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

Sterols. XLI. Reduction of Naphtholic Steroids to Phenolic Steroids. Equilenin

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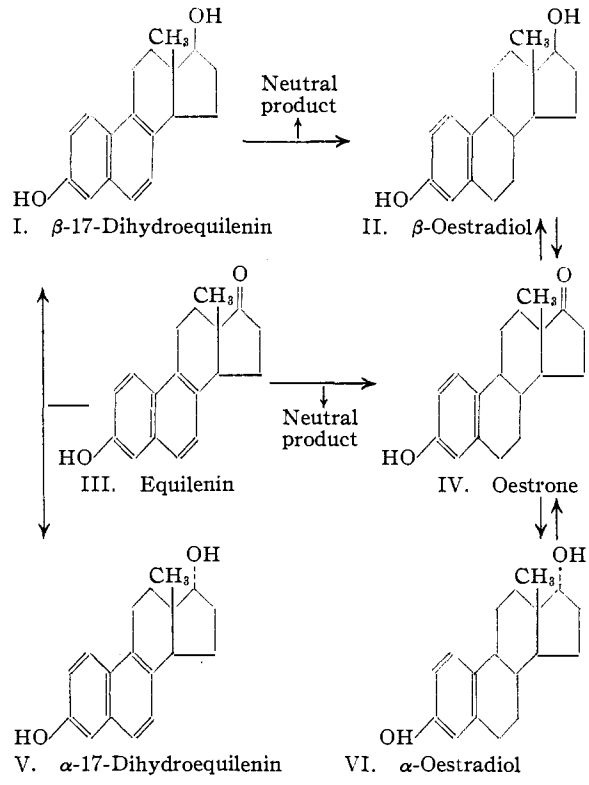
Some time ago we reported the preparation of oestrone from ergosterol.[1] Although no details of the synthesis could be included in that short note, we felt that since the entire synthesis had been checked by two of us, and since the oestrone was shown to be identical in all respects with the natural hormone, no doubt could exist as to the validity of our preparation. However, soon after the appearance of our note, Windaus and Deppe[2] reported that they had been unable to duplicate our preparation of the phenolic tetrahydrodehydroneoergosterol by the reduction of dehydroneoergosterol with sodium and amyl alcohol; in fact, they claimed to find almost no phenolic products in the reduction mixture. As a result of this paper, a number of authors[3-6] have questioned our claim to have prepared synthetic oestrone.

We are now prepared to present a series of papers showing that, contrary to the claims of Windaus and Deppe, β-naphtholic steroids can be reduced to give substantial yields of phenolic steroids as well as larger amounts of neutral steroids. These reductions, which have been carried out by the same general procedure as that used to prepare the phenolic steroid used in our synthesis of oestrone, are actually an extension of the classic studies of Bamberger and co-workers,[7] studies which apparently were overlooked by Windaus.

In this paper we shall describe the reduction of equilenin and of the isomeric 17-dihydroequilenins. The accompanying chart shows the transformation involved.



Since the products sought for in the reduction experiments included oestrone derivatives, great care was taken to purify the equilenin used in this work. Equilenin, m. p. 252°, obtained by repeated crystallizations was first converted into its picrate, and then into its benzoate. Hydrolysis of the equilenin benzoate after repeated recrystallization finally gave equilenin, m. p. 257–258°, $[\alpha]^{25}D + 89°$, of very high purity.

We have reported in a short note[8] that when equilenin is reduced with aluminum isopropylate and isopropyl alcohol, a mixture of α- and β-17-dihydroequilenins is formed. The β-17-dihydroequilenin, m. p. 215°, has been isolated as one component of the δ-follicular hormone, from mares' pregnancy urine, by Wintersteiner, Schwenk, Hirschmann and Whitman.[9] Since this substance does not precipitate with digitonin they suggested that it is configurationally related to β-oestradiol, for the latter, unlike α-oestradiol, also does not precipitate digitonin. Since Wintersteiner[10] has also suggested, on the basis of a com-

(1) Marker, Kamm, Oakwood and Laucius, THIS JOURNAL, **58**, 1503 (1936).

