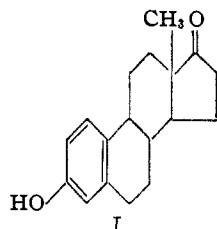


[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE UNIVERSITY OF MICHIGAN]

The Total Synthesis of a Stereoisomer of the Sex Hormone Estrone¹BY W. E. BACHMANN, S. KUSHNER AND A. C. STEVENSON²

The total synthesis of the sex hormone estrone (I) in its natural form has not yet been reported.



Through the application of the Diels-Alder reaction, Dane and Schmitt³ obtained a compound from which they prepared either a structural isomer or a stereoisomer of estrone. To our knowledge, the exact nature of the product has not yet been established. Robinson and Rydon⁴ synthesized *x-nor*-estrone, which lacked the angular methyl group which is present in the hormone. Three years ago the sex hormone equilenin and its three stereoisomers⁵ were synthesized, and since that time a number of homologs and analogs of this hormone have been prepared.⁶ Recently a structural isomer of estrone was synthesized in which the B ring rather than the A ring was phenolic.⁷ From the standpoint of synthesis, this compound was more closely related to equilenin than to estrone.

We have now synthesized a mixture of compounds which from the method of preparation should possess the structure of estrone. In our synthesis, β -*m*-anisylethyl bromide was condensed with sodio malonic ester to give β -*m*-anisylethylmalonic ester. By condensation of the sodio derivative of this compound with the acid chloride of ethyl hydrogen glutarate, the triester (II) was prepared. This ester was cyclized by warm 100% orthophosphoric acid, by a mixture of cold orthophosphoric acid and sulfuric acid, and by cold sulfuric acid to the important intermediate III. Alkaline hydrolysis of III yielded the corre-

sponding tricarboxylic acid (IIIa), which was decarboxylated in hot water to an unsaturated dicarboxylic acid to which the structure IV is assigned. The ethylenic double bond in this compound appears to be situated in the alicyclic ring in conjugation with the aromatic ring and the carbonyl group, although its position has not been established definitely. This structure accounts for the properties of the acid, although it is conceivable that a shift of the double bond takes place during the reactions. When the acid is heated to 190° it loses carbon dioxide; it is reduced readily in the form of its sodium salt by dilute sodium amalgam; and it can be converted to the known 7-methoxy-1-keto-1,2,3,4,9,10-hexahydrophenanthrene⁸ (IVa). The last operation can be carried out by cyclizing the dimethyl ester of the unsaturated acid by means of sodium methoxide and then hydrolyzing and decarboxylating the cyclic keto ester. A novel method of obtaining the same result consisted in refluxing the ester or the free acid with a mixture of acetic and hydrochloric acids. Even the triester (III) and the corresponding tricarboxylic acid yielded the unsaturated cyclic ketone under these conditions.

It was decided to retain the ethylenic bond in the molecule as long as possible in order to keep down the number of asymmetric carbon atoms in the intermediates to follow. It was anticipated that this procedure might yield crystalline intermediates, and this proved to be the case. The sodio derivative of the cyclic keto ester which was produced by the action of sodium methoxide on the dimethyl ester of IV was treated with methyl iodide. In this manner, the crystalline 7-methoxy-2-methyl-2-carbomethoxy-1-keto-hexahydrophenanthrene (V) was produced in good yield in practically one laboratory operation. The Reformatsky reaction with zinc and methyl bromoacetate usually proceeded smoothly and yielded the crystalline hydroxy ester (VI).⁹ Both the methyl keto ester (V) and the Re-

(1) Presented before the Organic Division at the Ninth Organic Symposium, Ann Arbor, Michigan, December 29, 1941.

(2) Part of the material in this paper is from the Ph.D. dissertations of S. Kushner and A. C. Stevenson.

(3) Dane and Schmitt, *Ann.*, **537**, 246 (1939).

(4) Robinson and Rydon, *J. Chem. Soc.*, 1394 (1939).

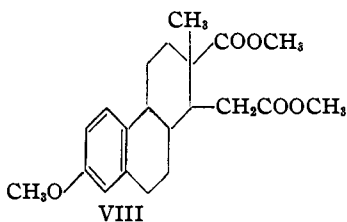
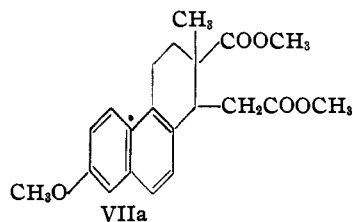
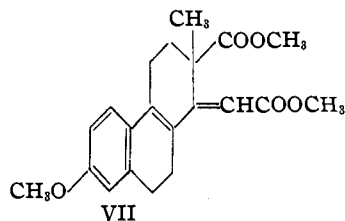
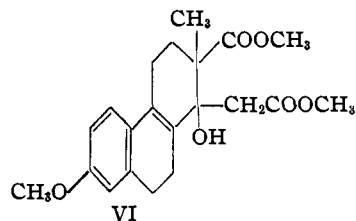
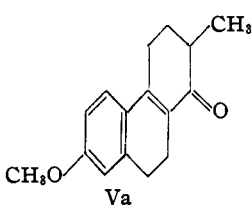
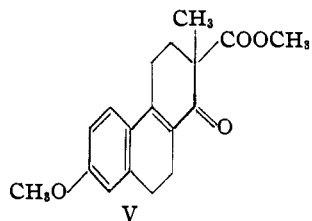
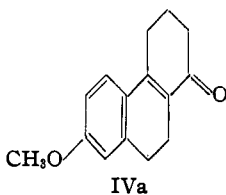
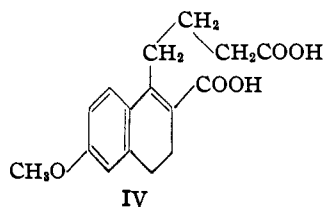
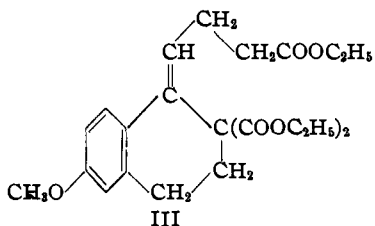
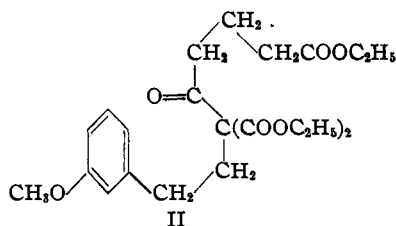
(5) (a) Bachmann, Cole and Wilds, *This Journal*, **61**, 974 (1939); (b) **62**, 824 (1940).

