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Rourinoside and rouremin, antimalarial constituents from *Rourea minor*

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Abstract

Bioassay-directed fractionation of the antimalarial active $CHCl_3$ extract of the dried stems of *Rourea minor* (Gaertn.) Aubl. (Connaraceae) liana led to isolation of two glycosides, rourinoside (**1**) and rouremin (**2**), as well as five known compounds, 1-(26-hydroxyhexacosanoyl)-glycerol (**3**), 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*-8*Z*)-2-*N*-(2′-hydroxypalmitoyl)-octadecasphinga-4,8-dienine, 9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoic acid, dihydrovomifoliol-9-β-D-glucopyranoside, and β-sitosterol glucoside. Compounds **1**–**3** showed weak *in vitro* activities against *Plasmodium falciparum*. Their structures and stereochemistry were elucidated by spectroscopic methods and selected enzyme hydrolysis.

Keywords: *Rourea minor*; Connaraceae; Rourinoside; Rouremin; Antimalarial activity; 2D NMR

1. Introduction

Rourea minor (Gaertn.) Aubl. (Connaraceae), a woody vine (liana), is known under a number of synonyms: *Aegiceras minus* Gaertn., *Connarus microphyllus* (Hook. et Arn.) Planch., *Pterotum procumbens* Lour., *R. santaloides* (Vahl) W. et A., *R. caudatum* Planch., *R. microphylla* (Hook. et Arn.) Planch., and *Santaloides microphyllum* (Hook. et Arn.) Schell (Wu et al., 1984; Wu and Raven, 2003). The leaves of this plant have been used in Chinese folk medicine as a styptic to treat minor abrasions and lesions (Jiangsu, 1986). Phytochemically, it has been reported to contain triterpenes, steroids, quinones, flavanes, flavones, anthracenediones, and fatty acids (Ramiah et al., 1976; Jiang et al., 1990). As part of the search for biologically active compounds from plants of Vietnam and Laos in our ICBG (International Cooperative Biodiversity Group) project (Soejarto et al., 1999), the stem sample of this liana, known as "Khua Ma Vo" and a decoction used locally to treat dengue fever (Bouamanivong, 1999, personal communication), was collected in Laos for biological evaluation. The $CHCl_3$ soluble fraction of this plant material showed *in vitro* inhibitory effect on *Plasmodium falciparum*. Previously, we reported on the isolation of antimalarial compounds from *Rhaphidophora decursiva* and *Ficus fistulosa* (Zhang et al., 2001, 2002a,b; He et al., 2005). Bioassay-directed fractionation of the aforementioned *R. minor* $CHCl_3$ extract by repeated flash column chromatography on Si gel, followed by Sephadex LH-20 and RP-18 column chromatography

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afforded two new compounds, rourinoside (**1**) and rouremin (**2**), as well as five known compounds, 1-(26-hydroxyhexacosanoyl)-glycerol (**3**) (Babady-Byla and Herz, 1996), 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*-8*Z*)-2-*N*-(2′-hydroxypalmitoyl)-octade casphinga-4,8-dienine (Shibuya et al., 1993), 9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoic acid (Kim et al., 2002), dihydrovomifoliol-9-β-D-glucopyranoside (Jakupovic et al., 1991), and β-sitosterol glucoside (Kojima et al., 1990) (Fig. 1). The known isolates were identified by comparing their spectroscopic data with those reported in the literature. Compounds **1**–**3** were shown to exhibit moderate antimalarial activities (350 times less than those of chloroquine). The present paper describes the bioassay-directed isolation of these compounds and the structural elucidation of the two new natural products.

