New Pregnane Glycosides from *Brucea javanica* and Their Antifeedant Activity

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Three new pregnane glycosides, 3-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3*β*,20-diol (**1**), 3-*O*-*α*-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3*β*,20-diol-20-*O*-*β*-D-glucopyranoside (**2**), 3-*O*-*α*-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3*β*,20-diol-20-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**3**) were isolated along with four known compounds, **4**–**7**, from the leaves and stems of *Brucea javanica*. Their structures were determined by detailed analyses of 1D- and 2D-NMR spectroscopic data. All of the compounds isolated from *Brucea javanica* were tested for the antifeedant activities against the larva of *Pieris rapae.* Compounds **1**, **3**, and **5** showed significant antifeedant activities after 72 h incubation.

Introduction. – *Brucea javanica* (L.) MERR. (Simaroubaceae) grows in the tropical and the subtropics areas of Asia, Indonesia, and Australia, and is mainly distributed in Guangdong and Guangxi provinces of China [1]. Its seeds have been used as antitumor agent and as insecticide against amoeba. Its leaves have been used as folk medicine in poultice against boils, ringworm, scurf, and centipede bites [2]. Quassinoids had been isolated as major constituents from the seeds and roots [3–9]; a pregnane glycoside had been reported in the leaves [10]. In the course of a screening, the crude extract from the leaves and stems of *Brucea javanica* showed antifeedant activity. Fractionation of the active extract afforded three new pregnane glycosides: 3-*O*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3*β*,20-diol (**1**), 3-*O*-*α*-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3*β*,20-diol-20-*O*-*β*-D-glucopyranoside (**2**), 3-*O*-*α*-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3*β*,20-diol-20-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**3**), together with four known compounds, **4**–**7** (*Fig. 1*). Here, we report the structure elucidation of **1**–**3** on the basis of spectroscopic analysis. In addition, the antifeedant activities against the larva of *Pieris rapae* are also described.

Results and Discussion. – The structures of the known compounds, (20*R*)-3-*O*-*α*-L-arabinopyranosylpregn-5-ene-3*β*,20-diol (**4**) [10], luteolin (**5**) [11], luteolin 7-*O*-*β*-D-glucopyranoside (**6**) [11], and apigenin 7-*O*-*β*-neospheroside (**7**) [12], were deduced by direct comparison of their spectroscopic data with those reported in the literature.

Compound **1**, isolated as white needles, showed a *quasi*-molecular-ion peak at *m*/*z* 635.6 ([*M*+Na]$^+$) in FAB-MS (positive-ion mode) and was assigned a molecular

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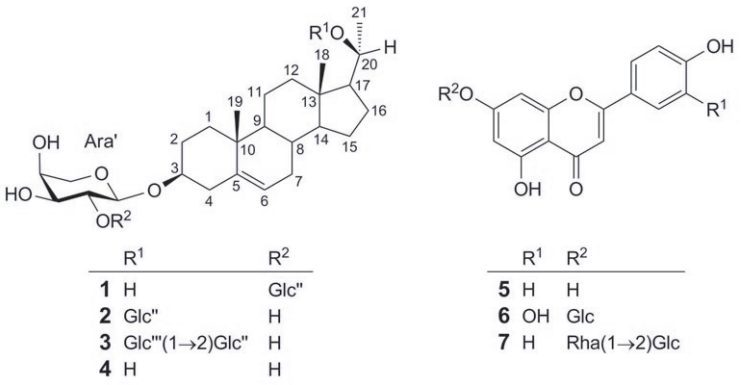

