Steroid Conjugates. Part V.[1] Glucuronosides of 19-Oxygenated 3β-Hydroxyandrost-5-en-17-ones

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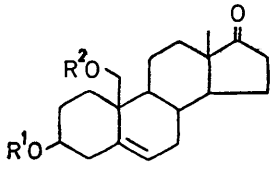
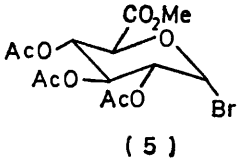
The monoglucuronosides of 19-hydroxy- and 19-oxo-3β-hydroxyandrost-5-en-17-one have been prepared by the Koenigs–Knorr procedure. Steric effects in these reactions are compared with those encountered in the preparation of the corresponding sulphates.

SELECTIVE routes have been developed for the synthesis of the sulphate conjugates of 3β,19-dihydroxyandrost-5-en-17-one (1).[2] We describe here the synthesis and some properties of the corresponding glucuronosides.

The 3β- and 19-monoacetates (2) and (3) of the diol (1) were coupled with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (5) by the standard





(5)

(1) $R^1 = R^2 = H$
(2) $R^1 = Ac$, $R^2 = H$
(3) $R^1 = H$, $R^2 = Ac$
(4) $R^1 = R^2 = Ac$

Koenigs–Knorr procedure,[3] to give the methyl 19- and 3β-glucosiduronate triacetates (10) and (12), in yields of 57 and 71%, respectively. Thus both hydroxy-groups were readily accessible to the bromo-ester. Although the isomers (10) and (12) had similar R_F values on t.l.c., their m.p.s differed by over 100°. This unexpected difference was due to solvation of the 3β-glucosiduronate (12), which melted with effervescence at *ca.* 110°. Its n.m.r. spectrum revealed the presence of benzene (estimated content 0·5 mol. equiv. by n.m.r.; confirmed by combustion analysis). This compound also tenaciously occluded acetone, ethyl acetate, and toluene, and the proportion of each solvent occluded was readily estimated by n.m.r. (see Experimental section). Solvates were only obtained with solvents containing π-bonds, suggesting that complex formation might involve charge-transfer interaction within the crystal structure. No such occlusion of solvents was observed with the isomeric 19-glucosiduronate (10), nor with the corresponding 19-hydroxy-3-glucosiduronate (see later).

The triacetate methyl esters (10) and (12) were readily hydrolysed with methanolic potassium hydroxide to the glucosiduronic acids (11) and (13), which were isolated and characterised as their potassium salts. When the tetra-acetate (12) was hydrolysed with sodium methoxide in methanol, a monoacetoxy-derivative of the methyl glucosiduronate was obtained. The n.m.r. spectrum

[1] D. Baxendale, D. N. Kirk, M. S. Rajagopalan, and A. B. Turner, *J. Chem. Soc.* (*C*), 1971, 2563.

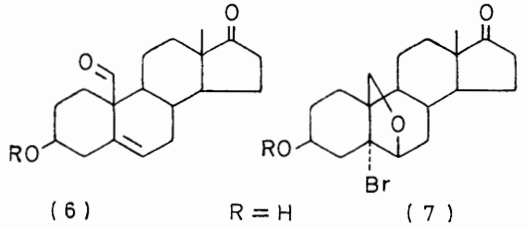
[2] M. S. Rajagopalan, D. S. H. Smith, and A. B. Turner, *J. Chem. Soc.* (*C*), 1971, 646.

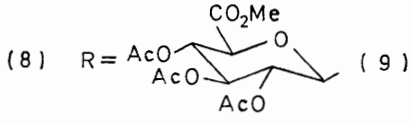
[3] W. Koenigs and E. Knorr, *Ber.*, 1901, **34**, 957.

of this compound suggested that the acetoxy-group was at C-19, as part of the AB quartet corresponding to the 19-methylene protons was visible (at τ *ca.* 5·5). The structure (14) was confirmed by its mass spectrum, which showed the conjugating system to be devoid of acetoxy-groups (*m/e* 191 and 173) while the *m/e* 328 steroidal fragment ion retained the 19-acetoxy-group. Loss of acetic acid from this ion gives the base peak, *m/e* 268. Selective hydrolysis of the sugar acetoxy-groups of the tetra-acetate (12) is not unexpected, since the primary 19-acetoxy group is much more sterically hindered than the secondary (equatorial) acetoxy-groups on the glucosiduronate residue. We have previously noted the selective hydrolysis of the secondary 3-acetoxy-group of the 3β,19-diacetate (4) by the hydrogen carbonate anion.[2]

