Chemical Conversion of Corticosteroids to 3α,5α-Tetrahydro Derivatives. Synthesis of Allotetrahydrocortisol Glucuronides and Allotetrahydrocortisone Glucuronides[1)]

Hiroshi HOSODA, Ken OSANAI, Ikuo FUKASAWA and Toshio NAMBARA*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan. *Received January 10, 1990*

The synthesis of the 3- and 21-glucuronides of allotetrahydrocortisol (allo-THF) and allotetrahydrocortisone (allo-THE) is described. 5α-Dihydrocortisol (5) was prepared by selective hydrogenation of 21-acetoxy-3,11β,17α-trihydroxy-3,5-pregnadien-20-one 3-ethyl ether (3), followed by acid hydrolysis and saponification. When 5α-dihydrocortisol 21-tetrahydropyranyl ether (6) was treated with potassium tri-*sec*-butylborohydride in tetrahydrofuran under mild conditions, regioselective and stereoselective reduction at C-3 took place to give allo-THF 21-tetrahydropyranyl ether (7). This compound was converted into the 3- and 21-monoacetates of allo-THF and allo-THE, key intermediates. Introduction of the glucuronyl residue at C-3 or C-21 was carried out by means of the Koenigs–Knorr reaction. Prior to saponification yielding the 3-glucuronides (20, 23), the alkali-sensitive ketol side chain at C-17 was protected as 20-semicarbazones.

Keywords allotetrahydrocortisol; allotetrahydrocortisone; allotetrahydrocortisol glucuronide; allotetrahydrocortisone glucuronide; cortisol; potassium tri-*sec*-butylborohydride; 5α-dihydrocortisol

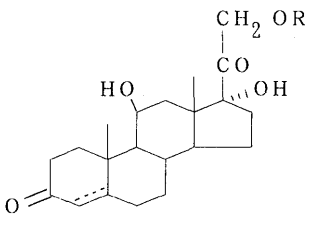
Cortisol metabolism in humans involves various transformations, for example, reduction of the Δ^4-3-keto group to form 3α-hydroxy products, oxidation at C-11, and reduction at C-20. The metabolites are tetrahydrocortisol (THF), its 5α-isomer (allo-THF), cortols, cortolic acids and their 11-keto derivatives. These are excreted in the urine mainly as conjugates with glucuronic acid.[2)] The existence of glucuronides in plasma has also been reported.[3)]

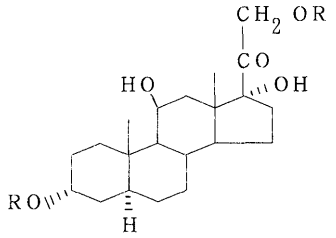
The level of urinary 17-hydroxycorticosteroids has long been an important index of adrenocortical activity.[4)] For use in metabolic studies and immunoassays of corticosteroids, we previously prepared the glucuronides of the 5β-metabolites.[5)] In the case of the 5α-steroid series, practical synthetic methods had not been available until we investigated the chemical conversion of Δ^4-3-keto corticosteroids to 3α-hydroxy-5α-steroids.[6)] This paper deals with the synthesis of the 3- and 21-monoglucuronides of allo-THF and allotetrahydrocortisone (allo-THE).

Cortisol (**1**) was selected as a starting material. Transformation of **1** into allo-THF 3-acetate (**9**) and allo-THF 21-acetate (**16**), key intermediates, involves the stereoselective reduction of the Δ^4-3-keto group. Hydrogenation of **1** or its 21-acetate (**2**) with palladium as a catalyst in ethyl acetate or ethanol is not stereospecific, yielding a mixture of C-5-isomers,[7)] and isolation of the 5α-compounds (**4**, **5**) is not always easy. Thus, we employed the route *via* the 3-ethoxy-3,5-diene (**3**).[6)]

