

Electrochemical Reduction of 1,1'-Ethylenebis(3-carbamidopyridinium bromide)<sup>1</sup>

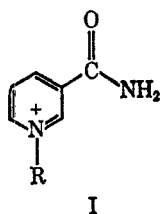
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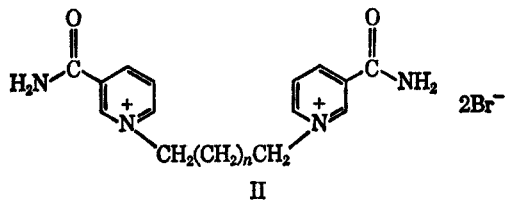
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1,1'-Ethylenebis(3-carbamidopyridinium bromide) undergoes a 2-electron reduction at a cathode of controlled potential to yield a product in which the two pyridine rings are linked by a new bond between the 6 positions. The original pyridinium salt is regenerated upon electrochemical oxidation at controlled anode potential, but partial oxidation with oxygen yields a stable, deep red free radical. The same radical is obtained in better yield by exhaustive oxidation with oxygen followed by a 1-electron electrochemical reduction. The esr spectrum of the radical is similar to that obtained upon reduction of 1,1'-ethylene-2,2'-dipyridylum dibromide ("Diquat"); differences between the spectra can be explained by the absence of carbamide groups in the "Diquat" case. Reasonable structures may be advanced for the various species on the basis of ultraviolet and esr spectra.

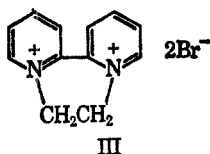
The electrochemical reduction of 1-substituted nicotinamide salts (I), including the biologically



important pyridine coenzymes di- and triphosphopyridine nucleotide, has been carefully studied in this laboratory.<sup>2-6</sup> Briefly, these compounds exhibit two reduction processes at well-separated potentials; the first represents free radical formation followed by dimerization, while the second leads to the two-electron reduction product, *viz.*, the substituted 1,4-dihydropyridine. Interest in biologically active pyridinium salts has led us to investigate the electrochemical reduction of several 1,1'-polymethylenebis(3-carbamidopyridinium bromides) (II), with particular emphasis



upon the ethylene compound (II,  $n = 0$ ). Interesting chemistry has developed during this study in the observation of quite stable free radicals. Parallel work on the herbicide 1,1'-ethylene-2,2'-dipyridylum



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(2) J. N. Burnett and A. L. Underwood, *J. Org. Chem.*, **30**, 1154 (1965).

(3) J. N. Burnett and A. L. Underwood, *Biochemistry*, **4**, 2060 (1965).

(4) A. J. Cunningham and A. L. Underwood, *Arch. Biochem. Biophys.*, **117**, 88 (1966).

(5) A. M. Wilson and D. G. Epple, *Biochemistry*, **5**, 3170 (1966).

(6) A. J. Cunningham and A. L. Underwood, *ibid.*, **6**, 266 (1967).

dibromide (known commercially as "Diquat") (III) has proved correlative and helpful.

## Experimental Section

**Materials.**—1,1'-Polymethylenebis(3-carbamidopyridinium bromides) (II, where  $n = 0, 1, 2,$  and  $3$ ) were prepared by a procedure similar to that of Hartwell and Pogorelskin<sup>7</sup> and of Hazard, *et al.*<sup>8</sup> The crude products were recrystallized twice from aqueous ethanol (1:1, v/v), washed with cold ethanol and then with ether, and dried *in vacuo* at 65°. Results of C, H, and N analyses<sup>9</sup> agreed well with calculated values. Melting points were as follows: II,  $n = 0$ , 307–309° dec; II,  $n = 1$ , 274–276° dec; II,  $n = 2$ , 287–288°; II,  $n = 3$ , 216–218°.

