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Elemanolide sesquiterpenes and eudesmane sesquiterpene glycosides from *Centaurea hierapolitana*

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Abstract

Two elemanolide sesquiterpenes and two eudesmane-type sesquiterpene glycosides named hierapolitanins A–D, were isolated, together with five known compounds, two flavones; hispidulin and jaceosidin, a flavon-*C*-glycoside, shaftoside, a flavonol glycoside, kaempferol-3-*O*-rutinoside and a neolignan, dehydrodiconiferyl alcohol from the aerial parts of *Centaurea hierapolitana* Boiss. (Asteraceae). Structure elucidations were based on spectroscopic evidence.

Keywords: *Centaurea hierapolitana*; Asteraceae; Sesquiterpene lactones; Flavones; Hierapolitanin A, B, C and D

1. Introduction

The genus *Centaurea* L. (Asteraceae) comprises about 187 species in the flora of Turkey (Wagenitz, 1975; Güner, 2000; Duran and Duman, 2002; Turkoglu et al., 2003). *Centaurea hierapolitana* Boiss. (Sect. Phalolepis) is an endemic species without close relatives (Wagenitz, 1975). The plant is not used in Turkish folk medicine; however, other species of the same genus (*C. pulchella*, *C. drabifolia* and *C. solstitialis* ssp. *solstitialis*) are used to treat abscess, hemorrhoid, peptic ulcer and common cold (Honda et al., 1996; Yeşilada et al., 1999; Sezik et al., 2001). Chemical investigations of various *Centaurea* species have revealed mainly sesquiterpene lactones (Gousiadou and Skaltsa, 2003), flavonoids (Akal et al., 2003) and lignans (Janaćković et al., 2004). To our knowledge, no phytochemical study has been reported on *C. hierapolitana*.

Fractionation of $CHCl_3$ extract of aerial parts of *C. hierapolitana* yielded two new elemanolide-type sesquiterpenes, hierapolitanins A and B (**1**, **2**) with two flavones; hispidulin (**3**) and jaceocidin (**4**), and a neolignan; dehydrodiconiferyl alcohol (**5**). The more polar *n*-BuOH extract afforded two new eudesmane-type sesquiterpene glycosides (**6**, **7**) named hierapolitanins C and D. Moreover a flavon-*C*-glycoside; shaftoside (**8**) and a flavonol glycoside; kaempferol-3-*O*-rutinoside (**9**), were also isolated. Herein, we describe the isolation and structural elucidation of **1**, **2** and **6**, **7**.

2. Results and discussion

Compound **1** had the molecular formula $C_{19}H_{24}O_6$ as determined by LC–MS (*m*/*z* 366.1893) $[M+NH_4]^+$ and 719.3097 $[2M+Na]^+$, and ^{13}C NMR measurements. Of the eight degrees of unsaturation indicated by the molecular formula of **1**, six were present as multiple bonds (four exocyclic double bonds and two carbonyl groups; δ112.5 *t*, 113.1 *t*, 117.8 *t*, 125.4 *t*, 136.7 *s*, 138.6 *s*, 145.1 *s*, 143.8 *s*, 166.2 *s*, 169.2 *s*) indicating the bicyclic nature of the

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molecule. Inspection of the ^{1}H and ^{13}C NMR spectra of **1** showed characteristic signals for methacrylate moiety, ^{1}H: (δ 1.96 *s*, H-3′, 3H; 5.68 and 6.12, each br*s*, H-4′, 2H, ^{13}C: 166.2, C-1′; 136.7, C-2′; 17.7, C-3′; 125.4, C-4′). The ^{13}C NMR spectrum of **1** showed 19 signals. After subtraction of the four carbon resonances of the methacrylate units, the remaining 15 signals were attributable to a 1,3,11(13)-elematrien-12,6-olide sesquiterpene framework based on detailed examination of the ^{1}H and ^{13}C NMR spectra and previously reported spectroscopic data of related metabolites (Stewart and Mabry, 1985; Rustaiyan et al., 1987; Rustaiyan and Ganji, 1987; Cuenca et al., 1993; Karioti et al., 2002). The covalent connectivities of the elema-trien aglycon of **1** were established by analysis of the DQF-COSY, and HMQC spectra, which revealed the aforementioned skeleton (Fig. 1). The connectivities between the partial structures and assignment of quaternary carbon atoms in the molecule were determined via long-range correlations in the HMBC spectrum. This not only connected the fragments but also facilitated the location of the methacrylate moiety based on the correlation between C-1′ (δ 166.2) and H-8 (δ 5.67, *ddd*, $J = 4.2$, 10.8 and 10.8 Hz).

