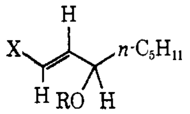
cuprate reagents **3**. A typical experimental procedure follows.

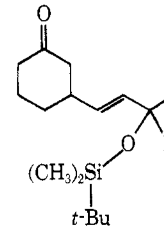
3-*n*-Butylcyclohexanone. A slurry of 0.64 g of dry *n*-propylethynylcopper[18] (4.90 mmol) in 10 ml of anhydrous ether was treated with 1.80 ml of dry hexamethylphosphorus triamide (9.80 mmol), and the mixture was stirred at room temperature under an argon atmosphere until a clear solution was obtained (5–10 min). To the cooled (−78°) solution was then added 3.10 ml of a 1.49 *M* solution of *n*-butyllithium (4.62 mmol) in hexane, and the resulting yellow solution was stirred for 15 min at −78°. The solution of mixed cuprate so formed was then treated with 2.50 ml of a 1.80 *M* solution of 2-cyclohexenone (4.50 mmol) in anhydrous ether, stirred for 15 min at −78°, quenched by pouring into ice-cold aqueous ammonium sulfate solution, and extracted with ether. The ethereal layers were extracted with ice-cold 2% (v/v) sulfuric acid, then filtered through Celite, and washed with aqueous sodium bicarbonate (5%). The dried (Na_2SO_4) extracts afforded almost pure 3-*n*-butylcyclohexanone (0.675 g, 97%), homogeneous by tlc analysis (R_f 0.40; ether–benzene, 1:10), and >99% pure by glpc analysis (glpc retention time, SE-30, 10-ft, 10% column, 170°, 5.2 min). The infrared and nuclear magnetic resonance spectra were also satisfactory for **4** (R_t = n-C_4H_9).

The demonstration that mixed cuprates of formula **3** are capable of highly efficient and selective transfer with R_t = primary alkyl, tertiary alkyl, and vinyl would seem to indicate utility through a large range of alkyl and substituted vinyl groups.[19] In connection with the question of selectivity of group transfer from mixed cuprates, it is relevant that G. Whitesides and coworkers have observed preferential transfer of the 2-norbornyl group (exo or endo) from a mixed cuprate having *tert*-butyl as the second group.[20]

The efficient generation and use of the mixed cuprate **3** in which R_t = **1** raised unexpected problems which were finally overcome by the following approach. *n*-Amyl *trans*-β-chlorovinyl ketone[21] was converted to the corresponding β-iodovinyl ketone, mp 36–37° (>95% yield), with dry sodium iodide in acetone at reflux for 7 hr and thence by treatment with sodium borohydride in ethanol (0°, 1 hr) to 3-hydroxy-*trans*-1-octenyl iodide (**5**) (95% yield).[22,23] This last intermediate was protected as the *tert*-butyl dimethylsilyl ether (**6**).[24] Reaction of **6** in ether at −78° with 2 equiv of *tert*-butyllithium (2.75 *M* reagent in pentane) for 2 hr led to formation of the desired lithium reagent **7** in *ca.* 90% yield as judged by tertiary alcohol formation with benzophenone. The use of 2 equiv of *tert*-butyllithium allowed the conversion of *tert*-butyl iodide, the initial product of lithium halogen exchange, to the innocuous products lithium iodide and isobutylene.[25,26] Treatment of an ethereal solution of the lithium reagent **7** so obtained with an ethereal solution of the 2:1 complex from hexamethylphosphorus triamide and 1-pentynylcopper at −78° afforded a yellow solution of the mixed cuprate **3**, R_t = **1**, which upon treatment with 1 equiv of 2-cyclohexenone gave the desired conjugate addition product **8** in 80% yield. This experiment shows the effectiveness of the mixed cuprate reagent **3** also holds for the O-protected 3-oxy-*trans*-1-octenyl group, an important result in connection with various synthetic approaches to prostaglandins.[27]



5, X = I; R = H
6, X = I; R = *t*-Bu(CH_3)$_2$Si
7, X = Li; R = *t*-Bu(CH_3)$_2$Si



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[18] C. E. Castro, E. J. Gaughan, and D. C. Owsley, *J. Org. Chem.*, **31**, 4071 (1966).

[19] It has previously been reported that nonmixed ethynyl cuprates do not transfer an ethynyl group to an enone under normal conditions; see H. O. House and W. F. Fischer, *J. Org. Chem.*, **34**, 3615 (1969).

