oxide catalyst, and 50 cc. of acetic acid was shaken with hydrogen at 45 pounds (3 atm.) pressure for four hours. The catalyst was removed by filtration, and the acetic acid by evaporation *in vacuo*, to leave a sirupy residue. This was hydrolyzed by heating for one-half hour on the steam-bath with alcoholic sodium hydroxide, and the solution cooled, diluted with water, and extracted with ether. The washed ethereal extract was evaporated, the residue clarified with Norit in alcohol, and crystallized from slightly diluted alcohol to give 110 mg. of *allo*-pregnanol-20(β), m. p. 140°, which did not depress with the sample obtained from *allo*-pregnanol-20(β)-one-3 acetate.

Summary

Convenient preparations of the pregnanone-20's and the pregnanol-20's stereoisomeric about C_5 and C_{20} are described.

STATE COLLEGE, PENNA. RECEIVED DECEMBER 7, 1938

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

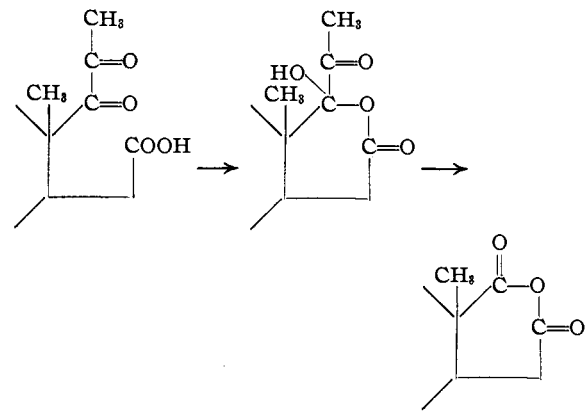
Sterols. LV. The Structure of Pregnanetriol-B

BY RUSSELL E. MARKER AND EUGENE L. WITTLE

Some years ago a carbinol, $C_{21}H_{36}O_3$, was isolated from mares pregnancy urine by Haslewood, Marrian and Smith.[1] Later[2] we obtained this carbinol, which was designated as pregnanetriol-B, together with another, pregnanetriol-A (uranetriol), from the same source and we were led to propose the structure I, 3(α),4(β),20(α)-trihydroxypregnane,[3] for the former compound. More recently Odell and Marrian[4] have published additional information concerning the structure of this triol which is not compatible with structure I, and these authors tentatively suggest that the substance is a pregnane-3(α),6,20-triol, (II). The present paper presents new information concerning the structure of pregnanetriol-B and it is now evident that both of the previously proposed structures are incorrect, and that the triol is a derivative of *allo*-pregnane having hydroxyl groups at 3, 16 and 20, (III).

Mild oxidation of triol-B was shown previously[3] to give as the major product a characteristic acid for which we were unable to propose a structure. The accumulated evidence now shows this acid to have structure IV. The acid is monobasic, forming a yellow crystalline benzylthiouronium salt from which it may be regenerated by hydrolysis. It reacts with periodic acid, indicating the $-\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}-$ grouping, gives the iodoform reaction, indicating the $-\overset{O}{\overset{\|}{C}}-CH_3$ grouping, and gives a positive Zimmermann test, indicating the presence of a 3-carbonyl group. In harmony with its structure as a 1,2-diketo compound, the acid is yellow in organic solvents and in the free state as an oil. The white crystalline acid contains a molecule of water and is obtained by acidifying a sodium carbonate solution of the acid, and allowing the cloudy mixture to stand. The molecular weight, 360, is in agreement with this structure.

Besides this acid we have now obtained from the same oxidation a small quantity of a second acid, m. p. 260°. While some of this acid may be obtained by crystallization of the total acid fraction removed from the oxidation mixture with sodium carbonate, the greater portion of the acid is obtained by further extraction of the neutral products with potassium hydroxide. This portion of the acid is formed in the reaction as an anhydride, and is probably derived from acid IV, the most readily formed product, by the following mechanism



The acid gives a positive Zimmermann test showing the presence of a 3-carbonyl group, and an analysis corresponding to the formula $C_{19}H_{28}O_5$.

