The above-described synthesis was repeated using the reaction of 40.42 g. (0.15 mole) of molybdenum pentachloride with 84.2 g. (0.30 mole) of stearic acid in 500 ml. of carbon tetrachloride. Instead of using room temperature for the initial reaction, the reaction mixture was heated at 50° for 16 hours. It was then filtered to separate 3.12 g. of black, crystalline solid. This solid was found to have the X-ray diffraction pattern reported for molybdenum tetrachloride.[10]

*Anal.* Calcd. for $Cl_4Mo$: Cl, 59.65; Mo, 40.35. Found: Cl, 60.06, 60.06; Mo, 38.52, 38.46, 38.76.

The reaction mixture filtrate was treated by various procedures to determine the most efficient purification method. One portion of the filtrate was vacuum evaporated to give a dark, gray-green solid, m.p. 67–72°.

*Anal.* Calcd. for $C_{36}H_{70}Cl_3MoO_4$: Cl, 13.83; Mo, 12.47. Found: Cl, 12.65, 12.63; Mo, 11.33, 11.30.

This solid dissolved in petroleum ether upon slight heating, and no precipitation occurred upon cooling. Addition of an excess of petroleum ether to a carbon tetrachloride solution of the product did not cause precipitation.

Partial vacuum evaporation of another portion of the reaction mixture filtrate caused precipitation of a green, gelatinous solid which slowly separated to form an indistinct top layer. This was crudely separated by decantation, washed with carbon tetrachloride, and separated as before. A portion of the separated layer was vacuum evaporated to dryness to give a light, gray-green solid, m.p. 73–75°.

*Anal.* Calcd. for $C_{36}H_{70}Cl_3MoO_4$: Cl, 13.83; Mo, 12.47. Found: Cl, 12.97, 13.01; Mo, 11.83, 11.79.

The remainder of the decanted solid was filtered and washed on the filter with carbon tetrachloride. After vacuum drying, a very light-green solid, m.p. 78–80°, was obtained. It evolved hydrogen chloride slightly when heated above 100°. The infrared spectrum of this solid was identical with that of the solid, m.p. 78–80°, separated by centrifugation, as described above.

*Anal.* Calcd. for $C_{36}H_{70}Cl_3MoO_4$: Cl, 13.83; Mo, 12.47. Found: Cl, 12.97, 12.90, 13.04; Mo, 12.47, 12.41.

**Reactions of Molybdenum Pentachloride with Excess Benzoic Acid in Benzene.**—These reactions were conducted using the procedure described above for the synthesis of molybdenum trichloride dibenzoate. The results are summarized in Table I.

The products of the 3- and 4- mole reactions were very similar. They were both sensitive to atmospheric moisture and several minutes exposure to water was required for noticeable hydrolysis to give benzoic acid. No appreciable benzoyl chloride could be detected in the reaction mixture filtrates by vacuum distillation or by treatment with concentrated ammonium hydroxide to give benzamide.

The 5-mole reaction was found to give a light-green, crystalline solid which possessed considerable hydrolytic stability. The same product, as confirmed by elemental, infrared and m-ray diffraction analyses, was obtained by the extended (five days) reflux of a benzene reaction mixture of molybdenum trichloride dibenzoate with three moles of benzoic acid. This product was washed with water and methanol without reaction. It is completely non-hygroscopic and resists attack by nitric and hydrochloric acids. It is completely soluble in concentrated sulfuric acid to release benzoic acid. Refluxing for several hours with 10% aqueous sodium hydroxide is required for saponification. The product is completely insoluble in common organic solvents with the exception of chloroform and pyridine, in which it reacts to replace benzoate.

Only trace benzoyl chloride and no benzoic anhydride or benzophenone could be detected in these reaction mixtures.

