Glucosiduronates of 3α,21-Dihydroxy-5β-pregnane-11,20-dione. Synthesis of C-3, C-21, and C-3,21 Derivatives[1]

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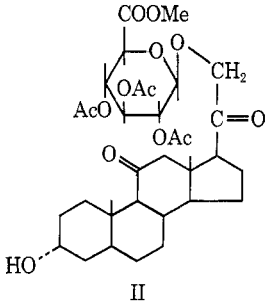
On treatment with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate and Ag_2CO_3, 3α,21-dihydroxy-5β-pregnane-11,20-dione (I) yielded the corresponding steroidal 21-glucosiduronate (II), the 3,21-diglucosiduronate (III), the 21-glucosiduronate 3-glucuronosyl orthoacetate (IV), and the 3-glucosiduronate (V) as methyl ester acetates. In dilute methanolic HCl, IV was converted into II. Acetyl groups were removed from II, III, and V in methanol containing a catalytic amount of NaOH, and corresponding crystalline methyl esters VI, VII, and VIII were obtained. Alkaline hydrolysis of esters VI and VII followed by acidification gave the corresponding glucosiduronic acids. The 3-glucosiduronate (V) was converted into the C-20 semicarbazone (IX) to stabilize the ketolic group during alkaline hydrolysis; alkaline cleavage of the ester groups in IX followed by hydrolysis of the semicarbazone group at pH 2.1 gave 21-hydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-glucopyranosiduronic acid in 90% yield. Treatment of the 21-acetate of I with Ag_2CO_3 in benzene gave the 3-oxo derivative of I and showed that oxidation may occur as a side reaction in the Koenigs–Knorr procedure.

One of the principal pathways of metabolism of the adrenocortical hormones in man involves reduction of the 3-oxo-Δ^4 function to the 3α-hydroxy-5β-pregnane



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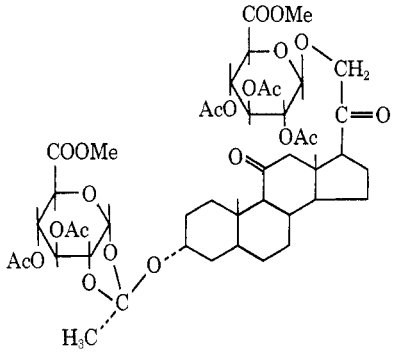

IV

structure. Subsequently, the 3α-hydroxy metabolite is joined to glucuronic acid and the conjugate is excreted in the urine. Although 3α-hydroxy-5β-pregnanes which have either a ketolic side chain or a dihydroxyacetone function at C-17 are excreted principally as the C-3 glucosiduronic acids,[2,3] small amounts of C-21 conjugates may also be present.[4,5] 3α,21-Dihydroxy-5β-pregnane-11,20-dione (**1**), a metabolite of corticosterone, occurs in human urine as a conjugate[6] with glucuronic acid; however, the position(s) of attachment of the conjugating group to the steroid has not been established unequivocally. In this paper we describe the synthesis of the C-3, the C-21, and the C-3,21 β-D-glucosyluronic acid derivatives of **1**.

Treatment of the 3,21-dihydroxy compound **1** with 3 eqiuv of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (hereafter referred to as methyl acetobromoglucuronate) under conditions reported previously[7] gave a mixture which was fractionated by column chromatography to yield four substances: the C-21 glucosiduronate (**4**), the C-3,21 diglucosiduronate (**12**), the C-3 orthoacetate of the C-21 glucosiduronate (**17**), and the C-3 glucosiduronate (**18**).

The β configuration for the glucosidic linkage in compounds **4**, **12**, **17**, **18**, and **19** is suggested by the mode of synthesis,[7] by the agreement of calculated and observed molecular rotations of the compounds (Table I), and by the hydrolysis of the glucosidic linkage of the corresponding free glucosiduronic acids (**7**, **14**, and **21**) with β-glucuronidase. In addition, compounds **4** and **12** lack the nmr doublet ($\delta \cong 5.1$; $J_{1',2'} \cong 3.2$ Hz) which is characteristic[8] of acetylated α-glycosides.

TABLE I

MOLECULAR ROTATIONS OF CONJUGATES

Compd	Calcd, deg[a] α-Glycoside	Calcd, deg[a] β-Glycoside	Found, deg
4	+950	+244	+159
5	+1058	+352	+276
12	+1555	+143	+98
18	+950	+244	+283
19	+1027	+321	+381

[a] Values calculated as previously described.[7]

The C-21 glucosiduronate (**4**) was obtained from diol **1** in yields of 32–38%. This conjugate (**4**), as well as orthoacetate **17** which is derived from it, must have the glucosiduronate function at C-21 because it (**4**) does not reduce alkaline tetrazolium blue in the manner typical of α-ketolic steroids. However, compounds **4**, **12**, and **17** give a yellow color with this reagent, a response characteristic of the C-21 glucosiduronates of 11-dehydrocorticosterone and 11-deoxycorticosterone.[9]

(1) (a) This investigation was supported in part by Research Grant AM-5452 from the National Institutes of Health, Public Health Service. (b) Part of this investigation was presented at the 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill., April 16–21, 1967, Abstract No. 945.

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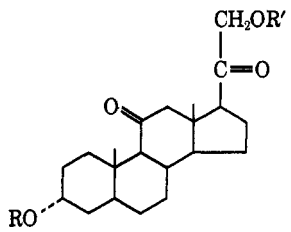
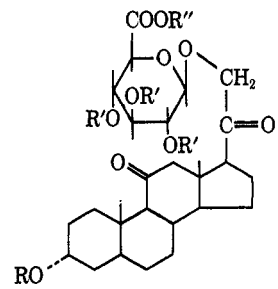
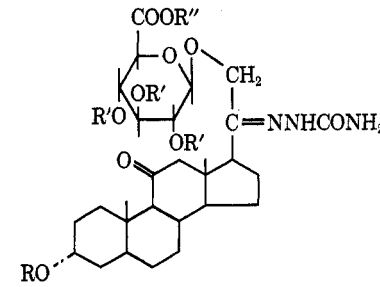


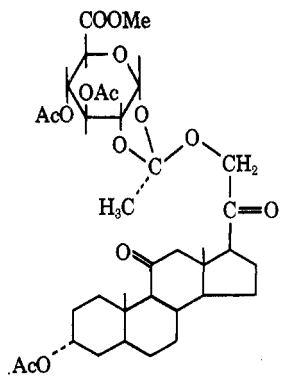
	R	R′
1	H	H
2	Ac	H
3	H	Ac



	R	R′	R″
4	H	Ac	Me
5	Ac	Ac	Me
6	H	H	Me
7	H	H	H
8	H	H	NH_4



	R	R′	R″
9	H	Ac	Me
10	Ac	Ac	Me



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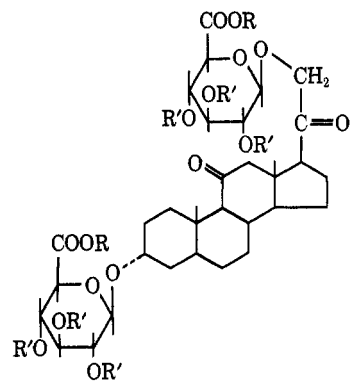
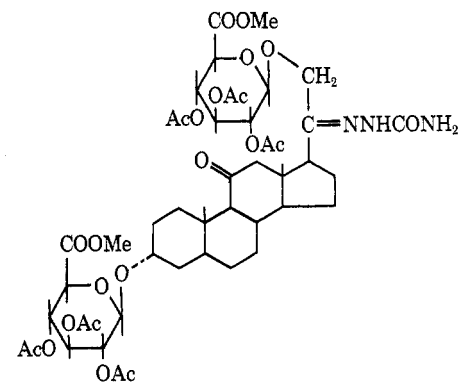


