Prenylated Phenols from *Sabia japonica*

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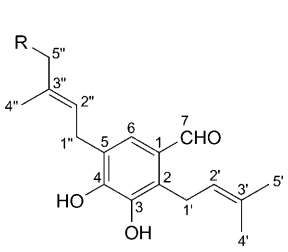
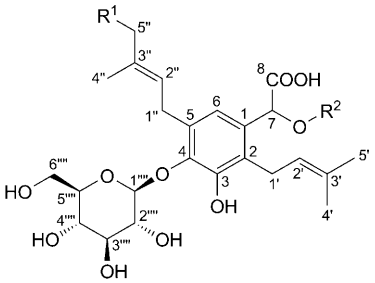
Six prenylated phenols, sabphenols A and B (**1** and **2**, resp.), and sabphenosides A–D (**3**–**6**, resp.), along with eight known constituents, were isolated from the rhizomes of *Sabia japonica* MAX. Their structures were established on the basis of 1D- and 2D-NMR spectral analysis.

Introduction. – In China, plants of the genus *Sabia* (Sabiaceae family) are extensively used for the treatment of lumbago [1]. The most commonly used species, *Sabia japonica* MAX., which is grown in the south of China, is well known for its efficacy in promoting blood circulation, relieving swelling, and alleviating pain. Its rhizomes are employed as an effective drug for hyperosteogeny and rheumatoid arthritis [1][2]. In previous research, the crude extract of *S. japonica* showed anti-inflammatory activity for mice paw edema [3], but the mechanistic actions remained unknown. As part of our ongoing effort to discover new bioactive agents from Chinese medicinal plants, the rhizomes of *S. japonica* were investigated. As a result, six prenylated phenolic compounds, sabphenols A and B (**1** and **2**, resp.) and sabphenosides A–D (**3**–**6**, resp.) were isolated. All of them were comprised by ordinary metabolite fragments as prenyls, benzaldehyde, mandelic acid (=2-hydroxy-2-phenylacetic acid) and HMG (=3-hydroxy-3-methylglutaric acid). Sabphenol A (**1**) was once prepared as an intermediate product by *Zhao* and *Li* during the synthesis of sigmoidin-A and the ^{1}H-NMR data were reported [4]. Sabpheol B (**2**) and sabphenosides A–D (**3**–**6**) were reported for the first time. Eight known constituents were isolated as well, and the structures were established by comparison with corresponding literature. Inhibitory activities of compounds **1**–**6** to NF-κB induced by TNF-α, STAT1 induced by IFN-γ, and STAT3 induced by IL-6 were also investigated, but all were inactive.

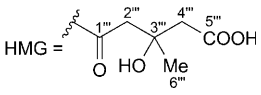
Results and Discussion. – The rhizomes of *S. japonica* were extracted with aqueous acetone (H_2O/acetone 3:7). After removal of the organic solvent under reduced pressure, the concentrated extract was centrifugated to afford a H_2O solution and precipitate. By chromatography on reversed-phase gels, sabphenol B (**2**), sabphenosides A–D (**3**–**6**, resp.), along with markhamioside F [5], (*2S*,*E*)-*N*-[2-hydroxy-2-(4-hydroxyphenyl)ethyl]ferulamide [6], hydroxytyrosol [7], tyrosol [8], helicianeoside B [9], and quercetin 3-(2″-β-D-glucosyl)-α-L-rhamnoside [10] were isolated from the H_2O solution. The precipitate was chromatographed on silica gel columns to afford sabphenol A (**1**), β-sitosterol [11], and fuseine [12].



1 R = H
2 R = OH



	R^1	R^2
3	H	H
4	H	HMG
5	H	HMG-5‴-Me ester
6	OH	HMG



Compound **1**, named sabphenol A, was obtained as colorless needles. The molecular formula of $C_{17}H_{22}O_3$ and seven degrees of unsaturation were determined based on the HR-ESI-MS (*m/z* 297.1475, $[M+Na]^+$; calc. 297.1467). The IR absorption bands implied the presence of an aromatic moiety (1585, 1571, 1479, 1452 cm^{-1}), OH groups (3353 cm^{-1}) and an aldehyde functionality (2820, 2750, 1647 cm^{-1}). The structure of **1** was established as 3,4-dihydroxy-2,5-bis(3-methylbut-2-en-1-yl)benzaldehyde by detailed analysis of the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*, resp.) and confirmed by HSQC, HMBC experiments.

