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Triterpene saponins from the fruits of *Hedera helix*

E. Bedir[a], H. Kırmızıpekmez[a], O. Sticher[b], İ. Çalış[a,*]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey*
[b]*Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, CH-8057 Zürich, Switzerland*

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Abstract

Six triterpene saponins, including two new compounds, were isolated from the fruits of *Hedera helix* L. (Araliaceae). The structures of the new compounds, named helixosides A and B, were established as 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, and 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, respectively, on the basis of chemical and spectral data.

Keywords: *Hedera helix*; Araliaceae; Triterpenoid saponins; Helixosides A and B

1. Introduction

Hederae Folium is one of the most frequently used saponin-containing plant drugs for the treatment of cough. Saponins from the leaves of *Hedera helix* L. (Araliaceae) have antifungal, anthelmintic, molluscicidal, antileishmanial, and antimutagenic activities (Timon-David, Julien, Gasquet, Balansard & Bernard, 1980; Julien, Gasquet, Maillard, Gasquet & Timon-David, 1985; Hostettmann, 1980; Majester-Savornin, Elias, Diaz-Lanza, Balansard, Gasquet & Delmas, 1991; Elias, Lanza, Vidal-Ollivier, Balansard & Faure, 1991). We herein report the isolation and structure elucidation of two new saponosides, helixosides A (**5**) and B (**6**) in addition to the known glycosides, 3-*O*-β-D-glucopyranosyl hederagenin (**1**), 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl oleanolic acid (**2**), 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl hederagenin (**3**) (Hostettmann, Hostettmann-Kaldas & Sticher, 1980) and 3-*O*-β-D-glucopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**4** = staunoside A) (Wang, Mayer & Rücker, 1993) from the fruits of *Hedera helix*.

2. Results and discussion

The IR spectra of the compounds showed strong hydroxyl (3400 cm^{-1}) for all of them, a carboxylic group absorption for **1**, **2** and **3** (1695 cm^{-1}), and ester group absorptions for **4**, **5** and **6** (1733, 1730 and 1731 cm^{-1}, respectively). The NMR spectral data and acid hydrolysis of **1**–**6** suggested that the aglycones were hederagenin for **1**, **3**, **4** and **5**, and oleanolic acid for **2** and **6**, and glucose as the only sugar unit for all compounds

Compounds **1**–**3** are known triterpene saponins and identified as 3-*O*-β-D-glucopyranosyl hederagenin (Hostettmann, 1980; Wang et al., 1993), 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl oleanolic acid, 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl hederagenin (Hostettmann et al., 1980). Compounds **1** and **3** have already been isolated from the same plant source.

The ^{13}C-NMR spectra of **4** and **5** indicated the presence of three anomeric carbon signals (δ 105.71, 104.64 and 95.77), and four anomeric carbon signals (δ

* Corresponding author. Tel.: +90-312-310-35-45; fax: +90-312-311-4777.
E-mail address: acalis@dominet.in.com.tr (İ. Çalış).

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104.64, 104.49, 104.44 and 95.76), respectively. The signals at δ 95.77 and 95.76 suggested that **4** and **5** have an 28-*O*-glycosidic linkage, which was further confirmed by their ester group absorptions (1733 and 1730 cm^{-1}) in their IR spectra, respectively. On the other hand, the ester linkages between the carboxy group located on C-28 of the aglycone (hederagenin) and the sugar moieties were also evident from the chemical shift values of the anomeric protons observed at δ 5.35 for **4** and δ 5.36 for **5**. The presence of a 3-*O*-glycosidic linkages was identified by the downfield shifts at δ 83.48 and 84.20 for C-3 of the aglycone moiety, respectively. Thus, compounds **4** and **5** are bisdesmosides.

