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# Synthesis of Cortoic Acid 3-Glucuronides[1)]

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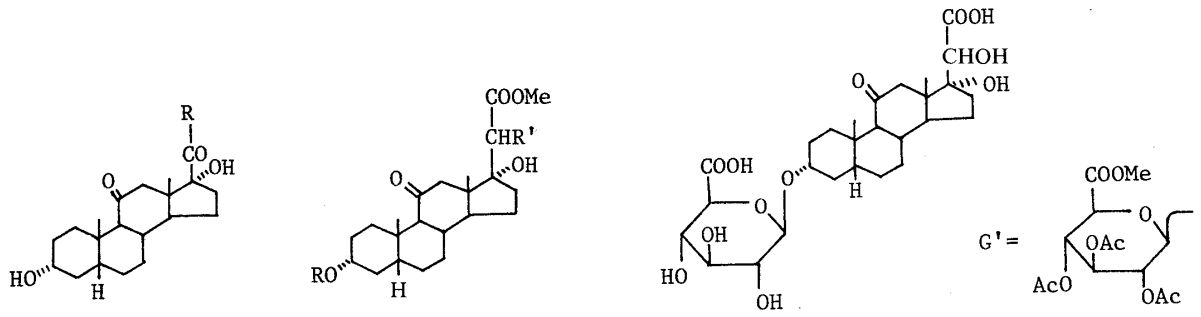
The synthesis of the 3-glucuronides of cortoic acids, metabolites of cortisol, is described. 20α-Cortolonic acid 3-glucuronide (**11**) and its 20β-epimer (**12**) were prepared starting from tetrahydrocortisone (**1**). The cortolonic acid 20-acetate methyl esters (**7**, **8**) were the key intermediates. The 20-oxo-21-aldehyde (**2**) obtained from **1** by oxidation with cupric acetate was derivatized into 20-epimeric methyl cortolonates (**3**). When **3** was treated with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide–pyridine, selective silylation of the hydroxyl group at C-3 took place. Subsequent acetylation with acetic anhydride in pyridine gave the separable 20-acetates (**5**, **6**), which, on removal of the silyl group at C-3 with sulfuric acid, were converted into the desired intermediates. Introduction of the glucuronyl residue into the C-3 position was carried out by means of the Koenigs–Knorr reaction. The cortolic acid 3-glucuronides (**21**, **22**) were synthesized from 5β-dihydrocortisol (**13**).

**Keywords**——cortisol metabolite; cortoic acid; cortolic acid 3-glucuronide; cortolonic acid 3-glucuronide; Koenigs–Knorr reaction

It has been reported that steroid 17-hydroxy-21-oic acids, collectively designated cortoic acids, together constitute 5—25% of the urinary metabolites of cortisol in humans.[2)] These compounds are 20-epimeric cortolic acids and cortolonic acids.[3)] Most of the neutral metabolites of cortisol, such as tetrahydrocortisol and tetrahydrocortisone, are excreted mainly as conjugates with glucuronic acid.[4)] It was also suggested that the cortoic acids may be excreted as glucuronides.[5)] For the purpose of developing methods for the analysis of urinary steroids, it is desirable to have authentic samples of these cortisol metabolites. We report here the chemical synthesis of the cortoic acid 3-glucuronides (**11**, **12**, **21**, **22**).

There may be many routes to the cortoic acid 20-monoacetates (**7**, **8**, **17**, **18**), key intermediates, since various related steroids are now commercially available. We have previously prepared several compounds which can be used as starting materials.[6)] Because of the convenience of separation of the epimeric 20-alcohols and the elimination of the step of oxidation or reduction of the oxygen function at C-11, the preparations of the cortolonic acid glucuronides (**11**, **12**) and cortolic acid glucuronides (**21**, **22**) were carried out starting from tetrahydrocortisone (**1**) and 5β-dihydrocortisol (**13**), respectively.