Table 1. *^{1}H-NMR* (400 MHz) *Data for Compounds* **1** *and* **2**. δ in ppm, *J* in Hz.

	1[a])		**2**[b])	
	δ(H)	HMBC	δ(H)	HMBC
H–C(6)	7.24 (*s*)	C(2), C(3), C(4), C(7), C(1″)	7.24 (*s*)	C(2), C(4)
H–C(7)	10.01 (*s*)	C(1), C(2), C(3)	10.00 (*s*)	C(1), C(2), C(3), C(6)
CH_2 (1′)	3.82 (*d*, *J* = 6.7)	C(1), C(3), C(2′), C(3′)	3.89 (*d*, *J* = 6.9)	C(2), C(3), C(2′), C(3′)
H–C(2′)	5.15 (*t*, *J* = 6.6)	C(1), C(2), C(1′), C(4′), C(5′)	5.23 (*t*, *J* = 7.0)	C(2), C(1′), C(4′), C(5′)
Me(4′)	1.76 (*s*)	C(2′), C(3′), C(5′)	1.84 (*s*)	C(2′), C(3′), C(5′)
Me(5′)	1.63 (*s*)	C(2′), C(3′), C(4′)	1.76 (*s*)	C(2′), C(3′), C(4′)
CH_2 (1″)	3.37 (*d*, *J* = 7.3)	C(4), C(6), C(2″), C(3″), C(4″)	3.43 (*d*, *J* = 7.3)	C(4), C(5), C(6), C(2″), C(3″)
H–C(2″)	5.33 (*t*, *J* = 7.4)	C(5), C(1″), C(4″), C(5″)	5.62 (*t*, *J* = 7.2)	C(5), C(1″), C(4″), C(5″)
Me(4″)	1.70 (*s*)	C(2″), C(3″), C(5″)	1.80 (*s*)	C(2″), C(3″), C(5″)
Me(5″) or CH_2(5″)	1.72 (*s*)	C(2″), C(3″), C(4″)	4.06 (*s*)	C(2″), C(3″), C(4″)

[a]) Recorded in (D_6)acetone. [b]) Recorded in $CDCl_3$.

Table 2. *^{13}C-NMR Data* (100 MHz) *for Compounds* **1**–**6**. δ in ppm.

	1[a])	**2**[b])	**3**[c])	**4**[c])	**5**[c])	**6**[d])
C(1)	127.2	126.6	136.7	131.8	134.3	134.9
C(2)	130.1	127.2	127.3	128.1	128.4	130.0
C(3)	143.2	142.4	149.0	149.3	148.9	149.5
C(4)	149.5	147.9	136.7	131.8	134.3	134.9
C(5)	126.4	125.0	134.7	135.2	134.5	135.5
C(6)	126.1	128.0	120.0	121.0	121.7	123.5
C(7)	191.6	192.0	71.1	73.2	76.0	76.9
C(8)			176.0	173.5	176.0	178.0
C(1′)	23.9	24.1	26.4	26.4	27.5	26.4
C(2′)	123.9	121.4	124.4	124.9	124.5	124.4
C(3′)	131.5	135.9	132.0	132.7	131.9	136.3
C(4′)	17.7	18.0	18.3	18.6	18.4	19.9
C(5′)	25.5	25.7	26.2	26.4	26.3	25.6
C(1″)	28.2	27.9	29.5	29.5	28.1	29.7
C(2″)	122.3	122.4	124.6	124.4	124.9	126.5
C(3″)	133.1	137.1	133.3	133.9	133.0	138.9
C(4″)	17.5	13.8	18.3	18.6	18.4	15.8
C(5″)	25.5	68.5	26.2	26.4	26.3	70.0
HMG						
C(1‴)				172.4	172.8	174.8
C(2‴)				47.0	46.2	48.4
C(3‴)				71.4	71.4	72.9
C(4‴)				46.3	47.3	50.5
C(5‴)				175.4	173.6	177.0
C(6‴)				27.9	28.1	28.8
COO*Me*					52.2	
Glc						
C(1⁗)			108.0	108.1	108.0	107.8
C(2⁗)			75.7	75.8	75.8	76.1
C(3⁗)			78.3	78.4	78.3	78.3
C(4⁗)			71.3	71.3	71.4	71.8
C(5⁗)			78.7	78.8	78.7	79.0
C(6⁗)			62.6	62.8	62.7	63.0

[a]) Recorded in (D_6)acetone. [b]) Recorded in $CDCl_3$. [c]) Recorded in CD_3OD. [d]) Recorded in D_2O.

