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Iridoid glucosides from roots of Vietnamese *Paederia scandens*

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Abstract

Four iridoid glucosides, three of which are dimeric were isolated from the methanol extract of roots of Vietnamese *Paederia scandens* (Lour) Merrill together with the five known glucosides, paederoside, asperuloside, paederosidic acid, asperulosidic acid and geniposide. Seven sulfur-containing iridoid glucosides were also isolated. The structures of the iridoid glucosides were determined by a combination of high-resolution NMR, MS, IR and UV spectra, and chemical reaction such as acetylation.

Keywords: *Paederia scandens*; Rubiaceae; Paederoside; Paederosidic acid; Asperuloside; Asperulosidic acid; Geniposide; Paederosidic acid methyl ester; Dimeric iridoid glucoside

1. Introduction

Paederia scandens (Lour) Merrill is a climbing plant, widely grown around Vietnam (Kadota, 2000) and also in India, China, Japan, Philippines and USA. The extracts of roots, leaves, bark and fruits are prescribed for toothache, chest pain, piles, inflammation of the spleen, diuretic, emetic, rheumatic arthritis and curing bacillary dysentery (Tran, 1987; Kapadia et al., 1996). The leaves of this plant are also used in soup and other food preparation in Vietnam. Until now, paederoside (**5**) and asperuloside (**6**) and their related glucosides, paederosidic acid (**7**), deacetylasperuloside and scandoside have been found in leaves and stem of *Paederia scandens* (Inouye et al., 1969a–c, 1988; Shukla et al., 1976; Kapadia et al., 1979). Sulfur-containing glucosides **5** and **6** from *P. scandens* exert an inhibitory effect on Epstein–Barr virus activation (Kapadia et al., 1996). Inoue et al. (1969b,c) reported that paederosidic acid (**7**) and asperulosidic acid (**8**) were obtained during the prolonged boiling of an aqueous solution of two lactone glucosides **5** and **6**, respectively and they suggested that both acids are artifacts (Inouye et al., 1969b,c). Paederosidic acid methyl ester (**3**) has also been obtained by methanolysis of **7** (Inoue et al., 1969c).

In this paper, we report the isolation and structural elucidation of two newly isolated iridoid glucosides (**1, 3**) and three dimeric iridoid glucosides (**10, 12, 14**), along with the previously known related compounds (**5–9**).

2. Results and discussion

The crude methanol extract of Vietnamese *P. scandens* (Lour) Merrill was partitioned between butanol and water. Butanol layer was concentrated in vacuo and purified using a combination of silica gel column chromatography and reversed-phase HPLC (C 18) to give a new iridoid glucosides (**1**), paederosidic acid methyl ester (**3**), which was previously reported as the reaction product with methanol, and three new dimeric iridoid glucosides (**10**, **12**, **14**) together with five iridoid glucosides (**5–9**).

Compound **1** was obtained as a brown powder, $[\alpha]_D^{20}$—8.6° (*c* 1.16, CH_3OH), with a *quasi*-molecular ion at *m/z* 501.1042 generated by HR-FABMS, indicating that the molecular formula of **1** was putatively $C_{19}H_{26}O_{12}S$. Its IR spectrum showed the presence of a hydroxyl (3334 cm^{-1}), a γ-lactone (1768 cm^{-1}) and an

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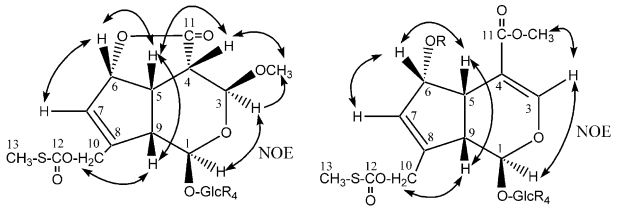

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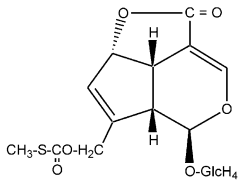

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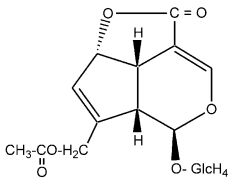

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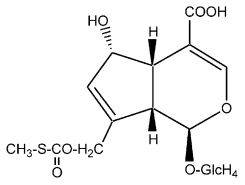

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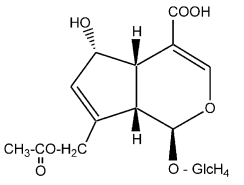

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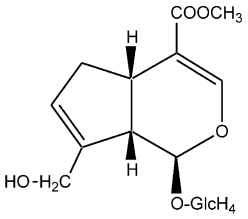

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ester (1714 cm^{-1}) group. The ^1H NMR spectrum of **1** (Table 1) showed an olefinic proton at δ 5.98 (*br*, *s*), one methoxyl group at δ 3.51 (*s*) and one sulfur methyl group at δ 2.35 (*s*), together with seven protons arising from sugar moiety. The ^{13}C NMR spectrum of **1** exhibited 19 carbon signals due to 10 carbons of the main iridoid skeleton (Damtoft et al., 1981) and six sugar carbons, including one ester carbonyl (δ 173.0) one methyl sulfur (δ 13.6) and one methoxy carbon (δ 56.5), as summarized in Table 1. The presence and position of sulfur atom were determined by HR-FABMS and comparison of the NMR data with those of paederoside (**5**) and asperuloside (**7**) (Suzuki et al., 1993) and the presence of higher frequency of ν 1717 cm^{-1}, corresponding to methyl thiocarbonate group (Suzuki et al., 1993).

From HMBC data of **1**, a proton at δ 3.25 showed correlation with C-3 and C-5, therefore, it was assigned to C-4. Other protons (δ 5.10, 5.01, 3.40, 5.37, 5.98 and 3.02) were assigned to positions C-1, 3, 5, 6, 7, and 9, respectively. The ester carbonyl (C-12) had HMBC correlations with sulfur methyl group and H-10 indicating the presence of the partial structure, CH_3–S–COO–CH_2–. The position of the methoxyl group was determined to be at C-3 by the presence of cross-peak between methoxyl group and C-3 in HMBC correlation of **1**.

Furthermore, the glycosidic linkage of glucopyranosyl moiety to C-1 position of aglycone was clearly indicated by the cross-peak between an anomeric carbon signal [δ 99.6, (C-1′)] of glucopyranose and H-1 [δ 5.10, (*d*, $J = 6.0$ Hz)] of the aglycone in HMBC correlation (Table 1).

The sugar moiety was confirmed to be β-glucose by comparing the ^1H NMR and ^{13}C NMR with those of previous papers (Lavaud et al., 2001; Muhammad et al., 2001).

The stereochemical assignment at C-4, C-5, C-6, C-9 of aglycone was based on 2D NMR NOESY experiment of **1** (Table 1), which showed the NOEs between (1) H-4; H-5 and 3-OCH_3, (2) H-5; H-4; H-6 and H-9, (3) H-6; H-5 and H-7, (4) H-9; H-5 and H-10, indicated that they were *cis* (β-face) to each other. In addition, the NOE correlation observed between H-1 and H-3, indicated that they are *cis* (α-face). These assignments were in agreement with those of 6β-*O*-substituted iridoid glucosides (Damtoft et al., 1981).

