Bacopaside III, Bacopasaponin G, and Bacopasides A, B, and C from *Bacopa monniera*

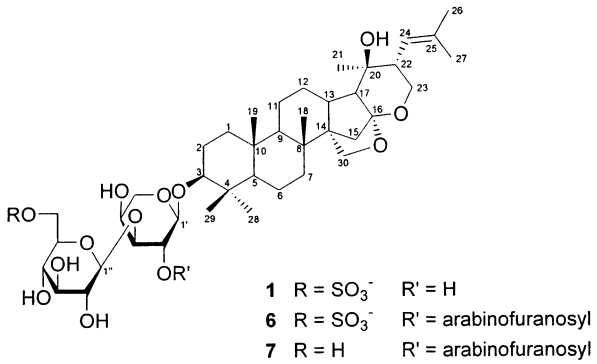
Chia-Chung Hou,[†] Shwu-Jiuan Lin,[†] Juei-Tang Cheng,[‡] and Feng-Lin Hsu*[,†]

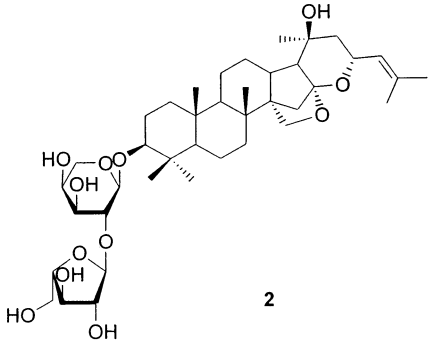
Department of Medicinal Chemistry, College of Pharmacy, Taipei Medical University, No. 250, Wu-Hsing Street, Taipei, Taiwan 110, Republic of China, and Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan, Taiwan 701, Republic of China

Received May 24, 2002

Two new saponins, 3-*O*-[6-*O*-sulfonyl-*β*-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl pseudojujubogenin (**1**) and 3-*O*-[α-L-arabinofuranosyl-(1→2)]-α-L-arabinopyranosyl jujubogenin (**2**), a new matsutaka alcohol derivative, (3*R*)-1-octan-3-yl-(6-*O*-sulfonyl)-*β*-D-glucopyranoside (**3**), a new phenylethanoid glycoside, 3,4-dihydroxyphenylethyl alcohol (2-*O*-feruloyl)-*β*-D-glucopyranoside (**4**), and a new glycoside, phenylethyl alcohol [5-*O*-*p*-hydroxybenzoyl-*β*-D-apiofuranosyl-(1→2)]-*β*-D-glucopyranoside (**5**), were isolated from *Bacopa monniera*. Their structures were established by NMR, MS, and chemical methods.

Bacopa monniera (Linn.) Wettestein (Scrophulariaceae) is a herbaceous plant widely distributed throughout tropical and subtropical areas.[1,2] It is used to cure various ailments including bronchitis, inflammation, and asthma in traditional medicine.[1,2] The alcohol extract of *B. monniera* has a relaxant effect on the trachea, pulmonary artery, and aorta of rabbit and guinea pig.[1] Recent results also suggest that the *B. monniera* extract acts as a nonselective calcium antagonist.[2] In earlier chemical investigations, four aglycones of ebelin lactone,[3] bacogenin A_1,[4] and partial characterization of bacogenin A_2 [5] and A_3,[6] jujubogenin,[7] and pseudojujubogenin[8] were reported. Attempts have been made to isolate and define other constituents of the saponin fraction, and the structures of some dammarane-type triterpenoid saponins have been reported thus far.[9−16] In our preliminary antidiabetic screening test, the activity is believed to be associated with the polar fraction. We are pursuing our studies on the isolation of the active compounds to provide a basis for discussion of their biological activity in relation to their chemical structures.





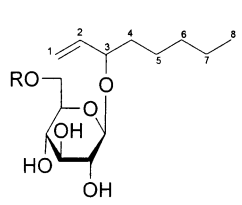
Results and Discussion

Repeated column chromatography of the *n*-BuOH-soluble fraction of the MeOH extract of the fresh whole plant of *Bacopa monniera* on silica gel and highly porous polymer gel produced five new compounds (**1**−**5**), as outlined in the Experimental Section.

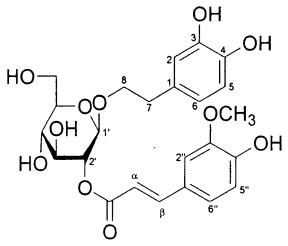
On acid hydrolysis, compound **1** yielded bacogenin A_1 [4] as the major sapogenin, which is an artifact formed from pseudojujubogenin[8] during acid hydrolysis. The sugar constituents were identified as glucose and arabinose. The absolute configuration of the sugars were D and L, respectively, as determined by the specific rotation of the isolated sugar. Treatment of **1** with periodate and alkali produced pseudojujubogenin.[8] The negative-ion FABMS of **1** showed a $[M - H]^-$ peak at *m*/*z* 845 as the base peak, 132 mass units less than that of **2**. The ^{13}C NMR spectrum of **1** displayed signals for 41 carbons, of which 30 carbons were assigned to the triterpene moiety and 11 carbons to the sugar units, which included two anomeric carbon signals at *δ* 107.2 and 105.0. Therefore, compound **1** consists of 1 mol each of pseudojujubogenin, D-glucose, and L-arabinose. The ^1H NMR spectrum of **1** indicated the presence of the pseudojujubogenin part [*δ* 0.74, 0.93, 1.07, 1.23, 1.37, 1.60, 1.68 (3H each, s, H-19, H-29, H-18, H-28, H-21, H-27, H-26), 1.53, 2.46, 2.59, 2.85, 3.33, 3.87, 4.71, 5.84], an arabinopyranosyl anomeric proton at *δ* 4.78, and a glucopyranosyl anomeric proton at *δ* 5.19. NMR techniques, DEPT, $^1H-^1H$ COSY, HMQC, and HMBC were used for unambiguous assignments of the ^1H and ^{13}C NMR spectra and determination of the sugar units. The sugar moiety contains a terminal glucose linked to an arabinose, which is attached to the aglycone. The diglycoside bonding to the 3-hydroxyl group of pseudojujubogenin in **1** was characterized by means of the HMBC experiment, which showed long-range correlations between Ara H-1′ (*δ* 4.78) and pseudojujubogenin C-3 (*δ* 88.5). The HMBC also confirmed the arabinose substituted at C-3 of the aglycone as well as the linkage of glucose to C-3 (*δ* 84.9) of arabinose. The

