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THE ANTHRAQUINONES OF *RHYNCHOTECHUM VESTITUM*

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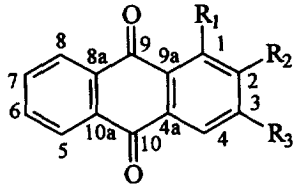
Key Word Index—*Rhynchotechum vestitum*; Gesnericeae; anthraquinones; munjistin-1-*O*-methyl ether; rubiadin-3-*O*-β-glucoside; lucidin-3-*O*-β-glucoside.

Abstract—From the hydrophilic fraction of the Chinese medicinal plant *Rhynchotechum vestitum*, three anthraquinones were isolated. Munjistin-1-*O*-methyl ether is a new compound, its structure was determined on the basis of spectral analysis. The occurrence of rubiadin-3-*O*-β-glucoside and lucidin-3-*O*-β-glucoside in the plant and some of their spectral data are reported. © 1997 Elsevier Science Ltd

INTRODUCTION

Rhynchotechum vestitum Hook. f. et Thoms, Chinese name Mao-Xian-Zhu-Ju-Tai, is distributed in the South of Yunnan Province of China. It is used in traditional Chinese folk medicine for the treatment of hepatitis A and B. Our previous investigations on the chemical constituents of the plant led to the isolation of anthraquinones, including a new compound rhynchotechol, from its lipophilic fraction [1]. In a continuation of our study, we now report on the isolation and structural elucidation of another new anthraquinone, munjistin-1-*O*-methyl ether (**1**), along with two anthraquinone glycosides, rubiadin-3-*O*-β-glucoside (**2**) and lucidin-3-*O*-β-glucoside (**3**), from the hydrophilic fraction of the whole plant.



	R_1	R_2	R_3
1	OMe	COOH	OH
2	OH	CH_3	*O*-β-glc
3	OH	CH_2OH	*O*-β-glc

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RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{16}H_{10}O_6$ ($[M]^+$ at *m/z* 298 and ^{13}C NMR data). A positive colouration with $FeCl_3$ and KOH and the UV maxima of 243.4 and 283.4 nm indicated a hydroxyanthraquinone. The fragment ion peaks at *m/z* 281 $[M\text{-}OH]^+$ and 254 $[M\text{-}CO_2]^+$ in the EI-mass spectrum showed the presence of OH and COOH groups. The peak at *m/z* 280 $[M\text{-}H_2O]^+$ suggested that OH was *ortho* to COOH, since the formation of a six membered transition state with the two substituents can facilitate loss of H_2O. The NMR signal at δ 3.798 (3H, *s*, MeO) showed the presence of the third substituent on the anthraquinone, and δ_H 7.156 (1H, *s*, isolated aromatic proton) and δ_H 7.789–7.871, 8.071–8.133 (4H, *m*, symmetrical AA′, BB′ type of aromatic protons) indicted that one aromatic ring of the anthraquinone was unsubstituted and the other substituted by OH, COOH and OMe groups. The signals at *v* 1675.9 and 1654.7 cm^{-1} and δ_C 179.0 and 183.2 indicated that the two quinone carbonyls were not chelated with the phenol group. Thus, the OH had to occupy a β position of the anthraquinone. The isolated proton at δ 7.156 could be assigned in the α-position (C-4), which was shifted upfield about 0.9 ppm relative to that characteristic of α-H (δ 8.07) because of the electron releasing effects of the OH and OMe groups in the *ortho* (C-3) and *para* (C-1) positions. As no NOE was observed between OMe and H-4, the methoxyl group must be at C-1 [2]. Thus, the structure of **1** was deduced as 2-carboxy-3-hydroxy-1-methoxy-9,10-anthraquinone, which we have named munjistin-1-*O*-methyl ether. The proposed structure of **1** was further elucidated by means of ^1H-^{13}C long range COSY (HMBC). The proton at δ 7.156 exhibited cross-peaks with carbons at δ 117.6, 114.5 and 183.2;