Comparison of the ^{13}C-NMR, DEPT, and HSQC spectra indicated the presence of four Me and two CH_2 groups, two trisubstituted olefinic functionalities, an aromatic moiety, and one aldehyde. The HMBC from Me(5′), Me(4′), and CH_2(1′) to the olefinic C-atoms C(2′) and C(3′) and from Me(5″), Me(4″) and CH_2(1″) to C(2″) and C(3″) helped to establish the two superimposed prenyl groups as 3-methylbut-2-en-1-yl groups. Chemical shifts of the H- and C-atoms in each Me group were assigned by comparing with corresponding literature [13]. The remaining C-atoms comprised a benzaldehyde, supported by consideration of ^{13}C-NMR chemical shifts and degrees of unsaturation. The HMBC from H–C(6) to C(7)=O suggested that the aldehyde functionality was attached *ortho* to H–C(6). Connections of all partial structures were

suggested by the HMBC from H–C(6) to C(1″), from CH_2(1″) to C(6), from H–C(2″) to C(5), from CH_2(1′) to C(1) and C(3), and from H–C(2′) to C(1) and C(2).

Compound **2**, named sabphenol B, was obtained as a white, amorphous powder. Its molecular formula $C_{17}H_{22}O_4$ was consistent with the *pseudo*-molecular ion at *m/z* 313.1412 ($[M+Na]^+$; calc. 313.1416) by the HR-ESI-MS. The IR spectrum showed absorptions for OH groups (3425 cm^{-1}) and a C=O group (1665 cm^{-1}). The structure was established as 3,4-dihydroxy-5-[(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl]-2-(3-methylbut-2-en-1-yl)benzaldehyde by comparison of the ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*, resp.) with those of **1** and confirmed by HSQC, HMBC, and NOESY experiments.

The ^{1}H- and ^{13}C-NMR data of **2** revealed the close structural relationship to **1**. A OH group at C(5″) was inferred from the signals at δ(C) 68.5 (C(5″)) and δ(H) 4.06 (*s*, CH_2(5″)). To determine the geometry of the olefinic bond between C(2″) and C(3″), a NOESY experiment was performed. Since H–C(2″) displayed an NOE correlation to CH_2(5″), but not to Me(4″), the CH_2(5″)OH group was assigned on the same side of the olefinic bond as H–C(2″). Thus, the geometry of the olefinic bond was assigned as (*E*), and the whole structure of compound **2** was confirmed by analysis of HSQC and HMBC spectra.

Compound **3**, named sabphenoside A, was obtained as a white, amorphous powder. The molecular formula $C_{24}H_{34}O_{10}$ was deduced from the HR-ESI-MS (*m/z* 505.2071, $[M+Na]^+$; calc. 505.2050). The IR spectrum also showed absorptions for the OH groups (3420 cm^{-1}) and a C=O group (1730 cm^{-1}). It was identified as 2-[4-(β-D-glucopyranosyloxy)-3-hydroxy-2,5-bis(3-methylbut-2-en-1-yl)phenyl]-2-hydroxyacetic acid based on 1D-, 2D-NMR spectra analysis and enzymatic hydrolysis.

In the ^{1}H- and ^{13}C-NMR spectra (*Tables 3* and *2*, resp.), characteristic signals due to a glucopyranosyl unit were observed and the D-configuration was confirmed by enzymatic hydrolysis (see *Exper. Part*). In the ^{1}H-NMR spectrum, the anomeric H-atom H–C(1‴) appeared as a *d* at 4.53 ppm ($J = 7.6$ Hz) which proved the β-configuration of the glucosidic linkage. The ^{13}C-NMR signals corresponding to the aglycon moiety were almost identical with those of compound **1**. The only difference was a glycolic acid attached to the Ph ring instead of the aldehyde, which was reflected by the signals at δ(C) 71.1 (HO–C(7)) and 176.0 (C(8)=O) in the ^{13}C-NMR spectrum. The position of the glycolic acid was confirmed by HMBCs from the H-atom signal at δ(H) 5.30 (*s*, H–C(7)) to the C(2), C(1), and C(6) signals, as well as to C(8)=O. The glycosylation of HO–C(4) was revealed by the HMBC between the anomeric H-atom H–C(1‴) and C(4).

