the spectrum of 11; mass spectrum (70 eV, glpc inlet, ion source 230°) (Figure 6).

Jones Oxidation of Cholest-5-en-4α-ol (4).—Jones reagent[14] (8 N, 0.85 ml) was added dropwise to a solution of 1.125 g (2.92 mmol) of alcohol **4** in 100 ml of acetone at 20°. The solution was warmed to room temperature and then diluted with an equal volume of water. The ether extract of this solution was washed with water (twice), saturated sodium bicarbonate solution, and brine. Evaporation of the solvent and crystallization from acetone gave 0.9 g (81%) of pure cholest-5-en-4-one **6**: mp 111–112°; $\lambda_{max}^{C_2H_5OH}$ 241 mμ (ε 6000) [lit.[15] mp 111–112°; $\lambda_{max}^{C_2H_5OH}$ 241 mμ (ε 7200)]; $\nu_{max}^{CHCl_3}$ 1675 and 1620 cm^{-1}.

Reaction of Sodium Hydride with 3β-*p*-Toluenesulfonoxy-cholest-5-en-4β-ol (1).—To a stirred solution of 500 mg (0.9 mmol) of tosylate **1** in 25 ml of tetrahydrofuran (thf) under a nitrogen atmosphere was added 41.0 g (0.9 mmol) of sodium hydride on mineral oil 52.8% NaH). The mixture was heated at reflux for 3 hr, cooled, and 1 equiv of water was added dropwise. The resulting solution was filtered and evaporated to dryness at reduced pressure. The brown residue which resulted was dissolved in ether and the ether solution was washed with water (twice), 5% sodium bicarbonate, and brine, and then dried over sodium sulfate. Evaporation of the solvent gave 0.45 g of crude product. Column chromatography on 20 g of silica gel (E. Merk), afforded 165 mg (48%) of cholest-5-en-4-one (**6**): mp 111–112° (from acetone); $\nu_{max}^{CHCl_3}$ identical with that of the material obtained from Jones oxidation of cholest-5-en-4α-ol (**4**).

Lithium Aluminum Hydride Reduction of Cholest-5-en-4-one (6).—To a slurry of 380 mg (10 mmol) of lithium aluminum hydride in 5.8 ml of refluxing anhydrous ether was added 200 mg (0.52 mmol) of ketone **6** in 3.0 ml of dry benzene. The resulting slurry was heated at reflux for 24 hr. The usual work-up gave a crude product that tlc identified as cholest-5-en-4α-ol (**4**) but no cholest-4-ene (**3**). Crystallization of the crude product from hexane gave 190 mg (95%) of **4**: 140–151°; $\nu_{max}^{CHCl_3}$ identical with that of the known **4**.

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Registry No.—Lithium aluminum hydride, 16853-85-3; **1**, 5847-14-3; **3**, 16732-86-8; **4**, 20230-16-4; **8**, 21537-62-2; **9**, 21537-63-3; **10**, 21537-64-4; **11**, 21537-65-5.

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Anomeric C-19-Steroid *N*-Acetylglucosaminides[1]

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The α and β anomers of C-19-steroid 2′-acetamido-2′-deoxy-D-glucopyranoside conjugated at C-3 or C-17 have been prepared from their corresponding 3′,4′,6′-tri-*O*-acetyl derivatives. The acetylated β-glucosaminides were synthesized from the steroid and 1α-chloro-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose with mercuric salts; anomerization was achieved by treatement of the acetylated β-glucosaminides with titanium tetrachloride. The differences in the molecular rotation and ir and nmr spectra between the anomers were studied. The α anomer had a sharply defined intense band between 1110 and 1125 cm^{-1} whereas the β anomer lacked this strong absorption between 1100–1200 cm^{-1}. In addition to the difference of the splitting patterns of the anomeric H-1′ protons in the nmr spectra, a broad multiplet for the H-6′ methylene protons, $W_{1/2} \sim 8.5$ cps, in the β anomer and a narrow multiplet, $W_{1/2} \sim 4.0$ cps, in the α anomer at δ 4.1–4.2 ppm were observed. For comparison purposes the synthesis and physical properties of the anomers of cyclohexyl *N*-acetylglucosaminides were investigated.

Three steroids conjugated with *N*-acetylglucosamine have been recently isolated: 3-hydroxy-$\Delta^{1,3,5(10)}$-estratrien-17α-yl 2′-acetamido-2′-deoxy-β-D-glucopyranoside,[4] $\Delta^{1,3,5(10)}$-estratriene-3,16α,17α-triol 2′-acetamido-2′-deoxy-β-D-glucopyranoside conjugated at C-16 or -17,[5] from rabbit urine, and 3β-hydroxy-Δ^5-pregnen-20α-yl 2′-acetamido-2′-deoxy-α-D-glucopyranoside from human urine.[6] All were present as the double conjugate, the first two as the 3-glucosiduronate and the last as the 3-sulfate ester. In order to study the properties of steriod *N*-acetylglucosaminides to aid in the isolation of this type of conjugate, the synthesis of various C-19 steroids conjugated with *N*-acetylglucosamine at C-3 or at C-17 in both the α- and β-glycosidic linkage were investigated.

