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Electron Transfer. 81. Reductions of Unsaturated Dicarboxylic Acids with Vitamin B_{12s} (Cob(I)alamin)[1]

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Vitamin B_{12s} (cob(I)alamin) in aqueous solution smoothly reduces maleic and fumaric acids to succinic acid. Acetylenedicarboxylic acid is rapidly reduced, first to fumaric and then to succinic acid. Reactions are first order each in carboxylic acid and B_{12s} and are accelerated by increases in $[H^+]$. Kinetic acidity patterns reflect partition of the oxidant acids into species H_2A, HA^-, and A^{2-} (the first of which is the most reactive) and partition of B_{12s} into a reactive protonated and an unreactive nonprotonated form. The rate ratio observed for the alkyne- and alkene-derived oxidants (10^3–10^5) is characteristic of heterolytic additions of strongly nucleophilic reagents, rather than free-radical additions, and indicates that these reductions by B_{12s} are initiated by a 2e rather than a 1e transaction. It is suggested that the stereochemical outcome of the acetylenedicarboxylate–fumarate conversion (a trans addition) is determined by the initial attack and that subsequent steps involve at least one olefinic carbanion-like species that maintains its structural integrity during the conversion.

Vitamin B_{12s} (cob(I)alamin) is the cobalt(I) analogue of the corrin-coordinated cobalt(III) derivative, vitamin B_{12}.[2] It is a strongly reducing species,[3] which reacts quickly and smoothly with a variety of inorganic and organic oxidants in polar media. Its versatility reflects, in part, its capacity to participate in both single-electron[4] and two-electron[5] transactions. Interest in its use in synthetic procedures, both as a reagent[6] and as a catalyst,[7] has quickened, but relatively few mechanistic studies[5b,8] of reactions with organic compounds have been described.

Preliminary examination of the reactions of B_{12s} with a number of diverse organic functions (aldehydes, keto acids, pyridinecarboxylic acids and the derived amides, and aromatic dinitriles) in aqueous solution indicates that these are stoichiometrically clean-cut but kinetically complex. Reductions of α,β-unsaturated dicarboxylic acids, with which this paper is concerned, are more straightforward, with the principal complications apparently reflecting partition of the redox partners between protonation levels.

Experimental Section

Materials. Maleic and fumaric acids (Matheson products), acetylenedicarboxylic and phthalic acids (Aldrich), sodium borohydride and glycine (Fisher), and hydroxocobalamin hydrochloride (Sigma) were used as received. Lithium perchlorate, for use in kinetic experiments, was prepared as described.[9] All reactions were carried out under oxygen-free N_2 in distilled water that had been previously boiled and sparged with N_2 for at least 4 h.

Cob(I)alamin was generated in solution from the cobalt(III) complex hydroxocobalamin hydrochloride by using zinc amalgam for reduction in acid solution[5a] and $NaBH_4$–$PdCl_4^{2-}$ in basic solution.[10] For stoichiometric experiments and kinetic experiments using conventional mixing, solutions were prepared in spectrophotometric cells;[5a] for stop-flow experiments, solutions of B_{12s} were prepared externally and then transferred under nitrogen to the reservoir syringe of the apparatus.

Table I. Stoichiometries of the Reactions of Vitamin B_{12s} (Cob(I)alamin) with Unsaturated Dicarboxylic Acids[a]

acid (H_2A)	pH	$10^5 \times [H_2A]$	$10^5 \times [B_{12s}]$	$10^5 \times \Delta[B_{12s}]$	$\Delta[B_{12s}]/\Delta[H_2A]$
maleic (I)	3.3	1.99	4.47	3.93	1.97
	3.3	2.45	6.47	5.15	2.10
	3.7	2.46	5.37	4.73	1.93
	3.8	1.30	3.87	2.76	2.12
	4.3	1.98	5.33	4.41	2.23
fumaric (II)	2.2	2.03	5.77	4.13	2.04
	2.4	2.22	6.67	4.88	2.20
	2.5	1.48	4.30	3.17	2.14
	2.9	1.99	5.13	3.86	1.94
acetylenedicarboxylic (III)	3.1	1.35	7.13	2.36,[b] 2.63[c]	1.75,[b] 1.96[c]
	3.2	0.69	5.80	1.28,[b] 1.23[c]	1.84,[b] 1.78[c]
	3.7	1.37	7.03	2.51,[b] 2.55[c]	1.83,[b] 1.86[c]

[a] Reactions were monitored at 387 nm. Concentrations are given in molarity. [b] Rapid reaction immediately on mixing reagents. [c] Slower reaction after initial absorbance drop.

