Metal Ion Complexes of Amino Acids. Part II [1]. The Copper Complexes of the α- and β-Isomers of N-oxalyl-L-α,β-diaminopropionic Acid.

P. O'BRIEN

Department of Chemistry, Chelsea College, Manresa Road, London SW3 6LX, U K.

and P. B. NUNN

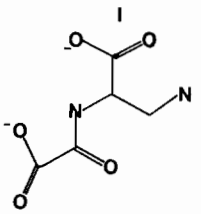
Department of Biochemistry, King's College London, Strand, London WC2R 2LS, U.K.

Received December 2, 1981

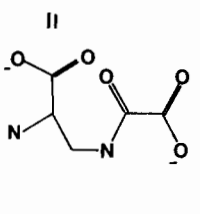
The copper complexes of the title amino acids of 1:1 stoichiometry have been synthesised. Their structure is discussed in relation to that of the aspartate and glutamate complexes of copper(II).

Introduction

The non-protein amino acid, β-N-oxalyl-L-α,β-diaminopropionic acid (II) (β-ODAP), has been isolated from, or detected in, the seed of many species of *Lathyrus, Crotalaria* and *Acacia* [2–4]. The substance undergoes spontaneous acyl transfer in aqueous solution [5] to form the corresponding α-isomer (I) (α-ODAP), which has been chemically synthesized [6, 7]. The β-isomer is acutely toxic to animals (see 8) but there is no generally accepted mechanism to explain this toxicity. The amino acid is considered by some to be an anti-metabolite of glutamate [9–12] but recent evidence suggests that the mechanism of action of β-ODAP may be against kainate binding receptors in the spinal cord [13]. This behaviour may implicate the amino acid in the chronic crippling human disease neurolathyrism. α-ODAP has a low toxicity [6, 7], but nothing is known of its long-term effects in animals. It has no appreciable electrophysiological activity in the *in vitro* frog spinal cord preparation [14].



I



II

Our interest in these compounds is two-fold. Firstly, a knowledge of the chemical similarities of α- and β-ODAP to aspartic or glutamic acids might give clues to the biochemical functions of the two oxalyl amino acids. Secondly, a difference in the solubilities of various salts or complexes of α- and β-ODAP might provide a facile method of separating the two isomers allowing an improved synthetic route to the α-isomer. At present a somewhat lengthy

TABLE I. Analytical Results and Parameters determined from Electronic and Electron Spin Resonance Spectra for Cuα-ODAP·H_2O and Cuβ-ODAP·H_2O

%C	%H	%N	%Cu	%H_2O	λ_{max}[a]	g_{av}	$g_{\parallel}$[d]	$g_{\perp}$[d]	$A_{\parallel}$[d]	Comment
23.49	3.15	10.96	24.85	7.05	–	–	–	–	–	calculated for CuODAP·H_2O
23.49	3.26	10.77	24.48[b] 24.40[c]	7.22	13 6	2 131	2.326	2.071	141	Cuβ-ODAP·H_2O
23.61	3.56	10.69	24.00[c]	7.20	13.0	2.130	–	–	–	Cuα-ODAP·H_2O

[a] $cm^{-1} \times 10^{-3}$, measured by diffuse reflectance, peak of a single broad absorption between 6 and 22 × 10^3 cm^{-1}. [b] Atomic absorption. [c] T.G.A. 10 °C min^{-1} as CuO. [d] 1% doped in ZnβODAP, $A_{\parallel}$ $cm^{-1} \times 10^4$.

0020-1693/82/0000-0000/$02.75

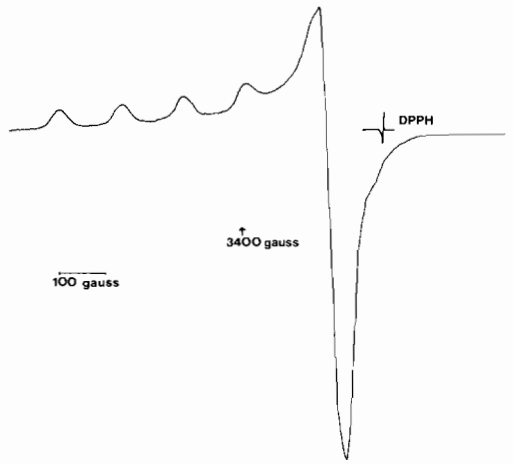


Fig. 1. ESR of Cuβ-ODAP doped in Zn complex.

chromatographic procedure is the only method available to separate the two isomers on a relatively large scale [7] and the direct synthesis of the α-isomer gives a poor yield [6].