**Acknowledgments.**—The author is grateful to the following personnel of the research staff of the Climax Molybdenum Co. of Michigan: Mr. Robert H. Maurer, Supervisor of Analytical Chemistry; Mr. Franz R. Fucek and Mr. Harry G. Monteith, for performing the elemental analyses; Mr. Robert E. Herfert for X-ray diffraction analyses, and both Mr. George A. Timmons, Vice President and Director of Research and Dr. Elbert C. Herrick, Supervisor of Chemical Research, for support of this research and helpful discussions.

The infrared spectra were determined by the Anderson Physical Laboratory, Champaign, Ill. Some of the elemental microanalyses were performed by the Huffman Microanalytical Laboratories, Wheatridge, Colo.

DETROIT, MICH.

(10) Private communication from Dwight E. Couch, National Bureau of Standards, to Robert E. Herfert, Climax Molybdenum Co., July 21, 1958.

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[CONTRIBUTION FROM DEPARTMENTS OF BIOCHEMISTRY AND OF OBSTETRICS AND GYNECOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

# The Cleavage of Androsterone β-D-glucopyranosiduronic Acid in Organic Media

BY SHLOMO BURSTEIN,[1] GERT M. JACOBSOHN[2] AND SEYMOUR LIEBERMAN

RECEIVED JULY 6, 1959

A kinetic study of the acid hydrolysis of androsterone β-D-glucopyranosiduronic acid under various conditions has revealed that rapid cleavage of this conjugate can be effected at low temperatures and low acid concentrations. At temperatures of 26–50°, this model conjugate was cleaved in good yield when dissolved in certain organic media containing between 0.01–0.1 *N* perchloric acid and less than 0.1–0.5% of water. Under these conditions other acids such as sulfuric acid and *p*-toluenesulfonic acid were ineffective.

In an effort to develop a general chemical procedure for the cleavage of urinary glucopyranosiduronides (glucuronosides) of at least certain classes of steroids, this study of the acid-catalyzed cleavage of androsterone glucuronoside was undertaken. Although other conjugates are present, most of the steroid hormone metabolites are excreted into the urine as glucuronosides. Because no mild chemical methods are known for the hydrolysis of these compounds,[3] the usual procedure involves the use of strong acid (1–10 *N*) and elevated temperatures. Even then, it is sometimes difficult to obtain complete hydrolysis. Furthermore, these drastic conditions simultaneously result in side reactions which alter many of the steroid aglycones. The use of the enzyme, β-glucuronidase, for the cleavage of these conjugates is considered the most satisfactory method available at present since it preserves the chemical integrity of the aglycones. Because of the possible occurrence of enzyme competitors and inhibitors in urine, which could lead to grossly erroneous results,

(1) Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

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(3) R. S. Teague, *Adv. Carbohydrate Chem.*, **9**, 199 (1954).

the enzymatic procedure is not entirely reliable, although high yields can often be obtained. Levvy,[4] in an excellent appraisal of the limitations of glucuronidase hydrolysis of urinary glucuronosides, has pointed out that it is a mistake to rely upon rapid hydrolysis until such interfering substances have been eliminated. Even in the absence of inhibitors, complete enzymatic hydrolysis in a given time cannot be ensured without proper control of the concentration of the substrates.

Few detailed studies of the hydrolysis of the glycoside derivatives of D-glucuronic acid are available. Our previous experience[5] on the rapid cleavage of steroid hydrogen sulfates in organic media of low ionizing power led us to investigate the behavior of a model steroid glucuronoside under similar conditions. Almost all previous studies of the kinetics and mechanism of hydrolysis of simple acetals[6] and glycosides[7] have been carried out in aqueous media, although recently Allen, *et al.*[8] have discovered that boron trichloride in methylene chloride solution can degrade (demethylate, deacetylate, etc.) carbohydrates and their derivatives. In this investigation, the cleavage of androsterone glucuronoside was studied in several, nearly anhydrous, organic solvents, in which urinary steroid glucuronosides are soluble.

