Notes

HYDROXYCHLOROTHRICIN, A NEW ANTITUMOR ANTIBIOTIC

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The cultured broth of *Streptomyces* sp. K818 showed antitumor activity and contained a new antibiotic identified as 2'''-hydroxychlorothricin (K818B). In this communication, the isolation and characterization of this novel antibiotic are reported.

Streptomyces sp. K818 was cultivated on a rotary shaker at 27°C for 5 days in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of glycerol 3.0%, corn steep liquor 1.0%, dry yeast 0.3%, $CaCO_3$ 0.35% and NaCl 0.5%.

The broth filtrate (5 liters) was adjusted to pH 3.0 and applied to a column of Diaion HP-20 (2 liters). The column was developed successively with water, 25% MeOH, and then eluted with MeOH (4 liters). The eluate was concentrated to a small volume under diminished pressure and extracted with EtOAc, at pH 3.0. The organic layer was back extracted with 5% sodium bicarbonate. The aqueous layer was then adjusted to pH 3.0 and extracted with EtOAc. The organic layer was evaporated to dryness under diminished pressure. The resulting residue contained two active substances, which were separated by a silica gel column chromatography with $CHCl_3$ - MeOH (40:1). Further purification was achieved by Toyopearl HW-40 column chromatography with MeOH to give chlorothricin (K818A) (200 mg) and 2'''-hydroxychlorothricin (180 mg).

The structure of chlorothricin[1] was confirmed by its UV, IR, mass, optical rotation and ^1H NMR spectral data.

2'''-Hydroxychlorothricin was isolated as a colorless powder, $C_{50}H_{83}O_{17}Cl$: MP 202°C; $[\alpha]_D^{25}$ +2.4° (*c* 0.5, MeOH); UV λ_{max}^{MeOH} nm (ε) 222 (10,000), 258 (3,700), 284 (1,600); secondary ion mass spectra (SI-MS) *m/z* 993 $(M+Na)^+$. The ^{13}C NMR spectrum of 2'''-hydroxychlorothricin was similar to that of chlorothricin but exhibited a new resonance due to the hydroxymethine carbon (δ 72.2) and lacked the signal due to the methylene carbon 2''' of chlorothricin. In accordance with these observation, the molecu-

Table 1. Antimicrobial activity.

Microorganisms	MIC (μg/ml)	
	K818A	K818B
Staphylococcus aureus FDA209P JC-1	25	100
Streptococcus pyogenes Cook	>100	>100
Bacillus subtilis ATCC 6633	25	>100
B. cereus IAM 1729	25	100
Micrococcus luteus ATCC 9341	50	>100
Staphylococcus aureus MS15009 (pI258)	25	100
Escherichia coli K-12 C600	>100	>100
Klebsiella pneumoniae PCI 602	>100	>100
Salmonella typhimurium IID971	>100	>100
Serratia marcescens IAM 1184	>100	>100
Pseudomonas aeruginosa NCTC 10490	>100	>100
Aspergillus fumigatus IFO 4400	>100	>100
Penicillium chrysogenum ATCC 10002	>100	>100
Trichophyton mentagrophytes	>100	>100
Candida albicans No. Yu1200	>100	>100

Fig. 1. The ^1H NMR spectrum of K818B (500 MHz, in $CDCl_3$).