The Koenigs–Knorr reaction has been successfully employed for the preparation of alkyl *N*-acetyl-β-D-glucosaminides.[7] An application of this reaction to steroids has been reported by Hirschmann and coworkers[8] without experimental detail in the preparation of 11β,17-dihydroxy-3,20-diketo-$\Delta^{1,4}$-pregnadien-21-yl 2′-acetamido-2′-deoxy-β-D-glucopyranoside. Preliminary studies in the reaction of 1-α-chloro-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose with various steroid alcohols in the presence of silver carbonate demonstrated that only the β-glycoside was isolated but in very poor yields. The yields were improved (10–25%) by carrying out the reaction with mercuric salts, mercuric chloride and mercuric cyanide, and the chloro sugar.[7a] In these cases, trace amounts of the α-glycosides were formed but were readily separated from the desired β epimer. Consequently the tetraacetyl β-glucosaminides conjugated at C-3 with 3α-hydroxy-5α-androstan-17-one, its 3β and 5β epimers, and 3β-hydroxy-Δ^5-androsten-17-one and those con-

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TABLE I

PHYSICAL CONSTANTS OF STEROID 2′-ACETAMIDO-2′-DEOXY-D-GLUCOPYRANOSIDES

	Compounds	Registry No.	Yield, %	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
					β-Glucopyranoside						
1βa	17-Keto-5α-androstan-3α-yl	21543-52-2		150–153°	$C_{27}H_{43}NO_7 \cdot H_2O$	63.36	8.87	2.74	63.83	8.74	2.77
1βb	Tri-*O*-acetyl	21543-53-3	14	229–231	$C_{33}H_{49}NO_{10}$	63.95	7.97	2.26	64.07	7.68	2.28
2βa	17-Keto-5α-androstan-3β-yl	21543-54-4		163–166/ 223–228	$C_{27}H_{43}NO_7 \cdot 0.5H_2O$	64.50	8.82	2.79	64.58	8.86	2.83
2βb	Tri-*O*-acetyl	21543-55-5	16	282–283	$C_{33}H_{49}NO_{10}$	63.95	7.97	2.26	63.71	7.93	2.32
3βa	17-Keto-5β-androstan-3α-yl	21543-56-6		161–163	$C_{27}H_{43}NO_7 \cdot 0.5H_2O$	64.50	8.82	2.79	64.15	8.64	2.74
3βb	Tri-*O*-acetyl	21543-57-7	24	179–181	$C_{33}H_{49}NO_{10} \cdot H_2O$	62.15	8.06	2.19	61.91	7.95	2.23
4βa	17β-Hydroxy-5β-androstan-3α-yl	21543-58-8		190–196	$C_{27}H_{45}NO_7 \cdot H_2O$	63.13	9.22	2.73	62.95	9.14	2.72
4βb	3′,4′,6′-Tri-*O*-acetyl	21543-59-9		133–135	$C_{33}H_{51}NO_{10} \cdot 1.5H_2O$	61.09	8.39	2.15	61.31	8.11	2.07
5βa	17-Keto-Δ⁵-androsten-3β-yl	21543-60-2		158–163	$C_{27}H_{41}NO_7 \cdot 0.5H_2O$	64.76	8.46	2.80	64.25	8.51	2.77
5βb	Tri-*O*-acetyl	21543-61-3	25	260–261	$C_{33}H_{47}NO_{10}$	64.20	7.66	2.27	63.70	7.57	2.34
6βa	3-Keto-5α-androstan-17β-yl	21543-62-4		220–230	$C_{27}H_{43}NO_7$	65.70	8.78	2.84	65.14	8.75	2.82
6βb	Tri-*O*-acetyl	21543-63-5	10	124–132	$C_{33}H_{49}NO_{10} \cdot 0.5H_2O$	62.99	8.01	2.23	62.82	7.87	2.37
7βa	3β-Hydroxy-Δ⁵-androsten-17β-yl	21543-64-6		271–278	$C_{27}H_{43}NO_7$	65.70	8.78	2.84	65.06	8.37	2.82
7βb	3,3′,4′,6′-Tetra-*O*-acetyl	21543-65-7	11	242	$C_{35}H_{51}NO_{11} \cdot 0.5H_2O$	62.64	7.81	2.09	62.52	7.60	2.19
8βa	3-Keto-Δ⁴-androsten-17β-yl	21559-58-0		261–263	$C_{27}H_{41}NO_7 \cdot 0.5H_2O$	64.76	8.46	2.80	64.25	8.18	2.66
8βb	Tri-*O*-acetyl	21559-59-1	25	192–193	$C_{33}H_{47}NO_{10}$	64.16	7.67	2.27	64.13	7.73	2.32
					α-Glucopyranoside						
1αa	17-Keto-5α-androstan-3α-yl	21559-60-4		277–279	$C_{27}H_{43}NO_7$	65.70	8.78	2.84	66.15	8.77	2.82
1αb	Tri-*O*-acetyl	21559-61-5		Amorph	$C_{33}H_{49}NO_{10}$	63.95	7.97	2.26	63.95	7.91	2.15
2αa	17-Keto-5α-androstan-3β-yl	21559-62-6		143–145	$C_{27}H_{43}NO_7 \cdot 1.5H_2O$	62.27	8.90	2.69	62.15	8.49	2.65
2αb	Tri-*O*-acetyl	21559-63-7		Amorph	$C_{33}H_{49}NO_{10} \cdot 0.5H_2O$	62.99	8.01	2.23	63.22	7.84	2.21
3αa	17-Keto-5β-androstan-3α-yl	21559-64-8		Amorph	$C_{27}H_{43}NO_7 \cdot H_2O$	63.36	8.87	2.74	63.40	8.65	2.66
3αb	Tri-*O*-acetyl	21559-65-9		Amorph	$C_{33}H_{49}NO_{10}$	63.95	7.97	2.26	64.31	7.88	2.02
4αa	17β-Hydroxy-5β-androstan-3α-yl	21559-66-0		170–186	$C_{27}H_{45}NO_7 \cdot 0.5H_2O$	64.25	9.19	2.77	64.26	9.52	2.60
4αb	3′,4′,6′-Tri-*O*-acetyl	21559-67-1		Amorph	$C_{33}H_{51}NO_{10}$	63.74	8.27	2.25	63.92	8.36	2.22
6αa	3-Keto-5α-androstan-17β-yl	21559-68-2		248–257	$C_{27}H_{43}NO_7$	65.70	8.78	2.84	66.20	8.90	2.75
6αb	Tri-*O*-acetyl	21559-69-3		Amorph	$C_{33}H_{49}NO_{10}$	63.95	7.97	2.26	63.58	7.93	2.28
8αa	3-Keto-Δ⁴-androsten-17β-yl	21559-70-6		271–274	$C_{27}H_{41}NO_7$	65.97	8.41	2.85	65.99	8.45	2.68
8αb	Tri-*O*-acetyl	21559-71-7		Amorph	$C_{33}H_{47}NO_{10}$	64.16	7.67	2.27	64.18	7.59	2.31