## Experimental

**Androsterone glucuronoside** (3α-O-(6β-D-glucopyranuronic acid)-androstane-17-one) was isolated from urine by Dr. John J. Schneider of the Jefferson Medical College, Philadelphia, who generously made available to us the crystalline, analytically pure material. *Anal.* Calcd. for $C_{25}H_{38}O_8$: C, 64.4; H, 8.16. Found: C, 64.57, 64.51; H, 8.43, 8.45. The absorbancy of androsterone glucuronoside on a molar basis in the Zimmermann reaction agreed within 5% with that of authentic androsterone. Extraction of an aqueous solution of the glucuronoside with benzene provided evidence that no Zimmermann chromogens contaminated the conjugate.

**Tetrahydrofuran** was refluxed over potassium hydroxide, distilled and stored. Before use, the solvent was heated under reflux with metallic sodium for two hours and then redistilled with the exclusion of moisture. The solvent purified in this manner was peroxide-free, as indicated by a colorless test with a 2% solution of $Ti(SO_4)_2$ in 20% sulfuric acid. It contained less than 0.03% of water as determined by a Karl Fischer titration.

**Ethyl acetate**, Mallinckrodt, analytical grade, was redistilled without further purification. It contained less than 0.1% of water.

**Perchloric acid** 70%, Mallinckrodt, analytical reagent, was used without purification.

The fission of androsterone glucuronoside was studied by dissolving 2.00 mg. in 10 ml. of the appropriate acidified solvent. Although androsterone glucuronoside was sparingly soluble in the organic solvents employed, it dissolved completely in the acidified media and, as a result, the data reported were obtained from homogeneous solutions. The ethyl acetate-containing media were equilibrated overnight or longer after the addition of the required amount of acid, and before the glucuronoside was added. The progress of androsterone formation was followed by removal of 2-ml. aliquots at various time intervals from the reaction mixture. Following neutralization of the acid with a few drops of pyridine the mixtures were blown to dryness under a stream of nitrogen. The dry residues were partitioned between 3 ml. of benzene and 3 ml. of a 1% $NaHCO_3$ solution. Under these conditions the liberated androsterone was quantitatively extracted into the organic phase, whereas the glucuronoside as well as glucuronic acid remained in the water phase. After equilibration, the benzene layers were filtered through cotton and 2-ml. aliquots of the filtrates were evaporated to dryness under nitrogen. The residues were applied to Whatman No. 2 paper and chromatographed for three hours, using the ligroin (b.p. 60–90°)-85% aqueous methanol system of Bush and Willoughby.[9] The areas corresponding to androsterone (determined by the $R_f$ of standard androsterone run on adjacent strips of paper and also by staining a narrow strip of each paper chromatogram with Zimmermann reagent) were eluted with 5 ml. of methanol. Four-ml. aliquots of these extracts were evaporated to dryness and the residue assayed quantitatively by the Zimmermann reaction.[10] Appropriate androsterone standards were simultaneously determined. In each run the concentration of steroid glucuronoside was $4.3 \times 10^{-4}$ *M* so that 5% hydrolysis could be readily estimated.

## Results

A detailed kinetic study of the cleavage of androsterone glucuronoside was made difficult because, under the conditions employed, other products as well as androsterone were formed. As a result, it was not possible to follow the reactions merely by measuring the benzene soluble Zimmermann-reacting material. Instead, the products from each run were separated by chromatography before quantification. The isolated androsterone was identified by means of its migration rate on paper and frequently by its infrared spectrum which, when determined, was always indistinguishable from that of the authentic material. When the progress of the reaction in tetrahydrofuran was followed in this way, a polar material, which did not move from the starting line of the paper chromatogram, was formed in addition to androsterone. It was extractable by benzene and was detected by a positive reaction in the Zimmermann test. In the carbonyl region of the infrared, this fraction exhibited (in chloroform) a broad absorption band at 1790 $cm.^{-1}$ and a more intense band at 1725 $cm^{-1}$. Its ultraviolet spectrum in methanol possessed an intense absorption maximum at 225 mμ and two weaker bands at 275 and 282 mμ. Upon addition of dilute alkali the two maxima at the higher wave lengths were obscured by the development of a single intense band at 278 mμ. Acidification led to the disappearance of this maximum and the reappearance of the two weaker bands at 275 and 282 mμ.

