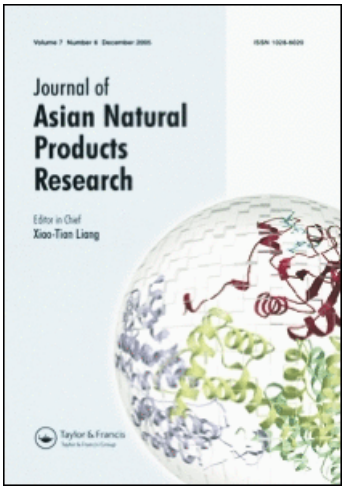

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Four new isoflavones from *Ampelopsis grossedentata*

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Online publication date: 09 September 2010

To cite this Article Wang, Ding-Yong , Zheng, Zong-Zhong , Xu, Su-Ying and Zheng, Shang-Zhen(2002) 'Four new isoflavones from *Ampelopsis grossedentata*', Journal of Asian Natural Products Research, 4: 4, 303 — 308
To link to this Article: DOI: 10.1080/1028602021000049104
URL: http://dx.doi.org/10.1080/1028602021000049104

Journal of Asian Natural Products Research, 2002 VOL. 4 (4), pp. 303–308

FOUR NEW ISOFLAVONES FROM *AMPELOPSIS GROSSEDENTATA*

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(Received 28 March 2002; Revised 9 April 2002; In final form 8 May 2002)

Four new isoflavones have been isolated from the BuOH extract of *Ampelopsis grossedentata* Hand.-Mazz. Based on spectral and chemical methods, their structures were elucidated as 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxy-isoflavone **1**; 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-β-D-glucopyranoside **2**; 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-α-L-rhamnopyranoside **3**; 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside **4**. This is the first report on isolation of isoflavones from this plant.

Keywords: *Ampelopsis grossedentata*; Vitaceae; Isoflavones

INTRODUCTION

Ampelopsis grossedentata Hand.-Mazz. (Vitaceae), which has strong antipyretic, antidote and anticancer effects, is widely distributed in South China [1]. Our previous paper reported the isolation and characterization of six limonoids from the EtOAc fraction obtained by partition of the MeOH extract [2]. In a continuation of the phytochemical research on this plant, four new isoflavones were obtained from the *n*-BuOH fraction of the MeOH extract. Here, we report the isolation and structural elucidation of these four new isoflavones from *A. grossedentata* Hand.-Mazz.

RESULTS AND DISCUSSION

Compound **1** was obtained as yellowish needles, mp. 171.5–172.8°C, and analyzed for $C_{17}H_{12}O_7$ by high resolution mass spectrometry. The UV (260, 325 nm), IR (1630 cm^{-1}, C = O), ^{1}HNMR (δ 8.05, 1H, s, H-2) and ^{13}CNMR (δ 153.0, CH, C-2 and 124.0, C, C-3)

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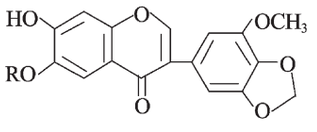
ISSN 1028-6020 print/ISSN 1477-2213 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1028602021000049104

