Iridoids and Anthraquinones from the Malaysian Medicinal Plant, *Saprosma scortechinii* (Rubiaceae)

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A further investigation of the leaves and stems of *Saprosma scortechinii* afforded 13 compounds, of which 10 are new compounds. These were elucidated as the bis-iridoid glucosides, saprosmosides G (1) and H (2), the iridoid glucoside, 6-*O*-*epi*-acetylscandoside (3), and the anthraquinones, 1-methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone (4), 1-methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-*β*-primeveroside (5), 1,3-dihydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-*β*-primeveroside (6), 1,3,6-trihydroxy-2-methoxymethyl-9,10-anthraquinone (7), 1-methoxy-3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone (8), 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone 3-*O*-*β*-primeveroside (9), and 3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone (10). Structure assignments for all compounds were established by means of mass and NMR spectroscopies, chemical methods, and comparison with published data. The new anthraquinones were derivatives of munjistin and lucidin.

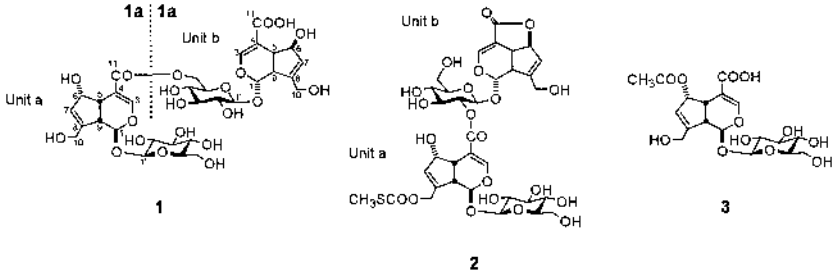
Key words *Saprosma scortechinii*; Rubiaceae; bis-iridoid glucoside; sulfur-containing iridoid; anthraquinone

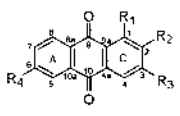
Saprosma scortechinii BL. KING & GAMBLE, a rubiaceous plant endemic in the Malay Peninsula,[1] is also known as 'sekentut', a local name associated with the unpleasant and fetid odor emitted by the bruised plant tissues. In the traditional medicinal system of Malaysia, the roots are employed in decoctions to treat fever by the native communities, while the young leaves are also eaten as a vegetable.[2] Our previous studies on the plant have resulted in the isolation of six sulfur-containing bis-iridoid glucosides (saprosmosides A—F) and 13 iridoid glucosides.[3] On-going studies on the same plant materials have resulted in the isolation of two new bis-iridoid glucosides, a new iridoid glucoside, and seven new anthraquinones, in addition to three known compounds. We wish to report herein the isolation and structural elucidation of these compounds.

Results and Discussion

Dried leaves and stems were separately extracted with methanol, and the resulting extracts were subjected to solvent–solvent partitioning. A combination of column chromatography followed by further purification led to the isolation of two new bis-iridoid glucosides (**1** and **2**) and a new iridoid glucoside (**3**) from the leaves, and seven new anthraquinones (**4**—**10**) from the stems, in addition to three known compounds identified as 6-*O*-acetylscandoside,[4] lucidin 3-*O*-*β*-primeveroside,[5] and damnacanthol 3-*O*-*β*-primeveroside.[6]

The ^{1}H- and ^{13}C-NMR spectra (Table 1) of compound **1** showed two sets of almost superimposable signals (16 carbons each), including signals from two glucopyranosyl moieties attributable to two iridoid glucoside units, indicating that **1** was a bis-iridoid glucoside. The $[M+H]^+$ ion peak (*m*/*z* 763) in the positive-ion FAB-MS supported this observation and the molecular mass was consistent with a molecular formula of $C_{32}H_{42}O_{21}$. The UV (235 nm) and IR (1696, 1635 cm^{-1}) spectra were characteristic for an iridoidic enol ether system conjugated with a carbonyl group.[7,8] The proton and carbon signals of the two units closely resembled





4: R_1 = OCH_3; R_2 = $COOCH_3$; R_3 = OH; R_4 = H
5: R_1 = OCH_3; R_2 = $COOCH_3$; R_3 = O-β-prim; R_4 = H
6: R_1 = OH; R_2 = $COOCH_3$; R_3 = O-β-prim; R_4 = H
7: R_1,R_3,R_4 = OH; R_2 = CH_2OCH_3
8: R_1 = OCH_3; R_2 = CH_2OH; R_3,R_4 = OH
9: R_1,R_4 = OH; R_2 = CH_2OH; R_3 = O-β-prim
10: R_1 = H; R_2 = CH_2OH; R_3,R_4 = OH

prim = glc(6'→1")-xyl

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1** and **2** (CD_3OD, 500 MHz for ^{1}H, 125 MHz for ^{13}C)

