[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE]

Observations on the Cleavage of Ring D in the Estratriene Series

BY JOSEPH C. TOUCHSTONE,[1] WILLIAM H. ELLIOTT, S. A. THAYER AND EDWARD A. DOISY

RECEIVED JULY 30, 1954

The ease with which ring D of certain estratrienes can be opened has been demonstrated by alkaline cleavage of 16-ketoestrone-3-methyl ether and 16-ketoestradiol-3-methyl ether to marrianolic acid-7-methyl ether. Cleavage of 16-ketoestrone-3-methyl ether has been effected with 2% potassium hydroxide in aqueous ethanol at room temperature, 0.3% sodium ethoxide in absolute ethanol for 18 hours, 13.3% potassium hydroxide in aqueous ethanol under reflux for 45 minutes, or by the action of 0.6% H_2O_2 or lead tetraacetate. Ozonization of 16-benzylidene-estrone-3-methyl ether also produced marrianolic acid-7-methyl ether.

During the course of an investigation designed to incorporate isotopic carbon into ring D of the female sex hormone, estrone, we have investigated several methods of opening the cyclopentane ring of 16-substituted estratrienes. Some years ago, Marrian and Haselwood[2] fused estriol with alkali and isolated a dicarboxylic acid, later called marrianolic acid.[3] More recently, Huffman, *et al.*,[4] have opened ring D of 16-ketoestradiol with lead tetraacetate. MacCorquodale, *et al.*,[5] have shown that alkaline fusion of estrone results in the formation of a monocarboxylic acid, later called doisynolic acid.[3] Westerfeld[6] and Jacobsen[7] have opened ring D of estrone using alkaline or acidic hydrogen peroxide at room temperature; Heer and Miescher[8] have formed marrianolic acid-7-methyl ether from estrone-3-methyl ether by oxidation with hypoiodite.

In the experiments reported here the hydroxyl group at position three of 16-ketoestrone, 16-ketoestradiol and 16-benzylidene estrone has been methylated to prevent reaction at that center. The ease with which the cyclopentane ring may be opened in these materials is illustrated by the ready conversion of 16-ketoestrone-3-methyl ether to marrianolic acid-7-methyl ether in 2% potassium hydroxide in aqueous ethanol at room temperature. When 16-ketoestrone-3-methyl ether was stirred at room temperature with 0.3% sodium ethoxide in absolute ethanol for 18 hours, an ethyl hemi-ester of marrianolic acid-7-methyl ether was formed. That this ester was the 2-ethyl ester was shown by the recovery of this material after alkaline hydrolysis under conditions known to hydrolyze only the 1-methyl ester of marrianolic acid-7-methyl ether dimethyl ester.

It has been demonstrated that atmospheric oxygen and an alkalinity greater than *p*H 8.0 are necessary for this type of cleavage of the 16,17-dioxyestratrienes. In an experiment in which 16-ketoestrone-3-methyl ether was allowed to stand at room temperature in the presence of 2% potassium hydroxide in aqueous ethanol and an atmosphere of nitrogen (oxygen-free), 94% of the estratriene was recovered unchanged. On the other hand, when air was bubbled through an alcoholic solution of this material buffered at *p*H 8.0, the 16-ketoestrone-3-methyl ether was recovered quantitatively.[9] Since marrianolic acid-7-methyl ether or its esters were the only products isolated from such a cleavage, it may be concluded that the mechanism of cleavage is different from those proposed by Doering and Haines[10] in their studies on alkoxide-catalyzed autoxidative cleavage of ketones and esters in the presence of two atmospheres of oxygen. Similar cleavage of 16-ketoestrone-3-methyl ether to marrianolic acid-7-methyl ether was observed using 13.3% potassium hydroxide in aqueous ethanol under reflux for 45 minutes, or the action of 0.6% hydrogen peroxide or lead tetraacetate. Ozonization of 16-benzylidene estrone-3-methyl ether also resulted in the formation of the 7-methyl ether of marrianolic acid.