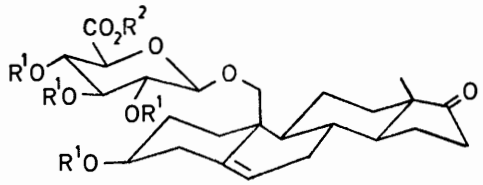
Attempts to prepare the glucuronoside of 19-oxodidehydroepiandrosterone (6) by coupling with the bromide (5) were unsuccessful. This may have been due to oxidation of the aldehyde by the silver oxide catalyst, as the starting steroid could not be recovered. The 19-oxo-conjugate (8) was synthesised by coupling of the 6β,19-epoxide (7) with the bromide (5) to give the glucosiduronate (9), followed by cleavage of the 6β,19-epoxide bridge with zinc and acetic acid in refluxing ethanol, and oxidation of the resulting 19-alcohol (15) with Jones reagent.

The 3β,19-diol (1) forms a disulphate without difficulty,[2] but the diglucuronoside could not be prepared by use of the Koenigs–Knorr procedure with an excess of the sugar halide (5). Instead, the 3β-monoglucuronoside (15) was obtained in 35% yield. It was identical with material prepared by cleavage of the epoxide (9). The structure was confirmed by oxidation to the 19-oxo-conjugate (8) with chromic acid in acetone. Thus

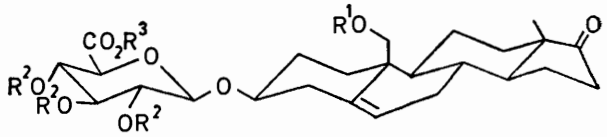


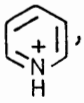


glucuronosylation of the 3β,19-diol (1) occurs preferentially at the 3β-hydroxy-group, in contrast to sulphation, which takes place at both hydroxy-groups. This emphasizes the great difference in bulk between the sulphur trioxide–pyridine complex and the sugar bromide (5), as was also apparent from the fact that the 3β-monoglucuronoside (15) could be sulphated by use of the sulphur trioxide–pyridine complex to give the pyridinium salt (16) of the mixed conjugate. The formation of the 19-conjugate (10) shows that conformational factors do not preclude attack of the bromo-sugar (5) at the 19-hydroxy-group, so that the preferential introduction of the glucuronoside residue at C-3 hinders further condensation at the angular hydroxymethyl group.[4]



(10) R^1=Ac, R^2=Me
(11) R^1=H , R^2=K



(12) R^1= R^2=Ac, R^3=Me
(13) R^1= R^2=H , R^3=K
(14) R^1=Ac , R^2=H, R^3=Me
(15) R^1=H , R^2=Ac , R^3=Me
(16) R^1=SO_3^-  , R^2=Ac , R^3=Me

In the mass spectra of the methyl glucosiduronate triacetates the fragments derived from the sugar residue (*m/e* 317, 257, 155, and 127) were very prominent, except in the case of the 19-alcohol (15). Molecular ions were only observed for the 19-oxo-conjugate (8) and the methyl ester (14), and accurate mass measurements were obtained on $M - 60$ ions for the latter and for the 19-glucosiduronate (10).

EXPERIMENTAL

For general directions and for preparation of starting steroid see ref. 2.

General Procedure for Conjugation.—The Shapiro modification [5] of the Koenigs–Knorr procedure was employed as follows. A solution of the hydroxy-steroid (1·0 mmol) and methyl 2,3,4-tri-*O*-acetyl-1α-bromo-1-deoxy-D-glucuronate (4) (3·0 mmol) in dry benzene (12·5 ml) containing freshly prepared silver oxide (1·6 mmol) and calcium sulphate granules (1·5 mmol) was stirred for 48 h in the dark at room temperature. After addition of Celite, the insoluble material was removed by filtration and most of the solvent was evaporated off. The residue was adsorbed on a column of Sephadex LH-20 (20 g) and the column was eluted with benzene (60 ml). Evaporation gave the crude product as a syrup, which was crystallised from ethanol unless otherwise stated.

Methyl [3β-*Acetoxy*-17-*oxoandrost*-5-*en*-19-*yl tri*-O-*acetyl*-β-D-*glucopyranosid*]*uronate* (10).—The 19-alcohol (2) (400 mg) gave needles of the *ester* (10) (360 mg, 47%), m.p. 237—238° (from ethanol–benzene) (Found: C, 61·7; H, 6·9. $C_{34}H_{46}O_{13}$ requires C, 61·6; H, 6·9%), *m/e* 602·2716 ($M^+ - C_2H_4O_2$) ($C_{32}H_{42}O_{11}$ requires 602·2727), $\nu_{max.}$ (KBr) 1765,

[4] *Cf.* S. Bernstein, J. P. Dusza, and J. P. Joseph, 'Chemical and Biological Aspects of Steroid Conjugation,' ed. S. Bernstein and S. Solomon, Springer New York, 1970, p. 10.

