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

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*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.*

Nitrogen heterocycles are an important category of compounds with large capabilities in various fields such as agriculture, analytical and medicinal chemistry, and opto-electronics [1-27]. Among them, diazoles, such as imidazole, benzimidazole and their analogues, proved remarkable biological activities, especially as anticancer, antibacterial, antifungal and antituberculosis agents [28-31]. On the other hand, very promising biological responses for these imidazole and benzimidazole derivatives come from the plants kingdom, responses that prefigures important applications in areas such agriculture and forestry. One of the most used way of synthesis of azaheterocyle involve ylides, as intermediary reactive species [32-40].

Previously [41] we used wheat seeds in order to highlight the biological response and that because the plant seed germination represents an excellent way to test the biological response to various chemical stimuli [4, 44]. The germination tests determines the actual germination potential of normal seeds within a seeds lot, which can be used to compare the quality of different lots and to estimate the field planting value. Nevertheless, germination tests are very simple, little time consuming, cheap and – therefore – could be perfect methods for testing the biological activity of some new synthesized compounds, including – in our case – the imidazolium salts. This paper reports the biological activity of spruce seeds. The spruce was chosen because, unlike the wheat, which is an angiosperm – the most evaluate phylum in the plant kingdom – the spruce is a gymnosperm, a more primitive species with completely different biological structure.

## Experimental part

*Materials and methods*

Apparatus

The germination test was performed in a growth chamber Conviron MP4030, model G30, with programmed temperature, humidity and light.

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 coleorhizae were considered as germinated. The seeds were periodically moistened and the percentages of germinated seeds (the germination rate, $\mathbf{G_R}$) were reported after 21 days. Young spruce plants were harvested from their seeds and measured (hypocotyls, $\mathbf{L_H}$ and radicles $\mathbf{L_R}$, 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

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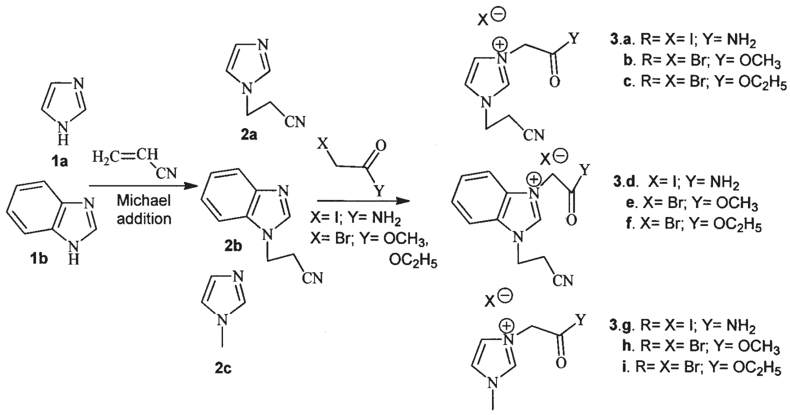


Fig. 1. Reaction pathway for the synthesis of imidazolium salts

**Table 1**
AVERAGE VALUES OF THE HYPOCOTYLS LENGTH ($L_H$), IN mm, OF GERMINATED SPRUCE SEEDS AS AN EFFECT OF THE TREATMENT WITH THE IMIDAZOLE DERIVATIVES AGAINST WATER

| Compound | Concentration (M) | | | | |
|---|---|---|---|---|---|
| | $1 \times 10^{-3}$ | $5 \times 10^{-4}$ | $1 \times 10^{-4}$ | $5 \times 10^{-5}$ | $1 \times 10^{-5}$ |
| | Average length of hypocotils (mm) | | | | |
| **Witness** | 42.04 | 42.04 | 42.04 | 42.04 | 42.04 |
| **1a** | 44.11 | 43.02 | 46.98 | 41.23 | 43.50 |
| **1b** | 41.39 | 42.32 | 42.02 | 39.98 | 39.03 |
| **2a** | 49.74 | 47.14 | 47.56 | 46.33 | 46.46 |
| **2b** | 42.35 | 41.12 | 41.33 | 39.25 | 38.63 |
| **2c** | 44.89 | 43.57 | 41.43 | 41.07 | 43.50 |
| **3a** | 28.44 | 33.19 | 40.44 | 45.23 | 46.55 |
| **3b** | 45.60 | 45.54 | 47.81 | 48.79 | 51.49 |
| **3c** | 48.34 | 55.27 | 54.46 | 58.32 | 56.46 |
| **3d** | 32.64 | 36.94 | 38.88 | 38.05 | 40.27 |
| **3e** | 44.63 | 44.88 | 44.08 | 44.07 | 43.58 |
| **3f** | 44.78 | 43.11 | 43.00 | 43.10 | 44.45 |
| **3g** | 29.12 | 36.64 | 41.87 | 42.06 | 44.49 |
| **3h** | 45.45 | 45.22 | 46.33 | 49.51 | 52.52 |
| **3i** | 43.14 | 48.88 | 48.38 | 57.12 | 57.45 |

