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Synthesis of Cortol 3-Glucuronides and Cortolone 3-Glucuronides[1)]

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The synthesis of the 3-glucuronides of 20α-cortol, 20α-cortolone and their 20β-epimers is described. The cortol 20,21-diacetates (**3**, **10**) and cortolone 20,21-diacetates (**6**, **12**) were the key intermediates. Sodium borohydride reduction of the carbonyl group at C-20 in 21-acetoxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one 3-*tert*-butyldimethylsilyl ether (**1**) or its 11-oxo derivative (**4**) followed by acetylation of the product with acetic anhydride gave the silyl ether-acetates (**2**, **5**), which, on removal of the protecting group at C-3 with sulfuric acid, were converted into the desired 20β-intermediates (**3**, **6**). On the other hand, the 20α-acetates, **10** and **12**, were synthesized from methyl 20α-acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-11-oxo-5β-pregnan-21-oate (**7**). Introduction of the glucuronyl residue at the C-3 position was carried out by means of the Koenigs–Knorr reaction.

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

20α-Cortol, 20α-cortolone and their 20β-epimers,[2)] which are metabolites of cortisol, are excreted mainly as the 3-monoglucuronides in human urine.[3)] The existence of these glucuronides in human plasma has also been reported.[4)] Recently, radioimmunoassays for the cortols and cortolones have been developed using antisera raised against the 21-hemisuccinate–bovine serum albumin conjugates.[5)] The assays have been done on urine samples treated with β-glucuronidase or unprocessed samples. Enzyme immunoassay is an attractive method, particularly if a direct assay procedure to measure the glucuronides in the biological fluids can be developed. For the purpose of developing immunoassays of these corticosteroids, it is desirable to have standard samples and appropriate haptenic derivatives. This paper deals with the synthesis of 20α-cortol 3-glucuronide, 20α-cortolone 3-glucuronide and their 20β-epimers.

Recently, Mattox *et al.* reported the preparation of these compounds by sodium borohydride reduction of the 3-glucuronide derivatives of tetrahydrocortisone or tetrahydrocortisol.[6)] In the present work, the 20,21-diacetates of cortol and cortolone were the key intermediates. We have previously reported the chemical conversion of cortisol to various tetrahydrocortisol[7)] and cortoic acid derivatives;[8)] some of these were used as starting materials.

First, the preparations of 20β-cortol 20,21-diacetate (**3**) and 20β-cortolone 20,21-diacetate (**6**) were carried out. It has been shown that reduction of 17α,21-dihydroxy-20-ketones with sodium borohydride yields predominantly 20β-alcohols.[9)] Treatment of 21-acetoxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one 3-*tert*-butyldimethylsilyl ether (**1**)[7)] with sodium borohydride in ethanol–tetrahydrofuran, followed by acetylation with acetic anhydride in pyridine, gave 20β-cortol 3-*tert*-butyldimethylsilyl ether 20,21-diacetate (**2**). Desilylation of **2** with sulfuric acid in acetone gave **3** in good yield. On the other hand, the 11-ketone (**6**) was prepared starting from 21-acetoxy-3α,17α-dihydroxy-5β-pregnane-11,20-dione 3-*tert*-butyldimethylsilyl ether (**4**).[7)] When **4** was treated with sodium borohydride under mild

