Direct Studies of Reactions of ^{17}O-Labelled Cobaloximes by ^{17}O N.M.R. Spectroscopy: Hydrolysis of 2-Acetoxyethyl(pyridine)cobaloxime and Hydration of Formylmethyl(pyridine)cobaloxime

Eirian H. Curzon, Bernard T. Golding,* and Ah Kee Wong

Department of Chemistry and Molecular Sciences, University of Warwick, Coventry CV4 7AL, U.K.

Using ^{17}O n.m.r. spectroscopy to monitor reactions, the hydrolysis of 2-acetyl[^{17}O]oxyethyl(pyridine)-cobaloxime is shown to proceed *via* the $B_{AL}1$ mechanism, whilst the hydration of [^{17}O]formylmethyl(pyridine) cobaloxime takes place with $\tau_{\frac{1}{2}}$ *ca.* 19 h in dioxan–aqueous 0·1 mol dm^{-3} phosphate (pH 6·8) (3:2) at 50 °C.

We have studied the hydrolysis of 2-acetoxyethyl(pyridine)-cobaloxime (1a) and the hydration of formylmethyl(pyridine)-cobaloxime (1b) using ^{17}O-enriched cobaloximes and ^{17}O n.m.r. spectroscopy. The hydrolysis is confirmed to be of the relatively uncommon $B_{AL}1$ type.[1] The hydration is shown to be exceptionally difficult.

R Co (dmgH)₂(py)

(1)

a; R = CH₂CH₂OCOMe   dmgH = monoanion of
b; R = CH₂CHO   dimethylglyoxime
c; R = CH₂CH(OH)₂   py = pyridine

Aqueous chemistry in which oxygen atoms are mobilised can be advantageously studied with ¹⁷O-labelled substrates in unlabelled water, because of the opportunity for direct monitoring by ¹⁷O n.m.r. spectroscopy.[2] For optimum application of this technique a substrate should be sufficiently enriched so that a high concentration of it and long times for data acquisition are avoided. Thus, efficient methods for preparing [¹⁷O]-compounds are required. We have described a convenient method for synthesising [¹⁷O]-aldehydes, -ketones, and -alcohols by which all the label in [¹⁷O]water is transferred to one of these compounds.[3] This method has now been applied to the study of some cobaloximes.

N-Ethyl-N-vinylbenzamide was brominated (1 equiv. of Br₂ in CCl₄; room temp.; 20 min) and then dehydrobrominated (1 equiv. of Et₃N; room temp.; 2 h) to give N-(2-bromovinyl)-N-ethylbenzamide, which was hydrolysed in ether containing 1 equiv. of [¹⁷O]water (7·3 atom % ¹⁷O) and 0·05 equiv. of HCl (30 h; room temp.).[3] The resulting [¹⁷O]-bromoethanal was reduced in situ with Zn(BH₄)₂ in ether[4] (10 min; room temp.) to afford [¹⁷O]bromoethanol, b.p. ca. 80 °C at 12 mmHg (kugelrohr), ¹⁷O n.m.r. (CDCl₃) δ 1·4, in an overall yield of 27%. Alkylation of [¹⁷O]bromoethanol with (pyridine)cobaloxime(i) [generated in de-aerated ethanol by reducing bromo(pyridine)cobaloxime with 1—2 mol. equiv. of NaBH₄ at room temp.] gave 2-[¹⁷O]hydroxyethyl(pyridine)-cobaloxime (42%), ¹⁷O n.m.r. (CDCl₃) δ 15·6, which was acetylated (Ac₂O in pyridine; room temp.; 24 h) to give 2-acetyl[¹⁷O]oxyethyl(pyridine)cobaloxime (35%). On incubating 0·02 mol dm⁻³ of this cobaloxime in water–dioxan (1:2, v/v) the ¹⁷O resonance† from the alkyl oxygen of the ester gradually declined and was replaced by a resonance from acetate (cf. Figure 1). The signals from water and dioxan did not change in intensity and no other signals were apparent. For an experiment performed at 323 K, $k = 5·5 \pm 0·1 \times 10^{-5}$ s⁻¹. After 3 half-lives the product, 2-hydroxyethyl-(pyridine)cobaloxime, free of ¹⁷O, was recovered. In a control experiment, it was found that 0·02 mol dm⁻³ of 2-[¹⁷O]-hydroxyethyl(pyridine)cobaloxime did not detectably lose ¹⁷O when heated at 323 K for 24 h in water–dioxan (1:2) containing 0·02 mol dm⁻³ of acetic acid.

The results obtained are consistent with a mechanism[5] in which loss of acetate from 2-acetoxyethyl(pyridine)cobaloxime (i.e. alkyl–oxygen, $B_{AL}1$ fission[1]) yields a π-ethene complex of cobaloxime(iii), which is either captured by water to give

Figure 1. ¹⁷O N.m.r. spectrum of aqueous dioxan containing 2-acetyl[¹⁷O]oxyethyl(pyridine)cobaloxime after 65 min incubation at 323 K.

