Pergamon

PII: S0031–9422(98)00079–X

Phytochemistry, Vol. 49, No. 4, pp. 1135–1137, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

ANTHRAQUINONE GLYCOSIDES FROM *RHYNCHOTECHUM VESTITUM*‡

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(*Received in revised form* 11 *December* 1997)

Key Word Index—*Rhynchotechum vestitum*; Gesneriaceae; anthraquinone glycosides; damnacanthol-11-*O*-*β*-glucoside; rubiadin-l-methyl ether-3-*O*-*β*-primeveroside; lucidine-3-*O*-*β*-primeveroside; rubiadin-3-*O*-*β*-primeveroside.

Abstract—A new anthraquinone glycoside was isolated from the hydrophilic fraction of the Chinese medicinal plant *Rhynchotechum vestitum*. Its structure was determined as damnacanthol-11-*O*-*β*-glucoside by spectroscopic evidence. The occurrence of rubiadin-l-methylether-3-*O*-*β*-primeveroside, lucidine-3-*O*-*β*-primeveroside and rubiadin-3-*O*-*β*-primeveroside in the plant is reported for the first time.

INTRODUCTION

Rhynchotechum vestitum Hook. f. et Thoms, Chinese name Mao-Xian-Zhu-Ju-Tai, is used as a traditional Chinese folk medicine for the treatment of hepatitis A and B in the South of Yunnan Province of China. Preliminary clinical observations showed that the oral administration of a decoction of the plant can reduce abnormal levels of serum glutamic-pyruvic transaminase and serum bilirubin found in hepatitis patients. In a search for the active constituents of the plant, several new anthraquinones along with known compounds have been reported in our previous papers [1, 2].

We now report on the isolation, from the hydrophilic fraction of the stems of the plant, of a new anthraquinone glycoside, damnacanthol-11-*O*-*β*-glucoside (**1**), in addition to the three known glycosides, rubiadin-l-methyl ether-3-*O*-*β*-primeveroside (**2**), lucidine-3-*O*-*β*-primeveroside (**3**) and rubiadin-3-*O*-*β*-primeveroside (**4**).

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{22}H_{22}O_{10}$ (EIMS, $[M]^+ = m/z$ 446 and ^1H and ^{13}C NMR). UV maxima at 241.3, 274.5 and 335.0 nm and IR bands at 3591, 1670 and 1583 cm^{-1} suggested the presence of a glycoside with a hydroxyanthraquinone aglycone. An isolated signal at δ_H 3.877 (3H, *s*), an AB spin system at δ_H 4.599 and 4.892 (each 1H, *d*, $J = 10.1$ Hz) and the signals at δ_C 162.0 and 162.5 (two oxygenated aromatic carbons) and δ_C 59.2 (one oxygenated secondary aliphatic carbon) indicated an anthraquinone aglycone possessing three substituents, a methoxy, a phenolic hydroxy and a hydroxymethyl. Furthermore, the carbon signals of the glycosyl group suggested a glucose residue attached to one of the hydroxyls. Four aromatic protons in a symmetrical AA′BB′ type pattern and one in an isolated *α* position indicated that one aromatic ring in the anthraquinone was unsubstituted and that the other was substituted at C-1, 2 and 3.

Most anthraquinones isolated from this plant have no substituent in one aromatic ring and always have a carbon substituent at position 2 in the other ring. Therefore, the hydroxymethyl group in **1** was placed at C-2 on comparative and biogenetic grounds [3]. The methoxy group was placed at C-1, since the possibility of its placement at C-3 was ruled out by the absence of a NOE between MeO and H-4 [4] and the δ_C of MeO (62.6 ppm i.e. over 60 ppm) was in agreement with an *ortho*, *ortho*-disubstituted arrangement. Thus, the third substituent, the phenolic hydroxy, had to be at C-3, which was in agreement with the lack of chelation between the phenol group and the quinone carbonyl (^1H and ^{13}C NMR).

In order to locate the glucosylated hydroxy group (OH-3 or OH-11), the ^{13}C NMR data of **1** were compared with those of the reference compound **5**, damnacanthol-3-*O*-*β*-primeveroside [5] (Table 1). Several significant differences involving the δ_C values of **1** were

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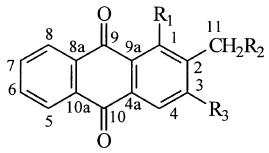
‡ Part 3 in the series 'Chemical studies of Chinese medicinal plant *Rhynchotechum vestitum*'. For part 2 see ref. [2].

