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TOTAL SYNTHESIS, ENZYME-SUBSTRATE INTERACTIONS AND HERBICIDAL ACTIVITY OF PLUMBEMICIN A AND B (N-1409)

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Abstract - The unusual natural amino acid 3,4-didehydro-5-phosphono-D-norvaline, D4, and the cyclic analogue 14 have been synthesized by four- and three-component condensation of the aldehydes 3 and 10, respectively. Strict selectivity has been observed in the enzyme-substrate interactions with the enzymes phosphodiesterase I, alkaline phosphatase, α-chymotrypsin, urease, and alkaline mesintericopeptidase. The tripeptide antibiotics Plumbemicin A, 21, and B, 25, have been synthesized by the methods of conventional peptide synthesis and enzyme-catalyzed removal of the protective groups. Biological tests of the newly synthesized compounds have shown that they possess herbicidal, fungicidal, and antitumour activity.

Plumbemicin A - N-(N-L-alanyl-L-α-aspartyl)-3,4-didehydro-5-phosphono-D-norvaline, 21 - and Plumbemicin B - N-(N-α-L-alanyl-L-asparaginyl)-3,4-didehydro-D-norvaline, 25 - are antibiotics, isolated from Streptomices plumbens strains.[1,2,3] To this moment, no reports have appeared in the literature about any method for the synthesis of either the unusual C-terminal aminocarboxylic acid 3,4-didehydro-5-phosphono-D-norvaline, D4, or the tripeptides Plumbemicin A and B.

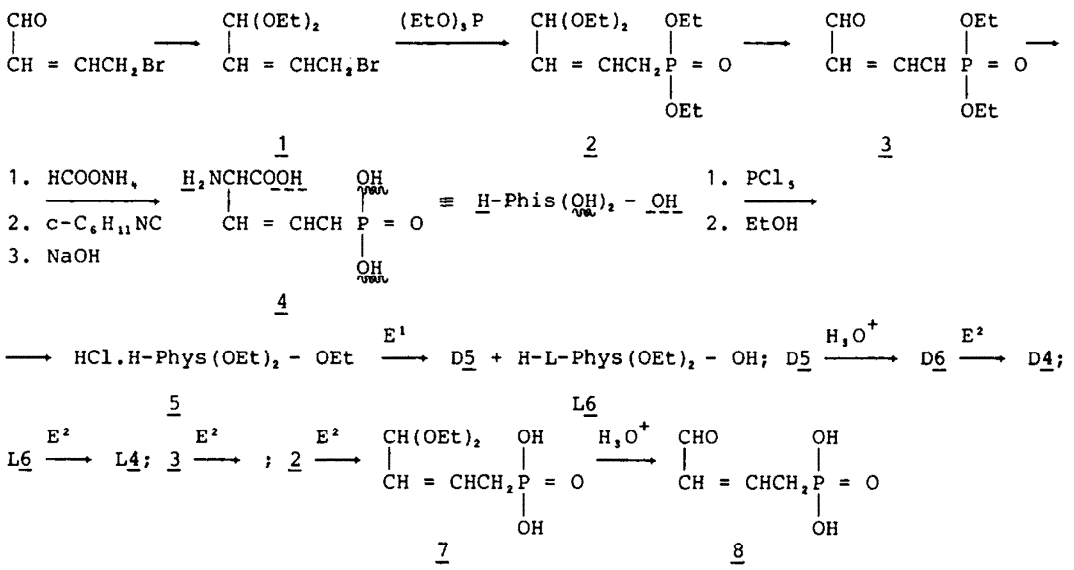
Our key approach to the synthesis of the starting norvaline D4 was four-component condensation after the reaction of Ugi.[4]

In view of the synthetic purposes in hand, we chose as our starting product 5-bromocrotonaldehyde,[5] which is easily converted into the diacetal 1 (cf. Scheme 1). The introduction of the diethoxyphosphinyl group was achieved by the reaction of Arbuzov-Michaelis, i.e. by treatment of 1 with triethylphosphite. The diethylphosphonate 2 was obtained in a yield of 48-52%. "Mild" mineral hydrolysis of the diacetal 2 affords the aldehyde 3 in a practically quantitative yield.

Four-component isonitrile condensation is achieved very simply. For convenience, the amino component, i.e. the ammonia, and the carboxyl component, i.e. formic acid, are applied in the form of formate. For better results, it is better that the formate, the aldehyde 3 and finally the isonitrile component, cyclohexyl isonitrile, be consecutively added to 80% aqueous methanol at intervals of 15 mins. at room temperature. The mixture is then boiled for 6 hours. Isolation of the condensation product is not necessary. After evaporation of the volatile components in vacuum to dryness, it would suffice to add to the residue 10% sodium hydroxide and heat the mixture in a water bath for 2.5-3.0 hours. Thus the norvaline 4 is isolated

with a yield of about 80%.

Scheme 1



E^1- α-chymotrypsin; E^2- phosphodiesterase I.

Enzymatic approaches were used to separate optically active isomers of the racemic mixture 4. Initially, 4 was treated with phosphorus pentchloride and then, without isolating the chlorinated product (a very unstable product, which is highly sensitive to ambient humidity), it was directly treated with ethanol. As a result, the hydrochloride of the triester 5 was isolated with a yield of approx. 60-65%. When 5 is employed as substrate in an enzyme-substrate interaction with the enzyme α-chymotrypsin, the unchanged D-form of the racemic mixture, D5, and the hydrolysis product - the free amino acid 6, are obtained in a practically quantitative yield.

"Mild" mineral acid hydrolysis of the ethoxycarbonyl group of D5 affords D6.

To hydrolyze the phosphonic ester groups, we used an original synthetic method, developed in our laboratory, i.e. enzyme-substrate interaction with the enzyme phosphodiesterase I (a detailed paper on this method is ready for publication). It has been empirically established that the most favourable conditions are created when 20g substrate are used with 5mg enzyme (or 10-15mg, if spread on a polymer carrier). Esters of phosphino and phosphono acids of the type $RPO(OR^1)H$, $RPO(OR^1)CH_3$ and $RPO(OR^1)_2$, where R is alkyl, aryl, and R^1 is alkyl, are stirred for 6 hours at 37°C in 500-800ml aqueous buffer (pH 8.8), which, as a rule, leads to the isolation of the corresponding acids in practically quantitative yields. Provided the used enzyme is spread on a polymer carrier, it can participate in at least ten interactions without any loss of activity towards the standard substrate bis(p-nitrophenyl) phosphate. This method is characteristic for its very strong selectivity: should the substrate contain other hydrolyzable groups, they remain unaffected. Naturally, it is possible to use bigger or smaller quantities of substrate provided the quantitative ratio enzyme/substrate is kept within the prescribed limits. The method is excellent when applied to optically active compounds. All experiments that have been conducted in our laboratory for mineral acid hydrolysis of optically active compounds (i.e. at least 6-8 hrs. boiling in 20-22% hydrochloric acid) have resulted in complete racemization. Furthermore, the enzyme-substrate interaction is not influenced by the type of optical activity and both the D-

and the L-forms in the residue R of the above-mentioned acids are hydrolyzed.

However, the enzyme approach should be used with certain reservations, when aryl, cyclic, tertiary butyl and trimethylsilyl esters of the discussed acids are involved. To this moment we have not observed any enzyme-substrate interaction between the enzyme phosphodiesterase I and substrates that contain such ester groups.

Studying the kinetics of the enzyme-catalyzed hydrolysis is outside the research scope of our laboratory. Probably other scientists engaged in the field will devote some time to finding out how much the empirically established quantity of phosphodiesterase I could be reduced.

