counter-current distribution in 70% methanol–carbon tetrachloride yielded 79 mg. of colorless solid with K = 0.31. This was combined with the "neutral" product and recrystallized from absolute ethanol. There was obtained 77 mg. (30%) of 2-hydroxyestrone 3-methyl ether as coarse prisms, m.p. 182–185°, and 24 mg. (9%), m.p. 180–184.5°. Further recrystallization gave a pure sample, rhombs, m.p. 182.5–185°, $[\alpha]^{23}D$ +158° (ethanol), λ_{max} 287.5 mμ (ε 4200), λ_{min}. 254 mμ (ε 670), λ_{infl} 310 mμ (ε 290).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 76.09; H, 8.04.

Although the distribution coefficient, melting point and ultraviolet spectrum of 2-hydroxyestrone 3-methyl ether were in agreement with those of 2-methoxyestrone 3-methyl ether, the specific rotations and the infrared spectra in potassium bromide dispersion were conclusively different.

4-Aminoestrone (IVa).—One gram of sodium hydrosulfite was added to a refluxing solution of 196 mg. of 4-nitroestrone in a mixture of 50 ml. of acetone, 10 ml. of water and 5 ml. of 1 N sodium hydroxide solution. After 30 minutes, 10 ml. of water and 0.7 g. of sodium hydrosulfite were added and refluxing was continued with periodic additions of alkali as before. After another 30 minutes, a final 0.3 g. of sodium hydrosulfite was added and heating was continued until the deep red color of the mixture had changed to light yellow. Following the addition of 25 ml. of water, most of the acetone was removed and the solution was neutralized with dilute acetic acid. After refrigeration for 2 hours a white precipitate was filtered off and washed with water. After drying, there was obtained 179 mg. of 4-aminoestrone, m.p. 254–255° dec., yield quantitative. For analysis, a sample was obtained by recrystallization from benzene–methanol as colorless needles, m.p. 260–262° dec., $[\alpha]^{24}D$ +139° (dioxane), λ_{max} 289 mμ (ε 2710), λ_{min}. 264 mμ (ε 850).

Anal. Calcd. for $C_{18}H_{23}NO_2$: C, 75.75; H, 8.12; N, 4.91. Found: C, 75.71; H, 8.23; N, 4.84.

4-Methoxyestrone (VIa).—A solution of 928 mg. of 4-aminoestrone in 15 ml. of 40% sulfuric acid was cooled to 0° and treated dropwise with a solution of 707 mg. of sodium nitrite in 15 ml. of water. The mixture was allowed to stand at 0° for 15 minutes and then 3 ml. of 40% aqueous urea solution was added. After 5 minutes at 0° the diazonium solution was added to 50 ml. of ice-cold absolute methanol and the solution was irradiated with ultraviolet light, with cooling and stirring, for 5 hours. Sodium hydroxide solution was added to neutrality and the solution was concentrated to about 100 ml. Water was added and after acidification the mixture was extracted thoroughly with ether. The ether layers were combined and washed with water. Extraction of the ether with 5% sodium bicarbonate solution followed by acidification and extraction with chloroform afforded 136 mg. of a brown oil. Extraction of the ether layer with 5% potassium hydroxide solution several times gave 344 mg. of a brown phenolic fraction. Evaporation of the ether layer gave 326 mg. of a brown neutral material.

The phenolic product was chromatographed on silica gel (100 g.) with petroleum ether containing increasing proportions of ethyl acetate. The desired 4-methoxyestrone was eluted with 1 liter of 25% ethyl acetate–75% petroleum ether. There was obtained 97 mg. of colorless solid, m.p. 185–212°, yield 10%. Recrystallization from ethyl acetate afforded 53 mg. of colorless platelets, m.p. 220–224°. Further recrystallization from ethyl acetate gave a pure sample, m.p. 224–225°, $[\alpha]^{24}D$ +146° (ethanol), λ_{max} 278 mμ (ε 1820), λ_{min}. 249 mμ (ε 490).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.96; H, 8.09.

Another sample had double m.p. 215–217° (prisms) and 222–225° (rectangular plates); K = 0.50 in the system 70% methanol–carbon tetrachloride.

