After drying (magnesium sulfate) and evaporation of the solvent there were obtained 400 mg. of crystalline material which appeared to consist (t.l.c.) of one major component and traces of a second compound. The crude product was chromatographed on basic alumina (20 g.). Elution with hexane–benzene (9:1) gave 8 mg. of crystalline material, m.p. 230°, which on the basis of comparative thin layer chromatography appears to be the diastereoisomeric dimer VIII. Elution with hexane–benzene (4:1) gave 342 mg. (80%) of crystals, m.p. 112° (lit.[9] m.p. 111–112° for ferrocenylphenylcarbinyl methyl ether). Further elution with benzene (50 ml.) gave 42 mg. of crystalline material whose infrared spectrum showed it to be benzoylferrocene.

Electrochemical Reduction of 1-Methyl-3-carbamidopyridinium Chloride[1a]

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1-Methyl-3-carbamidopyridinium chloride exhibits one polarographic wave at pH values below 7 ($E_{1/2} = -1.08$ v. *vs.* s.c.e. independent of pH), while a second, pH-dependent wave appears in basic solution ($E_{1/2} = -1.68$ to -1.78 v. *vs.* s.c.e.). The limiting currents for both waves are diffusion controlled. Coulometry during macroelectrolysis with controlled cathode potential on the second wave gives a value of two electrons for the reduction process. The product is 1-methyl-3-carbamido-1,4-dihydropyridine, proved by comparing ultraviolet and infrared spectra with those of authentic material. Electrolysis at a potential on the first wave involves the uptake of one electron and leads to the formation of a dimeric product. Its ultraviolet spectrum and other evidence strongly suggest that dimerization is at the 6-position. The dimer does not function as an intermediate in the formation of the 1,4-dihydro compound but is further reducible to a product which is still dimeric.

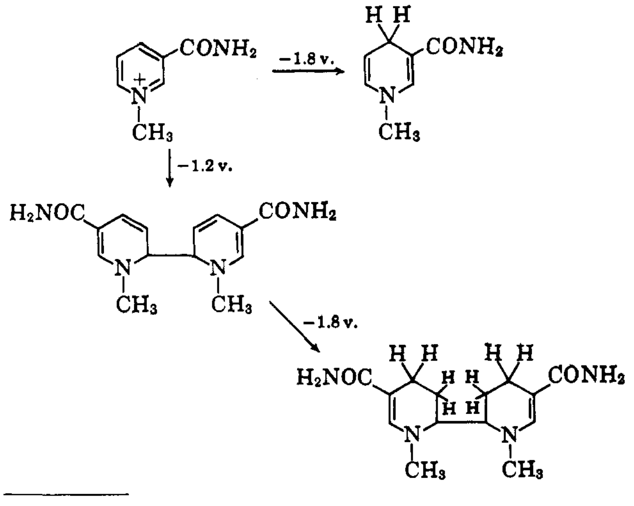
Several workers have attempted with varying success to prepare reduced nicotinamide adenine dinucleotide (NADH, DPNH) by electrolytic reduction of NAD at a cathode of controlled potential.[2–7] The reduction products varied in their coenzyme activity; the worst preparations were totally inactive,[3,4] while the best exhibited only 65–76% of the expected NADH activity.[7] Because electrode potentials may be precisely controlled and because no excess reducing agent is introduced, superior preparations were anticipated electrochemically; thus, even the best results were disappointing. It is apparent that a reduction product (or products) other than biologically active NADH may form in varying amount depending upon conditions, but neither its nature nor the manner of its formation has been elucidated. It is desirable to clarify the literature in this regard, if not indeed to circumvent the factors preventing exclusive reduction to NADH, by studying in detail the electrochemical behavior of NAD.