(1) Haslewood, Marrian and Smith, *Biochem. J.*, **28**, 1316 (1934).

(2) Marker, Kamm, Crooks, Oakwood, Wittle and Lawson, THIS JOURNAL, **60**, 210 (1938).

(3) Marker, Kamm, Wittle, Oakwood and Lawson, *ibid.*, **60**, 1067 (1938).

(4) Odell and Marrian, *Biochem. J.*, **125**, 333 (1938).

It forms an anhydride (VII), a dimethyl ester and a 2,4-dinitrophenylhydrazone. Accordingly, it must be 3-keto-*etio-allo*-bilianic acid (V). This is confirmed by its Clemmensen reduction to *etio-allo*-bilianic acid (VI) which no longer gives a Zimmermann test and which forms the characteristic anhydride upon treatment with acetic anhydride. The formation of *allo*-pregnanedione from triol-B shows that two of the hydroxyl groups must occupy positions 3 and 20, and the failure of the triol to react with lead tetracetate shows that the third hydroxyl group cannot be located at position 17. Therefore, the formation of acids (IV), (V) and (VI) constitutes definite proof that the third hydroxyl group occupies position 16 and the structure of triol-B is then *allo*-pregnanetriol-3(α),16,20, (III).

Some of the experimental work previously reported by us[3] is not compatible with this structure (III) and on reinvestigation of this earlier work we found it to be in error. The "pregnane" reported by us as prepared from triol B through the chloride was found in reality to be *allo*-pregnane, identical with the known compound, and with *allo*-pregnane prepared from the triol by a second method. This was due to an error in mixed melting points. Thus the only hydrocarbon obtained from the triol is *allo*-pregnane and this fact, together with the transformations to *allo*-pregnanedione and to *etio-allo*-bilianic acid definitely shows the configuration at C_5 to be that of the *allo*-series. We find that the conversion of the diacetate of pregnanone-3-diol-4,20 to 3,4,20-trihydroxypregnane followed by oxidation, gives an acid which differs from acid IV and are further investigating this reaction.

We reported that partial hydrolysis of triol-B triacetate, followed by oxidation, gave a product, m. p. 188°, which on the basis of its analysis was reported[3] as a monoketodiacetate. Odell and Marrian[4] also studied the partial hydrolysis of triol B triacetate and obtained as the only product of this reaction a triol monoacetate, m. p. 222–224°, which on oxidation gave a good yield of a diketomonoacetate, m. p. 191–192°, and they suggested that this product was identical with our "monoketodiacetate." We were aware of the fact that our keto diacetate was in reality a diketomonoacetate, our previously reported analysis being in error, and we thus confirm the finding of Odell and Marrian that partial hydrolysis of triol B triacetate forms a triol monoacetate, m. p. 235°, which on oxidation forms a diketomonoacetate, m. p. 191–192°. The lability of the acetoxy groups at C_3 and C_{16} would be expected to be quite similar and it is probable that the compound in question is a 3,16-diketo-20-acetoxy-*allo*-pregnane. We cannot agree, however, with the claim of Odell and Marrian that the diketomonoacetate is a γ-diketone, a structure postulated for this compound by them on the basis of its formation of a "pyridazine" derivative. This is contrary to structure III for triol B which we have put forth, since a γ relation of hydroxyl groups is not present. The properties of their "pyridazine" are such as to suggest that it is not a simple pyridazine, for they report that it is obtained in poor yields as a light colored amorphous solid, decomposing slowly from 210° upward, and not melting below 310°. In contrast to this, pyridazines of known 3,6-diketones, such as sitostanedione,[5] stigmastenedione,[5] ergostanedione,[6] and cholestanedione[7] are obtained, in excellent yields, as well-defined crystalline substances melting in the neighborhood of 200°. We find that the reaction of hydrazine hydrate on the diketoacetate leads to the formation of an ill-defined amorphous mixture separable into several components which, however, are still amorphous and still appear to be mixtures. This behavior, in view of the fact that hydrazine hydrate is known to give complex polymeric products with diketones[8] suggests that in the reaction of the diketoacetate with hydrazine hydrate, linear polymers are formed by the continued intermolecular condensation of the substances. In support of this view we have obtained similar products by the reaction of *allo*-pregnanedione-3,20 with hydrazine hydrate which we consider to be the same type of compound as Odell and Marrian's "pyridazine," namely, a linear polymer.

