[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF OREGON, AND THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF OREGON MEDICAL SCHOOL]

Studies on the Reactions between Formaldehyde and Enediols.[1a] I

BY F. J. REITHEL AND E. S. WEST

Krishnamurthy[1b] showed that ascorbic acid reacted with formaldehyde as evidenced by a marked decrease in reducing power when titrated with 2,6-dichlorophenol-indophenol or iodine. Lugg[2] found that ascorbic acid reacted readily with formaldehyde at *p*H 3.5 but slowly at *p*H 1.5, that some reducing substances reacted readily at both *p*H values, and still others, among them a crude preparation of reductone, reacted with formaldehyde at neither *p*H value. A critical study of Lugg's work was made by Snow and Zilva,[3] who found that reductone did react with formaldehyde but more slowly. Mapson[4] has also published some observations.

The prime purpose of these investigators was to develop a method for determining ascorbic acid in foods and biological products which would be more accurate than the simple method of dye titration. In addition, both Lugg[2] and Snow and Zilva[3] attempted to discover the nature of the reaction between enediols and formaldehyde. Extensive data were obtained on reaction velocities and equilibrium constants were determined but the reaction products could not be isolated.

In 1937, one of the present authors[5] noted the fact that formaldehyde abolished the reducing power of ascorbic acid. A study of this phenomenon produced evidence that *l*-ascorbic acid, *d*-isoascorbic acid, *d*-gluco-ascorbic acid, α-hydroxytetronic acid, reductone, and dihydroxymaleic acid lost the ability to reduce 2,6-dichlorophenol-indophenol when they were treated with formaldehyde. Furthermore, it was found that under the proper conditions mixtures of ascorbic acids and formaldehyde and similar mixtures of α-hydroxytetronic acid and formaldehyde slowly evolved carbon dioxide.

Like Snow and Zilva[3] we attempted to isolate a reaction product from mixtures of *l*-ascorbic acid and formaldehyde but no crystalline product was ever obtained. The sirupy material formed on removal of excess formaldehyde and evaporation of such reaction mixtures was investigated by many of the standard methods of carbohydrate chemistry such as methylation, acetylation, tritylation, periodic acid oxidation and permanganate oxidation. An acetone derivative and a semicarbazide were obtained in crystalline form but in very small yield. We concluded that a complex mixture of compounds was formed.

The following is a study of the reactions between enediols and formaldehyde at *p*H 3.5–6.0, utilizing carbon dioxide production as a criterion of reaction.

Experimental

1. **Carbon Dioxide Formation by Ascorbic Acid–Formaldehyde Mixtures.**—Two-hundred fifty milligrams of ascorbic acid was dissolved in 25 ml. of carbon dioxide- and oxygen-free water and neutralized to *p*H 7.2 with sodium hydroxide. Two and five-tenths milliliters of 37% formaldehyde was added and the mixture introduced into a small flask with a side arm arranged so that any carbon dioxide produced could be swept into an absorbing spiral immersed in barium hydroxide by a stream of nitrogen (purified over hot copper). The mixture was allowed to react at 60° for four hours, then acidified: carbon dioxide found, 52.8 mg.; theoretical (assuming one mole of carbon dioxide per mole of ascorbic acid), 62.5 mg.

In an exactly similar experiment calcium hydroxide was used to bring the *p*H to 7.65. Reaction was allowed to proceed for four hours at 60°, then acidified to liberate carbon dioxide from the calcium carbonate precipitate; carbon dioxide found, 57.7 mg.

A third experiment was done using the same quantities and conditions except that no alkali was added. After five hours at 60°, 62.2 mg. of carbon dioxide was produced.

Other variables were checked and the following observations made. Never was there obtained more than one mole of carbon dioxide per mole of ascorbic acid. The reaction proceeded somewhat more rapidly in alkaline than in acid solution. The reaction was slower at 30° than at 60° but the rate was appreciable. In blank experiments where no formaldehyde was added no carbon dioxide was obtained from ascorbic acid.

Several experiments were conducted to determine the effect of formaldehyde on the optical rotation of ascorbic acid. We found, as did Snow and Zilva, that the phenomenon was not simple. If the reaction mixture was allowed to remain in polariscope tubes for several days it was observed, upon opening them, that a considerable pressure of carbon dioxide had developed.

