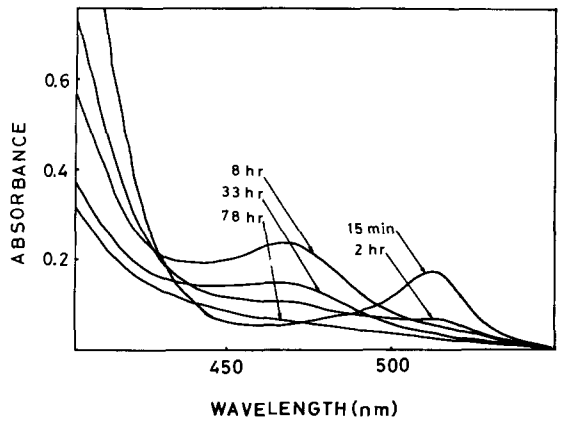


Figure 1. Spectral change accompanying the reaction of pyridoxal *N*-methochloride, tryptophan, and Al(III) nitrate in methanol containing KOH. Concentrations in the final mixture are described in the text. Times after initiating the reaction are indicated beside the spectral curves.

(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*-methylpyridoxylidene-tryptophan.[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.

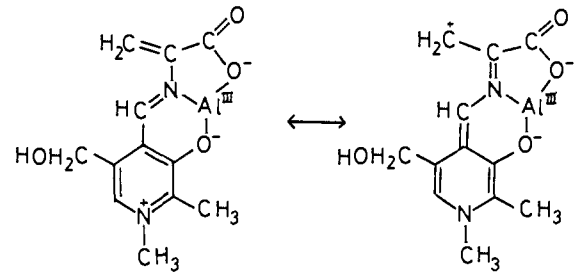
To a methanol solution of pyridoxal *N*-methochloride, tryptophan, and Al(III) absorbing at 467 nm, *p*-chlorothiophenol and, then, pyridoxamine were added. In the reaction mixture was found a considerable amount of *S*-(*p*-chlorophenyl)cysteine identified by comparison of the mass and ir spectra and 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.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan, for which the authors are grateful. Y.K. thanks Japan Securities Scholarship Foundation for a Predoctoral Fellowship.

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. Matsumoto 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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Received December 29, 1975