Thus, under the described conditions and with the phosphonic esters D6 and L6 as substrates, the free acids D4 and L4, respectively, are isolated with practically quantitative yields. However, all attempts at hydrolyzing the phosphonic ester 3 with phosphodiesterase I were unsuccessful - the enzyme was completely desactivated. If, however, the aldehyde group is protected, the interaction does take place and the phosphonic acid 7 is isolated with the acetal 2 as substrate. Mild mineral acid hydrolysis of 7 liberates the aldehyde group and results in the aldehydephosphonic acid 8.

A strictly selective hydrolysis of only one of the two ethoxyphosphinyl groups can be achieved by using the diphosphonic ester 2 as substrate of the enzyme alkaline phosphatase (cf. Scheme 2). 20g of the substrate 2 and 5mg enzyme are stirred for 6 hrs. in a buffer medium, ensuring the pH optimum of the enzyme (pH 10.2), at 37°C. Of course, bigger or smaller amounts of substrate could be used, provided the ratio enzyme/substrate remains within the prescribed limits. If the substrate exceeds 100g, it is better that the enzyme is spread on a polymer carrier. As a result of the enzyme-substrate interaction between alkaline phosphatase and the diphosphono ester 2, the monophosphono ester 9 is isolated in a practically quantitative yield. Mild mineral acid hydrolysis of the diacetal 9 releases the aldehydemonophosphonic ester 10.

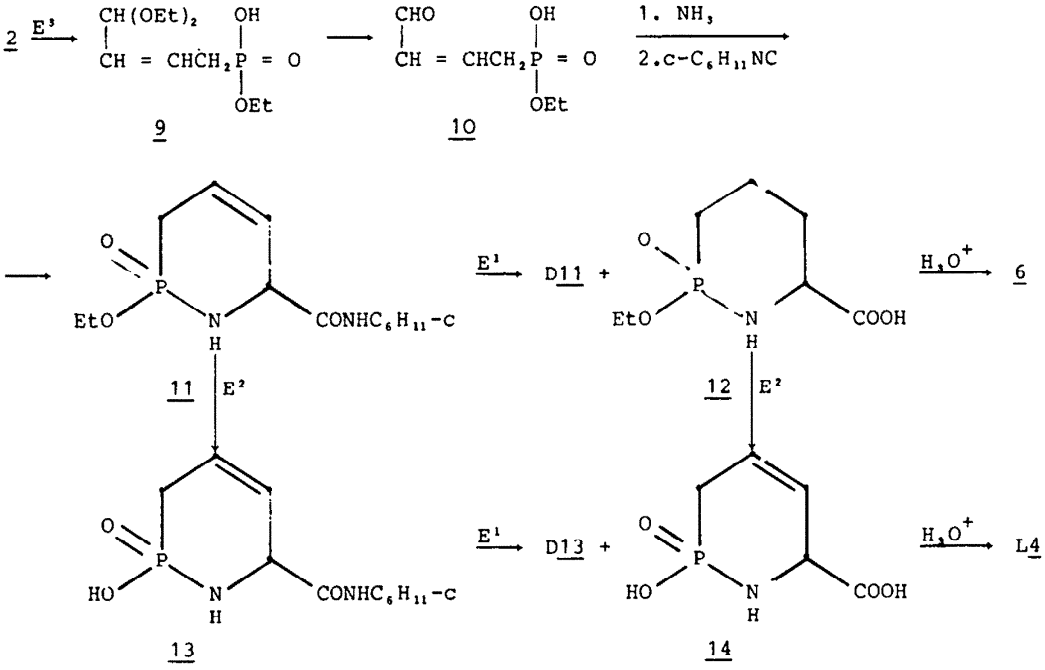
It is interesting to note that the enzyme alkaline phosphatase is quite inactive towards substrates of the type $RP(OR^1)H$ and $RP(OR^1)CH_3$: no enzyme-substrate interaction has to this moment been observed to occur. The enzyme does not affect the other hydrolyzable groups in the residue R as well. Much like the phosphodiesterase I, the enzyme-substrate interaction is not influenced by the type of the substrate optical activity: both the D- and the L-forms are hydrolyzed. The strict selectivity of the enzyme alkaline phosphatase makes it extremely suitable for synthesis of phospha C-peptides, which are very difficult to obtain by other methods.

An interesting cyclization is achieved when the aldehyde 10 is used in an isonitrile three-component condensation with no carboxyl component, whose functions are "taken over" by the monophosphonic group. Thus, when the aldehyde 10 is stirred for 15 mins. in 25% ammonium hydroxide, cyclohexylisonitrile in methanol is added and boiling is kept up for 3 hours, the unsaturated 1,2-azaphosphorinane 11 is obtained with a yield of 70%. A similar cyclization to the corresponding 1,2-azaphospholane occurs when 3-(hydroxymethylphosphinyl)propanal is used. The model compound 3-formylpropyonic acid does not take part in such cyclizations.

A detailed study of this phosphinic- and phosphonic-modification of the reaction of Ugi with compounds of the type $OHC(CH_2)_nPO(R)OH$ (n = 2-4, R = Me, OEt) is under way and the results will be published presently.

Apart from the very interesting physiological behaviour of azaphosphorinane (cf. below), the racemic mixture 11 can very comfortably be used to separate optically active antipodes and to obtain L4, while isolation of the triester 5 is thus avoided.

Scheme 2



E'- alkaline phosphatase.

The participation of 11 as substrate in an enzyme-catalyzed hydrolysis with α-chymotrypsin affords the unchanged D-form of the racemic mixture D11 and the free carboxylic acid 12 in an L-configuration.

We failed to hydrolyze the ethoxyphosphonyl group of 11, D11, and 12 by acid or alkali treatment without affecting the cyclic P-N bond - the cycle inevitably opened to the corresponding substituted norvalines. Enzyme-catalyzed hydrolysis with the phosphono ester 11 as substrate and the enzyme phosphodiesterase I leads to the isolation of the hydrolysis product 13 in a practically quantitative yield. The selectivity of the reaction makes it possible to preserve both the cyclic P-N bond and the carboxamide group, which creates opportunities to conduct condensation interactions with C-protected amino acids and peptides (cf. our subsequent publications).

The separation of optically active antipodes of the racemic mixture 13 is again achieved by enzyme-catalyzed hydrolysis of the cyclohexylcarboxamide group with the enzyme α-chymotrypsin to the unchanged D-form D13 and the hydrolyzed L-form 14. 14 can also be obtained by enzyme-catalyzed hydrolysis of the phosphonic ester group of the azaphosphorinane 12 with phosphodiesterase I.

No enzyme-substrate interaction occurs between the enzyme phosphatase and the four azaphosphorinanes 11-14. Mild mineral acid hydrolysis of the azaphosphorinanes 11-14 leads to the phosphonic ester 6 and the free norvaline L4, also obtained earlier, in practically quantitative yields.

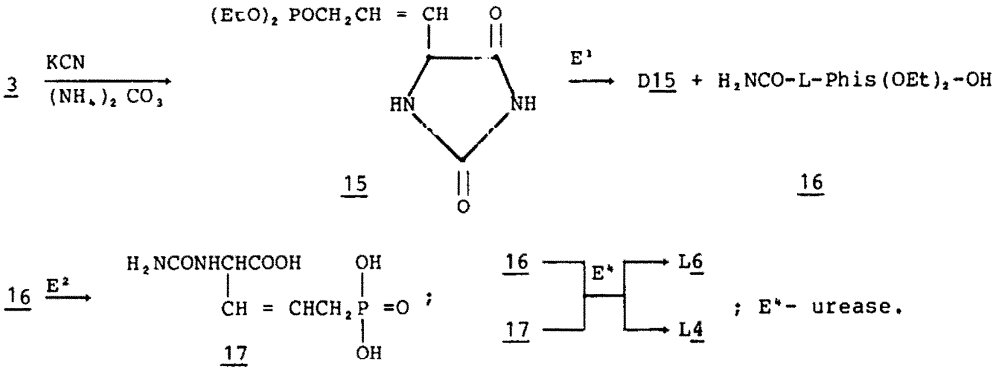
We did not succeed in synthesizing the D-antipode of 14, whereas attempted hydrolysis of the cyclohexylcarboxamide group of D13 always resulted in the hydrolysis of the cyclic P-N bond as well.

