Peptides Containing Aminophosphonic Acids. III. The Synthesis of Tripeptide Analogs Containing Aminomethylphosphonic Acid

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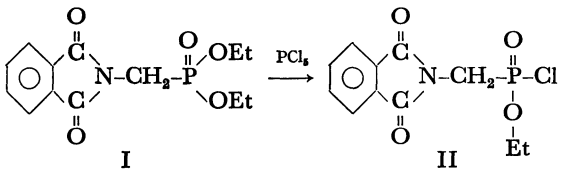
A synthetic method for tripeptide analogs containing aminomethylphosphonic acid has been described.

Since β-aminoethylphosphonic acid was discovered from marine organisms,[1] human beings, *etc.*,[2] its potential biological roles have been investigated actively; the results have suggested the occurence of the compound in lipid[3] as well as in peptide.[4] These aspects of an aminophosphonic acid have stimulated the study of the synthesis of peptides containing aminophosphonic acid;[5] we ourselves reported previously the first attempt at the preparation of a tripeptide containing aminomethylphosphonic acid in the center.[6]

In the present paper, we wish to describe the synthesis of tripeptide analogs containing more than one aminomethylphosphonic acid unit. Unlike an amino acid, a phosphonic acid group of aminophosphonic acid must be converted to the phosphonochloridate group in order to couple with an amino group of amino acid or aminophosphonic acid.

Thus, diethyl phthalimidomethylphosphonate (I), which could be easily prepared by the reaction of *N*-bromomethylphthalimide with triethyl phosphite, was chlorinated with a slight excess of phosphorus pentachloride to the corresponding phosphomonochloridate (II). On the other hand, Compound (I) was treated with one equivalent of hydrazine in ethanol at room temperature to generate diethyl aminomethylphosphonate (III) in a 70% yield. The chloride (II) was then allowed to react with the amine (III), affording a phosphorus analog of the dipeptide (IV) in a fairly good yield. The further extension of IV by aminomethylphosphonic acid was performed by the dephthalylation of IV with hydrazine to the corresponding amine



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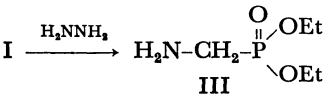

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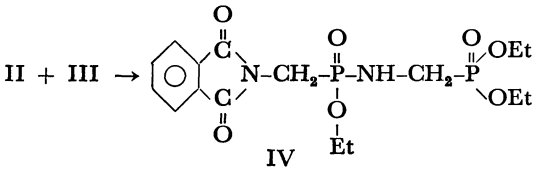

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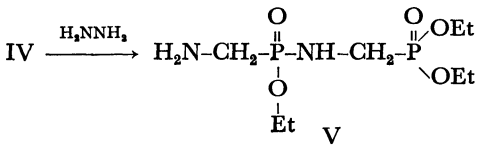

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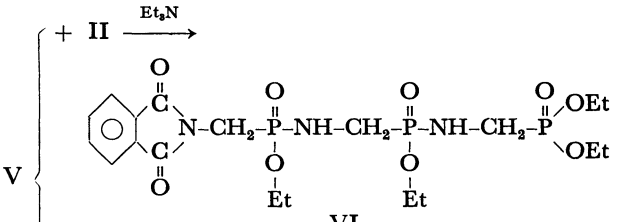

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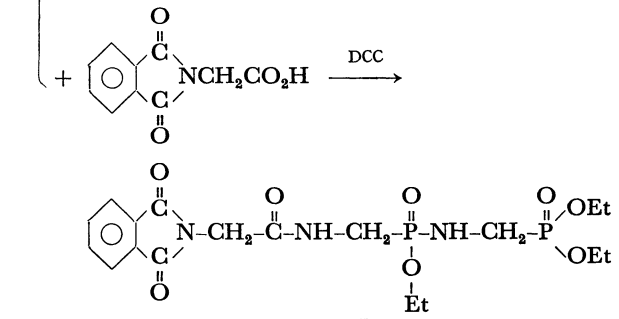
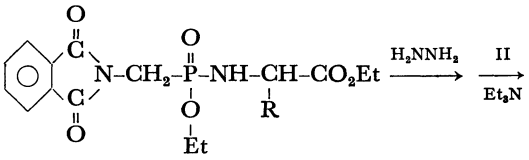
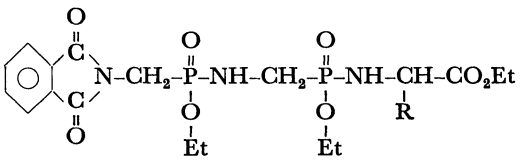


VII



VIII: R=H
IX: R=CH_3



X: R=H
XI: R=CH_3

(V), followed by a reaction with II to provide a tripeptide analog (VI). Compound (V) was coupled also with phthalylglycine by means of dicyclohexylcarbodiimide to form VII. Similarly, dipeptides (VIII and IX) could each be elongated by one aminomethylphosphonic acid unit to tripeptide analogs (X and XI).

The above successive processes enable us to synthesize the higher analogs of peptides containing aminophosphonic acid; our studies in this direction are continuing.

