4-Methylsemicarbazone of Pyruvic Acid. A. From 4,6-Dimethyl-*asym*-triazine-3,5(2,4)-dione (XI).—One-half gram (3.55 mmoles) of XI was dissolved in 3.75 cc. of 1 *N* sodium hydroxide and 1.7 cc. of 6 *N* sodium hydroxide. The solution was heated on a steam-bath for 5 hours in a sealed tube after which it was diluted to 25 cc. with water. Paper chromatography in water-saturated 1-butanol showed about 20% of the starting material (R_f 0.75) and a new spot (R_f 0.15) containing the other 80% of ultraviolet-absorbing material. The semicarbazone of pyruvic acid has an R_f value of 0.02 in this system. The solution was passed through a column containing 15 cc. of Dowex-50 resin (H^+) which was then washed with 120 cc. of water. The effluents were concentrated to dryness and the residue (0.45 g.) crystallized from 25 cc. of boiling ethanol to yield 0.075 g., m.p. 228°. This precipitate was recrystallized from 2 cc. of boiling ethanol to yield 40 mg., m.p. 229–230°. The melting point of this sample was not depressed upon admixture with an authentic sample of 4-methylsemicarbazone of pyruvic acid prepared as described below.

Anal. Calcd. for $C_5H_9N_3O_3$: C, 37.73; H, 5.70; N, 26.41. Found: C, 38.20; H, 5.81; N, 26.55.

B. From Pyruvic Acid and 4-Methylsemicarbazide.—A mixture of 0.3 g. (3.4 mmoles) of pyruvic acid and 0.3 g. (3.4 mmoles) of 4-methylsemicarbazide[18] in 5 cc. of water was refluxed 1.5 hours. On cooling, 0.3 g. (56% yield) of a product melting at 234° was obtained. This precipitate was recrystallized from 34 cc. of hot ethanol to give a product of melting point 229–230°.

Anal. Calcd. for $C_5H_9N_3O_3$: C, 37.73; H, 5.70; N, 26.41. Found: C, 38.00; H, 5.92; N, 26.50.

(18) C. Vogelsang, *Rec. trav. chim.*, **62**, 5 (1943).

Paper Chromatography.—The solvent system was ethyl acetate saturated with 3% acetic acid, run in a descending fashion with ethyl acetate saturated water in bottom of tank. The spots were visualized under a Gates ultraviolet lamp (germicidal bulb).

Derivative of *asym*-triazine-3,5(2,4)-dione	*R*
4,6-Dimethyl, XI	0.93
2,4,6-Trimethyl	.90
6-Methyl, VII	.84
2-(2′-Deoxy-D-ribosyl)-6-methyl	.63
4-D-Ribosyl-6-methyl, III	.63
2-D-Ribosyl-6-methyl, I	.49
Semicarbazone of pyruvic acid, X	.29
2,4-Di-D-ribosyl-6-methyl, II	.20
4-Ribosylsemicarbazone of pyruvic acid, IX	.05

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PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE CHEMICAL CORPS, CHEMICAL RESEARCH DIVISION, CHEMICAL WARFARE LABORATORIES]

Organic Phosphorus Compounds. II.[1] Isomeric Alkyl Phosphoro- and Phosphonothioates

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O,O-Diethyl O-(2-ethylthioethyl) phosphorothioate (I)(Systox[3]) was prepared by the transesterification of triethyl phosphite with 2-ethylthioethanol and subsequent addition of sulfur to the resulting diethyl 2-ethylthioethyl phosphite. The O-(2-isopropylthioethyl) homolog of I was obtained in an analogous manner using 2-isopropylthioethanol. The transesterification with 2-alkylthioethanols was extended successfully to dialkyl methylphosphonites; the resulting O-alkyl O-(2-alkylthioethyl) methylphosphonites upon addition of sulfur yielded analogs of I containing a carbon-to-phosphorus bond. In several instances, the O-alkyl O-(2-alkylthioethyl) methylphosphonothioates were found to undergo thermal rearrangement to the corresponding S-(2-alkylthioethyl) isomers under conditions reported[4] for the analogous isomerization of I.

