

The importance of such considerations becomes even more apparent if the dimensions of protein are compared with those of their respective dissociation and aggregation components.<sup>26</sup> From Table II it may be seen that dissociation of phycocyanin (*Ceramium*) for instance, affects only the length of the short axis, when the molecular weight is halved. Similarly, dissociation of hemocyanin (*Eledone*) only decreases the short axis when the molecular weight drops from 2,790,000 to 457,000. Such data immediately suggest that these molecules split in the direction parallel to the major molecular axis. In other cases, only the major axis is shortened during dissociation indicating that splitting takes place at right angles to the major axis. In Table II, the apparent direction of splitting is indicated by arrows, a horizontal arrow indicating cleavage along the major axis, and a vertical arrow cleavage along the minor axis.<sup>27</sup>

Comparison of the absolute dimensions of the anti-pneumococcus serum globulins indicates a similar relationship. These proteins seem to differ chiefly in respect to the length of the molecule, if the species from rabbit, man, and horse are compared with each other (Table I). An exact interpretation of the molecular dimensions in terms of chemical configuration would be premature at present. Only in a few isolated cases, such as

(26) Here the influence of hydration is most likely the same for the undissociated protein and its respective dissociation products.

(27) In the case of the dissociation of hemoglobin by urea, this regularity does not seem to hold exactly. It appears from such considerations, as well as from others,<sup>28</sup> that the value for the dissymmetry constant of this protein is somewhat too low.

(28) Neurath, *Symposia Quant. Biol.*, **6**, 23 (1938).

zein,<sup>7</sup> or hemoglobin,<sup>29</sup> has yet a satisfactory agreement between the shape factors determined by different independent methods been established. Further experimental work is needed before the values recorded in Tables I and II can be accepted as exact values.

The work of Astbury<sup>30</sup> and of Bergmann and Niemann<sup>31</sup> has furnished much evidence for the hypothesis that the difference between fibrous and globular proteins is merely a quantitative one. The data presented in this paper likewise illustrate the fibrous character of the globular proteins, thus lending support to the theory that both types of proteins are structurally closely related.

### Summary

A method for the estimation of the relative and absolute dimensions of protein molecules from the dissymmetry constant and Perrin's diffusion equation is outlined and its limitations discussed. The data indicate that most proteins are unsymmetric in shape. A certain regularity exists, independent of the molecular weight, when proteins are grouped on the basis of their molecular dimensions. Comparison of the dimensions of proteins with those of their respective dissociation and aggregation products indicates that cleavage occurs only in directions parallel to either the major or minor molecular axis.

(29) Arrhenius, *Physik. Z.*, **39**, 559 (1938).

(30) Astbury, *Nature*, **140**, 968 (1937).

(31) Bergmann and Niemann, *J. Biol. Chem.*, **115**, 77 (1936); **118**, 301 (1937).

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## The Hydroxylation of Unsaturated Substances. V. The Catalytic Hydroxylation of Certain Unsaturated Substances with Functional Groups

BY NICHOLAS A. MILAS, SIDNEY SUSSMAN AND HOWARD S. MASON

Further application of the recent hydroxylation method developed in this Laboratory<sup>1</sup> has led to the production of a number of useful products which hitherto have been rather difficult to procure in satisfactory yields. Of particular interest along this line is the production of glycolaldehyde from divinyl ether in yields as high as 96%. Furthermore, it has been found possible to add

hydroxyl groups to substances containing double bonds adjacent to carbonyl groups. Böeseken,<sup>2</sup> for example, has found that ethyl fumarate and ethyl maleate were not appreciably oxidized with perbenzoic acid, and in the case of unsaturated ketones having the same arrangement of groups he reports<sup>3</sup> oxidation of the ketones without the formation of epoxy derivatives but with cleavage

(2) Böeseken, *Rec. trav. chim.*, **45**, 838 (1926).

(1) Milas and Sussman, *THIS JOURNAL*, **58**, 1302 (1936); **59**, 2345 (1937).

(3) Böeseken and co-workers, *ibid.*, **50**, 827 (1931); **52**, 874 (1933); **55**, 786 (1936).

of the carbon-carbon bond next to the double bond or the carbonyl group. Table I summarizes some of the results obtained in the present investigation.