Table 1. ^{13}C NMR spectral data for compounds **1–3** (DMSO-d_6, 100.64 MHz)

C	1	2	3
1	165.3	160.1[a]	161.5[a]
2	117.6	120.8	123.5
3	167.1	161.2[a]	162.0[a]
4	113.1	105.7	106.2
4a	136.3	131.7[b]	132.8[b]
5	125.8	126.5[c]	126.5[c]
6	134.5	134.6[d]	134.7[d]
7	132.7	134.8[d]	134.8[d]
8	126.6	126.7[c]	126.8[c]
8a	135.4	131.8[b]	132.8[b]
9	179.0	188.5	187.0
9a	114.5	110.8	111.3
10	183.2	181.4	181.3
10a	132.0	132.7[b]	133.7[b]
1-OMe	61.4		
2-COOH	172.8		
2-Me		8.4	
2-CH_2OH			50.9
1′		100.2	100.9
2′		73.1	73.2
3′		76.2[e]	75.9[e]
4′		69.3	69.3
5′		77.3[e]	77.3[e]
6′		60.3	60.3

Assignments a, b, c, d, e may be reversed in the same column

the proton at δ 3.798 with the carbon at δ 165.3; the protons at δ 8.071 and 8.133 with the carbons at δ 183.2 and 179.0, respectively. Thus, all the ^1H and ^{13}C data were unambiguously assigned by application of HMQC and HMBC.

Compound **2** exhibited a characteristic anthraquinone UV band. The ^1H and ^{13}C NMR signals of the sugar moiety showed that **2** was a monoglycoside. In the EI-mass spectrum, the aglycone peak at m/z 254 in combination with ^{13}C NMR suggested the formula $C_{15}H_{10}O_4$. The fragment ion peaks resulting from successive loss of two CO indicated the presence of two phenol groups in addition to a methyl group (δ 2.179) [3]. An isolated α aryl proton and four adjacent aryl protons of an AA′,BB′ type in the ^1H NMR spectrum showed that the three groups are 1,2,3-trisubstituted. Since one phenol group was substituted at the α position (δ 13.00), another had to be at a β position, which was confirmed by the ^{13}C NMR signals of the chelated and unchelated carbonyl groups at δ 188.5 and 181.4. By comparison with the published ^{13}C NMR data of methyl-β-D-glucopyranoside [4] and rubiadin-3-*O*-β-primeveroside [5], the aglycone of **2** was elucidated as rubiadin and the sugar moiety was deduced to be 3-*O*-β-glucosyl. Thus, compound **2** was elucidated as 1,3-dihydroxy-2-methyl-9,10-anthraquinone 3-*O*-β-glucopyranoside, which we have named as rubiadin-3-*O*-β-glucoside.

The ^1H and ^{13}C NMR spectra of compound **3** were very similar to those of **2**. Thus, **3** was a 1,2,3-trisubstituted anthraquinone with a chelated 1-hydroxy group and a 3-*O*-β-glucosyl group, but the 2-methyl group in **2** was replaced by a hydroxymethyl groups of which the signals at δ_H 4.559, 4.649 and 4.890 were observed as an ABX system. Thus, the structure of compound **3** was elucidated as 1,3-dihydroxy-2-hydroxymethyl-9,10-anthraquinone 3-*O*-β-glucopyranoside, which we have named as lucidin-3-*O*-β-glucoside. Establishment of above identify was also conducted by comparison with published spectral data [6].

Compounds **2** and **3** were obtained previously as artifacts by partial hydrolysis of rubiadin-3-*O*-β-primeveroside [7] and lucidin-3-*O*-β-primeveroside [6], respectively. This is the first report of their isolation from a natural source.

EXPERIMENTAL

Plant material. Plants of *R. vestitum* (7 kg) were collected at Dali, Yunnan Province of China. A specimen has been verified by Prof. Xi-Wen Li (Kunming Institute of Botany, Chinese Academy of Sciences) and is deposited in the Department of Chemistry, Shanghai Second Medical University.

