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

Sterols. LII. Reduction Products of Progesterone and the Pregnanediones

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Several years ago Butenandt and Fleischer[1] hydrogenated progesterone and obtained a diol mixture which on oxidation yielded pregnanedione and *allo*-pregnanedione. We have repeated this work and find that the diol mixture can be separated readily by the use of digitonin to give the two principal products of the reaction, pregnanediol-3(α),20(β) and *allo*-pregnanediol-3(β),20(β).

Since the stereochemical course of the reduction of a carbonyl group at C-3 is governed by the Skita rule[2] so that in the presence of mineral acids the major product is that in which the 3-OH group is *cis* to the hydrogen atom at C-5, it was hoped that similar conditions might lead to the reduction of a C-20 carbonyl group to give a C-20 (α) hydroxy compound. It was known that in the absence of mineral acids C-20(β)-hydroxy compounds were produced almost exclusively. The following table shows the results of the hydrogenation in acetic acid of the steroids previously studied.

TABLE I

Substance hydrogenated	Product
allo-Pregnanedione[3]	*allo*-Pregnanediol-3(β),20(β)
Pregnanedione[4]	Pregnanediol-3(β),20(β)
allo-Pregnanol-3(α)-one-20[5]	*allo*-Pregnanediol-3(α),20(β)
Pregnanol-3(α)-one-20[6]	Pregnanediol-3(α),20(β)

In the case of the reduction in acetic acid of pregnanedione, the chief product is pregnanediol-3(β), 20(β), although one might expect, in the absence of mineral acid, that the chief product would be pregnanediol-3(α),20(β). A reëxamination of this reaction has shown that some of the latter is indeed formed, but in smaller amounts than its epimer. Apparently, the compounds of the regular and *allo* series do not conform in an exactly parallel way to the Skita rule. We have now found that the catalytic hydrogenation, in the presence of mineral acids, of pregnanedione or *allo*-pregnanedione yields mainly pregnanediol-3(β),20(β), or *allo*-pregnanediol-3(α),20(β), respectively. While very little pregnanediol-3(α),20(β) is produced in the former case, in the latter about 20% of *allo*-pregnanol-3(β),20(α) is also obtained. Thus in neither case has it been possible, by conducting the hydrogenation in the presence of mineral acid, to reverse the normal formation of C-20(β) hydroxy compounds.

The partial catalytic hydrogenation of pregnanedione already has been reported.[7]

In the presence of mineral acid the chief product is pregnanol-3(β)-one-20; but if the hydrogenation is done in alcohol in the absence of acid the chief product is pregnanol-3(α)-one-20. The reduction of *allo*-pregnanedione in the presence of mineral acid has been reported by Fleischer, Whitman and Schwenk,[8] who obtained *allo*-pregnanol-3(α)-one-20 and *allo*-pregnanol-3(β)-one-20 by this procedure. Our experiments are in complete accord with their findings, but we find that the desired products are obtained more readily in a pure form if the hydrogenation is done at room temperature. We also have found that *allo*-pregnanol-3(α)-one-20, which is ordinarily difficult to purify because of its tendency to separate as an oil from dilute alcohol, is readily purified by crystallization from carbon tetrachloride.

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

Experimental Part

Catalytic Reduction of Progesterone.—A solution of 100 mg. of progesterone in 50 cc. of ethyl alcohol was shaken with 100 g. of platinum oxide catalyst under a pressure of 45 pounds (3 atm.) of hydrogen for one hour. The catalyst was filtered and the solution concentrated to 20 cc. An excess of 2% alcoholic digitonin solution was added and the solution set aside overnight. The digitonide was collected and washed with cold alcohol. It was dried and then heated on a steam-bath for thirty minutes with 10 cc. of pyridine. Ether was added and the mixture filtered from digitonin. The filtrate was freed of pyridine by shaking with dilute hydrochloric acid. The ether was evaporated and the residue crystallized from 70% methanol to give *allo*-pregnanediol-3(β),20(β), m. p. 194°; yield 20 mg. Mixed with an authentic sample of *allo*-pregnanediol-3(β),20(β), it gave no depression in melting point.

(1) Butenandt and Fleischer, *Ber.*, **68**, 2094 (1935).

(2) Ruzicka, Brungger, Eichenberger and Meyer, *Helv. Chim. Acta*, **17**, 1407 (1934).

(3) Marker, Kamm, Jones and Oakwood, THIS JOURNAL, **59**, 614 (1937).

(4) Marker, Kamm and McGrew, *ibid.*, **59**, 616 (1937).

(5) Marker and Kamm, *ibid.*, **59**, 1393 (1937).

(6) Marker, Kamm and Jones, *ibid.*, **59**, 1595 (1937).

(7) Marker, Kamm and Wittle, *ibid.*, **59**, 1841 (1937).

(8) Fleischer, Whitman and Schwenk, *ibid.*, **60**, 79 (1938).

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.6; H, 11.2.