(6) For a recent paper in this series, see Bachmann and Thomas, *ibid.*, **64**, 94 (1942).

(7) Bachmann and Ness, *ibid.*, **64**, 526 (1942).

(8) Robinson and Schlittler, *J. Chem. Soc.*, 1288 (1935); Hewett, *ibid.*, 50 (1936).

(9) Litvan and Robinson, *ibid.*, 183 (1938), had little success in obtaining the desired product from the Reformatsky reaction employing the analogous saturated methyl keto ester.



formatsky ester (VI) can be converted in part to the unsaturated 2-methyl ketone (Va) by the action of a hot methanolic solution of potassium hydroxide.

After considerable experimentation, it was found that the most satisfactory method of dehydrating the Reformatsky ester consisted in treating a benzene solution of the compound with dry hydrogen chloride at room temperature. The doubly unsaturated ester (probably VII), isolated as pale-yellow crystals, was also produced by the action of anhydrous formic acid on the hydroxy ester. In order to determine whether dehydration had occurred without migration of the methyl group, a sample of the doubly unsaturated ester was heated with palladium on charcoal at 300°. By analogy with the behavior

of certain allyl derivatives of polycyclic compounds¹⁰ under similar conditions, it was expected that isomerization of the molecule (equivalent to simultaneous dehydrogenation of the ring and reduction of the side chain) would take place. This expectation was realized, for the product proved to be the dimethyl ester of α -7-methoxy-2-methyl-2-carboxy-1,2,3,4-tetrahydrophenanthrene-1-acetic acid (VIIa), an intermediate in the synthesis of isoequilenin.^{5b} This result proved that "normal" dehydration of the Reformatsky ester had taken place and furnished evidence in support of the structure assigned to the unsaturated ester.

The doubly unsaturated ester absorbed exactly two moles of hydrogen in the presence of palladium on charcoal and gave the saturated ester (VIII) as a colorless liquid. The first mole of hydrogen was absorbed rapidly by the conjugated system, while the second mole was taken up more slowly. The product, presumably a mixture of

stereoisomers, was employed in the succeeding step without separation into its components.

In constructing the cyclopentanone structure (ring D), the same procedures were employed which proved so successful in the synthesis of equilenin.⁵ The dimethyl ester was converted to the acid ester by preferential hydrolysis of the acetic ester group, the acetic acid chain was lengthened by one methylene group through the Arndt-Eistert synthesis, and the resulting product was cyclized by the Dieckmann method to the methyl ether of 16-carbomethoxyestrone (mixture of stereoisomers). By reaction with a boiling mixture of acetic acid and hydrochloric acid, the carbomethoxy group was removed and a mixture

(10) Bachmann and Wilds, *THIS JOURNAL*, **60**, 926 (1939); Bachmann and Chemerda, *ibid.*, **61**, 2858 (1939).

of stereoisomeric methyl ethers of the estrones was obtained as a viscous liquid (in an over-all yield of 75% from VII). Demethylation by hydrobromic acid in acetic acid yielded a mixture of stereoisomeric estrones as a resin-like mass. When this product was dissolved in methanol, colorless leaflets separated almost immediately, which melted at 214–214.5° after two recrystallizations.

All of the evidence accumulated thus far indicates that this crystalline compound possesses the structure of estrone. It appears to be a racemic mixture of one of the stereoisomers of the natural hormone, and to it we assign the name estrone-a. The presence of a carbonyl group was shown by the formation of a semicarbazone and reaction with a Grignard reagent, and the phenolic group was indicated by the solubility of the compound in aqueous sodium hydroxide but not in sodium bicarbonate solution. With concentrated sulfuric acid and phenol and water it gave the Kober¹¹ test like that shown by estrone. Its structure was confirmed by conversion of its methyl ether to 7-methoxy-3',3'-dimethyl-1,2-cyclopentenophenanthrene by the procedure employed by Cook and co-workers¹² on estrone to prove the structure of the hormone.

Further proof of the structure was obtained by means of the absorption spectrum curve, for

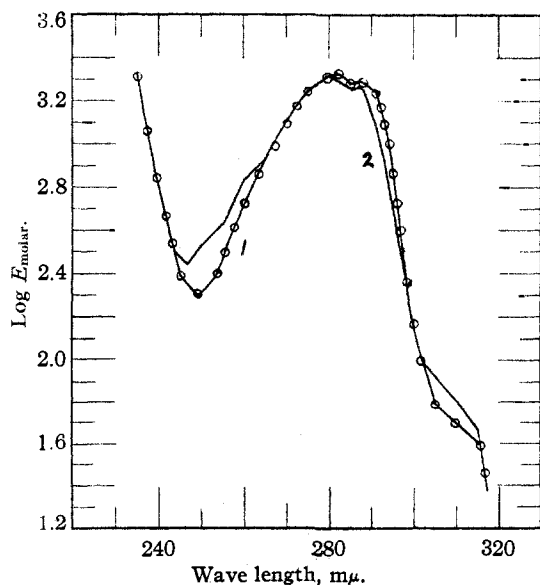


Fig. 1.—Absorption curves: Curve 1, with circles, estrone-a in 95% alcohol; Curve 2, estrone in alcohol (Mayneord and Roe¹³).