(2) Windaus and Deppe, *Ber.*, **70**, 76 (1937).

(3) Marrian, "The Chemistry of the Oestrogenic Hormones," in "Ergebnisse der Vitamin und Hormon-forschung," Ruzicka and Stepp, Akademische Verlagsgesellschaft m. b. H., Leipzig, pp. 446 ff.

(4) Remesov, *Rec. trav. chim.*, **56**, 1093 (1937).

(5) Strain, "The Sterols and Related Compounds," in Gilman's "Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1938, Chap. 15, pp. 1316 ff.

(6) Freedman, "Sterols and Related Compounds," Chemical Pub. Co., New York, N. Y., 1938.

(7) For example, Bamberger and Kitschelt, *Ber.*, **23**, 885–7 (1890), obtained *ar*-tetrahydro-β-naphthol by the reduction of β-naphthol with sodium and amyl alcohol (8% yield of purified product).

(8) Marker, Kamm, Oakwood and Tendick, THIS JOURNAL, **59**, 768 (1937).

(9) Wintersteiner, Schwenk, Hirschmann and Whitman, *ibid.*, **58**, 2652 (1936).

(10) Wintersteiner, *ibid.*, **59**, 765 (1937).

parison of physiological activities and melting points, that α-oestradiol and α-testosterone (*trans*-testosterone) are configurationally related, it follows that Wintersteiner's β-17-dihydroequilenin, m. p. 215–217°, has the structure I. We find that our β-17-dihydroequilenin, m. p. 215°, has the same properties as the natural hormone isolated by Wintersteiner; it forms a monobenzoate, m. p. 204°, and does not precipitate with digitonin. The less soluble α-17-dihydroequilenin, m. p. 248°, (V) forms a monobenzoate, m. p. 215°, and a diacetate, m. p. 124°. However, in contrast to the behavior of α-oestradiol, α-17-dihydroequilenin does not precipitate with digitonin. Tests[11] show that β-17-dihydroequilenin has an oestrogenic potency of 75–100 R. U. per mg. while the α-epimer has a somewhat greater potency, 250 R. U. per mg. Thus the two epimers are about one-fortieth to one-thirtieth, and one-twelfth as active as oestrone. This relatively low physiological activity of α-17-dihydroequilenin is contrary to the predictions of Wintersteiner, who thought he had isolated, in impure form, "α-dihydroequilenin" from mares' pregnancy urine. His product had a higher melting point than β-17-dihydroequilenin, and a high oestrogenic activity. Obviously, his product was contaminated with some highly active substance, possibly α-oestradiol. It is now evident that Wintersteiner's correlation of β-17-dihydroequilenin with β-oestradiol is correct, but his arguments —the non-precipitability with digitonin and low physiological activity of this, the lower melting epimeric 17-dihydroequilenin—have little bearing on the configuration, since the higher melting α-epimer is also not precipitated by digitonin, and also shows low oestrogenic potency. In this connection it should be emphasized that assignments of configuration based on a comparison of melting points are always to be regarded as only tentative, for the melting point relationships in a given type of epimeric steroids may be reversed by the introduction of double bonds. Thus while the melting points of *epi*-stigmastanol, *epi*-cholestanol, and similar *epi*-saturated steroids are considerably higher than for the corresponding β-epimers, this relationship is reversed in steroids of the type of cholesterol, sitosterol and stigmasterol, where the *epi*-forms melt somewhat lower than the β-forms. In most cases valid correlations of configurations can be made today only by actual chemical interconversions.

