Short Communication

Synthesis and Auxinic Behavior of 3-Ethyl-2-isopropyl-3,5-dimethyl-1,4,2-diazaphosphorine 2-Oxide

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Abstract

Alkyldiclorophosphines, as strong reducing agents, react with ketones in concentrated hydrochloric acid solution, to give the oxygen transfer products, which can be used for preparing cyclic compounds. In this respect, the synthesis and characterization of a new heterocyclic compound, 3-ethyl-2-isopropyl-3,5-dimethyl-1,4,2-diazaphosphorine 2-oxide (**4**), by a multi-component reaction, was realized. The new obtained compound was characterized by elemental analysis, MS, GC, IR and ^{1}H-NMR and it was subjected to biological tests on wheat by using different concentrations of the tested substance. The biometric measurements were judged in correlation with chlorophyll a and b development, so that the entire health of the plants can be monitored. The corroborated results showed that the compound has a very significantly stimulation effect on wheat, at the concentration of 50 ppm.

Key words: auxinic activity, phosphorus heterocycle, *i*-propyldichlorophosphine, chlorophyll a and b content, UV-vis spectrophotometry

Introduction

This paper tries to connect fine chemical synthesis, respectively a multi-component cyclization reaction, with agriculture science, and that is the reason why, we tried to obtain a synthetic compound capable to act as a plant hormone.

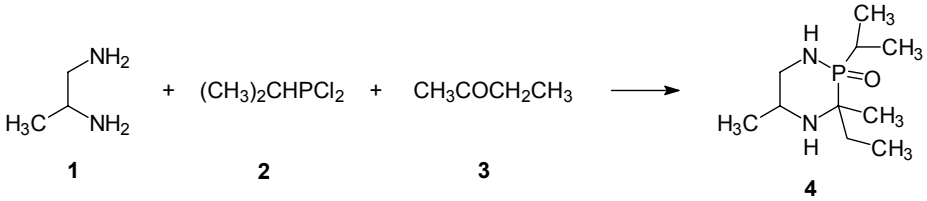
The aliphatic members of dichlorophosphines, are effective reducing agents being used as reagents for preparing cyclic compounds.[1] In connection to our previous studies,[2–5] we used *i*-propyldichlorophosphine (**2**) in a variant of Mannich reaction with methyl-ethyl ketone (**3**) and 1,2-diaminopropane (**1**), succeeding to obtain a new diazaphosphorine 2-oxide compound **4**, as shown by Scheme 1.

The compound **4** may be considered a phosphonic analog of naturally occuring α-aminoacids, and we expected that it may function similar to auxins.

Auxins[6–8] are a family of hormones commonly found in plants, which promote (and sometimes inhibit) growth. The term auxin is derived from the Greek word *auxein*, which means to grow. Compounds are generally considered auxins if they can be characterized by their ability to induce cell elongation in stems.

The significant auxinic effect of derivative **4**, was demonstrated by the Tsibulskaya - Vassilev general biotest,[9–11] which was applied on wheat (*Alex cultivar*), as monocotyledonous plant. Besides the biometric measurements it was determined the development of chlorophyll a and b from the tested plants, being aware that the chlorophyll, which is the photochemical and physical reaction center of photosynthesis, is the main indicator of the plants health.

The UV-vis spectrophotometric method was chosen for quantitative determination of chlorophyll a and



1 2 3 4

Scheme 1. Synthesis of compound **4**.

b. Because of the fact that the accuracy of the absorption coefficient used for the calculation is of main importance for the precision of this method, Lichtenthaler's standard extinction equations[12] were used.

Experimental

Equipment / reagents: Reactions were carried out with protection from atmospheric moisture. Melting points were determined on a Bőetius apparatus, and are uncorrected. The phosphorus content was determined by Schőniger method on a HERAUS apparatus. Elemental analysis was performed on a CARLO ERBA 1106 analyzer. ^{1}H-NMR spectra were determined in $CDCl_3$ solutions with a VARIAN GEMINI 300 apparatus. Chemical shifts (δ) are given in ppm downfield from internal TMS. IR spectrum was determined on a SPECORD M80 JENA. The mass spectrum was registered using a VARIAN FINNIGAN MAT 212 mass spectrometer. The GC analysis was performed on a CARLO ERBA, FRACTOVAP GT 200 gas chromatograph, with a double column system and a thermo conductibility detector, equipped with a DP700 FISONS INSTRUMENTS data station, by using two Pyrex glass columns, each 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 compound was investigated as toluene solution. The temperatures were: 260 °C for the columns, 290 °C for the injector, and 275 °C for the detector. The GC analyses certified the purity of the new product. All reactants 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.[13]

The statistical processing of the experimental data was carried out using the STATGRAPHICS software application.

