Two New Flavonoids from the Rhizomes of *Abacopteris penangiana*

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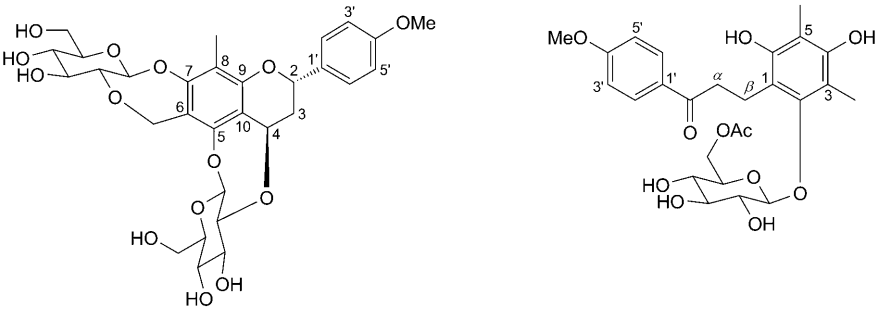
A new flavan-4-ol glycoside, abacopterin K (**1**), and a new dihydrochalcone glycoside, abacopterin L (**2**), were isolated from the rhizomes of *Abacopteris penangiana*. Their structures were elucidated on the basis of various spectroscopic analyses and chemical evidences. The novel glucosylation pattern of abacopterin K consisting of two 'glucose-fused' dioxepine ring moieties is found for the first time within natural flavonoids.

Introduction. – The genus *Abacopteris* (Thelypteridaceae family) consists of *ca.* 35 species of ferns, and some of them are widely distributed in tropical and subtropical zones. Phytochemical studies on this genus demonstrated that it is a source of the rather rare flavan-4-ol glycosides [1][2]. *Abacopteris penangiana* (HOOK.) CHING is widely distributed in the south of China, and is used as traditional Chinese medicine for the treatment of upper-respiratory-tract infections and dysentery [3][4]. As a part of the systematic chemical investigation on *Abacopteris* species, we initiated a chemical investigation of this plant. Our previous study on this species resulted in the isolation of nine new flavan-4-ol glycosides [5][6]. In this article, we report on the isolation and structural elucidation of two new flavonoids, abacopterins K[1]) (**1**) and L[1]) (**2**) (*Fig. 1*), from the rhizomes of *A. penangiana*.

Results and Discussion. – The rhizomes of *Abacopteris penangiana* were extracted with MeOH to give a residue which was suspended in H_2O and extracted sequentially with $CHCl_3$, AcOEt, and BuOH. After being chromatographed on silica gel, *Sephadex LH-20*, and ODS gel columns, the AcOEt fraction afforded **2**, and the BuOH fraction afforded **1**.

Compound **1** was obtained as optically active white needles, and the IR spectrum showed absorption peaks for OH groups (3430 cm^{-1}) and aromatic moieties (1616, 1516 cm^{-1}). The molecular formula was established as $C_{30}H_{36}O_{14}$ from the HR-ESI-MS (*m/z* 643.1981 ($[M+Na]^+$)). The ^{1}H-NMR spectrum of **1** (*Table*) showed signals characteristic for a Me group at δ(H) 2.37 (*s*), a MeO group at δ(H) 3.67 (*s*), two anomeric H-atoms at δ(H) 5.54 (*d*, $J=8.6$ Hz) and 4.97 (*d*, $J=7.4$ Hz), and four aromatic H-atoms at δ(H) 6.97 (*d*, $J=8.4$ Hz, 2 H) and 7.31 (*d*, $J=8.4$ Hz, 2 H),

