Triterpene Saponins from *Cyclamen mirabile* and Their Biological Activities

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Six saponins, cyclaminorin (**1**), deglucocyclamin (**2**), cyclacoumin (**3**), cyclamin (**4**), isocyclamin (**5**), and mirabilin (**6**) were isolated from the tubers of *Cyclamen mirabile*. Compound **6** is a new natural compound, and its structure was established as 3-{*O*-*β*-[[*β*-D-xylopyranosyl-(1→2)]-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl-(1→4)]-[*β*-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3*β*,16α,28-trihydroxyolean-12-en-30-oic acid (**6**). The structure elucidation of this compound was accomplished using both spectral and chemical methods. Antimicrobial and uterocontractile activities of the saponins were also investigated.

In a previous paper we reported on the isolation of four saponins from the tubers of *Cyclamen coum* Miller (Primulaceae), which are used in Turkish folk medicine against infertility.[1] These saponins were cyclaminorin (**1**), deglucocyclamin (**2**), and cyclacoumin (**3**) in addition to mirabilin lactone (**7**). In order to compare the chemical structures and biological activities of saponins from two species of the same genus, we have undertaken the study of the triterpenoid saponins of *C. mirabile* Hildebr., an endemic species growing in Turkey,[2] which resulted in the isolation of six saponins (**1**−**6**, Chart 1). Antimicrobial (antibacterial and antifungal) and uterocontractile activities of the saponins (**1**−**7**) obtained were also investigated.

The structures of the saponins **1**−**3** were established as cyclaminorin, deglucocyclamin, and cyclacoumin, respectively, on the basis of their ^{1}H-NMR spectral analyses and TLC comparison with authentic samples obtained from *Cyclamen coum*.[1] Compound **1** was the minor saponin, while **2** was the major saponin as observed in *C. coum*.[1] The FABMS of both the saponins **4** and **5** exhibited a peak at *m/z* 1245 $[M + Na]^+$ compatible with the molecular formula $C_{58}H_{94}O_{27}$ and suggesting the presence of a pentaglycosidic structure. The ^{1}H- and ^{13}C-NMR spectral data for **4** and **5** were in good agreement with those reported for cyclamin and isocyclamin, respectively.[3]

Saponin **6** was the most polar compound among the triterpenoid glycosides isolated. The FABMS of **6** showed a quasimolecular $[M + Na + H]^+$ peak at *m/z* 1262 corresponding to a molecular formula of $C_{58}H_{94}O_{28}$. IR absorptions at 3400, 1707, and 1646 cm^{-1} indicated the presence of OH, CO, and C=C functionalities. The ^{1}H-NMR spectra of **6** exhibited resonances for the anomeric protons of the sugar moiety at *δ* 4.42 (d, *J* = 6.0 Hz, H-1′ of the α-L-arabinose), 4.41 (d, *J* = 7.8 Hz, H-1′′′′ of the terminal *β*-D-glucose on the inner glucose), 4.73 (d, *J* = 7.6 Hz, H-1′′ of the terminal *β*-D-glucose on arabinose), 4.54 (d, *J* = 7.6 Hz, H-1′′′′ of the *β*-D-xylose), and 4.56 (d, *J* = 7.8 Hz, H-1′′′ of the inner *β*-D-glucose on arabinose). The ^{1}H- and ^{13}C-NMR spectral data indicated that **6** has a pentaglycosidic sugar chain as found for isocyclamin (**5**). Assignments for all proton and carbon resonances (see Experimental Section) were achieved by COSY, TOCSY, and HMQC experiments. The ^{13}C-NMR spectrum of **6** exhibited significant glycosidation shifts for C-3 (*δ* 91.3 d) of the aglycon, C-2′ (*δ* 79.5 d) and C-4′ (*δ* 80.0 d) of α-L-arabinose, and C-2′′′ (*δ* 85.1 d) and C-6′′′ (*δ* 70.3 t) of the inner *β*-D-glucose unit. A HMBC experiment performed on **6** established the interglycosidic connectivities, showing correlations between C-3 of the aglycon and the anomeric proton (H-1′; *δ* 4.42 d, *J* = 6.0 Hz) of α-L-arabinose, C-2′ of α-L-arabinose and the anomeric proton (H-1′′; *δ* 4.73 d (*J* = 7.6 Hz) of the terminal *β*-D-glucose, C-4′ of α-L-arabinose and the anomeric proton (H-1′′′; *δ* 4.56 d, *J* = 7.8 Hz) of the inner *β*-D-glucose, C-2′′′ of the inner *β*-D-glucose and the anomeric proton (H-1′′′′; *δ* 4.54 d, *J* = 7.6 Hz) of *β*-D-xylose, and C-6′′′ of the inner *β*-D-glucose and the anomeric proton (H-1′′′′′; *δ* 4.41 d, *J* = 7.8 Hz) of the second terminal *β*-D-glucose. In order to confirm the sites of interglycosidic linkages of the sugar moiety, **6** was acetylated, yielding **6a**. The ^{1}H-NMR spectrum of **6a** showed 15 acetoxy methyl resonances of which 14 were attributed to the sugar moiety. The signals for H-2′ (*δ* 4.0) and H-4′ (*δ* 4.06) of α-L-arabinose and H-2′′′ (*δ* 3.71) and H_2-6′′′ (*δ* 3.60 and 3.73) of the inner *β*-D-glucose units, whose assignments were based on the results of 2D ^{1}H−^{1}H homonuclear COSY and TOCSY experiments, showed no downfield shifts upon acetylation, confirming the sites of glycosidation. The FABMS of **6a** supported the proposed structure (*m/z* 1891 $[M + Na]^+$, calcd for $C_{88}H_{124}O_{43}$). Furthermore, mass spectral fragmentation peaks arising from the sugar moiety were observed at *m/z* 1339, 835, 331, and 259, confirming these deductions.

