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DITERPENE ALKALOID FROM *DELPHINIUM OREOPHILUM* AND ANTIOXIDANT ACTIVITY***DELPHINIUM OREOPHILUM* O'SIMLIGIDAN ANIQLANGAN DITERPEN ALKALOID VA ANTIOKSIDANT FAOLLI GI****ДИТЕРПЕНОВЫЙ АЛКАЛОИД ИЗ *DELPHINIUM OREOPHILUM* И ANTIOKSIDANTNAYA AKTIVNOST'Y****Ahunova Hilola Ibrohimovna¹**¹Namangan State University, PhD student, Department of Organic Chemistry**Kulonov Abdulaziz Ibragimovich²**²Chemistry International University in Tashkent, PhD, Tashkent, Uzbekistan**Abdullayev Shavkat Vohidovich³**³Namangan State University, Professor of the Department of Organic Chemistry, Academician of the Academy of Medicine of Uzbekistan**Annotatsiya**

Delphinium oreophilum o'simligining yer ustki qismi Zomin va Yangiqo'rg'on tumani tog' yonbag'irlaridan yig'ib olindi. O'simlik tarkibidagi alkaloidlarni o'rganish maqsadida o'simlik salqin joyda quritildi, maydalandi va 70% li etil spirit bilan ekstraksiya qilindi. O'simlikning spirtli ekstrakti etil atsetatli va xloroformli fraksiyalarga ajratildi. Xloroformli fraksiyadan diterpen alkaloidlar yig'indisi ajratib olindi. Olingan birikmalar MS, 1D, 2D YaMR spektrlari yorgamida analiz qilindi. Antioksidant faollik DPPH tahlili bilan amalga oshirildi. Olingan analiz natijalari tahlil qilindi va birikmalardan biri Lycoctonine ekanligi aniqlandi. Moddaning turli konsentratsiyalarda antioksidant faolligi o'rganildi.

Аннотация

Надземную часть растения *Delphinium oreophilum* заготовили со склонов гор Зоминского и Янеикурганского районов. Для изучения содержащихся в растении алкалоидов растение высушивали в прохладном месте, измельчали и экстрагировали 70%-ным этиловым спиртом. Спиртовой экстракт растения разделили на этилацетатную и хлороформную фракции. Из хлороформной фракции выделена сумма diterпеновых алкалоидов. Полученные соединения анализировали по спектрам MS, 1D, 2D ЯМР. Антиоксидантную активность определяли с помощью анализа DPPH. Результаты анализа были проанализированы и одним из соединений оказался Ликоктонин. Изучена антиоксидантная активность вещества в различных концентрациях.

Abstract

The aerial parts of the *Delphinium oreophilum* plant was harvested from the mountain slopes of Zomin and Yangikurgan districts. In order to study the alkaloids contained in the plant, the plant was dried in a cool place, crushed and extracted with 70% ethyl alcohol. The alcoholic extract of the plant was divided into ethyl acetate and chloroform fractions. A sum of diterpene alkaloids was isolated from the chloroform fraction. The obtained compounds were analyzed by 1H and 13C NMR spectra. Antioxidant activity was performed by DPPH assay. The results of the analysis were analyzed and one of the compounds was found to be Lycoctonine. The antioxidant activity of the compound in different concentrations was studied.

Kalit so'zlar: *Delphinium oreophilum*, Ranunculaceae, diterpene alkaloidlar, Lycoctonine, ¹H va ¹³C YaMR spektrlari, antioksidant faolligi.

Ключевые слова: *Delphinium oreophilum*, Ranunculaceae, diterпеновые алкалоиды, Ликоктонин, ¹H и ¹³C ЯМР-спектры, антиоксидантная активность.

Key words: *Delphinium oreophilum*, Ranunculaceae; diterpene alkaloids; Lycoctonine, ¹H and ¹³C NMR spectra, antioxidant activity

1. INTRODUCTION

Delphinium L. is a large plant belonging to the *Ranunculaceae* family. This species includes about 350 species, which of 22 species have been identified in Uzbekistan [1]. Most of the alkaloids contained in the *Delphinium* L. plant correspond to the flowering period of the plant. The amount of alkaloids was determined mainly from the above-ground part of the plant.