	R^1	R^2
1	H	Glc''
2	Glc''	H
3	Glc'''(1→2)Glc''	H
4	H	H

	R^1	R^2
5	H	H
6	OH	Glc
7	H	Rha(1→2)Glc

Fig. 1. *Structures of* **1**–**7**

formula of $C_{32}H_{52}O_{11}$, which was confirmed by HR-FAB-MS (positive-ion mode; 635.3459 ($[M+Na]^+$; calc. 635.3407)) and also deduced by analysis of the ^{13}C-NMR spectrum combined with the DEPT data (*Table 1*). The IR spectrum revealed the presence of OH groups (3400 cm^{-1}; br.), Me and CH_2 groups (2934 and 2869 cm^{-1}, resp.; br.), and C=O groups (1648 cm^{-1}). The ^{1}H-NMR spectrum of **1** displayed signals for two Me groups at δ(H) 0.74 and 1.00, one Me *doublet* signal at δ(H) 1.44 (d, $J=6.1$), suggesting a pregnane skeleton. Furthermore, two anomeric H-atom signals were observed at δ(H) 5.10 (d, $J=6.1$) and 5.15 (d, $J=7.9$). The ^{13}C-NMR spectrum combined with DEPT data (*Table 1*) of **1** exhibited 32 C-signals, including those corresponding to three Me (δ(C) 12.7, 19.5, and 24.8), ten CH_2, and 16 CH groups, and three quaternary C-atoms (δ(C) 141.1, 37.0, and 41.7). On acidic hydrolysis, **1** afforded a sugar mixture of D-glucose and L-arabinose, which were identified by GC analysis. The coupling constants of the anomeric H-atoms ($J=7.9$ and 6.1) indicated β-glucosidic and α-arabinosidic linkages, respectively. Signals of sugar units (*Table 1*) were assigned by HMQC, pyranosyl configuration of the arabinosyl moiety was identified by comparison ^{1}H- and ^{13}C-NMR spectroscopic data with those in the literature [10]. Further comparison of the NMR data with those of **4** showed that the two structures were essentially analogous except that **1** displayed additional signals arising from a β-glucopyranosyl moiety (anomeric C-atom signal at δ(C) 106.1, anomeric H-atom signal at δ(H) 5.15 (d, $J=7.9$)), and a glycosylation shift was observed for the signal of arabinosyl C(2′) (+9.0 ppm), suggesting that the attachment of the additional sugar was at C(2′) of the arabinosyl residue. This was confirmed by long-range correlations between H–C(1″) at δ(H) 5.15 (d, $J=7.9$) and C(2′) at δ(C) 81.6 in the HMBC spectrum of **1**. Thus, the structure of **1** was established as 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3β,20-diol.

Compound **2**, obtained as white needles, was assigned the molecular formula $C_{32}H_{52}O_{11}$ based on the HR-FAB-MS (positive-ion mode; m/z 635.3459 ($[M+Na]^+$; calc. 635.3407)), which was confirmed by the ^{13}C-NMR and DEPT spectrum (*Table 1*) and was the same as that of **1**. The IR spectrum indicated the presence of OH groups

Table 1. *^{13}C- and ^{1}H-NMR Data for* **1**–**3** *in (D$_5$)Pyridine*[a])[b])

