[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Chemistry of the Steroidal D-Ring Lactones[1]

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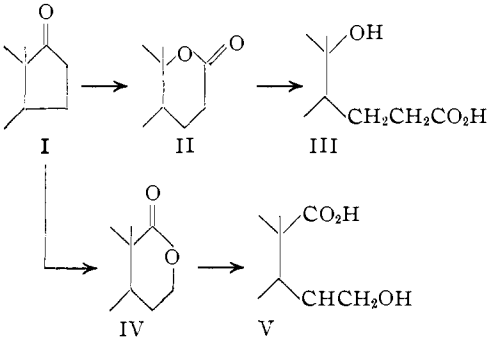
The problem as to whether 17-keto steroids are oxidized by peracids to give 13,17-seco steroidal lactones or 16,17-seco steroidal lactones has been settled by the peracetic acid oxidation of 3β-acetoxyandrostan-17-one. The structure of the product obtained from the oxidation has been determined as 3β-acetoxy-13α-hydroxy-13,17-secoandrostan-17-oic acid lactone.

The conversion of a 17-keto steroid (I) to a D-ring lactone was first reported by Westerfeld[2] who oxidized estrone with alkaline hydrogen peroxide. Since the hydroxy acid obtained from the lactone was easily converted to the methyl ester Westerfeld assigned the lactone the structure II, and the acid III.



Subsequently Jacobsen[3] oxidized estrone with peracetic acid in acetic acid to a lactone which was chemically similar to Westerfeld's lactone but differed in its physiological properties. Jacobsen assigned structure II to the D-ring of his lactone also. Related lactones have also been prepared recently by the microbiological oxidation of steroids.[4,5] Since the hydroxy acids derived from them resisted oxidation they too were assigned the D-ring structure III, and the lactones II. Moreover, the lactone originally obtained by Jacobsen[6] by the oxidation of dehydroepiandrosterone with peracetic acid was recently shown by Von Seemann and Grant[7] to be different than the corresponding lactone of structure IV which they prepared by a method which left no doubt as to its structure.

Jacques, Horeau and Courrier[8] concluded that Westerfeld's and Jacobsen's lactones were the same and differed only in degree of purity. However, in analogy to the peracid oxidation of camphor (VI) to the campholide (VII), they considered structure IV the more likely for their lactones.[9] Their subsequent work did not support this premise.

VI VII

In connection with exploratory studies directed toward the introduction of oxygen at C_{11}, we have investigated the structure of the D-ring lactone prepared by the peracetic acid oxidation of 3β-acetoxyandrostan-17-one (VIII). Our work has shown conclusively that oxidative cleavage of this 17-ketone by the method of Jacobsen occurred between carbon atoms 13 and 17 to give 3β-acetoxy-13α-hydroxy-13,17-secoandrostan-17-oic acid, lactone (IX). This compound has been previously prepared by Levy and Jacobsen[6] and named "Isoandrololactone" acetate. When this lactone was allowed to react with lithium aluminum hydride a "triol," subsequently proven to be 13,17-secoandrostan-3β,13α,17-triol (X), was obtained in 89% yield. When X was treated with benzoyl chloride and pyridine under mild conditions, two products were isolated: (1) the tribenzoate (XI) of X (2.5% yield) and (2) an "ene dibenzoate" (50%), later shown to be 3β,17-dibenzoxy-13,17-seco-13(18)-androstene (XII). In another experiment, conducted in a similar manner, the tribenzoate XI proved to be the major product (51%). XI was converted to XII in a 44% yield by refluxing in dimethylaniline for 3 hours. Ozonolysis of the "ene dibenzoate" gave a 65% yield of a product which formed no precipitate with Dimedon, and produced only a monosemicarbazone when treated with excess semicarbazide. In addition, formaldehyde was isolated as a by-product of the ozonolysis. These facts point unequivocally to XII as the correct structure of the starting material. In turn, XII could have come only from a lactone of the structure IX *via* X. Hydrolysis of 3β,17-dibenzoxy-13,17-seco-18-norandrostan-13-one (XIII), obtained from the ozonolysis of XII, with alcoholic sodium hydroxide gave a product which showed no carbonyl absorption by infrared analysis and must, therefore, have been the cyclic hemiketal XIV. Osmic acid oxidation of XII followed by hydrolysis of the product gave 3β,-13,17,18-tetrahydroxy-13,17-secoandrostane (XV).

