Hopane-Type Saponins from the Seeds of *Glinus lotoides*

Abebe Endale,[†] Victor Wray,[‡] Renato Murillo,[§] Peter C. Schmidt,[†] and Irmgard Merfort*[,⊥]

Pharmazeutisches Institut, Pharmazeutische Technologie, Auf der Morgenstelle 8, D-72076 Tübingen, Germany, GBF−Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, 38124 Braunschweig, Germany, Escuela de Quimica and CIPRONA, Universidad de Costa Rica, San José, Costa Rica, and Institut für Pharmazeutische Wissenschaften, Lehrstuhl für Pharmazeutische Biologie, Universität Freiburg, 79104 Freiburg, Germany

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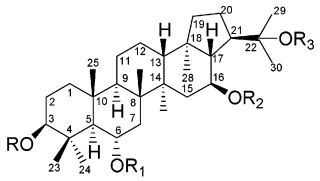
Four new hopane-type saponins, glinusides F, G, H, and I (**1**−**4**), and the known succulentoside B (**5**), as well as the two known flavones 5,7,4′-trihydroxyflavone-6,8-di-*C*-glucoside (vicenin-2) and 5,7,4′-trihydroxyflavone-8-*C*-sophoroside (vitexin-2″-*O*-glucoside), were isolated from the seeds of *Glinus lotoides* growing in Ethiopia. On the basis of the spectroscopic data analysis, including 2D NMR and HRESIMS, the new structures were characterized as 3*β*-*O*-*β*-D-xylopyranosyl-6α-*O*-*β*-D-xylopyranosyl-16*β*-*O*-*β*-D-xylopyranosyl-22-hydroxyhopane (**1**), 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl-6α,16*β*-dihydroxy-22-*O*-α-L-rhamnopyranosylhopane (**2**), 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl-6α-*O*-*β*-D-xylopyranosyl-16*β*-hydroxy-22-*O*-α-L-rhamnopyranosylhopane (**3**), and 3*β*-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-d-xylopyranosyl-6α-*O*-*β*-D-xylopyranosyl-16*β*-*O*-*β*-D-xylopyranosyl-22-hopane (**4**).

Glinus lotoides L. (Molluginaceae), locally known as "Mettere", is an annual or short-living perennial prostrate herb.[1] Its seeds are used traditionally in Ethiopia for the treatment of tapeworm infections.[2,3] Tenicidal activities have been shown in vitro and in vivo against *Tenia saginata* and *Hymenolepis nana* worms.[4−6] Preliminary pharmacological studies undertaken on the plant have indicated that a crude extract did not affect blood pressure, heart rate, or the ECG of anesthetized rabbits, bile production in guinea pigs, or contractions of frog isolated hearts after oral administration.[7,8] The cestocidal and pharmacological activities of the seeds of *G. lotoides* are probably due to its saponins.[4−8] Therefore, formulations of the seed extract as modern dosage forms have been developed and standardized based on its total saponin content.[9,10] However, as yet, only one triterpenoidal saponin with an oleanane skeleton has been isolated from the seeds of the plant.[11] Moreover, five hopane triterpenoidal saponins are known from the aerial parts of *G. lotoides* var. *dictamnoides* growing in Egypt.[12,13]

In this paper, we report the isolation and structure elucidation of one known (succulentoside B, **5**) and four new hopane-type saponins, glinuside F (**1**), G (**2**), H (**3**), and I (**4**), as well as the isolation of two known flavonoids, vicenin-2 and vitexin-2″-*O*-glucoside, from an extract of the seeds of *G. lotoides*.

Powdered seeds of *G. lotoides* were defatted with *n*-hexane and extracted with 60% aqueous methanol. The methanolic extract was concentrated under reduced pressure, and the aqueous part was extracted with an equal volume of *n*-butanol. The *n*-butanol fraction was dried, dissolved in a small volume of methanol, and poured into a large volume of diethyl ether. A precipitate formed, containing mainly saponins and flavonoids, and was separated by centrifugation and Sephadex LH-20 column chromatography. Flavonoid-containing fractions were further separated by RP-18 HPLC, affording two flavonoids. Their UV spectra in methanol using various diagnostic reagents



