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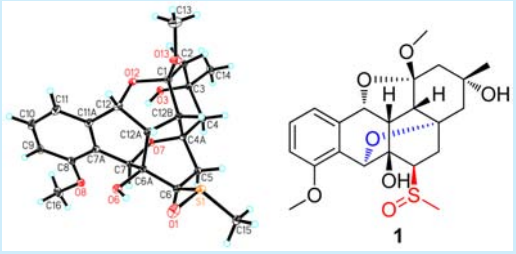
Grisemycin, a Bridged Angucyclinone with a Methylsulfinyl Moiety from a Marine-Derived *Streptomyces* sp.

Zeping Xie,[†] Ling Zhou,[†] Lin Guo,[‡] Xiaoping Yang,[†] Guiwu Qu,[§] Changjing Wu,[†] and Shumin Zhang*[,†]

[†]The Key Laboratory of Traditional Chinese Medicine Prescription Effect and Clinical Evaluation of State Administration of Traditional Chinese Medicine, School of Pharmacy, [‡]School of Enology, and [§]School of Gerontology, Binzhou Medical University, Yantai 264003, China

Supporting Information

ABSTRACT: Grisemycin (**1**), the first sulfur angucyclinone with an unusual ether-bridged system, was isolated from a marine-derived *Streptomyces griseus* strain M268. Its novel, here cage-like, structure was determined by spectroscopic analysis and single-crystal X-ray diffraction. Compound **1** exhibited modestly selective activity against the HL-60 cell line with an IC_{50} value of 31.54 μM. Futhermore, the absolute stereochemistry of kiamycin (**2**), an 1,12-epoxybenz[*a*]anthracene, previously obtained from the same strain, was established by X-ray diffraction analysis.



Actinomycetes, the filamentous bacteria, have proven to be the most prolific producers of antibiotics for the generation of 45% of all reported microbial active metabolities.[1,2] Chemical investigation of actinomycetes from terrestrial habitats has led to the development of anticancer drugs (daunorubicin, mitomycin, bleomycin), anti-infective agents (vancomycin, erythromycin, amphotericin B), immunosuppressants (rapamycin, tacrolimus), agricultural antibiotics (abamectin, blasticidin), etc.[3] However, over the past two decades increasing attention has been focused toward the marine ecosystems, as an important source of novel microbes and, hence, secondary metabolites, due to the development of drug resistance and frequent rediscovery of the same compounds from terrestrial organisms.[4] Studies of marine actinomycetes have prompted the discovery of more than 400 new bioactive metabolites with anticancer and antimicrobial activities.[5−7] For example, salinosporamide A, as a promising new drug for multiple myeloma, has been poised to enter phase II clinical trials.[8,9]

In an effort to identify new bioactive metabolites from marine actinomycetes, *Streptomyces griseus* strain M268 was isolated from sediment collected off Kiaochow Bay, China. Our previous chemical investigation of this strain enabled us to isolate a unique 1,12-epoxybenz[*a*]anthracene (**2**, kiamycin) (Figure 1). Further analysis of the extract by HPLC-UV revealed the production of several metabolites with similar UV absorptions to the angucyclinones isolated previously.[10] A larger scale fermentation (60 L) and repeated separation of the crude extract led to the discovery of a further novel, here cage-like, type of angucyclinone (**1**). In this paper, we report the isolation and characterization of **1**, as well as the absolute stereochemistry of **2**; a plausible biogenetic pathway of **1** and **2** from a common hypothetical precursor is also proposed.

Grisemycin (**1**) was obtained in an optially active form, $[\alpha]_D^{25}$ + 46.5 (MeOH, *c* 0.2). HRESIMS provided a molecular

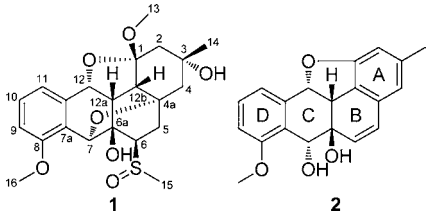


Figure 1. Structures of grisemycin (**1**) and kiamycin (**2**).

