Study on the Chemical Constituents of *Daphniphyllum angustifolium*

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Seven new iridoid glucosides, **1**–**7**, two new phenyl glucosides, **8** and **9**, along with eight known compounds were isolated from the bark of *Daphniphyllum angustifolium*. Their structures were established by ESI-MS and by 1D- and 2D-NMR spectroscopic methods.

Introduction. – Iridoids are of biogenetic and chemotaxonomic importance and are found mainly as glycosides in higher plants. A number of iridoid glucosides [1] and *Daphniphyllum* alkaloids [2] have been reported from the family Daphniphyllaceae. *Daphniphyllum angustifolium* is a shrub native to China. No study concerning its chemical constituents has been reported previously. We have examined the EtOH extract of the bark of *D. angustifolium*, and succeeded in isolating the seven new iridoid glucosides **1**–**7** and the two new phenyl glucosides **8** and **9**, along with eight known compounds.

Results and Discussion. – The dried plant material was extracted with 95% EtOH, and the concentrate was partitioned between H_2O and $CHCl_3$. The H_2O-soluble part was fractionated by means of a macroporous resin column, affording four groups of eluates, which yielded the seven new iridoid glucosides **1**–**7** and the two new phenyl glucosides **8** and **9** (see *Fig. 1*), together with four known compounds, after further chromatographic purification. The $CHCl_3$-soluble part yielded four known compounds after repeated chromatographic purification.

The known compounds were characterized by detailed NMR analyses to be daphylloside (**10**) [1], 10-*O*-deacetylasperulosidic acid methyl ester [3], (−)-epiafzelechin 7-*O*-*β*-D-glucopyranoside [4], urolignoside [5], concarpan [6], eupomatenoid-6 [6], 28-hydroxyllupen-3-one [7], and stigmast-5-ene-3,7,16-triol [8].

Compound **1** was obtained as an optically active white powder. Its IR spectrum showed the presence of OH groups (3411 cm^{-1}) and of a γ-lactone (1739 cm^{-1}). The ESI-MS exhibited quasi-molecular ions $[M+H]^+$ at m/z 519 and $[M-H]^-$ at m/z 517, suggesting a molecular formula of $C_{25}H_{26}O_{12}$, which was confirmed by the quasi-molecular ion $[M+H]^+$ at m/z 519.1510 generated by HR-ESI-MS. The ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, and HMBC spectra (see *Table 1*) suggested that **1** has a

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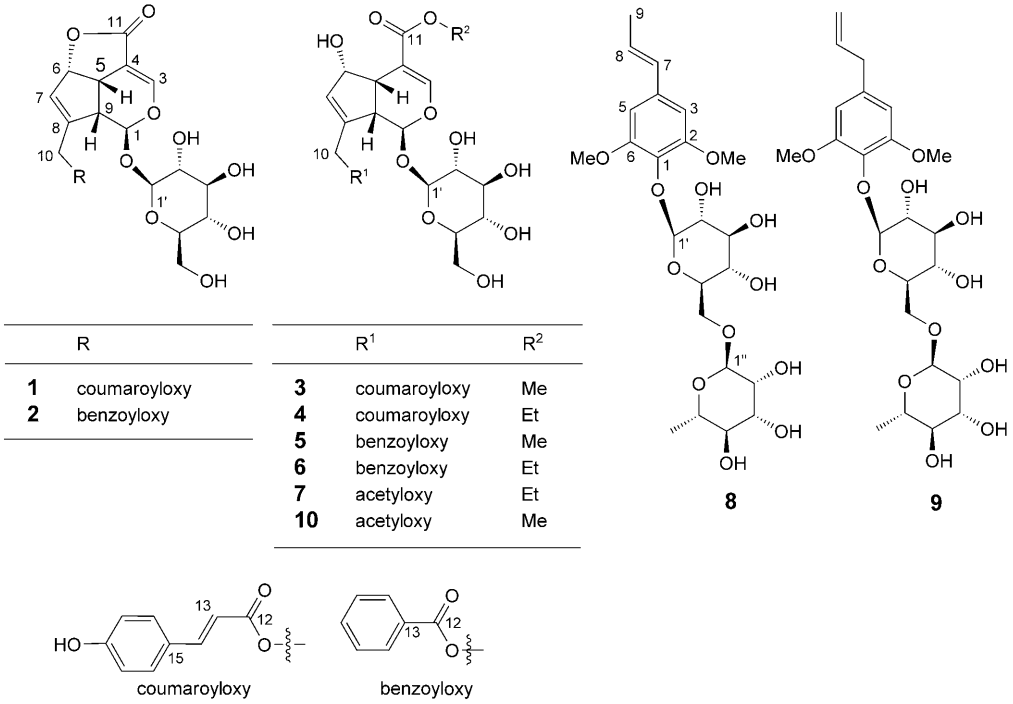

Fig. 1. *The structures of compounds* **1**–**10**

10-*O*-deacetylasperuloside skeleton [9]. Acid hydrolysis [10] of **1** yielded D-glucose. The structure of **1** was established as 10-*O*-coumaroyl-10-*O*-deacetylasperuloside[1]).

