Biological Effects of Some New Imidazole Derivatives on Spruce (Picea Abies) Germination

COSTEL MOLDOVEANU1, VIOLETA VASILACHE2, IOAN-MARIAN RISCA2*

1“Al. I. Cuza” University, Faculty of Chemistry, 11 Carol I Av., 700506, Iași, Romania
2“Ştefan cel Mare” University, Faculty of Silviculture, 13 Universitatii Str., 720229, Suceava, Romania

A new group of diazaheterocyclic salts derived from imidazole were synthesized by addition of acrylonitrile to N-1. The 1-(2-cyanoethyl) imidazole derivatives were quaternized with high-reacting halogen derivatives (halogen esters and halogen amides) and the structure of the obtained compounds were proved by spectral methods (IR, H-NMR, C-NMR, 2DCOSY, HITCOR). For all those compounds the biological effect on the spruce seeds germination were tested. The tests were conducted in controlled temperature room and results showed that the hypocotyls and radicles length varied as a function of concentration and structure of each investigated compound. The investigated compounds exhibited various biological activities, both on the hypocotyls and radicles growth, especially at lower concentrations. Various relationships between structure and biological activity are emphasised.

Keywords: imidazoles; N-alkylation; spruce; hypocotyls elongation; radicles elongation; auxines; chemical structure-biological activity relationship.

Biological material
There were used seed samples of spruce (Picea abies (L.) H.Karst), (yield 2011) achieved from the Forest District Moldovita, for which the germination rate was measured.

Treatment solutions
The imidazole derivatives (1a, 1b; 2 a-c and 3 a-i) prepared as above were previously prepared and there aqueous solutions were used for the seed treatments.

Procedure
The evaluations were conducted on three replicates of 100 seeds each. Surface sterilized seeds were treated with 5 mL of 10⁻⁵-10⁻³ molar solution of the imidazole derivatives for 1 hour, and sown in Petri dishes on filter paper together with the treatment solutions. As witness, a blank (3x 100 seeds) with bi-distilled water were also carried out. Seeds were maintained in the growth chamber at constant temperature, humidity and illumination regimes (20°C and 85%, respectively with a 12 h day/night alternation), without pre-refrigeration, until embryo elongation (hypocotyls and radicles) were established. A seed with visible coleorhizee were considered as germinated. The seedlings were periodically moistened and the percentages of germinated seeds (the germination rate, \( G_0 \)) were reported after 21 days. Young spruce plants were harvested from their seeds and measured (hypocotyls, \( L_h \) and radicles, \( L_r \) expressed as cm).

Statistics
The data were validated by the poly-factorial variance analysis [45, 46].

Results and discussions
The synthesis of imidazolium salts were described elsewhere [42,43] and consist in two steps: the N-cyanoethylation of the pyrrole nitrogen from the imidazole derivatives (imidazole and benzimidazole) by acrylonitrile addition followed by the quaternization of the pyridinic...
nitrogen with halogen derivatives with increased reactivity such as halogenated esters and halogenated amides, respectively (fig. 1). The quaternization were also performed for 1-methyl imidazole. For all the imidazolium salts the structure were proved by elemental and spectral methods: IR, 1H-NMR, 13C-NMR, 2D-COSY, 2D-HMQC, 2D-HMBC.

Experiments were conducted to determine the biological activity of the imidazole derivatives on the germination of spruce seeds. The obtained results are presented in the tables 1 and 5.

Average values of the hypocotyls length (LH), in mm, of germinated spruce seeds as an effect of the treatment with the imidazole derivatives against water.

A first remark is that the treatments did not influence the germination rates. For the witness, the average germination rate were $G = 58 \pm 1.2\%$ and for the treated seed $G$ varied between $66 \pm 2.0\%$ and $59 \pm 2.4\%$, depending on the used imidazolium derivatives.

On the other hand, according to the variance analysis (tables 2-4 and 6-8), the biological response of the spruce plantlets depends on the chemical structure and the concentration of the used imidazolium salts. For the hypocotyls and radicles the responses are also very different, namely:

- usually, the increased concentrations ($10^{-3}$ m and $5 \times 10^{-4}$ m) have toxic effects, both on spruce hypocotyls and radicles. The spruce radicles are more sensitive to the toxic action; the growth inhibition is very significant;
- imidazole and benzimidazole presented different effects. Thus, imidazole stimulates lightly the hypocotyls and induces a significant inhibition of the radicles. At its turn, benzimidazole acts oppositely, namely: an insignificant inhibition of the hypocotyls and a very significant stimulation of the rootlets;
- by adding acrylonitrile at N1 of imidazole a light stimulation of hypocotyls is observed; the stimulation level being opposite to the concentration;
- the presence of the methyl radical at N1 induces a stimulation effect, especially for the rootles; these results are very significant;
- through quaternization at N3 very interesting effects are obtained. Thus, the introduction of ethyl acetate or, especially, methyl acetate at the cyanoethyl imidazole molecule induces a very significant stimulation of the spruce hypocotyls. Their stimulating effect is more moderate for the rootlets. For benzimidazole, the quaternization effects are insignificant. For the compound which is quaternized with methyl acetate, only the hypocotyls reacted positively at the stimulation;
- if the quaternization was done with iodacetamide, all the obtained compounds – no matter the concentration – have showed very strong inhibition of the growth – both at hypocotyls and, especially, at rootlets.

Regarding the influence of the concentration, the main tendency is the increasing of the biological response with the decreasing of the concentration; the most biological active concentrations are those of $5 \times 10^{-5}$ m and $1 \times 10^{-5}$ m, respectively.

According to the variance analysis (tables 2-4 and 6-8), the biological effects, considered as an auxin-like action of the imidazolium derivatives [41], are linked with the
presence in the molecules of the methoxy, respectively ethoxy radicals (3b, 3c, 3h and 3i for hypocotyls and 3h and 3i for the rootlets 3i – tables 1 and 5, underlined values).

Table 2
VARIANCE ANALYSIS OF THE SPRUCE HYPOCOTYLs LENGTH

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>Degrees of freedom</th>
<th>Variance</th>
<th>F</th>
<th>F crit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = compounds</td>
<td>2748.7668</td>
<td>14</td>
<td>124.943976</td>
<td>13.19083</td>
<td>1.66448866</td>
</tr>
<tr>
<td>B = concentrations</td>
<td>488.59705</td>
<td>4</td>
<td>122.1492626</td>
<td>12.93941</td>
<td>2.47527741</td>
</tr>
<tr>
<td>Error</td>
<td>851.22081</td>
<td>56</td>
<td>9.672963756</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4068.5847</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DL 5% = 1.822; DL 1% = 2.414; DL 0.1% = 3.123

Table 3
AVERAGE VALUES FOR THE SPRUCE HYPOCOTYLs LENGTH DUE TO THE INFLUENCE OF IMIDAZOLE DERIVATIVES

Table 4
AVERAGE VALUES FOR THE SPRUCE HYPOCOTYLs LENGTH DUE TO THE INFLUENCE OF IMIDAZOLE DERIVATIVES CONCENTRATION

Table 5
AVERAGE VALUES OF THE RADICLES LENGTH ($L_R$), IN mm, OF GERMINATED SPRUCE SEEDS AS AN EFFECT OF THE TREATMENT WITH THE IMIDAZOLE DERIVATIVES AGAINST WATER

Table 6
VARIANCE ANALYSIS OF THE SPRUCE RADICLES LENGTH

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>Degrees of freedom</th>
<th>Variance</th>
<th>F</th>
<th>F crit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = compounds</td>
<td>14975.49923</td>
<td>14</td>
<td>680.7045104</td>
<td>13.485709</td>
<td>1.664488</td>
</tr>
<tr>
<td>B = concentrations</td>
<td>3372.069212</td>
<td>4</td>
<td>843.0173003</td>
<td>16.701353</td>
<td>2.475277</td>
</tr>
<tr>
<td>Error</td>
<td>4441.886788</td>
<td>56</td>
<td>50.47598623</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22789.45523</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DL 5% = 1.787; DL 1% = 2.368; DL 0.1% = 3.063
On the other hand, the presence of iodine in the imidazolium salts has a general inhibitory effect (3a, 3d and 3g, both for hypocotyls and rootlets). The same effects were observed also for the wheat seeds [41].

Conclusions
The effect of some new imidazole derivatives on germination and seedling growth of spruce were investigated. The length of hypocotyls varied as a function of concentration and structure of each investigated compound.

An obvious relationship between the chemical structure and the biological activity were observed at the derivatives obtained by the quaternization of 1-(2-cyanoethyl) imidazole and 1-methyl imidazole derivatives at N-3 with methyl- and ethyl bromoacetate. Those imidazolium salts present a very significant increased stimulatory activity on cell elongation, especially at lower concentrations (5 x 10^{-5} and 1 x 10^{-5} m). The presence of iodine in the imidazolium salts induces inhibitory effect, both for hypocotyls and rootlets. All the obtained results were statistical validated.

Acknowledgements. Authors are thankful to CNCS Bucharest, Romania, project PN-II-RU-TE-2011-3-0010, no. 79/05.10.2011, for financial support.

References

Manuscript received: 28.07.2014