Another synthetic approach to obtaining the unusual C-terminal amino acid of the natural tripeptides Plumbemicin A and B is to use the aldehyde 3 in the reaction of Bucherer-Bergs, i.e. to treat it in an aqueous alcohol solution with potassium cyanide and ammonium carbonate. The hydantoin 15 is obtained with a yield of about 80% (Scheme 3).

Mineral acid hydrolysis of the hydantoin ring is somewhat complicated, due to

the very poor hydrolyzability of the cyclic amide bonds and phosphonic ester groups and to the reactivity of the ethylene double bond. Indeed, when the hydantoin 15 is treated for 6 hours with a saturated aqueous solution of sodium carbonate at 150-160°C in a closed steel tube and in argon ambience, the desired norvaline 4 is isolated with a yield of about 5%. However, its isolation from the resinous reaction product is extremely difficult. The results are no better with other hydrolyzing agents, as barium hydroxide, sodium hydroxide, hydrochloric acid, etc.

Scheme 3



From the variety of enzymes used to catalyze the hydrolysis of the hydantoin 15, as urease, dihydropyrimidinase, mesintericopeptidase, alkaline phosphatase, and phosphodiesterase I, only the latter two enhance the hydrolysis of only one, or of both, phosphonic ester groups (not shown in the Scheme), without at the same time affecting the hydantoin cycle.

A specific enzyme that hydrolyzes the amide bond of 15 is α-chymotrypsin. Applied under the above conditions, it leads to the simultaneous separation of the racemic mixture to the unchanged D-form, D15, and to the hydrolysis of only the C^4-N^3 cyclic amid bond, thus affording the N-substituted urea 16 in a practically quantitative yield.

In order to investigate the rather unusual behaviour of α-chymotrypsin towards the hydantoin 15, we used 1-phosphonomethyl1-2,5-imidazolidinedione as substrate. This compound has been recently isolated in our laboratory by treatment of 1-hydroxymethyl-2,5-imidazolidinedione with phosphorus chloride and acetic acid (details will be published separately). In contrast to the hydantoin 15, here the enzyme-substrate interaction is different: glycine and aminomethylphosphonic acid are isolated and carbon dioxide is released.

Enzyme-catalyzed hydrolysis of the diphosphonic ester 16 with phosphodiesterase I affords the polyfunctional optically active product 17, which contains fragments of an N-substituted urea, of the unsaturated α-aminocarboxylic acid, and of a phosphonic acid.

Despite its structure, which is quite unusual for a natural product, the N-substituted urea 17 readily participates in enzyme-substrate interactions with the enzyme urease. As a result, the α-aminocarboxylic acid L4, also synthesized earlier is isolated with a very satisfactory yield of approx. 80%. Unlike the enzymes discussed above, some small desactivation of the enzyme is observed: after five consecutive experiments with the substrate 17 the yield of L4 goes down to 50%. On the other hand, already after the first hydrolysis reaction the regenerated enzyme urease does enter the enzyme-substrate interaction with the "normal" substrate - the unsubstituted urea - and no ammonia-release occurs when treatment is conducted under optimal conditions, i.e. pH 7.0, 27°C. Most probably, this partial deactivation of the enzyme, when used in combination with the substrate 17, is due to the

presence of a free phosphono group: if the diphosphonic ester 16 is employed as substrate, the diphosphono ester 6, also obtained earlier, is isolated with a practically quantitative yield, while the enzyme urease retains its activity towards the unsubstituted urea.

To synthesize the tripeptide antibiotics Plumbemicin A and B, we conducted conventional peptide synthesis, followed by liberation of the blocking groups by enzyme-catalyzed hydrolysis.

Our starting products were the amino acid D5, which is free in its amino group and protected in its carboxyl and phosphono functions, and the protected dipeptide N-trifluoroacetyl-L-alanyl-L-aspartic acid β-ethyl ester. Condensation by the DCC-method proceeds quite as expected and affords the protected tripeptide 18 in a yield of approx. 85%.

Scheme 4.

$$\text{D5} + \text{TFA-Ala-Asp(OEt)-OH} \xrightarrow{\text{DCC}} \underset{18}{\text{TFA-Ala-Asp(OEt)-D-Phis(OEt)}_2\text{-OEt}} \xrightarrow{E^5}$$

$$\longrightarrow \underset{19}{\text{TFA-Ala-Asp-D-Phis(OEt)}_2\text{-OEt}} \xrightarrow{E^2} \underset{20}{\text{TFA-Ala-Asp-D-Phis(OH)}_2\text{-OEt}} \xrightarrow{OH^-}$$

$$\longrightarrow \underset{21}{\text{H-Ala-Asp-D-Phis(OH)}_2\text{-OH}} \xrightarrow{E^1} \underset{22}{\text{H-Asp-D-Phis(OH)}_2\text{-OH}} + \text{H-Ala-OH}$$

E^5- alkaline mesintericopeptidase.

With a view to extending our studies on enzyme-substrate interactions with non-standard substrates for hydrolysis of the β-ethoxycarbonyl group of the tripeptide 18, we started experimenting with alkaline mesintericopeptidase. Despite the fact that the ethoxycarbonyl group is hydrolyzed several times faster than the peptide Ala-Asp bond, the hydrolysis process should by all means be controlled every 15 mins. The liberation of the β-carboxyl group could, of course, be achieved by mineral acid hydrolysis and this is one of a few cases where enzyme-catalyzed hydrolysis is not at any particular advantage. As a result of enzyme-substrate interaction of the tripeptide 18 with alkaline mesintericopeptidase, the tripeptide 19 is isolated with a yield of about 90%. A good thing about this enzyme is that, due to the D-configuration of the amino acid, it leaves the C-terminal ethoxycarbonyl group intact and thus the resultant free β-carboxyl function can, if protected functional groups were still available, be used for a subsequent peptide synthesis.

In a catalyzed hydrolysis process, the enzyme mesintericopeptidase can safely be replaced with an acid or a hydroxide, the hydrolysis of the diethoxyphosphonyl group would certainly require the presence of phosphodiesterase I because of the great hydrolytic stability of the POC bond, which is even greater than that of the peptide CONH bonds. A combination of the enzyme phosphodiesterase I and the tripeptide 19 as substrate leads to the selective hydrolysis of only the ethoxyphosphinyl groups without affecting the other hydrolyzable groups. The tripeptide 20 is isolated with a yield of over 90%.

Liberation of the blocking N-trifluoroacetyl and C-ester groups of the tripeptide 20 to the entirely free antibiotics Plumbemicin A, 21, is achieved by standard alkaline hydrolysis.

It is easy to explain the fact that, due to the D-configuration of the C-terminal amino acid, alanine and the dipeptide 22 are isolated from the combination of the tripeptide 21 as substrate and the enzyme α-chymotrypsin.

The close structural analogue Plumbemicin B is obtained analogously.

D5 is condensed with the dipeptide TFA-L-Ala-L-Asn-OH by the DCC-method to the completely protected tripeptide 23. The hydrolysis of the diethoxyphosphonyl group

was again catalyzed with the enzyme phosphodiesterase I and, with the tripeptide 23 as substrate, the tripeptide 24 was isolated. Alkaline hydrolysis of 24 releases the antibiotic Plumbemicin B, 25. Using the tripeptide 25 as substrate in an enzyme-substrate interaction with the enzyme α-chymotrypsin affords alanine and the dipeptide 26, much like the case with the tripeptide 21.

