BIOLOGICALLY ACTIVE COMPOUNDS FROM THE RHIZOMES OF IRIS HUNGARICA

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Species of Iris genus (Iridaceae) have a long history of traditional medicinal use in different countries as alternative aperient, tonic, cathartic, diuretic, gall bladder diseases, liver complaints, dyspops, purification of blood, venereal infections, fever, bilious infections and for a variety of heart diseases. The rhizomes of Iris are the rich source of the secondary metabolites, in which flavonoids predominate. The clinical studies of substances from irises gave positive results in the treatment of cancer, bacterial and viral infections. Continuing the search of new biologically active compounds from the plants of Iridaceae family for the first time three isoflavones that are new for this species – irigenin, iristectorigenin B and its glucoside iristectorin B have been isolated from the ethanolic extract of the rhizomes of Iris hungarica Waldst. et Kit., which is widespread in Ukraine. The structure of the compounds is described as 5,7,3'-trihydroxy-6,4',5'-trimethoxyisoflavone, 5,7,4'-trihydroxy-6,3'-dimethoxyisoflavone and iristectorigenin B-7-O-β-D-glucoside, respectively. The compounds were obtained from the ethyl acetate fraction of the iris rhizomes by column chromatography on silica gel with sequential elution of the chloroform – ethanol solvent with different concentrations. The structure of the compounds has been determined by chemical and spectral methods and in comparison with the literature data.

BIОЛОГИЧНО АКТИВНІ СПОЛУКИ КОРЕНЕВИЩ IRIS HUNGARICA

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Ключові слова: ізофлавоноїди; іригенін; іристекторигенін В; іристекторин В; Iris hungarica; УФ-, ІК-, ЯМР-, NOESY-спектроскопія; мас-спектрометрія

Рослини роду Iris (Iridaceae) мають давню історію застосування у традиційній медицині різних країн як альтернативний проносний, тонізуючий, відхаркувальний, сечовинний засіб, для лікування захворювань жовчного міхура, печінки, водянки, для очищення крові, венеричних інфекцій, лихоманок, жовчних інфекцій і для лікування захворювань серця. Кореневища ірисів мають велике джерело вторинних метаболітів, серед яких переважають флавоноїди. Клінічні дослідження речовин із ірисів дали позитивні результати при лікуванні різних рідкісних, інфекційних і вірусних інфекцій. Продовжуючи пошук нових біологічно активних сполук з рослин родини ірисових – Iris hungarica з етанольного екстракту кореневищ ірису венгрського – Iris hungarica Waldst. et Kit., поширених на території України, вперше відшлифовано три нових для даного вида ізофлавоноїди: іригенін, іристекторигенін В і його діглюкозид іристекторин В. Структура речовин охарактеризована як 5,7,3'-тригідрокси-6,4',5'-триметоксіізофлавон, 5,7,4'-тригідрокси-6,3'-диметоксіізофлавон та іристекторигенін В-7-O-β-D-глюкопіранозид, відповідно. Речовини були отримані методом колонкової хроматографії на силикагелі з етилацетатно-етанольної сіліка-гелевої фази. Структура речовин встановлена як хімічними та спектральними методами та у порівнянні з літературними даними.

БИОЛОГИЧЕСКИ АКТИВНЫЕ СОЕДИНЕНИЯ КОРНЕВИЩ IRIS HUNGARICA

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Ключевые слова: изофлавоноиды; иригенин; иристекторигенин В; иристекторин В; Iris hungarica; УФ-, ИК-, ЯМР-, NOESY-спектроскопия; масс-спектрометрия