Although the remainder of the molecule is important to its biological activity, the reduction of NAD to NADH involves only the nicotinamide moiety. Thus it is reasonable to begin an electrochemical study of NAD by investigating the reduction of simple 1-alkylnicotinamides. The chemical reduction of NAD was elucidated in a similar fashion, beginning with Karrer's study of the dithionite reduction of the model compound 1-methyl-3-carbamidopyridinium chloride.[8]

Results

Polarography.—One reduction wave is exhibited by 1-Methyl-3-carbamidopyridinium chloride at *ca.* -1.1 v.[9] in the pH range of 4–7, while in more alkaline solutions a second wave at -1.7 to -1.8 v. is seen in addition to the first one. The two waves are of about equal height. The first is independent of pH, while the half-wave potential for the second is shifted about -27 mv. per pH unit, not far from the expected value (59/2) for a 2-electron process involving one H^+. The heights of both waves vary directly with the square root of the mercury height, indicating that the limiting currents for both processes are diffusion controlled. Both reduction processes are irreversible under polarographic conditions. In strongly basic solution (*e.g.*, pH 13), the 1-methyl-3-carbamidopyridinium ion is quite rapidly altered, and the two waves give way to one wave at -1.5 v. as the solution stands. Catalytic hydrogen waves and adsorption waves, which frequently distort the polarograms of nitrogen heterocycles, are not encountered in the present case. The polarographic data are summarized in Table I.

Macroelectrolyses.—The electrochemical reduction processes of 1-methyl-3-carbamidopyridinium chloride are formulated as follows on the basis of large-scale



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[1] (a) Presented at the Southeastern Regional Meeting of the American Chemical Society, Charlotte, N. C., Nov. 1963. (b) Taken from a thesis presented by J. N. Burnett in partial fulfillment of the requirements for the M.S. degree, Emory University, 1963.

[2] B. Ke, *Biochim. Biophys. Acta*, **20**, 547 (1956).

[3] B. Ke, *J. Am. Chem. Soc.*, **78**, 3649 (1956).

[4] B. Ke, *Arch. Biochem. Biophys.*, **60**, 505 (1956).

[5] R. F. Powning and C. C. Kratzing, *ibid.*, **66**, 249 (1957).

[6] T. Kono, *Bull. Agr. Chem. Soc. Japan*, **21**, 115 (1957).

[7] T. Kono and S. Nakamura, *ibid.*, **22**, 399 (1958).

[8] P. Karrer, G. Schwarzenbach, F. Benz, and U. Solmssen, *Helv. Chim. Acta*. **19**, 811 (1936).

[9] All potentials mentioned in this paper are referred to an aqueous saturated calomel electrode (s.c.e.).

TABLE I

HALF-WAVE POTENTIALS AND WAVE HEIGHTS FOR 1-METHYL-3-CARBAMIDOPYRIDINIUM CHLORIDE[a]

		First wave		Second wave	
pH	Buffer	$E_{1/2}$	i_d/c	$E_{1/2}$	i_d/c
4	Acetate	−1.11	4.45	...	...
5	Acetate	−1.11	3.69	...	...
6	Citrate	−1.12	3.27	...	...
6	Phosphate	−1.10	3.27	...	...
7	Phosphate	−1.11	3.27	...	...
7	Tris	−1.11	3.46	...	...
7.5	Pyrophosphate	−1.11	3.27	−1.68	3.40
8	Phosphate	−1.11	3.18	−1.70	3.44
8.8	Pyrophosphate	−1.11	3.24	−1.70	3.18
9	Tris	−1.12	3.51	...	...
9	Phosphate	−1.10	3.68	−1.72	3.60
9.9	Ammonia	−1.09	3.76	−1.78	3.68
13	NaOH	(−1.12)[b]	(2.22)[b]	(−1.84)[b]	(1.20)[b]
		Mean[c]:	3.42 ± 0.12		3.46 ± 0.25

[a] 5×10^{-4} *M*. [b] Solutions are unstable; after 2 hr., both waves have been replaced by one wave at −1.52 v. [c] Values for pH 4 and 13 were omitted in calculating the confidence interval of the mean.

controlled-potential reductions and of concomitant data.