Synthesis of 3-ethyl-2-isopropyl-3,5-dimethyl-1,4,2-diazaphosphorine 2-oxide (4):

Diamino compound **1** (3.7 g, 0.05 mol) was dissolved in 100 mL anhydrous benzene and 7.25g (0.05 mol) of dichlorophosphine **2** was added at room temperature with vigorous stirring. With every drop of dichlorophosphine, a red color appears which transforms immediately into dark-yellow. After an hour, to the well-stirred solution was added dropwise ketone **3** at the same molar ratio 1:1:1. The stirring was kept for an hour and then the mixture was heated under reflux at 80–100 °C for 8 h to give the desired compound, which was separated by filtering the cooled reaction mixture. The product **4** shows as a colorless precipitate with m.p. 37–39 °C. It is to be noted that the reaction mixture includes some insoluble, in usual solvents, salt-like products.

4: colorless crystals, mp 37–39 °C, yield 39%. IR (KBr, cm^{-1}): 3410–3440(ν_{NH}); 2972–2855 ($\nu_{CH2,CH3}$); 1195(ν_{PO}); 1116(ν_{CN}); 967(ν_{PNC}); 910(ν_{PC}). ^{1}H-NMR ($CDCl_3$) δ 0.90 (t, 3H, CH_3; $^3J_{HH}$ 7.2 Hz); 1.02 (dd, 6H, CH_3; $^3J_{HH}$ 7.1 Hz); 1.21(d, 3H, CH_3; $^3J_{HH}$ 7.2 Hz); 1.46 (d, 3H, CH_3; $^3J_{PH}$ 12.8 Hz); 1.54–1.63 (m, 2H, CH_2); 2.38–2.42(m, 1H, CH); 2.88(m, 2H, CH_2); 3.12 (m, 1H, CH); 5.95(br, 2H, NH). MS (m/z) : 218($M^{+\cdot}$); Anal. Calcd for $C_{10}H_{23}N_2OP$: C 55.03, H 10.62, N 12.83%, P 14.19. Found: C 55.16, H 10.79, N 12.65, P 13.93.

Based on the retention data obtained in gas chromatographic analysis performed for the synthesized compound, in the above mentioned conditions, the corresponding retention indices on the two silicone stationary phases having different polarities (OV-1 and OV-17) were determined using the well known calculating formula of Kovats.[14] The compound (I_{OV-1}=1835 and I_{OV-17}=1970), proved to have stability, because during the elution time there has been obtained only one symmetric peak.

The method for testing growth regulator activity: To establish the auxinic effect activity of diazaphosphorine 2-oxide **4**, the Tsibulskaya-Vassilev general biotest[9–11] was used. Laboratory tests were carried out on wheat caryopses (*Alex cultivar*), comparatively with water control. The following concentrations were used: 10, 20, 50, 100 and 200 ppm. The seeds treated with the bioactive compound were held in Petri dishes (Φ=90 mm, 20 seeds/dish, 3 repetitions/concentration) on agar medium (5 g/L concentration) at 22 °C during six days. After that, 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 substance. The data were calculated in percentage and compared to the water control (Table 1).

The chlorophyll content determination: One g of fresh leaf tissue was weighted 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 small quantity of sea sand, 0.2–0.5 g $MgSO_4$ and ca 0.5 mL 100% acetone. We added 2–5 mL of 80% acetone to the fine powder and decanted the homogenate into a centrifugation tube through 2 x 5 mL 80% acetone. Centrifugation at 5000 rpm for 10 min separated solid compound elements.

The extracted solutions were kept in dark conditions and refrigerated for 30 minutes prior to measurement. The spectrophotometer was calibrated using

Table 1. The growth regulating activity of derivative **4** on wheat.

Variant	Average length of the seedling		Average length of the roots		Average number of roots		Dry substance	
	cm	%	cm	%	No.	%	g	%
Water control	7.21	100	4.95	100	4.06	100	0.0097	100
3-ethyl-2-isopropyl-3,5-dimethyl-1,4,2-diazaphosphorine 2-oxide (**4**)								
10 ppm	7.03	97.5^{000}	4.99	100.8	4.01	98.7	0.0108	111.3^{***}
20 ppm	7.85	108.8^{***}	5.14	103.8^{*}	4.37	107.6^{***}	0.0111	114.4^{***}
50 ppm	8.32	115.3^{***}	5.53	111.7^{***}	4.59	113.0^{***}	0.0113	116.5^{***}
100 ppm	7.63	105.8^{***}	5.06	102.2	4.28	105.4^{***}	0.0102	105.1
200 ppm	6.88	95.4^{000}	4.81	97.1	3.92	96.5^{0}	0.0084	85.6^{000}
	$DL_{5\%}$ =0.08808		$DL_{5\%}$ =0.18531		$DL_{5\%}$ =0.10530		$DL_{5\%}$ =0.00051	
	$DL_{1\%}$ =0.12347		$DL_{1\%}$ =0.36445		$DL_{1\%}$ =0.14859		$DL_{1\%}$ =0.00072	
	$DL_{0.1\%}$=0.17452		$DL_{0.1\%}$=0.54327		$DL_{0.1\%}$=0.21225		$DL_{0.1\%}$=0.00102	