Accordingly, to establish the relationship of the 17-dihydroequilenin to the oestradiols, and to provide additional examples of the reduction of naphtholic steroids to phenolic steroids, the 17-dihydroequilenins were treated with sodium in boiling amyl alcohol according to the procedure developed by Bamberger[7] and used by us in the preparation of oestrone from dehydroneoergosterol.[1] By this method α-dihydroequilenin gave α-oestradiol, m. p. 176°, as proved by comparison of the product and its benzoate with authentic α-oestradiol and its monobenzoate. In addition, a small amount of another phenolic substance, m. p. 151–154°, was isolated in an impure state. The non-phenolic fraction from the reduction constituted the major portion of the reaction products, and yielded a substance of m. p. 172°, which will be described in a subsequent paper. Similarly, the reduction of β-17-dihydroequilenin yielded, besides larger quantities of non-phenolic material, β-oestradiol. The mother liquors, after conversion to the monobenzoate, on oxidation yielded oestrone monobenzoate. From the mother liquors of this experiment and a similar experiment using the α-epimer, a phenolic ketone, m. p. 222–225°, which analyzed for four double bonds was obtained. This may be equilin or one of its isomers. When equilenin was reduced with sodium in amyl alcohol, and the phenolic reduction product benzoylated and oxidized, oestrone benzoate was obtained.

In connection with the reduction of equilenin it should be noted that David[12] found that the action of sodium in amyl alcohol gave an oil of high oestrogenic potency. Since the 17-dihydroequilenins have very little potency it seems likely that he had also obtained reduction to phenolic substances, oestradiols, which gave his oil its high activity.

The details of our preparation of oestrone, which have been repeated many times in this Laboratory by different workers, will be reported as the last paper of this series on the reduction of naphtholic steroids.

I wish to thank Dr. Oliver Kamm and Parke, Davis & Company for their generous help and assistance in various phases of this work. I also express my thanks to Drs. Elmer J. Lawson, Eugene L. Wittle, Thomas S. Oakwood, Harry

(11) Unpublished results from the laboratories of Parke, Davis & Co.

(12) David, *Acta brevia Neerland.* **4**, 63 (1934).

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Experimental

Purification of Equilenin.—The equilenin used in this work was separated from oestrone as well as possible by crystallization to give a product melting at 252°. Five grams of this product was dissolved in benzene and an equal volume of benzene saturated with picric acid was added. The solution was evaporated to one-half volume and let stand overnight in a refrigerator. The red picrate was filtered and recrystallized from a benzene solution saturated with picric acid. The picrate was dissolved in ether and shaken with ammonia diluted with an equal volume of water until it was washed free of picric acid. The ether was evaporated and the residue after one crystallization from 60% alcohol was shaken with 300 cc. of a 10% sodium hydroxide solution. At intervals benzoyl chloride was added until an excess was present. The insoluble benzoate was filtered, washed with water and recrystallized from ethanol to a melting point of 225°. This gave a depression in melting point when mixed with oestrone benzoate, m. p. 215°.

The purified equilenin benzoate was dissolved in ethanol and hydrolyzed by refluxing with an excess of potassium hydroxide for one hour. The equilenin was recrystallized from ethanol and then melted at 257–258° (uncorr.), $[\alpha]^{25}D$ +89°.

The Dihydroequilenins.—To a solution of 4 g. of purified equilenin in 70 cc. of dry isopropyl alcohol was added 4 g. of powdered aluminum isopropylate. The mixture was refluxed for five hours and then concentrated to 20 cc. by slow distillation over a five-hour period. While the mixture was still warm, 2 g. of potassium hydroxide dissolved in 60 cc. of methanol was added. The mixture was let stand for thirty minutes, and then was poured into water and acidified with hydrochloric acid. The product was extracted with ether, the solvent removed and the residue crystallized from 100 cc. of 80% ethanol. After three crystallizations a constant melting point of 248° was reached which did not increase upon further crystallization. When mixed with equilenin it gave a depression in melting point to 215°. This compound is designated as α-dihydroequilenin.

Anal. Calcd. for $C_{18}H_{20}O_2$: C, 80.5; H, 7.5. Found: C, 80.3; H, 7.6.

