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Synthesis of Haptens for Use in Immunoassays of Tetrahydrocortisol, Tetrahydrocortisone and Their Glucuronides[1]

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In order to develop specific and sensitive immunoassays, carboxylated derivatives of tetrahydrocortisol and tetrahydrocortisone were synthesized. The preparation of the 3-hemisuccinates (**23**, **27**), 21-hemisuccinates (**8**, **14**), 3-hemiglutarates (**24**, **28**), and 21-hemiglutarates (**9**, **15**) of these corticosteroids was carried out starting from cortisol 21-acetate (**1**). Tetrahydrocortisol monoglucuronides (**36**, **38**) and tetrahydrocortisone monoglucuronides (**37**, **39**) were also prepared.

Keywords——tetrahydrocortisol; tetrahydrocortisone; hapten for immunoassay; tetrahydrocortisol hemisuccinate; tetrahydrocortisol hemiglutarate; tetrahydrocortisone hemisuccinate; tetrahydrocortisone hemiglutarate; tetrahydrocortisol glucuronide, tetrahydrocortisone glucuronide

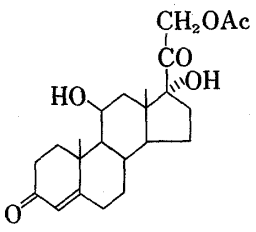
Tetrahydrocortisol (THF) and tetrahydrocortisone (THE), major metabolites of cortisol, are excreted mainly as the 3- and 21-monoglucuronides in human urine.[2] Recently, radioimmunoassays of these corticosteroids have been developed using antisera raised against the 20-carboxymethyloxime-[3] and 21-hemisuccinate[4]- bovine serum albumin conjugates. The assays have been done on urine samples treated with β-glucuronidase or unprocessed samples. Enzyme immunoassay is an attractive method, in particular when a direct assay procedure to measure the glucuronides in the urine can be developed.

It is reasonably well substantiated that the specificity of antibodies is significantly influenced by the position on the steroid molecule used for conjugation to the carrier. In radioimmunoassay using ^{125}I-radioligands and enzyme immunoassay, the combination of antibody and labeled antigen is an important factor determining the sensitivity, because the antibody has an affinity for the bridge between the label and antigen. Previously, we showed that the use of enzyme-labeled steroid prepared from a hapten having a bridge shorter than that used for antibody production is advantageous for obtaining increased sensitivity in enzyme immunoassay.[5] For the purpose of developing sensitive and specific immunoassays for THF, THE and their glucuronides, it is desirable to have appropriate haptenic derivatives. This paper deals with the synthesis of the hemisuccinates and hemiglutarates of THF and THE. The 3- and 21-monoglucuronides were also prepared.

There may be many routes leading to the final products, since various related steroids are now commercially available. In this work, cortisol (a well-known corticosteroid) was selected as a starting material. Transformation of cortisol into THF 21-acetate (**3**), a key intermediate, involves the stereochemistry of reduction of the Δ^4-3-keto group. It has been observed that the saturation of the 4,5-double bond in cortisol or its 21-acetate in ethyl acetate in the presence of palladium-on-charcoal[6] or palladium-on-barium sulfate[7] leads to the predominant formation of the 5α-derivatives. On the other hand, Combe *et al.*[8] found that hydrogenation of C_{19} Δ^4-3-ketosteroids over a palladium-calcium carbonate catalyst in solvents containing trivalent nitrogen groups afforded mainly 5β-compounds. This was the case in the reduction of cortisol 21-acetate (**1**). When **1** was hydrogenated with palladium-on-calcium carbonate in pyridine, reduction proceeded in the desired fashion, and 5β-dihydrocortisol 21-acetate (**2**) could be isolated in 64% yield by recrystallization. The formation of the 5α-isomer was less than 10%, as judged by thin-layer chromatography. Then, the Raney nickel hydrogenation of **2** was

carried out according to the method of Harnik.[6b] Separation of THF 21-acetate (**3**) from the 3β-epimer was achieved effectively by centrifugal liquid chromatography on silica gel. This compound was used for the preparation of the 21-hemisuccinates (**8**, **14**), 21-hemiglutarates (**9**, **15**) and 3-glucuronides (**36**, **37**).

Treatment of **3** with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide-pyridine gave the 3-silyl ether (**4**). Deacetylation of **4** with sodium methoxide in methanol gave THF 3-*tert*-butyldimethylsilyl ether (**5**) in good yield. On treatment with succinic anhydride in pyridine, **5** was transformed into the 21-hemisuccinate (**6**). Removal of the silyl group at C-3 in **6** with sulfuric acid in acetone furnished the desired compound (**8**). Next, oxidation of **4** was carried out with pyridinium chlorochromate[9] in methylene chloride to give



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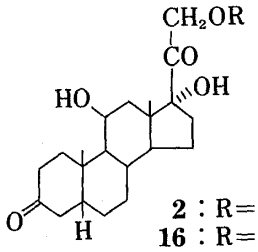
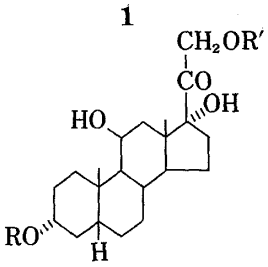
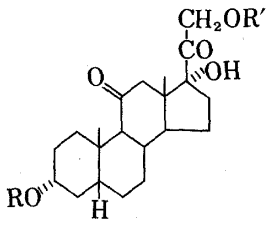


