Constituents of the Roots of *Rubia yunnanensis*

Meei-Jen LIOU,[a,b] Pei-Lin WU,[a] and Tian-Shung WU*,[a]

Department of Chemistry, National Cheng Kung University,[a] Tainan, Taiwan, Republic of China and Department of Applied Chemistry, Providence University,[b] Sha-Lu, Taichung Hsien, Taiwan, Republic of China.

Received August 23, 2001; accepted October 12, 2001

Four new naphthohydroquinones, rubinaphthins A (1), B (2), C (3), and D (4), together with 11 known compounds were isolated and characterized from the roots of *Rubia yunnanensis.* The structures of 1—4 were elucidated by spectral analysis and chemical transformation.

Key words *Rubia yunnanensis*; Rubiaceae; rubinaphthin A—D; naphthohydroquinone; anthraquinone; flavonoid

Rubia yunnanensis DIELS is a perennial climbing herb belonging to the family Rubiaceae.[1)] It is used as an alternate material for *Rubia cordifolia*, which is a well-known Chinese traditional medicine in Yunnan Province, China. The root extract of this plant has been found to enhance the quantity of ATP in the brain and the heart and to increase leukocytes, and has been used to treat psoriasis.[1)] Several major types of chemical constituents, including cyclopeptides,[2—6)] triterpenoids[1,2,7,8)] and anthraquinones[9,10)] have been isolated from *R. yunnanensis.* In this paper, we report the isolation and structural elucidation of four new naphthohydroquinones, rubinaphthins A—D (**1**—**4**), together with 11 known compounds, from the root of this plant. Those known compounds were identified as 1-hydroxy-2-methylanthraquinone,[11)] 1,3-dihydroxy-2-methylanthraquinone,[12)] xanthopurpurin,[12)] 1,4-dihydroxy-2-hydroxymethylanthraquinone,[13)] 2-hydroxymethylanthraquinone,[14)] 2-methyl-1,3,6-trihydroxyanthraquinone,[11)] 2-methyl-1,3,6-trihydroxyanthraquinone-3-*O*-α-L-rhamnopyranosy1-(1→2)-β-D-glucopyranoside,[9)] 2-methyl-1,3,6-trihydroxyanthraquinone-3-*O*-(6′-*O*-acetyl)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside,[9)] lucidin primeveroside,[15)] munjistin,[12)] and baicalin[16)] by comparing their physical and spectral data with those in the literature, or by direct comparison with authentic samples. 2-Hydroxymethylanthraquinone and baicalin were isolated for the first time from the genus *Rubia.*

Results and Discussion

Rubinaphthin A (**1**) was isolated as an optically active pale yellow powder. It showed a protonated molecular ion peak at *m/z* 367.1033 $[M+H]^+$ in high-resolution (HR)-FAB-MS, corresponding to the elemental composition $C_{17}H_{18}O_9$. A set of four mutually coupled aromatic protons at δ 7.62 (td, J=8.0, 1.2 Hz, H-7), 7.70 (td, J=8.0, 1.2 Hz, H-6), 8.27 (dd, J=8.0, 1.2 Hz, H-8), and 8.34 (dd, J=8.0, 1.2 Hz, H-5), together with two broad D_2O exchangeable signals at δ 12.40 and 14.00 in the ^{1}H-NMR spectrum, and IR absorption of the carbonyl group at 1667 cm^{-1}, provided evidence that **1** is a naphthohydroquinone derivative[11,12,17,18)] containing a hydroxyl group and carboxylic acid substituents. The downfield-shifted hydroxyl signal at δ 14.00, caused by intramolecular hydrogen bonding, led to the placement of the phenolic and carboxylic acid groups at the *ortho*-position. A lone singlet signal at δ 7.43 in the ^{1}H-NMR spectrum coupled with two downfield aromatic carbons at δ 145.7 and 156.1 in the ^{13}C-NMR spectrum indicated the presence of a further oxygenated substituent at C-4. The ^{1}H–^{13}C three-bond correlation between the proton signal at δ 8.27 (H-8) and the carbon signal at δ 156.1 (C-1) in the heteronuclear multiple bond connectivity (HMBC) experiment showed the hydroxyl group to be located at C-1. Thus a 1-hydroxyl-2-carboxyl-4-oxygenated naphthohydroquinone nucleus was established. A typical anomeric proton at δ 4.86 (d, J=7.6 Hz) in the ^{1}H-NMR spectrum, together with the six-carbon signals at δ 61.1, 70.1, 73.9, 76.8, 77.6, and 102.8 in the ^{13}C-NMR spectrum revealed the presence of a β-glucose moiety in the molecule. Furthermore, a nuclear Overhauser effect (NOE) between H-1′ (δ 4.86) and H-3 (δ 7.43) as well as the ^{1}H–^{13}C three-bond correlation between H-1′ and C-4 (δ 145.7) in the HMBC spectrum confirmed a C-4 glucoside. The complete proton and carbon signal assignments were established by correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), HMBC, and rotating frame Overhauser enhancement spectroscopy (ROESY) experiments. According to the above analysis, the structure of