**Apparatus.**—Polarograms were recorded at ambient temperature (controlled at  $24 \pm 1^\circ$ ) with a Sargent Model XV polarograph, using a conventional H cell with a saturated calomel anode (*see*). Macroscale electrolyses were performed using a Wenking Model 61TRS potentiostat with a large mercury pool cathode, silver-silver chloride anode, and *see* reference in a special cell which was purged with prepurified nitrogen. The anolyte was separated from the main solution, with electrolytic contact through a sintered disk. The solution was stirred by a magnetic bar at the mercury-solution interface. The coulometer was similar to that of Wise<sup>10</sup> and Bard.<sup>11</sup> The apparatus for cyclic voltammetry was similar to that used before<sup>9</sup> except that Philbrick chopper-stabilized operational amplifiers replaced Heath units. Absorption spectra were recorded with a Perkin-Elmer Model 202 spectrophotometer. Esr spectra were obtained with a Varian Model 4500 X-band spectrometer using 100-kc modulation; the sample was held in a special quartz flat cell. A Kewaunee glove box with nitrogen atmosphere was used for all operations involving reduction products, except, as noted, where oxygen was intentionally introduced.

## Results

**Polarography.**—All of the compounds in the series (II,  $n = 0-3$ ) exhibited a reduction wave at  $-0.7$  to  $-1.0$  V *vs.* sce. Halfwave potentials were independent of pH over the range studied (5–9 in 0.1 M acetate, phosphate, and pyrophosphate buffers). The dependence of limiting currents upon corrected mercury heights indicated diffusion control. Assuming on the basis of controlled potential coulometry (*see* below) that the wave represented a two-electron process, plots of potential *vs.*  $\log i/(i_d - i)$  showed irreversibility in all cases. The pentamethylene compound (II,  $n = 3$ ) exhibited an adsorption-controlled, concentration-

(7) J. L. Hartwell and M. A. Pogorelskin, *J. Amer. Chem. Soc.*, **72**, 2040 (1950).

(8) R. Hazard, J. Cheymol, J. A. Gautier, E. Cortegiani, and E. Leroi, *Arch. Int. Pharmacodyn. Ther.*, **90**, 271 (1952).

(9) Midwest Microlab Inc., Indianapolis, Ind.

(10) E. N. Wise, *Anal. Chem.*, **34**, 1181 (1962).

(11) A. J. Bard and E. Solon, *ibid.*, **34**, 1181 (1962).

TABLE I  
 POLAROGRAPHIC RESULTS

II, <i>n</i>	pH	Buffer	$E_{1/2}$ , V vs. sce	Diffusion current constant $I = i_d / (Cm^{2/3}t^{1/6})$
0	5.1	Acetate	-0.83	3.57
	7.0	Phosphate	-0.82	2.98
	9.0	Pyrophosphate	-0.82	2.69
1	5.1	Acetate	-0.78	2.92
	7.0	Phosphate	-0.78	2.86
	9.0	Pyrophosphate	-0.78	2.85
2	6.0	Acetate	-1.00	3.02
	7.0	Phosphate	-0.98	2.98
	9.0	Pyrophosphate	-0.98	2.81
3	6.0	Acetate	-1.0 <sup>a</sup>	2.90 <sup>b</sup>
	7.0	Phosphate	-1.0 <sup>a</sup>	3.20
	9.0	Pyrophosphate	-1.0 <sup>a</sup>	2.55

<sup>a</sup> Approximate; "prewave" at ca. -0.82 V vs. sce. <sup>b</sup> Based upon total wave height, i.e., "prewave" plus "normal" wave.

