rangement reactions which occur under enzyme control. It follows that conclusions drawn from model reactions lacking one or more of the above features[18] must be applied with circumspection to mechanistic questions surrounding the rearrangement reactions.

At the same time, it is proper to point out features of the model rearrangement reactions which have not yet been illuminated. Neither in this nor in our previous experiment[4] is the ionization state of either cobalt or carbon revealed.[19] We are just beginning to probe the possible salutory effects of the thioester grouping on the present rearrangement. The highly important question of stereochemistry remains completely open in the model series. These are examples and constitute only a few of the problems confronting us in attempting to understand the mechanism of the carbon-skeleton rearrangements from a chemical point of view. They will form the objectives of our continuing research effort in this area.

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References and Notes

(1) M. Flavin and S. Ochoa, *J. Biol. Chem.*, **229**, 965 (1957); J. Katz and I. L. Chaikoff, *J. Am. Chem. Soc.*, **77**, 2659 (1955); R. E. Swick and H. G. Wood, *Proc. Natl. Acad. Sci. U.S.A.*, **46**, 28 (1960); E. R. Stadtman, P. Overath, H. Eggerer, and F. Lynen, *Biochem. Biophys. Res. Commun.*, **2**, 1 (1960).

(2) For an excellent review see L. E. Rosenberg in "Metabolic Basis of Inherited Disorders," 3d ed, J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, Ed., McGraw-Hill, New York, N.Y., 1972, pp 440–458.

(3) We have recently presented a nonenzymic, chemical model reaction for part of an analogous transformation: the reversible, coenzyme B_{12} dependent, enzyme catalyzed interconversion of methylitaconic acid with α-methyleneglutaric acid.[4]

(4) P. Dowd, M. Shapiro, and K. Kang, *J. Am. Chem. Soc.*, **97**, 4754 (1975).

(5) H. Eggerer, E. R. Stadtman, P. Overath, and F. Lynen, *Biochem. Z.*, **333**, 1 (1960).

(6) R. W. Kellermeyer and H. G. Wood, *Biochemistry*, **1**, 1124 (1962); E. F. Phares, M. V. Long, and S. F. Carson, *Biochem. Biophys. Res. Commun.*, **8**, 142 (1962).

(7) R. H. Abeles and B. Zagalak, *J. Biol. Chem.*, **241**, 1245 (1966); J. Retey and D. Arigoni, *Experientia*, **22**, 783 (1966).

(8) M. Sprecher, M. J. Clark, and D. B. Sprinson, *Biochem. Biophys. Res. Commun.*, **15**, 581 (1964); *J. Biol. Chem.*, **241**, 872 (1966); J. Retey and F. Lynen, *Biochem. Biophys. Res. Commun.*, **16**, 358 (1964); J. Retey and B. Zagalak, *Angew. Chem.*, **85**, 721 (1973).

(9) Prepared by treatment of dimethyl methoxymethylmalonate (from dimethyl malonate and chloromethylmethyl ether) with 3 mole equiv of 32% HBr in acetic acid. Excess HBr and acetic acid were removed at room temperature and 0.01 mmHg. The residual oil was then distilled, bulb-to-bulb, at room temperature and 10^{-5} mmHg. This is a modification of the procedure of J. L. Simonsen, *J. Chem. Soc.*, **93**, 1777 (1908). Our procedure attempts, to a greater extent than that of Simonsen, to take into account the sensitive nature of the bromo compound III. The NMR spectrum ($CDCl_3$) of the bromomethylmalonate III shows only a single line, somewhat broadened at the base, at δ 3.8. The mass spectrum is more informative, showing equal intensity doublets at *m/e* (rel int): 224, 226 (6, parent); 193, 195 (15, $M^+ - OMe$); 165, 167 (73, $M^+ - COOMe$) and a singlet at *m/e* 113 (100, base peak, $M^+ - HBr - OMe$). The mass spectrum is free of extraneous peaks in the higher mass range, which might arise from impurities.

(10) Vitamin B_{12s} was prepared by sodium borohydride reduction of hydroxocobalamin.[11]

(11) A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, **4146** (1963); H. P. C. Hogenkamp and W. H. Pailes, *Biochem. Prep.*, **12**, 124 (1968).