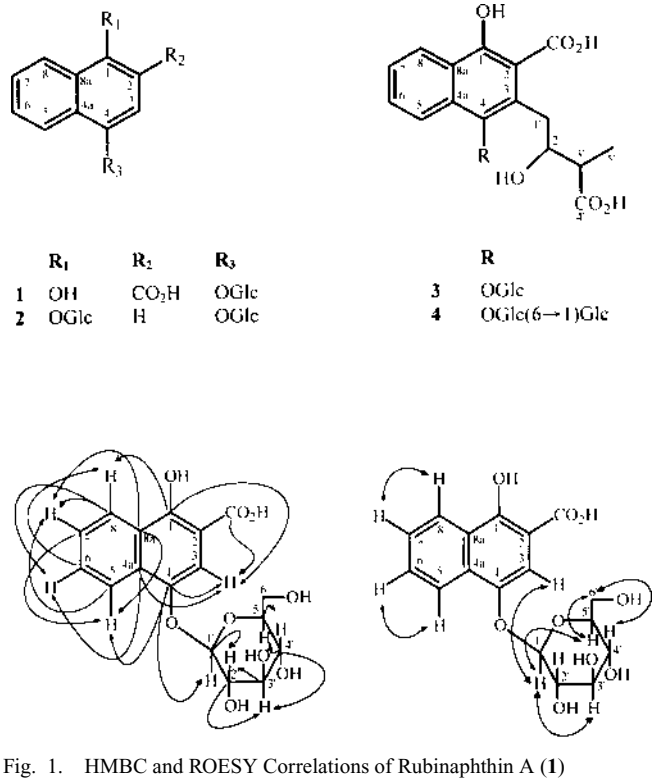

Fig. 1. HMBC and ROESY Correlations of Rubinaphthin A (**1**)

* To whom correspondence should be addressed. e-mail: tswu@mail.ncku.edu.tw

Table 1. ^{1}H, ^{13}C, HMBC, and ROESY Spectral Data of Compounds **1** and **2** in DMSO-d_6