TABLE I  
SUMMARY OF HYDROXYLATION EXPERIMENTS

Olefinic substance	Main product	% yield
Ethyl crotonate	Ethyl dihydroxybutyrate	56
Diethyl maleate	Diethyl mesotartrate	41
Diethyl fumarate	Diethyl racemate	58
Mesityl oxide	2-Methylpentanediol-2,3-one-4	23
Vinyl acetate	Glycolaldehyde	60
Divinyl ether	Glycolaldehyde	96.0
Vinyl bromide	Glycolaldehyde	12.5(?)
Oleic acid	9,10-Dihydroxystearic acid	60
Cinnamyl alcohol	$\beta$ -Phenylglycerol	12.2
	Di- $[\beta$ -phenylglycerol] ether	4.5

In the case of the vinyl compounds it was not found possible to isolate the corresponding glycols since these rearranged spontaneously to yield glycolaldehyde. With cinnamyl alcohol the greater portion of the  $\beta$ -phenylglycerol formed was further oxidized to benzaldehyde and glycolaldehyde, both of which were isolated.

### Experimental

**Ethyl Dihydroxybutyrate from Ethyl Crotonate.**—Ethyl crotonate was prepared in accordance with the method of Michael<sup>4</sup> and was purified by fractionation under reduced pressure; b. p. 43–45° (17 mm.). To 8.55 g. (0.075 mole) of this ester was added 42.9 cc. (0.075 mole) of 5.95% hydrogen peroxide reagent<sup>1</sup> in *t*-butyl alcohol, the solution cooled to 0° and to it added 1 cc. of osmium tetroxide solution likewise in *t*-butyl alcohol. The reaction was complete in three days at 0° when the solvent was removed by distillation at the temperature of the water-bath and the residue fractionated under reduced pressure. The fraction boiling at 123–125° (18 mm.) was collected and found to have the following properties. Unlike the ethyl crotonate, it was completely soluble in water and had a neutralization equivalent of 146 as compared with 148 for ethyl dihydroxybutyrate. A yield of 56% was obtained based on the amount of ethyl crotonate used.

**Diethyl Mesotartrate from Diethyl Maleate.**—Eight and six-tenths grams (0.05 mole) of diethyl maleate (Eastman Kodak Company) was dissolved in 28.6 cc. (0.05 mole) of a 5.95% solution of the hydrogen peroxide reagent, the solution cooled to 0° and to it added 1.5 cc. of the osmium tetroxide catalyst, but since no noticeable reaction occurred after four days at this temperature, the mixture was allowed to come to room temperature, at which the reaction was completed at the end of two days. The solvent was then removed by distillation and the residue fractionated under reduced pressure and the fraction boiling at 162–166° (18 mm.) collected. Kuhn and Wagner-Jauregg<sup>5</sup> reported a b. p. for diethyl mesotartrate of 157.5° (14 mm.) and a m. p. of 55°. Our compound was a highly viscous liquid which when crystallized from ether had a

m. p. of 53.5–55°. A neutralization equivalent gave a value of 107 as against 103 calculated for diethyl mesotartrate. A yield of 41% was obtained based on the amount of diethyl maleate consumed.

**Diethyl Racemate from Diethyl Fumarate.**—Eight and six-tenths grams of diethyl fumarate (Eastman Kodak Company) was dissolved in 28.6 cc. of a 5.95% solution of hydrogen peroxide reagent, the solution cooled to 0° and to it added 1.5 cc. of the osmium tetroxide reagent. As in the case of diethyl maleate the reaction mixture was brought, after four days at 0°, to room temperature, at which it was found to be complete at the end of one day. The solvent was then removed by distillation, the residue fractionated under reduced pressure and the fraction boiling at 163–167° (21 mm.) was collected. Anschuetz and Pictet<sup>6</sup> reported a b. p. of 162° (19 mm.) for diethyl *d*-tartrate and Perkin<sup>7</sup> has shown that the b. p. of this ester is identical with that of diethyl racemate. Attempts to crystallize this ester were unsuccessful. The saponification equivalent was found to be 106 as against 103 calculated for diethyl racemate. The yield of diethyl racemate was 58% based on the amount of diethyl fumarate used.

**2-Methylpentanediol-2,3-one-4 from Mesityl Oxide.**—To 9.8 g. (0.1 mole) of mesityl oxide (Eastman Kodak Company) was added 57.2 cc. (0.1 mole) of a 5.95% solution of hydrogen peroxide reagent, the solution cooled to 0° and to it added 0.5 cc. of the osmium tetroxide catalyst. After two days at 0° the reaction was complete, the solvent distilled, the residue fractionated under reduced pressure and the fraction boiling at 104–110° (16 mm.) collected. Harries and Pappos<sup>8</sup> reported a b. p. of 109° (19 mm.) for 2-methylpentanediol-2,3-one-4. Our product was found to reduce Fehling's solution instantly in the cold.

