcd for C₃₁H₅₄O₇Si: C, 65.69; H, 9.60. Found: C, 65.60; H, 9.63.

5 α -Cortol-20 α 20,21-Diacetate (12) i) A solution of 11 (260 mg, 0.46 mmol) and 47% HF (0.13 ml) in acetonitrile (6 ml) was allowed to stand at room temperature for 2 h. The resulting solution was diluted with AcOEt, washed with 5% NaHCO_3 and H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. Recrystallization of the crude product from MeOH gave 12 (205 mg, 99%) as colorless prisms. mp 242–245 °C. $[\alpha]_D^{25} - 18^\circ$ ($c=1.0$). $^1\text{H-NMR}$ (CDCl_3) δ : 1.02 (3H, s, 19-CH₃), 1.08 (3H,

s, 18-CH₃), 2.03, 2.11 (each 3H, s, -OCOCH₃), 3.9–4.7 (4H, 3 β -, 11 α -, 21-H), 5.32 (1H, dd, $J=3$, 9 Hz, 20 β -H). *Anal.* Calcd for C₂₅H₄₀O₇: C, 66.34; H, 8.91. Found: C, 66.30; H, 8.82.

ii) A solution of 3,11 β ,17 α ,21-tetrahydroxy-3,5-pregnadien-20-one 3-methyl ether 17,21-acetonide¹³ (2) (240 mg, 0.58 mmol) in AcOEt (5 ml)–EtOH (2 ml) was stirred under a hydrogen gas stream for 14 h at an atmospheric pressure in the presence of 5% Pd–C (30 mg). After addition of AcOEt (30 ml) followed by removal of the catalyst by filtration, the filtrate, which contained the enol ether (3), was treated with NaBH_4 (150 mg) in MeOH (5 ml) at 0 °C for 1 h. The crude product (4) obtained upon the usual workup was treated with 2N HCl (1 ml) in MeOH (5 ml). The resulting solution was diluted with AcOEt, washed with 5% NaHCO_3 , dried over anhydrous Na_2SO_4 , and evaporated down. Acetylation of the crude product was then carried out with acetic anhydride (1 ml) in pyridine (2 ml). After the usual workup, the crude product obtained was chromatographed on silica gel (20 g) with hexane–AcOEt (1:1) as an eluent, yielding the 20 α ,21-diacetoxy-3-one (5). A solution of 5 (60 mg, 0.13 mmol) and K-Selectride (1M in tetrahydrofuran, 0.2 ml) in dry tetrahydrofuran (3 ml) was stirred for 30 min at –78 °C under a nitrogen atmosphere. After addition of 30% H_2O_2 (0.3 ml), the resulting mixture was extracted with AcOEt. The organic layer was washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated down. The residue obtained was chromatographed on silica gel (15 g). Elution with hexane–AcOEt (1:2) and recrystallization of the product from MeOH gave 12 (55 mg, 21% from 2) as colorless prisms. mp 242–245 °C. Its infrared spectrum was identical with that of the sample obtained in i).

5 α -Cortolone-20 α 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (13) A mixture of 11 (400 mg, 0.71 mmol) and pyridinium chlorochromate (500 mg, 2.3 mmol) in CH_2Cl_2 (6 ml) was stirred at room temperature for 2.5 h. After addition of ether, the resulting solution was passed through Florisil on a sintered-glass funnel, and the filtrate was evaporated down. Recrystallization of the product from MeOH gave 13 (395 mg, 99%) as colorless leaflets. mp 203–205 °C. $[\alpha]_D^{25} + 14^\circ$ ($c=0.8$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.01 (6H, s, 3-OSi(CH₃)₂), 0.80 (3H, s, 18-CH₃), 0.88 (9H, s, 3-OSi-*tert*-Bu), 0.99 (3H, s, 19-CH₃), 2.02, 2.11 (each 3H, s, -OCOCH₃), 3.9–4.2 (2H, 3 β -H, one of 21-H), 4.38 (1H, dd, $J=3$, 12 Hz, one of 21-H), 5.26 (1H, dd, $J=3$, 9 Hz, 20 β -H). *Anal.* Calcd for C₃₁H₅₂O₇Si: C, 65.92; H, 9.28. Found: C, 65.80; H, 9.45.