* To whom correspondence should be addressed. Tel: 886-2-27361661, ext. 6132. Fax: 886-2-27370903. E-mail: hsu0320@tmu.edu.tw.
[†] Taipei Medical University.
[‡] National Cheng-Kung University.

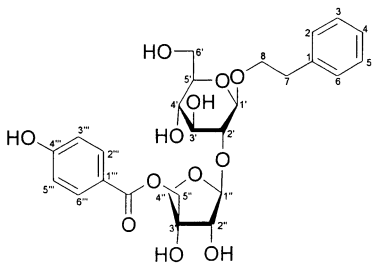
10.1021/np020238w CCC: $22.00
Published on Web 10/10/2002



3 R = SO_3H
8 R = H



4



5

signal of C-2 of arabinose was significantly shifted to a downfield value of δ 84.9. Elemental analysis indicated the presence of one sulfur atom and showed IR absorptions at 1221 and 814 cm^{-1} due to a sulfate group.[17] The position of the sulfate group was suggested by comparison of the ^1H and ^{13}C NMR (Table 1) spectra of **1** and **7**. The methylene protons (H-6″, δ 4.24, 4.49) and carbon (C-6″, δ 62.6) of the glucopyranose unit experienced deshielding to δ 5.02, 5.10 ($\Delta\delta$ + 0.78, 0.61 ppm) and δ 67.3 ($\Delta\delta$ + 4.7 ppm), demonstrating that the sulfate group must be linked to C-6″ of the glucopyranose unit.[16] This was also supported by the observed upfield shift of the C-5″ signal by 1.7 ppm (γ effect) as against that of **7**.[16] Methylation of **1** by the Hakomori method[18] gave 2″,3″,4″-tri-*O*-methyl-β-D-glucose and 2′,4′-di-*O*-methyl-α-D-arabinopyranose. This also proved that the sulfate group is linked to the C-6 of glucose. Furthermore, hydrolysis of **6** with hesperidinase produced **1**. Thus, on the basis of the above evidence, compound **1** was characterized as 3-*O*-[6-*O*-sulfonyl-β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl pseudojujubogenin and was named bacopaside III.

Compound **2** was obtained as an amorphous powder. Acid hydrolysis of **2** yielded ebelin lactone and L-arabinose. Treatment of **2** with periodate and alkali produced jujubogenin. The negative-ion FABMS of **2** exhibited the $[M - H]^-$ peak at *m/z* 735 as the base peak. Other discernible peaks were observed at *m/z* 603, 470, and 455 and are ascribable to $[M - H - \text{arabinosyl}]^-$, $[M - 2 \times \text{arabinosyl}]^-$, and $[\text{aglycone} - OH]^-$, respectively. The HRFABMS showed the molecular formula to be $C_{40}H_{64}O_{12}$. The ^{13}C and DEPT NMR spectra displayed signals for 40 carbons. Two anomeric carbon signals were evident at δ 106.0 and 109.9, and the ^1H NMR spectrum showed anomeric protons at δ 4.88 and 6.15. The $^1H-^1H$ COSY and HMQC spectra of **2** established each sugar's spin-coupling network, and complete assignments of the ^1H and ^{13}C NMR signals (Table 1) were accomplished by DEPT, $^1H-^1H$ COSY, and HMQC experiments. The linkage of the two sugars was deduced to be at the hydroxyl group of C-2 of arabinopyranose, because this carbon signal was shifted downfield from δ 73.0 to 76.2 in comparison to 3-β-*O*-α-L-arabinopyranosyl jujubogenin.[15] The proton signal at δ 4.88 (H-1′) was correlated to C-3 (δ 88.8), and the proton signal at δ 6.15 (H-1‴) was correlated to C-2′ (δ 76.2). These HMBC experimental data clearly indicate that a terminal arabinofuranosyl unit is joined to the inner arabinopyranosyl unit through a 1→2 linkage. The latter is joined to jujubogenin through an *O*-glycosidic linkage at C-3. The result was confirmed by a NOESY experiment. Correlations between the signals of H-3 (δ 3.24) and H-1′ (δ 4.88) as well as between H-2′ (δ 4.43) and H-1‴ (δ 6.15) were also observed. Thus, **2** was characterized as 3-*O*-[α-L-arabinofuranosyl-(1→2)]-α-L-arabinopyranosyl jujubogenin and was named bacopasaponin G.

