

C(5) methyl distance is 3.01 Å, and the C(4)–C(4) distance is 2.94 Å. For comparison the normal van der Waals separation between methyl groups of neighboring molecules is 4.0 Å,¹⁶ and between carbon atoms about 3.5 Å. Thus overcrowding and steric strain across the C(5)–C(5) cyclobutane bond are almost certainly present in photodimer A, and, although the crystal structure of dimer B has not been elucidated, one suspects that steric interference is also present there.

This stereochemical explanation of the relative radiation sensitivities of the pyrimidine photodimers may also

(16) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 260. The use of van der Waal's separation for methyl groups has been criticized by a referee who feels that in view of the fact that coordinates have been determined for the hydrogen atoms, the H...H distances should be quoted. Since it is our purpose to show qualitatively that steric crowding exists between the C(5) methyl groups, we felt nothing is gained by this distinction. Nonetheless, we have calculated the H...H separations involved and find there are two short H...H contacts of 2.08 and 2.24 Å (vdw distance = 2.40 Å) between the two methyl groups and two short C...H contacts of 2.48 and 2.71 Å (vdw distance = 2.80 Å).

shed some light on their relative sensitivities to acid-base hydrolysis. Thymine photodimers C and D are much more sensitive to hydrolysis than are dimers A and B (principal product is thymine in each case),¹⁷ and it may be that the steric overcrowding in the latter isomers obstructs attack by hydrolyzing agents, while the openness in the cyclobutane region in dimers C and D facilitates it.

Acknowledgments. The authors are indebted to Professors L. H. Jensen and R. F. Stewart for very kindly making available the photographic intensity data which were used in the refinement of the structure. We also thank Dr. D. Weinblum for supplying the material and Professor S. C. Nyburg for the use of facilities in his laboratory. Support at Toronto was from the Medical Research Council of Canada and at Seattle from National Institutes of Health Grant No. GM-13366.

(17) M. A. Herbert, J. C. LeBlanc, D. Weinblum, and H. E. Johns, *Photochem. Photobiol.*, **9**, 33 (1969).

Chemical Evolution. III. The Photochemical Conversion of Enaminonitriles to Imidazoles^{1,2a}

J. P. Ferris^{2b} and J. E. Kuder

*Contribution from the Department of Chemistry,
Rensselaer Polytechnic Institute, Troy, New York 12181.
Received October 9, 1969*

Abstract: A mechanistic investigation of the photocyclization of enaminonitriles to imidazoles is reported utilizing diaminomaleonitrile (HCN tetramer) (I) and β -aminocrotononitrile (ACN; V). The reaction proceeds from a π, π^* excited state which has some charge transfer character. The photocyclization must proceed *via* a singlet since the reaction is not sensitized by benzophenone or triphenylene and is not quenched by cyclohexadiene or piperylene. However, the *cis-trans* isomerization of ACN involves a triplet since it is sensitized by benzophenone and triphenylene. In addition, I quenches the phosphorescence of biacetyl. No luminescence was observed from the compounds studied. Flash photolysis studies revealed the presence of a long-lived transient ($t^{1/2} = 0.5$ msec); however, since the transient was quenched by dienes, it cannot be associated with the photocyclization. When the photolysis of ACN is carried out in D₂O solution, deuterium was not photolytically incorporated into the 5 position of 4-methylimidazole. Furthermore, only 4-methylimidazole and none of the 2-methyl isomer is the reaction product. These data show that the reaction proceeds by way of an intermediate in which the C–CN bond is broken by a process which does not involve a ketimine intermediate. An azetine intermediate, which undergoes further rearrangement to the imidazole, is suggested by the photochemical conversion of the vinylogous enaminonitrile XIII to the vinylogous azetine (pyridine) (XIV).

Hydrogen cyanide has been proposed as the starting point in the chemical evolution of purines and amino acids. Hydrolysis of a polymer formed from HCN yields fourteen amino acids.³ An intermediate in the polymerization is diaminomaleonitrile (HCN tetramer) (I), an essential compound in the proposed prebiotic synthesis of purines.⁴ The tetramer, which exists in the *cis* form in the ground state, is photo-

chemically converted to the *trans* isomer. The tetramer also undergoes a photochemical rearrangement to 4-aminoimidazole-5-carbonitrile⁵ (II) which, in a dark reaction with hydrogen cyanide, forms adenine (III). In addition, II or its hydrolysis product IV readily affords guanine, isoguanine, diaminopurine, hypoxanthine, and xanthine.⁶

The photochemical step is the only efficient pathway from hydrogen cyanide to the purine precursor II which is feasible in dilute aqueous solution. Because of its central role in the proposed prebiotic synthesis sequence, it was decided to investigate the photolysis of enaminonitriles further, with the aim of determining:

(5) J. P. Ferris and L. E. Orgel, *J. Amer. Chem. Soc.*, **88**, 1074 (1966).

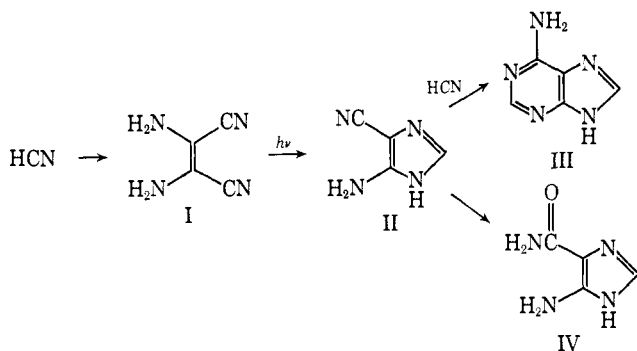
(6) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, **38**, 121 (1968).

(1) For the previous paper in this series see: J. P. Ferris, J. E. Kuder and A. Catalano, *Science*, **166**, 765 (1969). For the previous paper in a related series see ref 6.

(2) (a) Supported by Grant No. GM 15915 from the National Institutes of Health; (b) USPHS Research Career Development Awardee (GM 6380) of the National Institute of General Medical Sciences.

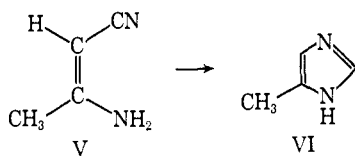
(3) C. N. Mathews and R. E. Moser, *Nature*, **215**, 1230 (1967).

(4) R. A. Sanchez, J. P. Ferris, and L. E. Orgel, *J. Mol. Biol.*, **30**, 223 (1967).

