

Study of Biologically Active Compounds of the Roots of *Prangos biebersteinii* Karjag.

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The roots of *Prangos biebersteinii* Karjag. collected from Beshbarmak mountains were investigated. Seven crystalline substances of the coumarin nature were isolated from an acetone extract of *P. biebersteinii* roots using the method of column chromatography (Al_2O_3 , III-IV degree activity): $\text{C}_{16}\text{H}_{14}\text{O}_4$, m.p. 108.0-109.0°C (1), $\text{C}_{15}\text{H}_{16}\text{O}_3$, m.p. 84.0-85.0°C (2), $\text{C}_{16}\text{H}_{14}\text{O}_5$, m.p. 145.0-146.0°C (3), $\text{C}_{16}\text{H}_{16}\text{O}_5$, m.p. 109.0-110.0°C (4), $\text{C}_{16}\text{H}_{14}\text{O}_5$, m.p. 141.0-143.0°C (5), $\text{C}_{16}\text{H}_{16}\text{O}_6$, m.p. 137.0-138.5°C (6), $\text{C}_{11}\text{H}_6\text{O}_4$, m.p. 286.0-279.5°C (7). On the basis of physical and chemical properties (elemental composition, melting temperature) and spectral (UR- and NMR-spectra) data they were identified as isoimperatorin (1), ostol (2), isooxypeucedanin (3), pranferol (4), peucedanin (5), oxypeucedanin hydrate (6) and bergaptol, respective (7).

Keywords: *Prangos*, roots, sum of substances, chromatography, individual, UR-spectroscopy, NMR-spectroscopy, chemical shift.

INTRODUCTION

The species which on morphological features quite differs from *Prangos ferulacea* species I.I.Karjagin in agreement with A.A.Grossheim named as *Prangos biebersteinii* in honor of M.Bieberstein who collected and separated it from the East Caucasian "race" for the first time (Karjagin, 1955). But recently, the species *P. biebersteinii* was merged with *Prangos ferulacea* (L.) Lindl. and represent as a synonym of the latter species (Menitsky, 2008).

Literature data on chemical studying of *P. biebersteinii* is relatively rare (Abyshev et al., 1973, 2003; Abyshev, Brodsky, 1974).

The studied plant species *P. biebersteinii* is characterized by the presence in their composition of biologically active coumarin derivatives as well as most representatives of *Apiaceae* family. Conversely there are a great number of scientific works devoted to the chemical study of *P. ferulacea* which is merged to this species (Abyshev, 1969, 1974; Abyshev et al., 1972, 1973, 1974; Kuznetsova, Abyshev, 1965a, 1965b).

MATERIAL AND METHODS

The research object representing the dried and finely ground roots of *Prangos biebersteinii* Karjag. (155 g) which were collected in 09.05.2014 from Beshbarmag mountains in the flowering phase was extracted with acetone. Output of the sum of extractive substances was 5.16%. For isolation of individual compounds the 8.0 g extractive substances subjected to the column chromatography method in

columns ($h=45$ cm, $d=2.5$ cm) filled with neutral (with III-IV activity degree) Al_2O_3 . An identity of obtained substances using of thin layer chromatography on Silufol UV-254 plates is confirmed. An individual compounds based on physical-chemical (elemental composition, melting point) properties and on information received at the detection of IR- and NMR-spectra were identified. IR-spectra in Varian 640-IR spectrometer, NMR-spectra in Bruker 300 spectrometer at the 300 MHz resonance frequency in DMSO-d_6 solvent were registered. The melting points (m.p.) of individual compounds in Boethius table were determined.

RESULTS AND DISCUSSION

From the fractions obtained as a result of elution of chromatographic column with solvents hexane, benzol, chloroform and their mixtures in different ratios from the sum of extractive substances of *Prangos biebersteinii* Karjag. roots 7 compounds in individual state have been isolated.

Compound-1. From fractions of 1-2 eluted with hexane a compound with elemental composition of $\text{C}_{16}\text{H}_{14}\text{O}_4$ and melting point (m.p.) of 108.0-109.0°C was obtained.

IR-spectrum of compound contains absorption bands relating to carbonyl group of δ -lactone ring (1723 cm^{-1}) and double bonds of aromatic system ($1626, 1601, 1579, 1544\text{ cm}^{-1}$). The studied compound 1 has been identified as izoimperatorin by comparison of its IR-spectra with IR-spectra of known coumarin derivative isoimperatorin (Serkerov and Aleskerova, 2006).

Compound-2. The elemental composition and melting point of the compound 2 obtained from fractions of 5-6 eluted by hexane were $C_{15}H_{16}O_3$ and m.p. of 84.0-85.0°C, respectively.