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, $^{1}$H-NMR, $^{13}$C-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 ($L_H$), 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 $G_R$ = 58 ± 1.2 % and for the treated seed $G_R$ varied between 66 ± 2.0 % and 59 ± 2.4 %, depending on the used imidazole 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 x $10^{-4}$ 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 $N_1$ of imidazole a light stimulation of hypocotyls is observed; the stimulation level being opposite to the concentration;
-the presence of the methyl radical at $N_1$ induces a stimulation effect, especially for the rootles were these results are very significant;
-through quaternization at $N_3$ very interesting effects are obtained. Thus, the introduction of ethyl acetate or, especially, methyl acetate in 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;
- a special remark must be done in the methyl imidazole‘s quaternization case with methyl-, and ethyl acetate, respectively, where for the $10^{-5}$m concentration, the growths were 25 - 36 % for hypocotyls and 27-38 % for rootlets, against the witness.
- 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 x $10^{-5}$ m and 1 x $10^{-5}$ m, respectively.

According to the variance analysis (tables 2-4 and 6-8), the biological effects, considered as an auxin-like action of the imidazole derivatives [41], are linked with the

| Source of variation | SS | Degrees of freedom | Variance | F | F crit. |
|---|---|---|---|---|---|
| A = compounds | 2748.7668 | 14 | 124.9439476 | 13.19083 | 1.66448866 |
| B = concentrations | 488.59705 | 4 | 122.1492626 | 12.93941 | 2.47527741 |
| Error | 851.22081 | 56 | 9.672963756 | | |
| Total | 4088.5847 | 74 | | | |

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

**Table 2**
VARIANCE ANALYSIS OF THE SPRUCE HYPOCOTYLS LENGTH

| Graduation | Average values (mm) | Differences (+/-) | Significance |
|---|---|---|---|
| Witness | 42.0 | 0 | - |
| 1a | 43.8 | 1.8 | - |
| 1b | 40.9 | -1.1 | - |
| 2a | 47.5 | 5.5 | *** |
| 2b | 40.5 | -1.5 | - |
| 2c | 42.9 | 0. 9 | - |
| 3a | 38.8 | -3.2 | 000 |
| 3b | 47.8 | 5.8 | *** |
| 3c | 54,6 | 12.6 | *** |
| 3d | 37.4 | -4.6 | 000 |
| 3e | 44.2 | 2.2 | * |
| 3f | 43.7 | 1.7 | - |
| 3g | 38.8 | -3.2 | 000 |
| 3h | 47.8 | 5.8 | *** |
| 3i | 50.9 | 8.9 | *** |

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

| Graduation | Average values (mm) | Differences (+/-) | Significance |
|---|---|---|---|
| Witness | 42.0 | 0 | - |
| $1 \times 10^{-3}$ m | 40.6 | -1,4 | - |
| $5 \times 10^{-4}$ m | 42.5 | 0,5 | - |
| $1 \times 10^{-4}$ m | 43.9 | 1,9 | * |
| $5 \times 10^{-5}$ m | 45.3 | 3,3 | *** |
| $1 \times 10^{-5}$ m | 46.4 | 4,4 | *** |

