Organophosphorus Analogues and Derivatives of the Natural L-Amino Carboxylic Acids and Peptides. IV.[1] A Phospha^{C}-Peptide Analogue of Plumbemycin A

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Synopsis. A condensation of 1,2-azaphosphorine **1** and the dipeptide **2** to the entirely protected phospha^{C}-tripeptide **3** was carried out by the DCC method. A high selectivity was achieved in the enzyme-catalyzed hydrolysis of the ethoxycarbonyl groups with alkaline mesintericopeptidase to **4** and of the peptide bond Ala-Asp with α-chymotrypsin to **5**, which in acid-catalyzed hydrolysis releases the norvaline **6**. Antitumor activity of the phospha^{C}-peptides **4** and **5** was found.

The tripeptide antibiotic Plumbemycin A was isolated from *Streptomices plumbeus* by Hirota and Sakai.[2,3] Its physiological activity was also studied.[4]

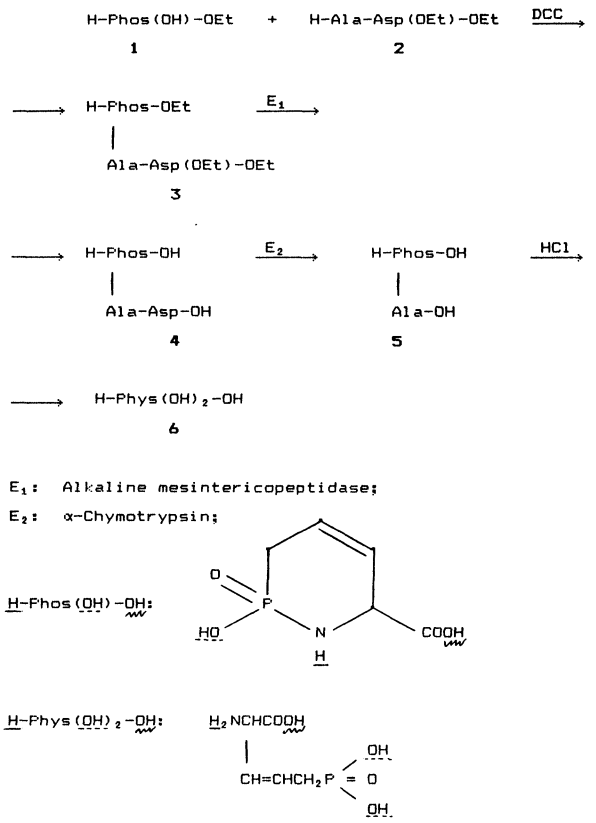
With a view to studying the relationship between the chemical structure and the physiological activity, in the present note the synthesis of phospha^{C}-peptide analogue of the above mentioned antibiotic is discussed.

Cyclic analogue of C-terminal-phosphonylated amino acid-3,4-didehydro-5-phosphono-L-norvaline= (*S*)-(6-ethoxycarbonyl-2-hydroxy-1,2,3,6-tetrahydro-1,2-azaphosphorine 2-oxide (**1**) recently synthesized (cf. later commun. of the same author) by us and the commercially available dipeptide H-Ala-Asp(OEt)-OEt (**2**) were chosen as starting substances. The condensation was carried out by DCC method. After the usual work-up of the reaction mixture, the fully protected phospha^{C}-tripeptide **3** was isolated with a yield of about 70%. At "mild" acid-catalyzed hydrolysis, the simultaneous hydrolytic cleavage of both PO-NH bonds (cyclic and exocyclic) was attained and the following products were obtained: 3,4-didehydro-5-phosphono-L-norvaline ethyl ester and the dipeptide H-Ala-Asp (OEt)-OEt (**2**) (see Scheme 1).

To ensure selective hydrolysis of the ethoxycarbonyl groups of the dipeptide **3** the alkaline mesintericopeptidase was used. Under the optimum conditions,[5] namely, 20 g substrate **3**, 5 mg enzyme in an aqueous buffer (500 ml, pH 7.8), stirring at 25 °C to a ninhydrin positive detection, the phospha^{C}-tripeptide **4** was isolated in about 90% yield.

The second enzyme we employed was α-chymotrypsin. Under the usual conditions (25 °C, pH 7.8) this enzyme enhances the hydrolysis of the peptide bond Ala-Asp selectively with no effect on the two PO-NH bonds. Thus, using 20 g substrate **4** and 5 mg enzyme α-chymotrypsin, the phospha^{C}-dipeptide **5** was isolated in about 70% yield. A "mild" acid hydrolysis (0.5 M HCl, 50 °C, 30 min) of the product **5** liberated the norvaline **6**.

The enzymes phosphodiesterase I and alkaline phosphatase did not exhibit enzyme-substrate interaction with the synthesized phospha^{C}-peptide analogues of Plumbemycin A.



Scheme 1.

