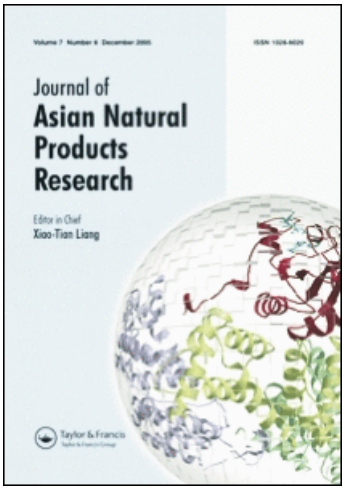

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Flavonoids from the seeds of *sphaerophysa salsula*

Zhongjun[a]; Isao Fujii[b]; Yutaka Ebizuka[b]; Xian Li[a]; Shigeki Shimomaki[b]; Yuichi Sakano[b]
[a] Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang, China [b] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan

To cite this Article Zhongjun, Fujii, Isao , Ebizuka, Yutaka , Li, Xian , Shimomaki, Shigeki and Sakano, Yuichi(2004) 'Flavonoids from the seeds of *sphaerophysa salsula*', Journal of Asian Natural Products Research, 6: 1, 69 — 73
To link to this Article: DOI: 10.1080/1028602031000135585
URL: http://dx.doi.org/10.1080/1028602031000135585

Journal of Asian Natural Products Research, March 2004, Vol. 6 (1), pp. 69–73

FLAVONOIDS FROM THE SEEDS OF *SPHAEROPHYSA SALSULA*

ZHONGJUN MA[a], ISAO FUJII[b], YUTAKA EBIZUKA[b], XIAN LI[a,*], SHIGEKI SHIMOMAKI[b] and YUICHI SAKANO[b]

[a]*Department of Natural Medicines, Shenyang Pharmaceutical University, Shenyang 110016, China;* [b]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan*

(Received 24 January 2003; Revised 20 March 2003; In final form 28 March 2003)

Two new flavonoids, named sphaerophyside SA and sphaerophyside SB, together with 15 known flavonoids were isolated from the ethanolic extract of the seeds of *Sphaerophysa salsula* (Pall.) DC. The structures of the new compounds were elucidated mainly on the basis of the 1D and 2D NMR data.

Keywords: Leguminosae; *Sphaerophysa salsula*; Seeds; Flavonoids; Sphaerophyside SA; Sphaerophyside SB

INTRODUCTION

Sphaerophysa salsula (Leguminosae) is widely distributed in the Middle-Asian and northwest of China. It has been used as a folk medicine to treat hypertension. In our previous study of the chemical constituents of the plant, some isoflavans [1] and stilbenes [2] were obtained from the whole herbs. However, there are no reports on the chemical constituents of the seeds. In the present investigation, we obtained 17 compounds (**1**–**17**) from the seeds, including two new flavonoids, namely isorhamnetin 3-*O*-(5-*O*-*trans*-feruloyl-β-D-apiofuranosyl)-(1→2)-β-D-glucopyranoside (**3**) and (3*R*)-isomucronulatol 2′-*O*-β-D-glucopyranoside (**15**).

RESULTS AND DISCUSSION

Column chromatography on silica gel, Sephadex LH-20, MPLC and preparative HPLC of the n-BuOH phase from the ethanolic extract of the seeds from *Sphaerophysa salsula* resulted in the isolation of 17 flavonoids. The 15 known compounds isorhamnetin (**1**), isorhamnetin 3-*O*-β-D-glucopyranoside (**2**), kaempferol (**4**), kaempferol 3-*O*-β-D-glucopyranoside (**5**), kaempferol 3-*O*-(6″-acetyl)-β-D-glucopyranoside (**6**), kaempferol 3-*O*-α-L-arabinopyranoside (**7**), kaempferol 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**8**), quercetin (**9**), quercetin

*Corresponding author. Tel.: +86-24-23902286. E-mail: lixian@mail.sy.ln.cn; chinamzj@yahoo.com

ISSN 1028-6020 print/ISSN 1477-2213 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/1028602031000135585

3-*O*-β-D-glucopyranoside (**10**), quercetin 3-*O*-α-L-arabinopyranoside (**11**), myricetin (**12**), myricetin 3-*O*-β-D-glucopyranoside (**13**), myricetin 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**14**), (3*R*)-isomucronulatol 7-*O*-β-glucopyranoside (**16**) [3], and 7,3′-dihydroxy-2′,4′-dimethoxyl-isoflavan 7-*O*-β-D-glucopyranoside) (**17**) [4] were identified by comparing their NMR data with those in the literature [5,6].