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

| Compound | Concentration (M) | | | | |
|---|---|---|---|---|---|
| | $1 \times 10^{-3}$ | $5 \times 10^{-4}$ | $1 \times 10^{-4}$ | $5 \times 10^{-5}$ | $1 \times 10^{-5}$ |
| | Average length of radicles (mm) | | | | |
| **Witness** | 41.13 | 41.13 | 41.13 | 41.13 | 41.13 |
| **1a** | 33.52 | 36.64 | 37.63 | 38.82 | 42.98 |
| **1b** | 39.45 | 48.33 | 42.88 | 44.06 | 48.81 |
| **2a** | 36.09 | 43.88 | 41.94 | 40.38 | 40.64 |
| **2b** | 40.81 | 45.57 | 43.17 | 43.70 | 44.93 |
| **2c** | 45.77 | 48.94 | 48.99 | 47.01 | 44.19 |
| **3a** | 17.43 | 15.48 | 17.04 | 20.25 | 28.11 |
| **3b** | 29.96 | 37.40 | 40.31 | 36.82 | 40.10 |
| **3c** | 34.14 | 42.23 | 43.64 | 42.87 | 44.33 |
| **3d** | 14.89 | 18.53 | 22.78 | 30.73 | 36.35 |
| **3e** | 33.64 | 38.39 | 39.35 | 41.14 | 41.82 |
| **3f** | 35.03 | 37.59 | 38.69 | 43.43 | 38.87 |
| **3g** | 22.64 | 27.77 | 28.33 | 36.53 | 40.88 |
| **3h** | 46.16 | 46.47 | 47.73 | 51.18 | 52.26 |
| **3i** | 48.67 | 50.38 | 52.34 | 55.57 | 57.02 |

**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

| Source of variation | SS | Degrees of freedom | Variance | F | F crit. |
|---|---|---|---|---|---|
| A = compounds | 14975.49923 | 14 | 680.7045104 | 13.485709 | 1.664488 |
| B = concentrations | 3372.069212 | 4 | 843.017303 | 16.701353 | 2.475277 |
| Error | 4441.886788 | 56 | 50.47598623 | | |
| Total | 22789.45523 | 74 | | | |

DL 5% = 1.787; DL 1% = 2.368; DL 0,1% = 3.063

**Table 6**
VARIANCE ANALYSIS OF THE SPRUCE RADICLES LENGTH

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 7**
AVERAGE VALUES FOR THE SPRUCE RADICLES LENGTH DUE TO THE INFLUENCE OF IMIDAZOLE DERIVATIVES

| Graduation | Average values (mm) | Differences (+/-) | Significance |
|---|---|---|---|
| Witness | 41.1 | 0 | - |
| 1a | 37.9 | -3.2 | 000 |
| 1b | 44.7 | 3.6 | *** |
| 2a | 40.6 | -0.5 | - |
| 2b | 43.6 | 2.5 | ** |
| 2c | 46.9 | 5.8 | *** |
| 3a | 19.7 | -21.5 | 000 |
| 3b | 36.9 | -4.2 | 000 |
| 3c | 41.4 | 0.3 | - |
| 3d | 24.7 | -16.5 | 000 |
| 3e | 38.9 | -2.3 | 0 |
| 3f | 38.7 | -2.4 | 00 |
| 3g | 31.2 | -9.9 | 000 |
| 3h | 48.8 | 7.6 | *** |
| 3i | 52.8 | 11.7 | *** |

**Table 8**
AVERAGE VALUES FOR THE SPRUCE RADICLES LENGTH DUE TO THE INFLUENCE OF IMIDAZOLE DERIVATIVES CONCENTRATION

| Graduation | Average values (mm) | Differences (+/-) | Significance |
|---|---|---|---|
| Witness | 41.1 | 0 | - |
| $1 \times 10^{-3}$ m | 34.1 | -7.1 | 000 |
| $5 \times 10^{-4}$ m | 37.5 | -3.6 | 000 |
| $1 \times 10^{-4}$ m | 38.8 | -2.3 | 0 |
| $5 \times 10^{-5}$ m | 41.1 | -0.1 | - |
| $1 \times 10^{-5}$ m | 42.9 | 1.7 | - |

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 ($5 \times 10^{-5}$ m and $1 \times 10^{-5}$ m). The presence of iodine in the imidazolium salts induces inhibitory effect, both for hypocotyls and rootlets. All the obtained results were statistical validated.

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