The structure of the original lactone IX has been further confirmed by oxidizing the triol X to 3-keto-

(1) Just previous to the time this manuscript was submitted for publication another paper was published [N. Wendler, D. Taub and H. L. Slates, THIS JOURNAL, **77**, 3559 (1955)] on the structure proof of the steroidal D-ring lactones. These workers have reached the same conclusions that we have but by an entirely independent route.

(2) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).

(3) R. P. Jacobsen, *ibid.*, **171**, 61 (1947).

(4) D. H. Peterson, S. H. Eppstein, P. D. Meister, H. C. Murray, H. M. Leigh, A. Weintraub and L. M. Reineke, THIS JOURNAL, **75**, 5768 (1953).

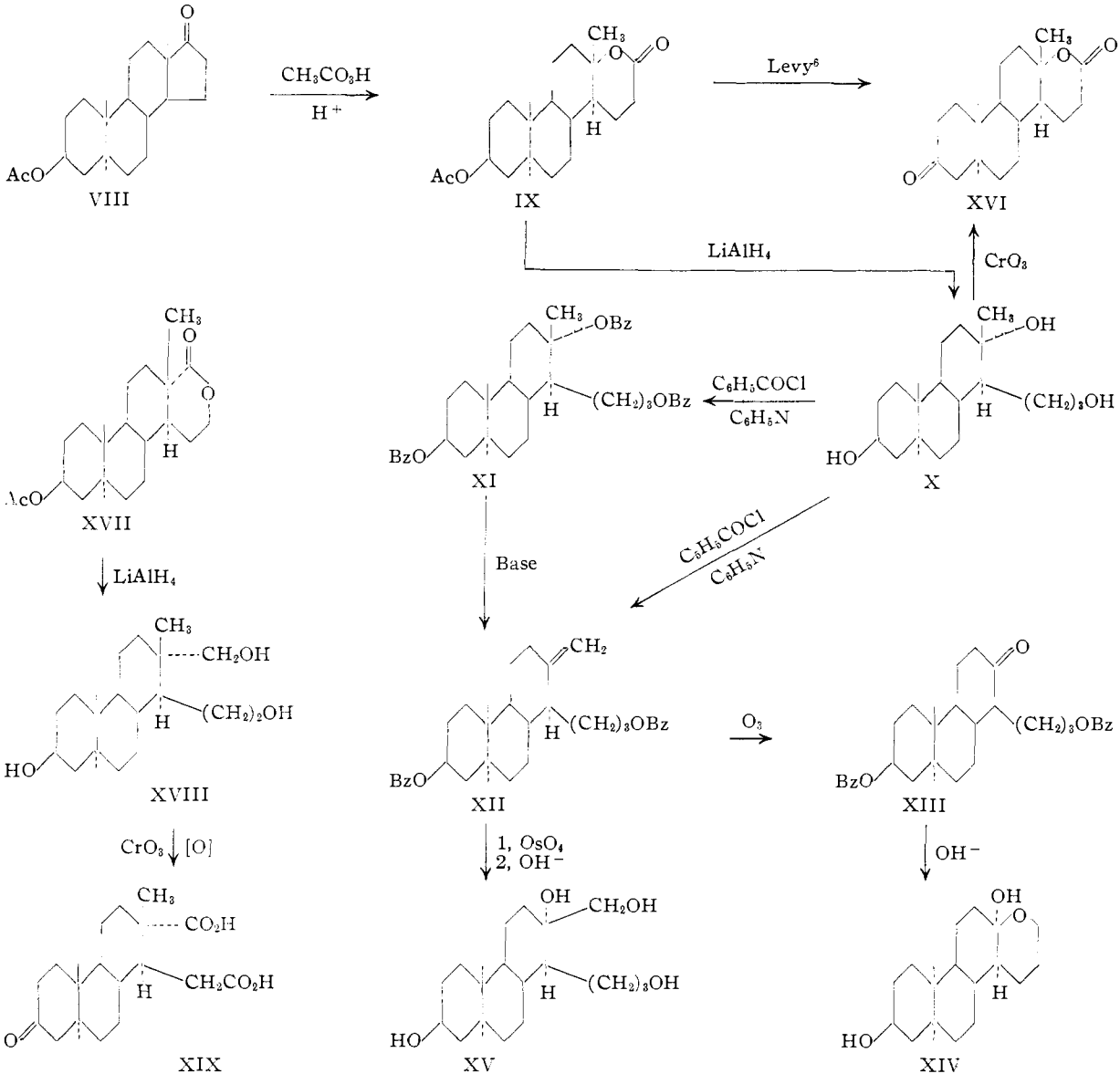
(5) J. Fried, R. V. Thoma and A. Klingsberg, *ibid.*, **75**, 5764 (1953).