Sabphenoside B (**4**) was also isolated as a white, amorphous powder. The molecular formula of $C_{30}H_{42}O_{14}$ was determined by the HR-ESI-MS (*m/z* 649.2506 ($[M+Na]^+$; calc. 649.2472). Absorptions for OH groups (3408 cm^{-1}) and a C=O group (1724 cm^{-1}) could be found in the IR spectrum. The combinatorial use of data of 1D- and 2D-NMR spectra allowed the structure assignment of **4** as 5-{carboxy[4-(β-D-glucopyranosyloxy)-3-hydroxy-2,5-bis(3-methylbut-2-en-1-yl)phenyl]methoxy}-3-hydroxy-3-methyl-5-oxopentanoic acid.

In the ^{13}C-NMR and DEPT spectra, compound **4** displayed additional signals to two C=O groups, two CH_2 groups, and one Me group, as well as one O-bearing quaternary C-atom, compared with compound **3**. The HMBC from CH_2(2‴) to C(1‴) and C(3‴),

Table 3. *^{1}H-NMR* (400 MHz) *Data for Compounds* **3** *and* **4**. In CD_3OD. δ in ppm, J in Hz.

	3		**4**	
	δ(H)	HMBC	δ(H)	HMBC
H–C(6)	6.78 (*s*)	C(2), C(4), C(7), C(1″)	6.77 (*s*)	C(2), C(4), C(7), C(1″)
H–C(7)	5.30 (*s*)	C(1), C(2), C(6), C(8)	6.15 (*s*)	C(1), C(2), C(6), C(8), C(1‴)
CH_2(1′)	3.46–3.56 (*m*)	C(1), C(3), C(3′), C(4′), C(5′)	3.40–3.43 (*m*)	C(1), C(3), C(3′)
H–C(2′)	5.20 (*t*, *J* = 6.2)	C(1′), C(4′), C(5′)	5.10 (overlapped)	C(4′), C(5′)
Me(4′)	1.81 (*s*)	C(1′), C(2′), C(3′), C(5′)	1.79 (*s*)	C(2′), C(3′), C(5′)
Me(5′)	1.71 (*s*)	C(1′), C(2′), C(3′), C(5′)	1.69 (*s*)	C(2′), C(3′), C(4′)
CH_2(1″)	3.48–3.58 (*m*)	C(4), C(6), C(2″), C(3″)	3.42–3.48 (*m*)	C(4), C(6), (2″), C(3″)
H–C(2″)	5.30 (*t*, *J* = 6.0)	C(1″), C(4″), C(5″)	5.30 (*t*, *J* = 7.0)	C(1″), C(4″), C(5″)
Me(4″)	1.74 (*s*)	C(1″), C(2″), C(3″), C(5″)	1.73 (*s*)	C(2″), C(3″), C(5″)
Me(5″)	1.75 (*s*)	C(1″), C(2″), C(3″), C(4″)	1.76 (*s*)	C(2″), C(3″), C(4″)
HMG				
CH_2(2‴)			2.88 (*d*, *J* = 14.8)	C(1‴), C(3‴), C(4‴), C(6‴)
			2.72 (*d*, *J* = 14.8)	
CH_2(4‴)			2.73 (*s*)	C(2‴), C(3‴), C(5‴), C(6‴)
Me(6‴)			1.45 (*s*)	C(2‴), C(3‴), C(4‴)
Glc				
H–C(1⁗)	4.53 (*d*, *J* = 7.6)	C(4)	4.47 (*d*, *J* = 7.7)	C(4)
H–C(2⁗)	3.48–3.56 (*m*)		3.50–3.54 (*m*)	
H–C(3⁗)	3.40–3.45 (*m*)		3.38–3.46 (*m*)	
H–C(4⁗)	3.43–3.47 (*m*)		3.40–3.46 (*m*)	
H–C(5⁗)	3.23–3.47 (*m*)		3.30 (overlapped)	C(3⁗), C(4⁗)
CH_2(6⁗)	3.88 (*dd*, *J* = 12.3, 2.3), 3.79 (*dd*, *J* = 12.2, 4.9)		3.90 (*dd*, *J* = 11.4, 2.2), 3.80 (*dd*, *J* = 11.4, 5.0)	C(4⁗), C(5⁗)

from CH_2(4‴) to C(3‴) and C(5‴), and from Me(6‴) to C(2‴), C(3‴), and C(4‴) established the assignment of the HMG (3-hydroxy-3-methylglutaric acid) residue. In comparison with compound **3**, the HO–C(7) C-atom signal of **4** was shifted downfield by 2.1 ppm for the C-atom and 0.85 ppm for the H-atom. These ^{1}H- and ^{13}C-NMR data suggested that the HMG moiety esterified HO–C(7). The correlation peak between H–C(7) and C(1‴) in the HMBC experiment also confirmed this connectivity.