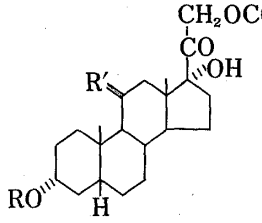
2 : R=Ac
16 : R=SiMe$_2$-*tert*-Bu

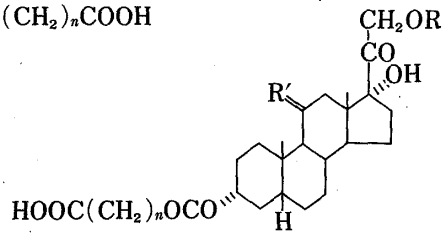


3 : R=H, R′=Ac
4 : R=SiMe$_2$-*tert*-Bu, R′=Ac
5 : R=SiMe$_2$-*tert*-Bu, R′=H
17 : R=H, R′=SiMe$_2$-*tert*-Bu
18 : R=Ac, R′=SiMe$_2$-*tert*-Bu
29 : R=Ac, R′=H



10 : R=SiMe$_2$-*tert*-Bu, R′=Ac
11 : R=SiMe$_2$-*tert*-Bu, R′=H
19 : R=Ac, R′=SiMe$_2$-*tert*-Bu
20 : R=H, R′=SiMe$_2$-*tert*-Bu
30 : R=Ac, R′=H
31 : R=H, R′=Ac





6 : R=SiMe$_2$-*tert*-Bu, R′=  , n=2
7 : R=SiMe$_2$-*tert*-Bu, R′=  , n=3
8 : R=H, R′=, n=2
9 : R=H, R′=, n=3
12 : R=SiMe$_2$-*tert*-Bu, R′=O, n=2
13 : R=SiMe$_2$-*tert*-Bu, R′=O, n=3
14 : R=H, R′=O, n=2
15 : R=H, R′=O, n=3

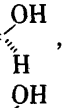
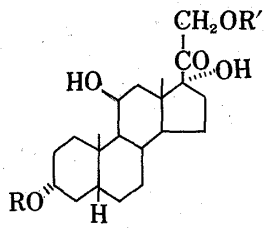
21 : R=SiMe$_2$-*tert*-Bu, R′=  , n=2
22 : R=SiMe$_2$-*tert*-Bu, R′=  , n=3
23 : R=H, R′=  , n=2
24 : R=H, R′=, n=3
25 : R=SiMe$_2$-*tert*-Bu, R′=O, n=2
26 : R=SiMe$_2$-*tert*-Bu, R′=O, n=3
27 : R=H, R′=O, n=2
28 : R=H, R′=O, n=3

Chart 1

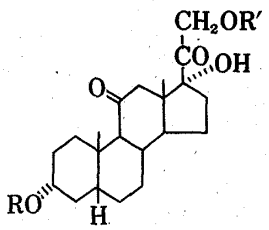
the 11-ketone (**10**), which, on transesterification of the acetyl group at C-21 with sodium methoxide, was converted into the THE 3-silyl ether (**11**). Hemisuccinoylation of **11** followed by desilylation with sufuric acid yielded THE 21-hemisuccinate (**14**). In a similar manner, the 21-hemiglutarates (**9**, **15**) were prepared.

The preparation of the 3-hemisuccinates (**23**, **27**) and 3-hemiglutarates (**24**, **28**) was then undertaken. 5β-Dihydrocortisol prepared from **2** was derivatized into 21-silyl ether (**16**). Selective reduction of the carbonyl group at C-3 in **16** was effected by hydrogenation with Raney nickel as a catalyst providing THF 21-*tert*-butyldimethylsilyl ether (**17**) and the 3β-epimer in the ratio of *ca.* 2: 1. The stereochemistry at C-3 was determined on the basis of the proton nuclear magnetic resonance (^{1}H-NMR) spectral data. The C-3 proton signal of **17** appeared at 3.66 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 the signal of $W_{1/2}$=*ca.* 10 Hz at 4.07 ppm. Oxidation of the 3-acetate (**18**) followed by deacetylation with sodium methoxide yielded the THE 21-silyl ether (**20**). For the removal of the acetyl group in **19**, saponification by treatment with methanolic sodium hydroxide was not satisfactory with respect to yield. Instability of the silyloxy group at C-21 under the basic conditions may be due to the nature of the ketol function of the side chain and/or to the participation of the hydroxyl group at C-17. On treatment with succinic anhydride or glutaric anhydride, and subsequent desilylation, the silyl ethers (**17**, **20**) were transformed into the desired haptens (**23**, **24**, **27**, **28**).

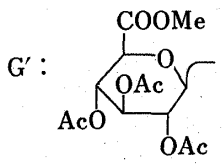
The glucuronides of THF and THE (**36**—**39**) can also be used as haptens as well as standard samples in immunoassays. Although these compounds have appeared in the literature,[2,3,10] to our knowledge, the methods of preparation were not described in detail. Therefore, the synthesis of the glucuronides was carried out. For this purpose, the 3- and 21-monoacetates (**29**—**31**) were derived from the silyl ethers (**18**, **19**, **10**) by desilylation with sulfuric acid in acetone. Introduction of the glucuronyl residue into **3** and **31** was achieved 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 (**32**, **33**). Prior to saponification of **32** and **33**, the alkali-sensitive ketol side chain at C-17 was protected by derivatization into the 20-semicarbazones.