No.	**1**				**2**			
	δ_C	δ_H	HMBC	ROESY	δ_C	δ_H	HMBC	ROESY
1	156.1 s		H-3, 8				H-2, 3, 1′	
2	105.6 s				109.8 d	7.11 (s)		H-1′
3	108.4 d	7.43 (s)		H-1′	109.8 d	7.11 (s)		H-1″
4	145.7 s		H-3, 5, 1′		148.2 s		H-2, 3, 1″	
5	123.0 d	8.34 (dd 8.0, 1.2)	H-7	H-6	122.2 d	8.30 (m)	H-6, 7	H-6
6	129.9 d	7.70 (td 8.0, 1.2)	H-8	H-5	125.9 d	7.51 (m)	H-5, 8	H-5
7	127.4 d	7.62 (td 8.0, 1.2)	H-5	H-8	125.9 d	7.51 (m)	H-5, 8	H-8
8	123.6 d	8.27 (dd 8.0, 1.2)	H-6, 7	H-7	122.2 d	8.30 (m)	H-6, 7	H-7
8a	125.1 s		H-5, 7		126.2 s		H-2, 5, 8	
4a	130.4 s		H-3, 6		126.2 s		H-3, 5, 8	
1′	102.8 d	4.86 (d 7.6)	H-2′	H-3, 3′, 5′	102.1 d	4.87 (d 7.6)	OH-2′	H-2, 3′, 5′
2′	73.9 d	3.16—3.40 (m)	H-3′		73.7 d	3.26—3.42 (m)	OH-3′	OH-3′
						5.45 (d 5.6, OH)		H-3
3′	76.8 d	3.16—3.40 (m)	H-2′	H-1′	76.8 d	3.26—3.42 (m)	H-1′, OH-4′	OH-2′
						5.10 (d 4.8, OH)		H-2
4′	70.1 d	3.16—3.40 (m)	H-3′, 5′	H-6′	70.0 d	3.19 (m)	H-6′, OH-3′	
						5.62 (d 5.2, OH)	H-5′	
5′	77.6 d	3.16—3.40 (m)	H-4′		77.3 d	3.26—3.42 (m)	H-1′, 6′	H-1′, 6′
							OH-6′	OH-4′, 6′
6′	61.1 t	3.51 (dd 10.4, 5.2)			61.0 t	3.48 (m)	OH-6′	
		3.68 (d 10.4)		H-4′		3.72 (dd 10.4, 5.2)		H-5′, OH-6′
						4.58 (t 5.2, OH)		
1″					102.1 d	4.87 (d 7.6)	OH-2″	H-3, 3″, 5″
2″					73.7 d	3.26—3.42 (m)	OH-3″	OH-3″
						5.45 (d 5.6, OH)		H-3″
3″					76.8 d	3.26—3.42 (m)	H-1″, OH-4″	OH-2″
						5.10 (d 4.8, OH)		H-2″
4″					70.0 d	3.19 (m)	H-6″, OH-3″	
						5.62 (d 5.2, OH)		H-5″
5″					77.3 d	3.26—3.42 (m)	H-1″, 6″,	H-1″, 6″
							OH-6″	OH-4″, 6″
6″					61.0 t	3.48 (m)	OH-6″	
		12.40 (s, OH-1)[a]				3.72 (dd 10.4, 5.2)		H-5″, OH-6″
		14.00 (s, CO_2H)[b]				4.58 (t 5.2, OH)		
2-CO_2H	173.0 s		H-3					

a, *b*) Assignments may be reversed.

rubinaphthin A was assigned to be 2-carboxyl-1,4-naphthohydroquinone-4-*O*-β-D-glucopyranoside (**1**).

Rubinaphthin B (**2**) was also isolated as an optically active pale yellow powder. Its molecular formula was determined to be $C_{22}H_{28}O_{12}$ by HR-FAB-MS (*m/z* 485.1654 $[M+H]^+$). Only 11 signals appeared in the ^{13}C-NMR spectrum, suggesting that the structure of **2** is highly symmetrical. The ^{1}H-NMR spectrum of **2** was similar to that of **1** except for the disappearance of the carboxylic acid, which also supported by the absence of a carbonyl group in the ^{13}C-NMR and IR spectra. The signals at δ 7.11 (2H, s), 7.51 (2H, m), and 8.30 (2H, m) in the aromatic region suggested that **2** is a 1,4-naphthohydroquinone. The signal at δ_H 4.87 (2H, d, J= 7.6 Hz) and the signals at δ_C 61.0, 70.0, 73.7, 76.8, 77.3, and 102.1 indicated the presence of a β-glucose unit in the molecule. COSY, HMQC, HMBC, and ROESY experiments gave complete ^{1}H- and ^{13}C-NMR signal assignments. Consequently, the structure 1,4-naphthohydroquinone-1,4-di-*O*-β-D-glucopyranoside (**2**) was assigned to rubinaphthin B.