2-Aminoestrone (IIIa).—Reduction of 2-nitroestrone as described for 4-nitroestrone gave 2-aminoestrone, which was crystallized from dilute methanol as long needles darkening at 220° with no real melting point; $[\alpha]^{24}D$ +178° (ethanol), λ_{max} 297 mμ (ε 4190). All manipulations were carried out in an atmosphere of nitrogen to avoid decomposition of the material.

Anal. Calcd. for $C_{18}H_{23}O_2N$: C, 75.75; H, 8.14; N, 4.91. Found: C, 75.62; H, 8.04; N, 4.67.

2-Methoxyestrone (Va).—The same procedure as used for 4-methoxyestrone gave an over-all yield of 0.8% of 2-methoxyestrone.[3]

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D-Glucopyranosiduronates. I. Steroidyl-β-D-glucopyranuronosides[1,2]

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The β-D-glucopyranosiduronic acids of 17β-hydroxy-androst-4-en-3-one, 3β-hydroxy-androst-5-en-17-one, estra-1,3,5(10)-triene-3,17β-diol, 3β-hydroxy-pregn-5-en-20-one and androst-4-ene-3,17-dione as well as the methyl (2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronates of the above steroids and of 3β-hydroxy-5α-androstan-17-one, 3-hydroxy-estra-1,3,5(10)-trien-17-one, 3β,17α,21-trihydroxy-5α-pregnan-20-one, 11,20-dioxo-3α,17α-dihydroxy-5β-pregnan-21-yl-acetate, 17α,21-dihydroxy-pregn-4-ene-3,11,20-trione, 3-hydroxy-pregna-3,5-dien-20-one and cholesta-3,5-dien-3-ol were prepared by the Koenigs–Knorr procedure. In several cases the method of Schapiro was compared with that described by Meystre, *et al.* Proof of structure was obtained from elemental analysis, ultraviolet and infrared absorption spectra, color reactions and enzymatic hydrolysis.

The preparation of methyl-2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate[3] by Goebel and Babers[4] opened the way for the synthesis of steroid glucosiduronates by Schapiro[5] in 1938 and by Huebner and co-workers[6] in 1944, using the Koenigs–Knorr reaction. More recently Schneider, *et al.*,[7]

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(2) Presented in part at the 131st National Meeting, American Chemical Society, Miami, Florida, April 7–12, 1957.

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reported the synthesis of methyl (21-acetoxy-11,20-dioxo-17α-hydroxy-5β-pregnan-3α-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate by the same procedure.

Steroids are excreted in the urine as conjugates, primarily as glucosiduronates and sulfates. Only a small fraction of urinary steroids are present in the non-conjugated form. The glucosiduronates of androgens have been measured in the urine by Jayle and Crepy[8] and by Wotiz, *et al.*[9] They have also been identified in tissues incubated with testosterone.[10-12]

Recently Schubert[13] showed the possible existence of the glucosiduronate of androst-4-ene-3,17-dione, following injection of testosterone into humans. Since this compound contains no hydroxyl groups it must be conjugated through an enolized ketone function. The stability of such compounds is demonstrated here through their synthesis.

The presence of the glucosiduronates was inferred by the detection of the steroid aglycone and of D-glucuronic acid after hydrolysis with β-glucuronidase, by chromatography and by colorimetric test for the steroid and glucuronic acid, respectively. Only in a few instances have steroid glucosiduronates been isolated as such and characterized.[14-16]

In order to undertake a project of isolating and characterizing steroid glucosiduronates from urine and tissues, it was necessary to obtain pure steroid-β-D-glucosiduronates. The synthesis of these reference compounds is the subject of this report. The methods used were essentially those described by Schapiro[17] and by Meystre, *et al.*[18]

A preliminary comparison of the two methods showed that while in some cases there was a decrease of up to 50% in the yield, a better than 20% increase occurred with others when utilizing the Schapiro procedure. Because of this and the relative ease of handling the latter procedure, it was more frequently employed. Deacetylation was carried out with methanolic barium hydroxide or by transesterification with methanolic sodium methoxide. The free acids are soluble in water, alcohols, acetone and to a lesser degree in chloroform.

