The reaction with phenyl isothiocyanate was carried out in pyridine–triethylamine–phenyl isothiocyanate (100:3:1)[5] at 40° for 4 hr. Excess reagent was removed under high vacuum. Trifluoroacetic acid,[6] within 1 hr., effected cyclization at room temperature to the phenylthiohydantoins (PTH) which were separated from the residual peptides by fractionation on a Dowex 50WX2 column with methanol and 1.0 *M* ammonia in methanol. The PTH-amino acids were identified by thin-layer chromatography[7] in chloroform–formic acid (20:1) and by gas chromatography[8] on 1% SE-30 on Gas-Chrom P. Aliquots of the residual peptides were hydrolyzed (0.3 ml. of AcOH–1.5 ml. of 6.0 *N* HCl, 110°, 49 hr.) and analyzed on an automatic amino acid analyzer (Phoenix)[9] (Fig. 1).

The combination of these three techniques gave consistent results, suggestive of the sequence of H–Val–Gly–Ala–Leu–Ala–Val–Val–Val–Try–Leu– for desformyl-valine-gramicidin A, and H–Ileu–Gly–. . . . . for desformylisoleucine–gramicidin A.[1,10] After the ninth degradation step, PTH-tryptophan was identified by thin-layer chromatography. After the tenth step, PTH-leucine was found using gas chromatographic analysis. The gradual destruction of tryptophan in the course of the multiple Edman degradation made its quantitative determination impossible. The degradations were therefore not pursued beyond the tenth step.

Oxidation of gramicidin A by N-bromosuccinimide does not lead to the liberation of the 5-bromodioxindole-spirolactone expected from a Try–Try sequence. This was ascertained by comparison of the electrophoretic mobility of the cleavage products as well as by their separation on a Sephadex G-25 column (50% aqueous acetic acid). A sample of authentic spirolactone, prepared by NBS treatment of Cbz–L-Try–L-Try–OH had properties completely different from the oxidation products of gramicidin A. The following structure for valine–gramicidin A at the present time best expresses the published observations, including the earlier findings on the optical configuration of the valine peptides[1,11]: HCO–L-Val–Gly–L-Ala–D-Leu–L-Ala–D-Val–L-Val–D-Val–L-Try–D-Leu–L-Try–D-Leu–L-Try–D-Leu–L-Try–NH–CH_2–CH_2–OH. Of the gramicidin A sample examined, 18% consisted of isoleucine–gramicidin A: HCO–L-Ileu–Gly–. . . . . This structure is in agreement with previously isolated peptides from partial hydrolysates, except for leucyl–glycine, which is probably isoleucyl–glycine.[12] These results also resolve doubts about the molecular weight[13] of gramicidin A and establish mol. wt. 1882 for valine–gramicidin A, a value independently confirmed by ultracentrifuge studies.[14] The unprecedented alternating pattern of L- and D-amino acids, the unusual accumulation of hydrophobic groups, and the complete insolubility in water readily explain the resistance of gramicidin A to attack by enzymes, such as nagarse, pronase, chymotrypsin, and pepsin.[2]

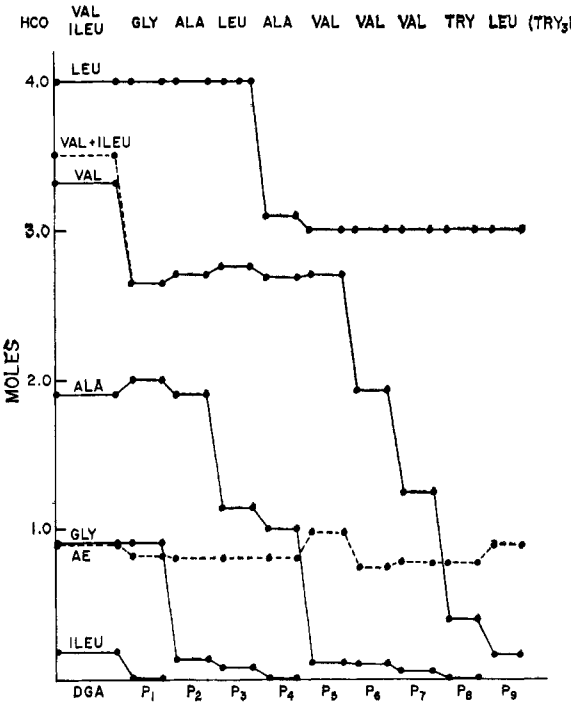

Fig. 1.—Edman degradation of Desformylgramicidin A (DGA). The abscissa lists the residual peptides P_1–P_9 resulting from 1–9 successive degradative steps; the ordinate shows the decrease in molar composition for the individual amino acids (except tryptophan). Full values for valine are not obtained unless the time of hydrolysis is extended from the practical time, *viz.*, 49 hr., to 72 hr.