The results of hydrolysis of the diketomonoacetate are also incompatible with a γ-ketone structure for the substance and strongly support formula III for triol B. Hydrolysis with alcoholic potassium hydroxide or even with the mild reagent sodium bicarbonate causes a removal not only of the acetate group but also of the hydroxyl group, forming an unsaturated diketone. This diketone is unaffected by acetic anhydride. That

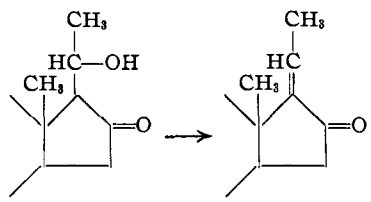
(5) Fernholz, *Ann.*, **508**, 215 (1934); Coffey, Heilbron and Spring, *J. Chem. Soc.*, 738 (1936).

(6) Windaus, Inhoffen and Reichel, *Ann.*, **510**, 248 (1934).

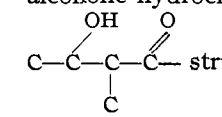
(7) Windaus, *Ber.*, **39**, 2249 (1906).

(8) Zimmerman and Lochte, THIS JOURNAL, **60**, 2456 (1938). This paper gives references to older studies.

the unsaturation is conjugated with the carbonyl group is not only expected from a consideration of formula III, but is also indicated by the forma-



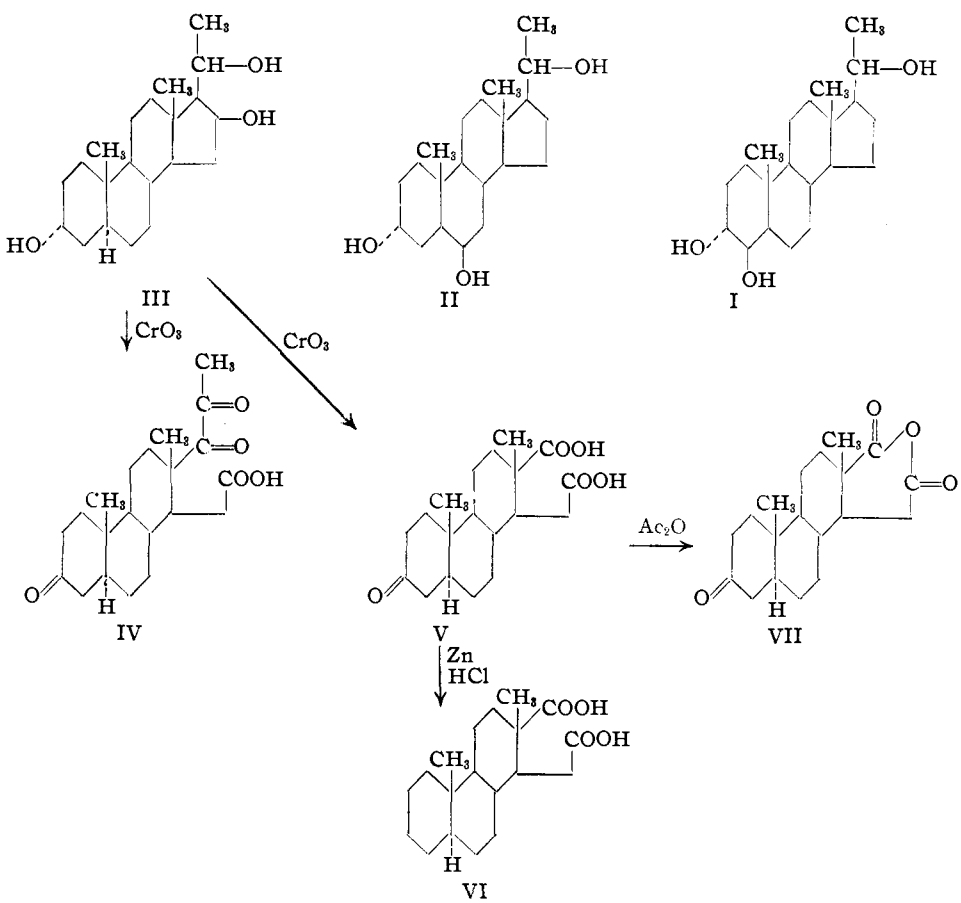
tion of a red *bis*-2,4-dinitrophenylhydrazone. This change is also brought about by hydrolysis with alcoholic hydrochloric acid and substantiates the