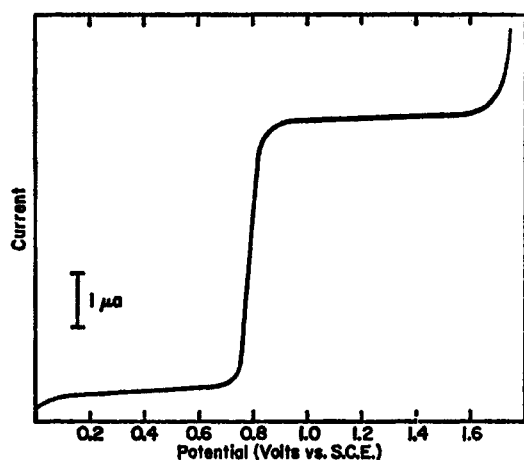


Figure 1.—Typical polarogram [ $1.0 \times 10^{-3}$  M 1,1'-ethylenebis(3-carbamidopyridinium bromide) in 0.1 M pyrophosphate buffer of pH 9.0].

independent "prewave." Polarographic data are summarized in Table I; Figure 1 shows a typical polarogram. At pH values above 8, an additional, poorly defined reduction process was seen near the breakdown of the background electrolyte solution. Severe maxima were present on this wave which did not yield to the usual suppressors, currents were irregular and nonreproducible, and coulometric values were scattered. The appearance and behavior of this wave suggested that it represented catalytic hydrogen evolution.

**Cyclic Voltammetry.**—Typically, solutions of the ethylene compound (II,  $n = 0$ ) exhibited two cathodic and two anodic peaks in cyclic scans. The less negative cathodic peak and the less positive anodic peak probably represented adsorption phenomena; these peaks disappeared upon the addition of the surfactant Triton X-100 (a common polarographic maximum suppressor) at a level of 0.004%, leaving one cathodic peak at -0.80 V and one anodic peak at -0.15 V vs. sce when the sweep rate was 1.0 V/sec in both directions. Linear graphs were obtained upon plotting peak current vs. concentration in the range  $1 \times 10^{-4}$  to  $1 \times 10^{-3}$  M at constant sweep rate, and peak current vs. the square root of the sweep rate from 0.05 to 1.0 V/sec at constant concentration. Results were essentially the same at pH values of 5, 7, and 9. The behavior of the other

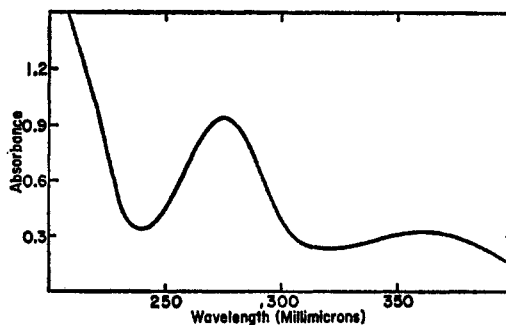
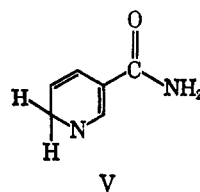


Figure 2.—Ultraviolet spectrum of initial reduction product [46.5 mg of 1,1'-ethylenebis(3-carbamidopyridinium bromide) in 0.1 M phosphate buffer of pH 7.2 electrolyzed at -1.0 V vs. sce; solution diluted tenfold to obtain the spectrum].

compounds in the series (II,  $n = 1, 2$ , and 3) was similar; these were not studied in detail. The large separation of cathodic and anodic peaks indicates, of course, a high degree of irreversibility for the observed process, the results essentially confirming the polarography. Reversible reoxidation of an intermediate radical was not seen at high sweep rates as it was with the nicotinamides.<sup>6</sup>

**Controlled Potential Electrolysis.**—Macroscale electrolyses were generally performed on 100–250-mg samples of the compounds in 200 ml of 0.1 M buffers of pH 5 (acetate), 7 (phosphate), and 9 (pyrophosphate) at potentials well on the plateaus of the polarographic waves and at least 0.2 V more positive than electrolyte breakdown (-1.0 V vs. sce for II,  $n = 0$  and 1; -1.20 V for II,  $n = 2$  and 3). Coulometry in all cases yielded a value of two electrons taken up per molecule of starting material.