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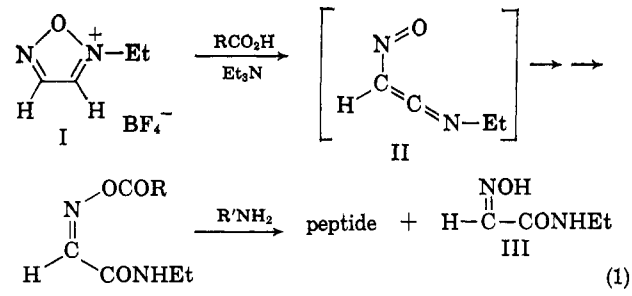
Furazans and Furazanium Salts[1]

Sir:

The utility of 3-unsubstituted isoxazolium salts as peptide-forming reagents has been amply demonstrated.[2] The commercially available reagent, N-ethyl-5-phenylisoxazolium-3′ sulfonate,[2] is expensive, and we entertained the idea of testing N-ethylfurazanium fluoroborate (I) as an economical substitute. We expected I to undergo peptide forming reactions according to eq. 1. The synthesis and characterization of furazan (IV)[3] and

(5) S. Ericson and J. Sjoquist, *Biochim. Biophys. Acta*, **45**, 290 (1960).
(6) W. Konigsberg and R. J. Hill, *J. Biol. Chem.*, **237**, 2547 (1962).
(7) M. Brenner, A. Niederwieser, and G. Pataki, *Experientia* (Basel), **17**, 145 (1961).
(8) J. J. Pisano, W. J. A. VandenHeuvel, and E. C. Horning, *Biochem. Biophys. Research Commun.*, **7**, 82 (1962).
(9) We are greatly indebted to Dr. E. Gross for the amino acid analyses.
(10) L. K. Ramachandran, *Biochemistry*, **2**, 1138 (1963).
(11) J. W. Hinman, E. L. Caron, and H. N. Christensen, *J. Am. Chem. Soc.*, **72**, 1620 (1950).
(12) R. L. M. Synge, *Biochem. J.*, **44**, 542 (1949).
(13) R. L. M. Synge, *Cold Spring Harbor Symp. Quant. Biol.*, **14**, 191 (1950).
(14) We are greatly indebted to Dr. Marc S. Lewis, National Institute of Dental Research, for these determinations.

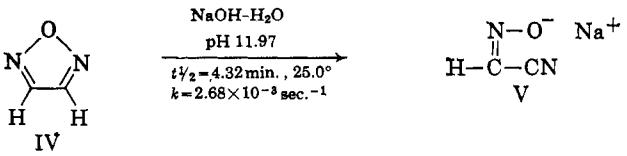
(1) This research was supported by grants from the U. S. Public Health Service (GM-09317) and the Milton Fund of Harvard University. Presented in part at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963, p. 63-Q.
(2) R. B. Woodward and R. A. Olofson, *J. Am. Chem. Soc.*, **83**, 1007 (1961); R. B. Woodward, R. A. Olofson, and H. Mayer, *ibid.*, **83**, 1010 (1961); C. H. Li, D. Chung, J. Ramachandran, and B. Gorup, *ibid.*, **84**, 2460 (1962); P. G. Katsoyannis and M. Tilak, *ibid.*, **85**, 4028 (1963).
(3) Though mono- and disubstituted 1,2,5-oxadiazoles have long been known, the parent compound, furazan, had eluded organic chemists for 80 years. B. Lach, *Chem. Ber.*, **17**, 1571 (1884); A. Hantzsch, *ibid.*, **25**, 705 (1892); L. Wolff, *ibid.*, **28**, 69 (1895); J. H. Boyer in "Heterocyclic Compounds," Vol. 7, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1961, p. 462 ff.; L. C. Behr in "Five and Six Membered Compounds with Nitrogen and Oxygen," R. H. Wiley, Ed., Interscience Publishers, New York, N. Y., 1962, p. 283 ff. (in the series "The Chemistry of Heterocyclic Compounds," A. Weissberger, Ed.).