In the area of characteristic IR-spectrum absorption frequencies the bands of lactone ring carbonyl group (1721 cm^{-1}) and aromatic system double bands ($1604, 1564, 1498\text{ cm}^{-1}$) have been revealed. The signals revealed in ^1H NMR-spectrum of compound: two singlets with 3H area of each (1.60; 1.80 ppm), doublet with 2H area (3.40 ppm, $J=4.5\text{ Hz}$) and triplet with 1H area (5.11 ppm, $J=4.5\text{ Hz}$) prove the existence of open side chain ($-\text{CH}_2-\text{CH}=\text{C}(\text{CH}_3)_2$) consisting 5 carbon atoms in molecule. The signals detected in lower magnetic field of spectrum: 6.20 (d., $J=9.65\text{ Hz}$, 1H, H-3), 7.91 (d., $J=9.65\text{ Hz}$, 1H, H-4), 7.50 (d., $J=9.10\text{ Hz}$, 1H, H-5) and 7.00 ppm (d., $J=9.10\text{ Hz}$, 1H, H-6) characterize double bonds of aromatic cycle of the compound. The singlet with chemical shift of 3.89 ppm in spectra is the evidence of methoxy-group ($-\text{OCH}_3$) in the structure of the studied compound.

Thus, the results obtained from the detection of IR- and ^1H NMR-spectra indicate that the structure of the compound 2 is identical with ostol (Gasimova, Serkerov, 2011).

Compound-3. The elemental composition and melting point of the compound obtained from fractions eluted by mixture of benzole and chloroform (2:1) were $C_{14}H_{14}O_5$ and of 145.0-146.0°C, respectively. In IR-spectrum of the compound absorption bands relating to δ -lactone cycle (1744 cm^{-1}), ketone group (1616 cm^{-1}) and double bonds of aromatic system ($1622, 1579, 1546, 1513\text{ cm}^{-1}$) have been revealed. By direct comparison of IR-spectrum of studied compound with IR-spectrum of isooxypeucedanin compound 3 was identified as isooxypeucedanin (Serkerov, Aleskerova, 2006).

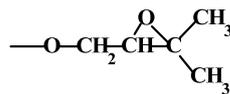
Compound-4. The elemental composition and melting point of the compound obtained from fractions of 78-80 of the chromatographic column eluted by mixture of benzole and chloroform (1:1) were $C_{16}H_{16}O_5$, m.p. 109.0-110.0°C, respectively. In the area of characteristic IR-spectrum absorption frequencies the bands characterizing of hydroxyl group ($3450-3200\text{ cm}^{-1}$), C=O group of δ -lactone cycle (1706 cm^{-1}) and double bonds of coumarin structure ($1628, 1619, 1575, 1547\text{ cm}^{-1}$) were detected.

In IR-spectrum of the studied compound there are two intensively bands in the area of $1600-1650\text{ cm}^{-1}$. This is characteristic for spectra of 5-monosubstituted furocoumarins, for example oxypeucedanin. However, for spectra of 8-monosubstituted furocoumarins, for example in the same area of spectra of the prangenin, prangenin hydrate, imperatorin only one weak band ($1625-1620\text{ cm}^{-1}$) appears (Kuznetsova, 1967).

Thus taking into account abovementioned facts and also directly comparing IR-spectra of pranferol and studied coumarin derivative the compound 3 was identified with 5-monosubstituted furocoumarin - pranferol (Sererkov, Aleskerova, 2006).

Compound-5. The elemental composition and melting point of the compound obtained from fractions of 88-91 of the chromatographic column eluted by mixture of benzole and chloroform (1:2) were $C_{16}H_{16}O_5$, m.p. 141.0-143.0°C correspondingly. In the area of characteristic IR-spectrum absorption frequencies the absorption bands relating to C=O group of δ -lactone cycle (1735 cm^{-1}) and double bonds of aromatic system ($1630, 1610, 1590\text{ cm}^{-1}$) have been revealed.

In ^1H NMR spectrum of studied compound 5 singlet signal (s., 1.30 and 1.40 ppm) attributed to 2 methyl group; quartet peak (3.20 ppm, $J_1=4.14, J_2=6.43\text{ Hz}$) attributed to proton bound with carbon atom of epoxy cycle; 2 quartet each with area of 1H (4.40 and 4.60 ppm, $J_1=4.14, J_2=11.03\text{ Hz}$) attributed to oxygen-bound methylene group ($-\text{CH}_2-$). These signals provide an opportunity to confirm that simple side ethereal chain as



-group is present in the structure of studied compound. The signals detected in in lower magnetic field of spectrum: doublets each 1H area (6.25, $J=9.50\text{ Hz}$ and 8.15 ppm, $J=9.50\text{ Hz}$; 6.95, $J=2.10\text{ Hz}$ and 7.60 ppm, $J=2.30\text{ Hz}$) and singlet (7.15 ppm) were attributed to protons in positions of C-3, C-4, C-2', C-3' and C-8 of the furocoumarin structure.