Acetylation of **1** with Ac_2O in pyridine yielded a tetraacetate (**2**), $C_{27}H_{34}O_{16}S$, the IR spectrum of which was similar to that of compound **1**, except for the disappearance of an absorption band corresponding to the hydroxyl group at 3334 cm^{-1} in place of the presence of a signal at 1725 cm^{-1} assignable to acetyl groups. The ^1H and ^{13}C NMR spectra of compound **2** were also similar to those of compound **1**, except for additional signals at δ 2.03, 2.03, 2.03 and 2.08 (4 CH_3) for four acetyl groups. Based on the above spectral and chemical evidence, compound **1** was determined as 3,4-dihydro-3-methoxypaederoside.

Böjthe-Horvath et al. (1982) reported the isolation of 3,4-dihydro-3-methoxyasperuloside without determination of stereochemistry of C-3 and they concluded that the formation of this compound during plant extraction can not be excluded although methanolysis of **6** is rather slow. We suggested that **1** might be a naturally occurring iridoid glucoside since the C-3 epimeric compound was detected in neither HPLC nor ^{13}C NMR experiments.

Compound **3** was obtained as a white powder, whose molecular formula ($C_{19}H_{26}O_{12}SNa$) was confirmed by HR-FABMS [m/z 501.1071, expected m/z 501.1043)]. The IR and UV spectra showed absorption bands at 3374 cm^{-1} (OH), 1700 cm^{-1}(C=O), 1633 cm^{-1}(C=C) and absorption maxima at 234 nm (log ϵ 4.16), respectively. Acetylation of **3** with Ac_2O in pyridine afforded a pentaacetate (**4**) [HR-FABMS: m/z 688.1687 ($C_{29}H_{36}O_{17}S$, expected m/z 688.1673)]; δ_H 1.94, 2.02, 2.04, 2.06, 2.07 indicating the presence of five hydroxyl groups in **3**. The ^1H NMR spectrum of **3** showed the presence of two

olefinic protons [δ 7.65, (*d*, $J=1.1$, H-3), and δ 6.02, (*d*, $J=1.7$, H-7)], and one methoxy [δ 3.74, (*s*, 11-OCH_3)] (Table 2). The UV, IR and NMR spectral data of **3** were similar to those of **6**–**7**, indicating that **3** was the same iridoid glucoside as **6**–**7** with the same absolute configuration. Further identification of the structure of **3** was carried out by HMBC and NOESY experiments. The cross peak between 11-OCH_3 and C-11 was clearly detected in HMBC spectrum indicating the presence of a carbomethoxyl group.

NOEs were observed between each H-5, H-6 and H-9 indicating that they were *cis* (β-face). Thus, the structure of compound **3** was established to be paederosidic acid methyl ester.

It has been demonstrated that methanolysis of paederoside (**5**) occurred when the methanolic solution of **5**

Table 1
^1H and ^{13}C NMR assignments, HMBC and NOESY correlations of **1**

Position	$^1H(\delta)$	$^{13}C(\delta)$	HMBC	NOESY
1	5.10 (*d*, 6.0)	96.7	H-3, H-9, H-1′	H-3, H-1′
3	5.01 (*d*, 3.6)	98.5	H-1, H-4, 3-OCH_3	H-1, H-4, 3-OCH_3
4	3.25 (*dd*, 3.6, 10.4)	44.4	H-5, H-9	H-5, 3-OCH_3
5	3.40 (*ddd*, 6.6, 9.1, 10.4)	37.6	H-4, H-6, H-9	H-4, H-6, H-9
6	5.37 (*br d*, 6.6)	87.7	H-7, H-9	H-4, H-5, H-7
7	5.98 (*br s*)	126.8	H-5, H-9, H-10	H-6, H-10
8		151.6	H-7, H-9, H-10	
9	3.02 (*ddd*, 0.8, 6.0, 9.1)	46.3	H-1, H-5, H-10	H-5, H-10
10	4.89 (*br d*, 15.7)	65.2	H-7, H-9	H-1, H-7, H-9
	5.08 (*br d*, 15.7)			
11		177.0	H-3, H-4, H-5	
12		173.0	H-10, H-13	
13	2.35 (*s*)	13.6		
3-OCH_3	3.51 (*s*)	56.5	H-3	H-3, H-4
1′	4.69 (*d*, 8.0)	99.6	H-1, H-2′	H-1, H-3′, H-5′
2′	3.21 (*dd*, 8.0, 9.3)	74.9	H-1′, H-3′	H-4′
3′	3.38 (*dd*, 9.3, 9.1)	78.0	H-2′, H-4′	H-1′, H-5′
4′	3.27 (*dd*, 9.1, 9.1)	71.5	H-3′, H-5′, H-6′	H-2′, H-6′
5′	3.31 (*m*)	78.2	H-4′, H-6′	H-1′, H-3′, H-6′
6′	3.68 (*ddd*, 1.6, 4.1, 11.8)	62.8	H-4′, H-5′	H-4′, H-5′
	3.88 (*dd*, 1.4, 11.8)			

Table 2
^1H and ^{13}C NMR assignments, HMBC and NOESY correlations of **3**

Position	$^1H(\delta)$	$^1H(\delta)$[a]	$^{13}C(\delta)$	HMBC	NOESY
1	5.06 (*d*, 8.5)		101.3	H-3, H-9, H-1′	H-3, H-9, H-1′
3	7.65 (*d*, 1.1)	7.71	155.4	H-5	H-1
4		108.1	H-3, H-5		
5	3.03 (*ddd*, 1.1, 6.0,7.4)	3.11	42.4	H-3, H-7, H-9	H-6, H-9
6	4.80 (*ddd*, 0.8, 2.5, 6.0)		75.3	H-5, H-7	H-5, H-7
7	6.02 (*d*, 1.7)	6.13	132.4	H-9, H-10	H-6, H-9, H-10
8		145.5	H-7, H-9, H-10		
9	2.62 (*dd*, 7.4, 8.5)	2.71	46.2	H-1, H-5, H-10	H-1, H-5, H-10
10	4.95 (*br d*, 14.6)	5.06	66.2	H-1, H-7, H-9	H-7, H-9
	5.10 (*dd*, 1.3, 14.6)				
11		172.9	11-OCH_3		
12		169.3	H-10, H-13		
13	2.34 (*s*)	2.38	13.5		
11-OCH_3	3.74 (*s*)	3.78	51.9	H-3	
1′	4.72 (*d*, 8.0)		100.7	H-2′	H-3′, H-5′
2′	3.24 (*dd*, 8.0, 9.1)		74.9	H-1′, H-3′	H-4′
3′	3.38 (*dd*, 8.8, 9.1)		77.9	H-2′, H-4′	H-1′, H-5′
4′	3.26 (*dd*, 8.8, 9.3)		71.6	H-3′, H-5′	H-2′, H-6′
5′	3.27 (*m*)		78.6	H-4′, H-6′	H-1′, H-3′, H-6′
6′	3.63 (*dd*, 1.9, 11.8)	3.46	63.0	H-5′	H-4′, H-5′
	3.85 (*dd*, 6.0, 11.8)				

[a] Measured in D_2O (Inouye et al., 1969c).

was refluxed for 100 h on a water bath to give several decompositon products of which paederosidic acid methyl ester (**3**) was obtained and its structure suggested by IR, UV and ^{1}H NMR spectroscopy (Inouye et al., 1969c). Indeed, the IR, UV and ^{1}H NMR data as well as specific rotation of **3** obtained by methanolysis resembled those of **3** isolated from the present *P. scandens*.

Although the present crude extract was not refluxed in methanol, it cannot be excluded that the methyl ester (**3**) is formed during fractionation of the crude extract. However, the C-6 epimeric compound of **5** has not been isolated from or detected in the crude extract.

