temperature for 1 hr, followed by filtration and neutralization in the usual way, $[\alpha]^{24}D$ +22.1°. A subsequent recrystallization of 1 g of this material from 500 ml of water gave 0.75 g melting at 241–244° dec, $[\alpha]^{24}D$ +23.2°.

Diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide was prepared as described.[7] From two runs with 50 g of diethyl acetamidomalonate, the yield of crude product melting at 151–156° was 76%. One recrystallization from ethanol gave 124 g (65%), mp 159–160°. For analysis a sample was further recrystallized. It melted at 167.5–168.5°,[8] lit.[4] mp 384°.

Anal.[8] Calcd for $C_{13}H_{25}IN_2O_5$: C, 37.5; H, 6.05; N, 6.73. Found: C, 37.6; H, 6.02; N, 6.83.

L-Cystathionine and D-Allocystathionine Mixture.—Once-recrystallized S-benzyl-L-homocysteine (16 g, 0.071 mole) and sodium (3.8 g, 0.17 g-atom) were added alternately in portions to 300 ml of liquid ammonia. The openings on the three-necked flask were protected with drying tubes of Mallcosorb, and a stream of dry nitrogen was led slowly over the mixture. The final permanent blue color was just discharged with a few crystals of ammonium chloride which sometimes left a brown solution. The ammonia was evaporated under the stream of nitrogen and then *in vacuo* with a water pump. The vacuum was released under nitrogen, and the residue was taken up in 300 ml of freshly boiled water. To the solution was added diethyl α-acetamido-α-dimethylaminomethylmalonate methiodide (I, 29.6 g, 0.071 mole, mp 159–160°), and the mixture was adjusted to pH 10 with air-free 2 *N* HCl if necessary. The solution was placed in an oil bath; the internal temperature was maintained at 80–85°. Introduction of nitrogen was continued. The solution was maintained at pH 8–9 with air-free 3 *N* LiOH as needed. Evolution of trimethylamine was followed by having an outlet tube dip into 75 ml of 1 *N* HCl. After 4 hr the yield of trimethylamine was 72% as determined by back-titration with 1 *N* NaOH (phenolphthalein). The reaction mixture (pH 6) gave a negative test for sulfhydryl and disulfide with nitroprusside reagent. It was filtered through a pad of Celite, and the clear solution was evaporated to dryness. The thick residue was allowed to stand under 500 ml of acetone in the cold overnight. The acetone was decanted, and the residue was triturated four times with 100 ml of acetone with gentle refluxing over a steam bath and decanting. A 1-ml sample of the last acetone triturate gave a negative test for iodide with acidic silver nitrate. The material was freed of residual acetone at the water pump and then taken up in 600 ml of 2 *N* HCl. The solution was refluxed for 2 hr. An aliquot placed on the amino acid analyzer showed cystathionine in 90% yield. The brown solution was concentrated to dryness, and the red solid residue was taken up in 50–75 ml of hot water. The resulting red solution was clarified with Darco (2–3 g). The pale yellow filtrate was concentrated to incipient precipitation (50 ml). The solid was dissolved by heating, and the solution was neutralized with 15–20 ml of concentrated ammonia to pH 6. Crystallization started from the hot solution. After standing at room temperature and chilling overnight, the white solid was filtered off and washed with cold water (about 200 ml) until free of chloride ion, then with cold absolute ethanol, and then dried (wt 10.4 g, 66%, $[\alpha]^{24}D$ +23.7°). Amino acid analysis showed 57% D-allocystathionine and 43% L-cystathionine. The material gave a negative cyanide–nitroprusside test for disulfide. The mother liquor and washings were concentrated to 75 ml, and two volumes of ethanol were added to incipient crystallization. After cooling, filtration, and washing, a second crop of 1.5 g was obtained (over-all yield 76%). Paper chromatography (ascending, Whatman No. 3MM, 70% phenol) showed for both crops a single ninhydrin-positive spot, R_F 0.28, agreeing with that of authentic DL- plus allocystathionine.

Fractionation of L-Cystathionine and D-Allocystathionine Mixture. 1A.—Cystathionine (9.4 g, 50% D-allo- and 50% L-cystathionine) was suspended in boiling water (100 ml) and dissolved by additon of concentrated ammonia (20 ml; pH of solution, 9.6). The solution was brought to turbidity by careful addition of hot ethanol (30 ml) and allowed to sit at room temperature whereupon crystallization began. After chilling overnight, the solid was collected by filtration and washed with cold water (wt 3.1 g, 66% of theoretical yield of D-allo-).