The progress of the hydrolysis of androsterone glucuronoside in tetrahydrofuran containing 0.01 *N* $HClO_4$ at 50° is shown in Fig. 1, wherein the rates of formation of androsterone (A) and the more polar material (B) are illustrated. The formation of B, it is apparent, rapidly increased and reached a maximum at a time when the concentration of liberated androsterone (A) was relatively small. The rate-curves of A and B are characteristic for those of the sequence: glucuronoside → B → A. A small amount, not more than 2%, of a compound less polar than androsterone was also detected.

The splitting of androsterone glucuronoside in ethyl acetate at 50° with 0.01 *N* $HClO_4$ followed

(4) G. A. Levvy, "Vitamins & Hormones," Vol. XIV, 1956, p. 268. Academic Press, New York, N. Y.

(5) S. Burstein and S. Lieberman, THIS JOURNAL, **80**, 5235 (1958).

(6) D. McIntyre and F. A. Long, *ibid.*, **76**, 3240 (1954).

(7) W. Pigman, "The Carbohydrates," Academic Press, Inc., New York, N. Y., 1957, p. 208.

(8) S. Allen, T. G. Bonner, E. J. Bourne and N. M. Saville, *Chemistry & Industry*, 630 (1958).

(9) I. E. Bush and M. Willoughby, *Biochem. J.*, **67**, 689 (1957).

(10) A. F. Holtorff and F. C. Koch, *J. Biol. Chem.*, **135**, 377 (1940).

TABLE I

$T_{1/2}$ OF CLEAVAGE OF ANDROSTERONE GLUCURONOSIDE UNDER VARIOUS CONDITIONS[a]

| Expt. | Temp., °C. | Solvent | $H_2O$, %[b] | Acid | N | $T_{1/2}$[c] |
|---|---|---|---|---|---|---|
| 1 | 50 | $H_2O$ | | $HClO_4$ | 1.0 | 10 hr. |
| 2 | 50 | $H_2O$ | | $H_2SO_4$ | 1.0 | 39 hr.[d] |
| 3 | 50 | EtOH | 0.1 | $HClO_4$ | 0.01 | 70 hr. |
| 4 | 26 | EtOAc | Eq. | $HClO_4$ | .001 | 7 hr. |
| 5 | 27 | EtOAc | Eq. | $HClO_4$ | .01 | 51 (63) min. |
| 6 | 50 | EtOAc | Eq. | $HClO_4$ | .001 | 84 min. |
| 7 | 50 | EtOAc | Eq. | $HClO_4$ | .01 | 14 (12, 22) min. |
| 8 | 50 | PrOAc | Eq. | $HClO_4$ | .01 | 120 min. |
| 9 | 50 | *i*-PrOAc | Eq. | $HClO_4$ | .01 | 130 min. |
| 10 | 50 | Dimethylformamide | 0.5 | $HClO_4$ | .1 | N.R., 120 min. |
| 11 | 1 | Tetrahydrofuran | .5 | $HClO_4$ | .1 | 262 hr. |
| 12 | 24 | Tetrahydrofuran | .1 | $HClO_4$ | .01 | 18 (14) hr. |
| 13 | 50 | Tetrahydrofuran | .03 | $HClO_4$ | .001 | 6.5 hr. |
| 14 | 50 | Tetrahydrofuran | .3 | $H_2SO_4$ | .01 | N.R., 240 min. |
| 15 | 50 | Tetrahydrofuran | .8 | $HClO_4$ | .01 | N.R., 240 min. |
| 16 | 50 | Tetrahydrofuran | .1 | $HClO_4$ | .01 | 45 min. |
| 17 | 50 | Tetrahydrofuran | .5 | $HClO_4$ | .1 | 25 (28) min. |
| 18 | 50 | Tetrahydrofuran | .5 | $H_2SO_4$ | .2 | N.R., 90 min. |
| 19 | 50 | Tetrahydrofuran-acetone[e] | .1 | $HClO_4$ | .01 | 84 min.[d] |
| 20 | 50 | Tetrahydrofuran-acetone | .1 | $HClO_4$ | .01 | 84 min.[d] |
| 21 | 50 | Tetrahydrofuran-EtOH[f] | .1 | $HClO_4$ | .01 | 106 min.[d] |
| 22 | 50 | Tetrahydrofuran-EtOH | .1 | $HClO_4$ | .01 | 8 hr.[d] |

