[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

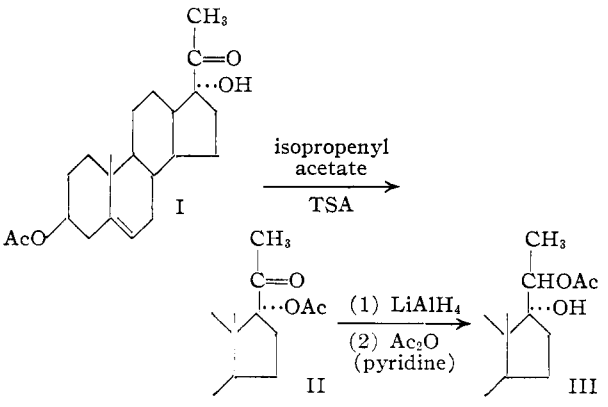
Acetylation of 17α-Hydroxy Steroids

BY ROBERT BRUCE MOFFETT AND HUGH V. ANDERSON

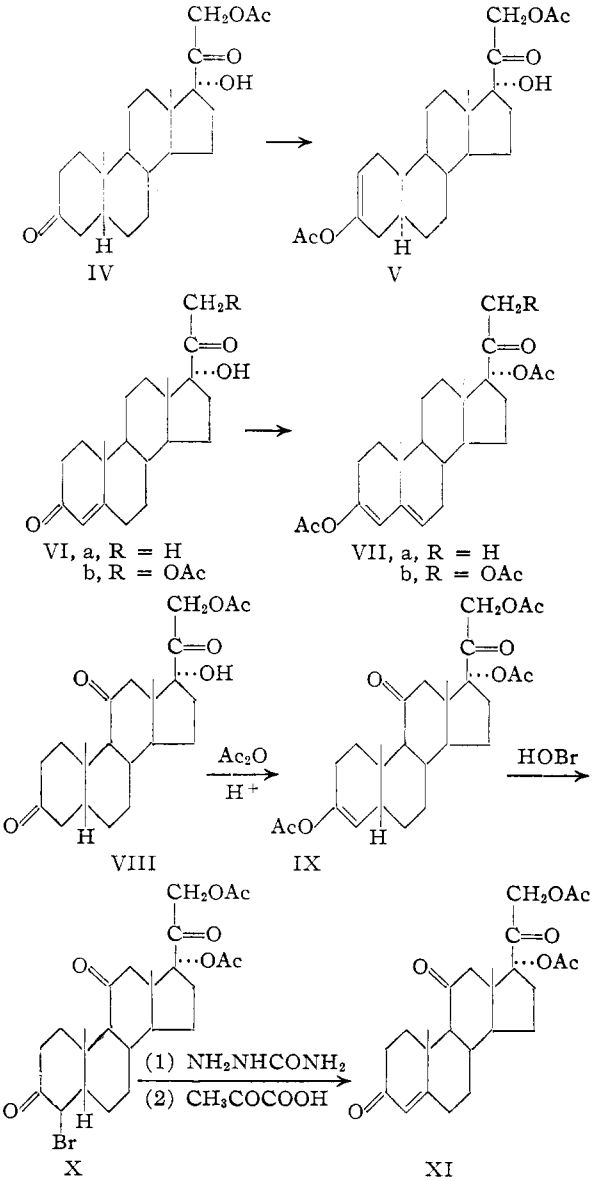
RECEIVED OCTOBER 12, 1953

Acetylation of 17α-hydroxy groups in several steroids has been carried out with isopropenyl acetate or acetic anhydride with an acid catalyst. The structure of one of these products was proved by removal of the 17α-acetate by lithium aluminum hydride. 3-Keto-17α-hydroxy steroids gave simultaneously the 3-enol acetate and 17α-acetate. One of these, 17α-hydroxy-21-acetoxypregnane-3,11,20-trione, was converted to cortisone-17α,21-diacetate.

