are reported in Table I. The reported uncertainties were calculated from formulas 12a and 12b of reference (3) with the addition of a scale factor uncertainty of two parts per thousand. The resulting uncertainties obtained from this procedure were multiplied by a factor of two since the structural data have not been refined completely. It should be pointed out that the error estimates do not include uncertainties from lack of exactness of the kinematic theory for electron scattering. In the analysis of the data the hydrogen parameters were assumed and bond length and bond angle data for the hydrogen positions were taken from an earlier electron diffraction structure determination of ethylene.[4]

TABLE I

PRELIMINARY STRUCTURAL PARAMETERS FOR 1,2,5-THIADIAZOLE BY ELECTRON DIFFRACTION

	$r_a(0)$	l	r
C—H	1.085[a]	0.077[a]	
S—N	1.628 ± 0.008 Å.	0.048 ± 0.008	1.60[b]
N—C	1.329 ± 0.010 Å.	0.040 ± 0.012	1.34[b]
C—C	1.400 ± 0.022 Å.	0.045 ± 0.026	1.41[b]
<N-S-N	99.4 ± 2°		102°[b]
<C—C—H	122°[a]		

[a] Assumed values taken from data on ethylene (see ref. 4). [b] See ref. 5.

The electron diffraction intensity data of thiadiazole and thiophene are almost identical feature for feature except for a scale factor shift of nearly 5%. The direction of the shift is such that thiadiazole must on the average possess distances shorter than those in thiophene by 5%. The fact that the shape of the two curves is similar is an indication that the molecule must possess nearly the same shape as the thiophene molecule.

A correlation study was made to determine the degree of planarity of the ring. This study indicated that the ring was planar to within 0.1Å. The preliminary parameters also suggest that the ring system is highly aromatic since the C–C bond is nearly the benzene value and the C–N bonds are in nearly the same relation to the C–N double and single bond distances as the benzene C–C distance is to the normal C–C double and single bond distances. The S–N distances appear to be quite short but are in good agreement with existing X-ray data for a similar ring system in the molecule benzo-2,1,3-thiadiazole.[5] A comparison of the relevant parameters between the two molecules is given in Table I.

Professor Louis Pierce of the University of Notre Dame has kindly furnished us with some preliminary results of a microwave determination of the molecular structure of 1,2,5-thiadiazole. The results to date indicate that the molecule is planar and the preliminary moments of inertia are I_A = 59.2, I_B = 79.8 and I_C = 139.1. The moments of inertia calculated from our preliminary electron parameters are I_A = 58.4, I_B = 79.6 and I_C = 138.0.

The authors wish to thank Professor Carmack for furnishing us with the chemical evidence for the structure and for providing a sample of thiadiazole.

(4) L. S. Bartell and R. A. Bonham, *J. Chem., Phys.*, **31**, 400 (1959).
(5) V. Luzzati, *Acta Cryst.*, **4**, 193 (1951).

We also wish to thank Professors Bartell and Brockway for the use of their equipment, Mr. Melvin Druelinger for his aid in reading some of the microphotometer traces and help with the computations, and Mr. Ralph Christofferson for his help in reading some of the traces. We also wish to thank Professor Pierce for his preliminary results and for kindly calculating the moments of inertia from our electron diffraction parameters.

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IMIDAZOLE- AND BASE-CATALYZED HYDROLYSIS OF PENICILLIN IN FROZEN SYSTEMS

Sir:

In studies on the penicillin amide bonds, we have observed an unusual lability of the β-lactam bond in frozen systems. Penicillin solutions containing imidazole or histidine lost antimicrobial activity when stored at −18°, but not when incubated for 17 hours at 38°. Further study showed that facile imidazole-catalyzed cleavage of the β-lactam in penicillin occurred only at temperatures between −5 and −30° in frozen, but not supercooled, systems (Table I). Splitting, which was routinely assayed by decreased hydroxamate formation at neutral pH,[1] was confirmed by changes in optical rotation, by formation of the hydroxamate under alkaline conditions, and by infrared and ultraviolet analyses of the products.

