TABLE III

Conditions for coupling	Yield, %	M.p., °C.	$[\alpha]^{25}D$ (*c* 5, methanol)
(1) 60° for 5 minutes	84	130–132	−35.1°
(2) 25° for 48 hours	79	132–133	−36.1
(3)[a] 90° for 10 minutes	75	131.5–132.5	−35.5

[a] Triethylamine hydrochloride was not removed.

19. **Ethyl Carbobenzoxyglycyl-L-phenylalanylglycinate.**[15]—Carbobenzoxyglycyl-L-phenylalanine[16] (1.78 g.) was dissolved in 50 cc. of benzene containing 0.6 g. of triethylamine. To this solution 0.88 g. of *o*-phenylene chlorophosphite was added and, after filtration, the solution was refluxed for 15 minutes with 0.5 g. of distilled ethyl glycinate.[14] The solution was cooled, 25 cc. of ethyl acetate was added to facilitate separation, and the organic layer was washed as usual. The residual oil after concentration was dissolved in ethanol to make a 2% solution and was seeded with *rac*-19. After storage at 5° for 2 hours, nothing crystallized and the solution was again concentrated. The residue rapidly solidified upon the addition of anhydrous ether (10 cc.) and 2.03 g. (92.5%) of 19 was obtained; m.p. 115–118°, $[\alpha]^{25}D$ −11.5° (*c* 2, ethanol). Recrystallization from 20 cc. of ethyl acetate–petroleum ether gave 1.85 g. (84%), m.p. 116–118°, $[\alpha]^{25}D$ −12.0° (*c* 2, ethanol). Subsequent recrystallization did not change the rotation. This work-up procedure permits the separation of any racemic tripeptide from the mixture.

Stability of Mixed Anhydrides.—Benzoic acid (2.44 g.) was dissolved in 50 cc. of benzene containing 2.02 g. of triethylamine; the solution then was treated with 3.5 g. of *o*-phenylene chlorophosphite. This solution was allowed to remain for 24 hours at 35°, after which time it was washed with 50 cc. of saturated sodium bicarbonate solution. Acidification of the bicarbonate extract afforded 2.0 g. (83%) of benzoic acid.

Benzoic anhydride (2.26 g.) when dissolved in 50 cc. of benzene and treated with 50 cc. of saturated sodium bicarbonate was quite stable, only 0.05 g. (2%) of benzoic acid being isolated. On the basis of this experiment, not more than 17% of the symmetrical anhydride could have been formed.

The mixed anhydride between phthaloylglycine and *o*-phenylene chlorophosphite, after standing for 2 days at 30°, deposited 20% of the symmetrical anhydride; m.p. 230–233°, reported[4] 240–241°.

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(15) Paper 3 in this series, THIS JOURNAL, **74**, 5309 (1952), reports m.p. 117–118°, $[\alpha]^{25}D$ −12.3° (*c* 2, ethanol).

(16) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940).

[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES, AMERICAN CYANAMID COMPANY]

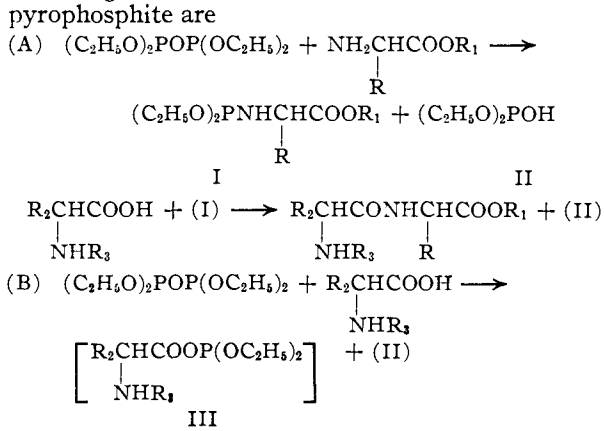
Tetraethyl Pyrophosphite as a Reagent for Peptide Syntheses[1]

BY GEORGE W. ANDERSON, JACK BLODINGER AND ALICE D. WELCHER

RECEIVED MARCH 3, 1952

Tetraethyl pyrophosphite has been found to be a useful reagent for making peptide derivatives. It is most conveniently used by adding it to a mixture of the amine (amino acid or peptide ester) and the acid (carbobenzoxy- or phthaloylamino acid or peptide), but may first react with either. No racemization of optically active compounds has been observed when one of the reactants is the acylamino acid. The synthesis of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate from the acyldipeptide and ethyl glycinate was found to give partial racemization under certain conditions.

