

## The Coumarins of the Roots of *Heracleum sosnowskyi*

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**6 coumarinic derivatives were isolated from the roots of *Heracleum sosnowskyi* Manden. collected in September 2013 in the vicinity of the village of Kuzun of the Gusar region of Azerbaijan by chromatography of the amount of extractives obtained by extracting plant material (shredded, air-dried roots) with acetone on a column of an  $Al_2O_3$  : I)  $C_{11}H_6O_3$ , m.p. 161-163°C; II)  $C_{11}H_6O_3$ , m.p. 138-139°C; III)  $C_{12}H_8O_4$ , m.p. 189-191°C; IV)  $C_{12}H_8O_4$ , m.p. 145-146°C; V)  $C_{13}H_{10}O_5$ , m.p. 116-118°C; VI)  $C_{13}H_{10}O_5$ , m.p. 118-120°C; Based on the data obtained, when interpreting IR and  $^1H$  NMR spectra, the obtained coumarin derivatives were identified with psoralen, angelicin, xanthotoxin, bergapten, pimpinellin and isopimpinellin, respectively.**

**Keywords:** *Heracleum*, coumarins, IR,  $^1H$  NMR spectrum, column chromatography

### INTRODUCTION

Coumarinic derivatives of *Heracleum sosnowskyi* Manden. have been studied by different authors (Kuznetsova, 1967; Kreyer, 1963). Previously, the amount of the coumarin derivatives obtained in the department Geobotany of the Institute of Botany NAS of Azerbaijan by H.Guliyeva allocated bergapten, izobergapten, and pimpinellin izopimpinellin (Serkerov, 1970), from roots *H.sosnowskyi* collected in the vicinity of the village Anikh Gusar region's (Abusheva and Denisenko, 1973) identified sfondin, pimpinellin, izopimpinellin, izobergapten, ostol, oksipeyседanin hydrate, marmezin and pangelin. Fruit plants Dagestan origin marked angelicin, bergapten and izopimpinellin, and from roots izobergapten, izopimpinellin and sfondin (Kerimov, 1980). From young leaves and shoots *H.sosnowskyi*, harvested at the flowering stage of the introductory nursery mountain-taiga station FEB RAS Yurlova et al. highlighted angelicin, sfondin, bergapten, xanthotoxin and umbelliferone (Yurlova et al., 2013).

### MATERIALS AND METHODS

The material for the study were air-dried roots of *Heracleum sosnowskyi*, collected in the vicinity of the village Kuzun Gusar region of Azerbaijan Republic. Herbarium specimens were determined by Z.S.Aliyeva and are stored in the herbarium collection of the Institute of Botany of the National Academy of Sciences of Azerbaijan.

Extraction (238.0 g roots) was carried out with acetone three times, each time for 3 days. Acetone was filtered and stripped off in a water bath. The residue is a dark brown resin (12.0 grams). The

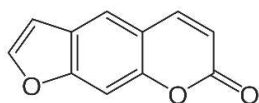
yield was 5.04%, the resin was mixed with 100.0 grams neutral  $Al_2O_3$  and left at room temperature. After evaporation of acetone to  $Al_2O_3$  resin was transferred to column (h = 45,0 d = 3,0sm) filled with neutral alumina (III-IV degree of activity) eluted with hexane, benzene, chloroform and mixtures thereof in various proportions: hexane (12 fractions ) mixture of hexane and benzene in a ratio of 2: 1 (18 fractions), hexane-benzene 1: 1 (20 fractions), with a mixture of benzene and chloroform (1: 1) and chloroform. Volume fractions of 100 ml each.. individual substances was determined on the plates Silufol UV 254. The melting point of crystalline substances was determined on the table Boethius. IR spectra were recorded on a Specord spectrometer in vaseline oil.  $^1H$  NMR spectra were recorded on a Bruker 300 spectrometer with a resonant frequency of 300 MHz for  $^1H$  MHz. Solvent dimethyl sulfoxide (DMSO), chemical shifts are given on the  $\delta$ -scale. The internal standard is TMS. Conditional designations: s - singlet, d - doublet, t - triplet, q - quartet, m - multiplet