Compound **3** was obtained as a white powder from methanol. It showed a positive reaction with $FeCl_3$. The high-resolution FAB mass spectrometry (HR-FAB-MS) of **3** exhibited a pseudo-molecular ion peak $[M + Na]^+$ at *m/z* 809.1998 (calcd. 809.2004), which is consistent with a molecular formula of $C_{37}H_{38}O_{19}Na$. The sugars were identified as D-glucose and D-apiose by acid hydrolysis and co-TLC with authentic samples. The sugars were both determined as β-type by the coupling constants of the anomeric protons (H-1″, $J = 8.0$ Hz and H-1‴, br.s) [7]. The ^1H and ^{13}C NMR data of the aglycon moiety of **3** were similar to those of **2**. Combined with the 2D NMR data, the aglycon of **3** was identified as isorhamnetin. DQFCOSY, HMQC and HMBC experiments were performed to determine the linkage sequence of the sugars. The long-range correlation between isorhamnetin C-3 (δ 132.6) and glucose H-1 (δ 5.70) showed the Glc unit to be attached at C-3 of isorhamnetin. Furthermore, long-range correlation between Glc C-2 (δ 75.7) and Api H-1 (δ 5.36), as well as reverse correlation, proved the sequence of the disaccharide moiety at C-3 to be [Api-(1→2)-Glc]. In the ^{13}C NMR of **3**, 9 more signals (δ 110.8, 114.0 115.4, 122.9, 125.4, 144.5, 147.8, 149.2 and 166.2) were observed apart from the carbons in the structural moiety mentioned above. They were assigned to a feruloyl group, on the basis of long-range $^{13}C-^1H$ correlations, that also has a *trans* double (δ 7.16 and δ 6.14) bond, as shown by the large coupling constant ($J = 16.0$ Hz). In the HMBC spectrum of **3**, the Api H-5 (δ 4.26 and 4.13) showed long-range correlations with the carbonyl group (δ 166.2) of the feruloyl moiety. This suggested that the carbonyl group was connected to the C-5 of sugar Api. From the above evidence, **3** was formulated as isorhamnetin 3-*O*-(5-*O*-*trans*-feruloyl-β-D-apiofuranosyl)-(1→2)-β-D-glucopyranoside, named sphaerophyside SA.