Rubinaphthin C (**3**), an optically active orange syrup, was determined to have the molecular formula $C_{22}H_{26}O_{12}$ (*m/z* 465.1399 $[M-H_2O+H]^+$). The ^{1}H-NMR spectrum showed the presence of a 1,4-naphthohydroquinone-2-carboxylic acid skeleton.[11,12,17,18] From the ^{13}C-NMR spectrum, in addition to the naphthohydroquinone signals, a 3′-carbroxy-2′-hydroxybutyl partial structure was determined based on the signals at δ_C 15.1, 29.4, 49.6, 83.6, and 181.5 together with δ_H 1.36 (3H, d, J=6.8 Hz, Me-3′), 2.67 (1H, dq, J=8.8, 6.8 Hz, H-3′), 3.04 (1H, dd, J=12.4, 16.4 Hz, H-1′), 3.66 (1H, br d, J=16.4 Hz, H-1′), and 4.46 (1H, br dd, J=12.4, 8.8 Hz, H-2′). The ^{1}H–^{13}C correlation of the two and three bonds between H-1′ (δ 3.66) and C-2 (δ 102.6), C-3 (δ 127.6); H-3′ (δ 2.67) and C-1′ (δ 29.4), C-2′ (δ 83.6), C-4′ (δ 181.5), and C-5′ (δ 15.1) suggested a 3′-carboxy-2′-hydroxybutyl group attached to the C-3 position.[11,12] This phenomenon seemed to be due to the oxidation and hydrolysis of the isoprenyl group. It was also supported by characteristic absorption of the carboxyl (1642 cm^{-1}) group in the IR and carbonyl carbon signal at δ 181.5 in the ^{13}C-NMR spectra. One oxygenated carbon signal at δ 159.9 in the ^{13}C-NMR spectrum indicated that a hydroxyl group was attached at C-1, and it was further confirmed by the correlation between C-1 (δ 159.9) and H-8 (δ 8.38) in HMBC experiments. A glucose unit could be located at C-4, and was further supported by the NOE between the anomeric proton (δ 4.64) and H-5 (δ 8.45). The full assignments of ^{1}H and ^{13}C were established by COSY, HMQC, HMBC, and ROESY NMR experiments. On the basis of the above results, the structure of rubinaph-

Table 2. ^{1}H, ^{13}C, HMBC, and ROESY Spectral Data of Compounds **3** and **4** in CD_3OD