(11) Kober, *Biochem. Z.*, **229**, 209 (1931).

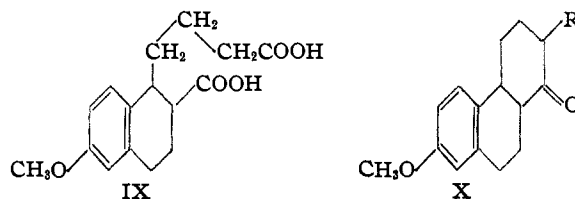
(12) Cohen, Cook, Hawett and Girard, *J. Chem. Soc.*, 445 (1935).

which we are indebted to Dr. L. C. Anderson of this Laboratory. In Fig. 1 there are shown the curve for estrone-a and the curve for estrone of Mayneord and Roe.¹³ It is apparent that there is a close agreement between the two curves.

In ovariectomized rats, estrone-a failed to show estrogenic activity in doses of 3.2γ or less (*d*-estrone is active in 0.8γ doses). It showed some activity at 100γ (positive response in 20% of the rats) and at 250γ its activity corresponded to that shown by 1γ of estrone. These results indicate that the compound is not *dl*-estrone but the racemic form of one of the stereoisomers of the hormone. This point will be settled definitely by resolution of the product into its optically active components.

It is of interest that only a single racemic form separated from the mixture of stereoisomers which was synthesized. The mixture of the remaining stereoisomers, which may possibly contain the hormone itself, is being subjected to various treatments in an attempt to separate it into its components. The mixture possesses greater estrogenic activity than the crystalline estrone-a; 50γ of the mixture is as active as 1γ of estrone in ovariectomized rats.

Various procedures for varying the relative proportions of the stereoisomeric estrones are under investigation. It has been observed that catalytic reduction of the unsaturated dicarboxylic acid (IV) in the presence of palladium on charcoal yielded nearly exclusively a crystalline reduced acid (IX), while reduction by sodium amalgam gave a mixture which consisted chiefly of a liquid reduced acid. By cyclization of the dimethyl



ester of catalytically reduced product, followed by hydrolysis and decarboxylation of the cyclic keto ester (X, R = COOCH₃), a mixture of ketones (X, R = H) was obtained which contained 63% of a solid ketone and 37% of a liquid ketone. The solid ketone was found to be identical with the 7-methoxy-1-keto-1,2,3,4,9,10,-

(13) Mayneord and Roe, *Proc. Roy. Soc. (London)*, **A158**, 634 (1937). See also Butenandt, *Z. physiol. Chem.*, **191**, 140 (1930); Dirscherl and Hanusch, *ibid.*, **223**, 13 (1935); Pedersen-Bjergaard and Schou, *Quart. J. Pharm. Pharmacol.*, **8**, 669 (1935); Callow, *Biochem. J.*, **30**, 906 (1936).

11,12-octahydrophenanthrene prepared by Robinson and Walker¹⁴ from the unsaturated ketone (IVa). From the acid produced by sodium amalgam reduction only 36% of the same solid ketone was obtained, the remainder being a liquid. From the cyclic keto ester (X, R = COOCH₃) the methylated keto ester was prepared readily. Other methods of approach involve the reduction of the doubly unsaturated ester (VII) by methods differing from that already employed and the reduction of the Reformatsky ester (VI).

We are grateful to Dr. Oliver Kamm of Parke, Davis & Co. for the estrogenic tests on estrone- α in low concentrations (up to 3.2 γ) and to Miss Helen McRae of the Department of Pediatrics of the Medical School of this University for tests on higher concentrations (100–250 γ) and on the mixture of stereoisomers. The method which has been developed is being extended to the preparation of other compounds related in structure to the sex hormones.

Experimental

β -*m*-Anisylethylmalonic Ester.—To a cooled solution of sodiomalonic ester prepared from 6.3 g. of sodium, 100 cc. of absolute alcohol and 53 g. of ethyl malonate was added 40 g. of β -*m*-anisylethyl bromide⁶ and 20 cc. of absolute alcohol. After the mixture had been refluxed for seven hours, the alcohol was removed in a current of air on a steam-bath. After addition of water to the cooled mixture to dissolve the sodium bromide, the mixture was acidified with dilute hydrochloric acid, saturated with salt, and extracted three times with benzene. From the dried extracts, the product was obtained by distillation under reduced pressure; b. p. 195–200° at 0.6–0.8 mm.; yield, 40–41.5 g. (75–77%).

An additional quantity of material was obtained from the forerun of the distillation by hydrolysis to β -*m*-anisylethylmalonic acid. The solid acid was collected and esterified with diazomethane.

Acid Chloride of Ethyl Hydrogen Glutarate.—A mixture of 179 g. of 1,1-dicarbethoxy-3-carbomethoxypropane,¹⁵ 200 cc. of water and 200 cc. of concentrated hydrochloric acid was refluxed for six hours. The liquids were removed by distillation, finally under reduced pressure at 100° and the residue of glutaric acid was heated with 200 cc. of acetyl chloride for only two hours. The mixture was distilled immediately and the glutaric anhydride was collected at 165–170° (20 mm.); m. p. 52–55°; yield, 77 g.