In the first two papers of this series,[1] it was shown that a diester chlorophosphite such as diethyl chlorophosphite can be used as reagent for forming a peptide link by reaction with either the amino function or the carboxylic function to form a phosphite amide or a phosphite anhydride, and this in turn reacts with the other function. We have now found that tetraethyl pyrophosphite can be used in place of the chlorophosphite with certain advantages. The reactions as written for the pyrophosphite are



(A) $(C_2H_5O)_2POP(OC_2H_5)_2 + NH_2CHCOOR_1 \longrightarrow (C_2H_5O)_2PNHCHCOOR_1 + (C_2H_5O)_2POH$ (R; I; II)

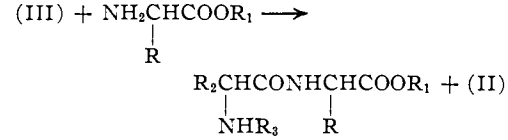
$R_2CHCOOH + (I) \longrightarrow R_2CHCONHCHCOOR_1 + (II)$ (NHR_3; NHR_3, R)

(B) $(C_2H_5O)_2POP(OC_2H_5)_2 + R_2CHCOOH \longrightarrow$ (NHR_3)

$\left[R_2CHCOOP(OC_2H_5)_2 \mid NHR_3\right]$ + (II)

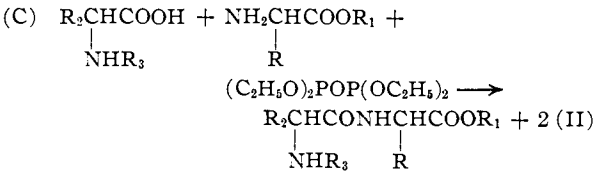
III

(1) Third paper in a series on phosphorus derivatives. See THIS JOURNAL, **74**, 5304 (1952) for previous papers; also presented in part at the September, 1951, Meeting of the American Chemical Society.



$(III) + NH_2CHCOOR_1 \longrightarrow R_2CHCONHCHCOOR_1 + (II)$ (R; NHR_3, R)

For convenience, (A) will be called the "amide" procedure and (B) the "anhydride" procedure. It has been found most convenient to add the reagent to a mixture of the acid and the amine, as indicated by Equation (C).



(C) $R_2CHCOOH + NH_2CHCOOR_1 + (C_2H_5O)_2POP(OC_2H_5)_2 \longrightarrow R_2CHCONHCHCOOR_1 + 2\,(II)$ (NHR_3; R; NHR_3, R)

This will be called the "standard" procedure. The validity of the first equations of (A) and (B) when tetraethyl pyrophosphite is used was shown by characterization of both products of a reaction using the (A) procedure and of diethyl phosphite from a (B) reaction. In the latter case, the anhydride was indirectly characterized by reaction with aniline to form a known anilide (see Experimental). When an amine hydrochloride is used, it is usually advantageous to add an equivalent of triethylamine, although this is not necessary.

TABLE I

PREPARATION OF PEPTIDE DERIVATIVES BY THE "STANDARD" PROCEDURE[a]

