Arch Pharm Res Vol 28, No 12, 1333-1336, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

A New Lavandulylated Flavonoid with Free Radical and $ONOO^-$ Scavenging Activities from *Sophora flavescens*

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(Received September 20, 2005)

A new lavandulylated flavonoid, 8-lavandulylkaempferol (**1**), was isolated from the roots of *Sophora flavescens* AITON (Leguminosae). The structure of this compound was determined via spectroscopic analysis. Compound **1** was determined to be a scavenger on both 1,1-diphenyl-2-picrylhydrazyl radicals and $ONOO^-$.

Key words: *Sophora flavescens*, Lavandulylated flavonoid, 8-Lavandulylkaempferol, 1,1-Diphenyl-2-picrylhydrazyl radicals, $ONOO^-$

INTRODUCTION

The dried roots of *Sophora flavescens* AITON (Leguminosae) are a well-known traditional Chinese herbal medicine, used for the treatment of acute dysentery, gastrointestinal symptoms, hemorrhaging, and eczema (Perry and Metzger, 1980; Huang, 1993). Phytochemical examinations of this plant have resulted in the isolation of a host of compounds, including: quinolizidine alkaloids (Okuda *et al.*, 1965; Murakoshi *et al.*, 1982; Saito *et al.*, 1990), pterocarpans (Wu *et al.*, 1985), saponins (Yoshikawa *et al.*, 1985; Ding *et al.*, 1992), and a variety of flavonoids (Kyogoku *et al.*, 1973; Woo *et al.*, 1998; Kuroyanagi *et al.*, 1999; Kang *et al.*, 2000; Ding *et al.*, 2005). In a previous study, we reported that the CH_2Cl_2-soluble fraction of the roots of *S. flavescens* exerts a scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. From this CH_2Cl_2-soluble fraction, *trans*-hexadecyl ferulic acid, *cis*-octadecyl ferulic acid, *trans*-hexadecyl sinapic acid, (−)-4-hydroxy-3-methoxy-(6a*R*,11a*R*)-8,9-methylendioxypterocarpan, and desmethylanhydroicaritin were isolated, and determined to be the active principles (Jung *et al.*, 2005). As the EtOAc fraction of the MeOH extract of this plant exhibited more profound scavenging activities on DPPH and $ONOO^-$ than the CH_2Cl_2 fraction, we conducted this study in order to identify the active compound(s) in the EtOAc fraction.

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MATERIALS AND METHODS

General experimental procedure

IR spectra were acquired using a Shimadzu FT-IR spectrometer with a KBr disc. Optical rotation was obtained using a Perkin-Elmer 341 Polarimeter. UV spectra were recorded on a Varian Carry UV-visible spectrophotometer. EI-MS and (HR)-FAB-MS data were obtained with QP-5050A (Shimadzu, Japan) and JEOL JMS-700 mass spectrometers, respectively. The ^1H- and ^{13}C-NMR spectra were determined using a JEOL JNM ECP-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) in DMSO-d_6. The HMBC spectra were recorded using pulsed field gradients. Column chromatography was conducted using silica (Si) gel 60 (70-230 mesh, Merck, Germany), RP-18 Lichroprep (40-63 μm, Merck, Germany), and Sephadex LH-20 (20-100 μm, Sigma, St. Louis, MO, U.S.A.). TLC was conducted on precoated Merck Kieselgel 60 F_{254} plates (20×20 cm, 0.25 mm) and a RP-18 F_{254s} plates (5×10 cm, Merck, Germany), using 50% H_2SO_4 as a spray reagent. All solvents used in the column chromatography were of reagent grade, and were purchased from commercial sources.

Chemicals

The DPPH (1,1-diphenyl-2-picrylhydrazyl), L-ascorbic acid, and DL-penicillamine (DL-2-amino-3-methylbutanoic acid) used in these experiments were purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.). The high-quality DCFH-DA (2',7'-dichlorofluorescin diacetate),

DHR 123 (dihydrorhodamine 123), and $ONOO^-$ were obtained from Molecular Probes (Eugene, Oregon, U.S.A.) and Cayman (Ann Arbor, MI, U.S.A.), respectively.

