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

The Use of Phosphite Amides in Peptide Syntheses[1]

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RECEIVED MARCH 3, 1952

A synthesis of peptide derivatives by the reaction of diester phosphite amides of amino acid esters or peptide esters with carbobenzoxyamino acids or phthaloylamino acids is reported. This new amide synthesis differs from the usual methods in that the intermediate is a reactive derivative of the amino moiety rather than the carboxylic fragment.

The general methods of peptide synthesis in use involve lengthening the chain by the reaction of carboxy derivatives (halides, azides, anhydrides) with an amino group

$$\begin{matrix} R_1CHCOX \\ | \\ NHR_2 \end{matrix} + \begin{matrix} H_2NCHR_4 \\ | \\ COOR_3 \end{matrix} \longrightarrow \begin{matrix} R_1CHCONHCHR_4 \\ | \quad\quad | \\ NHR_2 \quad COOR_3 \end{matrix} + HX$$

Since none of these methods is entirely satisfactory, the possibility of finding amine derivatives which would react with a carboxylic group was investigated

$$\begin{matrix} R_1CHCOOH \\ | \\ NHR_2 \end{matrix} + \begin{matrix} R_5NHCHR_4 \\ | \\ COOR_3 \end{matrix} \longrightarrow \begin{matrix} R_1CHCONHCHR_4 \\ | \quad\quad | \\ NHR_2 \quad COOR_3 \end{matrix} + R_5OH$$

Reactive amine derivatives which are stable, crystalline solids would be most desirable. This paper reports the use of reactive phosphite derivatives; although these are not crystalline, they are stable.

Grimmel, Guenther and Morgan[2] have prepared amides by the use of phosphazo derivatives according to equation (1). We have extended their work

$$RN{=}P{-}NHR + 2\ R'COOH \longrightarrow 2\ R'CONHR + HPO_2 \quad (1)$$

to the preparation of carbobenzoxy- and phthaloylamino acid anilides, and to the preparation of a (presumed) phosphazo derivative of ethyl DL-phenylalaninate. The latter was successfully combined with carbobenzoxyglycine to form the dipeptide derivative, but in poor yield.[3] A better yield was obtained when ethyl DL-phenylalaninate hydrochloride, carbobenzoxyglycine and triethylamine were mixed in toluene and phosphorus trichloride was then added.[4] However, this reaction might well proceed in other ways.

In order to obtain derivatives of lower molecular weight, we investigated phosphite esters of the type $(RO)_2PNHR_2$. Diethyl anilinophosphite, one of two compounds of this type recently reported,[5] was found to react readily with carbobenzoxyamino acids and phthaloylamino acids to form the carboxanilides in good yields and without destruction of optical activity.

The preparation of a number of diester phosphite amides of amino acid esters and of one dipeptide ester is reported in the Experimental section. In general, those of lower molecular weight were oils which were distilled at reduced pressures. The higher molecular weight phosphite amides were non-distillable oils, gums or low-melting solids. None has been successfully crystallized. Several of these compounds have been kept in a refrigerator for several months without apparent change in appearance or reactivity. They are decomposed by water.

The use of phosphite amides for the synthesis of peptide derivatives is illustrated by the example of equation (2).

$$C_6H_5CH_2OCONHCH_2COOH + (C_2H_5O)_2PNHCHCOOC_2H_5 \longrightarrow \begin{matrix} C_6H_5CH_2 \\ | \\ C_6H_5CH_2OCONHCH_2CONHCHCOOC_2H_5 \end{matrix} + \begin{matrix} C_6H_5CH_2 \\ | \\ (C_2H_5O)_2POH \end{matrix} \quad (2)$$

In general, best results were obtained by heating in an inert solvent such as toluene for periods of 15 minutes to an hour. Yields of the peptide derivatives are given in the Experimental section. The formation of diethyl phosphite has not actually been demonstrated experimentally.

