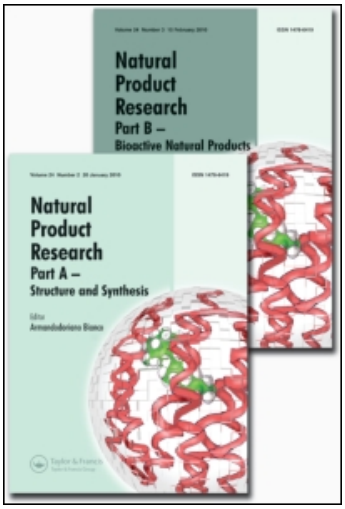

Natural Product Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713398545

Novel 4-(2-methylphenyl)-flavan, rhusjavanins A and B, from the roots of *Rhus semialata*

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Online publication date: 15 October 2010

To cite this Article Wu, Yan-Bing , Kuo, Yueh-Hsiung and Ouyang, Ming-An(2010) 'Novel 4-(2-methylphenyl)-flavan, rhusjavanins A and B, from the roots of *Rhus semialata*', Natural Product Research, 24: 17, 1643 — 1647
To link to this Article: DOI: 10.1080/14786419.2010.483528
URL: http://dx.doi.org/10.1080/14786419.2010.483528

Natural Product Research
Vol. 24, No. 17, 20 October 2010, 1643–1647

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(*Received 27 December 2009; final version received 3 April 2010*)

In this study, two novel flavan derivatives, namely, rhusjavanins A and B, along with two known flavans 2,3-*trans*-3,4-*trans*-3,4,7,4′-tetrahydroxyflavan and 2,3-*trans*-3,4-*cis*-3,4,7,4′-tetrahydroxyflavan, have been isolated from the roots of *Rhus javanica*. The structures were elucidated on the basis of spectroscopic data.

Keywords: *Rhus semialata*; 4-(2-methylphenyl) flavan; rhusjavanins A, B

1. Introduction

The roots of *Rhus semialata* (equivalent to *Rhus javanica* L. var. *roxburghiana*, Anacardiaceae) have been used in folk medicine as antitussives and in the treatment of anasarca, jaundice, snake bites, diarrhoea, spermatorrhoea and malaria (Jiang-Su Medicinal College, 1979; Kao, 1988). Recently, the plant was reported to possess inhibitory activity against IκBα kinase and on human cell proliferation activated by IL-1β and IL-6, as well as antifungal and antithrombin activities (Kuo et al., 1991, 1999; Prithiviraj, Manickam, Singh, & Ray, 1997; Ramakrishna et al., 2001). Earlier phytochemical studies on this species resulted in the isolation and characterisation of flavonoids, triterpenoids, phenols, tannins and an aromatic alkane (S. El & A. El, 1966; Kuo et al., 1991; Parveen & Khan, 1988; Parveen, Singh, Khan, Achari, & Logni, 1991; Sung, Akiyama, Sankawa, Iitaka, & Han, 1980; Taniguchi et al., 2000). The roots were collected from Beitou, Taiwan and extracted with MeOH. The MeOH extract was suspended in water and partitioned with EtOAc and *n*-BuOH. The EtOAc soluble fraction gave 37 pure compounds, two of which exhibited cytotoxic activity (Lee, Chiou, Lee, & Kuo, 2005). In this article, we wish to report the purification of the *n*-BuOH soluble fraction, which was subjected to Sephadex LH-20 (H_2O/MeOH) column chromatography and then separated by RP-18 and SiO_2 to yield two novel 4-(2-methylphenyl) flavans, rhusjavanins A (**1**) and B (**2**), along with two known compounds, 2,3-*trans*-3,4-*trans*-3,4,7,4′-tetrahydroxyflavan (**3**) and 2,3-*trans*-3,4-*cis*-3,4,7,4′-tetrahydroxyflavan (**4**) (Ali & Bhutani, 1993).