Experimental[11]

Estrone-3-methyl Ether.—This material was prepared in quantitative yield by the procedure of Huffman.[12] Recrystallization from a mixture of benzene–petroleum ether (b.p. 60–80°) was found to be superior to the use of aqueous solvents. The product thus obtained melted consistently at 175°. Melting points of samples reported previously are 169°,[13] 167.5–169°[14] and 165° (uncor.).[15]

16,17-Dioxyestratrienes.—16-Oximinoestrone-3-methyl ether was prepared in 90% yield by the procedure of Huffman[12] to give a product melting at 181.5–183°. Huffman[12] reported a melting point of 180–183°. 16-Ketoestrone-3-methyl ether and 16-ketoestradiol-3-methyl ether were prepared from this material by the procedure of Huffman[16] and identified by melting point and mixed melting points with authentic samples.

Marrianolic Acid-7-methyl Ether.—The procedure of Heer and Miescher[17] was modified to give a nearly quantitative yield of acid melting at 200–201°.

(1) Based on a portion of the thesis submitted by Joseph C. Touchstone in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry, St. Louis University, 1953.

(2) G. F. Marrian and G. A. D. Haselwood, *J. Soc. Chem. Ind.*, **51**, 279T (1932).

(3) K. Miescher, *Helv. Chim. Acta*, **27**, 1727 (1944).

(4) M. N. Huffman, M. H. Lott and James Ashmore, THIS JOURNAL, **196**, 367 (1952).

(5) D. W. MacCorquodale, S. A. Thayer and E. A. Doisy, *J. Biol. Chem.*, **99**, 327, **101**, 753 (1933).

(6) W. W. Westerfeld, *Biochem. J.*, **34**, 51 (1940); *J. Biol. Chem.*, **143**, 177 (1942).

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

(8) J. Heer and K. Miescher, *Helv. Chim. Acta*, **28**, 156 (1945).

(9) L. Velluz, A. Petit, M. Pesez and R. Berret, *Bull. soc. chim. France*, 123 (1947); *C.A.*, **41**, 5537 (1947), have shown that alcoholic KOH reacts with the α-ketols, 11-desoxycorticosterone acetate and 21-acetoxy-Δ^5-pregnen-3-ol-20-one, to form the corresponding etiocholenic acids.

(10) W. von E. Doering and R. M. Haines, THIS JOURNAL, **76**, 482 (1954).

(11) All melting points were taken on the Fisher–Johns apparatus and are recorded as read.

(12) M. N. Huffman, *J. Biol. Chem.*, **167**, 273 (1947).

(13) A. Butenandt, I. Stormer and U. Westphal, *Z. physiol. Chem.*, **208**, 167 (1932).

(14) A. Cohen, J. W. Cook and C. L. Hewett, *J. Chem. Soc.*, 445 (1935).

(15) S. A. Thayer, L. Levin and E. A. Doisy, *J. Biol. Chem.*, **91**, 796 (1931).

(16) M. N. Huffman and M. H. Lott, *ibid.*, **172**, 325 (1948).

(17) J. Heer and K. Miescher, *Helv. Chim. Acta*, **28**, 156 (1945).

Cleavage of 16-Ketoestrone-3-methyl Ether. (a) **Concentrated Alkali at Elevated Temperature.**—One hundred milligrams of 16-ketoestrone-3-methyl ether was dissolved in 35 ml. of ethanol and while refluxing this solution, 6 g. of KOH in 10 ml. of water was added slowly. Reflux was continued for 45 min., 10 ml. of alcohol was evaporated, 10 ml. of water was added and finally 20 ml. of alcohol was evaporated. The concentrate was cooled to room temperature and the neutral materials were removed by extraction with ether. Acidification of the alkaline phase with concentrated HCl caused precipitation of the acid which was extracted with ether. The ether was washed well with water and evaporated to give 48 mg. of oily residue. Recrystallization from acetone–petroleum ether (b.p. 60–80°) gave crystalline material melting at 195–198°. A mixed melting point with this material and authentic marrianolic acid-7-methyl ether was not depressed.

