

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

Sterols. LIV. Pregnanone-20, *allo*-Pregnanone-20 and their Reduction Products

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In continuation of our study of the stereoisomeric pregnanones and pregnanols¹ we have now prepared the 20-ketopregnanones epimeric at C₅, and the 20-hydroxypregnanones epimeric at C₅ and C₂₀.

The starting material for the preparation of pregnanol-20(α) was pregnanol-20(α)-one-3. Since in our preparation of pregnanol-3(α) from pregnanol-3(α)-one-20, we had found that the Wolff-Kishner method gave considerably higher yields than the Clemmensen method, the former was investigated first. To our surprise we found that the Wolff-Kishner mode of reduction yielded almost exclusively pregnanediol-3(α),20(α); in fact, no other product could be detected despite a careful examination of the mother liquors. When pregnanol-20(α)-one-3 acetate was refluxed for two hours with amalgamated zinc and a mixture of hydrochloric and acetic acids, reduction proceeded too far, for only a very small amount of pregnanol-20(α) could be isolated. However, good yields of the desired product were obtained when the reaction was conducted under milder conditions using an amalgamated zinc and a small amount of hydrochloric acid diluted with alcohol. The pregnanol-20(α) thus obtained formed an acetate and yielded pregnanone-20 on oxidation with chromic anhydride. In an attempt to prepare *etio*-cholic acid by more vigorous oxidation, pregnanol-20(α) was heated for an hour on a steam-bath with a large excess of chromic anhydride, but again pregnanone-20 was obtained in excellent yields, and only traces of acids were formed. This resistance of a carbonyl group at C-20 to further oxidation has been observed previously by Butenandt,² who showed that 20-keto-3||4-pregnane-3,4-dicarboxylic acid was unaffected by chromic anhydride.

Pregnanone-20 was prepared by Butenandt, Hildebrand, and Bruecher³ by the Wieland-Barbier degradation of cholic acid. We have found that it can be prepared very conveniently by the partial Clemmensen reduction of pregnanedione, using a procedure similar to that

employed by Wieland and co-workers⁴ for the preparation of 12-ketocholic acid and 7,12-diketocholic acid from dehydrodesoxycholic acid and dehydrocholic acid, respectively. When pregnanone is hydrogenated pregnanol-20(β) is formed together with about 20% of a mixture of hydrocarbons. Considerable difficulty was experienced in obtaining pregnanol-20(β) crystalline since it is extremely soluble in all solvents, and separates from mixed solvents as an oil. The sirup first obtained formed a crystalline acetate that on hydrolysis regenerated the oily carbinol. This crystallized after standing for three weeks. The formation of a mixture of hydrocarbons as a by-product of the hydrogenation of pregnanone-20 is important in providing an additional example of the complete reduction to a methylene group of a carbonyl group adjacent to a tertiary hydrogen atom.⁵ While pregnane was probably present in the hydrocarbon mixture, since seeding with a few crystals gave a crystalline deposit, the crystal crop was too small in quantity to permit further examination.

Using the same method of reduction which had been applied successfully to pregnanedione and pregnanol-20(α)-one-3 acetate, we obtained, from *allo*-pregnanedione, *allo*-pregnanol-20(α)-one-3 and *allo*-pregnanol-20(β)-one-3 acetate,⁶ the desired substances, *allo*-pregnanone-20, *allo*-pregnanol-20(α), and *allo*-pregnanol-20(β), in excellent yields. *allo*-Pregnanone-20, which was characterized by its semicarbazone and its 2,4-dinitrophenylhydrazone, was also obtained by the oxidation of *allo*-pregnanol-20(α). Catalytic hydrogenation of *allo*-pregnanone-20 yielded *allo*-pregnanol-20(β). These interconversions leave no doubt as to the correctness of our assigned structures for the new substances.

The authors express their thanks to Dr. Eugene L. Wittle for generously providing the pregnanol-20(α)-one-3 acetate and the *allo*-pregnanol-20(α)-one-3 used in this work, and to Dr. Oliver Kamm and Parke, Davis and Company for their financial support of this research program.