	R	R′
12	Me	Ac
13	Me	H
14	H	H
15	NH_4	H



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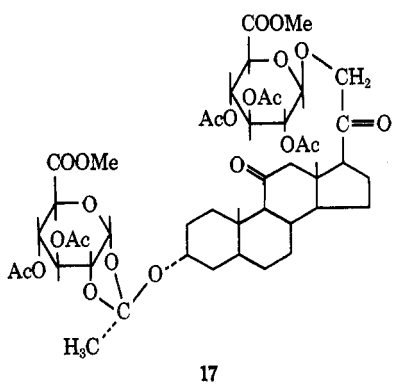

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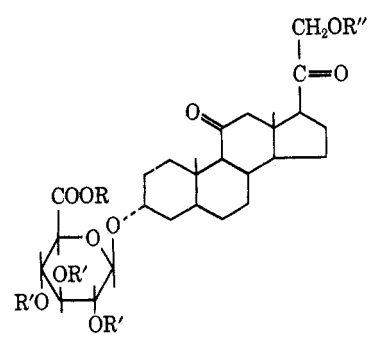
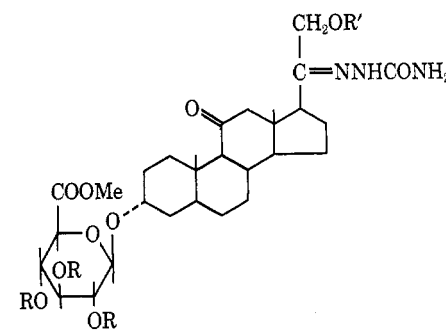
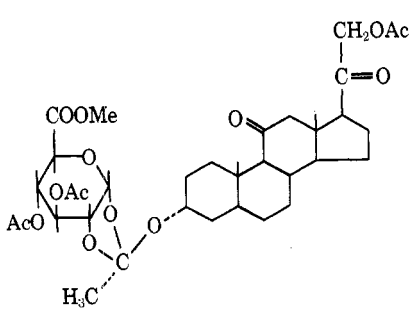


	R	R′	R″
18	Me	Ac	H
19	Me	Ac	Ac
20	Me	H	H
21	H	H	H



	R	R′
22	Ac	H
23	Ac	Ac
24	H	H



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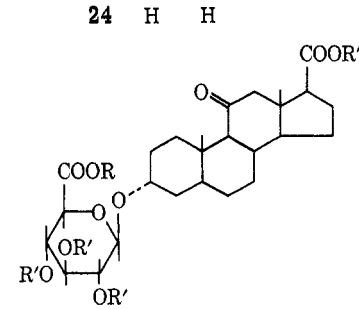

	R	R′	R″
26	H	H	H
27	Me	Ac	H
28	Me	Ac	Me

Conjugate **18**, obtained from **1** in 17% yield, has the glucosiduronate group at C-3; the substance reduces alkaline tetrazolium blue and is oxidizable by a catalytic amount of cupric acetate[10] to a product which gives a positive reaction with the Porter–Silber reagent. In addition, acetylation of **18** gave the C-21 acetate (**19**) which could be prepared in 49% yield by treatment of 21-acetoxy-3α-hydroxy-5β-pregnane-11,20-dione (**3**) with methyl acetobromoglucuronate.

Efforts to crystallize the steroidal C-3,21 diglucosiduronate (**12**) after its preparation from diol **1** or from the C-21 monoglucosiduronate (**4**) were not successful; however, the amorphous diglucosiduronate (**12**) gave a crystalline C-20 semicarbazone (**16**) in 16–18% yield from **1**. The semicarbazone could be converted to crystalline 20-oxo diglucosiduronate (**12**) by treatment[7] with pyruvic acid. Compounds **4**, **5**, **18**, **19**, and **20** also reacted with semicarbazide to give the corresponding semicarbazones which, on treatment with pyruvic acid, were converted to the 20-oxo starting materials.

The acetyl groups were removed from compounds **4**, **12**, **18**, and **19** by transesterification in methanol in the presence of dilute sodium methylate or sodium hydroxide, and the corresponding methyl esters were obtained in good yield. Removal of acetyl groups from a glucosiduronate moiety at C-3 occurs more slowly than from one at C-21.

Treatment of the C-21 glucosiduronate esters (**6** and **13**) with either ammonium hydroxide or sodium hydroxide followed by acidification gave the crystalline glucosiduronic acids (**7** and **14**). These acids were convertible back to the corresponding methyl esters (**6** and **13**) by treatment with diazomethane and to the parent dihydroxy steroid (**1**) by reaction with β-glucuronidase (Ketodase). The acetyl methyl ester glucosiduronates (**4** and **12**) were convertible to the corresponding free acids (**7** and **14**) in yields of about 80% without isolating the intermediate methyl esters.

When the acetylated glucosiduronate ester **19** was hydrolyzed with sodium hydroxide and the product was chromatographed on Celite in the presence of EDTA,[10] glucosiduronic acid **21** was obtained in 47% yield along with some etianic acid (**26**) which was formed by alkaline cleavage[11] of the ketolic side chain. In the absence of EDTA, the copper which was eluted from the Celite caused oxidation of the ketolic group, and glucosiduronic acid **21** could not be crystallized. When sodium bicarbonate was used to hydrolyze ester **20**, glucosiduronic acid **21** was obtained in 66% yield.

The structure of etianic acid **26** was demonstrated by the following transformations. Reaction of the ketolic conjugate **18** with periodate yielded acid **27**, which, on treatment with diazomethane, gave ester **28**. Similarly, the etianic acid **26** was converted into methyl ester triacetate **28** by esterification with diazomethane followed by acetylation. In addition, the 17-carboxy steroidal conjugate (**27**) could be hydrolyzed with alkali to produce **26**.

Wendler, *et al.*,[12] showed indirectly that the α-ketolic side chain of a steroid is stable toward alkali when this function is converted into a semicarbazone. We observed that, when semicarbazone **22** or **23** was treated with methanolic alkali, methyl ester semicarbazone **24** could be isolated in high yield. Reaction of this compound (**24**) with aqueous alkali followed by adjustment of the pH to 2.1 to hydrolyze the semicarbazone group gave crystalline glucosiduronic acid **21**. The yield of acid **21** from the acetate ester semicarbazone **23**, without isolation of the intermediates, was 88%. Removal of the semicarbazone function was also achieved by using the cation exchange resin, Dowex 50W-2X.

As a by-product of the Koenigs–Knorr reaction[13] orthoacetate **17** was recovered in 4% yield when dihydroxy steroid **1** was treated with methyl acetobromoglucuronate. In the preparation of diglucosiduronate **12** from monoglucosiduronate **4**, orthoacetate **17** was obtained in 9% yield. In addition to the function at C-21, this substance has a glucosyluronate group attached to the steroid at C-3 through an orthoacetate structure which involves the acetyl group at C-2 of the carbohydrate, an α linkage to C-1 of the carbohydrate, and the oxygen of the 3α-hydroxyl group of the steroid. The structure of orthoacetate **17** was suggested by its facile conversion to the C-21 glucosiduronate **4** during treatment with dilute acid,[13c] and by the analytical values for C, H, CH_3O, and CH_3CO. The structure was confirmed by the nmr spectrum,[13d,e] which has a band (δ 1.74 ppm; 3 protons) that is characteristic of the methyl group of an orthoacetate in the endo configuration relative to the carbohydrate group. This characteristic band was not present in either the C-21 glucosiduronate (**4**) or the C-3,21 diglucosiduronate (**12**).

