treated with ethyl acetate–(petroleum ether) and filtered. Crystallization was effected on concentration in a stream of dry air; yield of crude methyl α-D-glucopyranoside 2-(methyl xanthate),[13] 750 mg. (9.2%).

An amount of 446 mg. of the above product was acetylated at 0° for 20 hr. with pyridine (1 ml.) and acetic anhydride (1 ml.). The sirupy product obtained on pouring the reaction mixture into an excess of ice and water was separated by decantation and crystallized from methanol–water; yield 255 mg. (30%) of beautiful needle crystals, m.p. 72–73° undepressed on admixture with an authentic specimen of methyl α-D-glucopyranoside 3,4,6-triacetate 2-(methyl xanthate) of like melting point, $[\alpha]^{27}D$ +126° (*c* 2.81, 95% ethanol). Lieser and Leckzyck[13] cite the m.p. 75–76° and record no rotation.

Benzylation of Methyl Monosodio-α-D-glucopyranoside.—An amount of 5 g. of dry methyl monosodio-α-D-glucopyranoside was shaken with 90 ml. of benzyl chloride for 24 hr. at 90 ± 5°. The excess benzyl chloride was removed by steam distillation and the residual sirup, separated by decantation, was treated with 50 ml. of ethanol and filtered. Solvent removal under reduced pressure gave a sirup; yield 3.6 g. (55%).

An amount of 0.56 g. of the above sirup was dissolved in 50 ml. of 100% ethanol and added to the top of a 17.5 × 4.5 (diam.) cm.[14] column of Florex XXX[18]–Celite[16] (5:1 by wt.) and the chromatogram was developed with 100 ml. of ethanol–water (100:3 by vol.). The extruded column was wrapped with aluminum foil to leave an exposed area 15 mm. wide along the length of the column and after 24 hr. the dried exposed area was streaked with the permanganate indicator. Solvent removal from an eluted (with acetone) second zone (from the column top) appearing near the middle of the column, gave a light brown sirup; yield 0.11 g. (20%).

Anal. Calcd. for $C_7H_{13}O_5(OCH_2C_6H_5)$: C, 58.74; H, 7.04. Found: C, 58.62; H, 7.06; periodate assay (moles per mole of reductant, 0.05 *M* $NaIO_4$ in 0.002 *M* reductant, 25 ± 2°, extrapolated value, few min. required for complete reaction): oxidant consumed, 1.0; formic acid, absent; formaldehyde, absent.

An amount of 1.0 g. of the above original (not chromatographed) sirup was acetylated with pyridine (3.5 ml.) and acetic anhydride (3.5 ml.) at 0° for 24 hr. The product (0.64 g., 44%) was chromatographed on a 17.5 × 4.5 (diam.) cm. column of Magnesol–Celite (5:1) and developed with 600 ml. of benzene–(*t*-butyl alcohol) (200:1). The acetone eluate material from the main upper zone was a sirup; yield 0.52 g. (81%).

Anal. Calcd. for $C_{20}H_{26}O_9$: C, 58.53; H, 6.34. Found: C, 58.30; H, 6.57.

COLUMBUS 10, OHIO

(18) A fullers earth type of clay produced by the Floridin Co., Warren, Pa.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, COLLEGE OF MEDICINE, NEW YORK UNIVERSITY]

The Synthesis of 17β-Estradiol-16-C^{14}

BY MORTIMER LEVITZ

RECEIVED MAY 27, 1953

A method for the preparation of labeled dimethylmarrianolate methyl ether is described. Ring closure of this compound by the acyloin condensation and reduction of the C_{16}-carbonyl group gives 17β-estradiol-3-methyl ether-16-C^{14}. Demethylation with pyridine hydrochloride results in 17β-estradiol-16-C^{14}.

A study of the metabolism of an estrogen and its relation to cancer can be facilitated by the use of a compound labeled with radioactive carbon. Accordingly, the synthesis of labeled 17β-estradiol, the physiologically most potent estrogen,[1] was undertaken in this Laboratory.

