ORGANIC LETTERS

2012
Vol. 14, No. 23
5860–5863

Juniperolide A: A New Polyketide Isolated from a Terrestrial Actinomycete, *Streptomyces* sp.

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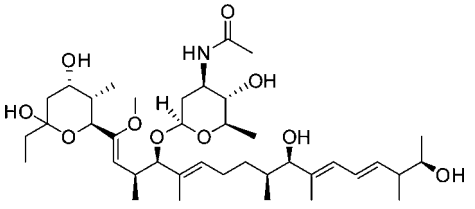
Received October 8, 2012

ABSTRACT



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A new linear polyketide, juniperolide A (1), was produced by the terrestrial actinomycete (Lv1-48) isolated from the rhizosphere of the plant *Juniperus excelsa*. The juniperolide A (1) structure contains a THP unit and a 3-amino-2,3,6-trideoxyhexose as the glycosidic moiety. Mosher's analysis was used for absolute stereochemistry determinations at C-2, C-8, C-20, and C-4′, while the relative stereochemistry assignments of the remaining stereocenters were based on ROESY correlations and *J*-based coupling.

A *Streptomyces* sp. (Lv1-48) cultivated from the rhizosphere of the plant *Juniperus excelsa* isolated in Ukraine presented an interesting chemical profile. HPLC-DAD-MS analysis of a small-scale liquid fermentation revealed the production of juniperolide A (**1**) (m/z 709, λ_{max} = 240 nm). In this letter we describe the isolation and structure elucidation of this novel linear polyketide. HRESI(+)MS analysis of **1** returned a molecular formula ($C_{39}H_{67}NO_{10}$) requiring seven double bond equivalents. The UV absorbance at 240 nm was suggestive of a diene functionality in the structure.

The NMR (methanol-d_4) data (Table 1) revealed resonances for an amide carbonyl (δ_C 173.5), and four double bonds (δ_C 118.9 − 154.7), requiring **1** to incorporate two rings. Interpretation of the COSY correlations identified six isolated spin systems, with the first one delineating the sequence from the secondary methyl H_3-1 (δ_H 1.11) through the oxymethine H-2 (δ_H 3.65) and extending through to the secondary methyl H_3-25 (δ_H 1.03) and the olefinic methines H-4 (δ_H 5.64) to H-6 (δ_H 5.99) (Figure 1). A second set of correlations defined the sequence from the oxymethine H-8 (δ_H 3.67) through to the secondary methyl H_3-27 (δ_H 0.95) and extending through a pair of methylenes H_2-10 and H_2-11 and terminating at the olefinic methine H-12 (δ_H 5.29). A third comparable set of correlations established the sequence from the oxymethine H-14 (δ_H 3.54) through the secondary methyl H_3-29 (δ_H 0.99) to the olefinic methine H-16 (δ_H 4.65). The fourth set of correlations delineated a sequence from the oxymethine H-18 (δ_H 4.20) through the secondary methyl H_3-30 (δ_H 0.85) and extending to a second oxymethine H-20 (δ_H 3.92) which in turn was correlated to the methylene H_2-21. COSY correlations also defined an ethyl group (H_3-24, δ_H 0.93; H_2-23, δ_H 1.60). Diagnostic two and three bond HMBC correlations from the tertiary methyl's (i) H_3-26 (δ_H 1.72) to C-8 (δ_C 83.0), C-7 (δ_C 138.2), and C-6 (δ_C 127.7) and (ii) H_3-28 (δ_H 1.57) to C-14 (δ_C 92.7), C-13 (δ_C 136.4), and C-12 (δ_C 129.5) led to the construction of the sequence C-1 to C-16. Further HMBC correlations from (i) H-18 to C-16 (δ_C 118.9) and

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10.1021/ol302766z
Published on Web 11/21/2012

