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66. Yoshihiro Nitta, Minoru Shindo, and Keiichi Takamura : Studies on Steroids. III.*1 The Preparation of Steroid-21-yl-glucopyranosiduronamides.

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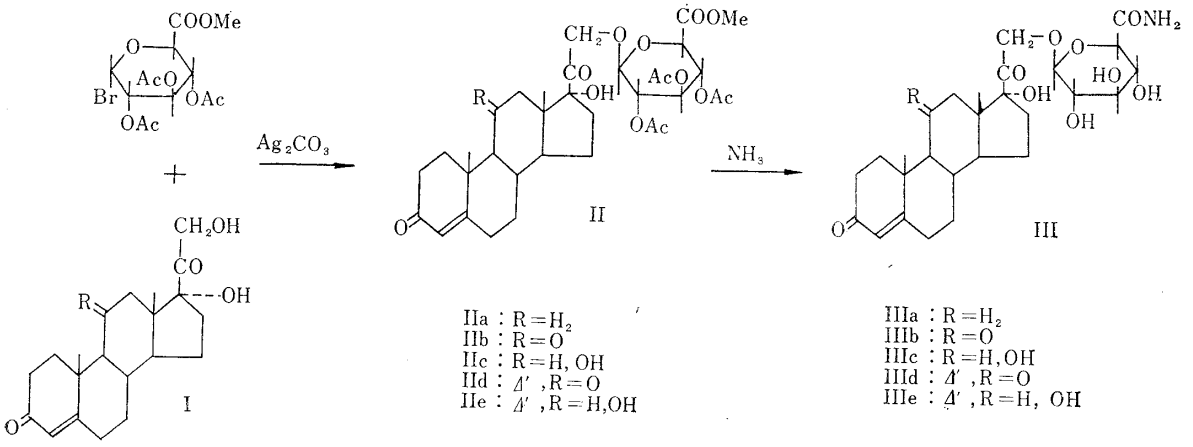
While reaction of steroid with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosiduronate is now well known, the example involving the reaction of a 21-OH group with the bromo compound is meager. Blocking of the 21-OH group may be not affect to the essentially biological properties. The investigation of preparation of the 21-methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronates has not been extended beyond that of only three compounds, *e.g.* deoxycorticosterone,[1] cortisone,[2] and 3β,17α,21-trihydroxyallopregnan-20-one.[2] Moreover, attempts to secure crystalline free acids of these compounds through the hydrolytic removal of the protecting groups from the sugar moiety were unsuccessful.

The present investigation was undertaken to prepare the 21-glucopyranosiduronates of some active adrenocortical steroids in order to obtain a water soluble derivatives of steroid which might display interesting biological properties.

Through hydrolytic removal of protecting groups, the objective free acid likewise was not isolated, but the free acid amide could be secured as a crystalline.



Ia : R=H_2
Ib : R=O
Ic : R=H,OH
Id : Δ^1, R=O
Ie : Δ^1, R=H,OH

IIa : R=H_2
IIb : R=O
IIc : R=H, OH
IId : Δ^1, R=O
IIe : Δ^1, R=H,OH

IIIa : R=H_2
IIIb : R=O
IIIc : R=H,OH
IIId : Δ^1, R=O
IIIe : Δ^1, R=H, OH

Treatment of the active adrenocortical steroids (I) with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosiduronate under conditions essentially the same as described by Mystre and Miescher[3] in their modification of the Königs-Knorr synthesis, gave the conjugation compounds (II). These products were always obtained as the oily substance after concentrating the reaction mixture. However, the white, crystalline

