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Coumarins of *Peucedanum baicalense* and cytotoxic activity of some isolated coumarins

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ABSTRACT: It was shown that the plant *Peucedanum baicalense* (Redow.) Koch is the source of valuable coumarins. Five linear furocoumarins – isoimperatorin, fellopterin, 8-(1,1-dimethylallyloxy) bergapten, deltoin and marmesin were isolated from the roots of *Peucedanum baicalense* (Redow.) Koch. The structures of these compounds were elucidated by spectroscopic methods. The cytotoxicity of isoimperatorin, fellopterin, 8-(1,1-dimethylallyloxy)bergapten and deltoin was studied on models of human CEM-13, MT-4 and U-937 tumor cells. Compound 8-(1,1-dimethylallyloxy)bergapten had the greatest cytotoxicity.

Keywords: Peucedanum baicalense, *furocoumarins, isoimperatorin, fellopterin, 8-(1,1-dimethylallyloxy)bergapten, deltoin, cytotoxic activity, tumor cells, X-ray analysis*

INTRODUCTION

Plants of the genus Peucedanum sp. attract an attention of many researchers as a source of coumarins. Previously, we isolated pyranocoumarin (+)-pterixin from *Peucedanum terebinthaceum* Fischer et Turcz. of the Mongolian flora [1]. Plants belonging to Peucedanum species characterized as the source of angular furocoumarins [2]. Coumarins are considered as phytoalexins since plants produce them as defence substances when wounded or attacked by other organisms. Coumarins can be suggested to be beneficial for the plants themselves as natural biocontrolling antipathogenic compounds as well as for humans as remedy for hyperproliferative skin deseases and as reference compounds in various bioactive tests. Furthermore, coumarin containing plants are valuable as dietary supplements on the basis of their mild antimicrobial and anti-inflammatory effects. Coumarins are also active in a plant metabolism, taking part in growth regulation.

Peucedanum baicalense (Redow.) Koch is a plant which widely spread throughout the Mongolian territory [3]. To our knowledge a systematic phytochemical investigation of this plant has not been properly carried out yet. The aim of this study was to investigate coumarins of *Peucedanum baicalense* (Redow.) Koch and cytoxic activity some of the pure coumarins.

EXPERIMENTAL

Plant material. Roots of *P. baicalense* were collected near the place Baruun buren, Selenge aimag, Mongolia in its butonization-flowering period in 2012.

Extraction and fractionation . Air-dried ground roots of *Peucedanum baicalense* (Redow.) Koch (400 g) were exhaustively extracted by maceration with 96% EtOH (3x500 ml) at room temperature. The EtOH extract was evaporated to an aqueous residue, which diluted with distilled water (1:1), and filtered. The filtrate was fractionated by solvents with increasing polarity, i. e., *n*-hexane, diethyl ether and ethylacetate, respectively. Each fraction was condensed by a rotatory evaporator. Then, the fractions were separated by column chromatography over silica gel.

Separation of the n-hexane fraction. The *n*-hexane fraction was subjected to a column chromatography (eluent: chloroform, chloroform-ethanol 100:1→10:1) which afforded successive fractions from which crystallization from Et_2O isolated diacetylenenic alcohol falcarinol (**7**) as form of oil (35 mg, 0.008%) [9], coumarins (5 fractions, totally ~0.8 g) and sterins. 130 mg isoimperatorin **1**, 156 mg fellopterin **2** and 176 mg 8-(1,1-dimethylallyloxy)bergapten **3**. ^{1}H NMR spectral data showed that the *n*- hexane fraction contains coumarins (~30%) and sterins (~12%) as main components.

Separation of the diethyl ether fraction. The column chromatography of the diethyl ether fraction afforded successive fractions from which 18 mg of 8-(1,1-dimethylallyloxy) bergapten (4) and 28 mg of deltoin (5) were crystallized from diethyl ether.

Hydrolysis of the ethyl acetate fraction. The EtOAc fraction (9.1 g) was dissolved in KOH (150 ml, 10%), treated with dioxane (150 ml), heated at 80^{o}C for 2 h. Then the mixture was cooled and neutralized with aqueous H_2SO_4 (10%). Coumarins were extracted with dichloromethane, which was removed by evaporating. The solid residue was chromatographed over silica gel to isolate marmesin (66 mg) **6.**

Structure elucidation of compounds. Freshly distilled solvents and pure grade reagents were used. Pure compounds were isolated by column chromatography over silica gel (Acros, 0.035-0.070 mm) with elution by chloroform: ethanol. The purity of the isolated compounds was monitored by TLC on Silufol UV-254 plates using $CHCl_3$:EtOH (10:1) and petroleum ether:Et_2O (4:1). Melting points were determined on Stuart SMF-38 instrument. The IR spectra were recorded on a Vector 22 spectrometer in KBr tablet, UV spectra were measured on a Specord UV-Vis spectrophotometer in ethanol ($c = 10^{-4}$ mol/l).

