Communications to the Editor

TRICHOMYCIN A, A HEPTAENE MACROLIDE: ISOLATION AND CHARACTERIZATION

Sir:

Trichomycin, a heptaenic macrolide, a potent and clinically useful antifungal drug, was discovered in the cultured broth of *Streptomyces hachijoensis*[1]. Two components, trichomycins A and B, were isolated by counter current distribution methods[2]. At the same time, the partial structure of the major constituent, trichomycin A, was deduced on the basis of chemical degradations by HATTORI[3]. However, it was found later that trichomycin A, originally thought to be homogeneous, was in the form of complex mixtures of closely related compounds.

This study is concerned with the isolation and characterization of new trichomycin A (**1**), the major constituent, from the complex mixtures using flash liquid chromatography (FLC) and HPLC.

The biological properties of trichomycin A (**1**) are also briefly discussed.

Isolation

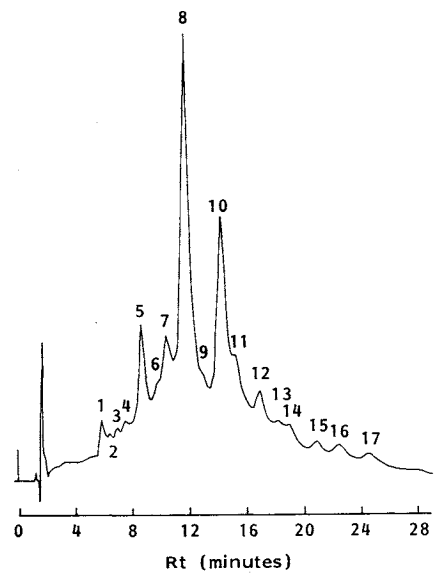
A sample of the complex mixture of trichomycins was obtained from the cultured mycelial cake of *S. hachijoensis*. The chromatogram of this complex mixture of trichomycin in HPLC is shown in Fig. 1. With peak number eight representing trichomycin A (**1**) in Fig. 1. The complex was dissolved in a mixture of DMF - 5 N-NaOH - acetone - water (6 : 2 : 27 : 3). The insoluble material was removed by filtration, and the filtrate was purified by column chromatography on alumina (acidic, Woelm) with acetone - water (9 : 1). The column was washed with 1/15 M phosphate buffer (pH 8.0) followed with water. Trichomycin was eluted with a mixture of butanol - pyridine - water (4 : 3 : 7). The eluate was concentrated *in vacuo* under 38°C in the dark, yielding an orange yellowish powder. It was further purified by column chromatography on silica gel 60 (Merck) with the lower phase of a mixture of chloroform - methanol - water (2 : 2 : 1). **1** was eluted from three times to seven times of the bed volume. The eluate was concentrated *in vacuo* in the dark to yield a yellow powder. Further purification was achieved by FLC on silica gel (Fuji gel, 30 ~ 50 μm) using the mentioned above solvent system, yielding a fine yellow powder. This powder was further purified by preparative chromatography on HPLC (μBondapak C18, Waters, i.d. 19 × 150 mm) with acetonitrile - phosphate citrate-buffer (36 : 64, pH 4.75 ~ 4.85), yielding a fine yellow powder of **1**.

Physical and Chemical Properties

The molecular formula, $C_{58}H_{84}N_2O_{18}$, was established by elementary analysis and positive ion mode FAB-MS ($(M+H)^+$ *m/z* 1,097). **1** has mp > 300°C, $[\alpha]_D^{20}$ +129.8° (*c* 0.1, DMF), and p*K'*a (in 66% DMF in water) 6.1 (-COOH) and 9.0 ($-NH_2$). **1** is soluble in pyridine, DMF, DMSO and alkali, slightly soluble in methanol and acetone, and insoluble in ethanol, ethyl acetate, chloroform and water. It shows positive color reactions with sulfuric acid, ninhydrin and antimony trichloride.

The UV-VIS spectra with the absorption maxima at 235 (ε 20,934), 289 (14,358), 344 (36,716), 360 (48,991), 379 (57,978) and 401 nm (44,607) in pH 2.08, 236 (21,482), 343 (35,620), 359 (38,470), 379 (38,689) and 400 nm (28,386) in pH 6.81, and 235 (28,444), 281 (27,948), 290 (28,934), 343 (40,223), 360 (61,595), 380 (85,050) and 402 nm (70,911) in pH 12.44 show typical absorption of a conjugated heptaene. The IR spectrum (Nujol) shows absorp-

Fig. 1. Chromatogram of crude trichomycin by HPLC.



Column: Nucleosil 5C8 (0.4 × 150 mm). Mobile phase: acetonitrile - phosphate citrate-buffer (32.5 : 67.5, pH 4.6). Flow rate: 1.0 ml/minute. Detection: 360 nm.

Fig. 2. ^{1}H NMR spectrum of trichomycin A (in DMSO-d_6).