(6) H. Levy and R. P. Jacobsen, *J. Biol. Chem.*, **171**, 171 (1947).

(7) C. Von Seemann and G. A. Grant, THIS JOURNAL, **72**, 4073 (1950).

(8) J. Jacques, A. Horeau and R. Courrier, *Compt. rend.*, **229**, 321 (1949).

(9) R. N. Jones, P. Humphries and K. Dobriner, THIS JOURNAL, **72**, 956 (1950), found some support for this view in infrared studies which indicated that the carbonyl group of the γ-lactone absorbed in the frequency range common to the 17-ketone, the ester and the carbomethoxy groups.



13α-hydroxy-13,17-secoandrostan-17-oic acid, lactone (XVI),[10] which had previously been prepared by Levy and Jacobsen[6] by the Oppenauer oxidation of "Isoandrololactone." Had the original lactone been 3β-acetoxy-16-hydroxy-16,17-secoandrostan-17-oic acid, lactone XVII, one would have obtained 3-keto-16,17-secoandrostane-16,17-dioic acid (XIX).

The seemingly contradictory course of the peracid oxidation of 17-keto steroids and camphor requires additional comment. Since the oxidation of ketones with peracids has been shown to occur with retention of configuration[11,12] the lactonic D-ring should possess the *trans* configuration whether rupture occurs at C_{13} or C_{16}. Moreover, in analogy with a large number of reactions in the steroid and terpene series, it may be assumed that the attack of the peracid on the carbonyl function occurs from the unhindered back side. The subsequent course of reaction is affected both by electronic and steric factors. In most cases[13] the group which migrates is that which is better able to accommodate a positive charge, *i.e.*, the more substituted alkyl group. However, particularly in alicyclic fused systems, steric effects may become important. It is interesting to note from an examination of models that migration of the highly substituted C_{13} to the electron deficient 17α-substituent proceeds with the least movement of atoms in space, through a transition state wherein the expanded D-ring possesses a chair form. Migration of the less substituted C_{16}, on the other hand, would pass through a higher energy transition state wherein the D-ring would possess the boat form.

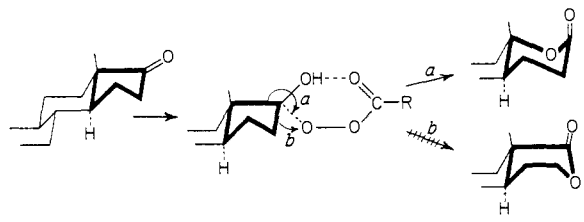
In camphor the situation is reversed since migra-

(10) This work was carried out by Dr. P. D. Meister of our laboratories in connection with an independent project. We are grateful to him for the use of this information.