I II III

the remarkable reasons why its N-alkyl salts *are not* useful peptide forming reagents is the subject of the present communication.

Furazan is easily prepared in 51% yield by melting glyoxime with succinic anhydride and allowing the heterocycle to distil from the mixture.[4] *Anal.* Found: C, 34.18; H, 2.96; N, 40.09; mol. wt., 70 (mass spectrometry).[5] It is a stable liquid [m.p. −28°, b.p. 98° (760 mm.), d^{20}_4 1.168, and $n^{20}D$ 1.4077] which has only end absorption in the ultraviolet and exhibits a single peak in the n.m.r. at τ 1.34 in the neat liquid or τ 1.81 at infinite dilution in CCl_4 ($J_{C^{13}-H}$ = 199 c.p.s.). Further confirmation of the structure is afforded by a complete structure determination by microwave spectroscopy[6] and by the base-induced ring scission of IV to yield an unstable and



IV V

explosive crystalline sodium salt (V, infrared absorption peak at 4.55 μ). This ring cleavage occurs 2000 times faster than the analogous reaction of 5-methylisoxazole.[7] That ionization of the C–H bond is involved in the rate-determining step is indicated by a primary isotope effect k_H/k_D = 2.9 (*vs.* dideuteriofurazan[8]).

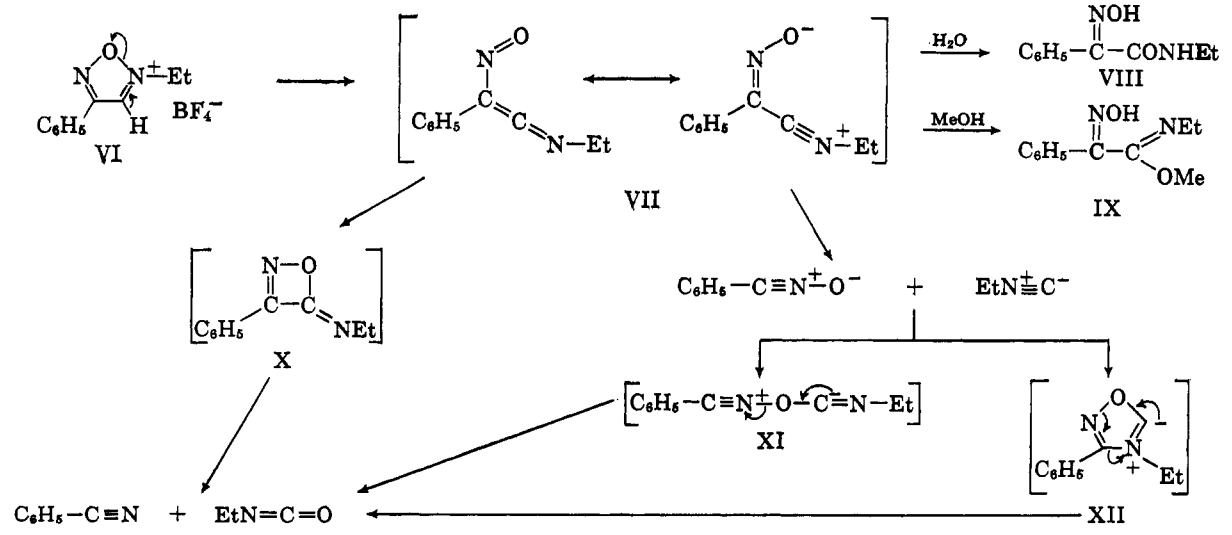
Alkylation of furazan with $EtO_3{}^+BF_4{}^-$ yielded the N-ethyl fluoroborate (I, m.p. 27°) which decomposed in water to the anticipated oximinoamide (III, m.p. 127–128°) undoubtedly by addition of water to the intermediate nitrosoketenimine (II).