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

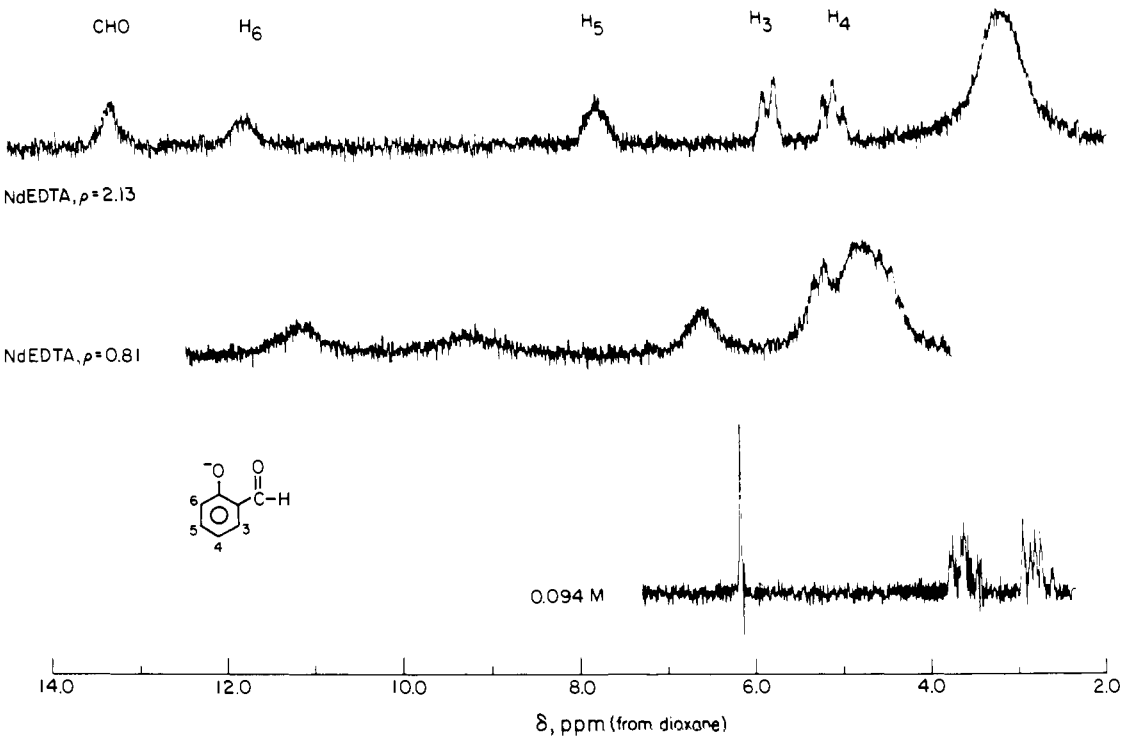


Figure 1. The 60-MHz spectra of the salicylaldehyde anion without (bottom) and with increasing concentrations of NdEDTA.

Table I. Lanthanide Induced Shifts[a] for the Anion of Salicylaldehyde

	H_3	H_4	H_5	H_6	CHO
PrEDTA, $\rho = 2.32$	4.87	5.35	8.87	21.25	3.52
Δ_i/Δ_4	0.91	[1.00]	1.66	3.97	0.66
NdEDTA, Δ_M[b]	2.3 ± 0.1	2.5 ± 0.1	4.5 ± 0.1	9.2 ± 0.2	7.2 ± 0.2
Δ_i/Δ_4	0.92	[1.00]	1.80	3.68	2.88
EuEDTA, $\rho = 2.53$	−1.98	−1.90	−5.03	−12.53	−12.48
Δ_i/Δ_4	1.04	[1.00]	2.65	6.60	6.57

[a] In parts per million, downfield shifts given as positive. [b] Intrinsic shifts obtained from a titration.

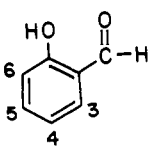
Table II. Lanthanide Induced Shifts[a] for *o*-Nitrophenolate

	H_3	H_4	H_5	H_6
PrEDTA, ρ = 2.48	1.02	1.54	2.86	7.60
Δ_i/Δ_4	0.66	[1.00]	1.86	4.94
NdEDTA, Δ_M[b]	0.53 ± 0.03	1.25 ± 0.1	2.8 ± 0.2	7.0 ± 0.3
Δ_i/Δ_4	0.42	[1.00]	2.24	5.60
EuEDTA, ρ = 2.53	−0.13	−0.67	−2.46	−5.53
Δ_i/Δ_4	0.20	[1.00]	3.69	8.30

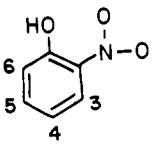
[a] In parts per million, downfield shifts given as positive. [b] Intrinsic shifts obtained from a titration.

obtained with the anions of salicylaldehyde ($pK_a = 8.14$)[2] and of *o*-nitrophenol ($pK_a = 7.06$).[2,3]

The effects of successive additions of NdEDTA on the proton spectrum of salicylaldehyde are shown in Figure 1. Considerable lanthanide induced shifts (LIS) are observed for all of the protons. The shifts are accompanied by line broadenings, the magnitude of which follows the same order as the LIS magnitude. At higher reagent concentrations, however, the line broadenings are reduced and the spin-spin splitting patterns are again discernible. This behavior of the line broadening is typical for relaxation effects arising from the modulation by chemical exchange of the shift difference, Δ_M, between the complexed and uncomplexed substrate molecules.[4] The broad and intense band seen in Figure 1 is due to one of the EDTA protons. Its position is also sensitive to complex formation and spectral interference may be avoided by changing the concentrations. The LIS's observed with the EDTA chelates of praseodymium, neodymium, and europium are summarized in Table I. The PrEDTA and NdEDTA chelates induce downfield shifts (denoted as positive), whereas EuEDTA induces upfield shifts. From a titration of a constant substrate concentration with NdEDTA and monitoring the LIS a dissociation constant of 6 ± 1 mM was obtained for the salicylaldehydo–NdEDTA complex.