Dimer **10**, $[\alpha]_D^{20}$ −5.7 (*c* 1.00, CH_3OH), was obtained as a yellow powder. The FAB-MS had a *quasi*-molecular ion peak at *m/z* 948 $[M+Na]^+$. Its UV and IR spectra showed maxima absorption at 236 nm based on unsaturated ester and the presence of a hydroxyl (3394 cm^{-1}), an ester (1702 cm^{-1}) and an olefinic (1633 cm^{-1}) group.

The ^{1}H and ^{13}C NMR data of **10** showed the presence of two paederosidic acid moieties, one of which (the 'b' unit) was esterified with methanol, the other ('a' unit) esterified with the 6b′-oxygen. This was proved partly by the down field position of C-6b′ (δ 63.8) and H-6b′ (δ 4.30 and 4.46); the up field position of C-5b′ (δ 75.7), partly by correlation between H-6b′ and C-11a in HMBC spectrum.

The configuration of carbon centers C-1a, C-5a, C-6a, C-9a and C-1b, C-5b, C-6b, C-9b was determined by NOE correlation as shown in the depicted structures and in Table 3. The NOEs between (1) H-5a; H-6a and

Table 3
^{1}H and ^{13}C NMR assignments, HMBC and NOESY correlations of compound **10**

Position	^{1}H(*δ*)	^{13}C(*δ*)	HMBC	NOESY
1a	5.06 (*d*, 9.1)	101.5	H-3a, H-9a, H-1a′	H-3a, H-1a′
3a	7.69 (*d*, 1.4)	155.8	H-1a, H-5a	H-1a
4a		108.0	H-3a, H-5a	
5a	3.07 (*ddd*, 1.4, 6.0, 7.4)	42.4	H-3a, H-7a, H-9a	H-6a, H-9a
6a	4.81 (*dd*, 1.9, 6.0)	75.5	H-5a, H-7a	H-5a
7a	6.06 (*d*, 1.9)	133.2	H-9a, H-10a	H-10a
8a		145.3	H-7a, H-9a, H-10a	
9a	2.64 (*dd*, 7.4, 9.1)	42.4	H-1a, H-5a, H-10a	H-5a, H-10a
10a	4.99 (*br d*, 14.8)	66.3	H-7a, H-9a	H-7a, H-9a
	5.09 (*dd*, 1.4, 14.8)			
11a		168.6	H-3a, H-5a, H-6b′	
12a		172.7	H-10a, H-13a	
13a	2.35 (*s*)	13.6		
1a′	4.73 (*d*, 8.0)	100.7	H-1a, H-2a′	H-1a, H-3a′, H-5a′
2a′	3.26 (*dd*, 8.0, 9.3)	74.9	H-1a′, H-3a′	H-4a′
3a′	3.84 (*dd*, 8.8, 9.3)	77.9	H-2a′, H-4a′	H-1a′, H-5a′
4a′	2.26 (*t*, 9.3)	71.6	H-3a′, H-5a′	H-2a′, H-6a′
5a′	3.28 (*m*)	78.6	H-4a′, H-6a′	H-1a′, H-3a′, H-6a′
6a′	3.64 (*dd*, 6.0, 12.1)	63.0	H-4a′, H-5a′	H-4a′, H-5a′
	3.86 (*dd*, 1.9, 12.1)			
1b	5.00 (*d*, 8.5)	102.1	H-3b, H-9b, H-1b′	H-3b, H-1b′
3b	7.65 (*d*, 1.1)	155.4	H-1b, H-5b	H-1b, 11b-OCH_3
4b		107.9	H-3b, H-5b	
5b	3.02 (*ddd*, 1.1, 6.0, 7.4)	42.3	H-3b, H-7b, H-9b	H-6b, H-9b
6b	4.79 (*dd*, 1.7, 6.0)	75.1	H-5b, H-7b	H-5b
7b	6.02 (*d*, 1.7)	132.7	H-9b, H-10b	H-10b
8b		145.3	H-7b, H-9b, H-10b	
9b	2.62 (*dd*, 7.4, 8.5)	42.3	H-1b, H-5b, H-10b	H-5b, H-10b
10b	4.96 (*dd*, 1.7, 14.6)	66.3	H-7b, H-9b	H-7b, H-9b
	5.03 (*br d*, 14.6)			
11b		169.3	H-3b, H-5b, 11b-OCH_3	
12b		172.9	H-10b, H-13b	
13b	2.34 (*s*)	13.7		
11b-OCH_3	3.74 (*s*)	51.9		H-3b
1b′	4.72 (*d*, 8.0)	101.4	H-1b, H-2b′	H-1b, H-3b′, H-5b′
2b′	3.27 (*dd*, 8.0, 9.6)	74.9	H-1b′, H-3b′	H-4b′
3b′	3.40 (*dd*, 8.8, 9.6)	77.6	H-2b′, H-4b′	H-1b′, H-5b′
4b′	3.93 (*dd*, 8.8, 9.3)	71.3	H-3b′, H-5b′	H-2b′, H-6b′
5b′	3.51 (*m*)	75.7	H-4b′, H-6b′	H-1b′, H-3b′, H-6b′
6b′	4.30 (*dd*, 4.8, 11.8)	63.8	H-4b′, H-5b′	H-4b′, H-5b′
	4.46 (*dd*, 1.9, 11.8)			

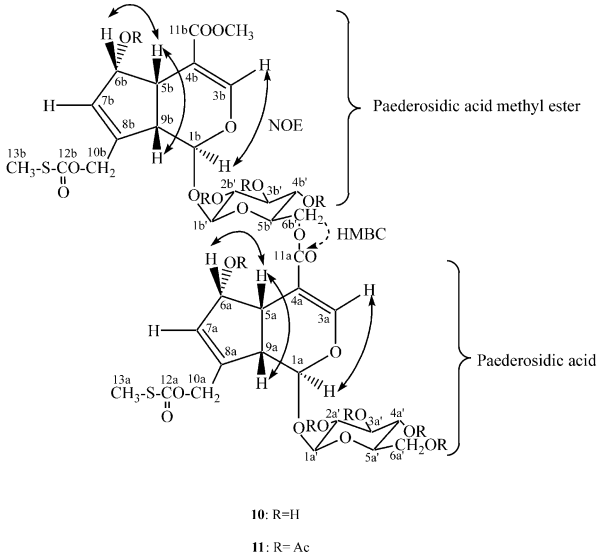

Fig. 1. Important HMBC and NOESY correlations of **10**.

H-9a, (2) H-5b; H-6b and H-9b indicated that they were *cis* (or β-face). On the other hand, the presence of NOEs between (1) H-1a and H-3a and (2) H-1b and H-3b and the absence of NOEs between (1) H-1a and H-9a and (2) H-1b and H-9b suggested that the configuration of H-1a and H-1b were α-face.

Acetylation of **10** with acetic anhydride in pyridine afforded compound **11** as oil, $[\alpha]_D^{20}$ +51.6 (c 0.30, $CHCl_3$), the IR spectrum of which was similar to those of **10** except for the disappearance of the absorption band of a hydroxyl group at 3394 cm^{-1} in place of the absorption bands at 1725 and 1223 cm^{-1} assignable to an acetyl group. The ^1H and ^{13}C NMR spectra of **11** were also similar to those of **10**, except for nine singlet signals (δ 1.92, 1.96, 2.02, 2.02, 2.03, 2.04, 2.06, 2.07, 2.07) due to nine acetyl groups. The HR-FABMS of **11** at *m*/*z* 1325.2900 corresponding to the molecular formula $C_{55}H_{66}O_{32}S_2Na$ supported the original dimeric structure of **10**.