### RESULTS AND DISCUSSION

Chromatographic separation using the solvent system specified in the "Materials and Methods" section of this work. From fractions 2-3, eluted with a mixture of hexane and benzene in a 2:1 ratio, crystalline substance (1) of the composition  $C_{11}H_6O_3$  (1) was isolated. m.p. 161-163°C. The yield is 0.08%. The IR spectrum of the substance has absorption bands of the  $\delta$ -lactone ring (1720  $cm^{-1}$ ) and 1630, 1568, 983  $cm^{-1}$  ( $-C=C-$  bonds of the aromatic system). In the  $^1H$  NMR spectrum of the substance, two single-proton doublets 6,37 (d., J =

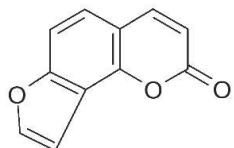
9,60 Hz, 1H, H-3), 7.80 (d., J=9.60 Hz, 1H, H-4) (d., J = 2.30 Hz, 1H, H-3'), 7.72 (d., J=2.30 Hz, 1H, H-2') The singlet at 7.70 (s., 1H, H-5) and 7.46 ppm (1H, H-8) are assigned to the proton H-5 and H-8, respectively.

Comparing the data obtained by us in interpreting the IR and <sup>1</sup>H NMR spectra of substance 1 with literature data, it is proved that the compound under study has a psoralen structure (Perelson et al., 1975).



**Psoralen (1)**

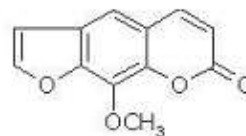
Fractions 4-5, also eluted with a mixture of hexane and benzene (2:1), contained a substance (with a small admixture of substance 1, which was purified by a different recrystallization from hexane) with the composition C<sub>11</sub>H<sub>6</sub>O<sub>3</sub> (2), m.p. 138-139°C. Yield 1.18%. In the range of characteristic frequencies, the IR spectrum has absorption bands of the δ-lactone cycle carbonyl (1720 cm<sup>-1</sup>) and the bands of the aromatic system (1629, 1565, 985 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum of substance 2 shows doublet H-3 and H-4 signals 6.38 (d., J = 9.60 Hz, 1H, H-3), 7.80 (d., J=9.60 Hz, 1H, H-4); H-3', H-2' furan ring 7.12 (d., J=1.98 Hz, 1H, H-3'), 7.70 (d., J=1.98 Hz, 1H, H-2') and protons H-5 - 7.36 ppm (d., J=8.50 Hz, 1H, H-5) and H-6, 7.42 ppm (d., J=8.50 Hz, 1H, H-6) differing from the signals of substance 1 and are characteristic of angular furocoumarin signals of angelilicin rather than linear psoralenic furocoumarin (Perelson et al., 1975). The spectra of substance 2 obtained by interpreting the IR and NMR spectra of substance 2 indicate the identity of substance 2 with angelicin (isopsoralene).



**Angelicin (2)**

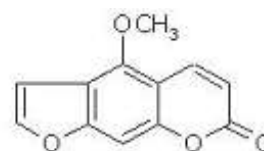
In fractions 15-17, obtained by elution of hexane with benzene in a ratio of 2:1, an individual compound of the composition C<sub>12</sub>H<sub>8</sub>O<sub>4</sub> (3) was isolated, m.p. 145-146°C. The yield is 0.62%. The IR spectrum of substance 3 has the -C=O- bands of the δ-lactone ring group (1730 cm<sup>-1</sup>) -C=C-bands of the aromatic system (1625, 1600, 1560 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum of the test compound is characteristic of methoxy-furocoumarins. Thus, the

detectable three-proton singlet 4.26 m.d. (OCH<sub>3</sub>) leaves no doubt about the presence of 3 methoxy groups in the molecule. The H-3 and H-4 proton signals in the spectrum are detected as two doublet signals (6.30, J=9.65 Hz, H-3 and 7.73 ppm J=9.65 Hz, H-4). The signals of protons H-3' and H-2' of the furan ring are also detected as doublets (6.80, J=2.00 Hz, 1H, H-3', 7.60 ppm J=2.00 Hz, 1H, H-2'). The single singlet signal at 7.32 ppm (s., 1H, H-8) belongs to the proton at C-8 coumarin. The data obtained in interpreting the IR and <sup>1</sup>H NMR spectra firmly demonstrate the identity of the substance 3 with xanthotoxin.



