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Isoflavans from *Sphaerophysa salsula*

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The total flavonoidal glycosides of the plant *Sphaerophysa salsula* (Pall.) DC have anti-hypertensive activity [1]. The aim of this study was to investigate the chemical constituents of the plant *S. salsula* (Pall.) DC. Chromatographic separation of the ethanol extract of the whole herbs of *S. salsula* (Pall.) DC afforded four isoflavans, namely sphaerosin s_1 (**1**), (+)-isomucronulatol (**2**), 3′,7-dihydroxy-2′,4′-dimethoxyisoflavan (**4**) and glyasperin H (**5**). Compounds **2**, **4** and **5** were the first time isolated from *S. salsula* (Pall.) DC. Compond **1** is unstable and degrades into several other compounds under standard conditions without adding any oxidant. The products of natural degradation of **1** were isolated by preparative TLC. One of then, sphaerosin s_3 (**3**), is a novel compound, the others were compounds **4** (0.8 mg) and **5** (1 mg).

Compound **4** (50 mg) was identified by comparing ^{1}H and ^{13}C NMR data with literature data [2].

Compound **1** (5 mg) had the molecular formula $C_{22}H_{26}O_5$, as determined by positive-ion ESIMS {m/z 371.2 $[M+H]^+$} and its ^{13}C NMR spectrum. It showed a positive reaction with $FeCl_3$ reagent. The ^{1}H and ^{13}C NMR spectra of **1** exhibited the signals of rings B and C similar to compound **4**; **1** has five more carbon signals than **4**, viz. δ 7.68 (C-4″), 22.21 (C-1″), 25.65 (C-5″), 122.17 (C-2″), 133.44 (C-3″), suggesting an isopentenyl group. The location of the isopentenyl group at C-8 was based on HMBC and HMQC spectra. The position of the hydroxyl groups at C-3′ was determined with designed deuterium induced isotope shift in ^{13}C NMR experiments. All the results led us to assign the structure of sphaerosin s_1 as 4′,7-dihydroxy-2′,3′-dimethoxy-8-isopentenylisoflavan.

Compound **3** (1 mg) showed a molecular ion peak $[M+H]^+$ at m/z 331.3 in the positive ESIMS and gave ^{13}C NMR data consistent with the molecular formula $C_{18}H_{18}O_6$. It showed positive $FeCl_3$ reaction. The ^{1}H NMR and ^{13}C NMR spectra of **3** were similar to those of **4**. Signal at δ 194.30 suggested a carbonyl group. The location of the carbonyl group was assigned by HMBC and HMQC spectra of **3**. In the ^{1}H NMR spectrum of **3**, two signals were existing at δ 10.33 and δ 11.80. Signal at δ 10.33 is a proton in aldehyde group. And the structure of sphaerosin s_3 was assigned as 4′,7-dihydroxy-2′,3′-dimethoxy-8-aldehydoisoflavan.

Compounds **2** (10 mg) and **5** (50 mg) were identified by comparison of their spectral data (UV, ^{1}H NMR, ^{13}C NMR, MS) with published data [3, 4]. The location of hydroxyl groups of compounds **4** and **5** were determined by acetylation of the compounds. Compounds **1** and **2** possessed selective toxicity to Gram-positive organism (*Staphylococcus aureus*) at 0.5 μg mL^{-1}; and showed no toxicity to a Gram-negative one (*Pseudomonas aeruginosa*).

Experimental

1. Plant material

The whole herbs of *S. salsula* were collected in the Western part of Autonomous Region of Inner Mongolia. The plant was authenticated by Shuanglong Kang, Professor of Autonomous Region of Inner Mongolia Institute for drug control.

2. Extraction and isolation

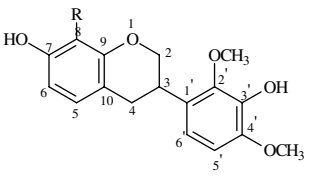
Dried whole herbs of the plant (1.8 kg) were extracted with ethanol. After filtration, the extract was concentrated to 1.8 l, and fractionated with $CHCl_3$, EtOAc and t-BuOH successively. The part of $CHCl_3$ extract was subjected to column chromatographic over silica gel and eluted with a mixture of Petroleum and Acetone.