structure for triol B. That this diketone has the carbonyl groups placed at 3 and 16 is indicated by the reduction with sodium and alcohol to a saturated dihydroxy compound, characterized by the formation of a diacetate, which on oxidation, forms a saturated diketone, m. p. 128°, not identical with *allo*-pregnanedione, m. p. 205° or iso-*allo*-pregnanedione, m. p. 135°.

The formation of *allo*-pregnane[3] by the Clemmensen reduction of the diketomonoacetate has been repeated by us and we find that besides this product there is also formed some unsaturated hydrocarbon which can be catalytically reduced to *allo*-pregnane. These products are readily explained from the knowledge that hydrochloric acid hydrolysis of the diketomonoacetate gives an α,β-unsaturated diketone. This process can also take place in the Clemmensen reduction mixture and the unsaturated diketone so formed would be expected to be reduced to *allo*-pregnane and an unsaturated hydrocarbon. This is found to be the case.

The isomerization of triol B with sodium in xylene to give a 3(β)-hydroxyl group takes place to only a slight extent, probably due to the insolubility of the triol in xylene. Using amyl alcohol as the solvent, most of the triol is isomerized to a substance having a 3(β)-hydroxy group as shown by the precipitation of digitonin, indicating that triol-B has the *epi-allo* configuration at C_3—C_5. From the digitonide so formed only



a complex mixture could be obtained, from which a small amount of a dihydroxy compound $C_{26}H_{46}O_2$, m. p. 209°, may be obtained. This diol, which forms a diacetate $C_{30}H_{50}O_4$, m. p. 150°, is formed by the loss of a hydroxyl group from the triol and the condensation with amyl alcohol, and may be comparable to the α-cholestanol $C_{32}H_{58}O$ obtained by the action of sodium and amyl alcohol on cholesterol[9] or cholestenone.[10]

We wish to thank Dr. Oliver Kamm and Parke, Davis and Company for their generous support and assistance in the various phases of this work.

Experimental Part

The Partial Hydrolysis of the Triacetate.—To a solution of 12 g. (0.026 mole) of triol triacetate in 3 liters of methanol at 15° was added 18.5 cc. of potassium hydroxide solution (0.0014 mole per cc.) (0.026 mole), and after vigorous shaking the solution was kept at 15–20° for four days. A quantity of sulfuric acid equivalent to the 18.5 cc. of base was then added, precipitating potassium sulfate, and the methanol was distilled off at reduced pressure. The residue was diluted with water and filtered with suction. After drying, the product was crystallized from ethyl alcohol to give 5 g. of a product, m. p. 230°. Recrystallization raised the melting point to 233–235° (Marrian gives m. p. 222–224°).

Anal. Calcd. for triol monoacetate, $C_{23}H_{38}O_4$: C, 72.9; H, 10.1. Found: C, 72.9; H, 10.4.

Treatment of the substance with acetic anhydride gave the original triol-B triacetate, m. p. 168°.

The mother liquor from the above crystallization contained other hydrolysis products which have not yet been thoroughly investigated. A partial hydrolysis parallel to the above reaction but without the addition of sulfuric acid led to the formation of the original triol.