This glycol has not been characterized fully and in order to characterize it, the *p*-nitrophenylsazone was prepared by allowing it to react, at the temperature of the water-bath, with *p*-nitrophenylhydrazine in dilute acetic acid. The dark red precipitate obtained from this reaction was recrystallized several times from pyridine; m. p. 251–253°, with decomposition. This substance was analyzed for nitrogen.

*Anal.* Calcd. for C<sub>13</sub>H<sub>20</sub>O<sub>6</sub>N<sub>6</sub>: N, 21.00. Found: N, 20.87.

A yield of 23% was obtained based on the amount of mesityl oxide consumed.

**Glycolaldehyde from Vinyl Acetate.**—The vinyl acetate (Eastman Kodak Company) used was fractionated just before the experiments through a Vigreux column and the fraction boiling at 72.5–73° collected. To 17.7 g. of this substance was added 110 cc. of 6.23% hydrogen peroxide reagent, the mixture cooled to 0° and to it added 1 cc. of the osmium tetroxide catalyst. After five days at 0° the reaction mixture turned brown and the peroxide had disappeared completely. The mixture was then distilled to remove the solvent and the unconverted vinyl acetate and the residue fractionated under reduced pressure and the fraction boiling at 90–98° (4.5 mm.) collected and examined for glycolaldehyde. Fischer and Feldmann<sup>9</sup>

(6) Anschuetz and Pictet, *ibid.*, **13**, 1177 (1880).

(7) Perkin, *J. Chem. Soc.*, **51**, 364 (1887).

(8) Harries and Pappos, *Ber.*, **34**, 2979 (1901).

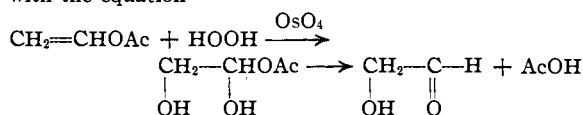
(9) Fischer and Feldmann, *Ber.*, **62**, 854 (1929).

(4) Michael, *Ber.*, **33**, 3766 (1900).

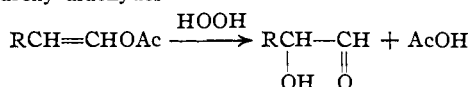
(5) Kuhn and Wagner-Jauregg, *ibid.*, **61**, 485 (1928).

reported a b. p. of 110–120° (12 mm.) for glycolaldehyde. Our product reduced Fehling's solution in the cold and with *p*-nitrophenylhydrazine formed an osazone which on recrystallization from pyridine had a m. p. of 306–307°. A m. p. of 308.5° is recorded in the literature<sup>10</sup> for this substance. Mixed m. p. with an authentic sample gave a m. p. of 306.5°. A yield of 60% of glycolaldehyde was obtained based on the amount of vinyl acetate consumed. Identical yields were obtained when the reaction was run with larger quantities: 86 g. of vinyl acetate and 630 cc. of the peroxide reagent.

In this reaction, the glycolaldehyde is formed as the result of a rearrangement of the initial glycol in accordance with the equation



and may serve as a general reaction for the preparation of  $\alpha$ -hydroxy aldehydes



**Glycolaldehyde from Divinyl Ether.**—The divinyl ether used in this work was kindly supplied by Merck and Co. through the courtesy of Dr. R. T. Major. It was fractionated through a Davis column and the fraction boiling at 28.3° collected and used in our experiments.

To 3.5 g. of divinyl ether was added 60 cc. of 6.23% hydrogen peroxide reagent, the mixture cooled to 0° and to it added 0.5 cc. of the osmium tetroxide catalyst. The mixture warmed up considerably so that the flask had to be connected to a reflux condenser and thence to a trap immersed in solid carbon dioxide so that any volatile products could be condensed and isolated. At the end of three days the reaction was over and the mixture fractionated to remove the unconverted ether as well as the acetaldehyde which was formed almost in equal quantities with glycolaldehyde showing that only one of the double bonds hydroxylated and the product thus formed rearranged spontaneously. In the low boiling fractions the acetaldehyde was estimated by preparing its *p*-nitrophenylhydrazone, whereas in the residue the glycolaldehyde was estimated by precipitating its *p*-nitrophenylosazone. On recrystallization from pyridine, the latter had a m. p. of 306.5–307°. A yield of 96% of glycolaldehyde was obtained based on the amount of divinyl ether reacted.

