10% solution of octyl alcohol in ethanol to the fermentation mixture and immerse the container in an actively boiling water-bath until the proteins are coagulated. Separate the coagulum by centrifugation or filtration (in a refrigerator), neutralize the clear filtrate to phenolphthalein by the addition of 4 *N* sodium hydroxide and immediately precipitate by the addition of a solution of 55 g. of calcium chloride in 100 cc. of water; *i. e.*, slightly more than one mol of this reagent is required for each mol of monosodium phosphate present in the initial mixture. Complete the precipitation by heating at a boiling water-bath for a short time, and filter the warm mixture with suction. Wash the precipitated calcium *d*-fructose-1,6-diphosphate with warm water; yield, 22 g.

An alternative procedure is the following: When phosphorylation is complete add to the fermentation mixture a solution of 40 g. of picric acid in 200 cc. of hot ethanol. Allow the mixture to stand in a refrigerator for two hours and filter. Add 4 *N* sodium hydroxide to the filtrate until neutral to phenolphthalein, then add calcium chloride as previously described. The precipitated calcium salt contains some picrate but this is removed by washing with hot water and subsequently with alcohol. An experiment conducted in this fashion with the quantities of material stated above yielded 24 g. of crude calcium *d*-fructose-1,6-diphosphate.

To purify the crude calcium salt obtained by either of these procedures, dissolve the moist crude product in 250 cc. of 2 *N* acetic acid, add 125 cc. of water, filter if necessary, and to the clear solution add 2 *N* sodium hydroxide until neutral to phenolphthalein. Heat the resulting mixture in a boiling water-bath for a short time, filter and wash the precipitated salt with warm water; yield about 80% of the crude product.

In analogous manner the barium salt can be prepared, but in this case the use of picric acid for the deproteinization is not advisable because of the difficult solubility of barium picrate.

As mentioned in the introduction, fresh Fleischmann's yeast is not satisfactory for the preparation of *d*-fructose-1,6-diphosphate in the manner described. If, however, this brand of yeast is well dried at room temperature in the usual manner, it may be employed, for with such dried yeast phosphorylation takes place rapidly. When a mixture of 30 g. of dried Fleischmann's yeast, 15 cc. of carbon tetrachloride and 100 cc. of the sugar–phosphate solution of the composition mentioned earlier was incubated at 37°, phosphorylation was found to be complete within one and one-half hours. Similar results were obtained with dried preparations of National Grain and Federal yeasts. The product of phosphorylation contains, as well as in the other cases described, in addition to hexose-diphosphate also sugar-monophosphates.

A purity test can be made using an observation published many years ago.[4] Calcium and barium hexose-diphosphate dissolve readily in solutions of ammonium tri-salts, such as acetate, chloride, nitrate, rhodanide, while calcium and barium phosphate are practically insoluble. Consequently the pure salts of hexose–diphosphate are completely dissolved in the ammonium salt solutions and added magnesia mixture does not cause a precipitate.

We are indebted to the manufacturers of the various brands of yeast mentioned for generous supplies of their respective products.

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(4) C. Neuberg and S. Sabetay, *Biochem. Z.*, **161**, 240 (1925).

The Catalytic Reduction of Cholesterol α-Oxide

BY HOMER E. STAVELY

The catalytic reduction of oxides of the ethylene oxide type has been suggested as a possible approach to certain synthetic problems in the steroid hormone field. Only a few such examples can be found in the literature. Triphenylethylene oxide,[1] ethylene oxide[2] and ethyl stearate oxide[3] have been catalytically reduced to alcohols. Fernholz[4] hydrogenated stigmasterol α-oxide-(5,6) acetate using platinum and acetic acid, and obtained stigmastanediol-3,5 monoacetate in 20% yield. According to a patent[5] 3-acetoxy-pregnadiene-5:6,20:21 can be converted into a dioxide, which can then be reduced in acetic acid with palladium and hydrogen to 3-acetoxypregnanediol-5,20.

Similar treatment of cholesterol α-oxide[6] should yield either 3,5- or 3,6-cholestanediol. The 3,5-diol was obtained by Chinaeva and Ushakov[7] when they treated cholesterol α-oxide with phenyllithium.

Cholesterol α-oxide was hydrogenated in acetic acid with palladium catalyst. The hydrogen uptake was slow, and was therefore allowed to proceed over a period of several days with intermittent shaking. The reaction products were acetylated and chromatographed on alumina. Three substances were isolated in pure form, cholestanediol-3,5 monoacetate,[8] cholestanol-3 acetate, and α-cholestanetriol-3,5,6 diacetate. The latter compound obviously is not a reduction product; it can be prepared by heating the oxide acetate with acetic acid. It is evident that the major part of the oxide is reduced to the 3,5-diol, from

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(1) Weill and Kayser, *Bull. soc. chim.*, [5] **3**, 841 (1936).

(2) Ushakov and Mikhaĭlov, *J. Gen. Chem.* (U. S. S. R.), **7**, 249 (1937); *C. A.*, **31**, 4645 (1937).

(3) Pigulevskiĭ and Rubashko, *J. Gen. Chem.* (U. S. S. R.), **9**, 829 (1939); *C. A.*, **34**, 378 (1940).

(4) Fernholz, *Ann.*, **508**, 215 (1934).

(5) Swiss Patent 214,540.

(6) Windaus and Westphalen, *Ber.*, **48**, 1064 (1915).

(7) Chinaeva and Ushakov, *J. Gen. Chem.* (U. S. S. R.), **11**, 335 (1941); *C. A.*, **35**, 5903 (1941).