NMR spectra of compounds in $CDCI_3$ or CD_3OD were obtained on Bruker AV-300 (operating frequency 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C and AV-600 (600.13 and 150.96 Mhz, respectively) spectrometers. Proton-proton and carbon-proton shift correlation spectroscopy COSY, COLOC, NOESY were used for structure elucidation of substances. The multiplicity of resonances in ^{13}C NMR spectra was determined by recording spectra in J-mode. A DFS Thermoscientific high-resolution mass spectrometer (ionizing electron energy 70 eV, vaporizer temperature 230-280 ^{o}C) was used to record mass spectra and determine molecular weights and elemental compositions. Specific rotation $[\alpha]_D$ was measured on PolAAr3005 polarimeter.

RESULTS AND DISCUSSION

The greatest coumarin content was found in the *n*-hexane and diethyl ether fractions of the ethanolic extract. The substances of the root of *Peucedanum baicalense* (Redow.) Koch were isolated by column chromatography over silica gel. The structures of the isolated compounds were established using spectral data and comparison to literature data. Results of our study showed that *Peucedanum baicalense* might be serve as a source of linear furocoumarins **1,2,3** which were known that for possessing valuable biological properties [4]. Thus, fellopterin **2** possesses an anti-inflammatory activity on animal inflammatory models [5]. Isoimperatorin **1** is proved as an inhibitor of β-secretase [6]. Deltoin **5** has an anti-tumor activity against Erlich cancer's ascite cells [7]. Isoimperatorin (1) (0.032% from sample weight), felloterin (2) (0.038%) and 8-(1,1-dimethylallyloxy) bergapten (3) (0.044%) were isolated from n-hexane fraction of the EtOH extract. Bergapten **(4)** (0.0045% from weight of the root) and deltoin (5) (0.007% from weight of the roots) were isolated from the diethyl ether fraction. Deltoin 5 was detected also in the ethyl acetate fraction. Sixty six mg (0.016% from root's weight) furocoumarin marmesin (6) was isolated after alkaline hydrolysis of the diethyl ether fraction and purified by subsequent column chromatography.

We obtained data on the cytotoxic activity of furocoumarins **1-3** and **5** for tumor cells CEM-13 (T-cellular leucosis), MT-4 (T-cellular leukemia) and U-937 (monocyte leukemia) human tumor cells. Cytotoxic activity has been established by means of determination of $CCID_{50}$ – concentration inhibiting viability of tumor cells by 50%. The standard MTT test according to published recommendations [8] was used to determine $CCID_{50}$. The selective