Растения рода Iris (Iridaceae) имеют давнюю историю применения в традиционной медицине различных стран как альтернативное слабительное, тонизирующее, отхаркивающее, мочегонное средство, для лечения заболеваний желчного пузыря, печени, водянки, для очищения крови, лечения венерических и желчных инфекций, и для лечения сердечных заболеваний. Корневища ирисов используются в качестве альтернативного слабительного, тонизирующего, отхаркивающего, мочегонного средства, для лечения заболеваний желчного пузыря, печени, водянки, для очищения крови, лечения венерических и желчных инфекций, и для лечения сердечных заболеваний. Корневища ирисов являются богатым источником вторичных метаболитов, среди которых преобладают флавоноиды. Клинические исследования веществ из ирисов дали положительные результаты при лечении рака, бактериальных и вирусных инфекций. Продолжая поиск новых биологически активных соединений из растений семейства ирисовые – Iridaceae из эфиричного экстракта корневищ ириса венгерского – Iris hungarica Waldst. et Kit., который широко распространен на территории Украины, впервые выделены три новых для данного вида изофлавоноида: иригенин, иристекторигенин В и его глукозид иристекторин В. Структура веществ охарактеризована как 5,7,3'-третиригидрокси-6,4',5'-триметоксиизофлавон, 5,7,4'-третиригидрокси-6,3'-диметоксиизофлавон и иристекторигенин В-7-О-β-D-глюкопиранозид, соответственно. Вещества были получены методом колоночной хроматографии на силикагеле с этилацетатно-этанольной фазой. Структура веществ установлена как химическими и спектральными методами и в сравнении с литературными данными.
Plants of *Iris* genus (the family Iridaceae) are perennial herbaceous plants of 30-100 cm in height, with well-developed ensiform leaves and peduncles, which are at the top of many large flowers: white, purple, violet. *Iris* is the genus of 260-300 species, which are mainly distributed across the Northern Hemisphere [1-2].

*Iris hungarica* Waldst. et Kit. (syn. *Iphya L.*) is widely distributed in most parts of the world, including the flora of Ukraine, Russia, Belarus [3-4], and is also cultivated as an ornamental plant [1]. The analysis of the chemical composition has not almost been carried out, but the component composition of essential oils (α-iron, squalene, β-damascenone, geranylacetone, eugenol, etc.) [5] and fatty acids (myristic, linoleic, palmitic and others) [6] in rhizomes has been determined; xanthone mangiferin has been identified [7].

Plants of *Iris* genus are the rich source of the secondary metabolites: flavonoids [8], isoflavonoids and their glycosides, xanthones, quinones, triterpenoids and stilbene glycosides [9-11]. The clinical studies of biologically active compounds of *irises* gave positive results in the treatment of cancer, bacterial and viral infections [12-13].

The aim of the work was to isolate and identify phenolic compounds from the rhizomes of *I. hungarica*. The EtOAc extract of the rhizomes of *I. hungarica* was subjected to repeated chromatography on columns of silica gel to obtain compounds 2, 6, 7. Compounds are soluble in ethanol, benzene, chloroform and are poorly soluble in water, diethyl ether and petroluem ether (Scheme).

The mass spectrum of compound 2 showed the molecular ion peak at m/z 360 (M+) in agreement with the molecular formula C18H16O8. The chromatographic analysis of compound 2 using the system of n-butanol – acetic acid – water (4:1:2) (Rf = 0.87) produced a spot with a dark fluorescence that was darkened by ammonia vapour. This was characteristic of 5-hydroxyisoflavones [14]. The UV spectrum 2 showed λ max absorptions at 269 and 337 nm (sh), suggesting the isoflavone skeleton. The IR spectrum showed intense absorptions at 269 and 337 nm (sh), suggesting the isoflavone moiety. In addition, the proton resonance for isoflavone C-2 was located at 8.35 ppm, which also confirmed the nature of the ring. The spectrum revealed the presence of three singlet signals of the hydroxyl groups at δ 13.0 (1H, s, 5-ОН), 10.75 (1H, s, 7-ОН), 9.21 (1H, s, 3′-ОН) ppm, and signals of three methoxy group at δ 3.3 (3H, s, 4′-OCH3), 3.55 (3H, s, 5′-OCH3), 3.8 (3H, s, 6-OCH3) ppm and a one proton singlet at δ 6.42 ppm for H-8. The spectrum also showed a pair of doublets at δ 6.65 ppm and 6.62 ppm characteristic of the p-substituted benzene ring (each 2H, J = 1.8 Hz). It also exhibited a signal at 5.62 (1H, s, 8-H) ppm, indicating that only one hydrogen atom was present on the A-ring of the isoflavone.