Table 2. The chlorophyll content development in wheat treated with compound **4**.

Wheat	A_{663}	A_{647}	Chlorophyll a		Chlorophyll b		Chlorophylls a+b ($mg.L^{-1}$)	Chlorophyll a/b ratio
			($mg.L^{-1}$)	%	($mg.L^{-1}$)	%		
Water control	0.589	0.257	6.4982	100.00	2.5216	100.00	9.01982	2.577023
3-ethyl-2-isopropyl-3,5-dimethyl-1,4,2-diazaphosphorine 2-oxide (**4**)								
10 ppm	0.511	0.217	5.6543	87.01^{000}	2.0594	81.67^{000}	7.71372	2.745615
20 ppm	0.597	0.274	6.5487	100.77^{***}	2.8463	112.87^{***}	9.39509	2.300808
50 ppm	0.691	0.323	7.5635	116.39^{***}	3.4204	135.64^{***}	10.98398	2.211314
100 ppm	0.542	0.239	5.9726	91.91^{000}	2.3743	94.15^{000}	8.34699	2.515558
200 ppm	0.531	0.232	5.8574	90.13^{000}	2.2799	90.41^{000}	8.13737	2.569178
			$DL_{5\%}$ =0.00141		$DL_{5\%}$ =0.00189			
			$DL_{1\%}$ =0.00198		$DL_{1\%}$ =0.00265			
			$DL_{0.1\%}$=0.00280		$DL_{0.1\%}$=0.00375			

80% acetone in a quartz cuvette. The extract (4 ml) was placed in a 1 cm quartz cuvette, and absorption was measured at two different wavelength positions. The quartz cuvette was rinsed between samples with 80% acetone and the spectrophotometer was recalibrated every 10 samples.

The absorbance of the samples was measured with a V-550 UV/VIS Jasco Spectrophotometer at 647 nm and 663 nm, these being the absorbance maxima in 80% acetone for chlorophyll a and chlorophyll b. Absorbance values were used to calculate pigment concentrations, as presented in Table 2, using standard extinction equations.[12]

$$chlorophyll\ a\ (mg\ L^{-1}) = (12.25 \cdot A_{663} - 2.79 \cdot A_{647}) \cdot D$$
$$chlorophyll\ b\ (mg\ L^{-1}) = (21.5 \cdot A_{647} - 5.1 \cdot A_{663}) \cdot D$$
$$chlorophyll\ a+b\ (mg\ L^{-1}) = (7.15 \cdot A_{663} + 18.71 \cdot A_{647}) \cdot D,$$

where: A = the absorption at given wavelengths;
D = the thickness of the used cuvette (cm).

Results and Discussions

From the experimental data, presented in Table 1 and drawn in Figure 1, it can be noticed that for wheat, which was treated with diazaphosphorine 2-oxide **4**, all the biometric measurements registered their best value at the concentration of 50 ppm.

Dry substance has recorded important growths of over 11% at variants treated with 10, 20 and 50 ppm (the best value of 16.5% has also been achieved at the concentration of 50 ppm), demonstrating that the new tested substance has stimulated the metabolism and thus the accumulation of protein substances. The results obtained for the dry substance are statistically assured, the recorded increases, compared with control, exhibiting very significantly differences for 10, 20 and 50 ppm concentrations.

Statistically assured results were also obtained for the average number of roots, which, compared to control, also displays three times very significantly differences for 20 ppm, 50 ppm and for 100 ppm.

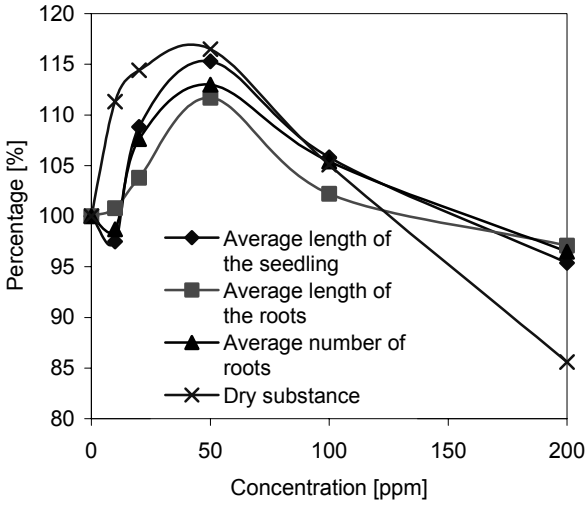


Figure 1. Effect of different concentrations of compound **4** on growth parameters of wheat seedlings.