Amino acid analysis showed 89% D-allocystathionine and 11% L-cystathionine (quantitative recovery).

1B.—This material was recrystallized in the same manner from 30 ml of hot water with addition of 14 ml of concentrated aqueous ammonia and 15 ml of hot ethanol (wt 2.2 g, 47% of theoretical yield of D-allo-).

Amino acid analysis showed 98% D-allocystathionine and 2% L-cystathionine (quantitative recovery).

For elemental analysis, a sample was recrystallized again at pH 9.6 and then at pH 5 by reprecipitation with ammonia from acidic solution: $[\alpha]^{26}D$ +25.3° ± 1.4% (*c* 1, 1 *N* HCl), lit.[17] $[\alpha]^{21}D$ +24.5°.

Anal. Calcd for $C_7H_{14}N_2O_4S$: C, 37.8; H, 6.35. Found: C, 37.5; H, 6.44.

Amino acid analysis showed less than 0.5% L-cystathionine with a quantitative recovery of D-allocystathionine.

IIA.—The filtrate and washings from 1A were concentrated to dryness, and the residue was dissolved in 60 ml of hot water by addition of concentrated HCl (4 ml). After clarification with charcoal, the hot solution was neutralized dropwise at the pH meter with concentrated ammonia. At pH 2 crystallization began. The solid was filtered after standing overnight at room temperature and washed on the filter with water until free of chloride ion (wt 2.2 g, 47% of theoretical yield of L-cystathionine).

Amino acid analysis showed 96% L-cystathionine, 4% D-allocystathionine (quantitative recovery).

For elemental analysis, a similar sample was recrystallized at pH 2: $[\alpha]^{27}D$ +23.9° ± 2% (*c* 1, 1 *N* HCl), lit. $[\alpha]^{20}D$ +23.9[23] and +23.7°.[16]

Anal. Calcd for $C_7H_{14}N_2O_4S$: C, 37.8; H, 6.35; N, 12.6; S, 14.4. Found: C, 37.5; H, 6.50; N, 12.3; S, 14.4.

Amino acid analysis showed quantitative recovery of L-cystathionine with no detectable D-allocystathionine.

IIB.—Neutralization of the mother liquor of IIA to pH 5 gave 2.4 g of cystathionine (47% D-allo-, 53% L-). Recovery of cystathionine in the three fractions, IA and IIA and B, amounted to 82%.

Acknowledgment.—We thank Miss Christine Lauinger and Mr. Arnold Benjamin for the amino acid analyses.

Reaction of Pyrrolidine with P-1-Aziridinyl-N,N,N′,N′-tetramethylphosphonic Diamides

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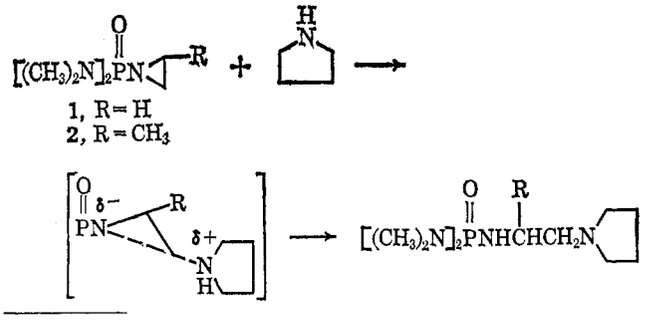
Recently Ham reported the bimolecular rate constants obtained from the reaction of 1-carboethoxyaziridine and substituted anilines in ethanol at 50°.[1] As part of our investigation of the chemistry of insect chemosterilants,[2] we wished to obtain comparable rate data for aziridines such as P-1-aziridinyl-N,N,N′,N′-tetramethylphosphonic diamide (**1**) and its 2-methyl analog (**2**). However, these compounds reacted



(1) G. E. Ham, *J. Org. Chem.*, **29**, 3052 (1965).

(2) A. B. Bořkovec, C. W. Woods, and R. T. Brown, *J. Med. Chem.*, **9**, 522 (1966).

only very slowly with aniline in ethanol under reflux, and pyrrolidine was chosen as the nucleophilic species instead. The lesser reactivity of **1** compared with 1-carboethoxyaziridine may be in part explained on the basis of a diminished ability of a phosphonyl group to afford resonance stabilization of the developing negative charge and/or a diminished inductive effect caused by the change in the other substituents.

