Seven New Sesquiterpene Glycosides from the Root Bark of *Dictamnus dasycarpus*

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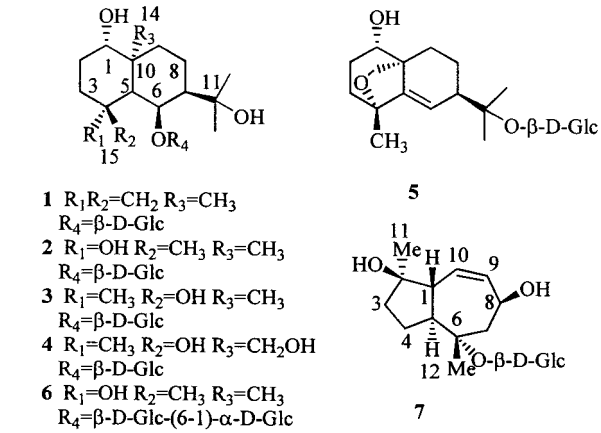
From the water-soluble constituents of the root bark of *Dictamnus dasycarpus*, six new eudesmane-type sesquiterpene glycosides, dictamnosides H−M (**1**−**6**), and a new trinorguaiane-type sesquiterpene glycoside, dictamnoside N (**7**), together with four known sesquiterpene glycosides, dictamnosides A (**8**), B (**9**), D (**10**), and G (**11**), were isolated. Their structures were elucidated by spectroscopic analyses and chemical evidence. In vitro tests for immunological activity showed dictamnoside A (**8**) to possess remarkable activity in stimulating the proliferation of T-cells.

As a traditional Chinese medicine, the root bark of *Dictamnus dasycarpus* Turcz. (Rutaceae) was used for the treatment for jaundice, cough, rheumatism, and some skin diseases. Moreover, its water extract was reported to inhibit the growth of many kinds of human pathogenic fungi in vitro.[1] Several kinds of compounds including limonoids,[2] furoquinoline alkaloids,[3,4] flavonoids,[5,6] coumarins,[7] sesquiterpene,[8] and sesquiterpene glycosides[9,10] have been isolated from *D. dasycarpus*. Recently, W. M. Zhao reported the isolation and identification of six components inhibitory to the plant pathogenic fungus *Cladosporium cucumerinum* from the dichloromethane extract of *D. dasycarpus*.[11] As a part of our study on the hydrophilic bioactive constituents from Chinese medicines, we systematically studied the water-soluble constituents of *D. dasycarpus*. The water-soluble fraction from 60% aqueous acetone extract of the root bark of *D. dasycarpus* was subjected to column chromatography on MCI gel CHP 20P, Cosmosil 75 C_{18}-OPN, and TSK gel Toyopearl HW-40F to afford seven novel sesquiterpene glycosides, dictamnosides H−N (**1**−**7**), together with four known sesquiterpene glycosides, dictamnosides A (**8**), B (**9**), D (**10**), and G (**11**).[9,10] Here, we report the isolation and structure elucidation of dictamnosides H−N, respectively.