The original solutions of the pyridinium salts all exhibited an absorption band at 266–267 mμ. Upon electrolysis to the completion of the two-electron reduction, the ethylene compound (II,  $n = 0$ ) yielded a two-banded spectrum, with absorption maxima at 275 mμ ( $\log \epsilon$  4.24) and 350 (3.77). The spectrum is shown in Figure 2 because it is important in establishing the structure of the reduction product. On the basis of previous work with the nicotinamides,<sup>2,3</sup> it was expected that reduction product IV (see Scheme I) would form, analogous to the nicotinamide dimers, with the preexisting ethylene bridge between the pyridine rings favoring the formation of the new bond at the 6 and 6' positions. The ultraviolet spectrum suggested that the reduction product was indeed IV. According to Wallenfels and Schüly,<sup>12</sup> as well as Schenker and Druey,<sup>13</sup> among the three isomeric dihydropyridines formed by the nicotinamides, 1,6-dihydro compound V is characterized by a two-banded

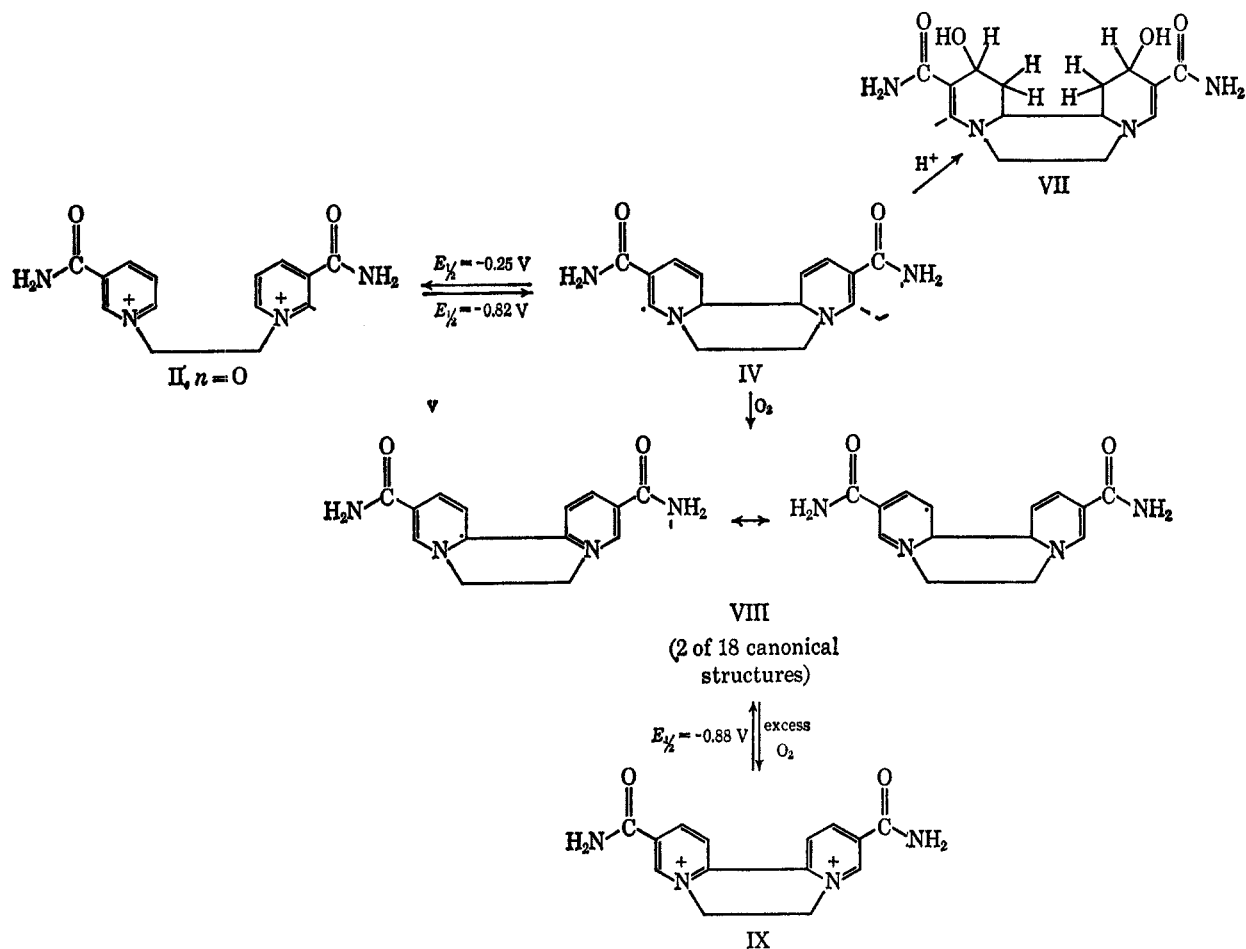


ultraviolet spectrum with absorption maxima in the

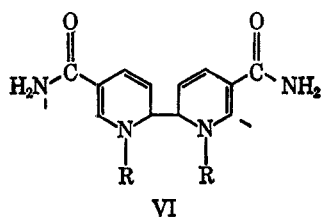
(12) K. Wallenfels and H. Schüly, *Angew. Chem.*, **67**, 517 (1955).

(13) K. Schenker and J. Druey, *Helv. Chim. Acta*, **42**, 1960 (1959).

SCHEME I



vicinity of 270 and 360  $m\mu$ . On the basis of their ultraviolet spectra, Wallenfels and Gellrich<sup>14</sup> assigned the 6,6' structure (VI) to dimers prepared by zinc

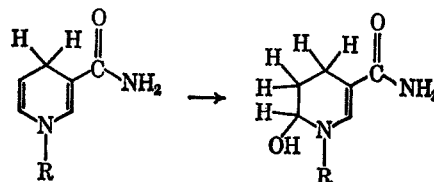


reduction of 1-substituted nicotinamides. The spectral data given<sup>14</sup> for VI where R = *n*-propyl were  $\lambda_{max}$  276  $m\mu$  ( $\log \epsilon$  3.59), 357 (3.92).