The presence of a coumaroyloxy group (C(12) to C(20)[1]) in **1** gave rise to ^{13}C-NMR signals at δ 168.6 (C(12)), 114.6 (CH(13)), 147.4 (CH(14)), 127.1 (C(15)), 131.5 (CH(16), CH(20)), 117.0 (CH(17), CH(19)), and 161.5 (C(18)). Signals of a β-D-glucosyl group (C(1′) to C(6′)) at δ 100.1 (CH(1′)), 74.7 (CH(2′)), 77.9 (CH(3′)), 71.6 (CH(4′)), 78.4 (CH(5′)), 62.8 (CH_2(6′)) and of an iridoid moiety (C(1) and C(3) to C(11)[1]) at δ 93.5 (CH(1)), 150.5 (CH(3)), 106.2 (C(4)), 37.4 (CH(5)), 86.4 (CH(6)), 129.1 (CH(7)), 144.5 (C(8)), 45.4 (CH(9)), 61.8 (CH_2(10)), and 173.0 (C(11)) were compatible with the proposed structure of **1**. The ^{1}H- and ^{13}C-NMR and HMBC spectra also indicated the presence of an ester C=O and two trisubstituted C=C moieties in the iridoid skeleton. Thirteen degrees of unsaturation were deduced from the molecular formula $C_{25}H_{26}O_{12}$. The ten degrees of unsaturation accounting for the coumaroyl β-D-glucosyl, ester C=O, and two C=C moieties left three degrees of unsaturation which were attributed to a tricyclic ring system, suggesting that **1** has a 10-*O*-deacetylasperuloside skeleton [9]. The hydrolysis product of **1** was identified as D-glucose by GC analysis of its leucine derivative, which was compared with a reference compound. The chemical shifts and the shape of the signal for the anomeric center, *i.e.*, δ 4.72 (*d*, J = 7.8 Hz, H–C(1′)) and δ 100.1 (C(1′)) indicated a β-D-configuration for the glucosyl unit. The cross-peaks in the HMBC spectrum of **1** between the CH_2(10) signals at δ 4.76 and 4.89 and the ester carbonyl signal at δ 168.6 (C(12)) established that the coumaroyloxy moiety is located at C(10), and the correlation between H–C(1′) at 4.72 and C(1) at δ 93.5 demonstrated that the β-D-glucosyloxy group is attachted at C(1).

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

Table 1. *^{1}H- and ^{13}C-NMR, HMBC, and NOESY Data of* **1**[1]). δ in ppm, J in Hz.

	δ(H)[a])	δ(C)[b])	HMBC[c])	^{1}H,^{1}H-COSY[a])	NOE[a])
H–C(1)	6.04 (br. *s*)	93.5	C(3), C(9), C(1′)	H–C(9)	CH_2(10), H–C(1′)
H–C(3)	7.29 (*s*)	150.5	C(1), C(4), C(11)	H–C(5)	
C(4)		106.2			
H–C(5)	3.33–3.40 (*m*)	37.4	C(1), C(3), C(7), C(8), C(9)	H–C(3), H–C(6), H–C(9)	H–C(6), H–C(9)
H–C(6)	5.55 (br. *d*, J=6.0)	86.4	C(5), C(7), C(8), C(11)	H–C(5), H–C(7), H–C(9)	H–C(5)
H–C(7)	5.77 (*s*)	129.1	C(8)	H–C(9), H–C(10)	
H–C(8)		144.5			
H–C(9)	3.22–3.27 (*m*)	45.4	C(10)	H–C(1), H–C(5), H–C(6), H–C(7)	H–C(5)
CH_2(10)	4.76 (br. *d*, J=14.3), 4.89 (br. *d*, J=14.3)	61.8	C(8), C(9), C(12)	H–C(7), H–C(9)	H–C(1)
C(11)		173.0			
C(12)		168.6			
H–C(13)	6.37 (*d*, J=15.9)	114.6	C(12), C(14), C(15)	H–C(14)	H–C(14)
H–C(14)	7.65 (*d*, J=15.9)	147.4	C(12), C(13), C(16)	H–C(13)	H–C(13)
C(15)		127.1			
H–C(16)	7.54 (*d*, J=8.4)	131.5	C(14), C(18)	H–C(17)	H–C(13), H–C(17)
H–C(17)	6.87 (*d*, J=8.4)	117.0	C(15), C(18)	H–C(16)	H–C(16)
C(18)		161.5			
H–C(19)	6.87 (*d*, J=8.4)	117.0	C(15), C(18)	H–C(20)	H–C(16)
H–C(20)	7.54 (*d*, J=8.4)	131.5	C(14), C(18)	H–C(19)	H–C(13), H–C(19)
H–C(1′)	4.72 (*d*, J=7.8)	100.1	C(1), C(6′)		H–C(1)
H–C(2′)	3.30–3.47 (*m*)	74.7			
H–C(3′)	3.30–3.47 (*m*)	77.9			
H–C(4′)	3.30–3.47 (*m*)	71.6			
H–C(5′)	3.30–3.47 (*m*)	78.4			
CH_2(6′)	3.64 (*dd*, J=12.0, 6.3), 3.86 (*d*, J=12.0)	62.8			

[a]) Recorded in CD_3OD at 300 MHz. [b])Recorded in CD_3OD at 75 MHz. [c]) Protons that correlate with C-atoms.

The configurations at C(1), C(5), C(6), and C(9) of **1** were determined by NOE correlations as shown in *Fig. 2* and in *Table 1*. The NOEs between H–C(5), H–C(6), and H–C(9) indicated that they were *cis*-positioned to each other (β-configuration), and the absence of NOEs between H–C(1) and H–C(9) suggested that they are located on opposite faces of the dihydro pyrane ring.

Compound **2** was obtained as an optically active white powder. The ESI-MS exhibited a quasi-molecular ion $[M+Na]^+$ at m/z 499 suggesting a molecular formula $C_{23}H_{24}O_{11}$, which was confirmed by a quasi-molecular ion $[M+Na]^+$ at m/z 499.1211 generated by HR-ESI-MS. Compound **2** had similar UV features to those of **1**. A com-

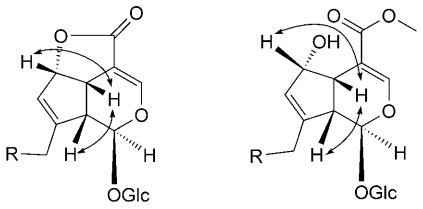

Fig. 2. *Important NOE correlations of* **1** *and* **3**

Table 2. *^{1}H- and ^{13}C-NMR, HMBC, and NOESY Data of* **2**[1]). δ in ppm, J in Hz.