To obtain the aglycons of compounds **3** and **4**, enzymatic hydrolysis was carried out. However, we did not afford enough aglycon material after the reaction (0.5 mg for aglycon of **4**, and even less for **3**), and the absolute configuration of the aglycons could not be established.

Compound **5**, obtained as a white, amorphous powder, had the molecular formula $C_{31}H_{44}O_{14}$ on the basis of the *pseudo*-molecular ion at *m*/*z* 663.2604 ($[M + Na]^+$; calc.

663.2629) by the HR-ESI-MS. In the IR spectrum, absorptions for OH groups (3410 cm^{-1}) and a C=O functionality (1735 cm^{-1}) were found. The ^1H- and ^{13}C-NMR data suggested it being a methyl ester of compound **4**, and this was confirmed by the HMBC spectrum. Thus the structure was elucidated as 2-[4-(β-D-glucopyranosyloxy)-3-hydroxy-2,5-bis(3-methylbut-2-en-1-yl)phenyl]-2-[(3-hydroxy-5-methoxy-3-methyl-5-oxopentanoyl)oxy]acetic acid, and it was named sabphenoside C.

Signals due to a MeO (δ(C) 52.2, δ(H) 3.66 (*s*)) group were observed in the ^1H- and ^{13}C-NMR spectra (*Tables 4* and *2*, resp.). The HMBCs from MeO to C(5‴)=O revealed that the MeO was esterified to the remaining carboxyl group (δ(C) 173.6) of HMG.

Compound **6**, named sabphenoside D, was isolated as a white, amorphous powder. The molecular formula of $C_{30}H_{42}O_{15}$ was determined from the HR-ESI-MS signal at *m/z* 665.2451 ($[M+Na]^+$; calc. 665.2421). The IR spectrum displayed absorptions for OH

Table 4. *^1H-NMR* (400 MHz) *Data for Compounds* **5** *and* **6**. δ in ppm, *J* in Hz.

	5[a])		**6**[b])	
	δ(H)	HMBC	δ(H)	HMBC
H–C(6)	6.87 (*s*)	C(2), C(4), C(7), C(1″)	6.92 (*s*)	C(2), C(4), C(7), C(1″)
H–C(7)	6.11 (*s*)	C(1), C(2), C(6), C(8), C(1‴)	6.05 (*s*)	C(1), C(2), C(6), C(8), C(1‴)
CH_2(1′)	3.46–3.54 (*m*)	C(2), C(2′), C(3′), C(4′), C(5′)	3.54 (*d*, *J* = 7.0)	C(2), C(2′), C(3′), C(4′)
H–C(2′)	5.15 (*t*, *J* = 7.1)	C(1′), C(4′), C(5′)	5.16 (*t*, *J* = 7.2)	C(1′), C(3′), C(4′), C(5′)
Me(4′)	1.77 (*s*)	C(2′), C(3′), C(5′)	1.74 (*s*)	C(1′), C(2′), C(3′), C(5′)
Me(5′)	1.68 (*s*)	C(2′), C(3′), C(4′)	1.83 (*s*)	C(1′), C(2′), C(3′), C(4′)
CH_2(1″)	3.45–3.57 (*m*)	C(5), C(6), C(2″), C(3″)	3.58–3.62 (*m*)	C(4), C(5), C(6), C(2″)
H–C(2″)	5.30 (*t*, *J* = 6.8)	C(1″), C(4″), C(5″)	5.65 (*t*, *J* = 7.6)	C(1″), C(4″), C(5″)
Me(4″)	1.73 (*s*)	C(2″), C(3″), C(5″)	1.80 (*s*)	C(2″), C(3″), C(5″)
Me(5″) or CH_2(5″)	1.73 (*s*)	C(2″), C(3″), C(4″)	4.10 (*s*)	C(2″), C(3″), C(4″)
HMG				
CH_2(2‴)	2.70 (*d*, *J* = 14.5), 2.77 (*d*, *J* = 14.5)	C(1‴), C(3‴), C(4‴)	2.74 (*d*, *J* = 14.1), 2.80 (*d*, *J* = 14.2)	C(1‴), C(3‴), C(6‴)
CH_2(4‴)	2.75 (*s*)	C(2‴), C(3‴), C(5‴)	2.47 (*d*, *J* = 14.8), 2.54 (*d*, *J* = 15.0)	C(3‴), C(5‴), C(6‴)
Me(6‴)	1.42 (*s*)	C(2‴), C(3‴), C(4‴)	1.10 (*s*)	C(2‴), C(3‴), C(4‴)
COO*Me*	3.66 (*s*)	C(5‴)		
Glc				
H–C(1⁗)	4.53 (*d*, *J* = 7.4)	C(4)	4.92 (*d*, *J* = 6.7)	C(4)
H–C(2⁗)	3.52–3.55 (*m*)		3.58–3.64 (*m*)	
H–C(3⁗)	3.45–3.50 (*m*)		3.53–3.60 (*m*)	
H–C(4⁗)	3.46–3.52 (*m*)		3.54–3.58 (*m*)	
H–C(5⁗)	3.26–3.32 (*m*)		3.48–3.55 (*m*)	
CH_2(6⁗)	3.87 (*dd*, *J* = 12.5, 2.1), 3.79 (*dd*, *J* = 12.4, 4.8)		3.95 (*dd*, *J* = 12.5, 2.3), 3.85 (*dd*, *J* = 12.3, 4.5)	

