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Ring Phosphorus (V) Compounds Obtained from *p*-isopropyl-phenyldichlorophosphine. Synthesis, Stereoisomerism and Biological Activity

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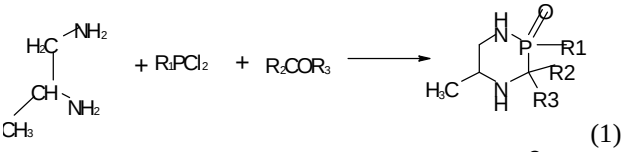
Abstract: In connection to our previous reports[1] regarding the auxine like effects of some phosphorin-2-oxide ring compounds, this study was concerned about a Mannich type syntheses involving *p*-isopropyl-phenyldichlorophosphine, *o*-aminophenol, 1,2-diaminopropan and acetone. A discussion about the products of the reactions, that are in fact a mixture of diastereomers, because of the existence of some stereogenic sites at the phosphorus and carbon atoms, was done.The newly obtained heterocyclic compounds were investigated regarding the development of chlorophyll a and b contents, in plants treated with different concentrations of the above-mentioned substances.

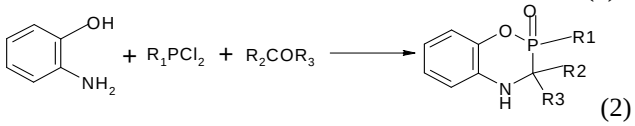
Keywords: *p*-isopropyl-phenyldichlorophosphine, Mannich reaction, stereogenic sites, chlorophyll a and b, auxine effect.

1. Introduction

p-(Isopropyl)-phenyldichlorophosphine, involved in a Mannich type reaction[2-3] together with *o*- aminophenol or 1,2- diaminopropane and acetone, generated two phosphorine-2-oxide ring compounds, containing oxaza or diaza groups, namely: 2-*p*(isopropyl)-phenyl-3,3-dimethyl-1,4,2-benzoxaza-phosphorine-2-oxide and 2-*p*(isopropyl)-phenyl-3,3,5-trimethyl-1,4,2-diaza-phosphorine-2-oxide.
The two compounds were characterized by elemental analysis, IR, ^{1}H-NMR, and gas chromatography.



(1)



(2)

where R_1= $C_6H_4CH(CH_3)_2$; R_2= R_3=CH_3;

Taking into consideration that chlorophyll contents in plants were revealed to have connection with nitrogen and phosphorus concentrations at growth early stage, and because the above mentioned compounds can be considered as phosphonic analogous of naturally occuring α-aminoacids, it was expected from them to develop biological activity. That is the reason why the present study was also developed to investigate the effects exhibited by different concentrations of the two phosphorus heterocyclic compounds on chlorophylls a and b contents, as well as on chlorophyll a/b ratio of wheat, after treatment with the above mentioned compounds.

Chlorophylls concentrations were determined by analysis of the UV-visible spectrum of the extracted pigments in 100% acetone. For calculations, Lichtenthaler and Wellburn[4] derived equations (3) and (4) were used. Similar equations exist for other solvents such as methanol and diethyl ether. The coefficients in equations (2) and (3) were determined for pure *chlorophyll a* and *b* in 100% acetone by finding the locations of their peaks in the red region (645 nm for *chlorophyll b*, 662 nm for *chlorophyll a*) and the values of their absorption coefficients at these peaks. Assuming that the absorbance by one pigment does not influence the absorbance by the other, the total absorbance in a mixture of *chlorophyll a* and *b* at the two peaks can be calculated as the sum of the individual absorbances of each pigment. This gives rise to two equations which can be solved simultaneously to give the concentration of each pigment in mg/l of extract as shown in equations (3) and (4) below. These equations are valid for standard square cuvettes with a path length of 1cm.

chlorophyll *a* ($mg \cdot l^{-1}$) = $11.75A_{662}$-$2.35A_{645}$ (3)

chlorophyll b ($mg \cdot l^{-1}$) = $18.61A_{645}$-$3.96A_{662}$ (4)

where: A is absorption at given wavelengths

Comparing the data resulting from the treatments with 2-*p*(isopropyl)-phenyl-3,3,5-trimethyl-1,4,2-diaza-phosphorine-2-oxide and 2- *p*(isopropyl)-phenyl -3,3-dimethyl-1,4,2-benzoxaza-phosphorine-2-oxide, we can conclude that both these substances exhibit biological activity in wheat, especially at 50 ppm concentration, when both chlorophyll a and b concentrations are significantly increased.

