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Lucknolides A and B, Tricyclic Ketal-lactone Metabolites from a Terrestrial *Streptomyces* sp

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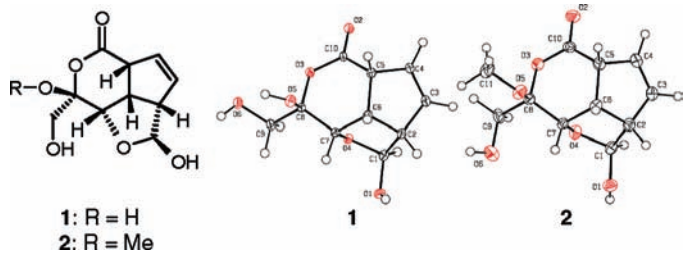
ABSTRACT



In a screening of micro-organisms for new secondary metabolites, two unprecedented tricyclic highly functionalized ketal-lactone metabolites, named lucknolide A (1) and lucknolide B (2), have been isolated, and the compounds were characterized by extensive NMR and mass spectroscopic studies. Single-crystal X-ray diffraction experiments on 1 and 2 were performed, and the absolute configuration of 1 was determined.

The emergence of resistance, new diseases, novel drug targets, and the concept of personalized medicine along with a declined rate of new chemical entities has created an urgent demand for new lead structures. To meet these challenges, an effective solution lies in the chemical and biological diversity of nature.[1] As a part of our ongoing program for biological and chemical screening of terrestrial bacteria, we have selected the *Streptomyces* sp. ANK-289, which showed violet to deep blue colored spots on TLC with an anisaldehyde/H_2SO_4 spray reagent in the chemical screening of the crude extract. Chromatographic purification of the extract led to isolation of two unprecedented tricyclic, highly functionalized ketal-lactone metabolites named lucknolides A and B (**1** and **2**). In addition to lucknolides, extensive chromatography of another fraction with a deep blue coloration with anisaldehyde/sulfuric acid spray reagent on TLC gave 2,7-dimethyl-nonane-1,3,4,8-tetrol (**3**) as a colorless oil.

Lucknolide A (**1**) was obtained as colorless crystals giving a deep violet color reaction on TLC with anisaldehyde/H_2SO_4. High-resolution electrospray ionization mass spectrometry (HRESIMS) of the pseudomolecular ion $[M + Na]^+$ confirmed the molecular formula to be $C_{10}H_{12}O_6$ (exptl *m*/*z* 251.05270, calcd for $C_{10}H_{12}O_6Na$, 251.05262). Dereplication using AntiBase[2] and the Chemical Abstracts showed that this formula could not be assigned to any other known metabolite.

Analysis of ^{13}C NMR data suggested the presence of one double bond and a number of other functional groups: one ester/lactone carbonyl, one acetal methine, one ketal, one oxymethine, one oxygenated methylene, along with three

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(1) Li, J. W.-H.; Vederas, J. C. *Science* **2009**, *325*, 161–165.

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Table 1. NMR Data of Compounds **1**, **2**, and **3**