Table 4
^1H and ^{13}C NMR assignments, HMBC and NOESY correlations of compound **12**

Position	$^1H(\delta)$	$^{13}C(\delta)$	HMBC	NOESY
1a	5.06 (*d*, 8.2)	101.6	H-3a, H-9a, H-1a′	H-3a, H-1a′
3a	7.71 (*d*, 1.1)	156.2	H-1a, H-5a	H-1a
4a		107.7	H-3a, H-5a	
5a	2.89 (*ddd*, 1.1, 6.1, 8.2)	42.8	H-6a, H-9a	H-6a, H-9a
6a	4.79 (*d*, 6.1)	72.5	H-5a, H-7a	H-5a, H-7a
7a	6.00 (*d*, 2.2)	131.7	H-9a, H-10a	H-6a, H-10a
8a		146.0	H-7a, H-9a, H-10a	
9a	2.72 (*dd*, 8.2, 8.2)	46.0	H-1a, H-7a, H-10a	H-5a, H-10a
10a	4.91 (*br d*, 14.8)	66.3	H-7a, H-9a	H-7a, H-9a
	5.14 (*dd*, 1.4, 14.8)			
11a		167.5	H-3a, H-2b′	
12a		172.9	H-10a, H-13a	
13a	2.34 (*s*)	13.6		
1a′	4.71 (*d*, 8.0)	100.9	H-1a, H-2a′	H-1a, H-3a′, H-5a′
2a′	3.27 (*dd*, 8.0, 9.1)	74.9	H-1a′, H-3a′	H-4a′
3a′	3.39 (*t*, 9.1)	77.8	H-2a′, H-4a′	H-1a′, H-5a′
4a′	3.29 (t, 8.8)	71.6	H-3a′, H-5a′	H-2a′, H-6a′
5a′	3.32(*m*)	78.6	H-4a′, H-6a′	H-1a′, H-3a′, H-6a′
6a′	3.64 (*dd*, 6.0, 12.0)	63.0	H-4a′, H-5a′	H-4a′, H-5a′
	3.85 (*br d*, 12.0)			
1b	5.85 (*d*, 1.4)	94.0	H-3b, H-9b, H-1b′	H-3b, H-1b′
3b	7.16 (*d*, 1.9)	150.1	H-1b, H-5b	H-1b
4b		106.2	H-3b, H-5b	
5b	3.47 (*ddd*, 1.9, 6.6, 8.8)	37.6	H-6b, H-9b	H-6b, H-9b
6b	5.52 (*br d*, 6.6)	86.0	H-5b, H-7b	H-5b, H-7b
7b	5.71 (*br s*)	129.8	H-9b, H-10b	H-6b, H-10b
8b		143.6	H-7b, H-9b, H-10b	
9b	3.28 (*dd*, 1.4, 8.8)	45.0	H-1b, H-7b, H-10b	H-5b, H-10b
10b	4.80 (*br d*, 14.0)	64.3	H-7b, H-9b	H-7b, H-9b
	4.89 (*dd*, 1.4, 14.0)			
11b		172.6	H-3b	
12b		172.1	H-10b, H-13b	
13b	2.35 (*s*)	13.6		
1b′	4.93 (*d*, 8.0)	98.6	H-1b, H-2b′	H-1b, H-3b′, H-5b′
2b′	4.79 (*dd*, 8.0, 9.6)	74.4	H-1b′, H-3b′	H-4b′
3b′	3.68 (*dd*, 8.8, 9.6)	75.5	H-2b′, H-4b′	H-1b′, H-5b′
4b′	3.38 (*t*, 8.8)	71.5	H-3b′, H-5b′	H-2b′, H-6b′
5b′	4.43 (*m*)	78.5	H-4b′, H-6b′	H-1b′, H-3b′, H-6b′
6b′	3.68 (*dd*, 1.4, 11.8)	62.6	H-4b′, H-5b′	H-4b′, H-5b′
	3.94 (*dd*, 1.9, 11.8)			

On the basis of the above spectroscopic and chemical data, the structure of **10** was determined to be the dimer depicted in Fig. 1.

Dimer **12** was obtained as a yellow powder, $[\alpha]_D^{20}$ −108.9° (c 0.54, CH_3OH). The FAB-MS of **12** gave a *quasi*-molecular ion peak at *m/z* 915 $[M+Na]^+$. Its UV and IR spectra showed absorption maximum at 239 nm and the presence of a hydroxyl (3318 cm^{-1}), an unsaturated γ-lactone (1746 cm^{-1}) and an α, β-unsaturated ester (1706 cm^{-1}) groups, respectively. The ^1H and ^{13}C NMR spectra of **12** (Table 4) resembled those of **10**, except the signal of H-6b was shifted to lower field at δ_H5.52 (*br d*, *J*=6.6) and chemical shifts of C-6b and C-11b appeared at higher frequency at δ_C 86.0 ppm and 172.6 ppm, respectively, indicating the presence of a five-membered lactone ring. The two partial structures of **12** were determined to be paederoside (**5**) and paederosidic acid (**7**) by comparing their spectral data with those of paederoside and paederosidic acid isolated from the same plant. Acetylation of **12** with acetic anhydride in pyridine afforded compound **13,** $[\alpha]_D^{20}$ −35.0° (*c* 0.40, $CHCl_3$). HR-FABMS of **13** at *m/z* 1251.2490 suggested a molecular formula of $C_{52}H_{60}O_{30}S_2Na$. The IR spectrum of **13** was similar to those of **12** except for the disappearance of the absorption band 3318 cm^{-1} due to a hydroxl group at in place of absorption bands at 1725 and 1226 cm^{-1} due to an acetyl group. The ^1H and ^{13}C NMR spectra of **13** were also similar to those of **12**, except for eight singlet signals (δ 1.91, 1.99, 2.02, 2.04, 2.05, 2.06, 2.10, 2.12) due to eight acetyl groups, indicating the presence of eight hydroxyl groups in **12**. In addition, the ester linkage between C-11a of paederosidic acid and C-2b′ of sugar moiety of paederoside was established by the correlation between H-2b′ and C-11a in HMBC spectrum (Table 4). The molecular ion peak at *m/z* 1251.2490 of **13** in HR-FABMS confirmed the proposed dimeric structure (Fig. 2).

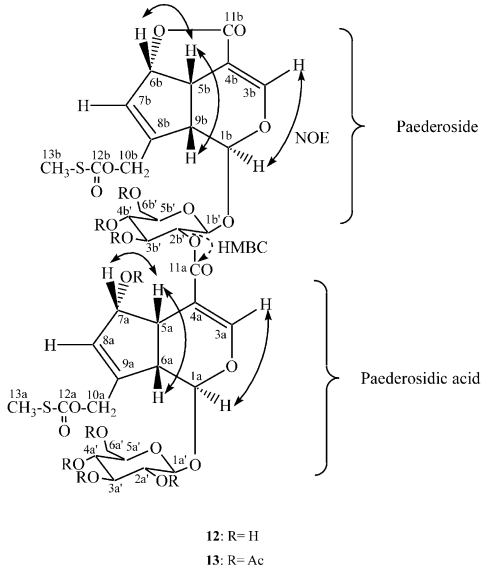

Fig. 2. Important HMBC and NOESY correlations of **12**.