Experimental

The following procedures describe typical experimental conditions employed for the various reactions. The physical data, yields and specific reactions for each individual compound (as described below) are shown in Table I. All melting points are corrected.

Koenigs-Knorr Reactions. A. Method of Schapiro.—Testosterone (5.80 g.) and methyl-2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate (15.40 g.) were dissolved in 300 ml. of dry benzene. Freshly prepared dry silver carbonate (13.79 g.) was added to the solution and the resultant mixture was shaken at room temperature for 20 hr. The silver salts were filtered off and the clear filtrate was evaporated to dryness *in vacuo* at 40–50°. The residue, a pale yellow gummy material, was recrystallized from 50% ethanol three times, until a constant melting range was attained.

B. **Method of Meystre and Miescher.**—Testosterone (3.024 g.) and 5.52 g. of dry silver carbonate were dissolved in 200 ml. of dry benzene. The solution was gently distilled and 5.96 g. of methyl-2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate was added slowly. The silver salts were filtered off and the solution was evaporated to a gummy residue *in vacuo*. The residue was dissolved in hot 70% ethanol and kept in a refrigerator. White crystals weighing 1.80 g. were obtained. Further evaporation of the mother liquor yielded another 33 mg. of material.

Deacetylation. C. Barium Hydroxide.—Five hundred mg. of methyl (3-oxo-androst-4-en-17β-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate was dissolved in 20 ml. of redistilled methanol and to this was added 20 ml. of 0.23 *N* aqueous barium hydroxide while shaking the mixture. The white crystalline precipitate which formed immediately was protected against carbon dioxide with an Ascarite tube and refluxed for 0.5 hr. The barium salt was filtered off after cooling, washed several times with cold distilled water and dried over calcium chloride. Three-tenths of a gram of the barium salt was dissolved in 30 ml. of methanol with the aid of a few drops of 0.1 *N* aqueous hydrochloric acid. The solution was heated and 20 ml. of 0.1 *N* aqueous sulfuric acid was added dropwise to precipitate the barium as the sulfate. The precipitate was filtered off and the methanolic filtrate was evaporated to a small volume *in vacuo*. The substance precipitated out during this process as fine needles and was filtered.

D. Sodium Methoxide.—To a solution of 4.1 g. of methyl-(3-hydroxy-estra-1,3,5(10)-trien-17β-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate in hot, dry methanol, were added six drops of a solution of 0.7 g. of sodium metal dissolved in 25 ml. of dry methanol. The solution was heated for 5 minutes and then allowed to cool. The methanol was evaporated and the residue dissolved in 50 ml. of distilled water. Addition of a few drops of glacial acetic acid resulted in a white flocculate precipitate which was centrifuged. The supernatant liquid was decanted and the residue was recrystallized from 60% ethanol yielding fine white crystals.

Infrared Spectroscopy.—Infrared investigations were carried out with a Perkin–Elmer model 21 spectrophotometer and are described in detail in a separate communication.

Ultraviolet Spectroscopy.—The ultraviolet absorption spectra for the various enolglucosiduronates were taken in methanol solution and recorded on a Beckman model DK-2 spectrophotometer between 200 and 260 mμ.

Tetranitromethane Reaction.—The compounds (2 to 3 mg.) tested were dissolved in a few drops of chloroform and mixed with 2 ml. of tetranitromethane. Substances with conjugated double bond systems produced a brownish color with the reagent. The results obtained for the glucosiduronates and their parent steroids are listed in Table II.

Incubation with β-Glucuronidase.—Approximately five mg. of each of the glucosiduronic acids and the glucosiduronates with the exception of the enol derivatives were incubated in 50 ml. of acetate buffer at *p*H 5.2 with 15,000 units of β-glucuronidase[19] for 24 hr. at 37°. Incubation was followed by extraction with ether, drying of the extract and paper chromatography of a portion of the extract corresponding to approximately 400 μg. of steroid in an appropriate system; (for androgens: ligroin–propylene glycol, for estrogens and corticosteroids: toluene–propylene glycol). Compounds II, V, VIII and XI gave virtually quantitative yields of the corresponding free steroids. None of the other substrates underwent cleavage to a detectable extent; all of these were fully acetylated. The inability of β-glucuronidase to cleave acetylated steroid glucosiduronates has already been demonstrated by Barlow.[20]