spectra indicated that **1** is an isoflavone [3–4] (Fig. 1). The ^{1}HNMR signals at δ 9.40 (s, 1H), δ 9.47 (s, 1H) and a singlet of three protons at δ 3.85, showed the presence of two hydroxyl groups (which disappeared upon addition of D_2O), and a methoxyl group, respectively. Moreover, the ^{1}HNMR signal at δ 6.02 (2H, s), ^{13}CNMR signal at δ 100.5 and IR absorption band at 932 cm^{-1} revealed the presence of a methylenedioxy group in **1**. In the aromatic proton region, two doublets at δ 7.65 (1H, $J = 2.5$ Hz, H-2′) and δ 7.58 (1H, $J = 2.5$ Hz, H-6′), and two singlets at δ 7.28 (1H, H-5) and δ 6.70 (1H, H-8), suggested B-ring substitution at positions 3′, 4′ and 5′, and A-ring substitution at positions 6 and 7, respectively. The fragments (EI-MS) at *m/z* 153 and 176 formed from *retro*-Diels-Alder cleavage, indicated **1** has two hydroxyl groups in A-ring, one methoxyl and one methylenedioxy group in B-ring [5]. Two hydroxyls in A-ring were assigned to 6,7-position by the facts of bathochromic shift of band I (+ 13 nm) upon addition of $NaOAc/H_3BO_3$ in UV spectrum and the singlet peaks of H-5 and H-8 in ^{1}HNMR spectrum [6]. The methylenedioxy group on B-ring was suggested to be between C-4′ and C-5′ from the evidence of chemical shifts and coupling constant of the aromatic protons at δ 7.65 ppm (d, $J = 2.5$ Hz, H-2′) and δ 7.58 ppm (d, $J = 2.5$ Hz, H-6′) [7]. Thus, the substitution pattern of B-ring was revealed to be 3′-methoxy-4′,5′-methylenedioxy. Based on the above spectral characteristics, the structure of **1** was established as 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone, which was confirmed by the ^{13}CNMR data (Table I) and HMBC (heteronuclear multiple-bond correlation) spectrum (Fig. 2). To our knowledge, **1** has not been reported previously from any plant source.

Compound **2**, $C_{23}H_{22}O_{12}$, was obtained as a yellowish amorphous solid. It gave a positive Molish reaction. The UV spectrum showed absorptions at 261 and 322 nm. The IR spectrum showed absorptions at 3350 (OH), 3026 (Ar–H), 1640 (C═O) and 1100–1000 cm^{-1} (glycoside function). The ^{1}HNMR spectrum of **2** was distinctly similar to that of **1** with additional signals for a sugar moiety. The anomeric signal appeared as a doublet at δ 5.18 ($J = 7.5$ Hz) indicating a β-linkage for the sugar. The ^{13}CNMR spectrum (Table I) and FAB-MS at *m/z* 329 [$M^+ + 1 - 162$] also indicated the presence of a sugar moiety in the molecule. Acid hydrolysis of **2** afforded an aglycone, which was identified as **1** (TLC, mp, IR, UV, ^{1}HNMR and ^{13}CNMR) along with D-glucose. The exact position of the glucose unit was determined by typical glycosylation shifts observed in the ^{13}CNMR spectrum with respect to the aglycone **1**: upfield shifts of C-5 (ca. 2.0 ppm) and C-7 (ca. 2.1 ppm), and downfield shift of C-6 (ca. 7.8 ppm) suggested the presence of a glucose unit at C-6 [8]. This was confirmed by HMBC experiment correlation between the glucose anomeric proton (δ_H 5.18) and the isoflavone C-6 (δ_C 153.3) carbon. Therefore, the structure of **2** was assigned as 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-β-D-glucopyranoside. To our knowledge, this compound has not been reported previously.



1 R = H
2 R = Glc
3 R = Rha
4 R = Glc $\overset{6}{-}$ Xyl

FIGURE 1 Chemical structure of compounds **1**–**4**.

TABLE I ^{13}CNMR data of compounds **1**–**4** in DMSO-d_6 (100 Hz, δ in ppm from TMS), DEPT