After the assignment of the ^{13}C-NMR signals of the sugar moiety, the resonances remaining for the aglycon

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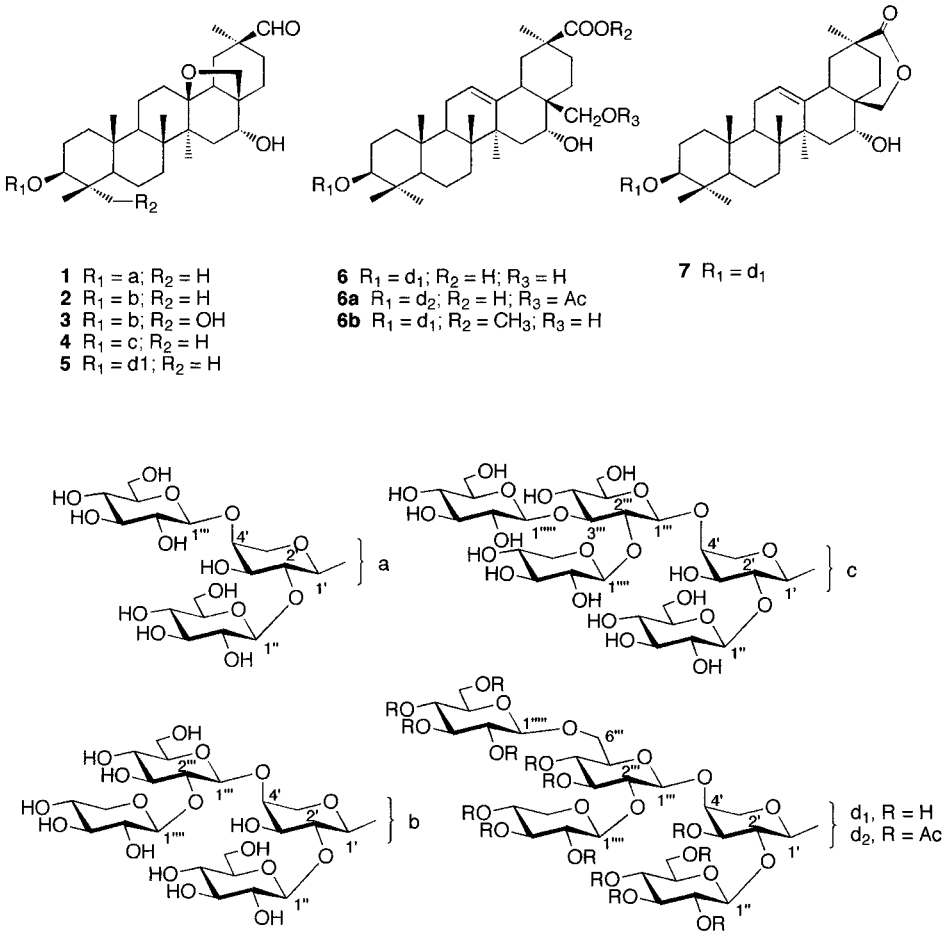
Chart 1