	δ(H)[a])	δ(C)[b])	HMBC[c])	NOE[a])
H–C(1)	6.06 (br. *s*)	93.6	C(3), C(9), C(1′)	CH_2(10), H–C(1′)
H–C(3)	7.31 (*d*, *J* = 1.8)	150.4	C(1), C(4), C(11)	
C(4)		106.4	C(4), C(7), C(8)	
H–C(5)	3.29–3.32 (*m*)	37.6	C(4), C(7), C(8)	H–C(6), H–C(9)
H–C(6)	5.58 (br. *d*, *J* = 6.0)	86.4	C(8), C(11)	H–C(5)
H–C(7)	5.82 (*s*)	129.5	C(5), C(6), C(9)	
C(8)		144.4		
H–C(9)	3.17–3.21 (*m*)	45.6	C(1), C(5), C(10)	H–C(5)
CH_2(10)	4.90 (*d*, *J* = 13.8), 5.03 (*d*, *J* = 13.8)	62.7	C(7), C(8), C(9), C(12)	H–C(1)
C(11)		172.7		
C(12)		167.5		
C(13)		131.0		
H–C(14)	8.03 (*d*, *J* = 7.2)	130.8	C(12), C(15), C(16)	H–C(15)
H–C(15)	7.49 (*t*, *J* = 7.2)	129.8	C(13), C(14)	H–C(14), H–C(16)
H–C(16)	7.61 (*t*, *J* = 7.2)	134.7	C(14)	H–C(15)
H–C(17)	7.49 (*t*, *J* = 7.2)	129.8	C(16), C(18)	H–C(16), H–C(18)
H–C(18)	8.03 (*d*, *J* = 7.2)	130.8	C(13), C(17)	H–C(17)
H–C(1′)	4.68 (*d*, *J* = 8.1)	100.2	C(1)	H–C(1)
H–C(2′)	3.18–3.40 (*m*)	74.7		
H–C(3′)	3.18–3.40 (*m*)	77.9		
H–C(4′)	3.18–3.40 (*m*)	71.6		
H–C(5′)	3.18–3.40 (*m*)	78.4		
CH_2(6′)	3.71 (*d*, *J* = 12.0), 3.86 (*d*, *J* = 12.0)	62.8		

[a]) Recorded in CD_3OD at 300 MHz. [b]) Recorded in CD_3OD at 75 MHz. [c]) Protons that correlate with C-atoms.

parison of the ^{1}H- and ^{13}C-NMR data of **2** (*Table 2*) with those of **1** revealed that the only difference was that the coumaroyloxy moiety in compound **1** is replaced by a benzoyloxy group in compound **2**. The position of the benzoyloxy group was also assigned to C(10), which was confirmed by HMBC experiments (correlation CH_2(10) (δ 4.90 and 5.03) C(12) (δ 165.2)). Thus, the structure of **2** was identified as 10-*O*-benzoyl-10-*O*-deacetylasperuloside[1]).

Compound **3** was obtained as an optically active white powder. The ESI-MS exhibited quasi-molecular ions $[M+Na]^+$ at m/z 573 and $[M-H]^-$ at m/z 549, suggesting a

Table 3. *^{1}H- and ^{13}C-NMR, HMBC, and NOESY Data of* **3**[1]). δ in ppm, J in Hz.

	δ(H)[a])	δ(C)[b])	HMBC[c])	NOE[a])
H–C(1)	4.99 (*d*, *J* = 9.0)	99.6	C(3), C(9), C(1′)	CH_2(10), H–C(1′)
H–C(3)	7.63 (*s*)	153.2	C(1), C(4), C(11)	
C(4)		107.2		
H–C(5)	2.91 (*t*, *J* = 6.6)	40.8	C(1), C(3), C(7), C(8), C(9)	H–C(6), H–C(9)
H–C(6)	4.64 (br. *d*, *J* = 6.0)	73.2	C(5), C(7), C(8), C(11)	H–C(5)
H–C(7)	6.00 (br. *s*)	131.8	C(8)	
C(8)		143.0		
H–C(9)	2.55 (*t*, *J* = 7.8)	44.8	C(10)	H–C(5)
CH_2(10)	4.80 (br. *d*, *J* = 15.3), 4.90 (br. *d*, *J* = 15.3)	61.0	C(8), C(9), C(12)	H–C(1)
C(11)		166.9		
C(12)		166.3		
H–C(13)	6.44 (*d*, *J* = 15.9)	113.9	C(12), C(14), C(15)	H–C(14)
H–C(14)	7.58–7.62 (*m*)	145.1	C(12), C(13), C(16)	H–C(13)
C(15)		125.1		
H–C(16)	7.54–7.58 (*m*)	130.5	C(14), C(18)	H–C(17)
H–C(17)	6.79 (*d*, *J* = 9.0)	115.9	C(15), C(18)	H–C(16)
C(18)		160.1		
H–C(19)	6.79 (*d*, *J* = 9.0)	115.9	C(15), C(18)	H–C(16)
H–C(20)	7.58–7.62 (*m*)	130.5	C(14), C(18)	H–C(19)
MeO	3.20 (*s*)	51.2	C(11)	
H–C(1′)	4.59 (*d*, *J* = 8.1)	99.1	C(1)	H–C(1)
H–C(2′)	3.02–3.22 (*m*)	73.4		
H–C(3′)	3.02–3.22 (*m*)	76.6		
H–C(4′)	3.02–3.22 (*m*)	69.9		
H–C(5′)	3.02–3.22 (*m*)	77.2		
CH_2(6′)	3.43 (*dd*, *J* = 11.9, 4.8), 3.64–3.66 (*m*)	62.0		

[a]) Recorded in (D_6)DMSO at 300 MHz. [b]) Recorded in DMSO at 75 MHz. [c]) Protons that correlate with C-atoms.

molecular formula $C_{26}H_{30}O_{13}$, which was confirmed by a quasi-molecular ion $[M+Na]^+$ at m/z 573.1580 generated by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR, DEPT, HMQC, and HMBC spectra (see *Table 3*) suggested the presence of a 10-*O*-deacetyldaphylloside skeleton. A direct comparison of the spectral data of **3** with those of daphylloside (**10**), which was also isolated from the title plant, indicated that **3** is an analogue of **10**, both compounds possessing the same daphylloside skeleton. The only difference between compound **3** and **10** was that the acetyloxy group at position 10 in compound **10** is replaced by a coumaroyloxy group in compound **3**. Thus, the structure of **3** was established 10-*O*-coumaroyl-10-*O*-deacetyldaphylloside.