Product[b]	Variations from general description in experimental	Recryst. solvent	M.p., °C. (cor.)	Yield, %	$[\alpha]^{23-25}D$
Z·Gly-Phe·OEt(DL)	15 min. heating	None	88–89[c]	94	
Z·Gly-Phe·OEt(DL)	20 hr. at 25–30°	None	88–89	74	
Ph·Gly-Leu·OEt(L)	H·Leu·OEt·HCl + Et_3N used	None	143–145[d]	68	
Ph·Gly-Leu·OEt(L)	H·Leu·OEt·HCl + Et_3N and 50% excess pyrophosphite used	EtOH–H_2O	144–145[d]	78	−26.5° (*c*, 2, EtOH)
Ph·Gly-Leu·OEt(L)	H·Leu·OEt·HCl and 50% excess pyrophosphite used	None	140–143	80	
Z·Gly-Tyr·OEt(L)	60 min. heating	EtOH	126–127[e]	65	+19.2 (*c*, 5, EtOH)
Z·Leu-Gly·OEt(L)	H·Gly·OEt·HCl and 100% excess pyrophosphite used, 15 min. heating	50% EtOH	102–103[f]	56	−27.2 (*c*, 5, EtOH)
Z·Leu-Gly·OEt(L)	H·Gly·OEt·HCl + Et_3N used	50% EtOH	101–102	58	
Z·Leu-Tyr·OEt(L–L)	None	EtOAc–pet. ether	112–114[g]	40	−14 (*c*, 5, EtOH)
Z·Val-Ala·OEt(DL–DL)	60 min. heating	EtOAc–pet. ether	109–111[h]	60	
Z·Val-Phe·OEt(DL–DL)	No excess pyrophosphite, 60 min. heating	EtOAc–pet. ether, ether	138–140[i]; 105–107[j]	11; 37	
Z·Gly-Gly-Gly·OEt	H·Gly-Gly·OEt·HCl + Et_3N used	EtOH–H_2O	167–168[k]	78	
Z·Tyr-Gly-Gly·OEt(L)	H·Gly-Gly·OEt·HCl + Et_3N used	EtOH–H_2O	165–167[l]	47	+ 6.5 (*c*, 2, HOAc)
Z·Gly-Phe-Gly-Gly·OEt(DL)	H·Gly-Gly·OEt·HCl + Et_3N used	EtOH–H_2O	174–176[m]	25	
Z·Gly-Leu-Gly·OMe(L)	H·Gly·OMe used	EtOAc–pet. ether	132–133[n]	61	−34.9 (*c*, 5, EtOH)

[a] Carbobenzoxy- or phthaloylamino acids were treated with distilled amino acid esters with exceptions noted. [b] The Brand abbreviation system is used. *Cf.* Erlanger and Brand, THIS JOURNAL, **73**, 3509 (1951). [c] H. Neurath, *et al.*, *J. Biol. Chem.*, **170**, 221 (1947), give m.p. 90–91° (cor.). [d] J. R. Vaughan, Jr., and R. L. Osato, THIS JOURNAL, **74**, 676 (1952), reported m.p. 143–145° and $[\alpha]^{21}D$ −28.5° (*c*, 2, EtOH). [e] Ref. *d* gives m.p. 124–125°, $[\alpha]^{24}D$ +19.8° (*c*, 5, EtOH). [f] Ref. *d* gives m.p. 104–105°, $[\alpha]^{24}D$ −25.6° (*c*, 5, EtOH). [g] Ref. *d* gives m.p. 116–118°, $[\alpha]^{24}D$ −14.9° (*c*, 10, EtOH). [h] Ref. *d* gives m.p. 111–113°. [i] Ref. *d* gives m.p. 138–140°. [j] Calcd. for $C_{24}H_{30}N_2O_5$: C, 67.6; H, 7.09; N, 6.6. Found: C, 67.5; H, 7.2; N, 6.7. [k] J. S. Fruton, *et al.*, *J. Biol. Chem.*, **173**, 467 (1948), give m.p. 165°. [l] M. Bergmann and J. S. Fruton, *ibid.*, 118, 413 (1937), give m.p. 165°. [m] Ref. *d* gives m.p. 172–174°. [n] No DL-form was isolated. Paper (2) [ref. (1)] gives $[\alpha]^{25}D$ −36.1° (*c*, 5, MeOH) and m.p. 132–133°.

The principal advantages of tetraethyl pyrophosphite over diethyl chlorophosphite are that it is simpler to use, it can be obtained initially in a pure form by distillation, and its purity can be checked readily from time to time by its refractive index. It can also be used in the peptide formation without a solvent.

Diethyl phosphite is a good solvent for the peptide-forming reaction. Since it is miscible with water and ordinary organic solvents, the product can be precipitated by the addition of one of these. In general, water is satisfactory.

Some of the peptide derivatives prepared by the use of tetraethyl pyrophosphite according to the "standard" procedure are listed in Table I. The optimum conditions for each compound have not been worked out. Usually 15 to 30 minutes heating on a steam-bath are adequate, but longer heating may be desirable in some cases. Use of a large excess of the pyrophosphite is occasionally advantageous. Several compounds were prepared in essentially the same yields by the "standard" procedure (C), by the "amide" procedure (A), and by the "anhydride" procedure (B). It is to be expected that special cases will arise when one procedure will give better results than others.

Examples which were not suitable for Table I are described in the Experimental section. An improved preparation of tetraethyl pyrophosphite is also given.