jugated at C-17 with 17β-hydroxy-5α-androstan-3-one, 17β-hydroxy-Δ⁴-androsten-3-one, and Δ⁵-androstene-3β,17β-diol 3-monoacetate have been prepared by the mercuric salt catalyzed reaction (Table I). O deacetylation by transesterification afforded the steroidal 2′-acetamido-2′-deoxy-β-D-glucopyranosides.

The α anomer of the tetraacetylglucosaminides of simple alcohols and phenols has been prepared by acid catalyzed condensation of *N*-acetylglucosamine or glucosamine pentaacetate with the alcohols and phenols in reasonable yields accompanied by appreciable amounts of the β-glycoside.[9] Attempts at modifying these methods for the synthesis of steroid *N*-acetylglucosaminides were unsuccessful. Zilliken and coworkers[10] employed a cation-exchange resin as the acidic catalyst. Since it was possible that this method could be applied to the synthesis of steroid *N*-acetylglucosaminide the reaction of cyclohexanol as a model substance and *N*-acetylglucosamine in the presence of Amberlite IR-120 (H^+) was undertaken. At 85°, both anomers of cyclohexyl 2′-acetamido-2′-deoxy-D-glycopyranoside were obtained with the α-glycoside predominating. For comparison purposes cyclohexyl 2′-acetamido-2′-deoxy-β-D-glucopyranoside was prepared from cyclohexanol and 1α-chloro-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose by the Koenigs–Knorr reaction with silver carbonate followed by O deacetylation. The β compounds obtained from the two routes were identical. Since the acid catalyzed condensation afforded predominant formation of the α anomer, treatment of cyclohexyl 2′-acetamido-2′-deoxy-β-D-glucopyranoside with acid should lead to anomerization. Indeed phenyl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside has been anomerized in good yield with zinc chloride in molten phenol.[9c] A more convenient method of anomerization appeared to be the use of titanium tetrachloride in chloroform solution previously described for acetylated β-glycosides.[11] Almost quantitative anomerization of cyclohexyl 2′-acetamido-2′-deoxy-3′,4′6′-tri-*O*-acetyl-β-D-glucopyranoside was obtained with titanium tetrachloride at room temperature. O deacetylation gave cyclohexyl 2′-acetamido-2′-deoxy-α-D-glucopyranoside, identical with the product obtained by condensation of cyclohexanol with *N*-acetylglucosamine in the presence of Amberlite IR-120 (H^+).

The *N*-acetyl-α-glucosaminides of the saturated steroids were prepared in equally high yields by the anomerization of the steroid 2′-acetamido-2′-deoxy-3′,-4′,6′-tri-*O*-acetyl-β-D-glucopyranoside with titanium tetrachloride followed by O deacetylation (Table I). The β-glucosaminides of the Δ⁵-unsaturated steroids **5βb** and **7βb** were unstable under the anomerization conditions and the α-anomer of these compounds could not be prepared by this method.

The assignment of configuration of the glucosaminides has been made from the method of preparation