In the preparation of the C-21 glucosiduronate (**5**) from the 21-hydroxy steroid (**2**), the corresponding steroid 21-yl glucuronylene orthoacetate (**11**) was obtained in 7% yield as a by-product. Similarly, orthoacetate **25** was obtained in 22% yield during preparation of **19**. These orthoacetates demonstrated characteristic spectral and chemical properties analogous to those of orthoacetate **17**.

Oxidation of a hydroxyl group to a ketone may occur in the Koenigs–Knorr reaction, as is shown by formation of 21-acetoxy-5β-pregnane-3,11,20-trione from **3** during the preparation of **19**. This 3,11,20-trione also was formed, and isolated in a yield of 47%, by the action of silver carbonate on the 3-hydroxy compound **3** in boiling benzene in the absence of methyl acetobromoglucuronate. It was reported[14] recently that primary and secondary alcohols are oxidized readily by silver carbonate on Celite in neutral media.

Experimental Section

Elemental analyses were carried out by Mr. Joseph F. Alicino, Metuchen, N. J.; samples were dried at 100° *in vacuo* immediately before analysis. Melting points were taken on a Fisher-Johns apparatus and are corrected. A substance synthesized by an optional procedure was identified by comparison of its melting point and ir spectrum with that of the authentic compound and by performing a mixture melting point determination. Infrared spectra were recorded with a Beckman IR-18 spectro-

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photometer; nmr measurements were made with a Varian A-60 spectrometer with deuteriochloroform as solvent and tetramethylsilane as internal standard. Rotations were taken at 26° ± 1° (c ~1). Evaporations were performed *in vacuo* on a rotary evaporator at a bath temperature of 40°. For column chromatography, Celite 545 was used as received from Johns-Manville and was impregnated with 40% of its weight of stationary (more dense) phase, using the solvent systems listed below. Holdback volume (HBV) represents the volume of the mobile phase retained by the packed portion of the column; the elution volume of a compound is expressed in HBV. Paper chromatography was performed as described previously;[7] Zaffaroni technique was used with systems S1–S7 and Bush technique was used for systems S8–S14. Semicarbazones were detected by viewing chromatograms over 254-mμ radiation or by treating the chromatograms with 5% ethanolic phosphomolybdic acid; other compounds were detected by treating the chromatograms with alkaline tetrazolium blue.[15] To detect acidic compounds which were not revealed by previously mentioned techniques, chromatograms were sprayed with a 0.04% solution of chlorophenol red in alcohol.[16]

Thin layer chromatography (tlc) was performed on silica gel G in 1:1 benzene–ether; compounds were detected by spraying the plates with water–sulfuric acid, 1:1, and charring.

Systems for Paper and Column Chromatography.—S1 = benzene–cyclohexane (25:75)–formamide–carbitol (1:1); S2 = benzene–cyclohexane (20:80)–formamide–carbitol (1:1); S3 = benzene–cyclohexane (25:75)–formamide; S4 = benzene–cyclohexane (50:50)–formamide; S5 = benzene–cyclohexane (75:25)–formamide; S6 = benzene–formamide; S7 = butyl acetate–formamide; mobile phase is butyl acetate saturated with formamide–water (1:1); S8 = cyclohexane–methanol–water (500:400:100); S9 = toluene–ethyl acetate–methanol–water (900:100:500:500); S10 = butyl acetate–butyl alcohol–water–acetic acid (90:10:90:10); S11 = butyl acetate–butyl alcohol–water–acetic acid (50:50:90:10); S12 = butyl acetate–butyl alcohol–water–acetic acid (25:75:90:10); S13 = butyl acetate–toluene–methanol–water–acetic acid (50:50:50:45:5); S14 = toluene–butyl acetate–methanol–water (25:75:50:50).

3α,21-Dihydroxy-5β-pregnane-11,20-dione (1).—Treatment of 1.0-mg amounts of **7**, **14**, and **21** in separate flasks with 25,000 units of β-glucuronidase (Ketodase) under conditions previously described[9] gave **1**, which was identified by its chromatographic mobility on paper in systems S6, S7, and S9, and, after acetylation, in systems S1 and S8. For a previously prepared sample[10] of **1**, $[\alpha]_D$ = +99 ± 2° ($CHCl_3$). This value is used in the calculation of MD for compound **4** (Table I).

3α-Acetoxy-21-hydroxy-5β-pregnane-11,20-dione (2). A.—To 8.65 g (20.0 mmol) of 3α,21-diacetoxy-5β-pregnane-11,20-dione[17] in 865 ml of methanol was added 8.65 g (86 mmol) of $KHCO_3$ in 288 ml of H_2O. After 1.5 hr the solution was acidified with acetic acid, concentrated, and extracted with chloroform. The extract was washed with $NaHCO_3$ solution and water and evaporated to dryness. The residue was chromatographed in system S1 on a column containing 700 g of Celite (HBV = 3.0), and crystals (4.52 g, mp 63–64°) were obtained from benzene. When a sample was dried at 1 mm and 100°, it melted and lost 15.3% (calcd for C_6H_6, 16.7%); $[\alpha]_D$ on the dried sample = +116 ± 2° ($CHCl_3$). A sample, dried to constant weight at 60° and 1 mm, lost 12.6% (calcd for loss of $^3/_4C_6H_6$, 12.5%), and absorbance of the dried product at 255 mμ indicated 4.4% benzene (calcd for $^1/_4C_6H_6$, 4.8%): ir (KBr) 3450 (OH), 1730 (acetate C=O), 1702 (ketone C=O), and 1237 cm^{-1} (ester COC). *Anal.* Calcd for $C_{23}H_{34}O_5 \cdot {}^1/_4C_6H_6$: C, 71.76; H, 8.73. Found: C, 71.65; H, 8.74.

B. From 11.—To 100 mg of orthoacetate **11** in 10 ml of benzene was added 1.0 ml of 0.1 N HCl in methanol.[13c] After 10 min, the solution was washed with 1.0 N Na_2CO_3 and then with water until neutral and taken to dryness. The residue was chromatographed on 30 g of Celite in system S1 (HBV 3.6). Crystals (18 mg, 28%, mp 63–65°) were obtained from benzene–cyclohexane and identified as **2**.

21-Acetoxy-3α-hydroxy-5β-pregnane-11,20-dione (3). A. From 1.—A pyridinium acetate buffer was prepared by diluting 121 ml of pyridine to 500 ml with glacial acetic acid. To 14.0 g of compound **1**[10] was added 500 ml of glacial acetic acid and 500 ml of pyridinium acetate buffer at 25°. While the flask was being shaken, 100 ml of acetic anhydride was added; the mixture became homogeneous in 15 min. One hour later the mixture was worked up, and crystals (8.2 g, 52%, mp 138–141°) were obtained from ethanol. In a similar preparation in which the product was chromatographed in system S5, **3** emerged at 2.0 HBV (yield 65%, mp 142–143°), $[\alpha]_D$ +108 ± 2° ($CHCl_3$) [reported[17] mp 137–138°; $[\alpha]_D$ 109° ($CHCl_3$)].