Plant materials

The *S. flavescens* AITON (Leguminosae) roots were collected at Young Chun, Kyeong Buk, Korea, in March 2004, and were authenticated by Prof. J. H. Lee, at the Kyung Hee University, in Seoul, Korea. A voucher specimen (no. 20040320) was deposited in the author's laboratory (J. S. Choi).

Extraction, fractionation and isolation

The roots (5 kg) of *S. flavescens* were refluxed for three hours with MeOH (3×10 L). The total filtrate was concentrated and dried *in vacuo* at 40°C, in order to render the MeOH extract (1.1 kg). This extract was then suspended in distilled water, and sequentially partitioned with CH_2Cl_2 (114 g), EtOAc (124 g), *n*-BuOH (305 g), and H_2O (524 g), in sequence. The EtOAc fraction (124 g) was initially chromatographed over a silica (Si) gel column, using CH_2Cl_2 -MeOH under gradient conditions (CH_2Cl_2 → CH_2Cl_2 : MeOH = 10:1 → 1:1, MeOH, gradient), thereby yielding 17 fractions (Fr.1 — Fr.17). Fraction 4 (2.29 g) was then further purified using Si gel with CH_2Cl_2 : MeOH (40:1 → 1:1, MeOH, gradient), ultimately yielding compound **1** (25 mg).

8-Lavandulylkaempferol (1)

Amorphous pale yellow powder, $[\alpha]_D^{20}$ + 1.96° (*c* 0.0158, MeOH); IR ν_{max} cm^{-1} : 3292, 2961, 2922, 1655, 1509; UV λ_{max} (MeOH): 272 (log ε 4.07), 303 (sh, 3.84), 373 (3.90) nm; NaOMe 283 (4.12), 333 (sh, 3.90), 426 (4.06); NaOAc 272 (4.06), 376 (3.89); NaOAc + H_3BO_3 272 (4.07), 374 (3.90); $AlCl_3$ 273 (4.06), 310 (sh, 3.70), 354 (3.71), 434 (3.94); $AlCl_3$ + HCl 273 (4.03), 311 (sh, 3.69), 355 (sh, 3.70), 432 (3.91); HR-FAB-MS $[M + H]^+$ found *m/z* 423.1808 (Calcd for $C_{25}H_{27}O_6$ 423.1813, Δ - 0.5 mmu); EI-MS *m/z* (rel. int.): 422 (32), 299 (100), 270 (5.9), 165 (35), 147 (8.3), 134 (3.5), 121 (30.9), 107 (15.5), 69 (20.5); ^1H- and ^{13}C-NMR data (DMSO-d_6) : see Table I.

DPPH radical scavenging effect

The DPPH radical scavenging effect was assessed according to the method described previously by Blois (1958), with some minor modifications. 160 μL of a methanolic sample solution at several concentrations, and 40 μL of a DPPH methanolic solution (1.5×10^{-4} M), were added to a 96-well microplate, in a total volume of 200 μL. After the reaction mixture was allowed to stand for 30 minutes at room temperature, its absorbance was determined at 520 nm with a microplate reader (VERSA max, Molecular Devices, CA, U.S.A.).

Table I. NMR Spectral data of **1** in DMSO-d_6 [a]

Position	δ_C	δ_H (J, Hz)	HMBC
2	146.5		
3	135.5		
4	176.1		
5	158.3		
6	97.6	6.27, s	C-5, C-10
7	161.7		
8	104.9		
9	153.8		
10	102.9		
1'	121.9		
2'	129.3	8.07, d, (8.6)	C- 2, C-4'
3'	115.4	6.93, d, (8.6)	C-1', C-4'
4'	159.1		C-3', C-5'
5'	115.4	6.93, d, (8.6)	C-1', C-4'
6'	129.3	8.07, d, (8.6)	C-2, C-4'
1"	26.8	2.81, m	C-7, C-8, C-9, C-3"
2"	46.6	2.49, m	
3"	30.8	2.07, m	C-4", C-5"
4"	122.9	4.97, br t, (6.9)	C-7"
5"	130.9		
6"	25.5	1.56, s	C-4", C-5", C-7"
7"	17.6	1.46, s	C-6"
8"	147.4		
9"	111.2	4.61, s	C-2"
		4.48, s	C-10"
10"	18.4	1.65, s	C-2", C-8", C-9"
3-OH		9.39, s	C-2
5-OH		12.42, s	C-5, C-6, C-10
7-OH		10.66, s	C-8
4'-OH		10.13, s	C-5'

[a] Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses.