(8) The melting points of the free diol and the diol acetate are somewhat higher than the figures given by Chinaeva and Ushakov. The two diols may be isomeric at C_6.

which the tertiary hydroxyl is then partially eliminated with the production of cholestanol-3. It is probable that the formation of the primary reduction product would have taken precedence over the side reactions if the experimental conditions had permitted a shortening of the reduction period.

The several examples now at hand permit the generalization that when an ethylene oxide ring is opened by hydrogenation the hydroxyl group formed will be attached to the carbon atom carrying the smaller number of hydrogen atoms,[9] as one would expect on theoretical grounds.

Experimental

Two hundred mg. of palladium catalyst in acetic acid was saturated with hydrogen, and a solution of 300 mg. of cholesterol α-oxide, m. p. 141°, in acetic acid was added. After three hours of shaking only two-thirds mole (10 cc.) of hydrogen had been absorbed. Intermittent shaking was continued for two days, and the total hydrogen uptake was 18 cc. (1.2 moles). After removal of the catalyst the acetic acid was removed by vacuum distillation and the residue acetylated at room temperature overnight with pyridine–acetic anhydride. The total acetylated reaction product was dissolved in hexane and chromatographed on a column of alumina 1 × 15 cm. Elution was carried out with hexane, then with hexane–benzene mixtures, and finally with benzene. The hexane fractions yielded 85 mg. After two recrystallizations from methanol the m. p. was 109–110°. The m. p. of a mixture with cholestanol-3 acetate was not depressed.

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.86; H, 11.70. Found: C, 80.80; H, 11.77.

The combined residues from the hexane–benzene (2/1) mixtures weighed 120 mg. After several recrystallizations the m. p. was constant at 181°.

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found: C, 77.81; H, 11.08.

Twenty-five mg. of the diol acetate was hydrolyzed with methanolic potassium hydroxide. The diol had a m. p. of 216–217°. Chinaeva and Ushakov[7] give a m. p. of 201° for the diol and 177° for the acetate.

The residue from the benzene fractions weighed 75 mg. After several recrystallizations from methanol the m. p. was 165–167°. The m. p. of a mixture with authentic α-cholestanetriol-3,5,6 diacetate was not depressed.

Anal. Calcd. for $C_{31}H_{52}O_5$: C, 73.77; H, 10.39. Found: C, 73.67; H, 10.63.

The triol was prepared by hydrolysis of the diacetate, m. p. 227–230°. The m. p. of a mixture with an authentic sample was not depressed.

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[9] Krassusky, *Chem. Ztg.*, **31**, 704 (1907), found that a similar rule applies when the alkene oxide ring is opened with ammonia to form α-amino alcohols.

Revised Constants for the Debye–Hückel Theory

BY H. I. STONEHILL AND M. A. BERRY

Using the newer values of certain fundamental constants, *viz.*, e (electronic charge) = 4.803×10^{-10} e. s. u., $k = 1.379 \times 10^{-16}$ erg/°C., $N = 6.028 \times 10^{23}$, T_0 (ice point) = 273.18°K.,[1] D (dielectric constant of water at 25°) = 78.54,[2] it may be calculated that the values of h and g in the first term of the Debye–Hückel expression for the log of the activity coefficient of a $z_1:z_2$ valent electrolyte in aqueous solution at 25°

$$\log \gamma = \frac{-hz_1z_2\sqrt{I}}{1 + ga\sqrt{I}} - \log\,(1 + 0.018\Sigma m_i)$$

are $h = 0.5103$ and $g = 0.3290 \times 10^8$.

The older values, $e = 4.774 \times 10^{-10}$, $k = 1.372 \times 10^{-16}$, $N = 6.061 \times 10^{23}$, $T_0 = 273.1$°K.,[3] lead to $h = 0.5065$, $g = 0.3287 \times 10^8$. Harned and Robinson in two recent publications[4,5] employed the values $h = 0.5065$, $g = 0.3288 \times 10^8$ and $h = 0.5056$, $g = 0.3288 \times 10^8$. While the value of g is practically unchanged, there are discrepancies of 1.0 and 0.8% between 0.5103 and the two values of h used by Harned and Robinson, leading to corresponding discrepancies in the first term of the Debye–Hückel equation. This term is the most important one even in extended forms of the equation containing arbitrary linear, quadratic or other additional functions of concentration, at least up to about $I = 1.0$. Thus a 0.9% error in h corresponds to an approximately equal error in log γ. A 0.9% error in log γ corresponds to % errors in γ of 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 1.1, 1.5, 2.1 when γ is, respectively, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1. Hence any equation purporting to give activity coefficients with an error not exceeding these limits should use the newer value of h. This will not affect the work of Harned and Robinson[5] since they only claimed an accuracy of 0.3% in γ where γ was always greater than 0.8; but there are many other calculations which will need revision, *e. g.*, the linear relation between log a and log B discovered by Harned and Robinson[4] for the alkali halides may not exist if values of a and B are recalculated with the newer value of h.

The authors on February 26, 1942, furnished the following supporting information: "Take the case of a 1:1 valent

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[1] Childs, "Physical Constants," London, 1939.

[2] Wyman, *Phys. Rev.*, **35**, 623 (1930).

[3] "International Critical Tables."

[4] Harned and Robinson, *Chem. Rev.*, **28**, 419 (1941).

[5] Harned and Robinson, *Trans. Faraday Soc.*, **37**, 302 (1941).