CHEMICAL CONSTITUENTS OF THE ROOTS OF FERULA SAMARKANDICA KOROVIN (OQ CHAIR) ANNOTATION

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ANNOTATION

Phytochemical study on the ethanolic extract of the dried roots of *Ferula samarkandica* Korovin led to the isolation of ten known sesquiterpenoid coumarins. Their structures were characterized by detailed spectroscopic analysis including NMR and HR-ESI-MS. Nevskin exhibited high inhibitory activity against MV-4-11 cell with IC50 values of 3.87 ± 0.10 μM, respectively, and nevskin and feshurin showed high inhibitory activity against mino cell with IC50 values of 1.48 ± 0.06 μM and 7.88 ± 0.60 μM, respectively.

keywords: UV, HPLC, Ferula samarkandica, coumarin, badrakemin, feshurin, neveskin, samarkandin, kovrak, BAA.

INTRODUCTION. The advent of modern science, especially chemical analysis, and the appropriate equipment have made it possible to isolate the active principles of medicinal plants. Today's medicine needs an industry that produces pharmaceutical drugs, which are mainly based on the active principles of plants and are often used as raw materials. Nevertheless, the underdeveloped world today does not have access to this modern medicine of synthetic origin, and therefore, traditional medicine based on the direct use of medicinal plants continues to be used in large areas of the world due to its low cost. One of such medicinal plants, Ferula L., is widespread in our country, and glue-tar is mainly obtained from this species. This medicinal plant is a biologically active substance that has a positive effect on the patient's body in treatment. Roots, leaves, bark, flowers, fruits, sap and other parts of plants are used as medicinals. Therefore, on the basis of the decision of the President of the Republic of Uzbekistan dated March 20, 2018, "On the establishment of carpet plantations in the Republic and measures to increase the volume of processing and export of their raw materials, PQ-3617 on the establishment of carpet plantations and an association for the processing of their raw materials was established.

ANALYSIS OF LITERATURE ON THE SUBJECT. Ferula belongs to the Apiaceae (Umbelliferae) family and is a perennial herbaceous plant. There are more than 160 species of cowries on earth, 104 species in Central Asian republics, and 50 species in our country. Kovrak grows in the sandy deserts, hills, mountains and sub-mountainous plains of our republic's Tashkent, Surkhandarya, Kashkadarya, Jizzakh, Navoi, Bukhara regions and the Republic of Karakalpakstan. Kovrak is also widely distributed in Kazakhstan, Tajikistan, Iran,

Afghanistan, Pakistan and India. In our region, this plant blooms in March-April, the fruit ripens in April-May. The plant is a perennial, the above-ground parts live for 1.5-2 months, depending on the growing environment and weather conditions. The height of the carpet is up to 1.5-2 meters, the root is up to 15-20 cm thick, it is deeply embedded like a beet. In the spring season, dishes such as koksomsa are prepared by local residents from the roots that are not yet fully ripe. In folk medicine, the glue-resin of kovrak is used for the treatment of pulmonary tuberculosis, plague, wounds, whooping cough, toothache, nerves and other diseases, as well as energizing, expectorant and used as an anthelmintic drug. In scientific medicine, carpet glue-resin under the name "assafetida" is used as a pain reliever, expectorant, energizer and sedative in the form of powder, emulsion and tincture (nastoika) and is included in the pharmacopoeia of many countries. In the eastern countries of Iran, Pakistan, Afghanistan and India, the juice and roots of the cocoon are used as a spice in the food industry, and in the production of cosmetics in the cosmetics industry [1]. Medicinal raw material of Kovarak is an oil-glutinous-resinous air-dried lump isolated from the genus Ferula. Its taste is bitter, its nature is hot, it is beneficial for the spleen and stomach. The rich phytochemical composition of the bark contains three main fractions, including resin (40-64%), gum (25%) and essential oil (10-17%). The resin fraction includes ferulic acid and its esters, coumarins, sesquiterpene coumarins and other terpenoids. The gum contains glucose, galactose, l-arabinose, rhamnose, glucuronic acid, polysaccharides, and glycoproteins, and the volatile fraction includes sulfur-preserving compounds, monoterpenes, and other volatile terpenoids. As a result of the research conducted in recent years, it is shown that the root of the carpet plant is also rich in bioactive substances, especially sesquiterpenoid coumarin compounds [2].