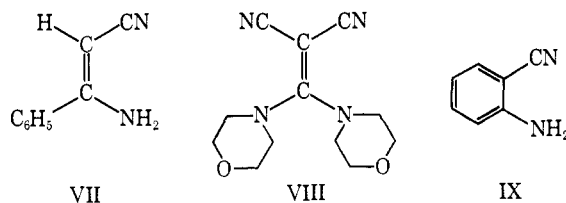


(1) the factors which affect the course of the rearrangement; (2) the scope of the reaction; and (3) the mechanism of the reaction.

The photocyclization discovered with hydrogen cyanide tetramer proceeds with β -aminocrotonitrile (V) and other enaminonitriles (e.g., 1,1-diamino-2,2-



dicyanoethylene and 1-amino-2-cyanoethylene) but not with β -aminocinnamionitrile (VII), 1,1-dimorpholino-2,2-dicyanoethylene (VIII), or aminobenzonitrile (IX).^{6a}



Of the examples given, we have studied the photolysis of HCN tetramer (I) and β -aminocrotonitrile (ACN; V) in detail. The photochemical rearrangement of these two compounds proceeds in a wide variety of solvents, including water, methanol, ethanol, ethyl acetate, ether, acetonitrile, tetrahydrofuran, and methylene chloride. The only solvent found to be unsuitable was chloroform, which when used as a solvent for ACN photolysis afforded ammonium chloride and an unidentified second product without the formation of an imidazole.⁷ The conversion of HCN tetramer to 4-aminoimidazole-5-carbonitrile (II) has been found to proceed in yields of greater than 90% if the photolysis is performed in the absence of oxygen. Furthermore, the imidazole produced is stable indefinitely to further irradiation. The photodestruction of II observed previously⁴ is apparently a photo-oxidation process.^{1,8}

Nature of the Excited State

The absorption spectrum of an enaminonitrile consists of a single broad, intense absorption band.⁹ In

(6a) NOTE ADDED IN PROOF. Mr. F. Antonucci recently observed that the photochemical conversion of VII to 4-phenylimidazole and VIII to benzimidazole and indazole proceeds in solutions which are rigorously degassed.

(7) Since lamps with principal emission at 254 nm were used for this experiment, it is likely that the reaction which occurred was due to chloroform, which itself absorbs at this wavelength.

(8) A. Catalano, B. S. Thesis, Rensselaer Polytechnic Institute, Troy, N. Y., 1968.

(9) S. Baldwin, *J. Org. Chem.*, **26**, 3288 (1961).

aqueous solution this is located at 257 nm ($\epsilon_{\max} 1.6 \times 10^4$) for ACN and at 295 nm ($\epsilon_{\max} 1.4 \times 10^4$)⁴ in the case of HCN tetramer (Table I). The ACN absorption band is shifted to 248 nm in cyclohexane while the tetramer band is shifted to 287 nm in benzene solution. Both the magnitude of the extinction co-

Table I. Effect of Solvent on the Absorption Spectrum of *cis*-ACN and of HCN Tetramer

Solvent	<i>cis</i> -ACN λ_{\max} ($\epsilon \times 10^4$)	Tetramer λ_{\max} ($\epsilon \times 10^4$)
Cyclohexane	248 (1.4)	Nd
Benzene	Nd ^a	287 (satd soln)
MeCN	254 (1.71)	294 (1.40)
THF	255 (1.72)	Nd
95% EtOH	257 (1.87)	Nd
H ₂ O	257 (1.60)	295 (1.35)
0.01 N HCl	Blank	293 (1.4)
1 N HCl	Nd	283 (1.2)
6 N HCl	Nd	281 (1.4)

^a Nd, not determined.

efficient and the direction of the solvent shift are consistent with a π - π^* transition.¹⁰ Furthermore, the observation that the absorption band of ACN is absent in acid solution, but reappears when the acid solution is neutralized, indicates that the amino group is an essential part of the chromophore. The absorption band of HCN tetramer in 6 N HCl is shifted to 281 nm, and is most likely that of the monoprotonated base, still containing the enaminonitrile moiety.

The result of HMO calculations¹¹ shown in Figure 1 indicate that the absorption of light by enaminonitriles is accompanied by a transfer of electron density from the amino to the cyano group. As suggested previously by Baldwin⁹ there is considerable charge transfer in the ground state of enaminonitriles and our calculations suggest further charge transfer in the excited state. According to Murrell,¹² "the term charge-transfer absorption may be used whenever there is a large electron displacement in going from the ground to the excited state." By this definition, it may be said that there is some charge-transfer character to the π , π^* absorption band of enaminonitriles.

Attempts were made to observe emission from the excited states of I and V. Neither ACN nor HCN tetramer exhibits luminescence however, either at room temperature or in ethanol-methanol glass at 77°K. Presumably vibrational relaxation (and/or rearrangement) must be preferred over emission as the major deexcitation mode.^{10b}

Since it was not possible to obtain information concerning excited states directly from luminescence studies, energy transfer experiments were undertaken

(10) (a) N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, Inc., New York, N. Y., 1967, p 46; (b) *ibid.*, p 69; (c) *ibid.*, p 132.

(11) HMO calculations were performed using a computer program provided by Professor S. C. Wait. The parameters used were those suggested by A. Streitwieser, "Molecular Orbital Theory for Organic Chemists," John Wiley & Sons, Inc., New York, N. Y., 1961, p 135. The parameters for the nitrile group were those determined by P. H. Rieger and G. K. Fraenkel, *J. Chem. Phys.*, **37**, 2795 (1962).^{11a}

(11a) NOTE ADDED IN PROOF. Similar calculations have recently been reported. J. Kuthan, *Coll. Czech. Chem. Commun.*, **34**, 2942 (1969).

(12) J. N. Murrell, *Quart. Rev. (London)*, **15**, 191 (1961). See also G. Porter and P. Suppan, *Pure Appl. Chem.*, **9**, 499 (1964), and S. Nagakura, *Mol. Phys.*, **3**, 152 (1960).