**Glycolaldehyde from Vinyl Bromide.**—Vinyl bromide was prepared from ethylene dibromide and alcoholic potash,<sup>11</sup> b. p. 16°.

To 10.7 g. of freshly prepared vinyl bromide was added 64 cc. of 5.27% hydrogen peroxide reagent, the mixture cooled to 0° and to it added 2 cc. of the osmium tetroxide catalyst and 10 g. of finely powdered calcium carbonate to take up the hydrogen bromide evolved during the reaction. The mixture was allowed to stand in an ice-box at 5–10° for twenty-four days, then worked up for the presence of glycolaldehyde; a yield of 12.5% of this aldehyde was obtained.

**9,10-Dihydroxystearic Acid from Oleic Acid.**—Seven grams of oleic acid, b. p. 230–235 (21 mm.), was mixed with 14.3 cc. of 5.95% solution of the hydrogen peroxide reagent, the mixture cooled to 0° and to it added 0.5 cc. of the osmium tetroxide catalyst; the solution became orange and after standing overnight at this temperature turned colorless and the reaction was complete. A considerable amount of the 9,10-dihydroxystearic acid had precipitated out. The solvent was removed under reduced pressure and the residue washed thoroughly with ether to remove the unconverted oleic acid and the crude solid recrystallized from absolute alcohol; m. p. 129–131° (corr.). Le Sueur<sup>12</sup> has reported a m. p. of 131–132° (corr.) for the high melting 9,10-dihydroxystearic acid. A neutralization equivalent was taken and found to be 319 as against 316 calculated for 9,10-dihydroxystearic acid. The yield obtained amounted to 60%.

**Phenylglycerol from Cinnamyl Alcohol.**—To 24.5 g. of cinnamyl alcohol (Eastman Kodak Company) was added 171 cc. of 5.43% hydrogen peroxide, the mixture stirred at 0° and to it added 2 cc. of the osmium tetroxide catalyst. If stirring were omitted in this experiment, it was not found possible to isolate phenylglycerol which, due to local heating, was rapidly oxidized to benzaldehyde and glycolaldehyde. The reaction was complete in four days, the solvent removed under reduced pressure, the highly viscous residue (26 g.) fractionated and the fraction coming over at 171° (1 mm.) failed to crystallize, but when treated with benzoyl chloride in pyridine it yielded chiefly a tribenzoate, m. p. 115.5–116°. The tribenzoate of  $\beta$ -phenylglycerol has been found by Prévost and Losson<sup>13</sup> to melt at 116.5°.

In the purification of the crude tribenzoate, a small amount of a product was found to be sparingly soluble in methanol. This was successively recrystallized from benzene, a 50–50 mixture of ethanol and methanol, and a 50–50 mixture of petroleum ether and benzene into fine white needles; m. p. 217°.

*Anal.* Calcd. for C<sub>48</sub>H<sub>38</sub>O<sub>9</sub>: C, 75.20; H, 5.18. Found: C, 75.11; H, 5.24.

This analysis seems to agree very well with that calculated for the tetrabenzoate of a mono-ether of  $\beta$ -phenylglycerol. In view of the small quantities available no further work was done on this product.

The viscous residue from the original fractionation, after the volatile products and the  $\beta$ -phenylglycerol had been removed, was extracted several times with hot water and the hot aqueous extract decolorized with charcoal whereby a white crystalline product soluble in hot but sparingly soluble in cold water separated out. It was recrystallized several times from water; m. p. 155.5–156°. It is soluble in ethanol and glacial acetic acid but sparingly soluble in ether and chloroform.

*Anal.* Calcd. for C<sub>18</sub>H<sub>22</sub>O<sub>6</sub>: C, 67.92; H, 6.91. Found: C, 67.36, 67.68; H, 6.86, 6.92.

A micromolecular weight determination in camphor gave a value of 345 as against 318 calculated for C<sub>18</sub>H<sub>22</sub>O<sub>6</sub>, another mono-ether of  $\beta$ -phenylglycerol.

This product was then benzoylated in pyridine and the

(10) Milas, *THIS JOURNAL*, **53**, 231 (1931).

(11) Marderosian, B.S. Thesis, M. I. T., 1935.

(12) Le Sueur, *J. Chem. Soc.*, **79**, 1315 (1901).

(13) Prévost and Losson, *Compt. rend.*, **198**, 659 (1934).

benzoate recrystallized from a 50-50 mixture of petroleum ether and benzene; m. p. 118-119°. A mixed m. p. with the tribenzoate of  $\beta$ -phenylglycerol gave a m. p. of 104-106°, showing that the two are different.