The rate data obtained are summarized in Table I. These reactions exhibited second-order kinetics to 75–90% conversion, and in one experiment a large change in concentration of pyrrolidine produced no large change in the calculated rate constant. Compound **1** reacts 3.3 times as fast as **2**. Since **2** appears to undergo nucleophilic attack virtually exclusively at the least substituted carbon atom,[3] the effect of a 2-methyl group is to slow the rate of reaction at the unsubstituted carbon atom of the aziridine ring by a factor of 1.6–1.7 The entropies of activation for the bimolecular hydrolyses of ethylenimine and 2-ethylethylenimine in dilute acid are −9.4 and −10.0 eu, respectively.[4] Such favorable values in cases of bimolecular substitution are generally associated with a decrease in rigidity of the transition state as opposed to the ground state, such as is encountered in the opening of a three-membered ring. The less favorable values which we found for the reactions described here must reflect the effect of charge separation in the transition state. Reactions of immonium salts with water, however, not only involve a favorable ring opening but also a delocalization of the positive charge in the transition state.

TABLE I

RATES OF REACTION FOR 1 AND 2 WITH PYRROLIDINE

Compd	$k \times 10^2$, l. mole^{-1} hr^{-1}	Temp, °C[a]	Solvent
1[b]	6.8 ± 0.3	50.0	95% ethanol
	5.2 ± 0.3[c]	50.0	Abs ethanol
	2.3 ± 0.1	40.0	Abs ethanol
	0.90 ± 0.1	30.0	Abs ethanol
2[d]	1.6 ± 0.1	50.0	Abs ethanol
	0.71 ± 0.06	40.0	Abs ethanol
	0.27 ± 0.02	30.0	Abs ethanol

[a] Temperatures were maintained at ±0.1°. [b] $\Delta H^* = 16.6 \pm 0.5$ kcal/mole, $\Delta S^* = 21.2$ eu. [c] This rate was obtained with initial concentrations of 0.2342 mole/l. of **1** and 0.8116 of pyrrolidine. With initial concentrations of 0.1843 of **1** and 1.9657 of pyrrolidine, the rate constant was $5.18 \pm 0.28 \times 10^{-2}$ l./mole hr. [d] $\Delta H^* = 17.3 \pm 1.3$ kcal/mole, $\Delta S^* = -23.2$ eu.

The method of analysis for aziridine concentration is described in detail in the Experimental Section. It is essentially the method employed by Earley and co-workers for determining the rate of reaction of ethyleneimmonium ions with thiosulfate.[4] Because of the possibility that water and acetate ion (sodium acetate is used as a buffer in this method) might compete more successfully with thiosulfate ion in this instance,[5] the purity of a sample of **2** was determined in solutions containing different concentrations of thiosulfate and acetate ion. The results are given in Table II. The impurity in these preparations is hexamethylphosphoramide and the nmr spectral data for **1** and **2** are given in the Experimental Section. It is evident that the concentration determinations are within 0.5–1.0% of the true absolute value (conditions described in B prevailed) and, in any case, errors in measurements of change in aziridine concentration due to competition by acetate and water would be negligible.

TABLE II

DETERMINATIONS OF PURITY OF 2

	Concn of thiosulfate (M)	Concn of acetate ion (M)	Purity detd[a]
A[b]	0.37	0.00	87.6 ± 0.1
B[c]	0.22	0.30	81.1 ± 0.4
C[c]	0.11	0.15	80.5 ± 0.4
D[c]	0.056	0.08	80.2 ± 0.1

[a] Each value is an average of two determinations. [b] Aqueous perchloric acid was added dropwise until the pH no longer rose with care taken to keep pH above 4. [c] Method described in Experimental Section.

Finally, a brief study was made of the reaction of diethyl(1-aziridinyl)phosphonate (EtO instead of Me_2N) with pyrrolidine at 30° in absolute ethanol. The calculated bimolecular rate constant showed a slight but definite drift upward. However, as an approximate value we obtained 13.5 ± 6% for $k \times 10^2$ (l./mole hr), which is 15 times as fast as **1**. This is ascribed to the increased electronegativity of the substituents on phosphorus.

Experimental Section

Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Nmr data were obtained on a Varian A-60 instrument. Tetramethylsilane was used as an internal standard in CCl_4 with compound concentrations of 20%.