First, **2** was treated with triethyl orthoformate in the presence of a catalytic amount of *p*-toluenesulfonic acid in ethanol–tetrahydrofuran, yielding **3**. Selective hydrogenation of the γ,δ-double bond in **3** over palladium, followed by treatment with hydrochloric acid, gave the desired 5α-compound (**4**). The overall yield from **2** was approximately 30%. The formation of the 5β-isomer was little, as judged by thin-layer chromatography. Hydrogenation of 3,11β,17α,21-tetrahydroxy-3,5-pregnadien-20-one 3-methyl ether 17,21-acetonide was also convenient for the preparation of **5**. It should be mentioned that the reaction of **2** or cortisol 21-tetrahydropyranyl ether with the methyl copper(I)–diisobutylaluminium hydride–hexamethylphosphoric triamide system[8)] as a reducing agent gave the 5α-derivative (**4**, **6**) and its 5β-isomers in the ratio of *ca.* 1 : 2.

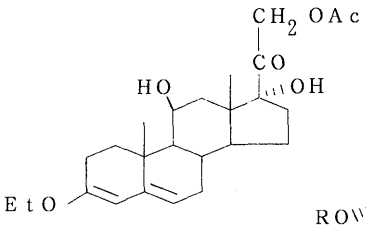
Next, there are several methods for the reduction of the carbonyl group at C-3 in 5α-steroids. However, practical routes leading to the desired 3α-alcohols are limited.[9)] Previous studies[6)] have shown that reduction with potassium tri-*sec*-butylborohydride (K-Selectride, Aldrich) is expected to give a satisfactory result with respect to regioselectivity





1 : Δ^4, R=H
2 : Δ^4, R=Ac
4 : 5α, R=Ac
5 : 5α, R=H
6 : 5α, R=THP

allo-THF : R=R′=H
7 : R=H, R′=THP
8 : R=Ac, R′=THP
9 : R=Ac, R′=H
12 : R=TBDMS, R′=THP
13 : R=TBDMS, R′=H
14 : R=TBDMS, R′=Ac
16 : R=H, R′=Ac



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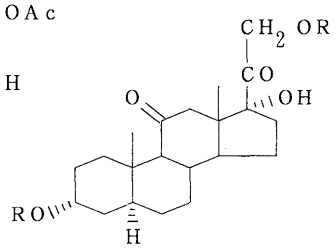


allo-THE : R=R′=H
10 : R=Ac, R′=THP
11 : R=Ac, R′=H
15 : R=TBDMS, R′=Ac
17 : R=H, R′=Ac

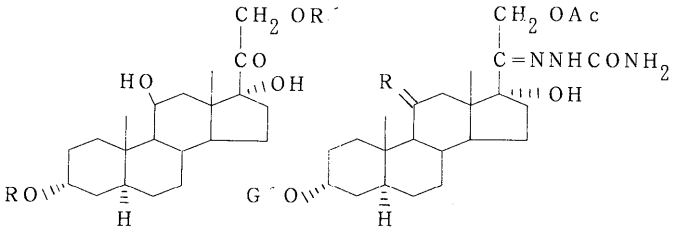
TBDMS = $-Si(CH_3)_2C(CH_3)_3$

Chart 1

between the carbonyl groups at C-3 and C-20 as well as stereoselectivity at C-3. Reaction of **6** with 1.5 eq of K-Selectride in dry tetrahydrofuran at −78 °C and a subsequent alkaline hydrogen peroxide work-up afforded the desired **7** in 72% yield. The large bulk of the tetrahydropyranyl group at C-21 should participate in the regioselectivity. The stereochemistry at C-3 was confirmed after derivatization of **7** into **9**.

On acetylation with acetic anhydride in pyridine, **7** was converted into the 3-acetate (**8**). Removal of the protecting group at C-21 in **8** furnished the desired intermediate (**9**). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **9** showed a characteristic signal assignable to the equatorial 3β-proton. Oxidation of **8** was then carried out with pyridinium chlorochromate in methylene chloride to give the 11-ketone (**10**), which, on removal of the protecting group at C-21 with acetic acid, was converted into allo-THE 3-acetate (**11**).