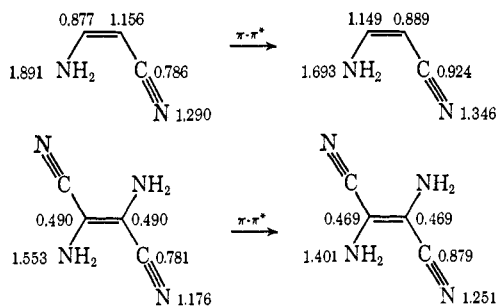


Figure 1. Calculated HMO electron densities in the ground and excited states.

between HCN tetramer and biacetyl. When solutions of biacetyl ($5 \times 10^{-3} M$) and varying concentrations of HCN tetramer in benzene were irradiated at 417 nm, it was observed that the biacetyl phosphorescence (524 nm) but not fluorescence (460 nm) was quenched. A relatively linear Stern-Volmer plot is obtained when the ratios of phosphorescence emission intensity, P_o/P_a , are plotted against tetramer concentration, (Figure 2). The slope gives a rate constant for energy transfer of $3 \times 10^8 \text{ l. mol}^{-1} \text{ sec}^{-1}$ as compared with a calculated rate constant of $10^{10} \text{ l. mol}^{-1} \text{ sec}^{-1}$ for a diffusion-controlled process. The difference between the observed and calculated rate constants may be due to a number of factors. It was assumed in calculating the energy-transfer rate constant that the lifetime of the biacetyl triplet is the same in our system as that reported previously for benzene solution.¹³ However, other factors, e.g., trace impurities, profoundly effect triplet lifetime measurements.¹³ Second, a part or all of the quenching of biacetyl phosphorescence may be due to hydrogen abstraction. The rate constant observed in this study is, in fact, similar to those reported for the quenching of triplet biacetyl by hydrogen transfer from amines.¹³ Third, it is possible that under the experimental conditions biacetyl and tetramer might condense to form 5,6-dimethylpyrazine-2,3-dicarbonitrile¹⁴ which might itself act as a triplet acceptor.

Since the results of biacetyl phosphorescence quenching were not conclusive, the study of energy transfer was made by a second approach. In the ground state HCN tetramer exists in the *cis* form but is photochemically converted to the *trans* isomer.⁴ Yamada and coworkers succeeded in isolating the *trans* tetramer (diaminofumaronitrile), but found that in acidic or basic solution it rapidly reverses to the *cis* isomer.¹⁵

Similarly, ACN isomerizes in solution or even in the solid state to produce a mixture of about 65% *trans* and 35% *cis* ACN.¹⁶ The two forms are readily distinguishable by nmr, since the *trans* form exhibits a H-CH₃ coupling of 0.8 Hz, while in the *cis* isomer this coupling is absent. Upon irradiation of either *cis*- or *trans*-ACN a photostationary state is rapidly achieved in which the *trans* isomer predominates. The photoequilibration may be followed by changes in the

(13) H. L. J. Bäckström and K. Sandros, *Acta Chem. Scand.*, **12**, 823 (1958); N. J. Turro and R. Engel, *Mol. Photochem.*, **1**, 143, 235 (1969).

(14) L. E. Hinkel, G. O. Richards, and O. Thomas, *J. Chem. Soc.*, 1432 (1937).

(15) Y. Yamada, N. Nagashima, Y. Iwashita, A. Nakamura, and I. Kumashiro, *Tetrahedron Lett.*, 4529 (1968).

(16) E. Bullock and B. Gregory, *Can. J. Chem.*, **43**, 332 (1965).

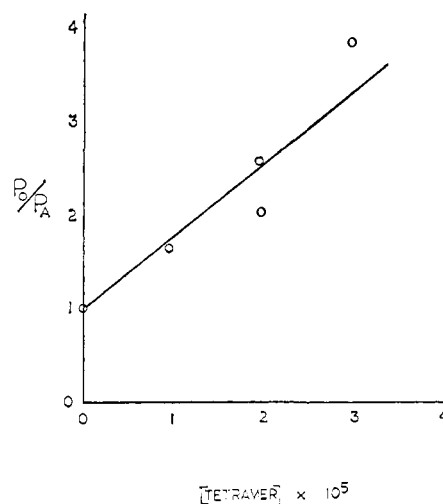


Figure 2. Quenching of biacetyl phosphorescence by HCN tetramer.

uv absorption spectrum since *cis*-ACN absorbs at slightly shorter wavelength and has a more intense absorption band ($\lambda_{\text{max}}^{\text{MeCN}} 254 \text{ nm}$, $\epsilon 1.77 \times 10^4$) than does the *trans* isomer ($\lambda_{\text{max}}^{\text{MeCN}} 255 \text{ nm}$, $\epsilon 1.54 \times 10^4$). The position of the photoequilibrium, as established by uv absorption data, is at 26% *cis* and 74% *trans*. The photoequilibrium has also been observed by nmr, in an experiment in which ACN was irradiated directly in quartz sample tubes. Thus, when samples of ACN initially containing either a 45:55 or a 27:73 *cis:trans* ratio were irradiated at 254 nm, the same final mixture of isomers (23% *cis* and 77% *trans*) was observed in both cases.

When a sample of ACN (64% *cis* and 36% *trans*) was irradiated in Pyrex in the presence of benzophenone using lamps with principal emission at 350 nm, the ratio changed to 33% *cis* and 67% *trans* after a total of 20 min of irradiation (see Table II). A control

Table II. Sensitized *cis-trans* Isomerization of ACN^a

Sample	<i>cis:trans</i> ratio after irradiation		
	0 min	10 min	20 min
ACN, Ph ₂ CO	64:36	42:58	33:67
ACN	64:36	63:37	60:40
ACN, Ph ₂ CO (not irradiated)	64:36		59:41
ACN, triphenylene	79:21	34:66	28:72
ACN	79:21	73:27	60:40
ACN, triphenylene (not irradiated)	79:21		72:26

^a Solutions of ACN and sensitizer 0.1 M in benzene irradiated in Pyrex with 350-nm lamps. Ratio determined from methyl protons at $\tau 8.50$ (*cis*) and 8.97 (*trans*).

sample irradiated in Pyrex without benzophenone contained 60% *cis* after the same time had elapsed. The possibility that the benzophenone sensitized *cis-trans* isomerization of the enamionitrile occurs by a combination-dissociation process was tested in a second experiment using triphenylene, a sensitizer of similar triplet energy but differing in structural type. A sample of ACN containing 79% *cis* and 21% *trans* was irradiated in Pyrex at 350 nm in the presence of triphenylene and was converted to a 28:72 *cis:trans*

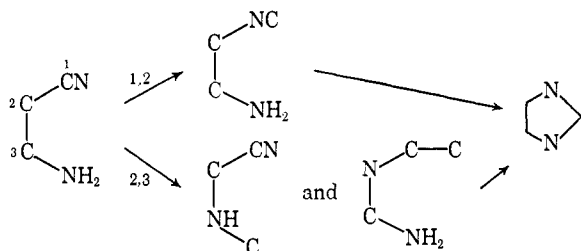
mixture after 20 min of irradiation. A control sample of ACN without triphenylene had a 60:40 *cis*:*trans* ratio after the same length of irradiation. It appears unlikely that *cis*-*trans* isomerization is by a combination-dissociation process since it occurs with two structurally different sensitizers. Since the photostationary state obtained by triplet energy transfer is essentially the same as that obtained upon direct irradiation, it may be concluded that the *cis*-*trans* isomerization proceeds by a triplet excited state.