Table 1. NMR (500 MHz, Methanol-d_4) Data for Juniperolide A (**1**)

position	δ_H, mult (*J* in Hz)	δ_C	COSY	HMBC	ROESY
1	1.11, d (*6.4*)	20.2	2	2, 3	4
2	3.65, m	72.1	1, 3	1, 3, 4, 25	3, 25
3	2.25, m	45.3	2, 4, 25	1, 2, 4, 5, 25	1, 2, 5, 6
4	5.64, dd (*15.2*, *8.4*)	136.9	3, 5	2, 3, 5, 6, 25	1, 6, 25
5	6.33, dd (*15.2*, *10.9*)	127.5	4, 6	3, 6, 7	3, 25, 26
6	5.99, d (*10.9*)	127.7	5	4, 5, 8, 26	3, 4, 8, 10a/b
7		138.2			
8	3.67, m	83.0	9	6, 9, 26	6, 10a, 27
9	1.65, m	36.7	8, 10b, 27		
10a	1.34, m	34.0	10b		6, 8, 12
10b	1.06, m		9, 10a, 11a	27	6
11a	2.10, m	25.9	10b, 11b, 12	12, 13	12, 28
11b	1.95, m		11a	12, 13	12
12	5.29, m	129.5	11a	10, 11, 14, 28	10a, 11a/b, 14, 16, 1′
13		136.4			
14	3.54, d (*9.1*)	92.7	15	12, 15, 16, 28, 29	1′, 12, 16, 29
15	2.85, m	33.9	14, 16, 29	14, 16, 17, 29	28
16	4.65, d (*9.7*)	118.9	15	14, 15, 17, 18, 29	12, 14, 18, 19, 29
17		154.7			
18	4.20, d (*10.8*)	73.2	19	16, 17, 19, 20, 22, 30	16, 17-OMe, 30
19	1.70, m	37.9	18, 20, 30		16
20	3.92, br s	70.7	19, 21		30
21a	2.08, m[a]	38.9	20	20	
21b	1.90, dd (*14.0*, *3.3*)			20	
22		99.9			
23	1.60, m	26.3	24	22, 24	
24	0.93, t (*7.5*)	8.0	23	22	
25	1.03, d (*6.9*)	16.4	3	2, 3, 4	2, 4, 5
26	1.72, s	12.5		6, 7, 8	5
27	0.95, d (*6.6*)	15.6	9	8, 9, 10	8
28	1.57, s	12.2		12, 13, 14	11a, 15
29	0.99, d (*6.6*)	18.3	15	14, 15, 16	14, 16
30	0.85, d (*6.7*)	14.4	19	18, 19, 20	18, 20
5′-Me	1.21, d (*6.2*)	18.4	5′	4′, 5′	4′
31	1.97, s	22.5		6′	
1′	4.50, dd (*9.8*, *1.5*)	101.1	2′a/b	2′, 14	2′a, 3′, 5′, 12, 14
2′a	2.08, m[a]	38.3	1′, 2′b, 3′	1′, 3′, 4′	1′, 3′
2′b	1.44, m		1′, 2′a, 3′	1′, 3′, 4′	4′
3′	3.80, m	52.6	2′a/b, 4′	2′, 4′, 5′, 6′	1′, 2′a, 5′
4′	2.93, dd (*9.4*, *9.3*)	75.8	3′, 5′	3′, 5′, 5′-Me	2′b, 5′-Me
5′	3.19, m	74.7	4′, 5′-Me	1′, 3′, 4′, 5′-Me	1′, 3′
6′		173.5			
17-OMe	3.64, s	59.9		17	18

[a] Overlapping signals. Assignments supported by HSQC and HMBC experiments.

C-17 (δ_C 154.7) and (ii) a methoxy H_3-17 (δ_H 3.64) to C-17 led to the extension of this sequence.

HMBC correlations from the oxymethine H-18 and the primary methyl H_3-24 to the hemiketal carbon C-22 (δ_C 99.9) suggested the presence of a tetrahydropyran ring (THP) leading to the construction of subunit A (Figure 1). A *trans*-diequatorial relationship of the substitutents at C-18 and C-19 was established based on a large coupling ($J_{18,19}$ = 10.8 Hz). The relative configuration of C-19 to C-20 was established as (*S,S,S*) based on the proton signal H-20 (δ_H 3.92) which appeared as a broad singlet supporting its equatorial orientation. A large coupling of ($J_{4,5}$ = 15.2 Hz) defined the *E* $\Delta^{4,5}$ configuration, while ROESY correlations from the olefinic methyl (i) H_3-26 (δ_H 1.72) to H-5 (δ_H 6.33) and (ii) H_3-28 (δ_H 1.57) to H-11a defined the *E* $\Delta^{6,7}$ and $\Delta^{12,13}$ configuration.

