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COPPER(II) COMPLEXES OF A HYDROXAMIC ACID FROM MAIZE

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Key Word Index—*Zea mays*; Gramineae; maize; hydroxamic acids; benzoxazinones; Cu(II) complexes; stability constants; metal transport.

Abstract—The thermodynamics of formation for DIMBOA–Cu(II) complexes (where DIMBOA = 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one, a hydroxamic acid from maize) has been investigated in aqueous solutions by a potentiometric method. DIMBOA forms 1:1 and 1:2 chelates with Cu(II) at ionic strength 0.05 M ($NaClO_4$). The stability constants measured were about 10^5 and 10^4 for the 1:1 and 1:2 complexes respectively, determined at 10, 20 and 30°. The contribution of ΔH and ΔS to the stability of complexes is examined and the pK values are compared with other ligands found in maize. Although DIMBOA has similar or higher constants to form copper complexes than other plant ligands, its possible role as a transport agent in maize remains to be established.

INTRODUCTION

Cyclic hydroxamic acids from *Zea mays* L. are important because of their ability to inhibit plant pathogens [1]. Other hydroxamic acids found in microorganisms which are important in transport of ions have a high affinity for iron, cobalt and other cations [2]. The possibility that DIMBOA (1), the main hydroxamic acid from maize extracts [3], plays a similar role in the transport of metal ions in plants has been suggested [4]. Tipton and Buell [5] measured the stability constants for the iron (III) complexes of DIMBOA and of its glucoside.

Copper is known to be an essential element for plants [6], and it is also known to form complexes with hydroxamic acids [7]. Therefore, we considered it of interest to study the possible formation of complexes between DIMBOA and copper. The experiments described in this paper were designed to determine the species in aqueous solutions, the stability constants of these complexes, as well as their thermodynamic stability parameters.

RESULTS

Titration curves of alkali with DIMBOA and DIMBOA plus Cu^{2+}, both in presence of perchloric acid are shown in Fig. 1. The discontinuity which may be seen in Fig. 1, around 0.7 equivalents of base per mole of DIMBOA, is a consequence of the appearance of a precipitate. For the calculations of stability constants only the points before this discontinuity were considered. These results suggest the formation of a complex between DIMBOA and Cu(II) in solution because: (a) there was a departure of curves a and b (Fig. 1) at pH lower than for the hydrolysis of Cu^{2+} ions (pH = 5.35 at 10^{-4} M Cu^{2+}). From the point of departure a pink colour appeared in the DIMBOA plus Cu^{2+} solution; (b) a pink-brown precipitate formed close to pH 6.0, whose analysis showed a DIMBOA–Cu relation 1:1. The possibility of a hydroxylated copper species as a part of the precipitate may not be rejected since a pH around 6 is required for its formation at $Cu^{2+} = 10^{-4}$ M. When DIMBOA was coordinated to Cu(II) the IR spectra of the solid precipitate formed at pH 5.5–6.0 presented the frequency associated to the C–O–C ring group

