New Constituents of *Artemisia monosperma*

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A new eudesmane sesquiterpene (**1**) and a C_{10} diyne (**2**) were isolated from the aerial parts of *Artemisia monosperma*. The structures of these compounds were determined as *rel*-1*β*,3α,6*β*-trihydroxyeudesm-4-ene (**1**) and 1,3*R*,8*R*-trihydroxydec-9-en-4,6-yne (**2**) on the basis of spectral data interpretation. The absolute stereochemistry of **2** was determined using Mosher ester methodology in which the terminal primary hydroxyl group was first protected to simplify the stereochemical analysis.

In an ongoing phytochemical study of selected species of the Kuwaiti flora,[1] the aerial parts of *Artemisia monosperma* Del. (Asteraceae) have been investigated. This species grows along the northwestern border with Iraq, and its distribution in Kuwait is restricted to the sandy gullies of Wadi Al-Batin. Previous investigation of this plant has led to the isolation of a series of acetylenes and acetophenones,[2] an insecticidal aromatic acetylene,[3] and flavones and flavanol glycosides.[4]

The aerial parts of *A. monosperma* were dried and extracted in a Soxhlet apparatus. Compound **1** was isolated as an oil from the hexane extract. Signals in the ^{1}H and ^{13}C NMR spectra (Table 1) were characteristic of a eudesmane sesquiterpene,[5,6] this class being commonly found in the genus *Artemisia*.[7] By inspection of the HMBC and COSY spectra it could be shown that compound **1** had the connectivities typical of a eudesmane skeleton. From HMBC and DEPT-135 data, a methyl singlet (C-14) exhibited a ^2J correlation to C-10 and ^3J correlations to C-9 (CH_2), C-5 (quaternary), and an oxymethine carbon (C-1). From the HMQC spectrum, the proton directly attached to this carbon was a broad doublet (J = 12.9 Hz) and therefore axial. This proton coupled to two protons of a methylene group (C-2) that further coupled to another oxymethine proton (H-3, δ_H = 4.38, δ_C = 85.1), which was a broad singlet and equatorial in orientation. In the HMBC spectrum, the carbon to which this proton was attached was correlated with the protons of a downfield methyl group (C-15), which was deshielded (δ_H = 1.88) being directly attached to a double bond. This was confirmed by a ^2J correlation between these methyl protons to a quaternary olefinic carbon (C-4) and a ^3J correlation to a further quaternary carbon (C-5, δ_C = 144.7), which was also coupled to H-3 and the methyl protons of C-14, placing this carbon at C-5 and not C-4.

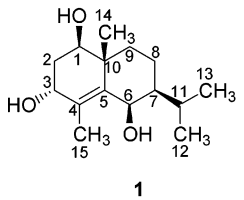
Further signals in the ^{1}H NMR spectrum included the most deshielded oxymethine proton (δ_H = 4.84), which gave HMBC correlations to carbons C-4 and C-5 and must therefore be placed at C-6. This resonance was a sharp singlet (equatorial) and coupled to a further methine proton (H-7) in the COSY spectrum. H-7 exhibited further couplings to a methine proton (H-11), which formed part of the typical eudesmane sesquiterpene isopropyl moiety commonly found at C-7 of this natural product class.[7] This was supported by the presence in the ^{1}H NMR spectrum of two methyl doublets, which in the COSY spectrum coupled to H-11. Additionally, H-7 coupled to a methylene group (C-8) that also correlated to the C-9 methylene group, therefore completing the B-ring and the 4-eudesmene skeleton.

From the coupling constant of 12.9 Hz for H-1 this proton must be axial, and lack of any discernible couplings for H-3 and H-6 (both singlets) implies that these protons are equatorial. Accurate mass determination indicated a molecular formula of $C_{15}H_{26}O_3$, which suggests that hydroxyl groups must be placed at C-1, C-3, and C-6. Compound **1** was therefore assigned as *rel*-1*β*,3α,6*β*-trihydroxyeudesm-4-ene and is reported here for the first time. A paucity of material prohibited determination of absolute stereochemistry.