The presence of a coumaroyloxy group (C(12) to C(20)[1])) in **3** was established by the ^{13}C-NMR signals at δ 166.3 (C(12)), 113.9 (CH(13)), 145.1 (CH(14)), 125.1 (C(15)), 130.5 (CH(16), CH(20)), 115.9 (CH(17), CH(19)), and 160.1 (C(18)) that of a β-D-glucosyl group (C(1′) to C(6′)) by the signals at δ 99.1 (CH(1′)), 73.4 (CH(2′)), 76.6 (CH(3′)), 69.9 (CH(4′)), 77.2 (CH(5′)), and 62.0 (CH_2(6′)), that of a MeO group by δ 51.2 and the 10 C-atoms of the iridoid skeleton (C(1) and C(3) to C(11)[1])) by the sig-

nals at δ 99.6 (CH(1)), 153.2 (CH(3)), 107.2 (C(4)), 40.8 (CH(5)), 73.2 (CH(6)), 131.8 (CH(7)), 143.0 (C(8)), 44.8 (CH(9)), 61.0 (CH_2(10)), and 166.9 (C(11)). The ^{1}H- and ^{13}C-NMR and HMBC spectra also indicated the presence of an ester C=O and two trisubstituted C=C moieties in the iridoid skeleton. The HMBC correlations MeO (δ 3.20)/C(11) (δ 166.9) and H–C(1′) (δ 4.59)/C(1) (δ 99.6) indicated that the MeO group was located at C(11) and the β-D-glucosyloxy group at C(1). The position of the coumaroyloxy group of **3** was assigned to C(10) by the HMBC cross-peaks CH_2(10) (δ 4.80 and 4.90)/C(12) (δ 166.3).

The configurations at C(1), C(5), C(6), and C(9) were determined by NOE correlations as shown in *Fig. 2* and in *Table 3*. The NOEs between H–C(5), H–C(6), and H–C(9) indicated that they were in *cis*-position to each other (β-configuration), and the absence of NOEs between H–C(1) and H–C(9) suggested the α-configuration of H–C(1).

Compound **4** was obtained as an optically active white powder. The molecular formula $C_{27}H_{32}O_{13}$ was confirmed by a quasi-molecular ion $[M-H]^-$ at m/z 563.1770 generated by HR-ESI-MS. Compound **4** had similar UV features to those of **3**. A comparison of the ^{1}H- and ^{13}C-NMR data of **4** (see *Tables 4* and *5*) with those of **3** revealed that the only difference consists in compound **4** having an EtO group at C(11) instead of the

Table 4. *^{1}H-NMR Data of* **4**–**7**[1]). δ in ppm, J in Hz.

	4[a])	**5**[b])	**6**[a])	**7**[a])
H–C(1)	5.09 (*d*, *J* = 9.0)	5.14 (*d*, *J* = 5.1)	5.10 (*d*, *J* = 9.0)	5.06 (*d*, *J* = 9.0)
H–C(3)	7.63 (*s*)	7.66 (*s*)	7.64 (*d*, *J* = 1.2)	7.65 (*d*, *J* = 1.2)
H–C(5)	3.02 (*t*, *J* = 6.6)	2.94 (*t*, *J* = 6.6)	3.07 (*t*, *J* = 6.6)	3.05 (*t*, *J* = 8.1)
H–C(6)	4.78 (br. *d*, *J* = 6.0)	4.74 (*d*, *J* = 6.0)	4.78–4.82 (*m*)	4.77–4.87 (*m*)
H–C(7)	6.03 (br. *s*)	6.08 (br. *s*)	6.09 (br. *s*)	6.02 (br. *s*)
H–C(9)	2.65 (*t*, *J* = 8.1)	2.61 (*t*, *J* = 8.1)	2.68 (*t*, *J* = 8.1)	2.63 (*t*, *J* = 8.1)
CH_2(10)	4.78–4.82 (*m*), 5.08 (*d*, *J* = 15.3)	4.99 (*d*, *J* = 15.3), 5.04 (*d*, *J* = 15.3)	5.01 (br. *d*, *J* = 15.3), 5.22 (br. *d*, *J* = 15.3)	4.77–4.87 (*m*), 4.94 (*d*, *J* = 15.3)
H–C(13)	6.35 (*d*, *J* = 16.2)			
H–C(14)	7.63 (*d*, *J* = 16.2)	8.03 (*d*, *J* = 7.2)	8.04 (*d*, *J* = 7.2)	
H–C(15)		7.55 (*t*, *J* = 7.2)	7.48 (*t*, *J* = 7.2)	
H–C(16)	7.45 (*d*, *J* = 9.0)	7.66 (*t*, *J* = 7.2)	7.60 (*t*, *J* = 7.2)	
H–C(17)	6.78 (*d*, *J* = 9.0)	7.55 (*t*, *J* = 7.2)	7.48 (*t*, *J* = 7.2)	
H–C(18)		8.03 (*d*, *J* = 7.2)	8.04 (*d*, *J* = 7.2)	
H–C(19)	6.78 (*d*, *J* = 9.0)			
H–C(20)	7.45 (*d*, *J* = 9.0)			
Me*C*H_2O or MeO	4.17 (*q*, *J* = 7.2)	3.35 (*s*)	4.17 (*q*, *J* = 7.2)	4.17 (*q*, *J* = 7.2)
MeCH_2O	1.27 (*t*, *J* = 7.2)		1.27 (*t*, *J* = 7.2)	1.27 (*t*, *J* = 7.2)
H–C(1′)	4.72 (*d*, *J* = 7.2)	4.59 (*d*, *J* = 7.5)	4.73 (*d*, *J* = 7.2)	4.72 (*d*, *J* = 7.5)
H–C(2′)	3.23–3.39 (*m*)	2.98–3.19 (*m*)	3.23–3.40 (*m*)	3.23–3.39 (*m*)
H–C(3′)	3.23–3.39 (*m*)	2.98–3.19 (*m*)	3.02–3.22 (*m*)	3.23–3.39 (*m*)
H–C(4′)	3.23–3.39 (*m*)	2.98–3.19 (*m*)	3.02–3.22 (*m*)	3.23–3.39 (*m*)
H–C(5′)	3.23–3.39 (*m*)	2.98–3.19 (*m*)	3.02–3.22 (*m*)	3.23–3.39 (*m*)
CH_2(6′)	3.57 (*dd*, *J* = 6.9, 2.0), 3.84 (*d*, *J* = 12.0)	3.39–3.42 (*m*), 3.60–3.64 (*m*)	3.60 (*dd*, *J* = 5.4, 12.0), 3.86 (*d*, *J* = 12.0)	3.61 (*dd*, *J* = 5.7, 11.7), 3.84 (*d*, *J* = 11.7)
AcO				2.08 (*s*)

[a]) Recorded in CD_3OD at 300 MHz. [b]) Recorded in (D_6)DMSO at 300 MHz.