It is to be underlined that a very good connection between chlorophyll content response and all the other changes concerning the general development of the monocotyledonous plant, was registered.

At the same concentration, a comparison between the effects obtained with the two compounds revealed that the increases in chlorophyll content is higher when 2-*p*(isopropyl)-phenyl-3,3,5-trimethyl-1,4,diazaphosphorine-2-oxide was used.

2. Experimental

- Reactions were carried out with protection from atmospheric moisture.
- Melting points are determined on a Bőetius apparatus, and are uncorrected.
- The phosphorus content was determined by Schőniger method on a Heraus apparatus.
- Elemental analysis was carried out on a Carlo Erba 1106 analyzer.
- ^{1}H-NMR spectra were determined in $CDCl_3$ solutions with a Varian Gemini 300 apparatus at 300 MHz. Chemical shifts (δ) are given in ppm downfield from internal TMS.
- IR spectra were determined on SPECORD M80 JENA as KBr pellets.
- UV spectra was determined on a V-550 UV/VIS Jasco Spectrophotometer.
- The GC analyses were performed on Carlo Erba, Fractovap GT 200 gas chromatograph, with double column system and thermo conductibility detector, equipped with DP700 Fisons Instruments data station, by using two Pyrex glass columns 2m long, filled with two silicone stationary phases having different polarities (OV-1 and OV-17) on Gas Chrom Q (80-100 mesh) support, at a flow rate of 80 mL/min hydrogen, as carrier gas. The compounds were investigated as toluene solutions. The temperatures were: 260^0 C for the columns, 290^0C for the injector, and 275^0C for the detector. The GC analyses certified the purity of the new products.
- All reactants excepting the aryldichlorophosphines were reagents for synthesis from Merck-Schuchardt, Germany and from Sigma-Aldrich Division, Germany. All chemicals used were predried and distilled from appropriate drying agents[5].
- The synthesis of *p*(isopropyl)-phenyldichlorophosphine involved in these reactions, were carried out in our laboratory, with improved yields[6] by Friedel-Crafts reaction[7].

General procedure for synthesis of new 1,4,2-diaza or benzoxaza-phosphorin-2-oxides (1-2)

o-Aminophenol(or 1,2-diaminopropane) was dissolved in anhydrous benzene (20 mL for each 10 mmol diamino or hydroxilamino compound) and p(isopropyl)-phenyldichlorophosphine was added at room temperature with vigorous stirring in a molar ratio 1:1 (dichlorophosphine: diamino or alkylamino compound)[1]. With every drop of dichlorophosphine added a dark red color appears which transforms immediately into dark-yellow. After an hour, to the well stirred solution was added dropwise acetone at the same molar ratio 1:1:1. The stirring was kept for an hour, then the mixture was heated under reflux at 80-100ºC for 12h to give the desired compounds 1and 2, which were separated by filtering the cooled reaction mixture, the two products showing as white precipitates. It is to be noted that the reaction mixture include intense yellow insoluble salt like products.

After recrystallization from benzene the new obtained products were subjected to IR, UV, MS and H^1-NMR analysis. The main results are given below:

