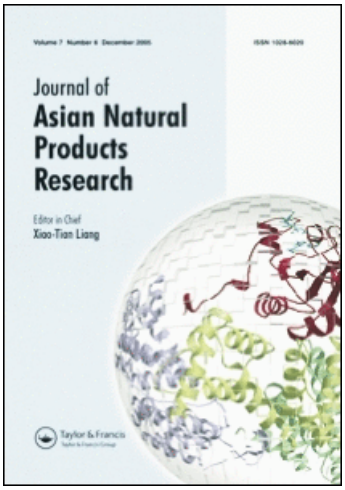

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A NEW LIGNAN FROM THE SEEDS OF *SPHAEROPHYSA SALSULA*

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A new compound, sphaerophysin A (**1**), together with 16 known compounds (**2**–**17**) were obtained from the ethanolic extract of the seeds of *Sphaerophysa salsula*. The structure of **1** was elucidated on the basis of spectral and chemical evidence. Compounds **2**–**17** were isolated from the plant for the first time.

Keywords: Leguminosae; *Sphaerophysa salsula*; Lignan; Sphaerophysin A

INTRODUCTION

Sphaerophysa salsula (Pall.) DC (Leguminosae) is widely distributed in Middle-Asian and northwest China. It has been used in China as a folk medicine to treat hypertension. We previously reported the isoflavans [1], stilbenes [2] and cycloartane [3] from the whole herbs of *S. salsula*, and several flavonols have also been obtained from the seeds of the plant. In our extended research, a new lignan, named sphaerophysin A (**1**), together with 16 known compounds were obtained from the ethanolic extract of the seeds of *S. salsula*. The known compounds were identified by comparison with the literature data and confirmed by 2D NMR data (DQFCOSY, HMQC and HMBC). This paper describes the isolation and structural elucidation of the novel compound.

RESULTS AND DISCUSSION

Column chromatography on silica gel, Sephaedex LH-20, MPLC and preparative HPLC of the n-BuOH phase from the ethanolic extract of the seeds from *S. salsula* resulted in the isolation of 17 compounds. The known compounds uracil (**2**) [4], 1,2-dihydro-[1,2,4] triazol-3-one (**3**) [5], uridine (**4**) [4], adenosine (**5**) [4], 5-isobutyl-imidazolidine-2,4-dione (**6**), 5-(2-hydroxyethyl)-imidazolidine-2,4-dione (**7**), methoxyhydroquinone-1-β-D-glucopyranoside

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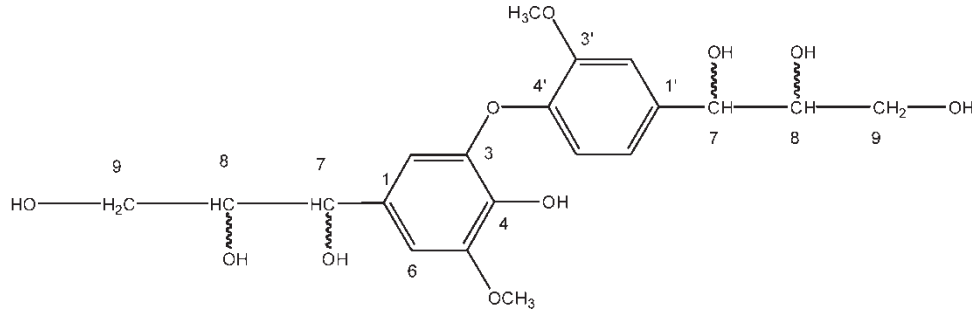

FIGURE 1 Structure of **1**.

(**8**) [6], methoxyhydroquinone-4-β-D-glucopyranoside (**9**) [6], 3, 4-dihydroxybenzoic acid (**10**) [7], benzyl alcohol *O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**11**) [8], calycosin 7-*O*-β-D-glucopyranoside (**12**) [9], 4′,7-dimethoxyisoflavone (**13**) [10], 3′-hydroxy-4′,7-dimethoxyisoflavone (**14**) [9], piceid (**15**) [9], *cis*-3,5,4′-trihydroxystilbene 3-*O*-β-D-glucopyranoside (**16**) and roseoside (**17**) [9] were identified by comparing their NMR data with those in the literature.