5 α -Cortolone-20 α 20,21-Diacetate (14) Hydrolysis of 13 (390 mg, 0.69 mmol) with HF was carried out in the manner described for 12. Recrystallization of the product from MeOH gave 14 (300 mg, 96%) as colorless leaflets. mp 271–273 °C. $[\alpha]_D^{25} + 5^\circ$ ($c=0.5$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.80 (3H, s, 18-CH₃), 1.00 (3H, s, 19-CH₃), 2.02, 2.11 (each 3H, s, -OCOCH₃), 3.9–4.2 (2H, 3 β -H, one of 21-H), 4.40 (1H, dd, $J=3$, 12 Hz, one of 21-H), 5.27 (1H, dd, $J=3$, 9 Hz, 20 β -H). *Anal.* Calcd for C₂₅H₃₈O₇: C, 66.64; H, 8.50. Found: C, 66.53; H, 8.37.

5 α -Cortol-20 β 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (16) Sodium borohydride reduction of 21-acetoxy-3 α ,11 β ,17 α -trihydroxy-5 α -pregnan-20-one 3-*tert*-butyldimethylsilyl ether (15) (570 mg, 1.1 mmol) was carried out in the manner described for 10. The product obtained was treated with acetic anhydride (2 ml) in pyridine (4 ml). Purification by chromatography on silica gel (20 g) using hexane–AcOEt (3:1) and recrystallization of the product from MeOH gave 16 (450 mg, 73% from 15) as colorless leaflets. mp 178–180 °C. $[\alpha]_D^{25} + 54^\circ$ ($c=0.7$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.02 (6H, s, 3-OSi(CH₃)₂), 0.88 (9H, s, 3-OSi-*tert*-Bu), 0.95 (3H, s, 18-CH₃), 0.99 (3H, s, 19-CH₃), 2.02, 2.11 (each 3H, s, -OCOCH₃), 3.94 (1H, m, 3 β -H), 4.13 (1H, dd, $J=8$, 12 Hz, one of 21-H), 4.3–4.6 (2H, 11 α -H, one of 21-H), 5.35 (1H, dd, $J=3$, 8 Hz, 20 α -H). *Anal.* Calcd for C₃₁H₅₄O₇Si: C, 65.69; H, 9.60. Found: C, 65.58; H, 9.40.

5 α -Cortol-20 β 20,21-Diacetate (17) Hydrolysis of 16 (73 mg, 0.13 mmol) with HF was carried out in the manner described for 12. Recrystallization of the product from MeOH gave 17 (57 mg, 98%) as colorless leaflets. mp 201–203 °C. $[\alpha]_D^{25} + 74^\circ$ ($c=0.5$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.95 (3H, s, 18-CH₃), 1.01 (3H, s, 19-CH₃), 2.03, 2.12 (each 3H, s, -OCOCH₃), 3.9–4.6 (4H, 3 β -, 11 α -, 21-H), 5.36 (1H, dd, $J=3$, 8 Hz, 20 α -H). *Anal.* Calcd for C₂₅H₄₀O₇: C, 66.34; H, 8.91. Found: C, 66.16; H, 8.93.

5 α -Cortolone-20 β 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (18) Oxidation of 16 (250 mg, 0.44 mmol) with pyridinium chlorochromate was carried out in the manner described for 13. Purification by chromatography on silica gel (20 g) using hexane–AcOEt (5:1) and recrystallization of the product from MeOH gave 18 (245 mg, 98%) as colorless leaflets. mp 167–169 °C. $[\alpha]_D^{25} + 56^\circ$ ($c=1.1$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.01 (6H, s, 3-OSi(CH₃)₂), 0.67 (3H, s, 18-CH₃), 0.88 (9H, s, 3-OSi-*tert*-Bu), 0.97 (3H, s, 19-CH₃), 2.02, 2.10 (each 3H, s, -OCOCH₃), 3.9–4.3 (2H, 3 β -H, one

of 21-H), 4.44 (1H, dd, $J=3$, 12 Hz, one of 21-H), 5.26 (1H, dd, $J=3$, 8 Hz, 20 α -H). *Anal.* Calcd for $C_{31}H_{52}O_7$: C, 65.92; H, 9.28. Found: C, 65.96; H, 9.34.