B. From 25.—To 100 mg of orthoacetate **25** in 10 ml of benzene was added 1.0 ml of 0.1 N HCl in methanol. After 10 min the solution was washed twice with 1.0 N Na_2CO_3 and then with water until neutral and taken to dryness. Crystalline **3** (83%, mp 141–143°) was obtained from benzene–cyclohexane.

Methyl (3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate (4). A. From 1.—A mixture of 348 mg (1.0 mmol) of **1** and 1.1 g (4.0 mmol) of Ag_2CO_3 in 150 ml of benzene was treated with 1.13 g (3.0 mmol) of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate by the general procedure described previously.[7] The product was chromatographed in solvent system S4; 9.2-ml fractions were collected. To monitor the effluent, alternate fractions were mixed with 1 ml of water and 50-μl samples of organic phase were removed, taken to dryness, and mixed with 1.0 ml of concentrated H_2SO_4. After 2 hr, the absorbance was read at 415 mμ and plotted against fraction number. Additional samples (25 μl) were removed, spotted on filter paper, and treated with alkaline tetrazolium blue. For fractions 23–37 (HBV 1.8) see **17** from **1**; for fractions 38–53 (HBV 2.6) see **16** from **1** *via* **12**; for fractions 69–94 (HBV 4.6), see **18** from **1**.

Fractions 189–259 (HBV 13.5) were combined, washed with water, and concentrated. Crystals of glucosiduronate **4** (215 mg, 32%) were obtained from ethanol: mp 108–111°; homogeneous in system S5; $[\alpha]_D$ +24 ± 2° ($CHCl_3$); ir (KBr) 3520 (OH), 1757 (ester C=O), 1705 (ketone C=O), and 1235 sh, 1215 cm^{-1} (ester COC); nmr ($CDCl_3$) δ 0.57 (C-18 CH_3), 1.15 (C-19 CH_3), 1.81 (C-3 OH?), 2.01 (two OAc), 2.08 (one OAc), 2.45 (C-12 protons), 3.75 (CH_3O of ester), and 4.50 ppm (C-21 CH_2O). *Anal.* Calcd for $C_{34}H_{48}O_{13}$: C, 61.43; H, 7.28. Found: C, 61.61; H, 7.29.

B. From 9.—Treatment of semicarbazone **9** (200 mg) in chloroform with pyruvic acid[7] gave crystals of **4**, mp 105–108°, in 79% yield.

C. From 17.—Orthoacetate **17** (30 mg) was treated as described for the preparation of **2** from **11**; crystals (4.9 mg, 24% yield, mp 103–105°) were obtained from cold ethanol and identified as glucosiduronate **4**.

Methyl (3α-Acetoxy-11,20-dioxo-5β-pregnan-21-yl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate (5). A. From 2.—A solution of **2** (3.38 g) in benzene was treated with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate by the procedure described for the preparation of **4** from **1**, and the product was chromatographed on 700 g of Celite in system S1. The band eluted at 2.0 HBV contained orthoacetate **11** (see **11** from **2**). The band eluted at 3.0 HBV gave crystals of **5** from benzene–cyclohexane (1.62 g, 27%, mp 168–169°, homogeneous by tlc).

B. From 4.—Treatment of 200 mg of **4** with a mixture of 1.0 ml each of acetic anhydride and pyridine at room temperature for 3 hr followed by crystallization of the product from methanol gave homogeneous (system S5) conjugate **5** (162 mg, 76%, mp 169–170°). The pure substance had mp 170–171°; $[\alpha]_D$ +39 ± 2° ($CHCl_3$); ir (KBr) 1775, 1750 (acetate CO), 1725 (ester CO), 1702 (ketone C=O), and 1245, 1215 cm^{-1} (ester COC). *Anal.* Calcd for $C_{36}H_{50}O_{14} \cdot {}^1/_2H_2O$: C, 60.40; H, 7.32; CH_3CO, 24.06. Found: C, 60.53; H, 6.94; CH_3CO, 24.78.

Compound **5** was prepared also by acetylation of **6** and by treatment[7] of **10** with pyruvic acid.

Methyl (3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-Glucopyranosid)uronate (6). A. From 4.—To a solution of 665 mg of triacetate **4** in 40 ml of dry methanol was added 0.30 ml of 1.2 N sodium methylate in methanol. After 30 min a slight excess of acetic acid was added, the solution was evaporated to dryness, and crystals (393 mg, 72%, mp 203–205°; homogeneous in system S13) were obtained from cold ethanol: $[\alpha]_D$ +37 ± 2° (CH_3OH); ir (KBr) 3420, 3300 (OH), 1746 (ester C=O), and 1708 cm^{-1} (C-11 + C-20, C=O). *Anal.* Calcd for $C_{28}H_{42}O_{10} \cdot {}^1/_2H_2O$: C, 61.41; H, 7.91; CH_3O, 5.66. Found: C, 61.78; H, 7.50; CH_3O, 6.03.

B. From 7.—Treatment of acid **7** with diazomethane gave an ester (mp 202–204°) which was identical with **6**.

(15) R. Neher, "Steroid Chromatography," 2nd ed, Elsevier, New York, N. Y., 1964, p 122.

(16) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 1779 (1963).

(17) R. Deghenghi and C. R. Engel, *J. Amer. Chem. Soc.*, **82**, 3201 (1960).

3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-Glucopyranosiduronic Acid (7). A. From 4.—To a solution of 382 mg of triacetate **4** in 7.5 ml of chloroform and 3.75 ml of methanol was added 3.75 ml of 0.04 *N* NaOH in methanol. The mixture stood at room temperature for 45 min; then 15 ml of methanol and 7.0 ml of 1.0 *N* aqueous NaOH were added. The mixture stood at room temperature for an additional 30 min, the pH was brought to 4 with dilute H_2SO_4, and the precipitate of sodium sulfate was filtered off and washed with 10 ml of methanol; the filtrate was concentrated to about 5 ml to remove the chloroform and methanol. Water was added to a volume of 80 ml, the pH was brought to 2.1 with H_2SO_4, and the homogeneous mixture was poured onto a column containing 40 g of Amberlite XAD-2.[7,18,19] The column was washed twice with 80 ml of water and three times with 80 ml of ethanol. The ethanol eluates contained an impurity which was removed by chromatography in system S10. A band which emerged at 2.1 HBV gave crystals (241 mg, 79%) from methanol–ethyl acetate (mp 155–157.5°) which were identical with **7** prepared from **8**.

B. From 8.—A solution of ammonium salt **8** (300 mg) in 30 ml of water was adjusted to pH 3.0 with H_2SO_4 and concentrated almost to dryness. The $(NH_4)_2SO_4$ was separated by its insolubility in dry butyl alcohol, and **7** was crystallized from water and then from methanol–ethyl acetate (yield 82%, mp 157–160°): homogeneous in S10; $[\alpha]_D$ +35 ± 2° (CH_3OH); ir (KBr) 3410 (OH), 1700 cm^{-1} (C=O). *Anal.* Calcd for $C_{27}H_{40}O_{10}\cdot{}^1/_2H_2O$: C, 60.77; H, 7.75. Found: C, 60.73; H, 7.97.

Ammonium (3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-Glucopyranosid)uronate (8). A. From 6.—A solution of methyl ester **6** (1.0 mmol) in 50 ml of 1 *N* NH_4OH stood at room temperature for 30 min and was evaporated to dryness. Crystals were obtained (82%, mp 167–170° dec) from methanol–ethyl acetate: homogeneous in system S10; $[\alpha]_D$ +35 ± 2° (CH_3OH); ir (KBr) 3400–3220 (OH and NH), 1703 (C-11 + C-20, C=O), 1600 (carboxyl C=O), and 1405 cm^{-1} (COO^-). *Anal.* Calcd for $C_{27}H_{43}O_{10}N$: C, 59.87; H, 8.00. Found: C, 59.40; H, 7.60.