In spite of the difficulties in acetylating the 17α-hydroxyl group in steroids, several papers[1-3] have appeared recently in which this was reported. Before the appearance of these reports we had independently accomplished similar results, using different compounds or conditions from those reported. In attempting to prepare the $\Delta^{20(21)}$-enol acetate from 3β-acetoxy-17α-hydroxy-5-pregnen-20-one (I) by the action of isopropenyl acetate and an acid catalyst,[4] a new compound was obtained. The analysis and infrared spectrum of this indicated that it was not the enol acetate, but contained two acetoxy groups and no hydroxyl group. Therefore, it was presumed to be the 17α-acetate II.[5] However, because of the possibility of rearrangement during formation, confirmatory evidence of its structure seemed necessary. Hydrolysis under either acidic or basic conditions might conceivably bring about rearrangement to a D-homo derivative or cleavage to an etio acid. Therefore the structure of this compound II was confirmed by reduction with lithium aluminum hydride and reacetylation with acetic anhydride in pyridine to 3β,20α-diacetoxy-5-pregnen-17α-ol (III) identical with that reported by Hirschmann and Hirschmann.[6] This demonstrates that although our conditions were more drastic than those employed by other workers no rearrangement occurs at the 17-position during the acetylation.



With 17α-hydroxy compounds containing a keto group in the 3-position, isopropenyl acetate, or acetic anhydride and an acid catalyst, gave enol acetylation at C-3 as well as acetylation of the 17α-hydroxyl function.[7] This is illustrated by the reaction of 21-acetoxy-17α-hydroxyallopregnane-3,20-dione (IV) to give 3,17α,21-triacetoxy-2-allopregnen-20-one (V). In the Δ^4-3-keto series compound VI gave the 3,5-diene VII, and in the 5-normal series



(1) R. B. Turner, THIS JOURNAL, **74**, 4220 (1952); **75**, 3489 (1953).

(2) Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, *ibid.*, **74**, 5394 (1952).

(3) E. P. Oliveto, C. Gerald and E. B. Hershberg, *Arch. Biochem. Biophys.*, **43**, 234 (1953).

(4) R. B. Moffett and D. I. Weisblat, THIS JOURNAL, **74**, 2183 (1952).

(5) This compound recently has been reported by R. B. Turner (ref. 1).

(6) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **187**, 137 (1950).

(7) It will be noted that under milder conditions R. B. Turner (ref. 1) did not obtain enol acetylation at C-3.

21-acetoxy-17α-hydroxypregnane-3,11,20-trione (VIII) gave 3,17α,21-triacetoxy-3-pregnene-11,20-dione (IX).

In the allo series the 3-enol acetate V is written with the double bond in the 2,3-position in conformity with the findings of Inhoffen, *et al.*,[8] and of Rubin and Armbrecht.[9] In the normal series (IX) the double bond has been placed in the 3,4-position.[8,9] This was confirmed by the addition of hypobromous acid to the enol acetate[10] to give the 4-bromo derivative (X) which was converted to cortisone 17,21-diacetate by the general method of Mattox and Kendall.[11]

Experimental[12,13]

3β,17α-Diacetoxy-5-pregnen-20-one (II).[1]—A solution of 0.9 g. of 3β-acetoxy-17α-hydroxy-5-pregnen-20-one (I) and 0.15 g. of *p*-toluenesulfonic acid monohydrate in 20 ml. of isopropenyl acetate was heated under reflux and slowly distilled through a fractionation column for about 12 hours. After cooling the reaction mixture, 1 g. of sodium bicarbonate was added and the excess isopropenyl acetate was removed by distillation under reduced pressure. The residue was taken up in ether and ice-water. The aqueous solution was extracted with ether, and the combined ether solution was washed with water and dried over sodium sulfate. Removal of the ether gave a crystalline solid which was recrystallized from methanol. The yield of the first crop of crystals was 0.6 g. (58.2%), m.p. 170–173°, $[\alpha]^{24}D$ −63.0° (1% in chloroform).

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71; CH_3CO, 20.66. Found: C, 72.26; H, 8.61; CH_3CO, 18.71.

3β,20α-Diacetoxy-5-pregnen-17α-ol (III).—A sample of the above diacetate II was reduced with lithium aluminum hydride essentially by the method described for an analogous compound by Hirschmann and Hirschmann.[6] After reacetylation of the product with acetic anhydride and pyridine and two crystallizations from methanol, 3β,20α-diacetoxy-5-pregnen-17α-ol (III) was obtained, m.p. 198–203°, $[\alpha]^{24}D$ −77° (0.64% in 95% ethanol). Hirschmann and Hirschmann[6] report for this compound m.p. 204–206°, $[\alpha]^{28}D$ −79° (alcohol).