	1			**2**			**3**		
no.	^{1}H (δ_H, mult., J Hz)[a]	^{13}C (δ_C)[b]	HMBC	^{1}H (δ_H, mult., J Hz)[a]	^{13}C (δ_C)[b]	HMBC	^{1}H (δ_H, mult., J Hz)[c]	^{13}C (δ_C)[d]	HMBC
1	5.58 (br s)	102.82	2, 3, 6, 7	5.58 (br s)	103.0	2, 3, 6, 7	3.51 (dd, 10.7, 5.6) 3.69 (dd, 6.4, 5.6)	64.8	2, 3, 11
2	3.63 (br d, 7.8)	60.3	1, 3, 4, 5, 6	3.61 (br d, 7.8)	60.6	3	1.92 (d, 5.4)	38.2	1, 3, 4, 11
3	6.16 (m)	132.46	1, 2, 4, 5, 6	5.94 (m)	133.0	2, 5, 6	3.36 (m)	78.7	1, 2, 4, 5, 11
4	5.91 (m)	132.41	2, 3, 5, 6	6.17 (m)	132.4	2, 5, 6	3.50 (m)	74.1	2, 3, 5, 6
5	4.01 (dt, 10.1, 2.4)	48.4	3, 4, 6, 10	3.89 (dt, 9.9, 2.2)	48.7	4, 10	1.72 (m) 1.32 (m)	31.4	3, 4, 6, 7
6	3.72 (m)	42.3	1, 2, 5, 10	3.57 (ddd, 9.9, 8.0, 5.4)	42.4	1, 3, 4, 10	1.73 (m) 1.17 (m)	30.3	4, 5, 7, 8
7	5.30 (d, 5.2)	74.3	5, 9	5.36 (d, 5.4)	73.3	5	1.41 (m)	41.4	5, 6, 8, 9
8	-	102.81	-	-	105.4	-	3.64 (m)	71.7	6, 7, 9
9	4.36 (d, 11) 4.34 (d, 11)	65.3	7, 8	4.34 (d, 12.2) 4.28 (d, 12.2)	59.2	7, 8	1.13 (d, 6.4)	20.4	6, 7, 8
10	-	171.9	-	-	171.4	-	0.92 (d, 6.8)	15.1	6, 7, 8, 9
OMe	-	-	-	3.60 (s)	50.2	8	-	-	-
11							0.97 (d, 7.1)	14.9	1, 2, 3, 4

[a] Pyridine-d_5, 600 MHz. [b] Pyridine-d_5, 125 MHz. [c] Methanol-d_4, 600 MHz. [d] Methanol-d_4, 125 MHz.

methine functionalities. These accounted for two out of the five double bond equivalents calculated from the molecular formula. Analysis of the HSQC spectrum allowed the assignment of protons to carbon signals as well as (indirectly) their multiplicities (Table 1). The ^{1}H−^{1}H COSY correlations of H-1−H-7 clearly indicated a fused cyclopentene system (fragment **1a** in Figure 1). A 2H AB signal at δ 4.36/4.34

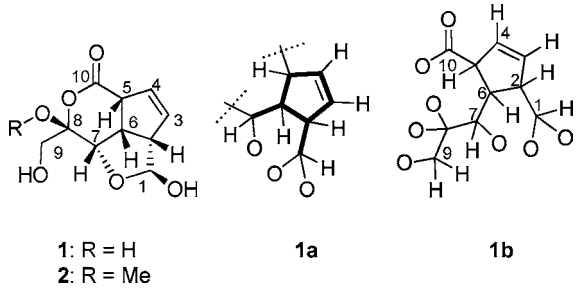

Figure 1. Structure of lucknolide A (**1**) and lucknolide B (**2**). Bold bonds in substructure **1a** indicate ^{1}H−^{1}H COSY correlations; partial structure **1b** was derived from ^{1}H−^{1}H COSY and HMBC correlations.

suggested isolated diastereotopic oxymethylene protons (H_2-9), which showed HMBC correlations with the quarternary ketal carbon C-8 at δ 102.81 and C-7 at δ 74.3, resulting in fragment **1b** (Figure 1).

The correlation of protons H-5 and H-6 with C-10 at δ 171.9 indicated the presence of a carbonyl group attached to C-5. Further HMBC correlations (Figure 2) of H-1 with C-7 suggested an oxygen bridge between C-1 and C-7. Moreover, quarternary ketal C-8 adjacent to oxymethylene C-9 and oxymethine C-7 had to be in a lactone ring to justify the presence of a ketal and to satisfy the remaining three double bond equivalents, resulting in a tricyclic structure as depicted in structure **1**. The conclusion was further reaffirmed by the results of a COCON simulation on the basis of the experimental 1D and 2D NMR data: We received again structure **1** as the only possibility.[3]

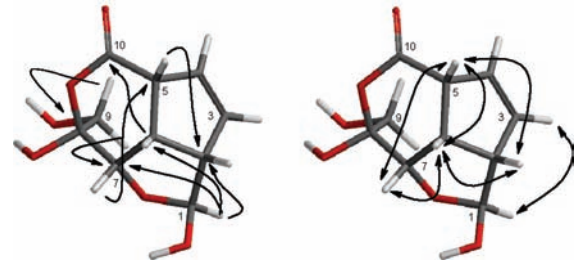

Figure 2. Selected HMBC (left) and NOESY (right) correlations of lucknolide A (**1**).