Oxidation of the Triol Monoacetate.—To a solution of 380 mg. of triol monoacetate, m. p. 235°, in 25 cc. of acetic acid was added a solution of 200 mg. of chromic anhydride in 10 cc. of 90% acetic acid, and the solution was allowed to stand at room temperature for one hour. After dilution with water the product was extracted with ether. The ether solution was washed with water, sodium carbonate solution and then with water and evaporated to yield 300 mg. of a diketomonoacetate, m. p. 191°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.7; H, 9.2. Found: C, 73.8; H, 9.0.

Hydrolysis of the Diketoacetate.—To a solution of 1.0 g. of diketomonoacetate, m. p. 190°, in 50 cc. of alcohol was added 1 g. of potassium hydroxide in 5 cc. of water and 25 cc. of ethyl alcohol. The solution was refluxed for a half hour, then diluted with water and the product was extracted with ether. The ether solution was washed thoroughly with water and evaporated to leave a white solid which was distilled in a molecular still. Crystallization of this product gave a small amount of an unidentified substance, m. p. 205–207°. The filtrate contained the major product which on crystallization from acetone melted at 188–190°; yield 400 mg.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.3; H, 9.8.

This substance gave a Zimmermann test showing a 3-keto group. On treatment with 2,4-dinitrophenylhydrazine this product gave a red *bis*-2,4-dinitrophenylhydrazine, m. p. 190°.

Anal. Calcd. for $C_{33}H_{38}N_8O_8$: C, 58.8; H, 5.7. Found: C, 59.2; H, 6.1.

Hydrolysis of the diketoacetate with sodium bicarbonate in dilute methyl alcohol gave an analogous result. A solution of 100 mg. of diketomonoacetate, m. p. 190°, in 400 cc. of methyl alcohol and 300 mg. of sodium bicarbonate in 10 cc. of water was refluxed for two hours. The methyl alcohol was then distilled off and the crystalline product was filtered and recrystallized from acetone, 80 mg., m. p. 190–192°. This gave a marked depression with the original diketomonoacetate.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 80.0; H, 9.7.

This product distilled completely in a molecular still at 130° and on crystallization from acetone, m. p. 190–192°.

Hydrolysis of the Diketoacetate with Hydrochloric Acid.—A solution of 100 mg. of the diketoacetate in 10 cc. of ethyl alcohol and 3 cc. of concentrated hydrochloric acid was refluxed for four hours on a steam-bath, diluted with water and the product was extracted with ether. The ether solution was washed with water and evaporated to dryness to leave a residue which was distilled in a molecular still. The product was impure and therefore purified by fractionation through a column of aluminum oxide to give 30 mg. of product melting at 189°.

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.2; H, 9.6. Found: C, 79.7; H, 9.6.

This product gave no depression in melting point with the alkaline hydrolysis product.

Reduction of the Unsaturated Diketone.—To a solution of 200 mg. of the above diketone, m. p. 190°, in 100 cc. of absolute ethyl alcohol was added 4 g. of sodium over a period of one-half hour. The solution was diluted with water and the product was extracted with ether. The ether solution after being thoroughly washed with water was evaporated to dryness, leaving a residue which after distillation was crystallized from dilute methyl alcohol, m. p. 255°.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.8; H, 11.3. Found: C, 78.9; H, 11.3.

This diol gave a diacetate on treatment with excess acetic anhydride which was crystallized from acetone, m. p. 140°.

Anal. Calcd. for $C_{25}H_{40}O_4$: C, 74.2; H, 10.0. Found: C, 74.0; H, 10.0.

Oxidation of the Dihydroxy Compound.—To a solution of 400 mg. of the dihydroxy compound in 5 cc. of acetic acid was added a solution of 600 mg. of chromic anhydride in 15 cc. of 80% acetic acid. After standing for fifteen minutes the solution was diluted with water and extracted with ether. The ether solution was washed with water dilute sodium carbonate and evaporated to dryness.

(9) Windaus, *Ber.*, **40**, 257, 2637 (1907).
(10) Diels and Linn, *ibid.*, **41**, 260 (1908).

The residue after distillation was purified by crystallization from dilute methyl alcohol, m. p. 128°.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.7; H, 10.2. Found: C, 80.1; H, 10.4.