The filtrate from the digitonide was evaporated to dryness, leached with ether, and filtered. The filtrate was evaporated to dryness and the residue crystallized from 70% acetone and 70% methanol to a constant melting point of 228°. The product, pregnanediol-3(α),20(β), gave no depression in melting point when mixed with an authentic sample; yield 30 mg.

Anal. Calcd. for $C_{21}H_{36}O_2$: C, 78.7; H, 11.3. Found: C, 78.5; H, 11.3.

Catalytic Reduction of *allo*-Pregnanedione in the Presence of Mineral Acids.—A solution of 1 g. of *allo*-pregnanedione in 100 cc. of glacial acetic acid was added to a suspension of 250 mg. of previously reduced platinum oxide in acetic acid. To this was added 2 cc. of hydrobromic acid and the mixture was shaken with hydrogen at 45 pounds (3 atm.) pressure for ninety minutes. The catalyst was filtered and the product precipitated by the addition of water. The precipitate was collected and dissolved in 100 cc. of alcohol. To this solution was added a solution of 2.5 g. of digitonin in 100 cc. of hot alcohol. After standing overnight, the digitonide was collected and washed with cold alcohol. The dried digitonide was worked up as usual to give *allo*-pregnanediol-3(β),20(β), m. p. 194°, after crystallization from dilute acetone. No depression was obtained on admixture with an authentic sample; yield 170 mg.

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

The filtrate from the digitonide was evaporated to dryness and the residue was shaken with ether and filtered. The filtrate was evaporated to dryness and the residue crystallized from 60% acetone to give *allo*-pregnanediol-3(α),20(β), m. p. 207°, which showed no depression in melting point with an authentic sample; yield 600 mg.

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

Catalytic Reduction of Pregnanedione in the Presence of Mineral Acid.—One gram of pregnanedione was hydrogenated in the presence of mineral acid in the same manner as that described above for the hydrogenation of *allo*-pregnanedione. The reaction mixture was worked up in the same way to give an insoluble digitonide which after decomposition and crystallization from 60% acetone yielded 820 mg. of pregnanediol-3(β),20(β), m. p. 178°. This substance did not depress with an authentic sample of pregnanediol-3(β),20(β).

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

The alcoholic filtrate from the digitonide above gave only a very small amount of pregnanediol-3(α),20(β).

Preparation of *allo*-Pregnanol-3(β)-one-20.—To a solution of 2 g. of *allo*-pregnanedione in 125 cc. of acetic acid was added 0.2 g. of platinum oxide catalyst, and the mixture shaken for fifteen minutes in a hydrogen atmosphere at 35 pounds (2.3 atm.) pressure. The catalyst was removed by filtration, and the filtrate concentrated *in vacuo* to a sirup. This sirup was dissolved in 40 cc. of alcohol, 2 g. of Girard's reagent added, and the mixture heated for twenty minutes on a steam-bath. The solution was diluted with water and extracted with ether. The aqueous solution was acidified strongly with hydrochloric acid and then heated for one hour on a steam-bath. The mixture was cooled, extracted with ether, and the ethereal solution washed with water. The ether was removed on a steam-bath, and the residue dissolved in 30 cc. of hot alcohol and added to a 2% solution of digitonin. The next day the digitonide was collected and washed with alcohol. The dried digitonide (weight 1.4 g.) was decomposed in the usual manner to give a product which after crystallization from alcohol melted at 193°. It gave no coloration with alcoholic *m*-dinitrobenzene and potassium hydroxide solution.

Anal. Calcd. for $C_{21}H_{34}O_2$: C, 79.2; H, 10.8. Found: C, 79.1; H, 10.9.

Forty milligrams of the *allo*-pregnanol-3(β)-one-20 obtained as described above was refluxed with 2 cc. of acetic anhydride for half an hour. The acetic anhydride was removed *in vacuo* and the residue recrystallized from diluted alcohol to give the acetate, m. p. 143°, of *allo*-pregnanol-3(β)-one-20.

Anal. Calcd. for $C_{23}H_{36}O_3$: C, 76.8; H, 10.1. Found: C, 76.5; H, 9.8.

Partial Catalytic Reduction of *allo*-Pregnanedione in the Presence of Mineral Acid.—A suspension of 1 g. of platinum oxide catalyst in 100 cc. of acetic acid was shaken with hydrogen for a few minutes, and a solution of 10 g. of *allo*-pregnanedione in 500 cc. of acetic acid containing 1.5 cc. of 48% aqueous hydrobromic acid added. The mixture was shaken with hydrogen at 40 pounds (2.6 atm.) pressure until 1 mol had been absorbed (twenty-three minutes). The mixture was filtered, the acetic acid removed *in vacuo*, and the sirupy residue separated into ketonic and non-ketonic fractions in the usual manner. The non-ketonic fraction, amounting to 3–4 g., proved to be mostly *allo*-pregnanediol-3(α),20(β). The ketonic fraction was separated into hydroxylic and non-hydroxylic fractions by means of the acid succinates. The non-hydroxylic fraction, amounting to 3–4 g., proved to be mostly *allo*-pregnanedione. The hydroxyl ketone fraction, amounting to 2 g., was crystallized from slightly diluted alcohol to give 1.1 g. of *allo*-pregnanol-3(β)-one-20, m. p. 191°. The mother liquor from this was crystallized from carbon tetrachloride, giving *epi-allo*-pregnanol-3(α)-one-20, m. p. 170°. It did not depress with an authentic sample.

