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Oleaceran: A Novel Spiro[isobenzofuran-1,2′-naptho[1,8-*bc*]furan] Isolated from a Terrestrial *Streptomyces* sp.

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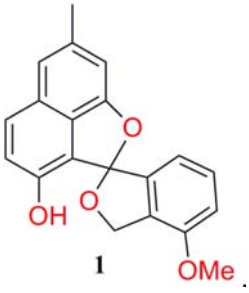
ABSTRACT



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Chemical analysis of a terrestrial-derived *Streptomyces* sp. Lv20−195 cultivated from the root zone of *Olea europea* yielded oleaceran, 1, possessing a novel spiro[isobenzofuran-1,2′-naptho[1,8-*b*,c]furan] carbon skeleton. The structure of 1 was determined by detailed spectroscopic analysis.

Actinomycetes have been an important source of novel secondary metabolites for many decades now, making this order of bacteria an important source for the discovery of rare and novel chemical entities.[1] Novel secondary metabolites reported from actinomycetes, cultivated from plant rhizospheres, have already led to some promising structural novelty. Noteworthy examples include alchivemycin A,[2] indotertine A,[3] juniperolide A,[4] and leopolic acid A.[5] One such strain (Lv20-195) came to our attention, based on our initial in-house chemical profiling.

Strain Lv20-195, later identified as a *Streptomyces* sp., was cultivated from the rhizosphere of the plant *Olea europea* showing an interesting chemical profile. The strain was cultivated in a 5 L Erlenmeyer flask containing 1.5 L of M1 media for 8 days at 150 rpm, after which the whole broth was extracted with an equal volume of EtOAc. The resulting crude extract (178 mg) was then sequentially triturated against hexane, dichloromethane, and methanol. The dichloromethane fraction was then later subjected to reversed-phase semipreparative HPLC (Zorbax, C_8, 9.6 mm × 250 mm, 5 μm; 10−100% MeCN/H_2O; 3 mL/min) to yield oleaceran, **1** (2.5 mg), as an amorphous brownish solid.

HRESI(+)MS analysis of **1** ($[\alpha]_D$ = +36.0) returned a pseudomolecular ion (321.1124 $[M + H]^+$) consistent with a molecular formula ($C_{20}H_{16}O_4$, Δmmu −0.4) requiring 13 double bond equivalents (DBE). The ^{13}C, edHSQC, and ^1H NMR established a total carbon count of 20 and 16 protons, represented by 1 methylene, 2 tertiary methyls,

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Table 1. NMR (500 MHz, DMSO-d_6) Data for Oleaceran **1**

pos	δ_H, mult (J in Hz)	δ_C[a]	COSY	HMBC	ROESY
1a	5.29, d (*13.1*)	71.2	1b	2, 2′, 3, 4, 5, 6, 7, 8	
1b	5.19, d (*13.1*)		1a	2, 3, 4, 5, 6, 7, 8	
2		127.2			
3		154.0			
4	7.08, d (*7.8*)	111.7	5	1, 2, 3, 6	3-OMe
5	7.30, dd (*7.8*, *7.5*)	130.6	4, 6	2, 3, 7, 8	
6	6.53, d (*7.5*)	114.7	5	2, 3, 4, 7, 8	
7		139.5			
8		123.2			
1′		155.8			
1′a		128.0			
2′		116.5			
3′		150.4			
4′	7.13, d (*8.5*)	121.4	5′	2′, 3′, 5′a, 8	5′
5′	7.66, d (*8.5*)	127.8	4′	1′, 1′a, 2′, 3′, 4′, 5′a, 6′	4′
5′a		125.1			
6′	7.07, s	115.3		1′, 1′a, 7′-Me, 8′	7′-Me
7′		135.9			
8′	6.47, s	103.2		1′, 1′a, 6′, 7′-Me	7′-Me
3-OMe	3.89, s	55.8		3	4
7′-Me	2.40, s	22.8		6′, 7′, 8′	6′, 8′
3-OH	9.84, brs				

[a] Assignments supported by HSQC and HMBC experiments.

7 methines, and 10 quaternary carbons. The NMR data (Table 1) revealed 16 sp^2 resonances, accounting for eight DBE and requiring five rings. Examination of the ^{1}H and COSY NMR data documented two isolated spin systems. The first spin system showed correlations from H-4 (δ_H 7.08) to H-6 (δ_H 6.53) extended by HMBC correlations to the quaternary carbons C-2 (δ_C 127.2), C-7 (δ_C 139.5), C-8 (δ_C 123.2) and the oxy quaternary carbon C-3 (δ_C 154.0) which was corraborated by an HMBC correlation from the methoxy signal (δ_H 3.89) to C-3. Additional long-range HMBC correlations were observed from an oxy methylene H_2-1 (δ_H 5.29, 5.19) to C-2, C-3, C-7, C-4 (δ_C 111.7), C-6 (δ_C 114.7), and C-8 revealing the presence of an isobenzofuran moiety as subunit A (Figure 1).