Position	**1**		**2**		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
1	37.7 (*t*)	1.03–1.05 (*m*), 1.75–1.77 (*m*)	37.7 (*t*)	1.03–1.06 (*m*), 1.75–1.76 (*m*)	37.7 (*t*)	1.06–1.08 (*m*), 1.74–1.76 (*m*)
2	30.3 (*t*)	1.80–1.83 (*m*), 2.12–2.14 (*m*)	30.4 (*t*)	1.72–1.75 (*m*), 2.13–2.15 (*m*)	30.4 (*t*)	1.72–1.74 (*m*), 2.13–2.15 (*m*)
3	78.3 (*d*)	3.93–3.95 (*m*)	78.1 (*d*)	3.86–3.89 (*m*)	78.1 (*d*)	3.94–3.96 (*m*)
4	39.2 (*t*)	2.56 (*d*, *J*=11.6), 2.73 (*d*, *J*=10.4)	39.3 (*t*)	2.43 (*d*, *J*=11.6), 2.66 (*d*, *J*=13.4)	39.3 (*t*)	2.43 (*d*, *J*=12.0), 2.66 (*d*, *J*=11.0)
5	141.1 (*s*)		141.1 (*s*)		141.0 (*s*)	
6	121.9 (*d*)	5.37 (br. *s*)	121.8 (*d*)	5.35 (br. *s*)	121.9 (*d*)	5.35 (br. *s*)
7	32.2 (*t*)	1.52–1.54 (*m*), 1.80–1.83 (*m*)	32.2 (*t*)	1.40–1.44 (*m*), 1.75–1.76 (*m*)	32.2 (*t*)	1.35–1.38 (*m*), 1.80–1.82 (*m*)
8	31.8 (*d*)	1.40–1.43 (*m*)	31.9 (*d*)	1.34–1.35 (*m*)	31.9 (*d*)	1.32–1.35 (*m*)
9	50.5 (*d*)	0.88–0.89 (*m*)	50.4 (*d*)	0.85–0.87 (*m*)	50.4 (*d*)	0.87–0.88 (*m*)
10	37.0 (*s*)		37.0 (*s*)		37.0 (*s*)	
11	21.1 (*t*)	1.47–1.48 (*m*)	21.1 (*t*)	1.36–1.40 (*m*)	21.1 (*t*)	1.30–1.32 (*m*)
12	39.1 (*t*)	1.10–1.12 (*m*), 1.89–1.91 (*m*)	39.2 (*t*)	1.06–1.08 (*m*), 1.83–1.85 (*m*)	39.2 (*t*)	1.11–1.14 (*m*), 1.79–1.80 (*m*)
13	41.7 (*s*)		41.6 (*s*)		41.7 (*s*)	
14	56.9 (*d*)	0.96–0.97 (*m*)	56.7 (*d*)	0.87–0.90 (*m*)	56.8 (*d*)	1.01–1.03 (*m*)
15	24.6 (*t*)	1.10–1.12 (*m*), 1.60–1.61 (*m*)	24.5 (*t*)	1.03–1.06 (*m*), 1.44–1.48 (*m*)	24.5 (*t*)	1.10–1.11 (*m*), 1.64–1.66 (*m*)
16	26.6 (*t*)	1.86–1.89 (*m*), 2.12–2.14 (*m*)	27.2 (*t*)	1.83–1.85 (*m*), 2.34–2.35 (*m*)	27.0 (*t*)	1.97–1.99 (*m*), 2.57–2.59 (*m*)
17	59.4 (*d*)	1.52–1.54 (*m*)	58.4 (*d*)	1.55–1.59 (*m*)	58.2 (*d*)	1.66–1.68 (*m*)
18	12.7 (*q*)	0.74 (*s*)	12.5 (*q*)	0.68 (*s*)	12.5 (*q*)	0.60 (*s*)
19	19.5 (*q*)	1.00 (*s*)	19.4 (*q*)	0.93 (*s*)	19.4 (*q*)	0.92 (*s*)
20	69.0 (*d*)	3.93–3.95 (*m*)	81.3 (*d*)	3.86–3.89 (*m*)	81.4 (*d*)	3.81–3.83 (*m*)
21	24.8 (*q*)	1.44 (*d*, *J*=6.1)	23.3 (*q*)	1.55 (*d*, *J*=6.1)	22.8 (*q*)	1.52 (*d*, *J*=6.1)
Ara′						
1′	100.9 (*d*)	5.10 (*d*, *J*=6.1)	103.1 (*d*)	4.84 (*d*, *J*=6.7)	103.2 (*d*)	4.84 (*d*, *J*=6.7)
2′	81.6 (*d*)	4.58 (*d*, *J*=6.5)	72.6 (*d*)	4.40–4.42 (*m*)	72.6 (*d*)	4.43–4.45 (*m*)
3′	73.0 (*d*)	4.42–4.44 (*m*)	74.6 (*d*)	4.19–4.21 (*m*)	74.6 (*d*)	4.16–4.18 (*m*)
4′	68.1 (*d*)	4.40–4.42 (*m*)	69.5 (*d*)	4.25–4.26 (*m*)	69.5 (*d*)	4.32–4.34 (*m*)
5′	65.0 (*t*)	3.76–3.79 (*m*), 4.27–4.29 (*m*)	66.8 (*t*)	3.79 (*d*, *J*=10.4), 4.30–4.32 (*m*)	66.8 (*t*)	3.79 (*d*, *J*=10.4), 4.33–4.35 (*m*)
Glc″						
1″	106.0 (*d*)	5.15 (*d*, *J*=7.9)	106.1 (*d*)	4.95 (*d*, *J*=7.9)	103.8 (*d*)	4.95 (*d*, *J*=7.3)
2″	76.2 (*d*)	4.11 (*t*, *J*=8.5)	75.8 (*d*)	3.88–3.91 (*m*)	83.1 (*d*)	4.19–4.21 (*m*)
3″	78.2 (*d*)	4.21 (*t*, *J*=8.9)	78.7 (*d*)	4.21–4.22 (*m*)	78.4 (*d*)	4.33–4.35 (*m*)
4″	71.7 (*d*)	4.27–4.28 (*m*)	71.9 (*d*)	4.19–4.20 (*m*)	71.7 (*d*)	4.15–4.17 (*m*)
5″	78.6 (*d*)	3.76–3.79 (*m*)	78.2 (*d*)	3.86–3.88 (*m*)	78.1 (*d*)	3.83–3.85 (*m*)
6″	62.8 (*t*)	4.42–4.43 (*m*), 4.53–4.56 (*m*)	63.1 (*t*)	4.30–4.33 (*m*), 4.56 (*d*, *J*=10.4)	63.0 (*t*)	4.28–4.30 (*m*), 4.48–4.50 (*m*)
Glc‴						
1‴					105.7 (*d*)	5.39 (*d*, *J*=7.3)
2‴					76.9 (*d*)	4.13–4.15 (*m*)
3‴					78.0 (*d*)	4.35–4.37 (*m*)
4‴					71.9 (*d*)	4.26–4.27 (*m*)
5‴					78.4 (*d*)	3.85–3.89 (*m*)
6‴					62.8 (*t*)	4.30–4.31 (*m*), 4.50–4.52 (*m*)