No.	**3**				**4**			
	δ_C	δ_H	HMBC	ROESY	δ_C	δ_H	HMBC	ROESY
1	159.9 s		H-8		159.9 s		H-8	
2	102.6 s		H-1′		102.8 s		H-1′	
3	127.6 s		H-1′		127.8 s		H-1′	
4	141.0 s		H-1′		141.1 s		H-5, 1′, 1″	
5	123.7 d	8.45 (d 8.0)	H-7	H-6, 1″	123.7 d	8.47 (br d 8.4)	H-7	H-6, 1″
6	131.0 d	7.60 (t 7.2)	H-8	H-5	131.2 d	7.68 (td 8.4, 1.2)	H-8	H-5, 7
7	126.5 d	7.44 (t 7.2)	H-6	H-8	126.7 d	7.52 (td 8.4,1.2)	H-5	H-6, 8
8	125.1 d	8.38 (d 8.0)		H-7	124.9 d	8.35 (br d 8.4)	H-6	H-7
8a	126.2 s		H-5		125.5 s		H-5, 7	
4a	133.6 s		H-6, 8		133.5 s		H-6, 8	
1′	29.4 t	3.04 (dd 12.4, 16.4)	H-3′	H-1′, 3′	29.2 t	3.14 (br t 16.8)		
		3.66 (br d 16.4)		H-1′, 2′		3.67 (m)		
2′	83.6 d	4.46 (br dd 12.4, 8.8)	H-3′, 5′	H-1′, 3′, 5′	83.6 d	4.58 (br t 9.2)	H-1′, 5′	
3′	49.6 d	2.67 (dq 8.8, 6.8)	H-5′	H-1′, 2′, 5′	48.4 d	2.76 (br)	H-5′	
4′	181.5 s		H-3′, 5′		180.8 s		H-5′	
5′	15.1 q	1.36 (d 6.8)	H-3′	H-2′, 3′	14.9 q	1.39 (d 6.8)		
1″	106.8 d	4.64 (d 7.6)	H-2″	H-5, 3″, 5″	106.4 d	4.67 (d 7.6)	H-2″	H-5, 3″, 5″
2″	75.6 d	3.58 (br t 7.6)	H-4″		75.7 d	3.61 (m)	H-3″	H-4″
3″	78.0 d	3.10 (br)	H-2″, 4″, 5″	H-1″	77.9 d	3.43 (m)	H-2″, 4″	H-1″, 5″
4″	71.6 d	3.38—3.43 (m)	H-5″	H-6″	71.5 d	3.43 (m)	H-3″, 6″	H-2″, 6″
5″	78.2 d	3.38—3.43 (m)	H-4″	H-1″	76.9 d	3.19—3.32 (m)		H-1″, 3″, 6″
6″	62.7 t	3.68 (dd 12.0, 3.2)			70.3 d	3.71 (dd 11.2, 6.0)	H-1‴	H-1‴
		3.81 (dd 12.0, 2.0)		H-4″		4.09 (dd 11.2 , 2.0)		H-4″, 5″
1‴					104.8 d	4.26 (d 7.6)	H-2‴	H-6″, 5‴
2‴					75.0 d	3.16 (dd 8.4, 7.6)	H-3‴	
3‴					77.8 d	3.19—3.32 (m)	H-2‴, 5‴	
4‴					71.7 d	3.19—3.32 (m)	H-5‴	H-6‴
5‴					78.0 d	3.19—3.32 (m)		H-1‴, 6‴
6‴					62.8 t	3.58 (dd 11.6, 4.8)		H-4‴
						3.82 (dd 11.6, 1.6)		H-5‴
2-CO_2H	172.7 s				172.9 s			

thin C was assigned to be 2-carboxyl-3-(3′-carboxyl-2′-hydroxy)butyl-1,4-naphthohydroquinone-4-*O*-*β*-D-glucopyranoside (**3**).

Rubinaphthin D (**4**) was obtained as an optically active orange syrup. Its molecular formula was determined to be $C_{28}H_{36}O_{17}$ by HR-FAB-MS (*m/z* 627.1924 $[M-H_2O+H]^+$). The ^{1}H- and ^{13}C-NMR spectra were very similar to those of **3** except for the presence of an additional glucose at δ_H 4.26 (1H, d, J=7.6 Hz, anomeric H-1‴) and δ_C 70.3, 71.7, 75.0, 77.8, 78.0, and 104.8. Because the carbon signal of C-6 in one of two glucoses was shifted downfield to δ 70.3, which had a three-bond correlation with δ_H 4.26 (H-1‴) in the HMBC spectrum, the sugar linkage was concluded to be glucopyranosyl (1→6) glucopyranoside. Hydrolysis of **4** with *β*-glucosidase gave two products. One was identical to **3** by comparison of the full two-dimensional (2D) NMR spectra, and the other was confirmed to be glucose by direct comparison of their TLC. Therefore the structure of rubinaphthin D was assigned to be 2-carboxyl-3-(3′-carboxyl-2′-hydroxy)butyl-1,4-naphthohydroquinone-4-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**4**).

Experimental

General Procedures Melting points (Yanagimoto apparatus) are uncorrected. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. UV spectra in MeOH solution were obtained on a Hitachi UV-3210 spectrophotometer. IR spectra in KBr disks were recorded on a Shimadzu FTIR-8501 spectrometer. ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker AMX-400 or a Varian Unity plus 400 NMR spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a VG 70-250S mass spectrometer.