	1				**2**			
	Unit a		Unit b		Unit a		Unit b	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.04 (d, 8.5)	101.7	5.02 (d, 9.0)	101.6	5.06 (d, 9.0)	101.6	5.86 (d, 2.0)	94.1
3	7.66 (d, 1.0)	155.8	7.65 (d, 1.5)	155.4	7.70 (d, 1.5)	156.2	7.15 (d, 2.0)	150.0
4	—	108.1	—	108.4	—	107.7	—	106.6
5	3.03 (ddt, 6.5, 6.5, 1.5)	42.7	2.98 (ddt, 6.0, 6.0, 1.5)	42.7	2.90 (ddt, 8.0, 6.0, 1.5)	42.8	3.45 (m)	37.6
6	4.80[a)]	75.6	4.80[a)]	75.2	4.80[a)]	75.1	5.51 (m)	86.4
7	6.04 (d, 2.0)	130.2	6.00 (d, 2.0)	130.6	6.01 (d, 2.0)	132.9	5.60 (dd, 2.0, 1.0)	128.6
8	—	151.2	—	151.0	—	146.1	—	149.5
9	2.58[a)]	45.8	2.58 (m)	45.8	2.71 (dd, 9.0, 8.0)	46.0	3.20[a)]	44.7
10	4.21[a)]	61.7	4.21[a)]	61.7	4.91 (d, 15.0)	66.2	4.16 (s)	60.1
	4.44 (dd, 12.5, 1.5)		4.41 (dd, 12.5, 1.5)		5.14 (dd, 15.0, 2.0)			
11	—	168.9	—	170.8	—	167.5	—	172.0
CO	—	—	—	—	—	172.9	—	—
CH_3	—	—	—	—	2.35 (s)	13.6	—	—
1′	4.72 (d, 7.5)	100.5	4.77 (d, 7.5)	100.5	4.72 (d, 7.5)	101.0	4.92 (d, 8.0)	98.4
2′	3.24 (dd, 9.0, 7.5)	75.0	3.24 (dd, 9.0, 7.5)	75.0	3.25 (dd, 9.0, 7.5)	74.8	4.79 (dd, 9.0, 8.0)	74.3
3′	3.40[a)]	77.8	3.40[a)]	77.6	3.28[a)]	78.6	3.67 (dd, 10.0, 9.0)	75.5
4′	3.28[a)]	71.6	3.38[a)]	71.6	3.28[a)]	71.6	3.38[a)]	71.4
5′	3.28[a)]	78.4	3.53[a)]	75.7	3.38[a)]	77.7	3.40[a)]	78.4
6′	3.63 (dd, 12.0, 5.5)	62.8	4.24 (dd, 12.0, 5.5)	64.1	3.64 (dd, 11.0, 5.5)	62.9	3.70 (dd, 12.0, 6.0)	62.6
	3.84 (dd, 12.0, 1.5)		4.47 (dd, 12.0, 2.0)		3.85 (dd, 11.0, 1.5)		3.94 (dd, 12.0, 2.0)	

a) Overlapped signals.

those of deacetylasperulosidic acid (**1a**),[3)] and alkaline treatment of **1** afforded deacetylasperulosidic acid. This observation confirmed that compound **1** is an ester dimer of deacetylasperulosidic acid. The position of the linkage between the two units was determined by comparing the ^{1}H- and ^{13}C-NMR chemical shifts of **1** with those of deacetylasperulosidic acid.[3)] The upfield shift of the carbonyl carbon signal (δ 168.9) of one unit (unit a), and the downfield shifts of the carbon and proton signals of C-6′ [δ_C 64.1, δ_H 4.24 (dd, J=12.0, 5.5 Hz), 4.47 (dd, J=12.0, 2.0 Hz)] of the other unit (unit b), indicated that the two identical units were connected through an ester bond between C-11 of unit a and C-6′ of unit b. This linkage was further confirmed by the heteronuclear multiple bond connectivity (HMBC) correlations between the protons of C-6′ of unit b and the carbonyl carbon of unit a. On the basis of the above data, the structure of the bis-iridoid was deduced to be as shown as **1** and designated as saprosmoside G.

Compound **2**, named saprosmoside H, was shown to have the molecular formula $C_{34}H_{42}O_{21}S$ as evidenced by positive-ion high resolution (HR)-FAB-MS and the ^{13}C-NMR spectral data. The functional groups of OH, C=O, and C=C were indicated by the IR absorptions at 3390, 1704, and 1656 cm^{-1}, respectively. The ^{1}H- and ^{13}C-NMR spectra (Table 1) displayed two sets of signals typical of a dimeric iridoid glucoside. One set of signals (unit a) showed similar chemical shifts as those of compound **1**, except for signals arising from a methyl (δ_C 13.6, δ_H 2.35, s) and a carbonyl (δ_C 172.9) group, the chemical shifts of which suggested the presence of a thiocarbonyl function.[9)] In the HMBC spectrum, the methyl proton signal (δ 2.35) was correlated with the carbonyl carbon (δ 172.9), and in turn was correlated with the C-10 methylene protons, which therefore supported the location of the thiocarbonyl function at C-10. The other iridoid unit (unit b) of the molecule demonstrated downfield shifts of the carbon (δ 86.4) and proton (δ 5.51, m) signals at C-6, and HMBC correlation between H-6 and the carbonyl carbon at C-11. These spectral characteristics strongly suggested that unit b was deacetylasperuloside.[3)] Alkaline hydrolysis of **2** generated deacetylasperulosidic acid and the characteristic odor of thiomethanol, which further supported the presence of the thiocarbonyl function and the identity of the two subunits. The C-2′ position of unit b was deduced to be the site of esterification with C-11 of unit a, on the basis of the upfield shift of the C-11 carbonyl carbon (δ 167.5) of unit a, and the downfield shift of C-2′ proton (δ 4.79, dd, J=9.0, 8.0 Hz) of unit b, as well as the existence of HMBC correlation between H-2′ of unit b and C-11 of unit a. The structure was further substantiated by comparing the spectra of **2** with those of saprosmoside E,[3)] one of the series of bis-iridoids isolated previously from the same plant, which was made up of the subunits paederosidic acid (unit a) and paederoside (unit b) esterified at C-2′ of unit b and C-11 of unit a. Accordingly, saprosmoside H was assigned the structure of **2**.