formula of $C_{22}H_{28}O_7S$ (*m*/*z* 459.1449 $[M + Na]^+$, Δ = −0.1 ppm) implying nine double bond equivalents (DBE). The ^1H NMR spectrum (Table 1) of **1** in $CDCl_3$ showed resonances for three *ortho*-coupled aromatic protons at δ 6.90 (d, J = 7.9 Hz), 7.34 (t, J = 7.9 Hz), and 7.03 (d, J = 7.9 Hz), two aliphatic hydroxyl groups at δ 4.92 (s) and 3.99 (s), two methoxyl groups at δ 3.82 (s) and 3.36 (s), and two methyls at δ 2.72 (s) and 1.17 (s). The ^{13}C and HSQC NMR spectra further revealed three sp^2 methines at δ 130.1, 122.0, and 111.2, and three sp^2 quaternary carbons at δ 157.3, 135.9, and 122.6. The aliphatic pattern displayed five methines, four oxygenated quaternary carbons, three methylenes, two methoxyls, and two methyls (Table 1). The NMR data indicated a total of six sp^2 resonances, accounting for three DBE and requiring **1** to incorporate six rings.

Examination of the ^1H and COSY NMR data permitted four fragments to be constructed (Figure 2a). The first fragment, a 1,2,3-trisubstituted benzene ring, was established by interpretation of COSY contacts from H-9 to H-11. The second fragment consisted of a methylene group and a methine proton coupled to one another, H-5α (δ 2.48, dd, J = 14.2, 11.2 Hz),

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for 1 in $CDCl_3$[a]

no.	δ_H mult (J in Hz)	δ_C[b]
1		106.5, C
2α	2.46, d (14.2)	41.4, CH_2
2β	1.56, d (14.2)	
3		69.3, C
4α	1.92, dd (14.2, 1.8)	43.8, CH_2
4β	1.36, d (14.2)	
4a		72.1, C
5α	2.48, dd (14.2, 11.2)	34.8, CH_2
5β	1.23, dd (14.2, 1.8)	
6	3.39, dd (11.2, 1.8)	63.1, CH
6a		72.5, C
7	5.27, d (2.2)	69.4, CH
7a		122.6, C
8		157.3, C
9	6.90, d (7.9)	111.2, CH
10	7.34, t (7.9)	130.1, CH
11	7.03, d (7.9)	122.0, CH
11a		135.9, C
12	5.28, d (9.0)	78.0, CH
12a	3.64, td (9.0, 2.2)	40.8, CH
12b	2.40, d (9.0)	51.0, CH
13	3.36, s	47.6, CH_3
14	1.17, s	30.4, CH_3
15	2.72, s	37.6, CH_3
16	3.82, s	55.6, CH_3
3-OH	3.99, s	
6a-OH	4.92, s	

[a]500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. [b]Attached protons were determined by analysis of 2D spectroscopic data.

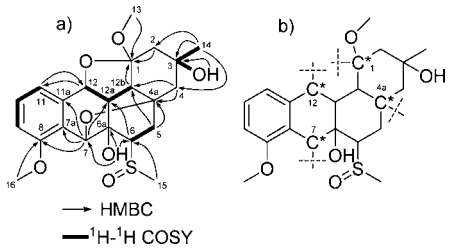

Figure 2. (a) Key COSY and HMBC correlations of 1. (b) Partial structure of 1 with four open valences; C* = oxygenated carbon.

H-5β (δ 1.23, dd, J = 14.2, 1.8 Hz), and H-6 (δ 3.39, dd, J = 11.2, 1.8 Hz). Cross signals of H-12a (δ 3.64, td, J = 9.0, 2.2 Hz) with H-12 (δ 5.28, d, J = 9.0 Hz) and H-12b (δ 2.40, d, J = 9.0 Hz), and considering the $^{1}H-^{1}H$ coupling constants of these three protons, allowed the construction of a third fragment from H-12 to H-12b. The last fragment was established by the COSY couplings between 3-OH and H_3-14 and flanked by a pair of methylenes H_2-2 (δ 2.46, 1.56) and H_2-4 (δ 1.92, 1.36) as indicated by the HMBC correlations from H_3-14 to C-2, C-4, and the oxygenated quaternary carbon C-3, which was further confirmed by an HMBC contact from 3-OH to C-3. These four fragments were subsequently linked based on a series of HMBC correlations: the correlation of H_2-2, H-12b, and the methoxyl group H_3-13 to C-1; of H-4α and H-5α to C-4a and C-12b; of H-6 and H-7 to C-12a; of 6a-OH to C-6, C-6a, and C-7; of H-7 to C-7a, C-8, and C-11a; and of H-11 to C-12 (and *vice versa*) established a nonquinonoid angucyclinone skeleton. In addition, the positions of the residual substituents were assigned by the HMBC correlations of H_3-16/C-8 and H_3-15/C-6; the chemical shifts of C-6 (δ 63.1) and C-15 (δ 37.6) indicated a sulfoxide group located at between C-6 and C-15 based on the molecular formula $C_{22}H_{28}O_7S$.[11] This highlights the structure of 1 for the incorporation of a methylsulfinyl moiety, in that no sulfur angucyclinone has been reported before.