A mixture of 113 g. of glutaric anhydride and 62 cc. of absolute ethanol was refluxed for two hours and then distilled from a Claisen flask bearing a short (20 cm.) Vigreux column. After a small forerun of diethyl glutarate (b. p. 137–155° (17 mm.)) 138 g. of ethyl hydrogen glutarate was collected at 159–165° (17 mm.), which was sufficiently pure for the next step. A mixture of 48 g.

of the product and 30 cc. of pure thionyl chloride was placed in a Claisen flask equipped with a reflux condenser bearing a calcium chloride tube. After the mixture had been warmed slightly to start the reaction, it was allowed to stand at room temperature for two hours and then warmed for ten minutes. The acid chloride was purified by distillation; b. p. 108–110° (15 mm.); yield, 69.2 g.

Ethyl 5-Keto-6,6-dicarbethoxy-8-*m*-anisyl octanoate (II).—A mixture of 3.45 g. of finely divided sodium, 100 cc. of dry benzene and 43.4 g. of β -*m*-anisylethylmalonic ester was refluxed with occasional swirling until all of the sodium had reacted (three to seven hours). To the cooled solution 33 g. of the acid chloride of ethyl hydrogen glutarate was added and the mixture was allowed to stand at room temperature for three hours and was then refluxed for thirty minutes. The cooled mixture was treated with water and salt, the benzene layer was separated and the aqueous solution was extracted twice with benzene. The benzene was removed from the combined extracts and low-boiling material was removed by distillation from a Claisen flask up to 185° at 20 mm. The residue was then flash-distilled at 0.05 mm. from a 50-cc. Claisen flask wrapped with asbestos and heated in a graphite bath (340°). The flask was fitted with a 7-cm. glass tube (6 mm. bore) one end of which was drawn to a capillary and extended to the bulb of the flask; to the other end was sealed a short length of 1.8 cm. tubing to which was attached a small dropping funnel whose delivery tube was drawn to a large capillary. The product distilled at 225° as fast as the liquid dripped into the flask. In this manner, 40 g. of a lemon-yellow oil was obtained in about two hours. It was redistilled from a Claisen flask with a short column in the usual manner; yield, 33.2 g. (52%); b. p. 210–220° (0.05 mm.). A forerun of 8 g. (b. p. 165–170° (0.05 mm.)) consisted chiefly of unreacted β -*m*-anisylethylmalonic ester and was used in another run.

γ -(6-Methoxy-2-carboxy-3,4-dihydro-1-naphthyl)-butyric Acid (IV).—Four mixtures, each containing 2.5 g. of II and 12 g. of 100% orthophosphoric acid,¹⁶ were kept at 42° in a constant temperature bath for four hours. The clear jelly-like solutions were treated with water and then combined for extraction with three 50-cc. portions of ether. The ethereal solution was washed with water, the ether was removed and the residue was refluxed on a steam-bath with a mixture of 20 cc. of methanol and 20 cc. of 45% aqueous potassium hydroxide for three hours. After the removal of the methanol in a current of air, insoluble organic material was extracted with ether, and the aqueous solution was stirred into dilute hydrochloric acid. The organic acid which precipitated as an oil was extracted with ether, the ether was removed in a current of air and then under reduced pressure, and the residual γ -(6-methoxy-2,2-dicarboxy-1,2,3,4-tetrahydronaphthylidene-1)-butyric acid (IIIa) was scratched until it crystallized. A sample of the crude acid (m. p. 178.5–180.5°) crystallized from acetone-petroleum ether in clusters of stout glistening prisms; m. p. 180.5–182° with vigorous evolution of carbon dioxide, when the tube was placed in a bath preheated to 175°.

(14) Robinson and Walker, *J. Chem. Soc.*, 747 (1936).

(15) Marvel and Stoddard, *J. Org. Chem.*, 8, 201 (1938).

(16) "Inorganic Syntheses," McGraw-Hill Book Co., New York, N. Y., Vol. I, p. 101, 1939.

Anal. Calcd. for $C_{17}H_{18}O_7$: C, 61.1; H, 5.4. Found: C, 61.4; H, 5.6. *Neutral equivalent.* Calcd.: 111. Found: 111.

Usually the crude tricarboxylic acid was converted to the dicarboxylic acid without purification. The crude acid was dissolved in 80 cc. of hot water and the solution was heated on a steam-bath for twelve hours. After a short time the dicarboxylic acid, which is much less soluble in water, began to precipitate from the solution. Most of the water was removed from the hot mixture in a current of air, and the dicarboxylic acid was filtered. After trituration with a small volume of ether to remove oily colored impurities, the acid was obtained as colorless crystals; yield, 3.8 g. (53% based on II); m. p. 183–187° with decomposition. After two recrystallizations from a mixture of benzene, methanol and acetone, a sample of the γ -(6-methoxy - 2 - carboxy - 3,4 - dihydro - 1 - naphthyl)-butyric acid (IV) formed colorless needles; m. p. 189–190° dec., when placed in a preheated bath.

Anal. Calcd. for $C_{16}H_{18}O_5$: C, 66.2; H, 6.2. Found: C, 65.8; H, 6.2.

In one run a mixture (cooled to -15°) of 3 g. of 95% sulfuric acid and 3 g. of 100% orthophosphoric acid was added to 0.96 g. of II which was cooled to -15° and the whole was kept at this temperature for three hours. Ice was added and the cyclized product was worked up as described. The yield of unsaturated dicarboxylic acid was 52%. A 42% yield of the dicarboxylic acid was obtained when 0.64 g. of II was treated with 6 cc. of 95% sulfuric acid at -15° for three and one-half hours and worked up as described.

If the tricarboxylic acid is decarboxylated at 180–190°, the yield of dicarboxylic acid (IV) is low, because the latter also suffers decarboxylation at this temperature, as was shown by experiment.

