Communication to the Editor

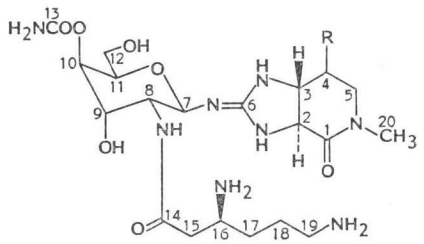
ALBOTHRICIN, A NEW STREPTOTHRICIN ANTIBIOTIC

Sir:

During the course of our screening program for new antibiotics, we have recently isolated albothricin (**1**), a streptothricin-group antibiotic, from the culture broth of a strain SIPI-2985, which was isolated from a soil sample collected in Hai-Nan island of China. Being different from the streptothricin producing organisms so far reported, the isolate was classified to belong to a rare actinomycete due to the cell wall component containing *meso*-diaminopimeric acid. A detailed taxonomic study is in progress. This paper describes the isolation, structural elucidation and some biological activities of **1**.

The producing organism SIPI-2985 was cultivated in a medium consisting of starch 2.0%, glucose 2.0%, soybean meal 1.0%, peanut meal 0.5%, corn steep liquor 0.5%, yeast extract 0.1%, $MgSO_4 \cdot 7H_2O$ 0.025%, K_2HPO_4 0.02%, NaCl 0.5%, $(NH_4)_2SO_4$ 0.25% and $CaCO_3$ 0.5% (pH 7.2) in a 50-liter jar fermentor. Fermentation was carried out at 28°C for 5 days. The maximum titer, 80 μg/ml, was estimated by the paper disc method using *Bacillus subtilis* ATCC 6633 as a test organism. The fermentation broth filtered at pH 2.0 was adjusted to pH 6.0 and passed through a column of Amberlite IRC-50 (Na^+, 2 liters). Active principle adsorbed on the column was eluted with 0.4 N HCl (10 liters). The eluate was adjusted to pH 6.0, adsorbed on a column of active carbon (400 ml) and eluted with 50% aq acetone. The eluate was concentrated and then applied to a column of CM-Sephadex C-25 (Na^+, 400 ml). The column was washed with 0.1 M, 0.2 M and 0.3 M NaCl successively, and finally developed with 0.4 M NaCl to give an active eluate which was desalted with active carbon and concentrated to dryness. Further purifications were carried out by Diaion CHP-20 and Sephadex G-10 column chromatographies. Lyophilization of the active effluent yielded albothricin (**1**) hydrochloride (880 mg). Attempts to prepare free base of albothricin were unsuccessful due to its alkaline instability.

Fig. 1. Structure of albothricin.



Albothricin (**1**) R=H
A37812 R=OH

The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was determined to be $C_{20}H_{36}N_8O_7$ on the basis of ^{13}C NMR and FAB-HRMS. Acid hydrolysis of **1** gave β-lysine which was identified by cellulose TLC in comparison with an authentic sample. The ^1H signals in Fig. 2 and the ^{13}C

Table 1. Physico-chemical properties of albothricin.

Appearance	White amorphous powder
MP	166~170°C (dec)
$[\alpha]_D^{20}$	−37.0° (*c* 1.0, H_2O)
Molecular formula	$C_{20}H_{36}N_8O_7$
FAB-HRMS *m/z* $(M+H)^+$	Calcd: 501.2757
	Found: 501.2760
UV (H_2O)	End absorption
IR (KBr) cm^{-1}	3400, 1710, 1642, 1390, 1310, 1070
Color reaction (+)	Ninhydrin, H_2SO_4, Lemieux
(−)	Sakaguchi
Silica gel TLC (Rf value)	Propanol - pyridine - CH_3COOH - H_2O (15: 10: 3: 12) 0.46
	Butanol - CH_3COOH - H_2O (2: 1: 1) 0.24

Fig. 2. 400 MHz ^{1}H NMR spectrum of albothricin in D_2O.