[a] The concentration of steroid was, in each case, $4.3 \times 10^{-4}$ *M*. [b] Eq. signifies that the solvent had been equilibrated with the specified acid overnight or longer at the temperature of the experiment. [c] Values in parentheses refer to replicate determinations; N.R. indicated no reaction at the time quoted. [d] $T_{1/2}$ for these experiments are calculated from a single determination. [e] Expt. 19, 1% acetone; expt. 20, 20% acetone. [f] Expt. 21, 1% EtOH; expt. 22, 20% EtOH.

a course similar to that illustrated in Fig. 1. As before, a benzene-soluble fraction, more polar than androsterone, was formed. It rapidly reached a maximum concentration (57% of the starting material) after five minutes and then declined so that only 5% was present at 40 minutes. At this time the yield of free androsterone was 80%. Thereafter the amount of androsterone diminished slightly due to the formation, by transesterification, of androsterone acetate which was identified by its infrared spectrum.

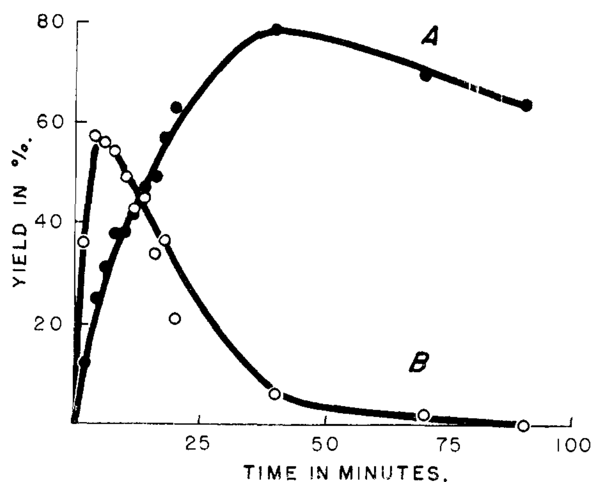


Fig. 1.—Progress of cleavage of androsterone glucuronoside in tetrahydrofuran containing 0.01 *N* $HClO_4$ at 50°; A is rate of formation of androsterone; B is rate of formation of polar material.

The effect of water upon the rate of cleavage of androsterone glucuronoside in ethyl acetate was demonstrated by comparing the rate in the presence of 0.5% water with that carried out in essentially anhydrous conditions. Water and 70% perchloric acid were added to ethyl acetate so that the final solution was 0.01 *N* with respect to acid and contained 0.5% water. To 10 ml. of this solution at 50°, androsterone glucuronoside was added and the resulting mixture kept at this temperature for about one hour, during which time, samples were removed for analysis of the benzene-soluble 17-ketosteroids (not resolved by paper chromatography). Another 10 ml. of the acidified media was equilibrated at 50° for 24 hours before the addition of androsterone glucuronoside. In the latter experiment, the results indicated that the rate of fission was rapid and constant throughout the 30 minutes required for 70% hydrolysis. In the presence of water, the initial rate was slow, but after half an hour, it was accelerated due to removal of water during the course of the reaction.

In ethanol (3 in Table I), the slow hydrolysis was accompanied by the rapid formation of large amounts of a fraction more polar than androsterone which remained at the origin of the paper chromatogram. After three hours, when only 3% of the androsterone had been formed, 63% of the reactant had been converted into this compound. With water as a solvent no benzene-soluble, polar compound was found (1 and 2 in Table I).