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



1 **2**

Fig. 1. *Compounds* **1** *and* **2**[1]*), isolated from* Abacopteris penangiana

suggesting a *para*-substituted phenyl group (*Table*). The ^{13}C-NMR spectrum of **1** (*Table*), analyzed with the aid of DEPT, HSQC, and ^{1}H,^{1}H-COSY data, revealed the presence of one *para*-substituted and one fully substituted benzene moiety, two sugar units, one aromatic Me, and one $CHCH_2CH$ substructure. Analysis of these structural characteristics indicated that **1** is a flavan-4-ol glycoside with two sugar units. The ^{1}H- and ^{13}C-NMR data of **1** were very close to those of the known abacopterin D (=(2*R*,3*S*,4*S*,4a*R*,8*R*,10*S*,13a*S*)-3,4,4a,9,10,13a-hexahydro-3,4,8-trihydroxy-2-(hydroxymethyl)-10-(4-methoxyphenyl)-12-methyl-2*H*,6*H*,8*H*-dipyrano[2,3-*b*:3′,2′*h*][1,4]benzodioxepin-7-yl *β*-D-glucopyranoside) [5], except for the signal of C(4) (*δ*(C) 65.6), which shifted downfield by *ca.* 9 ppm compared with that of abacopterin D.

On acid hydrolysis of **1**, D-glucose was detected. In conjunction with the ^{1}H- and ^{13}C-NMR data of the sugar moiety (*Table*), the absolute configurations of two sugar units were thus determined as *β*-D-glucose by GC. The sequence of the sugar and binding sites to the aglycone were determined by 2D-NMR experiments. As observed in the HMBC spectrum (*Fig. 2*), correlations of H–C(1″) (*δ*(H) 5.54) to C(5) (*δ*(C) 149.3) and of H–C(1‴) (*δ*(H) 4.97) to C(7) (*δ*(C) 155.7) suggested that the two D-glucose units were attached to C(5) and C(7). The correlation of H_a–C(11) (*δ*(H) 5.81) with C(2‴) (*δ*(C) 87.2) confirmed that C(2‴) of the sugar located at C(7) formed an ether linkage to C(11), and the correlation of H–C(2″) (*δ*(H) 3.89) to C(4) (*δ*(C) 65.6) indicated that C(2″) of the D-glucose located at C(5) also formed an ether linkage to C(4) of the aglycone. Thus, between the sugars and the aglycone, two unusual 'glucose-fused' dioxepine rings were formed.

To establish the configurations at C(2) and C(4), the CD spectrum of **1** was analyzed. The CD spectrum of **1** was very close to that of the previous reported abacopterin I (=1,2-*O*-[(2*S*,4*R*)-7-(*β*-D-glucopyranosyloxy)-3,4-dihydro-2-(4-methoxyphenyl)-6,8-dimethyl-2*H*-1-benzopyran-5,4-diyl *β*-D-glucopyranose) [6], and their CD spectra both showed a positive *Cotton* effect around 282 nm (*Fig. 3*), suggesting they have similar configurations at C(2) and C(4). In the CD spectra of flavan-4-ols, the presence of a positive *Cotton* effect in the region of 275–285 nm indicates that the absolute configuration at C(4) is (*R*) [7], and this was also supported by our previous

Table. *^{1}H- and ^{13}C-NMR Data* (C_5D_5N, 400 and 100 MHz) *of* **1** *and* **2**. δ in ppm, J in Hz.