First, **1** was treated with cupric acetate in methanol, according to the method of Lewbart and Mattox,[7)] yielding the 20-oxo-21-aldehyde (**2**). Subsequent treatment with sodium hydroxide in 5% methanol, followed by methylation with diazomethane, gave a mixture of 20-epimeric methyl cortolonates (**3**); separation of these epimers was carried out after acetylation of the hydroxyl group at C-20. For the derivatization of **3** to the key intermediates (**7**, **8**), selective protection of the hydroxyl group at C-3 is a prerequisite. In the previous paper,[8)] we have reported on the use of *tert*-butyldimethylsilyl chloride as a reagent for hydroxyl group protection in steroids. The large bulk of the reagent was found to be convenient for the present purpose. When **3** was treated with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide–pyridine, selective silylation took place, providing the desired 3-mono-



1: R=$CH_2OH$
2: R=CHO

3: R=H, R′=OH
4: R=*tert*-BDMS, R′=OH
5: R=*tert*-BDMS, R′=α-OAc
6: R=*tert*-BDMS, R′=β-OAc
7: R=H, R′=α-OAc
8: R=H, R′=β-OAc
9: R=G′, R′=α-OAc
10: R=G′, R′=β-OAc

11: 20α
12: 20β

*tert*-BDMS= $-Si(CH_3)_2C(CH_3)_3$

Chart 1

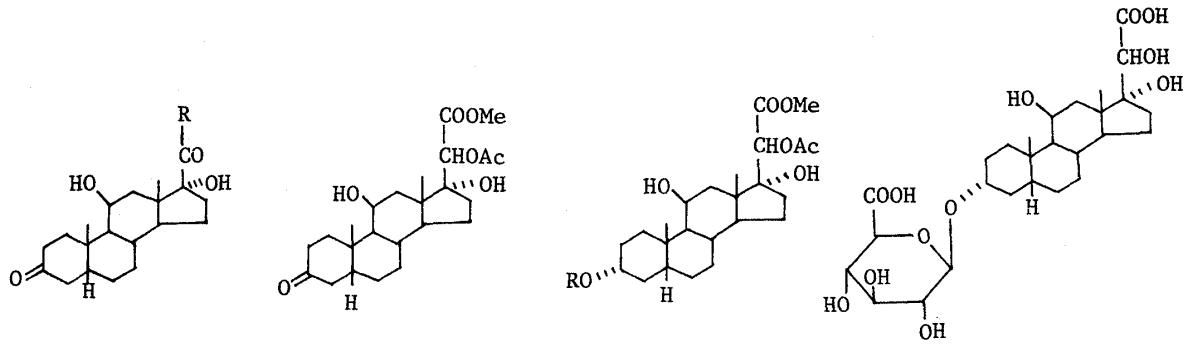
silyl ethers (**4**). Acetylation of **4** with acetic anhydride in pyridine gave the 20α-acetate (**5**) and its 20β-epimer (**6**) in the ratio of *ca.* 2:1; these products were separated by column chromatography on silica gel.

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.[2] The C-18 protons of **5** and **6** resonate at 0.83 ppm and 0.66 ppm, respectively, showing that the configuration at C-20 in **5** is α and that in **6** is β. This was confirmed by derivatization into cortol derivatives; the details will be reported elsewhere. It should be mentioned that application of the intramolecular Cannizzaro reaction to the 20-oxo-21-aldehyde derived from tetrahydrocortisone 3-*tert*-butyldimethylsilyl ether was not successful, probably owing to the sparing solubility of the compound.

Desilylation of **5** with sulfuric acid in acetone gave 20α-cortolonic acid 20-acetate methyl ester (**7**) in good yield. Introduction of the glucuronyl residue into **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, yielding the glucuronide acetate-methyl ester (**9**). Simultaneous removal of the protecting groups in both the steroid and sugar moieties with potassium hydroxide afforded 20α-cortolonic acid 3-glucuronide (**11**). The very polar product was isolated by the solid-phase extraction method using Amberlite XAD-2 as an adsorbent. Similarly, 20β-cortolonic acid 3-glucuronide (**12**) was prepared through the sequence of reactions **6**→**8**→**10**→**12**.