C	**1**	**2**	**3**	**4**
2	153.0(CH)	152.3(CH)	152.5(CH)	153.0(CH)
3	124.0(C)	124.0(C)	123.9(C)	123.7(C)
4	178.0(C)	178.2(C)	178.1(C)	178.5(C)
5	104.8(CH)	102.8(CH)	103.2(CH)	102.6(CH)
6	145.5(C)	153.3(C)	152.5(C)	153.8(C)
7	156.2(C)	154.1(C)	155.0(C)	154.3(C)
8	95.9(CH)	95.8(CH)	96.0(CH)	97.0(CH)
9	151.0(C)	151.0(C)	151.1(C)	151.0(C)
10	116.5(C)	117.2(C)	117.0(C)	116.8(C)
1′	121.5(C)	120.3(C)	120.5(C)	121.0(C)
2′	114.9(CH)	115.0(CH)	114.9(CH)	115.1(CH)
3′	146.4(C)	146.5(C)	146.3(C)	146.4(C)
4′	147.8(C)	147.8(C)	147.8(C)	148.2(C)
5′	140.6(C)	141.0(C)	140.5(C)	141.3(C)
6′	111.2(CH)	111.4(CH)	111.4(CH)	101.6(CH)
Glc-1		102.5(CH)		101.6(CH)
2		73.0(CH)		73.1(CH)
3		75.8(CH)		75.8(CH)
4		69.9(CH)		70.0(CH)
5		77.1(CH)		75.6(CH)
6		60.3(CH_2)		68.7(CH_2)
Rha-1			101.9(CH)	
2			71.0(CH)	
3			71.5(CH)	
4			73.1(CH)	
5			69.5(CH)	
6			18.4(CH)	
Xyl-1				105.1(CH)
2				73.8(CH)
3				76.5(CH)
4				70.4(CH)
5				66.3(CH)
$-OCH_3$	58.0(CH_3)	58.3(CH_3)	57.8(CH_3)	59.5(CH_3)
$-OCH_2O-$	100.5(CH_2)	100.3(CH_2)	98.9(CH_2)	102.0(CH_2)

Compound **3**, $C_{23}H_{22}O_{11}$, was obtained as a yellow powder, and gave a positive Molish reaction. Its UV and IR were very similar to those of **2**. ^{1}HNMR and ^{13}CNMR spectra showed similarity with those of **1** except for the additional signals of the sugar. The anomeric signal in ^{1}HNMR appeared as a doublet at δ 5.30 ($J = 1.5$ Hz). Acid hydrolysis of **3** gave **1** as the aglycone and L-rhamnose. The α-configuration of the rhamnosyl moiety was established by comparing its ^{13}CNMR assignments with published values for methyl

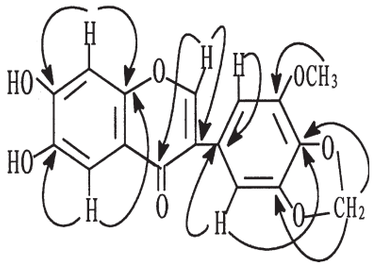


FIGURE 2 Important HMBC correlations for compound **1**.

TABLE II ^{1}H-NMR data of compounds **1**–**4** in DMSO-d_6 (400 MHz,δ in ppm from TMS)

H	**1**	**2**	**3**	**4**
2	8.05(s)	8.10(s)	7.98(s)	8.01(s)
5	7.28(s)	7.28(s)	7.30(s)	7.31(s)
8	6.70(s)	6.76(s)	6.75(s)	6.74(s)
2′	7.65(d,2.5*)	7.65(d,2.1)	7.68(d,2.5)	7.66(d,2.3)
6′	7.58(d,2.5)	7.58(d,2.1)	7.61(d,2.5)	7.58(d,2.3)
Glc-1		5.18(d,7.5)		5.15(d,7.5)
2		3.30(m)		3.33(m)
3		3.30(m)		3.31(m)
4		3.18(m)		3.20(m)
5		3.47(m)		3.45(m)
6-a		3.42(m)		3.62(m)
6-b		3.70(m)		3.91(m)
Rha-1			5.30(d,1.5)	
2			3.67(m)	
3			3.32(m)	
4			3.20(m)	
5			3.75(m)	
6			1.18(d,6.2)	
Xyl-1				4.20(d,7.2)
2				3.05(m)
3				3.07(m)
4				3.30(m)
5-a				2.95(m)
5-b				3.70(m)
$-OCH_3$	3.85(s)	3.88(s)	3.85(s)	3.90(s)
$-OCH_2O-$	6.02(s)	6.00(s)	6.00(s)	6.03(s)

*Coupling constant in Hz.