3,17α,21-Triacetoxy-2-allopregnen-20-one (V).—A 250-ml. three-necked flask was fitted with a 40-cm. column packed with glass helices and a still head which permitted total reflux. A mixture of 18.25 g. (0.180 mole) of acetic anhydride, 0.264 g. (0.001 mole) of sulfosalicylic acid and 140 ml. of toluene was placed in the flask and a mixture of acetic acid, acetic anhydride and toluene was slowly distilled for about 45 minutes when a head temperature of 109° indicated that all acetic acid had been removed. Then 5.858 g. (0.015 mole) of 17α-hydroxy-21-acetoxyallopregnane-3,20-dione (IV) was added, the reaction was again heated to reflux, the acetic acid formed was distilled. Titration of the acetic acid showed that one mole of acetic acid was formed in 10 minutes and a second mole in the next 2 hours. Heating was continued for a total of 5.5 hours, although little more acetic acid was formed.[14]

The mixture was diluted with 100 ml. of benzene and 275 ml. of ether, washed three times with ice-water, and dried over magnesium sulfate. Distillation of solvent under reduced pressure gave a yellow oil which crystallized on standing, m.p. 162–170°. This was recrystallized from ether-hexane giving 6.0 g. (84.4%) of colorless crystals, m.p. 170.5–174.5°. A sample recrystallized from methanol-water (8–1) gave material of m.p. 172–176°.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.32; H, 8.07. Found: C, 68.81; H, 8.04.

3β,17α,21-Triacetoxyallopregnan-20-one.—A mixture containing about 70% 17α-hydroxy-21-acetoxyallopregnane-3,20-dione and 30% 3β,17α-dihydroxy-21-acetoxyallopregnan-20-one was acetylated under conditions similar to those described for II above. The mixture of acetates was separated by chromatography on Florisil. The less polar fraction was 3,17α,21-triacetoxy-2-allopregnen-20-one (V) identical with that described above. The more polar fraction was 3β,17α,21-triacetoxyallopregnan-20-one. This was crystallized three times from ether-petroleum ether giving crystals, m.p. 204–205.5°.

Anal. Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found: C, 68.00; H, 8.36.

3,17α-Diacetoxy-3,5-pregnadien-20-one (VIIa).—In a manner similar to that described above for the preparation of II, 1 g. of 17α-hydroxyprogesterone (VIa) was treated with isopropenyl acetate and *p*-toluenesulfonic acid. The product was crystallized twice from methanol giving 0.13 g. of material, m.p. 179–184°. The ultraviolet spectrum (λ_{max}^{alc} 234.5 mμ, *E* 21000) confirms the $\Delta^{3,5}$-diene structure.

Anal. Calcd. for $C_{27}H_{36}O_7$: C, 68.62; H, 7.68; CH_3CO, 27.32. Found: C, 68.74; H, 7.81; CH_3CO, 26.59.

3,17α,21-Triacetoxy-3-pregnene-11,20-dione (IX).—In a manner similar to that described above for the preparation of V, 4.045 g. (0.01 mole) of 17α-hydroxy-21-acetoxypregnane-3,11,20-trione (VIII) was treated with acetic anhydride and sulfosalicylic acid in toluene. Titration of the acetic acid distilled from the reaction mixture indicated that two moles of anhydride had reacted in one hour. The reaction was then stopped and worked up as described above. Recrystallization of the crude product from aqueous methanol gave 3.727 g. (76.2%) of material, m.p. 144–149.3°, $[\alpha]^{25}D$ +30° (0.989% in $CHCl_3$).

Anal. Calcd. for $C_{27}H_{36}O_8$: C, 66.37; H, 7.43. Found: C, 66.60, 66.58; H, 7.73, 7.54.

17α,21-Diacetoxy-4-bromopregnane-3,11,20-trione (X).—To a solution of 1.954 g. (0.004 mole) of 3,17α,21-triacetoxy-3-pregnene-11,20-dione (IX) in 100 ml. of *t*-butyl alcohol was added a solution of 0.780 g. (0.0044 mole) of N-bromosuccinimide in 100 ml. of *t*-butyl alcohol and then 60 ml. (0.024 mole) of 0.8 *N* sulfuric acid. The mixture was allowed to stand at room temperature for 100 minutes and was then treated with a solution of 3.78 g. (0.030 mole) of sodium sulfite in 25 ml. of water. The mixture was distilled under reduced pressure to about 20 ml., diluted with 200 ml. of water, and left overnight in the refrigerator. The solid was collected on a filter and dried at 80° to give 2.08 g. of material, m.p. 180° dec. This crude bromide was recrystallized from acetone–water giving 1.76 g. (84.1%) of needles, m.p. 183.5–184.5° dec. A sample was twice recrystallized from ethyl acetate–hexane, m.p. 186–187° dec.