The stereochemistry and conformation of **1** were assigned by a combination of 2D-ROESY data, analysis of coupling constants, and molecular modeling studies (Fig. 2). Based on the assumption that H-7 is α, H-6 should be β-oriented as the coupling constants suggested antiperiplanar orientation ($J_{6,7} = 10.8$ Hz). Moreover, the coupling constant between H-6 and H-5 protons was also large ($J_{5,6} = 12.0$ Hz), indicating that H-5 and H-6 must be *trans*-diaxially oriented, which is a characteristic feature for elemane-type sesquiterpenes. In the ROESY spectrum, the strong correlation between H-5 and H-7 was observed supporting our assumption as H-5ax-α/H-7ax-α orientations. Furthermore, the allylic coupling $J_{7,13a}$ (3.0 Hz) and $J_{7,13b}$ (3.6 Hz) supported the above arguments suggesting a *trans*-fused lactone group. The ROESY correlation between H-6 and H-8 showed that these protons are in close proximity suggesting β-configuration of the latter. The α-configuration of C-8(OH) was also confirmed on the basis of the value of $J_{7,8}$ (10.8 Hz) implying antiperiplanar orientation of H-7 and H-8. Molecular modeling studies of **1** suggested a chair conformation of the cyclohexane ring for conformational minimum (Fig. 2, Conformer A). In the case of the aforementioned arrangement, the interatomic distances between H-6/H-14a and H-8/H-14a require a strong ROESY correlation (2.23 and 2.28 Å, respectively). However, in the ROESY spectrum, an extremely weak correlation was observed between only H-6 and H-14a. Owing to the conspicuous absence of a strong correlation, the chair conformation was not further considered. On the other hand, if cyclohexane ring adopts boat conformation (C-6, C-8, and C-9 are above the plane of C-5 and C-7 and C-10), the interatomic distances between H6/H-14a and H-8/H-14a remain reasonable for the observed lack of correlations (H-6/H-14a: 3.93 Å; H-8/H-14a: 4.61 Å) (Fig. 2, Conformer B). On the basis of these facts, it was possible to establish the dominant conformation, conformer B.

These results suggested that **1** had H-5(α)/H-6(β)/H-7(α)/H-8(β)/orientation and 5(*S*), 6(*R*), 7(*R*), 8(*S*), 10(*R*) absolute configuration.

On the basis of these findings, the structure of **1** was established as 5(*S*),6(*R*),7(*R*),8(*S*),10(*R*)-8-*O*-(2-methylene-propanoyloxy)-14,15-dihydroxy-elema-1,3,11(13)-triene-12,6-olide, named as hierapolitanin A.

LC–MS of **2** showed ion peaks for $[M+NH_4]^+$, and $[2M+Na]^+$ at *m*/*z* 424.1982 and 835.3177, respectively, in agreement with the molecular formula $C_{21}H_{26}O_8$. Detailed examination of 1D and 2D NMR spectra of **2** and comparison with those of **1** showed their considerable structural similarity. The main difference consisted in the signals of the side chain that was attached at C-8 of the sesquiterpene moiety. Additionally, the signals arising from an acetyl group were observed in the ^{1}H and ^{13}C NMR spectra (^{1}H: δ 2.07; 13C: δ 21.3, 170.6). ^{1}H and ^{13}C NMR data of the side chain were in accordance with 3-hydroxy-2-methylene-propanoyloxy moiety (^{1}H: δ 4.14, br*s*, H_2-3′; δ 5.68 and 6.12, each br*s*, H_2-4′, ^{13}C: δ 66.3, C-3′; δ 124.8, C-4′; δ 141.5, C-2′; δ 165.5, C-1′). Direct evidence of the linkage sites were derived by an HMBC experiment. Thus, key correlation peaks in the HMBC spectrum of **2** were observed between H_2-15 (δ 4.49) and the carbonyl carbon of the acetyl group at δ 170.6, and between H-8 (δ 5.50, *ddd*, $J = 4.2$, 10.8, 10.8 Hz) and C-1′ (δ 170.6), revealing the presence of an acetyl group at C-15 and a side chain unit (3-hydroxy-2-methylene-propanoyloxy) at C-8.

Based on these results, the structure of hierapolitanin B (**2**) was elucidated as 5(*S*),6(*R*),7(*R*),8(*S*),10(*R*)-3-acetoxy-8-*O*-(3-hydroxy-2-methylene-propanoyloxy)-14-15-dihydroxy-elema-1,3,11(13)-triene-12,6-olide.