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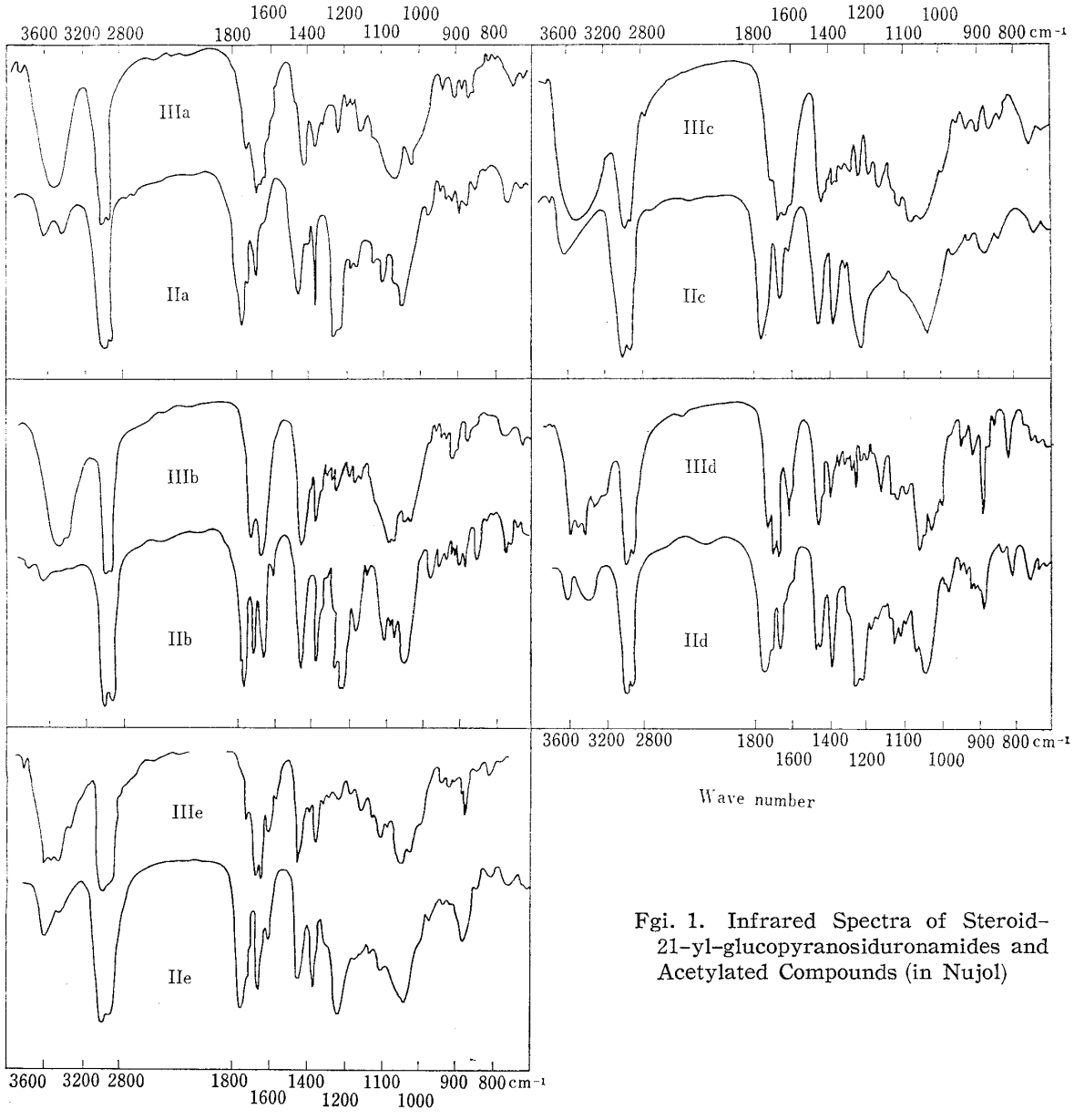
1) W. W. Zorbach : J. Org. Chem., **23**, 1797 (1958).
2) H. H. Wotiz, E. Smakula, N. N. Lichtin, J. H. Leftin : J. Am. Chem. Soc., **81**, 1704 (1959); *Idem* : *Ibid.*, **81**, 1708 (1959).
3) Ch. Mystre, K. Miescher : Helv. Chim. Acta, **27**, 231 (1944).

products were obtained by allowing the aqueous alcoholic solution to stand in the refrigerator for a few days or weeks. The above treatment of Ie afforded a poor yield of IIe, but the 60% of Ie could be recovered. On the other hand, in the case of other steroids the unreacted material were scarcely recovered.

Removal of the protecting groups from the sugar moiety of II by various hydrolytic measures, such as potassium carbonate, potassium hydrogen carbonate, sodium ethylate, sodium methylate, potassium hydroxide, barium hydroxide and anion exchange resines, always resulted in the unknown hygroscopic glass-like products, as reported up to the present. However, treatment of II with methanolic ammonia afforded a white needles, respectively. These products were positive for Liebermann-Burchardt reaction and for naphthoresorcine reaction, and negative for triphenyltetrazolium chloride reaction which is used to detect the α-ketol function, thus indicating that they are binding products of steroid with glucuronic acid derivative at the 21-OH group.