Compound **3** has the molecular formula $C_{18}H_{24}O_{12}$, as determined by positive-ion FAB-MS in combination with the ^{1}H- and ^{13}C-NMR spectroscopic data. The UV (229 nm) and IR (1712 and 1633 cm^{-1}) spectra indicated the presence of a conjugated enol-ether system of an iridoid.[7,8)] The ^{1}H-NMR spectrum was characterized by a doublet for the C-1 proton (δ 5.28, J=6.0 Hz), a doublet for the C-3 proton (δ 7.43, J=1.0 Hz), two doublets for the C-10 oxy-methylene (δ 4.19, 4.35, J=15.5 Hz), a doublet for the anomeric proton of glucose (δ 4.67, J=8.0 Hz) and a singlet for an acetoxyl group (δ 2.02). These spectral data were almost superimposable on those of 6-*O*-acetylscandoside.[10)] ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and HMBC analyses of the NMR signals enabled complete assignment of the planar structure of **3** to be the same as 6-*O*-acetylscandoside. The distinguishable features

between the two were the downfield shifts of the carbon signals at C-1 (+1.1 ppm) and C-3 (+4.4 ppm), and the upfield shifts of the carbon signals at C-4 (−4.8 ppm) and C-11 (−3.9 ppm) in **3**, in comparison with the later. This observation indicated that the 6-acetoxyl group in **3** was oriented in an α configuration instead of the β configuration as in 6-*O*-acetylscandoside.[11,12] These data were also in agreement with the previously isolated C-6 epimers, paederosidic acid (6α-OH) and 6-*epi*-paederosidic acid (6β-OH),[3] and asperulosidic acid (6α-OH) and 10-acetylscandoside (6β-OH),[3] where the 6α-OH epimers consistently displayed downfield shifts of the C-1 and C-3 carbon signals, and upfield shifts of the C-4 and C-11 carbon signals compared to those of their 6β-OH epimers. Compound **3** was therefore deduced as the 6α epimer of 6-*O*-acetylscandoside, and characterized as 6-*O*-*epi*-acetylscandoside.

Compounds **4**—**10** were obtained as yellow-orange powders which showed UV and IR absorption spectra characteristics of anthraquinone.[13] Compound **4** was assigned the molecular formula $C_{17}H_{12}O_6$ based on HR-electron impact (EI)-MS (*m*/*z* 312.0629 for $[M]^+$, Calcd 312.0634) and ^{13}C-NMR spectral data. The ^{1}H- and ^{13}C-NMR spectra (Tables 2, 3) of **4** resembled those of munjistin 1-*O*-methyl ether,[14] showing an AA′BB′ type of aromatic proton signals at δ 7.87—8.17, an isolated aromatic proton signal at δ 7.56, along with signals for one aromatic methoxyl group, and an aromatic hydroxyl group. However, compound **4** showed the presence of a methyl ester, as revealed by HMBC correlation of the methoxyl proton signal at δ 3.86 to the ester carbonyl (δ 164.9). ^{1}H–^{1}H COSY, HSQC and HMBC (Fig. 1) experiments led to the assignment of the structure as shown in **4**. Additionally, the ion peak at *m*/*z* 280 suggested the loss of a neutral molecule of CH_3OH, facilitated by the formation of a six membered transition state with the *ortho* disposition of the carbomethoxy and hydroxy substituents.[15] Accordingly, compound **4** was deduced to be 1-methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone.

The positive-ion FAB-MS of **5** revealed the $[M+2H]^+$ peak at *m*/*z* 608, characteristic of quinones due to the generation of a hydroquinone entity,[16] and was compatible with the molecular formula of $C_{28}H_{30}O_{15}$. Other fragment ion peaks at *m*/*z* 594 $[M+2H-14]^+$, 476 $[M+2H-132]^+$, 314 $[M+2H-132-162]^+$ indicated the respective elimination of one methyl, one pentosyl and one hexosyl moiety. The ^{1}H- and ^{13}C-NMR spectral data (Tables 2, 3) of **5** were almost superimposable on those of **4**, except for the presence of additional signals due to two glycosyl units in **5**, evidenced by two diaxial coupled anomeric protons [δ 5.22 (d, J=7.5 Hz), 4.13 (d, J=7.5 Hz)]. Acid hydrolysis of **5** and HPLC analysis of the sugar fraction established the identity of the glycosyl units as glucose and xylose. The anomeric proton signal (δ 5.22) of the glucose was correlated with C-3 of the anthraquinone moiety, which established the 3-*O*-β-glucosidic linkage. The signal attributable to C-6′ of the glucosyl unit was shifted downfield (δ 68.0), and correlated with H-1″ of the xylose moiety in the HMBC spectrum. Thus, the C-1″ of the terminal xylosyl unit should be linked to the C-6′ of the glucosyl part through a β-glycosidic linkage. These assignments were in agreement with those published for the sugar moieties of lucidin 3-*O*-β-primeveroside and damnacanthol 3-*O*-β-primeveroside.[5,6] Therefore, the identity of **5** was established

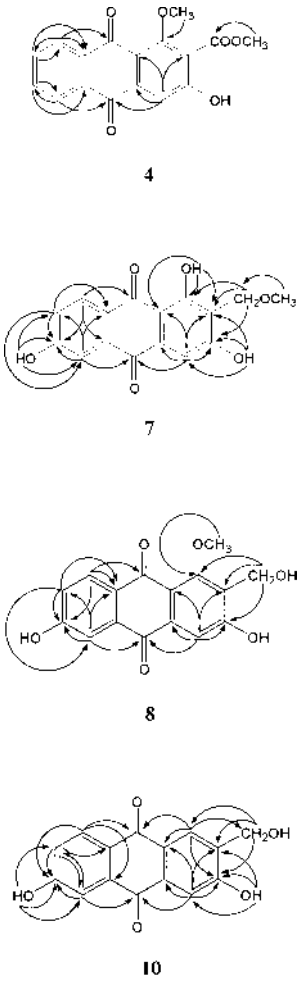

Fig. 1. Principal HMBC Correlations (H→C) of Compounds **4**, **7**, **8** and **10**

as 1-methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-β-primeveroside.