Preliminary studies showed that, in common with aspartic and glutamic acids, both isomers give soluble calcium salts that were insoluble in aqueous ethanol [15]. β-ODAP yielded an insoluble copper chelate (as does aspartate), a feature not shared with the α-isomer, while neither oxalyl derivative gave an insoluble hydrochloride, as is well known with glutamic acid. Apart from such preliminary findings, little is known of the chemistry of these amino acids. In this paper we report on the coordination chemistry of α- and β-ODAP with copper(II).

Results

Monohydrated complexes of 1.1 stoichiometry were prepared with α- and β-ODAP with copper(II) as described in the experimental section; analytical results are presented in Table I. Both complexes appear to be typical mononuclear copper(II) species: their reflectance spectra are dominated by a single broad absorption between 6.00 and 22 X 10^3 cm^{-1}, the β-ODAP complex absorbing at significantly higher energy than that of α-ODAP (Table I).

Electron spin resonance of the powdered undiluted complexes was uninformative; for both isomers the copper complex showed a single broad isotropic signal $g_{av} \sim 2.13$ indicative of exchange coupling [16]. For β-ODAP we obtained a sample of the copper complex doped at ~1 mole per cent into the zinc complex. The powder E.S.R. spectrum of this dilute sample is much more informative; g values of 2.32 and 2.07 are observed, typical of copper(II) in a square coplanar environment [16] (N_2O_2) possibly, with a distant ligator (H_2O) in the fifth position. This spectrum is very similar to those obtained for other copper(II) amino acid complexes [17, 18].

TABLE II. Major IR[a] Bands between 2,000 and 250 cm^{-1} for Cuβ-ODAP·H_2O and Cuα-ODAP·H_2O.

Cuβ-ODAP·H_2O	Cuα-ODAP·H_2O
1620 sb	1620 sb
1535 m	
1450 nj	
1420 w	
1400 nj	
1380 m	1390 m
1370 msh	
1310 w	1340 msh
1270 w	
1260 w	
1240 w	
1215 w	1190 w
1140 m	1160 m
1100 m	1080 m
1098 wsh	1030 m
1050 m	998 w
1040 m	960 w
950 w	915 w
915 w	860 w
865 m	800 m
830 w	770 m
810 w	750 w
795 w	740 w
770 m	730 w
720 w	
690 w	
630 m	650 w
600 w	
580 wsh	
560 wsh	
540 m	
500 w	
480 w	
450 w	
440 wsh	
355 w	
320 m	

[a] s = strong, m = medium, w = weak, b = broad, sh = shoulder, nj = nujol

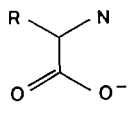
Infra-red spectra of both the α- and β-ODAP complexes were measured; the major features are summarized (Table II). The hygroscopic nature of the Cu α-ODAP complex made it difficult to obtain well resolved spectra, particularly below 650 cm^{-1}. The spectra of both complexes are typical of copper amino acid complexes, both containing a single broad antisymmetric carboxylate stretch at ν_{as} 1620 cm^{-1} and a single symmetric stretch at $\nu_s \cong 1390$ cm^{-1};

indicating both carboxylates on each amino acid are deprotonated and coordinated in a similar monodentate fashion [19]. A broad strong band in both complexes at 3600 cm^{-1} indicates the presence of coordinated water. The water of crystallization somewhat obscures the –NH stretching region in both complexes; in the β-complex bands at 3560, 3360 and 3300 cm^{-1} are observed, similar to those in β-ODAP [20].

Only for Cuβ-ODAP·H_2O is the low energy position of the spectrum sufficiently resolved for serious consideration. M–N and M–O stretches for copper amino acid complexes occur close to 480 cm^{-1} and 340 cm^{-1} respectively. The splitting of these bands in *cis* complexes is quite characteristic of this geometry [21]; a split band is observed at 500 cm^{-1} (weak), 480 cm^{-1} (weak) for the β-ODAP complex; no other bands are obviously split. We therefore tentatively assign the coordination geometry of Cuβ-ODAP·O_2O as *cis*.

Discussion

As the biochemistry of β-ODAP is more important than that of the α-isomer we will concentrate the discussion on this isomer. The structures of β-ODAP, glutamic and aspartic acids are illustrated below [III, IV, V]; the similarities in the relative orientations of carboxylate, amino and γ- and δ-carboxylates in β-ODAP and glutamic acid are apparent. The coordination chemistry of aspartic and glutamic