Delphinium oreophilum Huth is a perennial plant with a height of 30-60 cm and grows in the middle and upper mountain ranges of Jizzakh and Yangikurgan [1]. At first, the alkaloid content of this species was little studied [2,3]. For this reason, we started to study the above-ground part of *D. oreophilum* collected from Jizzakh mountain ranges during the flowering period. Essential oils, macro and microelements [4,5] and alkaloids contained in the alcoholic extract of the plant were studied during our work [6,7]. One of them is Lycoctonine, which belongs to the type of lycoctonine, which is a C₁₉-diterpene alkaloid [8,9]. The structure of the isolated compound was determined using 1D, 2D NMR spectroscopy, mass spectrometry (MS).

C₁₈, C₁₉, C₂₀-diterpene alkaloids were mainly isolated from *Delphinium L.* plant species. Lycoctonine-type alkaloids belong to the group of C₁₉-diterpene alkaloids. Alkaloids of this type are considered important for medicine and folk medicine, in particular, they have been used for a long time in folk medicine for the treatment of rheumatism and neuralgia. Diterpenoid alkaloids and their derivatives identified in this plant are cytotoxic against lung (A549), vincristine-resistant nasopharyngeal (KB-VIN), prostate (DU145) and triple negative breast cancer (MDA-MB-231) cancer cell lines. showed activity.

2. EXPERIMENTAL

2.1. General experimental procedures

Mass spectra were measured in an LC/MS-IT-TOF mass spectrometer (Shimadzu, Kyoto, Japan). 1D and 2D NMR spectra were taken with TMS internal standard on Avance III-500 or Avance III-600 spectrometers (Bruker, Bremerhaven, Germany). TLC monitoring used GF254 silica gel plates (Yantai Jiangyou Silicon Development Co., Yantai, China). Column chromatography (CC) used silica gel (200–300 mesh, Linyi Haixiang Co., Ltd., Linyi, China) and Sephadex LH-20 (Amersham Bioscience, Sweden).

2.2. Plant materials

D. oreophilum was collected in July 2018 in the Zomin and Yangikurgan mountains at an altitude of 800-1000 m. The plant passport is defined in the national herbarium fund of the Institute of Botany of the Academy of Sciences of Uzbekistan under the number DM0371.

2.3. Extraction and separation

The aboveground part of the air-dried plant *D. oreophilum* (2.8 kg) was extracted three times with 70% ethanol. The extracts were filtered and distilled under vacuum. The residue was dissolved in 1 liter of water and extracted with ethyl acetate. The mother cell was brought to pH = 9~10 by adding a dilute NaOH solution and extracted three times with chloroform. After solvent distillation, the resulting residue (22 g) was chromatographed over a column of silica gel with elution by CHCl₃-MeOH (19:1, 9:1, 7:1, 4:1) (Frac. A-F). Fraction F (2.63 g) was rechromatographed over a column of silica gel with elution by CHCl₃-MeOH-Et₂NH (49:1:0.5-18:2:0.2) (Frac. F 1-4) The resulting fraction F-4 (447 mg) was again rechromatographed over a column of silica gel with elution by CHCl₃-MeOH (19:1-4:1). As a result, compound (105 mg) was obtained

2.4. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay

Radical scavenging activity of the alkaloid was determined using DPPH as a reagent [10]. The reaction mixture containing test sample (1 mL) in different concentrations and DPPH (3 mL) (Sigma, 100 μM) in ethanol was taken and incubated in the dark for 30 min. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. A lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as positive control. All the chemicals used were of analytical grade (Sigma, USA). The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$$

where A_{control} is the absorbance of the control, and A_{sample} is the absorbance of the sample.

3. RESULTS AND DISCUSSION

Lycoctonine (Delsine; Royline) colorless needle-shaped crystals with m.p. 136-140 °C (EtOH) [8]. MS m/z 468.2977 [M + H]⁺(calcd for C₂₅H₄₁NO₇ 467.6) [11]. IUPAC name: 11-ethyl-13-(hydroxymethyl) -4,6,16,18-tetramethoxy-11-azahexacyclo (7.7.2.12, 5.01,10.03, 8.013, 17) nonadecane-8,9-diol (Fig. 1)