The preparation of the cortolic acid glucuronides (**21**, **22**) was then undertaken. Preliminary experiments showed that the route starting from 5β-dihydrocortisol (**13**) rather than tetrahydrocortisol was favorable for the separation of the 20-epimers. Treatment of **13** with cupric acetate gave the 20-oxo-21-aldehyde (**14**), which in turn was transformed into the epimeric 20-hydroxy-21-oic acids by the intramolecular Cannizzaro reaction. Methylation of the acids with diazomethane, followed by acetylation with acetic anhydride in pyridine, afforded the 20α- and 20β-acetates (**15**, **16**), which were separated by column chromatography on silica gel. The configuration at C-20 was determined by $^1$H-NMR spectral analysis as described above.

Reduction of **15** with sodium borohydride under mild conditions gave the desired 3α-hydroxyl derivative (**17**) and its 3β-epimer in the ratio of *ca.* 3:1. In the $^1$H-NMR spectra, the



**13**: R=$CH_2OH$
**14**: R=CHO

**15**: 20α
**16**: 20β

**17**: 20α, R=H
**18**: 20β, R=H
**19**: 20α, R=G′
**20**: 20β, R=G′

**21**: 20α
**22**: 20β

Chart 2

C-3 proton signal of **17** 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 4.11 ppm. The Koenigs–Knorr reaction of **17**, followed by removal of the protecting groups, furnished the desired 20α-cortolic acid 3-glucuronide (**21**). 20β-Cortolic acid 3-glucuronide (**22**) was prepared through the sequence of reactions **16**→**18**→**20**→**22**.

In the $^1$H-NMR spectra of **9**, **10**, **19** and **20**, the anomeric proton of the sugar moiety resonates as a doublet of $J$=7 Hz in the range of 4.59—4.66 ppm, showing β-configuration of the anomeric center. In the case of the free glucuronides (**11**, **12**, **21**, **22**), the anomeric proton signal in each was observed at 4.43 ppm as a doublet of $J$=7 Hz.

We have previously prepared the glucuronides of tetrahydrocortisol, tetrahydrocortisone[6] and tetrahydro-11-deoxycortisol.[9] The glucuronides obtained here should also be useful as standard samples in metabolic studies and immunoassays of 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.

**Methyl Cortolonates (3)**——A solution of **1** (2.15 g) (prepared from its 3-*tert*-butyldimethylsilyl ether)[6] and $Cu(OAc)_2 \cdot H_2O$ (300 mg) in MeOH (240 ml) was stirred at room temperature for 1.5 h while air was bubbled through it. After addition of ethylenediaminetetraacetic acid (EDTA)·2Na (580 mg) in $H_2O$ (80 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 (10 ml)–$H_2O$ (200 ml) was treated with 2 N NaOH (4.2 ml) under a nitrogen gas stream. After 50 min, the reaction mixture was extracted with AcOEt. 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 was treated with diazomethane. The crude product obtained was chromatographed on silica gel (40 g) with benzene–AcOEt (1 : 5) as an eluent, yielding a mixture of the 20-epimers (**3**) (1.92 g).

**Epimeric Methyl 20-Acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-11-oxo-5β-pregnan-21-oates (5, 6)**——A solution of **3** (1.9 g), imidazole (2.2 g), and *tert*-butyldimethylsilyl chloride (1.1 g) in pyridine (1 ml)–dimethylformamide (2 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. The product (**4**) was treated with acetic anhydride (2 ml) in pyridine (4 ml) 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. Purification of the crude product by column chromatography on silica gel with benzene–ether (4 : 1) as an eluent gave the 20α-acetate (**5**) (1.4 g) and 20β-acetate (**6**) (0.7 g).

**5**: Colorless needles from MeOH. mp 165—166 °C. $[\alpha]_D^{25}$ +39° ($c$=1.0). *Anal.* Calcd for $C_{30}H_{50}O_7Si$: C, 65.42;

H, 9.15. Found: C, 65.33; H, 9.15. $^1$H-NMR ($CDCl_3$) $\delta$: 0.04 (6H, s, 3-OSi($CH_3$)$_2$), 0.83 (3H, s, 18-$CH_3$), 0.88 (9H, s, 3-OSi-*tert*-Bu), 1.12 (3H, s, 19-$CH_3$), 2.13 (3H, s, 20α-OCO$CH_3$), 3.56 (1H, m, 3β-H), 3.76 (3H, s, 21-COO$CH_3$), 5.01 (1H, s, 20β-H).