salicylaldehyde



o-nitrophenol

The behavior observed with *o*-nitrophenol was similar to that of salicylaldehyde. The results are summarized in Table II. The dissociation constant for the *o*-nitrophenolato–NdEDTA complex obtained from a titration is 50 ± 10 mM. For comparison the complex formation between the anion of *p*-nitro-

phenol (pK_a = 7.15) and NdEDTA was also investigated. Significant LIS's were observed only for the protons ortho to the ionized hydroxyl group, their intrinsic LIS being 0.68 ppm. Also the complex formed was found to be relatively weak (dissociation constant of 160 ± 40 mM). The comparison between *p*- and *o*-nitrophenol suggests that chelation plays an important role in complex formation for the latter substrate. Both salicylaldehyde and *o*-nitrophenol contain, in addition to the ionizing hydroxyl group, oxygen containing functional groups that are well suited for the formation of a stable six-membered ring upon chelation.

In the case of chelation the complex formed will be devoid of axial symmetry. Moreover, also absent will be the possibility for internal rotation or stereochemical rearrangement of the substrate within the complex, i.e., absent will be the mechanisms leading to effective axial symmetry in many lanthanide shift reagent systems.[5] It is not surprising, therefore, that the internal shift ratios (given in Tables I and II as Δ_i/Δ_4) are different for the three lanthanides investigated. A similar behavior has recently been observed for the Pr^{3+} and Eu^{3+} complexes of the ethyl ester of *N*-acetyl-L-3-nitrotyrosine (ANTE).[6] For the analysis of the LIS's in such systems the complete pseudocontact shift equation (cf., e.g., ref 6 and references therein)

$$\Delta_M = r^{-3}[K_1(3\cos^2\theta - 1) + K_2(\sin^2\theta\cos 2\phi)] \quad (1)$$

has to be employed. One of the effects of rapid internal rotations or stereochemical rearrangements of the substrate in the complex is to average out the second term of eq 1, in which case the magnitude of the total shift is also reduced. Referring to the LIS values of *o*- and *p*-nitrophenol this effect is clearly revealed. We also note that the shifts induced by the LnEDTA chelates in the spectrum of *o*-nitrophenol are of similar magnitude as those reported for the corresponding aquo lanthanides and another substrate, ANTE, containing the *o*-nitrophenolate group.[6] In other instances, e.g. carboxylates, the LIS by the aquo ions have been found to be much larger than those by the EDTA chelates.[1] It seems that the difference between the carboxylates and the *o*-nitrophenolates arises from the chelating ability of the nitrophenolate moiety, which may prevent the averaging out of a substantial part of the pseudocontact shift.

Contact contributions to the observed LIS's cannot be ruled out altogether. They are likely to increase in the order Pr < Nd < Eu.[7] However, for an accurate separation between contact and pseudocontact contributions data for a larger number of lanthanides are needed. With the data presently available and using the tabulated theoretical estimates of the relative contact and pseudocontact LIS's (cf. ref 7 and references cited therein) one finds that only the LIS of CHO of salicylaldehyde could be largely of contact origin.

Acknowledgments. The technical assistance of Naomi Bauman and helpful discussions with Gabriel A. Elgavish are gratefully acknowledged. This work was supported in part by a grant from the United States-Israel Binational Science Foundation.