To our knowledge, the only previous method available was that of Heard.[2] Briefly, ring D in estrone is oxidatively cleaved[3] to marrianolic acid. It is reconstituted essentially by the method of Litvan and Robinson,[4] involving the use of diazomethane-C^{14} in the Arndt–Eistert reaction to extend the side chain of marrianolic acid and results in estrone-16-C^{14}. Finally, reduction with lithium aluminum hydride[5] gives 17β-estradiol-16-C^{14} (I).

We wish to report a novel synthesis which affords I in good yield, avoids the use of diazomethane-C^{14} and allows the direct utilization of the readily available and relatively inexpensive $C^{14}O_2$.

With dimethylmarrianolate methyl ether (II)[2,3] serving as the starting material for the introduction of C^{14}, the method of Hudson and Hauser[6] for the alkylation of esters using triphenylmethylsodium and alkyl halides was extended to carboxylate, with $C^{14}O_2$, the α-position of the primary ester group. The sodium enolate of II did not undergo intermolecular condensation as is the case with low molecular weight monosubstituted esters. After carboxylation with carbon dioxide (3.43 millicuries/millimole[7]) and saponification the presumed tricarboxylic acid III was obtained. Thermal decarboxylation yielded a mixture consisting chiefly of marrianolic acid anhydride methyl ether, with carbon dioxide (87% of theory, 1.75 millicuries/millimole[8]) being evolved and precipitated as barium carbonate. Treatment with alkali and esterification with diazomethane produced labeled dimethylmarrianolate (IV) in 83% yield (based on barium carbonate). Admixture of II and IV

(1) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publishing Corp., New York, N. Y., 1949, p. 329.

(2) R. D. H. Heard, J. Saffran and L. Thompson, private communication.

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

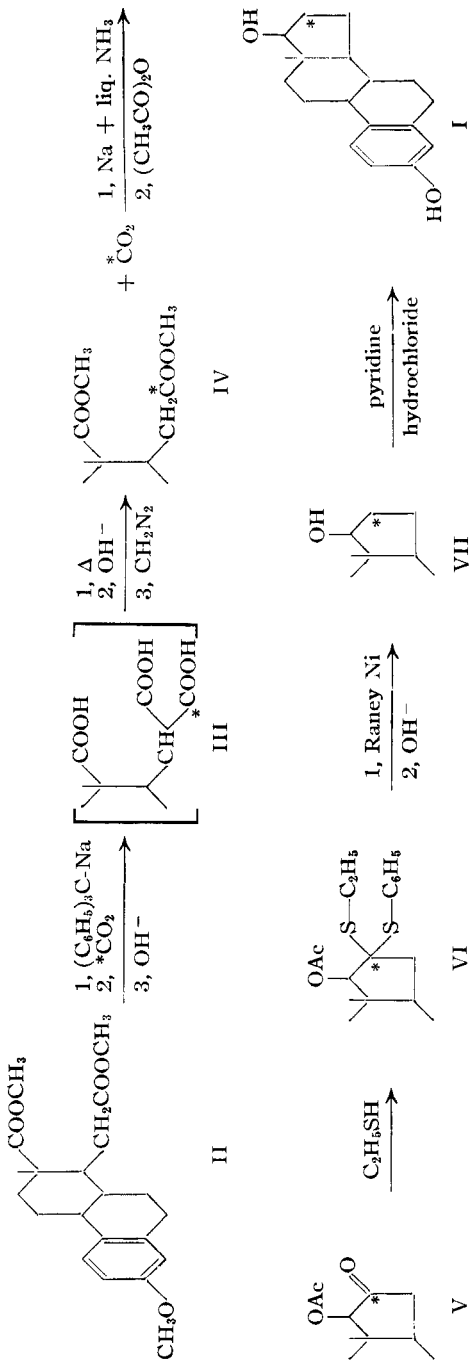
(4) F. Litvan and R. Robinson, *J. Chem. Soc.*, 1997 (1939).

(5) G. Papineau-Couture, E. M. Richardson and C. A. Grant, *Can. J. Research*, **27B**, 902 (1949).