Measurement of $ONOO^-$ scavenging activity

$ONOO^-$ scavenging activity was assessed by monitoring the oxidation of DHR 123, in accordance with a modified version of the method of Kooy *et al.* (1994). The DHR 123 (5 mM) in dimethylformamide, was purged with nitrogen, stored at –80°C, and used as a stock solution. This solution was then placed on ice and kept from exposure to light prior to the study. The buffer used in this experiment consisted of 90 mM sodium chloride, 50 mM sodium phosphate, 5 mM potassium chloride, at a pH of 7.4, and 100 μM diethylenetriaminepentaacetic acid, each of which had been prepared with high quality deionized water, and purged with nitrogen. The final concentration of the DHR 123 was 5 μM. The background and final fluo-

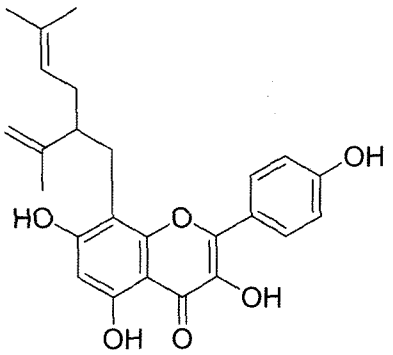
rescent intensities were determined 5 minutes after treatment, both with and without the addition of authentic $ONOO^-$. The DHR 123 was rapidly oxidized by the authentic $ONOO^-$, and its final fluorescent intensity remained unchanged over time. The fluorescent intensity of the oxidized DHR 123 was determined using an FLx 800 microplate fluorescence reader (Bio-Tek Instruments Inc.), with excitation and emission wavelengths of 480 and 530 nm, respectively. The results were then expressed as the means ± standard error (n = 3) of the final fluorescence intensity, minus the background fluorescence. The effects were expressed as the percentage of inhibition of the oxidation of DHR 123.

Statistical analysis

All values were expressed as the means ± standard error of three or five identical experiments.