Table 1. ^{13}C NMR Chemical Shifts of Compounds **1**, **2**, **6**, and **7** in C_5D_5N

carbon	1	2	6	7
1	38.8	38.7	38.8	38.8
2	26.8	26.7	26.8	26.8
3	88.5	88.8	88.5	88.7
4	39.8	39.6	39.9	39.9
5	56.1	56.1	56.2	56.2
6	18.3	18.3	18.3	18.3
7	36.1	36.0	36.1	36.1
8	37.5	37.5	37.5	37.5
9	53.1	53.0	53.1	53.1
10	37.3	37.2	37.3	37.2
11	21.8	21.8	21.8	21.8
12	28.6	28.6	28.6	28.6
13	37.1	37.1	37.1	37.1
14	53.5	53.8	53.5	53.5
15	36.9	36.9	36.9	36.9
16	110.4	110.6	110.4	110.4
17	51.3	51.3	51.3	51.3
18	18.9	18.9	18.9	18.9
19	16.4	16.3	16.4	16.4
20	71.9	71.9	71.9	71.9
21	27.2	27.3	27.2	27.2
22	46.3	45.5	46.3	46.3
23	66.2	68.6	66.2	66.2
24	124.2	127.2	124.2	124.2
25	133.0	134.1	133.0	132.9
26	26.1	25.6	26.1	26.1
27	18.5	18.4	18.5	18.5
28	28.0	28.1	27.8	27.8
29	16.8	16.8	16.6	16.6
30	65.9	66.0	65.9	66.0
1′	107.2	106.0	105.7	105.8
2′	71.3	76.2	76.9	77.1
3′	84.9	73.6	83.7	83.6
4′	68.3	68.6	68.6	68.7
5′	66.5	65.8	65.9	65.9
1″	105.0		105.0	105.0
2″	75.5		75.1	75.3
3″	77.6		77.5	78.2
4″	70.8		71.1	71.5
5″	76.8		76.0	78.5
6″	67.3		68.0	62.6
1‴		109.9	110.3	110.3
2‴		81.0	83.8	83.9
3‴		78.7	78.0	78.0
4‴		88.3	85.0	85.0
5‴		62.8	62.1	62.1

Compound **3** was obtained as an amorphous powder. The ^1H NMR spectral features of **3** were very similar to those of **8**, which was completely assigned with the aid of $^1H-^1H$ COSY and HMQC spectra. The ^1H NMR spectrum of **3** showed the presence of a 1-octen-3-ol (matsutake alcohol) moiety and a glucopyranose moiety. The ^{13}C NMR spectrum of **3** was also very similar to that of **8**, except that the signal of **3** at δ 62.9 (glc C-6) was downfield shifted to δ 67.7, and the signal at δ 78.4 (glc C-5) was upfield shifted to δ 76.2. Elemental analysis indicated the presence of one sulfur atom, and the IR showed absorptions characteristic of a sulfate group (1218 and 832 cm^{-1}). Solvolysis of **3** with a dioxane−pyridine mixture[19] gave **8**, and continued hy-

drolysis of **8** with β-glucosidase yielded 1-octen-3-ol.[20] This observation was consistent with the negative FABMS of **3**, which displayed an ion peak at *m/z* 369 $[M - H]^-$, 80 units higher than that of **8**. The site of the sugar linkage to the aglycon was considered to be C-3 from the HMBC experiment. The proton signal at δ 4.82 showed a cross-peak by the three-bond correlation with C-3 (δ 81.2). The configuration of **3** was suggested to be 3*R* by comparing the optical rotation values of the solvolysis and hydrolysis products with literature data.[20,21] Thus, **3** was identified as (3*R*)-1-octan-3-yl-(6-*O*-sulfonyl)-β-D-glucopyranoside and was named bacopaside A.

Compound **4** was obtained as an amorphous powder. In the ^{1}H NMR spectrum of **4**, there were of two groups of ABX-type aromatic signals. The ^{1}H NMR spectrum of **4** was very similar to that of plantainoside B,[22] except for the appearance of an additional methoxy signal at δ 3.93, which was further confirmed by an ion peak $[M - H]^-$ at *m/z* 491, 14 mass units more than that of plantainoside B in the negative FABMS of **4**. The glucose signals were assigned using the ^{1}H−^{1}H COSY and HMQC spectra. The site of the methoxy group in **4** was considered to be C-3″ from the result of the HMBC experiment. The methoxy signal at δ 3.93 showed a cross-peak with C-3″ (δ 148.7). Acid hydrolysis of **4** yielded 3,4-dihydroxyphenethyl alcohol, ferulic acid, and glucose. The HMBC spectrum showed a correlation between the proton signal of H-2′ (δ 4.86) and the carboxylic carbon signal (δ 166.6) of the feruloyl residue, as well as between the anomeric proton of glucose (δ 4.55) and the carbon signal (δ 71.0) of the 3,4-dihydroxylphenylethyl alcohol. Accordingly, **4** is 3,4-dihydroxyphenylethyl alcohol (2-*O*-feruloyl)-β-D-glucopyranoside and was named bacopaside B.