Table 5. *^{13}C-NMR Data of* **4**– **7**[1]). δ in ppm.

	4[a])	**5**[b])	**6**[a])	**7**[a])
C(1)	101.5	99.6	101.5	101.4
C(3)	155.4	153.2	155.4	155.3
C(4)	108.5	107.2	108.5	108.5
C(5)	42.6	40.7	42.6	42.5
C(6)	75.0	73.4	75.0	75.0
C(7)	131.8	131.9	131.4	131.9
C(8)	146.3	142.7	146.1	146.0
C(9)	48.3	44.8	46.6	46.3
C(10)	61.4	61.0	61.4	61.4
C(11)	169.0	166.9	169.1	172.6
C(12)	168.9	165.4	167.8	
C(13)	114.9	129.6	131.4	
C(14)	147.2	129.4	130.8	
C(15)	127.1	128.9	129.8	
C(16)	131.4	133.5	134.6	
C(17)	117.1	128.9	129.8	
C(18)	161.7	129.4	130.8	
C(19)	117.1			
C(20)	131.4			
C(1′)	100.8	99.1	100.8	100.7
C(2′)	75.6	73.4	75.6	75.6
C(3′)	77.9	76.5	78.0	78.0
C(4′)	71.6	69.9	71.6	71.6
C(5′)	78.7	77.2	78.7	78.7
C(6′)	63.1	62.8	63.1	63.1
Me*C*H$_2$O or MeO	63.8	51.1	64.4	63.9
*Me*CH$_2$O	14.7		14.7	14.7
–OAc				169.0, 20.9

[a]) Recorded in CD_3OD at 75 MHz. [b]) Recorded in (D_6)DMSO at 75 MHz.

MeO group. Its structure was established as 10-*O*-coumaroyl-10-*O*-deacetyl-11-demethoxy-11-daphylloside[1]).

Compound **5** was obtained as an optically active white powder. The molecular formula $C_{24}H_{28}O_{12}$ was confirmed by a quasi-molecular ion $[M+H]^+$ at *m*/*z* 509.1663 generated by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **5** (see *Tables 4* and *5*) were similar to those of **3**, except for the substituent at C(10). Comparison of the ^{1}H- and ^{13}C-NMR and HMBC spectra established that the only difference between **5** and **3** was that **5** had a benzoyloxy group at C(10) instead of the coumaroyloxy group. The structure of a 10-*O*-benzoyl-10-*O*-deacetyldaphylloside[1]) for **5** was confirmed by its ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY spectra.

Also compound **6** was optically active and obtained as a white powder. The molecular formula $C_{25}H_{30}O_{12}$ was confirmed by a quasi-molecular ion $[M+COOH]^-$ at *m*/*z* 567.1710 generated by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **6** (see *Tables 4* and *5*) were similar to those of **3**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **6**

and **3** established the structure of **6** as 10-*O*-benzoyl-10-*O*-deacetyl-11-demethoxy-11-ethoxydaphylloside[1]).

Compound **7** was optically active and obtained as a white powder. The molecular formula $C_{20}H_{28}O_{12}$ was confirmed by a quasi-molecular ion $[M+H]^+$ at *m/z* 461.1664 generated by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **7** (see *Tables 4* and *5*) were similar to those of daphylloside (**10**). A comparison of the ^{1}H- and ^{13}C-NMR spectra of **7** and **10** revealed that the only difference was the presence of an EtO group at C(11) in **7** compared to a MeO group in **10**. The configurations at C(1), C(5), C(6), and C(9) were confirmed by the NOESY data. Thus, the structure of **7** was established as 11-demethoxy-11-ethoxydaphylloside[1]).

Compound **8** was obtained as an optically active white powder. The molecular formula $C_{23}H_{34}O_{12}$ was confirmed by a quasi-molecular ion $[M+H]^+$ at *m/z* 503.2124 generated by HR-ESI-MS. Acid hydrolysis of **8** yielded D-glucose and L-rhamnose, identified by GC analysis of their leucine derivatives which were compared with reference samples. The ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments (*Table 6*) established the structure of **8** as 2,6-dimethoxy-4[(1*E*)-prop-1-enyl]phenyl *O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside.

The presence of a CH=CH−Me group (C(7) to C(9)[1])) in **8** was indicated by the ^{13}C-NMR signals at δ 132.2 (CH(7)), 126.5 (CH(8)), 18.7 (Me(9)). The tetrasubstituted benzene moiety (C(1) to C(6)[1])) gave rise to signals at δ 135.3 (C(1)), 154.5 (C(2)), 104.8 (CH(3)), 136.4 (C(4)), 104.8 (CH(5)), and 154.5 (C(6)), the β-D-glucosyl moiety (C(1′) to C(6′)) to δ 105.6 (CH(1′)), 75.7 (CH(2′)), 77.9 (CH(3′)), 71.8 (CH(4′)), 77.4 (CH(5′)), and 68.1 (CH_2(6′)), and the α-L-rhamnosyl moiety (C(1″) to C(6″)) to δ 102.2 (CH(1″)), 72.4 (CH(2″)), 72.2 (CH(3″)), 74.1 (CH(4″)), 69.8 (CH(5″)), and 18.2 (Me(6″)). Finally two MeO groups appeared at δ 57.1. The value of the coupling $J(7,8)=15.6$ Hz in the ^{1}H-NMR spectrum indicated an (*E*)-CH=CH−Me group. The chemical shifts and the shape of the signals for the anomeric centers (δ 4.75 (*d*, $J=7.8$ Hz, H−C(1′)), and 105.6 (CH(1′), δ 4.67 (*s*, H−C(1″)) and δ 102.2 (C(1″)) indicated a β-D-configuration for the glucosyl unit and a α-L-configuration for the rhamnosyl unit. Their positions were determined by ^{13}C,^{1}H long-range connectivities: *i*) The correlations H−C(7) (δ 6.34)/C(4) (δ 136.4) and C(3) and C(5) (δ 104.8), and H−C(8) (δ 6.23–6.26)/C(4) (δ 136.4) established that the (*E*)-CH=CH−Me moiety was located at C(4). *ii*) The correlation H−C(1′) (δ 4.75)/C(1) (δ 135.3) allowed to position the glucosyloxy unit at C(1). *iii*)) The correlation H−C(1″) (δ 4.67)/C(6′) (δ 68.1) elucidated that the rhamnosyloxy unit was located at C(6′). *iv*) The correlations of the 2 MeO (δ 3.85) with C(2) and C(6) (δ 154.5) established the position of the 2 MeO at C(2) and C(6).