1: R = xylose; R_1 = xylose; R_2 = xylose; R_3 = H

2: R = rhamnose 1 → 2 xylose; R_1 = H; R_2 = H; R_3 = rhamnose

3: R = rhamnose 1 → 2 xylose; R_1 = xylose; R_2 = H; R_3 = rhamnose

4: R = rhamnose 1 → 2 xylose; R_1 = xylose; R_2 = xylose; R_3 = H

5: R = xylose; R_1 = arabinose; R_2 = xylose; R_3 = H

revealed them to be flavones with free 4′-, 5-, and 7-hydroxyl groups.[14] Their ESIMS and ^{1}H and ^{13}C NMR spectra were identical to published data for the known *C*-flavone glycosides vicenin-2 and vitexin-2″-*O*-glucoside.[15,16] Fractions containing saponins were purified by RP-18 HPLC and preparative TLC, yielding four new triterpene saponins of the hopane type [glinusides F (**1**), G (**2**), H (**3**), I (**4**)], as well as the known 6α-*O*-α-L-arabinopyranosyl-3*β*-*O*-*β*-D-xylopyranosyl-16*β*-*O*-*β*-D-xylopyranosyl-22-hydroxyhopane, succulentoside B (**5**).[17] Interestingly, mollugogenol B, which was previously isolated from *Mollugo pentaphylla* as a natural compound,[18] was detected here after acid hydrolysis.

Glinuside F (**1**) was isolated as a white amorphous powder. The ESIMS of **1** in the positive and negative modes showed quasimolecular ion peaks at *m/z* 872.7 $[M + H]^+$ and 871.6 $[M - H]^-$, respectively, consistent with the molecular formula, $C_{45}H_{76}O_{16}$. This was confirmed by its HRESIMS, which showed a $[M + Na]^+$ at *m/z* 895.507. The ^{1}H NMR spectrum (CD_3OD) showed signals characteristic for eight tertiary methyls as singlets at *δ* 0.88, 0.96, 1.10, 1.11, 1.13, 1.15, 1.30, and 1.42 and three anomeric protons at *δ* 4.30 (d, *J* = 7.8 Hz), 4.45 (d, *J* = 7.8 Hz), and 4.50 (d, *J* = 7.8 Hz) (Table 1), demonstrating the presence of *β*-anomers of three sugar units. The ^{13}C NMR spectrum of **1** (CD_3OD) analyzed by the aid of DEPT and HMQC revealed the presence of 45 carbon atoms in the molecule (Table 1). Thirty carbon signals were indicated for the

* To whom correspondence should be addressed. Tel: (49) 761 203 8373. Fax: (49) 761 203 8383. E-mail: irmgard.merfort@pharmazie.uni-freiburg.de.
[†] Pharmazeutisches Institut, Tübingen.
[‡] GBF, Braunschweig.
[§] Universidad de Costa Rica.
[⊥] Institut für Pharmazeutische Wissenschaften, Freiburg.

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Table 1. ^{13}C and ^{1}H NMR Data of Glinusides F (**1**) and G (**2**) in CD_3OD