Table 1. ^{1}H and ^{13}C NMR Data (δ) for Compounds **1** and **2**[a]

	1			**2**	
position	^{1}H	^{13}C	position	^{1}H	^{13}C
1	3.65 bd (12.9)	73.3	1	3.70 m	59.1
2	1.83 m, 2.31 bd (14.0)	30.8	2	1.89 m	41.3
3	4.38 bs	85.1	3	4.56 t (6.9)	60.2
4		125.5	4		82.0
5		144.7	5		68.9
6	4.84 s	67.4	6		70.1
7	0.91 m	48.9	7		79.5
8	1.62 m	19.2	8	4.88 d (5.4)	63.8
9	1.11 m, 1.99 (12.6)	37.7	9	5.91 ddd (17.0, 10.1, 5.4)	138.1
10		39.2	10	5.19 d (10.1), 5.40 bd (17.0)	116.1
11	1.70 m	28.9			
12	1.00 d (6.3)	20.7			
13	0.97 d (6.3)	21.2			
14	1.16 s	18.0			
15	1.88 s	17.2			

[a] Measured in $CDCl_3$. Coupling constants (Hz) in parentheses.



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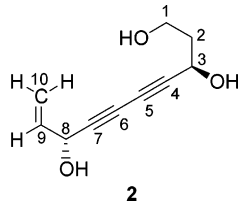

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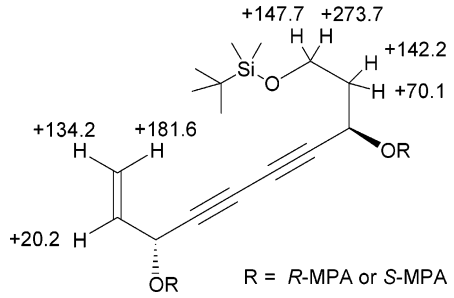

Figure 1. $\Delta\delta$ values [$\Delta\delta$ (in ppb) = $\delta_R - \delta_S$] obtained for the (*R*)- and (*S*)-MPA esters (**2a** and **2b**, respectively) of the TBDMS-protected **2**.

Compound **2** was isolated from the $CHCl_3$ extract by preparative TLC. Signals in the ^1H NMR and ^{13}C NMR spectra (Table 1) included two signals of an exomethylene, a highly coupled olefinic proton, two oxymethine protons, one oxymethylene group, and one methylene group. In addition to these resonances, the carbon spectrum showed the presence of four quaternary carbons, which were very similar to those seen in diacetylenic natural products such as falcarindiol;[8] in fact, the olefinic and oxymethine resonances were in close agreement with those reported for this diyne natural product.[8] The presence of triple bonds in compound **2** was confirmed by a weak absorption (2357 cm^{-1}) in the IR spectrum.

The COSY spectrum of **2** indicated that the exocyclic methylene protons coupled to the olefin, which in turn also coupled to an oxymethine proton (δ 4.88), and this exhibited ^2J and ^3J correlations in the HMBC spectrum to two acetylenic carbons (C-7 and C-6). Further couplings in the COSY spectrum included those between the oxymethylene, methylene, and remaining oxymethine proton, which resulted in a $CH(O)-CH_2CH_2OH$ spin system. In the HMBC spectrum the oxymethine resonance of this spin system also coupled to two acetylenic quaternary carbons (C-4 and C-5). The shielded nature of the two triple bonds suggested that they must be conjugated and connected, and this feature is commonly seen with other acetylenes such as falcarindiol.[8−10] HREIMS suggested a molecular formula of $C_{10}H_{12}O_3$, and therefore three hydroxyl groups must be placed at the oxymethine (C-3 and C-8) and oxymethylene carbons (C-1). Compound **2** was therefore assigned as 1,3,8-trihydroxydec-9-en-4,6-yne. The final problem that remained was the assignment of absolute stereochemistry at carbons 3 and 8, and this was resolved using a modified Mosher method.[11]

The *tert*-butyl dimethylsilyl (TBDMS)-protected **2** was treated with (*R*)-(−)- and (*S*)-(+) methoxyphenylacetic acid (MPA) in two separate reactions to give the bis-(*R*)- and bis-(*S*)-MPA esters (**2a** and **2b**, respectively). $\delta\Delta^{R,S}$ values ($\delta_R - \delta_S$) are shown in Figure 1. The $\delta\Delta^{R,S}$ values for H_2-1 and H_2-2 were positive, indicating *R* stereochemistry at C-3. By analogy, the $\delta\Delta^{R,S}$ values for H-9 and H_2-10 were positive, indicating *R* stereochemistry at C-8.