Compound **9**, an optically active white powder, exhibited a quasi-molecular ion $[M+Na]^+$ at *m/z* 525 in the ESI-MS, suggesting a molecular formula $C_{23}H_{34}O_{12}$, which was confirmed by a quasi-molecular ion $[M+Na]^+$ at *m/z* 525.1952 generated by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **9** (see *Table 6*) were similar to those of **8** (*Table 6*), except for the signals of the side chain C(7) to C(9)[1]). The ^{13}C-NMR and HMBC spectra of **9** suggested the side chain to be a CH_2=CH−CH_2 group (δ 41.5 (CH_2(7)), 137.6 (CH(8)), 116.4 (CH_2C(9)). The structure of **9** was confirmed by its ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra as 2,6-dimethoxy-4-(prop-2-enyl]phenyl *O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside.

The ethyl esters **4**, **6**, and **7** may be artifacts formed from **1**, **2**, and **10**, respectively, during the 7-day extraction of the plants with EtOH (see *Exper. Part*), and the methyl esters **3** and **5** may also be artifacts formed from **1** and **2** by nucleophilic opening of the γ-lactone moiety during column chromatography with MeOH.

Table 6. *^{1}H- and ^{13}C-NMR, HMBC, and ^{1}H,^{1}H-COSY Data of* **8** *and ^{1}H- and ^{13}C-NMR Data of* **9**[1]). δ in ppm, J in Hz.

	8				**9**	
	δ(H)[a])	δ(C)[b])	HMBC[c])	^{1}H,^{1}H-COSY[a])	δ(H)[a])	δ(C)[b])
C(1)		135.3				138.9
C(2)		154.5				154.3
H–C(3)	6.66 (*s*)	104.8	C(1), C(2), C(4), C(7)	MeO	6.48 (*s*)	107.4
C(4)		136.4				134.5
H–C(5)	6.66 (*s*)	104.8	C(1), C(2), C(4), C(7)	MeO	6.48 (*s*)	107.4
C(6)		154.5				154.3
H–C(7) or CH_2(7)	6.34 (*d*, *J* = 15.6)	132.2	C(3), C(4), C(9)		2.84 (*d*, *J* = 6.3)	41.5
H–C(8)	6.23–6.26 (*m*)	126.5	C(4), C(9)		5.05–5.07 (*m*)	137.6
Me(9) or CH_2(9)	1.85 (*d*, *J* = 6.3)	18.7	C(7), C(8)		4.59–4.61 (*m*)	116.4
MeO	3.85 (*s*)	57.1	C(2, C(3), C(5), C(6), C(3), C(5)		3.85 (*s*)	57.1
H–C(1′)	4.75 (*d*, *J* = 7.8)	105.6	C(1)		4.75 (*d*, *J* = 7.2)	105.6
H–C(2′)	3.30–3.91 (*m*)	75.7			3.30–3.91 (*m*)	75.7
H–C(3′)	3.30–3.91 (*m*)	77.9			3.30–3.91 (*m*)	77.9
H–C(4′)	3.30–3.91 (*m*)	71.8			3.30–3.91 (*m*)	71.8
H–C(5′)	3.30–3.91 (*m*)	77.4			3.30–3.91 (*m*)	77.4
CH_2(6′)	3.30–3.91 (*m*)	68.1			3.30–3.91 (*m*)	68.1
H–C(1″)	4.67 (*s*)	102.2	C(6′)		4.67 (*s*)	102.3
H–C(2″)	3.30–3.91 (*m*)	72.4			3.30–3.91 (*m*)	72.4
H–C(3″)	3.30–3.91 (*m*)	72.2			3.30–3.91 (*m*)	72.2
H–C(4″)	3.30–3.91 (*m*)	74.1			3.30–3.91 (*m*)	74.1
H–C(5″)	3.30–3.91 (*m*)	69.8			3.30–3.91 (*m*)	69.8
Me(6″)	1.19 (*d*, *J* = 6.6)	18.2			1.19 (*d*, *J* = 6.6)	18.2

[a]) Recorded in CD_3OD at 300 MHz. [b]) Recorded in CD_3OD at 75 MHz. [c]) Protons that correlate with C-atoms.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh), *Sephadex LH-20*, macroporous resin *AB-8* (from the Chemical Plant of Nankai University, Tianjin, China), *MCI Gel CHP20P* (75–150 μm ; *Mitsubishi Chemical Industry, Ltd.*), and *C-18* reversed-phase silica gel (*ODS*; 20–45 μm, *Fuji Silysia Chemical, Ltd.*). M.p.: *Yanagimoto* micromelting point apparatus; uncorrected. Optical rotations: in MeOH; *Perkin-Elmer-341* polarimeter. UV Spectra: λ_{max} (log ε) in nm. NMR Spectra: *Bruker AMX-500* spectrometer. MS: *Bruker Esquire-3000-plus* spectrometer for ESI and *Bruker Atex-III* instrument for HR-ESI in MeOH; in m/z.

Plant Material. Daphniphyllum Angustifolium was collected in Nanchuan County, Chongqing City, People's Republic of China, in May, 2001. A voucher specimen of the plant (No. PA0501) was identified by Mr. *Jin-Gui Shen* and deposited at the herbarium of the Chinese National Center for Drug Screening, Shanghai, P. R. China.