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ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786419.2010.483528
http://www.informaworld.com

2. Results and discussion

Rhusjavanin A (**1**) was obtained as colourless powder and had a molecular formula $C_{22}H_{20}O_6$, which gave 13 degrees of unsaturation, based on the HRFABMS [$(M+1)^+$ m/z 381.1337] and DEPT spectrum of ^{13}C-NMR. The IR absorption bands revealed the presence of hydroxyl (3337 cm^{-1}) and aromatic groups (1618 and 1508 cm^{-1}). The ^{1}H-NMR spectrum showed one set of an ABX system [δ_H 6.26 (1H, dd, $J=9.0$, 2.5 Hz, H-6), 6.42 (1H, d, $J=9.0$ Hz, H-5) and 6.47 (1H, d, $J=2.5$ Hz, H-8)], an AA'XX' system [δ_H 6.83 (2H, d, $J=8.6$ Hz, H-3′,-5′) and 7.19 (2H, d, $J=8.6$ Hz, H-2′,-6′)] and two *meta*-correlation protons [δ 6.20 (1H, d, $J=2.5$ Hz, H-5″) and 6.33 (1H, d, $J=2.5$ Hz, H-3″)]. In addition, the ^{1}H-NMR spectrum also exhibited four exchangeable phenolic protons at δ_H 8.11, 8.23, 8.48 and 8.67, and this evidence implies the presence of four phenolic groups in the aromatic rings. Because of the 13 degrees of unsaturation in compound **1**, it should have three aromatic rings in the molecule. The ^{13}C-NMR (DEPT) spectrum gave 22 signals, including 18 sp^2 carbons and 4 sp^3 carbons, and showed five oxygenated carbons in the aromatic rings (δ_C 155.0, 157.5, 157.8, 157.9 and 158.7); because of the presence of four phenolic groups in the aromatic rings, it is suggested that there were the other aliphatic rings in the molecule. Comparing the ^{1}H- and ^{13}C-NMR data of **1** with those of the reported compounds (**3** and **4**), the former is found to be a flavan-3-ol derivative. The HMBC spectrum revealed key correlations between H-2 and C-1′, C-2′, 6′ and C-9; between H-4 and C-5, C-9, C-10 and C-1″; and between H-7″ and C-1″, 5″ and 6″ (Figure 1, structure **1**); therefore, a 2,4-dihydroxy-6-methylphenyl group is located at C-4 in **1** instead of a hydoxy group as in **3** or **4**. The NOESY correlations (Figure 1, structure **1a**) of **1** can be illustrated by its relative stereochemistry. The NOESY correlations were H-4 with H-2′ and H-6′, which