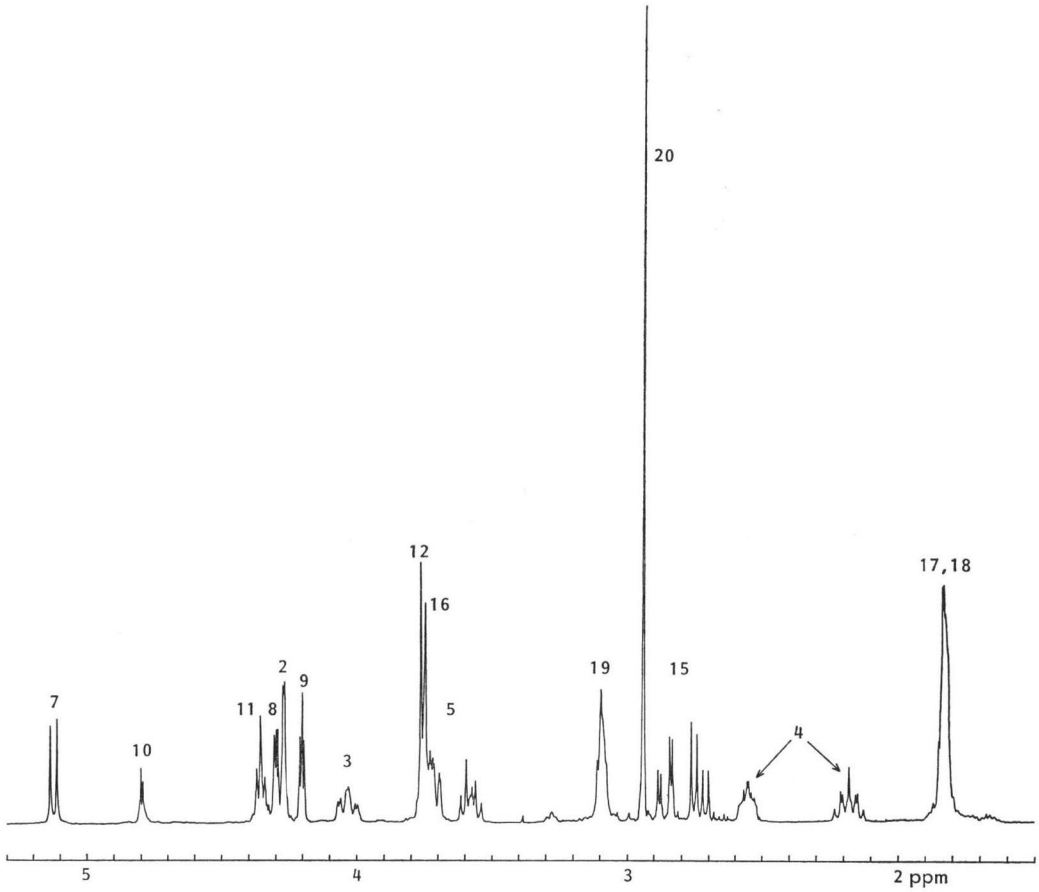


Table 2. ^{1}H NMR assignment of albothricin and A37812 in D_2O.

Position	Chemical shift, ppm (*J*, Hz)	
	Albothricin	A37812[1)]
2	4.29 d (14.0)	4.62 (14.5, small)
3	4.03 ddd (14.0, 12.5, 4.5)	4.09 (14.5, small)
4ax	2.18 dddd (12.5, 12.5, 8.5, 8.5)	4.71 (5.5, others)
4eq	2.55 dddd (12.5, 8.5, 4.5, 2.0)	
5ax	3.58 dt (13.5, 8.5)	3.50 (14.5, small)
5eq	3.72 ddd (13.5, 8.5, 2.0)	3.82 (14.5, small)
7	5.12 d (10.0)	5.08 (10)
8	4.28 dd (10.0, 3.0)	4.24 (10, 3)
9	4.20 dd (4.0, 3.0)	4.15 (3, 4)
10	4.80 d (4.0)	4.75 (4, small)
11	4.36 t (6.0, <1)	4.32 (6, small)
12	3.76 d (6.0)	3.71 (6)
15ax	2.73 dd (17.0, 8.5)	2.67 (17, 8)
15eq	2.86 dd (17.0, 4.0)	2.80 (17, 4)
16	3.74	3.69
17	1.83	1.78
18	1.83	1.78
19	3.10	3.04
20	2.94	2.89

Table 3. ^{13}C NMR chemical shifts for albothricin and A37812.