In order to obtain evidence for the type of linkage involved, the infrared absorption spectra and Barton's molecular rotation method were utilized.



Fgi. 1. Infrared Spectra of Steroid-21-yl-glucopyranosiduronamides and Acetylated Compounds (in Nujol)

Table I.

Compd.	Optical rotation $[\alpha]_D$	Molecular rotation $[M]_D$ Found	Molecular rotation $[M]_D$ Calcd.[a] α	β
Ia	+132 (D)	+457		
Ib	+209 (E)	+752		
Ic	+167 (E)	+606		
Id	+172 (D)	+616		
Ie	+102 (D)	+377		
Methyl (methyl-2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate	+174 (C)[b]	+605		
Methyl (methyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate	− 29 (C)[c]	−101		
IIa	+ 48 (E)	+317	+1062	+356
IIb	+ 95 (E)	+646	+1358	+652
IIc	+ 69 (E)	+468	+1210	+504
IId	+ 95 (E)	+639	+1221	+515
IIe	+ 59 (E)	+399	+ 973	+267
Methyl-α-D-glucuronamide	+135 (M)[b]	+279		
Methyl-β-D-glucuronamide	− 72 (M)[c]	−149		
IIIa	+ 52 (E-D)	+271	+ 736	+308
IIIb	+ 99 (E-D)	+531	+1031	+603
IIIc	+ 67 (E-D)	+360	+ 885	+457
IIId	+ 97 (E-D)	+517	+ 895	+467
IIIe	+ 62 (E-D)	+331	+ 656	+228

D: dioxane; E: EtOH; C: $CHCl_3$; M: MeOH; E-D: EtOH-dioxane

a) IIa~e: $[M]_D$ methyl(methyl-2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate+$[M]_D$ (Ia~e)
IIIa~e: $[M]_D$ (methyl-α-D-glucuronamide)+$[M]_D$ (Ia~e)
IIa~e: $[M]_D$ methyl(methyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate+$[M]_D$ (Ia~e)
IIIa~e: $[M]_D$ (methyl-β-D-glucuronamide)+$[M]_D$ (Ia~e)

b) E. Hardegger, D. Spitz: Helv. Chim. Acta, **32**, 2165 (1949).

c) *Idem*: *Ibid.*, **33**, 337 (1950).

Table II.

Compd.	m.p. (°C)	Optical rotation[a] $[\alpha]_D$	(c)	UV λ_{max}^{EtOH} mμ (ε)
IIa	134~136	+48±4	(0.32)	242 (1.040×10^4)
IIb	193	+95±3	(0.12)	237 (1.699×10^4)
IIc	118~120	+69±2	(1.81)	242 (1.779×10^4)
IId	135~137	+95±3	(0.28)	238 (1.906×10^4)
IIe	118~122	+59±1	(0.34)	244 (1.237×10^4)
IIIa	242.5~243.0 (decomp.)	+52±5	(0.90)	240 (1.017×10^4)
IIIb	244~245 (〃)	+99±4	(0.95)	241 (0.536×10^4)
IIIc	259~260 (〃)	+67±5	(0.49)	244 (1.204×10^4)
IIId	257~258 (〃)	+97±10	(0.30)	242 (0.941×10^4)
IIIe	253~254 (〃)	+62±10	(0.21)	248 (0.672×10^4)