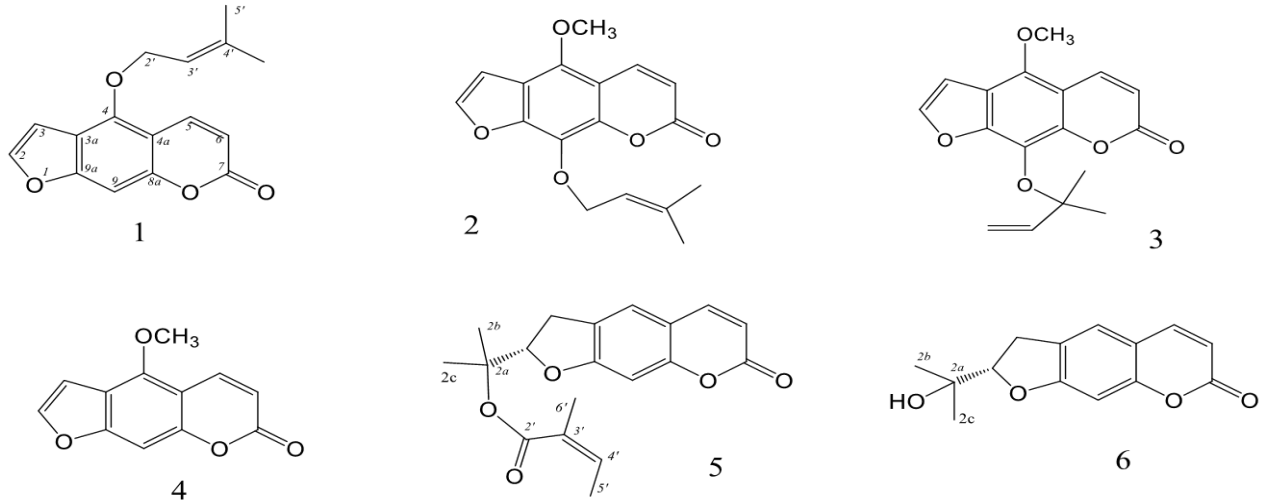

Figure 1. The molecular structures of isolated coumarins

cytotoxicity of linear furocoumarins **1-3** and **5** against U-937 is noteworthy. Compound 8-(1,1-dimethylallyloxy)bergapten **3** had the greatest cytotoxicity against all tumor cells. Compounds 2 had the least cytoxicity against CEM-13 cell, whereas compound 5 had the least cytotoxicity against MT-4 cell. We could not find the relationship between structure and activity. It need to provide more detailed investigations of more isolated pure compounds.

Table 1. Cytotoxic activity of linear furocoumerins 1-3 and 5.

Compound	Tumor cells CEM-13, $CCID_{50}$, (μM)	Tumor cells U-937, $CCID_{50}$ Tumor cells , (μM)	Tumor cells MT-4, $CCID_{50}$, (μM)
(**1**)	82.6±5.2	18.8±1.9	38.1±2.8
(**2**)	94.1±3.9	11.4±1.8	27.2±3.3
(**3**)	53.2±7.2	1.8±0.3	11.8±4.2
(**5**)	85.5±10.2	14.7±7.1	33.8±3.1

Characteristics of pure compounds.

Isoimperatorin {**4-(3-methylbut-2-ene-1-yl)oxy]-7*H*-furo[3,2-*g*]chromen-7-one**} (**1**). M. p. 104-107°C. UV and mass spectra are identical to data in paper [10]. ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz): 1.67 (3H, s, H-5′), 1.77 (3H, s, H-6′), 4.88 (2H, d, *J* = 7.0, H-2′), 5.50 (1H, m, H-3′), 6.23 (1H, d, *J* = 9.8, H-6), 6.92 (1H, d, *J* = 2.5, H-3), 7.11 (1H, s, H-9), 7.56 (1H, d, *J* = 2.5, H-2), 8.12 (1H, d, *J* = 9.8, H-5). ^{13}C NMR spectra, δ, ppm: 18.08 (C-5′), 25.66 (C-6′), 69.59 (C-2′), 94.03 (C-9), 104.91 (C-3), 107.34 (C-4a), 112.38 (C-6), 114.03 (C-3a), 118.96 (C-3′), 139.43 (C-5), 139.66 (C-4′), 144.74 (C-2), 148.82 (C-4), 152.51 (C-8a), 157.98 (C-9a), 161.14 (C-7).

Fellopterin {**9-[(3-methylbut-2-ene-1-yl)oxy]-4-methoxy-7*H*-furo[3,2-g]chromen-7-one**} (**2**). M.p. 101-104°C (from ether). In paper [11] m.p. 103.5-104.5°C. ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz): 1.68 (3H, s, H-5′), 1.72 (3H, s, H-6′), 4.15 (3H, s, CH_3O), 4.83 (2H, d, *J* = 7.2, H-2′), 5.59 (1H, m, H-3′), 6.26 (1H, d, *J* = 9.7, H-6), 6.97 (1H, d, *J* = 2.4, H-3), 7.60 (1H, d, *J* = 2.4, H-2), 8.10 (1H, d, *J* = 9.7, H-5). ^{13}C NMR spectra, δ, ppm: 17.97 (C-5′), 25.68 (C-6′), 60.71 ($\underline{C}H_3O$), 70.27 (C-2′), 104.92 (C-3), 107.51 (C-4a), 112.72 (C-6), 114.49 (C-3a), 119.77 (C-3′), 126.70 (C-9), 139.22 (C-5), 139.50 (C-4′), 144.26 (C-4), 144.28 (C-8a), 144.96 (C-2), 150.70 (C-9a), 160.37 (C-7). Mass-spectra, m/z ($I_{rel.}$, %): 302 (0.02), 301 (0.11), 264 (0.2), 246 (0.11), 232 (100), 217 (77), 203 (2), 189 (10), 188 (3), 161 (5), 76 (2), 69 (5). Found: [M^+-68] 232.0364. $C_{17}H_{16}O_5$. Calculated: M 300.0992.