	1			**2**	
	δ(H)	δ(C)		δ(H)	δ(C)
H–C(2)	5.05 (*d*, *J* = 11.7)	74.2	C=O		200.3
CH_2(3)	2.33–2.36 (*m*),	36.9	CH_2(α)	3.70–3.74 (*m*)	40.0
	2.08 (br. *t*, *J* = 11.0)		CH_2(β)	3.88–3.92 (*m*)	20.4
H–C(4)	5.23 (*d*, *J* = 3.1)	65.6	C(1)		116.0
C(5)		149.3	C(2)		153.0
C(6)		119.3	C(3)		110.9
C(7)		155.7	C(4)		154.1
C(8)		115.0	C(5)		110.5
C(9)		154.0	C(6)		152.97
C(10)		108.7	*Me*–C(3)	2.91 (*s*)	11.6
CH_2(11)	5.81 (*d*, *J* = 13.7),	65.1	*Me*–C(5)	2.67 (*s*)	10.7
	4.47 (*d*, *J* = 13.7)		C(1′)		130.5
Me–C(8)	2.37 (*s*)	9.2	H–C(2′,6′)	8.14 (*d*, *J* = 8.4)	130.8
C(1′)		133.0	H–C(3′,5′)	6.93 (*d*, *J* = 8.4)	114.0
H–C(2′,6′)	7.31 (*d*, *J* = 8.4)	128.2	C(4′)		163.7
H–C(3′,5′)	6.97 (*d*, *J* = 8.4)	114.3	*Me*O–C(4′)	3.64 (*s*)	55.2
C(4′)		160.0	2-*O*-Glc:		
*Me*O–C(4′)	3.67 (*s*)	55.2	C(1″)	5.44 (*d*, *J* = 7.7)	106.2
5-*O*-Glc:			H–C(2″)	4.38 (*t*, *J* = 9.1, 7.7)	75.8
C(1″)	5.54 (*d*, *J* = 8.6)	101.8	H–C(3″)	4.30 (*t*, *J* = 9.1, 8.9)	78.2
H–C(2″)	3.89 (*t*, *J* = 8.7)	76.0	H–C(4″)	4.16 (*t*, *J* = 9.6, 8.9)	71.1
H–C(3″)	4.30–4.34 (*m*)	76.3	H–C(5″)	3.91–3.94 (*m*)	75.2
H–C(4″)	4.04–4.08 (*m*)	71.3	CH_2(6″)	4.77 (*dd*, *J* = 11.7, 5.0),	63.9
H–C(5″)	3.98–4.02 (*m*)	79.9		4.71 (*dd*, *J* = 11.7, 2.3)	
CH_2(6″)	4.56 (br. *d*, *J* = 12.0),	62.5	*Ac*O–C(6″)	1.75 (*s*)	20.4,
	4.33–4.37 (*m*)				170.7
7-*O*-Glc:					
H–C(1‴)	4.97 (*d*, *J* = 7.4)	104.4			
H–C(2‴)	3.86 (*t*, *J* = 9.0)	87.2			
H–C(3‴)	4.24 (*t*, *J* = 9.0)	75.9			
H–C(4‴)	4.37–4.41 (*m*)	70.8			
H–C(5‴)	3.98–4.02 (*m*)	79.6			
CH_2(6‴)	4.52 (br. *d*, *J* = 12.0),	62.0			
	4.40–4.44 (*m*)				

studies [5][6]. Eruberin A (=1,2-*O*-[(2*S*,4*R*)-3,4-dihydro-7-hydroxy-2-(4-methoxyphenyl)-6,8-dimethyl-2*H*-1-benzopyran-5,4-diyl β-D-glucopyranose) and abacopterin C (=1,2-*O*-[(2*S*,4*S*)-3,4-dihydro-7-hydroxy-2-(4-methoxyphenyl)-6,8-dimethyl-2*H*-1-benzopyran-5,4-diyl β-D-glucopyranose), two known flavan-4-ol glycosides from *A. penangiana* [5][6], are a pair of stereoisomers, and the only difference between them is the configuration at C(4). The comparison of their CD spectra is shown in *Fig. 3*. Eruberin A with (4*R*) configuration showed a positive *Cotton* effect at 282 nm, while abacopterin C with (4*S*) configuration exhibited a negative *Cotton* effect at 278 nm. Furthermore, an NOE correlation of H–C(1″) to H–C(4), observed in the ROESY experiment (*Fig. 2*), also verified that the configuration at C(4) is (*R*). To determine