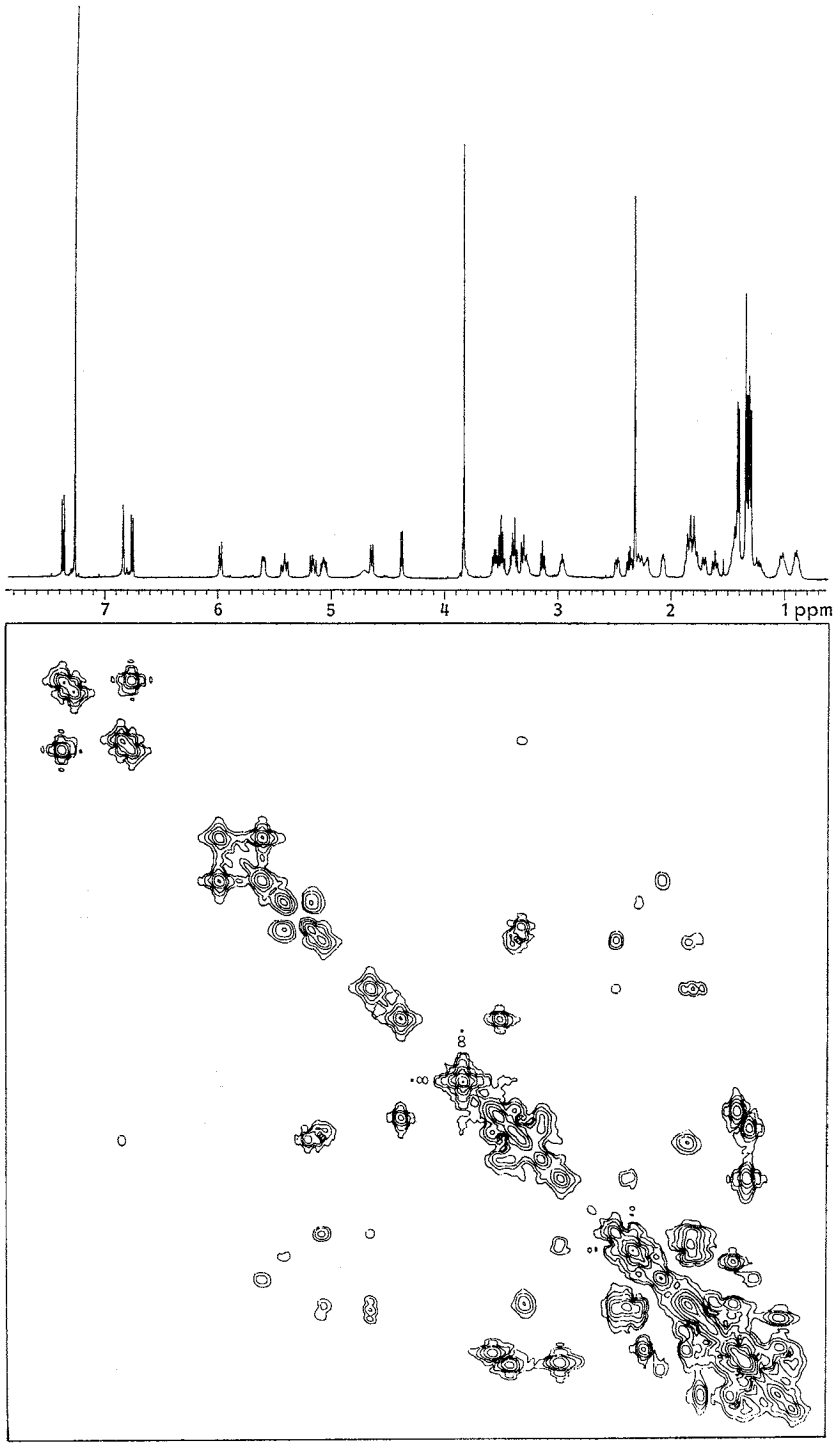
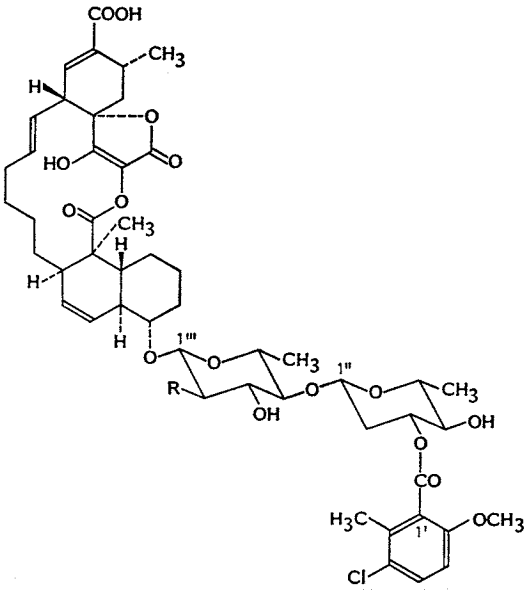


Fig. 2. The structures of K818A and K818B.



Chlorothricin R = H
Hydroxychlorothricin R = OH

lar weight of the new antibiotic was higher than that of chlorothricin by 16 mass units and proved the new substance to be a hydroxyl derivative of chlorothricin. Methanolysis of this antibiotic gave the methyl ester of the aglycone of chlorothricin. The ^{1}H NMR spectrum (Fig. 1) of hydroxychlorothricin showed the signals assigned to the 6-deoxyglucose moiety at δ 4.38 (1'''-H, d, J=7.5 Hz), 3.49 (2'''-H, t, J=7.5 and 8.5 Hz), 3.14 (3'''-H, t, J=8.5 and 8.5 Hz), 3.39 (4'''-H, overlapped with 4''-H), 3.56 (5'''-H, m) and 1.41 (6'''-H, d, J=6.5 Hz). The low field chemical shift of C-4''' (δ 85.5) of 6-deoxyglucose was explained in term of the glycosidation by 2-deoxyrhamnose[2]. Therefore, 6-deoxyglucose is attached to the aglycone, thus establishing the structure as 2'''-hydroxychlorothricin (Fig. 2). The configuration of the hydroxyl group was determined to be equatorial in view of $J_{1''',2'''}$=7.5 Hz.

The antimicrobial activity of hydroxychlorothricin was examined by the agar dilution method and the results are shown in Table 1. Hydroxychlorothricin prolonged the survival period of ICR mice intraperitoneally inoculated with Ehrlich carcinoma cells. Treatment with hydroxychlorothricin (40 mg/kg) resulted in more than 100% of the increase of life span. LD_{50} in mice was 295 mg/kg by the intraperitoneal route.

Acknowledgments

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