COMMUNICATIONS TO THE EDITOR

Alnumycin a New Naphthoquinone Antibiotic Produced by an Endophytic *Streptomyces* sp.

Sir:

During our recent investigations of the genus *Frankia* we isolated a novel endophytic actinomycete which was identified as *Streptomyces* sp.[1] Here we report the fermentation, isolation and structure elucidation as well as the biological activities of the new naphthoquinone antibiotic alnumycin (**I**; Fig. 1) which is produced by this strain.

The organism *Streptomyces* sp. (DSM 11575) was isolated from root nodules of *Alnus glutinosa* collected near Jena (Germany).

The fermentation was carried out for 14 days at 28°C in four fermentors containing 5 liters of a medium composed as follows: glucose 1%, $Na_2PO_4 \cdot 2H_2O$ 0.069%, KH_2PO_4 0.026%, $MgSO_4 \cdot 7H_2O$ 0.02%, Na(Fe)EDTA 0.001%, NaCl 0.03%, salt-solution 1 ml [H_3BO_4 $1.5 \cdot 10^{-4}$%, $MnSO_4$ $8 \cdot 10^{-5}$%, $ZnSO_4 \cdot 7H_2O$ $6 \cdot 10^{-5}$%, $CuSO_4 \cdot 5H_2O$ $1 \cdot 10^{-5}$%, $NaMoO_4 \cdot 2H_2O$ $2.5 \cdot 10^{-6}$% and $CoSO_4 \cdot 7H_2O$ $1 \cdot 10^{-7}$%], pH 6.8.

The culture broth (5 liters) was separated by filtration followed by concentration under reduced pressure (1 liter) and extraction with ethyl acetate (3 × 1 liter). After drying over Na_2SO_4 the organic layer was evaporated under reduced pressure. The residue of 667 mg was dissolved in 10 ml hexane/isopropanol (7:3) and fractionated on a silica gel column (Merck Silica gel 60, 0.1 ~ 0.063 mm, 1.4 × 15 cm) with the same solvent mixture as eluent. The bioactive fractions were determined using an agar plate diffusion assay with *Bacillus subtilis* as indicator for growth inhibition. Reddish bioactive fractions were pooled and concentrated under reduced pressure. The final purification of the residue (130 mg) by isocratic HPLC using a preparative column (Merck LiChrospher Si-60, 1.0 μm, 10 × 25 cm) 5 ml/minute hexane/isopropanol (7:3) (detection: 460 nm) provided 43 mg alnumycin (**I**) (retention time: 4.5 minutes) as red solid crystals melting at 134 ~ 136°C. The physico-chemical properties of **I** are summarized in Table 1, ^{13}C and ^{1}H NMR data are shown in Table 2.

The molecular formula of alnumycin ($C_{22}H_{24}O_8$) was readily inferred from the HREI-MS spectrum (M^+ *m/z* 416.1471). Analysis of the ^{1}H, ^{13}C, DEPT and COSY spectra of alnumycin revealed the presence of a naphtoquinone chromophore, three isolated olefinic and aromatic protons, a methyl group and two aliphatic fragments (CH_3–CH_2–CH_2CH–O and –O–CH_2–CH(OH)–CH(O–)–CH_2OH). The connection between these fragments was unambiguously settled by the HMBC spectrum (Fig. 2). For instance the long range correlations from 1′-H (δ 5.71) to C-3′ (δ 70.9) and C-5′ (δ 81.4) as well as to C-7 (δ 135.6), C-8 (δ 144.3) and C-9 (δ 186.9) were particular useful. They settled the structure of the 1,3-dioxan moiety as well as the attachment of this unit to the quinoid backbone. Further instructive heteronuclear long range correlations are provided by Fig. 2. Regarding the relative stereochemistry of the 1,3-dioxan moiety the occurrence of a NOE between 1′-H on the one hand and 3′-H_{ax} (δ 3.64)

Fig. 1. Chemical constitution of alnumycin (**I**).