Following are the data from a typical experiment. One gram of ascorbic acid was dissolved in 19% formaldehyde and made up exactly to 50 ml.

TABLE I

Time	$[\alpha]^{25}D$
0 hr.	+33.4
4	+51.5
24	+50.7
2 days	+45.0
5	+25.0
10	0.0
13	−11.2
17	−25.0
19	−22.5[a]

[a] Reading was made after releasing gas pressure in tube.

2. **Rates of Carbon Dioxide Production under Varying Conditions of *p*H, Temperature and Concentration.**—In

(1a) A portion of this work was taken from a thesis by F. J. Reithel presented to the Faculty of the University of Oregon Medical School in partial fulfillment of the requirements for the Ph.D. degree in June, 1942. This work was presented at the Northwest Regional Meeting of the A.C.S. at Pullman, Wash., May 2, 1947.

(1b) Krishnamurthy, *J. Indian Chem. Soc.*, **18**, 383 (1941).

(2) Lugg, *Nature*, **150**, 577 (1942); *Australian J. Exp. Biol. and Med. Sci.*, **20**, 273 (1942).

(3) Snow and Zilva, *Biochem. J.*, **37**, 630 (1943); **38**, 458 (1944).

(4) Mapson, *J. Soc. Chem. Ind.*, **62**, 223 (1943).

(5) West and Ney, *Proc. Am. Soc. Biol. Chem.*, cii, 31st Annual Meeting (1937).

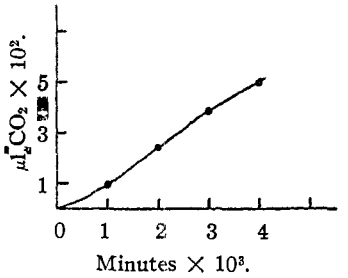

Fig. 1.—85 mg. of ascorbic acid in 10 ml. of 5.54% formaldehyde (0.0483 *M* ascorbic and 1.85 *M* formaldehyde), 40°, *p*H 3.5.

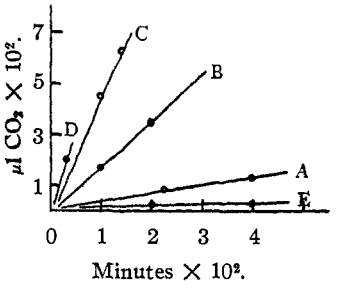

Fig. 2.—(A) 87.1 mg. of ascorbic acid in 10 ml. of formaldehyde (5% in 0.1 *M* phthalate buffer), *p*H 4.0; (B) 85.4 mg. of ascorbic acid in 10 ml. of formaldehyde (5% in 0.1 *M* phthalate buffer), *p*H 5.0; (C) 17 mg. of ascorbic acid in 2 ml. of formaldehyde (5.3% in *M* acetate buffer), *p*H 5.4; (D) 17 mg. of ascorbic acid in 2 ml. of formaldehyde (5% in 0.2 *M* phosphate buffer), *p*H 6.0; (E) portion of curve in Fig. 1 for comparison. All experiments carried out at 40°.

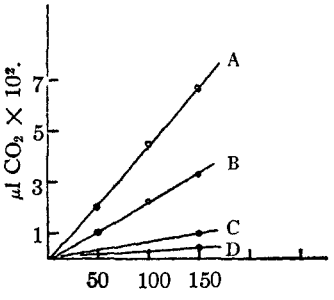

Fig. 3.—(A) 17 mg. of ascorbic acid in 2 ml. of formaldehyde (5.3% in *M* acetate buffer, *p*H 5.4), 0.0484 *M* ascorbic; (B) same except 0.0242 *M* ascorbic; (C) same except 0.0086 *M* ascorbic; (D) same except 0.0048 *M* ascorbic; all experiments carried out at 40°.

the following experiments the liberation of carbon dioxide from ascorbic acid–formaldehyde mixtures was followed in the Warburg manometric apparatus. All solutions were saturated with carbon dioxide before use and equilibrated with carbon dioxide while in the manometer flasks. The total volume of the reaction mixture was 2.0 ml. In most of the experiments the ascorbic acid was made up in buffer and tipped into the formaldehyde in the body of the Warburg flask after equilibration. (Figs. 1–5.)