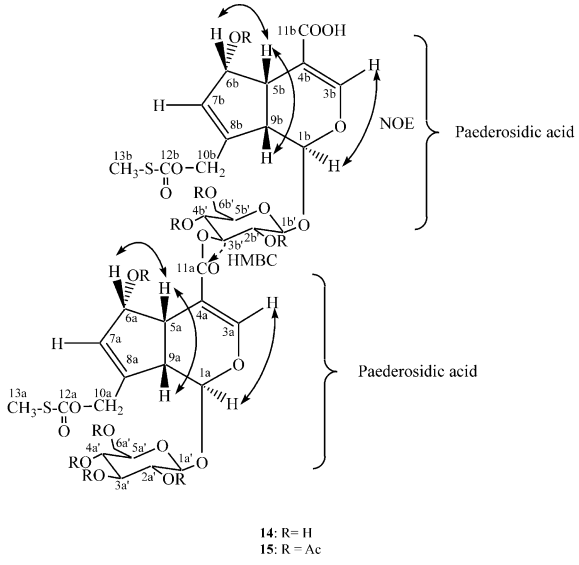

Fig. 3. Important HMBC and NOESY correlations of **14**.

Compound **14** was not obtained as pure state, thus it was acetylated after the absence of acetyl group was confirmed by NMR spectra, followed by purification of the reaction mixture using HPLC to afford **15**, HR-FABMS of which showed the molecular peak at *m/z* 1311.2730, suggesting the molecular formula $C_{54}H_{64}O_{32}S_2Na$. The ^1H and ^{13}C NMR spectra of **15** (Table 5) closely resembled those of **11**; they differed in the following points. It had no signal for methoxyl group and a cross-peak appeared between C-11a and H-3b′ in HMBC, indicating the presence of an ester linkage between C-11a of carboxyl group and C-3b′ of sugar moiety (Table 5). Based on the spectral data of dimer **15** relative to those of **11**, dimer **14** appeared to contain two monomers of paederosidic acid (Fig. 3). HR-FABMS (*m/z* 1311.2730) supported the proposed structure for **15**.

Compounds **5**, **6**, **7**, **8** and **9** were identified as paederoside (Inouye et al., 1969a–c; Kapadia et al., 1979; Suzuki et al., 1993; Calis et al., 2001), asperuloside (Otsuka et al., 1991; Suzuki et al., 1993; Calis et al., 2001), paederosidic acid, (Inouye et al., 1969a–c; Calis et al., 2001), asperulosidic acid (Otsuka et al., 1991; Calis et al., 2001) and geniposide (Inouye et al., 1969a–c; Jensen et al., 1973; Cameron et al., 1984; Inouye et al., 1988; Morota et al., 1989) by spectral data, respectively.

This is the first report of the isolation of a new iridoid glucoside (**1**) and three dimeric sulfur-containing iridoid glucosides (**10**, **12** and **14**). In addition, it is the first time that geniposide (**9**) has been isolated from *P. scandens* although plants belonging to Rubiaceae family are well known to contain various iridoid glucosides (Inouye et al., 1988).

Table 5
^{1}H and ^{13}C NMR assignments, HMBC and NOESY correlations of compound **15**

Position	^{1}H(δ)	^{13}C(δ)	HMBC	NOESY
1a	4.82 (*d*, 8.0)	100.3	H-3a, H-9a, H-1a′	H-3a, H-1a′
3a	7.53 (*d*, 1.4)	154.6	H-1a, H-5a	H-1a
4a		105.2	H-3a, H-5a	
5a	3.18 (*ddd*, 1.4, 6.1, 8.0)	38.9	H-3a, H-6a, H-9a	H-6a, H-9a
6a	5.64 (*dd*, 2.2, 6.1)	76.8	H-5a, H-7a, H-9a	H-5a, H-7a
7a	6.12 (*d*, 2.2)	128.6	H-6a, H-9a, H-10a	H-6a, H-10a
8a		146.6	H-7a, H-9a, H-10a	
9a	2.57 (*t*, 8.0)	44.7	H-5a, H-7a	H-5a, H-10a
10a	4.92 (*dd*, 2.2, 15.4)	64.4	H-7a, H-9a	H-7a, H-9a
	5.10 (*brd*, 15.4)			
11a		165.3	H-3a, H-3b′	
12a		171.1	H-10a, H-13a	
13a	2.36 (*s*)	13.5		
1a′	4.94 (*d*, 8.0)	97.8	H-1a, H-2a′	H-1a, H-3a′, H-5a′
2a′	5.06 (*dd*, 8.0, 9.6)	70.8	H-1a′, H-3a′	H-4a′
3a′	5.25 (*t*, 9.6)	72.6	H-2a′, H-4a′	H-1a′, H-5a′
4a′	5.14 (*dd*, 9.3, 9.6)	68.0	H-3a′, H-5a′	H-2a′, H-6a′
5a′	3.73 (*m*)	72.0	H-4a′	H-1a′, H-3a′, H-6a′
6a′	4.16 (*dd*, 2.2, 12.4)	61.5	H-5a′	H-4a′, H-5a′
	4.21 (*dd*, 4.4, 12.4)			
1b	4.83 (*d*, 8.2)	100.4	H-3b, H-9b, H-1b′	H-3b, H-1b′
3b	7.65 (*d*, 1.1)	154.6	H-1b, H-5b	H-1b
4b		105.2	H-3b, H-5b	
5b	3.25 (*ddd*, 1.1, 6.1, 8.2)	38.6	H-3b, H-6b, H-9b	H-6b, H-9b
6b	5.75 (*br d*, 6.1)	76.8	H-5b, H-7b, H-9b	H-5b, H-7b
7b	6.13 (*d*, 1.9)	128.6	H-6b, H-9b, H-10b	H-6b, H-10b
8b		146.6	H-7b, H-9b, H-10b	
9b	2.64 (*t*, 8.2)	45.1	H-5b, H-7b	H-5b, H-10b
10b	4.92 (*dd*, 2.2, 15.4)	64.4	H-7b, H-9b	H-7b, H-9b
	5.10 (*brd*, 15.4)			
11b		170.5	H-3b	
12b		171.1	H-10b, H-13b	
13b	2.35 (*s*)	13.5		
1b′	4.98 (*d*, 8.0)	97.7	H-1b, H-2b′	H-1b, H-3b′, H-5b′
2b′	5.06 (*dd*, 8.0, 9.6)	71.1	H-1b′, H-3b′	H-4b′
3b′	5.29 (*dd*, 9.3, 9.6)	72.4	H-2b′, H-4b′	H-1b′, H-5b′
4b′	5.18 (*t*, 9.6)	68.2	H-3b′, H-5b′	H-2b′, H-6b′
5b′	3.77 (*m*)	72.1	H-4b′	H-1b′, H-3b′, H-6b′
6b′	4.16 (*dd*, 2.5, 12.4)	61.5	H-5b′	H-4b′, H-5b′
	4.23 (*dd*, 4.4, 12.4)			
$\underline{CH_3}CO$	1.95 (*s*), 1.96 (*s*), 2.01 (*s*)	20.6, 20.6, 20.6	H-6b, H-6a, H-2b′	
	2.02 (*s*), 2.03 (*s*), 2.04 (*s*)	20.6, 20.7, 20.7	H-2a′, H-3a′, H-4b′	
	2.07 (*s*), 2.07 (*s*), 2.08 (*s*)	20.7, 21.1, 21.1	H-4a′, H-6b′, H-6a′	
$CH_3\underline{CO}$		169.3, 169.4, 169.4		
		169.5, 169.8, 170.2		
		170.2, 170.5, 170.5		