(b) **Dilute Alkali at Room Temperature.**—Three hundred mg. of 16-ketoestrone-3-methyl ether was added to a solution of 800 mg. of KOH in 10 ml. of water and 30 ml. of ethanol. This reaction mixture was stirred at room temperature for 22 hours; then the solution was acidified with concentrated hydrochloric acid and extracted in the manner described above. The reaction product amounting to 236 mg. was recrystallized from acetone–petroleum ether, benzene–petroleum ether and finally from aqueous ethanol and acetone. Glistening plates melting at 197–199° were obtained. On admixture with marrianolic acid-7-methyl ether, there was no depression of the melting point.

The acid was methylated with excess ethereal diazomethane to give an ester which melted at 70–73° after several recrystallizations from aqueous methanol. When mixed with authentic marrianolic acid-7-methyl ether dimethyl ester (m.p. 75–76°), this melting point was not depressed.

(c) **With Sodium Ethoxide.**—Eighty milligrams of sodium was dissolved in 80 ml. of absolute ethanol (freshly distilled from sodium). As soon as the sodium had gone into solution, 600 mg. of 16-ketoestrone-3-methyl ether was added, the flask was fitted with a drying tube, and the solution was stirred for 18 hours. The reaction mixture was extracted as described above to yield 549 mg. (76%) of acidic product. Crystallization from acetone–petroleum ether and finally from aqueous acetone gave glistening plates melting at 173–174°.

Anal. Calcd. for ($C_{21}H_{28}O_5$): C, 69.98; H, 7.82; neut. equiv., 358. Found: C, 69.83; H, 7.86; neut. equiv., 340.

Saponification experiments described below indicate that the carbethoxy group was attached to carbon 2 of marrianolic acid-7-methyl ether.

The acid was methylated with excess ethereal diazomethane to give the mixed methyl ethyl diester melting at 83–84° after several alternate recrystallizations from aqueous acetone and aqueous methanol.

Anal. Calcd. for ($C_{22}H_{30}O_5$): C, 70.56; H, 8.08. Found: C, 70.98; H, 8.13.

Alkaline Hydrolysis of the Product Obtained by Sodium Ethoxide Cleavage of 16-Ketoestrone-3-methyl Ether.—Fifty milligrams of the acid obtained by sodium ethoxide cleavage of 16-ketoestrone-3-methyl ether was dissolved in 5 ml. of methanol. After addition of 6 ml. of 0.8 *M* potassium carbonate solution, the reaction mixture was refluxed for 3 hours. On acidification with concentrated hydrochloric acid after cooling the reaction mixture, the unchanged acid was recovered quantitatively. This procedure has been shown to hydrolyze only the unhindered 1-methyl ester of marrianolic acid-7-methyl ether dimethyl ester.[18]

Fifty milligrams of the acid obtained by sodium ethoxide cleavage was dissolved in 10 ml. of ethanol and 10 ml. of alcoholic NaOH was added (10 g. of NaOH, 50 ml. of H_2O and 25 ml. of EtOH). The solution was refluxed for a total of 1.5 hours; aliquots of 5 ml. of the alkali were added every 30 minutes. After reflux was discontinued, 10 ml. of alkali was added, and the alcohol was removed. The precipitated sodium salt was dissolved in water, and the solution acidified with concentrated HCl, allowed to cool in the ice-box and filtered. After recrystallization of the residue from aqueous methanol, glistening plates were obtained, m.p. 200–201°. A mixed melting point with authentic marrianolic acid-7-methyl ether was not depressed. Thus, according to these results, the carbethoxy grouping is attached to the 2-carbon in the marrianolic acid nucleus.