An additional ROESY correlation of the olefinic methine H-16 (δ_H 4.65) to H-18 allowed the establishment of the *E* $\Delta^{16,17}$ configuration. From the 2D NMR correlations (Figure 1) the remaining resonances were attributed to a 3-amino 2,3,6-trideoxyhexose moiety (C-1′−C-6′). The conformation of the sugar was determined by vicinal $^1H-^1H$ coupling constants and through a well-defined set of ROESY correlations. The large coupling observed for the anomeric proton H-1′ ($J_{1',2'}$ = 9.8 Hz) and the oxymethine H-4′ ($J_{3',4'}$ = 9.3 Hz; $J_{4',5'}$ = 9.4 Hz) coupled with ROESY correlations between H-1, H-3, and H-5 (Figure 1) confirmed their axial orientations. The acetamide

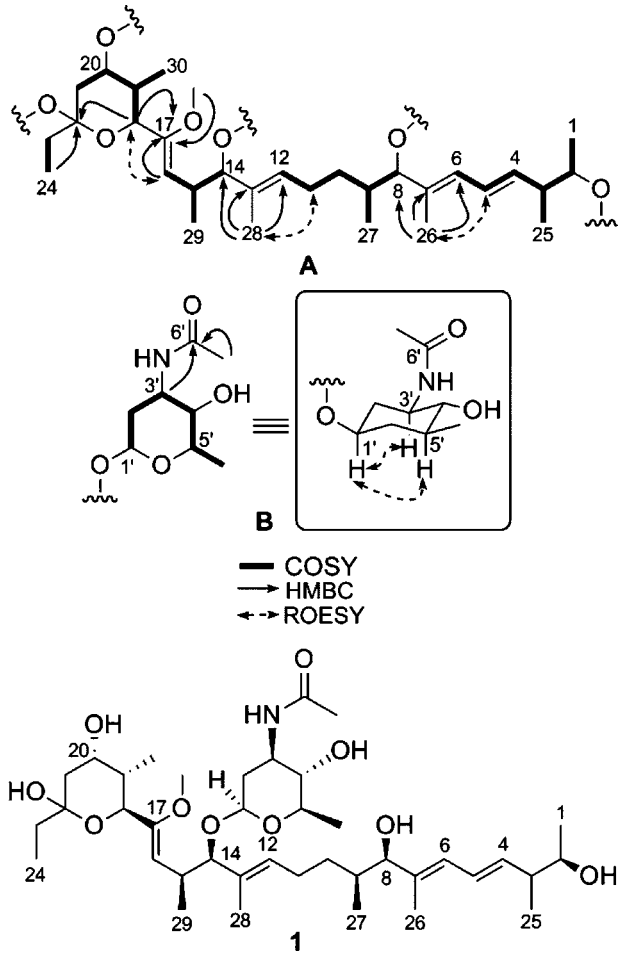

Figure 1. Key 2D NMR (500 MHz, methanol-d_4) correlations and the structure for **1**.

point of attachment on the sugar was confirmed by HMBC correlations from the methine H-3′ (δ_H 3.80, δ_C 52.6) to the acetamide carbonyl C-6′ (δ_C 173.5). The absolute stereochemistries of the hydroxyl-bearing carbons in **1** (C-2, C-8, C-20, and C-4′) were determined by the Mosher method.[1] Treatment of **1** with the (*R*)- and (*S*)-MTPA acid [α-methoxy-α-(trifluoro-methyl)phenyl-acetyl acid] and DCC gave the (*R*)- and (*S*)-MTPA penta esters (**1a** and **1b**) (Figures S8 and S11) in reasonable yields. This process also led to the opening of the sensitive (THP) ring which was confirmed by an HMBC correlation from the primary methyl H_3-24 to the ketone carbonyl C-22 (δ_C 210.0). The ^{1}H NMR spectra of the penta-Mosher ester derivatives led to the calculation of the $\Delta\delta_{S-R}$ values (Figure 2) allowing for the assignment of the absolute configurations of C-2, C-8, C-20, and C-4′ as 2*R*, 8*R*, 20*S*, and 4′S. The absolute configuration of C-18 could not be assigned based on the positive $\Delta\delta_{S-R}$ values on both sides of the stereogenic center.