The alkaline hydrolysis of **4** and **5** afforded **1** and **3**, respectively, which were identified by direct comparison by TLC. The positive ESI-mass spectra of **4** and **5** showed quasimolecular ions $[M + Na]^+$ at m/z 981.8 and 1143.8, respectively, while negative ESI-mass spectra of **4** and **5** exhibited molecular ions $[M - H]^-$ at m/z 957 and 1119.8, respectively, indicating a M_r of 958 (calcd for $C_{48}H_{78}O_{19}$) for **4** and 1120 (calcd for $C_{54}H_{88}O_{24}$) for **5**. The sites of sugar linkages were established by 1D and 2D NMR spectroscopy. The ^{1}H-NMR spectra exhibited three anomeric protons for **4** and four anomeric protons for **5**. Assignments for all proton and carbon resonances were achieved by COSY, HMQC, HSQC-TOCSY and HMBC experiments, which indicated the presence of three β-D-glucose units for **4** and four β-D-glucose units for **5** (Tables 1 and 2). The appearance of downfield signals at δ 69.52 for the carbon atoms of hydroxymethylene groups of the esterified glucose units revealed the presence of a (1 → 6)-glycosidic linkages between two glucose moieties in **4** and **5**. Additionally, downfield chemical shift of the inner glucose linked to C-3(OH) of the aglycone at δ 81.51 showed a (1 → 2)-glycosidic linkage between two glucose units in **5**.

HMBC experiments performed on **4** and **5** confirmed the interglycosidic connectivities. For the compound **5**, the correlations between C-3 (δ 84.20) of the aglycone and the anomeric proton at δ 4.44 (H-1′), C-2′ of the glucose glycosylated at C-3 and the anomeric proton at δ 4.69 (H-1″), C-28 (δ 178.20) of the aglycone and the anomeric proton at δ 5.36 (H-1‴), and C-6‴ (δ 69.52) of the glucose unit esterified and the anomeric proton at δ 4.35 (H-1⁗) were observed. In view of the above evidence, the structures of **4** and **5** were concluded to be 3-*O*-β-D-glucopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, and 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, respectively. Compound **4** has formerly been isolated from *Stauntonia hexaphylla* and named as staunoside A (Wang et al., 1993), while **5** is a new compound, for which the trivial name helixoside A is proposed.

Table 1
^{1}H-NMR spectral data for compounds **5** and **6** (CD_3OD, 300 MHz)

	5	**6***
H-Atom	δ ppm, J (Hz)	δ ppm, J (Hz)
H-3	3.64	3.18
H-12	5.25 *br s*	5.25 *br s*
H-18	2.86 *dd* (13.4, 3.2)	2.86 *dd* (13.2, 3.2)
H_2-23	3.64, 3.28	
(H_3-23)		1.08 *s*
H_3-24	0.73 *s*	0.86 *s*
H_3-25	0.99 *s*	0.96 *s*
H_3-26	0.81 *s*	0.80 *s*
H_3-27	1.17 *s*	1.15 *s*
H_3-29	0.91 *s*	0.91 *s*
H_3-30	0.94 *s*	0.94 *s*
H-1′	4.52 *d* (7.7)	4.44 *d* (7.5)
H-1″	4.69 *d* (7.7)	4.67 *d* (7.7)
H-1‴	5.36 *d* (7.9)	5.35 *d* (7.9)
H-1⁗	4.35 *d* (7.7)	4.35 *d* (7.8)

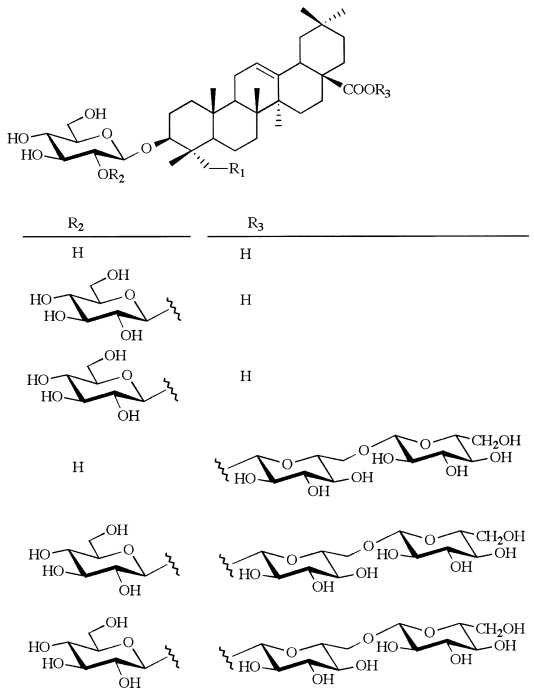
Table 2
^{13}C-NMR spectral data for compounds **5** and **6** (CD_3OD, 75.5 MHz)[a]