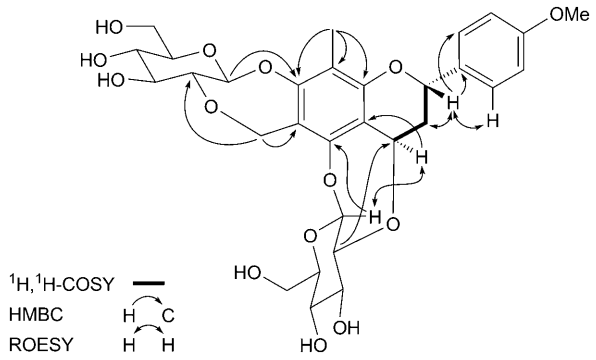


Fig. 2. *Selected 2D-NMR correlations of* **1**

the configuration at C(2), the chemical shifts and coupling constants of H–C(2) and H–C(4) were compared with those of known flavan-4-ol glycosides isolated from the thelypteridaceous ferns. The coupling constants of H–C(2) (*d*, *J*(2,3ax) = 11.7 Hz) and H–C(4) (*d*, *J*(4,3ax) = 3.1 Hz) of **1** were consistent with those of abacopterin I [6] and pneumatopterin C (= 1,2-*O*-[(2*S*,4*R*)-3,4-dihydro-7-hydroxy-6-(hydroxymethyl)-8-methyl-2-phenyl-2*H*-1-benzopyran-5,4-diyl β-D-glucopyranose) [2]; therefore, H–C(2) is in axial position and H–C(4) in quasi-equatorial position [8], establishing the relative *trans* configuration of H–C(2) and H–C(4) [8]. On the basis of the above evidences, the configuration at C(2) was finally deduced as (*S*). Thus, the structure of **1** was elucidated as (2*S*,4*R*)-4′-methoxy-6,8-dimethyl-4,2″-*O*:11,2‴-*O*-dicycloflavan-5,7-diol 5,7-bis(β-D-glucopyranoside)[1]), namely abacopterin K.

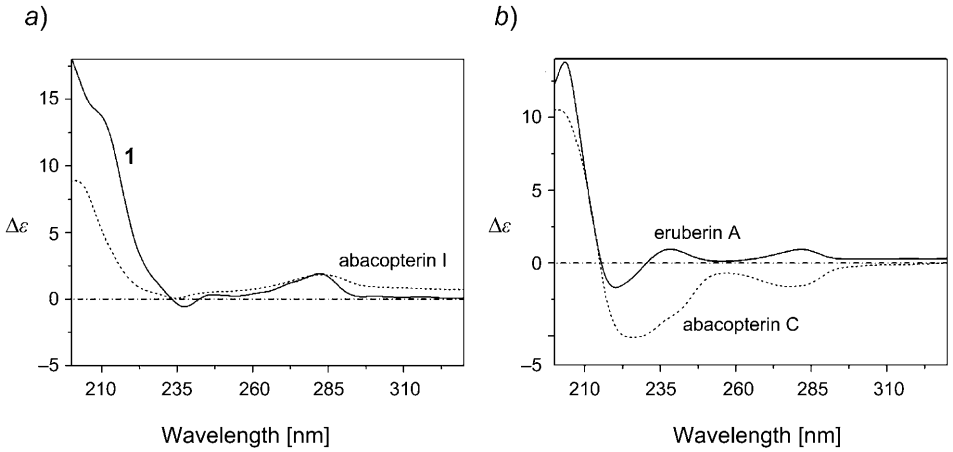


Fig. 3. *CD Spectra of four flavan-4-ol glycosides isolated from* A. penangiana: a) *CD of abacopterin K* (**1**) *and abacopterin I, and* b) *CD of eruberin A and abacopterin C*

In general, ethers resist hydrolysis, and ether bonds are relatively difficult to be cleaved by acids. However, our previous studies showed that the additional ether bond

between the aglycon and C(2) of the glucose residue could be easily cleaved by weak acids [5]. For example, when abacopterin C was hydrolyzed with 90% AcOH in 90° for 4 h, the ether linkage was broken [5]. These evidences suggested that the stability of the 'glucose-fused' dioxepine ring is poor, and this may be caused by its high ring strain. Within the 'glucose-fused' dioxepine rings in **1**, one C-atom (C(4) or C(11)), one O-atom (located at C(5) or C(7)) and two aromatic C-atoms lie in the plane of the benzene *A*-ring, two C-atoms of the remaining atoms are the members of the sugar ring, and this leads to the high ring strain of the seven-membered rings.