The other compounds in the series (II,  $n = 1, 2,$  and 3) behaved somewhat differently. To be sure, ultraviolet bands at  $\sim 276$  and 350  $m\mu$  appeared in the early stages of electrolysis, but during the time required to ensure complete reduction (about 1.5 hr) these bands were replaced by a new band at 294 (II,  $n = 1$ ) or 297  $m\mu$  (II,  $n = 2$  and 3). This new band appeared most rapidly at pH 5 and most slowly at pH 9, and the rate of its appearance increased as  $n$  increased in II. (The ethylene compound (II,  $n = 0$ ) yielded the same band in a reduction at pH 4, but at pH 5 or above it behaved as described in the previous paragraph.)

Numerous examples may be found in the literature

of absorption in the 290–300- $m\mu$  region associated with the chromophore  $>NC=CC=O$ . For example, the acid-catalyzed addition of water to 1,4-dihydropyridine derivatives, shown below, leads to such a band.<sup>15</sup> This reaction is favored by an acidic medium,



but occurs in neutral solution as well in the presence of certain anions including  $H_2PO_4^-$  which act catalytically.<sup>15,16</sup> The reaction is presumably initiated by protonation at the 5 position of the 1,4-dihydropyridine, reflecting the nucleophilicity of an enamine system. An acceptable interpretation of the results reported here is a similar addition of water to the 4,5-double bonds, involving protonation at the 5 positions. Products such as VII in Scheme I would be formed.

After electrolysis at a potential on the cathodic wave as described above, the solution of the ethylene compound (II,  $n = 0$ ) exhibited an anodic wave with a halfwave potential of  $-0.25$  V *vs.* sce. Electrolysis on this wave removed two electrons (the experimentally

(15) *E.g.*, see C. C. Johnston, J. L. Gardner, C. H. Suelter, and D. E. Metzler, *Biochemistry*, **2**, 689 (1963), and literature cited therein.