Experimental

The infrared spectra (IR) were measured on a Jasco Model IR-G spectrometer. The nuclear magnetic resonance spectra (NMR) were recorded with a Hitachi-Perkin Elmer R-20 spectrometer, with a dilute solution in deuteriochloroform and tetramethylsilane as the internal standard.

Preparation of O-Ethyl Phthalimidomethylphosphonochloridate (II). Compound (I, 4.34 g, 0.0146 mol) was refluxed with phosphorus pentachloride (3.06 g, 0.0147 mol) in benzene (50 ml) for 15 hr. The light-boiling substances were distilled out from the reaction mixture under reduced pressure to give a slightly yellow liquid, which, upon standing at room temperature, solidified as crystals; 3.58 g (85%) (tetrahydrofuran–ether); mp 92—94 °C; IR ($CHCl_3$); 1780 (w), 1725 (s), 1600 (w), 1385 (s), 1300 (m), 1250 (m), and 720 (m) cm^{-1}; NMR: τ 1.8 (m, 4, phthalyl), 5.40 (d, 2, $C\underline{H}_2P$), 5.50 (m, 2, $POC\underline{H}_2$), and 8.55 (qua, 3, $C\underline{H}_3$).

Found: C, 46.21; H, 4.03; N, 5.10; Cl, 12.76%. Calcd for $C_{11}H_{11}ClNO_4P$: C, 45.93; H, 3.85; N, 4.87; Cl, 12.33%.

Preparation of Diethyl Aminomethylphosphonate (III). A mixture of diethyl phthalylimidomethylphosphonate (I, 4.34 g, 0.0146 mol)[6] in ethanol (30 ml) and hydrazine hydrate (100%, 1.2 ml) was kept at room temperature overnight and then refluxed for 2 hr. The resulting phthalyl hydrazide was removed by filtration, and the filtrate was concentrated to give diethyl aminomethylphosphonate as a light-yellow liquid; 1.7 g (70%); IR (neat): 3300 (s), 2820 (w), 1600 (s), 1505 (m), 1195 (s), and 1005 (s) cm^{-1}; NMR: τ 5.87 (qua, 4, $2OC\underline{H}_2$), 6.75 (s, 2, $N\underline{H}_2$), 7.02 (d, 2, $C\underline{H}_2P$) and 8.70 (t, 6, $2C\underline{H}_3$). A subsequent purification by distillation under reduced pressure resulted only in decomposition. Therefore, III was utilized for the next reaction without further purification.

Preparation of Peptide Analogs (IV.) A THF solution of II (50 ml) was added, drop by drop, to an ice-cooled chloroform solution (50 ml) of a mixture of the amine (III) and triethylamine (4 ml) over a 15-min period; the solution was then stirred at room temperature for 10 hr. After the subsequent removal of the triethylammonium chloride, the solution was concentrated. The residue was dissolved in chloroform (100 ml), and the solution was washed with saturated sodium hydrogen carbonate, and water and then dried with Drierite. The evaporation of the solvent and the recrystallization of the residue from THF–diethyl ether afforded white crystals of IV; 3.44 g (56%); 108—109 °C; IR (KBr): 3350 (w), 3100 (w), 1770 (w), 1720 (s), 1410 (m), 1200 (s), 1020 (s), 960 (m), and 720 (m) cm^{-1}; NMR: τ 2.15 (m, 4, phthalyl), 5.60—6.10 (m, 6, 3 $OC\underline{H}_2$), 5.90 (d, 2, PHT-$C\underline{H}_2$), 6.60 (s, 2, NH-$C\underline{H}_2$-P), 7i70 (t, 6, $2C\underline{H}_3$), and 8.72 (t, 3, $C\underline{H}_3$).

Found: C, 45.49; H, 5.58; N, 6.62%. Calcd for $C_{16}H_{24}N_2O_7P_2$: C, 45.93; H, 5.79; N, 6.70%.

VI. Dipeptide (IV, 1.25 g, 0.0030 mol) in ethanol (15 ml) was first treated with hydrazine hydrate (0.3 ml) to give its free amine (V) as a yellow viscous liquid; IR (neat): 3170 (m), 2930 (m), 1745 (s), 1655 (m), 1480 (w), 1200 (s), 1035 (s) and 680 (w); NMR: τ 5.90 (qua, 2, $COC\underline{H}_2$), 5.94 (qua, 2, $PO\text{-}C\underline{H}_2$), 6.5 (b, 2, $N\underline{H}_2$), 8.68 (t, 3, CH_3), 8.65 (t, 3, $C\underline{H}_3$). The amine (V) was subsequently allowed to react with II (0.89 g, 0.0031 mol) in the presence of triethylamine (1.5 ml) in a manner similar to that mentioned above, thus giving 0.53 g (32%) of VI; mp 136—138 °C (THF-diethyl ether); IR (KBr): 3350 (w), 3150 (w), 1770 (w), 1710 (s), 1410 (m), 1305 (w), 1220 (s), 1030 (s), 960 (s), 830 (m), and 720 (m) cm^{-1}; NMR: τ 2.18 (m, 4, phthalyl), 5.60—6.10 (m, 8, $4OC\underline{H}_2$), 5.90 (d, 2, PHT-$C\underline{H}_2$), 6.30—6.80 (m, 6, $2NC\underline{H}_2P$, and $2N\underline{H}$), 8.70 (t, 6, $2C\underline{H}_3$), and 8.72 (t, 6, $2C\underline{H}_3$).