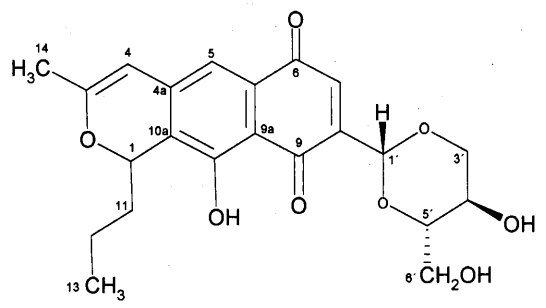

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Table 1. Physico-chemical properties of alnumycin.

Appearance	Reddish microcrystals
HREI-MS *m/z*	Calcd. 416.1477
	Found 416.1471
Molecular formula	$C_{22}H_{24}O_8$
MP	134 ~ 136°C
$[\alpha]_D^{20}$ (c=0.1, MeOH)	+170°
UV (λ_{max}) nm (ε)	201 (45.930), 237 (14.290),
in MeOH	301 (14.530), 471 (3.900)
IR (λ_{max}) cm^{-1}	771, 885, 952, 993, 1063, 1084, 1154, 1281, 1382, 1424, 1455, 1552, 1597, 1630, 2950, 3405
Rf (TLC, silica gel)[a]	0.52

[a] Hexane/isopropanol (7:3).

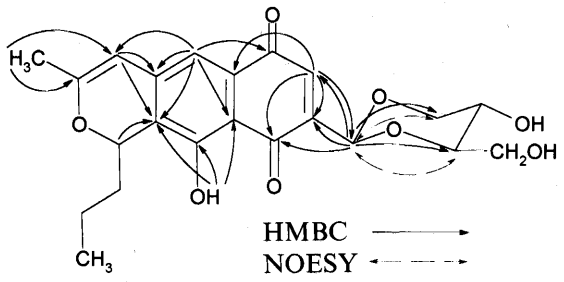
Table 2. ^{1}H and ^{13}C NMR data of alnumycin (300 MHz, $CDCl_3$). δ in ppm relative to internal TMS.

Position	δ_C	δ_H
1	73.0	5.65 (dd 7.0; 1.0)
3	158.6	—
4	100.0	5.60 (s)
4a	139.7	—
5	114.4	7.12 (s)
5a	131.5	—
6	184.7	—
7	135.6	7.07 (s)
8	144.3	—
9	186.9	—
9a	113.0	—
10	157.1	12.16 (OH)
10a	122.5	—
11	34.9	1.52 (m), 1.99 (m)
12	18.1	1.48 (m), 1.54 (m)
13	13.8	0.96 (t, 7.0)
14	20.4	1.95 (s)
1′	94.0	5.71 (s)
3′	70.9	3.64 (dd, 10.7, 10.2), 4.31 (dd, 10.7, 5.3)
4′	62.7	3.92 (ddd, 10.2, 9.0, 5.3)
5′	81.4	3.74 (dt, 9.0, 4.4)
6′	62.8	3.92 (m)

and 5′-H (δ 3.74), one the other, proved their synaxial orientation. This conclusion was supported by the occurrence of two large vicinal coupling constants (10.2 and 9.0 Hz) between 4′-H (δ 3.92), 3′-H_{ax}, and 5′-H, respectively, which additionally confirmed the axial position of H-4. Thus, the structure of alnumycin appears as closely related to naphthopyranomycin[2] and the exfoliamycins[3,4] but is distinguishable from the former by the missing side chain at C-7, and from the latter by the nature of the substituent at C-8.

Alnumycin displays antibacterial properties similar to the related naphthopyranomycin[2] and the exfoliamycins[3,4]. During the common agar plate diffusion assay, alnumycin showed narrow-spectrum antimicrobial activity against Gram-positive bacteria, such as *Bacillus subtilis* (20 mm diameter of inhibition zone caused by 6 μg alnumycin per agar well), *Arthrobacter crystallopoites*, *Micrococcus luteus* and *Rhodococcus* sp. but was inactive against Gram-negative species such as *Escherichia coli*, *Proteus rettgeri* and *Agrobacterium tumefaciens*.

Fig. 2. Selected long range couplings and nuclear overhauser effects (NOE).



Moreover, alnumycin inhibits the growth of K562 human leukemia cells (IC_{50}: 0.53 μg/ml) suggesting that its cytotoxicity is comparable to that reported for naphthopyranomycin[2].

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