III β-ODAP R = $CH_2NHCOCOOH$

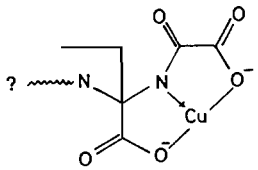
IV Glutamic Acid R = CH_2CH_2COOH

V Aspartic R = CH_2COOH

acids with copper has been studied for many years. The early literature is well reviewed by Greenstein and Winitz [22]. A more recent review of their coordination chemistry has appeared [23] and solution equilibria at low pH have been reinvestigated by E.S.R. spectroscopy [24]. From such work it may be concluded that at values of pH > 7.00 bis complexes of the kind $[Cu(AA^{2-})_2]^{2-}$ dominate solution chemistry, whereas at lower values of pH protonated complexes may become important. Complexes of a one-to-one stoichiometry are known and the only relevant crystal structure, that of CuGlu·$2H_2O$, falls into this category [25]. In this complex the copper atom is surrounded by a square planar NO_3 chromophore comprising of the nitrogen and oxygen of one glutamate, an oxygen of a second glutamate and a coordinated water molecule. Distant ligation (~2.5 Å) occurs from oxygen on adjacent glutamates at positions 5, 6 of the octahedron.

It was initially tempting to look for similarities between Cuβ-ODAP·H_2O and $CuAsPO^{-}$·n·H_2O based on the remarkable insolubility of these complexes [22, 23], a feature which is not shared with the glutamic acid complex. The copper aspartic acid 1:1 complex has been prepared on a number of occasions [22, 23] and may be formulated as $Cu[Cu(Asp\text{-}O^{-})_2]^{2-}$, the copper(II) within the square brackets being coordinated by the NO grouping from two different aspartates. The reasons for the gross insolubility of this complex remain unclear; its large size together with some as yet unknown mechanism of polymerization being presumed responsible. In marked contrast the insolubility of CuHDAP·H_2O may be explained by the ability of each β-ODAP molecule to coordinate to two different copper atoms with a nitrogen and an oxygen atom. Thus there are remarkably few structural similarities between the β-ODAP and the glutamic and aspartic acid complexes. All the spectral results are consistent with the Cuβ-ODAP·H_2O consisting of a 'ribbon' of CuN_2O_2 chromophores, somewhat reminiscent in structure to that of polymeric CuC_2O_4·xH_2O [26].

Cuα-ODAP·H_2O shows a weaker ligand field than Cuβ-ODAP·H_2O as judged from the electronic spectrum. The geometry of the α-isomer does not allow the easy polymerization which dominates Cuβ-ODAP chemistry. It seems possible that both α- and γ-COO^{-} groups coordinates to a single copper as shown below [VII]. The free amino group may coordinate to a second copper(II) ion.



VII

The remarkable insolubility of the Cuβ-ODAP·H_2O explains to some extent the poor yields obtained when attempts are made to chemically synthesize β-ODAP using the Cu(II) chelate of L-α,β-diaminopropionic acid [see 2, 27]. The existence of this insoluble chelate of β-ODAP may be of use in isolating β-ODAP from seed extracts, instead of the ion exchange separations currently available [5, 7].

Experimental

Copper acetate monohydrate was B.D.H. AnalaR grade. α- and β-ODAP were prepared as described previously [7]; both were free from isomeric and

other nonhydrin sensitive impurities as judged by high voltage electrophoresis [7].

Methods

Diffuse-reflectance spectra were recorded with a Beckman DK2a spectrometer. Infrared spectra were obtained for Nujol mulls between caesium iodide plates or for potassium bromide pellets (1% 200 mg discs) on Perkin-Elmer 457 and 257 spectrophotometers. Thermogravimetric analysis was performed using a Stanton Redcroft TG 750 instrument at a rate of 10 °C min^{-1} under dry nitrogen (10 ml min^{-1}), the sample size lying between 3 and 8 mg. E.S.R. spectra were recorded with a Varian E4 spectrometer, substantially the same results being obtained at *ca.* 77 K as at room temperature (*ca.* 293 K). Microanalysis (CHN) were performed by Butterworth Laboratories Ltd. copper content was determined by roasting the complex to CuO in air at 500 °C or by atomic absorption in dilute nitric acid.

Preparation of Cuβ-ODAP·H_2O

On mixing equimolar solutions of copper acetate monohydrate and β-ODAP monohydrate (typically 10 cm^3 of each 0.05 *M*). The complex Cuβ-ODAP·H_2O quantitatively separated in a few minutes as a blue microcrystalline powder; this was dried *in vacuo* over $CaCl_2$.

Cuα-ODAP·H_2O

Equimolar solutions were mixed as described above, no precipitate was obtained until the resulting solution was titrated with a few mls of absolute alcohol. The resulting solid was somewhat hygroscopic; it was rapidly filtered off and dried under *vacuo* over $CaCl_2$.

