

Short Note

Methyl-4-hydroxy-2-(2-hydroxypropan-2-yl)-6-methyl-2,3-dihydrobenzofuran-5-carboxylate

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Abstract: Microorganisms are an important source of compounds that are pharmaceutically active, both as leads and as scaffolds for synthesis. Often, interesting chemistry is uncovered by exploring new biomes, of which the Chilean Atacama Desert is a prime example. This paper describes the isolation and structural characterisation, using HR-LCMS and 1D and 2D NMR, of a new compound belonging to a family of compounds called radstrictins. The compound was isolated from a fungus, that had itself been isolated from a soil sample from the Atacama Desert. The compound was tested against pathogenic strains associated with bovine mastitis, but was found to be devoid of antimicrobial activity.

Keywords: radstrictins; natural products; Atacama Desert; fungi; high-resolution MS; 2D NMR; bovine mastitis

1. Introduction

Natural products are a source of diverse chemical scaffolds for drug discovery. However, due to issues such as limited availability and a high rate of rediscovery, their use has diminished over the past few decades. Nonetheless, new chemistry can be discovered in unexplored and extreme ecosystems, such as deserts. The Atacama Desert is a hyper-arid and ancient desert [1] in the north of Chile, characterised by a high salinity, high level of UV radiation, and long stretches of time with little to no precipitation. Often, little life is detected. However, several interesting, structurally diverse, and useful classes of secondary metabolites have been isolated from microorganisms found in this desert. This includes chaxamycins [2], lentzeosides [3], and lasso-peptides, among several others [4]. In search of new chemistry, soil samples were collected from various locations in the Atacama Desert. This paper reports the isolation and structural characterisation of radstrictin K (1), which was isolated from a fungal strain obtained from one of the soil samples collected, which is a new member of a family of prenylated aromatic compounds previously isolated from the Chinese liverwort *Radula constricta* [5]. This paper also reports activity against pathogenic strains associated with bovine mastitis, a highly infectious disease which causes inflammation in the udders of cows, leading to discarded milk; the related financial loss in the industry is often estimated to be in the hundreds of millions of USD per year [6].

2. Results and Discussion

The LCMS spectrum of 1 showed a [M+H]⁺ molecular ion at *m/z* 267.1233 and a [M+Na]⁺ ion at *m/z* 289.1038, consistent with a molecular formula of C₁₄H₁₈O₅ (Δ −0.1 ppm), and a double bond equivalence of six. The COSY spectrum (full spectrum in Figure S3) established the correlation between the triplet at δ_{H} 4.62 (H-2) and the doublet at δ_{H} 3.00 (H₂-1). The HSQC spectrum (full spectrum in Figure S4) revealed that the singlet at δ_{H} 6.25 (H 5') and the associated signal at δ_{C} 104.7 were part of the aromatic ring. The hydroxylated isopropyl moiety in the side chain was established based on the HMBC correlations (full spectrum in



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Figure S5) of δ_{H} 1.10 (H₃-4a/H₃-4b) and δ_{C} 90.9 (C-2), as well as δ_{C} 70.5 (C-3). The furan ring annelated to the aromatic ring was indicated by the correlations of δ_{H} 4.62 and δ_{C} 163.6 (C-6'), as well as H₂-1 and δ_{C} 111.5 (C-1'), δ_{C} 157.1 (C-2'), and C-6'. A sharp singlet at δ_{H} 10.85 was indicative of a phenolic hydroxy group chelated to the nearby carbonyl group C-7'. The methyl ester was revealed by the correlation of δ_{H} 3.83 (H₃-8') and δ_{C} 171.0 (C-7'). Further correlations of the aromatic methyl protons H₃-9' (δ_{H} 2.34) and 108.4 (C-3'), 141.5 (C-4'), and δ_{C} 104.7 (C-5') established the positions of the substituents on the aromatic ring. The full set of NMR data is summarised in Table 1 (full ¹H spectrum in Figure S1 and full ¹³C spectrum in Figure S2). On this basis, the planar structure of **1** was established as depicted in Figure 1, representing a new compound, for which the name radstrictin K is suggested. The key HMBC correlations used to elucidate structure are indicated in Figure 2.

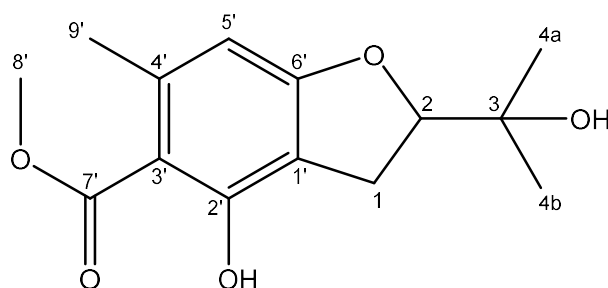


Figure 1. The structure of compound **1**.