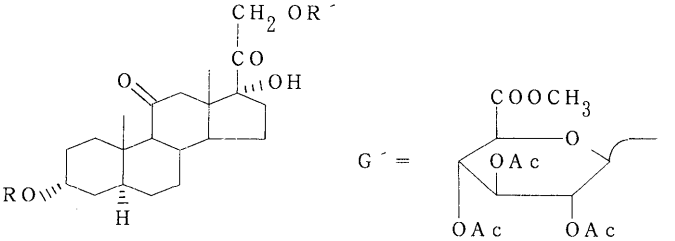
Allo-THF 21-acetate (**16**) and allo-THE 21-acetate (**17**) were then prepared. Treatment of the 3α-alcohol (**7**) with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide–pyridine gave the 3-silyl ether (**12**). When **12** was treated with 90% acetic acid, selective deprotection at C-21 took place to give allo-THF 3-*tert*-butyldimethylsilyl ether (**13**) in a satisfactory yield. Acetylation of **13** with acetic anhydride in pyridine gave the 3-silyl ether-21-acetate (**14**). On desilylation with 1% hydrofluoric acid in acetonitrile, **14** was converted into the desired **16** (53% yield from **7**). Oxidation of **14** with pyridinium chlorochromate in methylene chloride followed by desilylation with hydrofluoric acid gave **17**. For the preparation of **16**, the K-Selectride reduction of **4** seems also to be worthy of examination, since the alkaline hydrogen peroxide work-up is not always necessary.[6]



18 : R=G′, R′=Ac
20 : R=G, R′=H
24 : R=Ac, R′=G′
26 : R=H, R′=G

19 : R=OH (α) / H (β)

22 : R=O



21 : R=G′, R′=Ac
23 : R=G, R′=H
25 : R=Ac, R′=G′
27 : R=H, R′=G

G′ =

G =

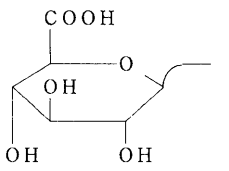


Chart 2

Finally, the preparations of allo-THF 3-glucuronide (**20**), allo-THE 3-glucuronide (**23**), allo-THF 21-glucuronide (**26**), and allo-THE 21-glucuronide (**27**) were carried out. Introduction of the glucuronyl residue into **16** and **17** 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 (**18**, **21**). Prior to saponification of **18** and **21**, the alkali-sensitive side chain at C-17 was protected by derivatization into the 20-semicarbazone (**19**, **22**). Sequential removal of the protecting groups was carried out by treatment with methanolic potassium hydroxide, and then with pyruvic acid–acetic acid to give **20** and **23** in satisfactory yields. The very polar glucuronides were isolated by the solid-phase extraction method using Amberlite XAD-2 as an adsorbent. The 21-glucuronides (**26**, **27**) were obtained by the Koenigs–Knorr reaction of **9** and **11** 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 **18**, **21**, **24** and **25**, the anomeric proton of the sugar moiety resonates in the range of 4.55—4.71 ppm as a doublet of J=7 Hz, showing β-configuration of the anomeric center. In the case of the free glucuronides (**20**, **23**, **26**, **27**), 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.

We have previously prepared the glucuronides of related cortisol[5] and 11-deoxycortisol[6,10] metabolites. Recently, liquid chromatography-mass spectrometry has been applied to the analysis of polar compounds. Thus, 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}H-NMR spectra were taken with a JEOL JNM-FX-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard.

