GLYOXYLUREA[1]

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This communication describes a chemical method for the synthesis of glyoxylurea, $NH_2-\overset{O}{\overset{\|}{C}}-\overset{H}{\overset{|}{N}}-\overset{OH}{\overset{|}{\underset{H}{\underset{|}{C}}}}-COOH$, (Blitz and Robl, 1920) and presents evidence that this compound is an intermediate in the degradation of allantoin by Streptococcus allantoicus (Valentine and Wolfe, 1960).

Methods and Results

Preparation and properies: Glyoxylurea was prepared as follows: 5 g of sodium glyoxylate (Sigma) and 7.5 g of urea were dissolved in 25 ml. of 0.05M potassium phosphate buffer at pH 7.0. The reaction mixture was incubated for 2 hr. at 30°. Upon the addition of 9 volumes of 95% ethanol to the vigorously-stirred reaction mixture large crystals were obtained. The white precipitate was collected on a Buchner funnel, dissolved in a minimal amount of water, and recrystallized as above. The crystals were washed while on the filter with ethanol and then with ether. Yields of approximately 5 g were obtained; the compound was stored as the dry white powder, and was identified as glyoxylurea by the following criteria:

1. The elemental analysis is shown in Table I. A known sample of allantoin was analyzed as a control.

The empirical formula, $C_3H_5N_2O_4Na$, was thus proposed for glyoxylurea.

2. Negative aldehyde tests indicated that the aldehyde group is bound.

3. Heating in dilute acid or alkali decomposes the compound to its constituents, glyoxylate and urea.

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TABLE I

Composition of Glyoxylurea

Element	Glyoxylurea	Allantoin
	per cent	per cent
C	22.6	30.4
H	2.8	3.7
N	16.4	37.3
O	42.7	28.5
Na	15.5	-

TABLE II

Ratio of Glyoxylate and Urea in Glyoxylurea

Compound	Ratio $\frac{\text{Glyoxylate}}{\text{Urea}}$
Glyoxylurea	1.1
Allantoin	0.5
Allantoic Acid	0.5

4. The ratio of glyoxylate and urea in glyoxylurea is presented in Table II; allantoin, allantoic acid, and glyoxylurea were degraded by the procedure of Young and Conway (1942). Glyoxylate was estimated colorimetrically by the Rimini-Schruyver reaction (Young and Conway, 1942); urea was determined in the method of Archibald (1944).

5. Figure 1 is an elution diagram obtained when 10 mg of glyoxylurea were chromatographed on Dowex-1X8 (formate). A linear salt gradient of 0-5% sodium formate was used for elution. After degradation, glyoxylurea was determined either as glyoxylate or urea.

Enzymic degradation. An ammonium-sulfate, dialized, lyophilized enzyme preparation from S. allantoicus catalyzes the cleavage of glyoxylurea. Figures 2 and 3 show the effect of enzyme concentration and time, respectively, on the degradation of glyoxylurea. Allantoic acid is not decomposed by this enzyme preparation. The reaction mixture for measuring glyoxylurea degradation consisted of a suitable amount of enzyme, 1 mg glyoxylurea, 10 μmoles phenylhydrazine, and 20 μmoles potassium phosphate (pH 7.0). Incubation was at 30° for 20 min.

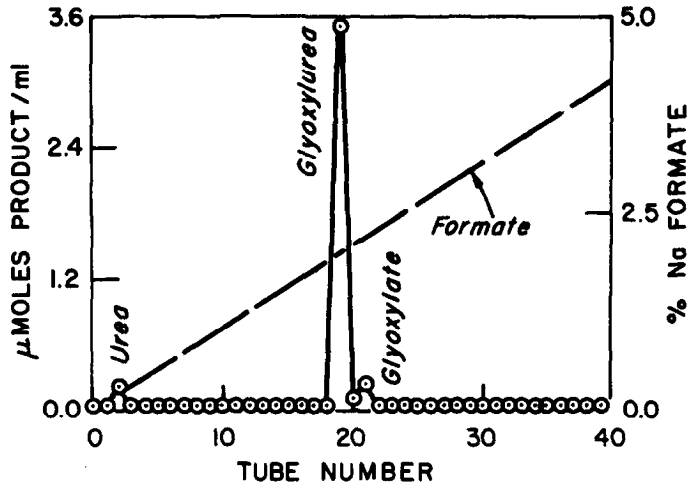

Figure 1. Chromatography of synthetic glyoxylurea on Dowex-1X8 (formate).

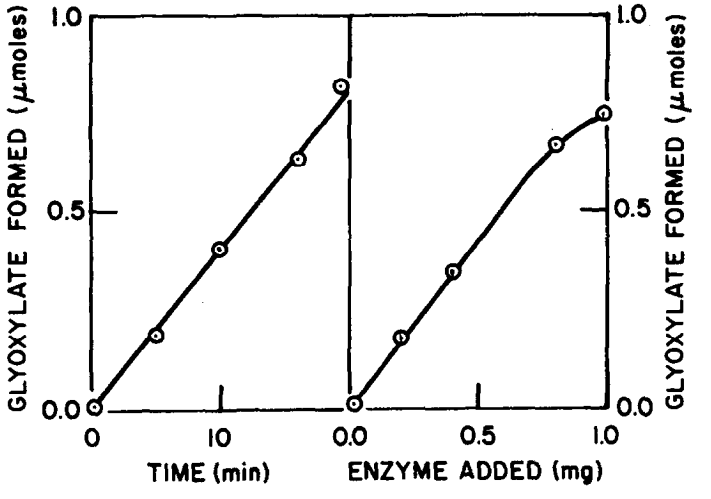

Figure 2 and Figure 3. Degradation of glyoxylurea by extracts of Streptococcus allantoicus as a function of time and of enzyme concentration.

Extracts of an unidentified Pseudomonas species grown on allantoin degraded glyoxylurea at about half the rate of S. allantoicus extracts. No cleavage of glyoxylurea (glyoxylurease activity) was detected in extracts of Sarcina ventriculi, Clostridium butyricum, Bacillus cereus, Leuconostoc mesenteroides, or Gluconobacter liquefaciens.

While the reaction of ammonia with carbonyl compounds is known to be biologically important, little is known about interactions of urea and aldehydes. The condensation of glyoxylate and urea occurs readily and appears to be an important step in allantoin decomposition.

References

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