No racemization of optically active compounds has been observed in the cases where one of the reactants was an acylamino acid. Comparison of rotations of several of these peptide derivatives with those of samples prepared by other methods disclosed no differences.

In a case where one reactant is an acyldipeptide such as carbobenzoxyglycyl-L-phenylalanine, the

TABLE II

RACEMIZATION IN THE REACTION OF CARBOBENZOXYGLYCYL-L-PHENYLALANINE WITH ETHYL GLYCINATE USING TETRAETHYLPYROPHOSPHITE[a]

Reaction	Procedure	Yields of the tripeptide deriv., % DL-Form	% L-Form	M.p. of DL-form,[b] °C.
1	Standard	..	74	
2	Amide	..	73	
3	Anhydride	17	34	130–131
4	Standard[c]	28	39	130–131
5	Amide[c]	21	35	124–130
6	Anhydride[c]	46	Trace	129–131
7	Standard[d]	27	Trace	130–131
8	Standard[e]	22	46	128–131

[a] A 10% excess of tetraethyl pyrophosphite was used in all except reaction (4). [b] M.p. of L-form was 117–118° (cor.) in every case. J. R. Vaughan, Jr., and R. Osato, THIS JOURNAL, **73**, 5555 (1951), report m.p. 132–133° for the DL-form. [c] Ethyl glycinate hydrochloride plus an equivalent of triethylamine was used in place of ethyl glycinate. [d] Ethyl glycinate hydrochloride was used in place of ethyl glycinate. [e] Ethyl glycinate hydrobromide plus an equivalent of triethylamine was used in place of ethyl glycinate.

possibilities of racemization are greater. Azlactone formation can occur,[2] and also the hydrogen on the assymmetric carbon is more easily ionized if an anhydride such as (III) is an intermediate in the peptide-forming reaction. It was found that the reaction of carbobenzoxyglycyl-L-phenylalanine with ethyl glycinate gave varying amounts of racemization when reaction conditions were changed (Table II). No racemization was found when distilled ethyl glycinate was used in the "standard" and "amide" procedures, and the most occurred when ethyl glycinate hydrochloride plus triethylamine was used by the "anhydride" procedure. It appears that both the heating of the mixed anhydride and the presence of a hydrochloride may cause racemization. A test indicated that triethylamine hydrochloride does not racemize the tripeptide product under the conditions of the reaction.

Experimental[3]

Tetraethyl Pyrophosphite.[4]—To a solution of 138 g. (1 mole) of diethyl phosphite[5] and 101 g. (1 mole) of triethylamine (redistilled) in 200 cc. of benzene (C.P.) at 0° there was added rapidly (10 minutes) with stirring 223 g. of diethyl chlorophosphite[1] (70% pure, equivalent to 1 mole of 100% diethyl chlorophosphite as determined by Volhard chlorine analysis) dissolved in 200 cc. of benzene (C.P.). The mixture was cooled for an additional 15 minutes and then filtered under nitrogen to remove triethylamine hydrochloride, and the latter was washed with 200 cc. of benzene. Distillation of the benzene solutions under vacuum gave a fraction; b.p. 79–81° (0.15 mm.), $n^{26}D$ 1.4313, d^{20} 1.053, yield 41%. It has been found that a bottle of the pyrophosphite may be opened a number of times for pipetting samples without a great drop of refractive index. Since water reacts to produce diethyl phosphite, mixtures of diethyl phosphite and tetraethyl pyrophosphite were made, and the refractive indices measured. Found: N (mol fraction of the pyrophosphite) 0.00, $n^{26}D$ 1.4050, N 0.21, n 1.4128, N 0.35, n 1.4160; N 0.52, n 1.4220; N 0.62, n 1.4235; N, 0.68, n 1.4258; N 1.00, n 1.4314. A curve plotted from these figures indicated that $n^{26}D$ 1.4300 would correspond to 93% of the pyrophosphite by weight. Consequently, the "standard" procedure (below) for the peptide reaction, which uses 10% excess of the pyrophosphite by weight, assures an adequate amount of this reagent if the $n^{26}D$ is 1.4300 or higher.