Compound **15** was isolated as a white powder from methanol and showed a positive reaction with $FeCl_3$. The molecular formula $C_{23}H_{28}O_{10}$ was determined on the basis of HR-EIMS and ^{13}C NMR data. After hydrolysis with acid, an isoflavan (**15a**) and a sugar were obtained, the sugar was determined as D-glucose on the basis of the same R_f value as an authentic sample, and **15a** was identified as (3*R*)-isomucronulatol by the same melting point, R_f value and ^1H NMR data as an authenticated sample [3]. In the ^1H NMR spectrum of **15**, the characteristic signals of an isoflavan appeared at δ 3.78 (1H, t, $J = 10.0$ Hz, H-2), 4.27 (1H, br. d, $J = 10.0$ Hz, H-2), 3.58 (1H, m), 2.64 (1H, dd, $J = 16.0$, 3.5 Hz, H-4) and δ 2.79 (1H, dd, $J = 16.0$, 11.5 Hz, H-4). There were also present an ABX [δ 6.83 (1H, d, $J = 8.0$ Hz, H-5), 6.26 (1H, dd, $J = 8.0$, 2.0 Hz, H-6) and δ 6.17 (1H, d, $J = 2.0$ Hz, H-8)] and an AB [δ 6.89 (1H, d, $J = 9.0$ Hz, H-6′) and δ 6.78 (1H, d, $J = 9.0$ Hz, H-5′)] spin system. The spectrum also showed two methoxy signals at δ 3.72 (3H, s, 3′-OCH_3) and 3.75 (3H, s, 4′-OCH_3). An anomeric proton was observed at δ 4.84 (1H, d, $J = 7.5$ Hz, H-2″), indicating that the configuration of anomeric carbon is β. In the ^{13}C NMR of **15**, 23 carbon signals were present, 12 of which were olefinic carbons and one for an anomeric carbon (C-1″, δ 103.4). Six signals are due to the sugar unit (δ 103.4, 74.0, 77.4, 69.6, 76.4 and 61.3). The signals at δ 70.2, 30.9 and 30.3 are ascribable to the ring-C carbons. It was observed that the anomeric proton was correlated with C-2′ (δ 147.7). Furthermore, the positions of the methoxy groups are determined on the basis of 2D NMR, especially the HMQC and HMBC (see Fig. 1) data. At the same time, from biogenesis considerations, the configuration of C-3 in **15** should be *R* (as in **16**). Thus, the structure was defined as (3*R*)-isomucronulatol 2′-*O*-β-D-glucopyranoside (**15**), named sphaerophyside SB.

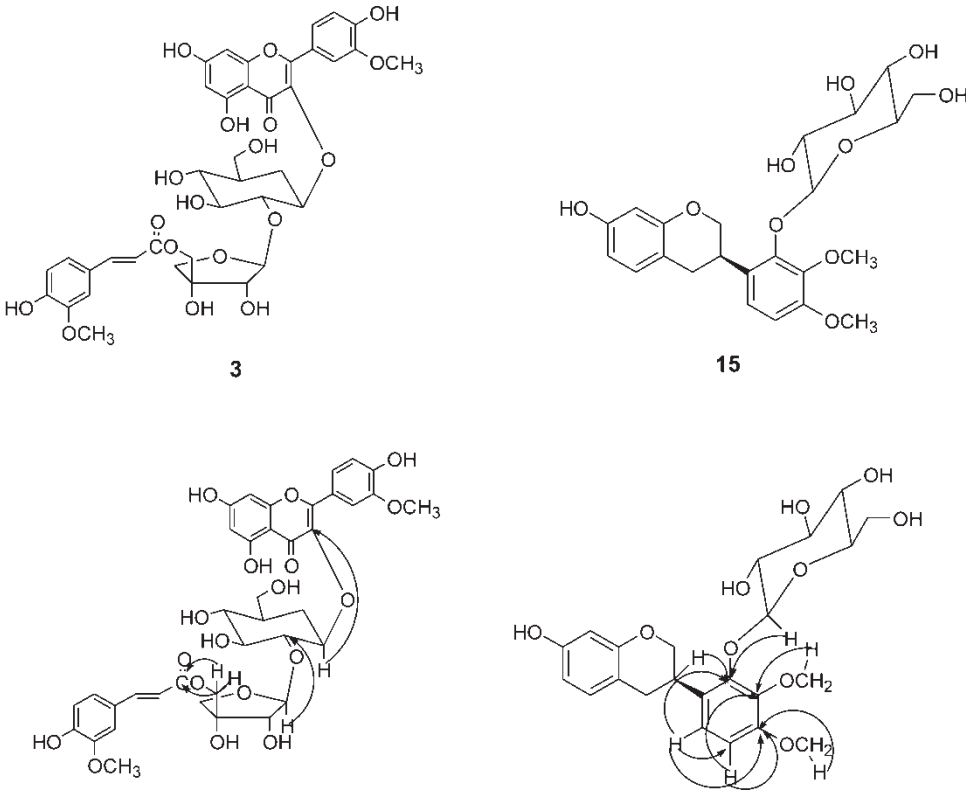

FIGURE 1 Important HMBC correlations of compounds **3** and **15**.