[a]) Me_4Si was used as an internal standard. [b]) Assignments were confirmed by HMQC, HMBC, and $^{1}H,^{1}H$-COSY experiments; due to severe overlapping, only detectable *J* values are reported; δ in ppm, *J* in Hz.

(3400 cm^{-1}; br.). On acidic hydrolysis, **2** afforded a sugar mixture of D-glucose and L-arabinose, which were identified by GC analysis. The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) of the aglycone portion of **2** were almost identical with those of **4**, except that the signal due to C(20) at δ(C) 69.0 was shifted to δ(C) 81.3, and the signals due to the C-atoms around C(20) were slightly changed: signal of C(16) was shifted downfield by 0.57 ppm, and those of C(17) and C(21) were shifted upfield by 0.97 and 1.49 ppm, respectively. In addition, the NMR spectra of **2** displayed additional signals arising from a β-glucopyranosyl moiety (anomeric C-atom signal at δ(C) 106.1, anomeric H-atom signal at δ(H) 4.95 (*d*, $J=7.9$)). These findings suggested that the additional glucopyranosyl group was at C(20) of the aglycone, which was further confirmed by HMBC spectrum showing long-range correlations between the H–C(1″) (δ(H) 4.95 (*d*, $J=7.9$)) and C(20) of the aglycone (δ(C) 81.3), and between H–C(1′) (δ(H) 4.84(*d*, $J=6.7$)) and C(3) of the aglycone (δ(C) 78.1). Accordingly, the structure of **2** was determined to be 3-*O*-α-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3β,20-diol-20-*O*-β-D-glucopyranoside.