(1) Marker and Lawson, *THIS JOURNAL*, **60**, 2438 (1938).

(2) Butenandt, *Ber.*, **63**, 660 (1930).

(3) Butenandt, Hildebrand and Bruecher, *ibid.*, **64**, 2529 (1931).

(4) Wieland and Schlichting, *Z. physiol. Chem.*, **160**, 267 (1925); Wieland, Dane and Kraft, *ibid.*, **210**, 281 (1932); Wieland and Dane, *ibid.*, **216**, 97 (1933).

(5) Marker, *THIS JOURNAL*, **60**, 1733 (1938).

(6) Marker, Kamm, Jones and Oakwood, *ibid.*, **59**, 614 (1937).

Experimental Part

Wolff-Kishner Reduction of Pregnanol-20(α)-one-3 Acetate.—A mixture of 1.1 g. of pregnanol-20(α)-one-3 acetate, 0.6 g. of semicarbazide hydrochloride, 0.9 g. of sodium acetate, and 20 cc. of alcohol was refluxed for three hours, and then diluted with water. After standing overnight in the refrigerator, the precipitate was collected and washed with water to give 1.34 g. of crude semicarbazone, m. p. 200–204°, dec.

Without further purification this semicarbazone (1.34 g.) was heated for seven hours at 180° with a solution of 1 g. of sodium in 12 cc. of absolute alcohol. The reaction mixture was washed out of the bomb tube with ether and water, and the insoluble crystalline material collected and washed with ether to obtain 0.67 g., m. p. 230°. After recrystallization from alcohol-acetone this product, pregnanediol-3(α),20(α) melted at 240° and did not depress with an authentic sample, m. p. 240°, but depressed to 205° with pregnanediol-3(α),20(β), m. p. 231°. The ethereal extract and the alcohol-acetone mother liquor were investigated further but no product other than pregnanediol-3(α),20(α) was present in appreciable quantities; yield 85%.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.4; H, 11.3.

The acetate, after crystallization from methanol, melted at 178° and gave no depression with pregnanediol-3(α),20(α) diacetate, m. p. 180°.

Anal. Calcd. for $C_{28}H_{46}O_4$: C, 74.2; H, 10.0. Found: C, 74.5; H, 10.0.

Clemmensen Reduction of Pregnanol-20(α)-one-3 Acetate. **A.**—A mixture of 1 g. of pregnanol-20(α)-one-3 acetate, 40 g. of previously amalgamated 40-mesh zinc, 100 cc. of concentrated hydrochloric acid, and 100 cc. of acetic acid was refluxed for two hours. The reaction mixture was diluted with water and the product isolated by extraction with ether. After removal of the ether the residual sirup was heated with an excess of alcoholic hydroxide solution, and the product isolated by extraction with ether. The ether was evaporated, and the residual sirup separated into hydroxy and non-hydroxy fractions by the acid-succinate method. The hydroxy fraction (25 mg.) was crystallized from slightly diluted alcohol to give pregnanol-20(α), m. p. 144°, which did not depress with a sample prepared as described below. The non-hydroxy fraction was a mixture of hydrocarbons from which no crystalline products could be separated.

B.—A mixture of 1 g. of pregnanol-20(α)-one-3 acetate, 10 g. of zinc strips (Baker c. p.), 10 cc. of alcohol, and 2 cc. of concentrated hydrochloric acid was refluxed for thirteen hours. At hourly intervals 1–2-cc. portions of a mixture of 15 cc. each of concentrated hydrochloric acid and alcohol were added. The mixture was cooled, diluted with water, extracted with ether, and the ethereal extract washed free of acid. After evaporating the ether, the solid residue was heated for one-half hour with a solution of 0.5 g. of sodium hydroxide in 15 cc. of alcohol. The solution was cooled, diluted with water, and extracted with ether. The ethereal extract was washed, the ether removed on the steam-bath, and the residue crystallized from petroleum ether to give 500 mg. of pregnanol-20(α) as needle-roses, m. p. 146°.