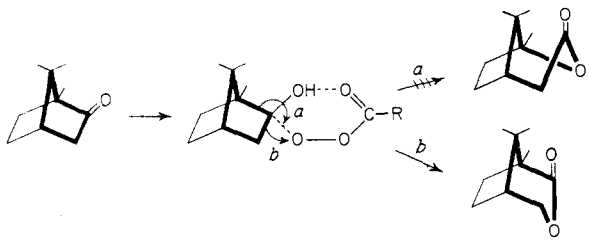
(11) T. F. Gallagher and T. H. Kritchevsky, THIS JOURNAL, **72**, 882 (1950).

(12) R. B. Turner, *ibid.*, **72**, 878 (1950).

(13) W. von E. Doering and L. Speers, *ibid.*, **72**, 5515 (1950).



tion of the tertiary carbon, C_1, involves a transition state of the boat form while migration of C_3 involves a lower energy transition state of the chair form.



Experimental[14,15]

3β,13α,17-Trihydroxy-13,17-secoandrostane (X).—A solution of 5.23 g. (0.015 mole) of 3β-acetoxy-13α-hydroxy-13,17-secoandrostan-17-oic acid, lactone (IX) (prepared by the method of Levy and Jacobsen[6] from dehydroepiandrosterone acetate) in 50 ml. of tetrahydrofuran was added to a solution of 4.25 g. (0.1125 mole) of lithium aluminum hydride in 500 ml. of tetrahydrofuran at room temperature, with good stirring, over a period of 25 minutes. The mixture was stirred an additional 10 minutes and the excess lithium aluminum hydride decomposed by the dropwise addition of 10 ml. of water. The complex was then decomposed by the addition of 120 ml. of 12% hydrochloric acid. The layers were separated and the aqueous layer extracted several times with 50-ml. portions of tetrahydrofuran. The organic extracts were combined, washed twice with 50-ml. portions of half-saturated sodium chloride solution and dried over sodium sulfate. After filtration the solvent was removed by distillation under slightly reduced pressure. Recrystallization of the residue from 250 ml. of absolute ethanol yielded 2.46 g. (53%) of material melting at 218–223°. An additional 1.67 g. (36%) of material melting at 218–222° was recovered from the mother liquors; yield 89%.

An analytical sample, prepared by further recrystallization from ethanol, had the following physical constants: m.p. 225–226°; $[\alpha]^{23}D$ +5° (95% ethanol).

Anal. Calcd. for $C_{19}H_{34}O_3$: C, 73.50; H, 11.04. Found: C, 73.51; H, 11.05.

3β,17-Dibenzoxy-13,17-seco-13(18)androstene (XII) and 3β,13α,17-Tribenzoxy-13,17-secoandrostane (XI) from 3β,13α,17-Trihydroxy-13,17-secoandrostane (X).—A solution of 9.33 g. (0.030 mole) of the 3β,13α,17-trihydroxy-13,17-secoandrostane (X) in 100 ml. of dry pyridine was cooled to 10° and 19.0 g. (0.135 mole) of benzoyl chloride was added dropwise, with stirring, in 20 minutes. After stirring an additional 15 minutes the mixture was allowed to warm to room temperature and was stirred an additional 8 hours. The mixture was then warmed to 45° and stirred 1 hour at 45–50°. After cooling, 300 ml. of a mixture of two parts ether to one part of methylene chloride was added and the organic solution washed successively with ice-cold 10% hydrochloric acid, water, ice-cold 5% sodium hydroxide and water. The combined organic extracts were dried over sodium sulfate, filtered, and the solvent removed by distillation. The residue was dissolved in 50 ml. of methylene chloride and diluted with 600 ml. of methanol. The solution was concentrated to 500 ml. and cooled in an ice-bath, and the precipitate so obtained was filtered, washed with cold methanol, and dried. The product, 3β,17-dibenzoxy-13,17-seco-13(18)androstene (XII), weighed 7.47 g. (49.7%) and melted at 128–132°. An analytical sample was prepared by recrystallization from methanol: m.p. 134–135°; $[\alpha]^{26}D$ −18° (chloroform).