7 - Methoxy - 1 - keto - 1,2,3,4,9,10 - hexahydrophenanthrene (IVa). (a) *From the Dicarboxylic Acid (IV).*—A mixture of 0.5 g. of γ -(6-methoxy-2-carboxy-3,4-dihydro-1-naphthyl)-butyric acid, 10 cc. of acetic acid, 5 cc. of concentrated hydrochloric acid and 1 cc. of water was refluxed in an atmosphere of nitrogen on a sand-bath for one hour. After the removal of the solvents under reduced pressure, the residue was dissolved in ether and the ethereal solution was shaken with 5% sodium hydroxide solution. Evaporation of the ether yielded 0.25 g. of the unsaturated cyclic ketone (IVa) as slightly yellowish crystals; m. p. 73.5–75°. Under similar conditions, the dimethyl ester prepared from 0.5 g. of IV and diazomethane gave 0.3 g. of IVa; m. p. 73.5–76°.

The dimethyl ester prepared from 0.5 g. of IV was cyclized by means of dry sodium methoxide from 0.1 g. of sodium in 10 cc. of benzene on a steam-bath (twelve hours). When the product, which was isolated in the usual manner, was heated with the acid mixture employed above, 0.335 g. of the unsaturated ketone (IVa) was produced.

(b) *From the Tricarboxylic Acid (IIIa).*—One-half gram of this acid gave 0.23 g. of IVa (m. p. 75–76.5°) when heated with the acid mixture in the manner described. Under similar conditions, the trimethyl ester prepared from 0.97 g. of the tricarboxylic acid and diazomethane yielded 0.53 g. of the ketone (m. p. 73–76°). By re-

crystallization from dilute methanol it was obtained as colorless prisms; m. p. 76–77.5°.

In one experiment, 1.11 g. of II was cyclized by 100% orthophosphoric acid as described, the resulting product was hydrolyzed by methanolic potassium hydroxide and the crude tricarboxylic acid was heated with the acetic acid and hydrochloric acid. From the mixture 0.3 g. of the unsaturated ketone (IVa) (m. p. 70–75°) was obtained.

All samples of the ketone were identified by mixed melting point determinations with the ketone of Robinson and Schlittler⁸ and of Hewett.⁸ We prepared the ketone by the following modifications of Robinson and Schlittler's procedure. To a suspension of sodium methoxide prepared from 2.18 g. of sodium (excess of methanol removed simply on a steam-bath under reduced pressure) in 70 cc. of benzene was added 10.46 g. of methyl 8-*m*-anisyl-5-ketoctanoate and 10 cc. of benzene. After four hours of refluxing on a water-bath, with frequent swirling, the cooled mixture was treated with ice and water and worked up as described. From the aqueous alkaline solution dilute hydrochloric acid precipitated 8.63 g. of β -*m*-anisylethylcyclohexane-2,6-dione (m. p. 138–143°). After trituration with ether, the product melted at 150–152° with slight previous softening; yield, 7.25 g. (78%).

A cyclizing mixture was made by dissolving 20 g. of metaphosphoric acid in 50 g. of hot 85% orthophosphoric acid; just before use 20 g. of metaphosphoric acid was dissolved in 25 g. of the resulting solution. A mixture of 30 g. of the final mixture and 3 g. of β -*m*-anisylethylcyclohexane-2,6-dione (m. p. 150°) was heated on a steam-bath for three hours, water was added, the mixture was extracted with ether and the ethereal extract was washed with aqueous sodium hydroxide. Removal of the ether left 2.75 g. (99%) of practically pure ketone; m. p. 75–76.5°. When only two hours was allowed for the reaction, the yield was 93%; after one hour it was only 70%.

7 - Methoxy - 2 - methyl - 2 - carbomethoxy - 1 - keto - 1,2,3,4,9,10 - hexahydrophenanthrene (V).—Sodium methoxide was prepared from 0.44 g. of sodium and 5 cc. of methanol and the excess methanol was removed under reduced pressure on a steam-bath. The sodium methoxide was powdered and to it were added 30 cc. of benzene and the dimethyl ester which had been prepared from 3 g. of the unsaturated dicarboxylic acid and diazomethane. The whole was refluxed in a nitrogen atmosphere on a steam-bath for three and one-half hours with frequent swirling and then for four hours on a water-bath. During this time copious precipitation of the sodio derivative of the cyclic keto ester took place. After the mixture had been cooled in ice-water, 10 cc. of methyl iodide and 10 cc. of methanol were added. After twelve hours at room temperature, the clear solution was refluxed for thirty minutes, cooled and just made acid with acetic acid. Water was added and the benzene layer, combined with an ether extract of the aqueous layer, was washed with water and with sodium bicarbonate solution and then dried over magnesium sulfate. The product obtained by removal of the solvents in a current of air crystallized when triturated with methanol; yield, 2.02 g.; m. p. 96.5–98.5° with slight previous softening. From the methanol filtrate an additional 0.23 g. of product (m. p. 95–96°) was isolated, and an additional 0.12 g. of product

was obtained by evaporatively distilling the material in the mother liquor under reduced pressure and recycling the distillate. The methyl keto ester gave no color with alcoholic ferric chloride solution; it dissolved in concentrated sulfuric acid to give a yellow solution with a brilliant green fluorescence. After two recrystallizations from methanol-acetone, a sample formed stout colorless prisms; m. p. 98-100°.

Anal. Calcd. for $C_{18}H_{20}O_4$: C, 72.0; H, 6.7. Found: C, 71.8; H, 6.6.