Compound **8** was also prepared from acid **7**.

Methyl (3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate 20-Semicarbazone (9).—A solution of compound **4** (200 mg) was treated[7] with semicarbazide hydrochloride to give crystals of **9** (homogeneous in system S6; 91% yield, mp 152–153°): uv max (CH_3OH) 236 mμ (ε 12,300); ir (KBr) 3590, 3495, 3350 (OH and NH), 1758 (ester C=O), 1700 (C-11 C=O), 1690 sh (amide C=O), 1562 (amide), and 1225 sh, 1207 cm^{-1} (ester COC). *Anal.* Calcd for $C_{35}H_{51}O_{13}N_3$: N, 5.82. Found: N, 5.65.

Methyl (3α-Acetoxy-11,20-dioxo-5β-pregnan-21-yl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosid)uronate 20-Semicarbazone (10).—**10** was obtained in 94% yield from **5**: hygroscopic crystals; mp 165–167° from methanol; homogeneous in S6; uv max (CH_3OH) 236 mμ (ε 13,000); ir (KBr) 3490, 3360 (NH), 1757 (acetate C=O), 1735 sh (ester C=O), 1702 (C-11 C=O), 1690 sh (amide C=O), 1570 (amide), and 1235, 1212 cm^{-1} (ester COC). *Anal.* Calcd for $C_{37}H_{53}O_{14}N_3\cdot{}^1/_2H_2O$: C, 57.50; H, 7.01; N, 5.44. Found: C, 57.25; H, 6.61; N, 5.21.

Methyl α-D-Glucopyranosiduronate Cyclic 1,2-(Hydrogen [*S*]-Orthoacetate) 3,4-Diacetate 21-Ester with 3α,21-Dihydroxy-5β-pregnane-11,20-dione 3-Acetate (11).—Fractions 93–144 (HBV 2.0), described under **5** from **2**, were combined, washed with water, and taken to dryness. The residue was chromatographed on 300 g of Celite in system S2 (HBV 2.8) and crystals of **11** (385 mg, 6.7%) were obtained from methanol–water: homogeneous by tlc and in system S1; mp 91–94°; $[\alpha]_D$ +68 ± 2° ($CHCl_3$); ir (KBr) 1753 (acetate C=O), 1735 sh (ester C=O), 1705 (C-11 + C-20, C=O), and 1232, 1208 sh cm^{-1} (ester COC); nmr ($CDCl_3$) δ 0.58 (C-18 methyl), 1.17 (C-19 methyl), 1.75 (orthoacetate methyl, endo), 2.02 (C-3 OAc), 2.08 and 2.12 (C-4′ and C-5′ OAc), 2.47 (C-12), 3.02 [C-17 (?)], 3.78 (CH_3O of ester), 3.97 (C-21 methylene), 4.75 (C-3 proton), and 5.85 ppm (J = 5 Hz) (C-1′ proton). *Anal.* Calcd for $C_{38}H_{50}O_{14}$: C, 61.17; H, 7.13; CH_3O, 4.39; CH_3CO, 24.36. Found: C, 60.98; H, 7.31; CH_3O, 4.35; CH_3CO, 22.53.

11,20-Dioxo-5β-pregnan-3α,21-ylene Di(methyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosiduronate) (12). A. From 13.—Acetylation of diglucosiduronate dimethyl ester **13** with 1:1 acetic anhydride–pyridine gave a product identical with ester **12** below.

B. From 16.—Semicarbazone **16** (2.08 g) was treated[7] with pyruvic acid as described previously to give **12**, which was crystallized from a mixture of ethanol–acetone–water (1.77 g, 90%, mp 125–128°): homogeneous in system S5 and by tlc; $[\alpha]_D$ +10 ± 2° ($CHCl_3$): ir (KBr) 1753 (ester C=O), 1706 (C-11 + C-20 C=O), and 1230 sh, 1210 cm^{-1} (ester COC). *Anal.* Calcd for $C_{47}H_{64}O_{22}$: C, 57.54; H, 6.58; CH_3CO, 26.32. Found: C, 57.19; H, 6.46; CH_3CO, 24.64.

Dimethyl 11,20-Dioxo-5β-pregnan-3α,21-ylene Di-β-D-glucopyranosiduronate (13). A. From 12.—Ester **13** was prepared from **12** in 86% yield as described for ester **6** from triacetate ester **4**. Crystals were obtained from a mixture of methanol and *tert*-butyl alcohol (mp 147–150°): homogeneous in S10; $[\alpha]_D$ +17 ± 2° (CH_3OH); ir (KBr) 3410 (OH), 1742 (ester C=O), and 1702 cm^{-1} (C-11 + C-20 C=O). *Anal.* Calcd for $C_{35}H_{52}O_{16}$: C, 57.68; H, 7.19. Found: C, 57.50; H, 7.61.

B. From 14.—Treatment of acid **14** with diazomethane also gave ester **13**.

11,20-Dioxo-5β-pregnan-3α,21-ylene Di(β-D-glucopyranosiduronic Acid) (14). A. From 12.—Compound **14** was prepared from diglucosiduronate **12** (0.25 mmol) as described for preparation of **7** from **4**. The residue from the Amberlite XAD-2 column contained material (chromatography system S12) which gave an atypical color (purple) with alkaline tetrazolium blue (compound **14** gave a yellow color). The residue was chromatographed on 50 g of Celite in system S11 (HBV 6.6) and the conjugate was crystallized from methanol–ethyl acetate (144 mg, 83%, mp 185° dec) and identified as **14**.

B. From 15.—A solution of ammonium salt **15** (200 mg) in water was acidified to pH 3 with H_2SO_4 and taken almost to dryness. The residue was extracted with two 100-ml portions of absolute ethanol, the solution was taken to dryness, and crystals of **14** were obtained from methanol–ethyl acetate (156 mg, 82%, mp 175° dec): homogeneous in system S11; $[\alpha]_D$ +17 ± 2° (CH_3OH); ir (KBr) 3430 (OH), 1720 sh (carboxyl C=O), and 1703 cm^{-1} (C-11 + C-20 C=O). *Anal.* Calcd for $C_{33}H_{48}O_{16}\cdot{}^1/_2H_2O$: C, 55.84; H, 6.96. Found: C, 55.77; H, 6.76.

C. From 16.—The diglucosiduronate semicarbazone **16** (1.038 g) was converted into acid **14** by the procedure used to prepare **7** from **4**, except for the following modification. The solution stood at pH 2.1 for 1 hr (in order to hydrolyze the semicarbazone) before it was poured into the column containing Amberlite XAD-2; the column was then washed with two 80-ml portions of 0.01 *N* H_2SO_4 before being washed with three 80-ml portions of water, etc. Crystals of **14** (529 mg, 75%, mp 170–174°) were obtained from methanol–ethyl acetate.

Diammonium 11,20-Dioxo-5β-pregnan-3α,21-ylene Di-β-D-Glucopyranosiduronate (15).—A solution of ester **13** (200 mg) in 20 ml of 1 *N* NH_4OH stood for 2 hr at 25° and was concentrated to dryness. Crystals (188 mg, 93%) of **15** were obtained from methanol–ethanol: mp 178–180° dec; homogeneous in system S11; $[\alpha]_D$ +10 ± 2° (CH_3OH); ir (KBr) 3400–3220 (OH and NH), 1700 (C-11 + C-20 C=O), 1600 (carboxyl C=O), and 1400 cm^{-1} (COO^-). *Anal.* Calcd for $C_{33}H_{54}O_{16}N_2\cdot H_2O$: C, 52.65; H, 7.44. Found: C, 52.66; H, 6.75.