Introduction

The highly effective systemic insecticide O,O-diethyl O-(2-ethylthioethyl) phosphorothioate[3] (I) and some of its homologs were prepared by several investigators by the reaction of the appropriate alkylthioethanol or its sodium derivative with a suitable dialkyl phosphorothiochloridate, $(RO)_2P(S)Cl$.[4-7] The thiolo isomers of I and of its homologs of the type $(RO)_2P(O)SCH_2CH_2SR'$ (II) were obtained either by thermal isomerization[4] of the corresponding thiono isomers $(RO)_2P(S)OCH_2CH_2SR'$ (III) or by the reaction of a suitable trialkyl phosphite or dialkyl sodium phosphonate with a 2-alkylthioethyl thiocyanate.[6,8]

The inhibitory effect on cholinesterase enzymes increases by several orders of magnitude upon isomerization of O,O-dimethyl O-(*p*-nitrophenyl) phosphorothioate and its dialkyl homologs to the corresponding O,O-dialkyl S-(*p*-nitrophenyl) phosphorothioates[9] and the systemic insecticidal activity of the commercial Systox[3] seems to be dependent upon its contamination by the more active isomer $(C_2H_5O)_2P(O)SCH_2CH_2SC_2H_5$ (IV).[4] Since samples of the thiono compounds III, prepared by any of the previously reported methods[5-7] and purified by ordinary high vacuum distillation were always found to contain considerable amounts of the isomeric thiolo derivative, the preparation of the III, free from contamination with the isomeric II, became desirable. The previously reported

(1) Paper I of this series: F. W. Hoffmann, T. C. Simmons and L. J. Glunz, III, THIS JOURNAL, **79**, 3570 (1957).

(2) To whom inquiries about this paper should be addressed.

(3) Compound I is marketed by Chemagro Corporation, New York, N. Y., under the trade-name Systox.

(4) T. R. Fukuto and R. L. Metcalf, THIS JOURNAL, **76**, 5103 (1954).

(5) G. Schrader, U. S. Patent 2,571,989 (1951).

(6) Farbenfabriken Bayer, German Patent 850,677 (1952).

(7) Ya. A. Mandelbaum, N. N. Mel'nikov and V. I. Lomakina, *Zhur. Obshchei Khim.*, **26**, 2581 (1956); *C.A.*, **51**, 1825e (1957).

(8) G. Schrader, U. S. Patents 2,597,534 (1952) and 2,640,847 (1953); German Patents 926,488 and 947,367 (1956).

(9) R. L. Metcalf and R. B. March, *J. Econ. Entomol.*, **46**, 288 (1953).

facile transesterification of trialkyl phosphites with aliphatic alcohols[10] suggested a simple alternate synthesis by which the pure II might be obtainable without distillation according to the equations

$$(RO)_3P + HOCH_2CH_2SR' \longrightarrow (RO)_2POCH_2CH_2SR' \text{ (V)} + ROH; \text{ and } V + S \longrightarrow III$$

Since I and three of its homologs were prepared successfully by this sequence of reactions, the transesterification reaction and subsequent sulfur addition appeared also applicable to the synthesis of analogous organophosphorus compounds[11] of the type $RO(R'')P(S)OCH_2CH_2SR'$ (VI). The thiono compounds VI were expected to be susceptible to thermal isomerization to the corresponding O-alkyl S-(2-alkylthioethyl) alkanephosphonothioates, $RO(R'')P(O)SCH_2CH_2SR'$ (VII), in analogy to the behavior of the thionophosphates.

Discussion of Results

The transesterification of trialkyl phosphites with 2-ethyl- and 2-isopropylthioethanol proceeded smoothly with the formation of the corresponding dialkyl 2-alkylthioethyl phosphites in good yields and does not differ from the reaction of the phosphites with unsubstituted alkanols.[10] As expected, smaller yields of the di-transesterification products, the di-(2-alkylthioethyl) alkyl phosphites, were also obtained in these exchange reactions and isolated in two instances.

The extension of the transesterification with 2-ethylthioethanol to dialkyl methylphosphonites proved to be successful[12] and proceeded in a fashion strictly analogous to that observed in the phosphite series. However, no added catalyst, such as sodium, was required in the exchange reactions employing diethyl, dipropyl or diisopropyl methylphosphonite and 2-ethylthioethanol. After removal of the exchanged alcohol, the reaction mixtures from these runs consisted of unreacted methylphosphonite, alkyl 2-ethylthioethyl and di-(2-ethylthioethyl) methylphosphonite, $R''P(OCH_2CH_2SR')_2$ (VIII). Since in these runs the methylphosphonite was treated with one mole equivalent of the alcohol the yield of the di-transesterification product VIII was much higher than that of the analogous product in the corresponding runs with trialkyl phosphites in which the molar phosphite to alcohol ratio of 2 or 3:1 favored the formation of the mono-transesterification product.

Both the dialkyl 2-alkylthioethyl phosphites and the alkyl 2-ethylthioethyl methylphosphonites added sulfur in an exothermic reaction to yield the corresponding thiono compounds in high yields if the temperature of the reaction mixture was maintained at about room temperature during the reaction with the sulfur. But even under these mild conditions the sulfur adducts still contained small amounts of the thiolo isomers. In runs in which no external cooling was applied during the sulfur addition the temperature of the reaction mixture rose frequently up to 100° depending on the rate of addition. The higher reaction temperatures resulted always in a considerable isomerization of the thiono compounds to the corresponding thiolo isomers II and VII, respectively.

