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Frigocyclinone, a Novel Angucyclinone Antibiotic Produced by a *Streptomyces griseus* Strain from Antarctica†

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Abstract A new angucyclinone antibiotic, frigocyclinone, was isolated from *Streptomyces griseus* strain NTK 97, consisting of a tetrangomycin moiety attached through a *C*-glycosidic linkage with the aminodeoxysugar ossamine. Frigocyclinone showed antibacterial activities against Gram-positive bacteria.

Keywords angucyclinone, screening, antibacterial, antibiotic, *Streptomyces griseus*

In our HPLC-diode array screening program to detect novel secondary metabolites from actinomycetes isolated from extreme habitats, extracts of culture filtrates of strain NTK 97 drew our attention. Strain NTK 97 was isolated from a terrestrial sample from Terra Nova Bay at Edmundson Point, Antarctica and produced a prominent metabolite whose UV-visible spectrum and retention time were compared with those of the more than 770 reference compounds stored in our HPLC-UV-Vis-Database [2]. The UV-visible spectrum of the dominant compound was highly similar to that of urdamycin B produced by *Streptomyces fradiae* [3], but their retention times differed significantly. The structure of the isolated metabolite was elucidated as a new angucyclinone with a *C*-glycosidic linked ossamine sugar moiety and was named frigocyclinone (**1**). The structure is shown in Fig. 1.

Strain NTK 97 was examined for a number of key properties known to be of value in streptomycete systematics [4]. The presence of LL-diaminopimelic acid in the peptidoglycan [5] together with its colonial characteristics [6] allowed its assignment to the genus *Streptomyces*. More detailed taxonomic studies showed that the organism was a *bone fide Streptomyces griseus* strain as it produced a grey aerial spore mass on oatmeal agar, formed straigth to flexuous chains of smooth-surfaced spores and shared very high 16S rRNA gene sequences (99.6~99.7%) with members of this taxon [7].

Batch fermentations of *Streptomyces griseus* strain NTK 97 were carried out in 10-liter stirred tank fermenters (New Brunswick). The production medium consisted of glucose 1%, starch 2%, Bacto peptone 0.3%, meat extract (Oxoid) 0.3%, yeast extract (Ohly Kat G, Deutsche Hefewerke) 0.5%, and $CaCO_3$ 0.3% in tap water (pH 7.0). The fermentation was conducted at 27°C for 118 hours with an aeration rate of 0.5 v/v/m and an agitation of 200 rpm. The

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production of **1** reached a maximal value of 22 mg/liter after 96 hours and decreased thereafter. Increasing the glucose concentration of the fermentation medium to 30 g/liter increased the maximum product concentration to 40 mg/liter observed after 120 hours.

The culture filtrate (21 liters) was loaded onto an Amberlite XAD-16 column (8×45 cm) and **1** was eluted by increasing concentrations of MeOH. The 100% MeOH-fraction, which contained **1**, was concentrated and dried (1.8 g). The raw product was dissolved in MeOH, dried with addition of 20 g of silica gel 60 under reduced pressure and was then subjected to adsorption chromatography using a column of silica gel 60 (2.5×33 cm). The separation was accomplished by gradient elution using increasing concentrations of MeOH in CH_2Cl_2. **1** containing fractions were combined and concentrated *in vacuo* to dryness (127 mg). Final purification of 70 mg of crude **1** on a Sephadex LH-20 column (2.5×70 cm) with MeOH afforded 47 mg of pure **1**.

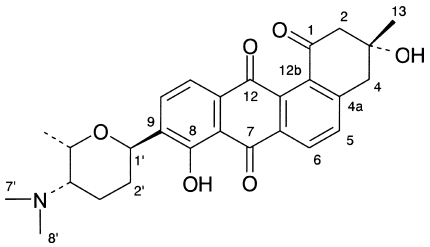

Fig. 1 Structure of frigocyclinone (**1**).

The UV-spectrum of compound **1** was in agreement with a substituted 1,2,3,4-tetrahydro-3-methyl-8-hydroxy-benz[α]anthraquinone chromophore with absorption maxima at 269, 322 and 408 nm. The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined to be $C_{27}H_{29}NO_6$ by the analysis of the ESI-TOF MS spectrum of **1** recorded with lockspray [$(M+H)^+$: found *m/z* 464.2052; calc 464.2073] as well as the NMR data including DEPT-135. 1D and 2D NMR data (Table 2) confirmed the presence of an angucyclinone ring system and showed this to be identical with tetrangomycin [8]. ^1H and ^{13}C chemical shifts were in very good agreement with the data reported [9]. In addition, an aminodeoxysugar was attached through a *C*-

Table 1 Physico-chemical properties of frigocyclinone (**1**)

Appearance	orange-brown solid
Molecular weight	463
Molecular formula	$C_{27}H_{29}NO_6$
ESI-TOF MS (*m/z*)	
Found	464.2052 $(M+H)^+$
Calcd	464.2073
UV λ_{max}^{EtOH} nm (ε)	269 (33300), 322 (3300), 408 (6400)
IR (ATR) ν_{max} cm^{-1}	3365 (br), 2961, 2870, 2821, 2774, 1702, 1672, 1630, 1589, 1426, 1269, 1096
CD $\lambda_{extreme}^{MeCN}$ (θ) deg · cm^2 · $decimole^{-1}$	(2700), 355 (−200), 325 (500), 269 (−21100), 218 (5000)

Table 2 ^1H and ^{13}C NMR data of frigocyclinone (**1**)