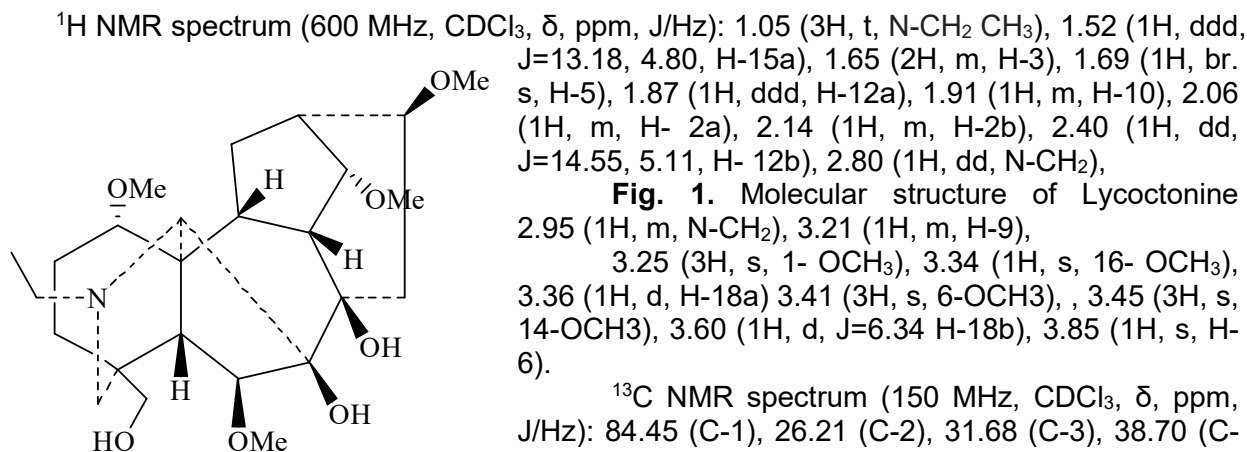


Fig. 1. Molecular structure of Lycoctonine

^{13}C NMR spectrum (150 MHz, CDCl_3 , δ , ppm, J/Hz): 84.45 (C-1), 26.21 (C-2), 31.68 (C-3), 38.70 (C-4), 43.38 (C-5), 90.69 (C-6), 88.54 (C-7), 77.70 (C-8), 49.67 (C-9), 38.13 (C-10), 49.02 (C-11), 28.88 (C-12), 46.21 (C-13), 84.05 (C-14), 33.71 (C-15), 82.76 (C-16), 65.08 (C-17), 67.81 (C-18), 52.85 (C-19), 51.34 (N- CH_2), 14.30 (CH_3), 55.99 (1- OCH_3), 57.96 (6- OCH_3), 58.06 (14- OCH_3), 56.44 (16- OCH_3). Based on the study of MS, ^1H , ^{13}C NMR spectra, compound 4 was identified as lycoctonine [8,9].

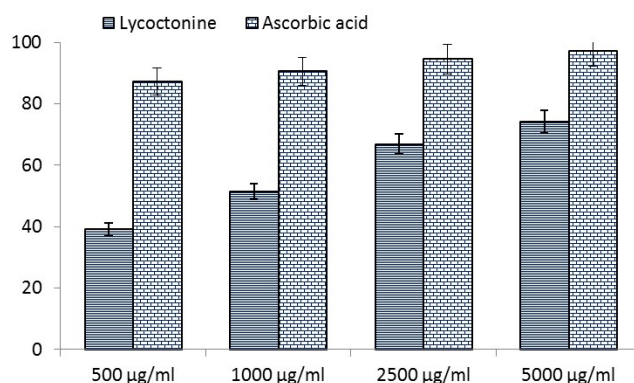


Fig. 2. Free radical scavenging activity of alkaloid (Lycoctonine) and ascorbic acid by DPPH radical.

Fig. 2 show the DPPH radical scavenging activity of Lycoctonine. Radical scavenging activity of these alkaloid increases with increasing dose. The difference between the tested alkaloid and control was statistically significant ($p < 0.05$). The scavenging effect of the alkaloid and standard on the DPPH radical decreased in the order ascorbic acid > Lycoctonine at all concentrations (500 $\mu\text{g/mL}$, 1000 $\mu\text{g/mL}$, 2500 $\mu\text{g/mL}$ and 5000 $\mu\text{g/mL}$), demonstrating a linearity with increasing concentration.

CONCLUSION

The chemical composition of the plant *D. oreophilum* belonging to the genus *Delphinium* was studied. Many diterpene alkaloids have been isolated from the chloroform fraction of the plant extract, one of which is Lycoctonine, a C_{19} -diterpene alkaloid. Its antioxidant activity was similar to that of ascorbic acid, which was obtained as a standard substance.

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