5 α -Cortolone-20 β , 20,21-Diacetate (19) Hydrolysis of **18** (240 mg, 0.43 mmol) with HF was carried out in the manner described for **12**. Recrystallization of the product from MeOH gave **19** (190 mg, 99%) as colorless leaflets. mp 209–211 °C. $[\alpha]_D^{25} + 77^\circ$ ($c=0.6$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.67 (3H, s, 18-CH₃), 0.99 (3H, s, 19-CH₃), 2.03, 2.11 (each 3H, s, -OCOCH₃), 3.9–4.3 (2H, 3 β -H, one of 21-H), 4.46 (1H, dd, $J=3$, 12 Hz, one of 21-H), 5.27 (1H, dd, $J=3$, 8 Hz, 20 α -H). *Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.58; H, 8.49.

Methyl (20 α ,21-Diacetoxy-11 β ,17 α -dihydroxy-5 α -pregnan-3 α -yl-2',3',4'-tri-*O*-acetyl- β -D-glucopyranosid)uronate (20) Freshly prepared Ag_2CO_3 (240 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl- α -D-glucopyranuronate (490 mg, 1.23 mmol) were added to a solution of **12** (280 mg, 0.62 mmol) in toluene (10 ml), and the suspension was stirred at room temperature for 2 d. After addition of AcOEt, the resulting solution was passed through Celite (30 g) on a sintered-glass funnel, and the filtrate was evaporated down. The residue was chromatographed on silica gel (30 g) with hexane–AcOEt (1:1) as an eluent, yielding a mixture of **20** and a sugar derivative. Separation of these products was achieved after acetylation of the latter compound. Repurification by chromatography using hexane–AcOEt (1:1) yielded **20** (110 mg, 23%) as colorless semi-crystals. $^1\text{H-NMR}$ (CDCl_3) δ : 1.01 (3H, s, 19-CH₃), 1.07 (3H, s, 18-CH₃), 2.03, 2.11 (15H, -OCOCH₃), 3.73 (3H, s, -COOCH₃), 3.8–4.7 (6H, 3 β -, 11 α -, 21-, 1'-, 5'-H), 4.8–5.4 (4H, 20 β -, 2'-, 3'-, 4'-H).

Methyl (20 α ,21-Diacetoxy-17 α -hydroxy-11-oxo-5 α -pregnan-3 α -yl-2',3',4'-tri-*O*-acetyl- β -D-glucopyranosid)uronate (21) The Koenigs–Knorr reaction of **14** (150 mg, 0.33 mmol) and purification were carried out in the manner described for **20**, yielding **21** (60 mg, 24%) as colorless semi-crystals. $^1\text{H-NMR}$ (CDCl_3) δ : 0.79 (3H, s, 18-CH₃), 0.98 (3H, s, 19-CH₃), 2.03, 2.11 (15H, -OCOCH₃), 3.74 (3H, s, -COOCH₃), 3.8–4.7 (5H, 3 β -, 21-, 1'-, 5'-H), 4.8–5.4 (4H, 20 β -, 2'-, 3'-, 4'-H).

Methyl (20 β ,21-Diacetoxy-11 β ,17 α -dihydroxy-5 α -pregnan-3 α -yl-2',3',4'-tri-*O*-acetyl- β -D-glucopyranosid)uronate (22) The Koenigs–Knorr reaction of **17** (300 mg, 0.66 mmol) was carried out in the manner described for **20**. The crude product was purified by chromatography on silica gel (30 g) using hexane–AcOEt (1:1) and benzene–AcOEt (1:1) as eluents. Recrystallization of the product from ether–hexane gave **22** (110 mg, 22%) as colorless leaflets. mp 175–178 °C. $[\alpha]_D^{20} + 25^\circ$ ($c=0.5$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.94 (3H, s, 18-CH₃), 1.00 (3H, s, 19-CH₃), 2.01, 2.11 (15H, -OCOCH₃), 3.74 (3H, s, -COOCH₃), 3.8–4.7 (6H, 3 β -, 11 α -, 21-, 1'-, 5'-H), 4.8–5.4 (4H, 20 α -, 2'-, 3'-, 4'-H). *Anal.* Calcd for $C_{38}H_{56}O_{16} \cdot 5/2\text{H}_2\text{O}$: C, 56.08; H, 7.55. Found: C, 56.30; H, 7.25.