Two experiments showed that at least some dipeptides can be formed under comparable experimental conditions by simply heating carbobenzoxyamino acids with amino acid esters in an inert solvent. The reactions are slower than those with comparable phosphite amino acid esters, and diketopiperazines are formed as by-products by the interaction of 2 molecules of amino acid ester.

The application of the use of phosphite amides for the preparation of simple carboxylic acid amides is illustrated by three examples (see Experimental).

Three experiments with diester phosphate amides and one with a diester thiophosphate amide showed that these will not react with carboxylic acids to form carboxamides under conditions where diester phosphite amides react readily.

Experimental[6]

Chlorophosphites.—Diethyl chlorophosphite was prepared by the method of Cook, *et al.*,[5] from phosphorus trichloride,

(1) First paper in a series on phosphorus derivatives. A preliminary publication appeared in THIS JOURNAL, **73**, 501 (1951); also presented in part at the September, 1951, Meeting of the American Chemical Society.

(2) H. W. Grimmel, A. Guenther and J. F. Morgan, THIS JOURNAL, **68**, 539 (1946).

(3) The application of the Grimmel, *et al.*, reaction to peptide syntheses has been independently proposed, with no experimental evidence, by St. Goldschmidt, *Z. angew. Chem.*, **62**, 538 (1950).

(4) O. Süs, *Ann.*, **572**, 96 (1951), has recently reported reactions of this type.

(5) H. G. Cook, *et al.*, *J. Chem. Soc.*, 2921 (1949).

(6) All melting points were taken on a Fisher–Johns block and are corrected. C, H, N and P analyses were obtained by our Microanalytical Laboratory under the direction of J. A. Kuck.

ethyl alcohol and diethylaniline in ether: b.p. 56–57.5° (30 mm.), $n^{25}D$ 1.4342; calcd. 22.7 Cl, found 22.7 Cl by Volhard titration. The average yield of a number of preparations was 50%. Samples kept at −5°, at room temperature and at room temperature over calcium carbonate for 2 weeks gave 53 to 54% yields of carbobenzoxyglycine anilide (through the undistilled phosphite anilide) under conditions where the freshly prepared compound yielded 58% of the anilide. **o-Phenylene chlorophosphite** was made by the method of Anschütz, *et al.*,[7] in 89% yield: b.p. 88–90° (15 mm.), and $n^{25}D$ 1.5687.

Phosphite Amides.—**Diethyl anilinophosphite** was prepared from diethyl chlorophosphite and aniline by the method of Cook, *et al.*,[5] in 54% yield: b.p. 106–108° (1 mm.), $n^{25}D$ 1.5248. Cuprous bromide reacted with evolution of heat to give a clear, viscous brown liquid. **o-Phenylene anilinophosphite** was prepared in a similar manner in 64% yield as an undistilled gum. **Diethyl morpholinophosphite**, b.p. 111–113° (17 mm.), $n^{23}D$ 1.4580, was obtained in 60% yield. *Anal.* Calcd. for $C_{18}H_{18}NO_3P$: N, 6.8; P, 15.0. Found: N, 6.6; P, 14.9.

Benzanilide,[8] m.p. 160–161°, crystallized in 83% yield on cooling from the reaction of diethyl anilinophosphite with benzoic acid in refluxing toluene for 2 hours. **Benzomorpholide**[9] was obtained in a similar reaction using diethyl morpholinophosphite; m.p. 72–73° after recrystallization from ether; yield 78%. **Salicyl-*p*-toluide** was obtained as follows: 1.75 g. (0.01 mole) of *o*-phenylene chlorophosphite was added to a solution of 1.07 g. (0.01 mole) of *p*-toluidine and 1.01 g. (0.01 mole) of triethylamine in 25 cc. of benzene. The triethylamine hydrochloride was filtered off and the filtrate refluxed with 1.38 g. (0.01 mole) of salicylic acid for 15 minutes. The solution was washed successively with 25 cc. of saturated sodium bicarbonate and 10 cc. of water. The product, isolated by concentrating the benzene solution in air, was recrystallized from 10 cc. of methanol and 2.1 g. (92%) was obtained; m.p. 153–155°.[10]