Scheme 5.

$$\underline{D5} + \text{TFA-Ala-Asn-OH} \xrightarrow{DCC} \underset{\underline{23}}{\text{TFA-Ala-Asn-D-Phis(OEt)}_2\text{-OEt}} \xrightarrow{E^2}$$

$$\longrightarrow \underset{\underline{24}}{\text{TFA-Ala-Asn-D-Phis(OH)}_2\text{-OEt}} \xrightarrow{OH^-} \underset{\underline{25}}{\text{H-Ala-Asn-D-Phis(OH)}_2\text{-OH}} \xrightarrow{E^1}$$

$$\longrightarrow \underset{\underline{26}}{\text{H-Asn-D-Phys(OH)}_2\text{-OH}} + \text{H-Ala-OH}$$

All newly synthesized peptides, 19-27, were subjected to total acidic hydrolysis (6N HCl, 110°C, 24°C in a sealed ampule) and the total hydrolysate was thoroughly investigated. It was found that these conditions cause changes in the 5-phosphono-3,4-didehydronorvaline - hence the method is not suitable for proving the content of di- and tripeptides.

With a view to clarifying the relationship between chemical structure and biological activity, tests were conducted for herbicidal, fungicidal, insecticidal, and antitumour activity of the following close structural analogues: N-phosphonomethylglycine, 27[6], 2-amino-4-hydroxymethylphosphonyl-L-butanoic acid, 28[6], its pyro-analogue 29[7], and the newly synthesized L4 and its pyro-analogue 14. All compounds were studied in the form of their water-soluble isopropylammonium salts.

The herbicidal activity was established by the method described in Ref.8, i.e. the germination of seeds from mono- and dicotyledons was subjected to careful observation. This method is discussed in details in the Sec. "Experimental" and comparisons are made with hot-house experiments (Item 16, Methods A and B). Comparing and estimating the results is made still easier by the availability of abundant literature data on numerous experiments with the method developed in Ref.8, as well as analytical parallels with field experiments. The laboratory method is thus rendered a fast and reliable qualitative and quantitative test for herbicidal activity.

We studied the selective activity index, introduced by Vassilev et al.[9], which represents the activity of the sample under study in relation to mono- and dicotyledons.

A curious fact (cf. Table 1.) is that, while the herbicidal activity of 27 and 28 towards mono- and dicotyledons is 70.6, 72.1, 100, and 100%, respectively, the pyrophosphinotricine 29 is completely inactive. The phosphono-substituted norvaline L4 has a pronounced herbicifal activity. Concentration of 10^{-3}-10^{-6} mols suppress germination in monocotyledons 100,100,84.3 and 69.3%, respectively. Towards dicotyledons, however, the activity of L4 is much weaker: 83.2,49.6,20.6 and 0%, respectively. The index of selective activity of L4 is estimated at 1.20,2.02,4.20 units. Selectivity is complete at concentrations of 10^{-6} mols. This rather rare selectivity renders L4 very promising for future post-germination herbicidal tests.

Similar to the pyro-analogue of phosphinotricine 29, however, engaging the phosphono and amino groups in an azaphosphorinane cycle (the pyro-analogue 14) results in a complete loss of the herbicidal activity.

Tests for fungicidal activity were carried out in connection with controlling rice sheath blight (Xanthomonas orysae) and rice blast (Pellicularia sasakii, Pyricularia orysae). It was found that the norvaline L4 and its pyro-analogue 14 are

most active, while N-phosphonomethylglycine 27 possesses no fungicidal activity at all.

Unlike 28 and L4, the pyro-analogues 29 and 14 display a better insecticidal activity, especially towards domestic insects. An aqueous solution of 0.1% 14 and 2.0% plain sugar, when placed in a tray on a farm-building window-sill, killed off fifty-three flies for an hour, after that no fly appeared for a whole week.

It is interesting to observe the effect of the products 29 and 14 on cockroaches. Aqueous solutions of 29 and 14, from 0.1 to 1.0%, do not affect the insects at all, cockroaches do not even react to the bait. However, if 0.2g of the isopropyl-ammonium salts of 29 and 14 and 2.0g plain sugar are dissolved in 100ml water, concentrated in vacuum and the syrup-like residue spread on a glass plate (10x10cm), the result is an excellent repellent.

These biological tests for insecticidal activity, though not conducted by standard methods, have nevertheless provided enough grounds to hope that any future experiments in this direction would be very successful.

Comparative anti-tumour tests were conducted of N-phosphonomethylglycine, 27, L-phosphinotricine, 28, norvaline, L4, its cyclic analogue, 14, and the tripeptides Bialphos (SF-1293), Plumbemicin A, 22, and B, 26. It was found that only the azaphosphorinane 14 has a pronounced activity towards an experimental tumour L1210 in rats. The strong toxicity of 14 (LD_{50}= 180mg/kg), however, would rather complicate further experiments, unless additional chemical modifications are made to reduce toxicity.

A detailed study of the discussed activities is under way and will be published soon.

EXPERIMENTAL

General notes: IR-spectra, elemental analysis, HPLC and optical activity - on a Perkin-Elmer instrument; ^{1}H NMR-spectra - on Joel-100MHz; mass-spectra - on LKB-900; m.p.'s - on a Köfler apparatus; TLC - silica gel film "Merk"; reagents and solvents - from "Aldrich" and "Merk"; enzymes and buffers - "Sigma".

The analytical data for all compounds, named in accordance with Chemical Abstracts and having their numbers in the text and chemical formulae, are given in the following order: yield; IR-spectrum (a: KBr, b: film, cm^{-1}); ^{1}H-NMR-spectrum taken in c: DMSO-d_6, d: $CDCl_3$, e: D_2O, TMS standard, δ scale, ppm (number of protons as calculated along the integral curve, multiplicity); mass-spectrum - M^+/e calculated/found; elemental analysis for % C, H, and N - calculated/found; R_f - phosphomolybdenite or ninhydrine (for NH_2-group containing compounds) detection in systems: A: n-BuOH: H_2O = 9:1:1, B: n-BuOH: 25% NH_4OH = 4:1, C: DMF: $CHCl_3$: MeOH = 5:1:2, and D: $CHCl_3$: MeOH = 9:1; m.p.; $[\alpha]_D^{20}$.

Spectral data of known compounds are compared with those of the natural counterparts. As optical antipodes have identical spectral data, only data for $[\alpha]_D^{20}$ are given.

1. Synthesis of the acetal 1.

A hot solution of 0.4g ammonium nitrate in 12.5ml ethanol is added to 14.89g (0.1M) 4-bromocrotoaldehyde[5], 17.78g (0.12M) triethoxymethane and 0.2g quinone. The mixture is boiled for 10 mins., filtered and added to a double volume of ether. The mixture is then washed with 5% ammonium hydroxide and water, dryed over $MgSO_4$ and distillated.

1,1-Diethoxy-4-bromo-2-buten, 1: $C_8H_{15}BrO_2$; 17.02g (76.3%); b: 3020, 2840, 1120, 975; 9: 1.18 and 1.29 (6H, t, J=7Hz, 2xCH_3), 3.40-3.60 (4H, m, 2xOCH_2), 4.18 (1H, d, J=7Hz, $BrCH_2$), 4.82 (1H, d, J=8Hz, CHO), 6.24 and 6.93 (2H, m, CH=CH); 223.1/223 (22%); 43.07/42.83, 6.78/7.01; D: 0.80; oil.