Compd.	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N	Yield based on I (%)
IIa	$C_{34}H_{46}O_{13}$	61.62	6.91		62.10	7.16		38.7
IIb	$C_{34}H_{44}O_{14}$	60.35	6.55		59.86	6.56		30.0
IIc	$C_{34}H_{46}O_{14}$	60.17	6.84		60.26	7.05		42.8
IId	$C_{34}H_{42}O_{14}\cdot H_2O$	58.95	6.40		58.97	6.52		26.5
IIe	$C_{34}H_{44}O_{14}\cdot H_2O$	58.78	6.67		59.08	6.74		11.7
IIIa	$C_{27}H_{39}NO_9\cdot H_2O$	60.10	7.66	2.60	59.82	7.99	2.76	29.0
IIIb	$C_{27}H_{37}NO_{10}\cdot H_2O$	58.58	7.10	2.53	58.83	7.49	2.46	20.2
IIIc	$C_{27}H_{39}NO_{10}\cdot H_2O$	58.37	7.43	2.52	58.05	8.08	2.35	19.4
IIId	$C_{27}H_{35}NO_{10}$	60.78	6.61	2.62	61.04	6.72	2.55	16.8
IIIe	$C_{27}H_{37}NO_{10}\cdot H_2O$	58.58	7.10	2.53	58.71	7.18	2.61	2.7

a) IIa~e: $[\alpha]_D^{19}$ in EtOH IIIa~e: $[\alpha]_D^{24}$ in EtOH-dioxane (1:1)

Their glucosidic linkages were considered to be in a β-configuration,[1,4] respectively, from the result calculated according to Barton's molecular rotation method,[5] as can be seen in the Table I. This is further proven by the fact that the acetylated compounds (II) showed no characteristic absorption bands[6] of α-anomer at around 940 cm^{-1}, as can be seen in the Fig. 1. The infrared spectra of II showed the strong absorption for the ester groups of the sugar moiety near 1750, 1220, and 1040 cm^{-1}. In the case of III, evidence for the removal of ester groups was derived from their infrared spectra which showed the absence of characteristic bands of ester groups and the present of strong band in the region of 3500~3300 cm^{-1}. Moreover, new bands near 1600 cm^{-1} for III are assigned to be absorptions due to the NH_2 deformation mode. Retention of the α,β-unsaturated ketone of II and III is substantiated by the ultraviolet spectra (λ_{max} 237~248 mμ) of these compounds.

Thus, III was identified as the β-D-glucopyranosiduronamide of steroid linked at 21-OH group.

Experimental*3

General Procedure——The physical data and yield for each individual product were shown in Table II.

1) **Methyl Steroid-21-yl-2',3',4'-tri-O-acetyl-β-D-glucopyranosiduronate (II)**——To a stirred solution of 14 mmoles of steroid I in 150 ml. of dioxane*4 and 1200 ml. of benzene was added 36 mmoles of dry Ag_2CO_3. One hundred milliliters of the solvent was gently distilled off at 80~90°, during which time a solution of 25 mmoles of methyl (1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate in 100 ml. of benzene was added to it. The mixture was allowed to reflux for 8 hr. During the reaction, 300 ml. of benzene was added gradually and at the same time the equal volume of solvent was distilled off. The Ag salts were filtered off and the filtrate was evaporated to dryness *in vacuo*. The aq. EtOH solution of the residual gummy material was crystallized after allowing to stand in the refrigerator for a few days or weeks, and the solid was filtered and recrystallized from the same solvent several times until a constant melting range was obtained.

2) **Steroid-21-yl-β-D-glucosiduronamide (III)**——Two and one-tenth mmoles of glucosiduronate (II) was added to 150 ml. of an abs. MeOH-NH_3 in an ice bath, and the dry NH_3 gas was passed into the mixture for 3 hr. The mixture was evaporated to dryness *in vacuo*, and the partially crystallized residue was treated with 10 ml. of MeOH and kept in a refrigerator. The precipitated crystal was recrystallized from EtOH several times. The products were obtained as white needles.

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Summary

The methyl steroid-21-yl-β-D-glucopyranosiduronates were prepared by treatment of Reichstein's compound S, cortisone, hydrocortisone, prednisone and prednisolone with methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-α-D-glucopyranosiduronate. Treatment of these compounds with methanolic ammonia afforded the corresponding steroid-21-yl-β-D-glucopyranosiduronamide.

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*3 Melting points are uncorrected.

*4 Benzene in Ia.

4) Ch. Mystre, K. Miescher : Helv. Chim. Acta, **34**, 2286 (1951).

5) D. H. R. Barton, E. R. H. Jones : J. Chem. Soc., **1944**, 659; D. H. R. Barton : *Ibid.*, **1945**, 813; *Ibid.*, **1946**, 512.

6) Y. Nitta, *et al.* : Yakugaku Zasshi, **81**, 1160 (1961).