The data of the chemical analysis, the spectral characteristics of compound 2 are identical with the literature data on the structure of 5,7,3′-trihydroxy-6,4′,5′-trimethoxy isoflavone or irigenin. It was first isolated from the rhizomes of *Iris hungarica* [12].

The chromatographic analysis of compound 6 using the system of 15% acetic acid (R, 0.49) produced a spot with a blue fluorescence. The mass spectrum of 6 showed the molecular ion peak at m/z 330 (M+) in agreement with the molecular formula C17H14O7. UV absorption 6 maxima at 272 and 341 nm (sh) suggested the presence of the isoflavone moiety. In addition, the proton resonance for isoflavone C-2 was located at δ 8.32 (1H, s) ppm, it also confirmed the nature of the ring. The IR-spectrum 6 showed intense absorptions at 3752 см⁻¹ (OH), 2960, 2836 см⁻¹ (OCH3), 1660 см⁻¹ (C=O), 1622, 1582, 1522 см⁻¹ (C=C), 1372, 1061, 1008 (OCH3).

The 1H NMR-spectrum 6 exhibited signals at δ 8.35 (1H, s), 7.12 (1H, d, J = 1.8 Hz), 6.60 (1H, d, J = 2.4 Hz), and 6.67 (1H, dd, J = 2.4, 1.8 Hz) ppm attributable to H-2 of the isoflavone and formed the spin-spin interaction (H-5′, H-2′ and H-6′). It also exhibited a signal at δ 6.40 (1H, s, 8-H) ppm, indicating that only one hydrogen atom was present on the A-ring of the isoflavone, and two signals each for two methoxy groups at δ 3.73 (3H, s, 3′-OCH3) and δ 3.75 (3H, s, 6-OCH3) ppm. The spectrum indicated the presence of three singlet signals of the hydroxyl groups at δ 13.05 (1Н, s, 5-ОН), 10.75 (1Н, s, 7-ОН), 9.21 (1H, s, 3′-ОН) ppm.

With NOESY spectrum 6 arrangements of the substituents at C-3′ and C-4′ were refined. The analysis showed the presence of two cross-peaks demonstrating the nuclear resonance, and they were spatially close (Nuclear Overhauser effect observed at a distance of 0.03-0.4 nm between atoms). The interaction of proton H-2 with protons H-2′ and H-6′ was observed (Fig.). Proton H-2′ gave a cross-peak with the protons of the methoxy group, thus, it was located in position 3′.

The MS-, 1H NMR-, NOESY-, IR- and UV-spectra of 6 indicate that compound 6 is 5,7,4′-trihydroxy-6,3′-dimethoxyisoflavone or iristectorigenin B first isolated from the rhizomes of *I. hungarica* [12].

The molecular formula of compound 7 C23H24O12 was determined by the molecular ion peak at m/z 6
β-(5,7,3’-hydroxy-6,3’-dimethoxyisoflavone-O-β-D-glucoside) isolated from the rhizomes of *I. hungarica* for the first time [12].

Irigenin, Iristectorigenin B and Iristectorin B were previously isolated from the rhizomes of *I. dichotoma* (2010), *I. tectorum* (1972), *I. kumaonensis* (1984), *I. florentina* L. (1973), *I. milesii* (1984) [12].

**Experimental Part**

**Devices and materials**

$^1$H NMR-spectra (200 MHz) were recorded on a VarianMercury-VX-200 instrument (USA) in DMSO-$d_6$ with TMS as an internal standard. Low-resolution mass spectra were measured on a GC-MS Varian 1200L (ionizing voltage – 70 eV) instrument (USA). UV spectra ($\lambda$, nm) were recorded on a Carl Zeiss Specord M-80 (Germany); Evolution 60S (USA); Spekol 1500 (Analytik Jena AG, Germany) spectrometers in EtOH. IR-spectra (KBr pellets) on a Tensor 27 UR-20 spectrometer (Germany). Column chromatography (CC) was carried out on silica gel, 100-200 (75-150 mesh) (USA). TLC used plates: silica gel 60 F$_{254}$, TLC plates (Merck), Silufol UV$_{254}$ and paper “Filtrak” (FN-1:4). Spots were detected in UV light (365 nm and 254 nm) after visualization by ammonia vapour. The melting point was determined on a Kofler bench (Franz Kustner Nacht KG, Dresden, Germany). The compound analyzed was dried in vacuo ($10^{-2}$ mm Hg) over $P_2$O$_5$ at 110-115°C for 5 hours.