The addition of sulfur to diethyl 2-ethylthioethyl phosphite (IX) below 25° gave a quantitative yield of the sulfur adduct which after the removal of a small amount of volatile material at 36° and 0.030 mm. pressure showed a refractive index of $n^{25}D$ 1.4856. This material contained, on the basis of its infrared spectrum (phosphoryl absorption at 7.97 μ), possibly up to 6% of O,O-diethyl S-(2-ethylthioethyl) phosphorothioate (IV), while the nuclear magnetic resonance spectrum[13] indicated the presence of approximately 3% of IV. The refractive index of a sample of I, which was prepared from O,O-diethyl phosphorothiochloridate and sodium 2-ethylthioethoxide below 25° and appeared to be free of IV on the basis of the infrared and NMR spectra, was 1.4852 at 25°. Taking as a standard the value of $n^{25}D$ 1.4922, shown by a sample of IV obtained by the thermal rearrangement of I, the content of IV in the I prepared by the transesterification procedure is about 6% confirming the infrared spectroscopic results.

It is interesting to note that the colorless I prepared by low temperature sulfur addition to IX did not discolor on standing for a period of one year in an ordinary Pyrex glass bottle with ground stopper, while the yellow commercial Systox and also the pale yellowish I prepared from the thiochloridate turned rapidly dark orange-brown under the same conditions. All other homologs and analogs of I synthesized by the addition of sulfur to the appropriate phosphites or phosphonites were colorless and displayed the same apparent stability on storage under ordinary conditions.

The rates of the isomerization of a sample of I, prepared by the transesterification method and evaporatively distilled at 58° and 10^{-4} mm, $n^{25}D$ 1.4865, were studied by Muller and Goldenson[13] and found to be intermediate between zero and first order. Following the isomerization of I, $n^{25}D$ 1.4865, at 68, 90 and 120° by the appearance of the infrared phosphoryl absorption band at 7.97 μ, the half-lifes of I at these temperatures were approximately 194, 24 and 1.5 hours, respectively.

While I and diethyl O-(2-isopropylthioethyl) phosphorothioate rearranged completely at 130–140° during a period of 3.5 hr., O,O-diisopropyl and O,O-dimethyl O-(2-ethylthioethyl) phosphorothioate underwent decomposition with the formation of tarry or waxy products when heated at 120° under a nitrogen atmosphere. No difficulties were encountered in the isomerization of the O-alkyl (2-ethylthioethyl) methylphosphonothioates (VI) to the corresponding thiolo isomers VII. The yields of the distilled thiol esters VII obtained by isomerization, their boiling points, refractive indices and analytical data are listed in Table II. In all examples, the VII exhibited higher refractive indices than the corresponding VI.

(10) F. W. Hoffmann, R. J. Ess and R. P. Usinger, Jr., THIS JOURNAL, **78**, 5817 (1956).

(11) Only compounds containing at least one carbon-to-phosphorus bond are regarded as *organophosphorus compounds*. In the opinion of the authors, esters of inorganic phosphorus acids should be classified as *organic compounds containing phosphorus*. This view-point follows the generally accepted Beilstein classification.

(12) A paper on the transesterification of dialkyl alkylphosphonites with aliphatic alcohols is in preparation.

(13) N. Muller and J. Goldenson, THIS JOURNAL, **78**, 5182 (1956).

The preparation of O-ethyl S-(2-ethylthioethyl) methylphosphonothioate by the general method of Kabachnik, *et al.*,[14] for the synthesis of O,S-dialkyl alkylphosphonothioates using ethyl hydrogen methylphosphonothioate and 2-ethylthioethyl chloride in the presence of dimethylaniline yielded about 50% of the desired product containing approximately 8% of the thiono isomer on the basis of the infrared spectrum. From the refractive index of $n^{25}D$ 1.5123 the thiono isomer content of this material is calculated to be 11.9%. Otherwise, the infrared and NMR spectra of the product were identical with those of a sample of the same compound prepared by the transesterification procedure.

Experimental

Starting Materials.—Commercial 2-ethylthioethanol (Sharples) was distilled under reduced pressure. Only a small amount of a lower boiling forerun was collected while the bulk of the material had a constant boiling point of 35° at 0.120 mm. A middle cut of the distillate, $n^{25}D$ 1.4853, was analyzed

Anal. Calcd. for $C_4H_{10}OS$: C, 45.24; H, 9.49; S, 30.19. Found: C, 45.22; H, 9.68; S, 29.95.

Because of the high purity of the commercial product, distillation is unnecessary. No effect on the purity of the reaction product was noticed in parallel runs using commercial grade and distilled 2-ethylthioethanol.

Commercial trimethyl, triethyl and triisopropyl phosphite (Virginia-Carolina Chemical Corp.) were purified by distillation from metallic sodium through a 4-ft. column packed with glass helices in order to remove any dialkyl phosphonate if present.