2. Synthesis of the diethyphosphonate 2.

22.31g (0.1M) of the acetal 1 and 24.92g (0.15M) triethyphosphite are heated for 2 hours at 135°C in an ambience of dry argon. The product is isolated by vacuum distillation.

4,4-Diethoxy-2-butenophosphonic acid ethyl ester, 2: $C_{12}H_{25}O_5P$; 17.04g (50.2%); b b: 3015, 2860, 1255, 1130-1000, 975, 865; d: 1.1-1.9 (12H, m, 4xCH_3), 2.83 (2H, m, PCH_2), 3.40-3.60 (4H, m, 2xOCH_2), 3.80-4.20 (4H, m, 2xOCH_2), 4.78 (1H, d, J=7Hz, CHO), 5.72 and 6.18 (2H, m, CH=CH); 280.3/280 (24%); 51.42/51.25, 8.99/9.18; D: 0.72; oil.

3. Synthesis of the aldehyde 3.

28.03g (0.1M) of the diacetal 2 and 50ml cold-saturated aqueous solution of tartaric acid are stirred until a homogeneous mixture is obtained. 500ml cold-satura-

ted aqueous solution of the calcium chloride are added, the mixture is left for 1 hour at 0°C and the resultant oil is decanted.

4-Oxo-2-butenophosphonic acid ethyl ester, 3: $C_6H_{15}O_4P$; 20.23g (98.6%) b: 3020, 1745,1255,1100-960; c: 1.20 and 1.36 (6H,t,J=6Hz,2xCH_3), 2.85 (2H,m,PCH_2), 3.39 and 3.66 (4H,q,2xOCH_2), 5.70 and 6.12 (2H,m,CH=CH), 9.73 (1H,d,CHO); 206.2/206 (16 %); 46.60/46.39, 7.33/7.41; A: 0.49; oil.

4. Synthesis of the norvaline 4.

12.61g (0.2M) ammonium formate, 20.62g (0.1M) of the aldehyde 3 and 13.10g (0.12M) cyclohexylisonitrile are consecutively added at intervals of 15mins. to 200ml 80% aqueous methanol and the mixture is boiled for 6 hours. The volatile components are evaporated in vacuum to dryness and 400ml 10% sodium hydroxide are added to the residue. The mixture is then heated at 70-80°C for 3 more hours. After acidification, the mixture is left for 24 hours at 0°C and filtered.

5-Phosphono-3,4-didehydro-DL-norvaline, 4: $C_5H_{10}NO_5P$; 15.47g (79.3%); spectral data IR and ^{1}H-NMR are identical with those of authentic sample; 195.1/195 (18%); 30.78:31.07, 5.17/4.99, 7.18/7.36; 170-172°C (decomp.).

5. Synthesis of the triester 5.

39.26g (0.33M) thionylchloride is added dropwise to 19.51g (0.1M) norvaline 4 in 100ml dry ethanol, containing 10ml dimethyformamide. The process is accompanied by cooling at a constant rate. A fast current of dry nitrogen is passed through the mixture, while gradually heating it to obtain a homogeneous solution. The solution is concentrated in vacuum, cooled down and filtered.

5-Diethoxyphosphinyl-3,4-didehydro-DL-norvaline ethyl ester, 5: $C_{11}H_{22}NO_5P.HCl$; 220.14g (63.8%); a: 3020,1740,1525,1255,1100-975; d(free base): 1.2-1.9 (9H,m,3xCH_3), 2.22 (2H,s,NH_2), 2.80 (2H,m,PCH_2), 3.20-3.60 (6H,m,3xOCH_2), 4.82 (1H,d,J=7Hz, CHN), 5.78 and 6.20 (2H,m,CH=CH); 279.3/279 (8%); 41.85/42.01, 7.34/7.12, 4.44/4.69; C: 0.55; 140-143°C (decomp.).

6. Enzyme hydrolysis - general method.

20g substrate and 5mg enzyme (or 10-15mg, if spred on a polymer carrier) are stirred for 6 hours in 500ml buffer at the temperature and pH optimum of the enzyme. After regeneration of the enzyme, acidification and extraction, or crystallization, the corresponding hydrolysis products are isolated in practically quantitative yields, plus the unchanged D-forms in case racemic mixtures are substrates and the enzyme is α-chymotrypsin. Using mesintericopeptidase requires strict control of the enzyme-substrate interaction.

The substrates of the enzyme α-chymotrypsin (pH 7.8, 25°C) and their hydrolysis products are as follows: 5 - D5 and L6; 11 - D11 and L12; 13 - D13 and 14; 15 - D15 and 16; 21 - 22; 25 - 26.

- of phosphodiesterase I (pH 8.8, 37°C): 2 - 7; D6 - D4; 6 - L4; 11 - 13; 12 - 14; 16 - 17; 19 - 20; 23 - 24.
- of alkaline phosphatase (pH 10.2, 37°C): 2 - 9.
- of urease (pH 7.0, 37°C): 16 - 6 and 17 - L4.
- of mesentericopeptidase (pH 8.0, 25°C): 18 - 19.

5-Phosphono-3,4-didehydro-D-norvaline, D4: $C_5H_{10}NO_5P$; spectral data IR and ^{1}H-NMR are identical with those of authentic sample; 195.1/195 (18%); 30.78/30.65, 5.17/5.33, 7.18/7.01; 184-186°C; (decomp.); c = 1(H_2O); $[\alpha]_D^{20}$: +52.3°.

5-Phosphono-3,4-didehydro-L-norvaline, L4: $C_5H_{10}NO_5P$; spectral data IR and ^{1}H-NMR are identical with those of authentic sample; 195.1/195 (18%); 30.78/30.56, 5.17/4.87, 7.18/7.21; 177-180°C; (decomp.); c = 1(H_2O); $[\alpha]_D^{20}$: -49.2°.

5-Diethoxyphosphinyl-3,4-didehydro-D-norvaline ethyl ester hydrochloride, D5: $C_{11}H_{22}NO_5P.HCl$; spectral data identical with those of 5; m.p. 156-160°C; (decomp.); c = 0.1(MeOH); $[\alpha]_D^{20}$: +48.6°.

5-Diethoxyphosphinyl-3,4-didehydro-L-norvaline, L6: $C_9H_{18}NO_5P$; a: 3520-2860, 1740,1520,1110-980; e + NaOD: 1.20 and 1.38 (6H,t,J=6Hz,2xCH_3), 2.83 (2H,m,PCH_2), 3.40 and 3.68 (4H,q,2xOCH_2), 4.78 (1H,d,J=7Hz,CHN), 5.64 and 6.14 (2H,m,CH=CH); 251.2/251 (18%); 43.03/42.96, 7.22/7.48, 5.58/5.42; B: -0.83; (decomp.); c = 0.1(H_2O) $[\alpha]_D^{20}$: +48.2°.

4,4-Diethoxy-2-butenophosphonic acid, 7: $C_8H_{17}O_5P$; a: 3020-2460,1270,1120; D_2O + NaOD: 1.16 and 1.28 (6H,t,J=7Hz,2xCH_3), 2.85 (2H,m,PCH_2), 3.46 and 3.70 (4H,q,2xOCH_2), 4.73 (1H,d,J=8Hz,CHO), 5.56 and 6.28 (2H,m,CH=CH); 224.2/224 (29%); 42.86/43.01, 7.64/7.49; C: 0.62; (decomp.); about 160°C.

4,4-Diethoxy-2-butenophosphonic acid monoethyl ester, 9: $C_{10}H_{21}O_5P$; b: 3025-2690, 2540-2470,1255,1100-960,840; c: 1.2-1.7 (9H,m,3xCH_3), 2.78 (3H,m,PCH_2), 3.4-3.6 (6H,m,3xOCH_2), 4.45 (1H,d,J=8Hz,CHO), 5.72 and 6.23 (2H,m,CH=CH), 10.2-10.4 (1H,br., POH); C: 0.72; 96-98°C.