	1		**2**			**1**		**2**	
aglycon	δ_C	δ_H	δ_C	δ_H	aglycon	δ_C	δ_H	δ_C	δ_H
1	40.1 t	1.00 m	40.1 t	1.01 m	30	27.1 q	1.29 s	26.1 q	1.42 s
		1.70 m		1.70 m	sugars				
2	26.8 t	1.84 m	26.9	1.86 m	Xyl C-3				
		1.70 m		1.70 m	1′	107.6 d	4.30 d (7.8)	106.5 d	4.42 d (6.6)
3	90.9 d	3.10 dd (12, 4.5)	90.2	3.09 dd (12, 4.5)	2′	75.5[a] d	3.25 m	78.6 d	3.46 m
4	40.8 s		40.9 s		3′	78.3[b] d	3.39−3.34 m	78.9 d	3.47 m
5	61.3 d	0.95 d (10.5)	61.9 d	0.94 d (10.5)	4′	71.3[c] d	3.52−3.48 m	71.6 d	3.52 m
6	81.1 d	4.05 ddd (10, 10, 4)	69.0 d	4.03 ddd (11, 10.5, 4)	5′ A	66.7[d] d	3.91−3.85 m	66.3 d	3.90 dd (5.0, 11.4)
7	45.1 t	1.62 m	46.1 t	1.70 m	5′ B		3.26−3.20 m		3.22 dd (9.3, 11.4)
		1.95 m		1.58 m	Xyl C-6				
8	44.0 s		44.8 s		1″	106.0 d	4.45 d (7.8)		
9	49.9[e] d	1.30 m	50.6 d	1.30 m	2″	75.0[a] d	3.25 m		
10	39.9 s		39.9 s		3″	77.9[b] d	3.39−3.34 m		
11	22.0 t	1.65 m	22.0 t	1.65 m	4″	71.0[c] d	3.52−3.48 m		
		1.65 m		1.65 m	5″ A	66.8[d] d	3.91−3.85 m		
12	24.6 t	1.60 m	24.7 t	1.60 m	5″ B		3.26−3.20 m		
		1.60 m		1.60 m	Xyl C-16				
13	50.6[e] d	1.45 dd (12, 4)	50.0 d	1.43 dd (12, 4)	1‴	104.1 d	4.50 d (7.8)		
14	44.0 s		43.9 s		2‴	75.2[a] d	3.25 m		
15	43.4 t	1.75 m	44.2 t	1.72 m	3‴	78.0[b] d	3.39−3.34 m		
		1.90 m		1.33 m	4‴	71.1[c] d	3.52−3.48 m		
16	80.8 d	4.30 ddd (10, 10, 4)	67.8 d	4.14 ddd (10, 10, 4)	5‴ A	67.2[d] d	3.91−3.85 m		
17	59.9 d	1.45 dd (10, 10)	62.7 d	1.55 dd (10, 10)	5‴ B		3.26−3.20 m		
18	47.5 s		46.6 s		Rha (1−2)				
19	42.6 t	1.10 m	42.7 t	1.10 m	1^{IV}			102.0 d	5.36 d (2)
		1.60 m		1.60 m	2^{IV}			72.1 d	3.98 m
20	29.1 t	1.90 m	28.7 t	1.90 m	3^{IV}			73.1 d	3.79 m
		1.60 m		1.60 m	4^{IV}			74.0 d	3.43 dd (9, 9)
21	53.4 d	2.60 ddd (10, 10, 9)	51.5 d	2.60 ddd (10, 10, 9)	5^{IV}			70.4 d	3.77 dq (9, 6.5)
22	74.6 s		84.5 s		6^{IV}			18.0 q	1.29 d (6.5)
23	31.2 q	1.41 s	31.2 q	1.38 s	Rha C-22				
24	16.8 q	1.10 s	17.0 q	1.07 s	1^{V}			97.0 d	5.21 d (2)
25	17.6 q	0.98 s	17.6 q	0.96 s	2^{V}			73.9 d	3.78 m
26	17.6 q	1.13 s	17.8 q	1.14 s	3^{V}			72.2 d	3.67 dd (9, 3.5)
27	18.5 q	1.11 s	18.7 q	1.13 s	4^{V}			74.0 d	3.42 dd (9, 9)
28	18.7 q	0.87 s	18.7 q	0.88 s	5^{V}			70.1 d	4.00 dq (9, 6.5)
29	30.7 q	1.15 s	25.5 q	1.37 s	6^{V}			18.0 q	1.26 d (6.5)

[a−e] Assignments of values with the same superscript may be interchanged.