3. Liberation of Carbon Dioxide from Mixtures of α-Hydroxytetronic Acid and Formaldehyde.—α-Hydroxy tetronic acid, $CH_2—COH=COH—CO$ (with O bridging the ring), was prepared by the method of Micheel and Jung.[6] (Fig. 6.)

4. The Effect of Formaldehyde on the Acidity of Solutions of Enediols.—A solution of 0.1000 *M* ascorbic acid in 37% formaldehyde was allowed to stand at 23° until it no longer reduced 2,6-dichlorophenolindophenol. As measured with a Beckmann *p*H meter this solution had a *p*H of 5.11. Five milliliters of this solution plus 2.50 ml. of 0.1000 *N* sodium hydroxide had a *p*H of 7.23. This corresponds to the *pK*. Our ascorbic acid (0.1000 *M* in water) had a *pK* of 4.08.

A similar experiment with reductone indicated a *pK* of 7.7 in 37% formaldehyde and 5.03 in water.

Two hundred and fifty milligrams of ascorbic acid was dissolved in 25 ml. of distilled water and 25 ml. of 37% formaldehyde added. The mixture was allowed to react in an atmosphere of nitrogen at 50°. At the beginning the *p*H was 4.4, at the end of eighteen hours the *p*H was 4.63.

Discussion

The foregoing data indicate that more than one reaction can occur in mixtures of formaldehyde and enediols. It is likely that Snow and Zilva were able to obtain reaction rates and equilibrium constants for an apparent single "condensation" reaction between enediols and formaldehyde because they worked with enediol concentrations of about 0.5 *M* and near room temperature. Under such conditions the liberation of carbon dioxide is very slow.

The hypothesis of Snow and Zilva that formaldehyde adds to carbons three and six of the ascorbic acid molecule is based on two lines of evidence. First, carbon number three was assumed to be involved because the *p*H of ascorbic acid solutions rose as reaction with formaldehyde progressed. This is confirmed by our data. Second, they found that six-substituted derivatives of ascorbic acid reacted at a different rate than ascorbic acid.

It would appear likely that such a reaction would lead to a product which is essentially neutral. Thus as reaction proceeds the *p*H should rise and sodium hydroxide titration values should decrease. However, Snow and Zilva found sodium hydroxide titration values to remain essentially the same throughout the course of reaction. They claim that this is due to reversal of the reaction by alkali. Krishnamurthy's data show that more ascorbic acid reacts at *p*H 6.0 than at 3.5 and our data show a marked acceleration of carbon dioxide liberation as the *p*H approaches 6.

Further we found that under our conditions little change in *p*H occurred during the course of carbon dioxide liberation. We interpret these findings as indicating that reaction with formaldehyde involves carbon number three and that the lactone ring breaks to free the potential carboxyl group. The carbon dioxide liberated probably arises from such a reaction product. That the lactone ring is involved is suggested by the fact that only those enediols possessing such a structure yield carbon dioxide when treated with formaldehyde.

It is anticipated that a more extended study of

(6) Micheel and Jung, *Ber.*, **66B**, 1291 (1933).

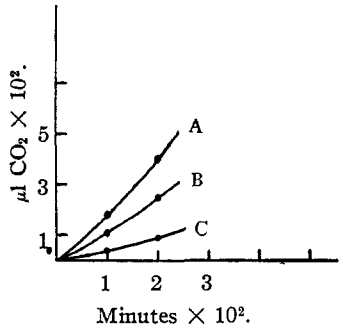

Fig. 4.—(A) 8.5 mg. of ascorbic acid in 2 ml. of formaldehyde (1.41 *M* in *M* acetate buffer, *p*H 5.4), 40° (B) same except 0.71 *M* formaldehyde; (C) same except 0.18 *M* formaldehyde.