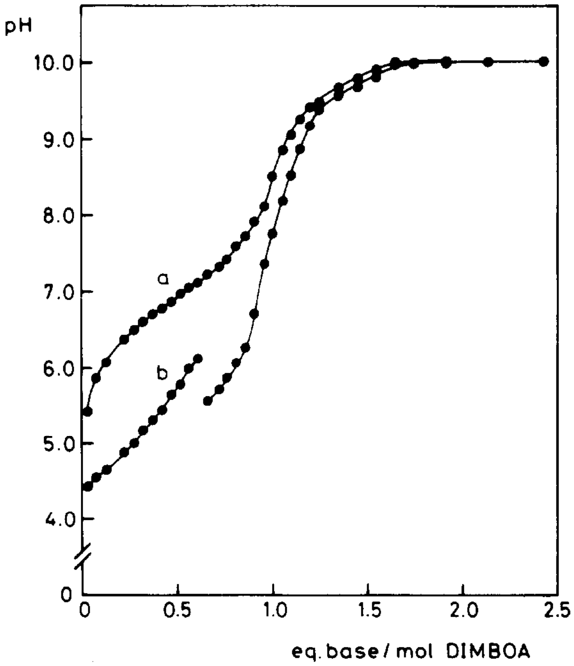

Fig. 1. Titration of solutions containing DIMBOA or DIMBOA and $Cu(ClO_4)_2$. (a) 10^{-3} M DIMBOA and 10^{-2} M $HClO_4$; (b) 10^{-3} M DIMBOA, 10^{-2} M $HClO_4$ and 5.10^{-4} M $Cu(ClO_4)_2$. Sections for the titration of $HClO_4$ are not shown. This titration was performed at 20°. Similar titrations were performed at 10° and 30° (not shown).

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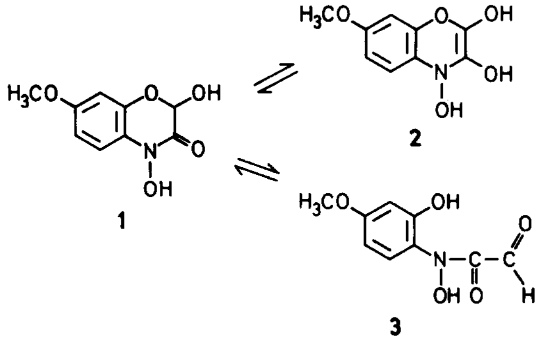


Scheme 1.

(1200–1260 cm^{-1} region) in the same position as free solid DIMBOA; the C=O was displaced about 60 cm^{-1}, and the OH of the N–O–H group was not present. Therefore the closed keto structure **1** seems to be the most appropriate to form metallic complexes, with the N–O–H and C=O functional groups as coordinating points. Attempts made to confirm these results by NMR in D_2O solutions were unsuccessful due to the perturbation by the paramagnetic centre. DIMBOA is known to decompose in aqueous solutions [8]. However, it is unlikely that our experiments were affected, since $t_{1/2}$ of decomposition of DIMBOA at 30°, pH 5.5, is 75 hr. Thus, during the titration time (30 min) at most 0.35% of DIMBOA would decompose. Moreover, the concentration of DIMBOA calculated from the equivalence point of a titration curve (Fig. 1, a) corresponded to the theoretical one.

Table 1 summarizes the values of the acidity, K_a and the stability constants K_1 and K_2 defined and calculated as described in the Experimental, together with the thermodynamic parameters, ΔH and ΔS. With the experimental values of K_a, K_1 and K_2, theoretical formation curves of $\bar{n}_t$ vs pL were obtained which were compared with the experimental ones. The standard deviation (with $N = 23$) fluctuated from 0.01 to 0.05 for all the curves compared at any of the three temperatures.

Distribution curves were obtained for the species in solution by means of COMICS, a computer program modified in our laboratory [9]. Besides the equilibrium concentration of the LCu^+ and L_2Cu complexes, the formation constants for basic species of Cu(II), K_3 and K_4 [10] as defined below, were considered in the computer calculations:

$$Cu^{2+} + H_2O \overset{K_3}{\rightleftharpoons} CuOH^+ + 2H^+$$

$$2Cu^{2+} + 2H_2O \overset{K_4}{\rightleftharpoons} Cu(OH)_2^{2+} + 2H^+.$$

Table 1. Stability constants and thermodynamic parameters of DIMBOA and its copper complexes

Temperature	$-\log K_a$	$\log K_1$	$\log K_2$
10°	6.93±0.03	5.51±0.03	4.11±0.03
20°	6.69±0.02	5.31±0.02	3.92±0.03
30°	6.36±0.02	5.02±0.08	3.80±0.10
Thermodynamic parameters			
ΔH (kJ/mol)	55.2	−46.5	−31.0
ΔS (J/g·mol)	59.0	−56.5	−30.1
r^*	0.997	0.995	0.994

Potentiometric titrations were done as described in the experimental section. The values of the constants are the average of three determinations at each temperature, and the uncertainty indicated in each value is the largest deviation of the log K with respect to log $\bar{K}$. r^* is linear regression coefficient for $\ln K = \frac{-\Delta H}{RT} + \frac{\Delta S}{R}$.