Carbobenzoxyglycine Anilide.—Carbobenzoxyglycine (3.0 g.) was refluxed with an equivalent of diethyl anilinophosphite (3.07 g.) in 50 cc. of dry toluene for an hour. The major portion of the anilide crystallized on cooling; a small portion was recovered by distillation of the solvent and crystallization of the residue from 50% alcohol. Recrystallization of the combined portions from 130 cc. of 50% alcohol yielded 2.87 g. (70%), m.p. 147–148°.[11] A preparation in dimethyl formamide as solvent gave only 24%. Another reaction of 0.01-mole quantities heated for 2 hours on a steam-bath in 75 cc. of benzonitrile yielded 62% of recrystallized anilide. For comparison, 0.02-mole quantities of carbobenzoxyglycine and aniline were refluxed in 25 cc. of toluene for an hour, giving 23% of the anilide, and 72% recovery of carbobenzoxyglycine.

Carbobenzoxy-L-phenylalanine Anilide.—Carbobenzoxy-L-phenylalanine (0.70 g.) and diethyl anilinophosphite (0.50 g.) were refluxed an hour in 45 cc. of anhydrous toluene. The major portion of the anilide crystallized on cooling and the remainder was obtained by evaporation of the solvent; yield 0.76 g. (87%), m.p. 168–169°. Recrystallization from alcohol and chloroform, and washing with bicarbonate solution gave needles, m.p. 169–170°, $[\alpha]^{23}D$ −3.7 (*c* 3, chloroform). *Anal.* Calcd. for $C_{23}H_{22}N_2O_3$: C, 73.8; H, 5.9; N, 7.5. Found: C, 73.9; H, 5.8; N, 7.5.

Carbobenzoxy-DL-phenylalanine anilide was similarly prepared in 72% yield, m.p. 158–160°; recrystallization from 70% alcohol gave m.p. 159–160° and analytical values of 73.7 C, 5.9 H and 7.5 N. When *o*-phenylene chlorophosphite was treated with aniline in ether, the crude anilinophosphite was obtained in 64% yield; this was then refluxed in toluene for 30 minutes with carbobenzoxy-DL-phenylalanine, and the anilide was obtained in 92% yield.

Phosphite Amides of Aminoacid and Peptide Esters.—In general, sufficient triethylamine to neutralize all hydrogen chloride present or formed in the reaction was added to a solution or suspension of an amino ester or hydrochloride in anhydrous ether, followed by an ether solution of a chlorophosphite (equimolar to the amino ester). Approximately 300 cc. of ether per 0.10 mole of reactants was used, and frequently the reaction mixture was externally chilled, since there was usually heat of reaction. After standing at room temperature for half an hour, the mixture was filtered to remove triethylamine hydrochloride, and the ether removed by vacuum distillation. The remaining phosphite amide was distilled when possible, otherwise used in the crude state. (1) The **diethyl phosphite amide of ethyl DL-phenylalaninate** was obtained in yields of 20 to 48%, b.p. 134–137° (0.05 mm.); $n^{26}D$ 1.4917; D^{25}_4 1.071. *Anal.* Calcd. for $C_{15}H_{24}NO_4P$: P, 9.9. Found: P, 9.7. (2) The ***o*-phenylenephosphite amide of ethyl DL-phenylalaninate** was obtained as an amorphous solid, m.p. 50–52° (91%), which could not be distilled at 10^{-4} mm., or crystallized. (3) The **diethyl phosphite amide of methyl L-leucinate** was an oil: b.p. 86–100° (0.1 mm.); $n^{25}D$ 1.4403; yield 50–65%. *Anal.* Calcd. for $C_{11}H_{24}NO_4P$: P, 11.7; N, 5.28. Found: P, 11.6; N, 5.28. (4) The ***o*-phenylenephosphite amide of methyl L-leucinate**, an oil, was made in 85% (crude) yield. (5) The **diethylphosphite amide of ethyl DL-alaninate** was an oil: b.p. 75–79° (0.15 mm.); $n^{27}D$ 1.4349; D^{27}_4 1.047; yield 41%. *Anal.* Calcd. for $C_9H_{20}NO_4P$: P, 12.7; N, 5.9. Found: P, 12.8; N, 5.5. (6) The **diethylphosphite amide of ethyl L-tyrosinate**, an oil, was obtained in 46% (crude) yield, and (7) the ***o*-phenylenephosphite amide of ethyl-L-tyrosinate**, a gum, in 67% (crude) yield. (8) The **diethylphosphite amide of ethyl glycylglycinate** was a yellow oil, $n^{28}D$ 1.4730, D^{28}_4 1.16, and was obtained in 59% (crude) yield.

