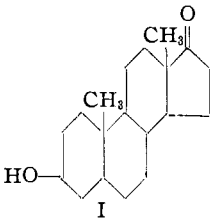
[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

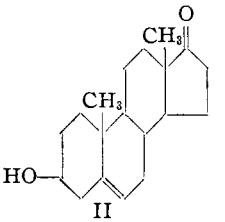
The Preparation of Dehydroandrosterone from Cholesterol

BY EVERETT S. WALLIS AND E. FERNHOLZ[1]

In the course of certain experiments on male urine Butenandt has isolated two hormones, androsterone, I,[2] and dehydroandrosterone, II.[3] The following structural formulas have been assigned by him.

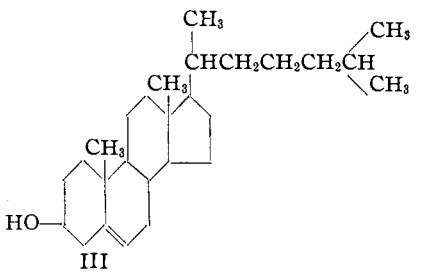


I



II

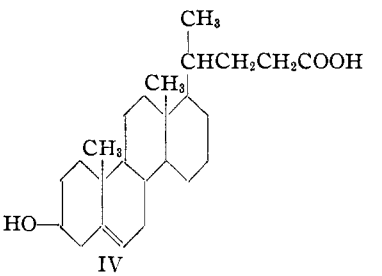
Androsterone has been prepared by Ruzicka and his co-workers.[4] In a communication to the Editor[5] we discussed the constitutional formula for dehydroandrosterone, and briefly described a method of preparation by means of which we had obtained it from cholesterol, III.



III

In this communication it also was pointed out that our preparation of this substance from cholesterol located definitely the position of the double bond, and also determined the spatial arrangement of the hydroxyl group, and it was concluded that dehydroandrosterone has the spatial arrangement of the hydroxyl group as in cholesterol and not as in androsterone.

Since these experiments were performed there has appeared a statement by Schoeller[6] that dehydroandrosterone has been prepared in his laboratory. No outline of his experimental methods, however, was given and no mention was made of the starting material used. A statement was made that unlike androsterone, the natural dehydroandrosterone is precipitated by digitonin.[7] Our compound is likewise precipitated by this substance, and there is now no doubt but that it is identical with the natural product. We are, therefore, publishing in detail our experimental method[7a] for preparing dehydroandrosterone, together with Δ^5-3-hydroxycholenic acid IV which is also formed in the oxidation process. Certain



IV

derivatives of this hydroxy acid have also been prepared. They are also described below.

Since our method of preparation gives a laboratory source for dehydroandrosterone, many new investigations can be carried out on this interesting compound. A description of some of these experiments will be published in a later issue of THIS JOURNAL.

Experimental Part

Preparation of Cholesteryl Acetate Dibromide.—One hundred grams of cholesterol was refluxed with 200 cc. of acetic anhydride for one hour. On cooling, the crystalline acetate separated. It was filtered, washed with glacial acetic acid, and then dissolved in 1000 cc. of ether. The contents were poured into an evaporating dish, and to the solution was added 16 cc. of bromine dissolved in 500 cc. of glacial acetic acid. Crystals began to deposit after a few minutes. The mixture was allowed to stand overnight in the evaporating dish in order that the ether might evaporate. The crystalline product was then filtered. It was washed with acetic acid and then several times

(1) Merck Fellow in Organic Chemistry.

(2) Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 167 (1934).

(3) Butenandt and Dannenbaum, *ibid.*, **229**, 192 (1934).

(4) Ruzicka, *Helv. Chim. Acta*, **17**, 1403 (1934).

(5) Wallis and Fernholz, THIS JOURNAL, **57**, 1379 (1935).

(6) Schoeller, Sereni and Gehrke, *Naturwissenschaften*, **23**, 337 (1935).

(7) Fernholz, *Z. phyiol. Chem.*, **232**, 97 (1935).

(7a) On May 20, 1935, a sample of our benzoate was sent to Professor Butenandt in Danzig with the request that he make a mixed melting point determination with his sample of the benzoate of the natural dehydroandrosterone. Since this manuscript was sent to the Editor one of us has received a letter from him stating that our product has the same melting point as his compound and gives no melting point depression when mixed with his substance. He has also informed us that he has prepared dehydroandrosterone from cholesterol and that an article on the chemistry and physiology of dehydroandrosterone and its transformation products is in press.

It is also to be added that in a recent issue of *Nature*, **135**, 1039 (1935), Oppenauer has announced that he has prepared dehydroandrosterone from γ-sitosterol.

with petroleum ether. It was dried at room temperature. It melted at 112–115°; yield 130 g.