Compound **1** (20 mg) was obtained as colorless needles from MeOH (Fig. 1). It showed a positive reaction with $FeCl_3$ reagent. The HR-EIMS exhibited a pseudo-molecular ion peak at *m/z* 408.1445 $[M - H_2O]^+$ which, together with the NMR data, gave a molecular formula of $C_{20}H_{26}O_{10}$. In the ^1H NMR spectrum of **1**, five olefinic protons, including an ABX system [δ 6.97 (1H, br.s, H-2′), 6.77 (1H, d, $J = 7.5$ Hz, H-6′) and δ 6.79 (1H, br.d, $J = 7.5$ Hz, H-5′)] and an AX system [δ 6.56 (1H, br.s, H-6) and 6.48 (1H, br.s, H-2)], were found. The upfield of the spectrum exhibited 8 proton signals [δ 4.85 (1H, br.s, H-7′), 4.40 (1H, br.s, H-7), 4.03 (1H, br.s, H-8′), 3.46 (1H, m, H-8), 3.55, 3.32 (each 1H, m, H-9) and δ 3.34, 3.16 (each 1H, m, H-9′)], the difference between $\delta_{H\text{-}8}$ and $\delta'_{H\text{-}8}$ may be caused by different configurations of 8-OH and 8′-OH. Two methoxy groups at δ 3.76 (3H, s, 5-OMe) and δ 3.77 (3H, s, 3′-OMe) were also found. In the ^{13}C NMR of **1**, 20 signals (12 olefinic, 6 oxygen-bearing and 2 methoxy carbon signals) were recorded. In the DQF COSY spectrum of **1**, the correlated peaks were found between proton H-7 and H-8, H-8 and H-9, H-7′ and H-8′, H-8′ and H-9′, H-3 and H-5, and H-6′ and H-2′, 5′. Correlated peaks were exhibited in the HMQC spectrum of **1** between H-2 and C-2 (δ 107.7), H-6 and C-6 (δ 103.2), H-2′ and C-2′ (δ 111.7), H-5′ and C-5′ (δ 120.5), H-6′ and C-6′ (δ 115.4), H-7,8,9 and C-7,8,9 (δ 72.8, 75.7, 60.2), H-7′,8′,9′ and C-7′,8′,9′ (δ 75.8, 77.9, 62.7). In the HMBC of **1**, the proton signals of H-2,6 and H-2′,6′ showed long-range correlations with carbon signal of C-7 and C-7′ respectively, which as well as the reverse correlations proved that C-1,1′ were substituted. From the 2D NMR data, two moieties (**A**, **B**) of the structure were determined (Fig. 2). The protons H-7,8,9 have no long-range correlations with the carbons of moiety **B** and so the connection between **A** and **B** is at C-4′ and C-4 or C-3 *via* an ether linkage. However, in the NOESY spectrum of **1**, NOEs between protons δ 3.77 (3′-OCH_3) and δ 6.97 (1H, br.s, H-2′), 6.48 (1H, br.s, H-2) were presented. The spectrum also exhibited a correlated peak between protons δ 3.76 (5-OCH_3) and δ 6.56 (1H, br.s, H-6). From the above evidence, the structure was established as **1**. The HMBC spectra of **1** also showed that protons 3′,5-OCH_3 (δ 3.77 and 3.76) correlated with carbon C-3′,5 (δ 147.0 and 148.0), and the positions of the methoxy and hydroxy substitutions were determined by 2D NMR. The main HMBC and NOESY correlations are shown in Fig. 3.

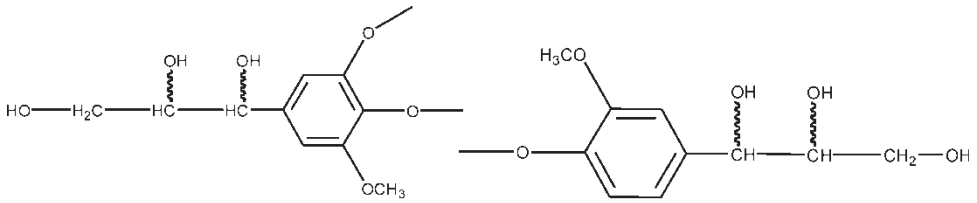

FIGURE 2 Moieties deduced from 2D NMR spectra of **1**.

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 with TMS as internal standard. Optical rotations were measured with a JASCO P-1010 polarimeter in CH_3OH. UV spectra were determined on a HITACHI U-2000 spectrophotometer. EI MS data 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). The 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 consisting of a TOYOSODA UV-8000 detector, a TOYOSODA CCPM pump, and a Lichroprep precolumn (310 × 25 mm) with the stationary phase RP-18 (40–63 μm, Merck).

Plant Material

The seeds of *S. salsula* (10.0 kg) were collected in August 1999, in Huhehaote City, Inner Mongolia, China. Authentication of the botanical material has been reported previously [2].