(6) B. E. Hudson and C. R. Hauser, THIS JOURNAL, **62**, 2457 (1940).

(7) Assays for radioactivity were performed by Tracerlab, Inc., Boston, Mass.

(8) The two carboxyl groups of the malonic acid fragment of III are chemically indistinguishable. Consequently, except for isotope effects (P. E. Yankwich and M. Calvin, *J. Chem. Phys.*, **17**, 109 (1949); J. Bigeleisen, *ibid.*, **17**, 425 (1949)) the molar specific activities of C^{14} in the precipitated barium carbonate and either in IV or each succeeding compound should be identical. The values obtained 1.75 for barium carbonate and 1.66 for I are in good agreement. No statement can be made concerning the apparent reverse isotope effect because information on the homogeneity of III is unavailable.



afforded no melting point depression. The identity of the infrared spectra firmly established the structure of IV. The position of the isotopic carbon was unequivocally placed, as depicted, on a mechanistic basis.

By applying the acyloin condensation as worked out by Sheehan and his co-workers[9,10] IV was then cyclized to a ketol, the acetate V[11] of which was purified by crystallization. The yield for these two steps was 77%. The reduction of the C_{16}-carbonyl to methylene was then effected by treating VI, the diethyl thioketal of V,[12] with W-7 Raney nickel.[13] Saponification of the reaction product afforded 17β-estradiol-3-methyl ether (VII)[14] (66%) of sufficient purity after one crystallization to proceed with the last step of the synthesis.

After a few attempts to oxidize VII to labeled estrone methyl ether in good yield failed, the possibility of a direct conversion to I was explored. When VII was treated with pyridine hydrochloride[15] for four hours at 180°, I, m.p. 177.5–178.5°, after one crystallization from 95% ethanol, was obtained in 64% yield. The specific activity was 6.1 microcuries per milligram. The diacetate[14] and dipropionate[16] served to characterize I. The melting points agreed with the corresponding derivatives of authentic 17β-estradiol, prepared for direct comparison, and no depression was observed upon admixture. Furthermore, the infrared spectra of the diacetates were identical. The results of a bioassay indicated that subcutaneous injections of a solution of 0.1 γ of I in oil compared favorably with similar injections of authentic 17β-estradiol in inducing estrus in ten ovariectomized mice.[17]

The over-all yield of I based on the weight of barium carbonate was 28%. The isotopic yield was 13%. However, if one considers that 517 mg. of barium carbonate of high specific activity (1.75 millicuries/millimole) was recovered, then the isotopic yield was 25%.

Acknowledgment.—This work was supported mainly by a grant from the National Cancer Institute, U. S. Public Health Service, and in part by the American Cancer Society. The author wishes to thank Dr. Gray H. Twombly for his encouragement and advice throughout the course of the work and Dr. E. Henderson, and the Schering Corporation, for supplying generous quantities of estrone and 17β-estradiol.

(9) J. C. Sheehan, R. C. Sodert, L. A. Cohen and R. C. O'Neill, THIS JOURNAL, **74**, 6155 (1952).

(10) The author wishes to thank Dr. Sheehan for disclosing the experimental details of this reaction in advance of publication.

(11) Subsequent to Dr. Sheehan's publication, we discovered that the 17-hydroxyl has the β-configuration and not the α as is reported. This structural assignment was unequivocally made on the basis of the facts presented later in this paper. Since the completion of our work, Dr. Sheehan has informed us that upon reinvestigation, they too feel that the β-configuration is valid.

(12) M. N. Huffman and M. H. Lott, THIS JOURNAL, **71**, 723 (1949).

(13) H. Adkins and H. R. Billica, *ibid.*, **70**, 695 (1948).