11,20-Dioxo-5β-pregnan-3α,21-ylene Di(methyl 2,3,4-Tri-*O*-acetyl-β-D-glucopyranosiduronate) 20-Semicarbazone (16). A. From 1 *via* 12.—Fractions 38–53 (HBV 2.6), described under the preparation of **4** from **1**, were combined, washed with water, and taken to dryness. The product (**12**) would not crystallize as the ketone; it was converted[7] into semicarbazone **16** (130 mg, 13% yield from **1**, mp 174–175°), a homogeneous (system S6), hygroscopic product. When recrystallized from methanol, it had mp 179–180°; uv max (CH_3OH) 236 mμ (ε 12,800); ir (KBr) 3510, 3380 (NH), 1757 (ester C=O), 1702 (C-11 + amide, C=O), 1570 (amide), and 1230 sh, 1212 cm^{-1} (ester COC). *Anal.* Calcd for $C_{48}H_{67}O_{22}N_3\cdot{}^1/_2H_2O$: C, 55.06; H, 6.55; N, 4.02; CH_3CO, 24.67. Found: C, 54.90; H, 6.23; N, 3.78; CH_3CO, 21.83.

B. From 4 *via* 12.—Fractions corresponding to 2.6 HBV (described under preparation of **17** from **4**) were combined and converted into **16** (254 mg, 24% from **4**, mp 176–177°) as described above.

(18) This resin is very useful for separating steroidal glucosiduronic acids from inorganic acids, salts, and various other water-soluble compounds. We have employed it to separate steroidal glucosiduronic acids from ammonium sulfate, sodium sulfate, ammonium chloride, semicarbazide (after acid hydrolysis of a semicarbazone), inorganic acids in general, and debris from the effluent of a Celite column when the solvent system contained aqueous acetic acid. Also, we have used it for converting ammonium, sodium, potassium, thallium, and barium glucosiduronates into free acids.

(19) H. L. Bradlow, *Steroids*, **11**, 265 (1968).

C. From 12.—Treatment[7] of **12** (490.5 mg) with semicarbazide hydrochloride yielded a product (512 mg, 99%, mp 177–179°) identical with authentic **16**.

Methyl 3α-Hydroxy-11,20-dioxo-5β-pregnan-21-yl β-D-Glucopyranosiduronate 2,3,4-Triacetate 3′-(Dihydrogen Orthoacetate) Cyclic 1,2-(*S*)-Ester with Methyl α-D-Glucopyranuronate 3,4-Diacetate (17). A. From 1.—Fractions 23–37 (HBV 1.8), obtained during preparation of **4** from **1**, gave a residue which was chromatographed on 100 g of Celite in solvent system S3. The appropriate fraction (HBV 13.5) gave crystals (43 mg, 4.4%, mp 110–115°) of orthoacetate **25** from methanol–water; this product was identical with orthoacetate **25** described in the following paragraph.

B. From 4.—One millimole of 3α-hydroxy conjugate **4** was treated with 3 mmol of methyl acetobromoglucuronate as described for the preparation of **4** from **1**. The product was chromatographed on 100 g of Celite (system S4). (For the substance eluted at 2.6 HBV, see **16** from **12**, B.) Fractions corresponding to 1.8 HBV were combined, washed with water, and taken to dryness. The residue was rechromatographed in solvent system S3 on 100 g of Celite. The residue from the principal band (HBV 16) weighed 127 mg, a 13% yield of orthoacetate **17**. Crystals (90 mg, 9.2%, mp 111–116°) from benzene–cyclohexane were homogeneous by tlc; ir (KBr) 1758 (ester C=O), 1705 (C-11 + C-20 C=O), and 1212 cm^{-1} (ester COC); nmr ($CDCl_3$) δ 0.55 (C-18 CH_3), 1.13 (C-19 CH_3), 1.74 (orthoacetate CH_3), 2.01 (two OAc groups of C-21 glucuronyl group), 2.09 (one OAc group of C-21 glucuronyl group; two OAc groups of C-3 glucuronyl function), 2.43 (probably C-12 protons), 3.75 (CH_3O of ester), and 4.50 ppm (C-21 methylene). *Anal.* Calcd for $C_{47}H_{64}O_{22}\cdot H_2O$: C, 56.50; H, 6.66; CH_3O, 6.21; CH_3CO, 25.85. Found: C, 56.40; H, 6.45; CH_3O, 7.05; CH_3CO, 24.78.

Methyl (21-Hydroxy-11,20-dioxo-5β-pregnan-3α-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate (18). A. From 1.—Fractions 69–94 (HBV 4.6), described under **4** from **1**, were combined, washed with water, and freed of solvent. Crystals (113 mg, 17%, mp 113–116°), obtained from acetone–methanol–water, were homogeneous in system S5; $[\alpha]_D$ +42 ± 2° ($CHCl_3$); ir (KBr) 3490 (OH), 1755 (ester C=O), 1702 (C-11 + C-20 C=O), and 1210 cm^{-1} (ester COC). *Anal.* Calcd for $C_{34}H_{48}O_{13}\cdot{}^1/_2H_2O$: C, 60.60; H, 7.33. Found: C, 60.68; H, 7.07.

Compound **18** reduced alkaline tetrazolium blue and reacted with cupric acetate[10] to produce a product which gave a yellow color with Porter–Silber reagent.

B. From 22.—Treatment of semicarbazone **22** (20 mg) with pyruvic acid[7] produced 9 mg (49%, mp 110–113°) of **18**.

Methyl (21-Acetoxy-11,20-dioxo-5β-pregnan-3α-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate (19). A. From 3.—Compound **3** (3.9 g, 10.0 mmol) was treated with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate as described under the preparation of **4** and the product was chromatographed in system S4. Fractions were combined to give residues designated as follows: 1.5 HBV (a mixture of glucosiduronate **19** and orthoacetate **25**; see below in this paragraph); 2.2 HBV (the 3-oxo compound, 21-acetoxy-5β-pregnane-3,11,20-trione; see below). The residue designated 1.5 HBV gave crystals (3.02 g, 43% mp 106–109°) of **19** from benzene–cyclohexane: homogeneous in system S1; $[\alpha]_D$ +54 ± 2° ($CHCl_3$); nmr ($CDCl_3$) δ 0.60 (C-18 CH_3), 1.12 (C-19 CH_3), 2.02 (2′,3′,4′ OAc groups), 2.16 (C-21 OAc), 2.50 (C-12 protons), 3.5 (CH_3O of ester), and 4.55 ppm (C-21 methylene). The ir spectrum was identical with that of compound **19**. (For isolation of the 3-orthoacetate **25** from the mother liquor of **19**, see **25** below.)

B. From 18.—Acetylation of **18** (200 mg) followed by crystallization from methanol–water gave **19** (174 mg, 81%, mp 106–108°): $[\alpha]_D$ +54 ± 2° ($CHCl_3$); ir (KBr) 1757 (ester C=O), 1728 sh (C-20 C=O), 1707 (C-11 C=O), and 1210 cm^{-1} (ester COC). The sample for analysis was dried *in vacuo* at 78°. *Anal.* Calcd for $C_{36}H_{50}O_{14}$: C, 61.17; H, 7.13; CH_3CO, 24.36. Found: C, 60.99; H, 7.05; CH_3CO, 23.31.