Compound **2** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{26}H_{32}O_{11}$ by HR-ESI-MS (*m/z* 543.1866 ($[M+Na]^+$)). Its UV spectrum exhibited absorption maxima at 221 and 274 nm. The IR spectrum of **2** showed the presence of OH groups (3431 cm^{-1}), C=O groups (1728, 1657 cm^{-1}), and aromatic rings (1600, 1513 cm^{-1}). In the ^{13}C-NMR spectrum of **2**, 26 C-atom signals including signals for one ketone C=O and one ester C=O group, eight sp^2 quaternary C-atoms, and four sp^2 CH, five sp^3 CH, three sp^3 CH_2, and four Me groups were observed (*Table*). The ^{1}H-NMR spectrum of **2** revealed the presence of an A_2B_2 spin system, *i.e.*, two *d* at δ(H) 8.14 ($J = 8.4$ Hz, 2 H) and 6.93 ($J = 8.4$ Hz, 2 H), an anomeric H-atom at δ(H) 5.44 (*d*, $J = 7.7$ Hz), a MeO group at δ(H) 3.64 (*s*), and three Me groups at δ(H) 2.91 (*s*), 2.67 (*s*), and 1.75 (*s*) (*Table*). Acidic hydrolysis of **2** afforded D-glucose, detected by GC. The ^{1}H,^{1}H-COSY data (*Fig. 4*) between $CH_2(\alpha)$ (δ(H) 3.70–3.74) and $CH_2(\beta)$ (δ(H) 3.88–3.92), and the HMBCs (*Fig. 4*) of $CH_2(\alpha)$ to C=O (δ(C) 200.3), C(β), and C(1), of $CH_2(\beta)$ to C=O, C(α), C(1), C(2), and C(6), of Me(3) to C(2), C(3), and C(4), of Me(5) to C(4), C(5), and C(6), of H–C(2′) to C=O, C(1′), and C(3′), and of *Me*O–C(4′) to C(4′) established the structure of the aglycone as α,β-dihydro-2,4,6-trihydroxy-4′-methoxy-3,5-dimethylchalcone (chalcone = (2*E*)-1,3-diphenylprop-2-en-1-one). The ROESY data also supported the substitution pattern of the aglycone by showing the correlations Me–C(4′)/H–C(3′) and H–C(5′), and $CH_2(\alpha)$/H–C(2′) and H–C(6′) (*Fig. 4*). An HMBC of H–C(1″) (δ(H) 5.44) to C(2) (δ(C) 153.0) suggested that the D-glucose was attached to C(2) of the aglycon, and an HMBC of CH_2(6″) (δ(H) 4.77 and 4.71) to the C=O group at δ(C) 170.7 suggested that the OH–C(6″) of the sugar moiety was acetylated. Therefore, abacopterin L[1]) (**2**) was unambiguously characterized as α,β-dihydro-2,4,6-trihydroxy-4′-methoxy-3,5-dimethylchalcone 2-(6-*O*-acetyl-β-D-glucopyranoside).