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TABLE II

MOLECULAR ROTATION DIFFERENCE OF N-ACETYLGLUCOSAMINIDES

2'-Acetamido-2'-deoxy-D-glucopyranoside	MD[a] Aglycone	MD[a] α	MD[a] β	ΔMD (α − aglycone)	ΔMD (β − aglycone)	ΔMD (α − β)
Methyl[b] tri-*O*-acetyl		+308	−104	+308	−104	+412
		+361	−87	+361	−87	+442
Ethyl[b]		+334	−106	+334	−106	+440
N-Propyl[b]		+368	−98	+368	−98	+466
Cyclohexyl tri-*O*-acetyl		+509	−67	+509	−67	+576
		+460	−116	+460	−116	+576
17-Keto-5α-androstan-3α-yl	+278	+844	+187	+566	−91	+657
tri-*O*-acetyl		+924	+211	+646	−67	+713
17-Keto-5α-androstan-3β-yl	+264	+840	+245	+576	−19	+595
tri-*O*-acetyl		+731	+216	+467	−48	+515
17-Keto-5β-androstan-3α-yl	+316	+950	+246	+634	−70	+704
Tri-*O*-acetyl		+862	+266	+546	−50	+596
17-Hydroxy-5β-androstan-3α-yl	+76	+701	+56	+625	−20	+645
tri-*O*-acetyl		+622	+23	+546	−53	+599
17-Keto-Δ5-androsten-3β-yl	+33		+84		+51	
tri-*O*-acetyl			+51		+18	
3-Keto-5α-androstan-17β-yl	+87	+721	+104	+634	+17	+617
tri-*O*-acetyl		+743	+94	+656	+7	+649
3β-Hydroxy-Δ5-androsten-17β-yl	−162		−155		+7	
tetra-*O*-acetyl	−186		−212		−26	
3-Keto-Δ4-androsten-17β-yl	+322	+940	+296	+618	−26	+644
tri-*O*-acetyl		+931	+265	+609	−57	+666

[a] In methanol. [b] In water: Zilliken, *et al.*[10]

and from optical rotation studies of the products. In accordance with analogous reactions, the β-glycoside linkage has been assigned to the products obtained by the Koenigs–Knorr reaction and the α configuration to the products of the anomerization with titanium tetrachloride. The optical rotations of the products are in agreement with the Hudson rule that the β anomers, the compounds prepared from the Koenigs–Knorr reaction, are more levorotatory. The molecular rotation differences between the anomers, ΔMD (α − β), were +515 to +713 for the steroid glucosaminides which were slightly greater than the differences, +412 to +466, for the lower alkyl derivatives (Table II). The cyclohexyl anomer had ΔMD (α − β) values, +576, within the ranges found for the steroid conjugates. Furthermore, the molecular rotation differences between the steroid glucosaminides and steroids, ΔMD (α − aglycone) and ΔMD (β − aglycone), were in agreement with Hudson's rule that change at the glycosidic carbon atom C-1 does not affect the rotation of the remainder of the sugar moiety and with Klyne's application of this rule to steroid glycosides.[12] Steroid α-D-glucosaminides all have very high positive ΔMD (α − aglycone) values ranging from +496 to +646 which are higher than those, +308 to +368, for the simple alkyl α-glycosides. These differences can undoubtedly be ascribed to the bulky nature of the steroid at C-1 of the sugar; the cyclohexyl derivatives have values, +460 and +509, more nearly like those of the steroid conjugates. The ΔMD (β − aglycone) values for the simple alkyl and the cyclohexyl β-D-glucosaminides were all negative as were the values for most of the steroid β-glycosidic conjugates. There were three steroid conjugates which had a positive ΔMD (β − aglycone) values but these values were very low and considerably lower than those positive values observed in the α anomers.

The utility of nmr spectrometry in carbohydrate chemistry has been well documented.[13] The chemical shifts of the methine protons in the nmr spectra of the anomers of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranoses have been assigned by Inch, Plummer, and Fletcher[14] and by Horton.[15] A difference in the chemical shifts of the anomeric protons, H-1, was observed. H-1 in the α anomer gave a doublet further downfield, δ 6.2 and a smaller coupling constant, J = 3.5 cps, than that in the β anomer, δ 5.8 and J = 8.5 cps. In a series more applicable to the present study Agahigian and coworkers[16] observed similar differences in the chemical shifts of H-1 in the anomers of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranosides. However the doublets for H-1 were further upfield, δ 4.75, J = 3.5 cps, and 4.65, J = 8.0 cps, for the α- and β-glycosides respectively. In the steroidal and cyclohexyl 2'-acetamido-2'-deoxy-3',4',6'-tri-*O*-acetyl-D-glucopyranosides the H-1' doublet appeared at δ 4.87–4.97, J = 3.5 cps, for the α anomers and 4.67–4.90, J = 8–8.5 cps, for the β-glycosides (Table III). In addition the nmr spectra of the methyl glycosides exhibited a small difference in the chemical shifts for the H-6 methylene protons; in the α anomer the signals appeared further upfield at δ 4.20 compared to those in the β anomer, δ 4.25. Similar relationship has been observed in this region in the present study. However a more striking difference has been noticed for the steroidal and cyclohexyl N-acetylglucosaminides which is not present in the methyl N-acetylglucosaminides. The multiplet centered at δ 4.2 in the β anomer of the cycloalkyl derivative is quite broad, half band width $W_{1/2}$ = 8.5 cps, whereas there is a narrower multiplet centered at 4.15, $W_{1/2}$ = 3.5 cps, in the α derivative. The broader band observed for the β anomer may be the result of restricted rotation of the C-6' primary al-

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TABLE III

CHEMICAL SHIFT (δ) OF C-1′ PROTON AND C-6′ METHYLENE PROTONS OF STEROID 2′-ACETAMIDO-2′-DEOXY-3′,4′,6′-TRI-*O*-ACETYL-D-GLUCOPYRANOSIDES