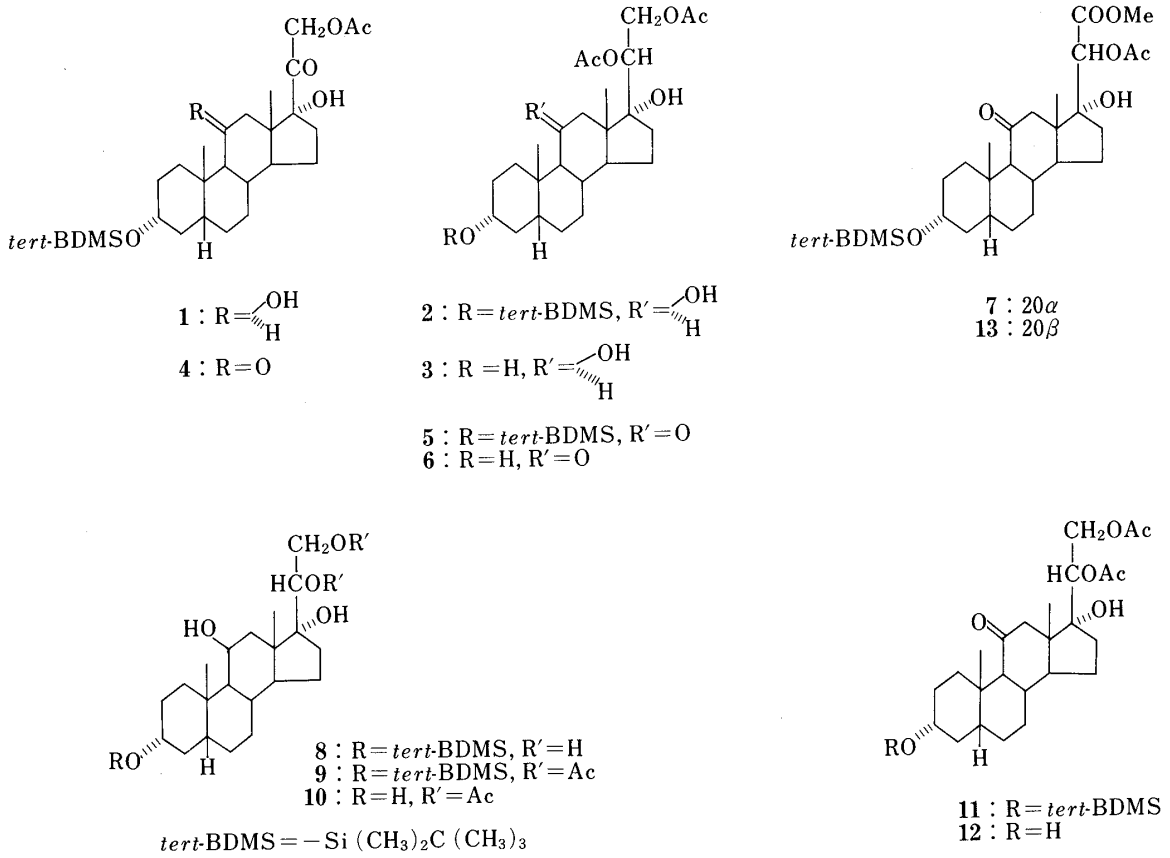


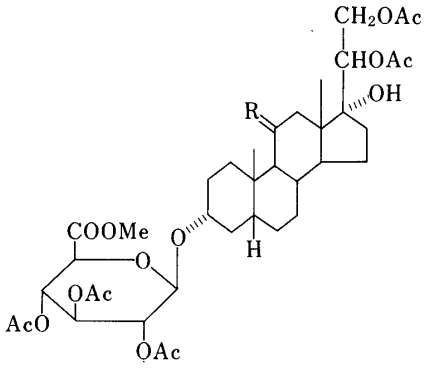
Chart 1

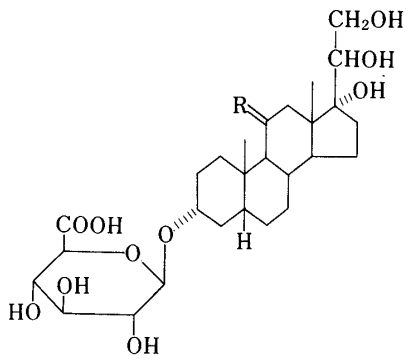
conditions, selective reduction of the carbonyl group at C-20 took place. Subsequent acetylation of the product gave the cortolone derivative (**5**), which in turn was desilylated to give **6**. Separate experiments showed that oxidation of **2** with chromium trioxide–pyridine reagent was suitable for preparing **5**. Compound **4**, which we have prepared from **1** by oxidation with pyridinium chlorochromate, can be derived from commercially available tetrahydrocortisone 21-acetate; therefore simultaneous reduction of the 11- and 20-carbonyl groups in **4** with metal hydrides is also useful in the preparation of the cortol derivatives.

