Three New Flavonol Galloylglycosides from Leaves of *Acacia confusa*

Tzong-Huei Lee,[†] Feng Qiu,[‡] George R. Waller,[§] and Chang-Hung Chou*[,†,⊥]

Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 11529, Republic of China, Department of Chemistry and Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma 74038-3035, and Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan 804, Republic of China

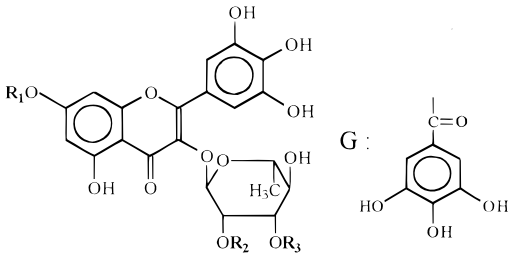
Received September 29, 1999

Myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**1**), myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**2**), and myricetin 3-*O*-(2″,3″-di-*O*-galloyl)-α-rhamnopyranoside (**3**), three new flavonol galloylglycosides, were isolated from leaves of *Acacia confusa* sampled from Chaoushi in the north of Taiwan. Their structures were established by analysis of spectroscopic data, and the compounds were evaluated for anti-hatch activity against brine shrimp.

Acacia confusa (Leguminosae), an erect shrub, is widely distributed on the hills and lowlands of Taiwan and often exhibits a unique pattern of weed exclusion under stands.[1] Although it has been reported that *A. confusa* is an allelopathic plant,[2] the leaves containing bioactive agents have not been investigated phytochemically except for some phenolic acids.[3] Because there have been no previous reports on the flavonoid constituents of *A. confusa*, a chemical investigation of the methanolic extract of the leaves was undertaken and has led to the isolation of three new flavonol galloylglycosides (**1**−**3**) as well as four known flavonoids (**4**−**7**). This study describes the isolation and structure elucidation of the new compounds as well as their biological activity toward brine shrimp.

From the methanolic extract of fresh leaves of *A. confusa* seven major flavonoids (Figure 1), including three new flavonol galloylrhamnosides (**1**−**3**), were identified. The compounds were isolated by a serial separation on Sephadex LH-20 column, Si-flash column, and reversed-phase HPLC. Spectroscopic data of myricitrin (**4**) were interpreted by comparison with those reported in the literature.[4] Myricitrin 7-methyl ether (**5**), was identical with the compound previously reported.[5] Compound **6** was obtained as a yellow powder whose UV, MS, and ^{1}H and ^{13}C NMR spectral data were consistent with those of the 2″-gallic acid ester of myricitrin,[6] which has been previously isolated from *Desmanthus illinoensis*,[6] *Myrica esculenta*,[7] and *Hexachlamys edulis*,[8] and reported as desmanthin-1.[6] Compound **7** was determined to be myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside, and its spectral data (^{1}H and ^{13}C NMR spectra and FABMS) were in good agreement with the published data.[7]

Compound **1** showed a prominent $[M + H]^+$ ion peak at *m*/*z* 631 in the positive FABMS. The IR spectrum indicated the presence of a hydroxyl group (3239 cm^{-1}) and a carbonyl group (1655 cm^{-1}). Its UV spectrum exhibited absorption maxima at 266 and 352 nm. In the ^{1}H and ^{13}C NMR spectra of **1**, complex signal patterns arising from the flavonol and rhamnose moiety, as well as the appearance of one galloyl signal, indicated that **1** was a flavonol substituted with a galloyl group and a rhamnose (Table 1). In the ^{1}H NMR spectrum of **1**, the observation of two aromatic doublets at δ 6.37 and 6.66 and an aromatic singlet at δ 6.95 suggested the flavonol moiety was myricetin.[9] Furthermore, the orientation of the methoxyl group, galloyl group, and rhamnose were determined by ^{1}H−^{13}C heteronuclear multi-bond correlation (HMBC) spectrum. In the HMBC spectrum, the methoxyl proton signal at δ 3.85 showed an interaction with myricetin C-7 resonance at δ 165.2, the rhamnose H-1 signal at δ 5.53 (J = 1.1, α-form) correlated with the myricetin C-3 resonance at δ 133.5 through a three-bond coupling, and the rhamnose H-2 signal at δ 5.47 correlated with the galloyl ester carbon resonance at δ 165.0. Accordingly, the galloylrhamnoside was determined to be attached to the myricetin C-3 position, and the galloyl moiety was attached to the rhamnose C-2 position. Thus, the structure of **1** was determined as myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether.