(14) The melting point of VII (120–121°) differed sufficiently from that reported for VII (97–98°) and for its α-isomer (109–110°) (A. Butenandt and C. Goergens, *Z. physiol. Chem.*, **248**, 129 (1937)) to warrant an investigation. Reduction of estrone methyl ether with lithium aluminum hydride, a reagent which converts the C_{17}-carbonyl to C_{17}β-hydroxyl,[5] produced VII. VII was again isolated when the phenolic hydroxyl in 17β-estradiol was methylated according to the directions of Butenandt. In both cases the melting point was 120–121° and admixture with a sample of VII obtained by our synthetic scheme did not depress the melting point. Furthermore, the infrared spectra of the three samples were identical. It appears that we have prepared a higher melting polymorphic modification of Butenandt's compound.

(15) Militating against the use of pyridine hydrochloride, a reagent used for cleaving phenolic ethers (V. Prey, *Ber.*, **75**, 350 (1942)), was the report of Wilds (A. L. Wilds and W. B. McCormack, THIS JOURNAL, **70**, 4127 (1948)) that 4-cyclohexylcyclohexanol is destroyed by that reagent at 180°. On the other hand, the measure of protection afforded the C_{17}-hydroxyl by the C_{18}-methyl could not be predicted. Controls were run in which the 17α (kindly donated by Dr. T. F. Gallagher) and 17β isomers of estradiol were submitted to the conditions of the demethylation reaction. The C_{17}β-hydroxy compound, suffering only a two degree diminution in the melting point, was recovered in 75% yield. The α-isomer afforded a colorless oil (80%) which could not be extracted from an ethereal solution with 1.5 *N* sodium hydroxide. The nature of the product(s) from 17α-estradiol is unknown.

(16) K. Miescher and C. Scholz, *Helv. Chim. Acta*, **20**, 268 (1937).

(17) Bioassay was kindly performed by Miss D. Meisel of this Laboratory.

Experimental[18]

Dimethylmarrianolate Methyl Ether (II).—A mixture of 200 ml. of distilled dimethyl sulfate and a cooled solution of 5 g. of estrone, m.p. 255–258°, in 1 l. of 30% potassium hydroxide was stirred for 90 minutes at room temperature. After being filtered, washed with water and dried, the estrone methyl ether[19] (5.2 g.), m.p. 171–173°, was converted to the corresponding marrianolic acid. The directions of Heer and Miescher[20] were followed except that 5.2 g. of estrone methyl ether was dissolved in 2 l. of methanol[2] prior to hypoiodite treatment. After one crystallization from dilute methanol the diacid, m.p. 190–194°, was converted with ethereal diazomethane to II. Purification by chromatography on silica gel, using a 25% solution of dry ether in petroleum ether (m.p. 30–60°) to elute, and one crystallization from dilute methanol afforded 3.8 g. (60%), m.p. 75.5–76.5°.

Labeled Dimethylmarrianolate Methyl Ether (IV).—The apparatus was essentially that described in "Isotopic Carbon"[21] with two modifications. A 60-ml. erlenmeyer flask sealed below the ground-glass joint to a small funnel, with a stopcock interposed, served as the carbon dioxide generator. A 25-ml. round-bottom flask sealed directly to the manifold near the top of its long neck was the reaction vessel. The flask was covered with a tight fitting rubber-capped stopper of the type used for serum bottles after being charged with 1.20 g. (0.00333 mole) of II and a glass-enclosed iron nail as a stirrer. Three millimoles (592 mg.) of barium carbonate containing 10.3 millicuries of C^{14} was then placed in the generator.

Prepurified nitrogen was admitted into the evacuated system when it proved to be free of leaks. The diester was dissolved, with the aid of magnetic stirring, in 5 ml. of dry ether injected with a hypodermic syringe. Next there was injected a solution of 0.00330 mole of triphenylmethylsodium[22] in 14.7 ml. of ether. After stirring for five minutes at room temperature the orange solution was surrounded by a Dry Ice-bath and the system was evacuated with an oil-pump. Carbon dioxide was generated by cautiously admitting 60% perchloric acid into the flask, care being taken to exclude air. Stirring was resumed after removing the ice-bath. Upon warming, a suspension separated, which partially dissolved when room temperature was reached. The mixture was replaced in the ice-bath and once again allowed to come to room temperature with stirring. This process was repeated a second time, the generator being gently heated to dissolve the salts.