Preparation of 2-Isopropylthioethanol.—To 500 cc. of absolute methanol in a 2-l., three-neck flask, fitted with a reflux condenser and a ball-joint stirrer, and protected from atmospheric moisture by a Drierite tube, was added 162 g. (3.0 moles) of powdered commercial sodium methoxide. The resulting solution was then treated with stirring and cooling in an ice-bath with 234 g. (3.0 moles) of 2-mercaptoethanol which was added in a thin stream from a dropping funnel over a period of 10 min. After completion of the addition, the reaction mixture was slowly heated to boiling and kept under reflux for 1 hr. before 369 g. (3.0 moles) of isopropyl bromide was added with stirring over a period of 1.5 hr. During the addition of the isopropyl bromide and for an additional period of 2 hr., the mixture was refluxed with vigorous stirring. The resulting suspension of sodium bromide was filtered by suction and the clear filtrate was distilled under reduced pressure to yield 265 g. (74%) of 2-isopropylthioethanol, b.p. 42–46° (1.5 mm.), $n^{25}D$ 1.4775.

Anal. Calcd. for $C_5H_{12}OS$: C, 49.95; H, 10.06; S, 26.67. Found: C, 49.7; H, 10.0; S, 27.06.

Preparation of Dialkyl Methylphosphonites.—A mixture of 2.1 mole equivalents each of the appropriate anhydrous alcohol and dry diethylaniline or pyridine was added dropwise with stirring to 1 mole equivalent of methylphosphonous dichloride, CH_3PCl_2,[15] b.p. 81–82°, in about 3–4 times its volume of dry diethyl ether. During the addition, the temperature of the reaction mixture was maintained by external cooling at 20–30°. Stirring of the mixture was continued for a period of 3 hr. at room temperature to ensure complete reaction. A slow stream of dry, oxygen-free nitrogen was passed through the reaction flask throughout the entire operation. The precipitated base hydrochloride was removed rapidly by filtration through a fritted glass filter with slight suction and washed with two 150-cc. portions of cold diethyl ether. The ether washings and the filtrate were combined and the solvent removed at room temperature under reduced pressure. The residual, pale yellow, liquid product was then fractionated under reduced pressure to yield the desired dialkyl methylphosphonite as a colorless mobile liquid of unpleasant odor. By this general procedure were prepared the following compounds:

Diethyl methylphosphonite, b.p. 47° (50 mm.), $n^{25}D$ 1.4168, 82% yield. *Anal.* Calcd. for $C_5H_{13}O_2P$: C, 44.11; H, 9.63; P, 22.76. Found: C, 44.5; H, 9.7; P, 22.78.

Di-*n*-propyl methylphosphonite, b.p. 76° (33 mm.), $n^{25}D$ 1.4243, 78% yield. *Anal.* Calcd. for $C_7H_{17}O_2P$: C, 51.20; H, 10.44; P, 18.87. Found: C, 50.7; H, 10.4; P, 19.17.

Diisopropyl methylphosphonite, b.p. 55° (36 mm.), $n^{25}D$ 1.4157, 79.7% yield. *Anal.* Calcd. for $C_7H_{17}O_2P$: C, 51.20; H, 10.44; P, 18.87. Found: C, 50.7; H, 10.3; P, 18.8.

Transesterification of Trialkyl Phosphites with 2-Ethylthioethanol and 2-Isopropylthioethanol.—The transesterification reactions with trialkyl phosphites were carried out by the general procedure reported previously[10] using as a catalyst 0.5 g. of metallic sodium dissolved in 1- to 2-mole batches of the mixture of the reactants.

2-Ethylthioethanol (216 g., 2 moles) and 665 g. (4 moles) of triethyl phosphite, b.p. 155–157°, $n^{25}D$ 1.4108, were heated during a period of 5 hr. from 103 to 205° with the overhead removal of 99 g. of crude ethanol, $n^{25}D$ 1.3610 (lit.[16] $n^{25}D$ 1.3594). The remaining reaction mixture was fractionated through an 8-inch packed column and yielded 360 g. (2.17 moles) of unchanged, recovered triethyl phosphite followed by 331 g. (71%) of **diethyl 2-ethylthioethyl phosphite**, colorless liquid, b.p. 95–98° (0.20 mm.), $n^{25}D$ 1.4608, d^{25}_4 1.0328.

Anal. Calcd. for $C_8H_{19}O_3PS$: C, 42.46; H, 8.46; P, 13.69; S, 14.17. Found: C, 42.39; H, 8.5; P, 14.00; S, 14.35.

Further distillation of the pot residue without a column gave 59.3 g. (21%) of **ethyl di-(2-ethylthioethyl) phosphite**, colorless oily liquid, b.p. 153–154° (0.20 mm.), $n^{25}D$ 1.4890, d^{25}_4 1.0758.