RESEARCH METHODOLOGY. The root (2kg) of Ferula Samarkandika Korovin plant, which is widespread in our republic, was collected and its chemical composition was studied. An optimal method for extracting the sum of sesquiterpene coumarins with high yield and quality was found at low temperature and low time. When extracting biologically active substances from plant raw materials, the extraction process was carried out in 70% ethanol alcohol. Methods used for qualitative analysis and separation included thin layer chromatography (TLC), Silica gel (200-300 bags, Qingdao Marine Chemical Company, China) and Sephadex (LH-20) column chromatography. Fractions were monitored by TLC and spots were observed by heating silica gel plates sprayed with $AlCl_3$, ammonia vapor, 5% H_2SO_4 in EtOH at 105^0C (Table 1).

Table 1

Chromatography for extraction and fractionation of Ferula Samarkandica plant

Column chromatography		**TLC**	
Hexane: acetone	8:1; 5:1; 3:1; 1:1	$CHCl_3$:MeOH:H_2O	65:35:5
$CHCl_3$:MeOH	20:1; 15:1; 9:1; 4:1	Hexane:EtOAc	4:1; 2:1
Hexane:EtOAc	10:1; 8:1; 4:1; 2:1	$CHCl_3$:MeOH	9:1; 4:1

Using the factors listed in the first table, based on the results obtained, as an optimal method for the extraction of total sesquiterpenoid coumarins from plant raw materials, the grinding level of the plant root is 1-5 mm and EtOH (95%, 4 × 12 L) at room temperature, the duration of 5 times of extraction (24 hours each) separated by The dark extract was successfully

fractionated with hexane (19.0 g), chloroform (16.0 g), ethyl acetate (32.0 g) and n-butanol (20.0 g) by suspending it with water in a 1:1 ratio.

ANALYSIS AND RESULTS. Based on the results of thin layer chromatography (TLC) and HPLC analysis, ethylacetate and n-butanol fractions were analyzed using silica gel column chromatography, hexane/acetone (10:1, 8:1, 6:1, 4:1, 2:1, 1:1) formed small fractions (Fs 1-4) through solvent mixtures. Rechromatography (n-hexane/ethyl acetate) of fraction Fs 1 (8.1 g) afforded pure compounds 1 (14 mg), 2 (17.0 mg). Fraction Fs-2 (CH_2Cl_2:CH_3OH, 1:1) was purified using Sephadex LH-20 column chromatography, and all fractions were analyzed by comparative chromatography (TLC) and sesquiterpene-rich fractions were pooled. Also, based on the analysis HPLC results, Fs 2-1 (110 mg), Fs 2-2 (100 mg), Fs 2-3 (130 mg), Fs 2-4 (100 mg) and Fs 2-5 (140 mg) small fractions were analyzed. Analysis of fraction Fs 2-2 (100 mg) by semi-preparative HPLC using optimized acetonitrile-water to isolate compounds 3 (3.5 mg), 4 (14.6 mg), and 5 (9.5 mg) in pure form was performed, from 20% to 100% acetonitrile in 60 min at a flow rate of 1 mL/min. Samples were prepared in methanol in a concentration range of 1–10 mg/ml. When fraction Fs 2-3 was passed through MeOH-H_2O solvent mixture using Sephadex (LH-20) column chromatography, pure compounds 6 (8.5 mg) and 7 (16 mg) were isolated. n-BuOH fraction of F. samarkandica plant (20 g) was separated into 6 fractions (Fsb-1-Fsb-6) was formed. According to the analysis results, Fsb-3, Fsb-5 fractions were formed using the ODS A-120 CC column chromatography method, using MeOH-H_2O (10-90% and 100-0%) solvents. All subfractions were analyzed by TLC and similar fractions were pooled. Fractions Fsb-3-1 and Fsb-5-1 were separated into pure compounds 8 (15 mg), 9 (50.4 mg) and 10 (80.5 mg) using Sephadex LH-20 (MeOH-H2O) column chromatography. (Scheme 1).