Reduction at a cathode potential on the second wave clearly leads to 1-methyl-3-carbamido-1,4-dihydropyridine. Coulometry shows an uptake of 2 electrons per molecule. The product isolated from the electrolysis solution exhibits the 360-mμ absorption band characteristic of the 1,4-dihydro compound, and its infrared spectrum is identical in every detail with that of authentic 1,4-dihydro compound prepared by dithionite reduction.[10]

At a cathode potential on the first wave, 1 electron is taken up per molecule of starting material, and a substance with the molecular weight of a dimer can be isolated from the solution. The product exhibits an absorption band at 298 mμ. In our opinion, dimerization occurs at the 6-position, as shown in the above scheme (see discussion below). After the electrolysis on the first wave is complete, the solution exhibits a polarographic wave with $E_{1/2}$ = −1.65 to −1.70 v., depending upon the pH. (The pH dependence is about −14 mv./pH unit.) This potential is near but not identical with the value for the second wave of the starting material. Electrolysis on this wave after prior electrolysis on the first of the two original waves leads to an uptake of 2 additional electrons per molecule of original pyridinium compound. The absorption band at about 298 mμ is still seen, and no new bands appear.

These facts clearly show that the dimeric 1-electron reduction product does not reduce to the 1,4-dihydro monomer, as this would require only 1 additional electron and also would result in a spectral shift. Thus, the dimer is reducible in its own right, but it is incapable of functioning as an intermediate in the formation of 1,4-dihydro monomer. In our view (see Discussion below), this reduction involves the double bond in the 4,5-position which is cross conjugated with the chromophore O=C—C=C—N, as shown in the scheme above.

Catalytic Hydrogenation.—A catalytic hydrogenation experiment is of interest in this connection. A solution of the dimer prepared electrolytically was subjected to hydrogenation with a palladium catalyst. Two overlapping stages of reduction were clearly observed by monitoring the hydrogen uptake and the ultraviolet spectrum during the reduction. In one experiment, 1.6 moles of hydrogen was consumed/mole of dimer in 5 hr.; no change in λ_{max} had occurred, but the 298-mμ band had decreased by about 15%. After 59 hr., the absorbance at 298 mμ had decreased by 25% and the hydrogen uptake was appropriate for complete reduction of two double bonds and 25% completion in the reduction of two more, per molecule of dimer. This experiment is at least compatible with the idea that the double bond in the 4,5-position is reduced fairly rapidly with no change in ultraviolet spectrum while simultaneously but much more slowly another double bond is reduced with the destruction of the O=C—C=C—N chromophore.

Discussion

Polarographic studies of 1-methyl-3-carbamidopyridinium salts have been reported by several other workers. $E_{1/2}$ values found in this study are in reasonable agreement with the literature values[11-15] except for those of Leach, *et al.*, which were about 0.1 v. more positive.[16] The wave at −1.4 to −1.5 v. attributed to nicotinic acid formed by alkaline hydrolysis of the amide has been described by others.[14,17]

Leach, *et al.*,[16] reported macroreductions of 1-methyl-3-carbamidopyridinium salts at −1.7 to −1.8 v. using manual potential control. Current efficiencies of only 50 to 80%, depending upon concentration, were reported, and it was suggested that a dimerization process competed with the 2-electron reduction. These workers electrolyzed, however, for only 1 hr., and their currents at the termination of the electrolyses were much larger than ours. Our electrolyses required 2 or 3 hr. for a 2×10^{-3} *M* solution, and as much as 9 hr. for a 1.5×10^{-2} *M* solution. By prolonging the electrolysis sufficiently, we were able to account coulometrically for all of the starting material in terms of exclusive reduction to either dimer or 1,4-dihydro compound, depending upon the potential.[18]

Paiss and Stein[19] described electrolysis experiments with 1-methyl-3-carbamidopyridinium salts, but their results are very difficult to correlate with the results reported here. Although the electrolyses were per-

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[10] The dithionite reduction product was thought by Karrer and his co-workers for many years to be the 1,2-dihydro compound, but there is now no doubt that it is the 1,4-dihydropyridine. See, *e.g.*, G. W. Rafter and S. P. Colowick, *J. Biol. Chem.*, **209**, 773 (1954); D. Mauzerall and F. H. Westheimer, *J. Am. Chem. Soc.*, **77**, 2261 (1955); R. F. Hutton and F. H. Westheimer, *Tetrahedron*, **3**, 73 (1958); H. E. Dubb, *et al.*, *J. Am. Chem. Soc.*, **80**, 1767 (1958).

[11] J. Nakaya, *Nippon Kagaku Zasshi*, **81**, 1459 (1960).