Anal. Calcd. for $C_{25}H_{33}BrO_7$: C, 57.01; H, 6.33; Br, 15.21. Found: C, 57.01; H, 6.02; Br, 14.76.

17α,21-Diacetoxy-4-pregnene-3,11,20-trione (XI).[1]—To a solution of 1.42 g. (0.0027 mole) of 17α,21-diacetoxy-4-bromopregnane-3,11,20-trione (X) in 114 ml. of dioxane and 28 ml. of water was added 0.605 g. (0.0054 mole) of semicarbazide hydrochloride and 0.443 g. (0.0054 mole) of sodium acetate. After standing at room temperature for 2 hours, the solution was treated with 1.4 ml. of an aqueous solution containing 0.019 mole of pyruvic acid and held at 60° for 3 hours. The reaction mixture was cooled to room temperature, diluted with 800 ml. of water, and extracted with four 200-ml. portions of methylene chloride. The extracts were combined, washed with 250 ml. of ice-cold 5% sodium hydroxide solution, then with 250 ml. of water, and dried over sodium sulfate. The solvent was removed by a stream of dry air, and the residue was dried at 110° for 30 minutes giving 1.1526 g. of solid, m.p. 199–200° dec. Re-

(8) H. H. Inhoffen, W. Becker and G. Kolling, *Ann.*, **568**, 181 (1950).

(9) M. Rubin and B. M. Armbrecht, THIS JOURNAL, **75**, 3513 (1953).

(10) This addition of hypobromous acid to steroid enol acetates to give α-bromo ketones was developed by Dr. B. J. Magerlein in these laboratories and will be described in a future publication.

(11) V. R. Mattox and E. C. Kendall, THIS JOURNAL, **72**, 2290 (1950).

(12) Melting points were taken on a Fisher–Johns block and are uncorrected. Analyses and rotations are by Mr. Wm. Struck and associates of our Analytical Chemistry Laboratory. Infrared and ultraviolet spectra are by Dr. J. L. Johnson and co-workers of our Department of Physics.

(13) Infrared spectra were obtained on all compounds herein reported, and were in all cases consistent with the proposed structures.

(14) This method for following the course of acetylations is published in more detail; H. V. Anderson, E. R. Garrett, F. H. Lincoln, Jr., A. H. Nathan and J. A. Hogg, THIS JOURNAL, **76**, 743 (1954).

crystallization from methanol–water, then from methanol using Darco, and finally from methanol, gave material of m.p. 216.5–218°.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.55; H, 7.26. Found: C, 66.98; H, 7.06.

KALAMAZOO, MICHIGAN

[CONTRIBUTION NO. 131 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TENNESSEE]

The Isomeric Mono- and Dibromohecogenin Acetates

BY GEORGE P. MUELLER[1] AND LILBURN L. NORTON

RECEIVED AUGUST 31, 1953

Bromination of hecogenin acetate gives rise to two isomeric monobromides in which the bromine atoms are located on the side chain. In the absence of direct evidence for the exact position and configuration of the halogen they are tentatively designated as 23a and 23b isomers. Further bromination of each isomer yields a mixture of dibromides which can be dehydrohalogenated to the isomeric 9,11-dehydro-23-bromo derivatives. In the 23a series this mixture was resolved, yielding the 11α,23a- and 11β,23a-dibromides.

Although dibromination of hecogenin acetate has been used in several laboratories[2–5] as a convenient route to the ring-C ketols, 11-ketosteroids and 9,11-dehydro derivatives, we have felt that a more detailed study of the bromides themselves was indicated by the complex nature of mixtures which are always obtained. We have previously commented on this situation and have reported the isolation of 11α- and 11β,23ξ-dibromohecogenin acetates from the mixture of dibromides.[5]

It now appears that there are two isomeric monobromohecogenin acetates; we have isolated these and derived the foregoing dibromides from one of them. The second pair of dibromides has defied separation but has yielded a second 9,11-dehydro-23ξ-bromohecogenin (IX) convertible to 9,11-dehydrohecogenin acetate, thus linking the isomerism to a bromine atom in the side chain.

