for gas chromatographic analysis, thiourea was added to the samples.

Butt and Hallaway [11] have reported that thiourea slowly disappears in an ascorbic acid solution, due to the hydrogen peroxide generated by the autoxidation of ascorbic acid. However, with the parameters used in the present experiments, no decrease in the inhibitory effect of thiourea was found (Table 1).

It was found that the non-enzymatic ethanol oxidation in fresh rat liver, extract, containing $0.3 - 0.6$ mM ascorbic acid, was completely inhibited by $25 - 50$ mM thiourea. The required concentration of thiourea depends primarily on the incubation time and the ascorbic acid level in the tissue sample. In blood samples which have a low ascorbic acid content, a much lower thiourea concentration was needed to inhibit the ethanol oxidation.

Experimental. The concentrations of acetaldehyde and ethanol in the samples were determined in a Perkin-Elmer automatic F 40 head-space gas liquid chromatograph with a hydrogen flame detector. The column used was 15 % polyethyleneglycol on celite (60/100). Each bottle in which the head-space was produced had 0.5 ml of the sample, containing either liver extract or ascorbic acid and 20 mM ethanol, added. The bottles were placed in a thermostatically controlled water bath at 65°C and kept at equilibrium for at least 15 min prior to the automatic analysis of the head-space gas. The analytical procedure was standardized with samples of diluted acetaldehyde and ethanol. The acetaldehyde was determined by using fresh redistilled acetaldehyde (50 μM) as a reference standard. The acetaldehyde concentration in the standard was checked daily with 0.025 ‰ *tert*.butanol as an internal standard.

The thiourea (of reagent grade from E. Merck, Darmstadt, Germany) was dried at 90°C for 12 h before the use to eliminate traces of volatile compounds which interfere with the acetaldehyde determination. The stability of thiourea in a 0.6 N perchloric acid (PCA) solution was tested but no decreased inhibitor effects was found even if the PCA-thiourea solution was one to two weeks old.

The rat liver was quickly removed from the animal after decapitation, and 5 g of the liver was homogenized in 20 ml 0.6 N PCA containing different amounts of thiourea. The precipitate was centrifugated, and 0.5 ml of the supernatant was analyzed by gas chromatography after incubation with 20 mM ethanol. The thiourea can also be added directly to the

acid supernatant after homogenization if the homogenization in PCA and the subsequent centrifugation are carried out quickly at a temperature of $+4°C$.

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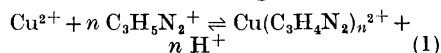
On the Existence of Aqueous Penta- and Hexaimidazole Complexes of Copper(II)

STAFFAN SJÖBERG

Department of Inorganic Chemistry, University of Umeå, S-901 87 Umeå, Sweden

In an earlier emf-investigation,[1] equilibria

$$Cu^{2+} + n\ C_3H_5N_2^+ \rightleftharpoons Cu(C_3H_4N_2)_n{}^{2+} + n\ H^+ \quad (1)$$

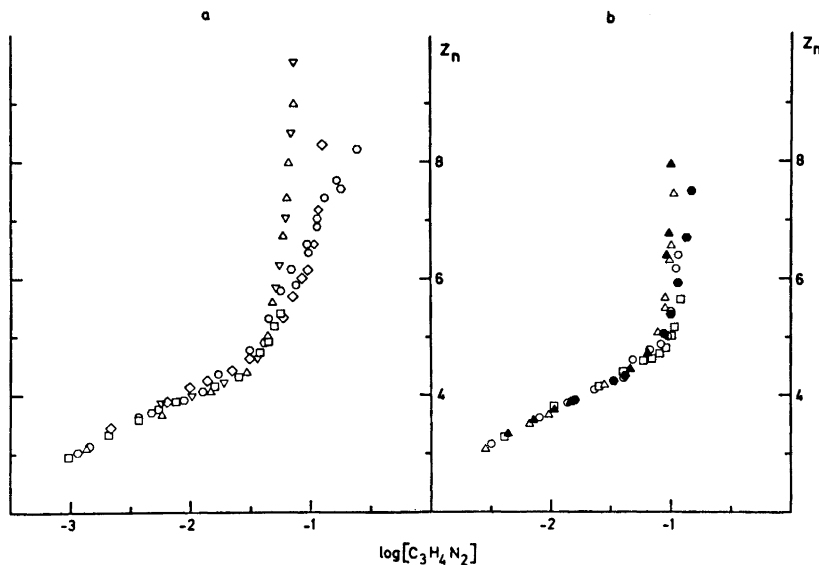were studied at 25°C and in 3.0 M (Na)ClO$_4$. It was then found that, Z_n, the