C-atom		**5**	**6**[a]	C-atom		**5**	**6**[a]
1	CH_2	39.59	39.88	1′	CH	104.49	105.42
2	CH_2	26.35	27.12	2′	CH	81.51	81.11
3	CH	84.20	91.51	3′	CH	77.66	77.67
4	C	44.10	40.40	4′	CH	71.73	71.92
5	CH	49.10	57.07	5′	CH	78.33	78.33
6	CH_2	18.90	19.35	6′	CH_2	62.75	63.12
7	CH_2	33.35	33.22				
8	C	40.69	40.73	1″	CH	104.44	104.51
9	CH	49.30	49.44	2″	CH	76.19	76.31
10	C	37.65	37.89	3″	CH	77.85	77.82
11	CH_2	24.60	24.59	4″	CH	71.50	71.55
12	CH	123.75	123.79	5″	CH	78.52	78.52
13	C	144.96	144.88	6″	CH_2	62.97	62.84
14	C	43.02	42.93				
15	CH_2	28.93	28.93	1‴	CH	95.76	95.78
16	CH_2	24.05	24.06	2‴	CH	73.85	73.85
17	C	48.80	49.00	3‴	CH	78.00	78.00
18	CH	42.56	42.56	4‴	CH	70.97	70.96
19	CH_2	47.27	47.26	5‴	CH	77.85	77.88
20	C	31.53	31.54	6‴	CH_2	69.52	69.51
21	CH_2	34.88	34.89				
22	CH_2	33.22	33.96	1⁗	CH	104.64	104.64
23	$CH_2(CH_3)$[a]	64.89	28.49	2⁗	CH	75.13	75.15
24	CH_3	13.36	16.93	3⁗	CH	77.85	78.00
25	CH_3	16.54	16.10	4⁗	CH	71.55	71.55
26	CH_3	17.88	17.83	5⁗	CH	78.00	78.18
27	CH_3	26.49	26.30	6⁗	CH_2	62.75	62.74
28	C	178.20	178.01				
29	CH_3	33.49	33.50				
30	CH_3	24.05	24.06				

[a] Assignments were based on COSY, TOCSY, HMQC and HMBC experiments.

The positive FAB-MS of **6** showed a quasimolecular $[M + Na]^+$ peak at m/z 1127.5 corresponding to a molecular formula of $C_{54}H_{88}O_{23}$, which was 16 mass unit less than that of helixoside A (**5**). The ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) of **6** were similar to those of **5** for the sugar moieties. The differences for the aglycone moieties were the presence of an additional tertiary methyl and the lack of the hydroxymethylene group resonances for **6**. The COSY, HMQC, HSQC-TOCSY and HMBC experiments performed on **6** indicated that it has the same oligosaccharidic sugar chains at C-3 and C-28 of the aglycone moiety. The ^{13}C-NMR spectral data assigned for the aglycone moiety were almost identical to those reported for oleanolic acid (Tian, Wu, Qiu & Nie, 1993). An HMBC experiment confirmed the interglycosidic connectivities as in **5**. Consequently, the structure of **6** was found to be a new compound and established as 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, for which the trivial name helixoside B is proposed.

