Phytochemistry, 1970, Vol. 9, pp. 1215 to 1217. Pergamon Press. Printed in England.

SHORT COMMUNICATION

FERRIC IRON COMPLEXES OF HYDROXAMIC ACIDS FROM MAIZE*

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(*Received* 24 *October* 1969)

Abstract—Stability constants for the Fe^{3+} complexes of a hydroxamic acid and its glucoside, isolated from maize seedlings, have been determined. Log *K* for the glucoside is 19·4 and, for the aglucone, 21·3.

INTRODUCTION

THE CEREAL grasses maize, wheat and rye have been found to contain glucosides of hydroxamic acids, the principle ones being 2-*O*-glucosyl-4-hydroxy-1,4-benzoxazin-3-one (I) and 2-*O*-glucosyl-4-hydroxy-7-methoxy-1,4-benzoxazin-3-one(II).[1-3] Upon injury of the plant cells, these glucosides are hydrolyzed enzymatically, releasing the corresponding aglucones III and IV (Fig. 1). The aglucone IV also occurs at low concentrations in intact maize plants.[4] Hydroxamic acids produced by micro-organisms have been shown to function in iron

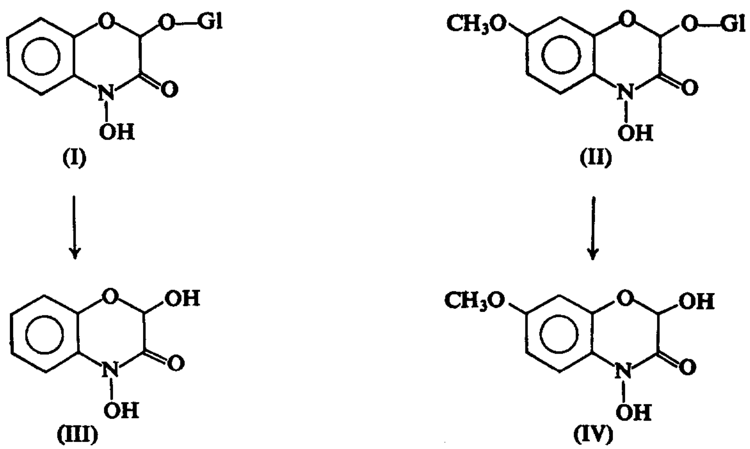

FIG. 1. STRUCTURES OF THE BENZOXAZINONE HYDROXAMIC ACIDS FROM MAIZE.

* Journal Paper No. J-6381 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, U.S.A. Project No. 1648. Taken from a dissertation submitted by E. L. B. in partial fulfilment of the requirements for the degree of Master of Science. Supported in part by the United States Department of Agriculture, Entomology Research Service.

[1] O. WAHLROOS and A. I. VIRTANEN, *Acta Chem. Scand.* **13**, 1906 (1959).
[2] A. I. VIRTANEN and P. K. HIETALA, *Acta Chem. Scand.* **14**, 499 (1960).
[3] P. K. HIETALA and A. I. VIRTANEN, *Acta Chem. Scand.* **14**, 502 (1960).
[4] O. WAHLROOS and A. I. VIRTANEN, *J. Pharm. Sci.* **53**, 844 (1964).

metabolism,[5] and the possibility has been suggested that the hydroxamic acids may play a similar role in higher plants.[6] As a means of evaluating the possibility that the cyclic hydroxamic acids of maize participate in iron metabolism, we have determined stability constants for the Fe^{3+} complexes of compounds II and IV.