(16) S. G. A. Alivisatos, F. Ungar, and G. J. Abraham, *ibid.*, **4**, 2616 (1965).

(14) K. Wallenfels and M. Gellrich, *Ber.*, **92**, 1406 (1959).

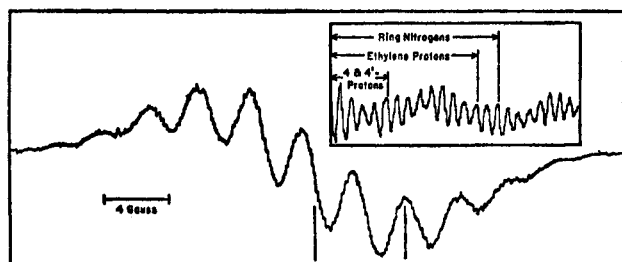


Figure 3.—Recorder trace of the 11-line esr spectrum of radical VIII prepared by electrochemical reduction of IX. Insert shows the portion of the spectrum between the vertical lines under conditions for higher resolution; assigned splittings are indicated.

measured value was 1.8) per initial molecule of II; the ultraviolet spectrum after this oxidation was identical with that of the original pyridinium salt.

**Esr Experiments.**—The above results were obtained under an atmosphere of nitrogen. If, after electrolysis on the cathodic wave at pH 5, 7, or 9, a little air or oxygen was introduced into the yellow reduced solution of the ethylene compound (II,  $n = 0$ ), a deep red color developed, and a new absorption band appeared in the visible region ( $545 \text{ m}\mu$ ). No photochemical phenomenon was involved; identical results were obtained under daytime lighting and in the dark. The red solutions yielded significant esr signals, although experiments at several concentrations failed to give complete resolution of the hyperfine structure. Eleven main lines could be discerned, with splittings of  $3.0 \pm 0.3 \text{ G}$  between peaks. The  $g$  factor of all observed spectra was measured and found to be  $2.004 \pm 0.002$ .

On the other hand, if the electrolytically reduced solution of the ethylene compound was allowed to stand for 30 min while saturated with oxygen, oxidation beyond the red free radical stage occurred to yield another compound. This oxidized solution, after nitrogen purging, exhibited a cathodic wave at  $-0.88 \text{ V vs. sce}$ . Coulometry during macroelectrolysis at a potential on this wave ( $-1.1 \text{ V vs. sce}$ ) indicated an uptake of one electron, and the resulting solution was deep red. The esr spectrum of this solution exhibited the same eleven main lines as above, but the signal was stronger and the resolution better. Typical recorder traces of the spectra are shown in Figure 3. The visible spectrum of the red solution showed a band at the same wavelength as before.

A detailed study of the electrochemistry of "Diquat" (III) and the esr spectrum of the electrogenerated "Diquat" radical will be reported separately. Briefly, "Diquat" exhibited (as others had reported<sup>17-20</sup>) a reversible, one-electron reduction ( $E_{1/2} = -0.62 \text{ V vs. sce}$ ) to yield a very stable free radical. A solution of this radical, conveniently prepared by macroelectrolysis at  $-0.75 \text{ V vs. sce}$ , yielded an excellent spectrum consisting of 133 lines. Reasonable assumptions regarding the relative magnitudes of hyperfine splittings by the nitrogen atoms and the several protons, and

consideration of published interpretations of the spectra of 2,2'-bitolyl and 2,2'-bipyridyl anion radicals, led to the assignment of hyperfine splitting constants enabling calculation of a "Diquat" radical spectrum which fitted well to the experimental one.