**6**: Colorless needles from MeOH. mp 208—209 °C. $[\alpha]_D^{25}$ +29° ($c$=0.99). *Anal.* Calcd for $C_{30}H_{50}O_7Si$: C, 65.42; H, 9.15. Found: C, 65.41; H, 9.19. $^1$H-NMR ($CDCl_3$) $\delta$: 0.04 (6H, s, 3-OSi($CH_3$)$_2$), 0.66 (3H, s, 18-$CH_3$), 0.88 (9H, s, 3-OSi-*tert*-Bu), 1.12 (3H, s, 19-$CH_3$), 2.14 (3H, s, 20β-OCO$CH_3$), 3.56 (1H, m, 3β-H), 3.76 (3H, s, 21-COO$CH_3$), 5.04 (1H, s, 20α-H).

**20α-Cortolonic Acid 20-Acetate Methyl Ester (7)**——A solution of **5** (640 mg) and 50% $H_2SO_4$ (0.7 ml) in acetone (5 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. Recrystallization of the crude product from acetone–hexane gave **7** (430 mg) as colorless prisms. mp 183—184 °C. $[\alpha]_D^{20}$ +34° ($c$=1.0). *Anal.* Calcd for $C_{24}H_{36}O_7$: C, 66.03; H, 8.31. Found: C, 65.68; H, 8.36. $^1$H-NMR ($CDCl_3$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 1.15 (3H, s, 19-$CH_3$), 2.16 (3H, s, 20α-OCO$CH_3$), 3.60 (1H, m, 3β-H), 3.79 (3H, s, 21-COO$CH_3$), 5.03 (1H, s, 20β-H).

**20β-Cortolonic Acid 20-Acetate Methyl Ester (8)**——Desilylation of **6** (150 mg) with $H_2SO_4$ was carried out in the manner described for **7**. After usual work-up, the crude product obtained was recrystallized from acetone–hexane to give **8** (110 mg) as colorless leaflets. mp 207—208 °C. $[\alpha]_D^{20}$ +16° ($c$=0.74). *Anal.* Calcd for $C_{24}H_{36}O_7$: C, 66.03; H, 8.31. Found: C, 65.84; H, 8.56. $^1$H-NMR ($CDCl_3$–$CD_3OD$ (10:1)) $\delta$: 0.67 (3H, s, 18-$CH_3$), 1.15 (3H, s, 19-$CH_3$), 2.18 (3H, s, 20β-OCO$CH_3$), 3.60 (1H, m, 3β-H), 3.76 (3H, s, 21-COO$CH_3$), 5.03 (1H, s, 20α-H).

**Methyl [(Methyl 20α-Acetoxy-17α-hydroxy-11-oxo-5β-pregnan-21-oat)-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (9)**——Freshly prepared $Ag_2CO_3$ (470 mg) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (680 mg) were added to a solution of **7** (150 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 oily residue was subjected to column chromatography on silica gel (25 g) with benzene–ether (2:3) as an eluent, yielding a mixture of **9** and a sugar derivative. Separation of these products was achieved after acetylation of the latter compound. Purification by chromatography on silica gel (20 g) with benzene–ether (1:1) as an eluent gave **9** (210 mg) as colorless semi-crystals. $^1$H-NMR ($CDCl_3$) $\delta$: 0.83 (3H, s, 18-$CH_3$), 1.12 (3H, s, 19-$CH_3$), 2.00 and 2.14 (12H, –OCO$CH_3$), 3.60 (1H, m, 3β-H), 3.72 and 3.75 (each 3H, s, –COO$CH_3$), 4.00 (1H, m, 5′-H), 4.60 (1H, d, $J$=7 Hz, 1′-H), 4.7—5.3 (3H, 2′-, 3′-, and 4′-H), 4.97 (1H, s, 20β-H).