aglycon part with signals characteristic for eight tertiary methyls, eight methylenes, eight methines, and six saturated quaternary carbons, including a carbon signal at δ 74.6 for a quaternary carbinol carbon. From the above data and by comparing the ^{13}C NMR data of **1** with literature reports for triterpenoids, the aglycon was identified as mollugogenol A, a triterpene of the hopane type isolated form *Mollugo pentaphylla* L.[18,19] Fifteen carbon signals were left for the sugar moieties including three anomeric carbons at δ 107.6, 106.0, and 104.1, from which the presence of three pentose-monosaccharide moieties was indicated. Accordingly, the ESIMS of **1** showed fragment ion peaks at *m/z* 740 $[(M + H) - 132]^+$, 608 $[(M + H) - 2(132)]^+$, and 476 $[(M + H) - 3(132)]^+$, due to subsequent loss of three pentose units. Acid hydrolysis of **1** on TLC in a hydrochloric acid vapor afforded only xylose,[17] which was supported by GC-MS analysis.[20] The identities of the monosaccharides were confirmed by a detailed inspection of 1D and 2D NMR experiments ($^1H-^1H$ COSY and HMQC), where information on the respective sugar residues and the anomeric configurations could be obtained. The downfield shift of three oxygen-bearing methines at C-3 (δ 90.9), C-6 (δ 81.1), and C-16 (δ 80.8), indicated the sites of glycosylation (Table 1).[18,19] The above proposed interglycosidic linkages were further substantiated by the long-range correlation observed in the HMBC spectra of **1**. Cross-peaks between H-1′/C-1′ (δ 4.30/δ 107.6) of one xylose unit and C-3/H-3 (δ 90.9/δ 3.10) of the aglycon showed one xylose to be bound to C-3 of the aglycon. Similarly, H-1″/C-1″ (δ 4.45/δ 106.0) of the second xylose unit correlated with C-6/H-6 (δ 81.1/δ 4.05), and H-1‴/C-1‴ (δ 4.50/δ 104.1) of the third xylose correlated with C-16/H-16 (δ 80.8/δ 4.30) of the aglycon. The ^1H and ^{13}C NMR data (Table 1) indicated the β-configuration at the anomeric positions for the xylose units ($^3J_{H1,H2}$ = 7.8 Hz).[18] The multiplicities and coupling constants of the axial protons at C-3 ($J_{2a,3\alpha}$ = 12.0; $J_{2e,3\alpha}$ = 4.5 Hz), C-6 ($J_{5a,6\beta}$ = 12.0; $J_{5e,6\beta}$ = 4.5 Hz), and C-16 ($J_{15a,16\alpha}$ = 10.0; $J_{15e,16\alpha}$ = 4.0; $J_{17a,16\alpha}$ = 10.0 Hz) confirmed the equatorial orientation of all three substitutents in the aglycon part.[18,21] Further analysis of the HMBC spectrum provided independent confirmatory evidence for the nature of the aglycon and allowed unambiguous assignment of both the ^{13}C and ^1H NMR chemical shifts of most of the relevant signals.[20] On the basis of the above evidence, the structure of glinuside F (**1**) was characterized as 3β-*O*-β-D-xylopyranosyl-6α-*O*-β-D-xylopyranosyl-16β-*O*-β-D-xylopyranosyl-22-hydroxyhopane.

Glinuside G (**2**) was isolated as a white amorphous powder. The molecular formula of compound **2**, $C_{47}H_{79}O_{16}$, followed from its HRESIMS, which showed a $[M + Na]^+$ at *m/z* 923.538. The ^1H, ^1H COSY, ^{13}C, DEPT, and HMBC spectra suggested the aglycon to be mollugogenol A, the same moiety as in saponin **1**. The only differences were changes in the chemical shifts associated with differences in the glycosylation pattern at C-6, C-16, and C-22. Sugar analysis revealed two rhamnose moieties and one xylose moiety as sugar components. The occurrence of these sugars was further supported by the fragmentation pattern in the ESIMS of **2**, taken in the negative mode, which

showed fragment ions at *m/z* 753 $[(M - H) - 146]^-$, 607 $[(M - H) - (146 + 146)]^-$, and 475 $[(M - H) - (146 + 146 + 132)]^-$, due to the subsequent loss of rhamnose, and two molecules of rhamnose and xylose, respectively. Additionally, these three sugars were confirmed from their ^{1}H and ^{13}C NMR data.[21] Glycosylation at C-3 with xylose was deduced from the correlation in the HMBC spectrum between the signals at δ_C 106.5/δ_H 4.42 of xylose and the signal at δ_C 90.2/δ_H 3.09 (C-3) of the aglycon moiety. The downfield shift of C-2 of xylose at δ_C 78.6 and δ_H 3.47 suggested further glycosylation at this position. Long-range correlations between C-2 (δ 78.6) of xylose and H-1^{IV} (δ 5.36) of rhamnose confirmed the linkage at this position. The second rhamnose moiety was attached to C-22 from long-range correlations between the signals at δ_C 97.0/δ_H 5.21 from C-1 of rhamnose and δ_C 84.5 of the aglycon at C-22. Analysis of the HMBC spectrum afforded confirmatory evidence of the structure and allowed assignment of the ^{13}C and ^{1}H NMR data.[20] Accordingly, glinuside G (**2**) was identified as 3β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-6α,16β-dihydroxy-22-*O*-α-L-rhamnopyranosylhopane.