"Standard" Procedure for Peptide Derivatives.—The carbobenzoxy- or phthaloylamino acid or peptide (0.010 mole) and the amino acid ester or peptide ester (0.010 mole) were added to 7 cc. (0.050 mole) of diethyl phosphite. Usually the reactants partially dissolved. If an ester hydrochloride was used, triethylamine (0.011 mole) was added in some cases. Then tetraethyl pyrophosphite (2.84 g., 0.011 mole) was added; occasionally some warming would occur. The mixture was heated on a steam-bath for 30 minutes and in most cases complete solution occurred. Water (25–50 cc.) was added to precipitate the peptide derivative. In a number of cases the mixture was tested and found to be acidic. After cooling in ice-water, the product was separated and washed with sodium bicarbonate solution (usually 10 cc. of 5%). Many of the oily products crystallized during this treatment. Following water washing (2 × 5 cc.), the crude peptide derivative was dried; in some cases it was crystallized directly from alcohol–water. Suitable recrystallization followed. Frequently, acidification of the bicarbonate wash precipitated unreacted acid; material balance with the product usually ran 80 to 95%.

"Amide" and "Anhydride" Procedures.—In the "amide" procedure, the amino acid ester or peptide ester hydrochloride was added to the diethyl phosphite followed by triethylamine and tetraethyl pyrophosphite. If a distilled amino acid ester was used, the triethylamine was omitted After heating about 2 minutes on a steam-bath, the acylamino acid or acylpeptide was added and heating continued for 30 minutes, followed by workup as in the "standard" procedure. In the "anhydride" procedure, the acylamino acid or acylpeptide was first treated with the tetraethyl pyrophosphite by heating about 2 minutes in diethyl phosphite, followed by addition of the amino acid ester (plus triethylamine if a hydrochloride was used). The subsequent treatment was then the same as in the "standard" procedure. Specific examples of these modifications are given below.

Ethyl Carbobenzoxyglycylphenylalanylglycinate. Method A. (Also See Table II).—Carbobenzoxyglycyl-L-phenylalanine, m.p. 123–125°, $[\alpha]^{22}D$ +38 2° (c 5, alcohol),[6] and distilled ethyl glycinate[7] reacted in 0.010-mole quantities in 7 cc. of diethyl phosphite by the above general procedures; in three examples, ethyl glycinate hydrochloride plus an equivalent of triethylamine were used in place of the distilled ethyl glycinate. After the reaction was complete, the product was precipitated by dilution of the solution with 50 cc. of water Following washing with two 10-cc. portions of 5% sodium bicarbonate and 10 cc. of water and drying, the crude material was taken up in enough boiling absolute alcohol to make a 2% solution, treated with Darco, filtered hot and chilled in a refrigerator for an hour. The DL-form crystallized here, and was separated. Concentration of the filtrate to dryness caused crystallization of the L-form; this was washed with anhydrous ether. From the various reactions, the $[\alpha]^{22-26}D$ was in the range −11° to −12° ± 0.5° (c 2, alcohol), and the m.p. consistently 117–118°. Repeated recrystallization in several cases did not appreciably change the m.p. or specific rotation. These properties are essentially the same as for the analyzed sample from method B. The effect of triethylamine hydrochloride on ethyl carbobenzoxyglycyl-L-phenylalanylglycinate obtained in these reactions was tested by heating equivalents in diethyl phosphite for 30 minutes on a steam-bath and working up as usual; 83% of the L-form was recovered, and a trace of DL-form. The latter could have been present at the beginning.

Method B.—Ethyl L-phenylalanylglycinate hydrobromide was prepared by dissolving 3.84 g. (0.010 mole) of ethyl carbobenzoxy-L-phenylalanylglycinate[8] in 30 cc. of a 1 N solution of hydrogen bromide in acetic acid and heating on a steam-bath until carbon dioxide evolution ceased. Dilution of the solution with 200 cc. of anhydrous ether and chilling yielded 3.15 g. (95% of theory) of the crude hydrobromide, m.p. 125–131°. This was heated to a gentle boil in 20 cc. of hot 1 N hydrogen bromide in absolute ethanol for 5 minutes to re-esterify small amounts of L-phenylalanylglycine, and the pure ester hydrobromide was caused to crystallize by the addition of 125 cc. of anhydrous ether: yield 2.72 g. (82% over-all) of small, shiny plates; m.p. 135–136°, $[\alpha]^{24}D$ +40° (c 2, water). *Anal.* Calcd. for $C_{13}H_{19}BrN_2O_3$: C, 47.1; H, 5.8; N, 8.5. Found: C, 47.4; H, 5.9; N, 8.2.[9] This compound reacted with carbobenzoxyglycine in 0.010-mole quantities by the "standard" procedure, using an equivalent of triethylamine and a 10% excess of tetraethylpyrophosphite and heating on a steam-bath in 7 cc. of diethyl phosphite for 30 minutes. The workup was the same as in method A. No DL-form of ethyl carbobenzoxyglycylphenylalanylglycinate was obtained, and concentration of the alcohol solution and washing of the residual crystals with anhydrous ether gave 59% of the theoretical

(2) H. E. Carter, *Org. Reactions*, **3**, 198 (1946).