Compound **5** was obtained as an amorphous powder. The HRFABMS analysis agreed with the molecular formula $C_{26}H_{32}O_{12}$. In the ^{1}H NMR spectrum, signals at δ 7.94 and 6.90 could be assigned as aromatic AX-type protons, together with five aromatic proton signals between δ 7.12 and 7.23. In addition, the ^{1}H NMR spectrum exhibited signals at δ 3.27−5.48 for two sugar moieties and at δ 2.84 (2H, t, J = 7.5 Hz) for one methylene group. The ^{13}C and DEPT NMR spectra of **5** showed 26 carbons, including one carbonyl carbon (δ 166.5), two methylene carbons (δ 37.0 and 70.7), two aromatic rings, and a five-carbon sugar, as well as a six-carbon sugar. The ^{1}H−^{1}H COSY and HMQC spectra led us to assign the glycosidic moieties as apiofuranose and glucopyranose. The HMBC experiment showed correlations between H-7 (δ 2.84) and C-2, -6 (δ 129.0), as well as between H-8 (δ 3.98) and C-1 (δ 139.6), indicating the presence of a phenylethyl alcohol unit. HMBC correlations were observed between the proton signal at δ 7.94 (H-2‴, -6‴) and the carbon signal at δ 162.8 (C-4‴), as well as signals at δ 7.94 and 166.5 (−COO−), suggesting the presence of a *p*-hydroxybenzoic acid unit in the compound. The HMBC spectrum showed correlations between H-5″ (δ 4.33 and 4.41) of the apiose moiety and −COO− (δ 166.5) of the *p*-hydroxybenzoic acid unit, as well as H-1′ (δ 4.36) of the glucose moiety and C-8 (δ 70.7) of the phenylethyl alcohol unit. Furthermore, the NOESY spectrum indicated the proximity of glucose H-1 to oxymethylene H-8. These correlations confirmed that the phenylethyl alcohol was linked to C-1 of glucose and that *p*-hydroxybenzoic acid was substituted at C-5 by the apiose moiety. The apiosyl-(1→2)-glucosyl linkage of the glycosidic moieties was assigned from the cross-peaks observed between apiose H-1″ and glucose H-2′ in the NOESY spectrum. Also in the HMBC spectrum of **5**, a correlation was evident between glucose H-2′ (δ 3.43) and apiose C-1″ (δ 109.5), as well as between apiose H-1″ (δ 5.48) and glucose C-2′ (δ 77.0). The position was confirmed by the chemical shift (δ 77.0) of glucose C-2, as compared with a nonsubstituted C-2, which is ca. δ 72.0. The β-configuration of C-1 in the sugar moieties was determined on the basis of the coupling constants (glucopyranose, 7.7 Hz; apiofuranose, 1.0 Hz) of the anomeric protons.[22] Since only the D-configuration is known to exist in naturally occurring glucose and apiose,[23] the sugars in **5** were tentatively assigned the D-configuration. Therefore, the structure of **5** was established to be phenylethyl alcohol [5-*O*-*p*-hydroxybenzoyl-β-D-apiofuranosyl-(1→2)]-β-D-glucopyranoside, and it was named bacopaside C.

We have tested 24 compounds isolated from this plant for antihyperglycemic activity in vivo using STZ-diabetic rats.[24] Three compounds showed moderate lowering of plasma glucose at a dose of 1 mM/kg: calcerorioside B[25] (Δ −26.49 ± 9.38%), marytynoside[26] (Δ −20.60 ± 5.71%), and luteolin 7-*O*-glucuronic acid[27] (Δ −25.54 ± 10.02%).

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi B-545 melting point apparatus and were uncorrected. Optical rotations were measured with a JASCO P-1020 digital polarimeter. IR spectra were recorded on a BIO-RAD 165 FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer. ^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz, respectively. All chemical shifts were recorded in ppm (δ) with respect to the corresponding solvent as the internal standard. FABMS spectra were recorded on JEOL JMS-SX102A spectrometers.

Plant Material. Fresh samples of *Bacopa monniera* (5 kg) were collected in Tainan, Taiwan, in February 1999. A voucher specimen is deposited at the Department of Medicinal Chemistry, College of Pharmacy, Taipei Medical University, Taipei, Taiwan.

Extraction and Isolation. Fresh whole plants were successively extracted with MeOH. The MeOH extract was concentrated and partitioned between H_2O and *n*-BuOH. The *n*-BuOH residue was chromatographed on a Sephadex LH-20 column eluted with EtOH to produce two fractions. Fraction 1 was separated using a Diaion HP-20 column (H_2O−MeOH) to give three fractions. Fraction 1-1 was successively purified by MCI-gel CHP 20P and silica gel to produce **6** (98 mg), **1** (41 mg), and **8** (203 mg). Fractions 1-2 and 1-3 were purified by silica gel column chromatography with $CHCl_3$−MeOH (50:1 to 0:1) to obtain **7** (951 mg), 3-β-*O*-α-L-arabinopyranosyl jujubogenin[28] (132 mg), **2** (46 mg), bacoside A_3 [11] (583 mg), and 1-linolenyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol[29] (359 mg). Fraction 2 was passed through a Diaion HP-20 column eluted with H_2O−MeOH (1:0 to 0:1) to give fractions 2-1 and 2-2. Fraction 2-1 was repeatedly chromatographed on MCI-gel CHP 20P (H_2O−MeOH, 1:0 to 0:1), a Sephadex LH-20 column (60% MeOH), and Cosmosil C_{18}-OPN (H_2O−MeOH, 1:0 to 1:1) to provide **3** (36 mg), β-(3,4-dihydroxyphenyl)ethyl-*O*-β-D-glucose[30] (203 mg), martynoside[26] (52 mg), and **5** (24 mg). Chromatography of fraction 2-2 over a Sephadex LH-20 column and Cosmosil C_{18}-OPN with H_2O−MeOH (1:0 to 0:1) yielded **4** (36 mg), plantainoside B[31] (47 mg), plantainoside A[31] (575 mg), calcerorioside B[25] (164 mg), acetoside[26] (100 mg), apigenin[32] (2 mg), and luteolin[32] (5 mg). The known compounds were identified from literature values or by comparion with standard material.

Bacopaside III (1): white amorphous powder; $[\alpha]^{25}_D$ −17.4° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3400 (OH), 1221, 814 (−SO_3H) cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.70 (1H, d, J = 11.8 Hz, H-5), 0.74 (3H, s, Me-19), 0.81 (1H, t, J = 11.8 Hz, H-1), 0.88 (1H, d, J = 13.6 Hz, H-9), 0.93 (3H, s, Me-29), 1.07 (3H, s, Me-18), 1.23 (3H, s, Me-28), 1.30−1.39 (3H, m, H-6, H-7, H-11), 1.37 (3H, s, Me-21), 1.45−1.56 (4H, m, H-1, H-6, H-7, H-11), 1.53 (1H, d, J = 8.3 Hz, H-15), 1.60 (3H, s, Me-27), 1.65 (1H, d, J = 7.0 Hz, H-17), 1.68 (3H, s, Me-26), 1.78−1.93 (3H, m,