Materials.—The P-1-aziridinylphosphonic diamides, **1** and **2**, were prepared as previously described from N,N,N′,N-tetramethylphosphorodiamidic chloride and the appropriate aziridine.[3b] These aziridines were frequently contaminated with hexamethylphosphoramide (as an alternate to contamination with the bisaziridinyl compound). The proton nmr spectrum of **1**, for example, exhibited a doublet at τ 8.07 (J = 14.5 cps) ascribed to the ring protons and a doublet at 7.34 (J = 9.4) assigned to the N-methyl protons. A doublet at τ 7.41 (J = 9.2) was attributed to hexamethylphosphoramide. The spectrum of **2** was more complex: C-methyl doublet at τ 8.88 (J = 5.0) and N-methyl doublet at 7.36 (J = 9.5). In the case of the sample of **2** titrated for the purposes of obtaining the data of Table II, an integration was calibrated with respect to the C-methyl absorption and the mole per cent of the aziridine component found to be *ca.* 78. The weight per cent was therefore *ca.* 79. The P-(1-aziridinyl)diethylphosphonate was prepared from aziridine and diethyl chlorophosphoridate in 77.2% yield by a procedure similar to that described in the literature,[6] bp 49–51° (0.06 mm), n^{25}D 1.4337; lit.[6] n^{20}D 1.4375. This material showed contamination with triethyl phosphate by nmr: when the area of the absorptions for the ring protons was taken as 4.0 H, the CH_2 and CH_3 absorptions were greater than they should have been.

The products of ring opening with pyrrolidine for **1** and **2** have been characterized previously.[3b] The pyrrolidine adduct of diethyl (1-aziridinyl)phosphonate was prepared by heating a solution of 1 equiv of this compound and 2 equiv of pyrrolidine in absolute ethanol under reflux for 18 hr. The solution was concentrated *in vacuo* and the residue was distilled under reduced pressure to produce a colorless liquid in 64.2% yield, bp 116–117° (0.10 mm), n^{25}D 1.4598. The nmr spectrum revealed a triplet at τ 8.72 and multiplet at 5.0 (presence of some triethylphosphate)

(3) (a) L. B. Clapp, *J. Am. Chem. Soc.*, **70**, 184 (1948); (b) P. E. Sonnet and A. B. Bořkovec, *J. Org. Chem.*, **31**, 2962 (1966).

(4) J. E. Earley, C. E. O'Rourke, L. B. Clapp, J. O. Edwards, and B. C. Lawes, *J. Am. Chem. Soc.*, **80**, 3458 (1958).

(5) One referee reported evidence which indicated that treatment of related aziridines with sodium thiosulfate at low concentrations would not result in a quantitative reaction.

(6) N. P. Grechkin, *Izv. Akad. Nauk. SSSR*, **5**, 538 (1956).

for the ethyl structural unit, the β-methylene protons of the ring occurred as a distinct unit at 8.3, and the remaining methylene proton absorption occurred in the region 6.8–7.7. The integration supports the assignment of structure based on the presence of 5 mole %, or less, of triethylphosphate.

Anal. Calcd for $C_{10}H_{23}N_2O_3P$: C, 47.99; H, 9.26; N, 11.20; P, 12.38. Found: C, 47.81; H, 9.31; N, 11.32; P, 12.52.

Kinetic Experiments.—Freshly prepared solutions of **1** or **2** and of pyrolidine were pipetted into reaction tubes. These were then placed into a constant temperature bath and periodically withdrawn. The concentration at time t (the first sample was taken as $t = 0$) was determined by transferring the contents of the tube with a little ethanol into a mixture of cracked ice and 1 equiv of $HClO_4$ which neutralized the amines present (pH was characteristically 7–8). Aqueous $Na_2S_2O_3$ (15 ml of 0.5 *M*) was added directly, 13 ml of 1.2 *M* NaOAc, and an amount of $HClO_4$ (0.72 g) which brought the pH of the mixture to 4–5. The concentrations of **1** or **2** and pyrrolidine in the reaction tubes (10-ml aliquots) were *ca.* 0.3 and 1 *M*, respectively. The next day the remaining thiosulfate was titrated with standard iodine to the starch end point. This end point showed a tendency to fade and was yellow-brown rather than deep blue, but it was sufficiently definite that reproducibility was good. Bimolecular rate constants were calculated from the integrated form of the second-order rate equation for each pair of samples. The method of weighted averages based on probable random error for each constant was used to determine the final value of the constant.[7]

Acknowledgment.—The author wishes to express his gratitude to Dr. Alexej B. Bořkovec and Dr. Nobel Wakabayashi of this division for generously reading and commenting upon this manuscript.