Results and Discussion

Compound **1** was obtained as white amorphous powder. ESIMS showed a quasimolecular ion peak at *m*/*z* 439 $[M + Na]^+$, indicating a molecular weight of 416. According to the MS and ^1H and ^{13}C NMR spectral data, the molecular formula of **1** is $C_{21}H_{36}O_8$. In the ^1H NMR spectrum of **1** (Table 1), two olefinic proton signals at δ 5.04 (1H, br s) and 5.00 (1H, br s) indicated the presence of an exocyclic group, which was confirmed by signals for a quaternary carbon at δ 149.6 and a methylene at δ 109.7 in the ^{13}C NMR spectrum (Table 2). Apart from six signals typical of hexose, the ^{13}C NMR spectrum shows an additional 15 carbon signals, similar to those of **9** (dictamnoside B). However, the signal of C-14 was changed from δ_C 63.7 in **9** to δ_C 17.5 in **1**, indicating that the position-14 is a methyl. The sugar moiety was determined as β-D-glucose by acid hydrolysis, comparison with an authentic sample, and the chemical shift of C-1′ (δ 104.6) and coupling constant (J = 7.9 Hz) of the anomeric proton (δ 4.65).[12] The glycosidic site was established unambiguously by the HMBC experiment in which a long-range correlation between H-1′ (δ 4.65) and C-6 (δ 80.9) was observed. The relative configuration, deduced from the results of the NOESY spectrum, is the same as that of **9**. Consequently, **1** was identified as a new *trans*-fused eudesmane-type sesquiterpene glycoside, named dictamnoside H. Compounds **2** and **3** were isolated as white amorphous powders. ESI mass spectra of **2** and **3** gave the same quasimolecular ion peak at *m*/*z* 457 $[M + Na]^+$. On the basis of the ESIMS and ^1H and ^{13}C NMR spectral data, the molecular formulas of **2** and **3** were deduced to be $C_{21}H_{38}O_9$. The molecular mass of **2** and **3** was 18 Da higher than that of **1**. In addition, the signal of C-4 was changed from δ_C 149.6 in **1** to δ_C 76.3 in **2** and δ_C 74.8 in **3**, respectively. On the basis of the degrees of unsaturation and from a biogenetic point of view, compounds **2** and **3** must be analogues of **1,** in which the olefinic bond is reduced, and C-4 is oxidized to a hydroxy group. The sugar moiety was determined as β-D-glucose by acid hydrolysis, comparison with an authentic sample, and the coupling constant of the anomeric proton (Table 1) and chemical shift of C-1′ (Table 2). The glycosidic site was established by the HMBC experiment, which revealed a long-range correlation between the anomeric proton and C-6. While cross-peaks between H-15 (δ 1.28) and H-3α (δ 1.85), H-6 (δ 4.70), H-14 (δ 1.00) were observed in the NOESY spectrum of **2**, cross-peaks between H-15 (δ 1.44) and H-5 (δ 2.01) were observed in that of **3**. Therefore, as a pair of

Chart 1



1 $R_1R_2=CH_2$ $R_3=CH_3$ R_4=β-D-Glc
2 R_1=OH $R_2=CH_3$ $R_3=CH_3$ R_4=β-D-Glc
3 $R_1=CH_3$ R_2=OH $R_3=CH_3$ R_4=β-D-Glc
4 $R_1=CH_3$ R_2=OH $R_3=CH_2OH$ R_4=β-D-Glc
6 R_1=OH $R_2=CH_3$ $R_3=CH_3$ R_4=β-D-Glc-(6-1)-α-D-Glc

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Table 1. ^{1}H NMR Data for Compounds **1**−**5**[a−c]

position	**1**	**2**	**3**	**4**	**5**
1	3.57 dd (11.6, 4.6)	3.43 dd (9.6, 2.0)	3.36 m	3.55 dd (11.5, 3.3)	3.93 dd (9.7, 6.0)
2α	1.54 m	1.90 m	1.85 m	2.00 m	1.65 m
2*β*	1.86 m	1.72 m	1.56 m	1.77 m	2.00 m
3α	2.37 dd (13.0, 3.3)	1.85 m	1.75 m	1.75 m	1.79 m
3*β*	2.15 dd (13.0, 5.0)	1.55 m	1.72 m	1.73 m	1.53 m
5	2.44 d (6.4)	2.10 d (5.8)	2.01 d (7.9)	2.13 d (6.3)	
6	4.63 m	4.70 m	4.60 dd (7.6, 4.0)	4.56 dd (6.0, 4.1)	5.79 br s
7	2.08 m	2.18 m	2.22 m	2.13 m	2.52 dd (10.1, 5.5)
8α	1.75 m	1.68 m	1.87 m	1.88 m	1.80 m
8*β*	1.75 m	1.68 m	1.87 m	1.88 m	1.40 m
9α	1.70 m	1.65 m	1.60 m	2.21 dd (13.5, 4.8)	2.14 br d (13.1)
9*β*	1.62 m	1.32 m	1.28 m	1.25 m	1.26 m
12	1.38 s	1.38 s	1.39 s	1.37 s	1.31 s
13	1.38 s	1.37 s	1.35 s	1.37 s	1.19 s
14a	0.80 s	1.00 s	1.10 s	4.48 d (12.6)	4.21 d (8.1)
14b				3.92 br d (12.6)	3.30 d (8.1)
15a	5.04 br s	1.28 s	1.44 s	1.42 s	1.36 s
15b	5.00 br s				
1′	4.65 d (7.9)	4.76 d (8.1)	4.69 d (7.9)	4.68 d (8.0)	4.72 d (8.0)
2′	3.26 dd (8.7, 7.9)	3.29 dd (9.2, 8.2)	3.34 dd (8.5, 8.0)	3.32 dd (8.7, 8.5)	3.27 dd (8.9, 8.0)
3′	3.47 dd (9.0, 7.1)	3.51 dd (9.0, 9.0)	3.51 dd (8.9, 8.8)	3.49 dd (9.1, 8.7)	3.52 dd (8.9, 8.8)
4′	3.43 dd (9.0, 7.4)	3.37 dd (9.7, 8.7)	3.43 dd (9.3, 8.8)	3.39 dd (9.3, 8.5)	3.47 m
5′	3.30 m	3.41 m	3.48 m	3.45 dd (8.7, 8.5)	3.49 m
6′a	3.84 dd (12.3, 2.4)	3.96 dd (12.0, 2.0)	3.92 br d (11.5)	3.92 br d (12.6)	3.87 br d (12.0)
6′b	3.76 dd (12.3, 4.6)	3.69 dd (12.0, 6.8)	3.74 dd (12.2, 5.2)	3.72 dd (12.2, 5.3)	3.72 dd (12.0,5.0)