[12] W. Ciusa, P. M. Strocchi, and G. Adamo, *Gazz. chim. ital.*, **80**, 604 (1950).

[13] H. Yasuda and S. Kitagawa, *Yagukaku Kenkyu*, **27**, 779 (1955).

[14] P. C. Tompkins and C. L. A. Schmidt, *Univ. Calif.* (Berkeley) *Publ. Physiol.*, **8**, 237 (1943).

[15] F. Šorm and Z. Šormova, *Chem. Listy*, **42**, 82 (1948).

[16] S. J. Leach, J. H. Baxendale, and M. G. Evans, *Australian J. Chem.*, **6**, 395 (1953).

[17] A. G. Anderson and G. Berkelhammer, *J. Org. Chem.*, **23**, 1109 (1958).

[18] During the review of this paper, it was suggested that the differences between the results of Leach, *et al.*, and the present findings may have resulted from differences in stirring rates. However, we have found no tendency of stirring rate to influence the *course* of the reduction, only its *rate*.

[19] Y. Paiss and G. Stein, *J. Chem. Soc.*, 2905 (1958).

formed at constant current, the cathode potential was measured with respect to a calomel reference electrode, and the reductions were terminated when it reached −1.1 v. At this potential, which is about the same as $E_{1/2}$ for the first, 1-electron reduction step, a dihydro compound was formed, according to Paiss and Stein, which was designated "dihydro-compound B" and postulated to be 1-methyl-3-carbamido-1,2-dihydropyridine. By electrolysis of 1-*n*-propyl-3-carbamidopyridinium iodide, Paiss and Stein obtained a product again designated "dihydro-compound B" when the solution was 2×10^{-3} *M* in starting material; when the concentration was increased to 3×10^{-2} *M*, the same dihydro compound was found in the electrolyzed solution, but, in addition, crystals representing about 15% of the starting material and identified as a dimeric 1-electron reduction product appeared during the electrolysis.

On the other hand, preliminary studies in this laboratory indicate that 1-*n*-propyl-3-carbamidopyridinium salts (and NAD itself) are electrochemically similar to the 1-methyl compound, except that dimerization occurs at the 4-position rather than at the 2-position and that the formation of 1,4-dihydro compound is very much slower owing to strong adsorption effects at the cathode surface. In particular, only 1-electron reductions leading exclusively to dimeric products have been observed with any of these compounds at −1.2 v. at any concentration including those employed by Paiss and Stein.

Thus it is difficult to explain Paiss and Stein's results. The catholyte composition, including the pH, is not given in their paper. Considering the large current employed (1 amp.), it is possible that generation of hydrogen at the cathode may have led to reductions of a different sort than ours. The compositions of the possible compounds involved are such that the reported carbon and hydrogen analyses are not definitive. It may be noted that the ultraviolet spectrum of Paiss and Stein's "dihydro-compound B" is considerably different from that of a 1-alkyl-3-carbamido-1,2-dihydropyridine as assigned by Wallenfels.[20] The ultraviolet spectrum shown for the Paiss and Stein dimer of the 1-*n*-propyl compound is very similar to spectra obtained in this laboratory for dimers of that compound and of NAD, and their interpretation of this spectrum as suggesting dimerization at the 4-position is correct in our view. We are unable to explain, however, their low yield of the dimer and especially the formation of a dihydro compound at the potential employed.

It may be supposed that the dimer produced at −1.2 v. results from the combination of free radicals. In electro reductions of 1-alkylpyridinium salts with electron-withdrawing groups at the 4-position, Schwarz, *et al.*,[21] observed the generation of e.p.r.-detectable free radicals. Such radicals have not been observed in the present study. No e.p.r. signal was obtained in several electrolyses at −1.2 v. with a specially designed electrolysis cell in the microwave cavity. It is possible to rationalize on resonance considerations a greater stability for 4- than for 3-carbamidopyridinyl radicals. In any case, failure to detect the radicals does not establish their nonexistence.