Stoichiometric Studies. Stoichiometries of the reactions, with B_{12s} in excess, were determined in buffered media by generating B_{12s} in acid solution, buffering the solution, adding a deficiency of the dicarboxylic acid in water, waiting an appropriate interval (generally 20 min), and then measuring the decrease in absorbance at 387 nm. These changes were compared with those occurring when B_{12s} was treated with excess oxidant. Corrections were made for the measurable loss in absorbance (about 10%) when the corresponding volume of aqueous buffer, devoid of dicarboxylic acid, was added to the B_{12s} solution. Representative results are given in Table I.

With acetylenedicarboxylic acid, reaction occurred in two separable stages. A very rapid drop in absorbance was observed almost immediately after mixing, and this was followed by a more gradual decrease of very nearly equal magnitude. This behavior was in accord with evidence from kinetic studies (see below) that both the acetylenic acid and its primary reduction product are reducible with B_{12s}. The slight negative deviations from 2:1 stoichiometry recorded for the first stage in reduction of the acetylenic acid probably reflect uncertainties in the "blank" correction; they are not considered significant.

Estimation of Acidity Constants. Acidity constants (25 °C, 0.2 M $LiClO_4$) for each of the dicarboxylic acids were estimated by using results from partial titration with NaOH. For both fumaric and acetylenedicarboxylic acids, pK_1 and pK_2 were separated by a small interval; hence, data (pH readings) for these acids were treated by Speakman's procedure.[11]

Kinetic Studies. Rates were estimated from measurements of decreases of absorbance at 387 nm. Reactions of fumaric acid (II) were carried out on a Beckman Model 5260 recording spectrophotometer, whereas the more rapid reductions of acetylenedicarboxylic acid (III) were run in a Durrum-Gibson stopped-flow spectrophotometer. Studies with maleic acid (I) utilized both instruments. Acidities were regulated

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(1) Sponsorship of this work by the National Science Foundation (Grant No. 8313253) is gratefully acknowledged.

(2) For reviews, see: (a) Pratt, J. M. *Inorganic Chemistry of Vitamin B_{12}*; Academic: London, 1972; p 96. (b) Toscano, P. J.; Marzilli, L. G. *Prog. Inorg. Chem.* **1984**, *31*, 105. (c) Halpern, J. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, p 501.

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(4) See, for example: Balasubramanian, P. N.; Gould, E. S. *Inorg. Chem.* **1985**, *24*, 1791.

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(6) See, for example: (a) Schrauzer, G. N.; Holland, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 4060. (b) Dolphin, D. *Methods Enzymol.* **1971**, *18C*, 34.

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(11) (a) Speakman, J. C. *J. Chem. Soc.* **1940**, 855. (b) See also: Sarjeant, E. P. *Potentiometry and Potentiometric Titrations*; Wiley: New York, 1984; p 342.

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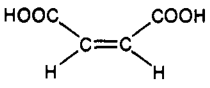

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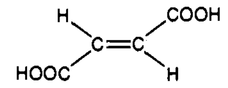

II

HOOC—C≡C—COOH

III

by addition of known concentrations of glycine, phthalate, or phosphate buffers to the oxidant or reductant solutions before mixing; pH values of the resulting solutions were checked experimentally. Total ionic strengths were, in most instances, maintained near 0.2 M by addition of $LiClO_4$.[12] Reactions were first order each in dicarboxylic acid and B_{12s} but were generally carried out with the oxidant in greater than 10-fold excess.[13]

Conversions were followed for at least 5 half-lives. Specific rates from successive half-life values within a run generally agreed to within 6%, and average values did not differ significantly from those obtained from slopes of logarithmic plots of absorbance differences against reaction time. Specific rates from replicate runs checked to better than 10%.