2-hydroxyethyl(pyridine) cobaloxime or loses ethene to produce hydroxy(pyridine)cobaloxime. The relative amounts of these cobaloximes were 3:1 according to a ¹H n.m.r. spectrum of material recovered from a hydrolysis by evaporation.

Formylmethyl(pyridine)cobaloxime (1b) and 2,2-dihydroxy-ethyl(pyridine)cobaloxime (1c) are important model compounds for the corresponding cobalamins, which are possible intermediates in the conversion of ethane-1,2-diol into ethanal catalysed by adenosylcobalamin-dependent diol dehydratase.[6] [¹⁷O]Formylmethyl(pyridine)cobaloxime, ¹⁷O n.m.r. (CDCl₃) δ 544, was obtained by hydrolysing 2,2-diethoxyethyl-(pyridine)cobaloxime with 1 equiv. of [¹⁷O]water (containing ca. 30 atom % ¹⁷O and 50 atom % ¹⁸O) and 0·05 equiv. of HCl in CH₂Cl₂ (2 h; room temp.). [¹⁷O]Pentanal (31 atom % ¹⁷O) was prepared as described.[3] Loss of ¹⁷O from these aldehydes, presumably via hydration then dehydration,[7] was monitored by ¹⁷O n.m.r. spectroscopy of aqueous solutions. The half-life of [¹⁷O]pentanal in 0·1 mol dm⁻³ phosphate buffer (pH 6·8) was ca. 1 min at 278 K. At 323 K in aqueous phosphate(pH 6·8)–dioxan (2:3) $k = 1·0 \pm 0·1 \times 10^{-4}$ s⁻¹ ($\tau_{\frac{1}{2}}$ 2 h) for loss of ¹⁷O from [¹⁷O]formylmethyl(pyridine) cobaloxime (0·02 mol dm⁻³), presumably via [hydroxy-¹⁷O]-2,2-dihydroxyethyl(pyridine)cobaloxime. The material recovered from such an experiment, after disappearance of the formyl resonance in the ¹⁷O n.m.r. spectrum, was shown to be > 90% formylmethyl(pyridine)cobaloxime by ¹H n.m.r. spectroscopy, and to contain only ¹⁶O by i.r. spectroscopy.

We have previously observed the lack of reactivity of the aldehyde group in formylmethyl(pyridine)cobaloxime to NaBH₄ and LiAlH₄, although conversion into 2-hydroxyethyl-(pyridine)cobaloxime can be achieved with diborane.[8] Brown[9] has recently discussed the relatively high pK_a values of carboxymethyl- and carboxyphenyl-cobaloximes and the low reactivity of their esters to alkaline hydrolysis. These properties were explained as a consequence of σ–π hyperconjugation involving the Co–C σ-bond and the carbonyl group. A similar effect could operate with formylmethyl(pyridine)cobaloxime, lowering νmax for the carbonyl stretching vibration and rendering its carbonyl carbon relatively unreactive to nucleophilic attack. This interaction is implied by the upfield shift

of the formyl oxygen ^{17}O n.m.r. resonance (δ 544) compared with that for, *e.g.* pentanal (δ 583).

Received, 17th August 1981; Com. 1000

References

1 *Cf.* R. A. Y. Jones, 'Physical and Mechanistic Organic Chemistry,' Cambridge University Press, Cambridge, 1979, ch. 12.
2 W. G. Klemperer, *Angew. Chem., Int. Ed. Engl.*, 1978, **17**, 246.
3 B. T. Golding and A. K. Wong, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 89.
4 W. J. Gensler, F. A. Johnson, and D. B. Sloan, *J. Am. Chem. Soc.*, 1960, **82**, 6074.
5 B. T. Golding, H. L. Holland, U. Horn, and S. Sakrikar, *Angew. Chem., Int. Ed. Engl.*, 1970, **9**, 959; J. W. Espenson and D. M. Wang, *Inorg. Chem.*, 1979, **18**, 2853 and references cited therein.
6 D. Dolphin, A. R. Banks, W. R. Cullen, A. R. Cutler, and R. B. Silverman, in 'Vitamin B_{12},' eds. B. Zagalak and W. Friedrich, Walter de Gruyter, Berlin, 1979, pp. 575—586.
7 P. Greenzaid, Z. Luz, and D. Samuel, *J. Am. Chem. Soc.*, 1967, **89**, 756; D. Samuel and B. L. Silver, *Adv. Phys. Org. Chem.*, 1965, **3**, 123; H. Dahn, H. P. Schlunke, and J. Temler, *Helv. Chim. Acta.*, 1972, **55**, 907; R. P. Bell, *Adv. Phys. Org. Chem.*, 1966, **4**, 1.
8 B. T. Golding and U. Horn, unpublished results.
9 K. L. Brown, *J. Am. Chem. Soc.*, 1978, **100**, 823.