Plant Material The roots of *R. yunnanensis* used in this investigation were collected in Jiu Jiang Xian, Yunnan Province, Peoples' Republic of China in July 1996, and identified by Professor C. S. Kuoh. A voucher specimen (TSWu 96021) has been deposited in the Herbarium of the National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation The dried root of *R. yunnanensis* (4.2 kg) was extracted with methanol (30 l). The combined methanol extracts were concentrated under reduced pressure to give a brown syrup (1.0 kg) which was partitioned in succession between $CHCl_3$ and water, and then EtOAc and water. Then the water layer was extracted with *n*-BuOH. The $CHCl_3$ layer (185.0 g) was subjected to column chromatography over silica gel eluted with a gradient of $CHCl_3$ and MeOH to afford 21 fractions. Fractions 4 and 5 were combined and chromatographed on a silica gel column and eluted with *n*-hexane–EtOAc (40 : 1) to give 1-hydroxy-2-methylanthraquinone (10.0 mg). Fractions 12—14 were rechromatographed on a silica gel column eluted with $CHCl_3$–MeOH (100 : 1) to yield 1,3-dihydroxy-2-methylanthraquinone (2.0 mg), xanthopurpurin (20.0 mg), 1,4-dihydroxy-2-hydroxymethylanthraquinone (1.0 mg), and 2-hydroxymethylanthraquinone (1.0 mg), in succession. Fractions 15—17 were also combined and subjected to Diaion HP-20 column chromatography eluted with a gradient of H_2O and MeOH to give 2-methyl-1,3,6-trihydroxyanthraquinone (10.0 mg).

The EtOAc layer (83.0 g) was separated by Diaion HP-20 column chromatography and eluted with a gradient of H_2O and MeOH to afford 21 fractions. Further RP-18 column chromatography of fractions 12—14 eluted with a gradient of H_2O and MeOH furnished rubinaphthin A (**1**) (10.0 mg). Fractions 15—17 were combined and chromatographed on an RP-18 column eluted with H_2O–MeOH (50 : 50) to give munjistin (1.0 mg) and rubinaphthin C (**3**) (3.0 mg). Fraction 21 was also rechromatographed on an RP-18 column eluted with a gradient of H_2O and MeOH and afforded 2-methyl-1,3,6-trihydroxyanthraquinone-3-*O*-*α*-L-rhamnopyranosy1-(1→2)-*β*-D-glucopyranoside (5.0 mg), and 2-methyl-1,3,6-trihydroxyanthraquinone-3-*O*-

(6′-*O*-acetyl)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (5.0 mg), respectively.

The *n*-BuOH layer (120.0 g) was chromatographed on Diaion HP-20 and eluted with H_2O–MeOH in a gradient to afford 40 fractions. Fraction 22 was recrystallized with MeOH and yielded a further quantity of rubinaphthin A (**1**) (2.0 g). Fraction 26 was further chromatographed on RP-18 gel and eluted with a gradient of H_2O–MeOH to give rubinaphthin D (**4**) (12.0 mg). Fraction 30 was also chromatographed on RP-18 gel and eluted with H_2O–MeOH (50 : 50) to afford lucidin primeveroside (3.0 mg), baicalin (3.0 mg), and munjistin (2.0 mg).

The water layer (530.0 g) was chromatographed on an active carbon column, eluted with a gradient of H_2O–MeOH, and then eluted with MeOH–$CHCl_3$ by a gradient elution to give 14 fractions. Fractions 11—13 were combined, passed over a Sephadex LH-20 column, and eluted with a gradient of H_2O–MeOH to provide additional rubinaphthin A (**1**) (3.0 mg) and rubinaphthin B (**2**) (15.0 mg).