[a] 400 MHz, D_2O; chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz. [b] Assignments were made by ^{1}H−^{1}H COSY, HMBC, and HMQC data. [c] Chemical shifts of all the α and *β* protons of each methylene were assigned on the basis of NOE results.

Table 2. ^{13}C NMR Data for Compounds **1**−**5**[a]

C	**1**	**2**	**3**	**4**	**5**
1	82.4 d	81.5 d	81.6 d	82.6 d	73.4 d
2	33.8 t	29.8 t	28.5 t	29.3 t	30.5 t
3	37.6 t	42.6 t	42.1 t	42.1 t	40.7 t
4	149.6 s	76.3 s	74.8 s	74.4 s	83.9 s
5	54.2 d	58.3 d	54.4 d	56.4 d	147.5 s
6	80.9 d	83.0 d	82.4 d	81.9 d	118.4 d
7	46.5 d	46.5 d	47.4 d	47.5 d	47.1 d
8	20.8 t	20.7 t	22.3 t	21.3 t	22.7 t
9	35.5 t	36.5 t	35.7 t	29.2 t	27.6 t
10	42.3 s	42.3 s	43.0 s	46.0 s	50.3 s
11	77.0 s	76.8 s	77.5 s	77.0 s	84.7 s
12	31.3 q	31.7 q	31.8 q	31.3 q	26.9 q
13	30.3 q	31.4 q	32.2 q	31.7 q	24.1 q
14	17.5 q	17.2 q	15.2 q	64.0 t	75.9 t
15	109.7 t	24.5 q	33.7 q	32.3 q	22.5 q
1′	104.6 d	104.5 d	104.4 d	104.9 d	99.2 d
2′	76.0 d	76.2 d	75.9 d	75.9 d	76.1 d
3′	79.0 d	78.6 d	78.6 d	78.6 d	78.8 d
4′	72.0 d	72.6 d	72.4 d	72.3 d	72.5 d
5′	78.4 d	78.8 d	78.6 d	78.5 d	78.5 d
6′	63.1 t	63.5 t	63.4 t	63.3 t	63.6 t

[a] 100 MHz, D_2O; multiplicity was established from DEPT data.

epimers, **2** and **3** were named dictamnosides I and J, respectively.

Compound **4** was obtained as a white amorphous powder. Positive FAB mass spectra of **4** gave the quasimolecular ion peaks at *m*/*z* 473 $[M + Na]^+$ and 489 $[M + K]^+$. On the basis of the ^{1}H and ^{13}C NMR spectra, the molecular formula of **4** was deduced to be $C_{21}H_{38}O_{10}$. Comparison of the ^{13}C NMR data of **4** with those of **3** revealed the signal for C-14 was changed from δ_C 15.2 in **3** to δ_C 64.0 in **4**, indicating that the position-14 is a hydroxymethyl. The sugar moiety was determined as *β*-D-glucose by acid hydrolysis, comparison with an authentic sample, and the coupling constant ($J = 8.0$ Hz) of anomeric proton (δ 4.68) and chemical shift of C-1′ (δ 104.9). The glycosidic site was established by an HMBC experiment in which long-range correlation was observed between the H-1′ (δ 4.68) and C-6 (δ 81.9). The relative configuration was deduced from the results of a NOESY spectrum, in which NOE signals were observed between H-15 (δ 1.42) and H-5 (δ 2.13). Consequently, **4** was named dictamnoside K.