[a]) Recorded in CD_3OD. [b]) Recorded in D_2O.

groups (3420 cm^{-1}) and a C=O group (1740 cm^{-1}). Detailed analysis of the 1D- and 2D-NMR spectra established the structure of **6** as 5-{carboxy[4-(β-D-glucopyranosyloxy)-3-hydroxy-5-[(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl]-2-(3-methylbut-2-en-1-yl)-phenyl]methoxy}-3-hydroxy-3-methyl-5-oxopentanoic acid.

The ^{1}H- and ^{13}C-NMR data of **6** (*Tables 4* and *2*, resp.) were generally similar to those of **4**. The only chemical-shift differences for some resonances were due to a OH group attached to C(5″) in compound **6**. The HMBC correlations from CH_2(5″) to C(2″), C(3″), and C(4″) confirmed the position of the OH group. A NOESY experiment showed that the geometry of the olefinic bond was identical with that of compound **2**.

HMG, a part of compounds **4**–**6**, was an important intermediate in the process of ketogenesis *in vivo*. Whether compounds **4**–**6** were derived *in vivo* by reaction of **3** with HMG-CoA, needs further investigation. If so, C(3‴) of the HMG residue must possess either (*R*)- or (*S*)-configuration. A previous study reported the elucidation of the absolute configuration by the selective reduction of the HMG ester group to give mevalonolactone and its conversion to a phenethylamine derivative. However, the low yield of this reaction prevented our experiment [14].

Since the aqueous extract of *S. japonica* was reported to have anti-inflammatory activity, we evaluated compounds **1**–**6** against NF-κB, STAT1, and STAT3, three factors responsible for regulation of inflammatory genes, using the luciferase assay. However, none of the compounds significantly inhibited these pathways. Whether they have other biological activities also needs further exploration.

Experimental Part

General. Thin-layer chromatography (TLC): precoated silica gel *GF*$_{254}$ (*Yantai Huiyou Inc.*, Yantai, P. R. China). Column chromatography (CC): silica gel *H* (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), *Cosmosil 75 C*$_{18}$*-OPN* (40–150 μm; *Nacalai Tesque, Inc.*), *C*$_8$*-OPN* (140 mesh; *Nacalai Tesque, Inc.*), *MCI* gel *CHP-20P* (75–150 μm; *Mitsubishi Chemical Industries Co., Ltd.*), *TSK* gel *Toyopearl HW-40F* (30–60 μm; *Toso Co., Ltd.*), *Diaion HP20 (Mitsubishi Chemical Industries Co., Ltd.*), *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*). Reversed-phase HPLC: *Agilent 1100* series system equipped with an *Eclipse XDB-C18* column (9.4 cm × 25 cm, *Zorbax* columns). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-2450 7* spectrophotometer. IR Spectra: *Perkin-Elmer 577* spectrophotometer. ^{1}H- and ^{13}C-NMR, HSQC, HMBC, and NOESY spectra: *Bruker AMX-400* spectrometer; δ in ppm, *J* in Hz.