Glinusides H (**3**) and I (**4**) were isolated as a mixture. Because of the small amount obtained, separation was omitted and identification was performed on the mixture. The HRESIMS showed the presence of two compounds with the molecular formula of $C_{52}H_{88}O_{20}$ (deduced from $[M + Na]^+$ at *m/z* 1055.581, indicating two rhamnose and two pentose units) for the major component and $C_{51}H_{86}O_{20}$ (calculated from $[M + Na]^+$ at *m/z* 1041.565, indicating three pentose and one rhamnose units) for the minor component. The presence of a mixture was confirmed from inspection of the 1D ^{1}H NMR spectrum, where the intensities of the signals for the anomeric protons indicated a 2:1 mixture of compounds **3** and **4**, respectively. Sugar analysis revealed rhamnose and xylose in the mixture. Inspection of the 1D and 2D COSY spectra of the anomeric protons indicated the major compound (**3**) possessed two rhamnose units from signals at δ_H 5.53 and 5.21 and two xylose units at δ_H 4.41 and 4.36 (Table S1, Supporting Information). From the integrals of these signals the second minor compound (**4**) had only one rhamnose signal at δ_H 5.53 and two separate signals for xylose units at δ_H 4.39 and 4.37. According to the integration, a third xylose signal overlapped with that of the major compound (**3**) at δ_H 4.41. Comparison of the data for compound **2** suggested both compounds in the mixture had a rhamnose (1→2) xylose unit at C-3 of the aglycon, while analogous comparison with saponin **1** suggested a β-xylopyranosyl unit at C-6. The difference between the two compounds resided in the position of a further α-rhamnopyranosyl unit in **3** and a β-xylopyranosyl unit in **4**. Long-range C−H correlations in the 2D HMBC spectrum provided further evidence for the substitution pattern of the aglycon. Thus, in both **3** and **4**, H-1 of the xylose moiety at δ_H 4.41 correlated with C-3 (δ_C 91.1) of the aglycon, as identified from its correlations with H-23 (δ_H 1.42) and H-24 (δ_H 1.10). Similarly, in both components H-1 of the xylose at δ_H 4.36 correlated with C-6 at δ_C 81.4 as in saponin **1**, and the rhamnose moiety at δ_H 5.53 correlated with a carbon signal at δ_C 77.9 attributed to C-2 of the same xylose moiety as shown for saponin **2**. For the major component (**3**), H-1 of the rhamnose moiety at δ_H 5.21 correlated with C-22 (δ_C 84.4) of the aglycon as determined from its correlations with H-30 (δ_H 1.43), H-29 (δ_H 1.36), H-17 (δ_H 1.52), and H-21 (δ_H 2.59). The remaining signal for the minor component of H-1 of the overlapping xylose moiety at δ_H 4.39 correlated with a carbon signal at δ_C 80.7, compatible to C-16 of the aglycon compared with saponin **1**. All these data permitted the identification of **3** as 3β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-6α-*O*-β-D-xylopyranosyl-16β-hydroxy-22-*O*-α-L-rhamnopyranosylhopane and **4** as 3β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-6α-*O*-β-D-xylopyranosyl-16β-*O*-β-D-xylopyranosyl-22-hopane.