2. Results and discussion

Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{20}$ −21.3° (MeOH), with a molecular formula of $C_{27}H_{38}O_{13}$ based on HRTOFMS (*m/s* 569.2268 $[M-H]^-$, calcd. 569.2234). The UV absorptions at λ_{max} 225, and 278 nm and the IR spectroscopic data at ν_{max} 1595, 1500 cm^{-1} showed the presence of aromatic groups, and the IR data of ν_{max} 3430 cm^{-1} indicated hydroxyl groups. The ^1H NMR spectrum of **1** showed the presence of three aromatic methoxyl groups at δ 3.86 (9H, s, $CH_3 \times 3$) and five aromatic protons at δ 6.09 (1H, dd, $J = 1.6$, 8.1 Hz, H-6), δ 6.75 (1H, dd, $J = 8.1$ Hz, H-5) and δ 7.08 (1H, d, $J = 1.6$ Hz, H-2), as well as δ 6.57 (2H, s, H-2′ and H-6′) (Table 1). In the ^1H-^1H COSY spectrum of **1**, couplings among H-8 [δ 4.25 (1H, dt, $J = 3.5$, 6.7 Hz)], H-7 [δ 5.13 (1H, d, $J = 6.7$ Hz)], and H_2-9 [δ 3.18 (2H, m)] were observed, indicating the presence of a glyceryl moiety. The existence of a 3-hydroxy-propyl group and a glucosyl group were also discerned from the ^1H-^1H COSY spectrum. On the basis of this evidence, as well as by comparison with the literature data (Zhang et al., 2003; Luyengi et al., 1996; Li et al., 1998), **1** was determined to be a neolignan glycoside. Compound **1** was shown to have very similar ^1H and ^{13}C NMR spectroscopic data to those of 4, 9, 9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-7-*O*-β-D-glucopyranoside, a neolignan glycoside, previously reported from *Lonicera gracilipes* var. *glandulosa* (Caprifoliaceae) (Matsuda and Kikuchi, 1996). It differs from the neolignan glycoside only by addition of a methoxy group at C-5′. The glucosyl group in **1** was assigned to C-7 based on the analysis of 2D NMR spectroscopic data, especially the HMBC spectrum (Fig. 2). The HMBC spectrum of **1** showed ^3J correlations between the anomeric proton signal at δ 4.60 (1H, d, $J = 7.7$ Hz, H-1″) and the ^{13}C resonance at δ 81.4 (C-7), and between the ^{13}C resonance at δ 104.4 (C-1″) and the proton resonance at δ 5.13 (1H, d, $J = 6.7$ Hz, H-7), thereby locating the glucosyl at C-7. The HMBC correlation of the proton resonance at δ 4.25 (1H, dt, $J = 3.5$, 6.7 Hz, H-8) to the ^{13}C resonance at δ 139.4 (C-4′) assigned the 4-(3-hydroxy-propyl)-2, 6-dimethoxy-phenoxy group at C-8. The locations of the three methoxy groups were further confirmed by the presence of ROE correlations between the methoxy proton [δ 3.86 (9H, s)] and their adjacent proton signals [(δ 7.08, 1H, d, $J = 1.6$ Hz, H-2), (δ 6.57, 2H, s, H-2′ and -6′)] in the ROESY spectrum. The stereochemistry of C-7 and C-8 was assigned as *threo* according to the coupling constant between H-7 and H-8 ($J = 6.7$ Hz) (Braga et al., 1984). The absolute stereochemistry of **1** was obtained on the basis of CD spectroscopic evidence. The positive Cotton effect at 242 (Δε +1.25) nm indicated that **1** has the *S* configurations at both C-7 and C-8 (Matsuda and Kikuchi, 1996). Thus, the structure of **1** was established as 1*S*,2*S*-1-(4-hydroxy-3-methoxy-phenyl)-2-[4-(3-hydroxy-propyl)-2,6-dimethoxy-phenoxy]-propane-1,3-diol 1-*O*-β-D-glucopyranoside, and was given the trivial name of rourinoside.

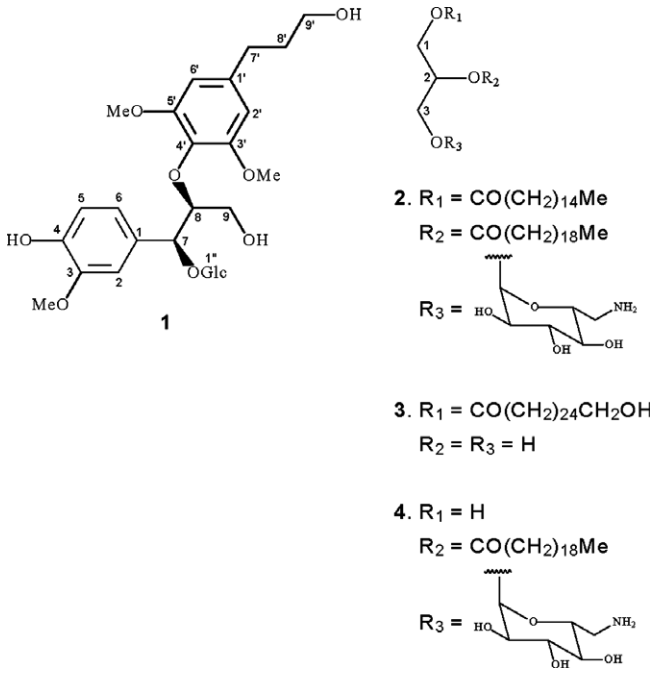

Fig. 1. Structures of compounds **1**–**4**.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{20}$ −4.1° (MeOH), with a molecular formula being determined as $C_{45}H_{87}NO_9$ by HRTOFMS ($[M+Na]^+$ *m/z* 808.6274, calcd. 808.6279; $[M+Li+H]^+$ *m/z* 793.6616, calcd. 793.6619). The IR spectrum of **2** showed the presence of a hydroxyl and an ester group (3400 and 1730 cm^{-1}). In the ^1H NMR spectrum of **2** taken in pyridine, the characteristic signals belonging to two terminal methyl signals at δ 0.89 (6H, t, $J = 6.6$ Hz, Me-16‴ and Me-20″) and methylene groups at δ 1.23 ($n \times H$, brs), δ 1.66 (4H, p, $J = 6.9$ Hz), δ 2.39 (2H, t, $J = 7.5$ Hz) and δ 2.42 (2H, t, $J = 7.5$ Hz), were ascribed to two fatty acid ester chains (Table 1). The carbonyl carbon signals of the two fatty ester groups were clearly observed [δ 172.3 (C-1″) and δ 172.4 (C-1‴)] when the NMR spectra were