This product gave a 2,4-dinitrophenylhydrazone (yellow), m. p. 245°.

Anal. Calcd. for $C_{33}H_{40}N_8O_8$: C, 58.6; H, 6.0. Found: C, 58.0; H, 6.0.

allo-Pregnane.—To a solution of 300 mg. of the diketomonoacetate in 30 cc. of acetic acid was added 10 g. of amalgamated zinc and 30 cc. of concentrated hydrochloric acid. The solution was refluxed for four hours, diluted with water and the product was extracted with ether. The ether solution was washed thoroughly with water and evaporated to dryness to leave a residue which was dissolved in acetone–methyl alcohol and allowed to stand overnight. The crystalline product so obtained was recrystallized from acetone–methyl alcohol to give *allo*-pregnane, m. p. 82°, 52 mg. This did not depress the melting point of *allo*-pregnane prepared by the Clemmensen reduction of *allo*-pregnanedione, but gave a 26° depression in melting point when mixed with pregnane. The hydrocarbon was saturated to bromine.

Anal. Calcd. for $C_{21}H_{36}$: C, 87.4; H, 12.6. Found: C, 87.0; H, 12.6.

The mother liquors contained the major product which was an oily hydrocarbon, unsaturated to bromine. It was readily distilled as an oil at 65–70° in high vacuum. Catalytic reduction of 150 mg. of this oil with Adams catalyst gave 100 mg. of impure *allo*-pregnane, m. p. 65–72°, which on recrystallization from acetone–methyl alcohol gave *allo*-pregnane, m. p. 80–82°. This gave no depression with the known *allo*-pregnane. It has been shown that the diketomonoacetate with hydrochloric acid gives an unsaturated diketone. Reductions of this diketone formed by the above acidic medium gave the above mixture of *allo*-pregnane and the unsaturated hydrocarbon.

Treatment of the Diketomonoacetate with Hydrazine.—A mixture of 100 mg. of diketomonoacetate, 10 cc. of 95% ethyl alcohol and 0.8 cc. of hydrazine hydrate was warmed at 80° for four hours. The solution was then evaporated to dryness and the residue was dissolved in hot benzene. Some insoluble material remained. The benzene solution was concentrated to 3 cc. and diluted with ligroin. This gave an amorphous powder which started to decompose at 210° but did not melt. This process of crystallization was repeated several times giving a product decomposing from 230 to 300°. The nitrogen in this sample was found to be 10.3%; calculated for pyridazine, 7.6. The mother liquors were concentrated and then diluted with ligroin to give a white powder decomposing about 250° but with no definite melting point. This gave an analysis for nitrogen of 8.2%.

Treatment of *allo*-pregnanedione in a parallel reaction gave similar white amorphous solid which started decomposing at 300° but was not melted at 325°. It analyzed for nitrogen, 10.5%, showing no simple definite product formed.

Oxidation of Pregnanetriol.—To a solution of 6 g. of pregnanetriol in 600 cc. of glacial acetic acid at 25° was added dropwise and with stirring a solution of 8 g. of chromic anhydride in 60 cc. of 80% acetic acid. The addition required two hours and the temperature of the solution remained at 35° during this time. The solution was allowed to stand at 25° overnight and then to it was added 10 cc. of methyl alcohol to remove the excess oxidizing agent. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in 500 cc. of water and 500 cc. of ether. The ether solution was washed with water several times and then extracted with sodium carbonate solution twice. It was further extracted with 10% potassium hydroxide solution, washed thoroughly with water and evaporated to dryness to leave a very small residue of a red oil which gave no crystalline material. Distillation of this product indicated it was mainly diketo compounds distilling at 120–130° in the molecular still.

The sodium carbonate solution was extracted with ether, acidified with hydrochloric acid and again extracted with ether to remove the acids. The ether solution was washed thoroughly with water and evaporated to dryness to leave a residue of 2 g. of yellow acids. This was taken up in acetone and benzene, allowed to stand for several days and yielded 200 mg. of a white crystalline acid, 3-keto-*etio-allo*-bilianic acid, m. p. 256°, with decomposition. Further crystallization gave the pure acid, m. p. 260°.