of **6** were six Me (all tertiary), ten methylene, six methine, and eight quaternary carbons. The carbon and proton resonances for the aglycon moiety of **6** indicated an elemental formula of $C_{30}H_{48}O_5$, implying seven degrees of unsaturation of which two were assigned to a double bond (^{1}H δ 5.36 br s, H-12; ^{13}C δ 123.8 d and 145.0 s, C-12 and C-13, respectively) and a carbonyl functionality (^{13}C δ 182.0 s, C-30), respectively, and the remaining indicated that **6** is pentacyclic. Additionally, two methine protons on oxygen-bearing carbons (^{1}H δ 3.32 and 4.11, H-3 and H-16; ^{13}C δ 91.3 d and 74.3 d, C-3 and C-16, respectively) and a pair of a hydroxymethylene protons (^{1}H δ 2.95 and 3.36, J_{AB} = 11.3 Hz, H_2-28; ^{13}C δ 71.8 t, C-28) were observed. These results clearly supported the presence of a pentacyclic olean-12-ene skeleton for **6**.

The HMBC spectrum of **6** exhibited correlations between C-30 (δ 180.5 s) and Me-29 (δ 1.17 s), C-30 and H-19 (δ 2.27 dd), and C-22 (δ 32.3 t) and H_2-28 (δ 3.36 and 2.95) for ring E of the molecule. Thus, **6** could be assigned as the acid analogue of mirabilin lactone (**7**), which has previously been isolated from *C. coum*.[1] Methylation of **6** with diazomethane afforded a monomethyl ester **6b** (FABMS: *m/z* 1275 $[M + Na]^+$, calcd for $C_{59}H_{96}O_{28}$). The ^{1}H-NMR spectrum of **6b** showed a three proton singlet at δ 3.72 assigned to a carbomethoxy signal, supporting the proposed structure. Thus, the structure of saponin **6** was established as 3-{*O*-β-[[β-D-xylopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)]-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α,28-trihydroxyolean-12-en-30-oic acid, for which the trivial name mirabilin is proposed.

Table 1. Antifungal Activities of Cyclaminorin (**1**), Deglucocyclamin (**2**), and Cylamin (**4**)

organism	compd MIC (μg/mL)		
	1	**2**	**3**
Candida albicans	160	160	160
Candida crusei	>320	>320	>320
Candida parapsilosis	80	80	80
Candida pseudotropicalis	160	160	160
Candida stellatoidea	160	160	160
Candida tropicalis	160	160	160
Cryptococcus neoformans	80	80	80

Saponins isolated from *C. mirabile* and *C. coum*[1] (**1**−**7**) were tested for their antimicrobial activities against Gram-positive (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) and Gram-negative (*Escherischia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria and yeasts (*Candida albicans*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, *C. tropicalis* and *Cryptococcus neoformans*). Antibacterial activity was very weak, and MIC values were over 400 μg/mL for **1**−**7**. However, significant antifungal activity was observed for all of the saponins tested. Among these, cyclaminorin (**1**), deglucocyclamin (**2**), and cyclamin (**4**) showed stronger activity than compounds **3** and **5**−**7** (Table 1). The common structural aglycon of these saponins is cyclamiretin A. Although isocyclamin has the same aglycon moiety, its activity was found to be weaker (>320 μg/mL).