32 : R=G′, R′=Ac
34 : R=Ac, R′=G′
36 : R=G, R′=H
38 : R=H, R′=G



33 : R=G′, R′=Ac
35 : R=Ac, R′=G′
37 : R=G, R′=H
39 : R=H, R′=G



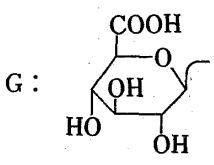


Chart 2

Sequential removal of the protecting groups was done by treatment with methanolic potassium hydroxide, and then with pyruvic acid–acetic acid to give the desired THF and THE 3-glucuronides (**36, 37**) in satisfactory yields. The 21-glucuronides (**38, 39**) were obtained by the Koenigs–Knorr reaction of **29** and **30** followed by simultaneous removal of the protecting groups in both the steroid and sugar moieties with methanolic potassium hydroxide.

In the ^{1}H-NMR spectra of **32, 33, 34** and **35**, the anomeric proton of the sugar moiety resonates in the range of 4.63—4.73 ppm as a doublet of $J=7$ Hz, showing β-configuration of the anomeric center. In the case of the free glucuronides (**36, 37, 39**), the signal of the methylene protons at C-21 and the anomeric proton could be easily assigned, when the water-eliminated Fourier transform method was employed. The anomeric protons were observed in the range of 4.43—4.48 ppm as a doublet of $J=7$ Hz.

The haptens and glucuronides obtained here may be useful in the development of practical immunoassays for THF and THE glucuronides in human urine. Production of antibodies to the corticosteroids by the use of these derivatives is now in progress 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.

5β-Dihydrocortisol 21-Acetate (2)——A solution of cortisol 21-acetate (**1**) (11.5 g) in pyridine (40 ml) was stirred under a hydrogen gas stream for 12 h at atmospheric pressure in the presence of palladium-on-calcium carbonate (30 g). After addition of acetone (50 ml) followed by removal of the catalyst by filtration, the filtrate was concentrated to one-third of its initial volume under reduced pressure. Upon addition of H_2O to the residue a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from EtOH gave **2** (7.4 g) as colorless needles. mp 210—212°C. Saponification of **2** gave 5β-dihydrocortisol. mp 206—208°C (lit. mp 206—208°C).[6a]

Tetrahydrocortisol 21-Acetate (3)——A solution of **2** (4.0 g) in dioxane (50 ml) was hydrogenated for 10 h in the presence of Raney nickel (W-2) (*ca.* 7 g). The filtered solution was evaporated down under reduced pressure. The crude product obtained was purified by centrifugal liquid chromatography on silica gel. Elution with benzene–AcOEt (2: 1) and recrystallization of the eluate from ether–acetone gave **3** (1.7 g) as colorless plates. mp 196—198°C (lit. mp 192—193°C).[6b] ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.85 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 2.18 (3H, s, 21-OCOCH_3), 3.60 (1H, m, 3β-H), 4.29 (1H, m, 11α-H), 4.86 and 5.06 (each 1H, d, $J=18$ Hz, 21-H).

21-Acetoxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one 3-*tert*-Butyldimethylsilyl Ether (4)——A solution of **3** (360 mg), imidazole (800 mg), and *tert*-butyldimethylsilyl chloride (400 mg) in pyridine (0.3 ml)–dimethylformamide (0.6 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 MeOH gave **4** (390 mg) as colorless leaflets. mp 197—199°C. $[\alpha]_D^{18}$ +63° ($c=0.34$). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi(CH_3)$_2$), 0.90 (12H, s, 18-CH_3 and 3-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 2.16 (3H, s, 21-OCOCH_3), 3.58 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.78 and 5.06 (each 1H, d, $J=18$ Hz, 21-H). *Anal.* Calcd for $C_{29}H_{50}O_6Si$: C, 66.63; H, 9.64. Found: C, 66.42; H, 9.62.

Tetrahydrocortisol 3-*tert*-Butyldimethylsilyl Ether (5)——A solution of **4** (500 mg) and MeONa (*ca.* 50 mg) in dry MeOH (10 ml) was stirred at room temperature for 15 min under a nitrogen gas stream. After neutralization with AcOH, the resulting solution was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4. The solution was passed through an Al_2O_3 (10 g) layer on a sintered-glass funnel. The filtrate was evaporated down under reduced pressure. Recrystallization of the crude product from ether–hexane gave **5** (330 mg) as colorless leaflets. mp 187—189°C. $[\alpha]_D^{14}$ +49° ($c=0.34$). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi(CH_3)$_2$), 0.90 (12H, s, 18-CH_3 and 3-OSi-*tert*-Bu), 1.14 (3H, s, 19-CH_3), 3.58 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.20 and 4.61 (each 1H, d, $J=19$ Hz, 21-H). *Anal.* Calcd for $C_{27}H_{48}O_5Si$: C, 67.46; H, 10.07. Found: C, 67.19; H, 10.22.

3α-*tert*-Butyldimethylsilyloxy-11β,17α,21-trihydroxy-5β-pregnan-20-one 21-Hemisuccinate (6)——A solution of **5** (200 mg) and succinic anhydride (400 mg) in pyridine (2 ml) was allowed to stand at 40°C for 10 h. After removal of the pyridine followed by addition of H_2O, 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 crude product obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt (1: 2) and recrystallization of the eluate from ether–hexane gave **6** (170 mg) as colorless leaflets. mp 171—173°C. $[\alpha]_D^{15}$ +55° ($c=0.30$). ^{1}H-NMR ($CDCl_3$) δ: 0.08 (6H, s, 3-OSi(CH_3)$_2$), 0.86 (3H, s, 18-CH_3), 0.90 (9H, s, 3-OSi-*tert*-Bu), 1.14 (3H, s, 19-CH_3), 2.73 (4H, m, –$COCH_2CH_2CO$–), 3.58 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.80 and 5.13

(each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{31}H_{52}O_8Si$: C, 64.10; H, 9.03. Found: C, 63.83; H, 9.22.