Rubinaphthin A (**1**): Pale yellow powder (MeOH), $C_{17}H_{18}O_9$, mp: 194—195 °C, $[\alpha]_D$ −96.0° (c=0.15 , MeOH). UV λ_{max} (MeOH) nm (log ε): 355 (3.73), 312 (3.49), 258 (4.07), 244 (4.09), 230 (4.07), 224 (4.09), 213 (4.06). IR (KBr) cm^{-1}: 3613 (OH), 3480 (OH), 3343 (OH), 2944, 2884, 1667 (C=O), 1634, 1603. ^{1}H- and ^{13}C-NMR: see Table 1. HR-FAB-MS m/z: 367.1033 $[M+H]^+$ (Calcd for $C_{17}H_{19}O_9$: 367.1029). FAB-MS m/z (rel. int. %): 367 ($[M+H]^+$, 6), 366 (M^+, 8).

Rubinaphthin B (**2**): Pale yellow powder (MeOH), $C_{22}H_{28}O_{12}$, mp: 272—273 °C, $[\alpha]_D$ −183.3° (c=0.075, MeOH). UV λ_{max} (MeOH) nm (log ε): 326 (sh, 3.63), 313 (sh, 3.79), 299 (3.88), 232 (4.33), 228 (4.33), 221(4.34). IR (KBr) cm^{-1}: 3362 (br OH), 2919, 2882, 1598. ^{1}H- and ^{13}C-NMR: see Table 1. HR-FAB-MS m/z: 485.1654 $[M+H]^+$ (Calcd for $C_{22}H_{29}O_{12}$: 485.1659). FAB-MS m/z (rel. int. %): 485 ($[M+H]^+$, 1), 484 (M^+, 7).

Rubinaphthin C (**3**): Orange syrup (MeOH), $C_{22}H_{26}O_{12}$, $[\alpha]_D$ −159.5° (c=0.47, MeOH). UV λ_{max} (MeOH) nm (log ε): 377 (sh, 3.43), 360 (3.51), 307 (sh, 3.27), 295 (sh, 3.59), 284 (sh, 3.49), 262 (4.29), 255 (4.27), 215 (4.28). IR (KBr) cm^{-1}: 3440 (br OH), 2857, 1642 (C=O), 1636, 1578, 1177, 1102. ^{1}H- and ^{13}C-NMR: see Table 2. HR-FAB-MS m/z: 465.1399 $[M-H_2O+H]^+$ (Calcd for $C_{22}H_{25}O_{11}$: 465.1397). FAB-MS m/z (rel. int. %): 465 ($[M-H_2O+H]^+$, 8). CD (c=1.6103×10^{-4} M, MeOH): $[\theta]_{214}$ +11950, $[\theta]_{220}$ +12680, $[\theta]_{236}$ +6160, $[\theta]_{254}$ +13160, $[\theta]_{278}$ 0, $[\theta]_{292}$ −1836, $[\theta]_{305}$ 0, $[\theta]_{313}$ +514.6, $[\theta]_{319}$ 0, $[\theta]_{362}$ −3262, $[\theta]_{399}$ 0, $[\theta]_{419}$ +297.6.

Rubinaphthin D (**4**): Dark orange syrup (MeOH), $C_{28}H_{36}O_{17}$, $[\alpha]_D$ −48.0° (c=0.38, MeOH). UV λ_{max} (MeOH) nm (log ε): 378 (sh, 3.59), 361 (3.68), 310 (sh, 3.53), 296 (sh, 3.67), 284 (sh, 3.75), 262 (4.44), 254 (4.42), 218 (4.44). IR (KBr) cm^{-1}: 3425 (br OH), 2936, 1650 (C=O), 1574, 1507,1175, 1071. ^{1}H- and ^{13}C-NMR: see Table 2. HR-FAB-MS m/z: 627.1924 $[M-H_2O+H]^+$ (Calcd for $C_{28}H_{35}O_{16}$: 627.1925). FAB-MS m/z (rel. int. %): 627 ($[M-H_2O+H]^+$, 7), 626 ($[M-H_2O]^+$, 13). CD (c=1.199×10^{-4} M, MeOH): $[\theta]_{224}$ +16840, $[\theta]_{236}$ +10550, $[\theta]_{252}$ +20100, $[\theta]_{280}$ 0, $[\theta]_{293}$ −2035, $[\theta]_{304}$ 0, $[\theta]_{312}$ +1023, $[\theta]_{323}$ 0, $[\theta]_{361}$ −2650, $[\theta]_{397}$ 0.