The IR spectrum of **6** indicated the presence of hydroxyl (3498 cm^{-1}), carbonyl (1697 cm^{-1}) and olefinic (1623 cm^{-1}) functionalities. The LC–ESI-MS of **6** provided $[M+Na]^+$ *m*/*z* 435.2145 indicating the molecular formula $C_{21}H_{32}O_8$. Inspection of the ^{1}H NMR of **6** showed a tertiary methyl group (δ 0.76, *s*), four olefinic protons (δ 4.59, *dd*, $J = 1.6$, 2.0 Hz; 4.95, *s*; 5.59, *d*, $J = 1.2$ Hz; 6.14, *d*, $J = 1.2$ Hz) suggesting two exocyclic double bonds, an oxygenated methine proton at δ 4.20 (*t*, $J = 2.0$ Hz), as well as one anomeric proton signal at δ 4.27 (*d*, $J = 8.0$ Hz) indicative of the presence of a β-linked sugar unit. The ^{13}C NMR spectrum of **6** displayed 21 signals, 6 of which were in good accordance with the presence of a β-glucopyranose moiety. The remaining 15 resonances of the aglycon moiety were consistent with a $C_{15}H_{22}O_3$ sesquiterpene framework, indicating the presence of five degrees of unsaturation, i.e. three double bonds (two exocyclic methylenes and one carbonyl functionality) and two ring systems. Taking into account the results from our comprehensive NMR studies and previous knowledge derived from metabolites isolated from the genus *Centaurea* (Medjroubi et al., 1998; Skaltsa et al., 2000a; Fortuna et al., 2001) it was inferred that **6** had eudesmane-type nucleus.

