

leading to lower energy requirements. Cv. Novbona plants accumulated 16.46 times more Cd in the roots than in the shoots and for cv. Goral plants this ratio was even higher (19.11). Siedlecka and Krupa [4] reported that of the total Cd uptake of the plant approximately 60% is stored in the root, 15% in the leaves, 10% in the stem and 15% in the seeds. A similar Cd distribution has also been found for *Zea mays*: 72% in the root, 13% in the leaves and 15% in the stem [5], and for *H. perforatum*: 77% in the root, 13% in the leaves and 10% in the stem [2].

Of the production parameters (root and shoot length and root and shoot dry mass) of *Ch. recutita* (cv. Goral) and *H. perforatum* plants the parameter most sensitive for metal treatment ($120 \mu\text{mol dm}^{-3}$ Cd and $240 \mu\text{mol dm}^{-3}$ Zn) and also to combined application of the same concentrations of both metals was found to be the root dry mass. This parameter was influenced to greater extent for the above mentioned chamomile cultivar than the root dry mass of *H. perforatum*. In spite of high added metal concentrations the shoots of both plant species grew without lethal symptoms. The addition of cadmium ($120 \mu\text{mol dm}^{-3}$) to the hydroponic solution containing $240 \mu\text{mol dm}^{-3}$ Zn did not cause significant changes to the production parameters observed after application of the $240 \mu\text{mol dm}^{-3}$ Zn. The added zinc concentration was very high and corresponded approximately to the maximum metal content, which can be bound by the relevant metal receptors in the plants.

From our results it could be concluded that the roots of *Chamomilla recutita* species contain a limited number of metal (Me) binding sites, which can be expressed as $\mu\text{mol Me(II)} \text{ g}^{-1}$ dry mass (Me = Cd or Zn in our experiments). Since the metal uptake in hydroponically cultivated plants occurs exclusively through the roots, at high added metal concentrations, these binding sites will be saturated and further addition of metal to the hydroponic medium cannot increase either metal accumulation in the roots or metal translocation into the shoots.

Experimental

The medicinal plants were kept in hydroponic solution for seven days under controlled conditions (mean air temperature: 25°C , relative air humidity: 80% and photosynthetic active radiation: $80 \mu\text{mol m}^{-2} \text{ s}^{-1}$). For both control and metal-treated specimens the length and dry mass of shoots and roots were estimated. Metal content of in dry mass the roots and shoots was determined using FAAS.

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Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Inhibition of UV-induced mutagenesis in *Euglena gracilis* by benzothiazole derivatives

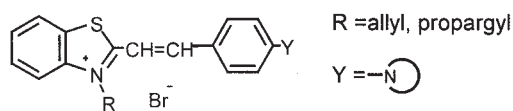
P. FOLTÍNOVÁ, P. MAGDOLEN, P. ZAHRADNÍK

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Dr. Pavol Zahradník, Faculty of Natural Sciences, Department of Organic Chemistry, Mlynská dolina CH-2, SK-84135 Bratislava, Slovak Republic
zahradnik@fns.uniba.sk

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This paper is a continuation of our previous work [1] where the toxicity of new Pharmacol 2-phenylethenylbenzothiazolium bromides substituted in *para*-position by cyclic amines (series A) against *Euglena gracilis* has been measured and QSAR study has been carried out.



In this article the effect of the compounds of the Series A (29 compounds) on the protection of the mutagenicity induced by UV-irradiation in *E. gracilis* has been studied. If the UV-irradiation is applied without the protective compound, a high number of white mutants of *E. gracilis* is induced. The protective effect of the compounds of Series A depends on the structure of the substituent Y. If the cycloamino substituent is a saturated amine, the UV-protective effect is negligible. On the other hand very effective influence on the UV mutagenicity is observed in the case of an aromatic heterocyclic substituent. These compounds exhibited a significant dose-dependent inhibitory effect on UV bleaching activity. At the highest concentrations tested ($100 \mu\text{g/ml}$) the percentage inhibition of the mutagenicity ranged from 68 to 99% and at the least concentration ($2 \mu\text{g/ml}$) the percentage inhibition reached 10–59%. The concentrations required to inhibit the UV-mutagenicity by 50% (IC_{50} values) of these effective substances 1–9 are summarized in the Table.

It can be seen that the substituted derivatives 2–8 are much more active than the unsubstituted compound 1, the most efficient substituent Y is 1,2,4-triazol-1-yl (compounds 4, 8). This activity can be attributed to the position of UV-vis absorption maxima as they are in the range

Table: Structure and anti-UV activity of the benzothiazolium salts studied

Compd.	R	Y	IC_{50} ($\mu\text{g/ml}$) ^a
1	allyl	H	37.03
2	allyl	imidazol-1-yl	3.82
3	allyl	benzimidazol-1-yl	4.15
4	allyl	1,2,4-triazol-1-yl	2.66
5	allyl	benzotriazol-1-yl	14.06
6	propargyl	imidazol-1-yl	12.59
7	propargyl	benzimidazol-1-yl	2.66
8	propargyl	1,2,4-triazol-1-yl	2.03
9	propargyl	benzotriazol-1-yl	13.21

^a The concentration required to inhibit the UV-mutagenicity (bleaching activity) by 50%

380–405 nm and they are shifted hypsochromic in the N-heteroaromatic substituted derivatives compared with other N-cycloalkyl compounds [2]. An important fact is that aromatic heterocycles alone have no significant inhibitory effect on mutagenicity of UV (inhibition 0–3%).