Kinetic profiles gave no indication of cobalt-containing intermediates formed or destroyed on a time scale comparable to that for disappearance of B_{12s}. The electronic spectrum of the cobalt product formed by oxidation with all three diacids in excess corresponded to that of B_{12r} (cob(II)alamin).[5a,14a]

Experiments carried out with B_{12s} in excess led to identification of the product formed from reduction of acetylenedicarboxylic acid. When this acid (1.0×10^{-5} M) was mixed with B_{12s} (7×10^{-5} M), a rapid drop of the B_{12s} absorbance occurred, followed by a near-exponential decrease having a specific rate near 9×10^{-3} s^{-1}. When analogous experiments were run with maleic and fumaric acids, there was no initial drop, but exponential decays having specific rates 3×10^{-2} (maleic) and 8×10^{-3} s^{-1} (fumaric) were observed.

Preliminary examination of the reactions of B_{12s} with additional carboxylic acids indicated the intrusion of complicating features. Reactions with pyruvic acid, 2-ketoglutaric acid, cinnamic acid, 2-methoxycinnamic acid (cis and trans isomers), 3-methoxycinnamic acid (trans isomer), (4-methoxyphenyl)glyoxylic acid, and *trans*-butadiene-1,4-dicarboxylic acid yielded biphasic kinetic curves, although each of these reactions exhibited isosbestic points (at 343, 416, and 543 nm) and each yielded vitamin B_{12r} as the cobalt oxidation product. The reactions with propiolic and phenylpropiolic acids appeared to be kinetically straightforward, but the spectrum of the oxidation product did not correspond to that of B_{12r}. Neither oxalic nor succinic acid reacted detectably with B_{12s} under our reaction conditions.

Results and Discussion

Reactions of both maleic acid (I) and fumaric acid (II) are found (Table I) to consume nearly 2 mol of Co(I)/mol of acid. The measured stoichiometry, in conjunction with the observed formation of B_{12r} (cob(II)alamin), allows us to represent the reactions as (1). With acetylenedicarboxylic acid (III), the reaction is seen to proceed in separable steps; 2 equiv of reductant is consumed rapidly, converting the triple bond to double, and the resulting alkene undergoes a second slower conversion to alkane. The kinetic behavior of the alkene intermediate corresponds to that of fumarate, rather than maleate.

$$\underset{\text{cis or trans}}{HOOC{-}CH{=}CH{-}COOH} + 2Co(I) + 2H^+ \rightarrow HOOC{-}CH_2CH_2{-}COOH + 2Co(II) \quad (1)$$

Table II. Representative Kinetic Data for the Reaction of Vitamin B_{12s}(Cob(I)alamin) with Maleic Acid (25 °C)[a]

$10^5 \times$ [H$^+$]	$10^{-2} \times$ k_{obsd}[b]	$10^{-2} \times$ k_{calcd}[c]	$10^5 \times$ [H$^+$]	$10^{-2} \times$ k_{obsd}[b]	$10^{-2} \times$ k_{calcd}[c]
0.016	0.056	0.059	1.48	4.1	4.1
0.087	0.40	0.49	3.0	4.4	4.9
0.158	0.81	0.96	8.7	5.8	5.7
0.195	1.06	1.19	23.4	6.2	6.6
0.245	1.37	1.47	288	18.1	17.8
0.437	1.97	2.31	437	23	23
0.63	2.7	2.9	646	29	29
1.02	3.5	3.6			

[a] The supporting electrolyte was $LiClO_4$ (μ = 0.20 M). Glycine, phthalate, and phosphate buffers were used (see Experimental Section). [B_{12s}] = 5×10^{-5}–9×10^{-5} M; [maleic acid] = 2×10^{-4}–6×10^{-3} M. Hydrogen concentrations are given in molarity. [b] Second-order rate constants, M^{-1} s^{-1}. [c] Calculated values obtained from eq 2 in the text, by using parameters listed in Table III.