Anal. Calcd. for $C_{21}H_{36}O$: C, 82.8; H, 11.8. Found: C, 82.6; H, 11.7.

The acetate melted at 130° after crystallization from methanol.

Anal. Calcd. for $C_{23}H_{38}O_2$: C, 79.7; H, 11.1. Found: C, 79.7; H, 10.8.

Oxidation of Pregnanol-20(α). **A.**—To a solution of 50 mg. of pregnanol-20(α) in 3 cc. of acetic acid was added a solution of 20 mg. of chromic anhydride in 2 cc. of 90% acetic acid. After an hour methanol was added and the solvents removed *in vacuo*. The neutral fraction, isolated in the usual manner, was crystallized from dilute alcohol to give 20 mg. of long needles of pregnanone-20, m. p. 115°. This sample did not depress with pregnanone-20 prepared from pregnanedione (*vide infra*).

B.—An attempt was made to obtain *etio*-cholic acid by oxidation of pregnanol-20(α) under more vigorous conditions. A solution of 120 mg. of pregnanol-20(α) and 180 mg. of chromic anhydride in 10 cc. of 95% acetic acid was heated for one hour on the steam-bath. The reaction mixture, worked up in the usual manner, yielded only traces of acid; instead pregnanone-20, m. p. 115°, was obtained in excellent yield.

Pregnanone-20 from Pregnedione.—To a refluxing mixture of 1 g. of pregnanedione, 10 g. of zinc strips, and 10 cc. of alcohol were added over a period of thirteen hours, 1–2-cc. portions of a mixture of 15 cc. each of hydrochloric acid and alcohol. The mixture was decanted while still hot from the zinc and the latter washed with a little slightly diluted boiling alcohol. The combined washings and solution yielded on cooling 450 mg. of long needles. The mother liquor was evaporated, and the ketones isolated by means of Girard's reagent to give an additional 50 mg. of needles. Recrystallization from diluted alcohol gave pure pregnanone-20, m. p. 116°. It did not give a coloration with alcoholic *m*-dinitrobenzene and alkali. When the reaction was run as above, but without diluting with alcohol the hydrochloric acid added, the yield dropped to 220 mg.

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.4; H, 11.4. Found: C, 83.0; H, 11.2.

The semicarbazone, prepared in the usual manner, after crystallization from methanol, melted at 237°, dec.

Anal. Calcd. for $C_{22}H_{37}ON_3$: C, 73.5; H, 10.4. Found: C, 73.5; H, 10.4.

The 2,4-dinitrophenylhydrazone melted at 240°, dec.

Anal. Calcd. for $C_{27}H_{38}O_4N_4$: C, 67.3; H, 8.0. Found: C, 67.4; H, 8.1.

Catalytic Hydrogenation of Pregnanone-20.—A mixture of 500 mg. of pregnanone-20, 500 mg. of platinum oxide catalyst, and 125 cc. of acetic acid was shaken for four hours in a hydrogen atmosphere at 40 pounds (2.67 atm.) pressure. The catalyst was removed by filtration and the filtrate evaporated *in vacuo*. The residual sirup was heated for one-half hour with 40 cc. of alcohol and 3 cc. of 50% sodium hydroxide solution, cooled and extracted with ether. After washing, the ethereal extract was evaporated to leave a sirup which would not crystallize; so it was separated into hydroxy and non-hydroxy fractions by means of pyridine and phthalic anhydride. The non-hydroxylated sirup, amounting to about 100 mg.,

had the solubility behavior of a hydrocarbon. In acetone solution it deposited, after seeding with pregnane, a crystal crop, which, however, was too small and too gummy to characterize further. The hydroxy fraction, amounting to about 400 mg. of sirup, could not be crystallized because of its extreme solubility in all ordinary solvents; so it was dissolved in benzene-petroleum ether and passed through a 40×8 mm. diameter column of activated aluminum. Fractions were collected by the "Durchlaufen" method, using petroleum ether to which increasing larger amounts of benzene were added. The first three elutions, on evaporation, gave about 350 mg. of colorless sirup which still resisted all efforts at crystallization. Accordingly, the sirupy carbinol was acetylated by refluxing it for one-half hour with 7 cc. of acetic anhydride. The sirupy residue, after removal of the acetic anhydride, was crystallized from dilute methanol to give 400 mg. of somewhat impure pregnanol-20(β) acetate, m. p. 78-82°. Recrystallization from dilute methanol raised the melting point to 85°.

