Registry No.—I, 18872-79-2; II, 18872-80-5; IV, 18872-88-3; VI, 18872-87-2.

Acknowledgment.—We wish to thank Dr. Howard Katzen for bringing to our attention the reported insulin activity of I. We wish to acknowledge the competent technical assistance of Mr. James E. Deak. We are indebted to Mr. Richard N. Boos and his associates for elemental analyses and to Mr. Carl Homnick for amino acid analyses.

Phosphonoacetaldehyde

A. F. ISBELL,[1] LEO F. ENGLERT,[2]

Department of Chemistry, Texas A & M University, College Station, Texas 77843

AND HARRY ROSENBERG

Department of Biochemistry, The John Curtin School of Medical Research, The Australian National University, A.C.T., Australia 2601

Received August 26, 1968

De Koning[3] has reported that the reaction of 2-aminoethylphosphonic acid (**1**, 2-AEP) with ninhydrin produces acetaldehyde and inorganic phosphate as a result of oxidation, followed by cleavage of the C–P bond. It was assumed that phosphonoacetaldehyde (**2**) was first formed when 2-AEP was heated with ninhydrin at 96° and at pH 5 for 8 hr. Attempts to

$$\underset{\mathbf{1}}{H_2NCH_2CH_2PO(OH)_2} \xrightarrow{\text{ninhydrin}} [\underset{\mathbf{2}}{OCHCH_2PO(OH)_2}] \xrightarrow[\text{pH 5}]{96^\circ} CH_3CHO + PO_4^{3-}$$

isolate and characterize phosphonoacetaldehyde (**2**) were not mentioned.

The decomposition of **2** is rather surprising because the C–P bond is usually stable with respect to hydrolytic cleavage, even under vigorous reaction conditions.[4] The normal procedure for the hydrolysis of a phosphonate ester is to heat the ester under reflux with 6 *N* hydrochloric acid for 48 hr or longer; normally no detectable C–P cleavage takes place. However, Chavane has stated that the polarity of the substituents on the alkyl chain has a large influence on the stability of phosphonic acids and predicted that **2** would be unstable.

We first tried to convert diethyl phosphonoacetaldehyde diethyl acetal[5] (**3**) to **2**. The problem was to remove the ester groups without destroying other portions of the molecule. One of the methods tried involved heating **3** with excess thionyl chloride in the hope of converting the diester to the phosphonyl dichloride. Instead, a thermally unstable compound was recovered, which proved to be monoethyl 2-ethoxyvinylphosphonochloridate (**4**) (Scheme I). The

SCHEME I

$$\underset{\mathbf{3}}{(C_2H_5O)_2CHCH_2PO(OC_2H_5)_2} \xrightarrow{SOCl_2} \underset{\mathbf{4}}{C_2H_5OCH{=}CHPO(OC_2H_5)Cl}$$

$$\downarrow SOCl_2 \qquad \downarrow H_2O$$

$$(C_2H_5O)_2CHCH_2POCl_2 \qquad \underset{\mathbf{5}}{OCHCH_2PO(OC_2H_5)OH}$$

structure of **4** was determined from its ir and its nmr spectra and from its chemical reactions.

This line of research was discontinued in favor of a second route that appeared to offer more promise. Lutsenko and Kirilov[6] synthesized 2-acetoxy-2-chloroethylphosphonyl dichloride (**6**) but for some reason they did not report the hydrolysis of **6** to **2**. The

$$CH_3CO_2CH{=}CH_2 + PCl_5 + SO_2 \longrightarrow \underset{\mathbf{6}}{CH_3CO_2CHClCH_2POCl_2} \xrightarrow{H_2O} \mathbf{2}$$

hydrolysis took place readily in an aqueous tetrahydrofuran solution at room temperature to give a nearly quantitative yield of **2** as a slightly yellow, viscous oil. This crude oil was shown to be at least 81% **2** by converting it to the crystalline 2,4-dinitrophenylhydrazone of **2**.