Oxidation of 16-Ketoestrone-3-methyl Ether with Hydrogen Peroxide.—Twenty-five milligrams of 16-ketoestrone-3-methyl ether was dissolved in 25 ml. of ethanol and adjusted to a *p*H of 8.5 with 25 ml. of phosphate buffer. To this solution, 1.0 ml. of hydrogen peroxide (30%) was added to give a final peroxide concentration of 0.6%. The yellow diketone solution became colorless immediately. After 12 hours at room temperature, the alcohol was removed without heating by means of a current of air; the aqueous solution was acidified to *p*H 1 with hydrochloric acid, diluted with 25 ml. of water and extracted twice with 50 ml. of ether. The ether extracts were combined and the acidic products removed by extraction with 3% bicarbonate solution; two extractions, 35 ml. each, served to remove quantitatively the acidic products. A yield of 24 mg. of marrianolic acid-7-methyl ether melting at 200–202° was obtained. On admixture with an authentic sample of marrianolic acid-7-methyl ether, this melting point was not depressed.

The above experiment was repeated without the use of buffer. The alcoholic 16-ketoestrone-3-methyl ether solution had a final peroxide concentration of 1.2%. After 30 minutes at room temperature, acidic products were isolated in the manner described above. A yield of 23 mg. of marrianolic acid-7-methyl ether identified by mixed melting point determination was obtained. Separate experiments showed that neither the bicarbonate extraction procedure used here nor a *p*H of 8.5 alone for 48 hr. had any effect on 16-ketoestrone-3-methyl ether itself.

Oxidation of 16-Ketoestrone-3-methyl Ether with Lead Tetraacetate.—Three hundred milligrams of 16-ketoestrone-3-methyl ether was dissolved in 25 ml. of 0.05 *M* lead tetraacetate in acetic acid and the last traces washed down with a small amount of 50% acetic acid. This solution was stirred for 30 minutes. The yellow diketone color disappeared. The reaction mixture was then diluted with water and placed in the ice-box. The acid was filtered and dissolved in 10 ml. of methanol and 10 ml. of 2 *N* KOH was added. This solution was refluxed 30 minutes, cooled, acidified with dilute HCl and allowed to crystallize in the ice-box. Two recrystallizations from aqueous acetone gave glistening plates of m.p. 200–202°. The yield of pure material was 211 mg. (63%). A mixed melting point with authentic marrianolic acid-7-methyl ether was not depressed.

The methyl ester made by use of ethereal diazomethane melted at 72–73°. On admixture with the authentic dimethyl ester of marrianolic acid-7-methyl ether, the melting point was unchanged.

Cleavage of 16-Ketoestradiol-3-methyl Ether with Sodium Ethoxide.—A solution of 35 mg. of sodium in 35 ml. of freshly distilled (from sodium) ethanol was prepared under dry nitrogen and 260 mg. of 16-ketoestradiol-3-methyl ether was added. This reaction mixture was stirred for 18 hr. at room temperature. Evaporation of the solvents to half-volume and dilution with 100 ml. of water followed by acidification with concentrated HCl gave a precipitate. The precipitate was extracted with ether and washed well with water. The ether layer was extracted two times with 50 ml. of 5% K_2CO_3. Acidification of the carbonate layer with HCl gave 261 mg. of acid which melted at 167–169° after several recrystallizations from aqueous ethanol and aqueous acetone. A mixed melting point with the ethyl hemi-ester obtained in experiments with 16-ketoestrone-3-methyl ether was not depressed.

Anal. Calcd. for ($C_{21}H_{28}O_5$): C, 69.98; H, 7.82. Found: C, 70.28; H, 7.69.