The rate of formation of androsterone from androsterone glucuronoside under various conditions is summarized in Table I. The rates are presented as half-times, the precision of which can be inferred from the few recorded replicate determinations which are given in parentheses in the last column. Except where noted, the $T_{1/2}$ data are

based on curves drawn from at least five points. Recovery from the paper chromatograms was of the order of 90 ± 5%.

Table II records the actual yield of androsterone isolated under the specified conditions. For comparison, the yield obtained when the enzyme β-glucuronidase was employed, is also given.

It is evident from Table I that the rate of formation of androsterone from androsterone glucuronoside is dependent upon the concentration and the nature of the acid employed, the temperature, and the nature of the medium. With sulfuric acid (and also *p*-toluenesulfonic acid) cleavage proceeds slowly and only at elevated temperatures. When $HClO_4$ is used in aqueous solution, the rate is accelerated (compare 1 with 2), but the most dramatic influence upon the rate appears to be the nature of the solvent. In water or ethanol (3) the reaction proceeded slowly even when the $HClO_4$ concentration was 1 *N* and the temperature, 50°. On the other hand, in ethyl acetate solution (4–7), the rate is appreciably greater even when the acid concentration is 0.001 *N* and the temperature 26° (6). At an acidity of 0.01 *N* $HClO_4$ the reaction carried out at 50° was about 90% complete in approximately one hour (7). Substitution of propyl acetate (8) or isopropyl acetate (9) for ethyl acetate retarded the reaction. In dimethylformamide, a polar solvent in which many glycosides are soluble, no hydrolysis occurred even at 0.1 *N* $HClO_4$ (10). Experiments 11–14 in Table I give the $T_{1/2}$ values when tetrahydrofuran was used as solvent with varying amounts of acid and water.

TABLE II

RECOVERY OF ANDROSTERONE BY $HClO_4$-CATALYZED CLEAVAGE OF ITS GLUCURONOSIDE UNDER VARIOUS CONDITIONS

| Temp., °C. | Solvent | $H_2O$, %[a] | $HClO_4$, *N* | Time | Yield, %[b] |
|---|---|---|---|---|---|
| 27 | EtOAc | Eq. | 0.01 | 150 min. | 93[c] |
| 50 | EtOAc | Eq. | .01 | 40 min. | 85[d] |
| 24 | Tetrahydrofuran | 0.1 | .01 | 27 hr. | 66 |
| 50 | Tetrahydrofuran | .1 | .01 | 180 min. | 81 |
| 50 | Tetrahydrofuran | .5 | .1 | 60 min. | 81 |
| 50 | $H_2O$ | | 1.0 | 24 hr. | 67 |
| 37 | β-Glucuronidase[e] at *p*H 5 | | | 2.5 days | 76 |

[a] Eq. signifies that the solvent had been equilibrated overnight or longer at the temperature of the experiment. [b] Yields are calculated from the total Zimmermann-reacting material migrating in the paper chromatograms at a rate equal to that of androsterone. [c] 13% isolated as androsterone acetate. [d] 6% isolated as androsterone acetate. [e] Ketodase, purchased from Warner–Chilcott Laboratories.