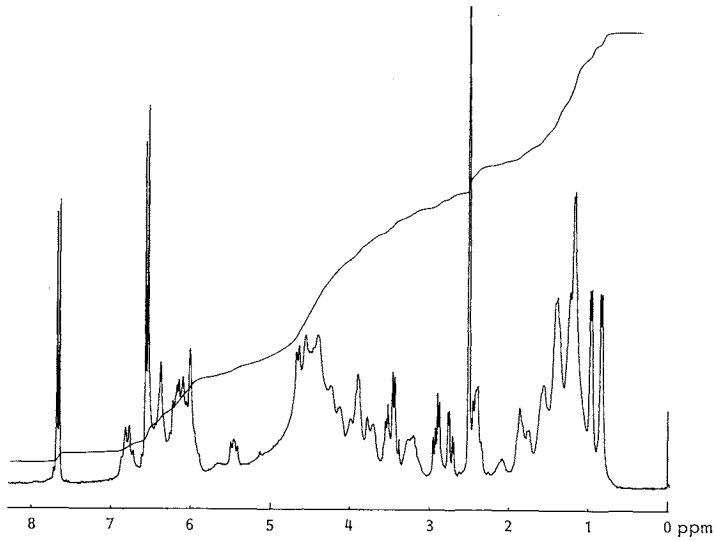
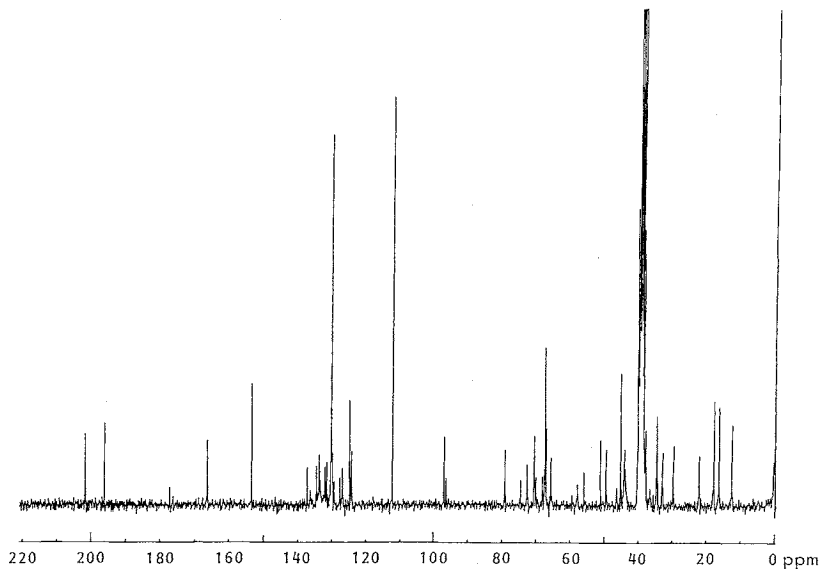


Fig. 3. ^{13}C NMR spectrum of trichomycin A (in DMSO-d_6).



tion bands at 3350 ($-NH_2$, $-OH$), 1730 (lactone), 1710 (C=O), 1630 (C=C), 1595 (carboxylate), 1560 (aromatic group), 1310, 1170, 1065, 1000, 930, 885, 845 and 760 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of **1** were shown in Figs. 2 and 3, respectively.

The structure determination of **1** was reported elsewhere[4].

Biological Activity

The antifungal, anticandidal and antitrichomonal activities of **1** together with those of amphotericin B (Sigma) by the agar dilution method are shown in Table 1. While trichomycin A (**1**) is more potent than amphothericin B, the well known antifungal drug, against diverse strains and species of *Candida*

Table 1. MIC and spectra of trichomycin A and amphotericin B against yeasts, fungi and trichomonas.

Organism	MIC (μg/ml)	
	Trichomycin A	Amphotericin B
Candida albicans (eight strains)	Mean 0.034	Mean 0.25
C. tropicalis FP583	0.1	0.78
C. pseudotropicalis FP584	<0.025	0.78
C. krusei FP585	0.1	0.78
C. parakrusei FP586	0.2	0.78
C. guilliermondii FP587	0.1	0.78
C. stellatoidea FP588	<0.025	<0.025
Aspergillus niger FP1398	0.1	1.56
A. flavus FP1022	6.25	1.56
A. fumigatus FP1305	6.25	1.56
Trichophyton rubrum FP596	12.5	3.13
T. mentagrophytes FP594	6.25	3.13
Cryptococcus neoformans FP1551	<0.025	<0.025
Trichomonas vaginalis 4FM[a]	0.2	>100
T. vaginalis ATCC 30001[a]	0.2	>100
T. vaginalis PCL110108[a]	0.2	>100

[a] V-Bouillon.

as well as strain of *Trichomonas vaginalis*, trichomycin A (**1**) is less potent against species of *Aspergillus*.

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