	α anomer			β anomer	
Compound	H-1′ (J, cps)	H-6, ($W_{1/2}$, cps)	Comp	H-1′ (J, cps)	H-6′ ($W_{1/2}$, cps)
1αb	4.97 (d, J = 3.5)	4.13 (nm, $W_{1/2}$ = 4)	1βb	4.83 (d, J = 8.5)	4.15 (m, $W_{1/2}$ = 8)
2αb	4.97 (d, J = 3.5)	4.13 (nm, $W_{1/2}$ = 4)	2βb	4.88 (d, J = 8.0)	4.17 (m, $W_{1/2}$ = 8)
3αb	4.97 (d, J = 3.5)	4.13 (nm, $W_{1/2}$ = 4)	3βb	4.90 (d, J = 8.5)	4.17 (m, $W_{1/2}$ = 8)
4αb	4.97 (d, J = 3.5)	4.15 (nm, $W_{1/2}$ = 4)	4βb	4.90 (d, J = 8.0)	4.15 (m, $W_{1/2}$ = 8)
6αb	4.87 (d, J = 3.5)	4.13 (nm, $W_{1/2}$ = 4)	5βb	4.90 (d, J = 8.0)	4.18 (m, $W_{1/2}$ = 8)
8αb	4.90 (d, J = 3.5)	4.17 (nm, $W_{1/2}$ = 4)	6βb	4.67 (d, J = 8.5)	4.17 (m, $W_{1/2}$ = 8)
			7βb	4.67 (d, J = 8.0)	4.18 (m, $W_{1/2}$ = 8)
			8βb	4.70 (d, J = 8.5)	4.18 (m, $W_{1/2}$ = 8)

TABLE IV

INFRARED ABSORPTION BANDS OF STEROID *N*-ACETYLGLUCOSAMINIDE

β-Glycosides				α-Glycosides			
Compound	Amide I, cm^{-1}	Amide II, cm^{-1}	1100–1200 cm^{-1}	Compound	Amide I, cm^{-1}	Amide II, cm^{-1}	1100–1200 cm^{-1}
1βa	1660	1555	1158, 1115	1αa	1644	1557	1120
1βb	1690 (sh), 1660	1560, 1530 (sh)	1168, 1120	1αb	1688, 1675 (sh)	1525	1126
2βa	1640	1572	1158, 1119, 1102	2αa	1657, 1640 (sh)	1558	1120
2βb	1680, 1655	1575, 1560 (sh)	1168, 1150, 1120	2αb	1687, 1680 (sh)	1527	1128
3βa	1667, 1635 (sh)	1570 (sh), 1540	1162, 1115	3αa	1662	1545	1120
3βb	1680 (sh), 1660	1550	1170, 1138, 1118	3αb	1690, 1670 (sh)	1520	1125
4βa	1635	1575	1179, 1155, 1142	4αa	1680	1523	1108
4βb	1668	1545 (sh), 1535	1175.1140	4αb	1654	1555	1128
5βa	1640, 1625 (sh)	1570, 1555 (sh)	1158, 1118, 1110				
5βb	1680, 1655	1575, 1550 (sh)	1168, 1135, 1122, 1110				
6βa	1660 (sh), 1627	1555	1170, 1124	6αa	1657 (sh), 1628	1579, 1530 (sh)	1123
6βb	1675	1545	1175, 1122	6αb	1685, 1670 (sh)	1525	1128
7βa	1630 (sh), 1620	1565	1163, 1101				
7βb	1690, 1670	1560 (sh), 1530	1180, 1120				
8βa	1664, 1620 (sh)	1545	1175, 1118	8αa	1646, 1610 (sh)	1545	1112
8βb	1678, 1620 (sh)	1550	1175, 1122	8αb	1678, 1618 (sh)	1522	1128

cohol ester by the bulky cyclohexyl ring at C-1′ affording greater coupling possibilities for the methylene protons. In the α anomer, there is freer rotation of the carbinol ester group and the H-6′ protons will give a sharper signal.

The chemical shifts of the methyl protons of the acetyl groups in all compounds studied were in agreement with those reported by Horton, Mast, and Philips.[17] The signals of the angular methyl protons of the steroids conjugated at C-3 appeared in positions expected for derivatization of the C-3 hydroxyl group; there was no significant difference between the anomers in the chemical shifts of the C-19 methyl protons. However when the amino sugar was attached at C-17, there was a 5-cps difference in the chemical shifts of the C-18 methyl protons with the signal in the α-glycoside appearing downfield, the δ for **6αb** and **6βb** were 0.83 and 0.75, respectively, and for **8αb** and **8βb** were 0.88 and 0.80.

Correlation of infrared absorption bands with anomeric configuration in the derivatives of D-glucopyranose has been discussed by Spedding.[18] Barker and coworkers[19] have reported characteristic absorption bands for the anomers of methyl 2-acetamido-2-deoxy-D-glucopyranoside in the region 730–960 cm^{-1}. In the present study these correlations were not apparent; the amide I and II bands were not definitive for a distinction between α and β anomer. However, in the *N*-acetyl-D-glucosamine derivatized with steroid, cyclohexyl or methyl at C-1′ and whether tri-O-acetylated or not, a distinction between α- and β-glycosides could be clearly discerned between 1100 and 1200 cm^{-1}. Here the α anomer was characterized by sharply defined, intense band between 1110 and 1125 cm^{-1}. The β anomers lack this sharply defined absorption and between 1100 and 1200 cm^{-1} have a group of bands of lesser intensity (Table IV). Similar differences in the infrared spectra of the anomeric pyranose sugar acetates[20] and glucopyranuronic acid derivatives[21] have been reported.