[20] Personal communication from Professor G. Whitesides, Oct 1971.

[21] Prepared from hexanoyl chloride, acetylene, and aluminum chloride in carbon tetrachloride solution; see C. C. Price and J. A. Pappalardo, "Organic Syntheses," Collect. Vol. IV, Wiley, New York, N. Y., 1963, p 186.

[22] Satisfactory infrared, nuclear magnetic resonance, and mass spectral data were obtained for all intermediates using chromatographically homogeneous samples.

[23] This sequence was first carried out in these laboratories by Dr. R. Noyori in 1969.

[24] E. J. Corey and A. Venkateswarlu, *J. Amer. Chem. Soc.*, **94**, 6190 (1972). The *tert*-butyldimethylsilyl protecting group was found to be definitely superior to the tetrahydropyranyl group in this instance.

[25] The generation of the lithium reagent **7** from the iodide **6** using lithium metal or lithium metal containing 2% sodium could not be effected in high yield despite numerous attempts. The reaction of **6** with 1 equiv of isopropyllithium afforded **7** in *ca.* 80% yield (ether, −78°); however, it was found that the isopropyl iodide formed as coproduct interfered seriously with subsequent generation and use of the cuprate reagent.

[26] The synthesis of the lithium reagent **6** with R = α-ethoxyethyl, in a yield of *ca.* 25%, has recently been reported.[18]

[27] Initial indications of the utility of mixed ethynyl cuprate reagents were obtained in this laboratory by Dr. Robert L. Carney. We are grateful to him and to Drs. John Mann and Philip Fuchs for many discussions which were helpful in dealing with the numerous obstacles which had to be overcome. Financial assistance from the U. S. Agency for International Development and the National Institutes of Health is also acknowledged.

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An Intermediate Species in Aluminum(III)-Catalyzed Transamination in Methanol

Sir:

The well-known mechanism for transamination between α-amino acids and α-keto acids catalyzed by vitamin B_6 involves formation and isomerization of Schiff bases, *i.e.*, aldimine and ketimine.[1] Studies of

$$\text{pyridoxal} + \text{RCH(NH}_2\text{)COOH} \underset{+\text{H}_2\text{O}}{\overset{-\text{H}_2\text{O}}{\rightleftharpoons}} \text{aldimine} \rightleftharpoons$$

$$\text{ketimine} \underset{-\text{H}_2\text{O}}{\overset{+\text{H}_2\text{O}}{\rightleftharpoons}} \text{pyridoxamine} + \text{RCOCOOH}$$

these steps in nonenzymatic systems have greatly helped

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(1) (a) A. E. Braunstein and M. M. Shemyakin, *Biokhimiya*, **18**, 393 (1953); (b) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Amer. Chem. Soc.*, **76**, 648 (1954).

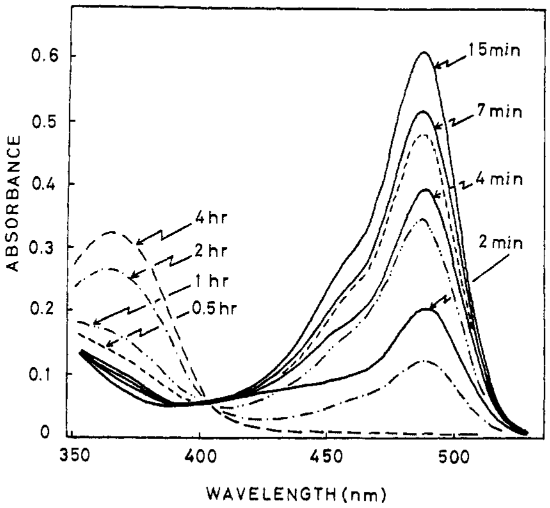

Figure 1. Changes of absorption spectra of a solution formed by adding aluminum nitrate in methanol to a mixture of pyridoxamine and ethyl pyruvate in methanol. The resulting solution contained 1×10^{-4} *M* pyridoxamine, 1×10^{-2} *M* ethyl pyruvate, and 1×10^{-4} *M* aluminum nitrate at the initiation of the reaction. Times after initiating the reaction are indicated beside the spectral curves.

in understanding the role of the coenzymes in enzymatic reactions.[1,2]