The ether solution was extracted twice with dilute acetic acid and then five times with 5% sodium carbonate. The dried neutral material was dissolved in a 5% solution of dry ether in petroleum ether (b.p. 30–60°) and chromatographed on 10 g. of silica gel to yield 683 mg. (0.00280 mole) of triphenylmethane, m.p. 90.5–92.5°. Further elution with a 25% solution resulted in the recovery of 190 mg. of starting material II, m.p. 70.5–74°.

The combined sodium carbonate extracts were acidified with dilute hydrochloric acid and the ether-extracted acid washed with water. The residue, after evaporation of the solvent, was refluxed in a solution of 7.5 g. of potassium hydroxide in 20 ml. of water containing 12 ml. of methanol for two hours. Most of the methanol was distilled and 50 ml. of water added. The acidic material III, isolated as described above, was a finely divided white powder when the last trace of ether was removed. It was heated in an oil-bath while a slow stream of nitrogen passed over and through a 0.5 *N* solution of carbonate-free sodium hydroxide. The evolution of gas commenced at 168°. The temperature was maintained at 180° for one hour. Nitrogen flushing was discontinued after an additional hour at room temperature. The carbonate was precipitated with barium chloride to afford 517 mg. (87%) of barium carbonate with a specific activity of 8.9 microcuries per milligram.

The residual yellow glassy substance was refluxed for 90 minutes in a solution of 2 g. of potassium hydroxide in 10 ml. of water and 3 ml. of methanol. An ethereal solution of the acidic material isolated in the usual way was treated with an excess of diazomethane. The ether was washed successively with 1% hydrochloric acid, water and saturated sodium chloride. The yellow waxy solid obtained upon evaporation of the ether was chromatographed on 10 g. of silica gel. The radioactive 25% ether in petroleum ether eluates were combined and evaporated to yield 898 mg. (83% based on barium carbonate) of IV, m.p. 73–75.5°. Admixture with II did not depress the melting point. The infrared spectra measured on the Baird double-beam spectrophotometer were identical.

3-Methoxy-17β-acetoxy-16-keto-1,3,5(10)-estratriene-16-C^{14} (V).—According to the directions of Sheehan,[9,10] 898 mg. of IV was submitted to the acyloin condensation. The crude ketol, m.p. 166–172°, was converted to the acetate V. One crystallization from 95% ethanol afforded 660 mg. (77%) of V as short white needles, m.p. 150.5–152°. The analytical sample melted at 153–154°.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.6; H, 7.6. Found: C, 74.0; H, 7.7.

Diethyl Thioketal of V (VI).—This was prepared from 660 mg. of V, using 25 ml. of ethyl mercaptan, 0.6 g. of freshly fused zinc chloride and 1.2 g. of anhydrous sodium sulfate as described by Huffman and Lott.[12] One crystallization from 15 ml. of 95% ethanol afforded 785 mg. (91%) of VI as white needles, m.p. 133–135.5°. Further crystallization raised the melting point to 137–138°.

Anal. Calcd. for $C_{25}H_{36}O_3S_2$: C, 66.9; H, 8.1; S, 14.3. Found: C, 67.1; H, 7.9; S, 14.1.

17β-Estradiol-3-Methyl Ether-16-C^{14} (VII).—The hydrogenolysis[12] of VI was effected with W-7 Raney nickel.[13] The crude solid residue was saponified[9] by refluxing it in 50 ml. of 5% ethanolic sodium hydroxide for two hours. The volume was reduced to 10 ml. and the precipitate thrown out with 75 ml. of cold water. The solid was filtered, washed with water and crystallized once by first dissolving it in 0.4 ml. of warm benzene and then adding 2.5 ml. of hexane. The filtered white needles (370 mg., 72%) melted at 115–116.5°. The analytical sample melted at 120–120.5°.

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.7; H, 9.2. Found: C, 79.8; H, 9.0.