Compound **6** had a very similar structure to **5** including the primeverosyl moiety. The differences shown in the NMR spectra (Tables 2, 3) were the absence of an aromatic methoxyl group and the presence of a phenolic hydroxyl proton signal at δ 12.80, as well as a chelated carbonyl carbon at δ 186.8 in **6** (IR, 1639 cm^{-1}),[17,18] which indicated that the methoxyl group at C-1 in compound **5** was replaced by a hydroxyl group in compound **6**. This assignment was in agreement with the molecular formula of $C_{27}H_{28}O_{15}$. On the basis of these data, compound **6** was characterized as 1,3-dihydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-β-primeveroside.

Compound **7** exhibited the molecular formula $C_{16}H_{12}O_6$ (FAB-MS, *m*/*z* 301 $[M+H]^+$). The ^{1}H-NMR spectrum (Table 2) showed three aromatic protons of an ABX pattern characteristic of a monosubstituted A-ring [δ 7.42 (d, J=2.5 Hz), 7.20 (dd, J=8.5, 2.5 Hz), 8.04 (d, J=8.5 Hz)], a proton singlet (δ 7.19) of a trisubstituted C-ring, one three-proton singlet for a methoxyl group (δ 3.27), one two-proton singlet attributed to an oxy-methylene (δ 4.42), and three hydroxyl protons, the chemical shift of one (δ 13.41) suggested a *peri* hydroxyl group. The ^{13}C-NMR data (Table 3) indicated three aromatic hydroxyl-substituted carbons (δ 163.1, 163.4, 163.5), while the presence of unchelated and chelated car-

Table 2. ^{1}H-NMR Spectral Data of Compounds **4**—**10** (DMSO-d_6, 500 MHz)

	4	**5**	**6**	**7**	**8**	**9**	**10**
1	—	—	—	—	—	—	8.22 (s)
4	7.56 (s)	7.75 (s)	7.50 (s)	7.19 (s)	7.48 (s)	7.45 (s)	7.51 (s)
5	8.14 (dd, 8.0, 1.5)	8.15[a)]	8.20 (dd, 9.0, 2.0)	7.42 (d, 2.5)	7.41 (d, 2.5)	7.48 (d, 2.5)	7.47 (d, 2.5)
6	7.92 (dt, 8.0, 1.5)	7.85 (dt, 8.0, 1.5)	7.96[a)]	—	—	—	—
7	7.87 (dt, 8.0, 1.5)	7.93 (dt, 8.0, 1.5)	7.96[a)]	7.20 (dd, 8.5, 2.5)	7.22 (dd, 8.5, 2.5)	7.23 (dd, 8.5, 2.5)	7.21 (dd, 8.5, 2.5)
8	8.17 (dd, 8.0, 1.5)	8.15[a)]	8.24 (dd, 9.0, 2.0)	8.04 (d, 8.5)	8.32 (d, 8.5)	8.11 (d, 8.5)	8.06 (d, 8.5)
1-OH	—	—	12.80 (s)	13.41 (s)	—	13.32 (br s)	—
1-OCH_3	3.84 (s)	3.84 (s)	—	—	3.85 (s)—	—	
2-CH_2OH	—	—	—	—	4.58 (s)	4.57 (d, 11.5)	4.59 (s)
						4.65 (d, 11.5)	
2-$COOCH_3$	3.86 (s)	3.88 (s)	3.87 (s)	—	—	—	—
2-$C\underline{H}_2OCH_3$	—	—	—	4.42 (s)	—	—	—
2-$CH_2OC\underline{H}_3$	—	—	—	3.27 (s)	—	—	—
3-OH	11.73 (s)	—	—	11.24 (s)	10.80 (br s)	—	10.95 (s)
6-OH	—	—	—	11.02 (s)	10.80 (br s)	ND	10.87 (s)
1′	—	5.22 (d, 7.5)	5.24 (d, 8.0)	—	—	5.08 (d, 7.0)	—
2′	—	3.25 (dd, 8.5, 7.5)	3.26 (dd , 8.5, 8.0)	—	—	3.40 (dd, 8.5, 7.0)	—
3′	—	3.38[a)]	3.35[a)]	—	—	3.37 (t, 8.5)	—
4′	—	3.30[a)]	3.30[a)]	—	—	3.32[a)]	—
5′	—	3.65 (m)	3.63 (m)	—	—	3.62 (m)	—
6′	—	3.70 (dd, 12.0, 5.5)	3.65 (dd, 12.0, 5.0)	—	—	3.67 (dd, 11.0, 5.5)	—
	—	3.95 (dd, 12.0, 2.0)	3.93 (dd, 12.0, 2.0)	—	—	3.97 (dd, 11.0, 2.0)	—
1″	—	4.13 (d, 7.5)	4.14 (d, 7.5)	—	—	4.16 (d, 7.5)	—
2″	—	3.00 (dd, 8.5, 7.5)	3.00 (dd, 8.5, 7.5)	—	—	3.02 (dd, 8.5, 7.5)	—
3″	—	3.08 (t, 8.5)	3.07 (t, 8.5)	—	—	3.10 (t, 8.5)	—
4″	—	3.30[a)]	3.30[a)]	—	—	3.32[a)]	—
5″	—	3.00 (dd, 11.5, 1.5)	3.05 (dd, 11.5, 1.5)	—	—	3.03 (dd, 11.0, 1.5)	—
	—	3.70 (dd, 11.5, 5.5)	3.70 (dd, 11.5, 5.5)	—	—	3.72 (dd, 11.0, 5.5)	—

a) Overlapped signals. ND, not determined.