Representative kinetic data for the reaction of maleic acid appear in Table II. For this oxidant, and for the others, specific rates increase with acidity, reflecting the protonation levels of the dicarboxylato system and, we are led to believe, of the reductant as well. If it is assumed that species H_2A, HA^-, and A^{2-} derived from the oxidant may contribute to the overall reaction but that only the protonated form of the reductant is reactive, we can describe the dependence of rate on acidity using (2), where k_{obsd}

$$k_{obsd} = \frac{(k_0K_1K_2 + k_1K_1[H^+] + k_2[H^+]^2)[H^+]}{(K_1K_2 + K_1[H^+] + [H^+]^2)(K_R + [H^+])} \quad (2)$$

is the second-order rate constant observed at a given acidity, k_0, k_1, and k_2 represent rate constants pertaining to the three protonation levels of the diacid, K_1 and K_2 are the acidity constants for H_2A and HA^-, and K_R is the acidity constant for the protonated form of B_{12s}. Nonlinear least-squares treatment[15] of the kinetic data for the three dicarboxylic acids, utilizing the experimental acidity constants in our medium, yields specific rates listed in Table III for the individual carboxylate species. Values of rate constants for reduction of maleic acid, calculated from (2), are compared with observed specific rates in Table II.

Although there are entries for just three carboxylic acids, we see considerable variation in kinetic acidity patterns. For the least reactive oxidant, fumaric acid, all three protonation levels contribute measurably to the overall reaction, whereas kinetic components attributable to the monoprotonated form of acetylenedicarboxylic acid and the nonprotonated form of maleic acid are not statistically different from zero in the acidity range examined (although studies at higher pH might detect k_0 for maleate).

The strikingly enhanced rate for acetylenedicarboxylic acid may be attributed, in large part, to the increased electronegativity of the sp-hybridized carbon atoms participating in the triple bond.[14b] The kinetic advantage that maleic acid enjoys over its trans isomer probably stems from internal hydrogen bonding between carboxyl groups of the cis acid. In maleic acid, rotation about the C—C single bonds, which would remove the coplanarity of the C=O and C=C units and erode conjugative interactions, is restricted. Beyond this, protonation of the oxidants is seen to accelerate reduction. This feature, which has been observed for B_{12s} reductions of disulfides[8] and for a wide variety of additional oxidations in solution, may be taken to reflect further electron withdrawal from the site that accommodates incoming electrons.

The pK_A for B_{12s} (5.35 in 0.2 M $LiClO_4$) obtained from refinement of our maleate data is in agreement with Rubinson's value (pK_A = 5.6 in 0.5 M KCl).[3c,16] Inclusion of the K_R terms

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(12) About a dozen kinetic runs with maleic acid were carried out at μ = 0.5 M, with buffer concentrations as high as 0.4 M. Although exponential curves were obtained, rate constants did not conform to the general relationship (2), which correlated data for the three carboxylic acids at μ = 0.2 M. Specific rates at the lowest pHs were unexpectedly high, suggesting the operation of general-acid catalysis, as has been observed for reactions of B_{12s} with oxyhalogen anions.[10b] This aspect of the problem was not pursued.

(13) As in previous stop-flow experiments with B_{12s},[8,10b] possible ambiguity resulted from reaction of traces of O_2 on mixing B_{12s} and coreagent solutions. In the absence of added oxidants, decay curves having half-life periods near 100 ms were observed near pH 3. An additional complication occurred with slower reactions involving B_{12s} that had been generated with excess BH_4^-; here, regeneration of B_{12s} at the conclusion of the primary reaction led to spurious "infinity absorbances" and, hence, to incorrect specific rates. Both types of difficulty were minimized by adjusting oxidant concentrations so that the reactions of interest were much more rapid than the complicating features, and the order in oxidant was checked. Even under the most favorable conditions, however, reliable stop-flow data for reduction of acetylenedicarboxylate at pHs greater than 5 could not be obtained.

(14) (a) Bonnet, R. *Chem. Rev.* **1963**, *63*, 573. (b) See, for example: Rutledge, T. F. *Acetylenic Compounds*; Reinhold: New York, 1968; Chapter 1.