Anal. Calcd. for $C_{33}H_{40}O_4$: C, 79.17; H, 8.05. Found: C, 78.92; H, 7.98.

From the mother liquors 0.48 g. of 3β,13α,17-tribenzoxy-13,17-secoandrostane (XI) melting at 171–173° was obtained. An analytical sample was obtained by recrystallization from acetone; m.p. 175–176°; $[\alpha]^{26}D$ +26° (chloroform).

Anal. Calcd. for $C_{40}H_{46}O_5$: C, 77.14; H, 7.45. Found: C, 77.33; H, 7.49.

In another experiment using similar conditions the tribenzoate (XI) proved to be the major product (50.8%).

3β,17-Dibenzoxy-13,17-seco-13(18)androstene (XII) from 3β,13α,17-Tribenzoxy-13,17-secoandrostane (XI).—A solution of 4.22 g. (0.0068 mole) of 3β,13α,17-tribenzoxy-13,17-secoandrostane (XI) in 25 ml. of dimethylaniline was refluxed 3 hours, cooled and diluted with 150 ml. of ether. The ether solution was washed three times with 50-ml. portions of 10% hydrochloric acid and twice with 25-ml. portions of 5% sodium carbonate. After drying over sodium sulfate the ether was removed by distillation and the residue was recrystallized from isopropyl ether to yield 1.50 g. (44%) of XII melting at 133–135° and 0.33 g. (4.8%) in a second crop melting at 128–132°.

3β,17-Dibenzoxy-13,17-seco-18-norandrostan-13-one (XIII).—A solution of 2.50 g. (0.005 mole) of 3β,17-dibenzoxy-13,17-seco-13(18)androstene (XII) in 100 ml. of methylene dichloride and 1.0 ml. of pyridine was cooled to −70° (Dry Ice–acetone bath) and ozonized with 6.5 mmoles of ozone (0.25 mmole of ozone/200 ml. oxygen/minute for 25.5 minutes). The ozonized solution was then poured onto 2.5 g. of zinc dust, warmed to 0°, 10 ml. of acetic acid added, and the mixture warmed to room temperature and stirred vigorously for 1.25 hours. The mixture was filtered and the filtrate was diluted with 1.5 volumes of Skellysolve B and washed twice with 100-ml. portions of water, three times with 50-ml. portions of cold 5% sodium hydroxide, and with water until neutral. The organic extracts were dried overnight over sodium sulfate, filtered, and the solvent removed by distillation. The glassy residue, after drying to constant weight at 50° under reduced pressure, weighed 2.56 g. (theory 2.66 g.). Two recrystallizations from isopropyl alcohol gave 1.67 g. (63%) of product which melted at 116–117°.

Anal. Calcd. for $C_{32}H_{38}O_5$: C, 76.44; H, 7.62. Found: C, 76.30; H, 7.47.

The product formed no "Dimedon" derivative and gave a negative iodoform test. A semicarbazone was formed under the usual conditions, m.p. 193–196°; $[\alpha]^{23}D$ −34° (chloroform). It showed the following infrared bands: 1717, 1600, 1584, 1491, 1279 and 713 cm.$^{-1}$.

Anal. Calcd. for $C_{33}H_{41}N_3O_5$: C, 70.81; H, 7.38; N, 7.51. Found: C, 70.63; H, 7.42; N, 7.51.