Compound **5** was obtained as an amorphous powder. ESIMS showed a quasimolecular ion peak at *m*/*z* 437 $[M + Na]^+$, indicating a molecular weight of 414. On the basis of the MS and ^{1}H and ^{13}C NMR spectral data, the molecular formula of **5** was deduced as $C_{21}H_{34}O_8$. In the ^{1}H NMR spectrum of **5**, one olefinic proton signal at δ 5.79 (1H, br s) indicated the 1,2,2-trisubstituted olefinic moiety, which was confirmed by signals of a quaternary carbon at δ 147.5 and a methine at δ 118.4 in the ^{13}C NMR spectrum. Moreover, the eudesmane-type skeleton was deduced from HMBC and HMQC experiments. Compared with **4**, the downfield shift of 11.9 ppm of the C-14 signal (δ 75.9) indicated etheration of the 14-position. This was supported by the correlation between the signal for H-14 (δ 4.21) and C-4 (δ 83.9) in the HMBC experiment. The sugar moiety was also determined as *β*-D-glucose by acid hydrolysis, comparison with an authentic sample, and the coupling constant ($J = 8.0$ Hz) of the anomeric proton (δ 4.72) and chemical shift of C-1′ (δ 99.2). The glycosidic site was also established by HMBC, in which a long-range correlation was observed between the H-1′ (δ 4.72) and C-11 (δ 84.7). The relative configuration was deduced from the results of a NOESY spectrum, in which NOE signals were observed not only between H-15 (δ 1.36) and H-1 (δ 3.93), H-6 (δ 5.79), but also between H-7 (δ 2.52) and H-1′ (δ 4.72), H-8α (δ 1.80). Consequently, **5** was named dictamnoside L.

Compound **6** was obtained as an amorphous powder. Its molecular formula, $C_{27}H_{48}O_{14}$, was deduced from positive FAB mass spectrometry and ^{1}H and ^{13}C NMR spectral data. The positive FAB mass spectrum showed quasimolecular ion peaks at *m*/*z* 619 $[M + Na]^+$ and 635 $[M + K]^+$. Apart from 15 typical signals of the sesquiterpene, the ^{13}C NMR spectrum exhibited 12 carbon signals, consistent with two hexoses; thus compound **6** is a sesquiterpene diglycoside. Comparison of the ^{13}C NMR data of **6** with those of **2** suggested that the aglycones of both compounds were identical. The sugar moieties were determined to be D-glucose by acid hydrolysis and comparison with an

Table 3. NMR Data for Compound **7**

position	$^1H^a$	$^{13}C^b$	HMBC	NOESY
1	2.35 br d (11.3)	52.5 d	H-3, H-9, H-10, H-11	H-7*β*, H-10, H-12
2		82.4 s	H-1, H-4, H-10, H-11	
3α	1.70 m	41.8 t	H-4, H-11	H-5, H-11
3*β*	1.70 m			H-1
4α	1.70 m	23.8 t	H-1, H-3, H-5	H-4*β*
4*β*	1.81 m			H-4α, H-12
5	2.11 ddd (10.6, 5.6, 5.0)	51.7 d	H-1, H-4, H-7, H-10, H-12	H-1′, H-4*a*, H-11
6		84.4 s	H-1′, H-1, H-4, H-7, H-8, H-12	
7α	2.18 br d (15.1)	49.8 t	H-9, H-12	H-1′, H-7*β*, H-8
7*β*	1.94 dd (14.8, 10.8)			H-1, H-7α
8	4.68 m	68.6 d	H-7, H-10	H-7α, H-9
9	5.79 br d (12.3)	138.6 d	H-7	H-8
10	5.84 br d (11.5)	132.2 d		H-1, H-11
11	1.16 s	23.7 q	H-1, H-3	H-4α, H-5, H-10
12	1.34 s	21.2 q	H-5, H-7	H-1′, H-1, H-4*β*
1′	4.68 d (7.9)	99.0 d	H-2′	H-3′, H-5, H-7α, H-12
2′	3.27 dd (9.0, 8.3)	75.8 d	H-3′	H-4′
3′	3.52 m	78.6 d	H-4′, H-5′	H-1′, H-5′
4′	3.32 dd (9.5, 9.4)	72.9 d	H-6′	H-2′, H-6′b
5′	3.48 m	78.6 d	H-3′, H-6′	H-3′, H-6′a
6′a	3.94 dd (12.0, 1.8)	64.2 t	H-4′, H-5′	H-5′, H-6′b
6′b	3.64 dd (12.0, 8.0)			H-4′, H-6′a

