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The Complex Cation [Co{tris(3,5-dimethyl-1-parazolylmethyl)amine}$H_2O]^{2+}$: a Model for Metalloenzymes containing Bipositive Zinc(II) Chromophores with a Water Molecule in the Coordination Sphere

IVANO BERTINI, GIORGIO CANTI, CLAUDIO LUCHINAT

Istituto di Chimica Generale ed Inorganica, Facoltà di Farmacia, Via G. Capponi 7, University of Florence, Italy

and FABRIZIO MANI

Istituto di Chimica Generale ed Inorganica, Via J. Nardi 39, University of Florence, Italy

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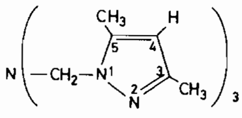
A cobalt(II) complex with a single water molecule bound to the metal ion has been prepared with formula [Co{tris(3,5-dimethyl-1-pyrazolylmethyl)amine}-$H_2O](ClO_4)_2$, where the ligand is:



The choice of the ligand is based on the consideration that pyrazolyl-containing ligands stabilize low oxidation states [1, 2]. This allows the investigation of the complex in water solution even at relatively high pH without oxidation of the metal ion.

The complex is soluble in water with an electronic absorption spectrum equal to that in the solid state. The molar conductance of a 10^{-3} *M* solution is 197 S cm^2 mol^{-1}, thus indicating a 1:2 electrolyte. The electronic spectrum shows a pH dependence which can be described in terms of two acid–base equilibria (Fig. 1). By plotting the absorption at 490 nm against pH a pK_a value can be located around 7.4, to which the detachment of the ligand corresponds. Indeed, at pH values < 5 the spectrum is that of the hexaaqua cobalt(II) complex. The group with such pK_a can be assigned as the apical nitrogen whose pK_a when free from coordination would be around 10. A second pK_a is observed whose value is about 8.8 ± 0.2; it can be attributed only to the water molecule, since the other candidates, *i.e.* the pyrazolyl nitrogens, are much more acidic (pK $\simeq 2$ when free from coordination). The pH dependence of the electronic spectra was investigated up to pH 9.65. This finding is meaningful with respect to the present debate on the pK_a values of water molecules bound to zinc atoms in pseudotetrahedral environments in metalloenzymes as well as on the effect of such acid-base equilibrium on the electronic absorption spectra of the cobalt analogues [3–6]. The coordination in the present case seems to be essentially five coordinate with trigonal bipyramidal geometry, although the steric requirements of the ligand may induce some tetrahedral distortion with larger cobalt-apical nitrogen distance and angles between the apical donor, the metal ion, and the pyrazolyl nitrogens smaller than 90°.

The pK_a of this bound water molecule is relatively low, probably on account of the low donor properties of the pyrazolyl ligands and of the hydrophobic methyl groups in position 3. The effect of the dissociation appears to be very large on the electronic

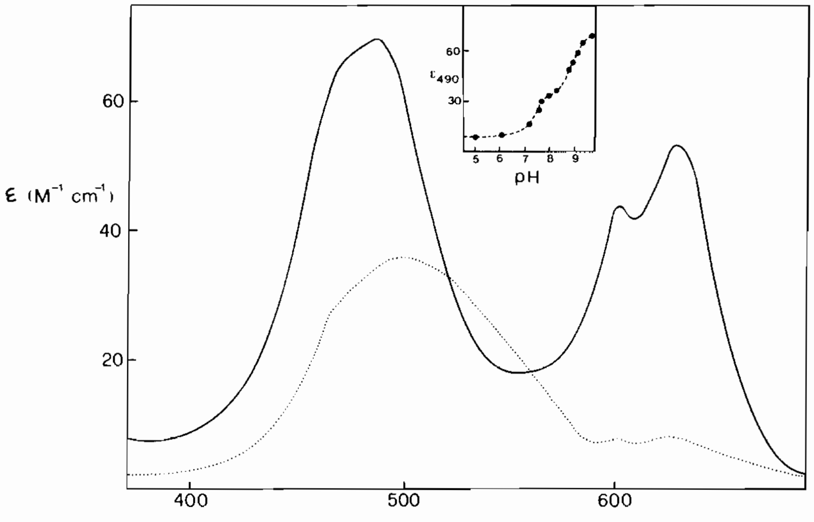


Fig. 1. Electronic spectra in the visible region of [Co{tris(3,5-dimethyl-1-pyrazolylmethyl)amine}$H_2O](ClO_4)_2$ dissolved in water as such (pH 8.3, ······), and brought to pH 9.65 by addition of sodium hydroxide (———). The inset shows the variation of ϵ_{490} with pH.

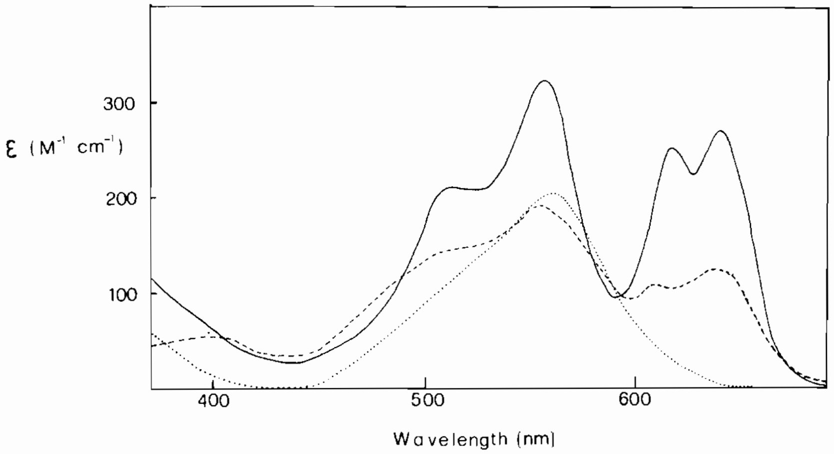


Fig. 2. Electronic spectra of cobalt(II) alkaline phosphatase from *Escherichia coli* at pH 8.0, monomer (– – –) (Ref. 7) and cobalt(II) human carbonic anhydrase B at pH 5.7 (······) and 9.1 (———) (Ref. 8).

spectra. Such considerations may be quite important if compared to the spectral properties of cobalt(II) substituted enzymes. In particular, the electronic spectrum of the high pH form of the model compound shows large splittings of the F → P transitions analogous to those displayed by alkaline phosphatase [7] and by the high pH form of carbonic anhydrase [8] (Fig. 2). Furthermore, the spectrum at pH 8.3 recalls the acidic spectrum of the latter cobalt enzyme derivative. It is felt, however, that the electronic spectra reflect similarities in the overall distortions experienced by the chromophores rather than in the coordination number. Analytical data. Calcd.: C, 35.0; H, 4.7; N, 15.9%. Found: C, 35.0; H, 4.9; N, 16.4%.

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