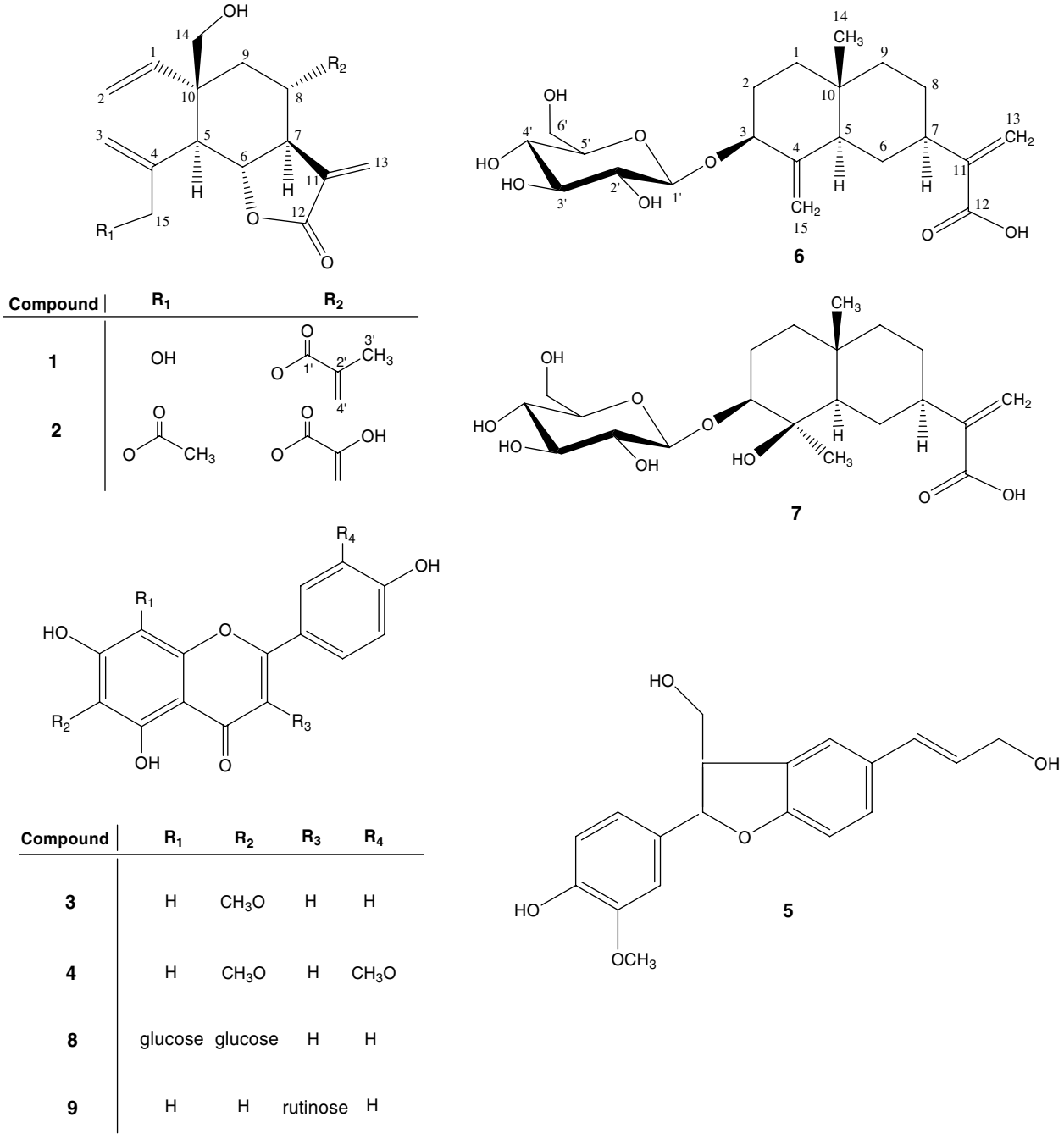

Fig. 1. Structures of compounds **1**–**9**.

On acid hydrolysis, **6** yielded D-glucose. A combination of 2D-NMR experiments (COSY, HMQC) allowed the complete assignment of all sugar signals in **6**. The covalent connectivities of the aglycone were unambiguously established by analysis of the DQF-COSY and HMQC experiments, which revealed the presence of three spin systems in the eudesmane framework: H_2-1 → H-3(0), H_a-15 (allylic coupling) → H-5 → H-9 and H_a-12 → H_b-12. An HMBC experiment showed J_3 correlation between C-7 (δ 39.8) and H_2-12, thus confirming the acrylic acid moiety at C-7. In addition, the HMBC spectrum showed key J_3 correlations between C-1/C-5/C-15 and H-3, C-14 and H-5, C-9/C-1 and H_2-14, thus allowing the assignments of a hydroxyl group at C-3, an exocyclic double band at C-4 (C-4/C-15) and a methyl group at C-10. Additionally in the HMBC spectrum, a long range correlation between H-1′ (δ 4.27) (glucose) and C-3 (δ 81.6) revealed the presence of sugar unit at C-3 position.

The stereochemistry of **6** was resolved by a combination of 2D-NOESY data and comparison with analogous compounds. The β-configuration assigned to Me-14 and the α configuration of H-5 is consistent with all other naturally occurring eudesmane-type sesquiterpenes present in the genus *Centaurea* (Medjroubi et al., 1998; Skaltsa et al.,

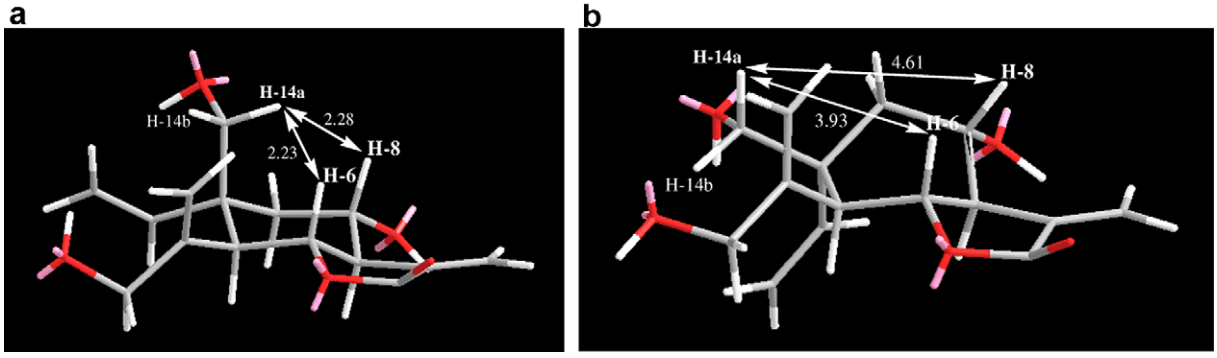

Fig. 2. Graphical representation of conformers A and B. Conformer A (steric energy: 11.006 kcal/mol), Conformer B (steric energy: 14.167 kcal/mol).

2000a; Fortuna et al., 2001). Cross peaks observed in the NOESY spectrum between H-5 and H-7 suggested that these protons were α-cofacial. Additionally, H-3 proton showed a cross peak with H-5 revealing C3(O) as β-oriented. On the other hand, missing NOESY correlations between Me-14 and the aforementioned protons revealed that the methyl group on C-14 was β-oriented. These results also suggested that compound **6** had the usual A/B *trans* ring junction. Based on these findings the structure of hierapolitanin C (**6**) was established as 3(*S*),5(*R*),7(*R*),10(*S*)-3hydroxyeudesma-4(15),11(13)-dien-12-oic acid 3-*O*-β-D-glucopyranoside.