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1**, **2** and **4** (500 MHz, *J* in Hz given in parentheses)

1			**2**					**4**	
C	δ_C	δ_H	C	δ_C		δ_H		δ_C	δ_H
	CD_3OD	CD_3OD		Pyridine	DMSO	Pyridine	DMSO	DMSO	DMSO
1	131.0		**1**	63.6	62.5	4.78 dd (2.7, 11.7) 4.57 dd (7.2, 10.1)	4.34 dd (1.2, 11.8) 4.14 dd (7.4, 11.9)	62.9	3.20 – 3.50
2	111.6	7.08 d (1.6)	**2**	69.8	69.7	5.71 m	5.13 m	69.6	3.67 m
3	147.7		**3**	66.5	64.6	4.66 dd (4.9, 10.6) 3.95 dd (5.9, 10.7)	3.89 dd (5.8, 10.4) 3.40 dd (5.6, 10.4)	65.3	3.91 dd (5.0, 10.7) 3.51 dd (5.0, 10.5)
4	146.4		**Sugar**						
5	114.8	6.75 d (8.1)	**1′**	100.4	98.3	5.33 d (3.4)	4.56 d (3.3)	98.8	4.57 (3.7)
6	120.4	6.09 dd (1.6, 8.1)	**2′**	73.6	71.6	4.12 dd (3.6, 9.5)	3.18 m	71.8	3.16 (3.4, 9.2)
7	81.4	5.13 d (6.7)	**3′**	75.1	72.8	4.55 t (9.2)	3.35 m	72.8	3.35 m
8	86.1	4.25 dt (3.51, 6.7)	**4′**	75.2	74.3	3.85 t (9.3)	2.90 m	74.3	2.90 m
9	60.2	3.18 m	**5′**	70.9	68.4	5.05 m	3.78 ddd (1.5, 1.5, 11.2)	68.3	3.80 brd (11.0)
1′	133.5		**6′**	54.9	54.7	4.21 bd (14.3) 3.68 dd (7.6, 14.5)	2.92 m 2.56 dd (6.5, 13.9)	54.8	2.92 m 2.56 dd (6.6, 13.9)
2′	105.9	6.57 s	**Fattyl**						
3′	153.4		**1″**	173.4	172.3			174.6	
4′	139.4		**2″**	34.5	33.3	2.42 t (7.5)	2.26 m	33.3	2.29 t (7.0)
5′	153.4		**3″**	24.4	24.4	1.66 p (6.9)	1.49 brs	24.3	1.51 m
6′	105.9	6.57 s	**4″**	29.7	28.7			28.6	
7′	32.5	2.65 t (7.4)	**5″**	29.7	28.8			28.8	
8′	34.6	1.83 q (8.3)	**6″**	29.9	28.9			28.9	
9′	61.2	3.57 t (6.4)	**7″**	29.45	28.6	1.23 brs	1.23 brs	28.6	1.24 brs
Glucosyl			**8″**	29.4	28.4			28.4	
1″	104	4.60 d (7.7)	**9″–17″**	30.0	29.0			28.9	
2″	74.7	3.32 m	**18″**	32.2	31.2			31.2	
3″	77.2	3.32 m	**19″**	23.0	22.0			22.0	
4″	70.5	3.32 m	**20″**	14.3	13.8	0.89 t (6.6)	0.85 t (6.1)	13.8	0.86 t (7.0)
5″	76.9	3.18 m	**1‴**	173.4	172.4				
6″	61.6	3.75 dd (2.2, 11.8) 3.61 dd (5.2, 12.0)	**2‴**	34.3	33.5	2.39 t (7.5)	2.26 m		
			3‴	25.3	24.4	1.66 p (6.9)	1.49 brs		
OMe			**4‴**	29.7	28.7				
	55.7 × 2	3.86 s × 3	**5‴**	29.7	28.9				
	55.5		**6‴**	29.9	28.6				
			7‴	30.0	28.6	1.23 brs	1.23 brs		
			8‴	30.0	28.4				
			9‴–13‴	30.1	29.0				
			14‴	32.2	31.2				
			15‴	23.0	22.0				
			16‴	14.3	13.8	0.89 t (6.6)	0.85 t (6.1)		