[5] E. Schapiro, *Biochem. J.*, 1939, **33**, 385.

1740, 1250, 1225, and 1045 cm^{-1}, τ ($CDCl_3$) 9·06 (s, 13-Me), 6·25 (s, OCH_3), and 4·36 (m, C-6 olefinic H), *m/e* 602, 317, 269, 268, 257, 255, 215, 155 (100%), and 127.

Potassium [3β-*Hydroxy*-17-*oxoandrost*-5-*en*-19-*yl* β-D-*glucopyranosid*]*uronate* (11).—To a hot solution of the tetra-acetate (10) (150 mg) in methanol (15 ml) was added methanolic 1M-potassium hydroxide (2 ml). The initial red colouration soon faded to yellow-orange, and after 24 h at room temperature benzene was added and the solution was concentrated *in vacuo*. The residual yellow syrup was dissolved in water (10 ml) and chromatographed on a column of Amberlite XAD-2 resin.[6,7] After elution with water until neutral, the glucuronoside was eluted with ethanol. Evaporation gave the *potassium salt* (75 mg, 64%) as a buff solid, m.p. 266—269°, which could not be crystallised (Found: C, 57·9; H, 7·1. $C_{25}H_{35}KO_9$ requires C, 57·7; H, 6·8%), $\nu_{max.}$ (KBr) 3480, 1742, and 1610 cm^{-1}, τ (CD_3·OD) 9·05 (s, 13-Me) and 4·40 (m, C-6 olefinic H).

Methyl [19-*Acetoxy*-17-*oxoandrost*-5-*en*-3β-*yl tri*-O-*acetyl* β-D-*glucopyranosid*]*uronate* (12).—3β-Hydroxy-19-acetoxyandrost-5-en-17-one (400 mg) gave the *ester* (12) (540 mg, 71%) as plates, m.p. 108—112° (Found: C, 63·3; H, 7·0. $C_{34}H_{46}O_{13}$,0·5C_6H_6 requires C, 63·3; H, 7·0%), or m.p. 103—108° (from acetone–hexane) (Found: C, 61·2; H, 7·3. $C_{34}H_{46}O_{13}$,Me_2CO requires C, 61·7; H, 7·2%), $\nu_{max.}$ (KBr) 1770, 1755, 1740, 1250—1220, and 1040 cm^{-1} for benzene solvate, τ 9·10 (s, 13-Me), 6·26 (s, OMe), 4·35 (m, C-6 olefinic H), and 2·65 (s, 0·5C_6H_6); acetone solvate, τ 7·83 (s, Me_2CO).

The results shown in the Table were obtained when this conjugate was crystallised from other solvent mixtures (ratio 1 : 1).

Solvent system	Solvent occluded	Molar proportion from n.m.r.	M.p. (°C)
Acetone–hexane	Acetone	1·0 *	103—108
Benzene–hexane	Benzene	1·0	101—110
		0·5 *	108—112
Chloroform–hexane			(Glass)
Dichloromethane–hexane			(Glass)
Ethyl acetate–hexane	Ethyl acetate	1·0	98—110
Toluene–hexane	Toluene	1·0	102—115

* After 6 h at 50° and 0·01 mmHg (analytical samples).

Potassium [19-*Hydroxy*-17-*oxoandrost*-5-*en*-3β-*yl* β-D-*glucopyranosid*]*uronate* (13).—Methanolic 1·0M-potassium hydroxide (3 ml) was added to a warm solution of the foregoing tetra-acetate (200 mg) in methanol (10 ml). The solution became pale yellow and after 24 h at room temperature had deposited colourless crystals. An equal volume of benzene was added and the crystals were collected and washed with a little cold methanol, giving the *potassium salt* (93 mg, 60%), m.p. 274—276° (Found: C, 54·2; H, 7·2. $C_{25}H_{35}KO_9$,H_2O,CH_3OH requires C, 54·9; H, 7·2%), $\nu_{max.}$ (KBr) 3430—3260, 1735, 1610, and 1040 cm^{-1}, τ [$(CD_3)_2SO$] 9·16 (s, 13-Me), 6·83 (s, MeOH), 6·67br (s, H_2O), and 4·43 (m, C-6 olefinic H).

Methyl [5α-*Bromo*-6β,19-*epoxy*-17-*oxoandrostan*-3β-*yl tri*-O-*acetyl*-β-D-*glycopyranosid*]*uronate* (9).—The 3β-alcohol (7) (500 mg), on coupling with the bromo-ester (5) gave the *glucosiduronate* (650 mg, 71%) as needles, m.p. 201—202·5° (Found: C, 55·1; H, 6·4; Br, 11·5. $C_{32}H_{43}BrO_{12}$ requires C, 54·9; H, 6·2; Br, 11·4%), $\nu_{max.}$ (KBr) 1765, 1755, 1740, 1255—1220, and 1060 cm^{-1}, τ ($CDCl_3$) 9·09 (s, 13-Me) and 6·24 (s, OMe), *m/e* 367 and 365(10%), 349 and 347(7), 317(20), 285(23), 267(10), 257(15), 255(13), 157(30), 155(67), 127(27), and 43(100).