The identity of the infrared and ultraviolet spectra of our 2-electron reduction product with those of the dithionite product, together with the coulometry, leaves no doubt that the 1,4-dihydropyridine is obtained quantitatively at 100% current efficiency from 1-methyl-3-carbamidopyridinium chloride. (In fact, this is a "cleaner" preparation than dithionite reduction because the solution is not contaminated with various sulfur compounds.) Likewise, the formation of a dimer in 100% yield cannot be questioned, as a result of the coulometry, the actual molecular weight determination on the isolated material, and the presence of a single ultraviolet absorption band. Much of the confusion in the literature regarding the electrochemical formation of these compounds can probably be traced to inadequate cathode potential control, failure to electrolyze for a sufficient length of time, lack of coulometric data, and failure to isolate reduction products.

Regarding the formulation of the dimer, in our opinion the predominance of the evidence favors dimerization at the 6-position. It may be noted that this is the same dimer postulated by Leach, *et al.*[16] Dimerization at the 4-position would lead to essentially the same chromophore as that of a 1-alkyl-3-carbamido-1,4-dihydropyridine; such compounds generally exhibit a single absorption band near 350 mμ.[22] A 1,2-dihydropyridine would be expected to absorb at an even longer wave length because of the additional double bond in the conjugated system, and indeed Wallenfels has presented evidence for a band near 400 mμ with one such compound.[20] Dimerization at the 2-position, then, is clearly incompatible with an absorption band at 298 mμ.

Many compounds may be found in the literature in which the chromophore O=C—C=C—N gives rise to absorption in the region of 290–310 mμ, and some examples which are pertinent in the present connection may be considered here. Karrer has presented evidence that hydrogen reduction using Raney nickel converted a cyano adduct of a 1-methyl-3-carbamidopyridinium salt into 1-methyl-3-carbamido-1,4,5,6-tetrahydropyridine (I); the product exhibited one absorption maximum at 295 mμ.[23] Burton and Kaplan have prepared II, III, and IV and discussed their spectra.[24] The dimer structure postulated here has a double bond in the 4,5-position which is cross conjugated with the above chromophore. An example from the work of Bowden, *et al.*, may be cited as a precedent for only a slight spectral shift caused by cross conjugation with this chromophore (V and VI).[25]

If it be agreed that cross conjugation in the present case would cause little shift in the ultraviolet absorption by the basic chromophore, then the dimer formulation proposed here is reasonable, and the reduction of the dimer without spectral shift may be interpreted as reduction of the double bond in the 4,5-position. The coulometry is correct for this reduction, and the cata-

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[20] K. Wallenfels and H. Schüly, *Ann.*, **621**, 215 (1959).

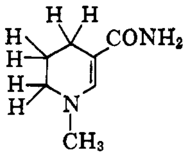
[21] W. M. Schwarz, E. M. Kosower, and I. Shain, *J. Am. Chem. Soc.*, **83**, 3164 (1961); see also E. M. Kosower and E. J. Poziomek, *ibid.*, **84**, 2035 (1963).

[22] K. Wallenfels and Schüly, *Ann.*, **621**, 106 (1959).

[23] M. Marti, M. Viscontini, and P. Karrer, *Helv. Chim. Acta*, **39**, 1451 (1965).

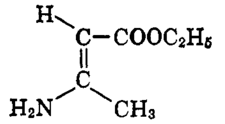
[24] R. M. Burton and N. O. Kaplan, *Arch. Biochem. Biophys.*, **101**, 150 (1963).

[25] K. Bowden, E. A. Braude, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 45 (1946).



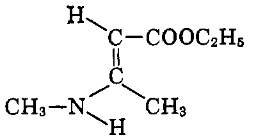
λ_{max} 295 mμ

I



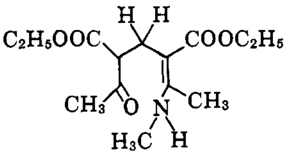
λ_{max} 290 mμ

II



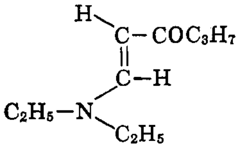
λ_{max} 290 mμ

III



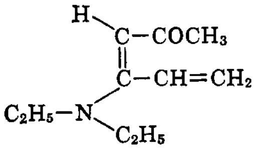
λ_{max} 290 mμ

IV



λ_{max} 307 mμ

V



λ_{max} 308 mμ

VI

lytic hydrogenation experiment is at least compatible with it.