Anal. Calcd. for $C_{23}H_{38}O_2$: C, 79.7; H, 11.1. Found: C, 79.6; H, 11.0.

Some of the purified acetate, m. p. 85°, was hydrolyzed, and worked up in the usual manner. The sirupy carbinol obtained crystallized as needle-roses after standing for three weeks.

allo-Pregnanol-20(α).—Seven hundred milligrams of *allo*-pregnanol-20(α)-one-3 was reduced by the partial Clemmensen method described for the preparation of pregnanone-20 from pregnanedione. The product, isolated by extraction with ether, was carried through the half-phthalate separation in the usual manner to yield about 100 mg. of non-hydroxylated sirup, and about 500 mg. of sirupy carbinol. The latter did not give a coloration with alcoholic *m*-dinitrobenzene and alkali. It was crystallized from benzene-petroleum ether to give 90 mg. of crystals, m. p. 200-215°. This was recrystallized from methanol to give 30 mg. of product melting at 229°, which depressed to 215° with *allo*-pregnandiol-3(α),20(α).

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.9; H, 11.4.

The acetate crystallized from dilute alcohol as needles, m. p. 162°.

Anal. Calcd. for $C_{25}H_{40}O_4$: C, 74.2; H, 10.0. Found: C, 74.1; H, 10.0.

The benzene-petroleum ether mother liquor deposited 250 mg. of crystals, m. p. 118-125°. This crop could not be purified by crystallization, so the substance, in benzene-petroleum ether, was filtered through a column of activated alumina and eluted with benzene-petroleum ether. The combined filtrate was evaporated to dryness. The residue crystallized as long needles from dilute methanol to give 70 mg. of *allo*-pregnanol-20(α), m. p. 136°.

Anal. Calcd. for $C_{21}H_{36}O$: C, 82.8; H, 11.8. Found: C, 82.9; H, 12.0.

The acetate formed needles, m. p. 94°, from dilute methanol.

Anal. Calcd. for $C_{23}H_{38}O_2$: C, 79.7; H, 11.1. Found: C, 79.7; H, 11.0.

The total mother liquors obtained both from the carbinol, m. p. 229°, and from the *allo*-pregnanol-20(α), were

combined, dissolved in benzene-petroleum ether and filtered through a tube of activated alumina. The filtrate gave about 0.3 g. of product which on crystallization gave 270 mg. of *allo*-pregnanol-20(α). The alumina was leached with boiling alcohol, and the latter evaporated to give about 50 mg. of solid which was recrystallized from benzene-petroleum ether to give an additional 20 mg. of carbinol, m. p. 229°. On oxidation of 20 mg. of this carbinol with an excess of chromic anhydride no acids were obtained, but only a small amount of an oily neutral material.

allo-Pregnanone-20 from *allo*-Pregnanol-20(α).—A solution of 50 mg. of chromic anhydride in 2 cc. of 90% acetic acid was added to a solution of 50 mg. of *allo*-pregnanol-20(α). After two hours some methanol was added and the solvents were removed *in vacuo*. The residue was taken up in dilute hydrochloric acid and ether, and the ether layer washed free of acids. The ether was evaporated and the residue crystallized from slightly diluted methanol to give 20 mg. of *allo*-pregnanone-20, m. p. 129°, which did not depress with a sample prepared by the partial Clemmensen reduction of *allo*-pregnanedione.

allo-Pregnanone-20 from *allo*-Pregnedione.—One gram of *allo*-pregnanedione was partially reduced by the procedure used for the preparation of pregnanone-20 from pregnanedione. The product was isolated by extraction with ether and recrystallized from slightly diluted alcohol and from methanol to give 430 mg. of *allo*-pregnanone-20, m. p. 129°. The mother liquor, after purification through the use of Girard's reagent, gave an additional 100 mg. *allo*-Pregnanone-20 does not give a red coloration with alcoholic *m*-dinitrobenzene and alkali.