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

δ_H	HMQC (δ_C)	HMBC (δ_C)				
7.156	113.1	183.2	114.5	117.6	136.3	167.1
(H-4)	(C-4)	(C-10)	(C-9a)	(C-2)	(C-4a)	(C-3)
8.017	125.8	183.2	135.4	132.7	134.5	
(H-5)	(C-5)	(C-10)	(C-8a)	(C-7)	(C-6)	
7.871	134.5	126.6	132.0			
(H-6)	(C-6)	(C-8)	(C-10a)			
7.789	132.7	125.8	135.4			
(H-7)	(C-7)	(C-5)	(C-8a)			
8.133	126.6	179.0	132.0	134.5	132.7	
(H-8)	(C-8)	(C-9)	(C-10a)	(C-6)	(C-7)	
3.798	61.4	165.3				
(OMe)	(OMe)	(C-1)				

Extraction and isolation. The whole plants were percolated with 95% EtOH. The combined extracts were concd *in vacuo* to yield a dark-brown syrup (173 g) which was successively partitioned between $CHCl_3$ and H_2O. The aq. layer was concd. The residue (59 g) was subjected to porous resin SIP-1400 (Shanghai Institute of Pharmaceutical Industry) CC and eluted with H_2O and then with a H_2O–EtOH gradient solvent system. The fr. eluted with 30% EtOH (3.0 g) was further chromatographed on silica gel eluted with $CHCl_3$–MeOH (4:1) to give compound **1** (12.5 mg), while that from 50% EtOH eluate (1.0 g) was subjected to silica gel CC and eluted with $CHCl_3$–MeOH (19:1) to give compound **2** (14 mg) and **3** (9.5 mg).

Munjistin-1-O-*methyl ether* (**1**). Yellow crystals, mp 270° (dec.) (MeOH). EIMS *m/z* (rel. int.): 298(29) $[M]^+$, 281(67) $[M\text{-}OH]^+$, 280(83) $[M\text{-}H_2O]^+$, 267(100) $[M\text{-}OMe]^+$, 254(85) $[M\text{-}CO_2]^+$, 240(95), 225(37), 196(33), 181(16), 168(25), 154(14), 139(32), 113(12), 76(13); UV λ_{max}^{MeOH} nm (log ε): 206.0(3.99), 243.4(4.02), 283.4(4.04); IR ν_{max}^{KBr} cm^{-1}: 3536.9, 3214.8, 1675.9, 1654.7, 1619.9, 1565.9, 1427.1, 1297.9, 1157.1, 943.0, 717.4, 617.1; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.798 (3H, *s*, OMe), 7.156 (1H, *s*, H-4), 7.789 (1H, *dd*, *J* = 7.2, 7.6 Hz, H-7), 7.871 (1H, *dd*, *J* = 7.2, 7.6 Hz, H-6) 8.071 (1H, *d*, *J* = 7.7 Hz, H-5), 8.133 (1H, *d*, *J* = 7.7 Hz, H-8); ^{13}C NMR: Table 1; HMQC and HMBC: Table 2. The ^1H and ^{13}C NMR spectra agreed with a molecular formula of $C_{16}H_{10}O_6$.