TABLE I

β-LACTAM HYDROLYSIS AT VARIOUS TEMPERATURES[a]

		% Hydrolysis					
Catalyst	Substrate	22°	0°[b]	−8°	−18°	−28°	−78°
Imidazole	pen-G	23	4	71	64	20	4
Histidine	pen-G	12	4	3[b]	14	0	6
Carnosine	pen-G	21	9	7[b]	26	14	9
Histamine	pen-G	30	10	6[b]	20	8	7
OH^-, pH 9.9	pen-G	75	14		28	12	4
Imidazole	pen-V	24	8	72	70	48	4
Imidazole	6-APA	2	0	0[b]	98	89	18
Histidine	6-APA	4	0	43	24	6	
Histamine	6-APA	9	0	0[b]	46	24	
OH^-, pH 9.9	6-APA	38	0		23	7	0

[a] Conditions are 65 hours, pH 7.7, 0.01 *M* catalyst and substrate. Buffer at pH 9.9 is 0.05 *M* borate. [b] Unfrozen.

The reaction occurred in imidazole-penicillin mixtures with pH levels as low as 6.2. With 0.01 *M* penicillin G, 0.02 *M* imidazole, −18°, and pH 7.7, the reaction was 52% complete in 5 hours and 92% complete in 17 hours. The rate in frozen H_2O was about twice that in frozen D_2O. Catalysis of ring opening by hydroxide ion, which proceeds readily at room temperature, could not be detected in borate-buffered (imidazole-free) solutions of pH lower than 9.6 which were kept at 0 or −28°. However, at −18°, penicillin and 6-aminopenicillanic acid (6-APA) were hydrolyzed significantly. Freezing penicillin in the absence of catalyst does not induce susceptibility to later hydrolysis by imidazole or increase the rate of penicillinase

(1) A. O. Niedermeyer, F. M. Russo-Alesi, C. A. Lendzian and J. M. Kelly, *Anal. Chem.*, **32**, 664 (1960).

attack. 6-APA in frozen solutions undergoes an autocatalytic hydrolysis of the β-lactam, probably owing to availability of the nucleophilic amino group.

That imidazole attack in the frozen state is not limited to a β-lactam structure is demonstrated by experiments with trypsin as substrate. In 42 hours at −18°, 80% of the tryptic activity[2] was lost, while at −7° or in the absence of imidazole at −18°, no loss occurred.

Penicillin hydrolysis rate and extent were independent of freezing rate. For example, after rapid freezing to −78°, systems warmed to and stored at −18° showed 78% penicillin destruction with imidazole and 38% destruction with pH 9.9 borate. Splitting was not influenced by the degree of stirring during freezing or by the container shape and dimensions.

When solid and liquid phases in equilibrium at −2° were separated and assayed, imidazole and control penicillin concentrations changed by less than 10% from their initial concentrations; subsequent storage of the solid at −2° led to as much as 77% hydrolysis while storage of the liquid at both −2° (unfrozen) and +22° gave no hydrolysis. Glycerol, ethanol, and acetone, at 5–10% by volume, all abolished imidazole-catalyzed hydrolysis in the frozen state at temperatures within the optimal range for controls, although these agents gave little or no protection to the β-lactam during lengthy storage at 22° in the absence of imidazole. With several substrates and imidazole–β-lactam concentration ratios, hydrolysis in frozen solutions was inhibited by Na^+ in proportion to its concentration (74% inhibition with penicillin G and 0.3 *M* NaCl), while the K^+ effect was far smaller and more dependent upon the imidazole concentration. This difference may result from the fact that Na^+, surrounded by a larger hydrate envelope than K^+, probably is more capable of disrupting ordered water structures.[3] Further experiments on structure forming and breaking solutes,[4] undertaken because of possible significance of crystal lattice order and dielectric properties of ice, showed inhibition of the hydrolysis by 10% formamide and no influence by NaI, $MgCl_2$, $CaCl_2$, or EDTA at 10^{-8} *M*, by NaCl, KCl, $NaNO_3$, NaBr, LiBr, or KSCN at 10^{-2} *M*, or by urea at 8 *M*.