**Methyl [(Methyl 20β-Acetoxy-17α-hydroxy-11-oxo-5β-pregnan-21-oat)-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (10)**——The Koenigs–Knorr reaction of **8** (150 mg) was carried out in the manner described for **9**. The crude product obtained was purified by column chromatography on silica gel (25 g) with benzene–ether (1:1) as an eluent and the product was recrystallized from aqueous MeOH to give **10** (185 mg) as colorless needles. mp 131—132 °C. $[\alpha]_D^{15}$ +3° ($c$=3.0). *Anal.* Calcd for $C_{37}H_{52}O_{16}$: C, 59.03; H, 6.96. Found: C, 59.04; H, 6.80. $^1$H-NMR ($CDCl_3$) $\delta$: 0.65 (3H, s, 18-$CH_3$), 1.11 (3H, s, 19-$CH_3$), 2.00, 2.01 and 2.15 (12H, –OCO$CH_3$), 3.60 (1H, m, 3β-H), 3.72 and 3.74 (each 3H, s, –COO$CH_3$), 3.98 (1H, m, 5′-H), 4.59 (1H, d, $J$=7 Hz, 1′-H), 4.7—5.3 (3H, 2′-, 3′-, and 4′-H), 4.98 (1H, s, 20α-H).

**20α-Cortolonic Acid 3-Glucuronide (11)**——A solution of **9** (530 mg) and 10% KOH (3 ml) in MeOH (10 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 **11** (360 mg) as colorless hygroscopic crystals. $^1$H-NMR ($CD_3OD$) $\delta$: 0.82 (3H, s, 18-$CH_3$), 1.15 (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. $[\alpha]_D^{12}$ −2.5° ($c$=0.20, AcOH–MeOH (1:4)). *Anal.* Calcd for $C_{27}H_{38}BaO_{12}\cdot 2H_2O$: C, 44.55, H, 5.82. Found: C, 44.24; H, 6.00.

**20β-Cortolonic Acid 3-Glucuronide (12)**——Saponification of **10** (75 mg) with KOH and chromatography on Amberlite XAD-2 were carried out in the manner described for **11**, yielding **12** (45 mg) as colorless hygroscopic crystals. $^1$H-NMR ($CD_3OD$) $\delta$: 0.80 (3H, s, 18-$CH_3$), 1.16 (3H, s, 19-$CH_3$), 4.24 (1H, s, 20α-H), 4.43 (1H, d, $J$=7 Hz, 1′-H). The barium salt: mp >250 °C. $[\alpha]_D^{16}$ −12° ($c$=0.20, AcOH–MeOH (1:4)). *Anal.* Calcd for $C_{27}H_{38}BaO_{12}\cdot 4H_2O$: C, 42.45, H, 6.07. Found: C, 42.23; H, 5.74.

**Epimeric Methyl 20-Acetoxy-11β,17α-dihydroxy-3-oxo-5β-pregnan-21-oates (15, 16)**——Treatment of **13** (2.9 g) (prepared from its 21-acetate)[6] with $Cu(OAc)_2\cdot H_2O$ to yield the 20-oxo-21-aldehyde (**14**) and the Cannizzaro reaction were carried out in the manner described for **3**. After methylation with diazomethane followed by acetylation with acetic anhydride in pyridine, the epimeric mixture obtained was chromatographed on silica gel with benzene–AcOEt (2:3) as an eluent, yielding the 20α-acetate (**15**) (940 mg) and 20β-acetate (**16**) (420 mg).

**15**: Colorless needles from acetone. mp 210—211 °C. $[\alpha]_D^{20}$ +45° ($c$=0.59). *Anal.* Calcd for $C_{24}H_{36}O_7$: C, 66.03; H, 8.31. Found: C, 65.88; H, 8.29. $^1$H-NMR ($CDCl_3$) $\delta$: 1.16 (3H, s, 18-$CH_3$), 1.26 (3H, s, 19-$CH_3$), 2.16 (3H, s, 20α-OCO$CH_3$), 3.78 (3H, s, 21-COO$CH_3$), 4.34 (1H, m, 11α-H), 5.10 (1H, s, 20β-H).