Experimental Section[22]

General Method. Synthesis of Steroid 2′-Acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranosides.—A solution of 731 mg of 1α-chloro-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose (2 mmol) in 25 ml of anhydrous benzene was added

(17) D. Horton, W. E. Mast, and K. D. Philips, *J. Org. Chem.*, **32**, 1471 (1967).

(18) H. Spedding, *Advan. Carbohyd. Chem.*, **19**, 23 (1964).

(19) S. A. Barker, E. J. Bourne, M. Stacey, and D. H. Whiffen, *J. Chem. Soc.*, 171 (1954).

(20) H. S. Isbell, F. A. Smith, E. C. Creitz, H. L. Frush, J. D. Moyer, and J. E. Stewart, *J. Res. Nat. Bur. Stand.*, **59**, 41 (1957).

(21) Y. Nitta, Y. Nakajima, M. Kuranari, A. Momose, and J. Ide, *Yakugaku Zasshi*, **81**, 1160 (1961).

(22) Melting points were taken on a micro hot stage apparatus and are corrected. Nmr spectra were determined in deuteriochloroform with tetramethylsilane as internal standard on a Varian A-60 spectrometer. Chemical shifts are expressed in δ (parts per million): d, doublet; m multiplet; nm, narrow multiplet; $W_{1/2}$ = half height width. Infrared, spectra were determined in potassium bromide dispersion on a Beckman IR-9 spectrophotometer: sh, shoulder. Optical rotations were determined in methanol at 24°.

within 3 hr at room temperature to a mixture of 580 mg of steroid (2 mmol), 250 mg of mercuric cyanide, and 270 mg of mercuric chloride in 20 ml of anhydrous benzene. The suspension was stirred at room temperature for 1–7 days with daily addition of all the reagents in the same portions. Chloroform was added and the extract was washed once with saturated sodium bicarbonate solution, and repeatedly with water. The solution was dried over sodium sulfate and the solvent evaporated. The residue was chromatographed on 150 g of Celite 545 in the system benzene–2,2,4-trimethylpentane–methanol–water (40:60:85:15). The steroid tetraacetylglucosaminides started to appear in about the twelfth hold back volume. The tetraacetyl compounds were recrystallized from ethyl acetate or ethanol. The R_f of these compounds on silica gel GF in ethyl acetate was about 0.5.

Preparation of Steroid 2′-Acetamido-2′-deoxy-D-glucopyranosides.—A solution of 200 mg of steroid 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-α or β-D-glucopyranoside in 30 ml of anhydrous methanol and 0.4 ml of 1 *N* sodium methoxide in methanol was allowed to stand at room temperature overnight. A suspension of 3 ml of Amberlite IR-120 (H^+) was added to the reaction mixture and stirred for 10 min. The mixture was filtered and washed with methanol. The solvent was removed under reduced pressure. Examination of the product by thin layer chromatography on silica gel GF (ethyl acetate–methanol 3:1, R_f about 0.3; the α anomer had a slightly higher mobility than the β anomer) demonstrated that the methanolysis was quantitative giving only one compound. The product was crystallized from aqueous ethanol, acetone or ethyl acetate depending on the steroid conjugate.

Anomerization of Steroid 2′-Acetamido-2′-deoxy-3′,4′6′-tri-*O*-acetyl-β-D-glucopyranoside.—A solution of 200 mg of steroid 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside and 1.0 ml of titanium tetrachloride in 100 ml of chloroform in a stoppered erlenmeyer flask was allowed to stand for 2 days at room temperature. The reaction mixture was shaken several times with a saturated solution of sodium bicarbonate and water. The chloroform solution was dried over sodium sulfate and the solvent evaporated. The reaction mixture was chromatographed on a thin layer of silica gel GF and the plates were developed twice in ethyl acetate. The tetraacetyl α-glucosaminides have slightly higher mobility than the corresponding β anomer. The conjugates were eluted with chloroform–methanol (95:5) and only amorphous products which could not be crystallized were obtained. The homogeneity of these compounds was demonstrated by thin layer chromatography and infrared and nmr spectrometry

17β-Hydroxy-5β-androstan-3α-yl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside (4βa).—17-Keto-5β-androstan-3α-yl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside (**3βb**, 600 mg) in 20 ml of methanol was reduced with 600 mg of sodium borohydride at 5° for 30 min. The solution was neutralized with acetic acid and poured into ice water. The precipitate (475 mg) was collected and recrystallized from aqueous acetone to give 372 mg of 17-hydroxy-5β-androstan-3α-yl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside (**4βb**), mp 129–133°. Further recrystallization from acetone gave an analytical sample of **4βb**, mp 133–135°. The O-acetyl groups from **4βb** were removed by transesterification with sodium methoxide as described in the general procedure to give **4βa**, mp 190–196°.

17β-Hydroxy-5β-androstan-3α-yl 2′-Acetamido-2′-deoxy-α-D-glucopyranoside (4αa). A.—17-Keto-5β-androstan-3α-yl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-α-D-glucopyranoside (**3αb**, 170 mg) in 10 ml of methanol was reduced with 170 mg of sodium borohydride as above for the β anomer. The product (142 mg) was purified by preparative thin layer chromatography on silica gel GF with ethyl acetate to give 105 mg of 17-hydroxy-5β-androstan-3α-yl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-α-D-glucopyranoside (**4αb**). The compound was further purified by dissolution in ether and precipitation with petroleum ether to give 86 mg of amorphous **4αb**. The O-acetyl group in **4αb** was removed by transesterification with sodium methoxide to give crystalline 17-hydroxy-5β-androstan-3α-yl 2′-acetamido-2′-deoxy-α-D-glucopyranoside (**4αa**), mp 170–176°.

