Humulene derivatives from Saharian Asteriscus graveolens

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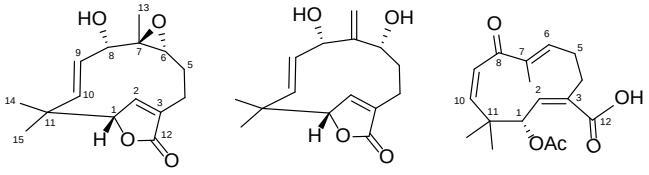
ABSTRACT

Three new sesquiterpene-humulenes, (-)- asteriscunolides I (**1**), J (**2**) and (-)-(2*Z*,6*E*,9*Z*)-8-oxo-1α-acetoxy-2,6,9-humulatrien-12-oic acid (**3**) were isolated from the leaves-flowers of the Saharan medicinal plant *Asteriscus graveolens* along with six known compounds. The structures of the compounds were determined on the basis of spectroscopic mono and bidimensional NMR, mass spectrometry and by single-crystal X-ray diffraction. Compounds **1**-**3** were evaluated for cytotoxic assay, no significant activity was detected.

Asteriscus graveolens (Forsk.) DC. syn. *Bubonium graveolens* (Forsk.) M., *Buphthalmum graveolens* (Forsk.) from Asteraceae family, is a medicinal plant found widely distributed in the Macaronesia region, Mediterranean basin and North Africa including Saharan desert.[1] It is used to treat fever, cephalic pain, gastrointestinal disorders, vomiting.[2] It has been also reported as antioxidant agent,[3] and to protect the skin tissues from damages caused by toxins present in the environmental pollution.[4] *A. graveolens* is characterized by high content of monoterpenes,[5] sesquiterpenes,[6] and flavonoids.[7]

Humulene skeleton is an essential scaffold involved in the sesquiterpene biosynthesis, humulyl cation is considered to be the biogenetic precursor of other important sesquiterpene skeletons,[8] and have been also inspire the synthesis of terpenoids-alkaloids.[9] Humulanolides are the predominant sesquiterpene lactones isolated from *A. graveolens*, which also include the asteriscanolide and aquatolide skeletons with a distinctive γ-lactone moiety.[10] These compounds show potential pharmacological activities, display intriguing chemical structures and serve as a precursor of more complex structures, eliciting the interest for synthetic chemist.[11]

In our continued examination of the North African-Macaronesia Asteraceae plants[6a] and as a part of our ongoing search of bioactive secondary metabolites from Algerian medicinal plants.[12] We were able to isolate three new sesquiterpenes, two new humulanolides (**1**-**2**) and one humulene derivative (**3**), which was previously isolated as methyl ester[13] (Fig. 1) along with six known compounds. The



chemical structures of the previous reported compounds were confirmed by comparing the NMR spectra with published data.

Fig. 1. Chemical structures of compounds **1**-**3** from *A. graveolens*

The ethanol-aqueous extract of *A. graveolens* was investigated, leading to the isolation of two new humulanolides, (-)-asteriscunolides I (**1**) and J (**2**); one new humulene derivative (-)-(2Z,6E,9Z)-8-oxo-1α-acetoxy-humulatrien-12-oic acid (**3**) and six known compounds, stigmasterol[14] and β-sitosterol[15] as a mixture, vanillin[16] and the (-)-asteriscunolides A,[17] B,[17] and F.[12]

Compound **1** was purified as a white crystals by recrystallization from methanol and dichloromethane, and with $[\alpha]^{21}_{D}$ -138° (*c* 0.018, MeOH). The molecular formula of

$C_{15}H_{20}O_4$ was determined according its pseudo-molecular peak at *m/z* 287.1256 $[M + Na]^+$ (calcd. for *m/z* 287.1259), on the basis of its (+) HRESIMS. The ^{1}H NMR (Table 1) spectrum of **1** exhibited the presence of three tertiary methyl carbons at δ_H 1.05 (3H, s), 1.14 (3H, s), and 1.15 (3H, s), a vinyl proton at δ_H 7.25 (1H, t, *J* = 1.6 Hz), the protons of a disubstituted double bond at δ_H 5.12 (1H, d, *J* = 16.1 Hz), and 5.28 (1H, dd, *J* = 8.5, 16.1 Hz), and the signals for three oxygenated methines at δ_H 2.42 (1H, dd, *J* = 1.1, 10.5 Hz), 3.43 (1H, d, *J* = 8.5 Hz), and 4.60 (1H, dd, *J* = 1.3, 2.9 Hz). The relationships between the proton signals in **1** were established from the ^{1}H-^{1}H COSY experiment (Fig. 2), which disclosed the following connectivities: H-1/H-2, H-4/H-5/H-6, H-8/H-9/H-10. The ^{13}C NMR (Table 1) and DEPT spectral data indicated the presence of a *γ*-lactone, as well as four olefinic carbons, three methyls, two methylenes, three methines and two quaternary carbons. The ^{1}H, ^{13}C, and DEPT NMR spectral data suggested that **1** has a humulanolide skeleton, the HMBC experiments (Fig. 2) established the following connectivities: H-8/ C-6, C-7, C-9, C-10 and C-13; H-1/ C-2, C-11, and C-14; H-6/ C-5, C-7, and C-8, the above data and the presence of a quaternary oxygenated carbon C-7, revealed that **1** possess a methyl-bearing trisubstituted epoxide between C-6/C-7. The presence of the epoxide was confirmed by the up-field chemical shifts of the CH_3-13 and H-6 signals observed in the ^{1}H NMR spectrum (Table 1).