Anal. Calcd. for $C_{10}H_{23}O_3PS_2$: C, 41.94; H, 8.10; P, 10.81. Found: C, 41.6; H, 8.1; P, 10.67.

Triethyl phosphite (332 g., 2 moles) and 120 g. (1 mole) of 2-isopropylthioethanol yielded in similar manner 145 g. (61%) of **diethyl 2-isopropylthioethyl phosphite**, colorless liquid, b.p. 104–106° (3 mm.), $n^{25}D$ 1.4588, d^{25}_4 1.0164.

Anal. Calcd. for $C_9H_{21}O_3PS$: C, 44.98; H, 8.81; P, 12.89; S, 13.34. Found: C, 44.8; H, 8.7; P, 13.07; S, 13.48.

Further distillation of the pot residue gave after an intercut of 24.5 g. of a colorless distillate, b.p. 100–113° (0.80 mm.), a fraction of 16.0 g. of impure **ethyl di-(2-isopropylthioethyl) phosphite**, colorless oily liquid, b.p. 113–118° (0.80 mm.), $n^{25}D$ 1.4861.

Anal. Calcd. for $C_{12}H_{27}O_3PS_2$: C, 45.83; H, 8.66; P, 9.85; S, 20.39. Found: C, 45.1; H, 8.5; P, 10.20; S, 20.32.

The transesterification of 416 g. (2 moles) of triisopropyl phosphite, b.p. 58–61° (9 mm.), $n^{25}D$ 1.4082, with 106 g. (0.88 mole) of 2-isopropylthioethanol gave by the general procedure 120 g. (51%) of **diisopropyl isopropylthioethyl phosphite**, colorless liquid, b.p. 87–92° (0.60 mm.), $n^{25}D$ 1.4358, d^{25}_4 0.9612, and 5.0 g. of a higher boiling residue which was discarded.

Anal. Calcd. for $C_{11}H_{25}O_3PS$: C, 49.23; H, 9.39. Found: C, 49.3; H, 9.1.

2-Ethylthioethanol (106 g., 1 mole) and 372 g. (3 moles) of trimethyl phosphite, b.p. 63° (159 mm.), $n^{25}D$ 1.4063, heated slowly with stirring during 6 hr. to 128° in the presence of 0.5 g. of dissolved metallic sodium with the overhead removal of 37.5 g. of crude methanol, b.p. 65–66°, yielded upon distillation under reduced pressure 146 g. (74%) of **dimethyl 2-ethylthioethyl phosphite**, colorless liquid, b.p. 86–89° (2.0 mm.), $n^{25}D$ 1.4692, d^{25}_4 1.0898, and left 20.0 g. of distillation residue which was not further investigated.

Anal. Calcd. for $C_6H_{15}O_3PS$: C, 36.35; H, 7.63; S, 16.17; P, 15.63. Found: C, 36.7; H, 7.8; S, 15.86; P, 16.04.

Transesterification of Dialkyl Methylphosphonites with 2-Ethylthioethanol.—These transesterifications were performed by the same procedure[10] which was used for the

(14) M. I. Kabachnik, T. A. Mastryukova, N. I. Kurochkin, N. P. Rodionova and E. M. Popov, *Zhur. Obshchei Khim.*, **26**, 2228 (1956); *C. A.*, **51**, 1823*f* (1957).

(15) The methylphosphonous dichloride was prepared by the reduction of the complex $[CH_3PCl_3][AlCl_4]$ in acetonitrile with aluminum following a method described by Shell Development Co., Emeryville, California, *Final Report, Chemical Corps Contract No. DA-18-108-CML-721* (1950).

(16) L. W. Andrews, THIS JOURNAL, **30**, 357 (1908).

TABLE I

YIELDS, PHYSICAL AND ANALYTICAL DATA OF O,O-DIALKYL O-(2-ALKYLTHIOETHYL) PHOSPHOROTHIOATES (III) AND O-ALKYL O-(2-ETHYLTHIOETHYL) METHYLPHOSPHONOTHIOATES (VI)

Compound	Yield,[m] %	$n^{22}D$	d^{25}_4	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Phosphorus, % Calcd.	Phosphorus, % Found
$(C_2H_5O)_2P(S)O(CH_2)_2SC_2H_5$[a,b]	50	1.4865	1.1114	37.19	37.1	7.41	7.3	11.99	12.06
$(C_2H_5O)_2P(S)O(CH_2)_2SCH(CH_3)_2$[a,c,d]	83	1.4805	1.0853	39.69	39.9	7.77	7.9	11.37	11.46
$(i\text{-}C_3H_7O)_2P(S)O(CH_2)_2SCH(CH_3)_2$[b,e]	70	1.4678	0.9612	...	..	..	..	10.31	10.65
$(CH_3O)_2P(S)O(CH_2)_2SC_2H_5$[f,g]	94	1.4949	1.1771	31.29	31.0	6.57	6.5	13.45	13.82
$CH_3P(S)(OC_2H_5)O(CH_2)_2SC_2H_5$[f,h]	94[n]	1.5027	0.9963	36.83	36.6	7.51	7.6	13.57	13.65
$CH_3P(S)(OC_3H_7)O(CH_2)_2SC_2H_5$[f,i,l]	95	1.4973	....	39.65	39.3	7.90	8.0	...	...
$CH_3P(S)(OC_3H_7\text{-}i)O(CH_2)_2SC_2H_5$[f,k,l]	90	1.4919	....	...	..	..	..	12.78	13.14