For evaluating the uterocontractile activity of saponins **1**−**7**, we used a rat uterus preparation.[4] At a concentration (in the bath) at 8 μg/mL of cyclaminorin

Table 2. Uterocontractile Activity of Compounds **1**−**7**[a]

compd	concn (M)	% ACh contraction	concn (M)	% ACh contraction
1	8.6×10^{-6}	46.2 ± 4.5		
2	7.5×10^{-6}	36.7 ± 1.7		
3	7.4×10^{-6}	58.2 ± 15.8		
4	6.5×10^{-6}	48.4 ± 4.3		
5	6.5×10^{-6}	0.1 ± 0.1	2.0×10^{-5}	28.3 ± 2.4
6	6.4×10^{-6}	0.7 ± 0.5	1.9×10^{-5}	1.1 ± 0.6
7	6.5×10^{-6}	0.6 ± 0.3	2.0×10^{-5}	5.7 ± 1.7

[a] Contractions were obtained using rat uterus preparation and were expressed as the percentage of maximum acetylcholine (10^{-4} M) contraction ($n = 6$).

(**1**) (8.6×10^{-6} M), deglucocyclamin (**2**) (7.5×10^{-6} M), cyclacoumin (**3**) (7.4×10^{-6} M), and cyclamin (**4**) (6.5×10^{-6} M), contractile responses were obtained that were equivalent to contractions obtained with 2.3 μg/mL ($10^{-4.9}$ M), 1.1 μg/mL $10^{-5.2}$ M), 1.1 μg/mL ($10^{-5.2}$ M), and 2.3 μg/mL ($10^{-4.9}$ M) acetylcholine (57.3 μg, 28.7 μg, 28.7 μg, and 57.3 μg in 25 mL of organ bath), respectively. Because low activity was observed with 8 μg/mL of isocyclamin (**5**), we also tested it at 24 μg/mL (2×10^{-5} M), which gave contractions equivalent to 1.1 μg/mL ($10^{-5.2}$ M) acetylcholine. Mirabilin (**6**) and mirabilin lactone (**7**) were also effective at the 24 μg/mL dose, and the contractions were equivalent to 0.1 μg ($10^{-6.3}$ M) and 0.2 μg/mL (10^{-6} M) acetylcholine, respectively (Table 2).

Similar saponins, especially **2**, have been reported from *Cyclamen* species as well as from *Ardisia* species (Myrsinaceae).[4−7] Deglucocyclamin (**2** = ardisiacrispin A) is one of the two uterocontracting saponins from *Ardisia crispa*. The roots of this plant are used in Thai traditional medicine to "wash out dirty blood" in women who suffer from menstrual pains.[4] Related use in traditional medicine of other plants of the genus *Ardisia* has been reported. Recently, Jia *et al*. have reported two new uterocontracting saponins, ardisicrenosides A and B, from the roots of *Ardisia crenata*.[7] The roots of this plant have been used in the treatment of respiratory tract infections and menstrual disorders in Chinese traditional medicine.[7] In the same study, it has also been reported that significant antifertility effects were observed in pharmacological studies.[7] As can be seen from these results, there are close similarities between the chemical structures of the saponins from *Cyclamen* and *Ardisia* species, as well as in their traditional uses.

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter using MeOH as solvent. IR spectra were measured on a Perkin-Elmer 2000 FT-IR as pressed KBr disks. 1D and 2D NMR spectra were recorded using Bruker AMX-300 and 500 instruments. FABMS were recorded using a ZAB2-SEQ mass spectrometer. A Büchi MPLC apparatus was used throughout this study for the separation of the compounds. Sepralyte, LiChroprep C_{18} (Merck) and Si gel 60 (70−230 mesh, Merck) were used as reversed- and normal-phases for chromatographic separations, respectively. Si gel 60 F_{254} precoated Al sheets (0.2 mm, Merck) were used for TLC controls.