Extraction and Isolation. The dried and powdered bark of *D. Angustifolium* (10.0 kg) were extracted with 95% EtOH (3 × 35 l) at r.t. for 7 days. The extract was evaporated and the residue partitioned between $CHCl_3$ (1.200 l) and H_2O (2.000 l). The H_2O-soluble portion (100 g) was then separated into

4 fractions by CC (macroporous resin, EtOH/H_2O): *Fr. 1* (15 g) with 15% EtOH (1 l), *Fr. 2* (15 g) with 30% EtOH (1 l), *Fr. 3* (30 g) with 60% EtOH (1 l), and *Fr. 4* (5 g) with 100% EtOH (1 l). *Fr. 4* (5 g) was subjected to CC (*MCI*, MeOH/H_2O): *Fr. 4.1* (850 mg) with 75% MeOH (1 l) and *Fr. 4.2* (3 g) with 90% MeOH (1 l). *Fr. 4.1* (850 mg) was further subjected to CC (*ODS*, 60% MeOH/H_2O (3 l): **1** (73 mg) and **2** (69 mg). *Fr. 3* (30 g) was subjected to CC (*MCI*, MeOH/H_2O): *Fr. 3.1* (1.5 g) with 45% MeOH (1 l), *Fr. 3.2* (700 mg) with 55% MeOH (1 l), *Fr. 3.3* (3 g) with 65% MeOH (1 l), and *Fr. 3.4* (700 mg) with 80% MeOH (1 l). *Fr. 3.1* (500 mg) was further subjected to CC (*ODS*, 20% (2 l), 25% MeOH/H_2O (1 l)): *10*-O-*deacetylasperulosidic acid methyl ester* (43 mg). *Fr. 3.2* (700 mg) was further subjected to CC (*ODS*, 25% MeOH/H_2O (3 l)): **3** (40 mg) and **4** (9 mg). *Fr. 3.3* (500 mg) was further subjected to CC (*ODS*, 30% MeOH/H_2O (2 l)): **5** (23 mg), **7** (12 mg), and **10** (100 mg). *Fr. 3.4* (700 mg) was further subjected to CC (silica gel, $CHCl_3$/MeOH 20 : 1): **6** (9 mg) and **10** (300 mg). *Fr. 2* (15 g) was subjected to CC (*MCI*, MeOH/H_2O): *Fr. 2.1* (150 mg) with 25% MeOH (1 l), *Fr. 2.2* (5 g) with 35% MeOH (2 l), and *Fr. 2.3* (6 g) with 45% MeOH (1 l). *Fr. 2.1* (150 mg) was further subjected to CC (*ODS*, 25% MeOH/H_2O (2 l)): **8** (28 mg). *Fr. 2.2* (500 mg) was further subjected to CC (*ODS*, 25% MeOH/H_2O (2 l)): **9** (18 mg) and *(−)-epiafzelechin 7*-O-*β*-D-*glucopyranoside* (55 mg). *Fr. 2.3* (100 mg) was further subjected to CC (*ODS*, 25% MeOH/H_2O (2 l): *urolignoside* (30 mg). The $CHCl_3$-soluble portion (20 g) was subjected to CC (silica gel, $CHCl_3$/MeOH): *Fr. A* (250 mg) with $CHCl_3$ (1 l), *Fr. B* (6 g) with $CHCl_3$/MeOH 100 : 2 (1 l), and *Fr. C* (1 g) with $CHCl_3$/MeOH 100 : 5 (1 l). *Fr. A* (250 mg) was subjected to CC (silica gel, petroleum ether/AcOEt 10 : 1: *concarpan* (34 mg). *Fr. B* (6 g) was subjected to CC (silica gel, petroleum ether/acetone): *Fr. A.1* (120 mg) with petroleum ether/acetone 20 : 1 (1 l) and *Fr. A.2* (90 mg) with petroleum ether/acetone 10 : 1 (2 l). *Fr. A.1* was further subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1 : 1 (1 l): *28-hydroxylupen-3-one* (11 mg) and *stigmast-5-ene-3,7,16-triol* (13 mg). *Fr. A.2* (90 mg) was further subjected to CC (*ODS*, 55% MeOH/H_2O (2 l): *eupomatenoid-6* (29 mg).

10-O-*Coumaroyl-10*-O-*deacetylasperuloside* (=*[(2aS,4aS,5S,7bS)-5-(β*-D-*Glucopyranosyloxy)-2a,4a,5,7b,-tetrahydro-1-oxo-1*H-*2,6-dioxacyclopent[*cd*]inden-4-yl]methyl (2*E*)-3-(4-Hydroxyphenyl)prop-2-enoate*; **1**): White powder. M.p. 65–66°. $[\alpha]_D^{20} = -96.1$ ($c = 0.60$, MeOH). UV (MeOH): 222.8 (3.25), 312.5 (3.08). IR (KBr): 3411, 2923, 1739, 1656, 1604, 1516, 1261, 1168, 1074, 1020, 982, 833. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 519.1510 ($[M+H]^+$, $C_{25}H_{27}O_{12}^+$; calc. 519.1502).

10-O-*Benzoyl-10*-O-*deacetylasperuloside* (=*(2aS,4aS,5S,7bS)-4-[(Benzoyloxy)methyl]-5-(β*-D-*glucopyranosyloxy)-2a,4a,5,7b,-tetrahydro-1*H-*2,6-dioxacyclopent[*cd*]inden-1-one*; **2**): White powder. M.p. 78–80°. $[\alpha]_D^{20} = -88$ ($c = 0.07$, MeOH). UV (MeOH): 202.5 (1.25), 231.0 (1.80). ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 499.1211 ($[M+Na]^+$, $C_{23}H_{24}NaO_{11}^+$; calc. 499.1216).

10-O-*Coumaroyl-10*-O-*deacetyldaphylloside* (=*Methyl (1S,4aS,5S,7aS)-1-(β*-D-*Glucopyranosyloxy)-1,4a,5,7a,-tetrahydro-5-hydroxy-7-{{[(2*E*)-3-(4-hydroxyphenyl)-1-oxoprop-2-enyl]oxy}methyl}cyclopenta[c]pyran-4-carboxylate*; **3**): White powder. M.p. 67–68°. $[\alpha]_D^{20} = +5$ ($c = 0.23$, MeOH). UV (MeOH): 225.8 (0.71), 316.5 (0.75). IR (KBr): 3427, 2922, 1695, 1633, 1604, 1516, 1440, 1275, 1169, 1076, 833, 519. ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-MS: 573.1580 ($[M+Na]^+$, $C_{26}H_{31}NaO_{13}^+$; calc. 573.1579).