Table 3. ^{13}C-NMR Spectral Data of Compounds **4**—**10** (DMSO-d_6, 125 MHz)

	4	**5**	**6**	**7**	**8**	**9**	**10**
1	159.5	158.7	159.9	163.4	161.4	161.7	127.5
2	123.3	125.4	117.2	116.7	128.7	123.9	137.6
3	159.6	157.8	159.7	163.4	161.6	161.5	160.5
4	110.1	109.0	105.7	107.3	109.7	106.3	112.6
5	126.3	126.5[a)]	127.1	112.5	111.2	112.9	113.5
6	134.7	133.8	134.8[a)]	163.1	161.9	164.0	163.9
7	133.6	134.8	135.0[a)]	121.3	121.6	121.6	122.6
8	126.7	126.7[a)]	126.5	129.3	129.5	129.6	130.9
9	179.6	180.0	186.8	185.5	179.3	186.2	182.0
10	182.1	181.8	181.2	181.8	182.7	181.7	184.2
4a	136.9	137.2	135.3	133.9	135.4	133.8	126.6
10a	134.3	132.1	132.9	135.0	134.0	135.2	136.7
8a	132.0	134.2	132.7	124.6	126.8	124.2	126.8
9a	117.7	120.2	111.6	108.6	117.8	111.1	134.8
1-OCH_3	62.6	62.9	—	—	62.3	—	—
2-CH_2OH	—	—	—	—	52.3	51.0	59.3
2-$\underline{C}OOCH_3$	164.9	164.2	163.7	—	—	—	—
2-$COO\underline{C}H_3$	52.4	52.7	52.6	—	—	—	—
2-$\underline{C}H_2OCH_3$	—	—	—	61.1	—	—	—
2-$CH_2O\underline{C}H_3$	—	—	—	57.5	—	—	—
1′	—	100.1	100.0	—	—	101.0	—
2′	—	73.0	73.0	—	—	73.2	—
3′	—	76.3	76.3	—	—	75.9	—
4′	—	69.2	69.1	—	—	69.5	—
5′	—	75.8	75.8	—	—	75.7	—
6′	—	68.0	68.0	—	—	68.0	—
1″	—	104.1	104.0	—	—	104.0	—
2″	—	73.2	73.2	—	—	73.2	—
3″	—	76.4	76.4	—	—	76.3	—
4″	—	69.5	69.5	—	—	69.2	—
5″	—	65.6	65.6	—	—	65.6	—

a) Interchangeable in each column.

bonyl carbons (δ 181.8, 185.5) was supported by the IR absorptions at 1662 and 1630 cm^{-1}, respectively.[17,18] The isolated proton (δ 7.19) was assigned to the C-4 position on the basis of its chemical shift and HMBC spectral analysis (Fig. 1). Similarly, the assignments of the hydroxyl groups at C-1, C-3, and C-6 were confirmed by the observed two- or three-bond correlations in the HMBC spectrum. Additional HMBC correlations of the methoxyl signal (δ 3.27) to the methylene carbon (δ 61.1), and of the methylene proton to the carbons at C-1, C-2, and C-3 established the location of this methoxymethyl group at C-2. The structure of **7** was therefore elucidated as 1,3,6-trihydroxy-2-methoxymethyl-9,10-anthraquinone.

Compound **8** was shown to have the same molecular formula as **7** by positive-ion FAB-MS. Their NMR data (Tables 2, 3) were closely related. However, in compound **8**, the methoxyl signal (δ_C 62.3, δ_H 3.85) resonated at lower field, and two unchelated carbonyl signals (δ 182.7, 179.3), as well as only two aromatic hydroxyl groups (δ 10.80, 2H, br s) were observed. The methoxyl group, on the basis of its chemical shift and the lack of C-9 carbonyl carbon chelation, was assigned to the C-1 position with the aid of HMBC spectral analysis (Fig. 1), while the hydroxymethyl substitution at C-2 was confirmed by cross-peaks observed between the methylene protons with C-1, C-2 and C-3. The two hydroxyl groups must occupy the two remaining sites on the aromatic rings, thereby permitting assignment of structure **8** as 1-methoxy-3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone.

The positive-ion FAB-MS of compound **9** displayed a protonated molecular ion at *m*/*z* 581, consistent with $C_{26}H_{28}O_{15}$. The NMR data (Tables 2, 3) in the aromatic region closely

resembled those of **7** or **8**, demonstrating the monosubstituted A-ring and trisubstituted C-ring. However, compound **9** showed additional signals due to a β-primeveroside moiety, as confirmed by acid hydrolysis of **9** and HPLC analysis of the sugar fraction. The primeverosyl moiety was deduced to be attached to the 3-hydroxyl because the anomeric proton, H-1′ (δ 5.08) of the primeverosyl moiety was correlated with C-3 in the HMBC spectrum. Additionally, the signal pattern of the oxy-methylene protons which appeared as two doublets [δ 4.57, 4.65 (both d, J=11.5 Hz)] were indicative of the restricted orientation imposed by the neighboring substitutions. Hence, the structure of **9** was elucidated as 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone 3-*O*-β-primeveroside.