Hydrolysis of Rubiaphthin D (4) Rubiaphthin D (**4**) (10.31 mg) was hydrolyzed with β-glucosidase (5000 units) in H_2O (6 ml), first warmed for 3 h at 37 °C and then stored for 64 h at room temperature. Two hydrolyzed products were isolated by Sephadex LH-20 column chromatography, one of which was identical to rubinaphthin C (**3**) by comparison of ^{1}H- and ^{13}C-NMR spectra. The other compound was identical to glucose on TLC on cellulose [*Rf* value of glucose, 0.34, *n*-BuOH–pyridine–H_2O (60 : 40 : 30)].

Acknowledgment We thank the National Science Council, Republic of China (NSC 88-2113-M-006-004) for supporting this research. We are thankful to Miss S. Zhang (Institute of Materia Medica, Chinese Academy of Medicinal Sciences, Beijing, People's Republic of China) and Professor C. S. Kuoh (Department of Biology, National Cheng Kung University, Tainan, Taiwan) for the plant collection and identification, respectively.

References and Notes

1) Xu X. Y., Zhou J. Y., Fang Q. C., *J. Chin. Pharm. Sci*., **4**, 157—159 (1995), and references cited therein.
2) Cheng Z., Hao X. J., Chen C. X., Jun Z., *Acta Botan. Yun*., **14**, 114—114 (1992).
3) Tan N. H., Wang D., Zhang H. G., Chen C. G., Zhou J., Zhao S. X., *Chin. J. Mag. Reson*., **10**, 245—250 (1993).
4) Zou C., Hao X. J., Zhou J., *Acta Botan. Yun*., **15**, 399—402 (1993).
5) He M., Zou C., Hao X. J., Zhou J., *Acta Botan. Yun*., **15**, 408—408 (1993).
6) Shen X. Y., Wu H. M., He M., Hao X. J., Zhou J., *Acta Chim. Sin*., **54**, 1194—1199 (1996).
7) Zou C., Hao X. J., Chen C. X., Zhou J., *Acta Botan. Yun*., **15**, 89—91 (1993).
8) Xu X. Y., Zhou J. Y., Fang Q. C., *Acta Pharm. Sin*., **29**, 237—240 (1994).
9) Chen Y. Q., Luo Y. R., *Youji Huaxue*, **11**, 523—524 (1991).
10) Chen B., Chen S., Dong X., *Nat. Prod. Res. Devel*., **4**, 5—10 (1992).
11) Itokawa H., Qiao Y., Takeya K., *Phytochemistry*, **30**, 637—640 (1991).
12) Itokawa H., Qiao Y., Takeya K., *Phytochemistry*, **28**, 3465—3468 (1989).
13) Berg W., Hesse A., Herrmann M., Kraft R., *Pharmazie*, **30**, 330—334 (1975).
14) Chang P., Lee K. H., *Phytochemistry*, **23**, 1733—1736 (1984).
15) Itokawa H., Mihara K., Takeya K., *Chem. Pharm. Bull*., **31**, 2353—2358 (1983).
16) Wang Y. Q., Matsuzaki K., Takahashi K., Okuyama T., Shibata S., *Chem. Pharm. Bull*., **36**, 3206—3209 (1988).
17) Itokawa H., Ibraheim Z. Z., Qiao Y., Takeya K., *Chem. Pharm. Bull*., **41**, 1869—1872 (1993).
18) Qiao Y. F., Takeya K., Itokawa H., Iitaka Y., *Chem. Pharm. Bull*., **38**, 2896—2898 (1990).