Dimethyl Ester of 7-Methoxy-2-methyl-2-carboxy-1-hydroxy-1,2,3,4,9,10-hexahydrophenanthrene-1-acetic Acid (VI).—To 3 g. of granulated zinc (20 mesh, previously treated with concentrated hydrochloric acid, water, acetone and dried) and 0.1 g. of iodine in 30 cc. of anhydrous ether and 30 cc. of benzene was added 2 g. of the methyl keto ester (V) and 1 cc. of freshly distilled methyl bromoacetate. The mixture was stirred mechanically with a small Hershberg stirrer¹⁷ made from platinum wire and refluxed in a water-bath held at 60-70°. In some runs the reaction started in about fifteen minutes (as indicated by the development of turbidity and the instantaneous discharge of the iodine color on addition of a crystal of iodine), while in other runs several hours were required. After the reaction had started, 4-g. portions of zinc and a crystal of iodine were added every forty-five minutes; in all five additions were made. During the reaction a crystalline addition product separated from the solution. The mixture was cooled, and the suspension was decanted from the zinc into water; a little benzene to which were added a few drops of methanol and acetic acid was used to dissolve the addition product mixed with the zinc. The hydrolysis mixture was acidified with acetic acid, the ether-benzene layer was separated, the aqueous solution was extracted with benzene, and the combined solutions were washed with dilute ammonium hydroxide in order to remove the color which was present. The product remaining after the removal of the solvents from the dried solution in a current of air crystallized when triturated with methanol; yield, 1.72 g. (69%); m. p. 111-112°. After three recrystallizations from methanol, a sample formed clusters of short colorless needles; m. p. 112-113°. It gave a yellowish-orange color with concentrated sulfuric acid.

Anal. Calcd. for $C_{21}H_{26}O_6$: C, 67.4; H, 7.0. Found: C, 67.1; H, 7.0.

A mixture of 65 mg. of the Reformatsky ester, 2 cc. of methanol and 1 cc. of 45% aqueous potassium hydroxide was heated on a steam-bath for two hours. Addition of water precipitated 20 mg. (47%) of 7-methoxy-2-methyl-1-keto-1,2,3,4,9,10-hexahydrophenanthrene (Va); m. p. 65-67°. After recrystallization from ether-petroleum ether (by cooling the solution in acetone-dry-ice), it melted at 66-67°. The same product was obtained in 62% yield from the methyl keto ester (V) under similar conditions. Robinson and Walker¹⁸ reported a m. p. of 67-68° for the same ketone, which they prepared by a different method.

Dehydration of the Reformatsky Ester.—To 17 cc. of dry benzene saturated with dry hydrogen chloride at 15°

was added 0.5 g. of calcium chloride and then 1.7 g. of the Reformatsky ester (VI). As the latter dissolved, a yellow color was imparted to the solution and the solution became turbid through the separation of water. Additional hydrogen chloride was passed into the solution until the latter acquired a greenish hue (an excess of hydrogen chloride engenders a red color). After fifteen minutes, the filtered solution was evaporated in a current of air; during this process the solution became yellow-orange in color. A solution of the residue in a minimum volume of methanol deposited the dimethyl ester of 7-methoxy-2-carboxy-2-methyl-1,2,3,4,9,10-hexahydrophenanthrylidene-1-acetic acid (VII) in light-yellow needles; yield, 1.2 g.; m. p. 135-136°. From the filtrate an additional 0.2 g. of slightly less pure product was isolated. For reduction, the product was usually recrystallized once from methanol, with only slight loss. After two recrystallizations a sample melted at 136.5-138°. A sample possessed only a pale cream color after a benzene solution of the ester had been passed through a short column of alumina; the m. p. was not changed by this treatment. The same ester was obtained by warming a benzene solution of the Reformatsky ester with anhydrous formic acid.

Anal. Calcd. for $C_{21}H_{24}O_6$: C, 70.8; H, 6.7. Found: C, 70.6; H, 6.5.

Isomerization of VII to VIIa.—A mixture of 50 mg. of the unsaturated ester (VII) and 20 mg. of palladium-charcoal catalyst¹⁹ was heated in a small test-tube at 300° for thirty minutes. The product was separated from the catalyst by means of acetone and the acetone was evaporated. A solution of the product in methanol deposited 30 mg. of the dimethyl ester of α -7-methoxy-2-carboxy-2-methyl-1,2,3,4-tetrahydrophenanthrene-1-acetic acid (VIIa)^{5b} when seeded with a crystal of that compound; m. p. 124-124.5°. After one recrystallization from methanol it formed colorless plates which melted at 126-126.5°, alone and when mixed with an authentic specimen.

Catalytic Reduction of the Unsaturated Ester (VII).—A suspension of 1.265 g. of once-recrystallized VII and 0.12 g. of palladium-charcoal catalyst¹⁹ in 30 cc. of absolute alcohol was shaken with hydrogen at atmospheric pressure and room temperature. The solid and the yellow color of the solution had disappeared when one mole equivalent of hydrogen had been absorbed (twenty to thirty minutes). The second mole equivalent of hydrogen was taken up quite rapidly at first and then more slowly near the end. Usually the mixture was allowed to shake for six to eight hours, when the calculated amount (178 cc.) of hydrogen had been absorbed. Evaporation of the filtered solution yielded the dimethyl ester of 7-methoxy-2-methyl-2-carboxy-1,2,3,4,9,10,11,12-octahydrophenanthrene-1-acetic acid (VIII) (mixture of stereoisomers) as a colorless liquid. It was used directly in the next step. Preliminary experiments indicate that a separation of the dicarboxylic acids obtained by hydrolysis can be effected.

Methyl Ethers of the Estrones.—A mixture of the aforementioned product (1.27 g.), 20 cc. of methanol and 3.08 cc. of 1.19 *N* sodium hydroxide was refluxed in a nitrogen atmosphere for eight hours. The methanol was removed

(17) Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 313 (1936).