The acetate was prepared by allowing a solution of 40 mg. of VII in 1 ml. of dry pyridine and 0.5 ml. of acetic anhydride to stand for 18 hours. The solution was pipetted onto 25 ml. of ice-water. The separated solid was crystallized several times from 95% ethanol to yield glistening white plates, m.p. 101–102.4°.

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.8; H, 8.6. Found: C, 76.6; H, 8.5.

Preparation of VII by the Reduction of Estrone Methyl Ether with Lithium Aluminum Hydride.—To a solution of 50 mg. of lithium aluminum hydride in 5 ml. of dry ether was added, with stirring, a suspension of 75 mg. of estrone methyl ether in 10 ml. of dry ether. The reaction was then allowed to proceed as described by Nystrom and Brown[23] except that gentle reflux was maintained for one hour after the addition. The crude product (40 mg.), m.p. 114–116.5°, was crystallized from benzene–hexane as needles, m. p. 120–121°. Admixture with VII prepared by our synthetic scheme produced no depression in the melting point. The infrared spectra were identical. The infrared spectrum of the acetate, m.p. 101–102.5° (no depression upon admixture), also coincided.

Preparation of VII by the Methylation of 17β-Estradiol.—Following the direction of Butenandt,[14] except that a 5% solution of sodium hydroxide sufficed to dissolve the steroid, 100 mg. of 17β-estradiol was converted by dimethyl sulfate to 40 mg. of X, m.p. 115–117°. One crystallization from benzene–hexane produced white needles, m.p. 119–120.2°. The identity with VII was established by the mixed melting point and infrared spectrum.

17β-Estradiol-16-C^{14} (I).—A mixture of 370 mg. of VII and 4.0 g. of pyridine hydrochloride[24] was sealed under nitrogen in a 12-mm. glass tube. The tube was heated to 100° and

(18) Analyses by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. Melting points were taken on uncalibrated Anschütz thermometers in the Hershberg apparatus.

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

(20) J. Heer and K. Miescher, *Helv. Chim. Acta*, **28**, 160 (1945).

(21) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 178.

(22) W. B. Renfrow, Jr., and C. R. Hauser, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 607.

(23) F. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 2549 (1947).

(24) G. Anner and K. Miescher, *Helv. Chim. Acta*, **31**, 2173 (1948).

vigorously shaken to ensure homogeneity. It was then maintained at 180 ± 2° for four hours. The cooled solid mixture was treated with dilute hydrochloric acid and the separated white solid extracted with ether. The combined alkaline extracts after six extractions with 1 *N* sodium hydroxide was acidified and extracted with ether. The dried ether solution was evaporated to afford 264 mg. of a slightly yellow solid, m.p. 176–178°. After one crystallization from 2 ml. of 95% ethanol, 230 mg. (64%) of I, m.p. 177.5–178.5°, with a specific activity of 6.1 microcuries per milligram, was obtained. Admixture with an authentic sample of I did not depress the melting point. The diacetate,[14] m.p. 125–127° and dipropionate,[16] m.p. 106–107°, were prepared and proved to be identical with authentic samples.

Effect of Pyridine Hydrochloride on 17β-Estradiol and 17α-Estradiol.—Solutions of 25 mg. of 17α-estradiol, m.p. 219–222°, and 17β-estradiol in 350 mg. of pyridine hydrochloride were maintained at 180° for four hours. Each was treated as described in the previous section. In the former case, the alkali soluble material was less than 1 mg. of a colorless oil, whereas the neutral fraction was 17 mg. of a colorless oil which turned yellow on standing in the refrigerator. In the latter case, 16 mg. of 17β-estradiol, m.p. 173–175.5°, was recovered in the alkaline fraction. Admixture with starting material produced no depression in the melting point.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