Oxidation of **2** with aqueous, alkaline permanganate gave phosphonoacetic acid in 68% yield, identical with the acid produced by the hydrolysis of triethyl phosphonoacetate. We have converted diethyl phosphonoacetaldehyde (**7**) to the biologically important 2-AEP (**1**) by the sequence shown in Scheme II. Phono-

SCHEME II

$$\underset{\mathbf{7}}{OCHCH_2PO(OC_2H_5)_2} \longrightarrow [HON{=}CHCH_2PO(OC_2H_5)_2] \longrightarrow$$

$$[CH_3CONHCH_2CH_2PO(OC_2H_5)_2] \longrightarrow \underset{\mathbf{1}}{H_2NCH_2CH_2PO(OH)_2}$$

acetaldehyde (**2**) has recently been shown to be a normal constituent of at least one biological system and there is good evidence that it is an enzymic breakdown product of **1**.[7]

The authentic **2** also provided the opportunity to determine its stability under the conditions reported by De Koning.[3] Heating a solution of **2** in aqueous acetate buffer (pH 5) for 8 hr at 90° produced good yields of acetaldehyde, isolated as the 2,4-dinitrophenylhydrazone, and of phosphate, recovered as the $MgNH_4$ salt. Thus, even though phosphonoacetic acid is stable to prolonged heating with 6 *N* hydrochloric acid, **2** contains a C–P bond which hydrolyzes at a moderate rate under much milder conditions, even though it appears to have good stability in aqueous solutions at low and at high pH values at 25°.

Experimental Section

Melting points were determined with a Hershberg[8] melting point apparatus and are corrected; boiling points are uncorrected. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., except for the Cl analysis of **4** which was determined by adding a sample of **4** to water, followed by HNO_3

(1) To whom inquiries should be addressed.
(2) Taken in part from the Ph.D. dissertation of L. F. E., 1968.
(3) A. J. De Koning, *Biochim. Biophys. Acta*, **130**, 521 (1966).
(4) V. Chavane, *Ann. Chim.* (Paris), **49**, 365 (1949).
(5) N. D. Dawson and A. Berger, *J. Am. Chem. Soc.*, **74**, 5312 (1952).
(6) I. F. Lutsenko and M. Kirilov, *Dokl. Akad. Nauk SSSR*, **132**, 842 (1960).
(7) J. M. La Nauze and H. Rosenberg, *Biochim. Biophys. Acta*, **165**, 438 (1968). This article contains the infrared and spectra of purified synthetic **2**.
(8) E. B. Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 312 (1936).

and $AgNO_3$, collecting the precipitate of AgCl, and weighing it. The ir spectra were determined with a Beckman IR-8 spectrometer and the nmr spectra were determined with a Varian HA-100 spectrometer. Hydrolysis equivalents, neutralization equivalents, and titration curves were determined by titrating the samples with standard base with the aid of a Sargent Model D recording titrator.

Monoethyl 2-Ethoxyvinylphosphonochloridate (4).—Thionyl chloride (82.5 g, 0.8 mol) was added dropwise with stirring to diethyl phosphonoacetaldehyde diethyl acetal[5] (3) (50.8 g, 0.2 mol), the addition being at a rate to maintain a reaction temperature of 40°. The solution was heated under reflux for 12 hr and was distilled, giving 12.2 g (30%) of crude **4**: bp 90–95° (1 mm); ir (neat), 6.2 (vinyl ether), 8.0 (P=O), 9.8 μ (P–O–C); nmr (neat), δ 1.32 (m, CH_3), 4.10 (m, CH_2), 5.20 (d of d, J_{HH} = 13 cps, J_{PH} = 15 cps, PCH), and 7.34 (t, $J_{HH} = J_{PH}$ = 13 cps, CHOR). *Anal.* Calcd for $C_6H_{12}ClO_3P$: Cl, 17.85; hydrolysis equiv, 99.3. Found: Cl, 17.9; hydrolysis equiv, 106. The hydrolysis equiv was determined by dissolving a weighed sample in water, allowing the solution to stand for 30 min, followed by titrating with standard base. It was assumed that **4** was converted to **5**. The analyses indicate that this compound was not analytically pure. However, the thermal instability of **4** prevented its careful fractional distillation. It was necessary to distil it without a column and preferably at a temperature below 100°. Above 100° decomposition seemed to increase rapidly and a violent decomposition occurred once at 115–120°.