Several enzymes containing pyridoxal 5′-phosphate as a cofactor have been reported to exhibit an intense absorption band in the 500-nm region of the spectrum on addition of substrate or pseudosubstrate.[3] Aldimine and ketimine derived from the cofactor are not likely to absorb in this region of the spectrum. Accordingly, this absorption has been ascribed to the intermediate species, in which the α carbon of the amino acid in the aldimine is deprotonated.[3]

On the other hand, there have been a few reports of similar absorption spectra in nonenzymatic systems.[4] Schirch and Slotter[4b] observed a species with an absorption peak at 480 nm in an ethanol solution of pyridoxal *N*-methochloride and diethyl aminomalonate. Maley and Bruice[4c] reported a similar species in the reaction of 1-methyl-4-formylpyridinium iodide with α-amino acids in water. However, these investigators failed to find corresponding species with compounds lacking a quaternized pyridine nitrogen.

In the present communication, we wish to report a new nonenzymatic system giving species absorbing in the 500-nm region. This species, we believe, is the first observed intermediate in the isomerization of Schiff bases derived from vitamin B_6 and should help in determining the structure of enzyme–substrate complexes.

The experimental procedure was: neutral methanol solutions of pyridoxamine and ethyl pyruvate were mixed and allowed to stand for 2 hr. Then a methanolic solution of aluminum nitrate was added. Concentrations in the final solution were 1×10^{-4} *M* for pyridoxamine, 1×10^{-2} *M* for ethyl pyruvate, and 1×10^{-4} *M* for Al(III). The spectrum of the mixture was measured over a period of time. Preparation and mixing of the solution and spectral measurements were carried out at room temperature.

As shown in Figure 1, a new absorption peak appeared at 488 nm with a shoulder at around 460 nm. Its intensity reached a maximum 15 min after the addition of Al(III). Then, the 488-nm band decreased gradually and disappeared in 4 hr. In the final spectrum, an absorption band was observed at 365 nm and was assigned to the Al(III) chelate of the aldimine[5] (ethyl pyridoxylidenealaninate). The absorption bands assigned to the Al(III) chelate of the ketimine (ethyl pyridoximinopyruvate) are in the uv region. The results indicate that the species assignable to the 488-nm band is a metastable intermediate in the Al(III) chelate mediated interconversion of ketimine and aldimine and corresponds to the intermediate of the same spectral character in enzymatic reactions.[3]

Preequilibration of pyridoxamine and ethyl pyruvate was necessary for formation of the 488-nm species. An increase in the concentration of ethyl pyruvate gave an increase in absorption at this wavelength. That Al(III) is involved in the species was confirmed by adding chelating agents such as tetrasodium ethylenediaminetetraacetate and acetylacetone to the methanol solution absorbing at 488 nm. The absorption peak disappeared instantly with the addition. From the method of continuous variation of the concentrations of the reactants, the ratio of pyridoxamine to Al(III) in the species was found to be 2:1.

The species was destroyed immediately in alkaline methanol, whereas it was fairly stable in weakly acidic methanol solution. In aqueous media the corresponding species was not observed, probably because of decreased formation constants of Schiff bases. We did not observe any signal in an esr measurement of a solution containing the species.

3-Hydroxy-4-aminomethylpyridine served as a substitute for pyridoxamine in the formation of the species, though the intensity of the band was somewhat decreased. 4-Aminomethylpyridine, 3-hydroxypyridine, *o*-hydroxybenzylamine, and benzylamine did not form a 488-nm band under the same conditions. Ethyl α-ketobutyrate and ethyl phenylglyoxylate could substitute for ethyl pyruvate, whereas sodium pyruvate and potassium α-ketobutyrate could not. Use of gallium trichloride in the place of aluminum nitrate resulted in the formation of a species of the same spectral character. This can be understood on the basis of the close similarity of Al(III) and Ga(III). All attempts failed to observe similar intermediates with other metal ions such as Mg(II), Ca(II), Mn(II), Fe(III), Ni(II), Cu(II), Zn(II), Cd(II), Sn(II), and Pb(II).