Peptide Derivatives.—Most of the reactions involved the refluxing of 0.01 or 0.02 mole of a carbobenzoxy- or phthaloylamino acid with an equivalent of a phosphite amide in a solvent. Usually the solvent was removed by vacuum distillation and the product recrystallized directly or washed with saturated sodium becarbonate solution and with water before recrystallization; products soluble in the cold reaction solvent were occasionally washed before removal of the solvent. In the following, the phosphite amides are identified by the numbers from the above section on their preparation: (a) Carbobenzoxyglycine was allowed to react with (1) for an hour in toluene, and the residue after distillation of the solvent was recrystallized from 50% alcohol; yield 65% of **ethyl carbobenzoxyglycyl-DL-phenylalaninate**,[12] m.p. 90–91°. Reaction times of 15 to 120 minutes in toluene, benzene, chloroform or benzonitrile as solvents gave comparable yields; ethyl acetate, tetrahydrofuran and methyl isobutyl ketone gave poor yields; and acetone, 95% alcohol and methanol gave none. A reaction in toluene with 50% excess of (1) yielded 90%, m.p. 89–90°. A 24% yield was obtained by reaction of carbobenzoxyglycine with (2) in tetrahydrofuran. (b) Phthaloylglycine was allowed to react with (1) for an hour in benzene and the product recrystallized from alcohol–water to give **ethyl phthaloylglycyl-DL-phenylalaninate**,[13] m.p. 148–150°, in 63% yield. (c) **Methyl carbobenzoxy-L-leucyl-L-leucinate**[14] was prepared in 74% yield by refluxing (3) with carbobenzoxy-L-leucine for 1 hour in toluene, washing with 10% sodium bicarbonate and water, concentrating and recrystallizing from ether–petroleum ether; m.p. 91–93°. The same compound was prepared from (4) in 77% crude yield, but the product was difficult to purify; several crystallizations from ether–petroleum ether yielded 27%, m.p. 93–95°, $[\alpha]^{21}D$ −36.2° (*c* 5, ethanol). (d) Phthaloylglycine was refluxed an hour in toluene with (5), and the precipitated product washed with sodium bicarbonate solution, then recrystallized twice from alcohol to yield 43% of **ethyl phthaloylglycyl-DL-alaninate**, m.p. 165–166°. *Anal.* Calcd. for $C_{15}H_{17}N_2O_5$: C, 59.0; H, 5.61; N, 9.18. Found: C, 59.1; H, 5.24; N, 9.35. (e) **Ethyl carbobenzoxyglycyl-L-tyrosinate**[15] was obtained by refluxing (6) with carbobenzoxyglycine for an hour in toluene. The oily product was washed with sodium bicarbonate solution, dilute hydrochloric acid, water, and then recrystallized from alcohol; yield 51%, m.p. 123–124°. A 40% yield was obtained when (7) was used in a similar reaction in benzene; m.p. 123–125°, $[\alpha]^{25}D$ +18.6° (*c* 5, ethanol). Tetrahydrofuran as solvent gave a poorer yield.