Color Reactions.—A few micrograms of the various glucosiduronates were dissolved in an appropriate solvent,

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TABLE I

Name of Compound	No. of compound	m.p., °C.	Optical rotation		Empirical formula	Analyses, % Calculated C	Calculated H	Found C	Found H	Method[e]	Yield, %
Methyl (3-oxo-androst-4-en-17β-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	I	188.2–188.5 (186–189)	$[\alpha]^{25.5}D$ +45.5 ± 5° $[\alpha]^{22}D$ +28.3° a[a]	a	$C_{32}H_{44}O_{11}\cdot C_2H_5OH$	62.73	7.74	62.50	7.51	A B	19 29
3-Oxo-androst-4-en-17β-yl-β-D-glucopyranosiduronic acid	II	182–183.5	$[\alpha]^{23}D$ +31.7 ± 3.5°	b	$C_{25}H_{36}O_8\cdot H_2O$	62.21	7.94	62.33	8.03	C	60
Methyl (17-oxo-androstan-3β-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	III	168–170	$[\alpha]^{22}D$ +27.8 ± 3.5°	a	$C_{32}H_{46}O_{11}$	63.35	7.64	63.47	7.39	A	11
Methyl (17-oxo-androst-5-en-3β-yl-2,3,4-tri-*O*-acetyl β-D-glucosid)-uronate	IV	195–195.5 (193–196)	$[\alpha]^{23}D$ −11.3 ± 2.5° $[\alpha]^{20}D$ −19.7°[b]	a	$C_{32}H_{44}O_{11}\cdot C_2H_5OH$	62.73	7.74	62.66	7.24	A	23
17-Oxo-androst-5-en-3β-yl-β-D-glucopyranosiduronic acid	V	230–232 262–264	$[\alpha]^{25}D$ −35.5 ± 1.3°	b	$C_{25}H_{36}O_8\cdot CH_3OH$	62.88	8.12	63.29	7.72	C	89
Methyl (estra-1,3,5(10)-trien-3,17β-ylene-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	VI	215–216.5	$[\alpha]^{25}D$ −5.1 ± 1.7°	c	$C_{44}H_{56}O_{20}$	58.46	6.13	57.81	6.24	A B	2.0 0.0
Methyl (3-hydroxy-estra-1,3,5(10)-trien-17β-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	VII	122–124	$[\alpha]^{25}D$ −6.5 ± 2.2°	a	$C_{31}H_{40}O_{11}\cdot H_2O$	62.40	7.09	62.21	6.71	A B	30 49
3-Hydroxy-estra-1,3,5(10)-trien-17β-yl-β-D-glucopyranosiduronic acid	VIII	198–202	$[\alpha]^{24}D$ −3.9 ± 0.9°	d	$C_{24}H_{32}O_8$	64.27	7.19	63.99	7.50	D	53
Methyl (17-oxo-estra-1,3,5(10)-trien-3-yl-2;3,4-tri-*O*-acetyl-β-D glucosid)-uronate	IX	212–215 (225.5–228)	$[\alpha]^{23}D$ +53.6 ± 0.4° $[\alpha]^{25}D$ +57.1 a	a[b]	$C_{31}H_{38}O_{11}\cdot 2H_2O$	59.79	6.80	59.97	6.30	A B	7.4 5.2
Methyl (20-oxo-pregn-5-en-3β-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	X	183–184	$[\alpha]^{23}D$ +4.9 ± 4.9°	a	$C_{34}H_{48}O_{11}$	64.53	7.65	64.54	7.67	A	17
20-Oxo-pregn-5-en-3β-yl-β-D-glucopyranosiduronic acid	XI	245–248	$[\alpha]^{21}D$ −40.0 ± 8°	c	$C_{27}H_{40}O_8\cdot H_2O$	63.50	8.29	63.22	7.98	D	63
Methyl (20-oxo-17α-hydroxy-5α-pregnan-3β,21-ylene-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	XII	176–177	$[\alpha]^{28}D$ −15.3 ± 3.8°	a	$C_{47}H_{66}O_{22}$	57.42	6.77	56.88	6.77	A	15
Methyl (21-acetoxy-11,20-dioxo-17α-hydroxy-5β-pregnan-3α-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	XIII	193–194 (209–212)[c]	$[\alpha]^{23}D$ +64.3 ± 6° $[\alpha]^{30}D$ +37.8 ± 1.5°[c]	a	$C_{36}H_{50}O_{15}$	59.82	6.97	59.04	6.91	A B	28 55
Methyl (3,11,20-trioxo-17α-hydroxy-pregn-4-en-21-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	XIV	105–107	$[\alpha]^{24}D$ +120.1 ± 0.7°	a	$C_{34}H_{46}O_{14}$	60.16	6.83	59.89	6.53	B	23
Methyl (17-oxo-androsta-3,5-dien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	XV	199–201	$[\alpha]^{23}D$ +118.7 ± 6.8°	a	$C_{32}H_{42}O_{11}\ C_2H_5OH$	62.94	7.46	62.46	6.87	A B	18 4.2
17-Oxo-androsta-3,5-dien-3-yl-β-D-glucopyranosiduronic acid	XVI	176–178	$[\alpha]^{23}D$ −119.1 ± 9.5°	a	$C_{25}H_{34}O_8$	64.63	7.81	64.01	7.52	C D	0.0 86
Methyl (20-oxo-pregna-3,5-dien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	XVII	191–194	$[\alpha]^{27}D$ 0 ± 2° a		$C_{34}H_{46}O_{11}$	64.75	7.35	64.29	6.91	A B	12 15
20-Oxo-pregna-3,5-dien-3-yl-β-D-glucopyranosidurono-γ-lactone	XVIII	216–224 with dec.			$C_{27}H_{36}O_7$	68.62	7.68	68.72	7.91	D	83
Methyl (cholesta-3,5-dien-3-yl-tri-*O*-acetyl-β-D-glucosid)-uronate	XIX	156–158	$[\alpha]^{28}D$ −55.3 ± 2.5°	a	$C_{40}H_{60}O_{10}$	66.82	8.77	66.49	8.40	A B	9.1 16