Compound **3** was shown to have the molecular formula of $C_{38}H_{62}O_{16}$ on the basis of the HR-FAB-MS (positive-ion mode; 797.4078 ($[M+Na]^+$; calc. 797.3936)), and the ^{13}C-NMR and DEPT spectrum (*Table 1*). The ^{1}H-NMR spectrum of **3** exhibited signals for three anomeric H-atoms at δ(H) 4.84 (*d*, $J=6.7$), 4.95 (*d*, $J=7.3$), and 5.39 (*d*, $J=7.3$), as well as signals for three Me groups at δ(H) 0.60, 0.92, and 1.52 and one Me *doublet* at δ(H) 1.52 (*d*, $J=6.1$), also suggesting a pregnane skeleton. Comparison of the ^{13}C-NMR spectrum of **3** with that of **4** suggested that the two structures were similar except that the spectra of **3** displayed additional signals arising from two β-glucopyranosyl moieties (anomeric C-atom signals at δ(C) 103.8, 105.7, and anomeric H-atom signals at δ(H) 4.95 (*d*, $J=7.3$), 5.39 (*d*, $J=7.3$)). In addition the signal due to C(20) was shifted downfield by 12.41 ppm, and the signals due to the C-atoms around C(20) were somewhat displaced: signals due to C(16) was shifted downfield by 0.40 ppm, and those due to C(17) and C(21) were shifted upfield by 1.17 and 1.96 ppm, respectively. On the other hand, the signal due to C(3) was unchanged, and the signals of the α-L-arabinopyranosyl moiety (anomeric C-atom signal at δ(C) 103.2 and anomeric H-atom signal at δ(H) 4.84 (*d*, $J=6.7$)) remained almost unshifted, suggesting that **3** is related to **4** with two additional sugar moieties at C(20) of the aglycone. As compared to **2**, the signal due to C(2″) was shifted downfield by 7.28 ppm, suggesting that the sugar chain at C(20) was β-glucopyranosyl-(1$\rightarrow$2)-β-glucopyranosyl. The glycosylation sites and the interglycosidic linkage were confirmed by the HMBC spectrum showing long-range correlations of δ(H) 5.39 (H–C(1‴)) with δ(C) 83.1 (C(2″)), of δ(H) 4.95 (H–C(1″)) with δ(C) 83.1 (C(2″)), of δ(H) 4.95 (H–C(1″)) with δ(C) 81.4 (C(20)), and of δ(H) 4.84 (H–C(1′)) with δ(C) δ78.1 (C(3)) (*Fig. 2*). Hence, the structure of **3** was elucidated as 3-*O*-α-L-arabinopyranosyl-(20*R*)-pregn-5-ene-3β,20-diol-20-*O*-β-D-glucopyranosyl-(1$\rightarrow$2)-β-D-glucopyranoside.

Pregnanes have been already reported to possess antifeedant activities [13][14]. It is assumed that their antifeedant activity is related to the effects on chemoreceptors for glucose, sucrose, and inositol [15]. In this study, the antifeedant activities of compounds **1**–**7** were tested against the larva of *Pieris rapae* (*Table 2*). Treated and control leaves were incubated 24, 48, and 72 h with the larvae. As a result, compounds **1**, **3**, and **5** showed significant antifeedant activities after 72 h incubation, as well as **1** and **5**, after

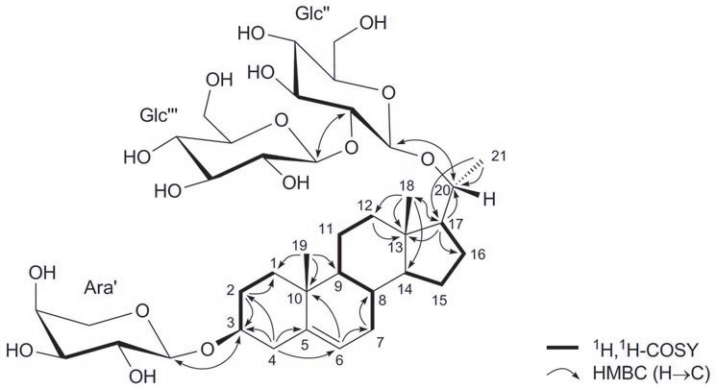

Fig. 2. *Selected ^{1}H,^{1}H-COSY and HMBC correlations of compound* **3**

Table 2. *Antifeedant Activity of* **1**–**7** *against the 3rd Larva of* Pieris Rapae

