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The Formation Constants of Complexes of L-*trans*-2,3-dihydro-3-hydroxyanthranilic Acid with H^+ and Cu^{2+}

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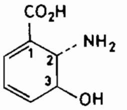
It has been found that cultures of a mutant (S-652) of *streptomices aureofaciens* accumulate the unusual β-amino acid, L-*trans*-2,3-dihydro-3-hydroxyanthranilic acid (DHAA) [1].



This compound arises manifestly by a shunt from the normal biosynthetic conversion of shikimic acid to anthranilic acid. The *cis* analogue is thought not to exist as it would immediately dehydrate internally to form a phenyl ring.

While it may be assumed from the structure that a large proportion exists as a zwitterion at intermediate pH values, the ligand cannot be totally analogous to other β-amino acids as the relative positions of the amino and carboxylic acid groups are fixed with the groups unable to approach as near to each other as is possible in simpler β-amino acids. Hence its ability to form complexes with metal ions would be expected to differ from that of other β-amino acids.

The ligand may also be prepared by hydrolysis of oryzoxymycin [2]. Hydrogenation of the ligand yields 2-amino-3-hydroxycyclohexane-1-carboxylic acid which may be esterified to form the methoxycarbonyl derivative [3]. The Cu(II) complex of this last compound has been used to confim the absolute configuration of oryzoxymycin and hence DHAA.

Experimental

Streptomyces aureofaciens S-652 was cultured and *trans*-2,3-dihydro-3-hydroxyanthranilic acid was obtained, as described previously [1]. It had m.p. 190–1 °C (dec.), and m/e 155 (14%, M^+), 138 (100%, $M^+ - NH_3$), 137 (10% $M^+ - H_2O$), 121 (73%, 138 – OH) 119 (13%, 137 – H_2O), 108 (40%), 93 (40%, 121 – CO).

TABLE I. Formation Constants of Complexes of Dihydrohydroxyanthranilic Acid at 25 °C and I = 0.10 *M* (K[NO₃]). Standard derivations (σ values) given in parentheses.

Proton Complexes		
log k_{HL}		8.563(3)
log k_{H_2L}		3.362(3)
Cu(II) Complexes		
	Model 1	Model 2
log β_{ML}	6.25(1)	6.25(1)
log β_{ML_2}	11 25(1)	11 28(1)
log $\beta_{ML_2H_{-2}}$	–8.14(1)	–8.19(1)
log $\beta_{MLH_{-1}}$	–1.63(1)	
log $\beta_{ML_2H_{-1}}$		1.67(1)
log $K_{ML_2}^{ML_2H_{-1}}$		9.61
log $K_{ML_2H_{-1}}^{ML_2H_{-2}}$		9.85

Complex formation constants of proton and Cu(II) complexes were calculated from potentiometric titration curves at 25 °C and I = 0.10 mol dm^{-3} (K[NO₃]) using a Radiometer pHM 64 pH meter. The glass electrode was calibrated in terms of hydrogen ion concentrations and formation constants were calculated with the aid of the MINIQUAD computer program [4].

Results

Calculated formation constnts are given in Table I. The ligand contains one ionizable proton (pK = 8.56) and is able to coordinate an additional proton in excess acid (pK = 3.36). These values are significantly closer than in comparable amino acids (*e.g.* pK values for β-alanine are 10.26 and 3.52) presumably the result of the greater charge separation in the zwitterion compared to β-alanine causing less zwitterion stabilization.

In the presence of Cu(II) ions a range of complexes is formed. With a metal·ligand ratio of 1:1 a precipitate forms at about pH 5 but with an excess of ligand a deep blue solution forms and the experimental data up to pH 11 are entirely reproducible. The data could be satisfied satisfactorily by two different models which differed only in detail. In both models the major species were $[CuL]^+$ and $[CuL_2]$ with $[CuL_2H_{-2}]^{2-}$ as a minor species. The formation constants for all three complexes species were effectively the same (see Table I). The species

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$[CuLH_{-1}]$ was present in the first model while in the second model it was rejected in favour of $[CuL_2H_{-1}]^-$. The statistics of the fit of the second model were slightly better, but the values were not conclusive. However, the species were only minor contributors to the overall equilibrium.

The $[CuL]^+$ and $[CuL_2]$ species would be expected to be β-amino acid-like in bonding, forming non-planar six-membered chelate rings. The $[CuL_2]$ complex is capable of losing 2 more protons with pK values of 9.61 and 9.85 (model 2). Comparison with other amino acids suggest that these cannot originate from coordinated water molecules, but must be the result of ionization of the hydroxy group of the ligand. It is impossible sterically, however, for the hydroxy oxygen to coordinate to the copper ion. Loss of a proton could also arise from an internal rearrangement of the ligand molecule, eliminating both a proton from C(2) and the hydroxyl group from C(3) forming an aromatic system. This was shown not to occur since the titration curve was reversible and the unchanged β-amino acid was shown by paper chromatography to be present at high pH.

References

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