Methyl [19-*Hydroxy*-17-*oxoandrost*-5-*en*-3β-*yl tri*-O-*acetyl*-β-D-*glucopyranosid*]*uronate* (15).—(*a*) A solution of the ester (9) (300 mg) in ethanol (100 ml) containing glacial acetic acid (0·3 ml) was refluxed with zinc dust (500 mg) for 4 h. The solution was decanted, the solvent was removed *in vacuo*, and the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with aqueous sodium hydrogen carbonate, then with water, and dried. The residue left after evaporation gave the *methyl ester* (191 mg, 70%) as needles, m.p. 193—195° (raised upon recrystallisation to 200—201°) (Found: C, 61·5; H, 7·2. $C_{32}H_{44}O_{12}$ requires C, 61·9; H, 7·1%), $\nu_{max.}$ (KBr) 3540, 1770, 1755, 1745, 1732, 1250—1220, and 1047 cm^{-1}, τ ($CDCl_3$) 9·06 (s, 13-Me), 6·25 (s, ester Me), and 4·20 (m, C-6 olefinic H).

When this reaction was repeated, using methanol instead of ethanol, the ester was obtained in 86% yield, m.p. 195—197°.

(*b*) 3β,19-Dihydroxyandrost-5-en-17-one (115 mg) in dry benzene (10 ml) and anhydrous ether (5 ml) was coupled with the bromo-ester (5) (920 mg). Crystallisation of the crude product gave rosettes of the methyl ester (82 mg, 35%), m.p. 200—201·5°, identical with material prepared by method (*a*).

Methyl [17,19-*Dioxandrost*-5-*en*-3β-*yl tri*-O-*acetyl*-β-D-*glucopyranosid*]*uronate* (8).—A solution of the foregoing 19-hydroxy-3β-glucosiduronate (45 mg) in purified acetone (25 ml) was treated with standard Jones reagent (0·2 ml) at room temperature. After 10 min the solution was diluted with water and decanted from the chromium salt sludge, and most of the acetone was removed *in vacuo*. The resulting suspension was extracted with ethyl acetate; the organic layer was washed well with water, dried ($MgSO_4$), and evaporated. Crystallisation of the residual oil from ethanol–methanol (1 : 1) gave the 19-*aldehyde* (23 mg, 51%), m.p. 116—117° (Found: C, 60·8; H, 6·9. $C_{32}H_{42}O_{12}$,H_2O requires C, 60·4; H, 6·9%), $\nu_{max.}$ (KBr) 1760, 1745, 1717, 1242, 1250—1210, and 1040 cm^{-1}, τ ($CDCl_3$) 9·17 (s, 13-Me), 6·25 (s, OMe), 4·12 (m, C-6 olefinic H), and 0·34 (s, C-19 aldehyde H).

Methyl [17-*Oxo*-19-*sulpho*-*oxyandrost*-5-*en*-3β-*yl tri*-O-*acetyl*-β-D-*glucopyranosid*]*uronate*, *Pyridinium Salt* (16).—Pyridine–sulphur trioxide complex (110 mg) was added to a solution of the foregoing 19-hydroxy-glucosiduronate (55 mg) in pyridine (1 ml) and the mixture was warmed until a clear solution was obtained. After 15 min at room temperate, water (5 ml) was added and the solution was extracted with chloroform. The dried extracts were evaporated and the residual oil (70 mg) was crystallised from dichloromethane–hexane to give the *pyridinium salt of the mixed conjugate* (55 mg, 73%) as an amorphous powder, m.p. 135—136° (Found: C, 56·7; H, 6·3; N, 1·7; S, 3·9. $C_{37}H_{49}NO_{15}S$ requires C, 57·0; H, 6·3; N, 1·8; S, 4·1%), $\nu_{max.}$ (KBr) 1760, 1223, and 1050 cm^{-1}, τ 9·06 (s, 13-Me), 7·97 and 7·93 (2′-, 3′-, and 4′-OAc), 6·26 (s, OMe), and 4·40 (m, C-6 olefinic H).

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[6] T. Nambara and S. Honma, *Chem. and Pharm. Bull.* (*Japan*), 1970, **18**, 1191.
[7] Y. Osawa and W. R. Slaunwhite, *Steroids*, 1970. **15**. 73.