measured in DMSO. Additional groups including a glyceroyl group and a glycosyl moiety were also deduced for **2** by analysis of the ^{1}H-^{1}H COSY and HMQC spectral data. Compound **2** was therefore deduced to be a glyceroglycolipid (Baruah et al., 1983; Murakami et al., 1990; Jung et al., 1996). The glycosyl moiety in **2** was shown to be a six-carbon sugar unit according to the ^{1}H and ^{13}C NMR spectroscopic data. However, the C-6′ signal appeared abnormally upfield (δ 54.7), suggesting the sugar unit of **2** to be an aminosugar (Yamaoka et al., 1974) in which the NMR signal of a sugar unit would be affected when the 6-hydroxyl group was changed to a 6-ammonium moiety of aminosugar derivatives (Yamaoka et al., 1974). In comparison with the reported value, the glycosyl moiety in **2** had identical ^{13}C NMR data as those reported for a 6-amino-6-deoxy-β-D-glucopyranosyl moiety (Yamaoka et al., 1974). This finding was confirmed by a HMBC experiment. Analysis of the HMBC spectrum showed the aminosugar unit to be attached to C-3 of **2**, due to the presence of a long-range correlation between H-3 and the anomeric carbon signal of the aminosugar unit. The two fatty acid ester groups in **2** were assigned to C-1 and C-2, respectively, based on the presence of HMBC correlations of H-1 and H-2 to the carbonyl carbons (C-1″ and 1‴) of the fatty acid ester groups. Compound **2** was thus determined to be a diacyl glyceryl aminoglycoside.

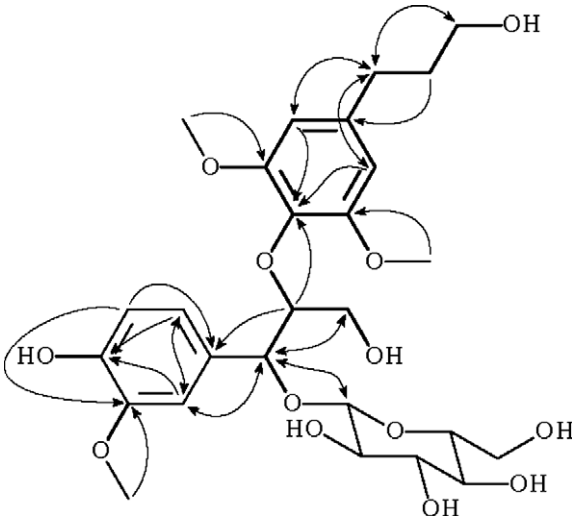

Fig. 2. Selected HMBC correlations of compound **1** (CD_3OD).

In order to determine the length and the location of the two fatty acid ester chains of **2**, a selective deacylation experiment was performed. Treatment (Murakami et al., 1991) with lipase type XI from *Rhizopus arrhizus*, **2** yielded compound **4** (Fig. 3). ^1H and ^{13}C NMR spectra of **4** (Table 1) showed the presence of a fatty acid ester, an aminoglycosyl group and a glyceryl moiety. In comparison with **2**, the proton signals of H_2-1 of **4** dramatically shifted upfield, indicating that the remaining fatty acid ester chain in **4** was located at C-2. The molecular formula of **4** was established as $C_{29}H_{57}NO_8$ on the basis of HRTOFMS [*m/z* 555.4333 $[M + Li + H]^+$ (calcd. for $C_{29}H_{58}NO_8Li$, 555.4322), 547.4088 $[M]^+$ (calcd. for $C_{29}H_{57}NO_8$, 547.4084)], which fixed the fatty acid ester chain in **4** as an eicosanoyl chain. By subtracting the molecular weight of **4** from that of **2**, the second fatty acid ester chain in **2** was deduced to be a palmitoyl group, which was attached at C-1. The structure of **2** was further confirmed by the representative full scan MS, MS^1, MS^2 and MS^3 spectra (Fig. 4) (Xia et al., 2003; Hsu and Turk, 2001), from which the key fragmentations were demonstrated to be in good agreement with the above elucidated structure. Accordingly, **2** was determined to be 1-*O*-palmitoyl-β-*O*-eicosanoyl-3-*O*-(6-amino-6-deoxy)-β-D-glucopyranosyl-glycerol, and was given the trivial name of rouremin.

In addition to compounds **1** and **2**, five known compounds, 1-(26-hydroxyhexacosanoyl)-glycerol (**3**) (Babady-Byla and Herz, 1996), 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*-8*Z*)-2-N-(2′-hydroxypalmitoyl)-octade casphinga-4,8-dienine (Shibuya et al., 1993), 9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoic acid (Kim et al., 2002), dihydrovomifoliol-9-β-D-glucopyranoside (Jakupovic et al., 1991), and β-sitosterol glucoside (Kojima et al., 1990) were also isolated from the active fractions of the $CHCl_3$ extract by activity-guided fractionation. The known compounds were identified by comparison of their physical and spectroscopic properties to those reported in the literature.

All isolates were tested for antimalarial activity on cultures of *P. falciparum* clones D6 and W2, which were characterized as chloroquine-sensitive and chloroquine-resistant, respectively. Of the isolates, compounds **1**–**3** exhibited much lower antimalarial activity (Table 2) than those of chloroquine. It should be noted that compound **1** is a neolignan. In our previous studies, lignans and neolignans isolated from other plant species were also found to possess antimalarial activity (Ma et al., in press; Zhang et al., 2001). With the exception of polysyphorin, all our previously isolated lignans and the neolignans showed a similar level of antimalarial activity to that of **1**. Polysyphorin, a neolignan with a structure similar to that of **1**, was approximately 10-fold more active (Zhang et al., 2001). Considering the low level of antimalarial activity observed, compounds **1**–**3** do not appear to be promising for further development at this time.