Rubiadin-3-O-β-*glucoside* (**2**). Orange yellow crystals, mp 250–253° (MeOH). EIMS *m/z* (rel. int.): 254(100) ($[M]^+$ of aglycone $[Ma]^+$), 253(24) $[Ma\text{-}H]^+$, 239(16), 238(19), 226(11) $[Ma\text{-}CO]^+$, 225(9) $[Ma\text{-}H\text{-}CO]^+$, 197(8) $[Ma\text{-}H\text{-}2CO]^+$, 181(10), 152(14), 141(5), 115(9); UV λ_{max}^{MeOH} nm (log ε): 288.2(4.04), 328.3(3.70); IR λ_{max}^{KBr} cm^{-1}: 3417.3, 2919.7, 2881.2, 1670.1(unchelated CO), 1631.5(chelated CO), 1589.1, 1577.5, 1330.7, 1286.3, 1230.4, 1195.7, 1157.1, 1076.1, 777.2, 756.0, 713.5(4 adj. ArH) and 588.2; ^1H NMR (DMSO-d_6, 400 MHz): δ 2.179 (3H, *s*, Me-2), 3.241–3.538 (5H, *m*), 3.689 (1H, *d*, *J* = 11.6 Hz), 5.140 (1H, *d*, *J* = 5.8 Hz, H-1′glc), 7.402 (1H, *s*, H-4), 7.919–7.929 (2H, *d*, *J* = 4.2 Hz, H-6 and H-7), 8.163–8.266 (2H, *d*, *J* = 4.6 Hz, H-5 and H-8), 12.983 (1H, *s*, HO-1); ^{13}C NMR: Table 1. The ^1H and ^{13}C NMR spectra agreed with a molecular formula of $C_{21}H_{20}O_9$.

Lucidin-3-O-β-*glucoside* (**3**). Yellow crystals, mp 220–221° (MeOH). EIMS *m/z* (rel. int.): 254(100) ($[M]^+$ of aglycone − 16), 207(78), 97(17), 85(16); UV λ_{max}^{MeOH} nm (log ε): 289.2(4.22), 301.6(3.74), 404.2(3.91); IR ν_{max}^{KBr} cm^{-1}: 3384.5, 2919.7, 1668.2(unchelated CO), 1633.4(chelated CO), 1589.1, 1484.9, 1371.2, 1332.6, 1294.0, 1074.2, 715.5(4 adj. ArH), and 540.0; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.152–3.556 (5H, *m*), 3.703 (1H, *d*, *J* = 11.1 Hz), 4.559, 4.649 (each 1H, *d*, *J* = 11.0 Hz), 4.890 (1H, *t*, ω-OH), 5.117 (1H, *d*, *J* = 6.9 Hz, H-1′ glc), 7.474 (1H, *s*, H-4), 7.931–7.950 (2H, *m*, H-6 and H-7), 8.173–8.232 (each 1H, *dd*, *J* = 5.9, 2.8 Hz, H-5 and H-8), 13.051 (1H, *s*, HO-1); ^{13}C NMR: Table 1. The ^1H and ^{13}C NMR spectra agreed with a molecular formula of $C_{21}H_{20}O_{10}$.

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REFERENCES

1. Liu, G. M., Chen, Z. N., Yao, T. R. and Ding, W. G., *Yao Xue Xue Bao* (*Acta Pharmaceutica Sinica*), 1990, **25**, 699.
2. Kawasaki, Y., Goda, Y. and Yoshihira, K., *Shoyakugaku Zasshi*, 1988, **42**, 166.
3. Tu, D. J., Pang, Z. H. and Bi, N. J., *Yao Xue Xue Bao* (*Acta Pharmaceutica Sinica*), 1981, **16**, 631.
4. Gong, Y. H., *^{13}C Chemical Shifts of Organic Natural Products*. Yunnan Scientific and Technological Publishing Co., Kunming, Yunnan Province of China, 1986, P. 398.
5. Kusamba, C., Federici, E., De Vicente, Y. and Galeffi, C., *Fitoterapia*, 1993, **LXIV**, 18.
6. Kawasaki, Y., Goda, Y., Maitani, T., Yoshihira, K. and Takeda, M., *Journal of the Food Hygiene Society of Japan* (*Shokuhin Eiseigaku Zasshi*), 1992, **33**, 563.
7. Briggs, L. H., Beachen, J. F., Cambie, R. C., Dudman, N. P. B., Steggles, A. W. and Rutledge, P. S., *Journal of the Chemical Society, Perkin Transactions I*, 1976, 1789.