**16**: Colorless leaflets from acetone. mp 251—252 °C. $[\alpha]_D^{25}$ +24° ($c$=0.33, MeOH). *Anal.* Calcd for $C_{24}H_{36}O_7$: C, 66.03; H, 8.31. Found: C, 66.36; H, 8.50. $^1$H-NMR ($CDCl_3$) $\delta$: 1.01 (3H, s, 18-$CH_3$), 1.27 (3H, s, 19-$CH_3$), 2.18 (3H, s, 20β-OCO$CH_3$), 3.78 (3H, s, 21-COO$CH_3$), 4.36 (1H, m, 11α-H), 5.11 (1H, s, 20α-H).

**20α-Cortolic Acid 20-Acetate Methyl Ester (17)**——A solution of **15** (230 mg) and $NaBH_4$ (20 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 with benzene–AcOEt (3 : 4) as an eluent, yielding **17** (140 mg) and its 3β-epimer (50 mg).

**17**: Colorless leaflets from acetone–hexane. mp 187—188 °C. $[\alpha]_D^{20}$ +36° ($c$=0.61, MeOH). *Anal.* Calcd for $C_{24}H_{38}O_7$: C, 65.73; H, 8.73. Found: C, 65.46; H, 9.00. $^1$H-NMR ($CDCl_3$) δ: 1.12 (3H, s, 18-$CH_3$), 1.17 (3H, s, 19-$CH_3$), 2.14 (3H, s, 20α-$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.78 (3H, s, 21-$COOCH_3$), 4.24 (1H, m, 11α-H), 5.08 (1H, s, 20β-H).

The 3β-Epimer: $^1$H-NMR ($CDCl_3$) δ: 1.12 (3H, s, 18-$CH_3$), 1.21 (3H, s, 19-$CH_3$), 2.14 (3H, s, 20α-$OCOCH_3$), 3.79 (3H, s, 21-$COOCH_3$), 4.11 (1H, m, 3α-H), 4.25 (1H, m, 11α-H), 5.12 (1H, s, 20β-H).

**20β-Cortolic Acid 20-Acetate Methyl Ester (18)**——Reduction of **16** (520 mg) with $NaBH_4$ in MeOH was carried out in the manner described for **17**. After usual work-up, the epimeric mixture obtained was purified by column chromatography on silica gel with benzene–AcOEt (1 : 2) as an eluent to give **18** (310 mg) and its 3β-epimer (100 mg).

**18**: Colorless leaflets from acetone–hexane. mp 227—228 °C. $[\alpha]_D^{20}$ +20° ($c$=0.27, MeOH). *Anal.* Calcd for $C_{24}H_{38}O_7$: C, 65.73; H, 8.73. Found: C, 65.65; H, 8.74. $^1$H-NMR ($CDCl_3$) δ: 0.96 (3H, s, 18-$CH_3$), 1.16 (3H, s, 19-$CH_3$), 2.16 (3H, s, 20β-$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.75 (3H, s, 21-$COOCH_3$), 4.26 (1H, m, 11α-H), 5.09 (1H, s, 20α-H).

The 3β-Epimer: $^1$H-NMR ($CDCl_3$) δ: 0.97 (3H, s, 18-$CH_3$), 1.22 (3H, s, 19-$CH_3$), 2.18 (3H, s, 20β-$OCOCH_3$), 3.76 (3H, s, 21-$COOCH_3$), 4.12 (1H, m, 3α-H), 4.28 (1H, m, 11α-H), 5.10 (1H, m, 20α-H).