Introduction of two bromine atoms at C-23 in sarsasapogenin acetate[2] suggested that either of these hydrogens might also be replaceable in the isosapogenins. Substantiation of this possibility appears in the same publication where a preparation of 9,11-dehydro-23-bromohecogenin acetate showed a lower melting point, 209–213°. We have found this melting range characteristic of a mixture of equal parts of 9,11-dehydro-23a-bromohecogenin acetate and the 23b isomer. Finally, the isolation earlier[5] of two bromoketols, each yielding 3β,12β-dihydroxy-5α,22a-spirostan-11-one on debromination, pointed toward this type of isomerism.

In the absence of any direct evidence for the position or configuration of the halogen in the side chain, the parent compounds of the two series have been arbitrarily designated as 23a- and 23b-bromides, II and III. The usual placement of bromine at C-23 is based on analogy with Marker's proof that this is the position occupied in bromosarsasapogenin acetate, a 22b-spirostane.[6]

We attempted a similar oxidation of 23a-bromohecogenin acetate (II) but had no success in isolating a twenty-two carbon diketo acid. We also tried making a C-20 bromide by adding bromine to pseudohecogenin in the cold, followed by gentle warming with acid. No product was isolated which corresponded to either of the bromohecogenins. The investigation of these structures is being continued.

Crystallization techniques appear particularly advantageous for the separation of these isomers, since mixtures of II and III as well as VIII and IX were not separable by chromatography. Due to the relative insolubility of the 23a series, VI and VII could be crystallized from the mixture obtained by dibromination of hecogenin acetate (I).[5] The melting point of VI and rotation of VII differ slightly from the dibromides previously isolated; these isomers were prepared by bromination of pure 23a-bromohecogenin acetate. Separations were in all cases followed by rotations as well as melting points, and the crystal forms, though similar, are distinctive. Differentiation of VI and VII was made through dehydrohalogenation, VI being stable in boiling pyridine; however, both compounds yield VIII when refluxed with collidine.[7]

The mixed dibromides in the 23b series were lower melting and resisted separation. Dehydrohalogenation with pyridine and chromatography were used in the hope of isolating 11α,23b-dibromohecogenin acetate and 9,11-dehydro-23b-bromohecogenin acetate. Only the latter, IX, was obtained in these experiments.

Acknowledgment.—We wish to thank Research Corporation, New York, and the National Institutes of Health for their generous financial support.

Experimental

23a-Bromohecogenin Acetate (II) and 23b-Bromohecogenin Acetate (III).—Hecogenin acetate, 2.0 g., in 100 ml. of glacial acetic acid was stirred at 20° and treated successively with one drop of hydrogen bromide saturated acetic acid and one equivalent of purified bromine in 25 ml. of acetic acid during ten minutes. Decolorization was complete in this time and the mixture was stirred into 1.5 l. of water. The flocculent white precipitate was washed thoroughly and dried at 55°, yielding 2.26 g. (97%) of product, m.p. 220–223° dec., $[\alpha]^{26}D$ −21.1° (dioxane). This mixture was not resolved by the various chromatographic procedures

(1) G. D. Searle & Co., Skokie, Illinois.

(2) C. Djerassi, H. Martinez and G. Rosenkranz, *J. Org. Chem.*, **16**, 303 (1951).

(3) G. P. Mueller, R. E. Stobaugh and R. S. Winniford, THIS JOURNAL, **73**, 2400 (1951).

(4) R. Hirschmann, C. S. Snoddy, Jr., and N. L. Wendler, *ibid.*, **75**, 3252 (1953).

(5) G. P. Mueller, L. L. Norton, R. E. Stobaugh, L. Tsai and R. S. Winniford, *ibid.*, **75**, 4892 (1953).

(6) R. E. Marker, D. L. Turner, A. C. Shabica and P. R. Ulshafer, *ibid.*, **63**, 1032 (1941).

(7) H. B. Alther and T. Reichstein, *Helv. Chim. Acta*, **26**, 492 (1943); E. Seebeck and T. Reichstein, *ibid.*, **26**, 536 (1943).