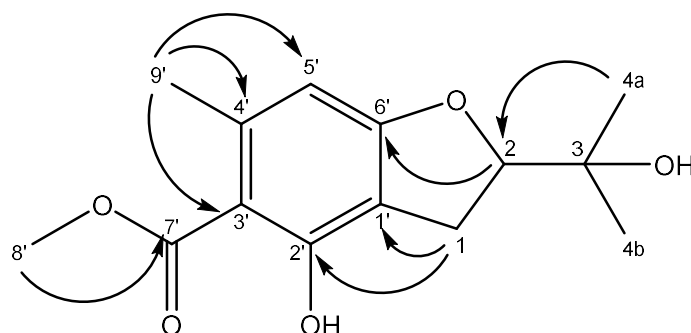


Figure 2. The key HMBC correlations observed for radstrictin K (**1**).

Table 1. The NMR data of radstrictin K (**1**) in DMSO-*d*₆.

Position	δ_{C} (mult)	δ_{H} (J/Hz)	COSY	HMBC (H→C)
1	27.6 CH ₂	3.00 (d, 8.2)	2	1', 2, 2', 3, 6'
2	90.9 CH	4.62 (t, 8.5)	1	4a/b, 6'
3	70.5 C			
3-OH		4.63 (br s)		2, 3, 4a/b
4a	25.1 CH ₃	1.10 (s)		2, 3, 4b
4b	26.4 CH ₃	1.11 (s)		2, 3, 4a
1'	111.5 C			
2'	157.1 C			
2'-OH		10.85 (s)		1', 2', 3'
3'	108.4 C			
4'	141.5 C			
5'	104.7 CH	6.25 (s)		1', 3', 6', 9'
6'	164.4 C			
7'	171.0 C			
8'	52.39 CH ₃	3.83 (s)		7'
9'	23.1 CH ₃	2.34 (s)		3', 4', 5'

The optical rotation of **1** was measured in methanol at 0.81 mg/mL as $^{21}[\alpha]_D +24.6^\circ$. It is interesting to note that the closest structural relative to **1** is radstrictin B (**2**) (Figure 3), which was previously isolated from the Chinese liverwort *Radula constricta*, and which was concluded to be present as a scalemic mixture, based on an optical rotation value of $+1.1^\circ$, in combination with an in-depth analysis of its CD spectrum [5].

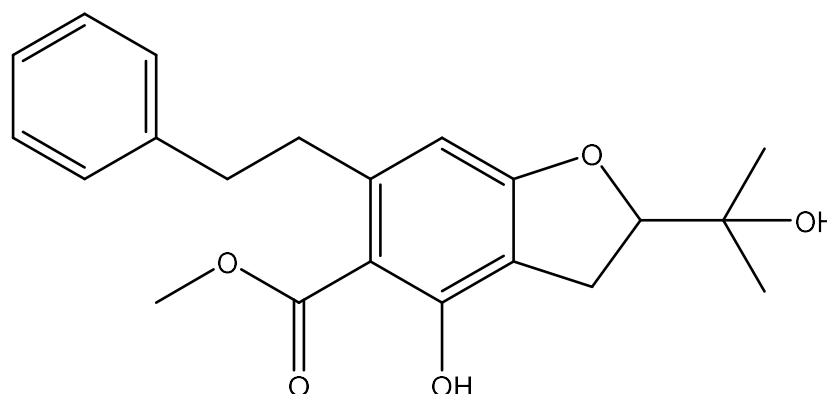


Figure 3. The structure of radstrictin B (**2**), isolated from the Chinese liverwort *Radula constricta*.

In this previous study, the authors assigned the absolute configuration at C-2 as R for the dextrorotatory enantiomer, and S for the levorotatory enantiomer, based on the circular dichroism spectrum of the mixture, and assumed that the overall low absolute value they measured was indicative of a scalemic mixture [5]. The compound was dissolved in methanol, and resolved using a chiral HPLC column, which gave a single peak, confirming that compound **1** is enantiomerically pure.

Compound **1** was tested against five strains, *Enterococcus pseudoavium* NCIMB 13084, *Escherichia coli* NCIMB 701266, *Klebsiella oxytoca* NCIMB 701361, *Staphylococcus aureus subsp. Aureus* NCIMB 701494, and *Streptococcus bovis* NCIMB 702087, using a disk diffusion assay at 100 $\mu\text{g/mL}$, but was found to be inactive. These strains are associated with bovine mastitis, a devastating infectious disease affecting cattle worldwide, and causing losses estimated to be around USD 500 million per year in the USA alone [6,7].