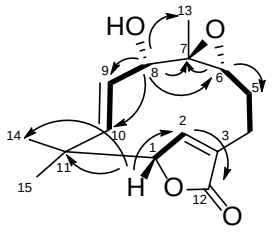


Fig. 2. Selected COSY (bold bond) and HMBC (H→C) correlations for compound **1**.

The relative stereochemistry of **1** was deduced from a 2D ROESY experiment, which indicated correlations of H-8 and H-6, clearly showing that these protons were on the same side. The absence of the correlation between the methyl C-13 and the protons H-6 or H-8 indicate that the epoxide ring was in *trans* form. The structural model of compound **1** (Fig. 3) was confirmed by X-ray diffraction analysis. Thus, single crystal was collected with a Rigaku SuperNOVA diffractometer with microfocus X-ray using Cu Kα radiation (λ = 1.54184 Å). CrysAlisPro[18] software was used to collect, index, scale and apply numerical absorption correction based on gaussian integration over a multifaceted crystal model and empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm into CrysAlisPro. The structure was solved applying the novel dual-space algorithm implemented in SHELXT program.[19] Fourier recycling and least-squares refinement were used for the model completion with SHELXL-2014.[20] All non-hydrogen atoms have been refined anisotropically, and all hydrogen atoms have been placed in geometrically suitable positions and refined riding with isotropic thermal parameter related to the equivalent isotropic thermal parameter of the parent atom. The geometrical analysis of interactions in the structure was performed with Olex2 program.[21] The hydrogen atoms were geometrically positioned with C-H = 0.93Å and Uiso(H) = 1.2 Ueq(C). Crystal data, collection procedures and refinement results are summarized in supplementary information. Crystallographic data for the structures reported in this contribution have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication with number 1840628. Upon consideration of all the above data, **1** was identified as (-)-(2*Z*, 9*E*) 8α-hydroxy-6α,7β-epoxy-2,9-humuladien-1(12)-olide and was named (-)-asteriscunolide I, the next consecutive designation in this nomenclature.

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Table 1. NMR spectroscopic data of compounds 1-3 in CD_3OD.[a]

Compounds	**1**		**2**		**3**	
Position	δ_C	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)[a]	δ_C[b]	δ_H mult. (*J*, Hz)
1	89.0	4.60, dd (1.3, 2.9)	89.8	4.79, dd (1.4)	79.8	6.09, d (9.1)
2	151.6	7.25, t (1.6)	150.1	7.20, s	143.6	5.81, d (9.1)
3	134.3		135.5		132.1	
4a	23.9	2.50, m	23.8	2.43, m	35.3	2.83, m
4b		2.40, m		2.33, td (2.3, 12.5)		2.35, m
5a	22.7	1.90, m	35.5	1.95, br t (13.5)	26.6	2.76, m
5b		1.79, m		1.55, m		2.35, m
6	62.0	2.42, dd (1.1, 10.5)	70.0	3.92, d (7.1)	153.5	6.67, dd (1.0, 11.1)
7	68.2		154.9		140.0	
8	81.9	3.43, d (8.5)	75.1	4.71 br s	203.2	
9	130.8	5.28, dd (8.5, 16.1)	132.2	5.30, dd (3.5, 16.2)	130.5	6.14, d (13.5)
10	137.2	5.12, d (16.1)	132.8	5.41, dd (1.6, 16.2)	140.0	5.88, d (13.5)
11	40.6		41.5		44.8	
12	175.1		176.0		169.3	
13	11.6	1.05, s	113.7	5.22, s 5.34, t (1.5)	11.2	1.69, s
14	26.3	1.15, s	26.5	1.29, s	23.2	0.86, s
15	22.8	1.14, s	22.8	1.23, s	27.9	1.07, s
OAc					20.8	2.09, s
					171.9	

[a] Spectra measured at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR.

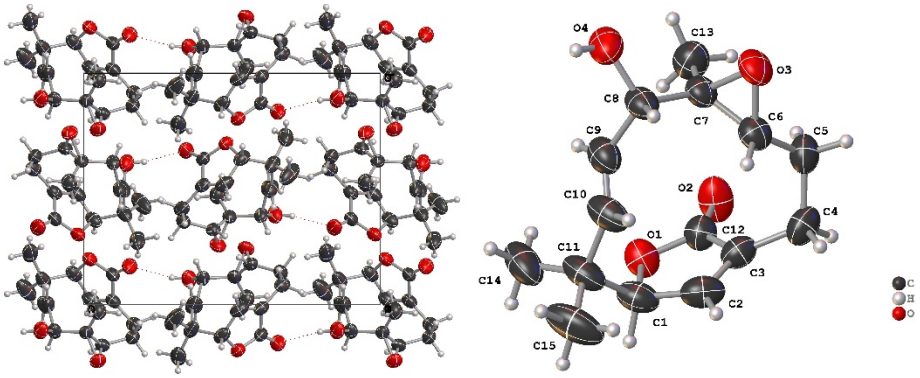

Fig. 3. Crystal packing and X ray structure for compound **1**.

