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NOTES

Lactonamycin Z, an Antibiotic and Antitumor Compound Produced by *Streptomyces sanglieri* Strain AK 623†

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Alkaliphilic streptomycetes strains were included in our HPLC-diode array screening program for detection of novel secondary metabolites. Strain AK 623, which was isolated from a pine wood soil collected at Hamsterley Forest, County Durham, UK, became attractive because of the appearance of a prominent peak in the HPLC chromatogram which did not correspond to any of the 700 reference compounds stored in our HPLC-UV-Vis-Database[2]. The structure of the isolated metabolite was elucidated as a new derivative of lactonamycin[3~5] and named lactonamycin Z (**1**). The structure is shown in Fig. 1.

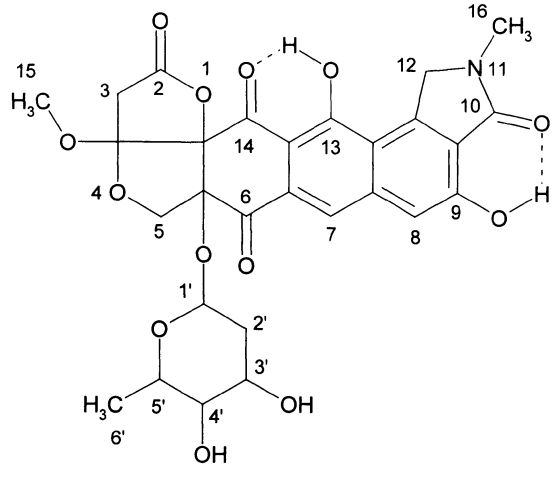
Strain AK 623 was examined for a number of key properties known to be of value in streptomycete systematic[6,7]. It was apparent from the resultant 16S rRNA gene sequence, and morphological and associated data that the organism should be classified as *Streptomyces sanglieri*[8].

Batch fermentations of strain AK 623 were carried out in 20-liter fermentors equipped with an intensor system (b20; Giovanola). The medium consisted of starch 1%, glucose 1%, glycerol 1%, corn steep powder 0.25%, casein peptone 0.5%, yeast extract 0.2%, and NaCl 0.1% in tap water. Although the growth of strain AK 623 was significantly increased in the alkaline range at pH 9, production of **1** was achieved during fermentations in the neutral pH range. The culture reached a maximal biomass of 60 vol-% after 30 hours, and production of 3.2 mg/liter of **1** was obtained after 66 hours.

Compound **1** was isolated from 18 liter culture filtrate (adjusted to pH 5) by ethyl acetate extraction (4×1 liter) and separation on a LiChroprep Diol column (2.6×40 cm; Merck) applying a linear gradient CH_2Cl_2 to a content of 10% MeOH within 3 hours at a flow rate of 5 ml/minute. Fractions containing **1** were concentrated to dryness, dissolved in a small volume MeOH and separated by Fractogel TSK HW-40 chromatography (2.5×90 cm; Merck) using MeOH as eluent at a flow rate of 30 ml/hour. Pure **1** was obtained by preparative reversed-phase HPLC column (16×250 mm) filled with 10 μm-Nucleosil-100 C-18 material and a linear gradient with 0.5% HCOOH-MeOH starting at 40% MeOH to 80% MeOH within 20 minutes at a flow rate of 20 ml/minute. **1** was obtained as a yellow powder (4.2 mg) after lyophilisation, soluble in DMSO and MeOH.

The molecular formula of **1** was determined as $C_{28}H_{27}NO_{13}$ (found: *m/z* $[M+H]^+$ 586.15554, calcd. *m/z*

Fig. 1. Structure of lactonamycin Z (**1**).