Plant Material. *Cyclamen mirabile* Hildebr. was collected from Barla, Isparta, 8 km south of Barla, Turkey, in October 1993. A voucher specimen (no. 93002) has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The freeze-dried tubers (300 g) were chopped and extracted twice with MeOH under reflux. The MeOH extract was evaporated under reduced pressure to dryness (25 g; yield 8.3%), which was fractionated by VLC (Sepralyte 40 μm, 100 g) eluting with increasing amounts of MeOH in H_2O (H_2O, 10% MeOH, 20% MeOH, MeOH), and the fractions were combined into three groups (I, II, III). Group III (7.99 g), eluted with MeOH, was rich in saponins and was further fractionated by VLC (Sepralyte 40 μm, 100 g) eluting with 40% MeOH and MeOH to give two fractions, A (782 mg) and B (4.97 g). Fraction A was subjected to MPLC (Sepralyte 40 μm; column dimensions 352 × 18.5 mm) eluting with increasing amounts of MeOH in H_2O (40−60% MeOH) to yield **6** (112 mg). A part of fraction B (2.75 g) was subjected to Si gel (150 g) column chromatography using mixtures of $CHCl_3$−MeOH−H_2O, [80:20:2 (1500 mL), 70:30:3 (1500 mL), and 60:40:4 (500 mL)] to give eight fractions, B1−B8. Compound **1** (28 mg) and **2** (300 mg) were obtained in pure form from fractions B1 and B3, respectively. Fraction B4 (329 mg) was reapplied to Si gel (40 g) column chromatography using mixtures of $CHCl_3$−MeOH−H_2O, [80:20:1 (250 mL), 80:20:2 (250 mL), 70:30:3 (600 mL), and 60:40:4 (100 mL)] to give compounds **2** (28 mg) and **3** (39 mg), along with a mixture of **2** and **3** (195 mg). Using similar chromatographic conditions, fraction B6 (218 mg) yielded compounds **2** (6 mg), **3** (8 mg), **4** (63 mg), and **5** (38 mg).

Mirabilin (6): amorphous powder; $[\alpha]^{20}_D$ +5.2° (*c* 0.42, MeOH); IR ν_{max} (KBr) 3400 (OH), 2925 (CH), 1707 (C=O), 1646 (C=C) cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 5.36 (1H, br s, H-12), 4.73 (1H, d, J = 7.6 Hz, H-1″), 4.56 (1H, d, J = 7.8 Hz, H-1‴), 4.54 (1H, d, J = 7.6 Hz, H-1⁗), 4.42 (1H, d, J = 6.0 Hz, H-1′), 4.41 (1H, d, J = 7.8 Hz, H-1⁗′), 4.28 (1H, dd, J = 12.5, 2.6 Hz, H_a-5′), 4.18 (1H, dd, J = 12.0, 2.0 Hz, H_a-6‴), 4.11 (1H, br s, H-16), 4.04 (1H, dd, J = 11.0, 5.0 Hz, H_a-5⁗), 3.94 (1H, overlapped, H-4′), 3.88 (2H, overlapped, H_a-6″, H_a-6⁗′), 3.83 (2H, overlapped, H-2′, H-3′), 3.80 (1H, overlapped, H_b-6‴), 3.68 (1H, overlapped, H_b-6″), 3.64 (1H, overlapped, H_b-5⁗′), 3.60−3.25 (7H, overlapped, H-3″, H-4″, H-5″, H-3‴, H-4‴, H-5‴, H-3⁗′), 3.57 (1H, overlapped, H_b-5′), 3.55 (1H, overlapped, H-4⁗′), 3.50 (1H, overlapped, H-5⁗′), 3.45 (1H, overlapped, H-3⁗′), 3.43 (1H, overlapped, H-2⁗′), 3.36 (2H, overlapped, H_a-28, H_b-5⁗′), 3.33 (1H, overlapped, H-4⁗′), 3.32 (1H, overlapped, H-3), 3.29 (1H, overlapped, H-2⁗′), 3.25 (2H, overlapped, H-2″, H-2⁗′), 2.95 (1H, d, J = 11.3 Hz, H_b-28), 1.45 (3H, s, H_3C-27), 1.17 (3H, s, H_3C-29), 1.12 (3H, s, H_3C-23), 1.03 (3H, s, H_3C-25), 0.99 (3H, s, H_3C-26), 0.92 (3H, s, H_3C-24); ^{13}C NMR (75.5 MHz, CD_3OD) δ 182.0 s (C-30), 145.0 s (C-13), 123.8 d (C-12), 107.3 d (C-1⁗′), 105.6 d (C-1′), 105.1 d (C-1⁗), 104.7 d (C-1⁗′), 104.4 d (C-1″), 91.3 d (C-3), 85.1 d (C-2⁗), 80.0 (C-4′), 79.5 d (C-2′), 78.0 d (C-3″), 78.0 d (C-3⁗), 78.0 d (C-3⁗′), 77.8 d (C-5⁗), 77.6 d (C-5″), 77.5 d (C-3⁗), 77.0 d (C-5⁗′), 76.0 d (C-2⁗′), 75.9 d (C-2″), 75.1 d (C-2⁗′), 74.3 d (C-16), 74.3 d (C-3′), 72.0 d (C-4⁗), 71.8 t (C-28), 71.6 d (C-4⁗′), 71.3 d (C-4″), 71.3 d (C-4⁗′), 70.3 t (C-6⁗), 67.4 (C-5⁗′), 66.0 t (C-5′), 63.3 t (C-6⁗′), 62.8 t (C-6″), 57.1 d (C-5), 48.2 d (C-9), 44.9 t (C-19), 44.9 d (C-18), 44.9 s (C-8), 42.5 s (C-20), 40.9 s (C-14), 40.6 s (C-17), 40.5 s (C-4), 40.1 t (C-1), 37.8 s (C-10), 35.0 t (C-15), 34.0 t (C-21), 33.8 t