(-)-Asteriscunolide J (**2**) was isolated as white crystals with $[\alpha]^{21}_{D}$ -49° (*c* 0.334, MeOH). Its molecular formula $C_{15}H_{20}O_4$ was established by (+) HRESIMS based on its $[M + Na]^+$ ion at *m/z* 287.1261 (calcd. for 287.1259). Its IR spectrum showed the presence of a hydroxyl groups (3437, 3285 cm^{-1}), an α,β unsaturated γ-lactone (1736 and 1720 cm^{-1}).The ^1H NMR spectrum of **2** (Table 1) exhibited the presence of two tertiary methyl carbons at δ_H 1.23 (3H, s), and 1.29 (3H, s), a vinyl proton at δ_H 7.20 (1H, s), the protons of a disubstituted double bond at δ_H 5.30 (1H, dd, *J* = 3.5, 16.2 Hz), and 5.41 (1H, dd, *J* = 1.6, 16.2 Hz), the signals for three oxygenated methines at δ_H 3.92 (1H, d, *J* = 7.1 Hz), 4.71 (1H, br s), and 4.79 (1H, t, *J* = 1.4 Hz) and exocyclic methylene protons at δ_H 5.22 (1H, s), and 5.34 (1H, t, *J* = 1.5 Hz). The ^1H and ^{13}C NMR spectra of **2** and **1** (Table 1) were similar with regard to their functional group analysis. However, compound **2** has three double bonds one of them exocyclic methylene assigned to C-13, led us to conclude that the epoxide is open in **2**. The above data was confirmed with the HMBC experiment, thus the protons at C-13 showed correlations with C-7, C-6 and C-8. The ROESY experiment showed analog correlations than compound 1. Therefore, the chemical structure of **2** was elucidated as (-)-(2*Z*, 9*E*) 6α,8α-dihydroxy-2,9,7(13)-humulatrien-1(12)-olide.

Compound **3**, was isolated as white crystals with mp 180 °C. Its (+) HRESIMS spectrum showed a pseudomolecular ion *m/z* 329.1371$[M + Na]^+$, compatible with the molecular formula $C_{17}H_{22}O_5$ (calcd. for $C_{17}H_{22}O_5Na$: 329.1365). The IR spectrum showed the presence of carboxylic acid absorptions at 3017-2875 and 1730 cm^{-1}, as well as a conjugated carbonyl group absorbing at 1690 cm^{-1} and acetyloxy group at 1243 cm^{-1}. The ^1H NMR of **3** showed the presence of three double bonds, two three substituted at δ_H 5.81 (1H, d, *J* = 9.1 Hz), and 6.67 (1H, dd, J = 1.0, 11.1 Hz), and one disubstituted at δ_H 5.88 (1H, d, *J* = 13.5 Hz), and 6.14 (1H, d, *J* = 13.5 Hz), one oxygenated methine at δ_H 6.09 (1H, d, *J* = 9.1 Hz) and four tertiary methyls at δ_H 0.86, 1.07, 1.69, and 2.09 ppm. The ^{13}C NMR and DEPT data indicated the presence of three carbonyl groups corresponding to an α, β unsaturated ketone, an acetyl group and an acid group, two aliphatic methylene, six olefinic, four methyl, one methine, and one quaternary carbons. Carefully study of the 2D NMR and comparison with the reported data of the methyl ester isolated for El Dahmny et al.,[13] led us to the conclusion that compound **3** could be identified as (-)-(2*Z*,6*E*,9Z)-8-oxo-1α-acetoxy-2,6,9-humulatrien-12-oic acid.

In summary, three new compounds were isolated from *A. graveolens*, to the best of our knowledge, compound **1**-**2** are the first example the humulanolides with C-8 reduced to hydroxyl. Compounds **1** - **3** were also tested against cancer cell lines HL-60, but compounds **1**-**3** did not show significant activity.

Acknowledgments

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Supplementary data

Detailed description of the experimental procedures, ^1H and ^{13}C NMR spectrum and 2D spectrum of compounds **1**–**3** are found in supplementary data.

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Graphical Abstract

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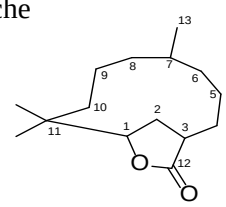
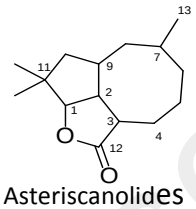


Humulenolides



Asteriscanolides