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### Summary

1. The rate constants for the hydrolysis of the three butadiene monochlorohydrins by water and by base have been determined.

2. The conversion of the two butadiene  $\alpha$ -chlorohydrins to the epoxide by base is extremely rapid. Their second order rate constants have been compared with those of ethylene and propylene chlorohydrins and have been discussed with regard to the mechanism involved.

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[CONTRIBUTION FROM THE DIVISION OF COTTON AND OTHER FIBER CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE]

## Identity of Some Electrodialyzable Constituents of Cotton Root Bark

BY DAVID R. EGGLE, L. E. HESSLER AND J. E. ADAMS

The Soil Fertility unit of the present Bureau of Plant Industry, Soils, and Agricultural Engineering for several seasons studied the effects of soils and fertilizer treatment on the composition of the cotton plant in relation to the incidence of phymatotrichum root rot. This paper presents the results of a systematic attempt, quantitatively to isolate and identify definite organic compounds, principally nitrogenous, from the dialyzates of cotton root bark. Electrodialysis<sup>1,2,3</sup> was used to separate the nitrogen that was subsequently determined as ammonia, amide, amino and basic nitrogen. While these experiments were not completed with respect to determining the relationship to root rot resistance of these organic constituents, it is felt that a record of the occurrence of the indicated compounds in the cotton plant may be of value to other workers.

### Experimental<sup>4</sup>

One kilogram of oven-dry, finely ground root bark from mature upland cotton plants was electro-dialyzed.<sup>1</sup> The catholyte, containing practically all of the dialyzable organic nitrogen, was investigated for nitrogenous compounds while the anolyte was examined to a limited extent for solid organic acids. The relative importance of the two fractions as investigative materials is shown by their organic nitrogen contents; the catholyte yielded 12.07 g. of nitrogen while the anolyte contained only 0.20 g.

Fractionation of the catholyte into groups suitable for isolation studies was accomplished by methods used by Vickery.<sup>5</sup> Only enough of the methods are given to ensure clarity of presentation.

A partial segregation and concentration of the catholyte nitrogen was effected with aqueous mercuric acetate. The precipitate, after decomposition with hydrogen sulfide, contained the following amounts of nitrogen

Nitrogen	Grams
Ammonia	0.474
Amide	2.978
$\alpha$ -Amino	2.746
Basic	1.049
Other	2.133
Total	9.380

(1) Collins, *et al.*, *Soil Sci.*, **44**, 217-229 (1937).

(2) Adams, *et al.*, *Proc. Soil Sci. Soc. Am.*, **4**, 329-332 (1939).

(3) Hessler, *et al.*, *J. Am. Soc. Agron.*, **31**, 528-540 (1939).

(4) All melting points uncorrected.

(5) Vickery, *Agr. Exp. Sta. Bull.* (Conn.), **339**, 639-645 (1932).

**Purines.**—The purine fraction contained only 0.024 g. of nitrogen from which no crystalline isolate was made. If either adenine or guanine was present, the amount was small.

**Histidine.**—Histidine was present as indicated by the diazo reaction,<sup>6</sup> but actual isolation was not accomplished. The nitrogen content of this fraction was 0.104 g.

**Arginine.**—The amount of nitrogen, 0.707 g., in this group was relatively large and from it arginine was obtained as the monoflavinate, equivalent to 0.555 g. of free arginine.

*Anal.* Calcd. for  $C_{16}H_{20}O_{10}N_6S$ : S, 6.56. Found: S, 6.45.

**Lysine.**—This fraction, containing 0.138 g. of nitrogen, failed to give a crystalline product when treated with picric acid in the conventional manner.

**Asparagine.**—The solution left after separation of the lysine fraction, when freed of phosphotungstic acid and barium, was concentrated by evaporation and ethanol added until a slight turbidity developed. After standing at 8-10° for several days, asparagine monohydrate separated as transparent rhombic prisms. Total yield of the recrystallized product was 26.5 g. Identification was made both by analysis and by preparation of copper asparaginate.

*Anal.* (1) Calcd. for  $C_8H_{12}O_4N_2$ : N, 21.20. Found: N, 21.21.

*Anal.* (2) Calcd. for  $C_8H_{14}O_4N_2Cu$ : N, 17.20. Found: N, 17.11.

**Betaine.**—The filtrate from the mercuric acetate precipitation, after removal of mercury, contained 1.360 g. of nitrogen. Of this amount, precipitation with phosphotungstic acid accounted for 1.03 g. of basic nitrogen. After removal of reagents and concentrating, part of the betaine was isolated as the picrate. For the remaining betaine, a second and better method was the precipitation of betaine from an alcoholic solution with mercuric chloride. Betaine hydrochloride in fairly pure form was thus obtained after removal of mercury and evaporation to dryness. The combined yield of free betaine by the two methods was 4.46 g. Identification was made by analysis of both derivatives.