The LC–ESI MS of **7** showed an ion peak for $[M+Na]^+$ at *m*/*z* 453.2353, in agreement with the molecular formula $C_{21}H_{34}O_9$. Detailed examination of the 1D- and 2D-NMR spectra of **7** and comparison with those of **6** showed their considerable structural similarity. The signals due to the sugar moieties and acrylic acid chain were superimposable. The differences consisted only in the signals of C-4/C-15 of ring A. In the ^1H NMR spectrum of **7**, the exocyclic methylene signals of **6**, were replaced by a tertiary methyl at δ 1.09. The ^{13}C NMR spectrum exhibited an oxygenated quaternary carbon at δ 72.2 which was assigned to C-4 based on the HMBC data: correlations between C-4 and H_2-2, H_3-15 and H-3 (Fig. 3).

The stereochemistry of the chiral centers in **7** was assigned by 2D-NOESY data. The strong NOESY correlations between H-3/Me-15, Me-15/H-5 and H-5/H-7 showed that these protons are in close proximity suggesting α-configuration. The β-configuration of Me-14 was also confirmed on the basis of missing NOESY correlations between abovementioned protons and Me-14.

Consequently, the structure of hierapolitanin D (**7**) was elucidated as 3(*S*),4(*R*),5(*R*),7(*R*),10(*S*)-3,4-dihydroxyeudesma-4(15),11(13)-dien-12-oic acid 3-*O*-β-D-glucopyranoside.

Hispidulin (**3**), jaceocidin (**4**), dehydrodiconiferyl alcohol (**5**), shaftoside (**8**), and kaempferol-3-*O*-rutinoside (**9**) were also isolated and identified by comparison of their ^1H and ^{13}C NMR spectral data with those in the literature (Agrawal et al., 1989; Tan et al., 1990) (Fig. 1).

Secondary metabolites of *Centaurea* species are mainly sesquiterpene lactones with guaiane, germacrane, and eudesmane skeletons (Bentamène et al., 2005; Marco et al., 2005). Elemane-type sesquiterpenes have also been isolated from *C. chilensis* (Negrete et al., 1993), *C. paui* (Cardona et al., 1997), *C. cuneifolia* (Aslan and Oksuz, 1999), *C. achaia* (Skaltsa et al., 2000b) and *C. aspera* (Marco et al., 2005). Our investigation demonstrated that elemane-type metabolites are the main sesquiterpenes in *C. hierapolitana* which is a member of Phalolepis section. This suggests that the presence of elemanolides which is rather unusual in *Centaurea* genus could be of taxonomic importance.

Moreover, to our knowledge, sesquiterpene glycosides have been encountered for the first time in the genus *Centaurea*.

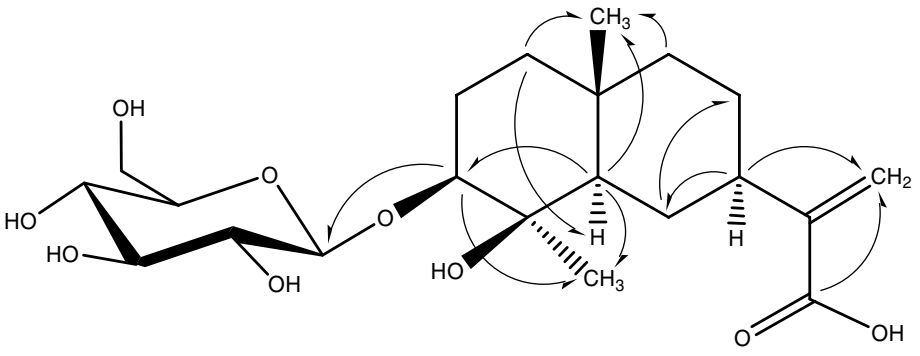

Fig. 3. Key HMBC of compound **7** (arrows from C to H).