H-2, H-12, H-12), 2.13 (1H, m, H-2), 2.46 (1H, d, J = 8.3 Hz, H-15), 2.59 (1H, br d, J = 10.4 Hz, H-22), 2.85 (1H, m, H-13), 3.33 (1H, dd, J = 4.2, 11.6 Hz, H-3), 3.77 (1H, d, J = 11.9 Hz, H-5′), 3.81 (1H, t, J = 7.4 Hz, H-2″), 3.87 (1H, br d, J = 9.5 Hz, H-23), 4.06 (1H, m, H-5″), 4.15 (2H, m, H-3″, H-4″), 4.19 (1H, d, J = 7.0 Hz, H-30), 4.24−4.29 (3H, m, H-30, H-3′, H-5′), 4.56 (2H, m, H-2′, H-4′), 4.71 (1H, br d, J = 9.5 Hz, H-23), 4.78 (1H, d, J = 7.0 Hz, H-1′), 5.02 (1H, dd, J = 4.8, 10.5 Hz, H-6″), 5.10 (1H, d, J = 10.5 Hz, H-6″), 5.19 (1H, d, J = 7.8 Hz, H-1″), 5.84 (1H, br d, J = 10.3 Hz, H-24); ^{13}C NMR (C_5D_5N, 125 MHz) (Table 1); FABMS m/z 845 $[M - H]^-$ (100), 765 (2); HRFABMS m/z 845.3993 $[M - H]^-$ (calcd for $C_{41}H_{65}O_{16}S$, 845.3993); *anal*. C 50.78%, H 7.85%, S 3.19%, calcd for $C_{41}H_{65}O_{16}S \cdot 7H_2O$, C 50.70%, H 8.2%, S 3.3%.

Acid Hydrolysis of 1. Compound **1** (10 mg) was hydrolyzed with 2 N HCl in aqueous MeOH (5 mL) in a H_2O bath for 5 h and was worked up in the usual way. The major aglycone was found to be identical with bacogenin A_1,[4] mp 241−242 °C.

The filtrate from the hydrolysate was neutralized with DOWEX HCR-S ion-exchange resin and filtered, and a portion of the filtrate was concentrated under reduced pressure and examined for carbohydrates by silica gel TLC [Kieselgel 60 (Merck Art 5554), *i*-PrOH−Me_2CO−H_2O (5:3:1)] using authentic samples. Two spots were detected, and these were identified as D-glucose, $[\alpha]^{25}_D$ +52.5° (*c* 0.4, H_2O), and L-arabinose, $[\alpha]^{25}_D$ +100.8° (*c* 0.4, H_2O), by comparison with authentic samples.

Periodate Oxidation of 1. Compound **1** (10 mg) was oxidized with $NaIO_4$ (40 mg in 20 mL of 50% aqueous MeOH) at room temperature for 48 h, and a few drops of ethylene glycol were added to destroy any excess $NaIO_4$. The reaction mixture was extracted with *n*-BuOH, and the butanolic layer was refluxed with 5% KOH in 20% aqueous EtOH for 3 h and then extracted with *n*-BuOH. The above procedure was repeated twice. The final products were chromatographed on silica gel eluted with *n*-hexane−acetone (10:1) to give pseudojujubogenin (2 mg). Its ^{1}H NMR spectrum was confirmed using data from the literature.[8]

Methylation of 1 by the Hakamori Method. Compound **1** (10 mg) was treated with NaH (100 mg) and MeI (1 mL) in DMSO (5 mL) under a N_2 atmosphere at room temperature for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed, dried, and evaporated. The residue was chromatographed on silica gel eluted with *n*-hexane−Me_2CO (3:1) to give **1a** (5 mg) as an amorphous powder: ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.74 (3H, s, Me-19), 0.93 (3H, s, Me-29), 1.07 (3H, s, Me-18), 1.13 (3H, s, Me-28), 1.30 (3H, s, Me-21), 1.60 (3H, s, Me-27), 1.67 (3H, s, Me-26), 2.47 (1H, d, J = 8.3 Hz, H-15), 2.59 (1H, d, J = 10.4 Hz, H-22), 2.85 (1H, m, H-13), 3.51, 3.55, 3.57, 3.67, 3.74 (3H each, s, 5 × OMe), 4.50 (1H, d, J = 7.0 Hz, H-1′), 4.86 (1H, d, J = 7.6 Hz, H-1″), 5.84 (1H, br d, J = 10.3 Hz, H-24).

Partial Hydrolysis of 6 with Hesperidinase. Compound **6** (10 mg) in H_2O was incubated with hesperidinase at 40 °C for 14 days, and the product was extracted with *n*-BuOH. The extracts were evaporated and chromatographed on silica gel to give **1** as an amorphous powder (2 mg).