Attempted Separation of α- and β-ODAP

Crude ODAP ~50% of each isomer was reacted with one equivalent of copper acetate in aqueous solution at a concentration of ~0.1 *M*. On standing a solid separated identical by IR and electronic spectra with Cuβ-ODAP·H_2O. High voltage electrophoresis revealed this compound to be contaminated ~5% with α-ODAP. However, the supernatant contained α-ODAP and β-ODAP in the approximate ratio of 3.1. Thus although the use of the Cu(II) chelate is a useful method of precipitating the β-isomer, the method does not give a sufficiently pure preparation of the α-isomer.

Cuβ-ODAP doped in Znβ-ODAP

A sample of Znβ-ODAP containing ~1 mole per cent Cu(II) was prepared by the method described for Cuα-ODAP·H_2O. Although it was necessary to precipitate this complex with ethanol its IR spectrum was almost identical with Cuβ-ODAP prepared as described above.

Acknowledgements

We thank the Wellcome Trust for financial support (P.B.N.), both the Wellcome Trust and the University of London Central Research Funds Committee for the purchase of a freeze drier (P.B.N.). Thanks are also due to (P.O'B.) to Prof. B. J. Hathaway (U. C. Cork, Eire) for his hospitality and providing access to the Beckman DK2a spectrometer used for this work.

References

1 Part 1 of this series: P. O'Brien, *Inorg Chim. Acta, 55,* L27 (1981)
2 S. L. N. Rao, P. R. Adiga and P. S. Sarma, *Biochemistry, 3,* 432–436 (1964).
3 E. A. Bell, *Nature (Lond.), 203,* 378–380 (1964).
4 M Y. Qureshi, D. J. Pilbeam, C. S. Evans and E. A. Bell, *Phytochemistry, 16,* 477–479 (1977).
5 E. A. Bell and J. P. O'Donovan, *Phytochemistry, 5,* 1211–1219 (1966).
6 G. Wu, S. B. Bowlus, K. S. Kim and B. E. Haskell, *Phytochemistry, 15,* 1257–1259 (1976).
7 F L. Harrison, P. B. Nunn and R. R. Hill, *Phytochemistry, 16,* 1211–1215 (1977).
8 G. Padmanaban, in *Lathyrogens in Toxic Constituents of Plant Foodstuffs,* 2nd edition, ed. by I. E. Liener, Academic Press, New York (1980).
9 T. Mehta, A.-F. Hsu and B. E. Haskell, *Biochemistry, 11,* 4053–4063 (1972).
10 J. Lakshmanan and G. Padmanaban, *Biochem. Biophys. Res. Comm., 58,* 690–698 (1974).
11 M. C. Duque Magalhaes and L. Packer, *FEBS Lett., 23,* 188–90 (1972).
12 E. Jacob, A. J. Patel and C. V. Ramakrishnan, *J. Neurochem., 14,* 1091 (1967).
13 S. Pearson and P. B. Nunn, *Brian Research, 206,* 178–182 (1981).
14 S. Pearson and P B. Nunn, unpublished data.
15 R. J. Block and D. Bolling, The Amino Acid Composition of Proteins and Foods, C. C. Thomas, Springfield, Illinois (1951).
16 D. F. Billing and B. J. Hathaway, *Coord Chem. Rev., 5,* 143 (1970).
17 H Yokoi, M. Sai, T. Isobe and S Ohsawa, *Bull. Chem. Soc. Japan, 45,* 2189 (1972).
18 A. K. Wiersema and J. J. Windle, *J. Phys. Chem., 68,* 2316 (1964).
19 K. Nakamoto, 'Infra Red and Raman Spectra of Coordination Compounds', 3rd Edn, J. Wiley, p. 305, 1978.
20 P. O'Brien and P. B. Nunn, *Phytochem.,* in press.
21 A. W. Herlinger, S. L. Wenhold and T. V. Long, II, *J. Am. Chem. Soc., 92,* 6474 (1970).
22 'Chemistry of the Amino Acids', p. 585 et seq., J. P. Greenstein and M. Winitz, J. Wiley and Sons 1961 and references therein.
23 C. A. Evans, R. Gueuremont and D. L. Rabenstein, p. 41 in 'Metal Ions in Biological Systems', Vol. 9, ed. H. Sigel, Marcel Dekker, 1979, and references therein.
24 R. F. C. Claridge, J. J. Kilpatrick and H. K. J. Powell, *Aus. J. Chem., 33,* 2757 (1980).
25 C. M. Gramaccioli and R. E. Marsh, *Acta Cryst., 21,* 594 (1966).
26 J. J. Girerd, O. Kahn and M. Verdaguer, *Inorg. Chem., 19,* 274 (1980) and references therein contained.
27 S. L. N. Rao, *Biochemistry, 14,* 5218–21 (1975).