Compound **10** showed IR bands at 3337, 1655 cm^{-1} for free hydroxyl and unchelated carbonyl groups, respectively. The molecular formula $C_{15}H_{10}O_5$ was deduced from the HR-EI-MS, showing an exact mass of 270.0521. The ^{1}H-NMR spectrum (Table 2) showed two isolated aromatic protons (δ 7.51, 8.22, each s), indicative of their *para* disposition in the ring system, in addition to a singlet for a hydroxymethyl group (δ 4.59), two aromatic hydroxyl protons (δ 10.87, 10.95, both s), and an ABX pattern for three aromatic protons as in compounds **7**—**9**. The chemical shifts of the hydroxyl protons and the C-9 and C-10 carbons suggested that no hydroxyl group is in the α position (Tables 2, 3). HMBC spectral analysis established the location of the hydroxyl groups at C-3 and C-6, and the hydroxymethyl moiety at C-2 (Fig. 1). An nuclear Overhauser effect (NOE) experiment provided further evidence for the substitution pattern in the ring-C where irradiation of the methylene at δ 4.59 resulted in enhancement of H-1 (δ 8.22). Accordingly, compound **10** was determined to be 3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone.

Many species of the family Rubiaceae are known to contain substantial amounts of anthraquinones, both free and as glycosides, in addition to the high occurrence of iridoids, usually as glucosides. It is noteworthy that a total of eight bis-iridoid glucosides and 14 iridoid glucosides have been isolated from the leaves and stems of *S. scortechinii*, while nine anthraquinone derivatives of munjistin or lucidin have been isolated from the stems. Among them, 18 are reported for the first time. The occurrence of the iridoids and anthraquinones as the dominant secondary metabolites in *S. scortechinii* gives chemotaxonomical proof for the relationship existing between the genus *Saprosma* and the other genera of the family Rubiaceae.

Experimental

Optical rotations were measured on a JASCO DIP-370 digital polarimeter for **1**, **2** and **3**, and on a JASCO P-1020 polarimeter for **5** and **6**. UV and IR spectra were recorded with JASCO V-560 and JASCO FT/IR-410 spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded in ppm (δ) in CD_3OD or DMSO-d_6 with TMS as the internal standard, employing a Varian Unity *plus* 500 and Varian Gemini 300 spectrometers operating at 500 or 300 MHz for ^{1}H, and 125 or 75 MHz for ^{13}C. HR-EI-MS, EI-MS and FAB-MS were recorded using a JEOL JMS DX-303 spectrometer while HR-FAB-MS were recorded using a JEOL JMS HX-110 spectrometer, with glycerol or *m*-NBA as the matrix. Column chromatography was performed with MCI gel CHP 20P (75—150 μm, Mitsubishi Chemical Industries, Ltd.), Chromatorex ODS (Fuji Silysia), Toyopearl HW-40F (Tosoh Co., Ltd.), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.), and silica gel 60 (0.040—0.063 mm, 0.063—0.200 mm, Merck). Medium pressure liquid chromatography (MPLC) was carried out with a prepacked column; C_{18}-20, equipped with a JASCO PU-986 preparative pump. HPLC was performed with a JASCO 880-PU pump equipped with an 830-RI RI detector, using a column of TOSOH TSK gel NH_2 60 (4.5×250 mm) with a solvent system of H_2O–CH_3CN (4 : 6), and flow rate, 1 ml/min. TLC was performed on precoated silica gel 60 F_{254} plates (0.2 mm thick, Merck) with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2 or 7 : 3 : 0.5 or 6 : 4 : 0.5), and spots were detected by UV illumination and by spraying with 10% H_2SO_4 followed by heating.

Plant Material *Saprosma scortechinii* was collected from the Pasoh Forest Reserve, Negeri Sembilan, Malaysia, in March 2000. A voucher specimen (FRI 45413) has been deposited at the Herbarium of the Forest Research Institute Malaysia (FRIM), Kuala Lumpur, Malaysia.