(15) In these refinements, individual observed specific rates were weighted in inverse proportion to the square roots of their uncertainties.

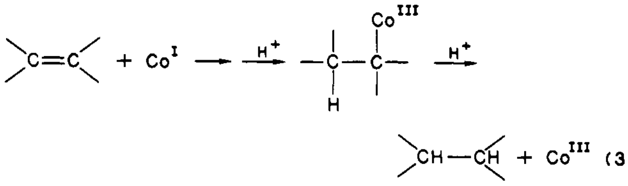
Table III. Kinetic Parameters for the Reactions of Vitamin B_{12s} (Cob(I)alamin) with Unsaturated Dicarboxylic Acids[a]

acid (H_2A)	k_0[b]	k_1[b]	k_2[b]	pK_1	pK_1	pK_R[c]	n[d]
maleic	*e*	$(5.7 \pm 0.1) \times 10^2$	$(1.1 \pm 0.1) \times 10^4$	1.66	5.90	5.35	50
fumaric	3.0 ± 0.5	59 ± 3	$(5.5 \pm 0.6) \times 10^2$	2.73	4.06	(5.35)[f]	18
acetylenedicarboxylic[g]	62 ± 15	*e*	$(4.3 \pm 0.3) \times 10^7$	0.94	2.17	5.28	19

[a] Reactions were carried out at 25 °C, $\mu = 0.2$ M ($LiClO_4$). [b] Specific rates ($M^{-1}\ s^{-1}$) pertaining to A^{2-} (k_0), HA^- (k_1), and H_2A (k_2) forms of the dicarboxylic acid oxidants, obtained by nonlinear least-squares treatment of kinetic data using eq 2 (see text). [c] pK_A values for B_{12s}, obtained from least-squares treatment of kinetic data. [d] Number of runs. [e] Value statistically indistinguishable from zero. [f] Taken from refinement of data from maleic acid and kept constant throughout refinement procedure. [g] 22 °C.

in eq 2 implies that the protonated form of B_{12s} is a much more effective reductant than the nonprotonated, an unusual situation for a reductant but one which has been previously observed for the oxidations of B_{12s} by oxyhalogen anions.[10b] This trend, in both instances, may reflect a conformational change in B_{12s} in which protonation of a proximal benzimidazole nitrogen results in electrostatic repulsion from cobalt(I), thus increasing the accessibility of the reducing center. Why this partition of the reductant between protonation levels affects rates in the present systems but not in reductions of disulfides[8] remains a puzzling point.[17]

A mechanistic ambiguity was encountered in earlier studies[8,10b] of the reactions of B_{12s} with even-electron oxidants, in which kinetic data allowed no choice between a reaction sequence initiated by a 1e (homolytic) or by a 2e (heterolytic) reduction. We believe that the present results strongly favor the latter alternative. The observed 2:1 stoichiometry (Table I) is consistent with sequence (3)–(4), in which rate-determining nucleophilic attack by Co^I on

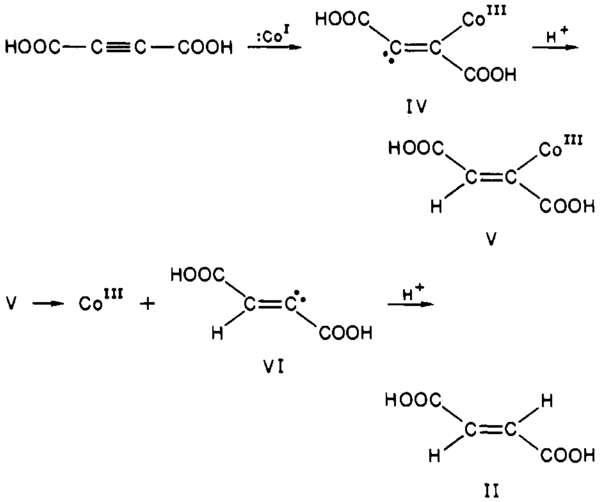


(3)