(7) S. W. Benson, "The Foundations of Chemical Kinetics," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p 86.

Nitration and Denitration in Hydrogen Fluoride

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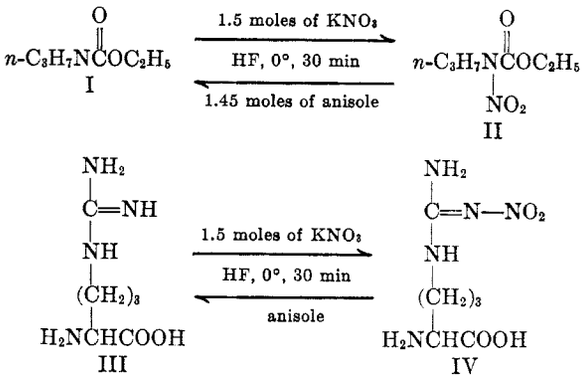
Recently, Sakakibara and Shimonishi[2] have shown that treatment of the synthetic, fully blocked peptide oxytocin with anhydrous hydrogen fluoride and excess anisole under mild conditions effectively removes the N-carbobenzyloxy, S-benzyl, and S-*p*-methoxybenzyl protecting groups from the peptide. Similar conditions have also been successfully applied to a wide variety of other peptide blocking groups.[3]

In the course of applying this procedure to the simultaneous unblocking and removing from the resin of bradykinin synthesized by the Merrifield solid-phase method,[4] it was observed that during the HF reaction the N-nitro group was quantitatively removed from the nitroarginine residues of the peptide.[5]

The transfer of the nitro group in HF from nitroarginine residues to anisole suggested that HF might be a suitable medium not only for denitration of a variety of compounds, but for nitration as well. This possibility was supported by the observations of Del Greco and Gryder,[6] who showed in an infrared and Raman spectral study that solutions of KNO_3 or HNO_3 in anhydrous HF contain appreciable concentrations of the nitrating species, NO_2^+.

This report describes the nitration, in HF under very mild conditions and in high yield, of a carbamate, N-*n*-propyl ethyl carbamate (I), and a guanidine derivative, L-arginine (III). The quantitative denitration of the corresponding N-nitro compounds (II and IV, respectively) by HF and anisole is also described.



Nitration and denitration of both carbamates and guanidines in sulfuric acid has been previously reported.[7] The use of HF for these reactions affords the advantages of very high yields, mild reaction conditions, ease of removal of HF (bp 19.5°), and apparent lack of side reactions.

Experimental Section

Reagents.—HF was obtained from the Matheson Chemical Co. It was purified on a vacuum line (see below) by distillation into a vessel containing cobalt trifluoride as drying agent. It was distilled directly from this vessel into the reaction vessel.

N-*n*-propyl ethyl carbamate (I) and N-*n*-propyl-N-nitro ethyl carbamate (II) were a gift of Mr. Lester A. Dolak, The Johns Hopkins University. The synthesis of these compounds has been reported.[8] L-Arginine (III) was obtained from Calbiochem. L-Nitroarginine (IV) was obtained from Cyclo Chemical Co., and was also prepared as described below. No differences in the denitration of these two samples were observed. Anisole, *o*-nitroanisole, and *p*-nitroanisole were products of Matheson Coleman and Bell.

Apparatus.—Experiments with HF were carried out on a monel vacuum line fitted with Hoke nickel diaphragm valves.[9] The reaction vessels (1.5 × 15 cm) were translucent Fluorothene tubes attached to the line by standard S.A.E. refrigeration flare fittings.

Vapor phase chromatography was carried out on an Aerograph 200 instrument. Chromatography of the amino acids was performed on a Beckman automatic amino acid analyzer, as modified by Dus, *et al.*[10]

Reactions in HF.—HF was introduced into the reaction vessel containing the reagents by freezing the vessel in liquid nitrogen and distilling the desired amount of HF into the vessel under vacuum from an oil pump. After allowing the mixture to stand for 30 min at 0°, the HF was removed by evaporation while stirring with a Teflon-coated magnetic stirring bar. A water aspirator rather than an oil pump was used to remove HF from reactions containing **I** and **II** owing to the volatility of these substances. After evaporation of the HF in this manner for about 30 min, the remaining HF and HNO_3 were removed by storing the vessel overnight in an evacuated desiccator containing NaOH.

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