3α-*tert*-Butyldimethylsilyloxy-11β,17α,21-trihydroxy-5β-pregnan-20-one 21-Hemiglutarate (7)——A solution of **5** (190 mg) and glutaric anhydride (380 mg) in pyridine (1 ml) was allowed to stand at 40°C for 10 h. Upon addition of H_2O a precipitate was formed; this was collected by filtration and dried. Recrystallization of the crude product from acetone–hexane gave **7** (200 mg) as colorless leaflets. mp 167—169°C. $[\alpha]_D^{18}$ +61° (c=0.35). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi$(CH_3)_2$), 0.90 (12H, s, 18-CH_3 and 3-OSi-*tert*-Bu), 1.14 (3H, s, 19-CH_3), 3.58 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.80 and 5.10 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{32}H_{54}O_8Si$: C, 64.61; H, 9.15. Found: C, 64.31; H, 9.33.

Tetrahydrocortisol 21-Hemisuccinate (8)——A solution of **6** (100 mg) and 30% H_2SO_4 (0.1 ml) in acetone was stirred at room temperature for 30 min. Upon addition of H_2O a precipitate was formed; this was collected by filtration and dried. Recrystallization of the crude product from aqueous MeOH gave **8** (50 mg) as colorless leaflets. mp 158—160°C. $[\alpha]_D^{14}$ +59° (c=0.28, EtOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.83 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 2.71 (4H, m, –$COCH_2CH_2CO$–), 3.60 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.93 and 5.05 (each 1H, d, J=19 Hz, 21-H). *Anal.* Calcd for $C_{25}H_{38}O_8 \cdot 3/4H_2O$: C, 62.55; H, 8.29. Found: C, 62.33; H, 8.23.

Tetrahydrocortisol 21-Hemiglutarate (9)——Desilylation of **7** (150 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The crude product obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt–AcOH (10: 40: 0.1) and recrystallization of the eluate from ether–hexane gave **9** (100 mg) as colorless leaflets. mp 178—180°C. $[\alpha]_D^{13}$ +70° (c=0.28, EtOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.84 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 3.60 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.90 and 5.04 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{26}H_{40}O_8$: C, 64.98; H, 8.39. Found: C, 65.20; H, 8.52.

21-Acetoxy-3α,17α-dihydroxy-5β-pregnane-11,20-dione 3-*tert*-Butyldimethylsilyl Ether (10)——A mixture of **4** (500 mg) and pyridinium chlorochromate (600 mg) in CH_2Cl_2 (9 ml) was stirred at room temperature for 2 h. After addition of ether, the resulting solution was passed through Florisil (20 g) on a sintered-glass funnel, and the filtrate was evaporated down. Recrystallization of the crude product from MeOH gave **10** (400 mg) as colorless leaflets. mp 214—216°C. $[\alpha]_D^{18}$ +80° (c=0.28). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi$(CH_3)_2$), 0.58 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.13 (3H, s, 19-CH_3), 2.16 (3H, s, 21-$OCOCH_3$), 3.54 (1H, m, 3β-H), 4.61 and 5.11 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{29}H_{48}O_6Si$: C, 66.88; H, 9.29. Found: C, 66.59; H, 9.22.

Tetrahydrocortisone 3-*tert*-Butyldimethylsilyl Ether (11)——Deacetylation of **10** (610 mg) with MeONa was carried out in the manner described for **5**. After usual work-up, the crude product obtained was recrystallized from acetone–hexane to give **11** (400 mg) as colorless leaflets. mp 208—210°C. $[\alpha]_D^{22}$ +56° (c=0.34). ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-OSi$(CH_3)_2$), 0.57 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.13 (3H, s, 19-CH_3), 3.54 (1H, m, 3β-H), 4.22 and 4.62 (each 1H, d, J=19 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{46}O_5Si$: C, 67.74; H, 9.68. Found: C, 67.62; H, 9.85.

3α-*tert*-Butyldimethylsilyloxy-17α,21-dihydroxy-5β-pregnane-11,20-dione 21-Hemisuccinate (12)——Hemisuccinoylation of **11** (250 mg) with succinic anhydride was carried out in the manner described for **6**. Upon addition of H_2O the precipitate formed was collected by filtration and dried. Recrystallization of the crude product from acetone–hexane gave **12** (200 mg) as colorless leaflets. mp 188—190°C. $[\alpha]_D^{14}$ +67° (c=0.32). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi$(CH_3)_2$), 0.58 (3H, s, 18-CH_3), 0.90 (9H, s, 3-OSi-*tert*-Bu), 1.13 (3H, s, 19-CH_3), 2.73 (4H, m, –$COCH_2CH_2CO$–), 3.55 (1H, m, 3β-H), 4.64 and 5.18 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{31}H_{50}O_8Si$: C, 64.33; H, 8.71. Found: C, 64.38; H, 8.74.