Cytotoxic activity. Pure compounds were tested for potent growth inhibitory activity in MV-4-11 and Mino cells by MTS assay. As a result, compounds 8 and 9 were found to exhibit high activity against MV-4-11 cell with IC50 values of 3.94 ± 0.06 μM and 3.87 ± 0.10 μM, respectively.

Hydroquinone Fs-1, $C_6H_6O_2$, white powder, liquefaction temperature 170-171^{0}C, ^{1}H NMR spectrum (400 MHz, CD_3OD, d, ppm, J/Hz): 7.02 (ddd, J = 8.7; 2.3; 0.4 Hz, H-2, 3, 5, 6). ^{13}C NMR spectrum (100 MHz, CD_3OD, d, ppm): 152.6 (C-1, 4), 117.1 (C-2, 3, 5, 6) [3].

4-isobutylbenzoic acid Fs-2, $C_6H_6O_2$, colorless oil. ^{1}H NMR spectrum (400 MHz, CD_3OD, d, ppm, J/Hz): 7.51 (dd, J = 5.7, 3.3 Hz, H-2,6), 7.71 (dd, J = 5.7, 3.3 Hz, H-3, 5), 4.07 (2H, d, J = 6.7 Hz, H-7), 2.02 (1H, dt, J = 13.4, 6.7 Hz, H-8) , 0.98 (3H s, CH_3), 0.96 (3H, s, CH_3). ^{13}C NMR spectrum (100 MHz, CD_3OD, d, ppm): 167.6 (COOH), 132.5 (C-1,4), 131.0 (C-2,6), 128.9 (C-3,5), 71.3 (C- 7), 27.8 (C-8), 19.3 (C-9,10) [4].

Teucladiol (Fs-3), $C_{15}H_{26}O_2$, colorless oil, ^{1}H NMR spectrum (400 MHz, CD_3OD, d, ppm, J/Hz): 2.26-2.34 (m, H-1), 1.72-1.76 (m, H-2), 175-1.81 (m , H-3), 1.87-1.89 (m, H-5), 4.08 (dd, J = 9.5; 3.7, H-6), 1.23 (d, J = 3;7 H-7), 1.57-1.63 (m, H-8), 2.14-2.60 (m, H-9), 1.71 (d, J = 1.9, H-11), 1.03 (d, J = 6.6, H-12), 0.97 (d, J = 6.6, H-13), 4.74 (d, J = 16.1, H-14), 1.63 (s, H-15). ^{13}C NMR spectrum (100 MHz, CD_3OD, d, ppm): 42.8 (C-1), 27.3 (C-2), 40.6 (C-3), 80.8 (C-4), 59.5 (C-5), 72.7 (C-6), 48.6 (C-7), 24.1 (C-8), 36.6 (C-9), 152.6 (C-10), 29.0 (C-11), 21.7 (C-12), 21.7 (C-13), 108.2 (C-14), 23.2 (C-15) [5].