Preparation of 16-Benzylidene-estrone-3-methyl Ether.—A solution of 300 mg. of sodium in 200 ml. of freshly distilled absolute ethanol was prepared in an atmosphere of dry nitrogen; 2.26 g. of estrone-3-methyl ether was added and after most of it had gone into solution, 2 ml. of freshly distilled benzaldehyde was added. Stirring was continued overnight; the reaction mixture was cooled several hours in an ice-box and filtered. Several recrystallizations from hot ethanol gave long needles melting at 176–177° (ϵ_{2950} 21,000). The compound was obtained in a quantitative yield.

Anal. Calcd. for ($C_{26}H_{28}O_2$): C, 83.83; H, 7.58. Found: C, 83.63; H, 7.83.

The maximum at 295 mμ for 16-benzylidene-estrone-3-methyl ether is comparable to that reported by Long, *et*

(18) See also the expts. of E. B. Hershberg, E. Schwenk and E. Stahl, *Arch. Biochem.*, **19**, 305 (1948).

al.,[19] for the 21-benzylidene derivative of 3α,11α-dihydroxy-20-ketopregnane (ϵ_{2940} 24,000). 21-Benzal-3α-hydroxy-11,-20-diketopregnane[20] also exhibited a maximum at 295 mμ. Attempts to prepare the 2,4-dinitrophenylhydrazone were unsuccessful, due possibly to the instability of the reaction product which seemed to resinify and defied attempts at purification. Several attempts to prepare the oxime have been unsuccessful.

Ozonolysis of 16-Benzylidene-estrone-3-methyl Ether.—One millimole (366 mg.) of 16-benzylidene-estrone-3-methyl ether was dissolved in 25 ml. of glacial acetic acid, 2 ml. of water and 8 ml. of ethyl acetate. Ozone was bubbled through this solution for one hour. The yellow solution obtained was diluted with 30 ml. of water after 15 minutes at room temperature. The yellow color faded at once. The solution was allowed to stand at room temperature for 12 hours.

Most of the solvent was removed *in vacuo* with a minimum of heating. The concentrate was dissolved in ether which was then washed twice with water. The ether was extracted with 5% potassium carbonate solution and acidification of the carbonate extracts gave an oil which was dissolved in ether. After washing with water, the ether was evaporated to give 253 mg. (76%) of solid material. Several recrystallizations from aqueous acetone and aqueous ethanol gave glistening leaflets melting at 200–201°. A mixed melting point with an authentic sample of marrianolic acid-7-methyl ether (m.p. 200–201°) was not depressed.

ST. LOUIS 4, MISSOURI

(19) W. P. Long, C. W. Marshall and T. F. Gallagher, *J. Biol. Chem.*, **165**, 197 (1946).

(20) Kindly supplied by Dr. S. L. Hsia of this department.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH AND DEVELOPMENT DIVISION OF SCHERING CORPORATION]

11-Oxygenated Steroids. XII. The Preparation of 17α-Hydroxycorticosterone 21-Acetate (Kendall's Compound F Acetate) via 11β-Formates[1]

BY EUGENE P. OLIVETO, CORINNE GEROLD, RICHARD RAUSSER AND E. B. HERSHBERG

RECEIVED FEBRUARY 1, 1955

The synthesis of 17α-hydroxycorticosterone acetate from 3α,11β,17α-trihydroxypregnan-20-one *via* 11β-formates is described.

The 11β-hydroxyl group, long considered to be unreactive toward the usual esterifying agents,[2] was found by us to be acetylated in good yield under very mild conditions, using acetic anhydride and an acid catalyst.[3,4] This discovery led to the relatively simple synthesis of Compound F 11,21-diacetate and 11-acetate, but further hydrolysis to Compound F was not achieved. Attention was next turned to the corresponding 11β-formates, which were found to be not only hydrolysable to the parent 11β-hydroxy compound, but also to survive the reactions necessary to elaborate the dihydroxyacetone side-chain and the α,β-unsaturated ketone in the A-ring.[5]

Our preliminary studies were made on 11β,17α-dihydroxypregnane-3,20-dione (I). While formic acid alone produced no significant change, the addition of an acid catalyst (either *p*-toluenesulfonic acid or perchloric acid) gave a new compound, lacking in free hydroxyl groups, and therefore assumed to be 11β,17α-dihydroxypregnane-3,20-dione diformate (II). This compound was stable to attempted acid hydrolysis, but easily reverted to I in good yield on treatment with sodium hydroxide or potassium carbonate at room temperature overnight.