[a] Still temperature 57°. [b] Pressure 1×10^{-4} mm. [c] Pressure 2×10^{-5} mm. [d] Calcd.: S, 23.54. Found: S, 23.28. [e] Still temperature 65°. [f] Undistilled material. [g] Calcd.: S, 27.84. Found: S, 28.21. [h] Calcd.: S, 28.08. Found: S, 28.09. [i] Calcd.: S, 26.25. Found: S, 26.04. [k] Calcd.: S, 26.25. Found: S, 25.55. [l] This material had a purity of approximately 96% calcd. on the basis of its analysis. [m] The yields given for the evaporatively distilled compounds represent the amounts of distillate obtained in a single pass through the still; the yields given for the undistilled products represent the amounts of material obtained after removal of the excess sulfur and differ from the actual quantitative yields by the mechanical losses suffered during the filtration. [n] Distillation of this material under reduced pressure gave a distillate, b.p. 88° (0.60 mm.), $n^{22}D$ 1.5080; the infrared and NMR spectra of the distillate indicated several per cent. of isomerization. *Anal.* Found: C, 36.7; H, 7.4.

TABLE II

YIELDS, PHYSICAL DATA AND ANALYSES OF THE O,O-DIALKYL S-(2-ALKYLTHIOETHYL) PHOSPHOROTHIOATES (II) AND O-ALKYL S-(2-ETHYLTHIOETHYL) METHYLPHOSPHONOTHIOATES (VII)

Compounds	Yield, %	d^{25}_4	B.p. °C.	B.p. Mm.	$n^{25}D$	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Phosphorus, % Calcd.	Phosphorus, % Found	Sulfur, % Calcd.	Sulfur, % Found
$(C_2H_5O)_2P(O)S(CH_2)_2SC_2H_5$	42	1.1269	100–105	0.025	1.4922	37.19	37.4	7.41	7.3	11.99	12.09	24.82	25.02
$(C_2H_5O)_2P(O)S(CH_2)_2SCH(CH_3)_2$	92	1.0164	96–97	.040	1.4881	39.69	40.1	7.77	7.7	11.37	11.46	23.54	23.28
$CH_3P(OC_2H_5)(O)S(CH_2)_2SC_2H_5$	53	1.1292	79–82	.050	1.5136	36.82	36.66	7.51	7.50	13.57	13.93	28.08	28.10
$CH_3P(OC_3H_7)(O)S(CH_2)_2SC_2H_5$	57	1.0875	90–100	.050	1.5020	39.65	39.3	7.90	8.0	12.78	13.16	26.46	26.20
$CH_3P(OC_3H_7\text{-}i)(O)S(CH_2)_2SC_2H_5$	89	1.0832	90–96	.500	1.4970	39.65	39.5	7.90	7.9	12.78	13.14	26.46	26.10

analogous runs employing trialkyl phosphites as the starting materials, except that a slow stream of oxygen-free nitrogen was passed through the apparatus during the entire operation; the addition of catalytic amounts of sodium was unnecessary since the reactions with the dialkyl methylphosphonites proceeded smoothly without an added catalyst.

2-Ethylthioethanol (106 g., 1.0 mole) and 136 g. (1.0 mole) of diethyl methylphosphonite yielded during a 3.5-hr. heating period 58 g. of a colorless, unpleasantly smelling distillate, b.p. 78–80°, $n^{25}D$ 1.3718, which consisted of ethanol contaminated with some of the starting methylphosphonite. A 150-g. portion of the residual reaction mixture was distilled under reduced pressure to give 52 g. (34%) of **ethyl 2-ethylthioethyl methylphosphonite**, colorless liquid, b.p. 42–43° (0.040 mm.), $n^{25}D$ 1.4782, d^{25}_4 0.9963, and 21 g. of crude di-(**2-ethylthioethyl**) **methylphosphonite (X)**, colorless liquid, b.p. 89–94° (0.025 mm.), $n^{25}D$ 1.5067, d^{25}_4 1.0675.