Diacetate of α-Dihydroequilenin.—α-Dihydroequilenin (100 mg.) was refluxed for thirty minutes with 5 cc. of acetic anhydride. After removal of the excess acetic anhydride, the residue was crystallized from 50% ethanol to give needles melting at 124°.

Anal. Calcd. for $C_{22}H_{24}O_4$: C, 74.9; H, 6.9. Found: C, 74.8; H, 7.0.

Monobenzoate of α-Dihydroequilenin.—α-Dihydroequilenin (25 mg.) was shaken with 6 cc. of 10% sodium hydroxide solution and an excess of benzoyl chloride for twenty minutes. The benzoate was filtered and recrystallized from ethanol; m. p. 215°.

Anal. Calcd. for $C_{25}H_{24}O_3$: C, 80.4; H, 6.7. Found: C, 80.5; H, 6.7.

β-Dihydroequilenin (δ-Follicular Hormone).—The mother liquor from the crystallization of α-dihydroequilenin was diluted while hot with water until a concentration of 50% alcohol was reached. It was allowed to cool slowly to room temperature for one hour, and the precipitate filtered. This consisted chiefly of some additional α-dihydroequilenin. The filtrate was cooled in a freezing mixture of ice and hydrochloric acid. The product was filtered and recrystallized from 50% ethanol to give a product melting at 215°, which gave a depression in melting point to 180° when mixed with α-dihydroequilenin.

Anal. Calcd. for $C_{18}H_{20}O_2$: C, 80.5; H, 7.5. Found: C, 80.4; H, 7.7.

Monobenzoate of β-Dihydroequilenin.—This was prepared in a way similar to that described for the α-form. It was crystallized from alcohol to a constant melting point of 204°.

Anal. Calcd. for $C_{25}H_{24}O_3$: C, 80.4; H, 6.7. Found: C, 80.4; H, 6.7.

Reduction of α-Dihydroequilenin by Sodium in Amyl Alcohol.—To a solution of 1 g. of α-dihydroequilenin, m. p. 248°, dissolved in 100 cc. of *n*-amyl alcohol (dry) was added 8 g. of sodium. The amyl alcohol was refluxed until the sodium had dissolved (two hours). Water was added, and the alkaline solution was acidified with hydrochloric acid. The product was extracted with ether, and the solvent removed *in vacuo*. The residue was dissolved in ether and the ethereal solution was washed well six times with 100-cc. portions of 2% sodium hydroxide solution. The alkaline solution was acidified and extracted with ether. The ether was evaporated and the phenolic residue was sublimed in a high vacuum at 175° bath temperature. The sublimate weighed 194 mg. A small amount of tarry phenolic material did not sublime. The sublimate was crystallized from dilute ethanol to give a product melting at 176°. This was purified only with difficulty as there was present a second phenolic product which could not be crystallized beyond 151–154° (unsharply) because of the small amount of product and some difficulty of separation. This product is being investigated further. The 176° product gave no depression in melting point when mixed with oestradiol, m. p. 178°.

The total product was converted to its monobenzoate by shaking with sodium hydroxide solution and benzoyl chloride. The product after crystallization from dilute ethanol melted at 194°. When mixed with the monobenzoate of α-dihydrooestrone it gave no depression in melting point; yield 41 mg.

Anal. Calcd. for $C_{25}H_{28}O_3$: C, 79.5; H, 7.5. Found: C, 79.5; H, 7.5.

The neutral fraction from the reduction which was not extractable with alkali from an ethereal solution was recrystallized from acetone–water to give a product melting at 172°. A study of this compound will be reported later.

Reduction of β-Dihydroequilenin with Sodium in Amyl Alcohol.—One gram of β-dihydroequilenin was reduced with sodium in amyl alcohol as described for the α-compound. The phenolic material was removed by shaking an ethereal solution of the reduced product with 2% sodium hydroxide. This phenolic fraction was sublimed in high vacuum at 175° (bath temp.) to give 205 mg. of sublimate.