(+)-4β, 6β-dihydroxy-1α, 5β (H)-guai-9-ene (Fs-4), $C_{15}H_{26}O_2$, colorless crystal. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, d, ppm, J/Hz); 2.27 (dd, J = 16.8;10.5, H-1), 1.74 (m, H-2), 1.70 (m, H-3), 2.08 (m, H-5), 4.13 (dd, J = 9.3; 3.7, H-6), 1.26 (td, J = 9.4;4.0, H-7), 1.23 (m, H-

8), 5.50 (d, J = 8.6, H-9), 1.58-1.65 (m, H-11), 1.02 (d, J = 6.6, H-12), 0.95 (d, J = 6.6, H-13), 1.83 (d, J = 9.1, H-14), 1.66 (s, H- 15). ^{13}C NMR spectrum (100 MHz, CD_3OD, d, ppm): 42.4 (C-1), 26.7 (C-2), 40.0 (C-3), 81.2 (C-4), 57.6 (C-5), 72.5 (C-6), 50.6 (C-7), 24.3 (C-8), 126.3 (C-9), 139.3 (C-10), 28.6 (C-11), 21.5 (C-12), 21.5 (C-13), 24.5 (C-14), 23.2 (C-15) [6].

Umbelliferone Fs-5, $C_{10}H_8O_4$, white powder, liquefaction temperature 232^{0}C.^{1}H NMR spectrum (400 MHz, $CDCl_3$, d, ppm, J/Hz): 6.28 (1H, d, J = 9.4, H-3), 7.67 (1H, d, J = 9.5, H-4), 7.42 (1H, d, J = 8.1, H-5), 7.05 (1H, d, J = 2.3 Hz, H-6), 7.03 (1H s, H-8). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, d, ppm): 161.6 (C-2), 112.4 (C-3), 144.6 (C-4), 130.3 (C-5), 114.4 (C-6), 163.4 (C-7), 103.4 (C-8), 157.2 (C-9), 112.6 (C-10) [7].

Badrakemin Fs-6, $C_{24}H_{30}O_4$, white powder, liquefaction temperature 200^{0}C, ^{1}H NMR spectrum (400 MHz, $CDCl_3$, d, ppm, J/Hz): 6.24 (1H, d, J = 9.3, H-3), 7.62 (1H, d, J = 9.3, H-4), 7.34 (1H, m, H-5), 6.84 (1H, dd, J = 7.3; 2.5 Hz H-6), 6.82 (1H, s, H-8), 1.51 (1H, dt, J = 12.7 :3.4 Hz, H-1'). 1.86 (1H, td, J = 12.7; 2.9 Hz, H-1'), 1.66 (1H, m, H-2'), 1.97 (1H, tt, J = 14.2; 2.9 Hz. H-2'), 3.47 (1H , m, brt J = 2.5 Hz, H-3', 1.66, (1H, m, H-5'), 1.43 (1H, dd, J = 13.2: 3.9 Hz, H-6'), 1.68, (1H, m, H-6'), 2.14 (1H, td, J = 13.2; 4.9 Hz, H-7'), 2.46 (1H, ddd J = 13.2; 3.9; 2.4 Hz, H-7'), 2.33 (1H , m, H-9'), 4.18 (1H, dd, J=9.8; 7.8 Hz, H-11'), 4.24 (1H, dd, J = 9.8; 3.9 Hz, H-11'), 4.54 (1H, br s , H-12), 4.90 (1H, br s, H-12'), 0.87 (3H, s H-13'), 0.88 (3H, s, H-14'), 0.86 (3H, s, H-15') ^{13}C NMR spectrum (100 MHz, $CDCl_3$, d, ppm): 161.4 (C-2), 113.1 (C-3), 143.6 (C-4), 128.8 (C-5), 113.4 (C-6), 162.5 (C-7), 156.1 (C-9), 112.6 (C-10), 107.7 (C-12'), 101.5 (C-8), 75.9 (C-3'), 65.9 (C-11'), 37.9 (C -4', 48.3 (C-5'), 146.9 (C-8'), 54.9 (C-9'), 39.0 (C-10'), 37.7 (C-7'), 32.0 (C-1'), 28.0 (C-13', 25.9 (C-2'), 23.6 (C-6'), 22.5 (C-14'), 15.4 (C-15') [8].