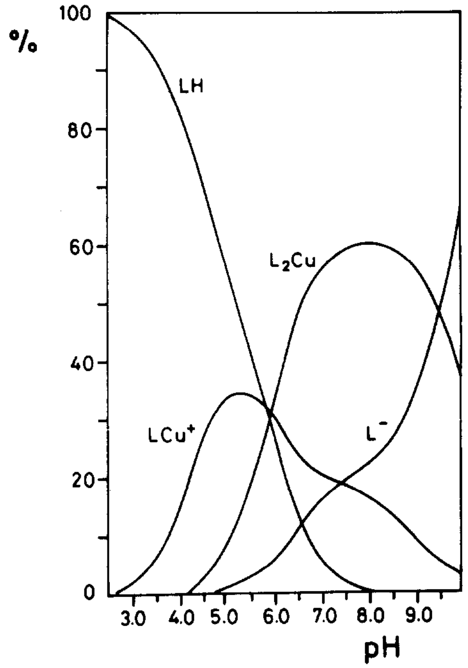
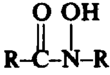


Fig. 2. Distribution curves for DIMBOA and DIMBOA–Cu(II) complexes as % of total ligand. This diagram was calculated from stability and acidity constants by means of COMICS, a computer program modified in our laboratory [17]. LH, DIMBOA; LCu and L_2Cu, DIMBOA copper complexes; L, DIMBOA anion formed by dissociation of the hydroxamic acid.

Figure 2 shows the distribution results with respect to ligand. It must be pointed out that distribution curves are valid only up to the pH where precipitation takes place.

DISCUSSION

Hydroxamic acids have the general formula  $R\text{–}\overset{O}{\overset{\|}{C}}\text{–}\overset{OH}{\overset{|}{N}}\text{–}R'$. DIMBOA should present a tautomeric equilibrium in solution (**1**), and the information published [11–13] suggests that the keto form (**1**) may be predominant.

Two K_a values, at 7 and at 10.5 have been found for the two dissociable hydrogens, which may be ascribed to the dissociation of the N–OH and C–OH protons respectively [14]. Considering substitution on the R groups, N–OH in DIMBOA has an acidity comparable to hydroxamic acids with a highly electrophilic R group [15, 16]. The log K values given in Table 1 compared with results for other hydroxamic acid–Cu(II) complexes are up to

10^4–10^5 times lower, which may be in agreement with the high acidity of DIMBOA.

There are several reports on stability constants of hydroxamic acid complexes with metal ions; however few thermodynamic studies have been carried out. In mixed solvents for example [17], the values of ΔH and ΔS are negative and positive respectively; as the polarity increases the exothermicity decreases and the entropy change is less favourable. It may be seen in Table 1, that the formation of the species Cu(II)–DIMBOA and Cu(II)–$(DIMBOA)_2$ in aqueous solution are essentially stabilized by the enthalpy change. The negative values for ΔH and ΔS may be explained according to Nancollas [18] as a predominantly-B-character interaction between ligand and metal, indicating some degree of covalency of the coordinate bond [18]. The negative values of $\Delta S(ML^+)$ and $\Delta S(ML_2)$ may indicate the importance of the degree of freedom lost by the ligand; the removal of the tautomeric equilibria of the ligand on coordination may also be relevant.

According to the 'hard' and 'soft' nomenclature, Cu^{2+} and DIMBOA may be classified as limiting behaviour [7], however, the results obtained here for the DIMBOA–Cu(II) complex makes them appear to have a definitive B-character.