(18) Robinson and Walker, *J. Chem. Soc.*, 192 (1936).

(19) Zelinsky and Turova-Pellak, *Ber.*, **66**, 1295 (1936).

in a current of air and water was added to dissolve the sodium salt of the acid ester (1.2 cc. of 0.1 *N* hydrochloric acid was required to neutralize the excess of alkali). Unreacted dimethyl ester (0.06 g.) was extracted with ether and the aqueous solution was acidified with hydrochloric acid. The liquid acid ester which precipitated was extracted with three portions of ether, the solution was dried with magnesium sulfate and the solvent was removed.

The Arndt-Eistert synthesis was carried out as described for an analogous compound.^{5b} A solution of the acid ester (1.2 g.) in 5 cc. of benzene was added to an ice cold solution of 2 drops of pyridine and 1.2 cc. of purified thionyl chloride in 3 cc. of benzene. The light-yellow solution gradually became orange-red during the course of the reaction. For reaction with the acid chloride, the diazomethane obtained from 5 g. of *N*-nitrosomethylurea was employed and the reaction mixture was allowed to stand at room temperature for six hours. A solution of the liquid yellow diazo-ketone in 15 cc. of methanol was refluxed with 0.1 g. of silver oxide for one-half hour. In order to hasten the reaction, which appeared to be slow in starting, 0.1 g. of silver oxide and a few cc. of methanol were refluxed in a small test-tube until a silver mirror became visible; the methanol was decanted, the silvered part of the tube was pulverized and the pieces were introduced into the reaction mixture, whereupon evolution of nitrogen appeared to take place. After several additions of silver oxide had been made over the course of four hours, the mixture was filtered and the solution heated with an additional 0.2 g. of silver oxide for one hour. Evaporation of the filtered solution yielded a liquid, presumably a mixture of stereoisomers of the dimethyl ester of 7-methoxy-2-methyl-2-carboxy-1,2,3,4,9,10,11,12-octahydrophenanthrene-1- β -propionic acid, which was used without further purification in the next step.

The product was cyclized by heating it with the sodium methoxide prepared from 0.3 g. of sodium in 10 cc. of benzene for four hours in an atmosphere of nitrogen in the manner described for an analogous compound.^{5b} The product which was isolated was a liquid which gave a deep bluish-purple color with alcoholic ferric chloride solution. The methyl ether of 16-carbomethoxyestrone (mixture of stereoisomers), 10 cc. of acetic acid, 5 cc. of concentrated hydrochloric acid and 1 cc. of water were refluxed for forty-five minutes in an atmosphere of nitrogen. Hydrolysis and decarboxylation took place as indicated by the formation of barium carbonate when the evolved gas was passed into a saturated solution of barium hydroxide; the reaction appeared to be complete in about twenty minutes. The solvents were removed under reduced pressure, the residue was dissolved in benzene and the solution was shaken with dilute sodium bicarbonate solution and then with 5% sodium hydroxide. The residue remaining after removal of the benzene was evaporatively distilled at 180° and 0.05 mm. The distillate (0.67 g.), a mixture of the methyl ethers of the estrones, was a colorless viscous liquid. It gave no color with an alcoholic solution of ferric chloride.

Estrone-a (I).—A mixture of 0.2 g. of the aforementioned mixture of methyl ethers, 1 cc. of 48% aqueous hydrobromic acid and 1 cc. of acetic acid was refluxed in an atmosphere of nitrogen for fifty minutes. The red

mixture was cooled, diluted with 10 cc. of water and treated dropwise with 45% aqueous potassium hydroxide until it was distinctly alkaline. This procedure brought the phenolic material into solution and left unreacted material (about 0.1 g.) undissolved. After the latter had been removed by an ether extraction, the clear aqueous solution was acidified with hydrochloric acid, the gum which precipitated was taken up in ether and benzene, the solution was washed with sodium bicarbonate solution, dried with magnesium sulfate and then evaporated. Evaporative distillation of the residue at 200° and 0.05 mm. gave a resin-like mass (when cool) which was readily powdered by means of a spatula and could be handled like a solid. When it was dissolved in methanol, the solution quickly deposited colorless leaflets of estrone-a; yield, 20 mg.; m. p. 213–214°. When crystallized slowly from methanol it formed thick colorless plates; from aqueous alcohol it crystallized in colorless leaflets. After two recrystallizations it melted at 214–214.5°. It gave an orange color with concentrated sulfuric acid which changed to a clear green-fluorescing red on warming with water; on addition of a little phenol the green fluorescence disappeared and a clear red or pink color remained.¹¹ For analysis a sample was sublimed at 200° and 0.05 mm.

Anal. Calcd. for $C_{18}H_{26}O_2$: C, 79.96; H, 8.20. Found: C, 79.98, 80.22; H, 8.19, 8.09.

The benzoate of estrone-a was prepared by allowing a mixture of 23 mg. of estrone-a, 1 cc. of benzene, 0.5 cc. of pyridine and 10 drops of benzoyl chloride to stand at room temperature for twenty-four hours. Ten more drops of benzoyl chloride was added and the mixture was warmed for a few minutes on a steam-bath. After evaporation of the liquids in a current of air, the residue was shaken with 10% aqueous sodium hydroxide to destroy the excess of acid chloride and the crystalline benzoate was extracted with benzene; yield, 28 mg. From acetone-alcohol it crystallized in colorless needles; m. p. 175–176° with slight previous softening. The melting point was not raised by a second recrystallization.

Anal. Calcd. for $C_{25}H_{36}O_3$: C, 80.18; H, 7.0. Found: C, 80.56, 80.31; H, 7.04, 6.98.