Compounds **1**–**6** (each 20 and 40 μg) were tested against *Candida albicans*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Escherichia coli*, using TLC autographic assay (Hamburger & Hostettmann, 1991; Rahalison, Hamburger, Hostettmann, Monod & Frenk, 1991). Compound **2**, which is a monodesmosidic saponin with oleanolic acid as aglycone, showed activity against *C. albicans*. Weak inhibition at 20 μg was also observed only by compound **2** against *S. epidermidis* and *B. cereus*. The MIC values of **2** were 64 ppm against *C. albicans*, and 128 ppm against *S. epidermidis* and *B. cereus*. The MIC value of the reference compound miconazol was 4 ppm against *C. albicans*, while the MIC value of chloramphenicol was 16 ppm for both bacteria, *B. cereus* and *S. epidermidis*. The other saponins (**1**, **3**–**6**) have showed no activity in the disk assay even at 40 μg. Thus, the antibacterial activity seems to be related to the presence of a monodesmosidic saponin structure.



3. Experimental

Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. IR spectra were measured on a Perkin Elmer 2000 FT-IR spectrometer in KBr pellets. NMR measurements in CD_3OD at room temp. were performed on a Bruker AMX 300 spectrometer operating at 300 and 75.5 MHz for ^{1}H and ^{13}C, respectively. The ^{1}H–^{1}H DQF-COSY, TOCSY, ^{1}H–^{13}C HMQC, and HMBC, experiments were recorded by employing conventional pulse sequences. ESIMS and FABMS were recorded in the positive and/or negative ion mode on a Finnigan TSQ 7000 and ZAB2-SEQ mass spectrometers, respectively. The materials for the biological assays were Mueller-Hinton agar (Oxoid, Hampshire, England) and nutrient broth No. 3 (Fluka, Buchs, Switzerland).

3.1. Plant material

The fruits of *Hedera helix* L. (Araliaceae) were collected from Zonguldak, Hisarönü (North Anatolia) in February 1996. Voucher specimens (96-002) are deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

3.2. Extraction and isolation

Air-dried powdered fruits of the plant (200 g) were extracted with MeOH (500 ml × 3) for 4 h at 25°C and filtered. The filtrate was concentrated to dryness in vacuo (54 g, yield 27%). An aliquot of the extract (13 g) was subjected to vacuum liquid chromatography (VLC) using reversed phase material (Sepralyte 40 μm, 150 g), employing H_2O (200 ml), H_2O–MeOH (90 : 10, 200 ml; 85 : 15, 200 ml; 80 : 20, 200 ml; 70 : 30, 200 ml; 60 : 40; 200 ml) and MeOH (200 ml) as eluents to yield thirteen main fractions (fraction volume: 100 ml). Fractions 6–10 were combined (7.5 g), and an aliquot (6.5 g) of the these fractions was subjected again to VLC using reversed phase material (Sepralyte 40 μm, 150 g), employing H_2O–MeOH mixtures (50 : 50, 40 : 60, 37.5 : 62.5, 35 : 65, 32.5 : 67.5, 30 : 70, 27.5 : 72.5, 25 : 75, 22.5 : 77.5; volume of each solvent mixture:

200 ml) and MeOH as eluents and 15 fractions were collected (fractions A–O) of which fractions D (490 mg), F (450 mg), H (800 mg) and J–K (combined, 820 mg) were studied for the isolation of saponins. Fraction D was chromatographed on a Si gel (30 g) column eluted with $CHCl_3$–MeOH–H_2O (70 : 30 : 3; 600 ml) to give compounds **4** (22 mg) and **5** (89 mg), respectively. Fraction F was subjected to a Si gel column (30 g) using $CHCl_3$–MeOH–H_2O (70 : 30 : 3; 700 ml) to yield compounds **2** (4 mg), **4** (110 mg), and **6** (23 mg), respectively. Repeated chromatography of fraction H on a si gel (35 g) column using the mixtures of $CHCl_3$–MeOH (99 : 1, 200 ml; 98 : 2, 100 ml; 97 : 3, 50 ml; 95 : 5, 100 ml, 90 : 10, 100 ml and 80 : 20, 250 ml) afforded **1** (9 mg), **2** (300 mg) and **3** (25 mg). Fraction J and K were combined and then subjected to a Si gel column using $CHCl_3$–MeOH–H_2O (85 : 15 : 0.5, 100 ml; 80 : 20 : 1, 200 ml; 80 : 20 : 2, 400 ml) to yield compound **1** (98 mg) and the crude saponin **2** (160 mg) which was further chromatographed on a Si gel (20 g) column eluting with $CHCl_3$–MeOH–H_2O (80 : 20 : 1, 200 ml; 80 : 20 : 1.5, 200 ml) to give compound **2** (46 mg).