β-L-rhamnoside and methyl α-L-rhamnoside [9], which were in agreement with an α-configuration. The connectivity of the sugar residue to the aglycone was deduced from HMBC, which showed a correlation between the rhamnose anomeric proton (δ 5.30) and the isoflavone C-6 (δ 152.5). FAB-MS at *m/z* 327 [$M^+ + 1 - 148$] and ^{13}CNMR data (Table I) also supported the above conclusion. Therefore, compound **3** was assigned to be 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-α-L-rhamnopyranoside. All of these results allow us to report the first isolation of this compound from nature.

Compound **4**, $C_{28}H_{30}O_{16}$, was obtained as a yellowish amorphous powder, and also gave a positive Molish reaction. Its UV and IR were very similar to those of **2**. ^{1}HNMR and ^{13}CNMR spectra showed similarity with those of **1** except for the additional signals of the two sugar moieties. Acid hydrolysis of **4** gave an aglycone, D-glucose and D-xylose. Spectral data for the aglycone were identical to **1**. The nature and stereochemistry of the glycosyl moieties, *viz.* β-D-xylosyl and β-D-glucosyl, were determined from anomeric proton resonances at δ_H 4.20 (1H, d, $J = 7.2$ Hz, xyl-1-H) and δ_H 5.15 (1H, d, $J = 7.5$ Hz, glc-1-H), respectively. The connectivity of the sugar residues to the aglycone nucleus was deduced from HMBC, which showed a correlation between the glucose anomeric proton (δ_H 5.15) and the isoflavone C-6 (δ_C 153.8) carbon. Furthermore, the xylose anomeric proton (δ_H 4.20) showed an HMBC correlation with the glucose C-6, indicating 1–6 linkage between xylose and glucose. The sequence of sugar residues was further confirmed by FAB-MS, which showed loss of xylose (*m/z* 489 [$M^+ -$ xylose]) before loss of glucose (*m/z* 327 [$M^+ -$ xylose $-$ glucose]). Thus, compound **4** was identified as 6,7-dihydroxy-3′-methoxy-4′,5′-methylenedioxyisoflavone 6-*O*-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside, and is reported for the first time.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on an XT4-100X micro-melting point apparatus and are uncorrected. Optical rotation was measured with a DIP-181 instrument. NMR spectra were run on a Bruker AM-400 spectrometer. Mass spectra were obtained on a MAT-112 mass spectrometer. IR and UV spectra were recorded on Perkin–Elmer 599B and Shimadzu UV-250 spectrometers, respectively. TLC was performed on silica gel GF and HRTLC on silica gel H (5–7 μm). Separation and purification were performed by column chromatography on silica gel (160–200 and 200–300 mesh).

Plant Material

The whole plants of *A. grossedentata* Hand.-Mazz. were collected from Fujian Province of China in September 2000, and identified by Prof. Yongtian Zhang, Fujian Institute of Subtropical Botany, China. A voucher specimen (No. 2000912) has been deposited in the author's laboratory, Department of Chemistry, Zhangzhou Teacher's College, Zhangzhou 363000, China.

Extraction and Isolation

Crushed plant material (1.5 kg, 20–30 mesh) was extracted three times with boiling MeOH. The MeOH extract was filtered and concentrated under reduced pressure to give a viscous residue (485 g). This was suspended in H_2O and partitioned with $CHCl_3$ (102 g), EtOAc (48 g) and *n*-BuOH (210 g), successively. A part of the *n*-BuOH fraction (35 g) was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (10:3.5:1) to give four fractions. Recrystallization of the fractions 1–3 from MeOH yielded compounds **1** (45 mg), **3** (26 mg) and **2** (78 mg). Compound **4** (51 mg) was isolated from fraction 4 by reverse phase column chromatography [MeOH–H_2O (2:3) as eluent].