3. Experimental

3.1. General procedures

IR spectra were obtained on an ATI Mattson Genesis series and Jasco 430 FT-IR spectrometers. Mass spectra analysis were carried out on an Agilent series 1100 SL equipped with an ESI source. Optical rotation measurements were done on an Autopol IV polarimeter in EtOH at 25 °C. NMR spectra were recorded on Varian Oxford AS400 and Varian Oxford AS600 NMR spectrometers. Proton and carbon chemical shifts are relative to TMS. Multiplicity determinations (DEPT) and 2D NMR spectra (COSY, NOESY, HMQC, HMBC) were run using standard Bruker pulse programs. Column chromatography was carried out on Silica gel (JT Baker, 40 μm), Sephadex LH-20 (Amersham Biosciences, 17-0090-02) and RP (C-18, 40 μm) (Merck). TLC analyses were carried out on Silica gel 60 F_{254} (Merck) and RP-18 F_{254s} (Merck) plates. Compounds were detected by UV and 1% vanillin/H_2SO_4 spraying reagent followed by heating at 105 °C for 1–2 min.

3.2. Plant material

C. hierapolitana was collected from Saraykoy-Babadag, Denizli, Turkey in June 2004 (37°54′41.2″N; 28°54′39.4″E). The plant was identified by Prof. Dr. Ozcan Secmen, from Section of Botany, Department of Biology, Faculty of Science, Ege University and a voucher specimen was deposited in the Herbarium of Ege University, Faculty of Pharmacy, Izmir, Turkey (IZEF 5669).

3.3. Extraction and isolation

Dried and ground aerial parts (1350 g) of the plant were extracted with MeOH (3 × 5 l), and the combined extracts were evaporated under reduced pressure to dryness (yield 159 g). A portion of dried MeOH extract (78.6 g) was suspended in a 1 l H_2O and then partitioned successively with *n*-hexane, $CHCl_3$ and *n*-BuOH (each 2 l × 3 times). The combined *n*-hexane, $CHCl_3$ and *n*-BuOH fractions were separately evaporated which afforded 9.5, 11.0, and 23.1 g extracts, respectively. $CHCl_3$ fraction (10.3 g) was chromatographed on Si gel column (40 μm, 300 g), eluted initially with *n*-hexane and then with *n*-hexane:$CHCl_3$ mixtures (10% → 100%$CHCl_3$; each 600 ml), $CHCl_3$:EtOAc mixtures (10% → 100% EtOAc; each 600 ml), $CHCl_3$:MeOH mixture (50:50, 600 ml), and finally with %100 MeOH (600 ml). One hundred and fourty-two fractions were collected and monitored by TLC (Frs. 1–142). Based on TLC profiles, 5 fractions; A (Frs. 49–51, 172 mg), B (Frs. 60–64, 307 mg), C (Frs. 78–83, 338.2 mg), D (Frs. 86–88, 67.8 mg) and E (Frs. 105–114, 717 mg) were selected for further purification. 300 mg of Fr. C was chromatographed over silica gel column (75 g) using *n*-hexane–EtOAc–MeOH mixtures (10:10:0.25, 10:10:0.50, 10:10:0.75, and 10:10:1, each 300 ml) to afford 134 fractions. Fractions C52–60 were combined (17.5 mg) and rechromatographed over silica gel column (Supelco, 2 g) using isocratic $CHCl_3$–MeOH (99:1, 100 ml) to afford compound **1** (2.6 mg). Part of Fr. D (60 mg) was subjected to silica gel column (15 g) eluted with *n*-hexane–EtOAc–MeOH mixture (10:10:0.25, 500 ml) to afford 80 fractions. Fractions D25–30 were combined (7.1 mg) and rechromatographed over silica gel column (Supelco, 2 g) using $CHCl_3$–MeOH mixtures (100:0 → 90:10, with 1% increasing polarity, each 10 ml) to afford 20 fractions. Fractions 6–14 were combined to yield **2** (9.8 mg). Part of Fr. B (280 mg) was chromatographed over silica gel column (70 g) using mixtures of *n*-hexane–$CHCl_3$–MeOH (5:5:0.25, 1 l; 5:5:0.5, 100 ml; and 5:5:1, 200 ml) to afford 210 fractions. Fractions B168–196 were combined to yield **3** (30 mg). Fr. A (172 mg) was chromatographed over silica gel column (35 g) eluted with *n*-hexane–EtOAc–MeOH mixtures (10:10:0, 200 ml; 10:10:0.25, 200 ml and 10:10:0.5, 125 ml) to give 60 fractions. Fractions A32–41 were combined (124.8 mg) and rechromatographed over Sephadex LH 20 (35 g) using MeOH (100%, 500 ml) to afford 82 fractions. Fractions A62–69 were combined to yield compound **4** (3.6 mg). Part of Fr. E (650 mg) were subjected to Sephadex LH-20 column (100 g) using MeOH (100%, 1 l) as eluent. Fractions E83–92 were combined to yield compound **5** (14.3 mg).