20α-Cortol 20,21-diacetate (**10**) and 20α-cortolone 20,21-diacetate (**12**) were then synthesized. Isolation of the 20α-alcohols formed by the above reduction of the 20-ketones is not efficient, because of their poor yields. An alternative route was therefore explored. Treatment of methyl 20α-acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-11-oxo-5β-pregnan-21-oate (**7**)[8] with lithium aluminum hydride in ether afforded the 20α-cortol 3-silyl ether (**8**). Acetylation of **8** with acetic anhydride in pyridine gave the diacetate-silyl ether (**9**), which, on removal of the protecting group at C-3 with sulfuric acid, was converted into **10** in good yield. On the other hand, **9** was oxidized with the chromium trioxide–pyridine reagent to give the 11-ketone (**11**). Desilylation of **11** yielded the desired compound **12**.

Studies on the configuration at C-20 in these derivatives were also carried out. The stereochemistry of **7** and its 20β-epimer (**13**), which are obtained in the ratio of *ca.* 2 : 1 from tetrahydrocortisone *via* the intramolecular Cannizzaro reaction of the 20-oxo-21-aldehyde, has been determined on the basis of the proton nuclear magnetic resonance (^{1}H-NMR) spectral data.[8] In order to confirm this, **13** was subjected to lithium aluminum hydride reduction, followed by acetylation with acetic anhydride in pyridine. The product was identical with the 20β-cortol derivative **2** prepared from **1**. On the other hand, saponification of **10** derived from **7** gave 20α-cortol which was identical with an authentic sample prepared

from tetrahydrocortisone 17,21-acetonide by reduction with sodium borohydride, followed by acid hydrolysis: the reduction is known to yield the 20α-alcohol.[10] Saponification of the other 20,21-diacetates (**3**, **6**, **12**) gave the corresponding cortol or cortolone. Thus, the stereochemistry at C-20 was confirmed.





14 : 20β, R = 20β-OH, H

15 : 20β, R = O

16 : 20α, R = 20α-OH, H

17 : 20α, R = O

18 : 20β, R = 20β-OH, H

19 : 20β, R = O

20 : 20α, R = 20α-OH, H

21 : 20α, R = O

Chart 2

Introduction of the glucuronyl residue into the C-3 position of **3**, **6**, **10** and **12** 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 glucuronide acetate-methyl esters (**14**, **15**, **16**, **17**, respectively). Subsequent removal of the protecting groups with potassium hydroxide provided 20β-cortol 3-glucuronide (**18**), 20β-cortolone 3-glucuronide (**19**), 20α-cortol 3-glucuronide (**20**) and 20α-cortolone 3-glucuronide (**21**).

In the ^{1}H-NMR spectra of **14**, **15**, **16** and **17**, the anomeric proton of the sugar moiety resonates as a doublet of $J=7$ Hz in the range of 4.62—4.64 ppm, showing β-configuration of the anomeric center. In the case of the free glucuronides (**18**—**21**), the water-eliminated Fourier transform method was employed. The anomeric proton signal in each compound was observed at 4.43 ppm as a doublet of $J=7$ Hz.

We have previously prepared the glucuronides of related cortisol metabolites, such as tetrahydrocortisol, tetrahydrocortisone[7] and cortoic acids.[8] The glucuronides obtained here should also be useful as haptens and standard samples in immunoassays for corticosteroids.

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.

20β-Cortol 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (2)——i) A solution of 21-acetoxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one 3-*tert*-butyldimethylsilyl ether (**1**)[7] (300 mg) and $NaBH_4$ (60 mg) in EtOH (3.5 ml)–tetrahydrofuran (3.5 ml) was stirred at room temperature for 30 min. After 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. The residue obtained was treated with acetic anhydride in pyridine. Purification of the product by column

chromatography on silica gel (20 g) with benzene–ether (2 : 1) as an eluent gave **2** (210 mg). mp 182—183 °C (colorless leaflets from ether–hexane). $[\alpha]_D^{22}$ +67° (*c*=1.2). *Anal.* Calcd for $C_{31}H_{54}O_7Si$: C, 65.69; H, 9.60. Found: C, 65.58; H, 9.67. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.90 (9H, s, 3-OSi-*tert*-Bu), 0.94 (3H, s, 18-CH_3), 1.14 (3H, s, 19-CH_3), 2.02 and 2.10 (each 3H, s, –$OCOCH_3$), 3.56 (1H, m, 3β-H), 4.0—4.3 (2H, 11α- and one of 21-H), 4.45 (1H, dd, *J*=3 and 12 Hz, one of 21-H), 5.35 (1H, dd, *J*=3 and 8 Hz, 20α-H).