Flash photolysis of HCN tetramer resulted in a large increase in absorption at 340 nm which was stable in the absence of further irradiation but which was slowly bleached by the analyzing beam. This change is undoubtedly due to the conversion of the *cis* to the *trans* isomer by the flash followed by the photochemical isomerization of *trans* to *cis* by the longer wavelength light of the analyzing beam. In addition, a transient species was observed with maximum intensity at 450 nm. This transient which was of extremely low intensity, had a half-life of about 0.5 msec and was quenched by the addition of 2×10^{-4} M 1,3-cyclohexadiene. However, in view of the fact that the photocyclization of tetramer proceeds in the presence of a large excess of diene (0.1 M), the 450-nm transient cannot be due to an intermediate in this conversion. The increase in absorption at 340 nm was not quenched by the addition of 2×10^{-4} M cyclohexadiene. Presumably the triplet involved in the *cis*-*trans* isomerization of HCN tetramer is of lower energy than that of cyclohexadiene ($E_{\text{triplet}} = 54$ kcal).¹⁷ This conclusion is consistent with a triplet-energy transfer process in the quenching of biacetyl phosphorescence ($E_{\text{triplet}} \text{ biacetyl} = 55$ kcal).^{10c}

While *cis*-*trans* photoisomerization of enamionitriles occurs *via* the triplet manifold, the photocyclization does not. The formation of 4-methylimidazole from ACN in degassed solutions is not sensitized by either benzophenone or triphenylene. Further, the photocyclization of tetramer in degassed solution is not retarded by 0.1 M cyclohexadiene and 1 M piperylene. It may be concluded then that the photocyclization proceeds *via* the singlet state.¹⁸

Mechanism of the Cyclization

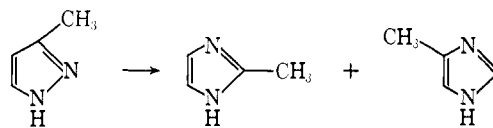
The conversion of an enamionitrile to an imidazole involves cleavage of a carbon-carbon bond and the formation of a carbon-nitrogen bond at some stage of the reaction. *A priori*, either 1,2 or 2,3 cleavage is possible. Processes involving 2,3 cleavage are analogous to the photochemical isomerization of hetero-



(17) R. E. Kellogg and W. T. Simpson, *J. Amer. Chem. Soc.*, **87**, 4230 (1965).

(18) Other examples of triplet *cis*-*trans* isomerization and singlet rearrangement are known; cf. E. Ullman, *Accounts Chem. Res.*, **1**, 353 (1968).

cyclic compounds,¹⁹ such as the conversion of 3-methylpyrazole to a 1:1 mixture of 2- and 4-methylimidazole.¹⁹



The formation of 2-methylimidazole must take place by a process involving 2,3 cleavage while the 4-methylimidazole requires 1,2 cleavage.

The possibility that the enamionitrile rearrangement proceeds *via* a pyrazole intermediate (2,3 cleavage) has been eliminated in two ways. First, careful product analysis has shown that 4-methylimidazole is the only imidazole produced when ACN is irradiated. None of the 2-methyl isomer nor any 3-methylpyrazole could be detected by nmr analysis of the reaction mixture. Furthermore, when 3-methylpyrazole was irradiated under conditions which result in the conversion of ACN to imidazole, only starting material was recovered.²⁰

The rearrangement of enamionitriles must involve a 1,2 cleavage step and several possibilities have been considered. The simplest involves heterolytic or homolytic cleavage of the 1,2 bond, rearrangement to the isonitrile, and cyclization (Scheme II). A 20% conversion of methacrylonitrile to the corresponding isonitrile by γ irradiation has been reported.²¹ In addition, photolysis of nitriles to cyanide ion and cyano radicals has been reported.²²

An experiment designed to observe the photochemical conversion of a nitrile to an isonitrile was not successful. Irradiation of acrylonitrile with 253.7-nm lamps for 30 min resulted in a 50% conversion to an insoluble polymer. The infrared spectrum of the supernatant liquid did not exhibit absorption at 2120-2185 cm^{-1} for the isonitrile group.²³

If a nitrile-isonitrile inversion were involved in imidazole formation one might expect that tautomerism occurs prior to bond breaking (homolytic or heterolytic) so that a fragment stabilized by allylic-type resonance might result. If this were the case it should be possible to detect it by a hydrogen-deuterium exchange of the vinyl proton of ACN as shown in Scheme I.

Two samples of ACN, one in the presence of a 10-fold molar excess of D_2O , the other with a 100-fold excess, were irradiated until all the starting material was converted to product (22 hr). In each case the 4-methylimidazole produced was isolated as the oxalate salt and its nmr spectrum determined. The ratios

(19) B. Singh and E. F. Ullman, *J. Amer. Chem. Soc.*, **89**, 6911 (1967); H. Tiefenthaler, W. Dörscheln, H. Göth, and H. Schmid, *Helv. Chim. Acta*, **50**, 2244 (1967), and references contained therein.