Compound	AI% [a])		
	24 h	48 h	72 h
1	94.4 ± 3.3**[b])	84.4 ± 1.5**	70.1 ± 0.6**
2	0.0 ± 0.0	−1.7 ± 0.7	29.7 ± 1.8
3	61.1 ± 3.4**	50.6 ± 2.3*	58.2 ± 2.0*
4	31.1 ± 4.2*	−10.9 ± 3.2	6.6 ± 0.6
5	72.2 ± 5.6**	53.3 ± 2.9*	53.7 ± 4.6*
6	77.8 ± 4.6**	54.4 ± 3.1*	41.9 ± 6.8
7	66.7 ± 2.5**	18.1 ± 4.2	29.5 ± 1.8

[a]) Antifeedant index calculated as AI [%] = [1 − (*T*/*C*)] × 100, data expressed as means ± SD of ten replicates. Significant differences between consumption of treated and control leaves (*Wilcoxon* signed rank test). [b]) *: $P < 0.05$; **: $P < 0.01$.

24 and 48 h. Compound **1** was the most active with an antifeedant index exceeding 70% for all incubation times.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 mesh and 200–300 mesh; *Qingdao Haiyang Chemical Company*, Qingdao, P. R. China), macroporous absorption resin *D101* (*Tianjin Chemical Industry*, Tianjin, P. R. China), and *Chromatorex ODS* (30–50 mm, *Fuji Silysia Chemical Ltd.*). GC: *Agilent 6890N* gas chromatograph, cap. column (28 m × 0.32 mm i.d., *HP-5*), FID detector, operated at 260° (column temp. 180°); N_2 as carrier gas (40 ml/min). M.p.: *Yanaco-50949* micro melting point apparatus; uncorrected. Optical rotations: *P-1010* polarimeter (*JASCO*, Japan) at 25°. IR Spectra: *5-DX* spectrometer (*Nicolet*), in KBr pellets. NMR Spectra: at 500 MHz for ^{1}H and 125 MHz for ^{13}C; *JNC-A500* NMR spectrometer; chemical shifts given on a δ [ppm] scale with Me_4Si as internal

standard; standard pulse sequences for DEPT and HMBC experiments. HR-FAB-MS: *JEOL JMS-DX303 HF* spectrometer; in a glycerol matrix containing NaI.

Plant Material. Leaves and stems of *Brucea javanica* were obtained from Jingdao County, Fangcheng City of Guangxi, P. R. China, in September 2004 and identified by Professor *Yan Liu* (Guangxi Institute of Botany). A voucher specimen (BJ040912) of the plant is deposited with the Herbarium of Guangxi Institute of Botany, P. R. China.