	R_1	R_2	R_3
1	CH_3	G	H
2	CH_3	H	G
3	H	G	G
4	H	H	H
5	CH_3	H	H
6	H	G	H
7	H	H	G

Figure 1. Flavonoids isolated from leaves of *Acacia confusa*.

Compound **2**, with the same molecular formula as **1**, also exhibited a UV spectrum with λ_{max} (MeOH) at 266 and 352 nm. In the positive FABMS, **2** exhibited an $[M + H]^+$ ion peak at *m*/*z* 631, which was identical to that of **1**. Its IR spectrum indicated the presence of a carbonyl group (1653 cm^{-1}). Compound **2** gave ^{1}H and ^{13}C NMR spectra (Table 1) closely related to those of **1**, except for the complexity of the rhamnose moiety signals. When the ^{1}H NMR spectrum of **2** was compared with that of **1**, the significant lower field

* To whom correspondence should be addressed. Tel.: (886) 2-2789-9590, ext. 451. Fax: (886) 2-2782-7954. E-mail: chou@gate.sinica.edu.tw.
[†] Institute of Botany.
[‡] Department of Chemistry.
[§] Department of Biochemistry and Molecular Biology.
[⊥] Department of Biological Sciences.

10.1021/np990482w CCC: $19.00
Published on Web 04/19/2000

Table 1. NMR Spectral Data (DMSO-d_6, 500 MHz) for Compounds **1**−**3** [d in ppm, mult. (J in Hz)]

	1		**2**		**3**[a]	
carbon no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
flavonol						
C-2	157.9		158.1		159.4	
C-3	133.5		135.1		135.8	
C-4	177.6		178.0		179.3	
C-5	160.9		161.0		163.2	
C-6	98.0	6.37 d (2.1)	97.9	6.38 d (2.1)	99.9	6.19 d (2.0)
C-7	165.2		165.2		166.1	
C-8	92.2	6.66 d (2.0)	92.3	6.67 d (1.9)	94.8	6.37 d (2.0)
C-9	156.4		156.4		158.5	
C-10	105.0		105.0		105.8	
OCH_3−7	56.1	3.85 s	56.1	3.86 s		
C-1′	119.2		119.5		121.8	
C-2′,−6′	108.1	6.95 s	108.0	6.91 s	109.5	7.02 s
C-3′, −5′	145.9		145.9		146.5	
C-4′	136.8		136.7		138.0	
rhamnoside						
C-1	98.3	5.53 d (1.1)	102.7	5.05 br s	100.7	5.48 d (1.6)
C-2	71.7	5.47 t (1.6)	67.8	4.31 br s	71.1[b]	5.93 dd (1.8, 3.1)
C-3	68.5	3.82 dd (1.6, 5.1)	73.8	5.02 dd (3.1, 9.6)	73.5	5.43 dd (3.1, 6.1)
C-4	71.7	3.28−3.30 t (−)	68.6	3.51 t (9.6)	71.4[b]	3.72−3.73 t (−)
C-5	70.7	3.28−3.30 m (−)	71.1	3.69 m (−)	72.4	3.72−3.73 m (−)
C-6	17.6	0.93 d (5.1)	17.5	0.90 d (6.1)	17.8	1.09 d (4.7)
2″-*O*-galloyl						
C-1	119.2				120.8	
C-2, −6	108.9	6.94 s			110.3	7.03 s
C-3, −5	145.5				146.3	
C-4	138.6				140.2	
C═O	165.0				166.8	
3″-*O*-galloyl						
C-1			120.0		121.2	
C-2, −6			109.0	7.03 s	110.4	7.00 s
C-3, −5			145.4		147.0	
C-4			138.3		140.0	
C═O			165.7		168.1	

[a] Measured in CD_3OD. [b] Values interchangeable.

and higher field shifts of the rhamnose H-3 and H-2 signals, respectively, suggested the galloyl group was located at the rhamnose C-3 position, which was further confirmed by HMBC experiment. Based on these findings, the structure of **2** was concluded to be myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether.