EXPERIMENTAL

General Experimental Procedures

NMR spectra were recorded at 500 MHz for ^{1}H and 125.0 MHz for ^{13}C on a JNM-A-500 spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Optical rotations were measured in a JASCO P-1010 polarimeter in CH_3OH. UV spectra were performed on an Hitachi U-2000 spectrophotometer. EI, FAB-MS were recorded on a Jeol JMS-SX 102A spectrometer. Silica gel (Wakogel C-200, Wako pure chemistry Co. Ltd., Japan) was used for column chromatography, and Sephadex LH-20 (Pharmacia) was used for molecular exclusion chromatography. TLC employed precoated Si gel 60F 254 plates (Merck) and RPTLC employed precoated RP-18F 254s plates (Merck). Preparative HPLC was performed on a TOSOH liquid chromatograph coupled to a TOSOH UV-8011 UV detector. The MPLC separations were performed on a system with a TOYOSODA UV-8000 detector, a TOYOSODA CCPM pump, and a Lichroprep precolumn (310 × 25 mm) with the stationary phase of RP-18 (40–63 μm, Merck).

Plant Material

The seeds of *Sphaerophysa salsula* (10.0 kg) were collected in August 1999, in Huhehaote City, Inner Mongolia, China. Authentication of the botanical material has been reported previously. A voucher specimen (No. 990801) has been deposited in the Herbarium of Department of Natural Medicines, Shenyang Pharmaceutical University.

Extraction and Isolation

The seeds were air-dried and de-fatted with light petroleum, and extracted with 95% EtOH to give a black crude material (436.0 g), which was partitioned with light petroleum and n-BuOH successively. The n-BuOH extract (212.0 g) was subjected to silica gel chromatography, using a gradient mixture of $CHCl_3$–CH_3OH as eluent, to give eight fractions. Fraction 1 was subjected to a silica gel column using a gradient mixture of $CHCl_3$–CH_3OH as eluent to yield compounds **1** (1 mg) and **4** (150 mg). Fraction 2 was separated by CC on silica gel with a gradient mixture of $CHCl_3$–CH_3OH to give four sub-fractions. Sub-fraction 1 was subjected to MPLC to give compound **9** (90 mg). Sub-fraction 2 was chromatographed on a Lobar column, and eluted with 20%, 40%, 60% and 100% CH_3OH–H_2O successively; the 60% eluate was subjected to preparative HPLC and eluted with 60% CH_3OH–H_2O to yield compounds **2** (23.7 mg, 32 min), **5** (17 mg, 16 min), **6** (3 mg, 36 min) and **7** (2.6 mg, 38 min). Sub-fraction 3 was chromatographed on a Sephadex LH-20 column, eluting with H_2O, 20%, 40% and 100% CH_3OH–H_2O successively; the 40% eluate was chromatographed on preparative HPLC, eluting with 50% CH_3OH, to produce compounds **15** (4.4 mg, 55 min), **16** (17 mg, 40 min) and **17** (6 mg, 50 min). Sub-fraction 4 was chromatographed on MPLC and dealt with in the same way as sub-fraction 2, and the 40% CH_3OH–H_2O part was subjected to preparative HPLC, eluting with 50% CH_3OH–H_2O, to obtain compounds **11** (12 mg, 35 min) and **12** (5 mg, 42 min). Fraction 3 was chromatographed on a silica gel column to produce five sub-fractions, of which sub-fraction 4 was chromatographed by preparative HPLC, using 45% CH_3OH–H_2O as eluent, to yield compounds **10** (27 mg, 32 min) and **3** (6.6 mg, 45 min). Fraction 4 was also chromatographed over a silica gel column, with a mixture of CH_3OH–$CHCl_3$ as eluent. From this, 3 sub-fractions were obtained, and sub-fraction 2 was chromatographed on a Sephadex LH-20 column, eluting with 20%, 40%, 60% and 100% CH_3OH–H_2O successively; eluting with 100% CH_3OH gave compound **13** (3 mg). The 40% CH_3OH–H_2O eluate was separated by preparative HPLC, with CH_3OH–H_2O as eluent, to afford compounds **8** (2 mg, 40 min) and **14** (98 mg, 24 min).