Extraction and Isolation. The air-dried cut leaves and stems of *Brucea javanica* (2.34 kg) were extracted three times for 2 h with 95% EtOH (15.0 l) under reflux. The extract was concentrated under reduced pressure to afford EtOH extract (205 g). The extract was suspended in H_2O (0.6 l) and successively partitioned with petroleum ether (PE; 0.5 l × 2), $CHCl_3$ (0.5 l × 3), and BuOH (0.5 l × 3), resp. The $CHCl_3$ extracts were concentrated *in vacuo* to afford a residue (18 g) which was subjected to CC (SiO_2; $CHCl_3$/MeOH from 20:1 to 1:1 (*v*/*v*)), to afford five fractions. *Fr. 2* (2.25 g) was subjected repeatedly to CC (SiO_2; $CHCl_3$/MeOH 10:1 (*v*/*v*)) to afford **5** (225.2 mg) and **4** (73.8 mg). *Fr. 3* (1.85 g) was repeatedly subjected to CC (SiO_2; $CHCl_3$/MeOH 5:1 (*v*/*v*)), followed by further purification with CC (*ODS*; 50–60% MeOH) to afford **2** (25.6 mg) and **1** (11.3 mg). The BuOH extract (75 g) was dissolved in H_2O and subjected to macroporous absorption resin *D101* (EtOH/H_2O 0:100, 50:50, 80:20). The fraction (35.8 g) eluted with EtOH/H_2O 50:50 (*v*/*v*) was repeatedly subjected to CC (SiO_2; $CHCl_3$/MeOH from 10:1 to 1:1 (*v*/*v*)) to afford nine fractions. Further purification of *Frs. 6* (1.5 g), *7* (2.4 g), and *8* (1.1 g) by CC (SiO_2; $CHCl_3$/MeOH 7:3 (*v*/*v*)) gave **6** (32.0 mg), **3** (195.8 mg), and **7** (25.0 mg), resp.

*(3β,20*R*)-20-Hydroxypregn-5-en-3-yl 2-*O*-β-*D*-Glucopyranosyl-α-*L*-arabinopyranoside* (**1**). White needles. M.p. 290.5–292.0°. $[\alpha]_D^{25} = -13.4$ ($c = -0.10$, MeOH). IR (KBr): 3415, 2934, 2869, 1455, 1379. ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS (pos.): 635.6 ($[M+Na]^+$). HR-FAB-MS (pos.): 635.3459 ($[M+Na]^+$, $C_{32}H_{52}NaO_{11}^+$; calc. 635.3407).

*(3β,20*R*)-3-(α-L-Arabinopyranosyloxy)pregn-5-en-20-yl β-D-Glucopyranoside* (**2**). White needles. M.p. 254.5–255.5°. $[\alpha]_D^{25} = -36.2$ ($c = 0.12$, MeOH). IR (KBr): 3393, 2934, 2869, 1455, 1379. ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS (pos.): 635.6 ($[M+Na]^+$). HR-FAB-MS (pos.): 635.3459 ($[M+Na]^+$, $C_{32}H_{52}NaO_{11}^+$; calc. 635.3407).

*(3β,20*R*)-3-(α-L-Arabinopyranosyloxy)pregn-5-en-20-yl 2-*O*-β-*D*-Glucopyranosyl-β-D-glucopyranoside* (**3**). White needles. M.p. 236–237°. $[\alpha]_D^{25} = -13.4$ ($c = 0.1$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS (pos.): 797.1 ($[M+Na]^+$). HR-FAB-MS (pos.): 797.4078 ($[M+Na]^+$, $C_{38}H_{62}NaO_{16}^+$; calc. 797.3936).

Antifeedant Activity Tests. Antifeedant activity of compounds **1**–**7** (*Fig. 1*) was studied using leaf disc no-choice method [16]. Fresh *Brassica alboglabra* leaf discs of 2 cm in diameter were punched using cork borer and were dipped in 1 mg/ml test compound, respectively. Leaf discs treated with acetone and H_2O were used as negative control. Treated and control leaf discs were then placed in two separate *Petri* dishes (1.5 cm × 7 cm) covered with a piece of wet filter paper, and one third larvae of *Pieris rapae* (starved for 4 h) were introduced into each *Petri* dish. Progressive consumption of treated and control leaf by the larvae after 24, 48, 72 h was recorded using leaf-area meter. Leaf area, eaten by larvae in treatment, was compared to that from the negative control. Tests were performed in ten replicates. The measurements were always performed by the same operator. The antifeedant index (AI [%]) was calculated as $[1-(T/C)] \times 100$, where T and C represent the consumption of treated and control leaf areas, resp.