**Xanthotoxin (3)**

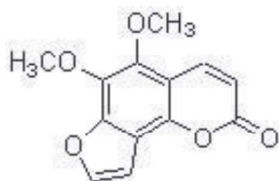
A substance of the composition C<sub>12</sub>H<sub>8</sub>O<sub>4</sub> (4) was isolated from fraction 18-19, obtained by elution (hexane-benzene, 1:2) of the chromatographic column, m.p. 189-191°C. Yield 1.30%, the IR spectrum shows the absorption bands of the -C=O group of the δ-lactone cycle (1730 cm<sup>-1</sup>) and the aromatic system (1620, 1580 cm<sup>-1</sup>). In the <sup>1</sup>H NMR spectrum of the compound, signals of H-3, H-4 of the pyro cycle 6.30 (d., J=9.65 Hz, 1H, H-3), 8.20 (d., J=9.65 Hz, 1H, H-4); H-2 furan ring 8.00 (d., J=2.30 Hz, 1H, H-2'), H-3' - 7.40 (d., J=2.30 Hz, 1H, H-3'), H-8, 7.30 s, 1H, H-8) and methoxy groups 4.22 ppm (s., 1H, -OCH<sub>3</sub>), which are characteristic of methoxyfurocoumarin - bergapten.



**Bergapten (4)**

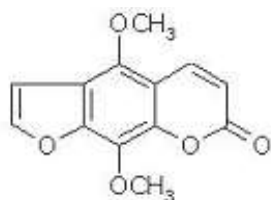
From fractions 32-35 obtained by eluting the chromatographic column with a mixture of benzene and hexane (1: 1), an individual crystalline substance of the composition C<sub>13</sub>H<sub>10</sub>O<sub>5</sub> (5) was obtained, m.p. 116-118°C. Yield 1.82%. The absorption bands of the C=O group of the δ-lactone cycle (1760 cm<sup>-1</sup>), double bonds of the aromatic system (1628, 1580, 1545 cm<sup>-1</sup>) were found in the IR spectrum of the substance under study. In the weak magnetic field of the <sup>1</sup>H NMR spectrum of substance 5, signals H-3 and H-4 protons were detected (6.45, d., J=11.00 Hz, H-3, 8.15 ppm d., J=11.00 Hz, H-4) of the furan ring H-2' and H-3' (8.14, d., J=2.30 Hz, 1H, H-2', 7.25 ppm, d., J=2.30 Hz, 1H, H-3'). Three proton singlet

signals 3.95 (3H, -OCH<sub>3</sub>) and 4.10 ppm (3H, -OCH<sub>3</sub>) indicate that the test substance belongs to the group of linear furocoumarins containing two methoxyl groups, hence the substance is identical to pimpinellin.



**Pimpinellin (5)**

From the fraction 37-38, eluting with a mixture of hexane and benzene, crystallized an individual substance of the composition C<sub>13</sub>H<sub>10</sub>O<sub>5</sub> (6), m.p. 118-120°C. Yield 0.91%. The absorption bands of the δ-lactone cycle (1755 and 1720 cm<sup>-1</sup>) and double bonds of the aromatic system (1660, 1558 cm<sup>-1</sup>) were found in the IR spectrum of the substance. The singlet signals of methoxyl groups of 4.05 and 4.15 ppm appear in the <sup>1</sup>H NMR spectrum of the compound. The signals detected by the <sup>1</sup>H NMR spectrum are 6.35 (d., J=10.11 Hz) and 8.15 ppm (d., J=10.11 Hz) are characteristic for protons H-3 and H-4. Signals of the protons of the furan ring appear as two doublets at 8.10 (d., J=2.30 Hz, H-2') and 7.40 ppm (d., J=2.30 Hz, H-3'). The given data of the <sup>1</sup>H NMR spectrum coincide completely with those of isopimpinellin (Perelson et al., 1975).



**Isopimpinellin (6)**

Thus, a comparison of our data on the coumarin composition of *Heracleum sosnowskyi* Manden. with literary ones (Kuznetsova, 1967; Kreyer, 1963; Kerimov, 1980; Abushev and Denisenko, 1973; Serkerov, 1973; Yurlova et al., 2013) revealed that furocoumarin psoralen isolated from *H.Sosnowskyi* plant material was not found in either one specimen of *H.sosnowskyi* by other authors of the work (Kreyer, 1963; Kerimov, 1980; Abushev, Denisenko, 1973; Serkerov, 1973; Yurlova et al., 2013). Angelicin found in some

plant samples (Y.Yurlova et al., 2013) together with psoralen was found only in our sample. It should be noted that the xanthoxin isolated by us was also obtained by the authors of the works (Yurlova et al., 2013). The obtained data on the coumarin composition of *H.sosnowskyi* suggest that there may be ecotypes of *H.sosnowskyi* with slightly modified compositions of coumarin derivatives in nature.