B.—17β-Hydroxy-5β-androstan-3α-yl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside (**4βb**, 230 mg) was anomerized with 1.0 ml of titanium tetrachloride in 100 ml of chloroform as described in the general procedure. The α anomer **4αb** (135 mg) was obtained again as an amorphous powder identical with that obtained in procedure A as judged by ir and nmr spectrometry.

Cyclohexyl 2′-Acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside.—A mixture of 500 mg of 1α-chloro-2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranose, 1.0 g of silver carbonate, and 1.0 g of Drierite in 5 ml of dry cyclohexanol was stirred at room temperature overnight. After filtration the excess cyclohexanol was evaporated under reduced pressure. The residue was crystallized from methanol to give 358 mg of cyclohexyl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside: mp 181–182°; ir 1668, 1545, series of small bands with no distinguishing absorption between 1200–1100 cm^{-1}; nmr, δ 4.20 (m, $W_{1/2}$ = 8.5 cps, H-6′) 4.90 (d, J = 9 cps, H-1′).

Anal. Calcd for $C_{20}H_{31}NO_9$: C, 56.05; H, 7.28; N, 3.26. Found: C, 56.19; H, 7.29; N, 3.28.

Cyclohexyl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside.—A solution of 50 mg of cyclohexyl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside in 50 ml of anhydrous methanol and 50 μl of 1 *N* sodium methoxide solution was left overnight at room temperature. One drop of acetic acid was added to the mixture and the solvent evaporated. The product was crystallized by dissolving in ethanol and addition of ether to give 31 mg of cyclohexyl 2′-acetamido-2′-deoxy-β-D-glucopyranoside: mp 205–206°; ir 1635, 1567 (sh), 1558, 1160, 1115 cm^{-1}.

Anal. Calcd for $C_{14}H_{25}NO_6 \cdot 0.5H_2O$: C, 53.87; H, 8.38; N, 4.48. Found: C, 54.13; H, 7.91; N, 4.32.

Cyclohexyl 2′-Acetamido-2′-deoxy-α-D-glucopyranoside. A.—A mixture of 1.0 g of 2-acetamido-2-deoxy-D-glucopyranose and 1.0 g of Amberlite IR-120 (H^+) in 50 ml of cyclohexanol was heated at 85° for 15 hr. The solids were filtered off and washed with cyclohexanol. The solvent from the filtrate was removed under reduced pressure and the residue crystallized from ethanol–ether affording 111 mg of cyclohexyl 2′-acetamido-2′-deoxy-α-D-glucopyranoside, mp 198–213°. The analytical sample melted at 215–217°: ir 1637, 1552, 1122 cm^{-1}.

Anal. Calcd for $C_{14}H_{25}NO_6$: C, 55.45; H, 8.30; N, 4.62. Found: C, 55.36; H, 8.23; N, 4.63.

The mother liquor was chromatographed on a column of silica gel and eluted with ethyl acetate–ethanol. An additional 120 mg of the α anomer, mp 216–218°, was eluted first followed by 100 mg of the β anomer, mp 205–206°, identical with the product obtained by the Koenigs–Knorr method.

B.—A solution of 30 mg of cyclohexyl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranoside and 0.15 ml of titanium tetrachloride in 15 ml of chloroform was sealed in an ampoule for 20 hr at room temperature. The reaction mixture was worked up as above for the anomerization of the steroid conjugate. The product, 29 mg, could not be crystallized. It was purified by thin layer chromatography on silica gel G in chloroform–acetone (10:1) R_f 0.3, and precipitation with cyclohexane from an ether solution. Cyclohexyl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl α-D-glucopyranoside was obtained as an amorphous powder: ir 1688, 1675, 1545, 1128 cm^{-1}; nmr δ 4.15 (nm, $W_{1/2}$ = 3.5 cps, H_2-6′), 4.98 (d, J = 3.5 cps, H-1′).

Anal. Calcd for $C_{20}H_{31}NO_9$: C, 56.05; H, 7.28; N, 3.26. Found: C, 55.99; H, 7.24; N, 3.16.

Deacetylation in 30 ml of anhydrous methanol and 30 μl of 1 *N* sodium methoxide solution gave cyclohexyl 2′-acetamido-2′-deoxy-α-D-glucopyranoside, mp 216–219°, the infrared spectrum of which was identical with that of the product obtained by the cation exchange method.

Method for Detection of Acetamido Compounds on Thin Layer Chromatograms.—The method of Rydon and Smith[23] has been applied. Following the chromatography of the glucosaminide on a thin layer of silica gel G, the plate was dried at 90° for several minutes and cooled to room temperature. The plate was placed in a chlorine atmosphere for 10 min avoiding any radiation by direct sunlight (artificial light does not interfere with the reaction). Excess chlorine gas was then allowed to evaporate from the plate in the hood. The plate was stained with a solution of 1% starch and 1% potassium iodide in water. Acetamido compounds give blue spots which are quite stable for several days. The sensitivity of the reaction is at least 1 μg per 1 cm^2 spot.