2.1. Sphaerosin s_1 (**1**)

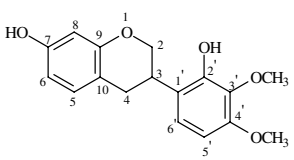
Colorless needles from $CHCl_3$, m.p. 120–122 °C, ESIMS: m/z 371.2 $[M+H]^+$, UV δ_{Max} (CH_3OH) nm 235, 279. $[\alpha]_D$ + 38.6° (20 °C, c = 0.065, CH_3OH); ^{1}H NMR (300 MHz, in $CDCl_3$) δ: 4.32 (1 H, br. d, J = 10.0 Hz, H-2e), 3.95 (1 H, t, J = 10.0 Hz, H-2a), 3.54 (1 H, m, H-3), 2.88 (2 H, m, H-4), 6.78 (1 H, d, J = 8.2 Hz, H-5), 6.39 (1 H, d, J = 8.2 Hz, H-6), 6.62 (1 H, d, J = 8.6 Hz, H-6′), 6.58 (1 H, d, J = 8.6 Hz, H-5′), 3.40 (2 H, d, J = 6.6 Hz, H-2″), 5.27 (1 H, br t, J = 6.6 Hz, H-1″), 1.71 (3 H, s, 5″-C$\underline{H_3}$), 1.79 (3 H, s, 4″-C$\underline{H_3}$), 3.88 (3 H, s, 4′-OC$\underline{H_3}$), 3.83 (3 H, s, 2′-OC$\underline{H_3}$), ^{13}C NMR, see Table.

Table: ^{13}C NMR spectral data for compounds 1, 2, 3, 4 and 5 (75.0 MHz, in $CDCl_3$)

Carbon No.	1	2	3	4	5 (in DMSO-d_6)
2	70.31	70.42	70.85	70.46	70.14
3	31.42	32.07	31.48	31.74	31.07
4	31.85	30.09	30.83	31.37	31.42
5	127.27	130.33	138.87	130.38	129.35
6	107.89	107.98	108.99	107.88	108.29
7	152.21	155.07	161.91	155.14	151.37
8	114.53	130.63	110.39	103.21	109.16
9	153.34	154.98	156.97	154.86	149.32
10	114.12	114.42	112.63	114.70	114.59
1′	127.54	120.29	126.60	127.45	126.65
2′	146.49	147.41	146.93	146.67	147.86
3′	138.53	135.35	138.76	138.68	139.37
4′	145.17	151.06	145.32	145.30	146.15
5′	106.53	103.20	106.42	106.48	107.67
6′	116.81	121.82	116.93	116.96	116.28
1″	22.18		194.30		27.45, 27.26
2″	122.24				75.32
3″	133.43				116.42
4″	17.62				129.35
5″	25.59				
2′-OCH_3	55.95	55.74	56.27	56.22	56.02
4′-OCH_3	60.84	60.39	61.02	61.01	60.33



1 R= $CH_2CH{=}C(CH_3)_2$



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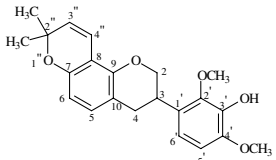

5

3 R=(C=O)H

4 R=H

2.2. (+)*Isomucronulatol* (**2**)

Colorless needles from $CHCl_3$, m.p. 137–140 °C, ESIMS: m/z 303.2 $[M + H]^+$, UV λ_{Max} (CH_3OH) nm 213, 279. $[\alpha]_D$ +28.6° (20 °C, c = 0.070, CH_3OH); ^{13}C NMR, see Table.

2.3. Sphaerosin s_3 (**3**)

Colorless needles from $CHCl_3$, m.p. 65–68 °C, ESIMS: m/z 331.3 $[M + H]^+$, UV λ_{Max} (CH_3OH) nm 235, 279. $[\alpha]_D$ +30.6° (20 °C, c = 0.035, CH_3OH); ^1H NMR (300 MHz, in $CDCl_3$) δ: 4.41 (1 H, br d, J = 10.0 Hz, H-2e), 4.06 (1 H, t, J = 10.0 Hz, H-2a), 3.56 (1 H, m, H-3), 2.88 (2 H, m, H-4), 7.19 (1 H, d, J = 8.6 Hz, H-5), 6.46 (1 H, d, J = 8.6 Hz, H-6), 6.60 (1 H, d, J = 8.6 Hz, H-6′), 6.65 (1 H, d, J = 8.6 Hz, H-5′), 3.88 (3 H, s, 2′-OC<u>H</u>$_3$), 3.83 (3 H, s, 4′-OC<u>H</u>$_3$), 10.33 (1 H, s, C<u>H</u>O), 11.80 (1 H, 7-OH). ^{13}C NMR, see Table.

2.4. 3′,7-Dihydroxy-2′,4′-dimethoxyisoflavan (**4**)

Colorless needles from $CHCl_3$, m.p. 132–134 °C, ESIMS: m/z 302.3 $[M + H]^+$, UV λ_{Max} (CH_3OH) nm 212, 281 $[\alpha]_D$ +30.6° (20 °C, c = 0.035, CH_3OH); ^{13}C NMR, see Table.

2.5. Glyasperin H (**5**)

Colorless needles from $CHCl_3$, m.p. 78–80 °C, EIMS: m/z 368.2 $[M]^+$, UV λ_{Max} (CH_3OH) nm 205, 228, 278 8.6° (20 °C, c = 0.050, $CHCl_3$); ^{13}C NMR, see Table.

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