As there was present in the sublimate a product other than β-dihydrooestrone, separation by direct crystallization could not be accomplished. The total product was converted into the benzoate by shaking with sodium hydroxide and benzoyl chloride. This was crystallized from dilute ethanol to give a substance of indefinite melting point; upon hydrolysis of this product with potassium hydroxide in ethyl alcohol a product was obtained which after crystallization from dilute ethanol gave a melting point of 215–219°. When this substance was mixed with β-dihydrooestrone, m. p. 222°, no depression in melting point was observed. Only 11 mg. of this product was obtained. It gave a depression in melting point of 24° when mixed with β-dihydroequilenin, m. p. 215°.

The total mother liquors from this product was converted again into the benzoate and this was oxidized with 40 mg. of chromic oxide in acetic acid for one hour. Water was added and the product extracted with ether and washed with water and sodium carbonate solution. After removal of the ether, the residue was dissolved in 50 cc. of ethanol and refluxed for thirty minutes with 1 g. of Girard's reagent. Water and ether were added, the layers separated and the aqueous layer heated on a steam-bath for a few minutes with an excess of hydrochloric acid. The ketonic material was extracted with ether, and the residue after removal of the ether was hydrolyzed with alcoholic potassium hydroxide. The product thus obtained was sublimed in high vacuum at 160°. From the sublimate after crystallization from ethanol was obtained 28 mg. of oestrone, m. p. 255°. When it was mixed with authentic oestrone it showed no depression in melting point.

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.9; H, 8.2. Found: C, 79.9; H, 8.3.

The mother liquors of this gave a product which after several crystallizations from dilute ethanol melted unsharply at 222–225°. This gave a depression in melting point when mixed with both oestrone and equilenin. The same product was obtained by the oxidation of the mother liquors from the α-dihydrooestrone obtained from the reduction of α-dihydroequilenin. This gave an analysis for a compound more unsaturated than oestrone, and is being studied further.

Anal. Calcd. for $C_{18}H_{20}O_2$: C, 80.5; H, 7.5. Found: C, 80.3; H, 7.5.

Reduction of Equilenin by Sodium in Amyl Alcohol.—One gram of equilenin was reduced by sodium in amyl alcohol as described for dihydroequilenin. The phenolic product of reduction was distilled in high vacuum, giving 123 mg. of distillate. This was converted to the benzoate and oxidized by chromic acid to give 31 mg. of oestrone after hydrolysis. It melted at 250–251°, and gave no depression in melting point when mixed with oestrone.

Reduction of Oestrone Benzoate with Aluminum Isopropylate.—To a solution of 500 mg. of oestrone benzoate dissolved in 20 cc. of dry isopropyl alcohol was added 1 g. of aluminum isopropylate and the reduction was carried out as described for the reduction of equilenin. The product which was obtained was dissolved in 25 cc. of ethanol and a solution of 2 g. of digitonin in 100 cc. of 65% alcohol was added. After standing overnight, the digitonide was filtered and washed with 70% ethanol. This after drying was warmed with pyridine on a steam-bath for thirty minutes. After removal of the pyridine and digitonin the product was crystallized from dilute ethanol and then melted at 195°. Upon hydrolysis it gave oestradiol of m. p. 178°.

The mother liquors from the digitonide of the α-form were concentrated to a small volume and shaken with water and ether. The ethereal solution was filtered and concentrated. The residue was hydrolyzed with alcoholic potassium hydroxide and gave after crystallization from dilute ethanol a product melting at 222°, which was β-oestradiol as shown by mixed melting points.

Summary

The reduction of equilenin by aluminum isopropylate gives a mixture of α- and β-dihydroequilenin which can be separated readily by crystallization. The latter compound is identical with the δ-follicular hormone. Upon reduction of either isomeric dihydroequilenin by sodium in amyl alcohol about 20% of phenolic substances are formed and from the α-form is obtained α-oestradiol whereas the β-dihydroequilenin yields β-oestradiol. Both yield about 75% of non-phenolic products which are being studied.

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