A somewhat smaller, although still rapid, rate was achieved when tetrahydrofuran was used as the solvent. In this medium, however, the presence of small amounts of water (15) seriously inhibited the reaction. As previously mentioned, a similar influence of water was observed in experiments with ethyl acetate as the solvent, but in determinations 4 through 7 (Table I) the amount of water was always much less than that in the corresponding runs with tetrahydrofuran. In the ethyl acetate experiments, water present in the solvent or in the 70% perchloric acid was consumed by the hydrolysis of the solvent which occurred during equilibration, so that the reaction was conducted in a nearly anhydrous medium. After the addition of $HClO_4$ to ethyl acetate (water content determined by Karl Fischer reagent), constant acid titer was reached after 2 hours. By this time, the water content was approximately 0.1% as determined by titration of the acetic acid produced. The concentration of water in the tetrahydrofuran determinations was always greater than that present in the ethyl acetate runs and consequently it is not possible to attribute the faster rates in ethyl acetate to the chemical nature of the solvent. Studies in tetrahydrofuran solution in the complete absence of water were not carried out. In any case, of the media studied, ethyl acetate afforded the most rapid rate. The addition of small amounts of water to either ethyl acetate or tetrahydrofuran retarded the reaction significantly. At 0.1% concentration of water (16) the reaction is complete in four hours. Increasing the concentration of water to 0.8% (15), resulted in no hydrolysis at all during the same period.

Dilution of tetrahydrofuran with small amounts of more polar solvents, such as acetone or ethanol, did not reduce drastically the rate of hydrolysis (compare 16 with 19, 20 and 21). With 20% ethanol (22) on the other hand, the $T_{1/2}$ of cleavage was increased about ten times.

## Discussion

It is generally accepted that the hydrolysis of simple acetals in aqueous media proceeds through the formation of a carbonium ion intermediate which reacts with water to complete the hydrolysis.[11] It seems unlikely that this rationalization is directly applicable to the cleavage of glucuronosides in organic media where water has a powerful inhibitory effect upon the rate-determining step. Although a detailed study of the mechanism of the cleavage of androsterone glucuronoside in organic media has not been made, the demonstration of the enormous role played by the solvent in determining the rate suggests the involvement of a transition state complex which is favored by non-polar media. Whether the ion-solvating capacity of the solvent participates in promoting such a complex, as has been suggested[5] by the more extensive studies on the solvolysis of alkyl hydrogen sulfates, has not been established, although this is a strong likelihood.

The spectral characteristics of the fraction more polar than androsterone, which was formed during the course of perchloric acid hydrolysis in tetrahydrofuran and ethyl acetate, are difficult to account for as the properties of a single entity. While the absorption band at 225 mμ suggests an α,β-unsaturated-γ-lactone,[12] the alteration in the spectrum in the region of 280 mμ resulting from the treatment with alkali is characteristic of the saturated γ-lactone of methyl glucofururonoside.[13] However, this latter compound does not exhibit maxima at 275 and 282 mμ in neutral or acid solu-

(11) C. A. Bunton, T. A. Lewis, D. R. Llewellyn and C. A. Vernon, *J. Chem. Soc.*, 4419 (1955).

(12) W. G. Dauben and P. D. Hance, THIS JOURNAL, **75**, 3352 (1953).

(13) L. N. Owen, S. Peat and W. J. G. Jones, *J. Chem. Soc.*, 339 (1941).

tion, as does the polar material. The absorption band at 1790 cm.$^{-1}$ which characterizes the unknown material appears to be at a higher frequency than that usually associated with $\gamma$-lactones (saturated $\gamma$-lactones absorb at 1775 cm.$^{-1}$, $\alpha,\beta$-unsaturated-$\gamma$-lactones absorb at 1750 cm.$^{-1}$ and $\beta,\gamma$-unsaturated-$\gamma$-lactones absorb at about 1800 cm.$^{-1}$).[12] In a KBr pellet, glucuronolactone exhibits an absorption maximum at 1775 cm.$^{-1}$.

From the evidence presented, it is reasonably certain that some of the substances occurring in this polar fraction were lactones. Assuming that the naturally occurring androsterone glucuronoside is a pyranoside, as are other uronic acids, lactones formed from it must have involved a conversion to the more stable furanoside. Ever since the demonstration[14] that glucurone is the $\gamma$-lactone of a furanoside and thus contains two five-membered rings, it has been recognized that a $\gamma$-lactone cannot form in a pre-existing pyranose structure. An analogous rearrangement of a pyranoside to a furanoside structure has been illustrated by the conversion of methyl 3,6-anhydro-$\alpha$-D-glucopyranoside to methyl 3,6-anhydro-$\alpha$-D-glucofuranoside by ethereal as well as methanolic HCl.[15] Protonation of androsterone glucuronoside probably produced an open chain carbonium ion which lactonized under the acidic conditions to the $\gamma$-lactone which, in turn, reverted to the strainless furanoside instead of returning to the sterically impossible pyranoside. An acid-catalyzed elimination reaction could have resulted in a subsequent formation of the $\alpha,\beta$-unsaturated lactone.