n-BuOH fraction (15 g) was chromatographed on RP-C18 column (400 g), eluted initially with H_2O (2 l) and then with MeOH:H_2O mixtures (10% → 90% MeOH, each 1 l) and finally with 100% MeOH (1 l). Fifty-six fractions were collected and monitored by TLC (Frs. 1–56). Based on TLC profiles, 4 fractions; A (Frs. 26–27, 200 mg), B (Frs. 36–37, 250 mg), C (Frs. 41–42, 136.1 mg) and D (Frs. 45, 96 mg) were selected for further purification. Fr. C was chromatographed over Sephadex column (80 g) MeOH (100% 300 ml) to afford 48 fractions. Fractions C20–28 were combined (61.3 mg) and rechromatographed over RP-C18 column (55 g) using MeOH:H_2O mixture (50:50, 200 ml; 55:45, 300 ml; 60:40, 200 ml) to afford 89 fractions. Frs. 76–82 were combined to yield compound **6** (8.2 mg). A part of Fr. D (90 mg) was subjected to RP-C18 column (55 g) eluted with MeOH:H_2O mixture (55:45, 300 ml; 60:40, 100 ml) to afford 80 fractions. Frs. D77–79 were combined to yield compound **7** (15.6 mg). Fr. A (200 mg) was chromatographed over Sephadex column (80 g) using MeOH (100%, 350 ml) to afford 70 fractions. Fraction A52–59 were combined to yield compound **8** (47.8 mg). Fr. B (250 mg) was chromatographed over Sephadex column (80 g) eluted with MeOH (100%, 300 ml) to afford 55 fractions. Fractions B33–36 were combined to yield compound **9** (36.1 mg).

3.4. Hierapolitanin A (*1*)

Amorphous powder; $[\alpha]_D^{25}$ +48.5° (*c* 0.003, MeOH); IR (KBr): $\nu_{max} = 3435, 2926, 2360, 2341, 1717, 1653, 1259, 1167, 1027\ cm^{-1}$; LC–MS: $m/z = 366.1893$ $[M+NH_4]^+$ (calcd. for $C_{19}H_{28}N_1O_6$: 366.1917), 719.3097 $[2M+Na]^+$ (calcd. for $C_{38}H_{48}O_{12}Na$: 719.3044); ^1H NMR

Table 1
^{1}H and ^{13}C NMR assignments of **1** (in acetone-d_6) and **2** (in DMSO-d_6)[a]

H/C	**1** (δ_H)	**2** (δ_H)	**1** (δ_C)	**2** (δ_C)
1	5.79 *dd* (11.4, 18.0)	5.69 *dd* (11.4, 18.0)	143.8 *d*	143.8 *d*
2	5.01 *d* (18.0), 5.04 *d* (11.4)	5.00 *dd* (10.8, 17.4)	113.1 *t*	114.5 *t*
3	5.16 br*s*, 5.49 *d* (1.8)	5.30 br*s*, 5.12 br*s*	112.5 *t*	116.0 *t*
4			145.1 *s*	138.2 *s*
5	2.59 *d* (12.0)	2.65 *d* (13.0)	51.9 *d*	51.8 *d*
6	4.92 *dd* (10.8, 12.0)	4.85 *dd* (10.8, 12.0)	78.7 *d*	78.5 *d*
7	3.01 *ddt* (3.0, 10.8, 10.8)	3.10 *ddt* (3.0, 10.8, 10.8)	52.3 *d*	51.2 *d*
8	5.67 *ddd* (4.2, 10.8, 10.8)	5.50 *ddd* (4.2, 10.8, 10.8)	70.5 *d*	70.3 *d*
9	1.79 *t* (12.0), 2.36 *dd* (4.8, 13.2)	1.69 *t*, (12.6), 2.20 *dd* (4.8, 12.4)	42.2 *t*	41.8 *t*
10			46.3 *s*	46.4 *s*
11			138.6 *s*	139.7 *s*
12			169.2 *s*	169.8 *s*
13	5.50 *d* (3.0), 5.96 *d* (3.6)	5.46 *d*, 5.94 *d*	117.8 *t*	119.5 *t*
14	3.60 *d*, 3.94 *d*	3.38 *dd*, 3.67 *dd*	66.5 *t*	65.5 *t*
15	4.05 *d* (15.0), 4.10 *d* (15.0)	4.49 br*s*	65.5 *t*	66.5 *t*
1′			166.2 *s*	165.5 *s*
2′			136.7 *s*	141.5 *s*
3′	1.96 *s* br*s*	4.14 br*s*	17.7 *q*	60.3 *q*
4′	5.68 br*s*, 6.12 br*s*	6.12 d, 5.86 *d*	125.4 *t*	124.8 *t*
$CO\mathit{CH}_3$		2.07 *s*		21.3 *q*
$\mathit{C}OCH_3$				170.6 *s*

[a] Assignments confirmed by DEPT, DQF-COSY, HMQC, and HMBC experiments.