RESULTS AND DISCUSSION

The results are shown in Table 1. The various constants are defined as:

$$HA \rightarrow A^- + H^+$$

$$Fe^{+3} + A^- \rightarrow FeA^{+2}$$

$$FeA^{+2} + A^- \rightarrow FeA_2^+$$

$$FeA_2^+ + A^- \rightarrow FeA_3$$

$$K_1 = \frac{(FeA^{+2})}{(Fe^{+3})(A^-)}; \quad K_2 = \frac{(FeA_2^+)}{(FeA^{+2})(A^-)}; \quad K_3 = \frac{(FeA_3)}{(FeA_2^+)(A^-)};$$

$$K = K_1 K_2 K_3 = \frac{(FeA_3)}{(Fe^{+3})(A^-)^3}; \quad K_I K_{II} K_{III} = \frac{(FeA_3)(H^+)^3}{(Fe^{+3})(HA)^3};$$

$$\text{Log } K = \log K_I K_{II} K_{III} + 3\, pK_a.$$

TABLE 1. FORMATION CONSTANTS OF THE Fe^{3+} COMPLEXES OF SOME HYDROXAMIC ACIDS AND CITRIC ACID

Compound	pK_a	Log K_1	Log K_2	Log K_3	Log $K_I K_{II} K_{III}$	Log K	Ref.
Acetohydroxamic acid	9·37	11·37	10·16	7·1	0·45	28·63	
Acetohydroxamic acid	9·37	11·42	9·68	7·24	0·23	28·34	7*
II	6·40	9·25	6·49	3·7	0·23	19·4	
IV	6·95	9·39	6·51	5·4	0·45	21·3	
Deferriferrichrome						29·1	5
Citric acid						11·85	8

* Determined at 20°C.

The agreement between the values obtained for acetohydroxamic acid and those reported by Schwarzenbach and Schwarzenbach[7] is quite close. The difference between the overall formation constants for the benzoxazinene hydroxamic acids and that of acetohydroxamic acid is mainly a reflection of the difference in acidity, as can be seen from the values for $K_I K_{II} K_{III}$, which are virtually identical for the two types of hydroxamic acids.

The overall formation constants for the hydroxamic acids from maize are several orders of magnitude lower than those of the trihydroxamic acids which function in microbial iron metabolism,[5] but much higher than that of citric acid,[8] which is reported to function in iron absorption and transport in higher plants.[9] At the concentrations (approx 10^{-5}–10^{-3} M)[10]

[5] J. B. NIELANDS, *Science* **156**, 1443 (1967).
[6] E. R. PAGE, *Biochem. J.* **100**, 34P (1966).
[7] G. SCHWARZENBACH and K. SCHWARZENBACH, *Helv. Chim. Acta* **46**, 1390 (1963).
[8] L. G. SILLEN and A. E. MARTELL, *Stability Constants of Metal Ion Complexes*, 2nd ed., The Chemical Society, London (1964).
[9] J. C. BROWN and L. C. TIFFIN, *Plant Physiol.* **40**, 395 (1965).
[10] J. A. KLUN and J. F. ROBINSON, *J. Econ. Entomology* **62**, 244 (1969).

at which these compounds occur in young maize plants, a high proportion of the ferric iron present must be bound as complexes to the hydroxamic acids. Further study is required to determine if they do, in fact, play a significant role in iron metabolism.

EXPERIMENTAL

The cyclic hydroxamic acids II and IV were isolated from maize seedlings and purified according to published procedures.[1,11] Acetohydroxamic acid was synthesized from ethyl acetate and hydroxylamine.

Potentiometric measurements of free ferric ion concentration were made over a range of hydroxamic acid concentrations, at four pH values, with a platinum electrode and a Beckman Research pH meter.[12,13] All measurements were made at 25°, ionic strength 0·100 maintained with $NaClO_4$. From these measurements, stability constants were calculated as described by Schwarzenbach and Schwarzenbach.[8] pK_a values for the cyclic hydroxamic acids were determined by spectrophotometric titration.[14]

[11] C. L. Tipton, J. A. Klun, R. R. Husted and M. D. Pierson, *Biochemistry* **6**, 2866 (1967).
[12] H. W. Dodgen and G. K. Rollefson, *J. Am. Chem. Soc.* **71**, 2600 (1949).
[13] F. J. C. Rossotti and H. Rossotti, *The Determination of Stability Constants*, McGraw-Hill, New York (1961).
[14] R. R. Husted, Ph.D. Thesis, Iowa State University, 1968. (Mic 69-4244, Univ. Microfilms, Ann Arbor, Mich.)