D-(2-ethoxy-4,5-didehydro-1,2-azaphosphorinane-6-yl-2-oxide)cyclohexylcarboxamide, D11: $C_{13}H_{23}N_2O_3P$; spectral data coincide with those of 11; c = 0.1(MeOH); $[\alpha]_D^{20}$ +68.3°.

(2-Hydroxy-4,5-didehydro-1,2-azaphosphorinane-6-yl-2-oxide)cyclohexylcarboxamide, 13: $C_{11}H_{19}N_2O_3P$; a: 3020,2840-2460,1650,1385,1255,940,860,635; e+NaOD: 0.8-2.2 (10H,m,5xCH_2), 2.83 (2H,m,PCH_2), 4.05 (1H,m,CHN), 4.48 (1H,d,J=6Hz,CHCO), 5.78 and 6.30 (2H,m,CH=CH); 258.3/258 (18%); 51.16/50.89, 7.42/7.56, 10.85/10.63; B: 0.72; 183-185°C; (decomp.).

L-(2-ethoxy-4,5-didehydro-1,2-azaphosphorinane-6-yl-2-oxide)carboxylic acid, 12 $C_7H_{12}NO_4P$; a: 3480-3200,3025,1760,1390,1110-960,870,640; e+NaOD: 1.21 (3H,t,J=7Hz, CH_3), 2.36 (2H,m,PCH_2), 3.43 (2H,q,OCH_2), 4.51 (1H,d,J=8Hz,CHCO), 5.68 and 6.33 (2H,m,CH=CH), plus two exchangeable protons, NH and COOH; 205.1/205 (16%); 40.98/41.16, 5.90/5.71, 6.68/6.92; B: 0.65; 143-146°C; (decomp.); c = 1(1N NaOH); $[\alpha]_D^{20}$: -62.3°.

D-(2-hydroxy-4,5-didehydro-1,2-azaphosphorinane-6-yl-2-oxide)cyclohexylcarboxamide, D13: $C_{11}H_{19}N_2O_3P$; spectral data coincide with those of 13; m.p. 198-200°C; (decomp.); c = 1(1N NaOH); $[\alpha]_D^{20}$: +66.2°.

L-(2-hydroxy-4,5-didehydro-1,2-azaphosphorinane-6-yl-2-oxide)carboxylic acid, 14: $C_5H_8NO_4P$; a: 3530-3200,2840-2470,1760,1400,1255,870,630; e: 2.31 (2H,m,PCH_2), 4.52 (1H,d,J=8Hz,CHCO), 5.76 and 6.28 (2H,m,CH=CH), plus three exchangeable protons, NH, POH, and COOH; 177.1/177 (39%); 33.91/34.11, 4.56/4.38, 7.91/8.06; A: 0.75; approx. 230°C; (decomp.); c = 1(1N NaOH); $[\alpha]_D^{20}$: -59.3°.

D-3-(2,5-dioxo-imidazolidine)-2-propenophosphonic acid ethyl ester, D15: $C_{10}H_{17}N_2O_5P$; spectral data identical with those of 15; m.p. 196-198°C; c = 0.1(MeOH); $[\alpha]_D^{20}$: -70.1°.

N-aminocarbonyl-5-diethoxyphosphinyl-3,4-didehydro-L-norvaline, 16: $C_{10}H_{19}N_2O_6P$; a: 3200-2800,1740,1670,1560,1260,865,635; e+NaOD: 1.16 and 1.38 (6H,t,J=7Hz,CH_3), 2.80 (2H,m,PCH_2), 3.41 and 3.52 (4H,m,2×OCH_2), 4.36 (1H,d,J=7Hz,CHCO), 5.82-6.18 (2H,m,CH=CH), plus four exchangeable protons, NH_2, NH, COOH; 294.2/294 (18%); 40.82/40.59, 6.51/6.62, 9.52/9.39; A: 0.40; 215-218°C; (decomp.); c = 1(1N NaOH); $[\alpha]_D^{20}$ -66.3°.

N-aminocarbonyl-5-phosphono-3,4-didehydro-L-norvaline, 17: $C_6H_{11}N_2O_6P$; a: 3500-3180,3040,2840-2470,1740-1630,1250,910,870,630; c: 2.86 (2H,m,PCH_2), 4.32 (1H,d,J=7Hz,$CHCO_2H$), 5.24 (2H,br.s,NH_2), 5.70 (1H,s,NH), 5.84 and 6.21 (2H,m,CH=CH), 10.2-10.6 (2H, broad,POH), 10.9-11.2 (1H,broad,COOH); 238.1/238 (68%); 30.26/30.31, 4.66/4.50, 11.76/11.85; B: 0.62; approx. 300°C; (decomp.); c = 1(1N NaOH); $[\alpha]_D^{20}$: -63.6

N-(N-trifluoroacetyl-L-alanyl-L-α-aspartyl)-3,4-didehydro-5-dietoxyphosphinyl-D-norvaline ethyl ester, 19: $C_{20}H_{31}F_3N_3O_{10}P$; 16.82g (88.3%) - with 20g of the substrate 18 used; a: 3480-3000,1760,1660-1620,1255,1110-980,870,630; (the ^{1}H-NMR spectra of the peptides 18-26 are obtained on a Brucker 250MHz); c: 1.1-1.8 (12H,m,3×OCH_2-CH_3,$CHCH_3$), 2.18 (2H,d,J=8Hz,$CHCH_2CO_2H$), 2.66 (2H,m,PCH_2), 3.92 (1H,t,J=6Hz,$CHCH_2$-CO_2H), 4.1-4.4 (8H,m,3×OCH_2CH_3,2×CH), 5.80 and 6.13 (2H,m,CH=CH), 11.0-11.5 (1H, br.s,CO_2H), plus three broad singlets of 3×NH; (under conditions of mass spectral analysis the peptide 18-26 decompose and weight formula is determined on Perkin-Elmer): 566; 42.79/43.01, 5.57/5.43, 7.48/5.52; A: 0.50; 124-126°C; total hydrolysate content (6N HCl, 24 hours, 110°C in a sealed ampule): Ala, Asp (Phis is changed); c = 0.1(MeOH); $[\alpha]_D^{20}$: +110°.

L6 and L4, obtained from the substrates 16 and 17, respectively, and the enzyme urease, have the same spectral data, IR and ^{1}H-NMR, as the compounds described above.

N-(N-trifluoroacetyl-L-alanyl-L-α-aspartyl)-3,4didehydro-5-phosphono-D-norvaline ethyl ester, 20: $C_{16}H_{23}F_3N_3O_{10}P$; a: 3480-3020,2840-2460,1760,1660-1620,1250,865,630; (D_2O+NaOD): 1.18 (3H,t,J=8Hz,OCH_2CH_3), 1.33 (3H,d,J=7Hz,$CHCH_3$), 2.19 (2H,d,J=7Hz,$CHCH_2CO_2H$), 3.90 (1H,t,J=8Hz,$CHCH_2CO_2H$), 4.03 (2H,q,J=7.5Hz,OCH_2CH_3), 4.18 (1H,d,J=6Hz,CHCH=CH), 4.26 (1H,q,J=7.5Hz,$CHCH_3$), 5.72 and 6.15 (2H,m,CH=CH) and six exchangeable protons: CO_2H, PO_3H_2, 3×NH; 510; 38.03/38.19, 4.59/4.33, 8.32/8.60; 186-189°C; (decomp.); A: 0.39; c = 0.1(NaOH); $[\alpha]_D^{20}$: +98.3°.