The second spin system consisted of two aromatic protons coupled to one another, H-4′ (δ_H 7.13, d, *8.5*) and H-5′ (δ_H 7.66, d, *8.5*), extended by HMBC correlations from H-4′ to the quaternary carbons C-2′ (δ_C 116.5) and C-5′a (δ_C 125.1), while H-5′ showed correlations to C-1′a (δ_C 128.0), C-6′ (δ_C 115.3), and C-3′ (δ_C 150.4) with the downfield characteristic of C-3′ suggesting the attachment of an oxygen. Diagnostic HMBC correlations were observed from the olefinic tertiary methyl H_3-7′ (δ_H 2.40) to C-6′,C-7′ (δ_C 135.9) and C-8′ (δ_C 103.3). Furthermore the aromatic methine protons H-6′ (δ_H 7.07) were coupled to C-1′a and C-8′, while H-8′ (δ_H 6.47) showed correlations to C-1′ (δ_C 155.8), C-1′a, and C-6′. The deshielded character of C-1′ suggested the attachment of the fourth and final oxygen in the structure, leading to the construction of subunit B, a trisubstituted 7-methylnaphthalene moiety (Figure 1). Finally, selective four-bond HMBC correlations, using a long-range constant of J (XH) = 6 Hz,

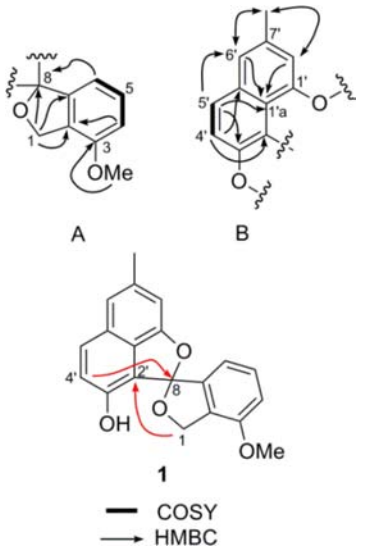

Figure 1. Key 2D NMR correlations (500 MHz, DMSO-d_6) for oleaceran (**1**); red arrows indicate selective four-bond HMBC correlations.

showed correlations from the oxymethylene H_2-1 to C-2′ and H-4′ to C-8 linking the two subunits together, combined with the need to account for the remaining 1 degree of unsaturation which could be satisfied by the generation of either a 1,6-dioxaspiro[4.4]nonane or a 1,5-dioxaspiro-[3.4]octane ring system. Therefore, to determine which of the ring systems lead to the planar structure of **1**, oleaceran was methylated using dry acetone and dimethyl sulfate in

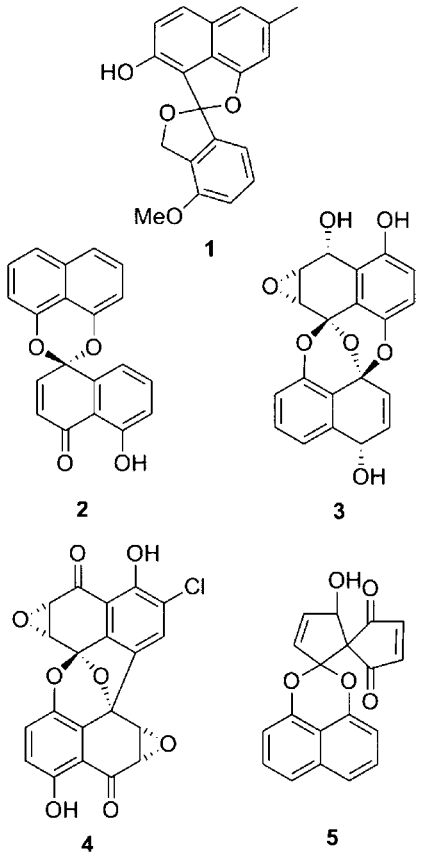

Figure 2. Structures of metabolites **1**–**5**.

the presence of potassium carbonate. The methylation at C-3′ (Figure S2a, Table S1) confirmed the presence of the former ring system, leading to the planar structure of **1**, with less absolute stereochemistry.

Oleaceran was screened for biological activity against a panel of Gram positive and Gram negative bacterial strains also including yeast and fungi and showed weak to moderate antifungal activity against *Mucor hiemalis* and *Pichia anomala* with an IC_{50} of 16.5 and 16.9 μg/mL respectively. A weak cytotoxic effect was also observed against HCT-116 cell lines with an IC_{50} of 20.5 μg/mL.

To the best of our knowledge oleaceran represents the first example of having a spiro[isobenzofuran-1,2′-naptho[1,8*b*,*c*]furan] carbon skeleton. The closest structural similarity was identified within the group of spirobisnaphthalenes, composed of palmarumycins CP_1[6] **2**, preussomerin A[7] **3**, spiroxin A[8] **4**, and the recently isolated spiro-mamakone A from a nonsporulating fungal endophyte derived from the New Zealand native tree *Knightia excelsa* (Figure 2).[9]

In summary we have isolated a novel metabolite, oleaceran, from a terrestrial actinomycete possessing a 1,6-dioxaspiro[4.4]nonane ring forming the core stucture of **1**, 3-methoxy-7′-methyl-3*H*-spiro[isobenzofuran-1,2′-naphtho[1,8-*bc*]furan]-3′-ol.

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Supporting Information Available. Full details of the collection, cultivation and taxonomy of strain Lv20-195, and the isolation, purification, and 1D and 2D NMR data of oleaceran. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.