

Fig. 2. High resolution NMR spectrum (Varian A60A) of 2-chlorobuten-3-yne. Ca. 0.3 M solution in CCl_4 . I: X part. II: Original AB part. III: The AB part after spin decoupling (field sweep mode) with a difference of -159.2 cps.

intensities and the remaining solution is:
 $\nu_X = 183.4$ cps, ($\delta = 3.06$ ppm), $\nu_{AB} = 342.9$ cps, ($\delta = 2.28$ ppm), $|J_{AB}| = 1.1$,
 $J_{AX} = J_{BX} = \pm 0.5$, $\nu_A - \nu_B = 3.6$ cps.

The low spin coupling constant J_{AB} no doubt indicates geminal hydrogen atoms as does the low value of the long range coupling constants. In monovinylacetylene a geminal coupling constant of 2.0 cps and long range coupling constants of 0.7 and 0.8 cps have been found⁷ but the coupling constants of hydrogen atoms in the *cis* and *trans* positions are as high as 11.5 and 17.5 cps. The chlorobutenyne derived from acetylene is thus identified as 2-chlorobuten-3-yne.

The two chlorobutenynes from 1,4-dichloro-2-butyne, however, have a different structure. Only the *cis* and *trans* forms can be considered. There is reason to believe that the more volatile substance, corresponding to the IR curve A, is *trans*-1-chlorobuten-3-yne.

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Model Systems for Copper-Protein Interaction: Polynuclear Copper(II) Complexes of Glycylhistidylglycine

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In an effort to gain a detailed understanding of the interactions in solution between Cu^{2+} ions and histidyl residues present in proteins,^{1,2} a quantitative study using a low-molecular-weight model compound, glycylhistidylglycine (H_2A^{2+}), has been performed at 25.0° in 3.0 M NaClO_4 medium.

Glycylhistidylglycine was obtained commercially from Yeda (Israel) and was tested for purity by paper chromatography, paper electrophoresis, and quantitative amino acid analysis. Acid-base data were recorded for values of the total concentrations of peptide (A), equal to 2.5, 10, and 20 mM, by using a glass electrode, calibrated against a hydrogen electrode. This gave the pK values 3.57, 7.54, and 8.62. When solutions of A~B (total concentration of Cu^{2+}), were titrated with OH^- in the pH range 3 to 7, it was found — in agreement with the results of Bryce, Roeske and Gurd³ — that the Cu(II)-induced proton dissociation of the peptide bond occurred at relatively low pH (\sim pH 5). Below pH 7 there was only one proton released in this manner per each peptide molecule.

Emf titrations using a copper amalgam electrode were carried out in order to give

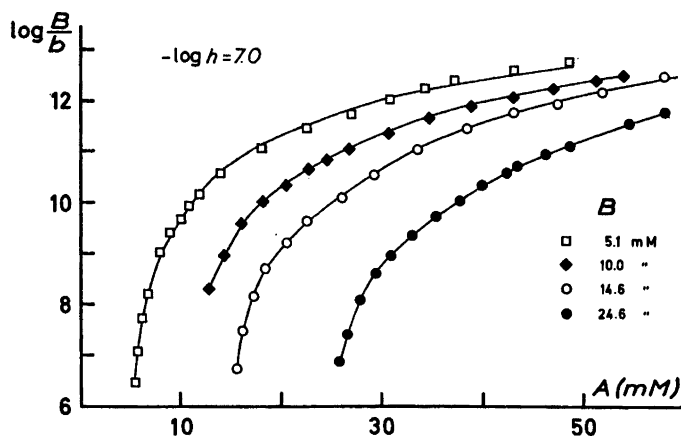


Fig. 1. Copper(II) complexes of glycylhistidylglycine in 3.0 M NaClO₄ medium at 25.0°. The symbols (circles etc.) are experimental points for the various values of B corresponding to $-\log h = 7.00$. The curves have been calculated assuming the species, CuL₂, Cu₃L₄, Cu₁₅L₁₆ and $\log \beta_1^* = 19.95$, $\log \beta_3^* = 53.7$, $\log \beta_{15}^* = 254.0$. For notations see the text.

the data $\log B/b(A)_{h,B}$. These were recorded for $B = 5, 10, 15$, and 25 mM and $\log h = -7.00$ (Fig. 1). $b = [\text{Cu}^{2+}]$, $h = [\text{H}^+]$. The apparatus was essentially that described previously.⁴ After the titrations the value on B was checked by electroanalysis.

The data were treated by first determining a , the free ligand concentration by graphical integration.⁴ Then $\log B/b$ was plotted against $\log a$. These data varied with B and thus indicated polynuclearity.⁵ When $\log (B/a)$ was plotted as function of $\log (ab)$ all the experimental points fell on a single curve. A comparison of this curve with normalized graphs⁶ showed that the data involving $A \geq 1.3 B$ could best be explained assuming the formation of CuL₂ and Cu₃L₄. This comparison also gave the first values of the equilibrium constants, β_1^* and β_3^* . These refer to $\log h = -7.00$ and $\beta_n^* = [\text{L}(\text{CuL})_n]/(b^n a^{n+1})$. L denotes the ligand and includes all different protonated forms that may enter the complexes. (The charges are omitted).