N-(L-α-aspartyl)-3,4-didehydro-5-phosphono-D-norvaline, 22: $C_9H_{15}N_2O_8P$; a: 3500-3000,2840-2460,1750,1640,1520,1250,980,740,630; (D_2O+NaOD): 2.18 (2H,d,J=7Hz,$CHCH_2$-CO_2H), 2.77 (2H,m,PCH_2), 4.06 (1H,d,J=8Hz,CHCH), 4.20 (1H,t,J=7Hz,$CHCH_2CO_2H$), 5.82 and 6.23 (2H,m,CH=CH), plus six exchangeable protons: CO_2H, NH_2, PO_3H_2, NH; 313; 34.85/35.00, 4.87/4.59, 9.03/9.18; A:0.43, B: 0.69; 188-190°C (decomp.); Asp. c = 0.1(NaOH); $[\alpha]_D^{20}$: +58.3°.

N-N^2-(N-trifluoroacetyl-L-alanyl-L-asparaginyl)-3,4-didehydro-5-phosphono-D-norvaline ethyl ester, 24: $C_{16}H_{24}F_3N_4O_9P$; a: 3345,3175,3030,2840-2460,1760,1660-1620,1255,870,635; c: 1.19(3H,t,J=7Hz,OCH_2CH_3), 1.35 (3H,d,J=7Hz,$CHCH_3$),2.20 (2H,d,J=7Hz,$CHCH_2$), 2.85 (2H,m,PCH_2), 4.1-4.4 (5H,m,OCH_2CH_3,3×CH), 5.78 and 6.15 (2H,m,CH=CH), 10.2-10.6 (2H,br.s,PO_3H_2), in a case of $CONH_2$ and 3×NHCO broad singlets are observed which after the exchange (D_2O + NaOD) disappear; 510; 38.10/38.42, 4.80/4.76, 11.11/11.24; A: 0.33; 169-171°C (decomp.); Ala, Asp; c = 0.1(NaOH); $[\alpha]_D^{20}$:+120

N-(L-asparaginyl)-3,4-didehydro-5-phosphono-D-norvaline, 26: $C_9H_{16}N_3O_7P$; a: 3480-3000,2840-2460,1750,1650,1640,1525,1250,980,740,630; (D_2O+NaOD): 2.18 (2H,d, J=7Hz,$CHCH_2$), 2.80 (2H,m,PCH_2), 3.91 (1H,t,J=7Hz,$CHCH_2$), 4.06 (1H,d,J=6Hz,CHCH), 5.8 and 6.18 (2H,m,CH=CH) and 8 exchangeable protons: CO_2H, PO_3H_2, NH, 2×NH_2; 313; 34.96/35.18, 5.22/5.10, 13.59/13.64; A: 0.55, B: 0.73; 173-176°C (decomp.), Asn; c = 0.1(NaOH); $[\alpha]_D^{20}$: +120°.

7. Hydrolysis of the diacetals 7 and 9.

22.42g (0.1M) of the diacetal 7 and 50ml cold-saturated aqueous solution of tartaric acid are stirred for 30 mins. at ambient temperature. An abrupt cooling down of the reaction mixture occurs, 500ml cold-saturated solution of calcium chloride are added and, after 1 hour at 0°C, the mixture is decanted.

4-Oxo-2-butenophosphonic acid, 8: $C_4H_7O_4P$; 14.21g (94.2%); a: 3045,2870-2460, 1745,1255,1050-940; e+NaOD: 2.83 (2H,m,PCH_2), 5.48 and 6.18 (2H,m,CH=CH), 9.42 (1H, d,CHO); 150.1/150 (53%); 32.01/32.39, 4.70/4.81; C: 0.40; decomposes at approx. 180°C.

9 is hydrolyzed quite analogously.

4-Oxo-2-butenophosphonic acid monoethyl ester, 10: $C_6H_{11}O_4P$; 17.16g(93.2%); a: 3025,2740-2640,1745,1250,1100-955,845; c: 1.19 (3H,t,J=7Hz,CH_3), 2.80 (2H,m,PCH_2), 3.41 (2H,q,OCH_2), 5.70 and 6.24 (2H,m,CH=CH), 9.74 (1H,d,CHO); 184.1/184(23%); 39.14/39.42, 6.02/5.98; A: 0.62; 143-146°C (decomposition).

8. Synthesis of the azaphosphorinane 11.

18.41g (0.1M) of the aldehyde 10 are added to 20.43g (0.3M) 25% ammonium hydroxide and the mixture is stirred for 15 mins. at room temperature. 13.10g (0.12M)

cyclohexylisonitrile in in 200ml methanol are added and the is boiled for 3 hours, evaporated in vacuum and the residue placed in a silica gel column (eluent chloroform : methanol = 9:1).

(2-Ethoxy-4,5-didehydro-1,2-azaphospharinane-6-yl-2-oxide)cyclohexylcarboxamide, 11: $C_{11}H_{23}N_2O_3P$; 20.11g(70.2%); b: 3040,1645,1250,1100-980,840,635; d: 0.7-2.2 (13H, m,5xCH_2 and CH_3), 2.39 (2H,m,PCH_2), 3.42 (2H,q,OCH_2), 4.01 (1H,m,CHN), 4.38 (1H,d, J=8Hz,CHCO), 5.0 and 5.4 (2H,broad s,2xNH), 5.74 and 6.38 (2H,m,CH=CH); 286.3/286 (13%); 54.54/54.40, 8.10/8.33, 9.78/9.82; D: 0.72; oil - cannot be distilled without decomposition up to 100°C/0.01 torr.

9. Mineral acid hydrolysis of the azaphosphorinanes 12 and 14.

0.1M each of 12 and 14 are heated for 30 mins. at 50°C in 100ml 1% hydrochloric acid and then evaporated in vacuum to dryness. The residue is worked up as above. Hydrolysis to L6 and L4 affords practically quantitative yields. Spectral data are identical to the spectra of the same compounds obtained by enzyme hydrolysis. L6: $[\alpha]_D^{20}$ +49.2°, c = 0.1(H_2O) and L4: $[\alpha]_D^{20}$ -50.1°, c = 0.1(H_2O).

10. Synthesis of the hydantoin 15

A mixture of 20.62g (0.1M) aldehyde 3, 6.51g (0.1M) potassium cyanide and 8.26g (0.1M) ammonium carbonate in 100ml 50% aqueous ethanol is stirred for 6 hours at 60°C. After cooling and filtration, the mother liquors are placed in a vent cabinet (possible separation of HCN!), acidified and cooled: an additional quantity of the product is thus filtered off. The product is crystallized from ethanol.

3-(2,4-Dioxo-imidazolidin-5-yl)-2-propenophosphonic acid ethyl ester, 15: $C_{10}H_{17}N_2O_5P$; 19.15g (69.3%); a: 3350-3200,3030,1750-1690,1300,1255,1110-980,865,630; c: 1.18 and 1.36 (6H,t,J=7Hz,2xCH_3), 2.80 (2H,m,PCH_2), 3.50 and 3.62 (4H,q,2xOCH_2), 4.18 (1H,d,J=8Hz,CHN), 5.62 and 6.12 (2H,m,CH=CH), 7.52 (1H,broad,NH), 9.60 (1H, broad,NH); 276.3/276 (8%); 43.48/43.56, 6.20/5.93, 10.14/10.16; D: 0.33, A: 0.83; 183-186°C.

12. Synthesis of the protected Plumbemicin A, 18.

A mixture of 27.93g (0.1M) norvaline analogue D5 (free base), 32.82g (0.1M) of the dipeptide N-trifluoroacetyl-L-alanyl-L-aspartic acid β-ethyl ester, and 24.76g (0.12M) N,N'-dicyclohexylcarbodiimide is kept for 24 hours at room temperature in 250ml dry ethylacetate. The N,N'-dicyclohexylureate is filtered out and the filtrate is washed consecutively with 5% aqueous sodium carbonate, water, 5% hydrochloric acid and with water again, dried over $MgSO_4$ and distilled to dryness. The residue is placed in a silica gel column and eluted with chloroform : methanol = 9:1.