Compound **1**. $C_{17}H_{12}O_7$, yellowish needles (MeOH), mp.171.5–172.3°C; IR (KBr) γ_{max}: 3410 (OH), 1630 (C═O), 1600, 1548, 1450, 1059, 932 cm^{-1}; UVλ_{max}(MeOH): 260, 325 nm; +NaOMe: 258, 350 nm; +$AlCl_3$: 236(sh), 256, 344; +$AlCl_3$/HCl: 261, 325; +NaOAc: 259, 340; +NaOAc/H_3BO_3: 259, 338. EI-MS (*m/z*, 70 ev): 328 (M^+), 327, 176, 153, 152, 125; HR-MS found: 328.0581, required: 328.0583; ^{1}H-NMR and ^{13}C-NMR data are listed in Tables II and I, respectively.

Compound **2**. $C_{23}H_{22}O_{12}$, yellowish amorphous solid (MeOH), mp. 198.2–199.0°C, $[\alpha]_D^{25}$ −55.8 (*c* 0.005, DMSO); IR (KBr) γ_{max}: 3350 (OH), 3026, 1640 (C═O), 1615, 1550, 1447, 1268, 1100, 1050, 935, 890 cm^{-1}; UV λ_{max}(MeOH): 261, 322 nm; FAB-MS (*m/z*): 491 ($M^+ + 1$), 329, 176, 163, 153; HR-MS found: 490.1101, required: 490.1111; ^{1}H-NMR and ^{13}C-NMR data are listed in Tables II and I, respectively.

Compound **3**. $C_{23}H_{22}O_{11}$, yellow powder (MeOH), mp.180.0–181.0°C, $[\alpha]_D^{25}$ −95.2 (*c* 0.005, DMSO); IRγ_{max} (KBr): 3405 (OH), 3026, 1638 (C═O), 1605, 1570,1452, 1275, 1190, 1025, 1075, 935, 840 cm^{-1}; UV λ_{max} (MeOH): 208, 263, 320 nm; FAB-MS (*m/z*): 475 ($M^+ + 1$), 327, 176, 153, 147; HR-MS (*m/z*) found: 474.1165, required: 474.1162; ^{1}H-NMR and ^{13}C-NMR data are listed in Tables II and I, respectively.

Compound **4**. $C_{28}H_{30}O_{16}$, yellowish amorphous powder (MeOH), mp. 208.5–210.1°C, $[\alpha]_D^{25}$ −148.0 (*c* 0.005, DMSO); IR γ_{max}(KBr): 3345 (OH), 3023, 1635 (C═O), 1610, 1546, 1460, 1257, 1218, 1100, 1047, 936, 892 cm^{-1}; UV λ_{max}(MeOH): 261, 325 nm;

FAB-MS (*m*/*z*): 623 ($M^+ + 1$), 489, 327, 176,153,133; HR-MS (*m*/*z*) found: 622.1518, required: 622.1534; ^{1}H-NMR and ^{13}C-NMR data are listed in Tables II and I, respectively.

Acid hydrolysis of **2**, **3**, **4**. These three compounds (5 mg, respectively) were hydrolyzed with 5% H_2SO_4 (5 ml) in MeOH–H_2O (1:1) under reflux for 3 h. The reaction mixture was then partitioned against EtOAc, the EtOAc fraction was concentrated under reduced pressure and recrystallized from MeOH to give the same aglycone, which was identified as **1** by direct comparison of spectral data **1**. The aqueous layers were neutralized with $NH_3{\cdot}H_2O$ and evaporated *in vacuo* and the resulting residues applied to a TLC plate and developed with EtOAc–MeOH–H_2O–HOAc (13:6:3:3). The R_f values of the sugars obtained from **2**, **3** were identical to those of D-glucose, L-rhamnose, respectively, and those obtained from **4** were identical to those of D-glucose and D-xylose, by co-TLC of standard sugars.

Acknowledgements

To Prof. Jia-Ming Liu for assistance during the early part of this work, and to the Educational Foundation of Fujian Province of China and Zhangzhou Teacher's College for financial support.

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