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NOTE

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A Highly Conjugated Dihydroxylated C_{28} Steroid from a Myxobacterium

Dnyaneshwar Gawas,[†,‡] Ronald Garcia,[†,‡] Volker Huch,[§] and Rolf Müller*[,†,‡]

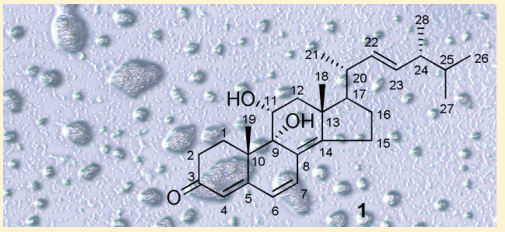
[†]Department of Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarland University, Campus C2 3, 66123 Saarbrücken, Germany

[‡]Department of Pharmaceutical Biotechnology, Saarland University, Campus C2 3, 66123 Saarbrücken, Germany

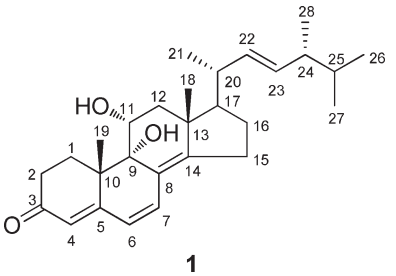
[§]Department of Inorganic Chemistry, Saarland University, Campus C4 1, 66123 Saarbrücken, Germany

Supporting Information

ABSTRACT: In the course of our search for novel secondary metabolites, the $CHCl_3$−MeOH extract of the novel myxobacterial strain *Sorangiineae* SBNa008 was shown to be active against human SW480 colon adinocarcinoma cells. Bioassay-guided fractionation of the extract yielded a highly conjugated novel, sterol 9α,11α-dihydroxyergosta-4,6,8(14),22-tetraen-3-one, **1**. The structure and the relative stereochemistry of **1** were established from interpretation of spectroscopic data and X-ray crystallography.



Myxobacteria are Gram-negative microorganisms capable of gliding and fruiting body formation. They are known to synthesize diverse and often novel compounds.[1,2] Most of the natural products known from this group of bacteria are antimicrobial and cytotoxic agents exhibiting unusual modes of action.[3] During our continuous search for biologically active secondary metabolites from myxobacteria, we discovered strain SBNa008 (*Sorangiineae*) producing a novel steroid **1**. This compound when tested in an MTT assay displayed cytotoxic activity against human colon adinocarcinoma cells (SW480) with an IC_{50} of 10 μM. To date, only a few groups of bacteria are known to synthesize steroids; in myxobacteria, they have been found in *Stigmatella*[4] and *Nannocystis*.[5] In this paper, we describe the isolation of a new ergostane derivative, 9α,11α-dihydroxyergosta-4,6,8(14),22-tetraen-3-one (**1**). The structure of compound **1** is based on extensive NMR studies, including particularly COSY, HSQC, HMBC, and NOE experiments, and was eventually proven by X-ray crystallography.



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The molecular formula of compound **1** isolated as an amorphous, white powder was determined to be $C_{28}H_{40}O_3$ on the basis of HREIMS at m/z 425.3049 $[M + H]^+$. Compound **1** showed fluorescence under UV light, which was confirmed by UV absorption at 348 nm, indicating the presence of a long conjugated system.[6] Fully decoupled ^{13}C and DEPT-135 NMR spectra of **1** indicated 28 carbons (Table 1) comprising six methyls, five methylenes, five methines, five vinylic carbonds, and seven quaternary carbons. The ^1H and DEPT-135 NMR spectra of compound **1** confirm the presence of six methyl groups containing two tertiary methyl groups at δ_H 1.16 and 1.05 and four secondary methyl groups at δ_H 1.12, 0.96, 0.88, and 0.86. This type of pattern is commonly observed in compounds containing an ergostane skeleton.[7,8] Other functionalities that were apparent from the ^1H and ^{13}C NMR spectroscopic data included two hydroxylated carbons, one tertiary carbon at δ_C 74.4, and another secondary carbon at δ_C 66.5. Comparisons of 2D-NMR (COSY, HSQC, and HMBC) analyses showed the secondary and tertiary hydroxyl groups to be at C-11 and C-9, respectively. The mass spectrum of compound **1** also showed an intense fragment ion at m/z 389, which should be derived from the loss of two water molecules. In addition, ^1H and ^{13}C NMR and HSQC experiments revealed the presence of four double bonds (δ_H 6.60, 6.15, 5.80, 5.30, and 5.27; δ_C 166.4, 158.6, 136.4, 134.0, 133.2, 127.6, 126.1, and 125.9). The two signals at δ_C 136.4 and 134.0 were assigned as a C_{22}−C_{23} double bond, since long-range couplings were observed for H-21 (δ 1.12) to C-22 (δ 136.4) and for H-28 (δ 0.96) to C-23 (δ 134.0). The coupling constant (J = 15.2 Hz) between H-22 and H-23 indicated an *E*-configuration of the carbon−carbon double bond at C-22. Out of the remaining six olefinic carbons, three are trisubstituted (δ 166.4, 158.6, and 127.6), while the other three carry vinylic protons (δ 133.2, 126.1, and 125.9). The ^1H NMR signals of these vinylic protons appear at δ 6.60 (H-7), 6.15 (H-6), and 5.80 (H-4), respectively, and the coupling constant of H-6 and H-7