ii) A mixture of methyl 20β-acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-11-oxo-5β-pregnan-21-oate (**13**)[8] (30 mg) and $LiAlH_4$ (20 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 20% Rochelle salt and H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from aqueous MeOH gave 20β-cortol 3-*tert*-butyldimethylsilyl ether (18 mg) as colorless needles. mp 217—219 °C. $[\alpha]_D^{22}$ +26° (*c*=0.69). *Anal.* Calcd for $C_{27}H_{50}O_5Si$: C, 67.17; H, 10.44. Found: C, 67.19; H, 10.22. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.01 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 3.4—3.9 (4H, 3β-, 20α-, and 21-H), 4.23 (1H, m, 11α-H). Acetylation of the silyl ether with acetic anhydride in pyridine gave **2**. The infrared spectra of the two samples obtained in i) and ii) were identical.

20β-Cortol 20,21-Diacetate (3)——A solution of **2** (250 mg) and 50% H_2SO_4 (0.2 ml) in acetone (3 ml) was stirred at room temperature for 10 min. 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 (15 g) with AcOEt–hexane (2 : 1) as an eluent, yielding **3** (180 mg) as colorless semi-crystals. ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 2.02 and 2.11 (each 3H, s, –$OCOCH_3$), 3.62 (1H, m, 3β-H), 4.0—4.3 (2H, 11α- and one of 21-H), 4.45 (1H, dd, *J*=3 and 12 Hz, one of 21-H), 5.35 (1H, dd, *J*=3 and 8 Hz, 20α-H). Saponification of **3** gave 20β-cortol. mp 268—270 °C (from MeOH) (lit. mp 266—269 °C).[11]

20β-Cortolone 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (5)——A solution of 21-acetoxy-3α,17α-dihydroxy-5β-pregnane-11,20-dione 3-*tert*-butyldimethylsilyl ether (**4**)[7] (300 mg) and $NaBH_4$ (8 mg) in EtOH (2 ml)–tetrahydrofuran (2 ml) was stirred at room temperature for 2.5 h. After usual work-up, the product obtained was acetylated with acetic anhydride in pyridine. Recrystallization of the crude product from hexane gave **5** (220 mg) as colorless needles. mp 145—148 °C. $[\alpha]_D^{20}$ +51° (*c*=0.30). *Anal.* Calcd for $C_{31}H_{52}O_7Si$: C, 65.92; H, 9.28. Found: C, 65.69; H, 9.09. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.66 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.12 (3H, s, 19-CH_3), 2.02 and 2.09 (each 3H, s, –$OCOCH_3$), 3.56 (1H, m, 3β-H), 4.10 (1H, dd, *J*=8 and 12 Hz, one of 21-H), 4.47 (1H, dd, *J*=3 and 12 Hz, one of 21-H), 5.26 (1H, dd, *J*=3 and 8 Hz, 20α-H).

20β-Cortolone 20,21-Diacetate (6)——Desilylation of **5** (210 mg) with H_2SO_4 was carried out in the manner described for **3**. Recrystallization of the product obtained from acetone–ether gave **6** (190 mg) as colorless leaflets. mp 182—183 °C. $[\alpha]_D^{22}$ +69° (*c*=1.5). *Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.38; H, 8.55. ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 2.02 and 2.10 (each 3H, s, –$OCOCH_3$), 3.60 (1H, m, 3β-H), 4.11 (1H, dd, *J*=8 and 12 Hz, one of 21-H), 4.45 (1H, dd, *J*=3 and 12 Hz, one of 21-H), 5.27 (1H, dd, *J*=3 and 8 Hz, 20α-H). Saponification of **6** gave 20β-cortolone. mp 258—260 °C (from MeOH) (lit. mp 263—264 °C).[12]