	R_1	R_2	R_3
1	OMe	*O*-*β*-glc	OH
2	OMe	H	*O*-*β*-prim
3	OH	OH	*O*-*β*-prim
4	OH	H	*O*-*β*-prim



prim = 6-*O*-*β*-D-xylopyranosyl-D-glucopyranosyl

Table 1. ^{13}C NMR spectral data for compounds **1**–**5** (DMSO-d_6, 100.64 MHz)

C	**1**	**2**	**3**	**4**	**5**
1	162.5	160.1[a]	161.8	161.1[a]	160.7[a]
2	125.2	129.2	123.5	120.7	131.6
3	162.5	160.3[a]	161.8	161.3[a]	160.8[a]
4	109.6	108.4	106.4	105.9	109.2
4a	135.9	132.2[b]	132.7[a]	131.9[b]	132.0[b]
5	126.1	126.4[c]	126.5[b]	126.5[c]	126.2[c]
6	133.4	133.8[d]	134.7[c]	134.6[d]	133.6[d]
7	134.6	134.9[d]	134.8[c]	134.7[d]	134.8[d]
8	126.5	126.9[c]	126.9[b]	126.8[c]	126.7[c]
8a	134.5	134.1[b]	132.8[a]	132.7[b]	134.4[b]
9	180.0	180.8[e]	187.0	187.0	180.5[e]
9a	117.8	120.3	111.4	110.9	120.4
10	182.5	182.5[e]	181.4	181.5	182.2[e]
10a	132.0	134.4[b]	133.8[a]	132.9[b]	135.8[b]
11	59.2	9.5	51.0	8.4	52.0
OMe	62.6	61.1			62.8
1′	103.0	100.4	100.8	100.3	101.0
2′	73.4	73.5	73.2[d]	73.1	73.4
3′	76.6[a]	75.8[f]	75.7[e]	75.7[e]	75.8[f]
4′	70.0	69.4[g]	69.4[f]	69.1[f]	69.6[g]
5′	76.9[a]	76.3[f]	76.3[e]	76.0[e]	76.4[f]
6′	61.0	68.2	68.0	68.0	68.0
1″		104.1	104.0	104.0	104.4
2″		73.5	73.1[d]	73.1	73.4
3″		76.5[f]	75.7[e]	76.3[e]	75.7[f]
4″		69.7[g]	69.3[f]	69.4[f]	69.2[g]
5″		65.7	65.6	65.6	65.6

[a–g] assignments in the same column may be reversed.

Table 2. HMQC and HMBC spectral data for compound **1** (DMSO-d_6)

δ_H	HMQC (δ_C)	HMBC (δ_C)
3.689 (H-6′*β*)	61.0 (C-6′)	
3.877 (OMe)	62.6 (OMe)	162.5 (C-1)
4.310 (H-1′)	103.0 (C-1′)	59.2 (C-11), 76.6, 76.9 (C-3′, 5′), 73.4 (C-2′)
4.599, 4.892 (H-11)	59.2 (C-11)	103.0 (C-1′), 162.5 (C-1,3), 125.2 (C-2)
7.538 (H-4)	109.6 (C-4)	117.8 (C-9a), 125.2 (C-2), 182.5 (C-10), 135.9 (C-4a), 162.5 (C-3)
7.846 (H-6)	133.4 (C-6)	132.0 (C-10a), 126.5 (C-8)
7.925 (H-7)	134.6 (C-7)	126.1 (C-5), 134.5 (C-8a)
8.117 (H-5)	126.1 (C-5)	134.5 (C-8a), 134.6 (C-7), 182.5 (C-10)
8.164 (H-8)	126.5 (C-8)	133.4 (C-6), 132.0 (C-10a), 180.0 (C-9)

noted: (1) C-11 was shifted to lower field (δ 59.2 cf. δ 52.0 for **5**) and C-2 to a higher field (δ 125.2 cf. δ 131.6 for **5**). Such changes were in agreement with the α-carbinyl carbon of the aglycone moiety being lower field shifted (ca. 7 ppm), while the *β*-carbon is higher field shifted after glycosylation [6, 7]. (2) The downfield shift of C-3 and upfield shift of C-9a in **1** relative to the corresponding signals in **5** which can be attributed to the free OH-3 and its shielding effect on the *para* carbon C-9a. (3) C-1′ was shifted to lower field (δ 103.0 cf. δ 101.0 in **5**), this change was due to the decreased shielding of the anomeric carbon from the tertiary pyranoside in C-3 to the secondary pyranoside in C-11 [6].

Furthermore, the δ of the anomeric proton of **1** was shifted to δ 4.310 from δ 5.10 in **5**, which also strongly suggested that C-1′ was linked to an alcoholic oxygen rather than a phenolic one. The above data confirmed that OH-11 was glucosylated and that OH-3 was free. Based on the *J* value (7.8 Hz) of the doublet of H-1′, the anomeric configuration of **1** was *β*. Thus, **1** was deduced as 3-hydroxy-2-hydroxymethyl-l-methoxy-9,10-anthraquinone-11-*O*-*β*-glucopyranoside named as damnacanthol-11-*O*-*β*-glucoside. The proposed structure was further proved by HMBC. The proton at δ 4.310 exhibited cross-peaks with the carbons at δ 59.2, 76.6 and 76.9; the protons at δ 4.599 and 4.892 with the carbons at δ 103.0 and 162.5; the proton at δ 7.538 with the carbons at δ 117.8, 125.2 and 182.5, respectively. Thus, all the ^{1}H and ^{13}C data of the aglycone were unambiguously assigned by means of HMQC and HMBC (Table 2).