Preliminary physiological tests with the phospha^{C}-tri- and dipeptides **4** and **5** have been carried out. When applied to an experimental tumor L1210 in mice in doses of 20 and 28 mg/kg per day for 5 consecutive days, the T/L (T/L% is the ratio of the mean survival time, expressed as a percentage of untreated controls) was 184% and 172% for the tripeptide **4** and for the dipeptide **5**, respectively. The insecticidal activity of the two peptides was also studied and it has been established that the tripeptide **5** is the stronger. It is of interest to point out the following observation: when the tripeptide **5**, Plumbemycin A, L-norvaline **6**, and its cyclic analogue (*S*)-1,2,3,6-tetrahydro-1,2-azaphosphorine-6-carboxylic acid were compared, it was found that the 1,2-azaphosphorine analogue had the highest insecticidal activity, whereas in the L-norvaline **6** and in Plumbemycin A, it was entirely missing.

Detailed investigations on the insecticidal activity are now under way and the results will be published soon.

Experimental

General. IR spectra, elemental analysis, mp, Mw, $[\alpha]_D^{22}$ on Perkin-Elmer instruments; ^{1}H NMR—JEOL 100 MHz and Brucker 250 MHz; Mass spectra—LKB 900 and Barian; reagents and solvents from "Aldrich," and "Merck"; α-chymotrypsin—from Pharmachim, Bulgaria; alkaline mesintericopeptidase—Inst. Org. Chem., Bulg. Acad. Sci.; TLC-silica gel film—molybdophosphate or ninhydrin detection.

***N*-[(*S*)-(6-Ethoxycarbonyl-1,2,3,6-tetrahydro-1,2-azaphosphorin-2-yl)]alanylaspartic Acid α,β-Diethyl Ester (3).** A mixture of 1,2-azaphosphorine (**1**) (20.52 g, 0.1 mol), the dipeptide (**2**) (26.03 g, 0.1 mol) and DCC (22.69 g, 0.11 mol) in dry ethyl acetate (300 ml) was stirred in a closed Erlenmeyer flask at room temperature for 24 h. After filtration, the reaction mixture was washed consecutively with water, 5% aqueous sodium carbonate, water, 0.5% hydrochloric acid, water, and then dried over anhydrous $MgSO_4$. After distillation under vacuum to dryness, the oily residue was taken into a silica-gel column and eluted with chloroform: methanol from 9 : 1 to 7 : 3 v/v. The product was dissolved in cold dry ethyl acetate (250 ml) and dry hexane was added until it becomes turbid. After rubbing to the flask walls with a glass stick and after a continuous stay in refrigerator at −5 °C, the product **3** was slowly crystallized.

Compound 3: Yield, 29.17 g (67.3%); mp 78—81 °C (EtOAc/*n*-C_6H_{14}); IR (KBr) cm^{-1}; 1755—1730, 1640, 1520, 1330—1300, 1200; R_f: 0.68 ($CHCl_3$: MeOH=9 : 1); $[\alpha]_D^{20}$ −46.4° (*c* 1, MeOH).

Found: C, 48.60; H, 6.86; N, 9.07%. Calcd for $C_{18}H_{30}N_3O_8P$: C, 48,32; H, 6.76; N, 9.39%. Mw, Found/Calcd, 443/447.427.

The substance is soluble in the usual organic solvents and insoluble in water. When **3** (4.47 g, 0.01 mol) was heated in 0.5 M HCl (25 ml) at 50 °C for 30 min, the products isolated were: H-Ala-Asp(OEt)-OEt (**2**) in a yield of 2.11 g (81.2%) and 3,4-didehydro-5-phosphono-L-norvaline ethyl ester: yield 1.58 g (77.7% as HCl salt); mp 210 °C (decomp); IR (KBr) cm^{-1}: 1750, 1550, 1240, 1010, 970; ^{1}H NMR (DMSO-d_6, δ, free basse) δ=1.26 (3H, t, $OCH_2C\underline{H}_3$), 2.75 (2H, dd, $J_{P\text{-}CH_2}$=8.6 and 23 Hz), 4.03 (2H, q, $OC\underline{H}_2CH_3$), 4.80 (1H, d, *J*=8 Hz, CH), 5.65 (1H, m, C<u>H</u>=CH), 6.13 (1H, m, CH=C<u>H</u>CH_2), 10.4—10.8 (2H, br, s, PO_3H_2); MS-free base, *m/z*, Found/Calcd, 223 (18%)/223.166; R_f: 0.33 (*n*-BuOH : AcOH : H_2O=3 : 1 : 1); $[\alpha]_D^{20}$ −56.3° (*c* 1, 0.1 M NaOH).

Found: C, 32.71; H, 5.61; N, 5.42%. Calcd for $C_7H_{14}NO_5P \cdot HCl$: C, 32.38; H, 5.82; N, 5.40%.

***N*-[(*S*)-(6-Carboxyl-1,2,3,6-tetrahydro-1,2-azaphosphorin-2-yl)]alanylaspartic Acid (4).** A mixture of the tripeptide **3** (20 g, 0.04 mol) homogenized with 3—4 drops of "Tween-8-", and the enzyme alkaline mesintericopeptidase (5 mg) in an aqueous buffer [tris(hydroxymethyl)aminomethane-(Trisma^R), 500 ml, 0.05 M, pH 7.8 at 25 °C] was stirred until it gave a positive test on ninhydrin. After acidification (pH 6.5) and distillation under vacuum to dryness, the organic mass was extracted with boiling ethanol. After cooling, the product **4** was filtered.