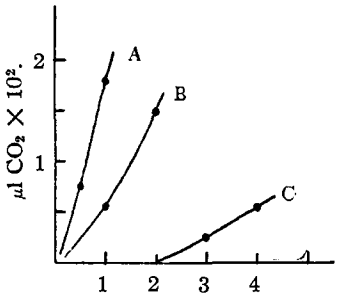

Fig. 5.—(A) 8.5 mg. of ascorbic acid in 2 ml. of formaldehyde (5.85% in *M* acetate buffer, *p*H 5.4), 40°; (B) same except 32°; (C) same except 25°.

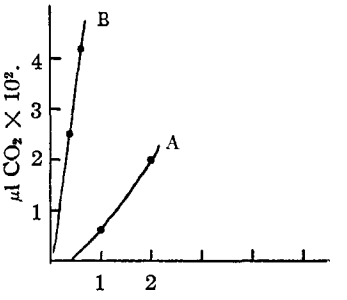

Fig. 6.—(A) 8.5 mg. of α-hydroxytetronic acid in 2 ml. of formaldehyde (5.85% in *M* acetate buffer, *p*H 5.4): 25.8° (B) same except 40°.

the phenomena described in this paper may lead to a quantitative method for determining ascorbic acid which is more specific than any yet devised.

Summary

1. It has been shown that ascorbic acid and α-hydroxytetronic acid react with formaldehyde and that under certain conditions one of the products of the reaction is carbon dioxide.

2. The rate of carbon dioxide formation in reaction mixtures of ascorbic acid and formaldehyde has been studied in relation to *p*H, temperature and concentration of reactants.

3. A similar study has been made on an α-hydroxytetronic acid–formaldehyde system.

4. The present work has been discussed in relation to previous studies which employed diminution of the reducing action of enediols as a criterion of reaction.

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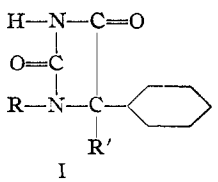
The Synthesis of 1-R-5-R'-5-Phenylhydantoins

BY LOREN M. LONG, C. A. MILLER AND H. D. TROUTMAN

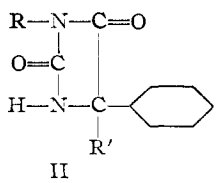
The discovery by Putnam and Merritt[1] of the efficacy of Dilantin[2] in the treatment of epilepsy has resulted in the synthesis and subsequent testing of a large number of related compounds. The activity of many of these against electrically induced convulsions has been reported by Merritt and Putnam.[3] On examining this report one notes that the majority of the hydantoins which have been studied are those substituted in the 5-position only. It was logical, then, to extend the types of hydantoins studied to include those containing an N-substituent.

Since a search of the literature revealed that an appreciable number of 3,5-substituted hydantoins have been prepared,[4] it seemed that the synthesis of hydantoins substituted in the 1- and 5-positions offered a better opportunity of finding new and useful anticonvulsants. Indeed, in reference to 3-substituted hydantoins, a derivative of 5-ethyl-5-phenylhydantoin (nirvanol) has been introduced recently[5] as being effective in reducing the number of convulsions exhibited by epileptic patients. However, the use of this drug, 3-methyl-5-ethyl-5-phenylhydantoin, may result in rash formation similar to that caused by nirvanol.

Another possible advantage of compounds unsubstituted in the 3-position, as in structure (I), was thought to be the retention of dilute alkali solubility in contrast to the dilute alkali insolubility of compounds of structure (II). Thus, in many



I



II

cases the sodium salts of cyclic ureides are preferred over the free compounds, *i. e.*, Dilantin[2] and phenobarbital.

A few derivatives of type (I) have been prepared

(1) Putnam and Merritt, *Science*, **85**, 525 (1937).

(2) Dilantin—registered trade-mark of Parke, Davis & Co. for 5,5-diphenylhydantoin.

(3) Merritt and Putnam, *Epilepsia*, **3**, 51 (1945).

(4) British Patents 430,255, 430,282, 430,283, 430,473; French Patent 769,667; German Patent 611,057; Swiss Patents 166,004, 168,947, 168,948, 169,509, 171,982, 176,827, 177,411, 179,255, 179,690, 179,692.

(5) Lascalzo, *J. Nerv. Ment. Dis.*, **101**, 537 (1945).