Anal. Calcd. for $C_{21}H_{34}O$: C, 83.4; H, 11.4. Found: C, 83.5; H, 11.4.

Its semicarbazone, prepared in the usual manner, melted at 260°, dec.

Anal. Calcd. for $C_{22}H_{37}ON_3$: C, 73.5; H, 10.4. Found: C, 73.6; H, 10.6.

The 2,4-dinitrophenylhydrazone melted at 220-223°.

Anal. Calcd. for $C_{27}H_{38}O_4N_4$: C, 67.4; H, 8.0. Found: C, 67.0; H, 7.8.

allo-Pregnanol-20(β) from *allo*-Pregnanol-20(β)-one-3 Acetate.—Five hundred milligrams of *allo*-pregnanol-20(β)-one-3 acetate, m. p. 156°, was reduced by the method described for the preparation of pregnanone-20 from pregnanedione. The product was removed from the reaction mixture with ether, hydrolyzed with alcoholic sodium hydroxide and crystallized from dilute alcohol and methanol to give needles of *allo*-pregnanol-20(β), m. p. 140°.

Anal. Calcd. for $C_{21}H_{36}O$: C, 82.8; H, 11.8. Found: C, 83.2; H, 12.0.

Its acetate crystallized from slightly diluted alcohol as needles, m. p. 156°. This acetate had the same superficial appearance and solubility behavior as the starting material, *allo*-pregnanol-20(β)-one-3 acetate, m. p. 156°, and depressed with it to only 152°, but in contrast to the latter it did not give a Zimmermann test.

Anal. Calcd. for $C_{23}H_{38}O_2$: C, 79.7; H, 11.1. Found: C, 79.4; H, 11.2.

allo-Pregnanol-20(β) from *allo*-Pregnanone-20.—A mixture of 200 mg. of *allo*-pregnanone-20, 200 mg. of platinum

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 C₅ and C₂₀ are described.

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Sterols. LV. The Structure of Pregnanetriol-B

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Some years ago a carbinol, C₂₁H₃₆O₃, was isolated from mares pregnancy urine by Haslewood, Marrian and Smith.¹ Later² we obtained this carbinol, which was designated as pregnanetriol-B, together with another, pregnanetriol-A (urane-triol), 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 Marrian⁴ 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 previously³ 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 benzylthiuronium salt from which it may be regenerated by hydrolysis. It reacts with periodic acid, indicating

the $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{C}- \\ \parallel \\ \text{O} \end{array}$ grouping, gives the iodoform reaction, indicating the $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{CH}_3 \end{array}$ grouping, and gives a positive Zimmermann test, indicating the pres-

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

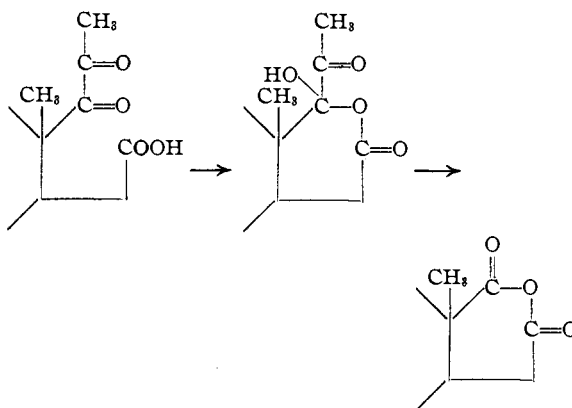
(2) Marker, Kamm, Crooks, Oakwood, Wittle and Lawson, *THIS JOURNAL*, **60**, 210 (1938).

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

(4) Odell and Marrian, *Biochem. J.*, **126**, 333 (1938).

ence 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 C₁₉H₂₈O₅.