Least squares refinement of the constants β_1^* and β_3^* was made using the computer program Letagropvrid,⁷ the special block "Ubbe" being slightly modified for the present problem. Here, we successively tested the L(CuL) _{n} species, having $n = 2, 4, 5$, and the species CuL and Cu₂L₂. However, only Cu₃L₄ gave an improved agreement with the experimental data. When the complete set of data ($A \geq 1.05$

B) was finally treated, it was necessary to introduce a complex of high nuclearity. This complex was found to be Cu₁₅L₁₆ from a series of L(CuL) _{n} species tested, having values of n from 7 to 19. Now, on eliminating Cu₅L₆, there was just a slight increase in the error square sum. The logarithms of the equilibrium constants, β_n^* , of our final set of species became (giving $3 \sigma(\log \beta_n^*)$):

$$\log \beta_1^* = 19.95 \pm 0.05, \\ \log \beta_3^* = 53.7 \pm 0.1, \log \beta_{15}^* = 254.0.$$

It is tempting to regard the species predominating in solution, CuL₂, Cu₃L₄, (Cu₅L₆), and Cu₁₅L₁₆, as fragments of an infinite chain -peptide-Cu-peptide-Cu-peptide-. Such chains are known to form when copper(II) ions crystallize with similar ligands such as triglycine⁸ and glycylhistidine.⁹ In our system, a copper ion within the chain may be coordinated either to the amino nitrogens of the two neighbouring peptides or to their imidazole nitrogens. Coordination *via* the amino nitrogen of one peptide molecule and an imidazole nitrogen of a second molecule would be more difficult to explain in view of the fact that no fragments L(CuL) _{n} having $n = 2$ and 4 could be detected.

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A New Methylated Uronic Acid from Paper Pulp Hydrolysates

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Recent examinations of the acidic components of the hydrolysates of paper pulps prepared from birch,¹ eucalyptus and pine^{2,3} woods, have shown that 4-*O*-methyl-D-glucuronic acid is the major monouronic acid present. A methylated uronic acid of unknown structure was shown to be an important component of the acids of birch sulphate pulp, whereas it could not be detected in birch hemicellulose. Eucalypt sulphate, pine sulphate, and eucalypt neutral sulphite pulps were also found to contain this acid. All four pulps had been prepared under alkaline conditions and it is noteworthy that no trace of the acid was found in a pine bisulphite pulp or in bisulphite cooking liquors.⁴ These results indicate that the new acid

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was formed by some rearrangement of the 4-*O*-methyl-D-glucuronic acid units in xylan under alkaline cooking conditions.

Results and discussion. As demonstrated by Perry and Hulyalkar,⁵ uronic acids can be reduced with sodium borohydride to the corresponding aldonic acids without detectable side reactions. It was expected that the new methylated uronic acid would yield a methylated aldonic acid on reduction and that demethylation of the latter would give a known aldonic acid. Perry and Hulyalkar have tested their method using a variety of uronic acids but no experiments were reported concerning its application to methylated acids. Trial experiments were therefore conducted with 4-*O*-methyl-D-glucuronic acid to determine suitable conditions for the reduction and demethylation reactions.

Reduction of 4-*O*-methyl-D-glucuronic acid with sodium borohydride gave a single methylated aldonic acid in high yield. Demethylation of this compound with hydrobromic acid yielded gulonic acid as expected. Reduction of the unknown acid with sodium borohydride also gave a single acidic product, confirming that the original compound was a uronic acid and not a ketoaldonic acid. The product of the reduction gave no reaction with carbazole, and was assumed to be a methylated aldonic acid. On demethylation with hydrobromic acid it yielded idonic acid, identified by comparison of its chromatographic behaviour with that of an authentic sample. This comparison was made using automated column chromatography on Dowex 1-X8 (Ac⁻) in both sodium acetate (Fig. 1) and acetic acid, and by paper chromatography. Further confirmation was obtained by preparation of the tetra-*O*-trimethylsilyl-1,4-lactone and comparison

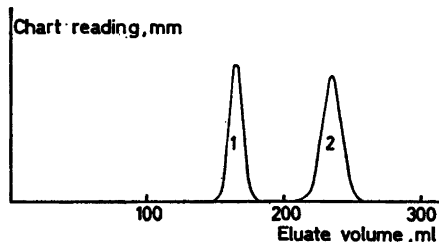


Fig. 1. Separation of 3-*O*-methyl-L-idonic acid (1) from the demethylated acid (2) in 0.05 M sodium acetate on an anion exchange column, 6 × 835 mm.