3. Experimental

3.1. General

Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. Circular dichroism (CD) spectra were recorded with a Jasco 710 CD Spectropolarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. 1D and 2D NMR spectra were recorded on a

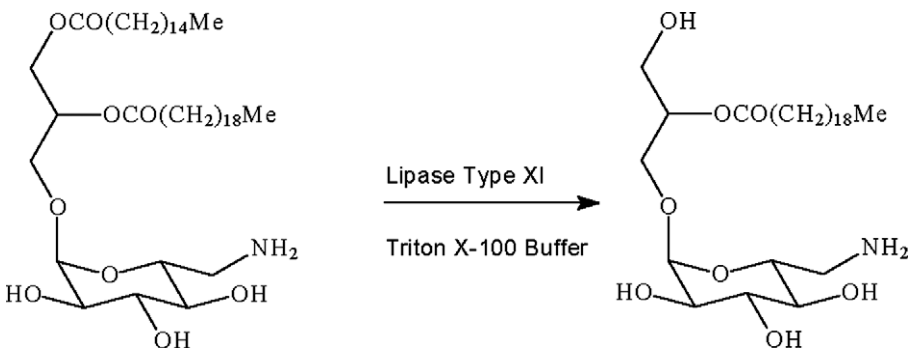

Fig. 3. Selective hydrolysis of **2**.

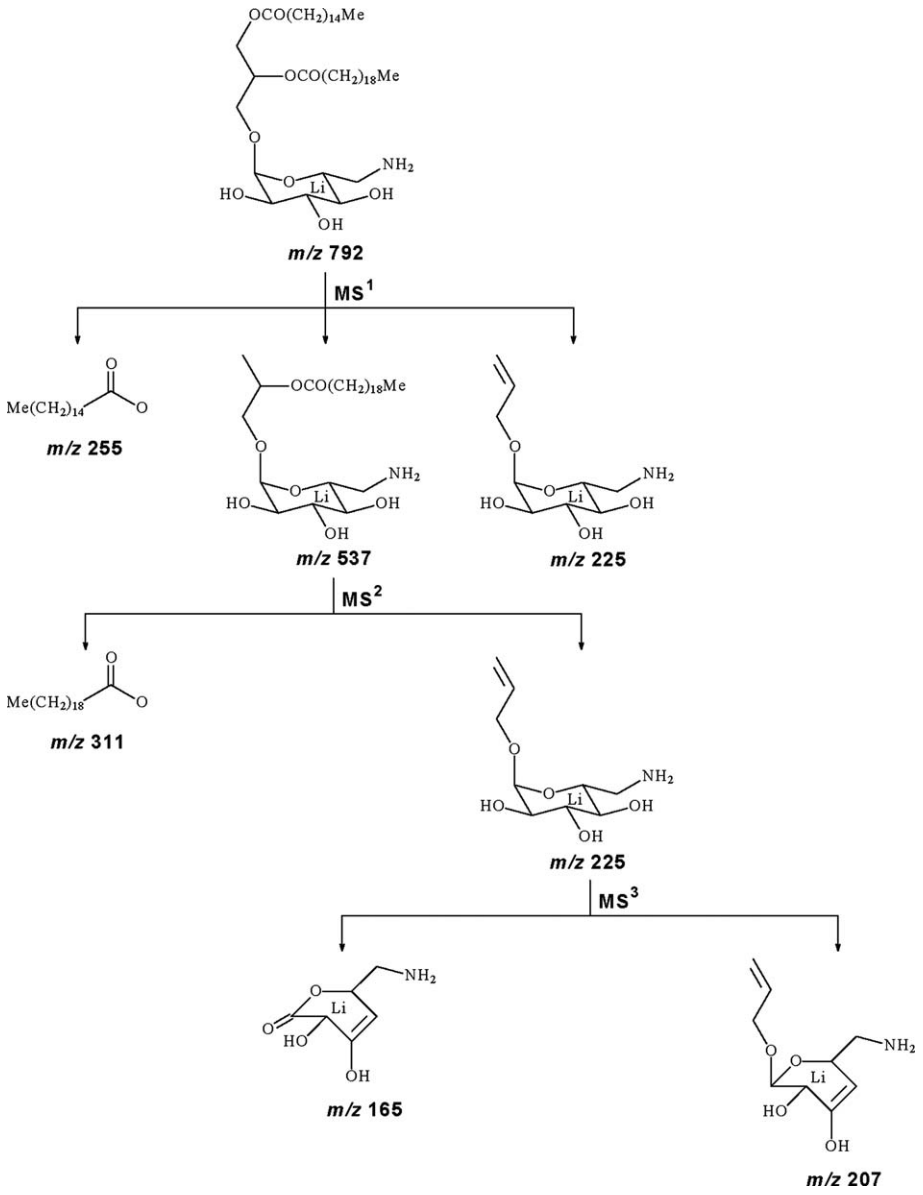

Fig. 4. Triple stage TOFMS fragmentation of **2**.