*Anal.* (1) Calcd. for  $C_5H_{12}O_2NCl$ : N, 9.12. Found: N, 9.25.

*Anal.* (2) Calcd. for  $C_{11}H_{14}O_8N_4$ : N, 16.18. Found: N, 16.17.

As shown in Table I these isolations accounted for 46.87% of the total organic nitrogen content of the catholyte.

**Oxalic Acid.**—The anolyte was examined for solid organic acids amenable to separation by differential solubility of their copper salts.

Concentrated to 150 ml. by evaporation at reduced pressure, the anolyte was treated while hot with a slight

(6) Kapeller-Adler, *Biochem. Z.*, **271**, 206-208 (1934).

TABLE I

Compound	Wt., g.	Nitrogen, g.	% of total N in catholyte
Arginine	0.555	0.179	1.48
Asparagine	26.50	4.945	40.97
Betaine	4.46	0.533	4.42
Total	31.515	5.657	46.87

excess of cupric carbonate and filtered. The soluble fraction containing principally inorganic cupric salts was discarded. The insoluble residue on the filter was freed of copper with hydrogen sulfide and the aqueous solution concentrated by evaporation. On standing at 8–10° the nearly colorless solution yielded finally 4.91 g. of rhombic crystals which reduced potassium permanganate readily and melted at 190°. Characterization as oxalic acid was completed when the substance reacted with an excess of aniline to form oxanilide, m. p. 253°.

### Discussion

Although the number of nitrogenous compounds isolated in this study was small, a high percentage of the nitrogen in certain fractions was accounted for as definite, crystalline substances, and nearly half of the total amount of catholyte nitrogen was similarly identified. Asparagine, for example, comprised 83% of the amide and 90% of the  $\alpha$ -amino nitrogen found to be present. If the  $\alpha$ -amino nitrogen of arginine is added to that of asparagine, the latter value is increased to 92%. Therefore the data show that the remaining amounts of these two forms of nitrogen as possible unidentified compounds do not exceed 0.2 g., respectively.

The possibility of the coexistence of the homolog of asparagine, glutamine, was realized but indicative tests for this common plant constituent were negative. However, another related compound, aspartic acid, was present in an amount too small for consideration and was of doubtful origin. Its presence may have been due to the hydrolysis of a

small amount of asparagine during the isolation of the latter compound.

Identification of substances in the two basic nitrogen fractions (A, basic nitrogen in mercuric acetate precipitate, and B, basic nitrogen in filtrate from mercuric acetate precipitation) was less successful. Arginine, the only isolate from (A), comprised less than 20% of the nitrogen known to be present. In the (B) fraction the isolation of betaine identified more than half of the total amount of nitrogen indicated. Choline, a closely related plant constituent, if present, occurred in an amount too small for positive identification.

The "other nitrogen," segregated from the various nitrogenous groupings in the catholyte, constituted a substantial part of the total nitrogen but little or no knowledge of its composition was obtained in this study.

According to the isolation data asparagine and betaine were present to the extent of 2.65 and 0.45%, respectively, of the oven-dry weight of root bark. These concentrations appear sufficiently large to suggest that they may have some bearing on the chemistry and physiology of nitrogen reserves in the cotton plant. One may conjecture that part of the nitrogen accumulating at plant maturity is stored in the root bark in the form of these compounds.

### Summary

Arginine, asparagine, betaine and oxalic acid were isolated in significant quantities from the electrolysates of extracts from cotton root bark.

Approximately half of the total organic nitrogen content of the catholyte was accounted for as definite compounds of which asparagine was predominant.

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## A Relation between the Oxidation-Reduction Potentials of Quinones and the Resonance Energies of Quinones and of Hydroquinones

BY ERNST BERLINER

The normal oxidation-reduction potential of a quinone is a measure of the free energy of reduction by hydrogen of the quinone to the hydroquinone, and expresses the oxidizing power of the quinone with reference to that of hydrogen ions. Measurements of potentials of many quinones have shown that substitution in the quinone changes the potential in a manner fairly well predictable by means of resonance or inductive effects of the substituent group.<sup>1,2</sup> Also, fusion of one

or more benzene rings to the quinone ring invariably lowers the potential.

The main structural difference between a quinone and the corresponding hydroquinone is the cyclic unsaturated ketonic structure in the quinone and the aromatic ring in the hydroquinone. Although both compounds are stabilized by a certain amount of resonance energy, the stabilizing energy inherent in the aromatic ring system certainly outweighs the amount of resonance energy possessed by the quinone. Since almost all quinones have a positive potential, *i. e.*, they are reducible by hydrogen on a platinum electrode, the assumption is reasonable that the driv-

(1) Branch and Calvin, "The Theory of Organic Chemistry," Prentice-Hall, Inc., New York, N. Y., 1941, p. 315.

(2) Fieser and Fieser, "Organic Chemistry," D. C. Heath and Company, Boston, Mass., 1944, p. 729.