8-(1,1-Dimethylallyloxy)bergapten {**9-[(2-methylbutyl-3-ene-2-yl)oxy]-4-methoxy-7*H*-furo[3,2-*g*]chromen-7-one**} (**3**). M.p. 88-90°C (from ether). UV-spectra (ethanol), λ_{max}/nm (lgε): 222 (4.32), 248 (4.17), 265 (4.17), 306 (4.13). ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz): 1.54 (3H, s, H-5′), 1.56 (3H, s, H-6′), 4.17 (3H, s, CH_3O), 4.99 (1H, dd, *J* = 10.0, 1.2, H-4′a), 5.11 (1H, dd, *J* = 16.8, 1.2, H-4′б), 6.23 (1H, d, *J* = 9.7, H-6), 6.29 (1H, dd, *J* = 16.8 and 10.0, H-3′), 6.96 (1H, d, *J* = 2.4, H-3), 7.55 (1H, d, *J* = 2.4, H-2), 8.08 (1H, d, *J* = 9.7, H-5). ^{13}C NMR spectra, δ, ppm: 26.38 (C-5′,6′), 60.48 ($\underline{C}H_3O$), 84.12 (C-2′), 104.94 (C-3), 107.24 (C-4a), 112.72 (C-6), 113.71 (C-4′), 113.98 (C-3a), 124.61 (C-9), 139.21 (C-5), 142.70 (C-3′), 144.67 (C-4), 144.90 (C-8a), 146.26 (C-2),

152.84 (C-9a), 160.45 (C-7). Mass-spectra, m/z ($I_{rel.}$, %): 302 (0.03), 301 (0.08), 300 (0.02), 243 (4), 233 (13), 232 (100), 231 (4), 218 (9), 217 (72), 189 (10), 188 (3), 175 (2), 161 (5), 133 (2), 104 (1.4), 95 (1.3), 89 (1.1), 69 (3). Found: [M^+] 301.1069. $C_{17}H_{16}O_5$. Calculated: M 300.0992.

Bergapten {4 – methoxy – 7 *H*-furo[3,2-*g*] chromen-7-one} (**4**). M.p. 185-187°C (from ether). ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz): 4.28 (3H, s, CH_3O), 6.28 (1H, d, *J* = 9.8, H-6), 7.00 (1H, d, *J* = 2.4, H-3), 7.15 (1H, s, H-9), 7.61 (1H, d, *J* = 2.4, H-2), 8.18 (1H, d, *J* = 9.8, H-5). ^{13}C NMR spectra, δ, ppm: 60.00 ($\underline{C}H_3O$), 94.3 (C-9), 105.04 (C-3), 106.42 (C-4a), 112.32 (C-3a), 112.52 (C-6), 139.43 (C-5), 144.56 (C-2), 144.92 (C-4), 148.88 (C-8a), 152.89 (C-9a), 161.35 (C-7). Mass-spectra, m/z ($I_{rel.}$, %): 216: [M^+] (100), 201 (28), 188 (10), 145 (40), 89 (18), 51 (30). $C_{12}H_8O_4$.

Deltoin (2*S*)-2-{2-[((Z)-2-methylbut-2-enoyl)oxy]propan-2-yl}-2,3-dihydro-7*H*-furo[3,2-*g*]chromen-7-one (**5**). M.p. 105-107°C, $[\alpha]_D$ = -38.8 (c 0.9, $CHCl_3$). In paper [10] M.p. 106-107°C $[\alpha]_D$ = -42.3 (c 0.62, $CHCl_3$). UV and mass spectra are identical to data in paper [11]. ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz): 1.66 (3H, s, H-2b), 1.67 (3H, s, H-2c), 1.81 (3H, s, H-6′), 1.87 (3H, broad d, *J* = 7.0, H-5′), 3.25 (2H, m, H-3), 5.06 (1H, dd, *J* = 9.0 and 7.8, H-2), 5.71 (1H, m, H-4′), 6.18 (1H, d, *J* = 9.6, H-6), 6.68 (1H, s, H-9), 7.20 (1H, s, H-4), 7.55 (1H, d, *J* = 9.6, H-5). ^{13}C NMR spectra, δ, ppm: 15.81 (C-5′), 20.78 (C-6′), 21.71 ($\underline{C}H_3$), 22.45 ($\underline{C}H_3$), 29.86 (C-3), 89.19 (C-2), 82.12 (C-2a), 97.82 (C-9), 112.32 (C-4a), 112.62 (C-6), 123.81 (C-3a), 124.16 (C-4), 127.65 (C-3′), 138.71 (C-4′), 143.02 (C-5), 155.62 (C-8a), 161.25 (C-7), 163.73 (C-9a), 166.41 (C-2′). Mass-spectra, m/z (I_{rel}, %): 328 [M^+] (2), 229 (26), 228 (41), 213 (100), 214 (17), 185 (19), 176 (15), 159 (21), 115 (15), 103 (24), 102 (17), 83 (65), 55 (49). Found: [M^+] 328.1309. $C_{19}H_{20}O_5$. Calculated: M 328.1311.