Compound 4: Yield, 14.18 g (87.3%); mp 162—165 °C (EtOH); IR (KBr) cm^{-1}; 3860—2800, 1760, 1640, 1520, 1340, 1265, 970—840, 630; ^{1}H NMR (D_2O+Na_2CO_3) δ=1.52 (3H, d, *J*=8 Hz, $CHC\underline{H}_3$), 2.26 (2H, d, CH_2CO), 4.2—4.6 (3H, m, 3×CH), 2.72 (2H, m, PCH_2), 5.68 and 6.21 (2H, m, C<u>H</u>=C<u>H</u>), R_f: 0.56 (DMF: dioxane: $CHCl_3$=9 : 1 : 1); $[\alpha]_D^{20}$ 63.8° (*c* 0.1, 0.1 M NaOH).

Found: C, 40.00; H, 5.11; N, 11.38%. Calcd for $C_{12}H_{18}N_3O_8P$: C, 39,67; H, 4.99; N, 11.57%. Mw, Found/Calcd, 360/363.327.

The substance is highly soluble in DMF, DMSO, hexamethylphosphoric triamide, and water, less in dioxane and ethanol and is insoluble in chloroform, ether, and hexane. When **4** (3.60 g, 0.01 mol) was heated in 0.5 M HCl (25 ml) at 50 °C for 30 min, the following products were isolated: the dipeptide H-Ala-Asp-OH (1.70 g, 83.4%) and 3,4-didehydro-5-phosphono-L-norvaline (**6**): yield 1.52 g (78.3%) mp 188—190 °C (decomp) [for the D-form mp: 183—185 °C[1]]; IR (KBr) cm^{-1}: 1735, 1630, 1520, 1240, 1150 1040, 930, 860, 725, 630; ^{1}H NMR (D_2O+NaOD) δ=2.75 (2H, dd, $J_{P\text{-}CH_2}$=8.3 and 23 Hz), 4.03 (2H, q, $OC\underline{H}_2CH_3$), 4.81 (1H, d, *J*=8 Hz, CH), 5.66 (1H, m, C<u>H</u>=CH), 6.12 (1H, m, CH=C<u>H</u>CH_2) and five exchangeable protons NH_2, COOH, PO_3H_2; MS, *m/z*, Found/Calcd, 195/195.112; R_f: 0.33 (*n*-BuOH : Pyr : AcOH : H_2O=15 : 12 : 3 : 10) and 0.10 (*n*-BuOH : AcOH : H_2O=3 : 1 : 1); $[\alpha]_D^{20}$ −53.2° (*c* 1, H_2O).

Found: C, 30.53; H, 5.28; N, 7.06%. Calcd for $C_5H_{10}NO_5P \cdot HCl$: C, 30.78; H, 5.17; N, 7.18%.

***N*-[(*S*)-(6-Carboxyl-1,2,3,6-tetrahydro-1,2-azaphosphorin-2-yl)]alanine (5).** A mixture of the tripeptide **4** (20 g, 0.55 mol) and α-chymotrypsin (5 mg) in an aqueous buffer [tris-(hydroxymethyl)aminomethane(Trisma^R), 500 ml, 0.05 M, pH 7.8 at 25 °C] was stirred for 6 h. After acidification (pH 5.8) and vacuum distillation to dryness, the organic residue was extracted with boiling ethanol. After cooling, the product **5** was filtered.

Compound 5: Yield, 11.04 g (82.8%); mp approx. 200 °C (decomp); IR (KBr) cm^{-1}; 3800—3000, 1750—1725, 1360, 1280, 960, 840, 720, 630; ^{1}H NMR (D_2O+Na_2CO_3) δ=1.48 (3H, d, *J*=8 Hz, $CHC\underline{H}_3$), 4.18 and 4.42 (2H, m, 2×CH), 2.77 (2H, m, PCH_2), 5.72 and 6.33 (2H, m, C<u>H</u>=C<u>H</u>), and four exchangeable protons; MS, *m/z*: Found/Calcd, 248 (18%)/248.175; R_f: 0.68 (DMF: dioxane : $CHCl_3$=9 : 1 : 3); $[\alpha]_D^{20}$ −63.7° (*c* 1, H_2O).

Found: C, 38.66; H, 5.41; N, 11.36%. Calcd for $C_8H_{13}N_2O_8P$: C, 37,72; H, 5.28; N, 11.29%. Mw, Found/Calcd, 251/248.175.

The substance is very soluble in DMF, DMSO, hexamethylphosphoric triamide, comparatively in dioxane and ethanol and is insoluble in chloroform, ether and hexane. When **5** (2.51 g, 0.01 mol) was heated in 0.5 M HCl (25 ml) at 50 °C for 30 min, the product 3,4-didehydro-5-phosphono-L-norvaline (**6**) (1.74 g, 89.1%) was isolated and has spectral data identical with those obtained by based-hydrolysis of the peptide **4** (cf. the above section).

References

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