Registry No.—Cyclohexyl 2′-acetamido-2′-deoxy-2-β-D-glucopyranoside, 21559-72-8; cyclohexyl 2′-acet-

(23) H. N. Rydon and P. W. G. Smith, *Nature*, **169**, 922 (1952).

amido - 2′ - deoxy - 3′,4′,6′ - tri - *O* - acetyl - β - D - glucopyranoside, 21588-60-3; cyclohexyl 2′-acetamido-2′-deoxy-α-D-glucopyranoside, 21559-73-9; cyclohexyl 2′-acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-α-D-glucopyranoside, 21559-74-0.

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Microbial Hydroxylations. IV. Differential Metabolism of 19-Nor Steroid Antipodes by *Curvularia lunata*[1]

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In order to explore the generality of prior findings that microbial systems hydroxylate both the natural *d* and the unnatural *l* enantiomers of racemic 19-norsteroids, the metabolic disposition of *d*- and *dl*-19-nortestosterone and its *dl*-13β-ethyl homolog by vegetative cell cultures of *Curvularia lunata* NRRL 2380 was examined. From *d*-19-nortestosterone there was obtained the *d*-10β-hydroxy, *d*-11β-hydroxy, *d*-14α-hydroxy, *d*-6β-hydroxy, and *d*-10β,11β-dihydroxy derivatives. From *dl*-19-nortestosterone there wss obtained the *d*-10β-hydroxy, *d*-14α-hydroxy, *dl*-11β-hydroxy, *dl*-6β-hydroxy, *l*-12α-hydroxy, and *dl*-10β,11β-dihydroxy derivatives together with *l*-10β,11β,17β-trihydroxy-5α-estran-3-one. From *dl*-13β-ethyl-17β-hydroxygon-4-en-3-one there was obtained the *dl*-10β-hydroxy, *l*-12α-hydroxy, *d*-14α-hydroxy, *dl*-6β-hydroxy, and *d*-6β,10β-dihydroxy derivatives. Structures of each product were established by elemental analysis, spectral behavior in ethanol, alkaline ethanol, and concentrated sulfuric acid, proton spectra, derivitization, and optical rotatory dispersion. These results support and expand on our prior similar results using *Aspergillus ochraceus* NRRL 405 on these same substrates.

The previously held notion that microbial systems metabolize only the natural *d* antipode[3] of racemic steroid preparations and reject the unnatural *l* antipode[4] is compromised by our prior findings,[5] wherein microbial hydroxylase and dehydrogenase systems were demonstrate to metabolize both the *d* and the *l* enantiomers of racemic 19-nortestosterone (*dl*-17β-hydroxyestr-4-en-3-one) (Ia) and its 13β-alkyl homologs. In order to establish the generality of the differential hydroxylation of the antipodes of racemic steroids in other microbial systems, we have examined the metbolism of natural and racemic Ia and of its racemic homolog *dl*-13β-ethyl-17β-hydroxygon-4-en-3-one (IIa) by vegetative cell cultures of *Curvularia lunata* NRRL 2380, a well-known microorganism used broadly to introduce the 11β-hydroxyl group into a wide variety of steroids.[6]

We have carefully reexamined the transformation of *d*-Ia by *C. lunata* NRRL 2380 and confirmed that the 10β-hydroxy derivative *d*-IIIa is the major product, with diminished amounts of the 11β-hydroxy, 14α-hydroxy, and 10β,11β-dihydroxy products *d*-IVa, *d*-Va, and *d*-VIa.[7] In addition to these products, we isolated the 6β-hydroxysteroid *d*-VIIa, not previously noted in these fermentations.[7] Estimation of the composition of the product mixture by gas chromatography[8] gave *d*-IIIa, 55.5% (52.0%); *d*-IVa, 17.5% (2.6%); *d*-Va, 13.3% (9.5%); *d*-VIa, 0.8% (0.5%); *d*-VIIa, 4.4% (1.4%); recovered *d*-Ia, 5.9%.

Fermentation of racemic Ia with *C. lunata* NRRL 2380 under the same conditions yielded a similar thin-layer and gas chromatographic pattern of hydroxylated products. However, fractionation of the product mixture afforded two additional minor products, VIIIa and IXa, not found in the *d*-Ia fermentations. The fortuitous superposition on thin layer chromatograms of the trace product IXa with Va and of VIIIa with IVa and VIa gave no hint of the formation of these components during fermentation, and their presence in the product mixture was noted only after isolation. Gas chromatographic analysis[8] of the product mixture from *dl*-Ia fermentation (with optical configurations given) gave *d*-IIIa, 50.3% (18.9%); *d*-Va, 7.3% (0.5%); *dl*-VIa, 10.3% (1.3%); *dl*-VIIa, 4.8% (4.3%); *l*-IXa, 4.8% (1.3%); *dl*-IVa and *l*-VIIIa, 19.7%[9] (6.1% and 1.4%); and recovered substrate, 2.8%.

The structures for the known steroids IIIa, IVa, Va, and VIIa were assigned on the basis of their spectral behavior in ethanol, in alkaline ethanol, and in concentrated sulfuric acid by systematic comparisons,[10]

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(2) Robert A. Welch Foundation Postdoctoral Fellow, 1968–1969.

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(8) The composition figures are not absolute yields of product based on substrate but are the relative proportions for each component in the crude product mixture. The yield figures in parenthesis following each gas chromatographic proportion are actual isolated yields for each component, based on substrate.

(9) Resolution of *dl*-IVa and *l*-VIIIa was not achieved on 3% QF-1 columns. Accordingly, the value of 19.7% represents both components together.

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