Preparation of Dehydroandrosterone Acetate Semicarbazone.—A solution of 30 g. of cholesteryl acetate dibromide in 700 cc. of glacial acetic acid was heated in a water-bath which was kept at 65°. The solution was stirred mechanically, and to it was added in about four hours a solution of 50 g. of chromic oxide in 50 cc. of water and 200 cc. of acetic acid. This mixture was then kept at the same temperature for six hours at the end of which time it still contained some unreduced chromic oxide. On cooling, the solution was diluted with three liters of water and the organic matter was taken up in ether. The ether solution was washed with dilute hydrochloric acid and with water to free it from chromic compounds. Thirty grams of zinc dust and 200 cc. of acetic acid were then added and the ether was distilled. The mixture remaining behind was then heated in the water-bath for two hours to finish the debromination. The organic products were again taken up in ether and the ether solution was treated with 2 *N* sodium hydroxide. In this manner an insoluble sodium salt was formed which yielded a crystalline acid. The ether extract was then evaporated and the neutral oxidation products were steam distilled to remove volatile ketones such as methyl isohexyl ketone. The light brown resinous material which remained, and which contained some unchanged cholesteryl acetate, was dissolved in 100 cc. of ethyl alcohol. This solution was treated with a solution of 2 g. of semicarbazide hydrochloride and 3 g. of sodium acetate in a small amount of water. The mixture was boiled for two hours, the alcohol was distilled off and the residue was treated with hot water to remove all water-soluble material. The product was dried thoroughly and then it was treated with 100 cc. of ether. The insoluble acetate semicarbazone separated. This compound was recrystallized from a mixture of benzene and alcohol with an extractor.

Dehydroandrosterone acetate semicarbazone crystallizes in the form of narrow leaflets, almost insoluble in ether, but easily soluble in chloroform. It melts at 270° with decomposition; yield, 0.35 g.

Anal. Calcd. for $C_{22}H_{33}N_3O_3$: C, 68.16; H, 8.59; N, 10.84. Found: C, 68.33, 68.27; H, 8.49, 8.48; N, 10.64, 10.75.

Preparation of Dehydroandrosterone.—0.65 gram of crude dehydroandrosterone acetate semicarbazone was heated on the water-bath with 20 cc. of alcohol and 10 cc. of 5 *N* sulfuric acid for three hours. The free hydroxy ketone[8] was taken up in ether. Evaporation of the ether extract yielded a crude crystalline product. This material was purified by sublimation in high vacuum at 140°. It was recrystallized from a mixture of benzene and petroleum ether.

This product melts at 148°, a melting point which is identical to that of the natural product. It is precipitated by digitonin. If crystallized from solvents containing water it forms needles containing water of crystallization which have an unsharp melting point. This water of crystallization is very difficultly removed on drying. A solution of 17 mg. in 2 cc. of chloroform solution showed no appreciable rotation in a 1-dm. semi-micro tube, D line.

(8) Hydrolysis of the acetyl group also occurs at this point.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.11; H, 9.79. Found: C, 79.25, 79.01; H, 9.51, 9.58.

Preparation of Dehydroandrosterone Benzoate.—0.4 gram of dehydroandrosterone was dissolved in 5 cc. of pyridine and to this solution was added 1.5 cc. of benzoyl chloride. This mixture was kept at room temperature overnight. Water was then added and the benzoate was taken up in a large quantity of ether, in which it is but sparingly soluble. The ether solution was then freed from pyridine and benzoic acid. On evaporation of the ether a crystalline residue was obtained. After one recrystallization from chloroform and alcohol it showed a constant melting point of 250° (uncorr.). This is identical with that of the benzoate of the natural product.

Dehydroandrosterone benzoate crystallizes in small needles, sparingly soluble in most solvents; easily soluble in pyridine and chloroform. 16.1 mg. in 2 cc. of chloroform solutions gave $\alpha^{30}D$ +0.21°, 1-dm. semi-micro tube, $[\alpha]^{30}D$ +26.1°.

Anal. Calcd. for $C_{26}H_{32}O_3$: C, 79.53; H, 8.22. Found: C, 79.67; H, 8.26.

Preparation of Δ^5-3-Hydroxycholenic Acid.—The alkaline extracts from two oxidations were combined and heated on the water-bath for two hours. After cooling, the precipitated sodium salt was collected on a filter and washed with 2 *N* sodium hydroxide. It was then decomposed with hydrochloric acid in the presence of a large amount of ether. (The free acid is sparingly soluble in ether.) On evaporation of the ether solution an amorphous residue was obtained. This was treated with acetone. The acid in crystalline form soon separated. The crude product melted at 200–210°; yield 1.7 g.

Recrystallization from ethyl acetate with an extractor gave a product which melted at 230° (with decomposition). An acid of a slightly higher melting point was prepared by hydrolysis of the methyl ester of the acetoxy acid described below.

Δ^5-3-Hydroxycholenic acid forms small prisms which are sparingly soluble in ethyl acetate, but soluble in ethyl alcohol and methyl alcohol. The pure acid melts at 236° with decomposition.[9] It is precipitated by digitonin.