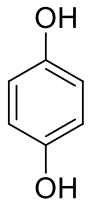
Colladonin Fs-7, $C_{24}H_{30}O_4$, white powder, liquefaction temperature 160^{0}C.^{1}H NMR spectrum (600 MHz, $CDCl_3$, d, ppm, J/Hz): 6.24 (1H, d, J = 9.3, H-3), 7.63 (1H, d, J = 9.3, H-4), 7.35 (1H, m, H-5), 6.83 (1H, dd, J = 7.3:2.5 Hz, H-6), 6.82 (1H, s, H-8), 2.10 (1H, dt J = 12.7 :3.4 Hz, H-1'). 2.45 (1H, td, J = 12.7; 2.9 Hz, H-1'), 1.64 (1H, m, H-2'), 1.73 (1H, tt, J = 14.2; 2.9 Hz. H-2'), 3.30 (1H , m, brt J = 2.5 Hz, H-3', 1.18, (1H, m, H-5'), 1.44 (1H, dd, J = 13.2: 3.9 Hz, H-6'), 1.77, (1H , m, H-6'), 1.81 (1H, td, J = 13.2; 4.9 Hz, H-7'), 1.45 (1H, ddd J = 13.2; 3.9; 2.4 Hz, H-7'), 2.21 (1H , m, H-9'), 4.19 (1H, dd, J = 9.8; 7.8 Hz, H-11'), 4.03 (1H, dd, J = 9.8; 3.9 Hz, H-11'), 4.92 (1H, br s , H-12'), 4.54 (1H, br s, H-12'), 0.85 (3H, s H-13'), 1.03 (3H, s, H-14'), 0.82 (3H, s, H-15'). ^{13}C NMR spectrum (150 MHz, $CDCl_3$, d, ppm): 161.3 (C-2), 113.1 (C-3), 143.5 (C-4), 128.8 (C-5), 113.1 (C-6), 162.3 (C-7), 101.5 (C-8), 156.0 (C-9), 112.6 (C-10), 37.5 (C-1'), 27.8 (C-2'), 78.1 (C-3'), 39.3 (C-4', 54.4 (C-5'), 23.6 (C-6'), 37.3 (C-7'), 146.4 (C-8'), 54.9 (C-9'), 38.9 (C-10'), 65.8 (C-11'), 107.9 (C-12'), 28.4 (C-13'), 15.6 (C-14'), 15.5 (C-15') [9].

Feshurin Fs-8, $C_{24}H_{32}O_5$, colorless crystal, liquefaction temperature 212-214^{0}C, ^{1}H NMR spectrum (600 MHz, $CDCl_3$, d, ppm, J/Hz): 6.24 (1H, d, J = 9.5, H-3), 7.63 (1H, d, J = 9.4, H -4), 7.36 (1H, d, J = 8.2, H-5), 6.83 (1H, d (1H, J = 8.4) H-6), 6.85 (1H, s, H-8), 1.55 (1H , m, H-1'), 1.41 (1H, d, J = 12.0, H-1'), 1.99 (1H, d, J = 2.4 Hz, H-6'), 1.45 (1H, m, H-6'), 1.81 (1H, d, J = 2.5 Hz, H-7'), 1.55 (1H, br s, H-7'), 1.52 (1H, m, H-9'), 4.35 (1H, d, J = 9.6 Hz, H-11'), 4.18 (1H, dd, J = 9.6; 3.0 Hz, H-11'), 1.24 (1H, s, H-12'), 0.96 (3H, s H-13'), 0.87 (3H, s, H-14', 1.08 (3H, s, H-15'). ^{13}C NMR spectrum (150 MHz, $CDCl_3$, d, ppm): 161.9 (C-2), 113.3 (C-3), 143.4 (C-4), 128.8 (C-5), 113.1 (C-6), 161.3 (C-7), 101.7 (C-8), 156.0 (C-9), 112.7 (C-10), 37.7 (C-1'), 27.8 (C-2'), 75.7 (C-3'), 42.6 (C-4', 54.4 (C-5'), 18.0 (C-6'), 48.6 (C-7'), 72.7 (C-8'), 57.5 (C-9'), 38.0 (C-10'), 66.5 (C -11', 32.5 (C-12'), 18.0 (C-13'), 22.2 (C-14'), 16.4 (C-15') [10].