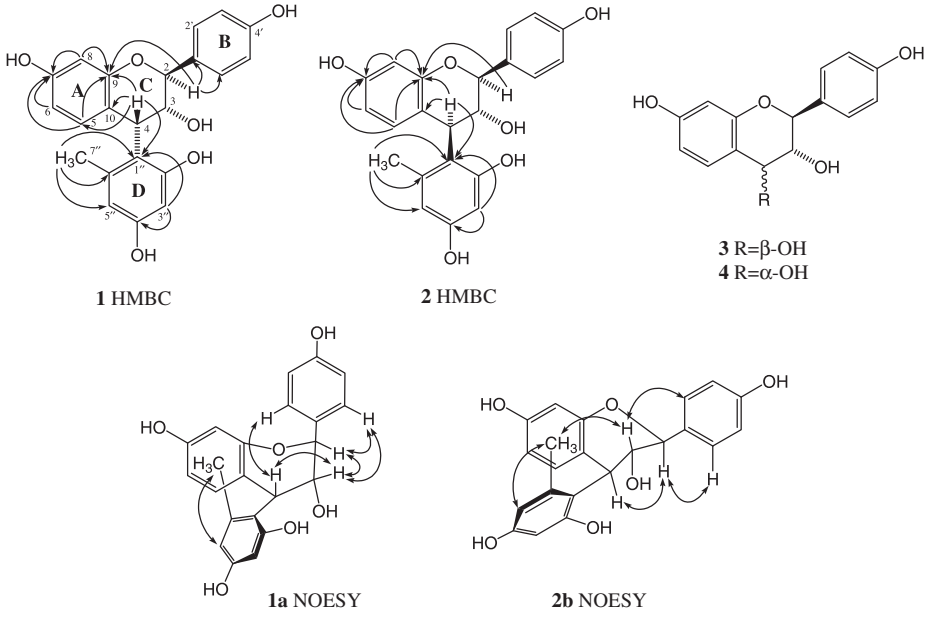

Figure 1. HMBC and NOESY experiment correlations of compounds **1** and **2**.

implied that H-4 and ring B are all in β-quasi-axial orientation, with ring C adapting to a twist-boat form; whereas H-3 is in β-quasi-equatorial orientation due to its correlation with H-2′, H-2 and H-4. Ascribed to shielding by ring D (Kuo, Lin, & Wu, 1989), the proton of H-5 (δ_H 6.42) gave higher shift than the corresponded protons in the compound **4** (about δ_H 6.8). For the same reason, the higher field signal of the methyl group (δ_H 1.78) should be explicated as the methyl group located under A ring receives a shielding effect from A ring. On combining the above evidence, the structure of **1** was determined as 3,7,4′-trihydroxy-4-(2,4-dihydroxy-6-methylphenyl)-flavan and the relative stereochemistry was assigned as structure **1a**, unambiguously.

On comparison with the ^{1}H- and ^{13}C-NMR data of (Figure 1, structure **2**) **1** and **2**, compound **2** was found to be a stereoisomer of compound **1**. The coupling values $J_{2,3}$ and $J_{3,4}$ expressed the same coupling constants as 9.6 Hz in compound **2**; these results suggested that the protons H-2, H-3 and H-4 were all in quasi-axial orientation. The C ring conformation was also proposed as a twist-boat form and the D ring was located at C-4 with β-quasi-equatorial orientation revealing from H-5 (δ_H 6.56) with upfield as in compound **3**; whereas, it led the methyl group to be shifted to a higher field than the normal case, due to the shielding effect from A ring. The NOESY spectrum showed correlations between H-3 and H-2′, H-6′ and H-7″; and between H-2 and H-4, H-2′ and H-6′ (Figure 1, structure **2b**). Based on the above results, the relative stereochemistry of rhusjavanin B (**2**) as the structure **2b** was determined.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured with a Jasco DIP-180 digital polarimeter spectrophotometer. IR spectra were recorded with a Perkin Elmer 1750 Fourier transform infrared (FT-IR) spectrometer, and the films of the all samples were measured on KBr discs. Mass spectra were recorded on a Jeol JMS-HX 110 instrument. The ^{1}H, ^{13}C, distortionless enhancement by polarisation transfer (DEPT), ^{1}H–^{1}H correlation spectroscopy (COSY), nuclear overhauser effect spectroscopy (NOESY), hetronuclear multiple quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC) and nuclear magnetic resonance (NMR) spectra were performed using a Bruker AM-400 spectrometer. Chemical shifts were reported using tetrametylsilane (TMS) as the internal standard. The chromatographic stationary phase used RP-18 (40–60 μm, Merck), silica gel (160–200 mesh, Qingdao Oceanic Chemical Co., Qingdao, China), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd.) and MCI-gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Ltd.). Compounds on a thin layer chromatograph (TLC) were detected by spraying with 5% H_2SO_4 followed heating.

3.2. *Plant material*

The roots of *R. semialata* var. *roxburghiana* were collected from the suburb of Taipei, Taiwan, in 1998. A voucher specimen (no. 191230) was deposited in the Department of Botany, National Taiwan University.