EXPERIMENTAL

Reagents. H_2O distilled (× 3) and CO_2-free was used in the preparation of all solns. $NaClO_4$ (Merck) was used to adjust the ionic strength to 0.05 M. The concn of dilute solns of $HClO_4$ (Mallinckrodt 70 %) was determined by potentiometric titrations with standard NaOH (CO_2-free); carbonate-free NaOH (Merck) solns were prepared by diluting a filtered 50 % NaOH soln with CO_2-free H_2O. The solns were standardized by titration with KH-phthalate [19]. Cu(II) perchlorate was prepared by treating a soln of $CuSO_4$ and $HClO_4$ with excess of $CuCO_3$. After filtration and evaporation to a small vol. the solid obtained was recrystallized twice from H_2O at 0°. The solns of $Cu(ClO_4)_2$ were standardized by complexometric titrations with Na_2EDTA using pyrocatechol violet as indicator in the presence of pyridine [20].

Isolation of DIMBOA. Seeds of maize (*Zea mays* L. cv. LH Rinconada) were planted on filter paper, dampened with H_2O and grown in the dark at 28° for 5–7 days. DIMBOA was isolated from the seedlings by a modification of the method of ref. [21] as described in ref. [8]. Recrystallization from Me_2CO by addition of C_6H_{12} gave light-pink needles. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm: 288 (shoulder), 262 ($\varepsilon = 10^5\ \mathrm{l\,mol^{-1}\,cm^{-1}}$), 206.5. NMR and IR data were the same as those reported in ref. [22] and ref. [21], respectively. Due to the thermal decomposition of DIMBOA [8], a stock soln to be diluted for titrations could not be used and fresh solns were prepared each time.

Bjerrum–Calvin titrations. Titrations were carried out using a Digital Radiometer PHM 62 standard pH-meter with glass and calomel electrodes (Radiometer G 202 and K 401, respectively). The soln to be titrated was made up to 20 ml and the beaker was immersed in H_2O thermostatted at ±0.1°. $NaClO_4$ was added to keep the ionic strength constant at 0.05 M. The pH titrations were carried out by the Bjerrum–Calvin method as described in ref. [23]. Titrations were performed for metal–ligand = 1:2 to allow the formation of higher complexes and carried out at 3 temp, 10, 20 and 30°; N_2 previously saturated with H_2O and free of O_2 was continuously bubbled into the soln to provide an inert atmosphere. Initial concns of $HClO_4$, DIMBOA and Cu^{2+} for titrations were 1.0×10^{-2}, 1.0×10^{-3} and 5×10^{-4} M, respectively; 1×10^{-2} M NaOH was used to titrate.

Calculations of the acid dissociation constant K_a *for the reaction* $LM \rightleftharpoons L^- + H^+$ (LH–DIMBOA). The practical acid dissociation constants K_a of the ligand acid were determined from the equation (calculated by the least squares method):

$$\mathrm{pH} = \log\frac{(1-\bar{n}_A)}{\bar{n}_A} + \log\frac{1}{K_a}.$$

Values of $\bar{n}_A$, the mean number of hydrogen ions bound per ligand molecule, were calculated from the method of ref. [24].

Stability constants of the complexes. The stoichiometric metal–ligand constants for 1:1 (K_1) and 1:2 (K_2) complexes in the range $0 < \bar{n} < 2$ were determined substituting the experimental values of $\bar{n}$ and L in the equation:

$$\frac{\bar{n}}{(1-\bar{n})L} = \frac{(2-\bar{n})\,LK_1K_2}{(1-\bar{n})} + K_1.$$

The value of $\bar{n}$, the mean number of ligands bound per metal ion present in whatever form and L the free ligand concentration were calculated as in ref. [28]. K_1 and K_2 correspond to the following equilibria:

$$Cu^{2+} + L^- \overset{K_1}{\rightleftharpoons} CuL^+$$

$$CuL^+ + L^- \overset{K_2}{\rightleftharpoons} CuL_2.$$

Since an approximate calculation showed that the value of $\log\frac{K_1}{K_2} < 1.78$, K_1 and K_2 values were calculated by the least squares method [13].

Thermodynamic parameters. The values of the enthalpy and entropy changes associated with the various equilibria were calculated from the expression (adjusted by the least squares method):

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

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