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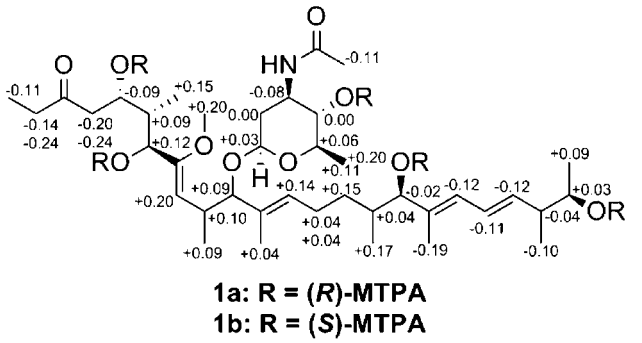

Figure 2. $\Delta\delta_{S-R}$ values for penta-MTPA derivatives (**1a** and **1b**) derived from juniperolide A (**1**).

Next, from the 1D TOCSY a large coupling of J = 7.9 Hz between H-8 and H-9 suggested an *anti*-relationship between these protons. These data together with ROESY correlations between H_3-27 and H-8, between H-8 and H-10a, and between H-6 and H-10a revealed a *threo* configuration between Me-27 and the hydroxyl group at C-8 (Figure 3).[6] Therefore, the configuration at C-9 was established as *S*. A medium coupling of $^3J_{H\text{-}2\text{-}H\text{-}3}$ = 4.8 Hz obtained from an E.(COSY) spectrum suggested an equilibrium between *gauche* and *anti* rotamers between C-2 and C-3. Unfortunately medium coupling constant values for $^3J_{H\text{-}2,C\text{-}4}$ (3.8 Hz) and $^3J_{H\text{-}2,C\text{-}25}$ (3.6 Hz) were obtained from *J*-resolved (HMBC) experiments. Based on these data it was not possible to differentiate between *threo* and *erythro* configurations for chiral centers C-2 and C-3.[6]

A large vicinal coupling (J = 9.1 Hz) between H-14 and H-15 indicated their *anti*-relationship. ROESY correlations between H-14 and H_3-29, between H-14 and H-16, between H-16 and H-12, between H-1′ and H_3-29, and between H-15 and H_3-28 revealed a 14*R*, 15*S* configuration (Figure 3).

The 3-amino-2,3,6-trideoxyhexose glycosidic moiety has been previously observed from the well-known anthracycline antibiotics, such as daunorubicin[2] and adriamycin.[3]

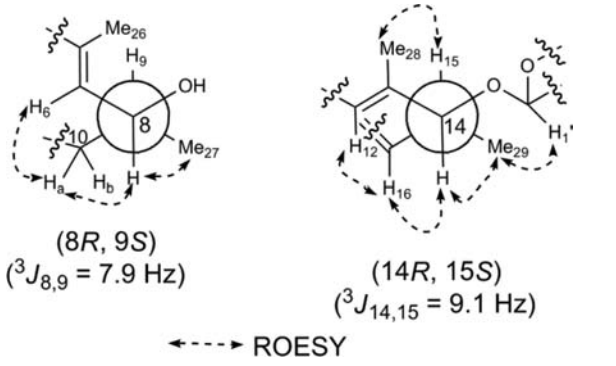

Figure 3. Newman projections showing ROESY correlations and $^3J_{H-H}$ values used to establish the relative configuration of C8/C9 and C14/C15 in **1**.

However, attachment of this hexose unit to a linear polyketide has not been reported to date to the best of our knowledge. Recently, analysis of plant-derived actinomycetes has led to the discovery of novel structural scaffolds. Noteworthy examples include the polycyclic polyketide alchivemycin A isolated from a *Streptomyces* sp. cultivated from a leaf of a Chinese chive[4] and the hybrid isoprenoids drimentines F and G, isolated from a reed rhizosphere soil derived *Streptomyces* sp.[5] Despite the structural novelty of juniperolide A (**1**), there was no significant antimicrobial or cytototoxic activity observed in initial assays. However, the screening of juniperolide A on several other bioassays is currently ongoing.

In conclusion, a novel linear polyketide harboring a 3-amino-2,3,6-trideoxy hexose glycosidic residue was isolated from a plant-derived actinomycete.

Acknowledgment. We thank Angela Kling (HIPS) for performing the biological assays. Research in R.M.'s laboratory was supported by grants from the BMBF and the DFG.

Supporting Information Available. Full details of the collection, cultivation, and taxonomy of strain Lv1-48 and of the isolation, purification, and 1D and 2D NMR data of leopolic acid A. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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