$$Co^{III} + Co^{I} \longrightarrow 2Co^{II} \qquad (4)$$

the double bond is followed by cleavage of the C–Co^{III} species and a rapid[10a,18] Co^{III}–Co^I comproportionation reaction forming Co^{II}. It may be argued that 1e initiation would lead instead to a free-radical intermediate that could undergo bimolecular termination, resulting in an overall stoichiometry nearer 1:1, but this implies that termination occurs principally by dimerization, rather than by radical disproportionation,[19] and that it proceeds much more rapidly than reduction of the radical by a second Co^I center. A more persuasive point supporting heterolytic initiation is the rate ratio here observed for the alkyne- and alkene-derived oxidants (10^3–10^5). In past instances, where the reactivities of C≡C and analogous C=C substrates toward a given free-radical species have been compared,[20] ratios much closer to unity have been reported, and in some cases, the double-bonded compound reacts more rapidly. In contrast, nucleophilic reagents greatly favor acetylenic acceptors.[21] The systems at hand then appear to fall into the latter category.

The observed reduction of acetylenedicarboxylate to fumarate, rather than to maleate, marks the overall transformation in this case as a trans addition. We suspect that the net stereochemical outcome reflects the nature of the intermediate(s), rather than the recognized greater stability[22] of the trans isomer. In proposed sequence (5)–(6), the initial attack by Co(I) yields a carbanion-like



(5)

(6)

Co(III) species, IV, which undergoes rapid protonation to adduct V. The latter then suffers heterolysis, possibly promoted by H^+, to product II and Co^{III} (which is then consumed by reaction 4). If this scheme, or one related to it, is correct, the resulting stereochemistry is very likely determined in the initial step, for there is considerable evidence that olefinic carbanions such as IV (and VI, if indeed it intervenes) maintain their structural integrity[23] and that substitution reactions involving such intermediates proceed with retention of configuration.[24]

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(16) The pK_A value for B_{12s} calculated from refinement of the maleate data was used as a nonadjustable parameter in the refinement for fumarate. This expedient appears to be reasonable, since the quality of fit in the latter refinement procedure is relatively insensitive to the value of K_R taken.

(17) In the case of B_{12s} reductions of amino- or carboxylate-substituted disulfides,[8] it might be aruged that rate increases resulting from protonation of the oxidants could obscure the effects of protonation of the reductant. This obviously cannot be so for reductions of dithiodiethanol and 1,2-dithiane-4,5-diol, which proceed just as rapidly at pH 8 as at pH 4.

(18) Ryan, D. A.; Espenson, J. H.; Meyerstein, D.; Mulac, W. A. *Inorg. Chem.* **1978**, *17*, 3725.

(19) Bimolecular chain termination by disproportionation should yield equivalent quantities of alkane and alkene ($2C—C^\bullet \rightarrow C—C + C{=}C$); the latter of which would undergo further reduction, thus maintaining 2:1 stoichiometry.

(20) See, for example: Julia, M. In *Chemistry of Acetylenes*; Viehe, H. G., Ed.; Dekker: New York, 1969; p 336.

(21) See, for example: (a) Acheson, R. M. *Adv. Heterocycl. Chem.* **1963**, *1*, 125. (b) Bergman, E. D.; Ginsburg, D.; Pappo, R. *Org. React.* **1959**, *10*, 519.

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(23) (a) Braude, E. A.; Coles, J. A. *J. Chem. Soc.* **1951**, 2078. (b) Curtin, D. Y.; Harris, E. E. *J. Am. Chem. Soc.* **1951**, *73*, 4519. (c) Dreiding, A. S.; Pratt, R. J. *J. Am. Chem. Soc.* **1954**, *76*, 1902. (d) Nesmeyanov, A. N.; Borisov, A. E. *Tetrahedron* **1957**, *1*, 158.

(24) This conclusion does not extend to reactions proceeding through aliphatic carbanions. With these, alteration of configuration about the reaction center is to be expected, especially in hydroxylic solvents. See, for example: Cram, D. J.; Langemann, A.; Allinger, J.; Kopecky, K. R. *J. Am. Chem. Soc.* **1959**, *81*, 5740.