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Table 1. ^{1}H and ^{13}CNMR Data for Compound 1 in CD_3OD

position	.	δ_H (J m Hz)	HMBC
1	31.0,CH_2	2.51, m	C-2, 3, 9, 10, 19
		2.17, m	C-2, 3,9, 10, 19
2	35.0,CH_2	2.55, m	C-1,3, 10
		2.41, m	C-1, 3, 10
3	202.6,C		
4	125.9,CH	5.80, s	C-2, 6, 10, 19
5	166.4,C		
6	126.1,CH	6.15, d (9.8)	C-4, 5, 8, 10
7	133.2,CH	6.60, d (9.8)	C-5,8,9, 10, 14
8	127.6,C		
9	74.4,C		
10	44.5,C		
11	66.5,CH	4.20, dd (12.2, 4.3)	C-9, 10, 12, 13
12	43.7,CH_2	2.08, dd (12.2, 4.3)	C-9, 11, 13, 14, 17, 18
		1.59, m	C-9, 11, 13, 14, 17, 18
13	46.6,C		
14	158.6,C		
15	26.5,CH_2	2.56, m	C-8, 13, 14, 16, 17
		2.38, m	C-8, 13, 14, 16, 17
16	28.7,CH_2	1.87, m	C-13, 14, 15, 17, 20
		1.56, m	C-13, 14, 15, 17, 20
17	56.9,CH	1.39, m	C-12, 13, 18, 20, 22
18	19.1,CH_3	1.05, s	C-12, 13, 14, 17
19	21.2,CH_3	1.16, s	C-1, 5, 9, 10
20	40.6,CH	2.20, m	C-12, 17, 21, 22, 23
21	21.7,CH_3	1.12, d (6.8)	C-17, 20, 22
22	136.4,CH	5.27, dd (15.2, 8.1)	C-17, 20, 21, 23, 24
23	134.0,CH	5.30, dd, (15.2, 7.8)	C-20, 22, 24, 25, 28
24	44.4,CH	1.89, m	C-22, 23, 25, 26, 27
25	34.4,CH	1.50, m	C-23, 24, 26, 27, 28
26	20.5,CH_3	0.88, d (6.7)	C-24, 25, 27
27	20.2,CH_3	0.86, d (6.7)	C-24, 25, 26
28	18.2,CH_3	0.96, d (6.8)	C-23, 24, 25

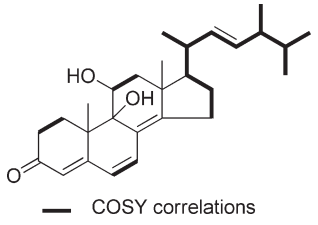

Figure 1. Structure of compound **1** with selected COSY correlations.

indicates a *Z*-configuration of the double bond. Besides the olefinic carbons, the ^{13}C NMR spectrum also showed the presence of a keto group at δ_C 202.6. The analysis of the COSY spectrum led to the determination of partial structures, C-1 to C-2, C-6 to C-7, C-11 to C-12, and C-15 to C-28, depicted by the bold bonds in Figure 1.