3. Materials and Methods

3.1. Isolation and Cultivation of Fungus

The soil sample was collected as described previously [8] from 23.00.489 S, 67.45.309 W, at an altitude of 5046 m. The isolated fungus (strain # GP-11) was submitted to NCIMB (Aberdeen, UK) for identification (GenBank accession # NC023088_GP-11), but did not reveal a sufficiently high species-level match in the MicroSeq database. Therefore, the sequence was searched against the non-validated EMBL public database, with the top matches *Chrysosporium merdarium* and *Geomyces pannorum* var. *pannorum* each displaying 99.66% similarity. The strain was stored on ISP2 plates at 4 $^\circ\text{C}$, and in 25% glycerol stock solutions at -80°C . The fungus was cultivated for 6 weeks, in a 2 L Erlenmeyer flask containing 160 g of rice and 160 mL of water autoclaved at 121 $^\circ\text{C}$ for 20 min.

3.2. Isolation and Structural Characterisation of Compound **1**

After cultivation, the culture was extracted three times, using 300 mL of methanol and ethyl acetate each time. The crude extract was dried under low-pressure conditions, dissolved in 100 mL of MilliQ water, and subjected to Kupchan partitioning [9]. The DCM fraction (200 mg) was subjected to HPLC, which used an Agilent 1100 series binary pump and 1100 series diode array detector with a Sunfire C18 (10 μm , 10 mm \times 250 mm) column, at 2 mL/min. The method used started at 100% A, and ended at 100% B after 90 min, followed by 15 min of the isocratic condition where A is 95% water and 5% methanol, and B is methanol, resulting in 2 mg of radstrictin K (**1**) (with a retention time of 78 min) being

isolated (the full HPLC trace is shown in Figure S7). Chiral HPLC was performed using the same HPLC system, with a Chiralcel OD-H column (5 μm , 4.6 mm \times 250 mm) from Daicell chemical industries, Ltd. The mobile phases used were methanol and ethanol at 1 mL/min, with the method starting from 100% methanol, and ending at 90% methanol at 20 min. Under these conditions, the compound has a retention time of 4.18 min (the full HPLC trace is shown in Figure S6).

LC-MS was performed using an Agilent 1290 infinity UHPLC and a Phenomenex Kinetex XB-C18 (2.6 μm , 100 \times 2.1 mm²) column, with the mobile phases as 5% acetonitrile + 0.1% formic acid and 94.9% water, to 100% acetonitrile + 0.1% formic acid, over the course of 15 min. The Bruker Maxis Q-tof II (Coventry, UK) was used to perform the MS, in which the mass range was set from 100–2000 and a 4.5 kV capillary voltage, the nebuliser gas was set to 4 bar, the dry gas was set to 9 L/min, and the dry temperature was set to 220 °C. The MS/MS experiments were conducted under Auto MS/MS scan mode, with a step collision energy from 80 to 200%. The NMR spectra were acquired at 25 °C using a Bruker Avance III HD 400 MHz system (Coventry, UK) and a Bruker Avance III 600 MHz system (Coventry, UK) equipped with a liquid helium-cooled Prodigy cryo-probe.

4. Conclusions

This paper reports the isolation and structural characterisation of radstrictin K (compound **1**), a new compound isolated from a fungus from the Atacama Desert. This compound belongs to the family of compounds isolated from the Chinese liverwort *radula constricta*, among which it is similar to radstrictin B; however, compound **1** lacks the phenyl ethyl chain at position 4'.

Supplementary Materials: Figure S1: ¹H NMR spectrum of **1** in DMSO-*d*₆ at 400 MHz; Figure S2: ¹³C NMR spectrum of **1** in DMSO-*d*₆ at 151 MHz; Figure S3: COSY spectrum of **1** in DMSO-*d*₆ at 400 MHz; Figure S4: HSQC spectrum of **1** in DMSO-*d*₆ at 400 MHz; Figure S5: HMBC spectrum of **1** in DMSO-*d*₆ at 600 MHz; Figure S6: HPLC trace of compound **1** using a chiral column. The second peak is the compound of interest, which was confirmed using the UV measured by the DAD; Figure S7: HPLC trace of the DCM fraction of the fungal extract.

Author Contributions: Conceptualisation, G.P. and R.V.A.; methodology, G.P. and R.V.A.; software, G.P. and R.V.A.; validation, R.E. and M.J.; formal analysis, all authors.; investigation, all authors; resources, M.J.; data curation, G.P. and R.V.A.; writing—original draft preparation, R.V.A.; writing—review and editing, G.P., R.E. and M.J.; visualisation, G.P. and R.V.A.; supervision, R.E. and M.J.; project administration, R.E. and M.J.; funding acquisition, R.E. and M.J. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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