Acid Hydrolysis of Compounds 15 and 16

A solution of **16** (5 mg) in 1 M HCl was refluxed on a water bath for 2 h. After extraction with $CHCl_3$, the aqueous layer was neutralized and then subjected to Sephadex LH-20, eluted with H_2O, to afford the sugar, which was analyzed on TLC by comparison with standard sugars. The $CHCl_3$ extract (2.5 mg) was subjected to co-TLC (light petroleum–acetone, 2:1, $R_f = 0.48$) with (3*R*)-isomucronulatol and has the same R_f value with the authenticated sample; the melting point and ^{1}H NMR data of **15a** were also identical to those reported in the literature [3].

A solution of **15** (2 mg) in 1 M HCl was refluxed on a water bath for 2 h. Following extraction with $CHCl_3$, the aqueous layer was analyzed by TLC through comparison with standard sugars. The $CHCl_3$ extract (1.1 mg) was subjected to co-TLC (light petroleum–acetone, 2:1, $R_f = 0.48$) with (3*R*)-isomucronulatol and has the same R_f value; the melting point and ^{1}H NMR were also similar to those of **15a**.

Isorhamnetin 3-*O*-(5-*O*-*trans*-Feruloyl-β-D-apiofuranosyl)-(1→2)-β-D-glucopyranoside (3)

Yellow powder, $[\alpha]_D$ −90.6 ($c = 0.07$, CH_3OH, 25°C), HR-FABMS (positive) *m/z*: 809.1998; calcd for $C_{37}H_{38}O_{19}Na$ $[(M + Na)^+]$ 809.2004. UV λ_{max} (nm) (CH_3OH): 330, 295, 271, 248, 220. ^{1}H NMR (in DMSO-d_6, 500.0 MHz) δ (ppm): 6.12 (1H, d, $J = 2.0$ Hz, H-6),

6.19 (1H, d, $J = 2.0$ Hz, H-8), 6.86 (1H, d, $J = 8.5$ Hz, H-5′), 7.36 (1H, dd, $J = 8.5$, 1.5 Hz, H-6′), 7.90 (1H, d, $J = 1.5$ Hz, H-2′), 5.70 (1H, d, $J = 8.0$ Hz, H-1″), 3.53 (1H, m, H-2″), 3.46 (1H, m, H-3″), 3.09 (1H, m, H-4″), 3.10 (1H, m, H-5″), 3.54 (1H, t, $J = 9.5$ Hz, H-6″), 3.32 (1H, m, H-6″), 5.36 (1H, br. s, H-1‴), 3.68 (1H, br. s, H-2‴), 3.92 (1H, d, $J = 9.5$ Hz, H-4‴), 3.50 (1H, d, $J = 9.5$ Hz, H-4‴), 4.13 (1H, d, $J = 11.0$ Hz, H-5‴), 4.26 (1H, d, $J = 11.0$ Hz, H-5‴), 7.10 (1H, d, $J = 1.5$ Hz, H-2⁗), 6.76 (1H, d, $J = 8.0$ Hz, H-5⁗), 6.89 (1H, dd, $J = 8.0$, 1.5 Hz, H-6⁗), 7.16 (1H, d, $J = 16.0$ Hz, H-7⁗), 6.14 (1H, d, $J = 16.0$ Hz, H-8⁗), 3.82 (3H, s, 3′-OCH_3), 3.79 (3H, s, 3⁗-OCH_3). ^{13}C NMR (in DMSO-d_6, 125.0 MHz) δ (ppm): 155.5 (C-2), 132.6 (C-3), 177.0 (C-4), 161.2 (C-5), 98.7 (C-6), 164.5 (C-7), 93.5 (C-8), 156.1 (C-9), 103.9 (C-10), 121.1 (C-1′), 113.3 (C-2′), 146.8 (C-3′), 149.2 (C-4′), 115.0 (C-5′), 121.8 (C-6′), 98.5 (C-1″), 75.7 (C-2″), 77.0 (C-3″), 70.2 (C-4‴), 77.4 (C-5‴), 60.5 (C-6″), 107.6 (C-1‴), 76.2 (C-2‴), 77.6 (C-3‴), 73.8 (C-4″), 68.0 (C-5‴), 125.4 (C-1⁗), 110.8 (C-2⁗), 147.8 (C-3⁗), 149.2 (C-4⁗), 115.4 (C-5⁗), 122.9 (C-6⁗), 144.5 (C-7⁗), 114.0 (C-8⁗), 166.2 (C-9⁗), 55.5 (× 2, 3″, 3⁗-O*C*H$_3$).