Compound **19** was prepared from **20** in a similar manner and from **23** by treatment with pyruvic acid.

Methyl (21-Hydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-Glucopyranosid)uronate (20). A. From 19.—Treatment of 353 mg of compound **19** with 0.01 *N* NaOH in 15 ml of chloroform–methanol, 1:1, for 45 min followed by addition of an excess of acetic acid and 1.0 mg of EDTA, concentration *in vacuo*, and separation from ethyl acetate–cyclohexane yielded amorphous **20** which was homogeneous in system S13 (232 mg, 86%): $[\alpha]_D$ +51 ± 2° (CH_3OH); ir (KBr) 3440 (OH), 1746 (ester C=O), and 1705 cm^{-1} (C-11 + C-20 C=O). *Anal.* Calcd for $C_{28}H_{42}O_{10}\cdot H_2O$: C, 60.41; H, 7.97; CH_3O, 5.57. Found: C, 60.58; H, 7.77; CH_3O, 5.62.

B. From 21.—Esterification of acid **21** with diazomethane also gave ester **20**.

21-Hydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-Glucopyranosiduronic Acid (21). A. From 19.—Compound **19** was treated as described below under procedure 1 for preparation of **21** from **23**. It was not possible to obtain crystals. The product was freed of salt and inorganic acid by use[7] of Amberlite XAD-2; to the alcoholic eluate was added 2 mg of EDTA, the solution was taken to dryness, and the residue was chromatographed on 100 g of Celite (system S10). Bands emerged at 1.14 (see **26** from **19**) and at 1.8 HBV. EDTA (2 mg) was added to the eluate containing the latter band, the solvent was removed, and crystals of **21** were obtained from 0.01 *N* H_2SO_4 and washed with H_2O (122 mg, 47%, mp 156–158°).

In two similar experiments on the preparation of **21** from **19** in which only 0.10 mg of EDTA was added to the alcoholic eluate from the Amberlite column and again after Celite chromatography, crystals could not be obtained. Several solvents were used during a period of 2 days. A paper chromatogram (system S10) showed that the original ketolic compound **21** (R_f 0.29) had disappeared and that a new Porter–Silber-positive[10] compound (R_f 0.70; presumably the 20-keto-21-aldehyde) had been formed.

In another experiment, the mobile phase was run through a column of Celite like that used for chromatography of **21**; the fraction corresponding to the band at 1.8 HBV was collected, taken to dryness, and shown to contain (by atomic absorption) 1.5 μg of copper. To one-tenth of this residue was added 17 mg of compound **21** in 0.10 ml of methanol; after 24 hr, the solution gave a strong color with the Porter–Silber reagent. Color with the Porter–Silber reagent was not obtained when 17 mg of compound **21** was treated similarly with 0.10 mg of the residue in the presence of 0.10 mg of EDTA.

B. From 23. Procedure 1.—Semicarbazone **23** (764 mg) was treated with alkali as described under **7** from **4**. Methanol[20] (10.0 ml) was added to dissolve the oil which separated when the pH was adjusted to 2.1. After 1 hr, the solution was concentrated *in vacuo* to 7 ml. Crystals, which formed slowly, were filtered, washed well with cold water, and dried at 100° (497 mg, 93%, mp 157–159°). In a second preparation the yield was 88% (mp 158–160°): homogeneous in system S10; $[\alpha]_D$ +59 ± 2° (CH_3OH); ir (KBr) 3400 (OH), 1748 sh (carboxyl C=O), and 1700 cm^{-1} (C-11 + C-20 C=O). *Anal.* Calcd for $C_{27}H_{40}O_{10}\cdot{}^1/_2H_2O$: C, 60.77; H, 7.75. Found: C, 60.76; H, 7.72.

B. From 23. Procedure 2. Ion-Exchange Column.—One millimole of **23** was treated with alkali as described in the previous paragraph and the solution was acidified with acetic acid, concentrated to dryness, and processed[7] on a column of Dowex 50W-X2 (40 g); acid **21** was obtained in 82% yield.

C. From 20.—A solution which contained 100 mg of **20** in 20 ml of 0.1 *M* $NaHCO_3$ stood at 26° for 24 hr. The pH was adjusted to 2.1 with 0.5 *M* H_2SO_4, and the solution was concentrated and cooled. Crystals of **21** (mp 157–159°) were obtained in 40% yield. By desalting the filtrate on Amberlite XAD-2 and chromatographing the steroid residue in system S10, an additional 26% of **21** was obtained.

Methyl (21-Hydroxy-11,20-dioxo-5β-pregnan-3α-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate 20-Semicarbazone (22).—**22** was obtained from **18** by the procedure described in the next paragraph and crystallized from aqueous ethanol (88% yield, mp 157–159°): homogeneous in system S6; uv max (CH_3OH) 236 mμ (ε 11,800); ir (KBr) 3510, 3385 (OH and NH), 1757 (ester C=O), 1706 (C-11 C=O), 1692 (amide C=O), 1573 (amide), and 1215 cm^{-1} (ester COC). The dried product gained 4.1% in weight when exposed to the atmosphere. *Anal.* Calcd for $C_{35}H_{51}O_{13}N_3$: N, 5.82. Found: N, 5.60.

Methyl (21-Acetoxy-11,20-dioxo-5β-pregnan-3α-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate 20-Semicarbazone (23). A. From 19.—A solution of 750 mg of semicarbazide hydrochloride and 504 mg of $NaHCO_3$ in 2 ml of water was added to 695 mg of 20-oxo conjugate **19** in 20 ml of methanol. After 18 hr, crystals (709 mg, 93%, mp 178–181°) of chromatographically pure (system

(20) If the pH is adjusted to 3.0 and the solution is stirred for a few minutes, it remains homogeneous when the pH is subsequently brought to 2.1; under these conditions, addition of methanol is not necessary.

S6) semicarbazone were obtained from aqueous methanol: uv max (CH_3OH) 237 mμ (ε 12,800); ir (KBr) 3515, 3395, 3285 br (NH), 1753 (ester C=O), 1705 (C-11 + amide, C=O), 1570 (amide), and 1217 cm^{-1} (ester COC). *Anal.* Calcd for $C_{37}H_{53}O_{14}N_3 \cdot {}^1/_2H_2O$: C, 57.50; H, 7.04; N, 5.44. Found: C, 57.55; H, 6.95; N, 5.38.

B. From 22 and 24.—Acetylation of **22** and **24** in 1:1 acetic anhydride–pyridine gave **23** (mp 178–181°).

Methyl (21-Hydroxy-11,20-dioxo-5β-pregnan-3α-yl β-D-Glucopyranosid)uronate 20-Semicarbazone (24). A. From 20.—Treatment of 50 mg of ester **20** with semicarbazide hydrochloride as described under the preparation of **23** from **19** gave crystals (30.8 mg, 55% yield) of **24** from butyl acetate.