Anal. Calcd. for $C_7H_{17}O_2PS$: C, 42.84; H, 8.73; P, 15.79; S, 16.34. Found: C, 42.7; H, 8.8; P, 15.60; S, 16.57. Calcd. for $C_9H_{21}O_2PS_2$: C, 42.16; H, 8.26; P, 12.10; S, 25.01. Found: C, 41.8; H, 8.2; P, 12.41; S, 24.64.

***n*-Propyl 2-ethylthioethyl methylphosphonite**, b.p. 53–56° (0.025 mm.), $n^{25}D$ 1.4715, was obtained similarly in 24% yield and approximately 96% purity from di-*n*-propyl methylphosphonite. In addition to the monotransesterification product was obtained 30% of crude X, b.p. 80–84° (0.025 mm.), $n^{25}D$ 1.5063.

Anal. Calcd. for $C_8H_{19}O_2PS$: C, 45.69; H, 9.11; P, 14.73; S, 15.25. Found: C, 44.9; H, 8.9; P, 15.37; S, 14.93

The transesterification of diisopropyl methylphosphonite by the standard procedure with one mole equivalent of 2-ethylthioethanol yielded 45% of **isopropyl 2-ethylthioethyl methylpnosphonite**, colorless liquid, b.p. 42–46° (0.100 mm.), $n^{25}D$ 1.4678, and 30% of X, b.p. 78–90°, $n^{24}D$ 1.5045.

Anal. Calcd. for $C_8H_{19}O_2PS$: P, 14.73; S, 15.25. Found: P, 14.55; S, 15.46.

Addition of Sulfur to the Mixed Phosphites and Phosphonites.—To the appropriate dialkyl 2-ethylthioethyl phosphite or alkyl 2-ethylthioethyl methylphosphonite in a round-bottom flask was added with stirring with a magnetic bar over a period of approximately 5 min. two mole equivalents (100% excess) of sulfur flowers in small portions. The reaction mixture was maintained during the addition at 15–25° by occasional cooling with a Dry Ice–acetone-bath. After the sulfur addition was completed, the mixture was allowed to stand at room temperature overnight. The excess, undissolved sulfur was then removed by filtration through a sintered glass filter. The colorless or faintly pale yellow filtrate consisted of the desired sulfur addition products III or VI, respectively. Since the usual vacuum distillation of these thiono compounds resulted in a partial rearrangement to the corresponding thiolo isomers II and VII, respectively, only evaporative distillation in a falling-film molecular still at relatively low temperatures allowed purification of the thiono compounds without extensive isomerization.

The percentage yields, refractive indices and analytical data of the thionophosphites and thionophosphonites prepared by transesterification and subsequent sulfur addition are listed in Table I.

Isomerization of the O,O-Dialkyl O-(2-Alkylthioethyl) Phosphorothioates (III) and O-Alkyl O-(2-Ethylthioethyl) (VI).—Compounds III and VI were isomerized by heating under a slow stream of oxygen-free nitrogen in an oil-bath of 140–150° for a period of 3 hr. Distillation of the charge under a reduced pressure gave the corresponding isomerization products II and VII, respectively. The physical constants and analytical data of these compounds are listed in Table II.

Preparation of O-Ethyl O-(2-Ethylthioethyl) Methylphosphonothioate from Ethyl Hydrogen Methylphosphonothioate.—A solution of 179 g. (1.5 moles) of thionyl chloride in 150 cc. of dry chloroform was added dropwise with cooling over a period of 40 min. to a stirred solution of 132.5 g. of commercial 2-ethylthioethanol in 150 cc. of dry chloroform. The mixture was stirred at room temperature for an additional two hours and then allowed to stand overnight. The chloroform was removed from the reaction mixture under reduced pressure and the dark brown liquid residue was distilled to yield 123.0 g. (80%) of 2-ethylthioethyl chloride, b.p. 45–46° (5 mm.), $n^{25}D$ 1.4865.

Anal. Calcd. for C_4H_9SCl: C, 38.54; H, 7.28; Cl, 28.45; S, 25.72. Found: C, 38.7; H, 7.4; Cl, 28.7; S, 25.6.

To a solution of 62.3 g. (0.5 mole) of 2-ethylthioethyl chloride and 74.6 g. (0.5 mole) of diethylaniline in 200 cc. of

dry refluxing benzene was added over a period of 1.5 hr. with stirring 70 g. (0.5 mole) of O-ethyl hydrogen methylphosphonothioate.[17] The mixture was refluxed for an additional period of 75 min. and then allowed to stand at room temperature overnight. Stirring and refluxing was resumed again for an additional 4 hr., after which 25 cc. of water was added to the stirred mixture in order to dissolve the white crystalline material which had formed upon cooling. The upper benzene layer was separated, dried with anhydrous magnesium sulfate and Drierite and filtered, and the benzene evaporated from the filtrate under reduced pressure. Distillation of the brownish oily residue resulted in some decomposition of the distillation charge while 17.5 g. of forerun was collected between 40 and 97° at 0.2–0.3 mm. pressure; a white crystalline solid, apparently diethylaniline hydrochloride, deposited in the still-head. The distillation was interrupted, the cooled liquid residue dissolved in 80 cc. of diethyl ether and the solution washed once with 50 cc. of water. After drying with Drierite, the ether was evaporated under reduced pressure and the orange-brown, liquid residue was distilled to yield 57.0 g. (50%) of O-ethyl O-(2-ethylthioethyl) methylphosphonothioate, b.p. 108–110° (0.100 mm.), $n^{25}D$ 1.5123. The brown liquid distillation residue (5 g.) was discarded.