**Biological Material**

The rhizomes of *I. hungarica* were collected from M.M.Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kyiv in May of 2015 and were air-dried. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department and Botany Department of the National University of Pharmacy, Kharkiv, Ukraine. The plant was identified by the Head of the Department of the Ornamental plants, Senior Researcher of M.M.Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv), Cand. Biol. Sci. Yu.V.Buydin.

**Extraction and Isolation of Compounds**

Air-dried rhizomes (2.5-3 mm) of *I. hungarica* (1.0 kg) were extracted with EtOH (70%, 5 L) in a percolator for 24 h. The extraction was repeated thrice under the same conditions. The aqueous EtOH extracts were combined, filtered, evaporated in a rotary evaporator to 0.5 L of the aqueous residue, and left for 1 day. The supernatant liquid was separated. The resulting extract was treated successively with CHCl$_3$, EtOAc and n-BuOH. The resulting extracts were evaporated in vacuo. The qualitative composition of CHCl$_3$, EtOAc and BuOH fractions was controlled by PC and TLC in the solvent system of n-butanol – acetic acid – water (4:1:2).

The EtOAc extract was evaporated by heating under vacuum to complete stripping of the solvent, subjected to CC (120×5 cm) on silica gel and eluted with...
the gradient: CHCl₃ and ethanol-mixtures (9:1; 8:2; 5:4; 1:5; 1:1), and ethanol to obtain 110 fractions by 50 ml. Compound 2 (600 mg) was detected in fractions of chloroform-ethanol (9:1), the compound 6 (80 mg) and 7 (55 mg) – in chloroform-ethanol (8:2).

Chromatographic analysis of 2 using n-butanol – acetic acid – water (4:1:2) produced a spot with a dark-blue fluorescence, compounds 6 and 7 gave a dark fluorescence that was darkened by ammonia vapour.

Spectral Data

Iristectorigenin B (6) – 5,7,3'-trihydroxy-6,4',5'-trimethoxyisoflavone – C₁₇H₁₆O₇, a yellow powder. M. p. – 182-183°C. M 360,32 g/Mol. MS, m/z 330 (M+), 295, 284 (C₈H₇O₃), 1660 (С=О), 1622, 1582, 1522 (С=С), 1372, 1061, 1008 (С=Н), 9.15 (1Н, s, 4'-ОН), 8.32 (1Н, s, H-2), 7.15 (1Н, d, J = 1.8 Hz, H-2'), 6.90 (1Н, d, J = 8.2 Hz, H-5'), 6.70 (1Н, dd, J = 2.4; 1.8 Hz, H-2'), 6.47 (1Н, s, H-8), 3.75 (3Н, s, 3'-ОCH₃), 3.70 (3Н, s, 6-ОCH₃), 5.1 (1Н, d, J = 7.2 Hz, H-1”), 4.60 (1Н, t, J = 9.0 Hz, H-3”), 4.1 (1Н, dd, J = 9.0, 7.2 Hz, H-5”), 3.47 (1Н, t, J = 9.0 Hz, H-4”), 3.95 (1Н, d, J = 9.0 Hz, H-6”), 3.70 (1Н, dd, J = 9.0, 7.2 Hz, H-2”).

Iristectorigenin B (7) – iristectorigenin B-7-O-β-D-glucoside – C₃₂H₃₄O₁₂, a yellow powder. M. p. – 153-155°C.

Conclusions

Isoflavonoids – irigenin, iristectorigenin B and its glucoside iristectorin B have been isolated from the ethyl acetate extract of the rhizomes of Iris hungarica Waldst. et Kit. by column chromatography for the first time. The structure of compounds has been determined by chemical and spectral methods.

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