The esr spectrum of the "Diquat" radical and that of the radical described above were similar. The large splittings by the ring nitrogens and the ethylene protons were clearly comparable in the two spectra. However, the splitting assigned to the protons at the 5 positions in the "Diquat" radical could not be observed in the spectrum of the radical described above, as would be expected when carbamide groups replace the single protons. Thus with guidance from the analysis of the well-resolved "Diquat" radical spectrum and measurements from spectra such as shown in Figure 3, hyperfine splitting constants for the radical reported here were assigned as follows: ring nitrogens, 3.56 G; ethylene protons, 2.80 G; protons at the 4 positions of the pyridine rings, 1.20 G; protons at the 3 and 6 positions, 0.20 G. Splittings due to the carbamide groups could not be assigned from the observed spectra. The additional lines due to these groups are thought to be responsible for the poorer resolution of the spectrum of the radical reported here as compared with that of the "Diquat" radical.

The magnetic field was scanned through 100 G on each side of the center line of the spectrum. Only the basic spectrum mentioned above, with eleven main lines, was ever observed. This permits the elimination of a possible diradical; a triplet would be expected to give rise to a signal in the "wings," and such was not seen in the present case.

## Discussion

All of the evidence is consistent with Scheme I. It would be difficult, in the light of previous work on dimer formation with the nicotinamides, to envision a two-electron reduction leading to any product other than IV. Furthermore, the ultraviolet spectrum of the reduction product is consistent with this formulation in terms of Wallenfels' generally accepted work on the spectra of the isomeric dihydropyridines and nicotinamide dimers. The esr spectrum of the radical obtained by partial oxidation of IV provides convincing evidence for formulation VIII in the scheme. It is plausible that a stronger esr signal resulted from complete oxidation to IX followed by the one-electron electrochemical reduction; production of the radical from IV by  $\text{O}_2$  doubtless overshoot considerably, forming IX as well as VIII, whereas at controlled cathode potential only one electron was added to IX. It is interesting although not surprising that the electrochemical reoxidation of IV led to a different product (II) than did oxidation with  $\text{O}_2$  (VIII and IX).

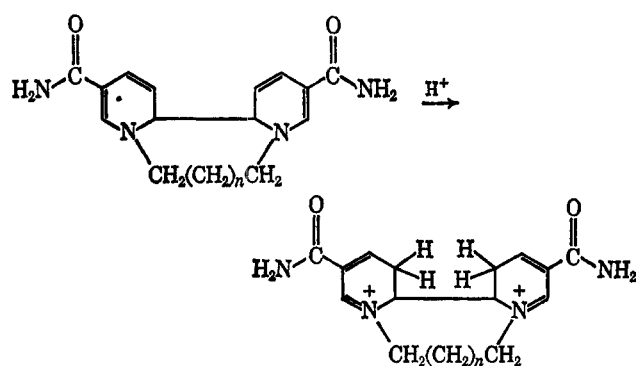
The heightened tendency to form products like VII as  $n$  is increased in II may be rationalized. The intuitive idea that increasing "flexibility" of the ring system may sterically facilitate attack at the two 5 positions is supported by the observation of models. Further, it may be argued that the relative reactivities of the compounds as  $n$  is increased could reflect the stabilities of the respective iminium salts generated by protonation. These stabilities could in turn be deter-

(17) R. F. Homer, G. C. Mees, and T. E. Tomlinson, *J. Sci. Food Agr.*, **11**, 209 (1960).

(18) R. F. Homer and T. E. Tomlinson, *Nature*, **184**, 20121 (1959).

(19) J. Engelhardt and W. P. McKinley, *J. Agr. Food Chem.*, **14**, 377 (1966).

(20) W. R. Boon, *Chem. Ind. (London)*, **752**, (1965).



mined by differences in energies of double bonds exocyclic to rings of various sizes.

Workers in the field of pyridine chemistry will recognize the difficulties in the classical approach of isolating and characterizing compounds of intermediate

oxidation state such as described here. Physical evidence like ultraviolet and esr spectra must be relied upon heavily. Obviously, the most convincing test of the present scheme would involve the unequivocal synthesis of the carbamido-substituted "Diquat" IX and a study of the radical obtained upon its reduction. Unhappily, attempts to prepare IX by a synthetic route independent of the work reported here have so far been unsuccessful.