3. Experimental

3.1. General

NMR spectra were recorded on Varian Unity 600 (600 MHz), using either $CDCl_3$ or CD_3OD as solvent. Chemical shifts were given in a value with TMS being used as internal standard (^{1}H NMR), and δ 77.03 (ppm) from $CDCl_3$ and δ 49.00 (ppm) from CD_3OD as a standard (^{13}C NMR). Mass spectra including high-resolution and high-resolution FAB mass spectra were recorded on a JOEL JMS AX-500 spectrometer. IR spectra were measured on JASCO FT/IR-5300 spectrophotometer. The UV spectra were obtained on a Hitachi U-3000 spectrophotometer or Shimadzu UV-1650PC in MeOH solution. The specific optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ or MeOH as solvents. HPLC was performed on Shimadzu liquid chromatography LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II or 5 SL-II column. TLC was performed on silica gel plates (Kiesegel 60 F254, Merck) and reversed phase C 18 silica gel plates (Merck), using

solvent system A: $CHCl_3$–MeOH–H_2O (65:35:10, lower phase) and solvent system B: MeOH–H_2O (60: 40). The spots of TLC were detected under UV 254 nm and by spraying with 10% H_2SO_4 or Godin reagent (Godin, 1954), followed by heating at 120 °C.

3.2. Materials

Fresh roots of *Paederia scandens* (Lour) Merrill were collected in Hanoi, Vietnam in July 2000 and then identified by Dr. Tran Ngoc Ninh (Ecological and Biological Resource Center, Hanoi, Vietnam). The voucher specimen (VN 02001) has been deposited in the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

3.3. Extraction and isolation

Roots of Vietnamese *P. scandens* (3.2 kg) were dried at room temperature and powdered, then extracted with MeOH using Soxhlet apparatus. The MeOH extract was concentrated in vacuo to give a residue (118.6 g) which was extracted again with butanol to give 57.2 g butanol extract. Silica gel column chromatography of butanol extract (19.79 g), using $CHCl_3$–MeOH–H_2O (65:15:10, lower phase) as eluent, partly resulted in the isolation of six fractions (Fraction 1–6). Fraction 1 and 2 contained compound **1** (190.7 mg) and compound **3** (382.5 mg), respectively as pure state. Fractions 3 (1477.4 mg) and 4 (389.8 mg) was subjected to reversed phase column HPLC (Shimadzu liquid chromatography LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II) with MeOH–H_2O (40:60) as solvent (flow rate 0.5–0.7 ml/min). Paederoside (**5**) (876.5 mg) was obtained from fraction 3, whereas asperuloside (**6**) (5.83 mg) and geniposide (**9**) (35.3 mg) were obtained from fraction 4. Fractions 5 (663 mg) and 6 (683 mg) were purified by HPLC with reversed phase column [flow rate 0.7 ml/min, solvent MeOH–H_2O (60: 40)] to give paederosidic acid (**7**) (333.7 mg) and dimer (**10**) (10.4 mg) from fraction 5 and asperulosidic acid (**8**) (11.7 mg), dimer **12** (7.3 mg) and a mixture containing **14** (27.3 mg) from fraction 6. The latter mixture was acetylated and purified by HPLC (C 18), using CH_3CN as solvent to obtain **15** (10.4 mg) as acetate of **14**.

3.3.1. Glucoside *1*

Brown powder, TLC solvent system A, R_f 0.60. $[\alpha]_D^{20}$ −8.6° (*c* 1.16, MeOH). Positive FAB-MS: 501 $[M+Na]^+$. HR-MS: *m/z* 501.1042 ($C_{19}H_{26}O_{12}SNa$, expected *m/z* 501.1043). IR (KBr): 3334, 2935, 1768, 1714, 1148, 1073, 944 cm^{-1}. ^1H and ^{13}C NMR (CD_3OD)(see Table 1).

3.3.2. Glucoside *3* (= Paederosidic acid methyl ester)

White powder, TLC solvent system A, R_f 0.52. $[\alpha]_D^{20}$ +12.4° [**c** 0.90, MeOH], lit. (Inouye et al., 1969c) +16.8° (**c** 1.20 MeOH)]; Positive FAB-MS: 501 $[M+Na]^+$. HR-MS: *m/z* 501.1071 ($C_{19}H_{26}O_{12}SNa$, expected *m/z* 501.1043). UV λ_{max} (CH_3OH) nm (logϵ): 234 (4.16), lit. (Inouye et al., 1969c) 236 (4.02); IR (KBr): 3374, 2929, 1700, 1633, 1440, 1308, 1159, 1077, 898 cm^{-1}, lit. (Inouye et al., 1969c) 1700, 1635 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD) (see Table 2).

3.3.3. Dimer *10*

Yellow powder, $[\alpha]_D^{20}$ −5.7° (*c* 1.00, CH_3OH). Positive FAB-MS: 948 $[M+Na]^+$. UV λ_{max} (CH_3OH) nm (logϵ): 236 (3.92). IR (Neat): 3394, 2920, 1702, 1633, 1440, 1379, 1294, 1161, 1102, 950 cm^{-1}. ^1H and ^{13}C NMR (CD_3OD) (see Table 3).

3.3.4. Dimer *12*

Yellow powder, $[\alpha]_D^{20}$ −108.9° (*c* 0.54, CH_3OH). Positive FAB-MS: 915 $[M+Na]^+$. UV λ_{max} (CH_3OH) nm (logϵ): 239 (4.05). IR (Neat): 3418, 2925, 1746, 1706, 1657, 1272, 1166, 1084, 901 cm^{-1}. ^1H and ^{13}C NMR (CD_3OD) (see Table 4).

3.3.5. Acetylation of *1*

Compound **1** (30 mg) in pyridine (2 ml) was acetylated with acetic anhydride (2 ml) and worked up as usual afforded tetraacetate (**2**)(35.1 mg) as oil, $[\alpha]_D^{20}$ −11.6° (*c* 1.18, $CHCl_3$). HR-MS: *m/z* 646.1526 ($C_{27}H_{34}O_{16}S$, requires *m/z* 646.1568). IR (KBr): 2939, 1755, 1725, 1435, 1368, 1227, 1145, 1037 cm^{-1}. ^1H-NMR ($CDCl_3$): δ 6.01 (1H, *br s*, H-7), 5.34 (1H, *br d*, *J* = 6.9 Hz, H-6), 5.22 (1H, *dd*, *J* = 9.3, 9.6 Hz, H-3′), 5.12 (1H, *t*, *J* = 9.3 Hz, H-4′), 5.05 (1H, *d*, *J* = 6.0 Hz, H-1), 5.02 (1H, *dd*, *J* = 8.2, 9.6 Hz, H-2′), 5.01 (1H, *d*, *J* = 3.9 Hz, H-3), 4.97 (1H, *br d*, *J* = 15.5 Hz, H-10), 4.92 (1H, *d*, *J* = 8.2 Hz, H-1′), 4.83 (1H, *br d*, *J* = 15.5 Hz, H-10), 4.23 (1H, *dd*, *J* = 4.7, 12.4 Hz, H-6′), 4.13 (1H, *dd*, *J* = 2.5, 12.4 Hz, H-6′), 3.72 (1H, *m*, H-5′), 3.45 (3H, *s*, 3-OCH_3), 3.32 (1H, *ddd*, *J* = 6.9, 9.1, 10.4 Hz, H-5), 3.02 (1H, *dd*, *J* = 3.9, 10.4 Hz, H-4), 3.01 (1H, *dd*, *J* = 6.0, 9.1 Hz, H-9), 2.35 (3H, *s*, H-13), 2.08 (3H, *s*, $\underline{CH_3}CO$), 2,03 (9H, *s*, 3×$\underline{CH_3}CO$); ^{13}C NMR ($CDCl_3$): δ 174.4 (C-11), 172.6 ($CH_3\underline{CO}$), 171.1 (C-12), 170.2 ($CH_3\underline{CO}$), 169.4 ($CH_3\underline{CO}$), 169.3 ($CH_3\underline{CO}$), 149.2 (C-8), 126.3 (C-7), 96.8 (C-3), 96.0 (C-1′), 95.3 (C-1), 85.7 (C-6), 72.9 (C-3′), 71.9 (C-5′), 71.0 (C-2′), 68.2 (C-4′), 63.5 (C-10), 61.7 (C-6′), 55.6 (3-OCH_3), 44.8 (C-9), 42.9 (C-4), 36.1 (C-5), 20.6 (3x$\underline{CH_3}CO$), 20.7 ($\underline{CH_3}CO$), 13.5 (C-13).