RESULTS AND DISCUSSION

The MeOH extract of the roots of *S. flavescens* was subjected to solvent partitioning, in CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O phases. The EtOAc fraction, which was found to exert DPPH and $ONOO^-$ scavenging effects at IC_{50} values of 23.57 ± 0.24 μg/mL (positive control, L-ascorbic acid, IC_{50} 1.94 ± 0.1 μg/mL) and 4.64 ± 1.78 μg/mL (positive control, penicillamine, IC_{50} 3.04 ± 0.74 μg/mL), respectively, was subjected to Si gel column chromatography, resulting in the isolation of compound **1**. Compound **1** appeared as a pale yellow amorphous powder, with a molecular formula of $C_{25}H_{26}O_6$, deduced from the $[M + H]^+$ peak at *m/z* 423.1808 (Calcd for $C_{25}H_{27}O_6$ *m/z* : 423.1813, Δ - 0.5 mmu) on HR-FAB-MS, and confirmed *via* ^{13}C-NMR spectrum analysis. It yielded a positive reaction with Mg-HCl, thereby indicating its flavonoid nature. The UV spectrum of compound **1** exhibited absorption maxima at 272 and 373 nm, indicative of a flavonol skeleton (Mabry *et al.*, 1970). The IR spectrum of this compound exhibited the characteristic absorptions associated with hydroxyl (3291 cm^{-1}) and aromatic (1655, 1509 cm^{-1}) groups. The EI-MS spectrum yielded a molecular ion peak at *m/z* 422, and other prominent fragments at *m/z* 299, 270, 165, 147, 134, 121, 107, and 69. Among these, *m/z* 299 [M^+ - 123 (C_9H_{15})] and 165 [M^+ - 134 ($C_8H_6O_2$) - 123] demonstrated that the A ring had been dioxygenated, and that a *C*-alkyl group had been substituted (Shirataki *et al.*, 1990; Ruangrungsi *et al.*, 1992). The observed peak at *m/z* 134 [M^+ - 165 ($C_8H_5O_4$) - 123] indicated that the B ring also harbored a hydroxyl group (Harborne *et al.*, 1975). The structure of compound **1** was also deduced from the ^{1}H-NMR spectrum. The presence of two two-proton doublets at δ 6.93 (H-3', 5') and 8.07 (H-2', 6') (each 2H, J = 8.6 Hz), and a one-proton singlet at δ 6.27 (H-6), showed that compound **1** must be based on kaempferol, with a *C*-alkyl substituent linked to C-8. In addition to the kaempferol moiety, three three-proton singlets at δ 1.46 (H-7"), 1.56 (H-6"), and 1.65 (H-10"), two two-proton multiplets, at δ 2.07 (H-3") and 2.81 (H-1"), a one-proton multiplet at δ 2.49 (H-2"), two one-proton broad singlets at δ 4.48 (H-9"a) and 4.61 (H-9"b), as well as a one-proton broad triplet at δ 4.97 (H-4"), indicated the presence of a lavandulyl (5-methyl-2-isopropenyl-hex-4-enyl) group, as the *C*-alkyl group. This conclusion was bolstered by the observation of signals at δ 26.8 assigned to C-1", 46.6 to C-2", 30.8 to C-3", 122.9 and 130.9 to C-4" and C-5", 25.5 to C-6", 17.6 to C-7", 147.4 and 111.2 to C-8" and C-9", and 18.4 to C-10" in the ^{13}C-NMR spectrum, in which all of the carbons were assigned by DEPT, ^{1}H-^{13}C COSY, and ^{1}H-^{13}C long range COSY. The linkage site of the lavandulyl group in the kaempferol moiety was identified as C-8, on the basis of HMBC spectrum analysis. In the



1

Fig. 1. Chemical structure of **1**

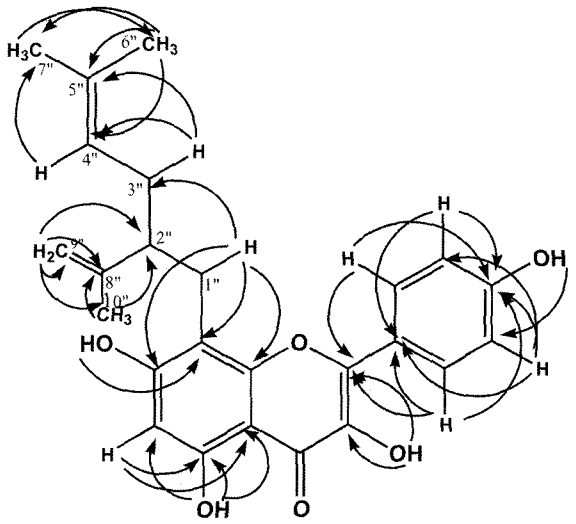


Fig. 2. HMBC Correlations of **1**

HMBC spectrum, the lavandulyl proton (H-1") peak, which was located at δ 2.81, was correlated with the C-8 at δ 104.9 (Fig. 2). On the basis of the above evidence, the structure of compound **1** was elucidated as 8-lavandulylkaempferol (Fig. 1, Table I). Although 8-prenylkaempferol (desmethylanhydroicaritin) has been previously isolated from *Epimedium davidii* (Berberidaceae) (Li *et al.*, 1988), to the best of our knowledge, this study contains the first report of compound **1** isolated from a natural source. Compound **1** exhibited profound DPPH radical and $ONOO^-$ scavenging activities, at IC_{50} values of 12.91 ± 0.21 μM (positive control, L-ascorbic acid, IC_{50} 12.70 ± 0.1 μM) and 4.06 ± 0.41 μM (positive control, DL-penicillamine, IC_{50} 3.04 ± 0.74 μM), respectively.

ACKNOWLEDGEMENTS

The (HR) FAB-MS spectra used in this study were provided by the Korea Basic Science Institute. This study was supported by a grant (05142HM625) from the National Center for Standardization of Herbal Medicine funded by the Food Drug & Administration, Republic of Korea (2005).

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