Isolation of Formaldehyde from the Ozonization of 3β,17-Dibenzoxy-13,17-seco-13(18)androstene (XII).—A solution of 0.745 g. (1.45 mmoles) of 3β,17-dibenzoxy-13,17-seco-13(18)androstene (XII) in 110 ml. of ethyl acetate was cooled in an ice-water bath and ozonized with 1.49 mmoles of ozone (0.079 mmole/80 ml. oxygen/minute for 19 minutes). The ozonization mixture was treated with 0.10 g. of 3% palladium–carbon catalyst and shaken for 2 hours in a hydrogen atmosphere. The solution at this stage still gave positive peroxide test with starch potassium iodide paper. The catalyst was removed by filtration, 5 ml. of water added, and the solution was heated under reflux for 2 hours using a Dry Ice–acetone condenser. After cooling to room temperature the layers were separated, and the ethyl acetate layer washed twice with 5-ml. portions of water. The combined aqueous extracts were treated with 0.28 g. of Dimedon in 15 ml. of absolute alcohol containing 1 drop of piperidine. After 10 minutes at room temperature the mixture was refluxed 10 minutes, cooled and allowed to stand overnight at 5°. The precipitated product, after washing with water and drying, weighed 0.138 g.; m.p. 196–197°.

(14) All melting points were taken on a Fisher–Johns block which had previously been calibrated with melting point standards.

(15) The authors are greatly indebted to Drs. J. C. Babcock, D. J. Cram and M. S. Newman for their helpful advice and criticism in connection with the writing of this paper. We are also grateful to Dr. J. L. Johnson and his associates for determining certain physicochemical data, and to W. A. Struck and associates for the microanalyses reported.

Recrystallization from aqueous isopropyl alcohol gave 0.108 g. of product melting at 196–197°. A mixed melting point with an authentic sample m.p. 196–197° of the Dimedon derivative of formaldehyde showed no depression. The infrared spectra of the unknown and the Dimedon derivative of formaldehyde were identical.

Hydrolysis of 3β,17-Dibenzoxy-13,17-seco-18-norandrostan-13-one (XIV).—A solution of 1.48 g. (0.00293 mole) of the glassy product obtained from the ozonization of XVII was dissolved in a solution containing 50 ml. of 3A ethanol, 5 ml. of water and 2.0 g. of potassium hydroxide, warmed to 45° and allowed to stand overnight. The solvent was then removed by distillation under reduced pressure and the residue slurried with 30 ml. of water, filtered and dried overnight at 50° under reduced pressure, weight 0.79 g. (theory 0.84 g.), m.p. 150–165°. Recrystallization from acetone gave 0.57 g. (68%) of material melting at 174–176°.

An analytical sample was prepared by recrystallization from acetone; m.p. 174–176°, $[\alpha]^{23}D$ +2° (95% ethanol).

Anal. Calcd. for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27. Found: C, 73.30; H, 10.33.

Since the infrared spectrum of this material showed strong hydroxyl but no carbonyl absorption, the probable structure is that of the hemiketal XVIII.

3β,13α,17,18-Tetrahydroxy-13,17-secoandrostane (XV).—To a slurry of 1.60 g. (0.0032 mole) of 3β,17-dibenzoxy-13,-17-seco-13,18-androstene (XVI) in 70 ml. of anhydrous diethyl ether was added, with stirring, 1.0 g. of osmic acid and 1.0 ml. of dry pyridine. The mixture was stirred an additional 3 hours and allowed to stand overnight. The ether was removed by distillation, 46 ml. of ethanol and a solution of 7.2 g. of sodium sulfite in 31 ml. of water was added, and the mixture was refluxed 3.5 hours with stirring. The reaction mixture was cooled and filtered and the solids were extracted several times with small portions of ethanol. The extracts were combined and the solvent was removed by distillation under reduced pressure. The residue was washed well with water and then redissolved in 30 ml. of ethanol. After adding 1.0 g. of potassium hydroxide dissolved in 6.0 ml. of water, the solution was heated on the steam-bath for 45 minutes. After diluting with 75 ml. of water and cooling in an ice-bath, the product was filtered, washed with water and dried at 60° *in vacuo;* weight 0.90 g. (86%), m.p. 224–227°.

An analytical sample was prepared by recrystallization from isopropyl alcohol; m.p. 229–230°, $[\alpha]^{23}D$ +23° (95% ethanol).

Anal. Calcd. for $C_{19}H_{34}O_4$: C, 69.90; H, 10.50. Found: C, 69.96; H, 10.41.