No	δ_C	δ_H	No	δ_C	δ_H
1	196.7	—	11a	133.5	—
2	53.1	3.05 (d, 14.0)	12	182.5	—
		2.74 (dd, 14.0, 1.8)	12a	135.5	—
3	71.5	—	12b	135.5	—
4	43.5	3.21 (d, 17.0)	13	29.7	1.35 (s)
		3.07 (dd, 17.0, 1.6)	1′	63.6	4.95_{ax} (m)
4a	149.3	—	2′	31.4	2.08_{eq} (m)
5	134.1	7.72 (d, 8.0)			1.28_{ax} (m)
6	128.5	8.21 (d, 8.0)	3′	22.3	1.91_{eq} (m)
6a	132.8	—			1.61_{ax} (m)
7	187.4	—	4′	63.5	2.20_{ax} (m)
7a	114.6	—	5′	70.9	4.42_{eq} (m)
8	157.6	—	6′	11.5	1.24 (d, 6.8)
9	137.7	—	7′, 8′	42.9	2.17 (s)
10	133.4	7.85 (dd, (7.8, 0.5)	3-OH		5.01 (br)
11	118.4	7.53 (d, 7.8)	8-OH		12.1 (s, br)

δ from TMS in DMSO-d_6; *J* in Hz

Table 3 Minimal inhibition concentrations (μM) of frigocyclinone (**1**), urdamycin B, erythromycin and vancomycin

Test organism	**1**	Urdamycin B	Erythromycin	Vancomycin
Bacillus subtilis DSM 10	10	100	1	0.33
Staphylococcus aureus DSM 20231	33	>100	1	1

glycosidic linkage at C-9, which was confirmed by the HC long-range couplings between carbon C-9 and the protons H-1′ and H-2′ ax of the tetrahydropyran moiety in the HC-HMBC spectrum. The sugar was identified as ossamine by detailed 1D- and 2D-NMR analysis. The tetrahydropyran spin systems could be identified from the HH-COSY spectrum. The assignment of the stereochemistry at carbon atoms C-1′, C-4′ and C-5′ was assigned by the intense ROESY cross peaks between the low-field proton H-1′ (ax, δ=4.95 ppm) and proton H-3′ (ax, δ=1.61 ppm) as well as to the methyl protons H-6′ (ax, δ=1.24 ppm) positioning these protons on the same site of the tetrahydropyran ring, whereas the strong ROESY effects between H-2′ (ax, δ=1.28 ppm) and H-4′ (ax, δ=2.20 ppm) describe their close spatial neighborhood on the other site of the sugar ring. Comparison with the NMR data reported for ossamine [10] and its stereoisomer forosamine [11], where both methyl and *N*,*N*-dimethyl substituents are in an equatorial orientation, confirmed the identification of the sugar moiety. The relative configuration at C-3 was established by an X-ray diffraction analysis, which at the same time confirmed the complete structure obtained from NMR data. Crystals were grown from a mixture of acetone and *n*-hexane. Crystal data were as follows: monoclinic, a=25.94 (1)Å, b=7,306 (2)Å, c=12,745 (5)Å, β=89,97 (3)°, Z=4, spacegroup $P2_1$. A total of 4944 reflections were measured by a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα-radiation (λ=1.54178 Å) using a yellow prismatic crystal, 0.15×0.15×0.1 mm at 100 K. The crystal structure was solved by direct methods with the program SHELXS86 [12], and refined by full-matrix least squares. Non-hydrogen atoms were refined with anisotropic temperature factors, and hydrogen atoms were included and not refined. The final R and Rw for 2725 reflections with $I>2s(I)$ were 0.064 and 0.078, respectively. The maximum and minimum peaks on the final difference fourier map were 0.28 e^-/Å^{-3} and −0.44 e^-/Å^{-3}, respectively. The absolute configuration was not established. However, 3*R*, 1′*R*, 4′*S*, 5′*S* configuration can be postulated, since only *R* configuration at C-3 is known in naturally occurring angucyclinones. The molecular structure of **1** is depicted in Fig 2.

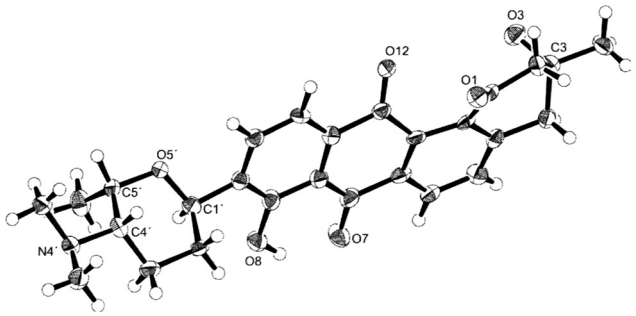

Fig. 2 Molecular structure of frigocyclinone (**1**) using ORTEP.

The antimicrobial spectrum of **1** was determined by agar plate diffusion assays and **1** revealed good inhibitory activity against Gram-positive bacteria, whereas Gram-negative bacteria like *Escherichia coli*, *Pseudomonas fluorescens* and *Proteus mirabilis* were not sensitive. Inhibitory activity of **1** was not detected against filamentous fungi like *Botrytis cinerea*, *Aspergillus viridinutans*, *Penicillium notatum* and *Paecilomyces variotii*. Yeasts such as *Saccharomyces cerevisiae* and *Candida albicans* were also insensitive to **1**. The minimal inhibition concentration of **1** was significantly higher in comparision to vancomycin and erythromycin, but **1** revealed more potent activity than urdamycin B (Table 3).

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