Acid Hydrolysis and Sugar Analysis by TLC and GC. Each soln. of the compounds **1**–**3** (each 5 mg) in a mixture of MeOH (2.0 ml) and 1M H_2SO_4 (2.0 ml) was refluxed for 2 h on a H_2O bath. The hydrolysate was allowed to cool, H_2O (8.0 ml) was added, and the soln. was extracted with AcOEt (3 × 8.0 ml). The aq. layer was neutralized with aq. $Ba(OH)_2$ and concentrated *in vacuo* to give a residue. The residues were analyzed by TLC (SiO_2) by comparison with standard sugars. The solvent system was $CHCl_3$/MeOH/H_2O 8:5:1 (*v*/*v*), and spots were visualized by spraying with 95% EtOH/H_2SO_4/anisaldehyde 9:0.5:0.5 (*v*/*v*/*v*), then heated at 120° for 10 min. For sugars of **1**–**3**, the R_f values of glucose and arabinose by TLC was 0.31, 0.55 resp. The results were further confirmed by GC analysis. The residues were dissolved in anh. pyridine (100 μl), then 0.1M L-cysteine methyl ester hydrochloride (200 μl; *Sigma*) was added, and the mixture was warmed at 60° for 1 h. The trimethysilylation reagent HMDS/TMCS (hexamethyldisilazane/trimethylchlorosilane/pyridine 2:1:10; *Acros Organics*, Belgium) was

added, and the mixture was warmed at 60° for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. The t_R values of L-arabinose (5.26 min) and D-glucose (12.43 min) were confirmed by comparison with authentic standards.

REFERENCES

[1] S. K. Chen, H. Li, B. Y. Chen, 'Flora of China', Beijing Science Press, Beijing, 1997, p. 11.
[2] L. M. Perry, 'Medicinal Plants of east and southeast Asia', MIT Press, Cambridge, 1989, p. 389.
[3] Y. Hitotsuyanagi, I. H. Kim, T. Hasuda, Y. Yamauchi, K. Takeya, *Tetrahedron* **2006**, *62*, 4262.
[4] T. Sakaki, S. Yoshimura, T. Tsuyuki, T. Takahashi, T. Honda, T. Nakanishi, *Tetrahedron Lett.* **1986**, *27*, 593.
[5] I. H. Kim, R. Suzuki, Y. Hitotsuyanagi, K. Takeya, *Tetrahedron* **2003**, *59*, 9985; I. H. Kim, Y. Hitotsuyanagi, K. Takeya, *Phytochemistry* **2004**, *65*, 3167.
[6] L.-Z. Lin, G. A. Cordell, C.-Z. Ni, J. Clardy, *Phytochemistry* **1990**, *29*, 2720.
[7] A. N. Shahida, T. W. Wong, C. Y. Choo, *J. Ethnopharmacol.* **2009**, *124*, 586.
[8] N. Fukamiya, M. Okano, M. Miyamoto, K. Tagahara, K.-H. Lee, *J. Nat. Prod.* **1992**, *55*, 468.
[9] C.-S. Liu Karin, S.-L. Yang, M. F. Roberts, J. D. Phillipson, *Phytochemistry* **1990**, *29*, 141.
[10] C. Kamperdick, T. V. Sung, T. T. Thuy, M. V. Tri, G. Adam, *Phytochemistry* **1995**, *38*, 699.
[11] K. R. Markham, B. Ternai, R. Stanley, H. Geiger, T. J. Mabry, *Tetrahedron* **1978**, *34*, 1389.
[12] S. Nishibe, A. Sakushima, T. Noro, S. Fukushima, *Shoyakugaku Zasshi* **1987**, *41*, 116.
[13] M. Nakatani, H. Takao, I. Miura, T. Hase, *Phytochemistry* **1985**, *24*, 1945.
[14] K. K. Purushohaman, A. Sarada, A. Saraswathy, *Can. J. Chem.* **1987**, *65*, 150.
[15] L. M. Schoonhoven, Y. Fu-Shun, *J. Insect Physiol.* **1989**, 35, 725.
[16] M. B. Isman, O. Koul, A. Luczynski, J. Kaminski, *J. Agric. Food Chem.* **1990**, *38*, 1406.

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