Table 2
^{1}H and ^{13}C NMR assignments of **6** and **7** (in CD_3OD[a])

H/C	**6** (δ_H)	**7** (δ_H)	**6** (δ_C)	**7** (δ_C)
1	1.20 *m*, 1.74 *m*	1.11 *m*, 1.68 *m*	35.9 *t*	34.0 *t*
2	1.98 *m*, 1.74 *m*	1.62 *m*	27.5 *t*	27.5 *t*
3	4.20 *t* (2.0)	3.64[†]	81.6 *d*	82.1 *d*
4			151 *s*	72.2 *s*
5	2.55[†]	1.70[†]	44.3 *d*	48.7 *d*
6	1.30*t* (12.4), 1.60 *m*	1.26 *dt* (12.4, 12.4), 1.89 *m*	29.8 *t*	26.1 *t*
7	2.54[†]	2.50 *ddt*	39.8 *d*	40.6 *d*
8	1.62 *m*, 1.42 *m*	1.86 *m*, 1.68 *m*	27.1 *t*	23.0 *t*
9	1.41 *m*, 1.50 *m*	1.42 *m*	40.7 *t*	44.3 *t*
10			35.5 *s*	33.7 *s*
11			147.0 *s*	147.1 *s*
12			169.9 *s*	170.0 *s*
13	6.14 *d* (1.2), 5.59 *d* (1.2)	6.10 *d* (1.2), 5.56 br*s*	121.5 *t*	121.2 *t*
14	0.76 *s*	0.96 *s*	15.3 *q*	18.0 *q*
15	4.95 *s*, 4.59 *t* (1.6, 2.0)	1.09 *s*	108.5 *t*	20.1 *q*
1′	4.27 *d* (8.0)	4.31 *d* (7.6)	101.4 *d*	101.5 *d*
2′	3.21 *t* (7.6)	3.24[†]	74.0 *d*	73.7 *d*
3′	3.33[†]	3.36 *t* (8.4)	77.2 *d*	76.9 *d*
4′	3.35[†]	3.30[†]	70.3 *d*	70.4 *d*
5′	3.11 *ddd* (5.2, 6.8, 8.8)	3.25[†]	76.4 *d*	76.8 *d*
6′	3.75 *dd* (6.8, 12.0), 3.66 *dd* (5.2, 11.6)	3.83 *dd* (2.4, 12.4), 3.64[†]	61.4 *t*	61.5 *t*

[a] Assignments confirmed by DEPT, DQF-COSY, HMQC, and HMBC experiments.
[†] Signal pattern was unclear due to overlapping.

(acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6, 100 MHz) data: see Table 1.

3.5. Hierapolitanin B (*2*)

Amorphous powder; $[\alpha]_D^{25}$ +64.2° (*c* 0.003, MeOH); IR (KBr): $\nu_{max} = 3435, 2918, 2360, 2341, 1715, 1635, 1236, 1050\ cm^{-1}$; LC–MS: $m/z = 424.1963$ $[M+NH_4]^+$ (calcd. for $C_{21}H_{30}N_1O_8$: 424.1972), 835.3143 $[2M+Na]^+$ (calcd. for $C_{42}H_{52}O_{16}Na$: 835.3177); ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) data: see Table 1.

3.6. Hierapolitanin C (*6*)

Amorphous powder; $[\alpha]_D^{25}$ −51.0° (*c* 0.002, MeOH); IR (KBr): $\nu_{max} = 3394, 2931, 2360, 2341, 1697, 1623, 1369, 1265, 1037\ cm^{-1}$; LC–MS: $m/z = 435.2145$ $[M+Na]^+$

(calcd. for $C_{21}H_{32}O_8Na$: 435.1995); ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Table 2.

3.7. Hierapolitanin D (7)

Amorphous powder; $[\alpha]_D^{25}-48.0°$ (c 0.002, MeOH); IR (KBr): $\nu_{max} = 3435$, 2926, 2360, 2341, 1717, 1653, 1259, 1167, 1027 cm^{-1}; LC–MS: $m/z = 453.2353$ $[M+Na]^+$ (calcd. for $C_{21}H_{34}O_9Na$: 453.2101); ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data: see Table 2.

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