Bacopasaponin G (2): white amorphous powder; $[\alpha]^{25}_D$ −54.5° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3253 (OH), 2944, 1462, 1288, 980 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.64 (1H, d, J = 11.7 Hz, H-5), 0.70 (3H, s, Me-19), 0.81 (1H, t, J = 11.8 Hz, H-1), 0.88 (1H, m, H-9), 0.96 (3H, s, Me-29), 1.04 (3H, s, Me-18), 1.23 (3H, s, Me-28), 1.30−1.34 (3H, m, H-6, H-7, H-11), 1.36 (3H, s, Me-21), 1.50−1.55 (6H, m, H-1, H-6, H-7, H-11, H-15, H-22), 1.65 (3H, s, Me-27), 1.67 (3H, s, Me-29), 1.74 (1H, dd, J = 1.2, 13.5 Hz, H-22), 1.79−1.80 (2H, m, H-2, H-12), 1.90, (1H, m, H-12), 2.12 (1H, dd, J = 13.5, 3.6 Hz, H-2), 2.46 (1H, d, J = 8.2 Hz, H-15), 2.80 (1H, m, H-13), 3.24 (1H, dd, J = 4.6, 11.6 Hz, H-3), 3.77 (1H, d, J = 10.4 Hz, H-30), 4.15−4.20 (3H, m, H-3′, H-5′, H-5‴), 4.26−4.28 (4H, m, H-30, H-4′, H-5′, H-5‴), 4.43 (1H, t, J = 7.0 Hz, H-2′), 4.85 (1H, m, H-3‴), 4.88 (1H, d, J = 6.0 Hz, H-1′), 4.94 (1H, dd, J = 8.3, 4.2 Hz, H-4‴), 5.02 (1H, br s, H-2‴), 5.18 (1H, t, J = 9.0 Hz, H-23), 5.51 (1H, *d-like*, J = 8.1 Hz, H-24), 6.15 (1H, br s, H-1‴); ^{13}C NMR (C_5D_5N, 125 MHz) (Table 1); FABMS m/z 735 $[M - H]^-$ (100), 603 (8), 455 (4); HRFABMS m/z 735.4317 $[M - H]^-$ (calcd for $C_{40}H_{64}O_{12}$, 735.4319).

Acid Hydrolysis of 2. Compound **2** (10 mg) was hydrolyzed with 2 N HCl in aqueous MeOH (5 mL) in a H_2O bath for 5 h and was worked up in the usual way. The purified major aglycone was identified as ebelin lactone: mp 173−175 °C. The ^{1}H, ^{13}C NMR, and mass spectral data were identical with data from the literature.[3]

The filtrate from the hydrolysate was neutralized with DOWEX HCR-S ion-exchange resin and then analyzed by silica gel TLC [Kieselgel 60 (Merck Art 5554), *i*PrOH−Me_2CO−H_2O (5:3:1)]. One spot was detected that corresponded to arabinose. The L-configuration was confirmed by isolation from preparative TLC and comparison of its specific rotation, L-arabinose, $[\alpha]^{25}_D$ +101.6° (*c* 0.5, H_2O), with an authentic sample.

Periodate Oxidation of 2. Compound **2** (10 mg) was oxidized with $NaIO_4$ (20 mg in 20 mL of 50% aqueous MeOH) at room temperature for 48 h, and a few drops of ethylene glycol were added to destroy excess $NaIO_4$. The reaction mixture was extracted with *n*-BuOH, and the butanolic layer was refluxed with 5% KOH in 20% aqueous EtOH for 3 h and then extracted with *n*-BuOH. The above procedure was repeated twice. The final products were chromatographed on silica gel eluted with *n*-hexane−acetone (10:1) to give jujubogenin (2 mg), identified by comparison of its ^{1}H and ^{13}C NMR spectra with data from the literature.[7]

Bacopaside A (3): off-white amorphous powder; $[\alpha]^{25}_D$ +17.7° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3385 (OH), 1274, 1218, 832 (sulfate) cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.77 (3H, t, J = 6.7 Hz, H-8), 1.09−1.20 (4H, m, H-6, 7), 1.40 (2H, m, H-5), 1.60 (1H, m, H-4), 1.72 (1H, m, H-4), 3.93 (1H, t, J = 8.2 Hz, glc H-2), 3.98 (1H, m, glc H-5), 4.17 (1H, t, J = 9.3 Hz, glc H-3), 4.25 (1H, t, J = 9.3 Hz, glc H-4), 4.36 (1H, m, H-3), 4.82 (1H, d, J = 7.7 Hz, glc H-1), 4.98 (2H, m, glc H-6), 5.10 (1H, d, J = 10.5 Hz, H-1), 5.31 (1H, d, J = 17.2 Hz, H-1), 6.06 (1H, ddd, J = 17.2, 10.5, 6.7 Hz, H-2); ^{13}C NMR (C_5D_5N, 125 MHz) δ 14.2 (C-8), 22.8 (C-7), 24.8 (C-5), 32.1 (C-6), 35.0 (C-4), 67.7 (glc C-6), 71.4 (glc C-4), 75.3 (glc C-2), 76.2 (glc C-5), 77.9 (glc C-3), 81.2 (C-3), 103.5 (glc C-1), 115.4 (C-1), 140.4 (C-2); FABMS m/z 369 $[M - H]^-$ (100), 257 (10); HRFABMS m/z 369.1224 $[M - H]^-$ (calcd for $C_{14}H_{26}O_9S$, 369.1219); *anal*. C 41.44%, H 7.2%, S 7.6%, calcd for $C_{14}H_{26}O_9S \cdot 3H_2O$, C 41.40%, H 7.4%, S 7.9%.

Solvolysis of 3. The solution of **3** (5 mg) in pyridine (5 mL) was refluxed for 10 min, then dioxane (20 mL) was added. The mixture was refluxed for a further 25 min. The reaction mixture was diluted with H_2O and extracted with *n*-BuOH, and the *n*-BuOH layer was washed and evaporated. The residue (solvolysate) was identified as (3*R*)-1-octan-3-yl-3-*O*-*β*-D-glucopyranoside (**8**) by comparison of its ^{1}H and ^{13}C NMR data with the literature,[17] and its specific rotation was $[\alpha]^{25}_D$ +11.4° (*c* 0.4, H_2O).