(C-7), 32.3 t (C-22), 29.1 q (C-29), 28.5 q (C-23), 27.6 q (C-27), 27.2 t (C-2), 24.5 t (C-11), 19.3 t (C-6), 17.4 q (C-26), 16.9 q (C-24), 16.2 q (C-25); positive FABMS *m/z* 1262 $[M + Na + H]^+$.

Acetylation of Mirabilin (6). Compound **6** (24 mg) was treated with Ac_2O (1 mL) and pyridine (1 mL) at room temperature for 24 h and purified on a Si gel column (10 g) eluting with C_6H_6–$(CH_3)_2CO$ (7:1, 6:1, 5:1 and 4:1 each 100 mL) to yield **6a** (25.5 mg).

Mirabilin Pentadecaacetate (6a): amorphous powder; IR ν_{max} (KBr) 3445 (OH), 2930 (CH), 1758 (C=O, ester) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.38 (1H, br s, H-12), 5.26 (1H, dd, J = 9.5, 9.5 Hz, H-3″), 5.17 (1H, dd, J = 9.5, 9.5 Hz, H-3‴″), 5.13 (1H, dd, J = 9.2, 9.1 Hz, H-3‴), 5.11 (1H, overlapped, H-3‴), 5.07 (2H, overlapped, H-4″ and H-4‴″), 4.98 (1H, dd, J = 8.0, 9.5 Hz, H-2‴″), 4.95 (1H, overlapped, H-2″), 4.93 (1H, ddd, J = 9.1, 5.1, 8.7 Hz, H-4‴), 4.85 (1H, dd, J = 7.2, 9.2 Hz, H-2‴), 4.84 (1H, dd, J = 9.2, 3.4 Hz, H-3′), 4.79 (1H, dd, J = 9.5, 9.5 Hz, H-4‴), 4.75 (1H, d, J = 8.0 Hz, H-1″), 4.67 (1H, d, J = 7.2 Hz, H-1‴), 4.52 (1H, d, J = 8.0 Hz, H-1‴″), 4.47 (1H, br s, H-1′), 4.43 (1H, d, J = 7.8 Hz, H-1‴), 4.26 (1H, overlapped, H_a-6″), 4.25 (1H, overlapped, H_a-28), 4.23 (1H, overlapped, H_a-6‴″), 4.16 (1H, dd, J = 12.1, 5.1 Hz, H_a-5‴), 4.14 (1H, overlapped, H_b-6‴″), 4.12 (1H, br d, H-16), 4.06 (1H, overlapped, H-4′), 4.05 (1H, overlapped, H_b-6″), 4.00 (3H, overlapped, H-2′, H_a-5′ and H_b-28), 3.75 (1H, m, H-5″), 3.73 (1H, dd, J = 12.0, 2.3 Hz, H_b-6‴), 3.71 (1H, dd, J = 7.8, 9.4 Hz, H-2‴), 3.67 (1H, m, H-5‴″), 3.60 (1H, overlapped, H_a-6‴), 3.57 (2H, overlapped, H_b-5′ and H-5‴), 3.36 (1H, dd, J = 12.1, 8.9 Hz, H_b-5‴″), 3.05 (1H, dd, J = 5.5, 11.5 Hz, H-3), 2.074, 2.065, 2.056, 2.043 (3H each, s), 2.039 (6H, s), 2.029 (9H, s), 2.021, 2.016 (3H, each, s), 2.007 (6H, s), 2.003, 1.999 (3H, each, s) (aliphatic acetoxy methyls × 15), 1.39, 1.27, 0.98, 0.91, 0.90, 0.77 (3H each, s, tertiary methyls × 6); positive FABMS *m/z* 1891 $[M + Na]^+$, 1339 [pentadecaacetyl − pentasaccharide oxonium]$^+$, 835 [octaacetyl − xylosyl − glucosyl − glucose oxonium]$^+$, 331 [tetraacetyl − glucose oxonium]$^+$, 259 [triacetyl − xylose oxonium]$^+$.