These partial structures were connected by long-range H−C correlations, obtained by means of a HMBC experiment, giving the final structure of **1** as shown in Figure 1. The major HMBC correlations were observed between H_3-19 and C-1, C-5, C-9, and C-10, between H_3-18 and C-12, C-13, C-14, and C-17, and between H-7 and C-5, C-6, C-8, C-9, C-10, and C-14, as summarized in Table 1.

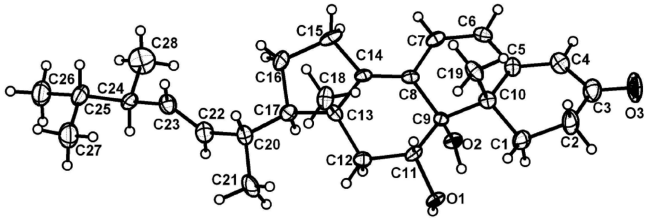

Figure 2. Crystal structure of compound **1**.

Comparison of spectroscopic data together with the UV absorption indicated that compound **1** is a steroid exhibiting a conjugated 3-oxo-4,6,8(14)-triunsaturated moiety.[9,10] Finally, from the above evidence the structure of compound **1** was elucidated as 9α,11α-dihydroxyergosta-4,6,8(14),22-tetraen-3-one, and this was proven unambiguously by X-ray crystallographic analysis of a crystal obtained by using DMSO as solvent (Figure 2).

EXPERIMENTAL SECTION

General Experimental Procedures. The NMR spectra, including ^{1}H, ^{13}C, and 2D NMR, were recorded on a Bruker Avance 500. The ^{1}H chemical shifts were assigned using a combination of data from COSY, HSQC, and HMBC experiments. Chemical shifts are given on the δ (ppm) scale, and coupling constants (J) are expressed in hertz. HREIMS were obtained on a Thermo LTQ Hybrid FT mass spectrometer. X-ray analysis was performed on a X8-Apex Bruker-AXS diffractometer.

Strain Cultivation and Production. Strain SBNa008 was a novel soil myxobacterium showing long rod-shaped vegetative cells with blunted ends. The colony spreads as a thin film on the surface of the agar, producing a colorless to pinkish film. A slight agar depression was produced on the surface of the colony, and the aggregation of cells led to the development of small and oval sporangioles. On the basis of morphology, chemophysiology characteristics, 16S rDNA, and phylogenetic tree analyses, SBNa008 belongs to the suborder *Sorangiineae*.[11] Its closest neighbor is *Phaselicystis flava*, a recently inaugurated species belonging to *Phaselicystidaceae*.[12] The strain was grown and maintained in buffered yeast agar.[13] A 10% preculture inoculum was prepared to seed 20 L flask cultures of the same liquid yeast medium. The culture was shaken at 150 rpm and incubated at 30 °C. Just like many *Sorangiineae*, the novel isolate grew as clumps, usually white to pink in color. On the fourth day of incubation, 2% (v/v) Amberlite XAD-16 resin (Sigma) was added and harvested by centrifugation at the end of 7 days together with the cell biomass.

Extraction and Isolation. The cell mass and the adsorber resin XAD-16 of the strain SBNa008 was extracted with $CHCl_3$−MeOH (5 L) at room temperature. After filtration and concentration of the solvent, the crude extract of SBNa008 was subjected to flash chromatography over a silica gel 60 (70−230, mesh) column using hexane−$CHCl_3$−MeOH mixtures of increasing polarity. Fractions enriched with compound **1** were obtained upon elution with $CHCl_3$−MeOH (8:2). Further purification of this sample was carried out using a Waters autopurifier system (XBridge prep C18 5 μm, 19 × 150 mm column, MeOH−H_2O gradient), which yielded 6.3 mg of compound **1**.

9α,11α-Dihydroxyergosta-4,6,8(14),22-tetraen-3-one (1): amorphous, white powder; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.60 (1H, d, J = 9.8 Hz, H-7), 6.15 (1H, d, J = 9.8 Hz, H-6), 5.80 (1H, s, H-4), 5.30 (1H, dd, J = 15.2, 7.8 Hz, H-23), 5.27 (1H, dd, J = 15.2, 8.1 Hz, H-22), 4.20 (1H, dd, J = 12.2, 4.3 Hz, H-11), 2.56 (1H, m, H-15a), 2.55 (1H, m,