Extraction and Isolation Dried ground leaves (357 g) and stems (3 kg) were separately extracted three times with MeOH by soaking. The concentrated extracts (68 and 253 g, respectively) were suspended in H_2O and successively partitioned with EtOAc in the case of the leaves, and EtOAc, and 1-BuOH in the case of the stems. The aqueous layer (48.6 g) of the leaves was chromatographed over MCI gel CHP 20P, using H_2O with increasing amounts of MeOH (20—100%) to afford 11 fractions. Fraction 2 (18.0 g) was subjected to MCI gel CHP 20P chromatography (0—100% MeOH) again to give eight subfractions. Subfraction 3 was then purified by Chromatorex ODS (0—100% MeOH), silica gel ($CHCl_3$–MeOH–H_2O (7 : 3 : 0.5—5 : 5 : 1)) and MCI gel CHP 20P (0—25% MeOH) column chromatography to afford saprosmoside G (**1**, 142.6 mg) and 6-*O*-*epi*-acetylscandoside (**3**, 100.6 mg). Fraction 5 (4.9 g) after Chromatorex ODS (0—100% MeOH) column chromatography was subjected to Toyopearl HW-40F (0—20% MeOH), followed by MCI gel CHP 20P (0—60% MeOH) and silica gel ($CHCl_3$–MeOH–H_2O (85 : 15 : 1—6 : 4 : 1)) column chromatography to give saprosmoside H (**2**, 10.3 mg). From the stems, the EtOAc layer (22.1 g) was chromatographed over silica gel with $CHCl_3$ as eluting solvent, followed by $CHCl_3$–MeOH (95 : 5), and $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1—5 : 5 : 1) to afford eight fractions. Fraction 3 (3.8 g) was subjected to further silica gel column chromatography with $CHCl_3$, $CHCl_3$–MeOH (95 : 5), and $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1—6 : 4 : 1) as eluting solvent to give six subfractions. Subfraction 1 (604.0 mg) was rechromatographed over silica gel with $CHCl_3$ and $CHCl_3$–MeOH (99 : 1—9 : 1), and Sephadex LH-20 ($CHCl_3$–MeOH; 97 : 3—1 : 1) to give 1-methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone (**4**, 2.0 mg), and 1,3,6-trihydroxy-2-methoxymethyl-9,10-anthraquinone (**7**, 20.0 mg). Subfraction 5 (137.0 mg) after repeated silica gel column chromatography with $CHCl_3$–EtOAc–MeOH (50 : 50 : 1—50 : 50 : 5) afforded 1-methoxy-3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone (**8**, 15.2 mg). The residue of fraction 4 (1.8 g) was resuspended in MeOH, and the MeOH soluble portion was chromatrographed over Sephadex LH-20 using $CHCl_3$–MeOH (1 : 1) to afford 3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone (**10**, 13.5 mg). On the other hand, the 1-BuOH layer (129.8 g) was chromatographed over MCI gel CHP 20P (0—100% MeOH) to give eight fractions. Fraction 3 (16.2 g) was rechromatographed over MCI gel CHP 20P (0—100% MeOH), followed by Chromatorex ODS (0—5% MeOH), and further purified by MPLC ODS (H_2O) to give 6-*O*-acetylscandoside (29.5 mg). Fraction 8 (5.7 g) was fractionated by Chromatorex ODS (50—100% MeOH) column chromatography into three subfractions. Subfraction 1 was then chromatographed over MCI gel CHP 20P (30—100% MeOH), followed by further purification using MPLC ODS (30—50% MeOH), and silica gel ($CHCl_3$, $CHCl_3$–MeOH (95 : 5), $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1—8 : 2 : 0.1)) to give 1-methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-β-primeveroside (**5**, 36.1 mg), 1,3-dihydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-β-primeveroside (**6**, 9.5 mg), 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone 3-*O*-β-primeveroside (**9**, 18.6 mg), lucidin 3-*O*-β-primeveroside (23.5 mg), and damnacanthol 3-*O*-β-primeveroside (17.6 mg). The known compounds were identified by comparison of their physical and spectral data with the literature values.

Saprosmoside G (**1**): White amorphous powder, $[\alpha]_D^{19}$ −1.2° (c=0.25, MeOH). UV λ_{max} (MeOH) nm (log ε): 235 (4.31). IR (dry film) cm^{-1}: 3466, 1696, 1635. ^{1}H- and ^{13}C-NMR data, Table 1. Positive FAB-MS *m/z*: 763 $[M+H]^+$. *Anal.* Calcd for $C_{32}H_{42}O_{21}\cdot 3H_2O$: C, 47.06; H, 5.92. Found: C, 47.26; H, 5.83.

Saprosmoside H (**2**): Yellow amorphous powder, $[\alpha]_D^{19}$ −46.4° (c=0.11, MeOH). UV λ_{max} (MeOH) nm (log ε): 233 (4.03). IR (dry film) cm^{-1}: 3390, 1704, 1656. ^{1}H- and ^{13}C-NMR data, Table 1. HR-FAB-MS (positive-ion mode) *m/z*: 841.1835 $[M+Na]^+$ (Calcd for $C_{34}H_{42}O_{21}SNa$: 841.1835). Positive FAB-MS *m/z*: 841 $[M+Na]^+$.

6-*O*-*epi*-Acetylscandoside (**3**): White amorphous powder, $[\alpha]_D^{19}$ −94.6° (c=0.19, MeOH). UV λ_{max} (MeOH) nm (log ε): 229 (3.91). IR (dry film) cm^{-1}: 3388, 1712, 1633. ^{1}H-NMR (CD_3OD) δ: 7.43 (1H, d, J=1.0 Hz, H-3), 5.79 (1H, t, J=2.0 Hz, H-7), 5.58 (1H, dd, J=3.0, 2.0 Hz, H-6), 5.28 (1H, d,

J=6.0 Hz, H-1), 4.67 (1H, d, J=8.0 Hz, H-1′), 4.35 (1H, d, J=15.5 Hz, H-10a), 4.19 (1H, d, J=15.5 Hz, H-10b), 3.87 (1H, dd, J=12.0, 1.5 Hz, H-6a′), 3.65 (1H, dd, J=12.0, 5.5 Hz, H-6b′), 3.38 (1H, t, J=9.0 Hz, H-3′), 3.21 (1H, dd, J=9.0, 8.0 Hz, H-2′), 3.16 (2H, m, H-4′, 5′), 3.14 (1H, m, H-5), 3.05 (1H, br t, J=6.0 Hz, H-9), 2.02 (3H, s, CH_3). ^{13}C-NMR (CD_3OD) δ: 172.7 (COO), 171.2 (C-11), 153.0 (C-3), 150.5 (C-8), 127.0 (C-7), 111.2 (C-4), 100.2 (C-1′), 97.4 (C-1), 83.8 (C-6), 78.3 (C-5′), 77.8 (C-3′), 74.7 (C-2′), 71.5 (C-4′), 62.7 (C-6′), 60.9 (C-10), 47.0 (C-9), 42.1 (C-5), 21.2 (CH_3). Positive FAB-MS m/z: 455 $[M+Na]^+$. *Anal.* Calcd for $C_{18}H_{24}O_{12}\cdot H_2O$: C, 48.00; H, 5.82. Found: C, 48.44; H, 5.99.

1-Methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone (**4**): Yellow powder. UV λ_{max} (MeOH) nm (log ε): 370 (3.48), 273 (4.50), 240 (4.32). IR (dry film) cm^{-1}: 3326, 1734, 1667, 1560. ^{1}H-NMR data, Table 2. ^{13}C-NMR data, Table 3. HR-EI-MS m/z: 312.0629 (Calcd for $C_{17}H_{12}O_6$: 312.0634). EI-MS m/z: 312 $[M]^+$, 280 $[M-CH_3OH]^+$. Positive FAB-MS m/z: 314 $[M+2H]^+$, 335 $[M+Na]^+$.