Comparing the hopane type saponins known from the aerial parts of *Glinus lotoides*[12,13] with those isolated from its seeds, it is evident that the major saponins of the seeds are mainly glycosylated with xylose and to a lesser degree with arabinose and rhamnose, but not with glucose. It may be supposed that saponins **1**−**5** are the effective compounds responsible for the beneficial effects in the treatment of prevalent tapeworm infections in traditional medicine in Ethiopia using *G. lotoides* seeds. However, this will need to be demonstrated conclusively in future experiments. Anthelmintic activity has been demonstrated for other triterpene saponins.[22,23]

Experimental Section

General Experimental Procedures. UV spectra of the flavonoids were recorded on a Perkin-Elmer Lambda 16 UV/vis spectrometer. 1D (^{1}H, ^{13}C, and DEPT-135) and 2D (^{1}H COSY, HMQC, and HMBC) NMR spectra were obtained at 300 K on Bruker AVANCE DMX-600 or ARX-400 NMR spectrometers locked to the major deuterium signal of the solvent, CD_3OD. Chemical shifts are reported in δ ppm and coupling constants in Hz. ESIMS data were recorded on a Thermo Finnigan TSQ Quantum triple-quadrupole mass spectrometer. LC-10AD, Shimadzu Liquid Chromatography, Shimadzu Co., HPLC pump with Rheodyne, 7021, Rohnert Park injector and a Chromato-Integrator, D-2000, Merck, were used for HPLC isolation. A Millpore Waters Model 481 LC spectrophotometer (Milford, MA) and a Shimadzu RID-10A refractive index HPLC detector were employed for flavonoids and saponins, respectively.

The following materials and experimental conditions were used for chromatography: size-exclusion column chromatography, Sephadex LH-20 (Amersham Biosciences AB); reversed-phase HPLC column (CC 250/4 Nucleosil 100-7 C_{18}, Macherey-Nagel); guard column (CC 8/4 Nucleosil 100-5 C_{18}, Macherey-Nagel); analytical TLC, precoated silica gel 60 F_{254} and precoated RP-18 F_{254s} aluminum sheets, Merck; preparative TLC, Sil G-100, 1.0 mm silica gel, Macherey-Nagel. The following TLC solvent systems were used for saponins: MeOH−H_2O (4:1) and $CHCl_3$−MeOH−H_2O (70:50:4), flavonoids: HCOOH−H_2O−ethyl methyl ketone−EtOAc (1:1:3:5), and sugars: H_2O−MeOH−CH_3COOH−$C_2H_4Cl_2$ (10:15:25:50). The TLC spray reagents were 5% vanillin in EtOH−H_2SO_4 followed by heating for saponins; 5 mL of solution I (1% diphenylboryloxyethylamine in MeOH) followed by 5 mL of solution II (5% PEG 400 in EtOH) for flavonoids, and *p*-anisaldehyde−H_2SO_4 followed by heating for sugars. All solvents used were spectral grade.

Plant Material. Fruits of *Glinus lotoides* were purchased from a local market in Addis Ababa, Ethiopia, in September 2001. The identity was confirmed by the National Herbarium, Department of Biology, Faculty of Science, Addis Ababa University, Ethiopia. A voucher specimen (No. 003444) has been deposited at the National Herbarium, Department of Biology, Faculty of Science, Addis Ababa University, Ethiopia.

Extraction and Isolation. The seeds of *G. lotoides* were powdered and defatted with *n*-hexane. The defatted powdered seeds (500 g) were extracted three times with 60% methanol in water using Ultra-Turrax (9500 rpm, 10 min). The extracts were filtered, concentrated under reduced pressure, and dried. The dried extract (90.0 g) was dissolved in water and extracted with an equal volume of *n*-butanol. On separation, the *n*-butanol fraction was dried (16.0 g) and subsequently dissolved in a small volume of methanol and added to a large volume of

diethyl ether. The precipitate formed was separated by centrifugation and dried (12.0 g). Sephadex LH-20 column chromatograpy (28 × 1.5 cm i.d.) of the precipitate (1.2 g) using methanol at a rate of 4 mL/min provided 20 fractions. These were combined into two major fractions, 1 (352 mg) and 2 (600 mg), after TLC control on analytical RP-18 TLC plates using a mixture of MeOH−H_2O (4:1) and 5% vanillin−H_2SO_4 reagent for detection. Fraction 1 (256 mg), containing mainly flavonoids, was separated by reversed-phase HPLC (CC250/4 Nucleosil 100-7 C_{18} Macherey-Nagel with guard column ChroCART 4-4 Lichrospher 100 RP-18, 5 μm, 15% CH_3CN, UV 360 nm) to afford vicenin-2 (18.3 mg) and vitexin-2″-*O*-glucoside (10.8 mg). Fraction 2 (480 mg), containing mixtures of saponins, was separated by RP-18 HPLC (75% MeOH; refractive index detector) to furnish two fractions [S1 (24 mg) and S2 (29 mg)]. Preparative TLC of S1 on silica gel (Sil G-100, 1.0 mm) with $CHCl_3$−MeOH−H_2O (70:50:4) afforded glinuside F (**1**) (2.2 mg) and succulentoside B (**5**) (1.9 mg). Similar work with S2 gave glinuside G (**2**) (2.4 mg) and a mixture of glinusides H (**3**) and I (**4**) (3.0 mg).