Table 2
Antimalarial activity of compounds **1**–**7**

Extract and compounds	D6		W2		KB
	IC_{50} (μM)	SI[a]	IC_{50} (μM)	SI	ED_{50} (μM)
$CHCl_3$ extract	5 μg/ml	>4	2 μg/ml	>10	>20 μg/ml
1	3.66 ± 0.17	>9.5	2.10 ± 0.14	>16.7	>35.09
2	5.09 ± 0.20	>5.0	4.49 ± 0.73	>5.7	>25.48
3	9.48 ± 0.14	>4.3	12.65 ± 715	>3.2	>41.15
Chloroquine	0.011 ± 0.0002	4114	0.106 ± 0.003	423	45.06
Artemisinin	0.013 ± 0.0003	>5405	0.009 ± 0.0007	>8000	>70.92
Quinine	0.028 ± 0.003	>2222	0.14 ± 0.01	>435	>61.73

[a] SI = selectivity index = ED_{50} KB/IC_{50} *Plasmodium falciparum*.

Bruker DRX-500 MHz spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. All NMR experiments were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on silica gel (200-400, Natland International Corporation). Reversed-phase flash

chromatography was accomplished with RP-18 (40–63 μm, EM Science). Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of silica gel 60. Mass spectra were recorded on a Micromass Q-TOF-2 mass spectrometer electrospray ionization source.

3.2. Plant material

A sample (SL7006) consisting of the stems of the woody vine of *R. minor* was collected in Pakkadan Village (18° 33′ N lat; 103° 84′ E long.), Paksan District, Bolikhamsai Province, People's Democratic Republic of Laos on August 21, 1999. Voucher herbarium specimens (*Bouamanivong 006*) have been deposited in the Herbarium of the Traditional Medicine Research Center, Vientiane, Laos, and in the John G. Searle Herbarium, Field Museum, Chicago (acquisition FMNH #2222148). Recollection was performed by Bounkong Bounyavong at the same locality as SLA7006 on July 7, 2003.

3.3. Extraction and isolation

The sample of dried vines (5.0 kg) was extracted with MeOH, and the extract (155 g) was subsequently partitioned with petroleum ether and $CHCl_3$. The $CHCl_3$ soluble fraction (3.4 g) was subjected to a Si gel CC (176 g), eluted with $CHCl_3$ and increasing concentration of MeOH [$CHCl_3$; $CHCl_3$-MeOH/20:1, 10:1, 9:1, 8:2, and MeOH; each elution volume was 250 mL] to afford 12 fractions. Bioassay localized the anti-malarial activity in fractions F_2 and F_3. Fraction F_2 (444.4 mg) was separated on a Sephadex LH-20 (250 g) column, followed by a RP-18 column chromatographic (100 g) separation (eluted with MeOH–H_2O/6:4) to yield compound **1** (20.5 mg). Fractions F_3 and F_4, which showed similar TLC patterns, were combined (415.0 mg) and applied to a RP-18 column (100 g) eluted with MeOH–H_2O/5:5 to afford compounds **2** (20.4 mg), **3** [1-(26-hydroxyhexacosanoyl)-glycerol, 28.1 mg], 1-*O*-β-D-glucopyranosyl-(2S,3R,4E-8Z)-2-N-(2′-hydroxypalmitoyl)-octade casphinga-4,8-dienine (26.1 mg), 9*S*,12*S*,13*S*-trihydroxy-10*E*-octadecenoic acid (14.2 mg), dihydrovomifoliol-9-β-D-glucopyranoside (13.1 mg), and β-sitosterol glucoside (1.9 mg).

3.3.1. Rourinoside (*1*)

$[\alpha]_D^{20}$ −21.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 225 (2.17), 278 (3.54) nm; CD (Δε) 242 (+1.25) nm; for ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; negative ESIMS: *m*/*z* 569 $[M-H]^-$, 408 $[M-gly-H]^-$; HRTOFMS: *m*/*z* 569.2268 $[M-H]^-$ (calcd for $C_{27}H_{37}O_{13}$, 569.2234).

3.3.2. Rouremin (*1-O-palmitoyl-β-O-eicosanoyl-3-O-(6-amino-6-deoxy)-β-D-glucopyranosyl-glycerol* (*2*))

$[\alpha]_D^{20}$ −4.1 (*c* 0.12, MeOH); for ^1H (pyridine and DMSO, 500 MHz) and ^{13}C NMR (pyridine and DMSO, 125 MHz), see Table 1; positive ESIMS: *m*/*z* 793 $[M+Li+H]^+$, 537 $[M+Li-palmitoyl]^+$, 225 $[M+Li-palmitoyl-eicosanoyl]^+$; positive HRTOFMS: *m*/*z* 808.6274 $[M+Na]^+$ (calcd. for $C_{45}H_{87}NO_9Na$, 808.6279), 793.6616 $[M+Li+H]^+$ (calcd for $C_{45}H_{88}NO_9Li$, 793.6619).