Marmesin {(2*S*)-2-(2-hydroxypropan-2-el)-2,3-dihydro-7*H*-furo[3,2-g]chromen-7-one} (**6**). M.p. 186-189°C, $[\alpha]_D$ = +22.5 (c 0.8, $CHCl_3$). In paper [11] m.p. 190-191°C $[\alpha]_D$ = +25.6 (c 0.51, $CHCl_3$). ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz): 1.23 (3H, s, H-2b), 1.37 (3H, s, H-2c), 1.87 (1H, broad s, OH), 3.25 (2H, m, H-3), 4.76 (1H, dd, *J* = 9.0 and 7.8, H-2), 6.20 (1H, d, *J* = 9.6, H-6), 6.71 (1H, s, H-9), 7.20 (1H, s, H-4), 7.60 (1H, d, *J* = 9.6, H-5). ^{13}C NMR spectra, δ, ppm: 24.51 ($\underline{C}H_3$), 26.22 ($\underline{C}H_3$), 30.54 (C-3), 72.09 (C-2a), 91.12 (C-2), 98.61 (C-9), 113.11 (C-4a), 112.44 (C-6), 125.12 (C-3a), 122.96 (C-4), 143.56 (C-5), 155.81 (C-8a), 161.38 (C-7), 163.35 (C-9a). Mass-spectra, m/z (I_{rel}, %): 246 [M^+] (12), 231 (8), 230 (22), 229 (16), 213 (11), 201 (15), 199 (10), 189 (61), 187 (100), 175 (12), 160 (25), 131 (20), 77 (18), 59 (38). $C_{14}H_{14}O_4$.

Falcarinol (panaxinol), [3(*R*)-(9Z)-hepta-1,9-diene-4,6-diyn-3-ol, colourless oil, $[\alpha]_D$ = -33.7 (c 1.2, $CHCl_3$). According to [9] $[\alpha]_D$ = -5 (c 0.05, CH_2Cl_2), [12] – $[\alpha]_D$ = -34.6 (c 8.09, $CHCl_3$). ^{1}H NMR spectra ($CDCl_3$), δ, ppm, J (Hz):0.88 (t, 3H, J = 6.8, H-17), 1.22-1.38 (m, 10H, H-12,13,14,15,16), 1.96-2.18 (2H, m, H-11), 3.01 (2H, d, J = 6.9 H-8), 4.89 (1H, broad s, H-8), 5.21 (1H, broad d, J = 4.9, H-3), 5.32 (1H, m, J_{gem} = 10.3, H-1б), 5.39 (1H, m, H-9), 5.46 (1H, m, J_{gem} = 10.3, H-1a), 5.50 (1H, m, H-10), 5.90 (1H, ddd, J = 16.3, 11.2, 5.4, H-2). ^{13}C NMR spectra, δ, ppm: 136.8 (C-2), 133.0 (C-10), 122.2 (C-9), 117.3 (C-1), 80.6 (C-7), 74.9 (C-4), 71.6 (C-5), 64.5 (C-6), 63.2 (C-3), 31.6 (C-15), 28.8 (C-12), 29.0, 29.5 (C-13,14), 27.8 (C-11), 22.4 (C-16), 16.9 (C-8), 14.5 (C-17).

CONCLUSIONS

1. For the first time five linear furocoumarins– isoimperatorin, fellopterin, 8-(1,1-dimethylallyloxy)bergapten, deltoin and marmesin were isolated from the roots of *Peucedanum baicalense* (Redow.) growing in Mongolia. The molecular structures of these compounds were elucidated by spectroscopic methods.
2. The cytotoxicity of some compounds was studied on models of human CEM-13, MT-4 and U-937 tumor cells. Compound 8-(1,1-dimethylallyloxy)bergapten had the greatest cytotoxicity.

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