On the grounds mentioned above, we tentatively assign the 488-nm band to the Al(III) chelate of a carbanion, possible resonance structures of which are shown in Scheme I. The pyridine nitrogen may not be protonated, as forms lacking a proton on the pyridine nitrogen are the predominant species of pyridoxal and related substances in neutral methanol.[5,6] In such a

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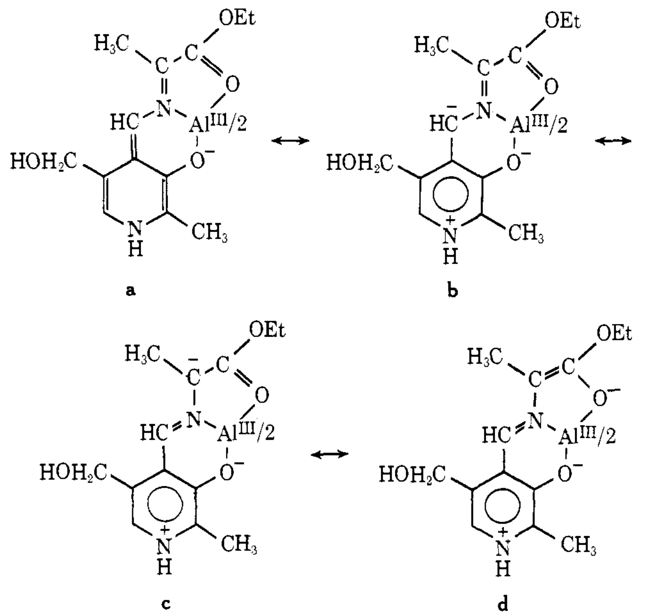
(2) See, for example, E. E. Snell, P. M. Fasella, A. E. Braunstein, and A. Rossi-Fanelli, Ed., "Chemical and Biological Aspects of Pyridoxal Catalysis," Macmillan, New York, N. Y., 1963; E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., "Pyridoxal Catalysis: Enzymes and Model Systems," Interscience, New York, N. Y., 1968.

(3) (a) W. T. Jenkins, *J. Biol. Chem.*, **239**, 1742 (1964); (b) L. V. Schirch and W. T. Jenkins, *ibid.*, **239**, 3801 (1964); (c) Y. Morino and E. E. Snell, *ibid.*, **242**, 2800 (1967); (d) M. Martinez-Carrion, D. C. Tiemeier, and D. L. Peterson, *Biochemistry*, **9**, 2574 (1970).

(4) (a) J. W. Thanassi and J. S. Fruton, *ibid.*, **1**, 975 (1962); (b) L. V. Schirch and R. A. Slotter, *ibid.*, **5**, 3175 (1966); (c) J. R. Maley and T. C. Bruice, *J. Amer. Chem. Soc.*, **90**, 2843 (1968); (d) E. H. Abbott, private communication.

(5) Y. Matsushima and A. E. Martell, *J. Amer. Chem. Soc.*, **89**, 1322 (1967).

Scheme I



case, the nitrogen would bear part of the negative charge of the carbanion. We assume a considerable contribution of structure d to the stability of the carbanion. This assumption comes from the approximate planarity of the ligand, the powerful affinity of aluminum ion for hydroxide ion and oxygen containing groups,[7] and the fact that the species could be observed with ester but not with carboxylate forms of α-keto acids.

Maley and Bruice, in their nonenzymatic transamination system with 1-methyl-3-hydroxy-4-formylpyridinium chloride, did not observe the species absorbing in the 500-nm region,[8] which they did observe with 1-methyl-4-formylpyridinium iodide.[4c]

In the study of the interactions of a variety of analogs of pyridoxal phosphate with apoaspartate aminotransferase, Metzler and coworkers[9] reported the appearance of a small peak at 485 nm on addition of glutamate to a mixture of the apoenzyme and *O*-methyl pyridoxal phosphate. With analogs having a 3-phenolic group, a corresponding absorption was not observed. These facts suggest that a free phenolic or phenolate group prevents the appearance of the carbanion. The role of aluminum ion in the present system would be to coordinate strongly to the phenolate oxygen and to the carbonyl oxygen of ester group and to stabilize the carbanion in the chelate form.

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(6) Y. Matsushima, *Chem. Pharm. Bull.*, 16, 2046 (1968).

(7) E. H. Abbott and A. E. Martell, *J. Amer. Chem. Soc.*, **91**, 6866 (1969); 92, 5845 (1970).

(8) J. R. Maley and T. C. Bruice, *Arch. Biochem. Biophys.*, **136**, 187 (1970).

(9) F. S. Furbish, M. L. Fonda, and D. E. Metzler, *Biochemistry*, **8**, 5169 (1969).