(12) λ_{max}^{water} 525 mμ(ϵ 6920), 500 (sh, ϵ 6450), 430 (ϵ 3590), 375 (ϵ 8000), 336 (ϵ 11 065), 315 (sh, ϵ 10 482), 285 (sh, ϵ 14 365), 282 (ϵ 15 530), and 260 (ϵ 17 280) with λ_{min}^{water} 410 (ϵ 3320). In acid solution (pH 3), the 525 mμ peak shifts to 274 mμ (ϵ 7740) and two peaks at 350 (ϵ 10 556) and 314 mμ (ϵ 11 494) become more apparent. This process is reversed upon the addition of base, but some decomposition can be observed, upon this relatively brief exposure to acid, in the rise of the peak at 352 mμ indicating the presence of increasing amounts of hydroxocobalamin. Since the alkyl cobalamin is a relatively unstable substance (see text), the ϵ values cited above could not be determined from a weighed sample. Instead, they were estimated by exposing a solution of the alkyl cobalamin to light, then calculating the ϵ values from those established prior[11] for hydroxocobalamin.

(13) In a typical reaction 1.346 g (1.0 mmol) of hydroxocobalamin *in* 85 ml of water was reduced under an atmosphere of nitrogen with 0.600 g (15.9 mmol) of sodium borohydride in 5 ml of water to the gray-green vitamin B_{12s}. This solution was treated in the dark with 0.700 g (3.1 mmol) of dimethyl bromomethylmalonate (III). After 6 min an ultraviolet spectrum showed the complete formation of the carbon–cobalt bond.

(14) The ether concentrate was a mixture of acids and esters. For this reason it was first stirred overnight with 1 ml of 10% sodium hydroxide, reacidified, and extracted again, continuously for 24 h, with ether. This ether concentrate was chromatographed on a 28 by 1.1 cm column of silicic acid, the products being eluted with a 55:45 mixture of ether–chloroform in 5-ml fractions.[15] Methylmalonic acid (V) was eluted first in fractions 7, 8, and 9; malonic acid (VII) was eluted next in fractions 10, 11 and 12; followed last by succinic acid (VI) in fractions 12, 13, and 14. Because of the overlap between malonic acid (VII) and succinic acid (VI), it was often necessary to combine fractions 12, 13, and 14 and to rechromatograph them on silicic acid. The yields cited in the text are those of recrystallized, sharp-melting solids, and are based on the limiting reagent, hydroxocobalamin. From 1.346 g (1.0 mmol) of hydroxocobalamin was isolated 16.1 mg (13.6%) of methylmalonic acid (V), 18.7 mg (18%) of malonic acid (VII), and 4.4 mg (3.7%) of succinic acid (VI). The spectral properties (ir, NMR, and MS) of the three crystalline solids were in excellent agreement with those of authentic samples.

(15) J. D. Erfle, J. M. Clark, Jr., and B. C. Johnson, *Ann. N.Y. Acad. Sci.*, **112**, 687 (1975).

(16) Malonic acid may arise in this system from hydrolysis of the starting bromomethylmalonate III to hydroxymethylmalonate followed by loss of formaldehyde through a reverse aldol reaction. Alternatively, the presence of malonic acid (VII) may have significance for the carbon-skeleton rearrangements (IV $\rightarrow$ VI and I $\rightleftarrows$ II) in a way not yet fully apprehended. From the point of view of chemical reactivity, the bond between the methyl and methine carbons is an interesting one to consider being broken after the attachment to cobalt. However, the possible significance of malonic acid (VII) in the rearrangement reactions is lessened, although not ruled out, by the observation that malonic acid (VII) is also observed in the control reaction, whereas succinic acid (VI) is not seen.[17]

(17) A control reaction in which cobalt: (II) nitrate was substituted for hydroxocobalamin yielded no detectable succinic acid (VI) following the identical reaction conditions and chromatographic workup as those described above.

(18) G. Bidlingmaier, H. Flohr, U. M. Kempe, T. Krebs, and J. Retey, *Angew. Chem.*, **87**, 877 (1975), have recently reported a similar model reaction using cobaloximes. The rearrangement product was not isolated and the yield of rearrangement product was unstated. Cf. J. N. Lowe and L. L. Ingraham, *J. Am. Chem. Soc.*, **93**, 3801 (1971).

(19) H. A. O. Hill in "Inorganic Biochemistry", Vol. 2, G. L. Eichorn, Ed., Elsevier, Amsterdam, 1973, p 1118, has pointed out that the existence of three stable valence states of cobalt, Co(I), Co(II), and Co(III), gives rise to the possibility of wide mechanistic variation ranging from carbonium ion to free radical to carbanion, depending upon substrate demand. This suggestion was made prior to ours,[4] and should have been referred to in our earlier paper.[4]

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α-Aminoacrylate Schiff Base in Nonenzymatic Pyridoxal Catalysis

Sir:

This report describes a species absorbing at 467 nm, which we conclude to be the titled compound and a possible model for an intermediate in pyridoxal catalyzed α,β-elimination and β-replacement reactions of amino acids.