The NOESY correlations (Figure 2) of proton H-6 with H-2, H-5, and H-7 indicated their *syn* orientation. An unambiguous proof of the assignment of the absolute configuration was achieved by a single-crystal X-ray diffraction (XRD) experiment (Figure 4).[4] Structure determination was possible from the anomalous signal mainly of the oxygen atoms by full-matrix least-squares refinement of the Flack parameter[5] after invariom refinement.[6] While in an independent atom model (IAM) refinement, the value and (in brackets) standard uncertainty of the Flack parameter were −0.06 (0.12), and they could be significantly reduced to −0.08 (0.08) using an aspherical scattering model. Hence,

Figure 3. Selected HMBC (left) and NOESY (right) correlations of lucknolide B (**2**).

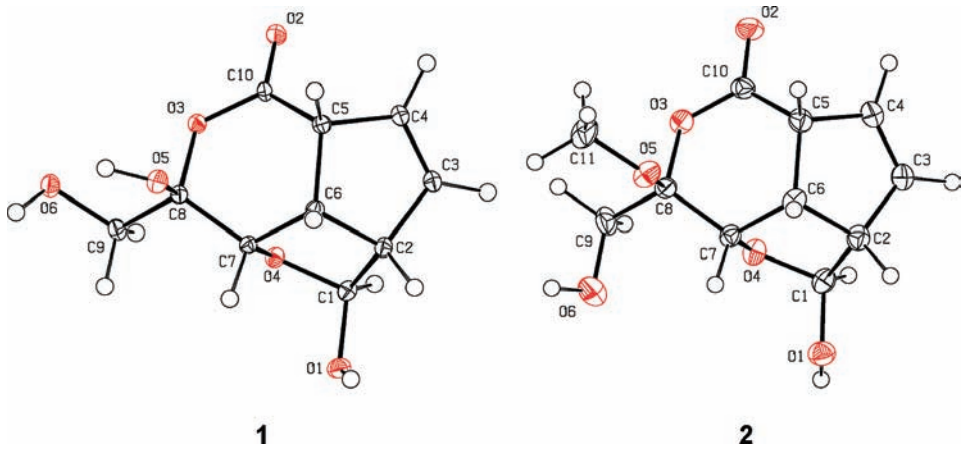

Figure 4. ORTEP representation of lucknolides A and B (**1** and **2**). Ellipsoids with 50% probability.

absolute stereochemistry of all the chiral centers was determined to be (1*S*,2*S*,5*R*,6*S*,7*S*,8*S*) (Figure 4).

Lucknolide B (**2**) was obtained as white crystalline solid and gave a deep violet color reaction on TLC with anisaldehyde/H_2SO_4 spray reagent as well. The molecular formula of $C_{11}H_{14}O_6$ was derived from HRESIMS by *m*/*z* 265.06838 $[M + Na]^+$ (calcd for $C_{11}H_{14}O_6Na$, 265.06827). ^1H and ^{13}C NMR data (Table 1) of **2** were similar to those of **1** and indicated the presence of an additional methoxy group, in agreement with the empirical formula.

Spectroscopic comparison of **2** with **1** revealed that both shared the same tricyclic structural framework with the same substitution pattern. The methoxyl group was placed at C-8 based on its HMBC correlations (Figure 3) with C-8 at δ 105.4. Further HMBC correlations (Figure 3) led to structure **2** (Figure 1). The same stereostructure for both compounds was revealed from the similar NOESY correlations (Figure 2 and 3) and from the X-ray diffraction experiment (Figure 4): The molecular connectivity of lucknolide B (**2**) could also be confirmed by XRD.[7] However, here values of the Flack parameter and its standard deviation did not allow an unambiguous assignment of absolute configuration due to less good scattering power of the crystals investigated. For all refinements, the program XDLSM[8] was used.