(3) Microanalyses were obtained under the direction of J. A. Kuck of these laboratories. Melting points are corrected and were taken on a Fisher–Johns block.

(4) Previously best prepared from sodium diethyl phosphite and diethyl chlorophosphite by A. E. Arbusov and B. A. Arbusov, *Ber.*, **65**, 195 (1932).

(5) We wish to thank the Victor Chemical Company and the Virginia-Carolina Chemical Corporation for generous samples of this chemical.

(6) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940).

(7) E. Fischer, *Ber.*, **34**, 436 (1901).

(8) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 414 (1937).

(9) This convenient method of removing a carbobenzoxy group was developed from an analogous use of hydrogen iodide in acetic acid by E. Waldschmidt-Leitz and K. Kühn (*Ber.*, **84**, 381 (1951)). It has been used in these laboratories for the removal of carbobenzoxy groups from carbobenzoxypeptides as well as their esters. The hydrobromides obtained were in general more readily crystallized and less likely to be hygroscopic than analogous hydrochlorides. They have an advantage over hydriodides in being less sensitive to discoloration.

yield of the L-form, m.p. 116–118°, $[\alpha]^{24}D$ −12.3° (*c* 2, ethanol). Recrystallization from alcohol-water gave m.p. 117–118°, $[\alpha]^{24}D$ −12.3° (*c* 2, ethanol). *Anal.* Calcd. for $C_{23}H_{27}N_3O_6$: C, 62.6; H, 6.2; N, 9.5. Found: C, 62.6; H, 6.2; N, 9.7.

Ethyl Carbobenzoxyglycyl-DL-phenylalaninate.—In addition to the examples in Table I, a number of experiments were done. It was found that use of 0.010-mole quantities of carbobenzoxyglycine, ethyl DL-phenylalaninate hydrochloride and tetraethyl pyrophosphite in 7 cc. of diethyl phosphite gave a 69% yield of the peptide derivative after 15 minutes heating on a steam-bath and 65% after 30 minutes heating. Use of 10% excess pyrophosphite gave 66% on heating 25 minutes, 28% when reacting at room temperature for 18 hours and 26% when reacting at room temperature for 120 hours. When a 50% excess of the pyrophosphite was used and the reaction mixture heated for 20 minutes, the yield was 85%. Thus, results with the amine hydrochloride are not quite as good as with the free base. The use of 0.010 mole of the free base with 0.010 mole quantities of the acid and the pyrophosphite, and heating 30 minutes in 7 cc. of diethyl phosphite, gave a yield of 91%. A similar reaction by the "amide" procedure wherein the DL-phenylalanine ester was heated to 90° with the pyrophosphite in diethyl phosphite, then the carbobenzoxyglycine added and all heated 30 minutes, yielded 81%. A reaction by the "anhydride" procedure, wherein the carbobenzoxyglycine and the pyrophosphite were heated in diethyl phosphite to 90°, then the phenylalanine ester added and all heated 30 minutes, yielded 79% of the dipeptide derivative. Reaction of 0.010-mole quantities of the acid, the amine and the pyrophosphite in 15 cc. of refluxing benzene for 30 minutes gave a 90% yield; a similar reaction without any solvent by heating on a steam-bath for 20 minutes yielded 91% of the dipeptide derivative.

Ethyl Carbobenzoxyglycyl-L-tyrosinate.—A number of reactions were run, comparable to those with ethyl DL-phenylalaninate. Heating for 15 minutes gave about the same yield as 60 minutes heating (see Table I). Use of 25% excess of tetraethyl pyrophosphite and 15 minutes heating gave a 71% yield. The "amide" procedure, wherein the ethyl L-tyrosinate was heated with a 10% excess of the pyrophosphite in 7 cc. of diethyl phosphite for 5 minutes on a steam-bath before the carbobenzoxyglycine was added and heated 15 minutes, yielded 63%, and a similar "anhydride" procedure gave 67%.