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586.15551, rel. error: 0.05 ppm) by ESI-FTICR-MS. The HPLC-UV-Vis spectrum of **1** with maxima at 210 (sh), 229 (sh), 258 (sh), 300, 398 (sh) and 415 (sh) nm was in close accordance with the UV-Vis data given for lactonamycin[4]. The structure of **1** was elucidated from its NMR data (Table 1) as a new derivative of lactonamycin with α-2,6-dideoxy-ribohexose instead of α-rhodinose as sugar moiety. ^{1}H, HMQC, and DEPTQ NMR experiments accounted for the presence of three methyl, four methylene, six methine, as well as fifteen quarternary carbons in the molecule. ^{1}H and ^{13}C chemical shifts indicated that one methyl (δ_C 52.8), one methylene (δ_C 73.8), and four methine groups (δ_C 65.4, 66.6, 72.1, 96.3) were oxygenated and two methine groups (δ_C 112.9, 120.9) were sp^2 hybridized. Two of the quarternary carbons were classified as ketocarbonyl carbons (δ_C 189.4, 192.1) and four as carbonyl or phenolic carbons (δ_C 157.6, 164.1, 168.9, 170.7). The presence of two hydrogen-bonded phenol groups in the molecule was suggested by two broad down field signals (δ_H 9.48, 13.72) in the ^{1}H NMR spectrum. The gross structure of the aglycon was determined as lactonamycinon from abundant HMBC connectivities including several $^4J_{CH}$ couplings (Fig. 2a). The gross structure of the sugar moiety was established from proton-proton couplings traced through a DQF-COSY spectrum and confirmed by HMBC connectivities (Fig. 2a). The *O*-glycosidic linkage between lactonamycinon and the sugar moiety was reflected in the HMBC cross peak between 1′-H and C-5a.

The relative stereochemistry of the sugar moiety was assigned as α-2,6-dideoxy-ribohexose from vicinal coupling constant information extracted from ^{1}H NMR (Table 1) and DQF-COSY spectra and corroborated by ROESY data (Fig. 2b). The small splitting of the dublet signal of 1′-H in the ^{1}H NMR spectrum (J=3.7 Hz) and the ^{13}C chemical shift of C-6′ (δ_C 17.3), respectively, indicated the equatorial positions of 1′-H and CH_3-6′, the latter necessitating an axial position of 5′-H which was confirmed by the large diaxial coupling constant observed between 5′-H and 4′-H (J=10.1 Hz). As both coupling constants observed between 3′-H and 2′-H_2 in the DQF-COSY spectrum were small (J=3.4 Hz), 3′-H was assigned an equatorial position. The diastereotopic assignment of 2′-H_2 was made on the basis of a slightly larger coupling constant between 1′-H_{eq} and 2′-H_{ax} (J=3.6 Hz) than between 1′-H_{eq} and 2′-H_{eq} (J=2.6 Hz) and observation of a strong ROE between 2′-H_{ax} and 4′-H_{ax}.

In summary, the structure elucidation revealed **1** to be closely related to lactonamycin, differing solely by the presence of an hydroxy group at C-3′ and the relative stereochemistry at C-4′ of the sugar moiety. Further

Table 1. ^{1}H and ^{13}C NMR data of **1** in $CDCl_3$.

position	δ_C[a]		δ_H[b]		J (Hz)[c]
2	170.7	s	-	-	-
3	35.1	t	2.96	d	16.9
			3.08	d	16.9
3a	112.8	s	-	-	-
5	73.8	t	4.33	d	9.7
			4.93	d	9.7
5a	86.4	s	-	-	-
6	189.4	s	-	-	-
6a	130.3	s	-	-	-
7	120.9	d	8.09	s	-
7a	141.8	s	-	-	-
8	112.9	d	7.36	s	-
9	157.6	s	-	-	-
9-OH	-	-	9.48	vbr	-
9a	121.1	s	-	-	-
10	168.9	s	-	-	-
12	55.0	t	5.02	s	-
12a	142.8	s	-	-	-
12b	116.7	s	-	-	-
13	164.1	s	-	-	-
13-OH	-	-	13.72	br	-
13a	109.1	s	-	-	-
14	192.1	s	-	-	-
14a	90.0	s	-	-	-
15	52.8	q	3.21	s	-
16	29.2	q	3.33	s	-
1'-eq	96.3	d	5.00	d	3.7
2'-ax	37.1	t	1.84	dt	15.0, 3.6
2'-eq			2.15	dd	15.0, 2.6
3'-eq	66.6	d	3.90	br	-
4'-ax	72.1	d	3.06	m	-
5'-ax	65.4	d	3.73	dq	10.1, 6.1
6'-eq	17.3	q	1.11	d	6.1