Fig. 1. Experimental data Z_n(log $[C_3H_4N_2]$) for copper(II)-imidazole equilibria in (a) 3 M (Na)ClO$_4$ and (b) 3 M (Na)Cl. The symbols stand for the following starting concentrations in B and C (in mM): a) $\triangle = 0.48 - 128.7$, $\bigtriangledown = 0.48 - 128.7$, $\Diamond = 2.39 - 258.2$, $\square = 5.12 - 323.7$, $\bigcirc = 4.78 - 516.1$, $\langle \rangle = 1.91 - 514.0$, b) $\blacktriangle = 1.26 - 150.0$, $\triangle = 1.26 - 150.0$, $\blacklozenge = 2.03 - 242.2$, $\bigcirc = 2.51 - 299.7$, $\square = 5.06 - 300.1$ Filled symbols represent back-titrations (decreasing pH).

average number of imidazole molecules bound per copper atom, reached values greater than four. Data obtained could be explained by assuming that besides complexes with $n = 1$, 2, 3, and 4 a complex with $n = 6$ also existed. However, an alternative explanation based upon a variation in the acidity constant for imidazole or/and an analytical error in the total concentration of imidazole was possible. Since the data given in Ref. 1 were too limited to provide a careful study, we found it necessary to perform supplementary measurements, and attempt a more complete interpretation.

Method and data. Measuring and titration procedures were the same as described earlier.[1] The hydrogen ion concentration was measured with a glass electrode at 25°C. In order to detect and prevent possible systematic errors caused by the ionic medium used, the study was carried out using two media, 3.0 M (Na)ClO$_4$ and 3.0 M (Na)Cl. The reversibility of the equilibria was tested by making both forward and backward titrations. The re-

producibility between different titrations using different stock solutions was good and no systematic errors caused by analysis or method of preparation could be detected.

The average number of imidazole molecules bound per copper (II) atom, Z_n, is given by the relationship:

$$Z_n = \frac{h - H - K_a h^{-1}(C + H - h)}{B} =$$

$$\frac{\sum n \beta_n (h^{-1}c)^n}{1 + \sum \beta_n (h^{-1}c)^n} \qquad (2)$$

where $h = [H^+]$, $c = [C_3H_5N_2^+]$ and B, $C =$ the total concentration of copper(II) and imidazole, respectively. H is the total analytical concentration of H^+ over the zero level Cu^{2+}, $C_3H_5N_2^+$ and H_2O. H may be negative. K_a is the acidity constant of $C_3H_5N_2^+$ and β_n the equilibrium constant of reaction (1). Evidently if the acidity constant, K_a is known, Z_n and log$[C_3H_4N_2]$ can be calculated from available emf-data $H(\log h)_{B,C}$ by using eqn. (2).

Table 1. "Best" acidity constants, $K_a(C_3H_5N_2{}^+)$ and $-\log \beta_n$ for the first four $Cu(C_3H_4N_2)_n{}^{2+}$ complexes in the media 3 M (Na)ClO$_4$ and 3 M (Na)Cl. The errors given are $\pm 3\sigma$ (log β_n).

Medium	$-\log K_a$ $\pm 3\sigma$	$-\log \beta_1$ $\pm 3\sigma$	$-\log \beta_2$ $\pm 3\sigma$	$-\log \beta_3$ $\pm 3\sigma$	$-\log \beta_4$ $\pm 3\sigma$
3 M (Na)ClO$_4$	7.913	3.25	7.18	11.79	17.04
	0.002	0.02	0.02	0.04	0.04
3 M (Na)Cl	7.637	3.24	7.21	11.85	17.17
	0.001	0.01	0.01	0.02	0.02

Table 2. Results of a LETAGROP[2] (version ETITR[3]) analysis in the range $Z_n \geq 3$. In the calculations the possibility of complexes $Cu(C_3H_4N_2)_5{}^{2+}$ and $Cu(C_3H_4N_2)_6{}^{2+}$ and corrections in the total imidazole concentration, ΔC and K_a, have been tested. For β_1, β_2, β_3, and β_4 the values given in Table 1 have been used and not varied. The error $\pm 3\sigma$(log β_n) is given only when the equilibrium constant has been varied. Range 1 refers to the data where $3 \leq Z_n \leq 5$ and range 2 to the whole data range ($Z_n \geq 3$). $\Delta C = ((C_{corr} - C_{exp})/C_{exp}) \times 100$ is given in the same order as the titrations are listed in Figs. 1 a and 1 b. The error squares sum has been defined as
$$U = \sum((H_{calc} - H_{exp})/B)^2.$$

Medium	Range	$U \times 10^{-5}$	$-\log K_a$ $\pm 3(\sigma)$	$-\log \beta_5$ $\pm 3(\sigma)$	$-\log \beta_6$ $\pm 3(\sigma)$	$\Delta C(\%)$
3 M (Na)ClO$_4$	1	2.10	7.913	—	—	a
			0.001			
		3.81	»	23.96	30.17	0
				0.30	0.13	
		2.00	»	23.84	30.32	b
				0.25	0.13	
	2	51.5	»	23.96	30.17	c
3 M (Na)Cl	1	3.13	7.629	—	—	d
			0.001			
		2.03	7.637	23.89	30.46	0
				0.19	0.11	
		1.69	»	23.90	30.48	e
				0.19	0.11	
	2	30.04	»	23.89	30.46	f

^{a} 0.6, 0.6, 4.0, 4.3, 9.0, 3.5, ^{b} -0.01, 0.01, 0.7, -0.2, 2.4, 0.7, ^{c} 0.4, 0.4, 0.6, -0.1, 1.4, 0.4, ^{d} -0.1, -0.07, 0.07, -0.04, 0.4, ^{e} 0.04, 0.07, 0.09, -0.01, -0.1, ^{f} 0.6, 0.5, 0.5, 0.1, -0.05.