3.4. Enzymatic hydrolysis of *2*

A solution of **2** (2.3 mg) and lipase type XI from *Rhizopus arrhizus* (448U) in the presence of Triton X-100 (0.8 mg) in a boric acid-borax buffer (0.2 mL, pH 7.7) was stirred at 38 °C for 20 min. The reaction was quenched with AcOH (32 μL), and then EtOH was added to the reaction mixture. The solvent was removed under reduced pressure and the residue was purified on a preparative silica gel TLC plate, developed with $CHCl_3$–MeOH (7:3). The developed band was scraped from the plate, eluted with MeOH, and concentrated to yield compound **4** (1.31 mg): white powder; for ^1H (DMSO, 500 MHz) and ^{13}C NMR (DMSO, 125 MHz), see Table 1; HRTOFMS: *m*/*z* 555.4333 $[M+Li+H]^+$ (calcd. for $C_{29}H_{58}NO_8Li$, 555.4322), 547.4088 $[M]^+$ (calcd. for $C_{29}H_{57}NO_8$, 547.4084).

3.5. Antimalarial and cytotoxicity assays

Antimalarial and cytotoxicity assays were conducted with cultured *P. falciparum* clones (W2, D6) and the oral epidermoid cancer cell line (KB) as previously described (Zhang et al., 2001). Chloroquine, artemisinin and quinine were employed as positive controls in the antimalarial assay.

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References

Babady-Byla, Herz, W., 1996. Triterpenes and 1-(ω-hydroxyceratyl) glycerols from *Pentaclethra eetveldenna* root bark. Phytochemistry 42, 501–504.

Baruah, P., Baruah, N.C., Sharma, R.P., Baruah, J.N., Kulanthaivel, P., Herz, W., 1983. A monoacyl galactosylglycerol from *Sonchus arvensis*. Phytochemistry 22, 1741–1744.

Bouamanivong, S., 1999. Information Communicated in a Letter in 1999, and as Noted on Herbarium Field Label of Specimen *Bouamanivong 006*. Somsanith Bouamanivong, Traditional Medicine Research Center, Ministry of Health, Vientiane, Lao People's Democratic Republic.

Braga, A.C.H., Zacchino, S., Badano, H., Sierra, M.G., Rüveda, E.A., 1984. ^{13}C NMR spectral and conformational analysis of 8-*O*-4′ neolignans. Phytochemistry 23, 2025–2028.

He, Z.D., Ma, C.Y., Zhang, H.J., Tan, G.T., Tamez, P., Sydara, K., Bouamanivong, S., Southavong, B., Soejarto, D.D., Pezzuto, J.M., Fong, H.H.S., 2005. Antimalarial constituents from *Nauclea orientalis* (L.) L. Chemistry & Biodiversity 2, 1378–1386.

Hsu, F.F., Turk, J., 2001. Structural determination of glycosphingolipids as lithiated 2adducts by electrospray ionization mass spectrometry using low-energy collisional-activated dissociation on a triple stage quadrupole instrument. Journal of the American Society for Mass Spectrometry 12, 61–79.

Jakupovic, J., Tan, R.X., Rohlmann, F., Jia, Z.J., Huneck, S., 1991. Seco- and nor-sesquiterpene lactones with a new carbon skeleton from *Artemisia santolinifolia*. Phytochemistry 30, 1941–1946.

Jiang, J.Q., Fang, S.D., Xu, C.F., Luo, J.T., 1990. Chemical constituents of *Rourea microphylla* (Hook. et Arn) Planch. Zhiwu Xuebao 32, 376–379.

Jiangsu Medical College, 1986. In: Zhongyaodacidian (A Dictionary of Traditional Chinese Medicines), first ed. Shanghai Science and Technology Publishers, Shanghai, PR China, p. 1616.

Jung, J.H., Lee, H.K., Kang, S.S., 1996. Diacylglycerylgalactoside from *Arisaema amurense*. Phytochemistry 42, 447–452.

Kim, H., Jang, Y.S., Ching, T., 2002. Effect of metal ions on the production of isomeric 9, 10, 13 (9, 12, 13)-trihydroxy-11*E* (10*E*)-octadecenoic acid from linoleic acid by *Pseudomonas aeruginosa* PR_3. Enzyme and Microbial Technology 30, 752–757.

Kojima, H., Sato, N., Hatano, A., Ogura, H., 1990. Sterol glucosides from *Prunella vulgaris*. Phytochemistry 29, 2351–2355.

Li, S.M., Lundquist, K., Wallis, A.F., 1998. Revised structure from a neolignan from *Brucea javanica*. Phytochemistry 49, 2125–2128.

Luyengi, L., Suh, N., Fong, H.H.S., Pezzuto, J.M., Kinghorn, A.D., 1996. A lignan and four terpenoids from *Brucea javanica* that induce differentiation with cultured HL-60 *Promyelocytic leukemia* cells. Phytochemistry 43, 409–412.