Many pyridoxal phosphate containing enzymes catalyze the elimination and/or replacement of a β-substituent of an amino acid. The generally accepted reaction sequence[1] involves two metastable intermediates: a quinoid (carbanion) intermediate, which is a Schiff base of the coenzyme and the amino acid deprotonated at the α-carbon atom, and a Schiff base of α-aminoacrylate formed from the quinoid intermediate with a loss of an electronegative group on the β-carbon atom.

Marked changes in spectra of enzymes during reactions with substrate or pseudosubstrate provided evidence for the reaction sequence. Intermediate species with an intense absorption in the 500-nm region have been studied in enzymatic[1,2] and in nonenzymatic systems[3,4] and identified as the quinoid intermediate.

On the other hand, there is less spectral evidence for the α-aminoacrylate Schiff base. Transient species absorbing at 455–470 nm have been reported in a few pyridoxal enzymes catalyzing β-elimination or β-replacement and have been suggested to be this intermediate.[5] A similar species has not been reported so far in nonenzymatic reactions.

Pyridoxal *N*-methochloride (1×10^{-4} M) and tryptophan

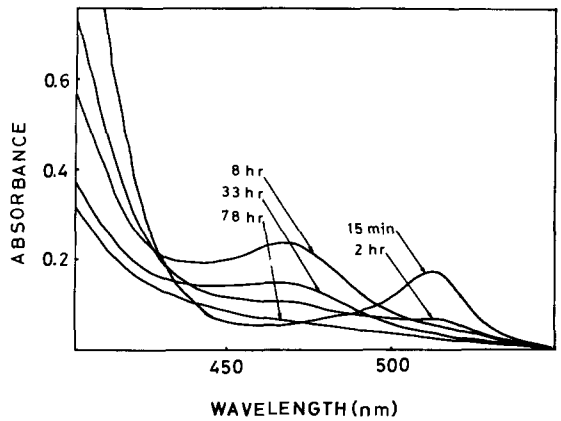


Figure 1. Spectral change accompanying the reaction of pyridoxal *N*-methochloride, tryptophan, and Al(III) nitrate in methanol containing KOH. Concentrations in the final mixture are described in the text. Times after initiating the reaction are indicated beside the spectral curves.

(1×10^{-3} M) were mixed in alkaline methanol (KOH, 2×10^{-3} M) and allowed to stand for 2 h. Then, a methanolic solution of aluminum nitrate (1×10^{-4} M) was added.

Figure 1 shows the spectral change observed in this system at room temperature. An absorption band appeared at 514 nm with a decrease of an absorption at 388 nm, ascribed to the Al(III) chelate of an aldimine, *N*-methylpyridoxylidenetryptophan.[6] The 514-nm band is assigned to the Al(III) chelate of a quinoid intermediate, the aldimine deprotonated at the α-carbon of tryptophan.[4] Its intensity reached a maximum 15 min after the addition of Al(III). With a decrease of the 514-nm band, a new absorption appeared at 467 nm.

The absorbance at 467 nm reached its maximum after about 8 h and was stable for about 10 h, before it decreased gradually and disappeared in several days. The disappearance was accelerated by addition of thiophenol, *p*-chlorothiophenol, or *N*,*N*-dimethylaminoethanethiol.

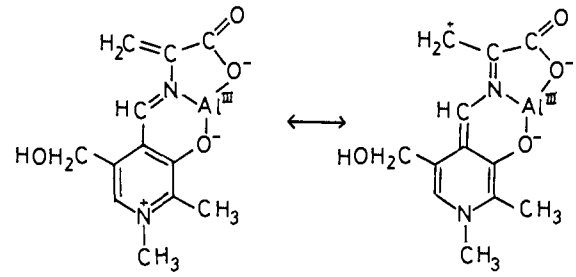
To a methanol solution of pyridoxal *N*-methochloride, tryptophan, and Al(III) absorbing at 467 nm, *p*-chlorothiophenol and, then, pyridoxamine were added. In the reaction mixture was found a considerable amount of *S*-(*p*-chlorophenyl)cysteine identified by comparison of the mass and ir spectra and gas chromatographic behavior with the authentic compound,[7] and by elemental analysis. The result suggests that the 467-nm species was converted to *S*-(*p*-chlorophenyl)cysteine.