To attain values $Z_n > 4$ it is necessary to keep the ratio $C/B > 60$, otherwise copper(II) imidazoles or copper(II) hydroxide precipitates. Furthermore, to avoid activity coefficient variations, ranges with rather low concentrations had to be studied. The ranges 0.5 mM $\leq B \leq$ 5 mM, 120 mM $\leq C \leq$ 320 mM (in some instances $C \leq$ 516 mM), $1 \leq -\log h \leq 9$ were investigated.

Figs. 1a and 1b show data Z_n (log [C$_3$H$_4$N$_2$]) for the 2 media, 3.0 M (Na)ClO$_4$ and 3.0 M (Na)Cl, respectively. According to eqn. (2), such a plot of Z_n would give points falling on a single curve independent of the values of B and C. This requirement is fulfilled for both media up to $Z_n \approx 5$, but for $Z_n > 5$ the curves start to deviate from each other. This behaviour must mean that below $Z_n \approx 5$ there exists a series of stepwise mononuclear complexes Cu-(C$_3$H$_4$N$_2$)$_n{}^{2+}$ with $n = 1, 2, 3, 4,$ and 5 or 6. The spread for $Z_n > 5$ indicates either formation of other types of complexes (polynuclear and/or mixed) or analytical and

experimental errors. That the activity coefficient variation should cause this spread we find less probable.

Separate studies of the range $Z_n < 4$ (assuming $n = 1$, 2, 3, and 4) and determinations of $K_a(C_3H_5N_2^+)$ gave the equilibrium constants and standard deviations collected in Table 1. In these separate investigations the upper limits for the concentration ranges (in mM) were: 3 M (Na)ClO$_4$, (3 M (Na)Cl); $B = 20$, (80); $C = 330$, (324) [C$_3$H$_4$N$_2$] = 281, (111).

Within these concentration ranges, the acidity constant of C$_3$H$_5$N$_2^+$ appeared to be independent of C, and the formation constants for the different copper complexes were independent of B and C as long as $C/B \geq 8$. This supports our assumption that activity factors cannot cause the spread at $Z_n > 5$.

Calculations and results. First we will try to explain the range $3 \leq Z_n \leq 5$, where the different Z_n-curves seem to coincide (*cf.* Figs. 1a and 1b). The results of the calculations of main interest are given in Table 2. A rather good fit to experimental data is obtained in 3 M (Na)ClO$_4$ as well as in 3 M (Na)Cl, if one introduces the formation constants β_5 and β_6 to the model with only four mononuclear complexes Cu(C$_3$H$_4$N$_2$)$_n^{2+}$, ($n = 1...4$). In 3 M (Na)Cl this model fits data better than a correction in C, ΔC and K_a without β_5 and β_6. In 3 M (Na)ClO$_4$ a good fit is obtained with a variation in C without β_5 and β_6. However, ΔC appears too great to be experimentally possible (in some instances 4 – 9 %). A somewhat better fit is obtained if, in addition to a correction in C, β_5 and β_6 were introduced. In this case the corrections in C become more tolerable (*cf.* Table 2).

We consider next the data range extended to include data where $Z_n > 5$. A calculation was made where log β_5 and log β_6 were kept constant in the two media, and had values determined in the range $Z_n \leq 5$. Assuming the deviations from the model Cu(C$_3$H$_4$N$_2$)$_n^{2+}$, ($n = 1...6$) to be due to an error in C, a good fit is obtained with corrections in C (usually < 0.5 %, *cf.* Table 2), which would seem to be reasonable, considering the experimental conditions. It is worth noticing that variations in K_a give K_a-values in very good agreement with those separately determined.

Calculations assuming mixed or polynuclear complexes were also carried out, but these calculations gave no further improvements.

Obviously the present complementary measurements and analysis at $Z_n > 4$ strongly confirmed the results earlier published. It has been clearly established that the effects at high Z_n-values cannot be explained solely by analytical or experimental errors. Additional complex formation must be taken into account. There is evidence for the formation of both the complexes Cu(C$_3$H$_4$N$_2$)$_5^{2+}$ and Cu(C$_3$H$_4$-N$_2$)$_6^{2+}$.

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Fatty Acid Composition of the Seed Fats of a Few Vacciniaceae and Empetraceae

AAGE JART and HANNE I. KRISTENSEN

Department of Organic Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark

No previous studies of fatty acids from seeds of plants belonging to the families Vacciniaceae and Empetraceae have been recorded.[1] As to the related family Ericaceae, (occasionally considered as including Vacciniaceae), a single analysis on *Arctostaphylos glauca* has been published,[2] *cf.* Table 1.