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Mixed Oxidation States in Osmium Ammine Dinitrogen Complexes

Sir:

In pursuing our interests in the coordination chemistry of dinitrogen, we have succeeded in preparing binuclear ammine complexes of osmium with dinitrogen as a bridging group. These are remarkable in the ease with which the [II–II] species are oxidizable to the $[OsNNOs]^{5+}$ unit, the kinetic stability of the oxidized forms, and the wealth of low-energy transitions which the mixed valence complexes exhibit. In contrast to the

unit, where electronic coupling between the metal centers appears to be quite weak,[1,2] in $[OsNNOs]^{5+}$ it is strong enough so that the molecules behave as Robin and Day class III systems.[3]

The green ions $[H_2O(NH_3)_4OsNNOs(NH_3)_5]^{5+}$ (**1**)[4] and $[Cl(NH_3)_4OsNNOs(NH_3)_5]^{4+}$ (**2**)[4] were synthesized by heating $[(NH_3)_5OsN_2]^{2+}$[5] (0.3 *M*) with *cis*-$[(NH_3)_4Os(N_2)_2]^{2+}$[6] (0.15 *M*) at 70° under argon for 36 hr. Following acidification with HCl and oxidation by air, the blue-green solution was subjected to ion exchange chromatography, using a column of Bio-Rad AG 50W-X2 resin in the acid form. Species **2** was elutable with 4 *M* HCl and **1** by 5 *M* HCl. Moving somewhat more slowly than **1** was a blue band and further ion exchange was necessary to resolve this band from **1**. Tosylate salts of **1** and **2** were precipitated and submitted for analysis. *Anal.* Calcd for $[H_2O(NH_3)_4OsNNOs(NH_3)_5](C_7H_7SO_3)_5\cdot H_2O$: C, 28.9; H, 4.6; N, 10.6. Found: C, 28.9; H, 4.6; N, 10.9. Calcd for $[Cl(NH_3)_4OsNNOs(NH_3)_5](C_7H_7SO_3)_4\cdot H_2O$: C, 25.8; H, 4.4; N, 11.8; Cl, 2.7. Found: C, 25.2; H, 4.3; N, 11.9; Cl, 2.6. Oxidation of **1** with an excess of Ce(IV) yielded 0.9 mol of N_2/mol of complex.

The green species $([Cl(NH_3)_4Os]_2N_2)^{3+}$ (**3**)[4] was prepared by heating *cis*-$[(NH_3)_4Os(N_2)_2]^{2+}$ in solution at 70° for 36 hr and then for *ca.* 0.5 hr at 90° after oxidation. The complex was eluted with 3 *M* HCl and was precipitated as the chloride salt. *Anal.* Calcd for $[(Cl(NH_3)_4Os)_2N_2]Cl_3\cdot H_2O$: H, 3.5; N, 18.9; Cl, 24.0. Found: H, 3.3; N, 19.1; Cl, 23.2.

When a solution containing the blue species mentioned above was heated in 1 *M* HCl under argon, **1** and **2** were obtained in about 20 and 50% yields, respectively. On the basis of this evidence, and the band structure in the ir in the N≡N stretch region, we take the blue species to be $[N_2(NH_3)_4OsNNOs(NH_3)_5]^{5+}$ (**4**).

A portion of the ir spectrum of compounds containing **2** and **3** is shown in Figure 1. It is clear that a band ascribable to the N≡N stretch is virtually absent in **3** but that it has a moderate intensity in **2** (the ir band of present interest is of intermediate intensity in **1**). The Raman spectrum of **3** in solution shows a strong

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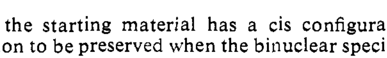
(1) C. Creutz and H. Taube, *J. Amer. Chem. Soc.*, **91**, 3988 (1969).

(2) C. Creutz, M. L. Good, and G. Chandra, private communication. Mössbauer spectroscopy shows two different kinds of Ru atoms to be present at low temperature in



(3) M. B. Robin and P. Day, *Advan. Inorg. Chem. Radiochem.*, **10**, 247 (1967).

(4) Because the starting material has a cis configuration, we take this configuration to be preserved when the binuclear species are formed. This assumption is in accord with the assignment of the far-visible–near-ir absorptions.

(5) A. D. Allen and J. R. Stevens, *Chem. Commun.*, 1147 (1967).

(6) H. A. Scheidegger, J. N. Armor, and H. Taube, *J. Amer. Chem. Soc.*, **90**, 3263 (1968).