Since peptides were not formed with I as predicted by eq. 1, the 4-phenyl salt (VI,[9] m.p. 98–99°) was prepared with the hope that the phenyl substituent would make the isolation of reaction products easier. VI undergoes base-catalyzed ring cleavage in water to yield the oximinoamide (VIII,[10] m.p. 137.5–138.5°) and in methanol to yield the imino ether (IX, m.p. 131–132°).

Two reasons are responsible for the fact that furazanium salts are not useful peptide-forming reagents. The first is the extreme lability of the C–H bond. Though VI is stable in concentrated hydrochloric or perchloric acid, the ring opening is too fast to measure at pH 7, pH 4, or even pH 1. Ring scission to the oximinoamide (VIII) in fact has a half-life of about 3 min. in 3.26 *N* perchloric acid at 25.0°,[11] making this one of the most facile base-catalyzed C–H bond ionizations known.[12] Probably water is a strong enough base to pull off the labile proton and in concentrated acid the number of comparatively free water molecules is decreased.

The second reason involves the extremely facile decomposition and rearrangement of the nitrosoketenimine (VII) when the added nucleophile is not the solvent and is thus in position to trap it. When VI is

SCHEME I



(4) Compare with the preparation of dimethylfurazan of L. C. Behr and J. T. Brent [*Org. Syn.*, **34**, 40 (1954)]. Most earlier attempts[3] involved dehydration in alkaline solution, conditions under which furazan would not be expected to survive if formed, *vide infra*.

(5) Satisfactory analyses and spectroscopic data were obtained for all new compounds. The compound is new if a melting point is reported.

(6) Work of E. B. Wilson and E. Saegebarth; see E. Saegebarth, Symposium on Molecular Structure and Spectroscopy, Ohio State University, Columbus, Ohio, June, 1963, Paper C7.

(7) P. Pino, A. Scartabelli, and E. Lombardi, *Rend. Ist. Lombardo Sci. Lettere*, **87**, 229 (1954).

(8) Dideuteriofurazan (mol. wt. 72, mass spectrometry) was prepared by decarboxylation of diketosuccinic acid in D_2O yielding dideuterioglyoxal as the starting material.

(9) Alkylation of phenylfurazan [A. Russanow, *Chem. Ber.*, **24**, 3497 (1891)] with $Et_3O^+BF_4^-$ proceeds only in the 2-position, presumably because alkylation at the 5-nitrogen is hindered by the phenyl group.

(10) As further proof of structure VIII, its more stable geometrical isomer was synthesized from the N-ethylamide of phenylglyoxalic acid (obtained from benzoyl chloride, ethyl isocyanide, and water), and VIII was converted into this isomer by refluxing in dioxane.

(11) Isoxazolium salts undergo ring cleavage at this rate at about pH 4 (R. B. Woodward and R. A. Olofson, unpublished results).

(12) This will be discussed further in the next communication in this series: R. A. Olofson, W. R. Thompson, and J. S. Michelman, *J. Am. Chem. Soc.*, **86**, 1865 (1964).

titrated with Et_3N in an inert solvent the products are benzonitrile and ethyl isocyanate (both isolated). These rearrangement products could be derived from an intermediate unsaturated β-lactim (X), or by decomposition of VII to benzonitrile oxide and ethyl isocyanide followed by recombination and oxygen transfer either through XI or the 1,3-dipolar addition product XII (Scheme I). Benzonitrile oxide and ethyl isocyanide do yield benzonitrile and ethyl isocyanate rapidly under the reaction conditions. The conjugate acid of XII (as BF_4^- salt, m.p. 110–113°)[13] also gives the same two products when titrated with Et_3N. Since these results do not exclude X as an intermediate and since there is the further possibility of solvent cage recombination here, a detailed study of the mechanism of this rearrangement is being undertaken.

(13) Prepared by alkylation of the known free base: R. Lenaers, C. Moussebois, and F. Eloy, *Helv. Chim. Acta*, **45**, 441 (1962).

(14) National Institutes of Health Predoctoral Fellow, 1962–1964.