Plant Material. The rhizomes of *Sabia japonica* (Sabiaceae) were collected from Sanjiang, in Guangxi Province, P. R. China, in May 2007, and authenticated by Prof. *Heming Yang*. A voucher specimen (No. SIMM61) is deposited with the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. Air-dried, pulverized rhizomes (5.0 kg) of *S. japonica* were extracted three times with aq. acetone (70%, 20 l) at r.t. for 24 h each. After concentration under reduced pressure, the suspension was centrifugated to afford a H_2O soln. and a precipitate. The H_2O soln. was separated on a column of *Dianion HP20* and eluted with H_2O and 25%, 50%, 75%, and 100% MeOH. The 25% MeOH fraction was further submitted to CC using *Toyopearl HW-40F*, *MCI* gel, *C*$_8$*-OPN*, repeatedly to afford markhamioside F (12 mg), hydroxytyrosol (4 mg), tyrosol (38 mg), and sabphenol B (**2**) (4 mg). As to the compounds exhibiting approximate R_f values (R_f 0.38–0.52) on co-TLC (AcOEt/MeOH/H_2O/AcOH, 6:1:1:1), it was rather difficult to separate them from each other by open CC, and at last they were purified with prep. reversed-phase HPLC, yielding sabphenoside A (**3**) (19 mg, 28% MeCN in H_2O, 2 ml/min, t_R = 14.3–17.7 min), sabphenoside B (**4**) (42 mg, 58% MeOH in H_2O, 2 ml/min, t_R = 10.0–

11.9 min), sabphenoside C (**5**) (3.5 mg, 58% MeOH in H_2O, 2 ml/min, $t_R = 13.3 - 14.6$ min), and sabphenoside D (**6**) (3 mg, 75% MeOH in H_2O, 2 ml/min, $t_R = 4.0 - 6.0$ min), resp. Isolation of the 50% MeOH fraction by repeated reversed-phase CC as above afforded (2*S*,*E*)-*N*-[2-hydroxy-2-(4-hydroxyphenyl)ethyl]ferulamide (40 mg), helicianeoside B (20 mg), and quercetin 3-(2″-β-D-glucosyl)-α-L-rhamnoside (14 mg). The precipitate was separated over a SiO_2 column using a petroleum ether (PE)/Me_2CO gradient (20:1–2:1) to give five fractions *(Frs. A–E)*. *Fr. A* was concentrated and kept undisturbed for one night and sabphenol A (**1**) (80 mg) crystallized in the form of colorless needles. β-Sitosterol (68 mg) was obtained from *Fr. B* by repeated SiO_2 CC with PE/$CHCl_3$ (1:1–1:4). *Fr. E* was successively subjected to SiO_2 CC with PE/Me_2CO gradient (3:1–1:5) and *Sephadex LH-20* with $CHCl_3$/MeOH (1:1) to afford fuseine (23 mg).

Sabphenol A (= *3,4-Dihydroxy-2,5-bis(3-methylbut-2-en-1-yl)benzaldehyde*; **1**). Colorless needles. UV (MeOH): 218 (4.3), 235 (sh, 4.1), 294 (4.0). IR (KBr): 3353, 3141, 2966, 2912, 2820, 2750, 1647, 1585, 1571, 1479, 1452, 1290, 1220, 1074, 721. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 297.1475 ($[M+Na]^+$, $C_{17}H_{22}NaO_3^+$; calc. 297.1467).

Sabphenol B (= *3,4-Dihydroxy-5-[(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl]-2-(3-methylbut-2-en-1-yl)benzaldehyde*; **2**). White, amorphous powder. UV (MeOH): 210 (4.4), 238 (sh, 4.2), 258 (4.1), 284 (4.1). IR (KBr): 3425 (br.), 2922, 2852, 1665, 1448, 1384, 1308, 1070. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 313.1412 ($[M+Na]^+$, $C_{17}H_{22}NaO_4^+$; calc. 313.1416).

Sabphenoside A (= *2-[4-(*β-D-*Glucopyranosyloxy)-3-hydroxy-2,5-bis(3-methylbut-2-en-1-yl)phenyl]-2-hydroxyacetic Acid*; **3**). White, amorphous powder. $[\alpha]_D^{20} = -59$ ($c = 0.1000$, H_2O). UV (H_2O): 204 (4.9), 230 (sh, 4.3), 281 (3.8). IR (KBr): 3420 (br.), 2968, 2926, 1730, 1638, 1440, 1386, 1208, 1072. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*, resp. HR-ESI-MS: 505.2071 ($[M+Na]^+$, $C_{24}H_{34}NaO_{10}^+$; calc. 505.2050).