Summary

Hydrogenation of progesterone gives pregnanediol-3(α),20(β) and *allo*-pregnanediol-3(β),-20(β). Hydrogenation in the presence of mineral acids of pregnanedione or *allo*-pregnanedione yields mainly pregnanediol-3(β),20(β), or *allo*-pregnanediol-3(α),20(β), respectively. The partial hydrogenation of *allo*-pregnanedione yields *allo*-pregnanol-3(α)-one-20 and *allo*-pregnanol-3(β)-one-20.

In every case studied the course of hydrogenation of a 20-carbonyl group leads to the

formation of 20(β) hydroxy compounds even when the reduction is done in the presence of mineral acids.

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The Keto-Enol Tautomerism of Pyruvate Ion Studied Polarographically*

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Introduction

Pyruvate ion holds a key position in practically all schemes of carbohydrate metabolism in the intermediary steps of which the enol form is often postulated. The only evidence for this enol form has been spectroscopic[1] as the usual methods for its determination have failed in this particular case.

In this paper, we present data regarding the keto/enol + enolate ratio and polymerization of pyruvic acid and of pyruvate ion in relation to *p*H, and determine the apparent reduction potential of both forms of pyruvate ion at different *p*H. These determinations are made electrochemically, using the polarographic method.

Method

Materials.—The pyruvate used in these experiments was kindly given to one of us (J. P. B.) by Dr. H. Borsook of Pasadena in the form of solid lithium pyruvate of great purity. All other chemicals were C. P. products. The McIlvane series of buffers was used for *p*H 2.2–8.0 and Clark and Lubs HCl–KCl for *p*H 1.0–2.2. The *p*H was checked by means of the glass electrode. The temperature at which these experiments were carried out was 25 ± 0.1° maintained in a water thermostat.

General Description of Polarographic Method.—The method makes use of a dropping mercury electrode as half-cell connected to a calomel half-cell by an agar bridge saturated with potassium chloride. A battery, slide wire potentiometer, and galvanometer are also in the circuit so that the voltage applied to the system may be varied and the resulting current measured. The procedure depends upon the fact that many compounds may be reduced at the surface of mercury dropping slowly from a capillary when the voltage applied is sufficiently negative. The resulting current is a function of the concentration of reducible substance, while the potential at which the reduction occurs is characteristic of the compound reduced. This principle has been incorporated into a very convenient apparatus, the polarograph, in which the current and voltage are automatically graphically coördinated on photographic paper to give a current–voltage curve, called a polarogram. J. Heyrovský and his school[2] are responsible for this development. On such a polarogram, the voltage is indicated by abscissas, and changes in current appear as waves.

Apparatus.—Our experiments were carried out with a Nejedlý polarograph Model VIII and a Nejedlý galvanometer with a sensitivity of 2.3×10^{-9} amp./mm./m. A special shunt permitted the modification of this sensitivity to any desired fraction, while critical damping was maintained.

The apparatus was so adjusted that the distance between two abscissas on the polarogram corresponded to 200 mv.

The dropping mercury electrode was made according to instructions given by Heyrovský[2]; its drop time in distilled water was six seconds when the mercury reservoir was 60 cm. above the capillary.

As oxygen is also reducible at the dropping mercury electrode, hydrogen gas was bubbled through all solutions before each experiment to remove the dissolved oxygen.

Instead of using a large layer of mercury at the bottom of the electrolysis vessel as non-polarizable anode, as is customary in polarographic work, we made use of a saturated calomel electrode with a large surface which was connected to the test solution by an agar bridge, saturated with potassium chloride. The potential of this separate electrode is constant[3] and its value is known, thus eliminating the troublesome measurement of the anode potential. All polarograms reproduced in this paper, therefore, show automatically the applied e. m. f. referred to the saturated calomel electrode.

For the plotting of graphs, liquid junction potentials have been neglected, while corrections for the iR drop[3] have been made in calculations of the half-wave potentials[4] which are all referred to the normal hydrogen electrode as zero. The accuracy of these values is ±20 mv., which seems sufficient for the present treatment. As we have shown[3] polarographic potentials may be read with an accuracy of ±1 mv. if proper precautions are taken.

Keto-Enol Tautomerism

This attempt to determine the concentration of keto and enol pyruvate ion was the direct outgrowth of the work on the coupled oxidation–reduction potential of pyruvate ⇄ lactate, carried out in this Laboratory.[5]

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