Hydrolysis of the Glinusides on TLC. Glinuside solutions (5 uL of 0.08 mg/mL corresponding to 0.4 μg of glinuside) were applied on precoated silica gel 60 F_{254} aluminum sheet TLC plates and left in a hydrochloric acid atmosphere at 100 °C for 1 h. HCl vapor was then eliminated under hot ventilation, and the authentic sugars, namely, arabinose (Fluka, Sigma-Aldrich), xylose (Merck), and rhamnose (Merck), were applied. The TLC plate was developed with H_2O−MeOH−CH_3-$COOH$−$C_2H_4Cl_2$ (10:15:25:50) and visualized after spraying with *p*-anisaldehyde−H_2SO_4 reagent followed by heating at 110 °C for 10 min. Arabinose, xylose, and rhamnose were observed at R_f values of 0.55, 0.65, and 0.68, respectively.

Methylation Analysis of the Sugar Constituents. This was performed as previously described.[20]

5,7,4′-Trihydroxyflavone-6,8-di-*C*-glucoside (Vicenin-2) and 5,7,4′-trihydroxy-flavone-8-*C*-sophoroside (Vitexin-2″-*O*-glucoside). These compunds were identified by UV, NMR, and mass spectral data comparison with literature values and by using authentic samples.

Glinuside F (1): ^1H and ^{13}C NMR, see Table 1; ESIMS (negative mode) *m/z* 871 (100) $[M - H]^-$, 739 (32) $[(M - H) - 132]^-$, 607 (100) $[(M - H) - (132 + 132)]^-$, 475 (44) $[(M - H) - (132 + 132 + 132)]^-$, (positve mode) *m/z* 872.7 (44) $[M + H]^+$, 740.5 (100) $[(M + H) - 132]^+$, 608.5 (42) $[(M + H) - (132 + 132)]^+$, 573.5 (41), 459.5 (15), 441.5 (64), 423.4 (76), 405.4 (45); HRESIMS *m/z* 895.508 $[M + Na]^+$ (calcd for $C_{45}H_{76}O_{16}$+Na, 895.5031); TLC R_f 0.48 ($CHCl_3$−MeOH−H_2O; 70:50:4).

Glinuside G (2): ^1H and ^{13}C NMR, see Table 1; ESIMS (negative mode) *m/z* 899 (100) $[M - H]^-$, 753 (38) $[(M - H) - 146]^-$, 607 (38) $[(M - H) - (146 + 146)]^-$, 475 (17) $[(M - H) - (146 + 146 + 132)]^-$; HRESIMS *m/z* 923.538 $[M + Na]^+$ (calcd for $C_{47}H_{80}O_{16}$+Na, 923.5344); TLC R_f 0.52 ($CHCl_3$−MeOH−H_2O; 70:50:4).

Glinuside H (3): HRESIMS *m/z* 1055.581 $[M + Na]^+$ (calcd for $C_{52}H_{88}O_{20}$+Na, 1055.5766); TLC R_f 0.25 ($CHCl_3$−MeOH−H_2O; 70:50:4).

Glinuside I (4): HRESIMS *m/z* 1041.565 $[M + Na]^+$ (calcd for $C_{51}H_{86}O_{20}$+Na, 1041.5610); TLC R_f 0.33 ($CHCl_3$−MeOH−H_2O; 70:50:4).

Succulentoside B (5): HRESIMS *m/z* 895.507 $[M + Na]^+$ (calcd for $C_{45}H_{76}O_{16}$+Na, 895.5031 TLC R_f 0.41 ($CHCl_3$−MeOH−H_2O; 70:50:4).

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Supporting Information Available: Table of NMR data for glinusides H and I. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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