3α-*tert*-Butyldimethylsilyloxy-17α,21-dihydroxy-5β-pregnane-11,20-dione 21-Hemiglutarate (13)——Hemiglutaroylation of **11** (250 mg) with glutaric anhydride was carried out in the manner described for **7**. After addition of H_2O, the resulting mixture was extracted with ether. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from acetone–hexane gave **13** (230 mg) as colorless leaflets. mp 181—184°C. $[\alpha]_D^{14}$ +68° (c=0.31). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi$(CH_3)_2$), 0.57 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.12 (3H, s, 19-CH_3), 3.55 (1H, m, 3β-H), 4.58 and 5.18 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{32}H_{52}O_8Si$: C, 64.83; H, 8.84. Found: C, 64.58; H, 8.95.

Tetrahydrocortisone 21-Hemisuccinate (14)——Desilylation of **12** (170 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. Upon addition of H_2O the precipitate formed was collected by filtration and dried. Recrystallization of the crude product from aqueous MeOH gave **14** (120 mg) as colorless leaflets. mp 199—201°C. $[\alpha]_D^{13}$ +67° (c=0.35, EtOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.56 (3H, s, 18-CH_3), 1.14 (3H, s, 19-CH_3), 2.72 (4H, m, –$COCH_2CH_2CO$–), 3.58 (1H, m, 3β-H), 4.75 and 5.09 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{25}H_{36}O_8 \cdot 1/4H_2O$: C, 64.01; H, 7.84. Found: C, 63.79; H, 7.87.

Tetrahydrocortisone 21-Hemiglutarate (15)——Desilylation of **13** (150 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. After usual work-up, the crude product obtained was chromatographed on silica gel (10 g). Elution with hexane–AcOEt–AcOH (20: 40: 0.2) gave **15** (100 mg) as colorless semi-crystals. ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.56 (3H, s, 18-CH_3), 1.14 (3H, s, 19-CH_3), 3.58 (1H, m, 3β-H), 4.76 and 5.08 (each 1H, d, J=18 Hz, 21-H).

5β-Dihydrocortisol 21-*tert*-Butyldimethylsilyl Ether (16)——Silylation of 5β-dihydrocortisol (2 g) prepared above with *tert*-butyldimethylsilyl chloride was carried out in the manner described for **4**. After usual work-up, the crude product obtained was recrystallized from ether–hexane to give **16** (2.2 g) as colorless leaflets. mp 160—162°C. $[\alpha]_D^{26}$ +40° (c=0.26). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.90 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.26 (3H, s, 19-CH_3), 4.38 (1H, m, 11α-H), 4.41 and 4.62 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{46}O_5Si$: C, 67.74; H, 9.69. Found: C, 67.54; H, 9.90.

Tetrahydrocortisol 21-*tert*-Butyldimethylsilyl Ether (17)——Hydrogenation of **16** (1 g) with Raney nickel was carried out in the manner described for **3**. After usual work-up, the crude product obtained was chromatographed on silica gel (70 g). Elution with benzene–ether (1: 2) and recrystallization of the eluate from acetone–hexane gave 3β,11β,17α,21-tetrahydroxy-5β-pregnan-20-one 21-*tert*-butyldimethylsilyl ether (200 mg) as colorless leaflets. mp 180—182°C. $[\alpha]_D^{25}$ +31° (c=0.36). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.90 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.20 (3H, s, 19-CH_3), 4.07 (1H, m, 3α-H), 4.28 (1H, m, 11α-H), 4.38 and 4.52 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{48}O_5Si \cdot 1/4H_2O$: C, 66.83; H, 10.08. Found: C, 66.60; H, 10.14. Subsequent elution and recrystallization of the product from ether–hexane gave **17** (410 mg) as colorless leaflets. mp 97—98°C. $[\alpha]_D^{27}$ +41° (c=0.20). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.90 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.17 (3H, s, 19-CH_3), 3.66 (1H, m, 3β-H), 4.28 (1H, m, 11α-H), 4.43 and 4.55 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{48}O_5Si$: C, 67.46; H, 10.07. Found: C, 67.21; H, 9.95.

3α-Acetoxy-11β,17α,21-trihydroxy-5β-pregnan-20-one 21-*tert*-Butyldimethylsilyl Ether (18)——A solution of **17** (510 mg) and acetic anhydride (2 ml) in pyridine (4 ml) was allowed to stand at room temperature for 12 h. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from ether–hexane gave **18** (500 mg) as colorless needles. mp 157—159°C. $[\alpha]_D^{22}$ +52° (c=0.29). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.90 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.17 (3H, s, 19-CH_3), 2.04 (3H, s, 3-OCOCH_3), 4.29 (1H, m, 11α-H), 4.41 and 4.59 (each 1H, d, J=18 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{29}H_{50}O_6Si$: C, 66.63; H, 9.64. Found: C, 66.52; H, 9.70.

3α-Acetoxy-17α,21-dihydroxy-5β-pregnane-11,20-dione 21-*tert*-Butyldimethylsilyl Ether (19)——Oxidation of **18** (600 mg) with pyridinium chlorochromate was carried out in the manner described for **10**. After usual work-up, the crude product obtained was recrystallized from MeOH to give **19** (440 mg) as colorless leaflets. mp 190—191°C. $[\alpha]_D^{22}$ +41° (c=0.48). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.59 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 2.01 (3H, s, 3-OCOCH_3), 4.45 and 4.52 (each 1H, d, J=18 Hz, 21-H), 4.68 (3H, m, 3β-H). *Anal.* Calcd for $C_{29}H_{48}O_6Si$: C, 66.88; H, 9.29. Found: C, 66.98; H, 9.36.