For the appearance of the 467-nm band, pyridoxal *N*-methochloride could be replaced by 1-methyl-3-hydroxy-4-formylpyridinium chloride. Pyridoxal and 3-hydroxy-4-formylpyridine produce neither the 514- nor 467-nm species under the conditions. Aluminum nirate can be replaced by its chloride or perchlorate. Gallium nitrate also formed a similar absorption.

Tyrosine and cysteine, in the place of tryptophan, formed the 467-nm absorption, though it was a weak shoulder with cysteine. Histidine formed an imidazotetrahydropyridine derivative[8] and did not form the 467-nm species. Serine and O-succinylserine formed the 467-nm species in the presence of 2-mercaptoethanol, the role of which was not clear.

S-Methylcysteine and *S*-(*p*-chlorophenyl)cysteine produced a similar absorption under slightly different conditions. Without added KOH and in the presence of 5×10^{-4} M Al(III) nitrate, a band appeared gradually at around 458 nm with a decrease of the band of the quinoid intermediate. The 458-nm band was not observed in the presence of KOH equimolar to the amino acid, whereas the addition of a small amount of methanolic HCl increased the absorbance at 458 nm. Addition of HCl to a solution absorbing at 467 nm in the pyridoxal *N*-methochloride–tryptophan–Al(III) reaction

Scheme I



caused a blue shift to 458 nm and promoted the disappearance.

Amino acids without a good leaving group in the β-position, such as alanine, valine, phenylglycine, phenylalanine, methionine and aspartic acid, did not give rise to the 467- or 458-nm band.

On the grounds mentioned above, we assign the 467-nm band to the Al(III) chelate of a Schiff base, *N*-methylpyridoxylidene-α-aminoacrylate. Possible resonance structures are shown in Scheme I. The species absorbing at 458 nm may be a closely related one, presumably with an undissociated carboxyl group.

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References and Notes

(1) For a review, see L. Davis and D. E. Metzler, "The Enzymes", 3d ed, Vol. 7, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1972, Chapter 2.
(2) W. T. Jenkins, *J. Biol. Chem.*, **239**, 1742 (1964); Y. Morino and E. E. Snell, *ibid.*, **242**, 2800 (1967).
(3) L. Schirch and R. A. Slotter, *Biochemistry*, **5**, 3175 (1966); E. H. Abbott and M. A. Bobrik, *ibid.*, **12**, 846 (1973).
(4) S. Matsurr.oto and Y. Matsushima, *J. Am. Chem. Soc.*, **94**, 7211 (1972); **96**, 5228 (1974).
(5) M. E. Goldberg and R. L. Baldwin, *Biochemistry*, **6**, 2113 (1967); M. A. Becker, N. M. Kredich, and G. M. Tomkins, *J. Biol. Chem.*, **244**, 2418 (1969); S. Guggenheim and M. Flavin, *ibid.*, **246**, 3562 (1971); M. Tokushige and A. Nakazawa, *J. Biochem.*, **72**, 713 (1972).
(6) L. Davis, F. Roddy, and D. E. Metzler, *J. Am. Chem. Soc.*, **83**, 127 (1961); Y. Matsushima and A. E. Martell, *ibid.*, **89**, 1322 (1967); Y. Matsushima, *Chem. Pharm. Bull.*, **16**, 2143 (1968).
(7) Y. Murakami and H. Kondo, *Bull. Chem. Soc. Jpn.*, **48**, 125 (1975).
(8) D. Heyl, S. A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **70**, 3429 (1948); Y. Matsushima, *Chem. Pharm. Bull.*, **16**, 2046 (1968).

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Aqueous Lanthanide Shift Reagents. 2. Interaction of the Ethylenediaminetetraacetate Chelates with the Anions of Salicylaldehyde and *o*-Nitrophenol

Sir:

The use of the trivalent lanthanide ions as aqueous shift reagents is restricted to the acidic side of neutral pH due to hydrolysis and precipitation of hydroxides at higher pH values. It has recently been shown that the ethylenediaminetetraacetate (EDTA) chelates are suitable as aqueous shift reagents for carboxylate substrates up to pH values of ca. 10, above which their effectiveness is reduced due to the formation of hydroxo complexes.[1] Thus the LnEDTA chelates should be useful for a variety of substrates, the pK_a values of which are above 7 or which are water soluble only (or practically) in their ionized form. Presented in this communication are results