B. From 23.—To a solution of 764 mg of semicarbazone **23** in a mixture of 10 ml of chloroform and 5 ml of methanol was added 5 ml of 0.04 *N* methanolic NaOH. After 45 min, a slight excess of 1.0 *N* acetic acid in methanol was added and the solution was taken to dryness immediately to remove all acetic acid and prevent hydrolysis of the semicarbazone. Hygroscopic crystals (552 mg, 92%, mp 173° dec) were obtained from methanol–butyl acetate: homogeneous in system S14; uv max (CH_3OH) 236 mμ (ε 11,800); ir (KBr) 3500–3300 (OH and NH), 1742 (ester C=O), 1702 sh (C-11 C=O), 1680 (amide C=O), and 1565 cm^{-1} (amide). *Anal.* Calcd for $C_{29}H_{45}O_{10}N_3 \cdot {}^1/_2H_2O$: C, 57.59; H, 7.84; N, 6.95; CH_3O, 5.13. Found: C, 57.33; H, 7.64; N, 7.35; CH_3O, 5.53.

Methyl α-D-Glucopyranuronate Cyclic 1,2-(Hydrogen [*S*]-Orthoacetate) 3,4-Diacetate 3-Ester with 3α,21-Dihydroxy-5β-pregnane-11,20-dione 21-Acetate (25).—The filtrate from the crystallization of 3.02 g of compound **19** (described under **19** from **3**) was evaporated and the residue was chromatographed as described for the separation of **19** except that system S1 was used. The band at 3.1 HBV gave 1.64 g of residue which crystallized from methanol–water to yield 1.56 g (22%, mp 95–98°) of chromatographically pure (systems S1 and S4) orthoacetate **25**: $[\alpha]D$ +78 ± 2° ($CHCl_3$); ir (KBr) 1753 (ester C=O), 1708 (ketone C=O), and 1225 cm^{-1} (ester COC); nmr ($CDCl_3$) δ 0.61 (C-18 CH_3), 1.13 (C-19 CH_3), 1.75 (orthoacetate CH_3), 2.10 (3′,4′ OAc groups), 2.15 (C-21 OAc), 2.50 (C-12 protons), 3.78 (CH_3O of ester), 4.55 (C-21 methylene), and 5.81 ppm (doublet, 1-proton of glucuronyl group, $J_{1,2}$ = 5 Hz). *Anal.* Calcd for $C_{36}H_{50}O_{14}$: C, 61.17; H, 7.13; CH_3O, 4.39; CH_3CO, 24.36. Found: C, 61.31; H, 7.36; CH_3O, 4.58; CH_3CO, 22.96.

21-Acetoxy-5β-pregnane-3,11,20-trione.—Chromatography fractions 144–195 (HBV 2.2), described under **19** from **3**, afforded crystals (462 mg, 12%, mp 157–160°) from methanol; this product was identified as 21-acetoxy-5β-pregnane-3,11,20-trione[17] by comparison with an authentic sample. Paper chromatography of compound **3** (starting material in this preparation) in system S4 showed that it contained no 21-acetoxy-5β-pregnane-3,11,20-trione.

Compound **3** (781 mg, 2.0 mmol) was treated with 2.20 g (8.0 mmol) of freshly prepared silver carbonate in benzene as described for the preparation of **4** except that the methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate was omitted. Chromatography of the product on 300 g of Celite (system S4) separated a band (HBV 2.0) which gave crystals (385 mg, 50%, mp 157–158°) of 21-acetoxy-5β-pregnane-3,11,20-trione from aqueous methanol.

17β-Carboxy-11-oxo-5β-androstan-3α-yl β-D-Glucopyranosiduronic Acid (26). A. From 19.—The residue (71 mg) from the band eluted at 1.14 HBV (described under **21** from **19**) was dissolved in the mobile phase of system S13; crystals formed (7.5 mg, 3.0%, mp 214–216° dec). The mother liquor was chromatographed on 30 g of Celite in system S13. The band eluted at 7.5 HBV yielded crystals (12.5 mg, 5.0%, mp 214–215°) of **26** (from ethanol) identical with the sample prepared in the following paragraph.

B. From 27.—Compound **27** (325 mg) was hydrolyzed by treatment with alkali as described for the preparation of **21** from **23**, procedure 1, and the solution was desalted on a column of Amberlite XAD-2. Crystals (228 mg, mp 214–215°) of chromatographically pure (system S13) **26** were obtained from ethanol: $[\alpha]D$ +43 ± 2° (CH_3OH); ir (KBr) 3380 (OH), 1763 (carboxyl C=O), 1710 sh, and 1696 cm^{-1} (C-11 C=O). *Anal.* Calcd for $C_{26}H_{38}O_{10}$: C, 61.16; H, 7.50. Found: C, 61.18; H, 7.74.

Methyl (17β-Carboxy-11-oxo-5β-androstan-3α-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate (27).—To 333 mg (0.50 mmol) of **18** in 15 ml of glacial acetic acid was added 1.54 mmol of H_5IO_6 in 70 ml of 80% acetic acid. After 15 min, water was added, the solution was extracted with ethyl acetate, and the extract was washed with water and taken to dryness. Crystals (230 mg, mp 220–222°; 61.5 mg, mp 218–220°) were obtained from ethanol. The sample for analysis melted partially at 135–140°, recrystallized spontaneously, and remelted at 220–221°: $[\alpha]D$ +41 ± 2° (CH_3OH); ir (KBr) 3320 (OH), 1756 (ester + carboxyl, C=O), 1705 (C-11 C=O), and 1212 cm^{-1} (ester COC). *Anal.* Calcd for $C_{35}H_{46}O_{13} \cdot {}^1/_2H_2O$: C, 60.08; H, 7.18; CH_3O, 4.70. Found: C, 60.35; H, 7.08; CH_3O, 4.83.

Methyl (17β-Carbomethoxy-11-oxo-5β-androstan-3α-yl 2,3,4-Tri-*O*-acetyl-β-D-glucosid)uronate (28). A. From 27.—Treatment of **27** (100 mg) in methanol with diazomethane in ether gave ester **28** (101 mg, mp 172–173°), identical with the product prepared from **26**.

B. From 26.—Treatment of 100 mg of a methanolic solution of **26** (derived from **27**) with diazomethane in ether followed by acetylation with acetic anhydride–pyridine and crystallization from ethanol gave 121 mg (92%) of chromatographically pure (tlc) ester **28** (170–171°). The analytical sample had mp 172–173°; $[\alpha]D$ +36 ± 2° (CH_3OH); ir (KBr) 1770 sh, 1755 (acetate C=O), 1740 (ester C=O), 1705 (C-11 C=O), and 1210 cm^{-1} (ester COC). *Anal.* Calcd for $C_{34}H_{48}O_{13} \cdot H_2O$: C, 59.81; H, 7.38; CH_3O, 9.09. Found: C, 60.09; H, 7.16; CH_3O, 8.99.

When dicarboxylic acid **26** (derived from **18** with alkali) was esterified with diazomethane and acetylated, **28** was obtained.

Registry No.—**1**, 566-03-0; **2**, 2631-05-2; **3**, 2526-11-6; **4**, 36707-52-5; **5**, 36707-53-6; **6**, 36707-54-7; **7**, 36707-55-8; **8**, 36707-56-9; **9**, 36707-57-0; **10**, 36763-74-3; **11**, 36763-75-4; **12**, 36707-58-1; **13**, 36707-59-2; **14**, 36707-60-5; **15**, 36707-61-6; **16**, 36707-62-7; **17**, 36707-63-8; **18**, 36707-05-8; **19**, 36707-06-9; **20**, 36707-07-0; **21**, 35105-23-8; **22**, 36707-09-2; **23**, 36707-10-5; **24**, 36707-11-6; **25**, 36707-12-7; **26**, 36707-13-8; **27**, 36707-14-9; **28**, 36707-15-0; 21-acetoxy-5β-pregnane-3,11,20-trione, 36707-16-1.

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