2-p(isopropyl)-phenyl-3,3-5-trimethyl-1,4,2-diaza-phosphorine-2-oxide (1). Anal. calcd. for $C_{15}H_{25}N_2OP$: C, 64.28; H, 8.93; P, 11.07. Found: C, 63.87; H, 8.81; P, 11.34. M.p.60-62º C.Yield 31%. IR (KBr, cm^{-1}): 3074(ν_{CHAr}); 2955-2871 ($\nu_{CH3as,sym}$); 1429(ν_{PPh}); 1210(ν_{PO}); 1093(ν_{CN}); 1058(ν_{PNC}); 902(δ_{PC}); 835(δ_{CHpara}). ^{1}H-NMR spectrum (CDCl3), δ: 1.00 (d, 3H, CH_3, J_{HH} =7.3 Hz); 1,10(d, 6H, CH_3, J_{HH} =7.1 Hz); 1.24 (d, 3H, CH_3, $^3J_{PH}$ =15.7 Hz); 1.52 (d, 3H, CH_3, J_{PH}=12.9Hz); 2.77 (m, 2H, CH_2); 3.10(m, 1H, CH);3.49 (m, 1H, CH); 5.94 (br, 2H, NH); 6.95-7.18 (m, 4H, C_6H_4).

2-p(isopropyl)-phenyl-3,3-dimethyl-1,4,2-benzoxaza-phosphorine-2-oxide (2). Anal. calcd. for $C_{18}H_{22}NO_2P$: C, 68.57; H, 6.98; P, 9.84. Found: C, 68.14; H, 6.76; P, 10.11. M.p. 164-166º C.Yield 43%. IR (KBr, cm^{-1}): 3072(ν_{CHAr}); 1601(ν_{CCAr}); 1218 (ν_{PO}); 1198(ν_{POC}); 1100(ν_{PPh}). ^{1}H-NMR spectrum ($CDCl_3$), δ: 1.08 (d, 6H, CH_3, J_{HH} =7.2 Hz); 1.22 (d, 3H, CH_3, $^3J_{PH}$= 14.9 Hz,); 1.46 (d, 3H, CH_3, $^3J_{PH}$=12.8 Hz); 3.32(m, 1H, CH); 5.22 (brs, 1H, NH); 6.90-7.45 (m, 8H, C_6H_4).

The method for testing growth regulating activity

To establish the auxinic effect activity of the two new synthesized 1,4,2-benzoxaza-phosphorine-2-oxide, the Tsibulskaya-Vassilev general biotest [8-9]was used.

The biotest method was carried out on monocotyledonous - wheat caryopses (*Alex variety*) comparatively with water control. The concentrations of 1,4,2-benzoxaza-phosphorine-2-oxides were: 10 ppm, 20 ppm, 50 ppm, 100 ppm and 200 ppm. The seeds, previously disinfected with calcium hypochlorite solution, were treated with bioactive compounds and were held in plastic Petri dishes (Φ=90 mm, 20 seeds/dish, 2 repetitions/concentration) on agar medium (5 g/l concentration) at 22^0C for six days. Next, the biometrics measurements were carried out, watching for the average height of plants, the average number of the roots for one plant, the average length of the roots and the dry matter. The obtained data were calculated in percentage and compared to the water control.

The chlorophyll content determination

1 g of fresh leaf tissue was weighed and cut into small pieces (about 1 mm wide) with scissors or razor blade and ground with a mortar and pestle in the presence of a little sea sand, 0.2–0.5 g of $MgSO_4$ and ca 0.5 ml 100% of acetone. 2-5 ml of 100% acetone was added to the fine powder and the solid compound elements were separated by centrifugation at 5000 rpm for 10 min.

The extracted solutions were kept in dark conditions and refrigerated for 30 minutes prior to measurement. The spectrophotometer was calibrated using 100% acetone in a quartz cuvette. Four ml of extract were placed in a 1-cm quartz cuvette, and absorption was measured at two different wavelength positions. The quartz cuvette was rinsed between samples with 100% acetone and the spectrophotometer was recalibrated every 10 samples.

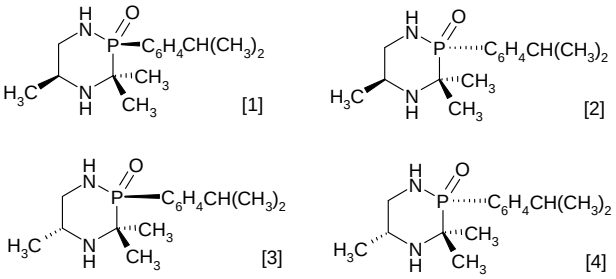
Extract solution absorbance was measured with a V-550 UV/VIS Jasco Spectrophotometer at 645 nm and 662

nm, these being the absorbance maxima in 100% acetone for chlorophyll a and chlorophyll b.