Tetrahydrocortisone 21-*tert*-Butyldimethylsilyl Ether (20)——Deacetylation of **19** (800 mg) with MeONa was carried out in the manner described for **5**. After usual work-up, the crude product obtained was chromatographed on silica gel (40 g). Elution with hexane–AcOEt (1: 1) and recrystallization of the product from acetone–hexane gave **20** (720 mg) as colorless leaflets. mp 168—170°C. $[\alpha]_D^{20}$ +35° (c=0.33). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.60 (3H, s, 18-CH_3), 0.95 (9H, s, 21-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 3.58 (1H, m, 3β-H), 4.35 and 4.47 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{46}O_5Si$: C, 67.74; H, 9.68. Found: C, 67.47; H, 9.71.

21-*tert*-Butyldimethylsilyloxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one 3-Hemisuccinate (21)——Hemisuccinoylation of **17** (200 mg) with succinic anhydride was carried out at 80°C in the manner described for **6**. After usual work-up, the crude product obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt (1: 3) and recrystallization of the eluate from acetone–hexane gave **21** (110 mg) as colorless leaflets. mp 194—196°C. $[\alpha]_D^{21}$ +47° (c=0.29). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.12 (6H, s, 21-OSi$(CH_3)_2$), 0.87 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.18 (3H, s, 19-CH_3), 2.60 (4H, s, –$COCH_2$-CH_2CO–), 4.28 (1H, m, 11α-H), 4.41 and 4.72 (each 1H, d, J=18 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{31}H_{52}O_8Si$: C, 64.10; H, 9.03. Found: C, 63.89; H, 9.11.

21-*tert*-Butyldimethylsilyloxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one 3-Hemiglutarate (22)——Hemiglutaroylation of **17** (380mg) was carried out at 80°C in the manner described for **7**. After usual work-up, the crude product obtained was chromatographed on silica gel (8 g) with hexane–AcOEt (1: 3) as an eluent to give **22** (300 mg) as colorless semi-crystals. ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi$(CH_3)_2$), 0.90 (3H, s, 18-CH_3), 0.95 (9H, s, 21-OSi-*tert*-Bu), 1.16 (3H, s, 19-CH_3), 4.28 (1H, m, 11α-H), 4.39 and 4.54 (each 1H, d, J=18 Hz, 21-H), 4.70 (1H, m, 3β-H).

Tetrahydrocortisol 3-Hemisuccinate (23)——Desilylation of **21** (200 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from aqueous MeOH gave **23** (120 mg) as colorless leaflets. mp 173—174°C. $[\alpha]_D^{20}$ +71° (c=0.28, MeOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.83 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 2.60 (4H, s, –$COCH_2CH_2CO$–), 4.28 (1H, m, 11α-H), 4.26 and 4.62 (each 1H, d, J=19 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{25}H_{38}O_8 \cdot 1/4H_2O$: C, 63.74; H, 8.24. Found: C, 63.83; H, 8.33.

Tetrahydrocortisol 3-Hemiglutarate (24)——Desilylation of **22** (90 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. After addition of H_2O, the resulting 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 aqueous MeOH gave **24** (50 mg) as colorless leaflets. mp 113—115°C. $[\alpha]_D^{21}$ +79° (c=0.31, MeOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.84 (3H, s, 18-CH_3), 1.19 (3H, s, 19-CH_3), 4.28 (1H, m, 11α-H), 4.24 and 4.62 (each 1H, d, J=19 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{26}H_{40}O_8$: C, 64.98; H, 8.39. Found: C, 64.81; H, 8.41.

21-*tert*-Butyldimethylsilyloxy-3α,17α-dihydroxy-5β-pregnane-11,20-dione 3-Hemisuccinate (25)——Hemisuccinolylation of **20** (250 mg) with succinic anhydride was carried out at 80°C in the manner described for **6**. After usual work-up, the crude product obtained was chromatographed on silica gel (7 g). Elution with hexane–AcOEt (1: 2) and recrystallization of the product from aqueous MeOH gave **25** (200 mg) as colorless leaflets. mp 150—151°C. $[\alpha]_D^{21}$ +45° (c=0.31). ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi(CH_3)$_2$), 0.59 (3H, s, 18-CH_3), 0.95 (9H, s, 21-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 2.63 (4H, m, –$COCH_2CH_2CO$–), 4.35 and 4.46 (each 1H, d, J=18 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{31}H_{50}O_8Si\cdot 3/4H_2O$: C, 62.87; H, 8.76. Found: C, 62.95; H, 8.56.

21-*tert*-Butyldimethylsilyloxy-3α,17α-dihydroxy-5β-pregnane-11,20-dione 3-Hemiglutarate (26)——Hemiglutaroylation of **20** (350 mg) with glutaric anhydride was carried out at 80°C in the manner described for **7**. After addition of H_2O, 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 crude product obtained was chromatographed on silica gel (7 g) with hexane–AcOEt (1: 1) as an eluent to give **26** (240 mg) as colorless semi-crystals. ^{1}H-NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-OSi(CH_3)$_2$), 0.60 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 4.36 and 4.48 (each 1H, d, J=18 Hz, 21-H), 4.70 (1H, m, 3β-H).

Tetrahydrocortisone 3-Hemisuccinate (27)——Desilylation of **25** (210 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from aqueous MeOH gave **27** (160 mg) as colorless leaflets. mp 160—161°C. $[\alpha]_D^{18}$ +66° (c=0.32, EtOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.56 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 2.60 (4H, s, –$COCH_2CH_2CO$–), 4.18 and 4.60 (each 1H, d, J=19 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{25}H_{36}O_8\cdot 3/4H_2O$: C, 62.81; H, 7.91. Found: C, 62.70; H, 7.48.