[a] Column 3 and 4 figures in parentheses refer to data reported by Schapiro.[13] Column 4: a, chloroform; b, ethanol; c, methanol; d, 0.5 *M* NaOH. [b] When calculated for $C_{31}H_{38}O_{11}$, $[\alpha]^{25}D$ +57.1°. [c] Figures in parentheses refer to data reported by Schneider, *et al.*[7] [d] Analyses were performed in the Microchemical Laboratory at Massachusetts Institute of Technology. [e] Letters refer to reactions from Experimental section.

TABLE II

COLOR REACTIONS OF $\Delta^{3,5}$-STEROIDYL-β-D-GLUCOPYRANURONOSIDES AND THEIR PARENT STEROIDS

Compound	Tetranitromethane color	Zimmermann color
Methyl (17-oxo-androsta-3,5-dien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	Brown	Purple
17-Oxo-androsta-3,5-dien-3-yl-β-D-glucopyranosid-uronic acid	Light yellow[a]	Purple
Methyl (20-oxo-pregna-3,5-dien-3-yl-2,3,4-tri-*O*-acetyl-β-D-glucosid)-uronate	Brown	Light brown
20-Oxo-pregna-3,5-dien-3-yl-β-D-glucopyranosiduronо-γ-lactone	Light yellow	Light brown
Methyl (cholesta-3,5-dien-3-yl-tri-*O*-acetyl-β-D-glucosid)-uronate	Brown	None
4-Androsten-3,17-dione	Light yellow	Purple
4-Pregnen-3,20-dione	Light yellow	Blue
4-Cholesten-3-one	Light yellow	Blue

[a] In some instances a transient brown coloration was observed.

applied to a small strip of paper and dried. The paper strips were dipped into a 2.5 *N* alcoholic solution of potassium hydroxide, blotted, dipped into a 2% alcoholic solution of *m*-dinitrobenzene and exposed to 70° heat for 1–2 minutes. Compounds I, II and XIV produced a deep blue color with Zimmermann's reagent, while compounds III, IV, V, IX, XV and XVI gave a purple color (Table II).