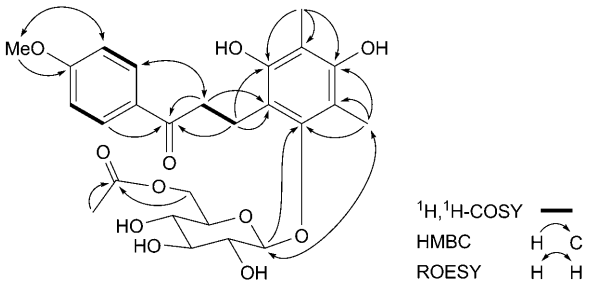


Fig. 4. *Selected 2D-NMR correlations of* **2**

Our phytochemical investigations on *A. penangiana* showed the presence of some unusual flavan-4-ol glycosides, such as abacopterins A–I [5][6], in which one glucose residue is 'fused' to the aglycone through a dioxepine ring. This type of flavonoids with a 'glucose-fused' skeleton has a very narrow distribution in nature, and has only been reported from four thelypteridaceous ferns, including one *Glaphyropteridopsis* species, *G. erubescens* [9], one *Pneumatopertris* species, *P. pennigera* [2], and two *Abacopteris* species, *A. penangiana* and *Pronephrium triphyllum* (synonymous with *Abacopteris triphylla*) [1][10]. These previously reported rare flavonoids only possess one 'fused-glucose' moiety located at either C(5) or C(7) of ring A. To the best of our knowledge, the particular glucosylation pattern of compound **1** consisting of two 'glucose-fused' dioxepine-ring moieties is found for the first time within natural flavonoids.

Experimental Part

General. TLC: Silica gel GF_{254} (SiO_2) pre-coated plates (*Qingdao Marine Chemical Co., Ltd.*). Column chromatography (CC): SiO_2 (200–300 mesh; *Qingdao Marine Chemical Co., Ltd.*), *Sephadex LH-20* (25–100 μm; *Fluka BioChemika*) and ODS gel (230–400 mesh; *Fluka BioChemika*). GC: *Shimadzu-GC-14C* gas chromatograph; cap. column (30 m × 0.25 mm × 0.25 μm; *DB-17*); detection, FID; injector and detector temp., 230°; N_2 as carrier gas; temp. gradient for the oven: 150° for 1 min, and then up to 300° at 5°/min. Melting points: *X4* micro melting-point apparatus. Optical rotations: *Perkin-Elmer Model 341* polarimeter. UV Spectra: *Shimadzu-UV-260* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. CD Spectra: *Jasco-J-810* spectropolarimeter. IR Spectra: *Perkin-Elmer-Spectrum-One* FT-IR spectrometer; KBr disk; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-AM-400 spectrometer*; at 400 (^1H) and 100 MHz (^{13}C); C_5D_5N as solvent; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-TOF-MS: *Mariner* spectrometer; in *m/z*.

Plant Material. The rhizomes of *Abacopteris penangiana* (HOOK.) CHING were collected in October 2004 from Wufeng County of Hubei Province, P. R. China; they were identified by *J. R.* A specimen (PZX0310) was deposited at the Tongji College of Pharmacy, Huazhong University of Science and Technology.

Extraction and Isolation. The air-dried rhizomes (5.0 kg) were ground and extracted five times with MeOH (10 l, 36 h each) at r.t. The MeOH extract was concentrated and the residue suspended in H_2O (3 l) and then extracted sequentially with $CHCl_3$ (3 × 3 l), AcOEt (3 × 3 l), and BuOH (3 × 3 l). A part of the AcOEt extract (20 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH gradient): *Frs. A1–A10*. *Fr. A4* (0.9 g) was separated by CC (SiO_2, petroleum ether/acetone 4:1 → 1:1), followed by CC (ODS, MeOH/H_2O 3:2): **2** (20 mg). A part of the BuOH extract (30 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH/H_2O 5:1:0.1 → 4:1:0.1): *Frs. B1–B10*. *Fr. B1* (1.2 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 3:1 → 2:1) and then purified by CC (*Sephadex LH-20*, MeOH/H_2O 2:1): **1** (10 mg).

Acidic Hydrolysis. Each compound (3 mg) was heated in 9% HCl soln. (1.5 ml) at 90° for 6 h. After being cooled, the mixture was filtered, and then the filtrate was freeze-dried. The residues were transformed into thiazolidine derivatives for GC analysis according to the methods described in [5].