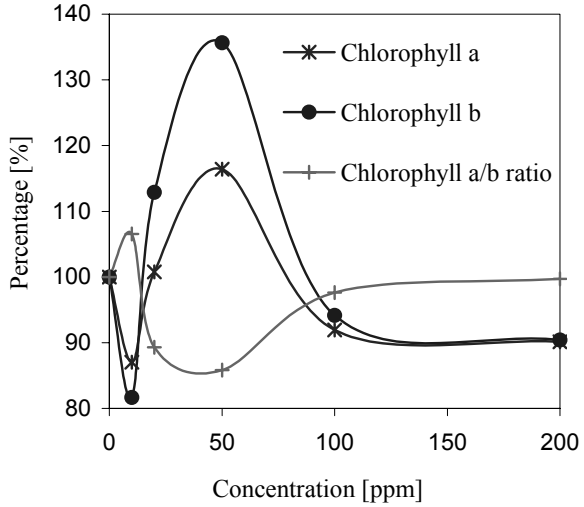


Figure 2. The influence of derivative **4** on chlorophyll a and b concentrations of wheat seedlings

With respect to average length of the seedling the tests have also given statistically assured results. The concentrations of 20, 50 and 100 ppm offer very significant differences compared to control, but the concentrations of 10 ppm and 200 ppm produced negative response from the plants, which denotes an inhibition effect at the lowest concentration, and even a toxic effect at the highest used concentration.

Regarding the average length of the roots, the experimental results display significant differences at 20 ppm and very significant differences with 50 ppm concentration.

In very good agreement with the general development of the wheat treated with diazaphosphorine 2-oxide **4**, the chlorophylls a and b concentrations exceed the values of control, at the same concentration value, that of 50 ppm, as it can be seen in Table 2. This concentration of 50 ppm proved to be the most benefic for all the plant evolution, probably because of the higher chlorophyll content, as presented in Figure 2.

At 50 ppm, the increase in chlorophyll b content was higher than the increase in chlorophyll a concentration. This is of great importance, because higher chlorophyll b levels allow light interception in wider wavelength bands. As a consequence, the transfer of a larger amount of energy to reaction centers is expected. For this reason the Chlorophyll a/b ratio, which is, (according to Figure 2) the single parameter registering a minimal value at 50 ppm, may indicate better acclimation to shade.[15] This situation was also noticed previously, at a lesser extent, when a benzoxazaphosphorine 2-oxide was tested.[2]

Conclusions

A new heterocyclic compound, 3-ethyl-2-isopropyl-3,5-dimethyl-1,4,2-diazaphosphorine 2-oxide (**4**), was obtained and fully characterized by IR, ^{1}H-NMR, GC, MS and elemental analysis, and we expected it to act as a plant growth regulator.

Regarding the auxinic activity exhibited by the above-mentioned compound, we could remark the following:

Comparing the experimental data resulting from the treatment with derivative **4**, we can conclude that this substance exhibits biological activity over wheat, especially at the 50 ppm concentration, when the chlorophyll a and b concentrations are also very significantly increased.

The increase of chlorophyll b concentration plays an important role during the development of the whole plant, because at 50 ppm the chlorophyll a/b ratio is the only parameter which registers a minimum value (Figure 2). The tested substance stimulated the metabolism acceleration and thus, the accumulation of protein substances up to 16.5%, these results being statistically assured.

This phosphorus heterocycle can be considered to act as an auxine, based on the statistically assured results obtained for the average height of plants, the average number of the roots for one plant and the average length of the roots, in comparison with control, which always registered very significant differences for 50 ppm.

The use of compound **4** in concentrations over 100 ppm, produced an important decrease both in chloro-

phyll content response and in all the other measured growth parameters of seedlings, probably because of an inhibition or even a toxic effect.

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Povzetek

Po večstopenjski sintezi smo pripravili novo heterociklično spojino, 3-etil-2-izopropil-3,5-dimetil-1,2,4-diazafosforin 2-oksid (**4**). Spojino smo karakterizirali z elementno analizo, MS, GC, IR in ^{1}H NMR. Njeno biološko aktivnost smo ovrednotili na pšenici.