The glucosiduronates derived from phenolic steroids were applied to paper strips in a similar fashion. The strips were dipped into an aqueous solution containing 1% of ferric chloride and 1% of ferricyanide. Compounds VII and VIII gave a blue color, while compounds VI and IX did not produce any color, since their phenolic hydroxyl groups were utilized for the glucosidic linkage.

Discussion

To date, most of the steroid glucosiduronates isolated from biologic media have been shown to be conjugated at carbon 3 of the steroid nucleus[7,14,16,21–23] with the exception of estriol glucosiduronate[24] and testosterone glucosiduronate.[12]

The first preparation of C-21 steroid glucosiduronate was reported by Schneider and co-workers[7] using the classical Koenigs–Knorr reaction. This compound was shown to be identical with the fully acetylated product isolated from the urine of a human being following ingestion of cortisone. The presence of a glucosiduronate of at least one other related adrenocortical steroid was also demonstrated.

Although mono-hydroxy steroids may logically be assumed to be conjugated at the position of the hydroxyl group, it is possible that the acetal linkage may also be formed through an enolic hydroxyl function from an α,β-unsaturated ketone.[25] The compounds prepared and reported in this communication and their physical constants are listed in Table I.

In order to obtain evidence for the position and type of linkage involved, the infrared and ultraviolet absorption spectra, enzymatic hydrolysis and certain color reactions were utilized.

The glucosiduronic acid derivatives of testosterone, dehydroepiandrosterone and estradiol were shown to be conjugated in a β-glucosidic linkage by their hydrolizability with the enzyme β-glucuronidase. Quantitative recovery of the parent steroids provides not only evidence for the nature of the linkage but also for the fact that during deacetylation of the derivative no degradation occurred.

The absence of O–H stretching bands in the infrared spectra of the glucosiduronates of the mono-hydroxy steroids assigns the position of the glucosidic linkage to carbon 17 for testosterone, to carbon 3 for dehydroepiandrosterone, epiandrosterone, estrone and pregnenolone and to carbons 3 and 17 for estradiol-bis-glucosiduronate. This is further proven by the retention of the ketone functions as shown by the blue Zimmermann color produced by the testosterone derivative and the purple Zimmermann color obtained for the other three mono-hydroxy C-19 steroid derivatives. Retention of the α,β-unsaturated ketone of testosterone and cortisone is further substantiated by the ultraviolet absorption (λ_{max} 240 mμ) of their respective glucosiduronates.

The existence of two different glucosiduronates of estradiol-17β is not surprising in view of the presence of two hydroxyl functions. Compound VII was identified as the mono-glucosiduronate of estradiol linked at carbon 17 because of its reaction with the ferric-chloride–ferricyanide reagent, showing retention of the phenolic group. To compounds VI and XII were assigned the structure of a bis-glucosiduronate on the basis of the elemental analysis as well as infrared spectroscopy.

The evidence presented by Schubert[13] for the possible *in vivo* existence of enol-glucosiduronates of active hormones allows the formulation of at least two pathways for the production of saturated steroidyl - β - D - glucopyranosiduronic acids. The first involves reduction of the double bond at C-4 and the ketone at C-3 followed by conjugation at this point. This appears to be the accepted pathway at the present time. The second pathway would involve first the enolization of the α,β-unsaturated ketone at C-3 and conjugation followed by reduction. The second mechanism is intriguing because urinary steroids with ring A/B *:cis* structures are primarily conjugated with glucuronic acid, while unsaturated and ring A/B *:trans* steroids are not at all or only in lesser amounts conjugated with glucuronic acid.[9] A partial enol-glucosiduronate formation of certain steroids may therefore yield a stereoselective reduction. In order to test this concept compounds XV–XIX were prepared.