Anal. Calcd. for $C_{19}H_{28}O_5$: C, 67.8; H, 8.4. Found: C, 68.0; H, 8.3.

The remainder of the acid fraction was the yellow α-diketo acid (IV). The potassium hydroxide solution was extracted with ether, then acidified with hydrochloric acid and the acid extracted with ether. The ether solution was washed thoroughly with water and evaporated to dryness to give a further quantity of 3-keto-*etio-allo*-bilianic acid which on crystallization from acetone melted at 260° with decomposition; yield 400 mg. It gave no depression with the above sample of the same acid.

Anal. Calcd. for $C_{19}H_{28}O_5$: C, 67.8; H, 8.4. Found: C, 67.6; H, 8.3.

This existed in the solution as an anhydride and was probably formed by lactonization of acid (IV) followed by oxidation.

3-Keto-etio-allo-Bilianic Acid.—The above acid, m. p. 260°, gave a Zimmermann test. It gave a yellow 2,4-dinitrophenylhydrazone, m. p. 260°.

Anal. Calcd. for $C_{25}H_{32}O_8N_4$: C, 58.1; C, 6.3. Found: C, 57.7; H, 6.4.

The acid was refluxed for one hour with acetic anhydride and then distilled in a molecular still at 160°. This gave an anhydride which on crystallization from ethyl alcohol gave heavy needles, m. p. 224°.

Anal. Calcd. for $C_{19}H_{26}O_4$: C, 71.7; H, 8.2. Found: C, 71.4; H, 8.3.

Treatment of the acid with diazomethane gave a dimethyl ester which was crystallized from dilute methyl alcohol, m. p. 135°.

Anal. Calcd. for $C_{21}H_{32}O_5$: C, 69.2; H, 8.9. Found: C, 69.2; H, 8.9.

These last two compounds gave Zimmermann tests.

The dimethyl ester gave a semicarbazone melting at 200°.

Anal. Calcd. for $C_{22}H_{35}O_5N_3$: C, 62.6; H, 8.4. Found: C, 62.8; H, 8.2.

etio-allo-Bilianic Acid.—A solution of 200 mg. of 3-keto-*etio-allo*-bilianic acid and 20 cc. of acetic acid was placed with 8 g. of amalgamated zinc and to it was added 20 cc. of concentrated hydrochloric acid. The solution was refluxed gently for six hours, cooled and poured into 250 cc. of water. The product was extracted with ether and the ethereal solution, after being thoroughly washed with water, was evaporated to dryness to leave a white solid, m. p. 256°. After crystallization from methyl alcohol it melted at 260° and gave no Zimmermann test.

Anal. Calcd. for $C_{19}H_{30}O_4$: C, 70.8; H, 9.4. Found: C, 70.9; H, 9.4.

An anhydride was prepared by refluxing a portion of this acid with excess acetic anhydride for two hours and then distilling the residue in a molecular still at 140°. Crystallization from methanol gave the anhydride, m. p. 184°.

Anal. Calcd. for $C_{19}H_{28}O_3$: C, 75.0; H, 9.3. Found: C, 75.3; H, 9.3.

Acid IV.—This acid[3] which existed as a yellow oil when in solution or precipitated from organic solvents can be obtained crystalline by dissolving it in sodium carbonate solution, acidifying the solution and allowing the precipitated acid to stand for several days, m. p. 95–98°.

Anal. Calcd. for $C_{21}H_{30}O_5 \cdot H_2O$: C, 66.3; H, 8.5. Found: C, 66.2; H, 8.4.

The benzylthiouronium salt was prepared by dissolving 50 mg. of the acid in 1 cc. of alcohol, neutralizing to phenolphthalein with sodium hydroxide and adding 28 mg. of benzylthiouronium chloride in 1 cc. of alcohol. The solution on dilution with water gave a solid which was readily crystallized from acetone giving yellow needles melting at 176°.

Anal. Calcd. for $C_{29}H_{40}O_5N_2S$: C, 65.9; H, 7.7. Found: C, 65.8; H, 7.8.