Abacopterin K (= *1,2-O-[(2R,3S,4S,4aR,8R,10S,13aS)-3,4,4a,9,10,13a-Hexahydro-3,4-dihydroxy-2-(hydroxymethyl)-10-(4-methoxyphenyl)-12-methyl-2H,6H,8H-dipyrano[2,3-b:3′,2′-h][1,4]benzodioxepin-7,8-diyl] β-D-Glucopyranose* = (*2R,3S,4S,4aR,5aR,7S,10aS,12R,13S,14S,14aR,17aS)-3,4,4a,5a,6,10a,13,14,14a,17a-Decahydro-2,12-bis(hydroxymethyl)-7-(4-methoxyphenyl)-9-methyl-2H,7H,12H,16H-dipyrano[2,3-b:4′,3′,2′-ef]pyrano[3‴,2‴:2″,3″][1,4]dioxepino[6″,5″-i][1,4]benzodioxepin-3,4,13,14-tetrol*; **1**): White needles (MeOH). M.p. > 300°. $[\alpha]_D^{25} = +128$ ($c = 0.05$, MeOH). CD ($c = 0.0024$, MeOH): 237 (−0.57), 282 (+1.91). UV (MeOH): 228 (4.50), 275 (3.61), 281 (3.60). IR (KBr): 3430, 2918, 1616, 1516, 1457, 1380, 1249, 1061, 837. ^1H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 643.1981 ($[M+Na]^+$, $C_{30}H_{36}NaO_{14}^+$; calc. 643.2003).

Abacopterin L (= *3-[2-(6-O-Acetyl-β-D-glucopyranosyl)oxy]-4,6-dihydroxy-3,5-dimethylphenyl]-1-(4-methoxyphenyl)propan-1-one*; **2**): Amorphous powder. $[\alpha]_D^{25} = -4$ ($c = 0.47$, MeOH). UV (MeOH):

221 (4.21), 274 (4.21). IR (KBr): 3431, 2928, 1728, 1657, 1600, 1575, 1513, 1461, 1256, 1172, 1082, 833. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 543.1866 ($[M+Na]^+$, $C_{26}H_{32}NaO_{11}^+$; calc. 543.1842).

REFERENCES

[1] N. Tanaka, T. Murakami, H. Wada, A. B. Gutierrez, Y. Saiki, C. M. Chen, *Chem. Pharm. Bull.* **1985**, *33*, 5231.
[2] N. Tanaka, T. Ushioda, H. Fuchino, J. E. Braggins, *Aust. J. Chem.* **1997**, *50*, 329.
[3] New Medical Jiangsu College, 'Dictionary of Chinese Traditional Medicine', Shanghai Scientific and Technical Publishers, Shanghai, 1977, p. 1633.
[4] H. S. Ding, 'The Medicinal Spore Plants of China', Shanghai Scientific and Technical Publishers, Shanghai, 1982, p. 124.
[5] Z. X. Zhao, J. L. Ruan, J. Jin, J. Zou, D. N. Zhou, W. Fang, F. B. Zeng, *J. Nat. Prod.* **2006**, *69*, 265.
[6] Z. X. Zhao, J. Jin, J. L. Ruan, C. C. Zhu, C. Z. Lin, W. Fang, Y. L. Cai, *J. Nat. Prod.* **2007**, *70*, 1683.
[7] D. Slade, D. Ferreira, J. P. J. Marais, *Phytochemistry* **2005**, *66*, 2177.
[8] C. Pouget, C. Fagnere, J. P. Basly, H. Leveque, A. J. Chulia, *Tetrahedron* **2000**, *56*, 6047.
[9] N. Tanaka, T. Sada, T. Murakami, Y. Saiki, C. M. Chen, *Chem. Pharm. Bull.* **1984**, *32*, 490.
[10] Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita, Flora Reipublicae Popularis Sinicae, Science Press, Beijing, 1999, Vol. 4, p. 296.

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