Found: C, 40.90; H, 5.69; N, 7.79%. Calcd for $C_{19}H_{32}N_3O_9P_3$: C, 41.44; H, 5.60; N, 7.71%.

VII. The amine (V), which was obtained *via* the dephthalylation of IV (1.34 g, 0.0032 mol), was stirred with phthalylglycine (0.65 g, 0.0032 mol) and DCC (0.68 g, 0.0033 mol) in THF (20 ml) at room temperature overnight. Aqueous acetic acid (40%, 1.5 ml) was then added to the reaction mixture, and the solution was kept at room temperature for 2 hr. The resulting dicyclohexylurea was filtered off, and the filtrate was concentrated. The residue was dissolved in chloroform, and the solution was washed with aqueous sodium hydrogen carbonate and water and dried with Drierite. The subsequent evaporation of the solvent afforded crystals of VII; 0.68 g (45 %); mp 149—150 °C (THF); IR (KBr): 3300 (w), 1770 (w), 1730 (s), 1660 (m), 1420 (s), 1200 (s), 1040 (s), 960 (s), and 710 (w) cm^{-1}; NMR: τ 1.65 (broad s, 1, $CON\underline{H}$), 2.15 (m, 4, phthalyl), 5.53 (s, 2, PHT-$C\underline{H}_2$), 5.5—6.10 (m, 8, $4OC\underline{H}_2$), 6.20—6.80 (m, 5, $2NC\underline{H}_2P$, and $PN\underline{H}$), 8.66 (t, 6, $2C\underline{H}_3$) and 8.69 (t, 3, $C\underline{H}_3$).

Found: C, 45.66; H, 5.73; N, 8.99%. Calcd for $C_{18}H_{27}N_3O_8P_2$: C, 45.48; H, 5.73; N, 8.84%.

X. A solution of the dipeptide (VIII, 1.95 g, 0.0055 mol) in ethanol (30 ml) was dephthalylated with hydrazine hydrate (0.4 ml) at room temperature overnight to give the corresponding amine through a procedure similar to that described in the preparation of III. The amine in THF (50 ml) was then added, drop by drop, to the chloride (II, 1.58 g, 0.0055 mol) in chloroform (50 ml), which had been precooled in an ice-water bath. After the mixture has been stirred further at room temperature overnight, it was processed in a manner essentially similar to that used in the preparation of IV, thus producing X; 0.56 g (22%); mp 159—160 °C (THF); IR (KBr): 3400 (w), 3150 (m), 2950 (w), 2900 (w), 1705 (s), 1410 (m), 1300 (m), 1060 (s), 960 (m), 900 (m), 830 (m), and 720 (m) cm^{-1}; NMR: τ 2.22 (m, 4, phthalyl), 5.60—6.10 (m, 9, $OC\underline{H}_2$, $2POC\underline{H}_2$, and $PN\underline{H}$,-$C\underline{H}_2C$) 5.90 (d, 2, PHT-$C\underline{H}_2$), 6.4—6.9 (m, 3, $PN\underline{H}C\underline{H}_2P$), 8.61 (t, 6, $2OC\underline{H}_3$), and 8.66 (t, 3, $C\underline{H}_3$).

Found: C, 45.55; H, 5.59; N, 8.96%. Calcd for $C_{18}H_{27}N_3O_8P_2$: C, 45.48; H, 5.73; N, 8.84%.

XI. The tripeptide analog (XI) was similarly synthesized from IX (1.19 g, 0.0032 mol) and II (0.92 g, 0.0032 mol), using triethylamine (0.7 ml); 0.185 g (12%); mp 140 °C; IR (KBr): 3300 (w), 1770 (w), 1740 (w), 1720 (s), 1405 (m), 1205 (s), 1160 (m), 1040 (s), 955 (m), 900 (w), and 720 (m) cm^{-1}; NMR: τ 2.20 (m, 4, phthalyl), 5.6—6.4 (m, 8, $3C\underline{H}_2$, and $N\underline{H}C\underline{H}CH_3$), 6.4—6.7 (m, 3, $N\underline{H}C\underline{H}_2P$), 5.90 (d, 2, PHT-$C\underline{H}_2$), 8.56 (d, 3, $CHC\underline{H}_3$), 8.70 (t, 6, $2OC\underline{H}_3$), and 8.73 (t, 3, $OC\underline{H}_3$).

Found: C, 45.21; H, 5.93; N, 8.62%. Calcd for $C_{19}H_{29}N_3O_8P_2$: C, 45.63; H, 5.97; N, 8.59%.

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