1-Methoxy-3-hydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-β-primeveroside (**5**): Orange powder. $[\alpha]_D^{23}$ −78.7° (c=0.13, MeOH). UV λ_{max} (MeOH) nm (log ε) 376 (3.44), 267 (4.41). IR (dry film) cm^{-1}: 3511, 1738, 1677, 1591. ^{1}H-NMR data, Table 2. ^{13}C-NMR data, Table 3. Positive FAB-MS m/z: 608 $[M+2H]^+$, 629 $[M+Na]^+$, 594 $[M+H-CH_3]^+$, 476 $[M+2H-C_5H_8O_4]^+$, 314 $[M+2H-C_5H_8O_4-C_6H_{10}O_5]^+$. *Anal.* Calcd for $C_{28}H_{30}O_{15}\cdot 1\,1/2H_2O$: C, 53.08; H, 5.25. Found: C, 52.95; H, 5.11.

1,3-Dihydroxy-2-carbomethoxy-9,10-anthraquinone 3-*O*-β-primeveroside (**6**): Yellow powder. $[\alpha]_D^{24}$ −184.9° (c=0.08, MeOH). UV λ_{max} (MeOH) nm (log ε) 397 (4.46), 335 (4.23), 262 (5.06), 246 (5.05), 227 (4.96). IR (dry film) cm^{-1}: 3399, 1724, 1675, 1639, 1592. ^{1}H-NMR data, Table 2. ^{13}C-NMR data, Table 3. Positive FAB-MS m/z: 594 $[M+2H]^+$, 615 $[M+Na]^+$, 461 $[M+H-C_5H_8O_4]^+$, 300 $[M+2H-C_5H_8O_4-C_6H_{10}O_5]^+$. *Anal.* Calcd for $C_{27}H_{28}O_{15}\cdot H_2O$: C, 53.12; H, 4.95. Found: C, 53.03; H, 4.96.

1,3,6-Trihydroxy-2-methoxymethyl-9,10-anthraquinone (**7**): Orange powder. UV λ_{max} (MeOH) nm (log ε) 428 (3.49), 341 (3.54), 279 (4.38), 215 (4.21). IR (dry film) cm^{-1}: 3373, 1662, 1630, 1585. ^{1}H-NMR data, Table 2. ^{13}C-NMR data, Table 3. Positive FAB-MS m/z: 301 $[M+H]^+$. *Anal.* Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.92; H, 4.32.

1-Methoxy-3,6-dihydroxy-2-hydroxymethyl-9,10-anthraquinone (**8**): Orange powder. UV λ_{max} (MeOH) nm (log ε) 383 (3.77), 335 (4.06), 279 (4.99), 214 (4.74). IR (dry film) cm^{-1}: 3296, 1667, 1574. ^{1}H-NMR data, Table 2. ^{13}C-NMR data, Table 3. Positive FAB-MS m/z: 301 $[M+H]^+$, 323 $[M+Na]^+$. *Anal.* Calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.79; H, 4.36.

1,3,6-Trihydroxy-2-hydroxymethyl-9,10-anthraquinone 3-*O*-β-primeveroside (**9**): Yellow powder. UV λ_{max} (MeOH) nm (log ε) 427 (3.87), 341 (3.69), 272 (4.63), 219 (4.52). IR (dry film) cm^{-1}: 3355, 1656, 1595. ^{1}H-NMR data, Table 2. ^{13}C-NMR data, Table 3. Positive FAB-MS m/z: 581 $[M+H]^+$, 603 $[M+Na]^+$, 564 $[M+H-OH]^+$. *Anal.* Calcd for $C_{26}H_{28}O_{15}\cdot 2\,1/2H_2O$: C, 49.92; H, 5.32. Found: C, 50.15; H, 5.20.

3,6-Dihydroxy-2-hydroxymethyl-9,10-anthraquinone (**10**): Yellow powder. UV λ_{max} (MeOH) nm (log ε) 384 (3.71), 335 (4.07), 277 (5.07), 212 (4.76). IR (dry film) cm^{-1}: 3337, 1655, 1596. ^{1}H-NMR data, Table 1. ^{13}C-NMR data, Table 3. HR-EI-MS m/z: 270.0521 (Calcd for $C_{15}H_{10}O_5$: 270.0528). EI-MS m/z: 270 $[M]^+$, 252 $[M-H_2O]^+$. Positive FAB-MS m/z: 271 $[M+H]^+$.

Alkaline Hydrolysis of 1 and 2 A solution of each of the compounds (1—2 mg) in 2% KOH (5 ml) was stirred at room temperature for 1 h and neutralized with 2 N HCl. The main product in each case was detected by TLC and identified by direct comparison with deacetylasperulosidic acid ($CHCl_3$–MeOH–H_2O, 6 : 4 : 0.5, *Rf*: 0.4). The reaction of **2** generated the characteristic odor of thiomethanol.

Acid Hydrolysis of 5, 6, and 9 A solution (2 ml) of the compound (1—4 mg) in 2 M H_2SO_4 was heated under reflux for 2 h. The reaction mixture was filtered and the filtrate was passed through an Amberlite IR 400 (OH^-) column, which was washed with H_2O. The eluate was evaporated to dryness and analyzed by HPLC. Identification of glucose and xylose was carried out by comparison of their retention time with those of authentic samples; t_R (glucose): 5.40 min, t_R (xylose): 4.65 min.

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