Treatment of 3α,11β,17α-trihydroxypregnan-20-one (III) with formic acid and an acid catalyst overnight gave the triformate IV in 43.5% yield. In the absence of the acid catalyst, the 3-monoformate V was obtained in 72% yield. Complete hydrolysis of IV to the starting triol III was accomplished with aqueous sodium hydroxide overnight at 30°. Reaction of IV with ethanol and *p*-toluenesulfonic acid gave the 11,17-diformate VI, which was oxidized easily with N-bromoacetamide in high yield to II. Partial hydrolysis of IV to 3α,11β,17α-trihydroxypregnan-20-one 11-formate (VII) was accomplished by either refluxing aqueous sodium bicarbonate, sodium methylate in tetrahydrofuran-methanol or Amberlite IRA-400 resin in methanol. The product VII, invariably a resin, was next brominated at C-21 with bromine in chloroform solution, and the bromine replaced by acetate by means of potassium acetate in refluxing acetone to give the 11-formate 21-acetate VIII in about 50% yield. Oxidation of VIII to 11β,17α,21-trihydroxypregnane-3,20-dione 11-formate 21-acetate (IX) was accomplished in good yield with N-bromoacetamide in *t*-butyl alcohol–methylene chloride.

The over-all yield of IX from IV was considerably improved if no purification of intermediates was attempted. Thus from 5 g. of IV there was obtained 3.37 g. of crude IX (*ca.* 67%) if no attempt was made to crystallize the intermediates V → VIII.

Hydrolysis of IX with sodium hydroxide in aqueous methanol at room temperature overnight, followed by acetylation with acetic anhydride and pyridine gave 11β,17α,21-trihydroxypregnane-3,-20-dione 21-acetate (X). This had been converted

(1) A preliminary account of this work has appeared in *Arch. Biochem. Biophys.*, **49**, 244 (1954).

(2) T. F. Gallagher, *J. Biol. Chem.*, **162**, 539 (1946); L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, pp. 408, 657. However, Dr. C. W. Shoppee has pointed out to us in a private communication that M. Steiger and T. Reichstein [*Helv. Chim. Acta*, **20**, 817 (1937)] converted 3β,11β-dihydroxyandrostan-17-one to its diacetate by warming with acetic anhydride and pyridine. See also A. Crawshaw, H. B. Henbest and E. R. H. Jones, *J. Chem. Soc.*, 731 (1954), and A. Kemp, A. Kappas, I. Salamon, F. Herling and T. F. Gallagher, *J. Biol. Chem.*, **210**, 123 (1954).

(3) (a) E. P. Oliveto, C. Gerold and E. B. Hershberg, *Arch. Biochem. Biophys.*, **43**, 234 (1953); (b) E. P. Oliveto, C. Gerold, L. Weber, H. E. Jorgensen, R. Rausser and E. B. Hershberg, THIS JOURNAL, **75**, 5486 (1953).

(4) Soon after our original communication, A. Crawshaw, H. B. Henbest and E. R. H. Jones [ref. 2] described the acetylation of 11β-hydroxy steroids with acetyl chloride–dimethylaniline in chloroform.

(5) At about the same time, A. Lardon and T. Reichstein [*Helv. Chim. Acta*, **37**, 443 (1954)] prepared an 11β-formate with an acetic anhydride–formic acid mixture, and found that it could not only be saponified, but also was stable during the sequence of reactions elaborating the side-chain from an etio acid *via* the diazo ketone synthesis.