H-2a), 2.51 (1H, m, H-1a), 2.41 (1H, m, H-2b), 2.38 (1H, m, H-15b), 2.20 (1H, m, H-20), 2.17 (1H, m, H-1b), 2.08 (1H, dd, J = 12.2, 4.3 Hz, H-12a), 1.89 (1H, m, H-24), 1.87 (1H, m, H-16a), 1.59 (1H, m, H-12b), 1.56 (1H, m, H-16b), 1.50 (1H, m, H-25), 1.39 (1H, m, H-17), 1.16 (3H, s, H-19), 1.12 (3H, d, J = 6.8 Hz, H-21), 1.05 (3H, s, H-18), 0.96 (3H, d, J = 6.8 Hz, H-28), 0.88 (3H, d, J = 6.7 Hz, H-26), 0.86 (3H, d, J = 6.8 Hz, H-27); ^{13}C NMR (CD_3OD, 125 MHz) δ 202.6 (C, C-3), 166.4 (C, C-5), 158.6 (C, C-14), 136.4 (CH, C-22), 134.0 (CH, C-23), 133.2 (CH, C-7), 127.6 (C, C-8), 126.1 (CH, C-6), 125.9 (CH, C-4), 74.4 (C, C-9), 66.5 (CH, C-11), 56.9 (CH, C-17), 46.6 (C, C-13), 44.5 (C, C-10), 44.4 (CH, C-24), 43.7 (CH_2, C-12), 40.6 (CH, C-20), 35.0 (CH_2, C-2), 34.4 (CH, C-25), 31.0 (CH_2, C-1), 28.7 (CH_2, C-16), 26.5 (CH_2, C-15), 21.7 (CH_3, C-21), 21.2 (CH_3, C-19), 20.5 (CH_3, C-26), 20.2 (CH_3, C-27), 19.1 (CH_3, C-18), 18.2 (CH_3, C-28); EIMS m/z 425 $[M + H]^+$ (100), 407 (4), 389 (10), 371 (1), 319 (2), 251 (1); HREIMS m/z 425.3049 $[M + H]^+$ (calcd for $C_{28}H_{41}O_3$ $[M + H]^+$, 425.3055).

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H, ^{13}C, 2D-NMR spectra and X-ray data of compound **1**. This information is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: rom@mx.uni-saarland.de. Tel: +49 681 302 70201. Fax: +49 681 302 70202.

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■ REFERENCES

(1) Wenzel, S. C.; Müller, R. *Mol. Biosyst.* **2009**, *5*, 567–574.
(2) Wenzel, S. C.; Müller, R. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 220–230.
(3) Weissman, K. J.; Müller, R. *Nat. Prod. Rev.* **2010**, *27*, 1276–1295.
(4) Bode, H. B.; Zeggel, B.; Silakowski, B.; Reichenbach, H.; Müller, R. *Mol. Microbiol.* **2003**, *47*, 471–481.
(5) Shimkets, L.; Dworkin, M.; Reichenbach, H. In *The Prokaryotes*; Dworkin, M.; Falkow, S.; Rosenberg, E.; Schleifer, K.-H., Eds.; Springer: New York, 2006; Vol. 7, Chapter 3.4.3, pp 31−115.
(6) Weng, Y.; Xiang, L.; Matsuura, A.; Zhang, Y.; Huang, Q.; Qi, J. *Bioorg. Med. Chem.* **2010**, *18*, 999–1002.
(7) Rama Rao, M.; Venkatesham, U.; Venkata Rami, R.; Venkateswarlu, Y. *J. Nat. Prod.* **1999**, *62*, 185–186.
(8) Rivera, A.; Benavides, O. L.; Rios-Motta, J. *Nat. Prod. Res.* **2009**, *23*, 293–300.
(9) Lee, W. Y.; Park, Y.; Ahn, J.; Park, S .; Lee, H. *Bull. Korean Chem. Soc.* **2005**, *26*, 1464–1466.
(10) Kawahara, N .; Sekita, S.; Satake, M. *Phytochemistry* **1994**, *37*, 213–215.
(11) Garcia, R.; Gerth, K.; Stadler M.; Dogma I. J.; Müller, R. *Mol. Phylogenet. Evol.* in press.
(12) Garcia, R.; Reichenbach, H.; Ring, M. W.; Müller, R. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 1524–1530.
(13) Garcia, R.; Krug, D.; Müller, R. In *Methods in Enzymology*; Hopwood, D., Ed.; Academic Press: Burlington, 2009; Vol. 458, Part A, Chapter 3, pp 59−91.