Conversion of the Methyl Ether of Estrone-a to 7-Methoxy-3',3'-dimethyl-1,2-cyclopentenophenanthrene.—This was carried out on 40 mg. of crude methyl ether prepared by the action of dimethyl sulfate on a solution of the sodium salt of estrone-a in water. (The methyl ether exists in dimorphic forms. From petroleum ether it was obtained as crystals melting at 81.5–82°; from dilute methanol it crystallized in colorless leaflets which melted at 101.5–102.5°. A mixture of the two forms melted at 101–102°.) The mixture of the methyl ether and the Grignard reagent from 0.5 cc. of methyl iodide was allowed to stand at room temperature for twelve hours and then refluxed for one hour. The methyl carbinol without purification was dehydrated by means of potassium acid sulfate¹² and the product was heated with 20 mg. of palladium-charcoal¹³ in a small test-tube at 300° for three hours. Several times during this period the heating was interrupted, the material which had condensed on the sides of the tube above the catalyst was washed down with a little acetone, the solvent was removed, and the heating was continued. The product was

leached from the catalyst by means of acetone, the solvent was evaporated and the residue was dissolved in a small amount of methanol. From the solution 10 mg. of 7-methoxy-3',3'-dimethyl-1,2-cyclopentenophenanthrene precipitated; m. p. 160–162°. After one recrystallization from methanol it melted at 162–163.5°. The identity of the compound was established by a mixed melting point determination with an authentic specimen which had been prepared from the methyl ether of equilenin.

Reduction of IV to γ -(6-Methoxy-2-carboxy-1,2,3,4-tetrahydro-1-naphthyl)-butyric Acid (IX). (a) **Catalytically.**—A solution of 1.7 g. of the unsaturated dicarboxylic acid (IV) in 50 cc. of acetic acid was shaken with 0.17 g. of palladium-charcoal catalyst¹⁹ and hydrogen at atmospheric pressure and room temperature. The volume of hydrogen corresponding to one mole per mole of the acid was absorbed in six hours. The reduced acid obtained from the filtered solution by evaporation of the solvent crystallized immediately; m. p. 150–154°. From benzene-methanol it was obtained as colorless crystals; m. p. 156–157.5°.

Anal. Calcd. for $C_{18}H_{20}O_3$: C, 65.7; H, 6.9. Found: C, 65.5; H, 6.8.

The dimethyl ester prepared from 0.5 g. of the acid (m. p. 150–154°) and diazomethane was cyclized by the procedure employed for making V. The cyclic keto ester which was isolated was a liquid which gave a reddish-purple color with alcoholic ferric chloride. It was refluxed with a mixture of 10 cc. of acetic acid, 5 cc. of concentrated hydrochloric acid and 1 cc. of water in an atmosphere of nitrogen for one-half hour. The crude ketone which remained after removal of the liquids was dissolved in benzene, the solution was washed with sodium bicarbonate solution, and the ketone obtained from the solution was evaporatively distilled at 0.05 mm. The product (0.39 g.), a mixture of a solid and a liquid ketone, was separated into its components by means of petroleum ether-methanol. The solid ketone, 7-methoxy-1-keto-1,2,3,4,9,10,11,12-octahydrophenanthrene (X, R = H) weighed 0.25 g. and melted at 103–106°. After recrystallization from dilute methanol the colorless needles melted at 107–108°, alone and when mixed with a sample prepared according to the procedure of Robinson and Walker.¹⁴

One gram of the acid (m. p. 150–154°) was esterified with diazomethane and the dimethyl ester was cyclized in benzene by sodium methoxide (five hours) according to

the procedure described. To the cooled mixture were added 5 cc. of methanol and 1.5 cc. of methyl iodide. After ten hours at room temperature, 1.5 cc. of methyl iodide was added and the mixture was refluxed for one hour. From the mixture there was isolated 0.89 g. of 7-methoxy-2-methyl-2-carbomethoxy-1-keto-1,2,3,4,9,10,11,12-octahydrophenanthrene as a colorless liquid. Robinson and Walker¹⁴ have prepared the corresponding carbomethoxy compound from the ketone (X, R = H) through the glyoxalate.

A mixture of 0.3 g. of the methyl keto ester, 5 cc. of methanol and 5 cc. of 45% aqueous potassium hydroxide was refluxed on a steam-bath for two hours. Water was added to the cooled solution, and after some time the 7-methoxy-2-methyl-1-keto-1,2,3,4,9,10,11,12-octahydrophenanthrene (X, R = CH_3) (0.205 g.) was filtered off and evaporatively distilled at 160° and 0.05 mm. After two recrystallizations from methanol, the ketone melted at 116–117.5° (reported, 119–120°⁹).

(b) **By Sodium Amalgam.**—A solution of 0.96 g. of IV in 2 *N* sodium hydroxide in an amount just sufficient to form the sodium salt was shaken vigorously with 16 g. of 2% sodium amalgam for ten minutes. Excess of alkali was neutralized with acetic acid and the mixture was then shaken mechanically for one hour (vigorous agitation). The acid which was obtained by acidification was a liquid which solidified only partly on standing. It did not react with dilute potassium permanganate solution.

Cyclization of the dimethyl ester prepared from 0.67 g. of the reduced acid yielded a liquid cyclo keto ester, which was hydrolyzed and decarboxylated in the manner described. From the mixture of liquid and solid ketones (0.45 g.) there was isolated 0.165 g. (m. p. 98–101°) of the solid ketone (X, R = CH_3). After one recrystallization from dilute methanol it melted at 107–108°, alone and when mixed with the ketone obtained in (a).

Summary

The synthesis of a mixture of stereoisomers possessing the structure of estrone is described. From the mixture the racemic form of one of the stereoisomers of estrone was isolated in crystalline form. The estrogenic activities of the products are reported.

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