Neveskin Fs-9, $C_{24}H_{32}O_5$, colorless crystal, liquefaction temperature 193-194^{0}C, ^{1}H NMR spectrum (600 MHz, $CDCl_3$, d, ppm, J/Hz): 6.25 (1H, d, J = 9.3, H-3), 7.62 (1H, d, J = 9.5, H

-4), 7.36 (1H, d, J = 8.6, H-5), 6.85 (1H, dd, J = 8.6; 9.5 H-6), 6.91 (1H, s, H-8), 1.75 (1H, d, J = 4.2, H-1'). 1.32 (1H, d, J = 3.7, H-1'eq), 1.68 (1H, d, J = 4.1, H-2'), 2.00 (1H, m. H-2'), 3.26 (1H, dd, J = 11.6 ; 4.6 Hz, H-3', 1.00 (1H, d, J = 1.8 Hz, H-5'), 1.72 (1H, dd, J = 2.6; Hz, H-6'), 1.37, (1H, d, J = 3.3 Hz, H-6'), 1.95(1H, dt, J = 12.6; 3.1 Hz, H-7'), 1.54 (1H, d, J = 4.0, H-7'eq), 1.74 (1H, s, H-9', 4.37 (1H, dd, J = 9.9; 4.8 Hz, H-11'), 4.19 (1H, dd, J = 9.9; 5.5 Hz, H-11'), 1.25 (1H, s, H-12'), 0.80 (3H, s H-13'), 1.03 (3H, s, H-14'), 0.95 (3H, s, H-15'). ^{13}C NMR spectrum (150 MHz, $CDCl_3$, d, ppm): 161.8 (C-2), 113.4 (C-3), 143.4 (C-4), 128.8 (C-5), 113.2 (C-6), 161.8 (C-7), 101.8 (C-8), 156.0 (C-9), 112.8 (C-10), 38.2 (C-1'), 27.2 (C-2'), 78.5 (C-3'), 39.0 (C-4', 54.9 (C-5'), 20.1 (C-6'), 44.2(C-7'), 72.6 (C-8'), 59.4 (C-9'), 29.8 (C-10'), 66.7 (C -11', 24.9 (C-12'), 15.6 (C-13'), 28.3 (C-14'), 16.2 (C-15') [10].

Samarkandin Fs-10, $C_{24}H_{32}O_5$, colorless crystal, liquefaction temperature 176-177^{0}C, ^{1}H NMR spectrum (600 MHz, $CDCl_3$, d, ppm, J/Hz): 6.22 (1H, d, J = 9.5, H- 3), 7.61(1H, d, J = 9.5, H-4), 7.33 (1H, d, J = 8.6, H-5), 6.83 (1H, dd (J = 8.6; 9.5) H-6), 6.87 (1H, s, H-8'), 1.44 (1H, d J = 13.0, H-1'). 1.69 (1H, d J = 3.7, H-1'), 1.59 (1H, d, J = 4.1, H-2'), 1.93 (1H, m. H-2'), 3.42 (1H, s, H-3' , 1.52 (1H, d, J = 12.2 Hz, H-5'), 1.34 (1H, d, J = 13.00; Hz, H-6'), 1.69, (1H, dt, J = 13.0; 2.5 Hz, H 6'), 1.58 (1H, m, H-7'), 1.95 (1H, m, H-7'), 1.86 (1H, t, J = 4.79, H-9'), 4.17 (1H, dd, J = 9.75; 5.55 Hz, H-11'), 4.37 (1H, d, J = 4.37, H-11'), 1.22 (1H, s, H-12'), 0.97 (3H, s H-13'), 0.84 (3H, s, H-14', 0.94 (3H, s, H-15'). ^{13}C NMR spectrum (150 MHz, $CDCl_3$, d, ppm): 161.3 (C-2), 113.3 (C-3), 143.5 (C-4), 128.8 (C-5), 113.1 (C-6), 161.9 (C-7), 101.7 (C-8), 155.9 (C-9), 112.6 (C-10), 32.9 (C-1'), 25.2 (C-2'), 75.7 (C-3'), 38.0 (C-4', 48.5 (C-5'), 20.0 (C-6'), 44.2 (C-7'), 72.7 (C-8'), 59.4 (C-9'), 37.5 (C-10'), 66.7 (C -11', 24.7 (C-12'), 22.2 (C-13'), 28.5 (C-14'), 16.1 (C-15') [10].