Enzymic Hydrolysis of (3*R*)-1-Octan-3-yl-3-*O*-*β*-D-glucopyranoside (8). Compound **8** (10 mg) in H_2O was incubated with *β*-glucosidase at 40 °C for 2 days, and the product was extracted with *n*-BuOH. The extracts were evaporated and chromatographed on a Sephadex LH-20 column with MeOH to give aglycone (2 mg), which was identified as 1-octen-3-ol by comparison of its ^{1}H and ^{13}C NMR spectra with data from the literature, and its specific rotation was $[\alpha]^{25}_D$ −3.5° (*c* 0.4, H_2O).[20] The H_2O layer was analyzed by silica gel TLC [Kieselgel 60 (Merck Art 5554), *i*-PrOH−Me_2CO−H_2O (5:3:1)] and showed a brown spot (R_f 0.45) after spraying an anilin-phthalate solution and heating, which was coincident with that of glucose, $[\alpha]^{25}_D$ +53.0° (*c* 0.4, H_2O).

Bacopaside B (4): off-white amorphous powder; $[\alpha]^{25}_D$ −209.5° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3253 (OH), 1699 (C=O), 1517, 1268, 1028 cm^{-1}; ^{1}H NMR (Me_2CO-d_6 + D_2O, 500 MHz) δ 2.65 (2H, td, J = 7.0, 3.0 Hz, H-7), 3.36 (1H, m, H-5′), 3.45 (1H, t, J = 9.0 Hz, H-4′), 3.58 (1H, m, H-8), 3.62 (1H, t, J = 9.0 Hz, H-3′), 3.68 (1H, dd, J = 5.6, 11.5 Hz, H-6′), 3.86 (1H, br d, J = 11.5 Hz, H-6′), 3.93 (3H, s, OCH_3), 3.90−3.94 (1H, m, H-8), 4.55 (1H, d, J = 8.0 Hz, H-1′), 4.86 (1H, dd, J = 8.0, 9.0 Hz, H-2′), 6.40 (1H, d, J = 15.9 Hz, H-α), 6.51 (1H, dd, J

= 1.8, 8.1 Hz, H-6), 6.65 (1H, d, J = 8.1 Hz, H-5), 6.69 (1H, d, J = 1.8 Hz, H-2), 6.86 (1H, d, J = 8.1 Hz, H-5′), 7.14 (1H, dd, J = 1.8, 8.1 Hz, H-6′), 7.35 (1H, d, J = 1.8 Hz, H-2′), 7.61 (1H, d, J = 15.9 Hz, H-β); ^{13}C NMR (Me_2CO-d_6 + D_2O, 125 MHz) δ 36.2 (C-7), 56.3 (OMe), 62.7 (C-6′), 71.0 (C-8), 71.8 (C-4′), 74.4 (C-2′), 76.1 (C-3′), 77.6 (C-5′), 101.8 (C-1′), 111.1 (C-2″), 115.8 (C-5), 115.9 (C-5″), 116.0 (C-α), 116.8 (C-2), 121.1 (C-6), 124.0 (C-6″), 127.5 (C-1″), 131.1 (C-1), 144.1 (C-4), 145.5 (C-3), 145.7 (C-β), 148.7 (C-3″), 150.0 (C-4″), 166.6 (COO); FABMS m/z 491 [M − H]$^-$ (100), 257 (10); HRFABMS m/z 491.1550 [M − H]$^-$ (calcd for $C_{24}H_{28}O_{11}$, 491.1553).

Acid Hydrolysis of 4. Compound 4 (5 mg) was hydrolyzed with 2 N HCl in aqueous MeOH (5 mL) in a water bath for 5 h and was worked up in the usual way. The purified major aglycones were identified as 3,4-dihydroxyphenylethyl alcohol and ferulic acid by comparison with authentic samples, and the sugar was identified as glucose.

Bacopaside C (5): off-white amorphous powder; $[\alpha]^{25}_D$ −13.8° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3253 (OH), 1685 (C═O), 1610, 1282, 1012 cm^{-1}; ^{1}H NMR (Me_2CO-d_6 + D_2O, 500 MHz) δ 2.84 (2H, t, J = 7.5 Hz, H-7), 3.27 (1H, m, H-5′), 3.32 (1H, t, J = 9.0 Hz, H-4′), 3.43 (1H, dd, J = 7.7, 9.0 Hz, H-2′), 3.55 (1H, t, J = 9.0 Hz, H-3′), 3.61−3.64 (2H, m, H-8, H-6′), 3.78 (1H, d, J = 9.6 Hz, H-4″), 3.80 (1H, dd, J = 2.4, 12.0 Hz, H-6′), 3.98 (1H, q, J = 7.6 Hz, H-8), 4.05 (1H, br s, H-2″), 4.17 (1H, d, J = 9.6 Hz, H-4″), 4.33 (1H, d, J = 11.3 Hz, H-5″), 4.36 (1H, d, J = 7.7 Hz, H-1′), 4.41 (1H, d, J = 11.3 Hz, H-5″), 5.48 (1H, br s, H-1″), 6.90 (2H, d, J = 8.7 Hz, H-3‴, H-5‴), 7.12−7.16 (3H, m, H-3, H-4, H-5), 7.20−7.23 (2H, m, H-2, H-6), 7.94 (2H, d, J = 8.7 Hz, H-2‴, H-6‴); ^{13}C NMR (Me_2CO-d_6 + D_2O, 125 MHz) δ 37.0 (C-7), 62.8 (C-6′), 68.1 (C-5″), 70.7 (C-8), 72.0 (C-4′), 75.2 (C-4″), 77.0 (C-2′), 77.3 (C-5′), 78.0 (C-2″), 78.6 (C-3″), 78.7 (C-3′), 102.6 (C-1′), 109.5 (C-1″), 116.0 (C-3‴, 5‴), 122.3 (C-1⁗), 126.8 (C-4), 129.0 (C-2, 6), 129.7 (C-3, 5), 132.7 (C-2⁗, 6‴), 139.6 (C-1), 162.8 (C-4‴), 166.5 (COO); FABMS m/z 535 [M − H]$^-$ (49), 415 (6), 279 (5), 137 (100), 121 (8); HRFABMS m/z 535.1815 [M − H]$^-$ (calcd for $C_{26}H_{32}O_{12}$, 535.1816).