Monoethyl Phosphonoacetaldehyde 2,4-Dinitrophenylhydrazone.—Crude **4** (2.45 g, 0.0125 mol) was added to 20 ml of water. The mixture was stirred to effect complete solution (about 5 min), it was added to 2.50 g (0.0126 mol) of 2,4-dinitrophenylhydrazine in 95 ml of water–ethanol–sulfuric acid (20:60:15 by vol), the solution was allowed to stand for 30 min, water was added to cause a cloudy solution, and the mixture was allowed to stand 12 hr longer. The resulting orange precipitate was collected and recrystallized from water–ethanol to give 3.70 g (89%) of the DNP derivative, mp 192–193° dec. *Anal.* Calcd for $C_{10}H_{13}N_4O_7P$: N, 16.87; P, 9.33; neut equiv, 332. Found: N, 16.86, 17.08; P, 9.37, 9.25; neut equiv, 328, 333.

2-Acetoxy-2-chloroethylphosphonyl Dichloride (6).—By following the procedure of Lutsenko and Kirilov,[6] **6** was prepared in 76% yield, bp 83° (0.3 mm), $n^{25}D$ 1.4861 (lit.[6] bp 99–100° (1.5 mm), $n^{20}D$ 1.4855).

Phosphonoacetaldehyde (2).—Five grams (0.021 mol) of **6** was dissolved in 50 ml of tetrahydrofuran and 1.2 g (0.067 mol) of water was added dropwise with stirring so that the temperature did not rise above 30°. The reaction flask was stoppered and allowed to stand for 4 days at room temperature. After the solvent was removed with a water aspirator, the residue was placed in a vacuum desiccator over solid KOH–$CaCl_2$ and at 1 mm pressure for 6 hr. The resulting residue was a viscous, slightly yellow oil, weighing 2.7 g (theor 2.6 g). No further purification of **2** was attempted.

Crude **2** (1.55 g, 0.0125 mol) in 10 ml of water was added to 2.5 g (0.0126 mol) of 2,4-dinitrophenylhydrazine in 95 ml of water–ethanol–sulfuric acid (20:60:15 by vol). The solution was allowed to stand overnight at room temperature, during which time a yellow-orange precipitate formed. After the solution was cooled to 5°, the solid was collected and recrystallized from water to give 3.1 g (81%) of yellow-orange crystals, mp 177–178° dec. *Anal.* Calcd for $C_8H_9O_7N_4P$: N, 18.42; P, 10.18; neut equiv, 304. Found: N, 18.38; P, 10.48; neut equiv, 305. The titration curve had two distinct breaks at pH 5.2 and pH 9.9.

The crude **2** from the hydrolysis of 1.0 g of **6** was dissolved in 200 ml of 0.1 *M* acetate buffer (pH 5). The solution was heated at 90° for 8 hr while a slow stream of nitrogen was passed through the solution and then through 200 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2 *M* HCl. In about 1 hr a precipitate began to separate from the HCl solution. After 8 hr, the solid was collected and recrystallized from ethanol–water to give 0.62 g (66%) of acetaldehyde dinitrophenylhydrazone, mp 147–148°. Authentic acetaldehyde dinitrophenylhydrazone and a mixture of the two solids showed the same melting point.

The acetate buffer solution was made alkaline by the addition of concentrated NH_4OH. To the alkaline solution was added 25 ml of "magnesia reagent" (0.246 *M* magnesium chloride and 1.87 *M* ammonium chloride) and the solution was chilled overnight in a refrigerator. The resulting white solid was collected and washed successively with dilute NH_4OH, 95% ethanol, and ether. The solid, assumed to be magnesium ammonium phosphate hexahydrate, was dried by pulling air through it, 0.985 g (98%).