Tetrahydrocortisone 3-Hemiglutarate (28)——Desilylation of **26** (240 mg) with 30% H_2SO_4 was carried out in the manner described for **8**. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from aqueous MeOH gave **28** (140 mg) as colorless needles. mp 98—102°C. $[\alpha]_D^{22}$ +85° (c=0.33, EtOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.56 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 4.20 and 4.61 (each 1H, d, J=19 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{26}H_{38}O_8$: C, 65.25; H, 8.00. Found: C, 64.98; H, 8.20.

Tetrahydrocortisol 3-Acetate (29)——Desilylation of **18** (600 mg) with sulfuric acid was carried out in the manner described for **8**. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from ether–hexane gave **29** (470 mg) as colorless needles. mp 196—198°C. $[\alpha]_D^{20}$ +65° (c=0.33). ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 1.18 (3H, s, 19-CH_3), 2.03 (3H, s, 3-$OCOCH_3$), 4.29 (1H, m, 11α-H), 4.27 and 4.74 (each 1H, d, J=19 Hz, 21-H), 4.70 (1H, m, 3β-H). *Anal.* Calcd for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.85; H, 8.90.

Tetrahydrocortisone 3-Acetate (30)——Desilylation of **19** (410 mg) with sulfuric acid was carried out in the manner described for **8**. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. Recrystallization of the crude product from AcOEt gave **30** (300 mg) as colorless needles. mp 196—198°C (lit. mp 209—212°C).[11] ^{1}H-NMR ($CDCl_3$) δ: 0.59 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 2.03 (3H, s, 3-$OCOCH_3$), 4.25 and 4.63 (each 1H, d, J=19 Hz, 21-H), 4.70 (1H, m, 3β-H).

Tetrahydrocortisone 21-Acetate (31)——Desilylation of **10** (500 mg) with sulfuric acid was carried out in the manner described for **8**. Upon addition of H_2O the precipitate formed was collected by filtration and dried. Recrystallization of the crude product from acetone–hexane gave **31** (380 mg) as colorless plates. mp 218—220°C. Its infrared spectrum was identical with that of an authentic sample obtained from Steraloids, Inc. (U.S.A.).

Methyl (21-Acetoxy-11β,17α-dihydroxy-20-oxo-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (32)——Freshly prepared Ag_2CO_3 (4.4 g) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (6.6 g) were added to a solution of **3** (1.6 g) in toluene (40 ml), and the suspension was stirred at room temperature for 12 h. After addition of AcOEt, the resulting solution was passed through Florisil (20 g) on a sintered-glass funnel, and the filtrate was evaporated down. The oily residue was subjected to column chromatography on silica gel (70 g) with hexane–AcOEt (2: 3) as an eluent, yielding a mixture of **32** 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 and recrystallization from ether–CH_2Cl_2 gave **32** (1.4 g) as colorless prisms. mp 183—185°C (lit. mp 191—194°C).[10a] ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 2.03, 2.06 and 2.18 (12H, –$OCOCH_3$), 3.60 (1H, m, 3β-H), 3.75 (3H, s, –$COOCH_3$), 4.02 (1H, m, 5′-H), 4.27 (1H, m, 11α-H), 4.65 (1H, d, J=7 Hz, 1′-H), 4.83 and 5.07 (each 1H, d, J=18 Hz, 21-H), 4.8—5.3 (3H, 2′-, 3′-, and 4′-H)

Methyl (21-Acetoxy-17α-hydroxy-11,20-dioxo-5β-pregnan-3α-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)-uronate (33)——The Koenigs–Knorr reaction of **31** (500 mg) was carried out in the manner described for **32**. After usual work-up, the crude product obtained was chromatographed on silica gel (80 g). Elution with

hexane–AcOEt (2: 3) and recrystallization of the product from ether–CH_2Cl_2 gave **33** (400 mg) as colorless leaflets. mp 201—205°C (lit. mp 209—212°C).[12] ^{1}H-NMR ($CDCl_3$) δ: 0.58 (3H, s, 18-CH_3), 1.13 (3H, s, 19-CH_3), 2.03 and 2.17 (12H, –$OCOCH_3$), 3.58 (1H, m, 3β-H), 3.75 (3H, s, –$COOCH_3$), 4.02 (1H, m, 5′-H), 4.63 (1H, d, J=7 Hz, 1′-H), 4.67 and 5.12 (each 1H, d, J=18 Hz, 21-H), 4.8—5.3 (3H, 2′-, 3′-, and 4′-H).

Methyl (3α-Acetoxy-11β,17α-dihydroxy-20-oxo-5β-pregnan-21-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)-uronate (34)——The Koenigs–Knorr reaction of **29** (300 mg) was carried out in the manner described for **32**. After usual work-up, the crude product obtained was subjected to column chromatography on silica gel (35 g) with hexane–AcOEt (2: 3) as an eluent. Repurification by chromatography using benzene–ether (1: 1) gave **34** (430 mg) as colorless semi-crystal. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 1.18 (3H, s, 19-CH_3), 2.02, 2.03 and 2.10 (12H, –$OCOCH_3$), 3.74 (3H, s, –$COOCH_3$), 4.02 (1H, m, 5′-H), 4.30 (1H, m, 11α-H), 4.53 and 4.69 (each 1H, d, J=18 Hz, 21-H), 4.73 (1H, d, J=7 Hz, 1′-H), 4.5—5.4 (4H, 3β-, 2′-, 3′-, and 4′-H).