A substructure search in AntiBase[2] for the carbon framework of **1b** gave echinosporin and deoxyechinosporin as related compounds,[9,10] which differ, however, in functionalities and the mode of cyclization. Literature reports showed that echinosporin biosynthesis follows the shikimate pathway via chorismate as a possible later intermediate. It can be speculated that lucknolides A and B (**1** and **2**) originate from a similar biosynthetic pathway.[10]

For a third compound **3** (2,7-dimethyl-nonane-1,3,4,8-tetraol), HRESI MS of the pseudomolecular ion at *m*/*z* 243.15702 ($[M + Na]^+$, calcd 243.157019 for $C_{11}H_{24}O_4Na$) confirmed the molecular formula as $C_{11}H_{24}O_4$. Analysis of ^{13}C NMR and HSQC data indicated the presence of three oxygenated methines and one oxygenated methylene group but no quarternary carbon atom. Analysis of the HSQC spectrum delivered the positions of protonated carbons and their hydrogen assignments as shown in Table 1. The $^1H-^1H$ COSY and HMBC couplings clearly indicated a straight carbon chain and confirmed the positions of the methyl groups. The bond between C-5/C-6 could be proven only by a weak HMBC correlation between these methylene groups, due to closely related proton shifts. However, as this is the only way to connect the fragments C-1−C5 and C-6−C9, structure **3** resulted as depicted in Figure 5.

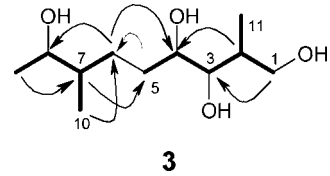

Figure 5. Structure and selected HMBC correlations of compound **3**. Bold: bonds were confirmed by $^1H-^1H$ COSY correlations.

Compounds **1** and **2** were found to be inactive in the agar diffusion test against *Escherichia coli*, *Bacillus subtilis*,

(2) Laatsch, H. *AntiBase 2010, A Data Base for Rapid Dereplication and Structure Determination of Microbial Natural Products*; Wiley VCH: Weinheim, Germany, 2010. See http://wwwuser.gwdg.de/~ucoc/laatsch/antibase.htm.

(3) Lindel, T.; Junker, J.; Köck, M. 2D-NMR-guided constitutional analysis of organic compounds employing the computer program COCON. *Eur. J. Org. Chem.* **1999**, 573–577.

(4) CCDC, reference number 754035.

(5) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–88.

(6) Dittrich, B.; Strumpel, M.; Schäfer, M.; Spackman, M. A.; Koritsánszky, T. *Acta Crystallogr.* **2006**, *A62*, 217–223.

(7) CCDC, reference number 754036.

(8) Koritsanszky, T.; Richter, T.; Macchi, P.; Volkov, A.; Gatti, C.; Howard, S.; Mallinson, P. R.; Farrugia, L.; Su, Z. W.; Hansen, N. K. *XD - Computer Program Package for Multipole Refinement and Topological Analysis of Electron Densities from Diffraction Data*; Freie Universität Berlin: Germany, 2003.

(9) Sato, T.; Kawamoto, I.; Oka, T.; Okachhi, R. *J. Antibiot.* **1982**, *35*, 266–271.

(10) Dübeler, A.; Krastel, P.; Floss, H. G.; Zeeck, A. *Eur. J. Org. Chem.* **2002**, 983–987.

Streptomyces viridochromogenes, and *Staphylococcus aureus*; antifungal activities against the oomycetes *Botrytis cinerea*, *Septoria tritici*, *Pyricularia oryzae*, and *Phytophthora infestans* were also not found. Compound **3** could not be tested due to insufficient amounts.

The unusual structural framework with a highly functionalized fused tricyclic ring system reported here for **1** and **2** has no counterpart in the literature. Their structures invite innovative approaches for synthesis and to explore their possible biological targets.

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Supporting Information Available: Experimental procedure, taxonomy of the producing strain, ^{1}H, ^{13}C, and 2D NMR data (HSQC, HMBC, and NOESY), HRESI MS, CD spectra, and crystallographic data of lucknolide A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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