20α-Cortol 3-*tert*-Butyldimethylsilyl Ether (8)——Reaction of methyl 20α-acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-11-oxo-5β-pregnan-21-oate (**7**) (600 mg) with $LiAlH_4$ (400 mg) was carried out in the manner described for **2**. The crude product obtained was chromatographed on silica gel (40 g) with AcOEt–hexane (3 : 1) as an eluent to give **8** (410 mg). mp 213—215 °C (colorless needles from ether–hexane). $[\alpha]_D^{21}$ +18° (*c*=0.31). *Anal.* Calcd for $C_{27}H_{50}O_5Si$: C, 67.17; H, 10.44. Found: C, 67.15; H, 10.70. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.89 (9H, s, 3-OSi-*tert*-Bu), 0.94 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 3.4—3.9 (4H, 3β-, 20β-, and 21-H), 4.23 (1H, m, 11α-H).

20α-Cortol 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (9)——A solution of **8** (280 mg) and acetic anhydride (1 ml) in pyridine (2 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 ether–hexane gave **9** (250 mg) as colorless leaflets. mp 194—196 °C. $[\alpha]_D^{21}$ −1° (*c*=1.0). *Anal.* Calcd for $C_{31}H_{54}O_7Si$: C, 65.69; H, 9.60. Found: C, 65.53; H, 9.74. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.07 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 2.02 and 2.10 (each 3H, s, –$OCOCH_3$), 3.56 (1H, m, 3β-H), 4.06 (1H, dd, *J*=9 and 12 Hz, one of 21-H), 4.26 (1H, m, 11α-H), 4.51 (1H, dd, *J*=3 and 12 Hz, one of 21-H), 5.31 (1H, dd, *J*=3 and 9 Hz, 20β-H).

20α-Cortol 20,21-Diacetate (10)——Desilylation of **9** (130 mg) with H_2SO_4 was carried out in the manner described for **3**. The crude product obtained was chromatographed on silica gel (10 g) with AcOEt–hexane (3 : 1) as an eluent to give **10** (100 mg). mp 110—111 °C (colorless needles from aqueous acetone). $[\alpha]_D^{23}$ −13° (*c*=1.4). *Anal.* Calcd for $C_{25}H_{40}O_7 \cdot 1/4H_2O$: C, 65.69; H, 8.93. Found: C, 65.64; H, 8.90. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 2.03 and 2.11 (each 3H, s, –$OCOCH_3$), 3.62 (1H, m, 3β-H), 4.04 (1H, dd, *J*=9 and 12 Hz, one of 21-H), 4.24 (1H, m, 11α-H), 4.51 (1H, dd, *J*=3 and 12 Hz, one of 21-H), 5.31 (1H, dd, *J*=3 and 9 Hz, 20β-H). Saponification of **10** gave 20α-cortol. mp 245—249 °C (from MeOH–AcOEt) (lit. mp 250.5—254 °C).[3a] Its infrared spectrum was identical with that of an authentic sample prepared from tetrahydrocortisone 17,21-acetonide by sodium borohydride reduction and subsequent acid hydrolysis.

20α-Cortolone 3-*tert*-Butyldimethylsilyl Ether 20,21-Diacetate (11)——A solution of **9** (130 mg) in pyridine (2 ml) and a 10% CrO_3–pyridine complex (2.5 ml) was allowed to stand at room temperature for 1 h. The reaction mixture

was diluted with ether, washed with H_2O, and dried over anhydrous Na_2SO_4. After removal of the solvent, the crude product was recrystallized from acetone–hexane to give **11** (110 mg) as colorless prisms. mp 229—230 °C. $[\alpha]_D^{23}$ +15° (c=1.26). *Anal.* Calcd for $C_{31}H_{52}O_7Si$: C, 65.92; H, 9.28. Found: C, 65.62; H, 9.49. ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.79 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.13 (3H, s, 19-CH_3), 2.02 and 2.10 (each 3H, s, –OCOCH_3), 3.54 (1H, m, 3β-H), 4.00 (1H, dd, J=9 and 12 Hz, one of 21-H), 4.38 (1H, dd, J=3 and 12 Hz, one of 21-H), 5.25 (1H, dd, J=3 and 9 Hz, 20β-H).