Presumably a dimer coupled at the 6-position should exhibit an ultraviolet spectrum very similar to that of a 1-alkyl-3-carbamido-1,6-dihydropyridine. Wallenfels has reported the preparation of such compounds by borohydride reduction. The products exhibited two absorption bands, at 265 and 350 mμ[22,26] admittedly not in harmony with the dimer structure proposed here. Furthermore, reduction of 1-*n*-propyl-, 1-benzyl-, and 1-(2,6-dichlorobenzyl)-3-carbamidopyridinium salts with chromous solutions was said to form radicals which dimerized at the 6-position on the basis of their spectra.[26,27] It may be noted that in another case, that of NAD itself, borohydride reduction led to a 50% yield of the 1,4-dihydro compound.[28,29] It is not completely clear why both 1,4- and 1,6-dihydro compounds should exhibit absorption maxima at 350 mμ, nor is the band at 265 mμ easily reconciled with a 1,6-dihydro structure (*e.g.*, 265 mμ is not appropriate for a homoannular conjugated diene adjacent to the hetero nitrogen, considering the other substituents, by a "Woodward's rules" approach).

Dimers formed electrochemically from 1-*n*-propyl-3-carbamidopyridinium salts and from NAD itself exhibit important differences from the dimer of the 1-methyl compound.[30] In agreement with the findings of Paiss and Stein[19] these dimers exhibit absorption bands in the wave-length region appropriate for 1,4-dihydro structures. In the case of NAD it has been found that the dimer is slowly reducible to NADH at -1.8 v. In our view, the 1-methyl compound differs from the others, then, in forming 6,6′ dimers rather than dimerizing at the 4-position and in not being reducible to the 1,4-dihydro monomer. Electrochemically, the 1-*n*-propyl compound is thus a more realistic NAD model than is the methyl derivative. In assessing the evidence for various postulated structures of pyridine derivatives in the literature, the reader should be reminded that the spectroscopy of this class of compounds is not in a completely satisfactory state.[31]

Experimental

Apparatus.—Polarograms were recorded with a Sargent Model XV Polarograph, using an H-cell like that of Lingane and Laitinen[32] with a saturated calomel anode. The cell was immersed in a constant-temperature water bath at 25 ± 0.05°. The dropping mercury electrode had the following characteristics with a mercury head of 54 cm.: m = 1.866 mg./sec., t = 4.6 sec./drop. Macroelectrolyses were performed with a potentiostat built in this laboratory from the design of Lingane and Jones.[33] The cathode was a mercury pool with an area of 33 cm.^{2}, the anode was a coil of silver wire, and the reference electrode was a saturated calomel electrode. The anolyte was isolated from the main solution by a porous alundum cup. The electrolysis cell was provided with a nitrogen purge, and the catholyte was stirred vigorously by a magnetic stirring bar at the cathode–solution interface. The coulometer used in conjunction with the potentiostat was of the type described by Wise[34] and Bard,[35] which integrates the current–time function by feeding the iR drop across a standard resistor into a voltage-to-frequency converter whose pulsed output is counted electronically by a scaling circuit with digital readout. pH values were measured with a Beckman Zeromatic pH meter with glass and calomel electrodes. Ultraviolet spectra were recorded with a Cary Model 14 spectrophotometer. Infrared spectra were obtained with a Perkin-Elmer Model 137 Infracord spectrophotometer. Molecular weights were measured with Mechrolab Model 301A vapor pressure osmometer.

Polarography.—Typical solutions for polarography were 5×10^{-4} M in 1-methyl-3-carbamidopyridinium chloride and 2×10^{-2} M in the various buffers shown in Table I, although a few polarograms were recorded at other pyridinium salt concentrations to ascertain that concentration-dependent effects were not being overlooked. Half-wave potentials and i_d/c values remained constant within experimental error from 1×10^{-4} M to 1.5×10^{-3} M for both waves.