[a] 400 MHz, D_2O; chemical shifts in ppm relative to TMS; coupling constants (*J*) in Hz. [b] 100 MHz, D_2O; multiplicity was established from DEPT data.

authentic sample. Two anomeric proton signals at δ 5.00 (1H, d, $J = 3.5$ Hz) and δ 4.84 (1H, d, $J = 8.1$ Hz) in the ^1H NMR indicated that two glucose units are in α and *β* glycosidic linkage, respectively. The glycosidic sites were established by the HMBC experiment in which long-range correlations were observed between the H-1′ (δ 4.84) and C-6 (δ 82.3) and between H-1″ (δ 5.00) and C-6′ (δ 69.4). Therefore, the linkage of two glucose units was α-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl. The relative configuration was deduced from a NOESY experiment in which cross-peaks between H-15 (δ 1.36) and H-3α (δ 1.90), H-6 (δ 4.84), H-14 (δ 0.99) were observed. Consequently, **6** was determined as a new sesquiterpene diglycoside, named dictamnoside M.

Compound **7** was obtained as an amorphous powder. Its molecular formula, $C_{18}H_{30}O_8$, was deduced from positive FAB mass spectrometry and ^1H and ^{13}C NMR spectral data (Table 3). The positive FAB mass spectrum showed quasi-molecular ion peaks at *m/z* 397 $[M + Na]^+$ and 413 $[M + K]^+$. In the ^1H NMR spectrum of **7**, two olefinic proton signals at δ 5.84 (1H, br d, $J = 11.5$ Hz) and 5.79 (1H, br d, $J = 12.3$ Hz) indicated the presence of a 1,2-disubstituted *cis*-form olefin, which was confirmed by two methine peaks at δ 132.2 and 138.6 in the ^{13}C NMR spectrum. In addition to six typical signals of a hexose, the ^{13}C NMR spectrum exhibited 12 carbon signals. Analysis of $^1H-^1H$ COSY, HMBC, and HMQC spectra of **7** established the following fragment: $CH_2-CH_2-CH-CH-CH=CH-CH(O)-CH_2$. The four remaining carbons of the aglycone were two methyls and two oxygen-bearing quarternary carbons. Moreover, on the basis of HMBC, two methyls were attached to two quaternary carbons. The sugar moiety was determined as *β*-D-glucose by acid hydrolysis, comparison with an authentic sample, and the coupling constant ($J = 7.9$ Hz) of anomeric proton (δ 4.68) and chemical shift of C-1′ (δ 99.0). The glycosidic site was established by an HMBC experiment in which long-range correlation was observed between the H-1′ (δ 4.68) and C-6 (δ 84.4). The ring juncture was proposed to be *trans* on the basis of the coupling constant ($J = 11.3$ Hz) between H-1 and H-5. Although the relative configurations of most sites were determined according to the results of the NOESY spectrum (D_2O), the relative configuration at C-8 was not determined because the signal of H-8 (δ 4.68) overlapped that of H-1′. When the ^1H NMR spectrum was run in C_5D_5N as solvent, the signal of H-8 shifted from δ 4.68 to δ 5.50, while the signal of H-1′ shifted to δ 5.15 (d, $J = 7.7$ Hz). Therefore, the relative configuration could be unambiguously deduced from a NOESY experiment (C_5D_5N) in which cross-peaks between H-8 (δ 5.50, m) and H-5 (δ 2.67, m), H-7α (δ 2.89, br d, $J = 14.5$ Hz), H-9 (δ 6.40 br d, $J = 10.9$ Hz) were observed. Consequently, **7** was identified as a new trinorguaiane-type sesquiterpene glycoside, named dictamnoside N.

Except for compound **7**, the other 10 eudesmane-type sesquiterpene glycosides appeared to be closely related biosynthetically. On the basis of the interesting pharmacological activities of the root bark of *Dictamnus dasycarpus*, compounds **1**−**11** were tested in an immunological assay, in which dictamnoside A (**8**) showed remarkable activity (10^{-5} mol/L, $P < 0.001$) in stimulating the proliferation of T-cells in vitro, compared with ConA.