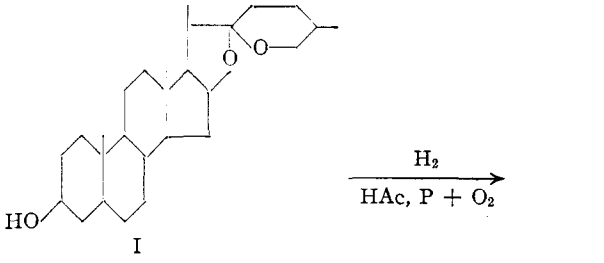
Acid-catalyzed Reduction of Spirostanols and Spirostenols by Lithium Aluminum Hydride

BY H. M. DOUKAS[2] AND T. D. FONTAINE

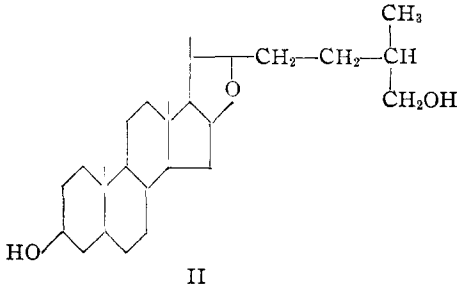
RECEIVED JUNE 12, 1953

A new method for cleaving ring F of steroidal sapogenins with lithium aluminum hydride in the presence of anhydrous hydrogen chloride and hydrogen bromide is reported whereby furostene and furostane diols can be prepared directly from spirostenols and spirostanols.

The conversion of sapogenins (I) to their corresponding dihydro compounds II by catalytic hydrogenation under acid conditions was first reported by Marker and Rohrmann.[3]



I



II

Catalytic hydrogenation of diosgenin (22a,5-spirosten-3β-ol) always resulted in first the saturation of the double bond to tigogenin, followed by the opening of ring F to yield dihydrotigogenin (5α,22a-furostane-3β,26-diol). The Clemmensen method of reduction was also utilized by Marker and Rohrmann[3] to open simultaneously both oxido rings (E and F) in the spiroketal side chain to yield triol compounds.

The use of $LiAlH_4$ in opening oxido rings in the steroidal secondary amines, tomatidine[4] and solasodine,[5] has been reported but it was found that under the same alkaline reaction conditions neither oxido ring of steroidal sapogenins opened. It has been reported in an earlier Communication,[6] however, that $LiAlH_4$, in the presence of anhydrous HCl, reduces both spirostanols and spirostenols to their corresponding furostane and furostene diols. Further investigation of this new reaction using $LiAlH_4$ and $NaBH_4$ as reducing agents and several anhydrous acids (HCl, HBr, H_2S, SO_2, *p*-toluenesulfonic acid) have been completed. It was found that only $LiAlH_4$ in the presence of either HCl or HBr would open the oxido linkage.

It is well established that catalytic reduction of sapogenins in an acidic medium, using platinum oxide catalyst, results in a cleavage of ring F.[7] Therefore, diosgenin acetate was hydrogenated according to the method of Marker, *et al.*,[7] and the product acetylated to yield dihydrotigogenin diacetate (5α,22a-furostane-3β,26-diol 3,26-diacetate) (VI). The product obtained by this method was identical with the acetylated $LiAlH_4$ reduction product of tigogenin. That both rings E and F of sapogenins did not open under $LiAlH_4$ reduction is supported by the fact that the reduced compounds yielded only diacetyl products which showed the complete absence of an unacetylated hydroxyl group in the infrared spectra.

Infrared spectra, obtained on all compounds, were used as an aid in confirming the structure of the dihydro compounds. Wall, *et al.*,[8,9] and Jones,

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) Part of a Thesis presented by H. M. Doukas to the Georgetown University, Washington, D. C., in partial fulfillment of the requirements for the degree of Ph.D.

(3) R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 846 (1939).

(4) T. D. Fontaine, J. S. Ard and R. M. Ma, *ibid.*, **73**, 878 (1951).

(5) L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, 3020 (1950).

(6) H. M. Doukas and T. D. Fontaine, THIS JOURNAL, **73**, 5917 (1951).

(7) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *ibid.*, **69**, 2167 (1947).

(8) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

(9) C. R. Eddy, M. E. Wall and M. K. Scott, *ibid.*, **25**, 266 (1953).