Both compounds were tested against methicillin- and multidrug-resistant strains of *Staphylococcus aureus* but were inactive (MIC > 128 μg/mL). This was surprising in the case of **2**, which shares some similarity with the anti-staphylococcal acetylene, falcarindiol.[12]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Bellingham and Stanley ADP 200 polarimeter. IR spectra were recorded on a Nicolet 360 FT-IR spectrophotometer. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ on a Bruker Avance 500 spectrometer. Chemical shift values (δ) are reported in parts per million (ppm) relative to NMR solvent $CDCl_3$ (δ_H = 7.27, δ_C = 77.0). Coupling constants (*J* values) are given in Hz. $^1H-^1H$ COSY, HMBC, and HMQC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. Accurate mass measurement was performed on a Finnigan MAT 95 high-resolution magnetic sector mass spectrometer using electron ionization and voltage scanning at 10 000 resolution. Vacuum-liquid chromatography on Merck silica gel 60 $PF_{254+366}$ was used for fractionation and isolation. TLC was performed using Kieselgel 60 F_{254} (Merck) precoated plates, and spots were visualized by spraying with vanillin−sulfuric acid spray followed by heating.

Plant Material. *Artemisia monosperma* was collected from the sandy gullies in northwestern Kuwait bordering Iraq, interspersed with sandstone ridges and opening westwards into the extensive plains of the Wadi Al-Batin. The material was identified by K.T.M. A voucher specimen (KTM 4225, collected by K.T.M. and S.G. in February 1999) is deposited at the Kuwait University Herbarium (KTUH).

Extraction and Isolation. The aerial parts were air-dried for 3 days and ground to a fine powder. The powdered plant material (285 g) was extracted sequentially in a Soxhlet apparatus (3 L each) with hexane, chloroform, and methanol. Vacuum-liquid chromatography (VLC) of the hexane extract (10 g) was performed using a step gradient of 10% EtOAc in hexane followed by a final methanol wash to yield 12 fractions. Flash chromatography of VLC fraction 5 (1.4 g, 6:4 hexane−EtOAc) employing an 8:2 hexane−EtOAc isocratic system, followed by multiple preparative TLC (96 mg, 7:3 hexane−EtOAc, 3 developments), afforded 3 mg of compound **1**.

The chloroform extract (10 g) was subjected to VLC as described above. LH-20 Sephadex chromatography of VLC fraction 10 (231 mg; eluted using 90% EtOAc in hexane) using dichloromethane yielded nine fractions. Fractions 6 and 7 were combined (29 mg) and subjected to preparative TLC (toluene−EtOAc−AcOH, 30:68:2) to afford compound **2** (10 mg).

Compound 1 (*rel*-1β,3α,6β-trihydroxyeudesm-4-ene): colorless oil, $[\alpha]_D^{25}$ +314° (*c* 0.05, $CHCl_3$); IR ν_{max} (thin film) 3362, 2939, 2868, 1738, 1217, 781 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table 1; HREIMS *m/z* 254.1864 (calcd for $C_{15}H_{26}O_3$, 254.1882).

Compound 2 (1,3*R*,8*R*-trihydroxydec-9-en-4,6-yne): colorless oil, $[\alpha]_D^{25}$ +127° (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (3.18), 255 (3.05), 282 (3.05) nm; IR ν_{max} (thin film) 3259, 2357, 1635, 1507, 792 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table 1; HREIMS *m/z* 180.0788 (calcd for $C_{10}H_{12}O_3$, 180.0786).

Determination of Absolute Stereochemistry of Compound 2. TBDMS Protection of 2. Compound **2** (500 μg, 2.8 μmol) was dissolved in 750 μL of $CDCl_3$. To this mixture were added 20 μL aliquots of a 2.5:1 mixture of imidazole and TBDMS-Cl (1 μmol/mL). The reaction was monitored by NMR, and when complete protection of the primary alcohol was observed, the mixture was applied directly to a preconditioned 3 mL silica gel solid-phase extraction cartridge (Bakerbond). The TBDMS-protected **2** was eluted with 50% EtOAc−hexane and evaporated to dryness.

MPA Esterification of TBDMS-Protected 2. To a vial containing the TBDMS-protected **2** were added 1.5 mg of *R*- or *S*-MPA, 15 mg of polystyrene-carbodiimide (Argonaut Inc., Foster City, CA), and 200 μg of DMAP. The reaction mixture was dissolved in 750 μL of $CDCl_3$ and placed on a rotary shaker overnight. The reaction mixture was applied directly to a preconditioned 3 mL silica gel solid-phase extraction cartridge (Bakerbond). The desired product was eluted with $CDCl_3$ and evaporated to dryness.

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