(7) L. Anschütz, *et al.*, *Ber.*, **76**, 222 (1943).

(8) O. Wallach and M. Hoffmann, *Ann.*, **184**, 80 (1883).

(9) L. Knorr, *ibid.*, **301**, 7 (1898), gives m.p. 74–75°.

(10) L. Anschütz, *ibid.*, **439**, 265 (1924).

(11) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937), give m.p. 144°.

(12) H. Neurath, *et al.*, *ibid.*, **170**, 222 (1947).

(13) J. R. Vaughan and R. Osato, THIS JOURNAL, **73**, 5553 (1951).

(14) M. Nyman and R. Herbst, *J. Org. Chem.*, **15**, 115 (1950), report m.p. 97–98°.

(15) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 412 (1937), report m.p. 118°.

(f) **Ethyl carbobenzoxydiglycylglycinate**[16] was obtained in 76% crude yield by refluxing (8) with carbobenzoxyglycine in toluene for 1.5 hours. Recrystallization from ethyl acetate, then alcohol–water yielded 34% pure product, m.p. 166–167°. A similar reaction with a portion of (8) which was kept in a refrigerator for 4 months gave a 66% crude yield.

Control Reactions for Peptide Derivatives (A).—Carbobenzoxyglycine (4.18 g., 0.02 mole) and ethyl DL-phenylalaninate (3.87 g., 0.02 mole) were refluxed in 50 cc. of toluene for an hour. After cooling, DL-phenylalanine anhydride[17] crystallized out (0.30 g., or 10% of the maximum possible). After recrystallization from acetic acid, then alcohol–water, the m.p. was 285-287° (uncor.). *Anal.* Calcd. for $C_{18}H_{18}N_2O_3$: C, 73.5; H, 6.2. Found: C, 73.0; H, 6.3. The toluene solution was washed with 25 cc. of 10% sodium bicarbonate and 10 cc. of water. Acidification of the washes caused precipitation of 2.24 g. (53% recovery) of carbobenzoxyglycine. Distillation of the toluene under vacuum left an oil which was crystallized from 40 cc. of 50% alcohol, yielding 2.92 g. (38%) of **ethyl carbobenzoxyglycyl-DL-phenylalaninate**, m.p. 87–89°. A similar experiment in which 2 hours reaction was used yielded 13% of DL-phenylalanine anhydride, 38% recovered carbobenzoxyglycine, and 55% of the peptide derivative.

(B).—Reaction of carbobenzoxyglycine with ethyl L-tyrosinate in refluxing toluene for an hour yielded 42% of **ethyl carbobenzoxyglycyl-L-tyrosinate**, 52% recovery of carbobenzoxyglycine, and 9% of (presumed) L-tyrosine anhydride, m.p. 245–260° dec.[18]

Phosphazoamides and Reactions.—Phenyl phosphazoanilide[2] (2.14 g., 0.01 mole) and carbobenzoxyglycine (4.18 g., 0.02 mole) were refluxed in 75 cc. of toluene for an hour, then filtered hot from an oily precipitate. **Carbobenzoxyglycine anilide** crystallized from the filtrate on cooling; yield 4.3 g. (75%). Recrystallization from alcohol gave 2.7 g. (48%); m.p. 147–148°. A similar reaction of phenyl phosphazoanilide with phthaloylglycine gave **phthaloylglycine anilide**; yield 53% after recrystallization from absolute alcohol, m.p. 237–238°.[19]