The benzylthiouronium salt was hydrolyzed with an aqueous solution of dilute hydrochloric acid and the acid was extracted with ether. The ethereal solution was extracted with sodium carbonate and this was acidified slowly and the cloudy solution was allowed to stand. The acid crystallized in white fibrous clusters covering the bottom of the flask. These were filtered and melted at 95–98°.

This acid gives a dioxime, m. p. 183°.

Anal. Calcd. for $C_{21}H_{32}O_5N_2$: C, 65.3; H, 8.2; N, 7.1. Found: C, 64.0; H, 8.3; N, 6.6.

To a solution of 100 mg. of acid IV in 3 cc. of dioxane and 2 cc. of 5% sodium hydroxide was added an iodine–potassium iodide solution until the color of iodine disappeared very slowly. On dilution with 10 cc. of water a precipitate was formed. This was filtered off as a yellow solid with the distinct odor of iodoform, m. p. 120–123°. The remaining product was not recovered.

To a solution of 50 mg. of the acid in 10 cc. of methyl alcohol was added 12.5 cc. of potassium periodate solution to stand for forty-eight hours and then it was treated with an excess of potassium iodide solution and the iodine liberated was titrated with 0.053 *N* sodium thiosulfate: required 37 cc. of sodium thiosulfate; calculated, 37.4 cc.

Treatment of Pregnanetriol with Sodium in Amyl Alcohol.—To a refluxing solution of 4 g. of pregnanetriol in 700 cc. of amyl alcohol was added 25 g. of sodium over a period of one-half an hour. The solution was then refluxed for ten hours and 500 cc. of alcohol was then distilled off. The residue was diluted with one liter of water and extracted with ether. The ether solution was washed thoroughly with water and then evaporated and the remaining amyl alcohol was steam distilled off. The water solution was decanted from the oily residue and this residue was dissolved in 400 cc. of alcohol. A solution of 14 g. of digitonin in alcohol was added and after twelve hours standing the digitonide was filtered off, and dried (11 g.). This was heated for one hour with 50 cc. of dry pyridine and the pyridine solution was poured into 500 cc. of ether. The digitonin which precipitated was filtered off, washed with ether and the ether solution was washed with water, dilute hydrochloric acid and water. Evaporation of the ether gave an oil which was very difficult to crystallize and was apparently a mixture. It was converted to the acetate with acetic anhydride and these were separated by fractionation through a column of aluminum oxide (25 g.), in a solution of benzene–ligroin. This gave 0.5 g., m. p. 146°, which was recrystallized from methyl alcohol to give plates melting at 150°.

This gives an analysis for the diacetate of a diol with an amyl group.

Anal. Calcd. for $C_{30}H_{50}O_4$: C, 76.0; H, 10.6. Found: C, 76.0; H, 10.8.

Hydrolysis of this acetate gave the corresponding diol which was crystallized from dilute methyl alcohol, m. p. 209°.

Anal. Calcd. for $C_{26}H_{46}O_2$: C, 79.9; H, 11.9. Found: C, 79.8; H, 12.0.

Summary

Oxidation of "pregnanetriol-B" gave a mixture of a triketomonobasic acid (IV) and 3-keto-*etio-allo*-bilianic acid. The latter was converted to *etio-allo*-bilianic acid. The formation of these acids, together with the formation of *allo*-pregnanedione from triol-B, and the failure of the triol to react with lead tetraacetate show the structure of "pregnanetriol-B" to be *allo*-pregnanetriol-3(α),16,20.

The hydrocarbon which we previously prepared from this triol was found to be *allo*-pregnane.

Partial hydrolysis of triol-B triacetate followed by oxidation gave a diketomonoacetate, confirming the findings of Odell and Marrian, but which cannot be a γ-diketo compound.

Hydrolysis of the diketo monoacetate gave an α,β-unsaturated diketone which is probably $\Delta^{17,20}$-*allo*-pregnanedione-3,16.

Isomerization of triol-B takes place on treatment with sodium in amyl alcohol and a digitonide is formed indicating the triol to be of the *allo* series.

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