References and Notes

(1) G. A. Elgavish and J. Reuben, *J. Am. Chem. Soc.*, in press.
(2) Cf., e.g., C. Postmus, Jr., L. B. Magnuson, and C. A. Craig, *Inorg. Chem.*, **5**, 1154 (1966).
(3) Spectra were recorded with a Varian T-60 spectrometer operating at the ambient probe temperature of 39 ± 1°. Solutions were made up in D_2O containing ca. 0.1% v/v dioxane, the resonance of which served as internal reference. Substrate concentrations were in the range 75-120 mM. The shift reagents were prepared by mixing equivalent amounts of the lanthanide trichloride and Li_4EDTA. The pH was adjusted to be in the range 8.6-9.3 using either LiOD or DCl.
(4) R. E. Lenkinski and J. Reuben, *J. Magn. Reson.*, **21**, 47 (1976), and references cited therein.
(5) (a) J. M. Briggs, G. P. Moss, E. W. Randall, and K. D. Sales, *J. Chem. Soc., Chem. Commun.*, 1180 (1972); (b) W. DeW. Horrocks, Jr., *J. Am. Chem. Soc.*, **96**, 3022 (1974).
(6) T. D. Marinetti, G. H. Snyder, and B. D. Sykes, *J. Am. Chem. Soc.*, **97**, 6562 (1975).
(7) J. Reuben, *J. Magn. Reson.*, **11**, 103 (1973).

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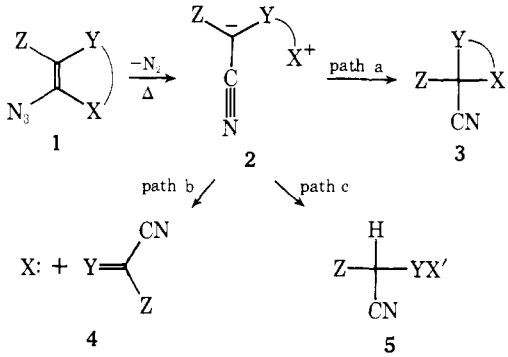
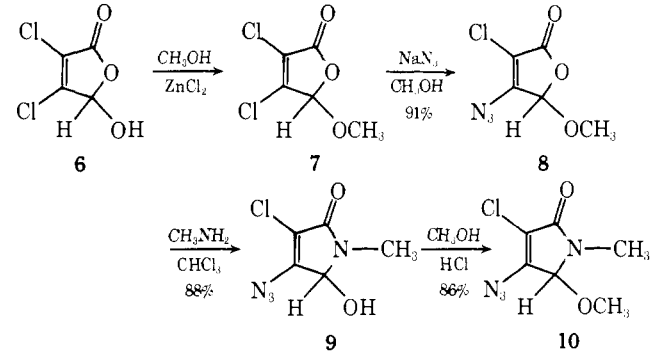
Chlorocyanoketene. A New β-Lactam Synthesis

Sir:

A working mechanistic model for the rearrangements of certain cyclic vinyl azides, which has significant predictive powers for synthetic objectives, can be formally viewed as outlined below. That is, we assume cleavage of **1** to **2** to be facile when X can easily carry a positive charge and Y and/or Z are anion stabilizing substituents. The zwitterionic intermediate, **2**, can then ring close to **3** (path a), cleave to **4** (path b), or, in cases where X carries an acidic proton, collapse to **5** (path c).[1]



Reported here is the thermal chemistry of some selected heterocyclic β-azidoeneones that undergo rearrangements and fragmentations which are predictable according to the above model. Of particular interest is the observed cleavage of β-azido-α-chloro-γ-methoxy-$\Delta^{\alpha,\beta}$-crotonolactone (**8**)[2] to the previously unknown chlorocyanoketene (**11**) and the ring contraction of 4-azido-3-chloro-1-methyl-5-methoxy-Δ^3-pyrrolinone (**10**) to the β-lactam (**15**). The potential versatility



of the cyanoketene and β-lactam syntheses described here is obvious when one considers the general synthetic route to their respective butenolide and pyrrolinone precursors. These were prepared from the commercially available mucochloric acid **6** as outlined below.[3] It is significant to note that a vast chemistry of mucohalic acids has been reported, and that synthetic methodology exists which allows their structural modifications at the α-, β-, and γ-positions.[4] Thus, a detailed study of the synthetic scope and mechanisms of these azide thermolyses is warranted and will appear subsequently. When