5α-Dihydrocortisol (5) i) A mixture of cortisol 21-acetate (**2**) (2.2 g, 5.4 mmol), triethyl orthoformate (5 ml, 30 mmol) and *p*-toluenesulfonic acid (60 mg, 0.3 mmol) in EtOH (2.5 ml)–tetrahydrofuran (25 ml) was vigorously stirred at room temperature for 4.5 h. After an addition of pyridine (2 ml), the mixture was diluted with AcOEt. The organic layer was washed with 5% $NaHCO_3$, dried over anhydrous Na_2SO_4, and evaporated down. Purification of the product by column chromatography on silica gel (30 g) with hexane–AcOEt (1 : 2) as an eluent gave the 3,5-diene (**3**) (1.5 g). This was dissolved in AcOEt (6 ml)–EtOH (6 ml) and the solution was stirred under a hydrogen gas stream for 14 h at atmospheric pressure in the presence of 5% Pd–C (130 mg). After addition of AcOEt (50 ml) followed by removal of the catalyst by filtration, the filtrate was treated with 2 N HCl (2 ml). The organic layer was washed with 5% $NaHCO_3$, dried over anhydrous Na_2SO_4, and evaporated down. The crude product obtained was recrystallized from acetone–hexane to give 5α-dihydrocortisol 21-acetate (**4**) (385 mg) as colorless prisms. mp 222—223 °C (lit.[7] mp 220—224 °C). This was freed from 5β-dihydrocortisol 21-acetate.[5a]

Chromatography of the mother liquor on silica gel using hexane–AcOEt (1 : 1) as an eluent gave an additional amount of **4** (270 mg, total 30%). Saponification of **4** with NaOH in MeOH gave **5**. mp 231—235 °C (lit.[7] mp 230—233 °C).

ii) Hydrogenation of 3,11β,17α,21-tetrahydroxy-3,5-pregnadien-20-one 3-methyl ether 17,21-acetonide[11] (1.2 g) and acid hydrolysis of the product were carried out in the manner described above. 5α-Dihydrocortisol was isolated as its 21-tetrahydropyranyl ether (**6**) (600 mg, 46%).

5α-Dihydrocortisol 21-Tetrahydropyranyl Ether (6) A mixture of **5** (800 mg, 2.2 mmol), 2,3-dihydropyran (2 ml, 22 mmol), and pyridinium *p*-toluenesulfonate (110 mg, 0.4 mmol) in CH_2Cl_2 (20 ml) was stirred at room temperature for 2 h. The resulting solution was concentrated to half of its initial volume under reduced pressure, diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from acetone–hexane gave **6** (750 mg, 76%) as colorless leaflets. mp 185—188 °C. $[\alpha]_D^{20}$ +18° (c=0.5). *Anal.* Calcd for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99. Found: C, 69.25; H, 8.92.

Allo-THF 21-Tetrahydropyranyl Ether (7) A solution of **6** (360 mg, 0.80 mmol) and K-Selectride (1 M in tetrahydrofuran, 1 ml) in dry tetrahydrofuran (2.5 ml) was stirred for 20 min at −78 °C under a nitrogen atmosphere, and then additional K-Selectride (0.1 ml) was added. After 1 h, the resulting solution was treated with 30% H_2O_2 (0.3 ml) and 10% NaOH (0.3 ml). The reaction 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 (20 g). Elution with hexane–AcOEt (2 : 5) and recrystallization of the product from acetone–hexane gave **7** (260 mg, 72%) as colorless leaflets. mp 182—184 °C. $[\alpha]_D^{20}$ +29° (c=0.5). *Anal.* Calcd for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40. Found: C, 69.07; H, 9.49.

3α-Acetoxy-11β,17α,21-trihydroxy-5α-pregnan-20-one 21-Tetrahydropyranyl Ether (8) A solution of **7** (135 mg, 0.30 mmol) and acetic anhydride (0.5 ml) in pyridine (1 ml) was allowed to stand overnight at room temperature. After addition of H_2O, the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue was chromatographed on silica gel. Elution with hexane–AcOEt (3 : 2) and recrystallization of the product from hexane–AcOEt gave **8** (125 mg, 85%) as colorless needles. mp 110—113 °C. $[\alpha]_D^{15}$ +46° (c=0.4). *Anal.* Calcd for $C_{28}H_{44}O_7 \cdot 1/4H_2O$: C, 67.64; H, 9.02. Found: C, 67.37; H, 9.01.