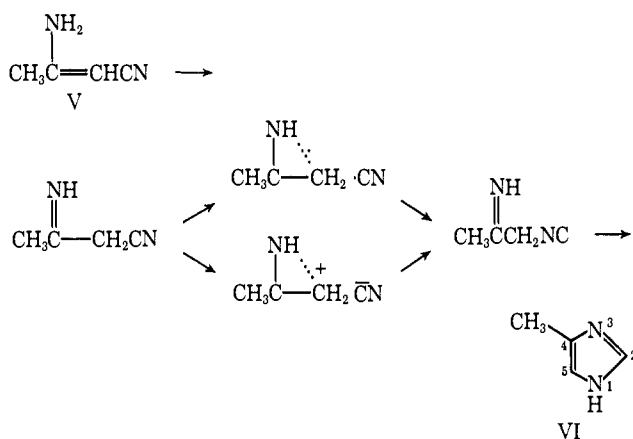
(20) The previously reported experiment to test for a pyrazole intermediate was designed incorrectly.⁵ 3-Amino 4 cyanopyrazole was considered as a potential intermediate in the conversion of HCN tetramer to 4-aminimidazole-5-carbonitrile. The compound which should have been investigated is 3-cyano-4-aminopyrazole.

(21) M. Talat-Erben, E. Ünseren, and N. Seber, AEC Accession No. 46326, Report No. CNAEM 28; *Chem. Abstr.*, **65**, 6565 (1966).

(22) (a) R. E. McElcheran, M. H. J. Wijnen, and E. W. R. Steacie, *Can. J. Chem.*, **36**, 321 (1958); (b) L. Harris and J. Kaminsky, *J. Amer. Chem. Soc.*, **57**, 1154 (1935); (c) E. O. Holmes, Jr., *J. Phys. Chem.*, **61**, 434 (1957).

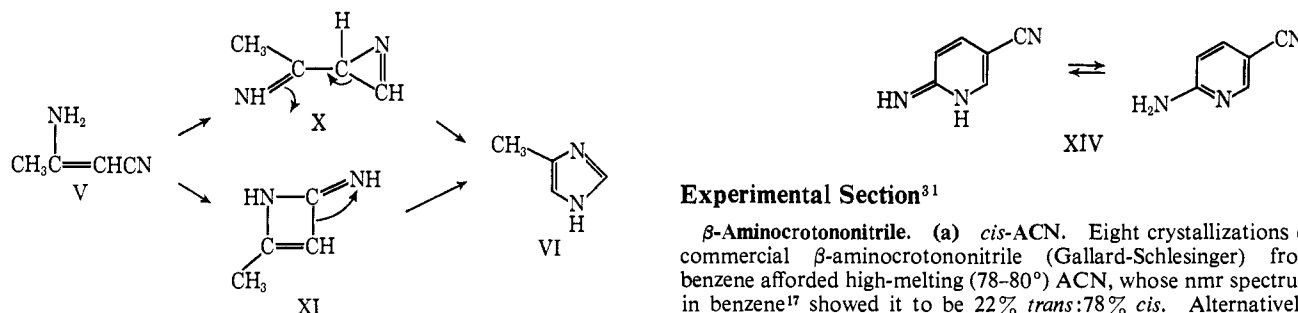
(23) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," John Wiley & Sons, Inc., New York, N. Y., 1963, p 68.

Scheme I



of the integration heights (compared to $\text{CH}_3 = 3.00$) were $\text{H}_2:\text{H}_5 = 0.60:0.81$ in the sample photolyzed with a 10 *M* excess of D_2O and $\text{H}_2:\text{H}_5 = 0.21:0.77$ in the sample irradiated with a 100 *M* excess of D_2O . In a control experiment it was observed that 76% of the vinyl hydrogen of ACN exchanged in 8 hr in the absence of ultraviolet light. The deuterium incorporation at C-5 of the imidazole is undoubtedly due to some ground-state exchange prior to photochemical rearrangement.²⁴ These data suggest that photochemical isomerization does not proceed *via* the tautomeric imine pathway outlined in Scheme I.

Scheme II



Two possible 1,2-cleavage mechanisms are shown in Scheme II. The photochemical formation of an azirine (X) and its subsequent conversion to an imidazole is completely analogous to the photochemical rearrangement of isoxazoles and related heterocyclic systems.¹⁹ The photochemical degradation of cytosine has been postulated to proceed *via* an azetine²⁵ and the subsequent conversion of XI to the imidazole is similar in nature to the photochemical conversion of ketones to cyclic ethers.²⁶

Infrared analysis during the course of the photolysis showed only absorption bands due to ACN or 4-methylimidazole. Trace amounts of other compounds were isolated from the photolysis reaction of ACN by preparative tlc; however, these were not converted to 4-methylimidazole on further irradiation. These data show that if either X or XI is the reaction intermediate

(24) It has been suggested that the hydrolysis of enamines involves a reversible tautomerization to the ketimine which is then hydrolyzed to the carbonyl grouping (J. Coward and T. Bruice, *J. Amer. Chem. Soc.*, **91**, 5329 (1969)). Our observation of a ground-state deuterium exchange is consistent with this proposal.

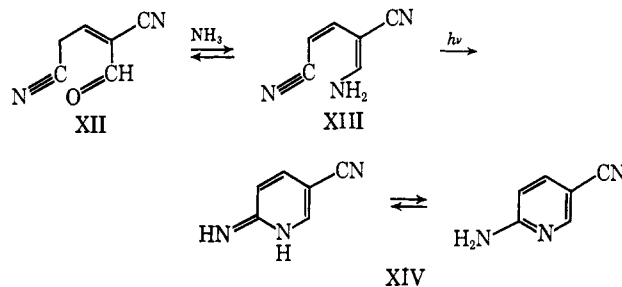
(25) K. Wierzchowski and D. Shugar, *Photochem. Photobiol.*, **2**, 377 (1963).

(26) N. J. Turro and R. M. Southam, *Tetrahedron Lett.*, 545 (1967); H. U. Hostettler, *Helv. Chem. Acta*, **49**, 2417 (1966).

it does not accumulate but rather rearranges directly to 4-methylimidazole.

There have been at least two literature reports of azirines with structures very similar to X.^{19,27} In both instances, the azirines were produced photochemically and in each instance the compounds were stable. To our knowledge, there are no literature reports of 2-iminoazetine derivatives. However, oxetenes, the oxygen analogs of azetines have been postulated as intermediates in the photoaddition of ketones to acetylenes. However, it was not possible to isolate these adducts.²⁸ The apparent absence of a long-lived reaction intermediate suggests but does not prove that the rearrangement proceeds by azetine XI.