3-Keto-13α-hydroxy-13,17-secoandrostane-17-oic Acid, Lactone[10] (XVI).—A solution of 0.310 g. (0.001 mole) of X in 20 ml. of glacial acetic acid was treated with a solution of 0.351 g. (5.25 milliequivalents) of chromium trioxide in 2 ml. of water and 10 ml. of glacial acetic acid and allowed to stand 15 hours at room temperature. It was then diluted with 20 ml. of methanol and concentrated to room temperature in a stream of air. After dilution with 50 ml. of water the steroid was extracted with three 50-ml. portions of chloroform. The combined chloroform extracts were washed five times with 20-ml. portions of 5% sodium hydroxide and water, dried over sodium sulfate, and concentrated to give 0.204 g. of neutral residue. Recrystallization from acetone–Skellysolve B gave 0.070 g. product melting at 165–168°. Three additional recrystallizations gave 0.047 g. of material melting at 169–171° (Levy and Jacobsen[5] reported a m.p. of 166–167.7° for this compound).

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 75.00; H, 9.27.

The infrared spectrum also supported the structure for 3-keto-13α-hydroxy-13,17-secoandrostan-17-oic acid, lactone (XVI).

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE MEDICINAL CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Steroidal Cyclic Ketals. XVIII.[1] The Preparation of 9α-Hydroxyhydrocortisone

BY RUDDY LITTELL AND SEYMOUR BERNSTEIN

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9α-Hydroxyhydrocortisone (VIIIa) has been prepared by two different pathways for evaluation of the influence of a 9α-hydroxyl group on the biological activities of hydrocortisone.

The recent Communications by Fried and Sabo[2] on the preparation and biological activities of 9α-fluorohydrocortisone and related compounds have stimulated efforts in the direction of obtaining other variants of hydrocortisone which may also have an increased activity.[3] This work immediately suggested to us that the unknown 9α-hydroxy derivative of hydrocortisone would be a highly desirable compound for evaluation in this direction. We wish to report here that this compound has now been synthesized by two different pathways.

The synthesis to which most of our efforts were directed was based essentially on the successful hydroxylation with osmium tetroxide of a $\Delta^{9(11)}$-5α-hydroxy-steroid. The structure of the previously unknown 9α-hydroxyhydrocortisone (VIIIa) was established unequivocally by a synthesis which involved a hydrolytic fission of the oxide ring in Δ^4-pregnene-17α,21-diol-3,20-dione-9β,11β-oxide (IX), one of the key intermediates described by the Squibb workers[2] in their preparation of 9α-halogenated hydrocortisones. We wish to state that this second synthesis *via* IX was executed solely for corroborative purposes, and was not studied in detail.

The starting material for our projected synthesis was pregnane-5α,11β,17α,21-tetrol-3,20-dione 21-acetate 3,20-bis-ethylene ketal (IIIb) recently prepared in this Laboratory.[4] However, a modified synthesis from the bis-ethylene ketal I of hydrocortisone was employed to circumvent a difficult chromatographic separation of oxides. This improved process involved the epoxidation of I in chloroform solution with peroxybenzoic acid in ethyl acetate solution to give a product which crystallized directly from the reaction mixture and consisted principally of the 5α,6α-oxide II (52% yield). Pure 5α,6α-oxide II was readily obtained

(1) Paper XVII, W. S. Allen, C. E. Linden and J. Clemente, THIS JOURNAL, **77**, 6612 (1955).

(2) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **76**, 1455 (1954); see also, J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer and P. Numerof, *ibid.*, **77**, 1068 (1955).

(3) In this connection, see H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg and P. L. Perlman, *Science*, **121**, 176 (1955), for a report on two compounds which are more "active" than cortisone or hydrocortisone, namely, metacortandracin ($\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione) and metacortandralone ($\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione).

(4) S. Bernstein and R. H. Lenhard, THIS JOURNAL, **77**, 2233 (1955).