(3R)-Isomucronulatol 2′-*O*-β-D-Glucopyranoside (15)

White powder, $[\alpha]_D$ −24.3 ($c = 0.08$, CH_3OH, 25°C), HR-EI-MS *m*/*z* 464.1678; calcd for $C_{23}H_{28}O_{10}$ 464.1682. UV λ_{max} (nm) (CH_3OH): 212, 281. ^{1}H NMR (in DMSO-d_6, 500.0 MHz) δ (ppm): 3.78 (1H, t, $J = 10.0$ Hz, H-2), 4.27 (1H, br. d, $J = 10.0$ Hz, H-2), 3.58 (1H, m), 2.64 (1H, dd, $J = 16.0$, 3.5 Hz, H-4), 2.79 (1H, dd, $J = 16.0$, 11.5 Hz, H-4), 6.83 (1H, d, $J = 8.0$ Hz, H-5), 6.26 (1H, dd, $J = 8.0$, 2.0 Hz, H-6), 6.17 (1H, d, $J = 2.0$ Hz, H-8), 6.89 (1H, d, $J = 9.0$ Hz, H-6′), 6.78 (1H, d, $J = 9.0$ Hz, H-5′), 3.72 (3H, s, 3′-OCH_3), 3.75 (3H, s, 4′-OCH_3), 4.84 (1H, d, $J = 7.5$ Hz, H-1″), 3.2–3.8 (H-2″-6″). ^{13}C NMR (in DMSO-d_6, 125.0 MHz) δ (ppm): 70.2 (C-2), 30.9 (C-3), 30.3 (C-4), 130.1 (C-5), 107.9 (C-6), 156.5 (C-7), 102.6 (C-8), 154.7 (C-9), 112.9 (C-10), 128.7 (C-1′), 147.7 (C-2′), 141.1 (C-3′), 152.1 (C-4′), 108.6 (C-5′), 121.6 (C-6′), 103.4 (C-1″), 74.0 (C-2″), 77.4 (C-3″), 69.6 (C-4″), 76.4 (C-5″), 61.3 (C-6″), 60.5 (3′-O*C*H$_3$), 55.8 (4′-O*C*H$_3$).

Acknowledgements

The authors are grateful for the support of the Taiwan Shenrong Research Foundation.

References

[1] Ma, Z.J., Hou, B.L., Wang, J.H. and Li, X. (2002), *Pharmazie* **57**, 75–76.
[2] Ma, Z.J., Li, X., Li, N. and Wang, J.H. (2002), *Fitoterapia* **73**, 313–315.
[3] He, Z.Q. and Findly, J.A. (1991), *J. Nat. Prod.* **54**, 810–815.
[4] He, Z.Q. and Wang, B.Q. (1990), *Acta Pharm. Sin.* **25**, 694–698.
[5] Cui, B.L., Nakamura, M. and Kinjo, J. (1993), *Chem. Pharm. Bull.* **41**, 178–182.
[6] Markham, K.R., Ternai, B. and Stanley, R. (1978), *Tetrahedron* **34**, 1389–1392.
[7] Semmar, N., Fenet, B. and Lacaille-dubois, M. (2001), *J. Nat. Prod.* **64**, 656–658.