Strong support for an iminoazetine intermediate comes from studies of compound XIII, a vinylogous enamionitrile. Compound XIII exists in equilibrium with the anion of cyanoacetaldehyde dimer (XII) in aqueous ammonia.²⁹ When an ammoniacal solution of XII is irradiated 6-aminonicotinonitrile is produced. The photocyclization of XIII to XIV is completely analogous to the cyclization of ACN to XI with the exception that XIV is stable and does not undergo further rearrangement. In this way the reaction intermediate has been trapped.³⁰



Experimental Section³¹

β -Aminocrotonitrile. (a) *cis*-ACN. Eight crystallizations of commercial β -aminocrotonitrile (Gallard-Schlesinger) from benzene afforded high-melting (78–80°) ACN, whose nmr spectrum in benzene¹⁷ showed it to be 22% *trans*:78% *cis*. Alternatively,

(27) G. R. Harvey and K. W. Ratts, *J. Org. Chem.*, **31**, 3907 (1966).

(28) G. Büchi, J. T. Kofron, E. Koller, and D. Rosenthal, *J. Amer. Chem. Soc.*, **78**, 876 (1956).

(29) J. P. Ferris, R. A. Sanchez, and L. E. Orgel, *J. Mol. Biol.*, **33**, 693 (1968).

(30) After completing this experiment we discovered that a synthesis of 2-amino-5-cyano-4,6-lutidine had been described in similar photochemical reaction.¹⁶

(31) Ultraviolet absorption spectra were obtained with a Unicam SP 800A ultraviolet spectrophotometer, infrared spectra with a Perkin-Elmer 137, and proton magnetic resonance spectra with a Varian A60. Melting points were determined on Mel-Temp melting point apparatus and are uncorrected. Methanol, tetrahydrofuran, and acetonitrile were purified by standard procedures; all other solvents were reagent grade and were used without further purification. Irradiations were carried out in a Rayonet photochemical reactor with fittings for 16 lamps having principal emission at 350, 300, or 254 nm. Samples were degassed before irradiation by five freeze-pump-thaw cycles. Paper chromatography was done by spotting the sample on Whatman 3MM paper and developing with butanol-acetic acid-water (4:1:1) (BAW) or butanol saturated with water (BW). Thin layer chromatography was done on microscope slides coated with Silic Gel AR using chloroform-methanol-ammonia (80:25:0.1) or ethyl acetate as solvent. Spots were visualized by uv light, iodine vapor, or spraying⁶ with Folin spray or diazotized sulfanilic acid. A special cell was constructed for degassing solutions to be irradiated and monitored spectrophotometrically. Degassing was done with the solution in the Pyrex side arm, and absorbance measurements with the solution in the quartz cell. The sample could be irradiated in either Pyrex or quartz. HCN tetramer was prepared from aminomalonnitrile.³² Piperylene and cyclohexadiene were purchased from Aldrich Chemical Co. and were distilled before use.

(32) J. P. Ferris and R. A. Sanchez, *Org. Syn.*, **48**, 60 (1968).

commercially ACN which had been recrystallized twice from benzene, was dissolved in water and freeze dried. As soon as the freeze-drying was complete, the sample was crystallized once from benzene to afford a mixture of 15% *trans*:85% *cis*. The procedure was suggested by the observation that when a mixture of ACN isomers is dissolved in D₂O only a one-proton singlet and a three-proton singlet are seen in the nmr spectrum. Since $J_{H/C}$ is absent, the only species present in aqueous solution must be *cis*-ACN.

(b) *trans*-ACN. Low-melting ACN was prepared by the method of Adkins and Whitman³³ by the reaction of sodium with acetonitrile. Fractional sublimation of the product afforded a mixture that was shown by nmr analysis to be 78% *trans*:22% *cis*.

β -Aminocinnamitrile (V, R = C₆H₅). β -Aminocinnamitrile was prepared according to Holtzworth³⁴ by the reaction of acetonitrile, sodium, and benzonitrile in ether. The product after recrystallization from benzene was obtained as pale yellow needles, mp 88.5–90° (lit.³⁴ 86°), λ_{max}^{MeCN} (log ϵ) 290 nm (4.06), 229 nm (4.05).

Irradiation of β -Aminocinnamitrile. A solution of β -aminocinnamitrile (1.0×10^{-4} M) in 95% ethanol (purged with nitrogen) was placed in a quartz uv cell and irradiated at 254 nm. A slight shift in the wavelength of the absorption maxima within the first minute of irradiation was followed by a decrease in intensity. After 100 min of irradiation about a third of the original sample remained. There was no evidence of the formation of 4-phenylimidazole (λ_{max} 260 nm).

Preliminary Irradiation of ACN in Various Solvents. Solutions of ACN (10^{-2} M) were irradiated in quartz tubes at 254 nm in the following solvents: water, methanol, ethanol, ethyl acetate, ether, acetonitrile, tetrahydrofuran, methylene chloride, and chloroform. The samples were examined periodically by tlc for the appearance of 4-methylimidazole. After 6 hr of irradiation, both ACN and 4-methylimidazole were detected in all the solvents except chloroform. A repetition of the irradiation in chloroform afforded a white solid whose ir spectrum showed it to be ammonium chloride³⁵ and a yellow oil which was neither ACN nor 4-methylimidazole, but was not investigated further.

Conversion of HCN Tetramer to AICN (II). (a) An aqueous solution of HCN tetramer (1.0×10^{-4} M)³¹ was degassed and irradiated with lamps having their major emission at 350 nm. The sample was monitored spectrophotometrically for the disappearance of HCN tetramer and appearance of AICN (II) (λ_{max} 245, ϵ 11,000). In a series of four runs, AICN yields of 82, 70, 80, and 82% were obtained, the reaction being complete in 9 hr.

(b) The observation that HCN tetramer decomposes at neutral pH but is stable in acid, prompted the irradiation of tetramer in aqueous acid solution. In 10^{-3} N HCl 93 and 83% yields of AICN were obtained in two runs, while yields of 89 and 94% were observed in 10^{-2} N HCl.

(c) When the photolysis was carried out in acetonitrile an 84% yield of AICN was obtained.

Luminescence Measurements.³⁶ No luminescence was observed from ACN or HCN tetramer (10^{-3} M) in ethanol-methanol 4:1 at room temperature or at 77°K.

Quenching of Biacetyl Phosphorescence by HCN Tetramer.³⁶ A series of samples containing biacetyl (5×10^{-3} M) and varying concentrations of tetramer in benzene were irradiated at 417 nm and the intensity of phosphorescence noted. When the ratios of biacetyl phosphorescence to phosphorescence in the presence of tetramer were plotted against tetramer concentration a straight line was obtained (Figure 2). The slope of this line and the phosphorescence lifetime of biacetyl (10^{-8} sec)¹³ were used to calculate the rate constant for energy transfer, 3×10^8 l. mol⁻¹ sec⁻¹.