3.3. Helixoside A (5)

Amorphous colorless; $[\alpha]_D^{20} = +3.2^\circ$ (*c* 0.6, MeOH); IR ν_{max} (KBr) cm^{-1}: 3392 (OH), 2926 (CH), 1730 (C=O, ester), 1163 (C–O–C); ^{1}H-NMR data (CD_3OD, 300 MHz) see Table 1; ^{13}C-NMR data (75.5 MHz, CD_3OD) see Table 2; positive ESIMS *m/z* 1143.8 $[M + H]^+$, negative ESIMS *m/z* 1119.8 $[M + H]^+$ (calculated for $C_{54}H_{88}O_{24}$).

3.4. Helixoside B (6)

Amorphous colorless; $[\alpha]_D^{20} = -7.2^\circ$ (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1}: 3402 (OH), 2942 (CH), 1731 (C=O, ester), 1077 (C–O–C); ^{1}H-NMR data (CD_3OD, 300 MHz) see Table 1; ^{13}C-NMR data (75.5 MHz, CD_3OD) see Table 2; positive FABMS *m/z* 1127.5 $[M + Na]^+$ (calculated for $C_{54}H_{88}O_{23}$).

3.5. Acid hydrolysis of 1–6

Compounds **1**–**6** (each 5 mg) were separately dissolved in 5 ml 5% HCl solution and heated at 100°C for 5 h, cooled, and filtered. The filtrates were neutralized by passing them through Dowex (Cl form) and evaporated. The residues were examined for sugars by TLC (cellulose) using EtOAc–pyridin–AcOH–H_2O (36 : 36 : 7 : 21) as solvent system and anilinphtalate as spray reagent.

3.6. Alkaline hydrolysis 4 and 5

Compounds **4** and **5** (each 5 mg) were separately refluxed in 5% KOH in MeOH at 80°C for 2 h. After neutralization with methanolic HCl, the reaction mixtures were evaporated to dryness. The residues were dissolved in H_2O and extracted three times with *n*-BuOH, and the combined *n*-BuOH extracts were concentrated and compared with compounds **1** and **3** by TLC using the mixtures of $CHCl_3$–MeOH–H_2O (61 : 32 : 7 and 80 : 20 : 2) as solvent systems and 5% H_2SO_4 in MeOH as spray reagent.

3.7. Microbiological assays

Detection of biological activity against microorganisms was performed by using paper disk diffusion method and agar (Mueller-Hinton agar) overlay method on a TLC plate (Hamburger & Hostettmann, 1991; Rahalison et al., 1991). The minimum inhibition concentration (MIC) was evaluated by a dilution assay in nutrient broth (Rios, Recio & Villar, 1988). One yeast, *Candida albicans* ATCC 26790, two Gram-positive bacteria, *Bacillus cereus* ATCC 10702 and *Staphylococcus epidermidis* ATCC 12228, and *Escherichia coli* were used as test organisms. Cultures were made visible by spraying with 1% MTT reagent. Saponins **1**–**6** were tested in the diffusion method in amounts of 20 and 40 μg on a paper disk which were applied on an inoculated agar plate. After storage for 4 h at 4°C the zone of inhibition against microorganisms was measured after 12 h incubation at 27°C. The measured inhibition zones were about 1–2 mm.

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