Methyl (20 β ,21-Diacetoxy-17 α -hydroxy-11-oxo-5 α -pregnan-3 α -yl-2',3',4'-tri-*O*-acetyl- β -D-glucopyranosid)uronate (23) The Koenigs–Knorr reaction of **19** (400 mg, 0.89 mmol) and purification were carried out in the manner described for **22**. Recrystallization of the product from ether–hexane gave **23** (230 mg, 34%) as colorless leaflets. mp 128–130 °C. $[\alpha]_D^{20} + 24^\circ$ ($c=1.1$). $^1\text{H-NMR}$ (CDCl_3) δ : 0.66 (3H, s, 18-CH₃), 0.97 (3H, s, 19-CH₃), 1.99, 2.03, 2.10 (15H, -OCOCH₃), 3.73 (3H, s, -COOCH₃), 3.8–4.7 (5H, 3 β -, 21-, 1'-, 5'-H), 4.8–5.4 (4H, 20 α -, 2'-, 3'-, 4'-H). *Anal.* Calcd for $C_{38}H_{54}O_{16} \cdot 3/2\text{H}_2\text{O}$: C, 57.49; H, 7.24. Found: C, 57.51; H, 6.82.

5 α -Cortol-20 α 3-Glucuronide (24) A solution of **20** (100 mg, 0.13 mmol) in 2% methanolic KOH (5 ml) was stirred at room temperature for 3 h. After addition of H₂O followed by neutralization with AcOH, the resulting solution was evaporated down under reduced pressure. The crude product obtained was subjected to column chromatography on Amberlite XAD-2. Elution with MeOH gave the crude product, which was chromatographed

on silica gel (10 g) with CHCl_3 –MeOH–H₂O–AcOH (100:30:1:0.5) as an eluent, and then on Amberlite XAD-2, yielding **24** (38 mg, 54%). mp 185 °C (dec.) (colorless leaflets from MeOH–AcOEt). $[\alpha]_D^{23} - 9^\circ$ ($c=0.5$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 0.97 (3H, s, 18-CH₃), 1.04 (3H, s, 19-CH₃), 3.93 (1H, m, 3 β -H), 4.32 (1H, m, 11 α -H), 4.35 (1H, d, $J=7$ Hz, 1'-H). *Anal.* Calcd for $C_{27}H_{44}O_{11}$: C, 59.54; H, 8.14. Found: C, 59.31; H, 8.36.

5 α -Cortolone-20 α 3-Glucuronide (25) Saponification of **21** (70 mg, 0.09 mmol) with 2% KOH and purification were carried out in the manner described for **24**, yielding **25** (31 mg, 63%). mp 196 °C (dec.) (colorless leaflets from MeOH–AcOEt). $[\alpha]_D^{18} + 5^\circ$ ($c=0.6$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 0.70 (3H, s, 18-CH₃), 1.02 (3H, s, 19-CH₃), 3.95 (1H, m, 3 β -H), 4.33 (1H, d, $J=7$ Hz, 1'-H). *Anal.* Calcd for $C_{27}H_{44}O_{11} \cdot 3/4\text{H}_2\text{O}$: C, 58.31; H, 7.88. Found: C, 58.22; H, 7.94.

5 α -Cortol-20 β 3-Glucuronide (26) Saponification of **23** (100 mg, 0.13 mmol) with 2% KOH and purification were carried out in the manner described for **24**, yielding **26** (36 mg, 51%). mp 195 °C (dec.) (colorless leaflets from MeOH–AcOEt). $[\alpha]_D^{17} + 6^\circ$ ($c=0.5$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 1.04 (6H, s, 18-, 19-CH₃), 3.98 (1H, m, 3 β -H), 4.31 (1H, m, 11 α -H), 4.35 (1H, d, $J=7$ Hz, 1'-H). *Anal.* Calcd for $C_{27}H_{44}O_{11} \cdot 3/2\text{H}_2\text{O}$: C, 56.73; H, 8.29. Found: C, 56.97; H, 8.08.

5 α -Cortolone-20 β 3-Glucuronide (27) Saponification of **24** (230 mg, 0.30 mmol) with 2% KOH and purification were carried out in the manner described for **24**, yielding **27** (125 mg, 76%). mp 190 °C (dec.) (colorless leaflets from MeOH–AcOEt). $[\alpha]_D^{15} + 14^\circ$ ($c=0.4$, MeOH). $^1\text{H-NMR}$ (CD_3OD) δ : 0.75 (3H, s, 18-CH₃), 1.03 (3H, s, 19-CH₃), 3.92 (1H, m, 3 β -H), 4.34 (1H, d, $J=7$ Hz, 1'-H). *Anal.* Calcd for $C_{27}H_{42}O_{11} \cdot 1/2\text{H}_2\text{O}$: C, 58.79; H, 7.86. Found: C, 58.66; H, 7.86.

References and Notes

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