***cis,trans* Isomerization of ACN.** A solution of ACN (1.00×10^{-4} M) in MeCN containing 78% *cis*:22% *trans* (as determined by nmr) was irradiated in quartz at 254 nm and its spectrum monitored periodically. The sample showed a sudden decrease in absorbance (from 1.72 to 1.60) after 30 sec of irradiation, followed by a more regular decrease thereafter. Similarly, a sample of ACN (1.00×10^{-4} M) containing 13% *cis*:87% *trans* showed an increase in absorbance (1.57 to 1.60) after 30 sec of irradiation, followed by a regular decrease. Since the absorption maxima of the two isomers differ by only one nanometer, the total absorbance at 254 nm may

be taken as the sum of the absorbance of the two species. Knowing the initial concentrations of the two isomers in the two samples, the molar extinction coefficients may be calculated, $\epsilon_{cis} = 1.77 \times 10^4$ and $\epsilon_{trans} = 1.54 \times 10^4$. Now the absorbance at the initially established photoequilibrium is 1.60, whence the equilibrium concentration of the isomers may be calculated, 26% *cis*:74% *trans*.

Flash Photolysis Studies of HCN Tetramer.³⁷ Experiments were performed using 10^{-3} M aqueous acid, methanol, or acetonitrile as solvent with no observable difference in results. Furthermore, the results were unchanged if the tetramer was purified by three vacuum sublimations. The degassed tetramer solution (3×10^{-5} M, 10-cm cell) was flashed through a NiSO₄-CoSO₄ filter solution which afforded a window to the tetramer absorption band. The sample was monitored for transient absorption from 340 to 650 nm. At 340 nm a large (20%) increase in absorbance was observed upon flashing. The change, which is attributed to the conversion of *cis* to *trans* tetramer, was permanent in the absence of light but was bleached with the analyzing beam turned on. A low-intensity transient (λ_{max} 450 nm) was observed with half-life of 0.5 msec. This transient was absent in the presence of 2×10^{-4} M 1,3-cyclohexadiene but was present in reduced intensity (with the same half-life) in 2×10^{-5} and 8×10^{-5} M diene. Since the intensity but not the lifetime of the transient was reduced, the diene must have quenched a precursor to the 450-nm transient rather than the transient itself. In a preliminary experiment, it was observed that 0.1 M piperylene also quenched the 450-nm transient but had no effect on the *cis-trans* isomerization observed at 340 nm.

Attempts at Sensitized Imidazole Formation. (a) **With Benzophenone.** The following solutions in ethyl acetate were prepared, each solute being 10^{-2} M in concentration: (i) ACN; (ii) ACN plus Ph₂CO; (iii) 4-methylimidazole; (iv) 4-methylimidazole plus Ph₂CO. About 1.5 ml of each was placed in a Pyrex tube degassed, sealed, and irradiated with 350-nm lamps for 48 hr. At this time the ampoules were opened and the solutions examined by tlc. Sample i showed only ACN. Sample ii, which had turned yellow, showed ACN and no benzophenone. Sample iii showed only 4-methylimidazole. Sample iv showed 4-methylimidazole, benzophenone plus one additional component. Thus, there is no evidence for sensitized imidazole formation, and further, 4-methylimidazole is inert to sensitized decomposition.

(b) **With Triphenylene.** The above experiment was repeated using triphenylene as sensitizer. Again neither sensitized formation of imidazole nor its sensitized destruction was observed.

Irradiation of HCN Tetramer in the Presence of Dienes. (a) Samples of tetramer (1×10^{-4} M) in acetonitrile with various amounts of piperylene were degassed and irradiated in Pyrex using 350-nm lamps. The approximate half-times for destruction of tetramer were: (no piperylene) 160 min; (10^{-1} M piperylene) 130 min; and (1 M piperylene) 140 min.

(b) Methanolic solutions of tetramer (10^{-2} M) and of tetramer (10^{-2} M) plus piperylene (1 M) were degassed and irradiated in Pyrex with 350-nm lamps for 24 hr and then examined by paper chromatography (BW). Both samples showed spots (uv, Folin's) due to HCN tetramer and AICN approximately equal in size. Thus, neither the formation of AICN nor the rate of HCN tetramer photolysis is inhibited by the presence of piperylene.

(c) Methanolic solutions of HCN tetramer (10^{-2} M) and of HCN tetramer (10^{-2} M) plus 1,3-cyclohexadiene (0.1 M) were degassed and irradiated in Pyrex with 350-nm lamps for 24 hr and then examined by paper chromatography (BW). Both samples showed spots of equal size (uv, Folin's) due to AICN, indicating that there is no quenching of the photocyclization by 1,3-cyclohexadiene.

Irradiation of Neat Acrylonitrile. About 4 ml of freshly distilled acrylonitrile in a quartz tube was irradiated at 254 nm. After 30 min of irradiation, over half the sample had been converted to a white granular polymer which does not melt but chars at ca. 300°. The ir spectra of the supernatant liquid and the starting material are identical and no peak which might be ascribed to an isonitrile group²³ was observed.

Deuterium Incorporation during Photolytic Imidazole Formation. A solution of ACN (0.041 g; 10^{-3} M) and D₂O (0.1 ml; 10^{-2} M) in 500 ml of tetrahydrofuran (distilled from sodium) was irradiated in quartz at 254 nm for 22 hr. Examination of the solution by tlc

(33) H. Adkins and G. M. Whitman, *J. Amer. Chem. Soc.*, **64**, 150 (1942).

(34) R. Holtzworth, *J. Prakt. Chem.*, **39**, 230 (1889).

(35) F. A. Miller and C. H. Wilkins, *Anal. Chem.*, **24**, 1253 (1952).

(36) The luminescence apparatus has been described in H. H. Richtol and F. H. Klappmeier, *J. Chem. Phys.*, **44**, 1519 (1966). We thank Dr. H. H. Richtol for the use of this apparatus.