Allo-THF 3-Acetate (9) A solution of **8** (760 mg, 1.5 mmol) in 60% AcOH (4 ml) was allowed to stand at 37 °C for 2 d. 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 residue obtained was chromatographed on silica gel. Elution with hexane–AcOEt (1 : 2) and recrystallization of the product from MeOH gave **9** (440 mg, 70%) as colorless plates. mp 228—230 °C. ^{1}H-NMR ($CDCl_3$–CD_3OD (10 : 1)) δ: 0.84 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.04 (3H, s, 3-$OCOCH_3$), 4.25, 4.59 (each 1H, d, J=19 Hz, 21-H), 4.38 (1H, m, 11α-H), 4.98 (1H, m, 3β-H). *Anal.* Calcd for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.49; H, 9.14.

Allo-THE 3-Acetate (11) A mixture of **8** (50 mg, 0.10 mmol) and pyridinium chlorochromate (100 mg, 0.46 mmol) in CH_2Cl_2 (1 ml) was stirred at room temperature for 1 h. After addition of ether, the resulting solution was passed through Florisil on a sintered-glass funnel, and the filtrate was evaporated down. The oily residue was chromatographed on silica gel using benzene–ether (2 : 1) as an eluent, yielding the 11-ketone (**10**) (40 mg, 80%) as semi-crystals. Hydrolysis of **10** in the same manner as with **8** and recrystallization of the crude product from acetone–hexane gave **11** as colorless leaflets. mp 187—190 °C. $[\alpha]_D^{14}$ +67° (c=0.5). ^{1}H-NMR ($CDCl_3$–CD_3OD (10 : 1)) δ: 0.56 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.05 (3H, s, 3-$OCOCH_3$), 4.18, 4.59 (each 1H, d, J=19 Hz, 21-H), 4.96 (1H, m, 3β-H). *Anal.* Calcd for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.82; H, 8.27.

Allo-THF 3-*tert*-Butyldimethylsilyl Ether (13) A solution of **7** (200 mg, 0.44 mmol), imidazole (600 mg, 8.8 mmol), and *tert*-butyldimethylsilyl chloride (300 mg, 2.0 mmol) in pyridine (0.2 ml)–dimethylformamide (0.4 ml) was allowed to stand at room temperature for 4 h. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The oily product (**12**) obtained was dissolved in 90% AcOH (4 ml) and the solution was stirred at room temperature for 4 h. After addition of H_2O followed by neutralization with NaOH, 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 (20 g) using hexane–AcOEt (1 : 1). Recrystallization of the crude product from acetone–hexane gave **13** (140 mg, 66%) as colorless needles. mp 184—186 °C. $[\alpha]_D^{20}$ +39° (c=0.5). ^{1}H-NMR ($CDCl_3$) δ: 0.02 (6H, s, 3-$OSi(CH_3)_2$), 0.87 (12H, s, 18-CH_3, 3-OSi-*tert*-Bu), 1.00 (3H, s, 19-CH_3), 3.94 (1H, m, 3β-H), 3.1—3.8 (3H, 11α-, 21-H). *Anal.* Calcd for $C_{27}H_{48}O_5$ Si: C, 67.45; H, 10.06. Found: C, 67.19; H, 10.22.