3.3. *Extraction and isolation*

The dry roots of *R. semialata* (8 kg) were extracted with methanol for two weeks. The extract was concentrated to dryness under reduced pressure. The residue (1.1 kg) was dissolved and suspended in water (2.5 L) and partitioned with ethyl acetate (3 × 3 L) and the water layer was then extracted with *n*-butanol (3 × 3 L). The *n*-butanol extract was evaporated *in vacuo* to give a residue of 130 g. The residue was subjected to dry column chromatography (DCC) on silica gel (1.0 kg), eluted with $CHCl_3$–MeOH (10 : 1), to obtain 13 fractions. Each fraction was subjected to Sephadex LH-20 and RP-18 columns were eluted with water–methanol (10–90%) and finally purified by a silica gel column with CH_2Cl_2–EtOAc–MeOH (10 : 10 : 1) and $CHCl_3$–EtOH (10 : 0.5–10 : 2) to yield **1** (24 mg), **2** (17 mg), **3** (41 mg) and **4** (23 mg).

3.3.1. *Rhusjavanin A (**1**)*

Amorphous powder, $C_{22}H_{20}O_6$, $[\alpha]_D^{21}$ +22°C (*c* 1.6, acetone); FAB-MS *m/z* 381 $[M+H]^+$, 369, 339, 313, 289, 245 and 154; HRFABMS: 381.1337 (Calcd for $C_{22}H_{20}O_6$: 381.1338); IR ν_{max}: 3337, 2912, 1618, 1597, 1508, 1458, 1149, 1120 and 824 cm^{-1}; see Table 1 for ^1H- and ^{13}C-NMR data.

Table 1. ^1H- and ^{13}C-NMR data of compounds **1** and **2.**

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
2	81.6 d	5.44 d (2.0)	83.3 d	4.69 d (9.6)
3	72.8 d	4.43 t (2.0)	72.4 d	4.16 t (9.6)
4	35.4 d	4.10 d (2.0)	42.2 d	4.91 d (9.6)
5	129.4 d	6.42 d (9.0)	129.7 d	6.56 d (8.0)
6	108.6 d	6.26 dd (9.0, 2.5)	103.4 d	6.33 dd (8.0, 2.0)
7	157.8 s		156.5 s	
8	103.0 d	6.47 d (2.5)	101.2 d	6.31 d (2.0)
9	155.0 s		156.9 s	
10	113.0 s		118.9 s	
1′	132.0 s		131.0 s	
2′	126.7 d	7.19 d (8.6)	130.0 d	7.33 d (9.4)
3′	116.1 d	6.83 d (8.6)	115.6 d	6.84 d (9.4)
4′	157.9 s		157.9 s	
5′	116.1 d	6.83 d (8.6)	115.6 d	6.84 d (9.4)
6′	126.7 d	7.19 d (8.6)	130.0 d	7.33 d (9.4)
1″	115.8 s		118.9 s	
2″	158.7 s		158.4 s	
3″	103.7 d	6.33 d (2.5)	111.5 d	6.31 d (2.4)
4″	157.5 s		157.0 s	
5″	110.1 d	6.20 d (2.5)	109.7 d	6.15 d (2.4)
6″	140.0 s		139.3 s	
CH_3	20.7 q	1.75 s	20.7 q	1.78 s

Note: 400 and 100 MHz in acetone-d_6 mult. (*J* in Hz).

3.3.2. *Rhusjavanin B (**2**)*

Amorphous powder, $C_{22}H_{20}O_6$, $[\alpha]_D^{21}$ −14°C (*c* 0.9, acetone); FAB-MS *m/z* 381 $[M+H]^+$, 339, 313, 289, 237, 154 and 137; HRFABMS: 381.1334 (Calcd for $C_{22}H_{20}O_6$: 381.1338); IR ν_{max}: 3331, 2920, 1612, 1600, 1502, 1465, 1147, 1120 and 878 cm^{-1}; see Table 1 for ^1H- and ^{13}C-NMR data.

Acknowledgements

This research was supported by Fujian Administration of Education (Grant numbers 2006NZ0002C4, 2008J0073 and 2009N0016). The authors thank the staff of the analytical group of the Department of Chemistry, Taiwan University, for the measurements of NMR and FAB-MS spectra.

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