Evidence for the structure of the enol-glucosiduronates was derived from their infrared spectra (as discussed in detail in the paper following) which showed no absorption near 1670 cm.$^{-1}$, where

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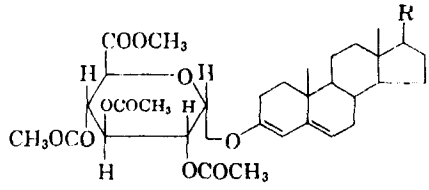
α,β-unsaturated ketone vibrations are known to absorb, concomitant with the appearance of two bands at 1655 and 1631 cm.$^{-1}$, known to be associated with conjugated -C=C- stretching vibrations.

Further evidence for the diene structure was obtained from the formation of the brown color of the polyacetylated derivatives of the three enol-glucosiduronates with tetranitromethane[26] (Table II) as well as the bathochromic shift of the ultraviolet absorption maximum from 240 mμ for the parent steroid to 237.5 mμ for the derivatives.

Compounds XV, XVII and XIX can be written as



XV, R = O
XVII, R = $-COCH_3$
XIX, R = $-CH(CH_3)(CH_2)_3CH(CH_3)_2$

The enol-glucosiduronates are assumed to be $\Delta^{3,5}$-dienes since they are the only heteroannular dienes that can be written for these substances. A homoannular diene system can be expected to have an ultraviolet absorption maximum near 275 mμ.[27] This is further substantiated by the increase of levo-rotation of these compounds. Callow and Young[28] have shown that introduction of a 5,6-double bond causes a strong increase in levo-rotation while the introduction of a 4,5-double bond causes a marked increase in dextrorotation.

The appearance of a purple color with Zimmermann's reagent also shows retention of the 17-ketone in XV, while a brownish coloration with XVII indicates a C-20 ketone. Compound XIX produced no color with this reagent due to the loss of its ketone group.

The acetylated methyl ester derivative of androst-4-ene-3,17-dione shows a bathochromic shift of 2.5 mμ which compares favorably to the shift produced for cholesta-3,5-dien-3-yl-acetate (from 240 to 238 mμ).[27] When compound XV was subjected to methanolysis (XVI), the ultraviolet absorption maximum underwent a hypsochromic shift of 2.5 to 240 mμ. The infrared characteristics of this substance were comparable to that of compound XV with respect to its absorption near 1655 and 1631 cm.$^{-1}$ and its lack of absorption near 1675 cm.$^{-1}$. Retention of the enol-glucosidic linkage was further borne out by the fact that on reacetylation of XVI the ultraviolet absorption maximum again showed a bathochromic shift to 237.5 mμ. This phenomenon may be caused by the inability of the acetylated glucuronic acid moiety to approach the diene system, while the deacetylated sugar residue may be able to approach more closely and exert a modifying influence on the electronic spectrum of this compound.

Compound XVIII was assigned the structure of a glucuronolactone, rather than the free acid, on the basis of its non-acidic properties, relative insolubility, elemental analysis and the infrared absorption spectrum.

The ultraviolet spectrum and optical rotation of this substance was not recorded because of its insolubility in the proper solvents.

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[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE, BOSTON UNIVERSITY SCHOOL OF MEDICINE]

D-Glucopyranosiduronates. II. Infrared Absorption Spectra of Some Methyl-[steroidyl-2,3,4-tri-*O*-acetyl-β-D-glucosid]-uronates[1]

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Eleven steroid glucosiduronates were investigated in the amorphous and crystal states. These two methods yielded complementary information in the difficult interpretation of the superficially simple yet complex spectra of these partially flexible molecules. The spectra uniformly showed bands of proportionally high intensity due to the sugar moiety common to all compounds and chiefly arising from its four ester groups. The spectra were differentiated by a low intensity proportion of bands arising from the functional groups of the parent steroids. Qualitative intensity evaluation of these bands permitted an estimation of the steroid/sugar ratio. The stretching vibrations of the glucosidic linkage were shown to be characteristically perturbed by the environmental influence of neighboring steroid bands.

Steroid glucosiduronates are large molecules flexible at the substituent ester linkages of the sugar residue and at the glucosidic linkage. The degree of freedom of rotation about the C–O–C bonds of the three equatorial acetoxy groups and the C–C–O linkage of the methyl ester group can be expected to be relatively high and nearly identical for all compounds. Since the glucosidic linkage connects two bulky molecular residues, steric barriers to free rotation are likely to exist.

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