3.3.6. Acetylation of *3*

Acetylation of **3** (30 mg) was carried out by the same method as described above to give pentaacetate (**4**) (39.1 mg) as oil, $[\alpha]_D^{20}$ +19.9° (*c* 2.34, $CHCl_3$). HR-MS: *m/z* 688.1687 ($C_{29}H_{36}O_{17}S$, requires *m/z* 688.1673). UV λ_{max} (CH_3OH) nm (logϵ): 235 (4.37). IR (KBr): 2954, 1725, 1637, 1436, 1370, 1227, 1148, 1042, 958 cm^{-1}. ^1HNMR ($CDCl_3$): δ 7.56 (1H, *d*, *J* = 1.7 Hz, H-3), 6.09 (1H, *br d*,

$J=1.9$ Hz, H-7), 5.76 (1H, *dd*, $J=1.9$, 6.3 Hz, H-6), 5.25 (1H, *t*, $J=9.6$ Hz, H-3′), 5.14 (1H, *dd*, $J=9.6$, 10.2 Hz, H-4′), 5.05 (1H, *dd*, $J=8.2$, 9.6 Hz, H-2′), 5.01 (1H, *dd*, $J=1.7$, 15.1 Hz, H-10), 4.94 (1H, *d*, $J=8.2$ Hz, H-1′), 4.92 (1H, *br d*, $J=15.1$ Hz, H-10), 4.79 (1H, *d*, $J=8.8$ Hz, H-1), 4.22 (1H, *dd*, $J=4.4$, 12.4 Hz, H-6′), 4.16 (1H, *dd*, $J=2.5$, 12.4 Hz, H-6′), 3.74 (1H, *m*, H-5′), 3.73 (3H, *s*, 3-OCH_3), 3.25 (1H, *ddd*, $J=1.7$, 6.3, 8.0 Hz, H-5), 2.62 (1H, *dd*, $J=8.2$, 8.8 Hz, H-9), 2.36 (3H, *s*, H-13), 2.07 (3H, *s*, $\underline{CH_3}CO$), 2.06 (3H, *s*, $\underline{CH_3}CO$), 2.04 (3H, *s*, $\underline{CH_3}CO$), 2.02 (3H, *s*, $\underline{CH_3}CO$), 1.94 (3H, *s*, $\underline{CH_3}CO$). ^{13}C NMR ($CDCl_3$): δ 171.1 (C-12), 170.5 ($CH_3\underline{CO}$), 170.2 ($CH_3\underline{CO}$), 169.8 ($CH_3\underline{CO}$), 169.3 ($CH_3\underline{CO}$), 169.2 ($CH_3\underline{CO}$), 166.8 (C-11), 153.1 (C-3), 146.2 (C-8), 128.4 (C-7), 106.6 (C-4), 100.2 (C-1), 97.8 (C-1′), 77.0 (C-6), 72.4 (C-3′), 72.1 (C-5′), 70.8 (C-2′), 68.2 (C-4′), 64.5 (C-10), 61.6 (C-6′), 51.5 (3-OCH_3), 45.2 (C-9), 38.8 (C-5), 21.0 ($\underline{CH_3}CO$), 20.7 ($\underline{CH_3}CO$), 20.6 (3×$\underline{CH_3}CO$), 13.5 (C-13).

3.3.7. Acetylation of *10*

Compound **10** (5.02 mg) in pyridine (1 ml) was acetylated with acetic anhydride (1 ml) and worked up as usual gave compound **11** (6.25 mg) as oil. $[\alpha]_D^{20}$ +51.6° (*c* 0.30, $CHCl_3$). Positive FAB-MS: 1325 $[M+Na]^+$. HR-FABMS: *m/z* 1325.2900 ($C_{55}H_{66}O_{32}S_2Na$, expected *m/z* 1325.2876). UV λ_{max} (CH_3OH) nm (logϵ): 235 (4.47). IR (KBr): 2935, 1725, 1714, 1636, 1437, 1372, 1223, 1148, 1070, 958 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.63 (1H, *d*, $J=1.7$ Hz, H-3a), 7.55 (1H, *d*, $J=1.7$ Hz, H-3b), 6.07 (1H, *d*, $J=1.9$ Hz, H-7a), 6.05 (1H, *d*, $J=1.9$ Hz, H-7b), 5.76 (1H, *dd*, $J=1.9$, 6.0 Hz, H-6b), 5.73 (1H, *dd*, $J=1.9$, 6.0 Hz, H-6a), 5.25 (2H, *t*, $J=9.6$ Hz, H-3a′ and H-3b′), 5.14 (1H, *dd*, $J=9.6$, 9.9 Hz, H-4b′), 5.10 (1H, *dd*, $J=9.6$, 9.9 Hz, H-4a′), 5.06 (1H, *dd*, $J=8.0$, 9.6 Hz, H-2a′), 5.05 (1H, *dd*, $J=8.0$, 9.6 Hz, H-2b′), 5.00 (2H, *br d*, $J=14.8$ Hz, H-10a and H-10b), 4.97 (1H, *d*, $J=8.0$ Hz, H-1b′), 4.96 (1H, *d*, $J=8.0$ Hz, H-1a′), 4.93 (2H, *dd*, $J=2.2$, 14.8 Hz, H-10a and H-10b), 4.83 (1H, *d*, $J=8.8$ Hz, H-1b), 4.82 (1H, *d*, $J=8.8$ Hz, H-1a), 4.40 (1H, *dd*, $J=2.2$, 12.4 Hz, H-6b′), 4.22 (1H, *dd*, $J=4.7$, 12.4 Hz, H-6a′), 4.17 (1H, *dd*, $J=4.1$, 12.4 Hz, H-6b′), 4.16 (1H, *dd*, $J=2.5$, 12.4 Hz, H-6a′), 3.75 (1H, *m*, H-5a′), 3.73 (3H, *s*, 11b-OCH_3), 3.71 (1H, *m*, H-5b′), 3.25 (2H, *ddd*, $J=1.7$, 6.0, 7.7 Hz, H-5a and H-5b), 2.63 (2H, *dd*, $J=7.7$, 8.8 Hz, H-9a and H-9b), 2.36 (6H, *s*, H-13a and H-13b), 2.07 (6H, *s*, 2×$\underline{CH_3}CO$), 2.06 (3H, *s*, $\underline{CH_3}CO$), 2.04 (3H, *s*, $\underline{CH_3}CO$), 2.03 (3H, *s*, $\underline{CH_3}CO$), 2.02 (6H, *s*, 2× $\underline{CH_3}CO$), 1.96 (3H, *s*, $\underline{CH_3}CO$), 1.92 (3H, *s*, $\underline{CH_3}CO$); ^{13}C-NMR ($CDCl_3$): δ 171.1 (C-12a and 12b), 170.5 (2× $CH_3\underline{CO}$), 170.2 ($CH_3\underline{CO}$), 170.1 ($CH_3\underline{CO}$), 169.8 ($CH_3\underline{CO}$), 169.7 ($CH_3\underline{CO}$), 169.3 (2×$CH_3\underline{CO}$), 169.2 ($CH_3\underline{CO}$), 166.8 (C-11b), 165.7 (C-11a), 154.0 (C-3a), 153.1 (C-3b), 146.3 (C-8a and C-8b), 128.5 (C-7a), 128.3 (C-7b), 106.5 (C-4b), 105.9 (C-4a), 100.7 (C-1b), 100.2 (C-1a), 98.5 (C-1b′), 97.6 (C-1a′), 77.0 (C-6a and C-6b), 72.5 (C-3a′), 72.4 (C-5a′ and C-3b′), 72.1 (C-5b′), 70.8 (C-2a′ and C-2b′), 68.2 (C-4a′), 67.9 (C-4b′), 64.5 (C-10a and C-10b), 61.6 (C-6a′), 61.0 (C-6b′), 51.6 (11b-OCH_3), 45.1 (C-9b), 45.0 (C-9a), 38.9 (C-5b), 38.8 (C-5a), 21.1 ($\underline{CH_3}CO$), 21.0 ($\underline{CH_3}CO$), 20.7 (3×$\underline{CH_3}CO$), 20.6 (4×$\underline{CH_3}CO$), 13.5 (C-13a and 13b).