20α-Cortolone 20,21-Diacetate (12)——Desilylation of **11** (100 mg) with H_2SO_4 was carried out in the manner described for **3**. Recrystallization of the crude product from aqueous acetone gave **12** (60 mg) as colorless needles. mp 200—201 °C. $[\alpha]_D^{23}$ +6° (c=0.56). *Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.71; H, 8.73. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 2.02 and 2.11 (each 3H, s, –OCOCH_3), 3.60 (1H, m, 3β-H), 4.02 (1H, dd, J=9 and 12 Hz, one of 21-H), 4.39 (1H, dd, J=3 and 12 Hz, one of 21-H), 5.26 (1H, dd, J=3 and 9 Hz, 20β-H). Saponification of **12** gave 20α-cortolone. mp 203—205 °C (from MeOH–AcOEt) (lit. mp 208—209 °C).[12]

Methyl (20β,21-Diacetoxy-11β,17α-dihydroxy-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (14)——Freshly prepared Ag_2CO_3 (760 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (900 mg) were added to a solution of **3** (200 mg) in toluene (10 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 was chromatographed on silica gel (40 g) with benzene–ether (2 : 3) as an eluent, yielding a mixture of **14** 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 (2 : 3) as an eluent gave **14** (180 mg) as colorless crystals. mp 115—120 °C. Mattox *et al.*[6] obtained this compound as a crystalline material of mp 204—205 °C. The product obtained here was pure, as judged by thin-layer chromatography and the ^{1}H-NMR spectrum. ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 2.02, 2.04 and 2.10 (15H, –OCOCH_3), 3.6 (1H, m, 3β-H), 3.75 (3H, s, –COOCH_3), 3.9—4.6 (4H, 11α-, 21-, and 5′-H), 4.64 (1H, d, J=7 Hz, 1′-H), 4.8—5.5 (4H, 20α-, 2′-, 3′-, and 4′-H).

Methyl (20β,21-Diacetoxy-17α-hydroxy-11-oxo-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)-uronate (15)——The Koenigs–Knorr reaction of **6** (280 mg) was carried out in the manner described for **14**. Purification of the crude product obtained by column chromatography on silica gel with benzene–ether (1 : 2) as an eluent gave **15** (480 mg) as colorless crystals. mp 100—105 °C (lit. mp 119—121 °C).[6] ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s, 18-CH_3), 1.12 (3H, s, 19-CH_3), 2.02, 2.04 and 2.10 (15H, –OCOCH_3), 3.6 (1H, m, 3β-H), 3.75 (3H, s, –COOCH_3), 3.9—4.6 (3H, 21- and 5′-H), 4.63 (1H, d, J=7 Hz, 1′-H), 4.8—5.4 (4H, 20α-, 2′-, 3′-, and 4′-H).

Methyl (20α,21-Diacetoxy-11β,17α-dihydroxy-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (16)——The Koenigs–Knorr reaction of **10** (150 mg) in CH_2Cl_2 (6 ml)–toluene (12 ml) and purification of the product were carried out in the manner described for **14**. Recrystallization of the crude product from acetone–hexane gave **16** (80 mg) as colorless leaflets. mp 214—215 °C (lit. mp 220.5—221.5 °C).[6] $[\alpha]_D^{22}$ −22° (c=1.2). *Anal.* Calcd for $C_{38}H_{56}O_{16}$: C, 59.36; H, 7.34. Found: C, 59.26; H, 7.09. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 2.01, 2.05 and 2.10 (15H, –OCOCH_3), 3.6 (1H, m, 3β-H), 3.75 (3H, s, –COOCH_3), 3.9—4.6 (4H, 11α-, 21-, and 5′-H), 4.65 (1H, d, J=7 Hz, 1′-H), 4.8—5.4 (4H, 20β-, 2′-, 3′-, and 4′-H).