10-O-*Coumaroyl-10*-O-*deacetyl-11-demethoxy-11-ethoxydaphylloside* (=*Ethyl (1S,4aS,5S,7aS)-1-(β*-D-*Glucopyranosyloxy)-1,4a,5,7a,-tetrahydro-5-hydroxy-7-{{[(2*E*)-3-(4-hydroxyphenyl)-1-oxoprop-2-enyl]oxy}methyl}cyclopenta[c]pyran-4-carboxylate*; **4**): White powder. M.p. 66–68°. $[\alpha]_D^{20} = -7$ ($c = 0.24$, MeOH). UV (MeOH): 225.0 (0.70), 316.2 (0.74). ^{1}H- and ^{13}C-NMR: *Tables 4* and *5*. HR-ESI-MS: 563.1770 ($[M-H]^-$, $C_{27}H_{33}O_{13}^-$; calc. 563.1765).

10-O-*Benzoyl-10*-O-*deacetyldaphylloside* (=*Methyl (1S,4aS,5S,7aS)-7-[(Benzoyloxy)methyl]-1-(β*-D-*glucopyranosyloxy)-1,4a,5,7a,-tetrahydro-5-hydroxycyclopenta[*c*]pyran-4-carboxylate*; **5**): White powder. M.p. 65–67°. $[\alpha]_D^{20} = +3$ ($c = 0.09$, MeOH). UV (MeOH): 202.0 (0.63), 232.0 (1.36). ^{1}H- and ^{13}C-NMR: *Tables 4* and *5*. HR-ESI-MS: 509.1663 ($[M+H]^+$, $C_{24}H_{27}O_{12}^-$; calc. 509.1659).

10-O-*Benzoyl-10*-O-*deacetyl-11-demethoxy-11-ethoxydaphylloside* (=*Ethyl (1S,4aS,5S,7aS)-7-[(Benzoyloxy)methyl]-1-(β*-D-*glucopyranosyloxy)-1,4a,5,7a,-tetrahydro-5-hydroxycyclopenta[*c*]pyran-4-carboxylate*; **6**): White powder. M.p. 70–72°. $[\alpha]_D^{20} = -9$ ($c = 0.16$, MeOH). UV (MeOH): 204.0 (0.73), 231.8 (1.18). ^{1}H- and ^{13}C-NMR: *Tables 4* and *5*. HR-ESI-MS: 567.1710 ($[M+COOH]^-$, $C_{26}H_{31}O_{14}^-$; calc. 567.1714).

11-Demethoxy-11-ethoxydaphylloside (= *Ethyl (1S,4aS,5S,7aS)-7-[(Acetyloxy)methyl]-1-(β-*D*-glucopyranosyloxy)-1,4a,5,7a,-tetrahydro-5-hydroxycyclopenta[c]pyran-4-carboxylate*; **7**): White powder. M.p. 63–65°. $[\alpha]_D^{20} = -10$ ($c = 0.20$, MeOH). UV (MeOH): 203.0 (0.46), 233.0 (0.53). ^{1}H- and ^{13}C-NMR: *Tables 4* and *5*. HR-ESI-MS: 461.1664 ($[M+H]^+$, $C_{20}H_{29}O_{12}^+$; calc. 461.1659).

*2,6-Dimethoxy-4-[(1*E*)-prop-1-enyl]phenyl α-*L*-Rhamnopyranosyl-(1 → 6)-β-*D*-glucopyranoside* (**8**): White powder. M.p. 180–182°. $[\alpha]_D^{20} = -37$ ($c = 0.16$, MeOH). UV (MeOH): 216.0 (2.40). ^{1}H- and ^{13}C-NMR: *Table 6*. HR-ESI-MS: 503.2124 ($[M+H]^+$, $C_{23}H_{35}O_{12}^+$; calc. 503.2129).

*2,6-Dimethoxy-4-(prop-2-enyl)phenyl α-*L*-Rhamnopyranosyl-(1 → 6)-β-*D*-glucopyranoside* (**9**): White powder. M.p. 206–207°. $[\alpha]_D^{20} = -62$ ($c = 0.26$, MeOH). UV (MeOH): 217.0 (2.40). ^{1}H- and ^{13}C-NMR: *Table 6*. HR-ESI-MS: 503.2124 ($[M+H]^+$, $C_{23}H_{35}O_{12}^+$; calc. 503.2129).

Acid Hydrolysis of Compounds **1**–**9** [10]. A compound **1**–**9** (4 mg) in 10% HCl soln./dioxane (1:1, (1 ml) was heated separately at 80° for 4 h in a water bath. The mixture was neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (3 × 1 ml). The H_2O layer was evaporated and the residue (monosaccharide portion) examined by TLC ($CHCl_3$/MeOH/H_2O 55:45:10) and compared with authentic samples.

Determination of the Sugar Components [10]. The monosaccharide units were obtained by hydrochloric acid hydrolysis as described above. The sugar residue was then dissolved in of H_2O (2 ml), $NaBH_4$ (15 mg) was added, and the mixture was left to stand for 2 h at r.t. Several drops of 25% AcOH were added until the pH value was 4–5. After co-distillation with MeOH to remove the extra boracic acid and H_2O, the resulting product was dried overnight in a vacuum desiccator and then heated at 110° for 15 min to further remove H_2O. Next, Ac_2O (3 ml) was added and the soln. kept at 100° for 1 h. Then the soln. was cooled and co-distilled with toluene several times. The acetate derivative was dissolved in $CHCl_3$ and the soln. washed with dist. H_2O, dried (Na_2SO_4), and then concentrated to 0.1 ml. The acetate derivatives were subjected to GC (column temp. 210°; injection temp. 250°; carrier gas N_2, flow rate 25 ml/min): t_R 17.38 min for derivative of D-glucose and 4.85 min for derivative of L-rhamnose.

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