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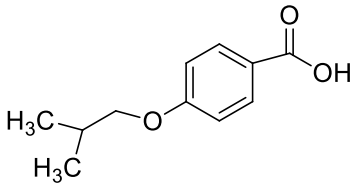

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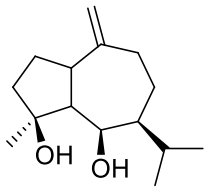

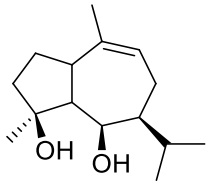

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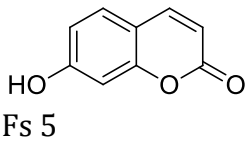

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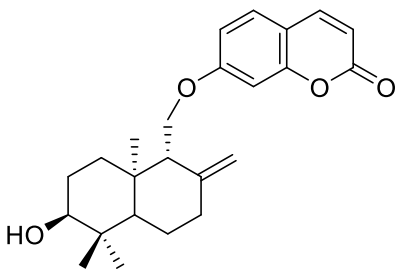

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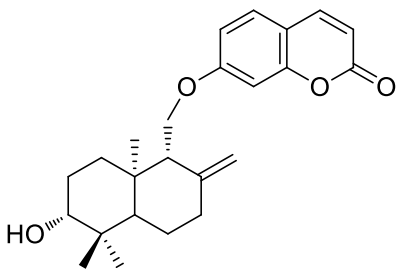

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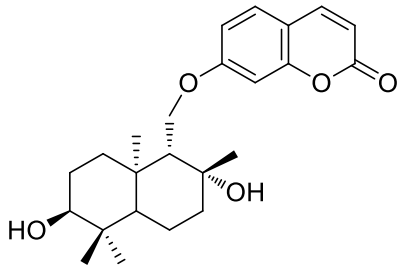

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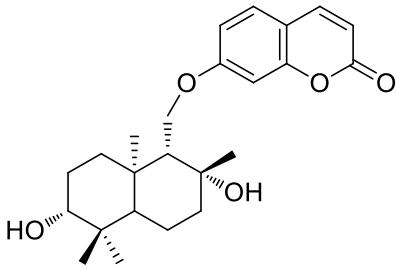
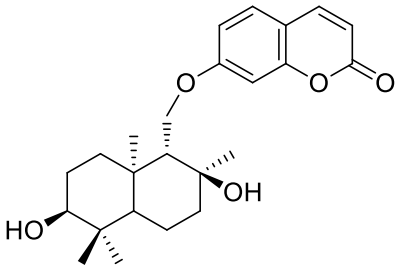


Fs 9



Fs 10

Scheme 1. Chemical formula of substances extracted from Ferula samarkandica plant

CONCLUSIONS AND SUGGESTIONS: The results of YSSX indicate that the root composition of Ferula samarkandica plant is rich in sesquiterpene and sesquiterpene coumarin substances, as well as feshurin and neveskin compounds have high cytotoxic activity, which opens up the prospects of creating drugs and BFQ based on this type of blanket in the future.

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