Methyl(20α,21-Diacetoxy-17α-hydroxy-11-oxo-5β-pregnan-3α-yl-2′,3′-4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (17)——The Koenigs–Knorr reaction of **12** (200 mg) was carried out in the manner described above. Purification of the crude product obtained by column chromatography on silica gel with AcOEt–CH_2Cl_2 (1 : 2) as an eluent and rechromatography after acetylation in the manner described for **14** gave **17** (250 mg). mp 207—208 °C (colorless leaflets from aqueous MeOH, lit. mp 205—206.5 °C).[6] $[\alpha]_D^{13}$ −8° (c=0.66). *Anal.* Calcd for $C_{38}H_{54}O_{16}$: C, 59.52; H, 7.10. Found: C, 59.54; H, 7.03. ^{1}H-NMR ($CDCl_3$) δ: 0.79 (3H, s, 18-CH_3), 1.14 (3H, s, 19-CH_3), 2.01, 2.04 and 2.11 (15H, –OCOCH_3), 3.6 (1H, m, 3β-H), 3.75 (3H, s, –COOCH_3), 3.9—4.5 (3H, 21-, and 5′-H), 4.62 (1H, d, J=7 Hz, 1′-H), 4.8—5.4 (4H, 20β-, 2′-, 3′-, and 4′-H).

20β-Cortol 3-Glucuronide (18)——A solution of **14** (180 mg) and 10% KOH (1.5 ml) in MeOH (7 ml) was stirred at room temperature for 3 h. 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 **18** (130 mg) as colorless crystals. mp 176—179 °C (lit. mp 179—182 °C).[6] $[\alpha]_D^{22}$ +2° (c=0.67, MeOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 1.01 (3H, s, 18-CH_3), 1.18 (3H, s, 19-CH_3), 4.22 (1H, m, 11α-H), 4.43 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250 °C. *Anal.* Calcd for $C_{27}H_{43}Ba_{1/2}O_{11}\cdot 3H_2O$: C, 48.66; H, 7.41. Found: C, 48.75; H, 7.36.

20β-Cortolone 3-Glucuronide (19)——Saponification of **15** (370 mg) with KOH and Amberlite XAD-2 chromatography were carried out in the manner described for **18**, yielding **19** (190 mg) as colorless crystals. mp 185—192 °C (lit. mp 181—183 °C).[6] $[\alpha]_D^{22}$ +12° (c=2.4, MeOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.74 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 4.43 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250 °C. *Anal.* Calcd for $C_{27}H_{41}Ba_{1/2}O_{11}\cdot 2H_2O$: C, 50.17; H, 7.02. Found: C, 49.96; H, 7.13.

20α-Cortol 3-Glucuronide (20)——Saponification of **16** (700 mg) with KOH and Amberlite XAD-2 chromatography were carried out in the manner described for **18**, yielding **20** (480 mg) as colorless crystals. mp 174—176 °C. $[\alpha]_D^{22}$ −6° (c=0.43, MeOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.96 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 4.22

(1H, m, 11α-H), 4.43 (1H, d, $J=7$ Hz, 1′-H). The barium salt: mp >250 °C. *Anal.* Calcd for $C_{27}H_{43}Ba_{1/2}O_{11}\cdot 2H_2O$: C, 50.02; H, 7.31. Found: C, 49.76; H, 7.45.

20α-Cortolone 3-Glucuronide (21)——Saponification of **17** (150 mg) with KOH and Amberlite XAD-2 chromatography were carried out in the manner described for **18**, yielding **21** (80 mg) as colorless crystals. mp 175—180 °C. $[\alpha]_D^{22}$ +4° ($c=1.0$). ^{1}H-NMR ($CDCl_3$–CD_3OD (4:1)) δ: 0.70 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 4.43 (1H, d, $J=7$ Hz, 1′-H). The barium salt: mp >250 °C. *Anal.* Calcd for $C_{27}H_{41}Ba_{1/2}O_{11}\cdot 2H_2O$: C, 50.17; H, 7.02. Found: C, 50.25; H, 6.78.

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References and Notes

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