Moreover, four oxygen-bearing carbons (C-1, 4a, 7, and 12) still had open bonds (Figure 2b), and only two oxygens were left to be assigned according to the molecular formula; it was deduced that these four oxygenated carbons should be linked via two additional ether bridges, indicating a significant difference from all epoxy angucyclinones.[12] Two ring closures through these ether bridges also achieved the hexacyclic requirement of 1; however, ether bridges could span between C-1/12 (C-4a/7), C-1/4a (C-7/12), or C-1/7 (C-4a/12), which made it problematic to elucidate the complete structure by NMR analysis, so we proposed the structure of 1 as depicted in Figure 1 by biogenetic reasoning; as mentioned above, the same organism provided an 1,12-epoxybenz[a]anthracene in parallel (2 in Figure 1).

The relative configuration of 1 is tentatively proposed based on the NOE correlations and coupling constants (Figure 3).

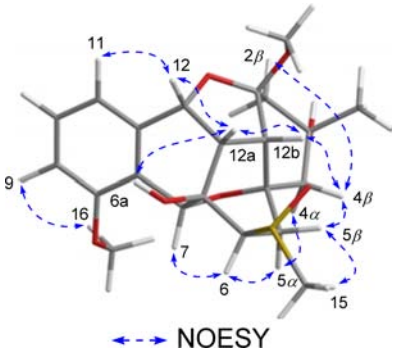

Figure 3. Key NOESY correlations of 1.

The NOEs of 6a-OH with H-12a, of H-12a with H-12 and H-12b, of H-12b with H-4β (δ 1.36, d, J = 14.2 Hz), of H-4β with H-2β (δ 1.56, d, J = 14.2 Hz) and H-5β, and of H-5β with H_3-15 indicate their common orientation that is randomly assigned to be β-oriented, requiring an α-orientation of both C-1/12 and C-4a/7 ether bonds, which further defines the β-orientation of H_3-13 at C-1 and the relative configuration of C-4a. Protons H-7 and H-12a exhibiting a strong W-coupling (J = 2.2 Hz) require a coplanar orientation of the connecting bonds; the NOEs of H-7 with H-6 and of H-6 with H-5α reveal these axially oriented hydrogens to be located on the same side of the subjacent pyran ring with an α-orientation. The relative configuration of the last chiral center at C-3 is apparently not available in the absence of diagnostic NOE data; thus, the methyl group H_3-14 at C-3 may be commonly β-oriented with the known derivatives 8-*O*-methylrabelomycin and 8-*O*-methyltetrangomycin also isolated from the same strain previously.[10]

To verify these assumptions, crystalline 1 was subjected to X-ray diffraction using Mo Kα radiation, as the structure contained a sulfur atom. Slow volatilization of a concentrated solution of 1 in $MeOH/CH_2Cl_2$ mixture afforded X-ray quality crystals. The X-ray experiment confirmed the structure assigned for 1 and unambiguously determined the absolute config-

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urations as 1*R*, 3*R*, 4a*R*, 6*R*, 6a*R*, 7*R*, 12*R*, 12a*S*, and 12b*S*, respectively (Figure 4). A striking difference between **1** and