Ma, C.Y., Zhang, H.J., Tan, G.T., Hung, N.V., Cuong, N.M., Soejarto, D.D., Fong, H.H.S., in press. Antimalarial compounds from *Grewia bilamellata*. Journal of Natural Products.

Matsuda, N., Kikuchi, M., 1996. Studies on the constituents of Lonicera species. X. Neolignan glycosides from the leaves of *Lonicera gracilipes* var. *glandulosa* Maxim. Chemical and Pharmaceutical Bulletin 44, 1676–1679.

Murakami, N., Imamura, H., Sakakibara, J., Yamada, N., 1990. Seven new monogalactosyl diacylglycerols isolated from the axenic cyanobacterium *Phormidium tenue*. Chemical and Pharmaceutical Bulletin 38, 3497–3499.

Murakami, N., Morimoto, T., Imamura, H., Ueda, T., Nagai, S.I., Sakakibara, J., 1991. Studies on glycolipids III. Glyceroglycolipids from an axenically cultured cyanobacterium, *Phormidium tenue*. Chemical and Pharmaceutical Bulletin 39, 2277–2281.

Ramiah, N., Prasad, N.B.R., Abraham, K., 1976. Rapanone and leucopelargonidin from the roots of *Rourea santaloides*. Journal of Institute of Chemists (India) 48 (Pt. 4), 196–197.

Shibuya, H., Kurosu, M., Minagawa, K., Katayama, S., Kitagawa, I., 1993. Sphingolipids and glycerolipids. IV. Syntheses and ionophoretic activities of several analogs of soya-cerebroside II, a calcium ionophoretic sphingoglycolipid isolated from soybean. Chemical and Pharmaceutical Bulletin 41, 1534–1544.

Soejarto, D.D., Gyllenhaal, C., Regalado, J., Pezzuto, J.M., Fong, H.H.S., Tan, G.T., Hiep, N.T., Xuan, L.T., Binh, D.Q., Hung, N.V., Bich, T.Q., Thin, N.N., Loc, P.K., Vu, B.M., Southavong, B.H., Sydara, K., Bouamanivong, S., O'Neill, M.J., Lewis, J., Xie, X., Dietzman, G., 1999. Studies on biodiversity of Vietnam and Laos: the UIC-based ICBG program. Pharmaceutical Biology 37 (Suppl.), 100–113.

Wu, Z.Y., Yin, W.Q., Bao, S.Y., Tao, D.D., Yuan, S.H., Deng, X.F.,Yuan, S.X., You, H.Z., Lin, Q., 1984. In Index Florae Yunnanensis, Kunming, Yuannan, PR China, first ed., Tomus I, pp. 869.

Wu, Z.Y., Raven, P.H., 2003In Flora of China. Sciences Press, Beijing and Missouri Botanical Garden Press, St. Louis, pp. 437.

Xia, Y.Q., Miller, J.D., Bakhtiar, R., Franklin, R.B., Liu, D.Q., 2003. Use of a quadrupole linear ion trap mass spectrometer in metabolite identification and bioanalysis. Rapid Communication of Mass Spectrometry 17, 1137–1145.

Yamaoka, N., Ushi, T., Sugiyama, H., Seto, S., 1974. C-13 NMR spectra of some aminosugars and sugar-antibiotics neomycin and kanamycin. Chemical and Pharmaceutical Bulletin 22, 2196–2200.

Zhang, H.J., Tamez, P.A., Hoang, V.D., Tan, G.T., Hung, N.V., Xuan, L.T., Huang, L.M., Cuong, N.M., Tao, D.T., Soejarto, D.D., Fong, H.H.S., Pezzuto, J.M., 2001. Antimalarial compounds from *Rhaphidophora decursiva*. Journal of Natural Products 64, 772–777.

Zhang, H.J., Qiu, S.X., Tamez, P.A., Hoang, V.D., Tan, G.T., Aydogmus, Z., Hung, N.V., Cuong, N.M., Angerhofer, C., Soejarto, D.D., Pezzuto, J.M., Fong, H.H.S., 2002a. Antimalarial agents from plants II. Decursivine, a new antimalarial indole alkaloid from *Rhaphidophora decursiva*. Pharmaceutical Biology 40, 221–224.

Zhang, H.J., Tamez, P.A., Aydogmus, Z., Tan, G.T., Saikawa, Y., Hashimoto, K., Nakata, M., Hung, N.V., Xuan, L.T., Cuong, N.M., Soejarto, D.D., Pezzuto, J.M., Fong, H.H.S., 2002b. Antimalarial agents from plants. Trichothecenes from *Ficus fistulosa* and *Rhaphidophora decursiva*. Planta Medica 68, 1088–1091.

Zhang, Z.Z., ElSohly, H.N., Li, X.C., Khan, S.I., Broedel Jr., S.E., Raulli, R.E., Cihlar, R.L., Burandt, C., Walker, L.A., 2003. Phenolic compounds from *Nymphaea odorata*. Journal of Natural Products 66, 548–550.