Compounds **2**, **3** and **4** also exhibited characteristic anthraquinone bands in their UV spectra. The ^{1}H and ^{13}C NMR signals showed that all sugar moieties were disaccharides. By comparison of the ^{1}H and ^{13}C NMR data of **2**, **3** and **4** with those of reference compound (Table 1), their structures were identified as those of rubiadin-l-methylether-3-*O*-*β*-primeveroside (**2**) [5], lucidin-3-*O*-*β*-primeveroside (**3**) [5, 8, 9] and rubiadin-3-*O*-*β*-primeveroside (**4**) [5] respectively. The occurrence of **2**, **3** and **4** in this plant is reported for the first time.

EXPERIMENTAL

Plant material

Aerial parts of *R. vestitum* were collected at Dali, Yunnan Province of China in the autumn of 1988. A voucher specimen, which was identified by Professor Xi-Wen Li (Kunming Institute of Botany, Chinese Academy of Sciences), is deposited in the Department of Chemistry, Shanghai Second Medical University, Shanghai, China.

Extraction and separation

The dry stems of the plants (7 kg) were cut into small pieces and percolated at room temp. with 95% EtOH. The combined extracts were concentrated under reduced pressure to yield a dark-brown syrup (173 g) which was partitioned with $CHCl_3$ and H_2O. The residue (59 g) from the concentrated aq. layer was subjected to porous resin SIP-1400 (Shanghai Institute of Pharmaceutical Industry) CC eluted with H_2O followed by an H_2O–EtOH gradient. The 30% EtOH eluate (3 g, 1/3 concentrate) was subjected to silica gel CC and eluted with $CHCl_3$–MeOH (4:1) to give munjistin-l-methylether reported previously [2] and three anthraquinone glycosides, **1** (11.3 mg), **2** (36.5 mg) and **3** (151 mg). From the 50% EtOH eluate (1 g, 1/4 concentrate), another anthraquinone glycoside **4** (14.1 mg) was separated by CC on silica gel with $CHCl_3$–MeOH (9:1) in a gradient system.

Damnacanthol-11-O-β-glucoside (**1**). Pale yellow needles, mp 218–220° (MeOH). EIMS *m/z* (rel. int.): 446 $[M]^+$ (10), 269(5), 268(5), 254(12), 85(13), 73(100), 61(49), 60(89); UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 205.3(3.96), 241.3(3.86), 274.5(4.00), 335.0(3.09); IR ν_{max}^{KBr} cm^{-1}: 3596.6 (ν_{OH}, free), 3320 (ν_{OH}, br, intermolecular hydrogen bonded), 3025, 3010 (ν_{C-H}, aromatic), 2920, 2880 (ν_{CH_2}), 1670.1 ($\nu_{C=O}$, unchelated), 1583.3, 1332.6, 1278.6, 1076.1 (ν_{C-O}, primary alc.), 980, 719.3 (δ_{C-H}, 4 adj. ArH); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.944–3.529 (5H, *m*), 3.686 (1H, *d*, $J = 10.9$ Hz, H-6′β), 3.877 (3H, *s*, MeO), 4.310 (1H, *d*, $J = 7.8$ Hz, H-1′), 4.599, 4.892 (each 1 H, *d*, $J = 10.1$ Hz, AB system), 7.538 (1H, *s*, H-4), 7.846–7.925 (2H, *dd*, $J = 7.7$, 6.9 Hz, H-6,7), 8.117, 8.164 (each 1 H, *d*, $J = 7.9$ Hz, H-5 and H-8); ^{13}C NMR: Table 1; HMQC and HMBC: Table 2. The ^{1}H and ^{13}C NMR spectra agreed with a molecular formula of $C_{22}H_{22}O_{10}$.

Rubiadin-l-methylether-3-O-β-primeveroside (**2**). $C_{27}H_{30}O_{13}$, yellow crystals, mp 158–160° (MeOH); Lucidine 3-*O*-β-primeveroside (**3**), $C_{26}H_{28}O_{14}$, yellow needles, mp 208–210° (MeOH-H_2O); Rubiadin-3-*O*-β-primeveroside (**4**), $C_{26}H_{28}O_{13}$, yellow crystals, mp 250–252° (MeOH). All the spectral data (MS, UV, IR, ^{1}H and ^{13}C NMR) of **2**, **3** and **4** agreed with their molecular formulae and characteristic structures.

Acknowledgements—The work was supported by the State Educational Committee and Shanghai Educational Committee, People's Republic of China.

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