3. Results and discussion

During the multicomponent cyclization reaction 4 stereoisomers of compound 1: 2-*p*(isopropyl)-phenyl-3,3-5-trimethyl-1,4,2-diaza-phosphorine-2-oxide, can be obtained because the carbon atom C5 is a stereogenic center as well as the phosphorus atom from the heterocycle.

A competition between anomerical and sterical effects could occur, so that a steric repulsion between two axial groups to be avoided. Therefore, it is not inappropriate for exocyclic oxygen to have a preference for axial disposal



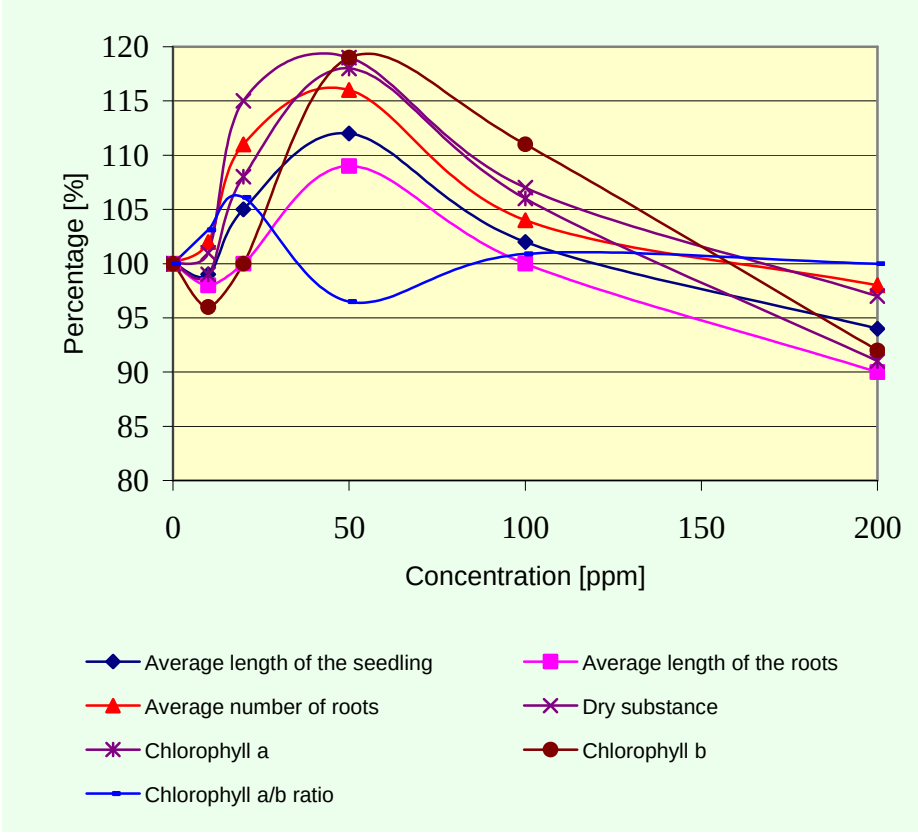


Fig. 1. The influence of 2-*p*(isopropyl)-phenyl -3,3,5-trimethyl-1,4,2-diaza-phosphorine-2-oxide on growth parameters and chlorophyll content of wheat seedlings

From Figure 1 and 2, it is obvious that the two substances manifested their most significant influence on all growth parameters and chlorophyll a and b content of wheat seedlings, at the concentration of 50 ppm. Nevertheless, for both tested compounds, the level of chlorophyll a/b ratio is minimum at the same concentration of 50 ppm, which means a faster growth of chlorophyll b level compared to that of chlorophyll a. The changes in chlorophyll content in wheat were also accompanied by changes in stem elongation.

Dry substance had recorded important growths at all applied concentrations (the best values increased with more than 14% at the concentration of 50 ppm), except for 200 ppm, demonstrating that the new tested substances have stimulated the metabolism and thus the accumulation of protein substances.