Compound **3** also showed UV absorption bands characteristic of a flavonol derivative. The ^{1}H NMR spectrum of **3** indicated the presence of a myricetin moiety as revealed by a 3,4,5-trisubstituted benzene proton signal at δ 7.02, and *meta*-coupled signals at δ 6.19 and 6.37. Besides these signals, two singlets attributable to two galloyl groups (δ 7.00, 7.03) were observed in the aromatic region. The coupling patterns of the aliphatic proton signals, which were assigned with the aid of ^{1}H−^{1}H correlation spectroscopy (COSY), were characteristic of rhamnopyranose.[7] The ^{13}C NMR resonances of the aglycon moiety also coincided with those of myricitrin (**4**). These data, coupled with the FABMS data, suggested that **3** is a digallate of myricitrin. The galloyl groups in **3** were located at C-2 and C-3 of the rhamnose residue based on the remarkable downfield shifts of the H-2″ (δ 5.93) and H-3″ (δ 5.43) signals, and they were confirmed by HMBC measurements. Thus, compound **3** was characterized as myricetin 3-*O*-(2″,3″-di-*O*-galloyl)-α-rhamnopyranoside. Although various acylated flavonol glycosides are commonly found in plants, digallates such as **3** are quite rare, and **3** is the first example of a natural flavonol galloylated at both C-2 and C-3 of the rhamnose residue.

Several biological activities have been reported for these types of flavonoids, including antiviral activity[10] and inhibition of xanthine oxidase[11] and of the intestinal α-glucosidases.[12] Compound **6**, the gallic acid ester at 2″ position of myricitrin, also showed a strong growth inhibition of the tobacco budworm, as well as antibacterial activity and plant growth regulation effect.[6]

We also found that the flavonol galloylrhamnosides exhibit anti-hatch activity on brine shrimp *Artemia salina*, with IC_{50} values for **1**, **2**, **6**, and **7** of 89, 50, 75, and 64 μg/mL, respectively. The IC_{50} value for **3** was not measured due to the small amount of compound available.

Experimental Section

General Experimental Procedures. UV spectra were obtained in MeOH on a Hitachi U-2000 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 (or MeOH-d_4) at room temperature on a Bruker DMX-500 SB spectrometer, and the solvent resonances were used as internal shift references. The ^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC spectra were recorded using standard pulse sequences. LRFAB-MS were recorded on a JEOL SX-102A instrument using *m*-nitrobenzyl alcohol as the matrix.

Plant Material. Leaves of *A. confusa* were sampled from Chaoushi in Ilan County in December 1998, and a voucher specimen has been deposited at the Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan. Fresh plant material was ground into powder in liquid nitrogen for MeOH extraction.

Extraction and Isolation. Fresh leaves (200 g) of *A. confusa* were extracted three times with 500 mL of MeOH for 30 min. The methanolic extract was adjusted to 85% in aqueous solution for hexane partition, which generated two fractions: the methanol solubles and the hexane solubles. Subsequently, the methanol solubles were then vacuum-evaporated to dryness and further partitioned between ethyl acetate and water. The ethyl acetate layer was vacuum-evaporated to dryness (6.5 g) and redissolved in 50 mL of

MeOH for batches of chromatographic separations. The first separation step was carried out using gel filtration chromatography on a Sephadex LH-20 column (Pharmacia Biotech, Sweden, 3 × 55 cm). A flow of 13 mL/min MeOH was used to elute the flavonoid fractions. Each fraction was analyzed by TLC using plates of Si gel 60, PF_{254}, 200 μm thickness (Merck, Germany), and a solution of dichloromethane−ethyl acetate−formic acid−H_2O (15:70:5:1, v/v/v/v) for development. Vanillin−sulfuric acid charring to form yellow spots, in addition to UV absorption, was used to detect the flavonoids. Subsequently, the fractions containing the same components were combined and chromatographed on a 2.5 cm i.d. flash column (36 g, Baker's Si gel for flash column) using the same solution in TLC as the eluent, flow rate 25 mL/min. The fractions were combined into major portions based on the result of TLC separation. Each portion was further purified by repetitive HPLC separations on a Hyperprep ODS semipreparative column (250 × 10 mm, Keystone Scientific Inc.) with acetonitrile−water from 15% to 40% in a linear gradient mode in 30 min to afford myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**1**) (80 mg), myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (**2**) (60 mg), myricetin 3-*O*-(2″,3″-di-*O*-galloyl)-α-rhamnopyranoside (**3**) (5.5 mg), myricitrin (**4**) (15 mg), myricitrin 7-methyl ether (**5**) (25 mg), myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside (**6**) (65 mg), and myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside (**7**) (50 mg). Purified compounds were vacuum-dried and stored at −20 °C in a freezer for further chemical structure analysis and the determination of related biological activity.