Methylation of Mirabilin (6) with Diazomethane. A saponin fraction (130 mg) containing compound **6** as a major saponin was treated with CH_2N_2. The product was purified on a Si gel (25 g) column using $CHCl_3$–MeOH–H_2O (80:20:1, 80:20:2, 70:30:3 and 60:40:4) as eluent to give compound **6b** (12 mg).

Mirabilin Methyl Ester (6b): amorphous powder; IR ν_{max} (KBr) 3390 (OH), 2925 (CH), 1735 (ester C=O), 1595 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 5.34 (1H, br s, H-12), 4.73 (1H, d, J = 7.6 Hz, H-1″), 4.56 (1H, d, J = 7.8 Hz, H-1‴), 4.54 (1H, d, J = 7.5 Hz, H-1‴″), 4.42 (1H, d, J = 6.0 Hz, H-1′), 4.41 (1H, d, J = 7.8 Hz, H-1‴″), 4.17 (1H, br s, H-16), 3.72 (3H, s, $-COOCH_3$), 3.40–3.30 (1H, overlapped, H_a-28), 3.30–3.20 (1H, overlapped, H-3), 2.93 (1H, d, J = 11.1 Hz, H_b-28), 1.45 (3H, s, H_3C-27), 1.14 (3H, s, H_3C-29), 1.12 (3H, s, H_3C-23), 1.03 (3H, s, H_3C-25), 0.99 (3H, s, H_3C-26), 0.91 (3H, s, H_3C-24); positive FABMS *m/z* 1275 $[M + Na]^+$.

Antibacterial Assays. Gram-positive (*S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) and Gram-negative *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) bacteria and yeasts (*C. albicans*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, *C. stellatoidea*, *C. tropicalis*, and *Cr. neoformans*) were used as test organisms. Two *Candida* species (*C. albicans* and *C. parapsilosis*) have previously been used in a similar study.[8] All fungi were the isolates of Microbiology Department of Faculty of Medicine, Hacettepe University. Mueller Hinton broth (Merck) and RPMI 1640 with L-glutamine (Difco) were used as media for bacteria and yeast, respectively. *Cr. neoformans* was cultured in Sabaroud Dextrose Broth (Difco). In order to evaluate antibacterial and antifungal activity, minimum inhibitory concentrations (MICs) were determined by microtiter-tube dilution procedure.[9,10]

Isolated Rat Uterus Test.[4] This test was used for the saponins **1**–**6** isolated from *C. mirabile* and saponin **7** from *C. coum*.[1] Female rats, weighing 120–200 g, were injected with 0.1 mg/kg of diethylstilbestrol 24 h prior to the experiment. The uterine horns were dissected from the sacrificed animals. Each horn was mounted in a 25-mL organ bath in De Jalon's solution and connected to a transducer (Maycom). Contractions were recorded on a Polwin 95 Maycom equipped with preamplifier, oscillograph, and event marker. A mixture of 95% O_2 and CO_2 was bubbled through the organ bath, which was kept at 30 °C.

The composition of De Jalon's solution is NaCl (18 g), KCl (0.84 g), $NaHCO_3$ (1.0 g), D-glucose (1.0 g), and $CaCl_2$ (0.06 g) (all reagents were dissolved in deionized H_2O to make 2 L).

Acetylcholine. Percent control dose–response curve was obtained by using 10^{-8}–10^{-4} M concentrations of acetylcholine.

Statistical Analysis. For statistical analysis the Student's *t*-test was employed.

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