21-Acetoxy-3α,11β,17α-trihydroxy-5α-pregnan-20-one 3-*tert*-Butyldimethylsilyl Ether (14) Acetylation of **13** (130 mg, 0.27 mmol) was carried out in the manner described for **8**. After the usual work-up, the crude product was recrystallized from MeOH to give **14** (115 mg, 81%) as colorless needles. mp 137—139 °C. $[\alpha]_D^{15}$ +69° (c=0.5). ^{1}H-NMR ($CDCl_3$) δ: 0.02 (6H, s, 3-$OSi(CH_3)_2$), 0.88 (12H, s, 18-CH_3, 3-OSi-*tert*-Bu), 1.00 (3H, s, 19-CH_3), 2.16 (3H, s, 21-$OCOCH_3$), 3.95 (1H, m, 3β-H), 4.44 (1H, m, 11α-H), 4.77, 5.09 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{29}H_{50}O_6Si \cdot 3/4H_2O$: C, 64.95; H, 9.68. Found: C, 65.12; H, 9.68.

21-Acetoxy-3α,21-dihydroxy-5α-pregnane-11,20-dione 3-*tert*-Butyldimethylsilyl Ether (15) Oxidation of **14** (50 mg, 0.10 mmol) with pyridinium chlorochromate was carried out in the manner described for **11**. After the usual work-up, the product obtained was recrystallized from MeOH to give **15** (40 mg, 80%) as colorless needles. mp 189—191 °C. $[\alpha]_D^{20}$ +78° (c=0.5). ^{1}H-NMR ($CDCl_3$) δ: 0.02 (6H, s, 3-$OSi(CH_3)_2$), 0.63 (3H, s, 18-CH_3), 0.88 (9H, s, 3-OSi-*tert*-Bu), 0.99 (3H, s, 19-CH_3), 2.16 (3H, s, 21-$OCOCH_3$), 3.94 (1H, m, 3β-H), 4.62, 5.13 (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, 66.30; H, 9.18.

Allo-THF 21-Acetate (16) A solution of **14** (100 mg, 0.19 mmol) and 47% HF (60 μl) in acetonitrile (3 ml) was allowed to stand at room temperature for 20 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 MeOH gave **16** (77 mg, 99%) as colorless needles. mp 217—220 °C (lit.[7] mp 221—223 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 2.17 (3H, s, 21-$OCOCH_3$), 4.05 (1H, m, 3β-H), 4.46 (1H, m, 11α-H), 4.78, 5.11 (each 1H, d, J=18 Hz, 21-H).

Allo-THE 21-Acetate (17) Hydrolysis of **15** (100 mg, 0.19 mmol) with HF was carried out in the manner described for **16**. Recrystallization of the product from MeOH gave **17** (70 mg, 90%) as colorless needles. mp 217—221 °C. $[\alpha]_D^{15}$ +61° (c=0.4). ^{1}H-NMR ($CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.15 (3H, s, 21-$OCOCH_3$), 4.04 (1H, m, 3β-H), 4.55, 5.20 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{23}H_{34}C_6$: C, 67.95; H, 8.43. Found: C, 67.81; H, 8.66.

Methyl (21-Acetoxy-11β,17α-dihydroxy-20-oxo-5α-pregnan-3α-yl-2′, 3′,4′-tri-*O*-acetyl-β-D-glucopyranosid)uronate (18) Freshly prepared Ag_2CO_3 (700 mg, 2.5 mmol) and methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate (1.2 g, 3.0 mmol) were added to a solution of **16** (220 mg, 0.54 mmol) in toluene (10 ml), and the suspension was stirred at room temperature for 5 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 (80 g) with hexane–AcOEt (1 : 1) as an eluent, yielding a mixture of **18** and a sugar derivative. Separation of these products was achieved after acetylation of the latter compound. Repurification by chromatography using hexane–AcOEt (1 : 1) and recrystallization of the product from acetone–hexane gave **18** (110 mg, 28%) as colorless leaflets. mp 223—225 °C. $[\alpha]_D^{25}$ +16° (c=0.9). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.02, 2.04, 2.16 (12H, –$OCOCH_3$), 3.75 (3H, s, –$COOCH_3$), 3.8—4.1 (2H, 3β-, 5′-H), 4.35 (1H, m, 11α-H), 4.5—5.3 (6H, 1′-, 21-, 2′-, 3′-, 4′-H). *Anal.* Calcd for $C_{36}H_{52}O_{15} \cdot H_2O$: C, 58.21; H, 7.33. Found: C, 58.44; H, 7.06.