The results obtained from IR- and ^1H NMR-spectra proves that a structure of compound-5 is identical with structure of oxypeucedanin.

Compound-6. The elemental composition and melting point of the compound obtained in individual state from fractions of 129-130 of the chromatographic column eluted by mixture of benzole and chloroform (1:3) and chloroform were $C_{16}H_{16}O_6$, m.p. 137.0-138.0°C, respectively.

In the area of characteristic IR-spectrum absorption frequencies the bands characterizing of hydroxyl group (3400 cm^{-1}), C=O group of δ -lactone cycle (1703 cm^{-1}) and double bonds of aromatic system ($1618, 1603, 1575, 1554\text{ cm}^{-1}$) are present.

In the ^1H NMR spectrum of the compound the signals relating to 2 methyl groups (s., 1.30 and 1.40 ppm), 2 hydroxyl groups (s., 2.35 and 3.05 ppm), to protons of methylene (t., 4.45 and 4.55 ppm) and gem-hydroxyl (d., 3.90 ppm) groups were detected.

The signals detected in in lower magnetic field of ^1H NMR spectrum: 6.30 (d., $J=9.65\text{ Hz}$, 1H, H-3),

8.20 (d., $J=9.65$ Hz, 1H, H-4), 7.30 (s., 1H, H-8), 7.00 (d., $J=2.30$ Hz, 1H, H-3'), 7.60 m.h. (d., $J=2.30$ Hz, 1H, H-2') characterize furocoumarin part of the molecule. In ^{13}C NMR spectrum of studied compound providing the presence of 16 carbon atoms in molecule 16 singlet signals (25.0; 27.0; 30.0; 71.0; 74.0; 94.0; 99.0; 104.0; 108.0; 111.5; 112.0; 119.0; 133.0; 139.0; 145.0; 161.0 ppm) were detected.

Seven signals that were not detected in ^{13}C Dept 135 spectrum prove that the number of non-protonated carbon atoms is seven. Based on above-mentioned spectral data it is proved that the structure of compound-6 is identical to structure of oxy-peucedanin hydrate.

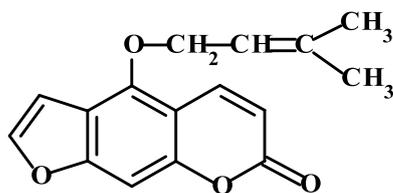
Compound-7. The elemental composition and melting point of the crystalline compound obtained in individual state from fractions of 129-131 of the chromatographic column eluted by mixture of chloroform and ethanol (95:5) were $\text{C}_{11}\text{H}_6\text{O}_4$, m.p. 286.0-289.5°C, respectively.

In the IR-spectrum the bands characterizing of hydroxyl group (3226 cm^{-1}), C=O group of δ -lactone cycle (1690 cm^{-1}) and double bonds of aromatic system ($1585, 1253, 824\text{ cm}^{-1}$) were detected. These bands allow attributing studied compound to simple linear furocoumarins (Li et al., 2006; Ghada et al., 2015).

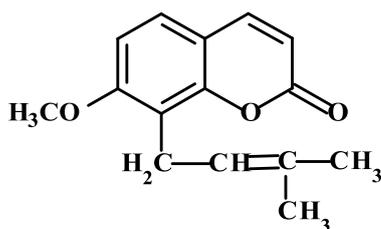
In ^1H NMR spectrum the signals were detected each with area of 1 proton unit: 6.19 (1H, d., $J=9.90$ Hz, H-3), 8.25 (1H, d., $J=9.90$ Hz, H-4), 11.10 (1H, HO-), 6.90 (1H, s., H-8), 7.76 (1H, d., $J=2.40$ Hz, H-2') and 7.48 ppm (1H, d., $J=2.40$ Hz, H-3').

The interpretation of IR- and ^1H NMR-spectral data prove that the structure of studied compound is identical to the structure of oxy-furocoumarin bergaptol.