Antihyperglycemic Testing. Antihyperglycemic activity was determined as described previously.[33]

Acknowledgment. We extend our appreciation to Mr. Mu-Thun Kou (Taipei Medical University) for identification of plant material. The authors are grateful to Ms. Shu-Yun Sun (Taipei Regional Analytical Instrumentation Center, NSC) and Mr. Shih-Jen Wang (Hsinchu Regional Analytical Instrumentation Center, NSC) for measurement of the high-resolution FABMS, and to Ms. Shwu-Hui Wang, Center for Instrumentation, Taipei Medical University, for acquisition of the NMR spectra. This work was supported in part by the National Science Council of the Republic of China (NSC 90-2323-B-038-001).

References and Notes

(1) Dar, A.; Channa, S. *Phytother. Res.* **1997**, *11*, 323−325.
(2) Dar, A.; Channa, S. *J. Ethnopharmacol.* **1999**, *66*, 167−174.
(3) Kulshreshtha, D. K.; Rastogi, R. P. *Phytochemistry* **1973**, *12*, 2074−2076.
(4) Kulshreshtha, D. K.; Rastogi, R. P. *Phytochemistry* **1973**, *12*, 887−892.
(5) Kulshreshtha, D. K.; Rastogi, R. P. *Phytochemistry* **1974**, *13*, 1205−1206.
(6) Chandel, R. S.; Kulshreshtha, D. K.; Rastogi, R. P. *Phytochemistry* **1977**, *16*, 141−143.
(7) Kawai, K. I.; Akiyama, T.; Ogihara, Y.; Shibata, S. *Phytochemistry* **1974**, *13*, 2829−2832.
(8) Kawai, K. I.; Shibata, S. *Phytochemistry* **1978**, *17*, 287−289.
(9) Jain, P.; Kulshreshtha, D. K. *Phytochemistry* **1993**, *33*, 449−451.
(10) Rastogi, S.; Kulshreshtha, D. K. *Indian J. Chem.* **1999**, *38*, 353−356.
(11) Rastogi, S.; Pal, R.; Kulshreshtha, D. K. *Phytochemistry* **1994**, *36*, 133−137.
(12) Garai, S.; Mahato, S. B.; Ohtani, K.; Yamasaki, K. *Phytochemistry* **1996**, *42*, 815−820.
(13) Garai, S.; Mahato, S. B.; Ohtani, K.; Yamasaki, K. *Phytochemistry* **2000**, *43*, 447−449.
(14) Mahato, S. B.; Garai, S.; Chakravarty, A. K. *Phytochemistry* **2000**, *53*, 711−714.
(15) Maurya, S. K.; Pandey, D. P.; Singh, J. P.; Pandey, V. B. *Pharmazie* **1995**, *50*, 372.
(16) Chakravarty, A. K.; Sarkar, T.; Masuda, K.; Shiojima, K.; Nakane, T.; Kawahara, N. *Phytochemistry* **2001**, *58*, 553−556.
(17) Higuchi, R.; Kubota, S.; Komori, T.; Kawsaki, T.; Pandey, V. B.; Singh, J. P.; Shah, A. H. *Phytochemistry* **1984**, *23*, 2597−2600.
(18) Hakomori, S. *J. Biochem. (Tokyo)* **1964**, *55*, 205−208.
(19) Kitagawa, I.; Kobayashi, M. *Chem. Pharm. Bull.* **1978**, *26*, 1864−1873.
(20) Takano, S.; Yanase, M.; Takahashi, M.; Ogasawara, K. *Chem. Lett.* **1987**, 2017−2020.
(21) Yamamura, S.; Ozawa, K.; Ohtani, K.; Kasai, R.; Yamasaki, K. *Phytochemistry* **1998**, *48*, 131−136.
(22) Ishii, T.; Yanagisawa, M. *Carbohydr. Res.* **1998**, *313*, 189−192.
(23) Abe, F.; Yamauchi, T. *Phytochemistry* **1989**, *28*, 1737−1741.
(24) Forman, L. J.; Estilow, S.; Mead, J.; Vasilenko, P. *Horm. Metabol. Res.* **1988**, *20*, 555−558.
(25) Shimomura, H.; Sashida, Y.; Adachi, T. *Phytochemistry* **1987**, *26*, 249−251.
(26) Miyase, T.; Koizumi, A.; Ueno, A.; Noro, T.; Kuroyanagi, M.; Fukushima, S.; Akiyama, Y.; Takemoto, T. *Chem. Pharm. Bull.* **1982**, *30*, 2732−2737.
(27) Stochmal, A.; Piacente, S.; Pizza, C.; De Riccardis, F.; Leitz, R.; Oleszek, W. *J. Agric. Food Chem.* **2001**, *49*, 753−758.
(28) Maurya, S. K.; Pandey, D. P.; Singh, J. P.; Pandey, V. B. *Pharmazie* **1995**, *50*, 372.
(29) Baruah, P.; Baruah, N. C.; Sharma, R. P.; Baruch, J. N.; Kulanthaivel, P.; Herz, W. *Phytochemistry* **1983**, *22*, 1741−1744.
(30) Yahara, S.; Satashiro, M.; Nishioka, I.; Nagasawa, T.; Oura, H. *Chem. Pharm. Bull.* **1985**, *33*, 527−531.
(31) Miyase, T.; Ishino, M.; Akahori, C.; Ueno, A.; Ohkawa, Y.; Tanizawa, H. *Phytochemistry* **1991**, *30*, 2015−2018.
(32) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389−1397.
(33) Hsu, F. L.; Chen, Y. C.; Cheng, J. T. *Planta Med.* **2000**, *66*, 228−230.

NP020238W