[a] 150 MHz, chemical shifts in ppm, multiplicity

[b] 600 MHz, chemical shifts in ppm, multiplicity

[c] coupling constants extracted from ^{1}H NMR spectrum

Fig. 2a. Structure elucidation of **1** by HMBC connectivities (arrows) and DQF-COSY couplings (bold lines).

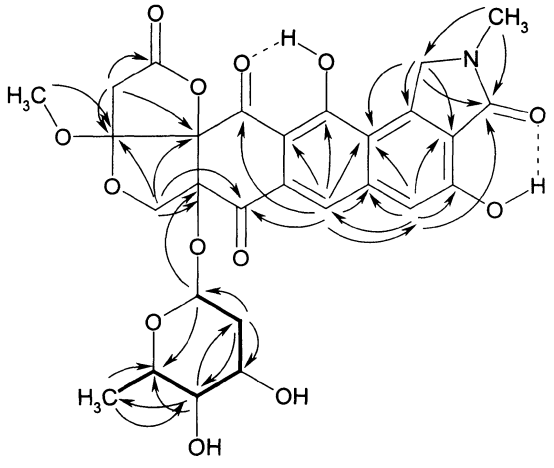
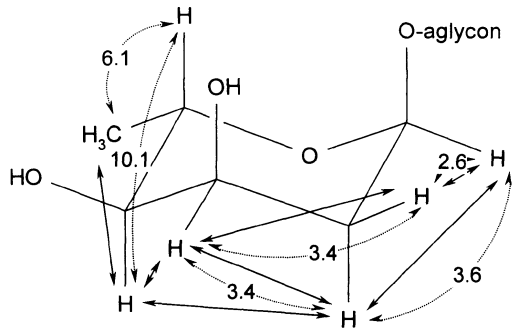


Fig. 2b. Relative stereochemistry assignment of the sugar moiety based on vicinal coupling constants extracted from a DQF-COSY spectrum (dashed arrows) and ROE information (straight arrows).



differences are possible with respect to the absolute configuration, which has been determined for lactonamycin by X-ray crystallographic and optical rotation analysis[5].

The antibacterial activities of **1** were determined by an agar plate diffusion assay. A weak activity against Gram-positive bacteria was detected as shown in Table 2. The antitumor activity of **1** was tested according to NCI guidelines[9] with human cell lines from gastric adenocarcinoma (HMO2), breast carcinoma (MCF 7), and hepatocellular carcinoma (Hep G2). **1** strongly inhibited the proliferation of HMO2 (IC50 value: 0.19 μg/ml), whereas the effects on MCF 7 and Hep G2 cell lines were less pronounced. The growth is inhibited in the G2/M cell cycle phase. The results are summarised in Table 3.

Table 2. Antimicrobial spectrum of **1** determined by the agar plate diffusion assay at a concentration of 1 mg/ml (inhibition zones in mm).

Test organism	**1**
Arthrobacter aurescens DSM 20116	10
Arthrobacter oxydans DSM 6612	24
Arthrobacter pascens DSM 20545	21
Rhodococcus erythropolis DSM 750	7
Staphylococcus aureus ATCC 12600	9
Streptomyces viridochromogenes Tü 57	17

Table 3. Activities (μg/ml) of **1** against selected human tumor cell lines.

	GI_{50}			TGI			LC_{50}		
	HMO2	MCF 7	Hep G2	HMO2	MCF 7	Hep G2	HMO2	MCF 7	Hep G2
1	1.9	0.85	5.1	>10[a]	9.5	>10[a]	>10	>10	>10

GI_{50}: 50% growth inhibition; TGI: 100% growth inhibition; LC_{50}: 50% reduction of cell amount after 24 hours compared to time point zero.

[a] 60% growth inhibition at a concentration of 10 μg/ml

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