Chemical Communications

NUMBER 15/1968 7 AUGUST

The Synthesis of a Cobaloxime with a Polymeric Ligand as a Cobalamin Model Compound

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It is well known that cobalamin compounds (vitamin B_{12} compounds) act as coenzymes in B_{12}-dependent enzymatic reactions. Most biochemically active B_{12} compounds contain Co–C bonds. The elucidation of the mechanism of coenzyme function involves determining whether the activity involves this bond: the resemblance of cobaloxime compounds [bis(dimethylglyoximato)cobalt complexes] to cobalamin compounds in chemical behaviour has also been described.[1]

We considered that the coenzyme activity of cobaloximes in enzymatic reactions, required some amide groups in the neighbourhood of the Co atom, since these groups in cobalamin must take part in the reactions *in vivo*, perhaps as the binding sites to the apo-enzyme. We are then comparing the coenzyme activity of cobaloximes having polymeric ligands, which are low molecular weight vinyl copolymer and/or oligopeptides, with that of cobalamins. The polymer as ligand must contain both some amide groups and a basic group such as a pyridine or imidazole residue.

We have chosen, initially, low molecular weight copolymers of acrylamide–4-vinylpyridine (Co-poly-AM–VPy) as polymeric ligands. Acrylamide and 4-vinylpyridine were copolymerized in the ratio of 20 : 1 with azobisisobutyronitrile (*ca.* $\frac{1}{5}$ mole of monomer) as initiator, under a nitrogen atmosphere. The crude polymer was fractionated by gel filtration on Sephadex LH-20. Four of the six fractions [M.W. 3000, 2400, 1400, and 830 (cryoscopic), AM : VPy ratio 11·9, 12·8, 13·7, and 17·5, respectively (absorption at 257 mμ, pyridine residue)] were used to prepare the cobaloximes, (hydroxocobalamin model compounds) as follows: a methanolic solution of dimethylglyoxime (DH_2) was mixed with an aqueous solution of $CoCl_2,6H_2O$ (mole ratio: DH_2 : Co = 2) for 1 hr.: an aqueous solution of the polymer was then added to the reaction mixture (Co : VPy-residue *ca.* 4), after adjusting the pH of the mixture to *ca.* 8·0—8·3. The reaction was complete within 3—4 hr. After concentration the reaction mixture was applied to the Sephadex column to purify the product, by removing by-products, *i.e.* hydroxoaquocobaloxime[$H_2O{\cdot}Co(DH)_2OH$] and NaCl. Alternatively the product was purified by repeated reprecipitation in water–acetone.

It appeared that all the pyridine residues of the original polymer co-ordinated with cobaloximes, while neither the amide or nitrile group did so. The structure of the product was determined as (Copoly-AM–VPy)$Co(DH_2)_2OH$, in which Copoly-AM-VPy co-ordinates with the Co-atom through a pyridine residue (i.r. and u.v. absorption and Co analysis, and comparison with the structure of hydroxoaquocobaloxime).

The polarography of cobaloxime compounds and cobalamin compounds have been studied.[2,3] We have observed that the half-wave potential of (Copoly-AM–VPy)$Co(DH)_2OH$ in the polarographic reduction was $-1{\cdot}24$ v *vs.* S.C.E. (in 0·1 N-K_2SO_4 at 25°), intermediate between $-1{\cdot}02$ v for hydroxocobalamin[2] and $-1{\cdot}52$ v for 5′-deoxyadenosylcobalamin:[3] the potential was not affected by the molecular weight of the polymer ligand.

Comparisons of the biological and coenzyme activity of these cobaloximes with cobalamins, and studies on cobaloximes with oligopeptides as ligands are in progress.

(*Received, May 6th*, 1968; *Com.* 557.)

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