*Anal.* Calcd. for  $C_{46}H_{38}O_6$ : C, 75.20; H, 5.18. Found: C, 75.52, 75.12; H, 4.99, 5.19.

Hydrolysis of this product gave back the mono-ether of  $\beta$ -phenylglycerol, m. p. 155.5-156°. It is therefore the tetrabenzoate of another mono-ether of  $\beta$ -phenylglycerol and is certainly different from the one obtained in small quantities from the distillate of the main reaction product.

The yield of the  $\beta$ -phenylglycerol amounted to about 12.2% while that of its mono-ethers amounted to about 4.5%. A considerable amount of the cinnamyl alcohol went over to a resin as well as benzaldehyde and glycerinaldehyde, both of which were isolated.

### Summary

1. The hydroxylation of certain unsaturated substances with functional groups using hydrogen

peroxide in *t*-butyl alcohol and osmium tetroxide as the catalyst has led to the production of a number of useful glycols.

2. From ethyl crotonate, diethyl maleate, and diethyl fumarate, the corresponding hydroxylated esters were obtained in fairly good yields.

3. From mesityl oxide, 2-methylpentanediol-2,3-one-4 was obtained in about 23% yield.

4. From vinyl acetate, divinyl ether, and vinyl bromide, glycolaldehyde was obtained in yields of 60, 96, and 12.5%, respectively.

5. From oleic acid a yield of 60% of the high melting dihydroxy stearic acid was obtained.

6. From cinnamyl alcohol was obtained  $\beta$ -phenylglycerol and two different new mono ethers of this glycerol. The tetrabenzoates of the ethers were prepared and analyzed.

CAMBRIDGE, MASS.

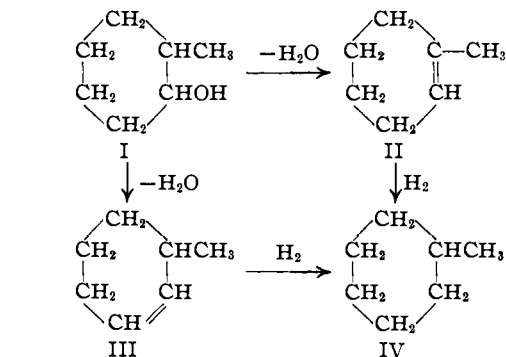
RECEIVED MAY 12, 1939

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

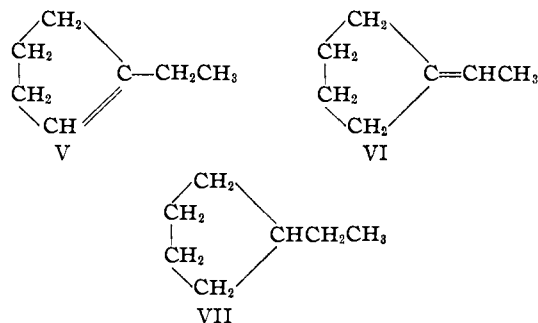
## The Dehydration of *trans*-2-Methylcyclohexanol

BY CHARLES C. PRICE

It recently has been reported<sup>1</sup> that the dehydration of 2-methylcyclohexanol with phosphoric anhydride, followed by hydrogenation of the olefin thus obtained, yielded a methylcyclohexane differing in its physical properties from any previously reported. It was suggested that this material was a new isomer, arising from the non-planar nature of the cyclohexane ring. Since the properties reported for the cyclo-olefin and cycloparaffin closely resembled those of 1-ethyl-1-cyclopentene and ethylcyclopentane, respectively, and since ring contraction could conceivably occur during the dehydration, the olefin intermediate in the transformation of the alcohol to the cycloparaffin has been examined carefully.



(1) Vogel, *J. Chem. Soc.*, 1323 (1938).



An examination of Table I reveals the similarity in physical properties of the cycloolefin obtained by Vogel with the cycloolefins III and V, as well as the similarity between his cycloparaffin and VII.

The dehydrations of *trans*-2-methylcyclohexanol carried out in this Laboratory, however, using phosphoric anhydride at temperatures varying from 140 to 230°, always resulted in the formation of a mixture containing only compounds II and III. The mixtures contained 35 to 50% of II; the components could be separated by fractional distillation.

The structures of the cycloolefins were determined by oxidation of the crude mixture with aqueous potassium permanganate. The products isolated were  $\epsilon$ -ketoheptanoic acid (as its semi-