1-Methyl-3-carbamidopyridinium Chloride.—The iodide was prepared from nicotinamide and methyl iodide according to the method of Karrer, *et al.*[8] This was converted to the chloride by passing an aqueous solution through a column of the anion exchanger Dowex 1-X8 in the chloride form, concentrating the solution, and precipitating the product with ethanol and ether. The compound was dried at room temperature *in vacuo*.

Dimer Preparation by Electrolysis on the First Wave.—Seven samples of 1-methyl-3-carbamidopyridinium chloride ranging from 100–500 mg. were electrolyzed at -1.2 v. in 200 ml. of 0.1 M phosphate buffers of pH 7–8.1 which were 0.1 M in potassium chloride. Careful blank corrections were made by pre-electrolyzing the buffer solution before the pyridinium salt was added, and then carrying the electrolysis of the latter to the same background current value. Polarograms of the solutions were recorded just before and immediately after the electrolyses, as were ultraviolet spectra. An atmosphere of nitrogen was maintained at all times. The *n*-value obtained from these seven experiments was 0.98 ± 0.05 (uncertainty expressed as a confidence interval for the mean at a probability level of 0.95).

The solutions from the electrolysis experiments were combined and evaporated to dryness at room temperature *in vacuo*. The residue, containing 0.9 g. of reduction product as well as phosphate buffer salts and potassium chloride, was leached with hot 1-butanol. The extract was filtered and cooled, and the reduction product was precipitated by the addition of cyclohexane. The solid was collected by centrifugation and dried *in vacuo* at room temperature. The molecular weight from measurements on aqueous solutions was 283 (calcd. for dimer: 274). Errors

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

(27) K. Wallenfels and M. Gellrich, *Ann.*, **621**, 198 (1959).

(28) M. B. Matthews, *J. Biol. Chem.*, **176**, 229 (1948).

(29) M. B. Matthews and E. E. Conn, *J. Am. Chem. Soc.*, **75**, 5428 (1953).

(30) J. N. Burnett and A. L. Underwood, work in progress.

(31) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 203.

(32) J. J. Lingane and H. A. Laitinen, *Ind. Eng. Chem., Anal. Ed.*, **11**, 504 (1939).

(33) J. J. Lingane and S. L. Jones, *Anal. Chem.*, **22**, 1169 (1950).

(34) E. N. Wise, *ibid.*, **34**, 1181 (1962).

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

of this magnitude (3–4%) were obtained in checking the osmometer with known materials.

Electrolytic Reduction of Dimer.—A sample of 1-methyl-3-carbamidopyridinium chloride was completely reduced to the dimer at −1.2 v. as described above. The resulting solution was then electrolyzed at −1.8 v., whereupon a 2-electron reduction occurred (measured value: 2.11 electrons/initial molecule of pyridinium salt). The ultraviolet spectrum retained the 298-mμ band observed with the original dimer.

Catalytic Hydrogenation of Dimer.—One millimole of 1-methyl-3-carbamidopyridinium chloride was completely reduced electrolytically to the dimer as described above. About 50 mg. of 10% palladium on charcoal was added to the solution, and hydrogenation was carried out at atmospheric pressure in an apparatus similar to that described by Wiberg.[36] Hydrogen uptake was rapid at first and gradually tapered to a very slow rate. After 5 hr., 0.8 mmole of hydrogen had been consumed and the absorbance at 298 mμ had decreased by 15%. After 59 hr., the absorbance at 298 mμ had decreased by 25% from its initial value, and hydrogen uptake was correct for the complete reduction of one double bond and 25% completion in the reduction of a second one. Thus, in the rapid stage, it appears that a double bond having little involvement with the chromophore is reduced while simultaneously but much more slowly a second reduction process destroys the chromophore.

In another experiment, a sample of the dimer, prepared electrolytically, was further reduced electrolytically as described above, and then subjected to hydrogenation. In this case, there was no rapid initial stage, only a very slow hydrogen uptake, and an absorbance decrease at the same rate as in the later stage of the previous experiment. Thus it appears that, early in the catalytic hydrogenation, the same reduction product is formed as in the electrolytic reduction but that the latter is "cleaner," stopping after one double bond is reduced.