Methyl (3α-Acetoxy-17α-hydroxy-11,20-dioxo-5β-pregnan-21-yl-2′,3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)-uronate (35)——The Koenigs-Knorr reaction of **30** (200 mg) and purification by chromatography were carried out in the manner described for **32** to give **35** (130 mg) as colorless semi-crystals. ^{1}H-NMR ($CDCl_3$) δ: 0.57 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 2.03 and 2.10 (12H, –$OCOCH_3$), 3.76 (3H, s, –$COOCH_3$), 4.02 (1H, m, 5′-H), 4.32 and 4.78 (each 1H, d, J=18 Hz, 21-H), 4.65 (1H, d, J=7 Hz, 1′-H), 4.5—5.4 (4H, 3β-, 2′-, 3′-, and 4′-H).

Tetrahydrocortisol 3-Glucuronide (36)——A mixture of **32** (300 mg), semicarbazide·HCl (770 mg), and AcONa (500 mg) in MeOH (8 ml) was stirred at room temperature for 12 h. Upon addition of H_2O a precipitate was formed, and this was collected by filtration and dried. The crude product was chromatographed on silica gel (35 g) with AcOEt as an eluent to give the 20-semicarbazone. This was dissolved in 2% methanolic KOH (25 ml) and allowed to stand at room temperature for 2 h. After addition of H_2O followed by neutralization with AcOH, the resulting solution was evaporated down under reduced pressure. The oily residue was dissolved in 80% pyruvic acid (4 ml)–AcOH (2 ml)–$CHCl_3$ (6 ml) and the solution was stirred at room temperature for 12 h. After addition of H_2O, the resulting mixture was extracted with $CHCl_3$. The aqueous layer was subjected to column chromatography on Amberlite XAD-2. Elution with MeOH gave the crude product, which was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O–AcOH (100: 20: 2: 0.1) as an eluent, and then on Amberlite XAD-2. Recrystallization of the product from MeOH–AcOEt gave **36** (150 mg) as colorless fine leaflets. mp 187—195°C (lit. mp 192—198°C).[10a] ^{1}H-NMR (CD_3OD) δ: 0.82 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 4.22 (1H, m, 11α-H), 4.25 and 4.61 (each 1H, d, J=19 Hz, 21-H), 4.45 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250°C. $[\alpha]_D^{20}$ +39° (c=0.33, MeOH–AcOH (10: 1)). *Anal.* Calcd for $C_{27}H_{41}O_{11}Ba_{1/2}\cdot 5/2H_2O$: C, 49.49; H, 7.08. Found: C, 49.40; H, 6.88.

Tetrahydrocortisone 3-Glucuronide (37)——Removal of the protecting groups in **33** (200 mg) and purification were carried out in the manner described for **36**, yielding **37** (110 mg) as colorless semi-crystals. $[\alpha]_D^{15}$ +42° (c=0.29, MeOH). ^{1}H-NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.55 (3H, s, 18-CH_3), 1.14 (3H, s, 19-CH_3), 4.20 and 4.60 (each 1H, d, J=19 Hz, 21-H), 4.43 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250°C. *Anal.* Calcd for $C_{27}H_{39}O_{11}Ba_{1/2}\cdot 5/2H_2O$: C, 49.63; H, 6.79. Found: C, 49.83; H, 6.51.

Tetrahydrocortisol 21-Glucuronide (38)——A solution of **36** (430 mg) and 5% KOH (5 ml) in MeOH (10 ml) was stirred at room temperature for 2 h. After removal of the MeOH followed by addition of H_2O, the resulting mixture was extracted with AcOEt. The aqueous layer was acidified with AcOH, and subjected to chromatography on Amberlite XAD-2. The eluate was purified by chromatography on silica gel (14 g) with $CHCl_3$–MeOH–H_2O–AcOH (60: 30: 2: 0.1) as an eluent, and then on Amberlite XAD-2 to give **38** (200 mg) as colorless semi-crystals. $[\alpha]_D^{14}$ +27° (c=0.32). ^{1}H-NMR (CD_3OD) δ: 0.83 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 4.1—4.6 (3H, 11α-, 1′-H and one of 21-H), 4.97 (1H, d, J=19 Hz, one of 21-H). The barium salt: mp >250°C. *Anal.* Calcd for $C_{27}H_{41}O_{11}Ba_{1/2}\cdot 1/2H_2O$: C, 52.36; H, 6.84. Found: C, 52.01; H, 7.06.

Tetrahydrocortisone 21-Glucuronide (39)——Saponification of **37** (210 mg) with 5% KOH and purification were carried out in the manner described for **38**. Recrystallization of the product from MeOH–AcOEt gave **39** (120 mg) as hygroscopic fine leaflets. mp >240°C. $[\alpha]_D^{24}$ +23° (c=0.37, EtOH). ^{1}H-NMR (CD_3OD) δ: 0.56 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 4.37 and 4.99 (each 1H, d, J=19 Hz, 21-H), 4.38 (1H, d, J=7 Hz, 1′-H). The barium salt: mp >250°C. *Anal.* Calcd for $C_{27}H_{39}O_{11}Ba_{1/2}\cdot H_2O$: C, 51.78; H, 6.60. Found: C, 52.14; H, 6.91.

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