Sabphenoside B (= *5-{Carboxy[4-(*β-D-*glucopyranosyloxy)-3-hydroxy-2,5-bis(3-methylbut-2-en-1-yl)phenyl]methoxy}-3-hydroxy-3-methyl-5-oxopentanoic Acid*; **4**). White, amorphous powder. $[\alpha]_D^{20} = -69$ ($c = 0.1050$, H_2O). UV (H_2O): 205 (4.6), 230 (sh, 4.3), 281 (3.8). IR (KBr): 3408 (br.), 2976, 2928, 1724, 1635, 1439, 1385, 1209, 1074. ^{1}H- and ^{13}C-NMR: *Tables 3* and *2*, resp. HR-ESI-MS: 649.2506 ($[M+Na]^+$, $C_{30}H_{42}NaO_{14}^+$; calc. 649.2472).

Sabphenoside C (= *2-[4-(*β-D-*Glucopyranosyloxy)-3-hydroxy-2,5-bis(3-methylbut-2-en-1-yl)phenyl]-2-[(3-hydroxy-5-methoxy-3-methyl-5-oxopentanoyl)oxy]acetic Acid*; **5**). White, amorphous powder. $[\alpha]_D^{20} = -56$ ($c = 0.1550$, H_2O). UV (H_2O): 202 (4.8), 228 (sh, 4.3), 274 (3.7). IR (KBr): 3410 (br.), 2970, 2928, 1735, 1436, 1379, 1210, 1076. ^{1}H- and ^{13}C-NMR: *Tables 4* and *2*, resp. HR-ESI-MS: 663.2604 ($[M+Na]^+$, $C_{31}H_{44}NaO_{14}^+$; calc. 663.2629).

Sabphenoside D (= *5-{Carboxy[4-(*β-D-*glucopyranosyloxy)-3-hydroxy-5-[(2*E*)-4-hydroxy-3-methylbut-2-en-1-yl]-2-(3-methylbut-2-en-1-yl)phenyl]methoxy}-3-hydroxy-3-methyl-5-oxopentanoic Acid*; **6**). White, amorphous powder. $[\alpha]_D^{20} = -21$ ($c = 0.3200$, H_2O). UV (H_2O): 205 (4.6), 231 (sh, 4.0), 276 (3.6). IR (KBr): 3420 (br.), 2972, 2930, 1740, 1440, 1386, 1210, 1074. ^{1}H- and ^{13}C-NMR: *Tables 4* and *2*, resp. HR-ESI-MS: 665.2451 ($[M+Na]^+$, $C_{30}H_{42}NaO_{15}^+$; calc. 665.2421).

Enzymatic Hydrolysis of Compounds **3** *and* **4**. To identify the glucoses and establish the absolute configuration of the aglycons, hydrolysis was carried out. Compounds **3** (8.2 mg) and **4** (14.7 mg) were dissolved in H_2O (10 ml for **3**, 20 ml for **4**, resp.), and β-cellulase (two folders substrate) was added to the soln. and kept at 37°, pH 5–6 for 45 h, in each case. After that, the mixture was evaporated and separated over a *C_8-OPN* column, resp. Glucoses were eluted with H_2O and compared with an authentic sample by co-TLC (AcOEt/MeOH/H_2O/AcOH, 13:3:3:4, R_f 0.48 for glucose). Identification of D-glucose in each compound was carried out by comparing the optical rotation of the liberated glucose with that of the authentic sample of D-glucose ($[\alpha]_D^{20} = +56$, $c = 0.1000$, H_2O). However, 0.5 mg of aglycon for compound **4** and even less of that for compound **3** were obtained after the hydrolysis, and the attempt to elucidate the absolute configurations was given up. Compounds **5** and **6** were not hydrolyzed for their small remaining quantity after the bioassay. However, according to the ^{1}H- and ^{13}C-NMR data and their biogenesis relationship to compounds **3** and **4**, the glucose moieties of compounds **5** and **6** were concluded to be β-D-glucose.

Bioassay Evaluation. A HEK293 cell line and a HepG2 cell line were stably transfected with a κB-luciferase reporter gene and a STAT-luciferase reporter gene, resp. For the luciferase assay, the cells were seeded into 24-well plate, grown to about 90% confluency and were pretreated with the tested

compounds (5 μM or 10 μM) for 1 h. Then the cells were stimulated by corresponding cytokines including TNF-α (25 IU/ml), IFN-γ (50 IU/ml), IL-6 (10 ng/ml) for 5 h. After that, equal cell numbers were collected for the assay and the luciferase activity was measured by a luminometer using a luciferase assay system (*Promega*, Shanghai, P. R. China).

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