Allo-THF 3-Glucuronide (20) A mixture of **18** (150 mg, 0.21 mmol), semicarbazide·HCl (530 mg, 4.8 mmol) and AcONa (500 mg, 6.1 mmol) in MeOH (4 ml) was stirred at room temperature for 2 d. 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 was chromatographed on silica gel (25 g) with AcOEt as an eluent to give the semicarbazone (**19**). This was dissolved in 2% methanolic KOH (3 ml) and allowed to stand at room temperature for 14 h. After addition of H_2O followed by neutralization with AcOH, the resulting solution was evaporated down under reduced pressure. The residue was dissolved in 80% pyruvic acid (1.5 ml)–AcOH (1 ml)–$CHCl_3$ (4 ml) and the solution was stirred at room temperature for 14 h. After removal of the organic solvent followed by the addition of H_2O, the resulting solution 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 : 10 : 2 : 0.1) as an eluent, and then on Amberlite XAD-2. Recrystallization of the product from MeOH–AcOEt gave **20** (48 mg, 43%) as colorless

leaflets. mp 195 °C (dec.) $[\alpha]_D^{25}$ +44° (c=0.9, MeOH). ^{1}H-NMR (CD_3OD) δ: 0.80 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.92 (1H, m, 3β-H), 4.1—4.8 (4H, 1'-, 11α-, 21-H). *Anal.* Calcd for $C_{27}H_{42}O_{11}$: C, 59.76; H, 7.80. Found: C, 59.57; H, 7.82.

Methyl (21-Acetoxy-17α-hydroxy-11,20-dioxo-5α-pregnan-3α-yl-2',3',4'-tri-*O*-acetyl-β-D-glucopyranosid)uronate (21) The Koenigs–Knorr reaction of **17** (150 mg, 0.37 mmol) and purification were carried out in the manner described for **18**. Recrystallization of the product from acetone–hexane gave **21** (115 mg, 43%) as colorless needles. mp 205—209 °C. $[\alpha]_D^{23}$ +33° (c=0.9). ^{1}H-NMR ($CDCl_3$) δ: 0.59 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 2.02, 2.04, 2.16 (12H, –OCOCH_3), 3.75 (3H, –COOCH_3), 3.8—4.1 (2H, 3β-, 5'-H), 4.5—5.3 (6H, 1'-, 21-, 2'-, 3'-, 4'-H). *Anal.* Calcd for $C_{36}H_{50}O_{15}$: C, 59.82; H, 6.97. Found: C, 59.76; H, 6.86.

Allo-THE 3-Glucuronide (23) Removal of the protecting groups in **21** (210 mg, 0.29 mmol) *via* the semicarbazone (**22**) and purification were carried out in the manner described for **20**, yielding **23** (95 mg, 61%). mp 195 °C (dec.) (colorless leaflets from MeOH–AcOEt). ^{1}H-NMR (CD_3OD) δ: 0.53 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 3.92 (1H, m, 3β-H), 4.0—5.5 (3H, 1'-, 21-H). *Anal.* Calcd for $C_{27}H_{40}O_{11}\cdot 1/2H_2O$: C, 59.00; H, 7.52. Found: C, 58.73; H, 7.74.