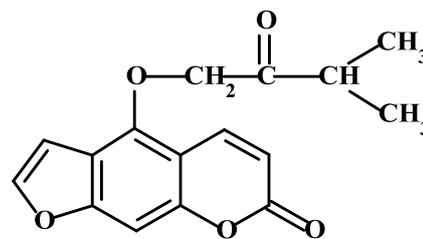
The chemical structure of compounds identified as a result of researches:



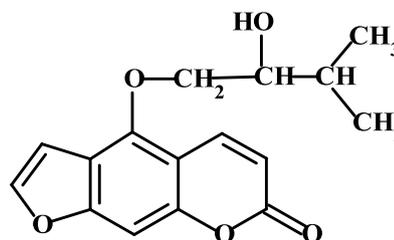
Isoimperatorin



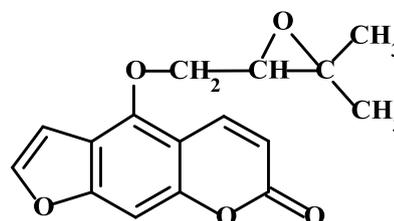
Osthol



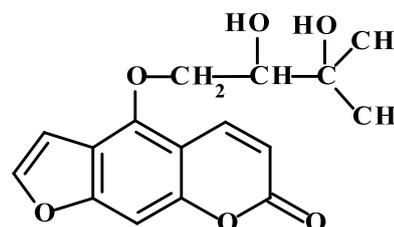
Isooxypeucedanin



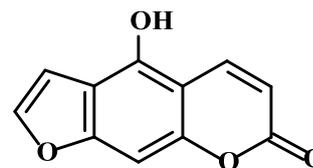
Pranferol



Oxypeucedanin



Oxypeucedanin hydrate



Bergaptol

CONCLUSIONS

1. From the roots of *Prangos biebersteinii* Karjag. collected in flowering phase from Beshbar-mag mountain 7 coumarin derivatives in individual state have been obtained.

2. Based on physicochemical (elemental composition, melting temperature) properties and spectral (IR- and NMR) data the obtained individual coumarin derivatives were identified as izoimperatorin, ostol, izooxypeucedanin, pranferol, oxypeucedanin, oxypeucedanin hydrate and bergaptol.

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Prangos biebersteinii Karjag. Növünün Köklərinin Bioloji Fəal Maddələrinin Öyrənilməsi

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Prangos biebersteinii Karjag. (Biberşteyn çəşiri) növü köklərindən alınmış ekstraktiv maddələr cəmindən sütunlu xromatoqrafiya metodundan istifadə edərək fərdi şəkildə 7 kristallik maddə alınmışdır: C₁₆H₁₄O₄, ə.t. 108,0-109,0°C (1), C₁₅H₁₆O₃, ə.t. 84,0-85,0°C (2), C₁₆H₁₄O₅, ə.t. 145,0-146,0°C (3), C₁₆H₁₆O₅, ə.t. 109,0-110,0°C (4), C₁₆H₁₄O₅, ə.t. 141,0-143,0°C (5), C₁₆H₁₆O₆, ə.t. 137,0-138,5°C (6) və C₁₁H₆O₄, ə.t. 286,0-289,5°C (7). Alınmış maddələrin fiziki-kimyəvi xassələrinin (tərkibi, ə.t.) və spektral (İQ- və NMR-) xüsusiyyətlərinin tədqiq edilməsindən alınan nəticələr əsasında onlar uyğun olaraq izoimperatorinlə (1), ostolla (2), izooksipepsedaninlə (3), pranferolla (4), oksipepsedaninlə (5), oksipepsedanin hidratla (6), berqatolla (7) identifikasiya edilmişdir.

Açar sözlər: *Prangos*, köklər, maddələr cəmi, xromatoqrafiya, fərdi, İQ spektroskopiyası, NMR spektroskopiyası, kimyəvi sürülmə

Изучение Биологически Активных Веществ Корней *Prangos biebersteinii* Karjag.

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Исследованы корни *Prangos biebersteinii* Karjag., собранные на горе Бешбармак. Из ацетонового экстракта корней *P. biebersteinii* методом колоночной хроматографии (Al_2O_3 , III-IV степени активности) выделены 5 кристаллических веществ кумариновой природы: $C_{16}H_{14}O_4$, т.пл. 108,0-109,0°C (1), $C_{15}H_{16}O_3$, т.пл. 84,0-85,0°C (2), $C_{16}H_{14}O_5$, т.пл. 145,0-146,0°C (3), $C_{16}H_{16}O_5$, т.пл. 109,0-110,0°C (4), $C_{16}H_{14}O_5$, т.пл. 141,0-143,0°C (5), $C_{16}H_{16}O_6$, т.пл. 137,0-138,5°C (6), $C_{11}H_6O_4$, т.пл. 286,0-289,5°C (7), которые на основании физико-химических свойств (элементный состав, температура плавления) и спектральных (ИК- и ЯМР-спектры) данных идентифицированы, соответственно, с изоимператорином (1), остолом (2), изооксипейцедином (3), пранферолом (4), оксипейцедином (5), оксипейцеданин гидратом (6) и бергаптолом (7).

Ключевые слова: *Прангос, корни, сумма веществ, хроматография, индивидуальный, ИК-спектроскопия, ЯМР-спектроскопия, химический сдвиг.*