Preparation of 1-Methyl-3-carbamido-1,4-dihydropyridine by Electrolysis on the Second Wave.—Six samples of 1-methyl-3-carbamidopyridinium chloride ranging from 100–960 mg. were reduced in phosphate buffers of pH 7.8–9.2 with the cathode potential controlled at −1.85 v. Both polarographic waves disappeared during the electrolysis. Coulometry showed that the reduction involved 2 electrons (1.93 ± 0.15; the precision is poor because of the high background current at this potential). The ultraviolet spectrum of the starting material (λ_{max} 264 mμ) gave way to one with a band at 360 mμ. In one of the experiments, the reduction product was isolated as follows. With all solutions carefully deoxygenated and kept under an atmosphere of nitrogen, the 200-ml. electrolyzed solution was extracted 20 times with 50-ml. portions of chloroform, and the combined extracts were evaporated *in vacuo*. The yellow, oily residue could not be crystallized, but was simply stored in the cold under nitrogen.[37] A portion of the material was spread in a thin, uniform layer between rock salt plates and its infrared spectrum was recorded. Ultraviolet spectra in aqueous Tris buffer of pH 9 were also obtained. The infrared spectrum of the electrolytic reduction product was identical, band for band including the fingerprint region, with that of authentic 1,4-dihydro compound prepared by dithionite reduction, and both materials exhibited the same λ_{max} values in the ultraviolet.

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(36) K. B. Wiberg, "Laboratory Technique in Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 227.

(37) Failure to obtain a crystalline product does not reflect unfavorably upon the electrolytic preparation; crystallization is often equally difficult with the dithionite product. Crystals have occasionally been obtained after electrolysis by treating the chloroform residue with ethyl acetate [H. Kuhnis, W. Traber, and P. Karrer, *Helv. Chim. Acta*, **40**, 751 (1957)], but often the oil is intractable.[8]

The Synthesis and Reactions of 2-Isocyanatoacyl Chlorides

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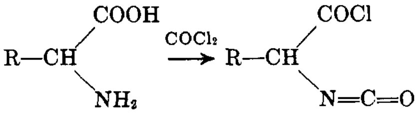
A series of 2-isocyanatoacyl chlorides was prepared by treating glycine, DL-alanine, L-valine, L-leucine, and L-phenylalanine with phosgene in an inert solvent such as dioxane. A reaction mechanism for 2-isocyanatoacyl chloride formation is proposed. The acid chloride group of the 2-isocyanatoacyl chloride is more reactive than the isocyanate group as an electrophile. In the reaction with an equimolar amount of ethanol or water, 2-isocyanatoacetyl chloride gave ethyl 2-isocyanatoacetate or 2,5-oxazolidinedione, respectively. With a molar excess of *p*-phenetidine, it gave 3-(*p*-phenetyl)hydantoin and with a 2 molar or greater excess of amine it gave the corresponding ureidoamide. Treatment with molar quantities of N-methylaniline in the presence of molar quantities of pyridine furnished N-methyl-2-isocyanatoacetanilide.

It has been well established that the reaction of α-amino acids with phosgene gives 2,5-oxazolidinediones in good yield under suitable conditions.[1] However, on prolonged exposure, a tendency to form an oily product instead of the 2,5-oxazolidinedione was noted.[2]

This work was undertaken to elucidate the nature of this oily product. During the course of the investigation, it was shown that the oil contains 2-(N-chloroformylamino)acyl chlorides (IV) and that they are converted to 2-isocyanatoacyl chlorides (V) with evolution of hydrogen chloride when distilled.



$$\mathrm{R{-}CH(COOH)(NH_2)} \xrightarrow{\mathrm{COCl_2}} \mathrm{R{-}CH(COCl)(N{=}C{=}O)}$$

I V

a, R = H
b, R = CH_3
c, R = $(CH_3)_2CH$
d, R = $(CH_3)_2CHCH_2$
e, R = $C_6H_5CH_2$

The formation of compounds of type V has briefly been described by Baird, Parry, and Robinson.[3]

When α-amino acids (I) were treated with phosgene in an inert solvent such as dioxane and the oil remaining

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(1) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 867.

(2) A. C. Farthing, *J. Chem. Soc.*, 3213 (1950).

(3) W. Baird, E. G. Parry, and S. Robinson, British Patent 646,033 (1950).