(14) F. Smith, *J. Chem. Soc.*, 157 (1944).

(15) W. N. Haworth, L. N. Owen and F. Smith, *ibid.*, 88 (1941).

The results described in this paper demonstrate that a gentle, chemical procedure for the hydrolysis of naturally occurring glycosides and glucuronosides is possible. To effect cleavage at low acid concentrations and low temperature requires that the glycosides first be solubilized in a suitable, nonionizing solvent. This requirement imposes a serious limitation since many glycosides are soluble only in polar media. Fortunately, the glucuronosides of the steroid hormone metabolites, as well as some other glycosides, can, under appropriate conditions, be extracted from aqueous solution into an organic solvent, such as ethyl acetate or tetrahydrofuran. Following reduction of the water content to tolerable levels (0.1–0.5%) by azeotropic distillation or other means, the hydrolysis can then be catalyzed by the addition of small amounts of $HClO_4$ (0.01–0.1 *N*). Whether this procedure will supplant the popular, albeit unreliable, hydrolysis using $\beta$-glucuronidase will depend upon the results of experiments presently being carried out to determine the extent of undesirable side reactions (acetate formation, dehydration, rearrangement, etc.), which the acidic conditions may produce simultaneously with cleavage.

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NEW YORK 32, N. Y.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

# Steroids. CXXXIV.[1] Derivatives of 17$\alpha$-Ethylnyltestosterone and 17$\alpha$-Ethynyl-19-nortestosterone

BY LAWRENCE H. KNOX, JOHN A. ZDERIC, J. PÉREZ RUELAS, CARL DJERASSI AND H. J. RINGOLD

RECEIVED JULY 27, 1959

The reaction of enol ethers of $\Delta^4$-androstene-3,17-dione and 19-nor-$\Delta^4$-androstene-3,17-dione with propynylmagnesium bromide followed by acid hydrolysis gave 17$\alpha$-propynyltestosterone (III) and the conjugated (VII) and unconjugated (VI) 17$\alpha$-propynyl-19-nortestosterone. The syntheses of 6-fluoro and 6-chloro analogs in the ethynyl-, propynyl- and 19-norethynyltestosterone series are described.

In recent years considerable attention has been devoted to chemical modification of the orally active progestational agent 17$\alpha$-ethynyltestosterone[2] in the hope of obtaining even more active compounds. Among the outstanding modifications leading to potentiated progestational activity have been replacement of the C-10 angular methyl group by hydrogen (17$\alpha$-ethynyl-19-nortestosterone[3] and 17$\alpha$ - ethynyl - $\Delta^{5(10)}$- estren-17$\beta$-ol-3-one[4]), substitution by methyl at C-6$\alpha$,[5,6] and conversion of the ethynyl group to a propynyl[6b] group. It has also been demonstrated that acetylation of the tertiary hydroxyl group[7] of 17$\alpha$-ethynyl-19-nortestosterone further potentiates progestational activity. Finally, it has recently been reported that substitution of 17$\alpha$-acetoxyprogesterone by fluorine,[8,9] chlorine[9] or bromine[9] at C-6 with and without additional double bond introduction at C-1 or C-6 leads to compounds of exceptional progestational potency. Thus it was of considerable interest to synthesize deriva-

(1) Paper CXXXIII, H. J. Ringold, THIS JOURNAL, **82**, 961 (1960).

(2) H. Inhoffen, W. Logemann, W. Hohlweg and A. Serini, *Ber.*, **71**, 1024 (1938).

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