Methyl (3α-Acetoxy-11β,17α-dihydroxy-20-oxo-5α-pregnan-21-yl-2',-3',4'-tri-*O*-acetyl-β-D-glucopyranosid)uronate (24) The Koenigs–Knorr reaction of **9** (210 mg, 0.51 mmol) and purification were carried out in the manner described for **18**, using benzene–ether (1:2) as an eluent for repurification chromatography. Recrystallization of the product from ether–hexane gave **24** (200 mg, 54%) as colorless leaflets. mp 107—110 °C. $[\alpha]_D^{15}$ +14° (c=0.4). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 2.00, 2.02, 2.04, 2.10 (12H, –OCOCH_3), 3.73 (3H, s, –COOCH_3), 4.00 (1H, m, 5'-H), 4.3—4.9 (4H, 11α-, 21-, 1'-H), 4.9—5.3 (4H, 3β-, 2'-, 3'-, 4'-H). *Anal.* Calcd for $C_{36}H_{52}O_{15}$: C, 59.63; H, 7.23. Found: C, 59.62; H, 7.31.

Methyl (3α-Acetoxy-17α-hydroxy-11,20-dioxo-5α-pregnan-21-yl-2',3',4'-tri-*O*-acetyl-β-D-glucopyranosid)uronate (25) The Koenigs–Knorr reaction of **11** (250 mg, 0.61 mmol) and purification were carried out in the manner described for **24**. Recrystallization of the product from acetone–hexane gave **25** (210 mg, 47%) as colorless leaflets. mp 235—239 °C. $[\alpha]_D^{19}$ +16° (c=0.7). ^{1}H-NMR ($CDCl_3$) δ: 0.58 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.0—2.1 (12H, –OCOCH_3), 3.75 (3H, s, –COOCH_3), 4.02 (1H, m, 5'-H), 4.30 (1H, d, J=18 Hz, one of 21-H), 4.63 (1H, d, J=7 Hz, 1'-H), 4.75 (1H, d, J=18 Hz, one of 21-H), 4.9—5.3 (4H, 3β-, 2'-, 3'-, 4'-H). *Anal.* Calcd for $C_{36}H_{50}O_{15}$: C, 59.82; H, 6.97. Found: C, 59.52; H, 6.77.

Allo-THF 21-Glucuronide (26) A solution of **24** (120 mg, 0.17 mmol) in 2% methanolic KOH (15 ml) was stirred at room temperature for 12 h. After removal of the MeOH followed by the addition of H_2O, the resulting mixture was extracted with AcOEt. The aqueous layer was acidified with AcOH, and subjected to column chromatography on Amberlite XAD-2. Elution with EtOH gave the crude product, which was chromatographed on silica gel (8 g) with $CHCl_3$–MeOH–H_2O–AcOH (80:20:2:0.1) as an eluent, and then on Amberlite XAD-2, yielding **26** (80 mg, 89%). mp 186 °C (dec.) (colorless leaflets from MeOH–ether). $[\alpha]_D^{15}$ +16° (c=0.5, EtOH). ^{1}H-NMR (CD_3OD) δ: 0.82 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 3.93 (1H, m, 3β-H), 4.2—5.1 (4H, 11α-, 21-, 1'-H). *Anal.* Calcd for $C_{27}H_{42}O_{11}\cdot 3/2H_2O$: C, 56.93; H, 7.96. Found: C, 56.71; H, 7.67.

Allo-THE 21-Glucuronide (27) Saponification of **25** (270 mg, 0.37 mmol) with 2% KOH and purification were carried out in the manner described for **26**, yielding **27** (140 mg, 69%). mp 195 °C (dec.) (colorless leaflets from MeOH–AcOEt). $[\alpha]_D^{15}$ +17° (c=0.5, EtOH). ^{1}H-NMR (CD_3OD) δ: 0.56 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 3.93 (1H, m, 3β-H), 4.2—5.1 (3H, 21-, 1'-H). *Anal.* Calcd for $C_{27}H_{40}O_{11}\cdot 3/2H_2O$: C, 57.13; H, 7.64. Found: C, 57.04; H, 7.24.

References and Notes

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