3.3.8. Acetylation of *12*

Acetylation of **12** (2.8 mg) in the same manner as described above gave compound **13** (3.8 mg) as oil. $[\alpha]_D^{20}$ −35.0° (*c* 0.40, $CHCl_3$). Positive FAB-MS: 1251 $[M+Na]^+$. HR-FABMS: *m/z* 1251.2490 ($C_{52}H_{60}O_{30}S_2Na$, expected *m/z* 1251.2509). UV λ_{max} (CH_3OH) nm (logϵ): 234 (4.05). IR (KBr): 2935, 1755, 1725, 1661, 1634, 1435, 1370, 1226, 1145, 1041, 908 cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 7.48 (1H, *d*, $J=1.4$ Hz, H-3a), 7.12 (1H, *d*, $J=1.9$ Hz, H-3b), 6.08 (1H, *d*, $J=2.2$ Hz, H-7a), 5.76 (1H, *br s*, H-7b), 5.68 (1H, *d*, $J=1.4$ Hz, H-1b), 5.67 (1H, *dd*, $J=2.2$, 6.0 Hz, H-6a), 5.47 (1H, *br d*, $J=6.3$ Hz, H-6b), 5.29 (1H, *t*, $J=9.6$ Hz, H-3a′), 5.25 (1H, *t*, $J=9.6$ Hz, H-3b′), 5.14 (2H, *t*, $J=9.6$ Hz, H-4a′ and H-4b′), 5.05 (2H, *dd*, $J=8.0$, 9.6 Hz, H-2a′ and H-2b′), 5.01 (1H, *dd*, $J=1.9$, 15.4 Hz, H-10a), 4.98 (1H, *d*, $J=8.0$ Hz, H-1a′), 4.91 (1H, *d*, $J=8.0$ Hz, H-1b′), 4.90 (1H, *br d*, $J=15.4$ Hz, H-10a), 4.82 (1H, *d*, $J=8.8$ Hz, H-1a), 4.81 (1H, *dd*, $J=1.4$, 13.7 Hz, H-10b), 4.76 (1H, *dd*, $J=1.1$, 13.7 Hz, H-10b), 4.36 (2H, *dd*, $J=4.7$, 12.4 Hz, H-6a′ and H-6b′), 4.20 (1H, *dd*, $J=4.4$, 12.4 Hz, H-6a′), 4.16 (1H, *dd*, $J=2.5$, 12.4 Hz, H-6b′), 3.80 (1H, *m*, H-5b′), 3.74 (1H, *m*, H-5a′), 3.46 (1H, ddd, $J=1.9$, 6.3, 6.9 Hz, H-5b), 3.25 (1H, *br d*, $J=6.9$ Hz, H-9b), 3.09 (1H, *ddd*, $J=1.4$, 6.0, 8.0 Hz, H-5a), 2.67 (1H, *dd*, $J=8.0$, 8.8 Hz, H-9a), 2.36 (3H, *s*, H-13a), 2.35 (3H, *s*, H-13b), 2.12 (3H, *s*, $\underline{CH_3}CO$), 2.10 (3H, *s*, $\underline{CH_3}CO$), 2.06 (3H, *s*, $\underline{CH_3}CO$), 2.05 (3H, *s*, $\underline{CH_3}CO$), 2.04 (3H, *s*, $\underline{CH_3}CO$), 2.02 (3H, *s*, $\underline{CH_3}CO$), 1.99 (3H, *s*, $\underline{CH_3}CO$), 1.91 (3H, *s*, $\underline{CH_3}CO$). ^{13}CNMR ($CDCl_3$): δ 171.3 (C-12b), 171.0 (C-12a), 170.6 (2×$CH_3\underline{CO}$), 170.2 (2× $CH_3\underline{CO}$), 169.6 ($CH_3\underline{CO}$), 169.4 ($CH_3\underline{CO}$), 169.3 (2× $CH_3\underline{CO}$), 168.9 (C-11b), 164.3 (C-11a), 154.1 (C-3a), 147.6 (C-3b), 146.7 (C-8a), 140.7 (C-8b), 129.8 (C-7b), 127.9 (C-7a), 105.6 (C-4a), 105.3 (C-4b), 99.8 (C-1a), 97.0 (C-1a′), 96.6 (C-1b′), 91.8 (C-1b), 83.8 (C-6b), 77.5 (C-6a), 72.5 (C-5a′), 72.3 (C-5b′), 72.2 (C-3b′), 72.1 (C-3a′), 70.7 (C-2b′), 70.2 (C-2a′), 68.2 (C-4a′), 68.1 (C-4b′), 64.5 (C-10a), 62.9 (C-10b), 61.7 (C-6b′), 61.6 (C-6a′), 44.8 (C-9a), 43.5 (C-9b), 39.1 (C-5a), 36.0 (C-5b), 20.6 (8× $\underline{CH_3}CO$), 13.5 (C-13a and C-13b).

3.3.9. Acetylation of a mixture containing *14*

A mixture (27.3 mg) containing **14** was acetylated with Ac_2O in pyridine. Work up as usual gave compound **15** (10.4 mg) as oil, $[\alpha]_D^{20}$ +17.9° (*c* 0.35, $CHCl_3$). Positive FAB-MS: 1311 $[M+Na]^+$. HR-FABMS: *m/z* 1311.2730 ($C_{54}H_{64}O_{32}S_2Na$, expected *m/z* 1311.2720). UV λ_{max} (CH_3OH) nm (logϵ): 234 (4.36). IR (KBr):

2940, 1725, 1634, 1435, 1372, 1231, 1149, 1070, 956 cm^{-1}. ^1H and ^{13}C NMR ($CDCl_3$) (see Table 5).

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