Anal. Calcd. for $C_{24}H_{38}O_3$: C, 76.94; H, 10.23. Found: C, 76.89, 76.89; H, 10.31, 10.04.

Preparation of the Methyl Ester.—Two grams of the acid described above was boiled with 50 cc. of methyl alcohol, and 8 drops of concd. sulfuric acid. After one hour, the ester was precipitated with water. The crystalline product was filtered and dried in a desiccator. It was recrystallized from acetone.

The methyl ester crystallizes from acetone in the form of needles which melt at 144°. The ester is very soluble in chloroform. It is precipitated by digitonin.

Anal. Calcd. for $C_{25}H_{40}O_3$: C, 77.26; H, 10.38. Found: C, 77.43; H, 10.35.

Preparation of the Methyl Ester of Δ^5-3-Acetoxycholenic Acid.—Two grams of the above methyl ester was heated on the water-bath with 20 cc. of acetic anhydride for two hours. On cooling the crystalline acetate separated. It was recrystallized from ethyl acetate in the

(9) When heated at 240° it loses one-half a molecule of water and yields a solid product. Experiments are being carried out on the constitution of this compound.

form of flat needles which melted at 156°. This compound is less soluble than the methyl ester of the hydroxy acid. Hydrolysis with potassium hydroxide in methyl alcohol gave the hydroxy acid, m. p. 236°; 26.8 mg. in 2 cc. of chloroform solution gave $\alpha^{20}D$ −0.25°, 1-dm. semi-micro tube, $[\alpha]^{20}D$ −18.7°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.30; H, 9.84. Found: C, 75.44; H, 9.85.

The authors wish to express their thanks to Merck and Company, Inc., Rahway, N. J., for all the analyses published in this article, and for a grant-in-aid for this work.

Summary

Dehydroandrosterone and Δ^5-3-hydroxycholenic acid have been prepared from cholesterol by oxidation. This oxidation is possible provided both the double bond and the hydroxyl group in cholesterol are protected against oxidation. This has been accomplished by oxidation of cholesteryl acetate dibromide with chromic acid. A detailed description of the oxidation process has been given. This method of preparation locates definitely the double bond and determines the spatial arrangement of the hydroxyl group in dehydroandrosterone.

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NOTES

Apparent Molal Heat Capacities of Amino Acids and Other Organic Compounds

BY JOHN T. EDSALL

The heat capacities of organic substances in aqueous solution have never been extensively studied. Zwicky[1] pointed out that the apparent molal heat capacities of several organic molecules in water were nearly identical with their molal heat capacities in the pure crystalline state, in other words, that the law of the ideal solution is approximately obeyed. For other substances, however, this is far from true.[2] A survey of the available data in "International Critical Tables," and of the recent data of Zittle and Schmidt[3] on amino acids in water, reveals certain relations between chemical structure and apparent molal heat capacity in water which appear worthy of consideration. In Table I are recorded the apparent molal heat capacities (ϕ) in dilute aqueous solution, and the molal heat capacities (Cp) in the pure state, for a number of substances.[4]

(1) Zwicky, *Physik. Z.*, **27**, 271 (1926).

(2) See for instance Bose, *Z. physik. Chem.*, **58**, 585 (1907).

(3) Zittle and Schmidt, *J. Biol. Chem.*, **108**, 161 (1935).

(4) To make all the data comparable, the value of Cp should preferably be given for the pure *liquid* state. This is impossible for many substances, such as the amino acids which decompose on melting. In general, the value of Cp for any substance is distinctly higher in the liquid state than for the solid at or near the same temperature. This fact should be remembered in considering the data in Table I, but it does not essentially affect the arguments here advanced.

In the three homologous series recorded (alcohols, fatty acids, amino acids) it will be observed that the introduction of a CH_2 group increases ϕ by 20 to 30 calories per mole (see column headed Δ in Table I), while Cp increases by only 5 to 8 calories per mole for each CH_2 group introduced. Thus the presence of a hydrocarbon chain tends to produce a *positive* deviation from the ideal solution law with respect to heat capacity. This trend is approximately indicated by the series of values of $\phi - Cp$ given in the last column of Table I. On the other hand, substances such as glycerol, urea, dextrose and sucrose, which contain many polar groups and no hydrocarbon chain, behave much more nearly like ideal solutes in water. The substances cited by Zwicky all belong to this latter class.

It remains to be seen how far these suggested approximate rules will hold good when a wider range of substances has been investigated.

The apparent molal volumes of amino acids in water are known to be lower than those of most organic compounds, due to electrostriction of the solvent.[5] The calculations of Zwicky[1] on the heat capacities of electrolyte solutions suggest that the values of apparent molal heat capacity for the amino acids should be lowered by the charged NH_3^+ and COO^- groups which they con-

(5) Cohn, McMeekin, Edsall and Blanchard, THIS JOURNAL, **56**, 784 (1934).