(17) A paper describing the preparation of this compound and of analogs and homologs is in preparation.

Anal. Calcd. for $C_7H_{17}O_2PS_2$: C, 36.82; H, 7.51; S, 28.08. Found: C, 36.4; H, 7.6; S, 28.15.

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The Use of N-Formylamino Acids in Peptide Synthesis

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Several representative peptides and peptide derivatives have been synthesized using an N-formyl blocking group and the carbodiimide coupling method. Racemization was not observed, and optimum conditions for acidic hydrolysis of the N-formyl function were determined. Similar peptide syntheses employing the mixed anhydride procedure led to extensive racemization. It has been shown that an N-formyl peptide ester can be extended on either the amino or carboxyl end by selective hydrolysis.

The N-formyl group has long been known to undergo acid hydrolysis[2] and alcoholysis[3] under conditions which generally do not rupture a peptide linkage. Although the N-formyl group has been proposed previously[4,5] it has not gained wide acceptance as an amino protective function. Two major disadvantages of the use of an N-formylamino acid in peptide synthesis are tendency toward racemization, characteristic of many acylamino acids under treatment which may lead to azlactone formation, and instability toward reagents conventionally employed to "activate" the carbonyl function for coupling.[6] With the advent of the carbodiimide method of peptide bond formation,[7] which operates under exceptionally mild conditions, it becomes feasible to employ sensitive and labile blocking groups, including the N-formyl function.

In this communication, using N-formylamino acids, a comparison has been made between two of the recent methods of amide bond formation, namely, the mixed carbonic anhydride[8,9] and the carbodiimide procedures. Also, conditions for the removal of the N-formyl group have been studied carefully.

An N-formyl group can be introduced readily without racemization of the parent amino acids by formylation in the presence of acetic anhydride, using a modification of the procedure of du Vigneaud.[10] For the solvolysis studies of N-formyl derivatives, simple DL-formyl peptides including formyl-DL-valine anilide, formyl-DL-phenylalanine amide, racemic formylvalylphenylalanine and its methyl ester were prepared from the corresponding formyl-DL-amino acids *via* the mixed carbonic anhydride procedure. The solvolytic conditions employed are considerably milder than those reported previously, but the actual time required for complete deformylation varies with different amino acids. In general, an N-formyl group can be removed smoothly by treatment of the formyl peptide with a slight excess of 0.5 *N* hydrochloric acid in methanol (or in water–dioxane if the peptide free acid is used) at room temperature for a period of 48 hours or at reflux temperature for one hour. The yields are high, ranging from 80–95%.

In order to test the tendency toward racemization of the mixed carbonic anhydride procedure as applied to N-formylamino acids, we chose to prepare acetylphenylalanylglycine anilide for comparison with the optically pure product obtained previously.[11] The mixed carbonic anhydride derived from formyl-L-phenylalanine and ethyl chloroformate was treated with glycine anilide. The product N-formylphenylalanylglycine anilide had a

(1) Aided by a Contract from the Office of Naval Research, Washington, D. C.

(2) E. Fischer and O. Warburg, *Ber.*, **38**, 3997 (1905).

(3) T. Curtius, *ibid.*, **16**, 753 (1883).

(4) A. Hillmann and G. Hillmann, *Z. Naturforsch.*, **6B**, 340 (1951).

(5) S. G. Waley, *Chemistry & Industry*, 107 (1953).

(6) For example, F. R. King, J. W. Clark-Lewis, D. D. A. Kidd and G. R. Smith, *J. Chem. Soc.*, 1039 (1954), reported very low yields using an azide method of coupling; and unpublished results in this Laboratory indicate substantial decomposition of many N-formylamino acids by interaction with such reagents as thionyl chloride and phosphorus pentachloride.

(7) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(8) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(9) J. R. Vaughan, Jr., THIS JOURNAL, **73**, 3547 (1951).

(10) V. du Vigneaud, R. Dorfmann and H. S. Loring, *J. Biol. Chem.*, **98**, 577 (1932).

(11) J. C. Sheehan, D. W. Chapman and R. W. Roth, THIS JOURNAL, **74**, 3822 (1952).