**Methyl [(Methyl 20α-Acetoxy-11β,17α-dihydroxy-5β-pregnan-21-oat)-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (19)**——The Koenigs–Knorr reaction of **17** (200 mg) was carried out in the manner described for **9**. The crude product obtained was purified by column chromatography on silica gel with benzene–ether (1 : 1) as an eluent and recrystallized from acetone–hexane to give **19** (210 mg) as colorless leaflets. mp 207—208 °C. $[\alpha]_D^{20}$ +4° ($c$=0.47). *Anal.* Calcd for $C_{37}H_{54}O_{16}$: C, 58.79; H, 7.34. Found: C, 58.82; H, 7.18. $^1$H-NMR ($CDCl_3$) δ: 1.12 (3H, s, 18-$CH_3$), 1.16 (3H, s, 19-$CH_3$), 2.02, 2.04 and 2.14 (12H, -$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.74 and 3.77 (each 3H, s, -$COOCH_3$), 4.01 (1H, m, 5′-H), 4.20 (1H, m, 11α-H), 4.66 (1H, d, $J$=7 Hz, 1′-H), 4.8—5.3 (3H, 2′-, 3′-, and 4′-H), 5.08 (1H, s, 20β-H).

**Methyl [(Methyl 20β-Acetoxy-11β,17α-dihydroxy-5β-pregnan-21-oat)-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid]uronate (20)**——The Koenigs–Knorr reaction of **18** (170 mg) in $CH_2Cl_2$ (14 ml)–toluene (14 ml) was carried out in the manner described for **9**. The crude product obtained was purified by column chromatography on silica gel with benzene–AcOEt (2 : 3) as an eluent and recrystallized from $CHCl_3$–ether to give **20** (180 mg) as colorless needles. mp 235—236 °C. $[\alpha]_D^{14}$ −5° ($c$=0.28). *Anal.* Calcd for $C_{37}H_{54}O_{16}$: C, 58.79; H, 7.34. Found: C, 58.42; H, 7.15. $^1$H-NMR ($CDCl_3$) δ: 0.96 (3H, s, 18-$CH_3$), 1.14 (3H, s, 19-$CH_3$), 2.01, 2.04 and 2.16 (12H, -$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.74 and 3.75 (each 3H, s, -$COOCH_3$), 4.00 (1H, m, 5′-H), 4.22 (1H, m, 11α-H), 4.64 (1H, d, $J$=7 Hz, 1′-H), 4.8—5.3 (3H, 2′-, 3′-, and 4′-H), 5.08 (1H, s, 20α-H).

**20α-Cortolic Acid 3-Glucuronide (21)**——Saponification of **19** (170 mg) with KOH and chromatography on Amberlite XAD-2 were carried out in the manner described for **11**, yielding **21** (110 mg) as colorless hygroscopic crystals. $^1$H-NMR ($CD_3OD$) δ: 1.08 (3H, s, 18-$CH_3$), 1.17 (3H, s, 19-$CH_3$), 4.10 (1H, s, 20β-H), 4.17 (1H, m, 11α-H), 4.43 (1H, d, $J$=7 Hz, 1′-H). The barium salt: mp >250 °C. $[\alpha]_D^{13}$ −8° ($c$=0.20, AcOH–MeOH (1 : 5)). *Anal.* Calcd for $C_{27}H_{40}BaO_{12}\cdot 2H_2O$: C, 44.42; H, 6.08. Found: C, 43.99; H, 6.39.

**20β-Cortolic Acid 3-Glucuronide (22)**——Saponification of **20** (140 mg) with KOH and chromatography on Amberlite XAD-2 were carried out in the manner described for **11**, yielding **22** (100 mg) as colorless hygroscopic crystals. $^1$H-NMR ($CD_3OD$) δ: 1.07 (3H, s, 18-$CH_3$), 1.16 (3H, s, 19-$CH_3$), 4.17 (1H, m, 11α-H), 4.27 (1H, s, 20α-H), 4.43 (1H, d, $J$=7 Hz, 1′-H). The barium salt: mp >250 °C. $[\alpha]_D^{14}$ −11° ($c$=0.27, AcOH–MeOH (1 : 5)). *Anal.* Calcd for $C_{27}H_{40}BaO_{12}\cdot 3H_2O$: C, 43.35; H, 6.19. Found: C, 43.45; H, 5.94.

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## References and Notes

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