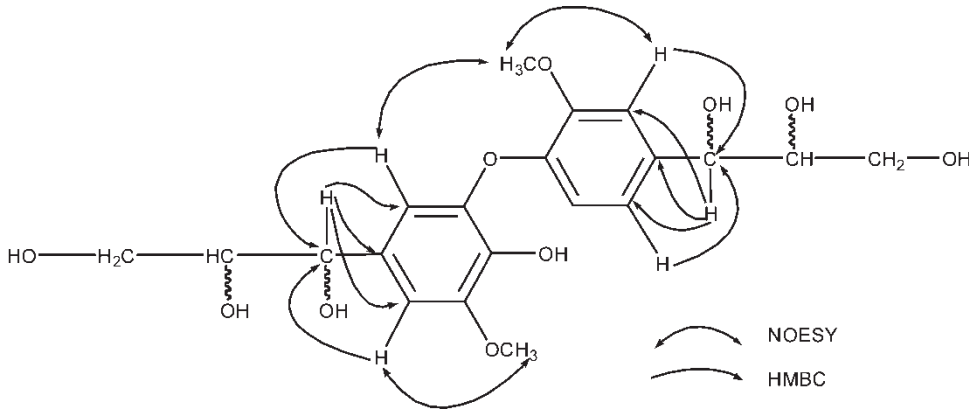

FIGURE 3 Important HMBC and NOESY correlations of **1**.

Extraction and Isolation

The seeds were air-dried, de-fatted with light petroleum, and extracted with 95% EtOH to give a black crude material (436.0 g), which was then partitioned with light petroleum and n-BuOH successively. The n-BuOH extract (212.0 g) was subjected to silica gel chromatography using a gradient eluent of $CHCl_3$–CH_3OH, 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 **6** (3 mg, 100: 0), **13** (5 mg, 100: 0), **14** (60 mg, 100: 1) and **2** (50 mg, 100: 5). Fraction 2 was separated on a silica gel column with a gradient mixture of $CHCl_3$–CH_3OH to give four sub-fractions. From sub-fraction 1, **7** (20 mg, 100:2 gradient mixture) was obtained. Sub-fraction 2 was chromatographed on a Lobar column, and eluted with 20, 40, 60 and 100% CH_3OH–H_2O successively; the 40% eluate was then subjected to preparative HPLC and eluted with 30% CH_3OH–H_2O to yield **1** (25 mg, 8 min) and **10** (6 mg, 10 min). Sub-fraction 3 was chromatographed on a Lobar column, and eluted with H_2O, 20%, 40% and 100% CH_3OH–H_2O successively; the 40% eluate was chromatographed by preparative HPLC, eluting with 15% CH_3CN–H_2O, to produce compound **17** (2.0 mg, 15 min). Fraction 3 was chromatographed on a silica gel column to produce five sub-fractions, of which sub-fraction 2 was chromatographed by preparative HPLC, using 15% CH_3OH–H_2O as eluent, to yield **8** (60 mg, 15 min), **9** (40 mg, 17 min) and **3** (80 mg, 27 min). Fraction 4 was also chromatographed over a silica gel column with a mixture of $CH_3OH\,CHCl_3$ as eluent. From this, three sub-fractions and **5** (6 mg, 100:8) were obtained, of which sub-fraction 1 was subjected to preparative HPLC and eluted with 10% $CH_3OH\,H_2O$ to furnish **11**; sub-fraction 2 was chromatographed on a Sephadex LH-20 column, eluted with 20%, 40%, 60% and 100% CH_3OH–H_2O successively, and the 20% part was chromatographed by preparative HPLC with 50% CH_3OH–H_2O as eluent to yield **15** (60 mg, 32 min) and **16** (20 mg, 28 min). The 40% part was subjected to silica gel CC, from which **12** was obtained.

Sphaerophysin A (1)

$C_{20}H_{26}O_{10}$, colorless needles, mp 98–100°C. $[\alpha]_D^{20}$ −2.6 (*c* 0.13, CH_3OH). UV (CH_3OH) λ_{max} 242, 279 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.97 (1H, br.s, H-2′), 6.77 (1H, d, $J = 7.5$ Hz, H-6′), 6.79 (1H, br.d, $J = 7.5$ Hz, H-5′), 6.56 (1H, br.s, H-6), 6.48 (1H, br.s, H-2), 4.85 (1H, br.s, H-7′), 4.40 (1H, br.s, H-7), 4.03 (1H, br.s, H-8′), 3.46 (1H, m, H-8), 3.55, 3.32 (each 1H, m, H-9) and 3.34, 3.16 (each 1H, m, H-9′), 3.76 (3H, s, 5-OMe) and 3.77 (3H, s, 3′-OMe). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 135.4 (C-1), 107.7 (C-2), 143.5 (C-3), 131.7 (C-4), 148.0 (C-5), 103.2 (C-6), 72.8 (C-7), 75.7 (C-8), 60.2 (C-9), 127.8 (C-1′), 111.7 (C-2′), 147.0 (C-3′), 147.6 (C-4′), 120.5 (C-5′), 115.4 (C-6′), 75.8 (C-7′), 77.9 (C-8′), δ 62.7 (C-9′), 55.6 (5-OMe) and 55.7 (3′-OMe). EIMS *m/z* 408 $[M - H_2O]^+$, HREIMS *m/z* 408.1445 $[M - H_2O]^+$ (calcd for $C_{20}H_{26}O_{10}$ 408.1420).

Acknowledgements

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