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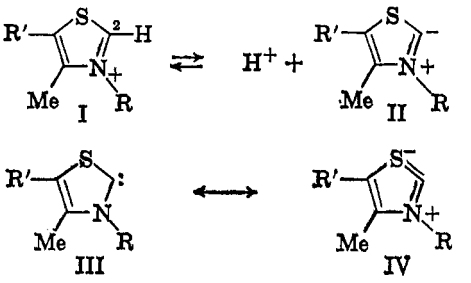
Heterocyclic Nitrogen Ylides[1]

Sir:

Studies of the mechanism of thiamine action first established the importance of heterocyclic ylides as reaction intermediates.[2] The lability of the 2-hydrogen in the thiazolium ring of thiamine (I) has been ascribed to the combined effect of a number of factors[2a] including (1) high s-character of the C–H bond (the reason acetylene is a stronger acid than ethane),

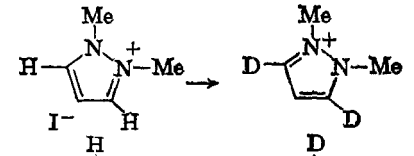
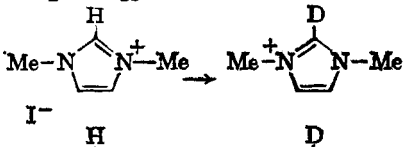
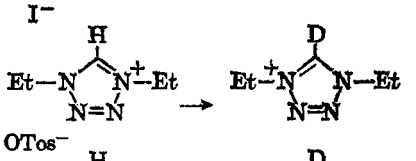
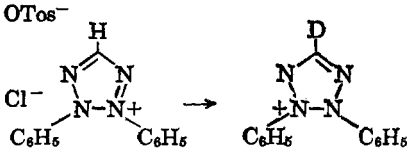


(2) an inductive effect (the reason HCN is a stronger acid than acetylene and $Et\overset{+}{N}\equiv C-H$ ($\rightarrow H^+ + EtNC$) is a stronger acid than either), (3) stabilization of the ylidic species (II) by a resonance contribution from the carbene-like structure (III), and (4) d–σ overlap of the electron pair of the anion with an empty d-orbital of sulfur (IV).

In a future communication[3] we shall describe some experiments designed to test the importance of d–σ overlap in these systems. We now wish to present kinetic evidence intended to give a qualitative estimate of the magnitude of the inductive and related effects. The experiments involve an n.m.r. study of the rates of deuterium incorporation in heavy water buffers, of a number of five-membered aromatic positively charged polynitrogen heterocyclic salts. Some of the preliminary results are summarized in Table I.

These results show first that acidity in these systems is strongly dependent on the positioning of the nitrogen atoms. In the imidazolium salt (VI) where a much greater percentage of the positive charge is localized at the two positions α to the forming carbanion, the exchange rate is 30,000 times that of the pyrazolium

TABLE I

		Ref.	Rate[a]	Relative rate[b]
V		*c*	$t_{1/2}$ = 14 min. at pD 12.95, NaOD–KCl buffer	1
VI		*d*	$t_{1/2}$ = 4.5 min. at pD 8.92, borate buffer	3×10^4
VII		*e*	$t_{1/2}$ = 5.1 min. in 2.04 *N* DCl–D_2O	(5×10^{18})
VIII		*f*	$t_{1/2}$ = 6.2 min. at pD 8.85, borate buffer	3×10^4

[a] The rates were measured by n.m.r. at approximately 31°; the reproducibility is about 20%. Under widely varying buffer concentrations and buffer types at a single pH the variation in rate constant is less than a factor of 2 for V, VI, and VIII. [b] Assuming the rate is first order in substrate and OH^-. Within the errors this is true for V, VI, and VIII. For VII the major contribution to the rate seems to come from other bases so a relative rate cannot really be reported. [c] G. Dedichen, *Chem. Ber.*, **39**, 1831 (1906). [d] O. Wallach, *ibid.*, **15**, 644 (1882). [e] New compound, prepared by ethylation of 1-ethyltetrazole [F. G. Fallon and R. M. Herbst, *J. Org. Chem.*, **22**, 933 (1957)]. A satisfactory analysis was obtained; see text for structure proof. [f] H. von Pechmann and P. Runge, *Chem. Ber.*, **27**, 2920 (1894).

(1) This research was supported by a grant from the U. S. Public Health Service (GM-09317).

(2) (a) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958); (b) F. H. Westheimer, *Advan. Enzymol.*, **24**, 467 (1962).

(3) R. A. Olofson and J. M. Landesberg, unpublished results; Abstracts, 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April, 1963, p. 45M.