Ethyl DL-phenylalaninate hydrochloride (4.47 g., 0.02 mole) was suspended in 50 cc. of toluene, then triethylamine (5.05 g., 0.05 mole) and phosphorus trichloride (1.36 g., 0.01 mole) were added. After refluxing an hour and filtering, the toluene was distilled under vacuum, leaving an oily residue. Attempts to crystallize this failed. A similar reaction was therefore run and the crude product reacted with carbobenzoxyglycine (4.18 g., 0.02 mole) by refluxing an hour in toluene. From this an oil, presumably crude **ethyl carbobenzoxyglycyl-DL-phenylalaninate**, was obtained. Since the latter was not successfully crystallized, it was saponified by reaction for an hour at room temperature with sodium hydroxide in alcohol–water. Acidification with hydrochloric acid gave **carbobenzoxyglycyl-DL-phenylalanine**. After some difficulties in recrystallization (chloroform–petroleum ether was finally used), a 16% yield, m.p. about 155°, was obtained.[20] Better success was met when ethyl DL-phenylalaninate hydrochloride (0.03 mole), carbobenzoxyglycine (0.03 mole), and triethylamine (0.09 mole) were mixed in toluene (100 cc.), then phosphorus trichloride (0.01 mole) in toluene was added. After standing 20 minutes, then refluxing 30 minutes, the triethylamine hydrochloride was filtered off, the toluene solution washed with 10% sodium bicarbonate, dilute hydrochloric acid and water. Removal of toluene under vacuum followed by recrystallization from alcohol–water yielded 5.98 g. (52%) of **ethyl carbobenzoxyglycyl-DL-phenylalaninate**; m.p. 85–87°.

Phosphate Amide Reactions.—**Diphenyl anilinophosphate**[21] (2.94 g., 0.009 mole) and carbobenzoxyglycine (2.09 g., 0.01 mole) were heated together in 75 cc. of refluxing toluene for 1.5 hours. The solvent was removed by concentration. Treatment with *N* sodium hydroxide and ether separated the unreacted compounds; a 92% recovery of the phosphate was obtained by evaporation of the ether, and a 91% recovery of the acid by acidification of the alkaline solution.

Diethyl anilinophosphate[22] was heated with carbobenzoxyglycine in toluene solution for an hour, with similar recoveries of reactants.

Diisopropyl anilinothionophosphate[23] and carbobenzoxyglycine (0.01-mole quantities) were treated in 50 cc. of refluxing toluene for 2.5 hours; 97% of the carbobenzoxyglycine crystallized on cooling.

The ethyl DL-phenylalaninate amide of diphenylphosphate[24] (1.0 g., 0.0023 mole) and carbobenzoxyglycine (0.4 g., 0.002 mole) were heated together in 25 cc. of refluxing toluene for 2 hours, then allowed to stand at room temperature for 65 hours. The solution was concentrated and treated with 5 cc. of 2 *N* sodium hydroxide and ether. Evaporation of the ether gave nothing, but acidification of the alkaline solution gave a 95% recovery of carbobenzoxyglycine.

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(16) J. S. Fruton, *et al.*, *J. Biol. Chem.*, **173**, 467 (1948), give m.p. 165°.

(17) E. Erlenmeyer and A. Lipp, *Ann.*, **219**, 206 (1883).

(18) E. Fischer and W. Schrauth, *ibid.*, **354**, 21 (1907), reported m.p. 277–280° dec. (cor.).

(19) J. Scheiber, *Ber.*, **46**, 1103 (1913), gave m.p. 231–232°.

(20) H. Neurath, *et al.* (ref. 12), gave m.p. 159.5–160.5°.

(21) F. R. Atherton, V. M. Clark and A. R. Todd, *Rec. trav. chim.*, **69**, 295 (1950).

(22) H. McCombie, B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 380 (1945).

(23) Prepared from diisopropyl thiophosphoryl chloride and aniline by Mr. L. C. Beegle of these laboratories; m.p. 89–92°. *Anal.* Calcd. for $C_{18}H_{22}NO_2PS$: N, 5.1; S, 11.7; P, 11.3. Found: N, 5.1; S, 12.3; P, 11.2.

(24) L. J. Sciarini and J. S. Fruton, THIS JOURNAL, **71**, 2940 (1949).