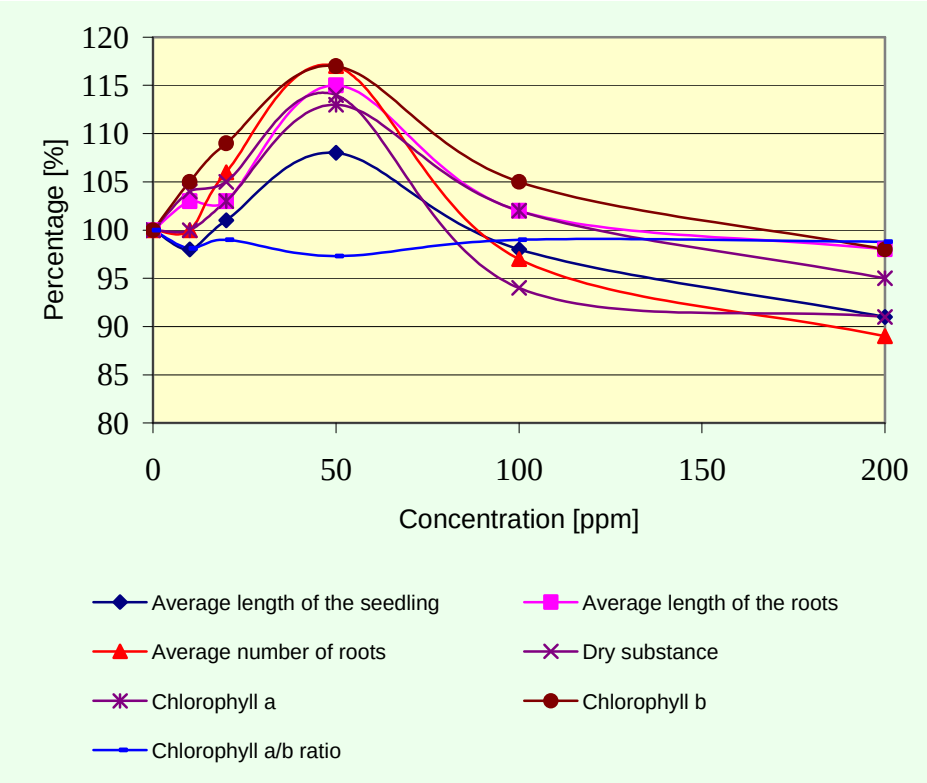


Fig. 2. Effect of different concentrations of 2-*p*(isopropyl)-phenyl-3,3-dimethyl-1,4,2-benzoxaza-phosphorine-2-oxide on growth parameters and chlorophyll content of wheat seedlings

For both tested substances, when using a concentration of 200 ppm, all the measured parameters present lower values comparatively with control, which may be a result of the toxicity of the products at higher concentrations.

In case of 2- p(isopropyl)-phenyl -3,3-dimethyl-1,4,2-benzoxaza-phosphorine-2-oxide, it is to underline the insignifiant variation of the chlorophyll a/b ratio, that means a similar response of chlorophyll a and b development to the different concentrations of the tested substance. Although it can be notify that the ratio value is always under 100%, that means a slight increase of chlorophyll b in comparison with chlorophyll a.

4. Conclusions

Two phosphorine-2-oxide ring compounds, containing oxaza or diaza groups, namely: 2-*p*(isopropyl)-phenyl-3,3-dimethyl-1,4,2-benzoxaza-phosphorine-2-oxide and 2-*p*(isopropyl)-phenyl-3,3,5-trimethyl-1,4,2-diaza-phosphorine-2-oxide were obtained by a Mannich type reaction and characterized by elemental analysis, IR, ^{1}H-NMR, and gas chromatography.

Both heterocycles exhibit auxine effect and manifested their most significant influence on all growth parameters and on chlorophyll a and b content of wheat seedlings, at the concentration of 50 ppm.

At the same concentration, a comparison between the effects obtained with the two compounds revealed that the increase in chlorophyll content is higher when the diaza-phosphorine-2-oxide was used.

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