**Registry No.**—II,  $n = 0$ , 19293-83-5; II,  $n = 1$ , 19293-84-6; I,  $n = 2$ , 19293-85-7; II,  $n = 3$ , 19293-86-8.

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## Application of the Hammett Equation to Nonaromatic Unsaturated Systems. VII. Heterovinylene Sets

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Ionization constants for 10 heterovinylene sets have been correlated with the extended Hammett equation  $Q_X = \alpha\sigma_{I,X} + \beta\sigma_{R,X} + h$ . Sets studied include disubstituted oximes, disubstituted glyoximes, *syn*- and *anti*-substituted aldoximes, substituted acetyl oximes, substituted methyl oximes, *syn*- and *anti*-substituted phenyl oximes, substituted phenylimines, and substituted ethoxyimines. Good results were generally obtained. The magnitude of  $\alpha$  is dependent upon molecular geometry. Assignment of configuration by means of correlation with the Hammett equation is discussed.

In previous papers of this series we have considered the application of the Hammett equation<sup>1</sup> to *trans*-<sup>2</sup> and *cis*-vinylene,<sup>3</sup> vinylidene,<sup>4</sup> and heterovinylidene<sup>4</sup> sets. Our purpose in this paper is to extend these studies to include *cis*- and *trans*-heterovinylene sets. Two types of heterovinylene sets have been investigated: (1) oximes, in which the reaction site is the hydroxyl group bonded to the nitrogen atom; (2) imines, in which the reaction site is a full nonbonding orbital on the nitrogen atom.

Ionization constants for oximes and imines taken from the literature have been correlated with the extended Hammett equation

$$Q_X = \alpha\sigma_{I,X} + \beta\sigma_{R,X} + h \quad (1)$$

by multiple linear regression analysis. The data used in the correlations are given in Table I. The  $\sigma_I$  constants were taken from our collection;<sup>5</sup> the  $\sigma_R$

constants were obtained from

$$\sigma_R = \sigma_p - \sigma_I \quad (2)$$

The  $\sigma_p$  constants were from the compilation of McDaniel and Brown.<sup>6</sup> In some cases values of  $\sigma$  were taken from previous papers in this series or were estimated by our method.<sup>7</sup>

The justification for the correlation of the ionization constants (as  $pK_a$  values) of the disubstituted oximes (set 1) with eq 1 is as follows. The effect of the substituent may be written

$$Q_X = \alpha_{syn}\sigma_{I,X^1} + \alpha_{anti}\sigma_{I,X^2} + \beta_{syn}\sigma_{R,X^1} + \beta_{anti}\sigma_{R,X^2} + h \quad (3)$$

As  $X^1 \equiv X^2$ , eq 3 becomes

$$Q_X = (\alpha_{syn} + \alpha_{anti})\sigma_{I,X} + (\beta_{syn} + \beta_{anti})\sigma_{R,X} \quad (4)$$

equivalent to eq 1 with

$$\alpha = \alpha_{syn} + \alpha_{anti}, \quad \beta = \beta_{syn} + \beta_{anti}$$

The correlation of the *anti*-disubstituted glyoximes II with eq 1 may be justified in the same manner. The

(1) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953); R. W. Taft, Jr., "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley & Sons, Inc., New York, N. Y. 1956, p 565; V. Palm, *Russ. Chem. Rev. (Eng. Transl.)* **31**, 471 (1961); P. R. Wells, *Chem. Rev.*, **63**, 171 (1963).

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(3) M. Charton, *ibid.*, **30**, 974 (1965).

(4) M. Charton, *ibid.*, **30**, 557 (1965).

(5) M. Charton, *ibid.*, **29**, 1222 (1964).

(6) D. H. McDaniel and H. C. Brown, *ibid.*, **23**, 420 (1958).

(7) M. Charton, *ibid.*, **28**, 3121 (1963).