Experimental Section

General Experimental Procedures. Optical rotation data were obtained on a Perkin-Elmer 241 automatic digital polarimeter. ^1H,^{13}C NMR, $^1H-^1H$ COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker DRX-400 spectrometer (^1H 400 MHz and ^{13}C 100 MHz). The carbon multiplicities were obtained by a DEPT experiment. FABMS data were obtained on a MAT-212 mass spectrometer with NBA, NaCl, and KCl as matrixes. ESIMS data were measured on a Quattro instrument. Elemental analysis was carried out on an Elementary Vario EL instrument. Gas chromatography (GC) was run on a HP 1890 gas chromatograph. Reversed-phase chromatography column: TSK gel Toyopearl HW-40F (30−60 *μ*m, Toso Co., Ltd.), MCI gel CHP 20P (75−150 *μ*m, Mitsubishi Chemical Industries Co., Ltd.), and Cosmosil 75 C_{18}-OPN (42−105 *μ*m, Nacalai Tesque Inc.) columns. TLC: precoated kieselgel 60 F_{254} plates (0.2 mm, Merck).

Plant Material. The root bark of *Dictamnus dasycarpus* was collected from Anhui province, People's Republic of China, in 1999 and was identified by J.C. A voucher specimen (No. BX001) has been deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The root bark of *Dictamnus dasycarpus* (2 kg) was extracted three times with 60% aqueous

acetone at room temperature (3 × 10 L). The solvent was evaporated under reduced pressure to 1 L and filtered with Celite. The filtrate was subjected to a reversed-phase chromatography column, such as TSK gel Toyopearl HW-40F, MCI gel CHP 20P, and Cosmosil 75 C_{18}-OPN eluting with MeOH−H_2O as gradient eluent. The 10−30% aqueous MeOH eluate from the MCI column was repeatedly chromatographed on Cosmosil 75 C_{18}-OPN (eluted with 20% MeOH), MCI gel CHP 20P (eluted with 10−30% MeOH), and Toyopearl HW-40F (H_2O) to give **4** (15 mg), **6** (13 mg), **7** (17 mg), **8** (25 mg), **10** (20 mg), and **11** (12 mg), respectively. The 40−60% aqueous MeOH eluate from the MCI column was repeatedly chromatographed on Cosmosil 75 C_{18}-OPN (eluted with 30% MeOH), MCI gel CHP 20P (eluted with 40−60% MeOH), and Toyopearl HW-40F (H_2O) to give **1** (20 mg), **2** (22 mg), **3** (25 mg), **5** (12 mg), and **9** (27 mg), respectively.

Dictamnoside H (1): white amorphous powder; $[\alpha]^{24}_D$ −13.2° (*c* 0.10 MeOH); ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 439 $[M + Na]^+$; *anal.* C 60.48%, H 8.65%, calcd for $C_{21}H_{36}O_8$, C 60.56%, H 8.71%.

Dictamnoside I (2): white amorphous powder; $[\alpha]^{24}_D$ −21.4° (*c* 0.10 MeOH); ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 457 $[M + Na]^+$; *anal.* C 57.94%, H 8.76%, calcd for $C_{21}H_{38}O_9$, C 58.05%, H 8.82%.

Dictamnoside J (3): white amorphous powder; $[\alpha]^{24}_D$ −15.7° (*c* 0.10 MeOH); ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 457 $[M + Na]^+$; *anal.* C 57.92%, H 8.77%, calcd for $C_{21}H_{38}O_9$, C 58.05%, H 8.82%.

Dictamnoside K (4): white amorphous powder; $[\alpha]^{24}_D$ −24.2° (*c* 0.10 MeOH); ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; FABMS *m*/*z* 473 $[M + Na]^+$, 489 $[M + K]^+$; *anal.* C 55.88%, H 8.42%, calcd for $C_{21}H_{38}O_{10}$, C 55.98%, H 8.50%.

Dictamnoside L (5): white amorphous powder; $[\alpha]^{24}_D$ −12.7° (*c* 0.10 MeOH); ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 437 $[M + Na]^+$; *anal.* C 60.72%, H 8.20%, calcd for $C_{21}H_{34}O_8$, C 60.85%, H 8.27%.