In attempts to determine whether the effect was from local concentration of the reactants on freezing, we studied the distribution of reactants in several ways, including the sectioning and assaying of solutions frozen from one end of capillaries; no evidence for local concentration was observed. These and a number of the experiments outlined make doubtful, but do not completely exclude, concentration effects as the basis for the hydrolysis observed.

The marked increase in efficiency in going from bimolecular to intramolecular to enzymatic catalysis has stressed the importance of spatial orientation.[5,6] The freezing effect in the present experiments suggests the possible imposition of a favorable substrate-catalyst positional constraint. In addition, the exceptionally high proton mobility in ice[7] may facilitate fast proton transfer in either nucleophilic or general base catalysis of β-lactam cleavage. Recent studies have suggested that ice-like water structures containing "cavities" may provide biological systems with fast proton transport mechanisms.[8]

(2) M. L. Anson, *J. Gen. Physiol.*, **22**, 79 (1938).

(3) H. S. Frank and W. Wen, *Discussions Faraday Soc.*, **24**, 133 (1957).

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(5) G. L. Schmir and T. C. Bruice, *J. Am. Chem. Soc.*, **80**, 1173 (1958).

(6) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960).

(7) M. Eigen and L. DeMaeyer, *Proc. Roy. Soc. (London)*, **247A**, 505 (1958).

(8) H. Fernandez-Moran, "Abstracts of Lectures on Fast Fundamental Transfer Process," M.I.T., Cambridge, Spring, 1960, pp. 26 and 33.

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ORGANOMERCURIALS. VIII. MERCURY AS THE LEAVING GROUP IN SOLVOLYSIS REACTIONS[1]

Sir:

In our studies of the stereochemistry of the electrophilic cleavage of organomercurials by acids, it was noted that the alkylmercuric salts produced in the reaction undergo decomposition in a solvolytic type reaction.[2] This decomposition reaction has been described by Winstein and Traylor[3] as "oxidation of the solvent," by Robson and Wright[4] to be "classed as auto oxidation of an alkylmercuric salt" since it doesn't require inorganic salt, and by Ichikawa and Ouchi[5] to occur by direct attack of the nucleophile on the ionized alkylmercuric salt. Robson and Wright[4] reported that the alkyl group is converted only to olefin and that the reaction is "not entirely a general one...as we have been unable yet to oxidize a primary alkylmercuric salt." The present communication reports the stoichiometry and the mechanism of the reaction.

The reaction has been found to be general for all alkyl groups thus far studied. In each instance, the only products are those expected for a solvolytic reaction.

$$RHgY + HS \longrightarrow HY + Hg + RS + \text{olefin}$$

Cyclohexylmercuric acetate in acetic acid reacts slowly by first order kinetics to give only olefin, alkyl acetate and mercury. If a catalytic amount of perchloric acid is added, a fast pseudo zero order reaction occurs whose rate constants are proportional to the concentration of acid added. When an equimolar amount of perchloric acid is added, a fast first order reaction results whose rate constant is equal to the first order rate constant obtained by dividing the zero order rate constant by the concentration of acid in those cases where a catalytic

(1) Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research.

(2) L. H. Gale, F. R. Jensen and J. A. Landgrebe, *Chem. and Ind.* (London), 118 (1960).

(3) S. Winstein and T. G. Traylor, *J. Am. Chem. Soc.*, **77**, 3748 (1955).

(4) J. H. Robson and G. F. Wright, *Can. J. Chem.*, **38**, 21 (1960). These workers have also classed the reaction as possibly being a free radical or solvolysis reaction.

(5) K. Ichikawa and H. Ouchi, *J. Am. Chem. Soc.*, **82**, 3876 (1960).