N-(N-trifluoroacetyl-L-alanyl-β-ethyl-L-α-aspartyl)-3,4-didehydro-5-diethoxyphosphinyl-D-norvaline ethyl ester, 18: $C_{22}H_{35}F_3N_3O_{10}P$; 43.80g (74.3%); b: 3345, 3175,3040,1760-1720,1660-1620,1255,1110-980,870,630; d: 1.1-1.8 (15H,m,4xOCH_2CH_3), 2.36 (2H,d,J=6Hz,$CHCH_2$), 2.68 (2H,m,PCH_2), 4.0-4.5 (11H,m,4xOCH_2CH_3), 5.75 and 6.22 (2H,m,CH=CH) and three broad singlets for 3xNHCO, which disappear after the reaction (D_2O + NaOD); 590; 44.82/44.69, 5.98/6.01, 7.13/7.41; A: 0.56; oil; $[\alpha]_D^{20}$ +102°, c=0.1(NaOH).

13. Synthesis of the protected Plumbemicin B, 23.

The above method is repeated with 0.1M TFA-L-Ala-L-Asn-OH.

N-(N²-L-trifluoroacetyl-L-alanyl-L-asparaginyl)-3,4-didehydro-5-diethoxyphosphinyl-D-norvaline ethyl ester, 23: $C_{20}H_{32}F_3N_4O_9P$; 36.50g (72.5%); a: 3345,3175,3040, 1760,1660-1620,1250,1110-970,850,635; c: 1.2-1.6 (12H,m,3xOCH_2CH_3,$CHCH_3$), 2.19 (2H, d,J=7Hz,$CHCH_2$), 2.86 (2H,m,PCH_2), 4.0-4.4 (9H,m,3xOCH_2CH_3,3xCH), 5.80 and 6.13 (2H, m,CH=CH), for $CONH_2$ and 3xNHCO broad singlets are observed, which after the exchange (D_2O + NaOD) disappear; 553; 42.86/43.02, 5.75/5.49, 10.00/9.78; A: 0.54; 96-99°C; c = 0.1(NaOH), $[\alpha]_D^{20}$: +92.4°.

14. Synthesis of Plumbemicin A, 21.

25.27g (0.05M) of the tripeptide 20 are left for 12 hours at 5°C in 100ml 2N sodium hydroxide. After acidification, the product is crystallized from methanol-dioxan.

N-(N-L-alanyl-L-asparty1)-3,4-didehydro-5-phosphono-D-norvaline, 21: $C_{12}H_{20}N_3O_9P$ 16.45g (86.3%); spectral data, IR and ^{1}H-NMR, coincide with those of an authentic sample; 384; 37.80/37.56, 5.29/5.40, 11.07/10.88; c = 0.1(NaOH), $[\alpha]_D^{20}$: +88.4°.

15. Synthesis of Plumbemicin B, 25.

The method in Section 14 is used with 25.22g (0.05M) of the tripeptide 24.

N-(N²-L-alanyl-L-asparaginyl)-3,4-didehydro-5-phosphono-D-norvaline, 25: $C_{12}H_{21}N_4O_8P$; 16.08g (84.6%); spectral data, IR and ^{1}H-NMR, are identical with those of an authentic sample; 383; 37.90/37.48, 5.57/5.71, 14.73/14.65; c = 0.1(NaOH), $[\alpha]_D^{20}$ +93.6°.

TEST FOR HERBICIDAL ACTIVITY

Method A: Seeds of mono- and dicotyledons (wheat and cucumbers) are placed into Petri dishes, which are then put on filter paper. Each dish should contain 30 wheat seeds and, separately, 20 cucumber seeds. Aqueous solutions are then prepared of the product to be treated, ranging from 10^{-3} to 10^{-6} mols. Three Petri dishes are used to test each concentration. The filter paper is soaked with the solution (approx. 5ml). A reference sample is also prepared with filter paper soaked with plain water and the same number of seeds in the dish. All samples as prepared are placed in an incubator, where they left in the dark at 25°C for 96 hours (soil conditions are simulated). When the dishes are taken out, the length of the main

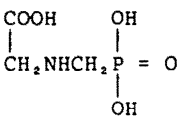
root of all germinated seeds is measured, registrating zero for all seeds that have not germinated. The activity of the tested product in relation to the reference sample is calculated by the formula:

$$A\% = 100(1 - l_1/l_2),$$

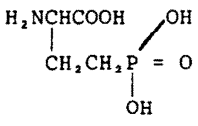
where A is the activity, l_1 - the length of the main root of the treated seeds, and l_2 - the length of the main root of the seeds from the reference sample. Active herbicides are those which have suppressed germination over 50% (100% means total inhibition). The results are represented in Table 1.

Table 1. Herbicidal activity of N-phosphonomethylglycine (27), 2-amino-4-hydroxymethylphosphonyl-L-butanoic acid (28), its pyro-analogue (29), and the newly synthesized L4 and its pyro-analogue 14. Here C denotes the concentration, F is the selective activity index, A_1 - activity, l_1, l_2 - the length of the main root of treated and reference, respectively, wheat corn, and A_2, l_3, l_4 correspond to the case of cucumber seeds.

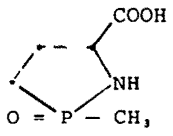
Compound	C(mol)	l_1(cm)	l_2(cm)	A_1(%)	l_3(cm)	l_4(cm)	A_2(%)	$F(A_1/A_2)$
27	10^{-3}	1.5		70.6	0.7		72.1	
Glyphosate	10^{-4}	1.9	5.1	62.8	1.4	3.9	64.2	
(Reference)	10^{-5}	3.9		23.6	3.5		10.3	
	10^{-6}	4.6		9.9	3.9		0.0	
28	10^{-3}	0.0		100.0	0.0		100.0	
L-phosphino-	10^{-4}	0.8	5.1	84.2	0.8	3.9	79.4	
tricine	10^{-5}	2.5		50.6	2.0		48.6	
(Reference)	10^{-6}	4.0		20.8	3.0		23.5	
29	10^{-3}	3.2		39.6	3.2		20.2	
	10^{-4}	4.2	5.2	18.0	3.6	4.0	9.8	
	10^{-5}	5.2		0.0	4.0		0.0	
	10^{-6}	5.2		0.0	4.0		0.0	
L4	10^{-3}	0.0		100.0	0.6		83.2	1.20
	10^{-4}	0.0	5.0	100.0	1.9	3.9	49.6	2.02
	10^{-5}	0.8		84.3	3.1		20.0	4.22
	10^{-6}	1.5		69.3	3.9		0.0	full selectivity
14	10^{-3}	5.2		0.0	4.0		0.0	
	10^{-4}	5.2	5.2	0.0	4.0	4.0	0.0	
	10^{-5}	5.2		0.0	4.0		0.0	
	10^{-6}	5.2		0.0	4.0		0.0	



27



28



29

Method B: The weeds couch-grass (Synodom dactylom) and cornthistle (Circium arvense) are planted in flowerpots and two weeks after germination are spray-treated with an aqueous solution of L4 as an isopropylammonium salt and a surfactant (35 parts butylammonium salt of dodecylbenzolsulphonic acid and 65 parts thallic oil, condensed with ethylene oxide in a ratio of 11 mols ethylene oxide per 1 mol thallic oil). The quantity of the active ingredient L4 corresponds to a dosage of 0.240kg/1000m². The treated plants are left in a vegetation chamber. They all perish in two weeks.

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