No.	Albothricin[a]	A37812[1),b]
1	168.1	168.7 s
2	62.3	55.6 d
3	57.7	61.7 d
4	26.1	62.3 d
5	49.3	58.4 t
6	163.3	163.8 s
7	79.8	80.0 d
8	49.5	50.0 d
9	67.6	67.5 d
10	71.1	71.1 d
11	74.5	74.4 d
12	61.4	61.2 t
13	158.3	158.7 s
14	172.6	173.2 s
15	37.5	37.7 t
16	49.3	49.2 d
17	30.2	30.3 t
18	24.1	23.9 t
19	40.2	40.0 t
20	34.5	34.0 q

[a] Spectrum in D_2O with internal dioxane; δ (dioxane)=67.4 ppm.
[b] Spectrum in D_2O containing 2% pyridine-d_5.

resonances of **1** are listed in Tables 2 and 3, respectively. The anomeric resonance at δ_H 5.12 and δ_C 79.8 suggested the presence of an *N*-glycoside. The presence of β-lysine and *N*-glycoside strongly indicated that **1** belonged to streptothricin-group antibiotics.

The ^1H and ^{13}C NMR spectra were analyzed mainly based on 2D-COSY, ^{13}C-^1H shift correlation and ^1H spin decoupling experiments. Consequently, two carbon skeletons corresponding to the β-lysine (δ_C 172.6, 37.5, 49.3, 30.2, 24.1 and 40.2) and the *N*-glycoside (δ_C 79.8, 49.5, 67.6, 71.1, 74.5, 61.4 and 158.3) were revealed. The latter sugar moiety showed close resemblance to 4-carbamoyl gulosamine. Accordingly the carbonyl resonance at δ_C 158.3 and 172.6 were assigned to the carbamoyl group attached to the gulosamine and the amide of β-lysine, respectively.

The sequence from C-2 to C-5 was straightforwardly revealed by ^1H spin decoupling experiments. The carbonyl signal (C-1) at δ_C 168.1 and guanidyl resonance (C-6) at δ_C 163.3 were in good agreement with those of A37812[1]. These indicate the absence of the hydroxyl group at 4-position in the streptolidine moiety. Compared to A37812, the upfield shifts of C-3 and C-5, and downfield shift of C-2 were reasonably explained by the β-effect and of γ-effect by the hydroxyl group, respectively. The remaining problem is to determine the position of a methyl group (δ_H 2.94 s). Since a nuclear Overhauser effect (NOE) enhancement was observed with H-5 methylene protons upon irradiation of CH_3 (δ_H 2.94), the methyl group was attached to the nitrogen of amide bond. The FAB-MS spectra data confirmed that albothricin ($C_{20}H_{36}N_8O_7$) differed in molecular ion peak from A37812 ($C_{20}H_{36}N_8O_8$) by 16 mass units equivalent to one oxygen atom. From all the results described above, the structure of albothricin was determined to be as depicted in Fig. 1.

Table 4. Antibacterial activity of albothricin.

Test organisms	MIC (μg/ml)
Staphylococcus aureus 209P JC-1	3.13
S. aureus Smith S-424	1.56
S. aureus No. 26	3.13
S. epidermidis ATCC 14990	3.13
S. epidermidis 109	3.13
Enterococcus faecalis ATCC 8043	100
Bacillus anthracis No. 119	12.5
Escherichia coli NIHJ JC-2	3.13
E. coli No. 29	6.25
E. coli W3630 RGN-823	3.13
E. coli JR66/W677	3.13
Citrobacter freundii GN346	1.56
Salmonella typhi 0-901-W	1.56
S. enteritidis No. 11	3.13
S. typhimurium LT-2	3.13
Salmonella sp. D-0001	3.13
Shigella sonnei EW33 Type 1	6.25
Klebsiella pneumoniae PCI 602	3.13
K. pneumoniae 22#3038	3.13
Proteus vulgalis OX19	1.56
P. rettgeri J-0026	6.25
P. mirabilis GN310	3.13
Morganella morganii Kono	1.56
Serratia marcescens MB-3848	25
Pseudomonas aeruginosa MB-3829	>100
P. cepacia M-0527	>100
P. maltophilia M-0627	50

Medium: Sensitivity disk agar (Nissui). Inoculum size: 10^6 cfu/ml.

Antimicrobial activities of albothricin by agar dilution method is given in Table 4. No acute toxicity of **1** was observed by administering the antibiotic at 200 mg/kg to ICR-mice intravenously. Necrotic symptoms characteristic to streptothricin-group antibiotics, however,

were observed on the tails of mice at the site of injection.

Recently, several streptothricin-group antibiotics have been reported[2~4]. It is however, to be noted that albothricin is the first example of streptothricin possessing the 4-deoxystreptolidine moiety and also the first example of streptothricin produced by rare actinomycetes.

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