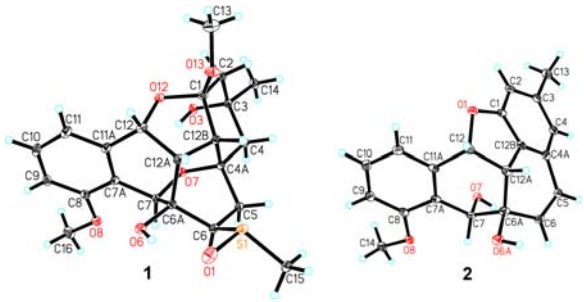
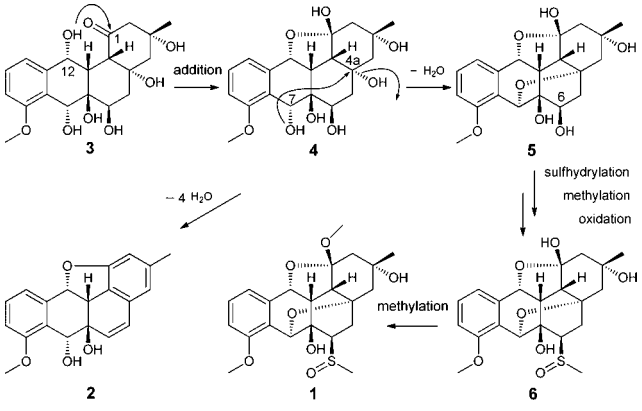


Figure 4. X-ray crystal structure of **1** and **2**.

other angucyclinones is the *R*-configuration at C-7; this may be attributed to the geometry imposed by the epoxy and/or bridged structure, for several carbonyl substituted angucyclinones, such as panglimycins A–E, have a conventional *S*-configuration at this position.[13] From an evolutionary point of view, it is worthy to note how, at first glance, a little configurational change, *R*-configuration at C-7, can lead to the constitution of a novel and even cage-like skeleton. Coincidentally, the absolute stereochemistry of **2**, an epoxybenz[*a*]anthracene from the same strain, was also assigned as 6a*R*, 7*R*, 12*R*, and 12a*S* based on the X-ray experiment, which were coincident with those of **1**, suggesting their common origination was probably from the same precursor.

A biosynthetic origin for **1** and **2** is proposed as shown in Scheme 1. Starting with a nucleophilic addition of keto group

Scheme 1. Plausible Biogenetic Pathway for 1 and 2



C-1 and 12-OH on the hypothetical precursor (**3**) results in the epoxy intermediate **4**, which on intramolecular cyclization between 4a-OH and 7-OH furnishes the bridged intermediate **5**, which continues through sulfhydrylation of the hydroxyl at C-6. Subsequent methylation and oxidation forms a methylsulfinyl moiety in intermediate **6**, which is further methylated into compound **1**.[14] In another pathway, a series of intramolecular dehydration on rings A and B of intermediate **4** leads to the formation of compound **2**. It can be deduced that the formation of the furan ring between C-1 and C-12 necessitates the ether bridge span from C-4a to C-7 only on the same side of the angucyclinone backbone for steric reasons, resulting in an unprecedented 7*R*-configuration as present only in **1** and **2**.

Compound **1** exhibited no significant activity against HepG2, SMMC-7721, and A-549 cell lines, but a moderate inhibitory effect to HL-60 cells with an IC_{50} value of 31.54 μM was observed. Trypan blue dye exclusion assay showed that **1** was not directly related to cell death, suggesting another antiproliferative mechanism, such as cell cycle arrest and forcing malignant cells to undergo terminal differentiation. However, the specific mechanism needs further investigation.

To date, more than 200 angucyclinones have been reported.[15−17] Grisemycin (**1**) represents the first example of a sulfur-containing angucyclinone. From other angucyclinones, it differs also in the unusually high degree of oxidation, including carbonyl substitutions, hydroxy substitutions, epoxidation, and even ether bridge formation, which seems to include participation of enzymatic steps; for instance, LolO, a nonheme iron oxygenase, has proven to be required for loline ether bridge formation.[18] Hence, it is interesting to pursue the biosynthetic machinery for the formation of an ether bridge in **1**.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00332.

Experimental details, complete NMR spectral data for **1**, and X-ray data for **1**–**2** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: zhsm_66@sohu.com.

Notes

The authors declare no competing financial interest.

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