Dictamnoside M (6): white amorphous powder; $[\alpha]^{24}_D$ −24.0° (*c* 0.10 MeOH); ^{1}H NMR (D_2O), δ 5.00 (1H, d, J = 3.5 Hz, H-1″), 4.84 (2H, d, J = 8.1 Hz, H-1′, H-6), 3.98 (1H, dd, J = 17.1, 5.5 Hz, H-6′a), 3.87 (1H, m, H-6″a), 3.77 (2H, m, H-6″b, H-6′b), 3.75 (1H, m, H-3″), 3.73 (1H, m, H-5″), 3.70 (1H, m, H-3′), 3.62 (1H, m, H-2″), 3.57 (1H, m, H-5′), 3.50 (1H, m, H-4′), 3.47 (1H, m, H-4″), 3.45 (1H, m, H-1), 3.32 (1H, dd, J = 8.0, 7.9 Hz, H-2′), 2.32 (1H, br s, H-7), 2.23 (1H, d, J = 6.0 Hz, H-5), 2.00 (2H, m, 2H-8), 1.90 (1H, m, H-3α), 1.81 (2H, m, 2H-2), 1.75 (1H, m, H-9α), 1.72 (1H, m, H-3β), 1.42 (3H, s, 3H-12), 1.38(3H, s, 3H-13), 1.36 (3H, s, 3H-15), 1.34 (1H, m, H-9β), 0.99 (3H, s, 3H-14); ^{13}C NMR (D_2O), δ 102.6 (C-1′), 101.3 (C-1″), 82.3 (C-6), 81.6 (C-1), 78.7 (C-5′), 77.6 (C-3′), 77.4 (C-11), 76.6 (C-4), 76.1 (C-3″), 75.9 (C-2′), 74.5 (C-5″), 74.2 (C-2″), 72.3 (C-4″), 72.2 (C-4′), 69.4 (C-6′), 63.2 (C-6″), 55.3 (C-5), 46.8 (C-7), 43.0 (C-10), 42.3 (C-3), 37.1 (C-9), 32.2 (C-12), 32.0 (C-13), 29.6 (C-2), 25.0 (C-15), 22.4 (C-8), 16.9 (C-14); FABMS *m*/*z* 619 $[M + Na]^+$, 635 $[M + K]^+$; *anal.* C 54.23%, H 8.07%, calcd for $C_{27}H_{48}O_{14}$, C 54.35%, H 8.11%.

Dictamnoside N (7): white amorphous powder; $[\alpha]^{24}_D$ −34.3° (*c* 0.10 MeOH); ^{1}H NMR and ^{13}C NMR, see Table 3; FABMS *m*/*z* 397 $[M + Na]^+$, 413 $[M + K]^+$; *anal.* C 57.70%, H 8.04%, calcd for $C_{18}H_{30}O_8$, C 57.74%, H 8.08%.

Acid Hydrolysis of 1−7. A solution of **1**−**7** (6 mg each) in 2 N HCl was heated (90 °C) for 2 h. After removing HCl by evaporation in a vacuum, the mixture was diluted with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 1 N NaOH and subjected to TLC analysis on Kieselgel 60 F_{254} (Merck) [using $CHCl_3$−MeOH−H_2O (30:12:9), 9 mL, and HOAc, 1 mL] and paper chromatography [using *n*-BuOH−HOAc−H_2O (4:1:5)] with standard sugars, in which the presence of glucose was established. The remaining aqueous layer was then passed through an Amberlite IRA-60E column, and the aqueous eluate was concentrated and derivated with thiazolidine as described previously.[13] Only the D-glucose derivative was detected by GC (GC conditions: column, Supelco SPB^{-1}, 0.25 mm × 27 m, column temperature 230 °C; carrier gas, N_2; t_R, D-glucose derivative 17.9 min, L-glucose derivative 17.3 min).

Bioassay Procedures. Compounds **1**−**11** (10^{-7} to -10^{-5} mol/L) were each incubated with mouse splenocytes in the presence of mitogen ConA (5 μg/mL) or LPS (20 μg/mL). After incubation for 44 h at 37 °C in a humidified 5% CO_2 incubator, T and B lymphocyte proliferation was tested by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetiazolium bromide] assay. The absorption was measured by DG-3022 ELISA at 570 nm.[14]

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