***Artemia* Bioassay.** Brine shrimp eggs (*A. salina*) were supplied by Ocean Star International, Inc. (Snowville, UT). NMR-confirmed pure compounds **1**, **2**, **6**, and **7** isolated from *A. confusa* were assayed with brine shrimp to observe their relative toxicity, expressed as IC_{50} in μg/mL. Each compound was diluted with seawater to give four assay concentrations of 1000, 500, 100, and 10 μg/mL. Assays were performed in a 96-well microtitration plate with 20−30 brine shrimp eggs in 100 μL of each concentration level per well, and the percentage of the residual eggs at 48 h was determined. The IC_{50} determined by probit analysis[13] was defined as the concentration that inhibited the hatch of half the tested brine shrimp eggs within 48 h.

Myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (1): yellow powder; UV λ_{max} (MeOH) 266 (log ϵ 4.3), 352 (log ϵ 4.0) nm; $[\alpha]^{25}_D$ −0.44° (*c* 0.0015, MeOH); IR (KBr) ν_{max} 3239, 1601, 1341, 1210 cm^{-1}; FABMS, *m*/*z* 631 [M + H]$^+$; ^{1}H and ^{13}C NMR data, see Table 1.

Myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether (2): yellow powder; UV λ_{max} (MeOH) 266 (log ϵ 4.2) and 352 (log ϵ 4.0) nm; $[\alpha]^{25}_D$ −76.31° (*c* 0.002, MeOH); IR (KBr) ν_{max} 3256, 1601, 1339, 1213 cm^{-1}; FABMS, *m*/*z* 631 [M + H]$^+$; ^{1}H and ^{13}C NMR data, see Table 1.

Myricetin 3-*O*-(2″,3″-di-*O*-galloyl)-α-rhamnopyranoside (3): yellow powder; UV λ_{max} (MeOH) 268 (log ϵ 4.5) and 351 (log ϵ 4.1) nm; $[\alpha]^{25}_D$ +16° (*c* 0.0013, MeOH); IR (KBr) ν_{max} 3387, 1610, 1344, 1201 cm^{-1}; FABMS, *m*/*z* 769 [M + H]$^+$; ^{1}H and ^{13}C NMR data, see Table 1.

Acknowledgment. This research was supported by grants to C.H.C. of the National Science Council (NSC-88-2311-B001-001; NSC-89-2311-B001-026) and a postdoctoral fellowship to T.H.L. of the Academia Sinica, Taiwan. Wide Shared NMR Facility was provided by the National Science Foundation (BIR-9512269), National Science Foundation through EPSCoR, the Oklahoma State Regents for Higher Education, the W.M. Keck Foundation, and Conoco, Inc.

References and Notes

(1) Kuo, Y. L.; Chiu, C. Y.; Chou, C. H. In *Phytochemical Ecology*; Chou, C. H., Waller, G. R., Eds.; Institute of Botany, Academia Sinica Monograph Series No. 9, Taipei, 1989; pp 303−314.
(2) Fu, C. Y. The Allelopathic Potential of *Acacia confusa* Merr. in Taiwan. MS Thesis. National Taiwan University, Taipei, Taiwan, 1995, pp 1−2.
(3) Chou, C. H.; Fu, C. Y.; Li, S. Y.; Wang Y. F. *J. Chem. Ecol.* **1998**, *24*, 2131−2149.
(4) Addae-Mensah, I.; Achenbach, H. *Phytochemistry* **1985**, *24*, 1817−1819.
(5) Harborne, J. B. *Phytochemistry* **1967**, *6*, 1415−1428.
(6) Nicollier, G.; Thompson, A. C. *J. Nat. Prod.* **1983**, *46*, 112−117.
(7) Sun, D.; Zhao, Z.; Foo, L. Y.; Wong H. *Chem. Ind. For. Prod.* **1991**, *11*, 251−257.
(8) Schmeda-Hirschmann G.; Zúñiga, J.; Dutra-Behrens M.; Habermehl, G. *Phytother. Res.* **1996**, *10*, 260−262.
(9) Kinoshita, T.; Firman, K. *Phytochemistry* **1997**, *45*, 179−181.
(10) Dirk, A. R.; Venden, B.; Achiel, H.; Arnold, J. V. In *Bioctive Natural Products—Detection, Isolation, and Structural Determination*; Steven, M. C., Russell, J. M., Eds.; CRC Press: Boca Raton, FL, 1993; pp 405−440.
(11) Lio, M.; Moriyama, A.; Matsumoto, Y.; Takai, N.; Fukumoto, M. *Agric. Biol. Chem.* **1985**, *49*, 2173−2176.
(12) Miwa, I.; Horie, T.; Nakayama, M. *Chem. Pharm. Bull.* (*Tokyo*) **1986**, *34*, 838−844.
(13) Finney, D. J. In *Probit Analysis*, 3rd ed. Cambridge University Press: London, 1971; p 333.

NP990482W