(37) We thank Professor R. Strong for these measurements and for assisting us in the interpretation of the results. The apparatus used is described in the doctoral dissertation of P. A. Carapellucci, Rensselaer Polytechnic Institute, Troy, N. Y., 1967.

showed the presence of 4-methylimidazole but no ACN. The solvent was removed under reduced pressure, and the residue taken up in 2 ml of ether and treated with a saturated solution of oxalic acid in ether. The precipitated oxalate salt, recrystallized once from acetone-water (3:1) had mp 205–206° dec; lit.³⁸ 205–206° dec. The nmr spectrum in D₂O of the salt obtained showed peaks at 1.45, 2.82, and 7.65 in the ratio 0.60:0.81:3.00. Authentic samples were prepared of oxalate salts of the following compounds and their nmr spectra in D₂O then recorded: 4-methylimidazole oxalate, τ H₂ 1.45, H₅ 2.82, CH₃ 7.65; 2-methylimidazole oxalate, τ H₄, H₅ 2.67; CH₃ 7.35; 3-methylpyrazole oxalate, τ H₄ 3.30, H₅ 1.82; CH₃ 7.47. Thus, the only product of the irradiation of ACN is 4-methylimidazole, the product having a certain amount of deuterium incorporated at positions two and five.

In a similar experiment using a 100-fold molar excess of D₂O the ratio of protons in the recovered oxalate salt of 4-methylimidazole was H₂:H₅:CH₃ = 0.21:0.77:3.00. Again tlc indicated the presence of 4-methylimidazole only.

Deuterium Exchange on ACN in the Absence of Irradiation. The nmr spectrum of a solution of ACN (0.1 M) in D₂O was determined at intervals and the disappearance of the vinyl proton monitored. The amount of vinyl proton (compared to CH₃ = 3.00) remaining after various times was: 0 hr, 1.00; 2 hr, 0.82; 4 hr, 0.71; 8 hr, 0.24.

Irradiation of 3-Methylpyrazole. A solution of 3-methylpyrazole (0.041 g; Aldrich) in 250 ml of tetrahydrofuran (distilled from sodium) was irradiated in quartz at 254 nm for 24 hr. The solvent was removed under reduced pressure and the residue examined by tlc and by nmr. Only 3-methylpyrazole was detected.

In a second experiment, 3-methylpyrazole (0.041 g) plus benzophenone (0.009 g) in 250 ml of tetrahydrofuran was irradiated in Pyrex with 350-nm lamps for 48 hr. The solvent was removed under reduced pressure and the residue examined by tlc. Only 3-methylpyrazole and benzophenone were detected.

These results are somewhat surprising, since our conditions are not too much different from those reported by Tiefenthaler, *et al.*,¹⁹ for the conversion of 3-methylpyrazole to 2- and 4-methylimidazole.

(38) A. Windaus and F. Knoop, *Ber.*, **338**, 1166 (1905).

Attempted Detection of Intermediates in the Photolysis of ACN. Five milliliters of a 5% (w/v) solution of ADN in acetonitrile was irradiated in quartz at 254 nm. Periodically, aliquots were withdrawn for examination by ir and tlc. After 155 hr of irradiation only bands due to ACN could be seen in the ir spectrum of the sample, these being somewhat diminished in intensity. Examination of the sample by tlc, however, indicated the presence of ACN and 4-methylimidazole (diazotized sulfanilic acid is about ten times as sensitive to imidazole as it is to ACN) as well as three other compounds whose R_f values lay between the origin and 4-methylimidazole. These minor components were isolated by preparative tlc and each was irradiated at 254 nm for 2 hr. No 4-methylimidazole was detected by tlc or by uv absorption spectrum in any of the samples.

Conversion of Cyanoacetaldehyde Dimer to 6-Aminonicotinonitrile. Duplicate samples of cyanoacetaldehyde dimer (5×10^{-5} M)²⁹ in 1 M NH₄OH were degassed with two freeze-pump-thaw cycles, irradiated in Pyrex and monitored spectrophotometrically. The disappearance of starting material (310 nm) and formation of product (263 nm) was complete after 12 days. Examination of the reaction mixture by paper chromatography in two solvent systems (BW and PA) showed that the R_f value of the major component matched that of an authentic sample of 6-aminonicotinonitrile. This spot was eluted from the paper and its uv spectrum determined: neutral, λ_{max} 263 nm; acid, 255 nm (lit.²⁹ for 6-aminonicotinonitrile; neutral, λ_{max} 263 nm; acid, 255 nm).

A control reaction was carried out in parallel with the above runs which was identical in all respects except that a 40° water bath was used in place of the ultraviolet light. Only a slight decrease in the absorption at 310 nm was observed.

Acknowledgments. We thank Professor R. Strong for the flash photolysis results and for assistance in their interpretation, Professor H. Richtol for use of his luminescence apparatus and for assistance in interpreting the data, and Professor S. Wait for the HMO program and for helpful discussions concerning the calculations.

An Enzyme Electrode for the Substrate Urea

G. G. Guilbault and J. G. Montalvo

*Contribution from the Department of Chemistry,
Louisiana State University in New Orleans, Lakefront Campus,
New Orleans, Louisiana 70122. Received August 13, 1969*

Abstract: The development of several types of a urea transducer suitable for rapid, continuous determination of urea is described. The transducer is called an enzyme electrode because it is made by placing a thin film of urease enzyme immobilized in acrylamide gel over the surface of a Beckman cationic electrode responsive to ammonium ions. The immobilized enzyme catalyzes the decomposition of urea to ammonium ion at the surface of the cationic electrode. The ammonium ion is sensed by the cation electrode; the steady-state potential developed is proportional to the logarithm of the urea concentration. The preparation of the various types of urease enzyme electrodes, the immobilization parameters that affect the response of the electrodes, factors that affect the stability of the immobilized enzyme, and the effect of foreign monovalent cations on electrode response are described. An electrode was made which could be used continuously at 25° for 14 days with no loss in activity. By placing a thin film of cellophane over the immobilized gel layer, an electrode could be used continuously at 25° for 3 weeks with no loss in activity.

Enzymes are finding increased use in analytical chemistry. Reviews on the use of these biological catalysts as analytical reagents have been published by Guilbault.^{1,2} One of the primary objections to the use

of enzymes in chemical analysis is the high cost of these materials. A continuous or semicontinuous routine assay using enzymes requires large amounts of these materials, quantities greater than can be reasonably supplied, and quantities that would represent a prohibitive expenditure in most instances. If, however,

(1) G. G. Guilbault, *Anal. Chem.*, **38**, 527R (1966).

(2) G. G. Guilbault, *ibid.*, **40**, 459R (1968).