One gram of crude **2** in 10 ml of water was cooled to 10° and was made basic to pH 10 by the addition of 6 *N* NaOH. A 5% aqueous solution of $KMnO_4$ was added dropwise with stirring until the characteristic permanganate color persisted; filtration removed MnO_2. To the filtrate, 5% aqueous $NaHSO_3$ was added until the solution was colorless and completely clear. The solution was evaporated to dryness, the residue was dissolved in a small amount of water, and the solution was passed through a column of Dowex 50 (H^+) ion-exchange resin. The strongly acidic eluate was collected and evaporated to dryness, leaving 0.85 g of a viscous oil that slowly crystallized. Recrystallization from glacial acetic acid gave 0.77 g (68%) of white crystals, mp 138–139°. A sample of authentic phosphonoacetic acid was prepared by the hydrolysis of triethyl phosphonoacetate[9] and was shown to melt also at 138–139°, as did a mixture of the two solids.

Conversion of Diethyl Phosphonoacetaldehyde (7) to 2-Aminoethylphosphonic Acid (1).—A 5-g quantity of **7**[6] was added to a solution of 5 g of hydroxylamine hydrochloride in 25 ml of absolute ethanol and 25 ml of anhydrous pyridine. After the solution was allowed to stand for 48 hr at room temperature, it was evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml of acetic anhydride and 150 ml of glacial acetic acid, 1 g of 5% Pd–C was added, and the mixture was shaken with 60 psi H_2 for 24 hr. The solution was filtered and the filtrate was evaporated to dryness, leaving a residue which was heated under reflux for 48 hr with 100 ml of 6 *N* HCl. Evaporation of the hydrolysate to dryness and boiling the residue in 100 ml of 2 *N* NaOH removed volatile basic impurities. Excess HCl was added, the solution was evaporated to dryness, and the solid residue was extracted with three 50-ml portions of hot ethyl alcohol–concentrated HCl (5:1 by vol). The extracts were evaporated to dryness, the residue was dissolved in 25 ml of water, and the resulting solution was passed through a column of Dowex 50 (H^+) ion-exchange resin. A strongly acidic eluate was discarded, followed some time later by a ninhydrin-positive eluate, which was evaporated to dryness. Recrystallization of the residue from water–ethanol gave 1.5 g (43%) of 2-aminoethylphosphonic acid (**1**). The **1** prepared in this manner had neut equiv 124.7 (calcd 125.1) and an ir spectrum identical with that reported for the metastable α form.[10]

Registry No.—**2**, 16051-76-6; **4**, 5607-01-2; monoethyl phosphonoacetaldehyde 2,4-dinitrophenylhydrazone, 18916-92-2; **2** 2,4-dinitrophenylhydrazone, 18910-31-1.

(9) P. Nylen, *Ber.*, **57**, 1023 (1924).
(10) M. Horiguchi and M. Kandatsu, *Agr. Biol. Chem.* (Tokyo), **28**, 408 (1964).

Monohydrazones of Thiocarbohydrazide. The Occurrence of 1,4,5,6-Tetrahydro-3(2H)-*s*-tetrazinethione Structures

ROBERT W. LAMON

Research Laboratories, Eastman Kodak Company, Rochester, New York 14650

Received August 26, 1968

Thiocarbohydrazide and its bishydrazones have been known for some time.[1–4] Examples of monohydrazones

(1) R. Stollé and R. E. Bowles, *Ber.*, **41**, 1099 (1908).
(2) P. C. Guha and S. C. Dey, *Quart. J. Ind. Chem. Soc.*, **2**, 225 (1925).
(3) H. W. Stephen and F. J. Wilson, *J. Chem. Soc.*, 2531 (1926).
(4) W. Reid and G. Oertel, *Ann.*, **590**, 136 (1954).