Ethyl Carbobenzoxy-L-tyrosylglycylglycinate.—Several attempts to improve the yield were made. Use of 150% excess of the pyrophosphite and 20 minutes heating increased the yield 5% over the standard procedure (see Table I). Other variations, such as longer heating with 50 to 150% excess of the pyrophosphite, or use of a 50% excess of either the acid or the amine with a 100% excess of the pyrophosphite, gave crude yields in the 50–65% range. Perhaps significant is that the "amide" procedure with a 10% excess of the pyrophosphite gave a 61% crude yield, m.p. 155–160°, and a comparable "anhydride" procedure yielded 41%, m.p. 155–160°. Acidification of the bicarbonate wash from the "amide" procedure yielded 20% of crude carbobenzoxy-L-tyrosine (presumed) as an oil; a similar oil in 41% recovery was obtained from the "anhydride" reaction. A repetition of the "amide" procedure with a 50% excess of the pyrophosphite gave 63% of the crude peptide derivative and a 29% recovery of the crude acid.

Diethyl Phosphite Amide of Ethyl DL-Phenylalaninate.—Tetraethyl pyrophosphite (10.38 g., 0.040 mole) and ethyl DL-phenylalaninate (freshly prepared from the hydrochloride and distilled; 7.72 g., 0.040 mole) were mixed, with no noticeable reaction. Fractionation through a 3-inch Vigreux distilling head yielded 5.30 g. (72%) of diethyl phosphite, b.p. 65–70° (10 mm.), $n^{27}D$ 1.4073[10]; 3.49 g. of an intermediate fraction, b.p. to 140° (1 mm.); and 7.61 g. (61%) of the phosphite amide, b.p. 140–150° (1 mm.), $n^{25}D$ 1.4952; refractionation gave 6.20 g. (50%) of the latter; b.p. 140–150° (1 mm.), $n^{28}D$ 1.4920.[11]

Reaction of Carbobenzoxyglycine with Tetraethyl Pyrophosphite.—Mixture of 0.050-mole quantities yielded a solution which was then distilled at 2 mm. from a water-bath, yielding 4.09 g. (59% yield) of diethyl phosphite, b.p. 50–52°, $n^{29}D$ 1.4042. The residue, presumably the mixed anhydride, was treated with 0.050 mole of aniline for an hour at room temperature. Distillation yielded a liquid with $n^{30}D$ 1.4520, indicating a mixture of diethyl phosphite and aniline. The residue was treated with alcohol-water, then 5% sodium bicarbonate, leaving 9.53 g. (67% of the theoretical) of crude carbobenzoxyglycylanilide, m.p. 135–140°. Recrystallization from alcohol gave 5.18 g. (36%), m.p. 145–146°, which did not depress the m.p. of an authentic sample.[1]

(10) Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 202, lists b.p. 72° (10 mm.), $n^{22}D$ 1.4080.

(11) $n^{25}D$ 1.4917 from a preparation through diethyl chlorophosphite. See paper 1, ref. 1.

STAMFORD, CONNECTICUT

[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

Some Alkyl Thiazolephosphonates

BY NORMAN D. DAWSON[1] AND ALFRED BURGER

RECEIVED MAY 26, 1952

The synthesis of ethyl esters of 2-amino-4-methyl- and 4-methylthiazole-5-phosphonic acids from diethyl phosphonate substituted aliphatic bromo carbonyl compounds is described.

The effect of thiazole-5-carboxylic acid and of thiazole-5-sulfonic acid on the multiplication of bacteria has been the subject of numerous investigations.[2–9] We are now reporting the synthesis of two esters of methyl- and amino-substituted thiazole-5-phosphonic acids which have been designed for comparable biological studies.

The routes to these compounds led through diethyl 1-halogeno-2-oxoalkanephosphonates which were cyclized to the substituted diethyl thiazole-5-phosphonates by condensation with thiourea or thioformamide. For the synthesis of diethyl 4-methylthiazole-5-phosphonates, chloroacetone (I) was allowed to react with triethyl phosphite (II) and after completion of the two-stage reaction the resulting diethyl acetylmethanephosphonate (III) was brominated. Treatment of diethyl 1-bromo-2-oxopropanephosphonate (IV) with thioformamide gave diethyl 4-methylthiazole-5-phosphonate (V) while the reaction of the same bromo ketone with thiourea furnished diethyl 2-amino-4-methylthiazole-5-phosphonate (VI).

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