## CONCLUSIONS

1. From the resin of the roots of *Heracleum Sosnowskyi* Manden. The method of column chromatography with aluminum oxide was used to isolate the coumarin derivatives C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, m.p. 161-163°C (1), C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, m.p. 138-139°C (2), C<sub>12</sub>H<sub>8</sub>O<sub>4</sub>, m.p. 189-191°C (3), C<sub>12</sub>H<sub>8</sub>O<sub>4</sub>, m.p. 145-146°C (4), C<sub>13</sub>H<sub>10</sub>O<sub>5</sub>, m.p. 116-118°C (5) and C<sub>13</sub>H<sub>10</sub>O<sub>5</sub>, m.p. 118-120°C (6).
2. Based on chemical and spectral (IR, <sup>1</sup>H NMR) data, it is proved that they have a structure identical to psoralen (1), angelicin (2), xanthoxin (3), bergapten (4), pimpinellin (5), isopimpinellin (6).
3. Psoralen from *Heracleum Sosnowskyi* Manden. isolated for the first time.

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## ***Heracleum sosnowskyi* Növü Köklərinin Kumarinləri**

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2013-ci ilin sentyabr ayında Azərbaycan Respublikası Qusar rayonunun Kuzun kəndi yaxınlığından toplanan *Heracleum sosnowskyi* Manden. növünün köklərindən xromatoqrafiya metodu vasitəsilə bitki materialından (döğranmış-xırdalanmış, havada qurudulmuş köklər) ekstraksiya yolu ilə ekstraktiv maddələr cəmindən neytral Al<sub>2</sub>O<sub>3</sub> doldurulmuş asetonlu sütunda fərdi halda altı kumarin törəmələri ayrılaraq alınmışdır: **I)** C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, ə.t. 161-163°C; **II)** C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, ə.t. 138-139°C; **III)** C<sub>12</sub>H<sub>8</sub>O<sub>4</sub>, ə.t.189-191°C; **IV)** C<sub>12</sub>H<sub>8</sub>O<sub>4</sub>, ə.t. 145-146°C; **V)** C<sub>13</sub>H<sub>10</sub>O<sub>5</sub>, ə.t. 116-118°C; **VI)** C<sub>13</sub>H<sub>10</sub>O<sub>5</sub>, ə.t.118-120°C; Alınmış nəticələrə əsaslanaraq, İQ və <sup>1</sup>H NMR spektrlərinin interpretasiyası zamanı alınmış kumarin törəmələri müvafiq olaraq psoralen, angelisin, ksantotoksin, berqapten, pimpinellin və izopimpinellinlə identifikasiya edilmişdir.

**Açar sözlər:** *Heracleum, kumarinlər, İQ, <sup>1</sup>H NMR spektrlər, sütünlu xromatoqrafiya*

## **Кумарины Корней *Heracleum sosnowskyi***

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Из корней *Heracleum sosnowskyi* Manden., собранных в сентябре 2013 года в окрестностях село Кузун Гусарского района Азербайджана методом хроматографии на колонке заполненной Al<sub>2</sub>O<sub>3</sub> суммы экстрактивных веществ, полученных путем экстракции растительного материала (измельченных, воздушно-сухих корней) ацетоном индивидуальном состоянии выделены 6 кумариновых производных: **I)** C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, т.пл. 161-163°C; **II)** C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, т.пл. 138-139°C; **III)** C<sub>12</sub>H<sub>8</sub>O<sub>4</sub>, т.пл. 189-191°C; **IV)** C<sub>12</sub>H<sub>8</sub>O<sub>4</sub>, т.пл. 145-146°C; **V)** C<sub>13</sub>H<sub>10</sub>O<sub>5</sub>, т.пл. 116-118°C; **VI)** C<sub>13</sub>H<sub>10</sub>O<sub>5</sub>, т.пл. 118-120°C; На основании полученных данных, при интерпритации ИК- и <sup>1</sup>H ЯМР- спектров полученные кумаринпроизводные идентифицировали с псораленом, ангелицином, ксантотоксином, бергаптенем, пимпипеллином и изопимпипеллином, соответственно.

**Ключевые слова:** *Heracleum, кумарины, ИК, <sup>1</sup>H ЯМР спектр, колоночная хроматография*