Experimental

1. Chemicals

The synthesis of the studied benzothiazolium salts was described previously [1]. DMSO was used as a solvent in all biological experiments.

2. Microorganisms

Euglena gracilis (strain Z) was maintained on a Cramer-Myers medium [3] supplemented with sodium acetate (0.5%) under static conditions at $26 \pm 2^\circ\text{C}$ and with the permanent lighting.

3. Mutagenicity and antimutagenicity assays

3.1. Preparation of cells

E. gracilis cells in the exponential growth phase in the C. M. medium were centrifuged, washed and resuspended in a phosphate buffer (pH 6.2), preincubated for 16–18 h under permanent illumination at 26°C and then used in the experiments.

Cell suspensions (about $0.8\text{--}1.0 \times 10^5$ cells/ml) were supplemented with tested substances in appropriate concentrations (2–100 $\mu\text{g/ml}$), incubated 8–10 h at 26°C (co-treatment) and then UV irradiated. In the experiments with the post-treatment the cell suspensions (without benzothiazoles) were irradiated and then inoculated and incubated in medium containing corresponding benzothiazoles.

3.2. UV irradiation

2 ml of *E. gracilis* cells were transferred into Petri-dishes (7 cm diameter), inserted into a UVC 508 crosslinker and exposed to UV irradiation (200 J/m², 254 nm). After 24 h in dark irradiated cells were diluted and plated onto agar plates. All procedures were carried out under green safe-light to prevent photoreactivation. After 10–14 days of cultivation (2 days in the dark, remaining days in the light) growing green and white (bleached) colonies were counted and bleaching effect (mutagenicity) was determined.

3.3. Antimutagenicity

The mutagenicity inhibition percentage (MI%) was calculated for all samples, the method has been described previously [4]. The IC₅₀ values ($\mu\text{g/ml}$) were interpolated from the cubic function for a relation between the concentration used and the MI %.

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Faculty of Science¹, Faculty of Pharmacy², Jamia Hamdard, Hamdard Nagar, New Delhi, India

A new sterol from the pseudobulb of *Desmotrichum fimbriatum* Blume

A. Ali¹, S. T. ABDULLAH¹, H. HAMMID¹, M. Ali², M. S. ALAM¹

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Prof. Mohammed Ali, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110062, India
mali_chem@rediffmail.com

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Desmotrichum fimbriatum Blume (Orchidaceae), popularly known as 'Jivanti', is an epiphytic orchid distributed up to 2,700 m. It is an important crude drug used in the indigenous system of medicine as astringent, aphrodisiac, expectorant, stimulant, cardio tonic, asthma, bronchitis, and throat infections [1, 2]. The presence of α - and β -jivantic acids from the aerial part of the plant has been reported [3]. The present paper describes the isolation and characterization of a new sterol.

Desmosterol (**1**) was isolated from petroleum ether-chloroform (55:45) mixture as colourless crystalline solid. It responded positively to the Lieberman-Burchard test for sterols. The IR spectrum contained absorption bands for hydroxyl group (3450 cm^{-1}), unsaturation (1640 cm^{-1}) and gem dimethyl isopropyl group (1380 , 1308 , 1080 cm^{-1}). The EIMS of **1** exhibited a molecular ion peak at m/z 412 as the base peak corresponding to the molecular formula $\text{C}_{29}\text{H}_{48}\text{O}$, which is supported by ¹³C and DEPT NMR spectra. It indicated six degrees of unsaturation, four of which fully adjusted to the four rings of the carbocyclic nucleus and the remaining two to the olefinic bonds.

The EIMS exhibited diagnostically important fragment ions at m/z 226, 197 due to C_8/C_{14} and $\text{C}_{11}/\text{C}_{12}$ fission and the ions at 248 and 164 arose due to C_8/C_{14} and C_9/C_{11} fission. These fragments suggested that the compound was a C_{29} sterol [4, 5] possessing one hydroxyl group at in ring A/B which was placed at C-3 on the basis of biogenetic consideration, a C-10 saturated side chain and a diunsaturated steroidal skeleton of which one of the unsaturation was at C-2. The presence of a prominent peak at m/z 69 and 83 were suggestive of the existence of one of the double bond at C-5 and confirmation of the location of the hydroxyl group at C-3 in the ring.

The ¹H NMR spectrum of **1** showed the presence of three downfield one-proton doublets at δ 5.35 ($J = 5.1\text{ Hz}$) assigned to H-6, at δ 5.14 ($J = 8.4\text{ Hz}$) attributed to H-1 and another at δ 5.02 ($J = 8.4$, 8.4 Hz) associated with H-2. A broad multiplet at δ 3.52 ($w_{1/2} = 15.6\text{ Hz}$) integrating for one proton was ascribed to H-3 axial carbino-lic proton. Four doublets integrating for three proton each at δ 0.97 ($J = 6.60\text{ Hz}$), 0.84 ($J = 6.0\text{ Hz}$), 0.82 ($J = 6.0\text{ Hz}$) and 0.88 ($J = 6.50\text{ Hz}$) were due to three secondary methyl functionalities at C-21, C-26, C-27 one primary methyl group and C-29 respectively. The remaining two methyls resonated as three proton broad singlets at δ 0.68 and 1.00 and were correspondingly assigned to the two tertiary methyls at C-18 and C-19. The appearance of