HOLOMYCIN AND N-PROPIONYL-HOLOTHIN, ANTIBIOTICS PRODUCED BY A CEPHAMYCIN C PRODUCER

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It was previously found that *Streptomyces* sp. P6621 which was isolated by us from soil produces cephamycin C.[1] A nitrosoguanidine mutant of this culture designated P6621-7N49 was found to produce additionally two antibiotics which were extractable with *n*-butanol, not inactivated by β-lactamase of *Citrobacter freundii* and active against a β-lactamase-producing strain of *Serratia marcescens*. After isolation and physico-chemical analysis, the new components were demonstrated to be holomycin and N-propionylholothin. Holomycin has been reported previously as a streptomyces product, while N-propionylholothin has been reported only as a chemical derivative.[2] This paper deals with isolation and identification of these two components.

One ml of spore suspension of *Streptomyces* sp. P6621-7N49 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium of the following composition: meat extract, 0.3%; Bacto-tryptone, 0.5%; glucose, 0.1%; soluble starch, 2.4%; yeast extract, 0.5%; soybean meal, 0.5% and $CaCO_3$, 0.5%. The pH was adjusted to 6.8 prior to sterilization. One ml of the seed culture was transferred after 48 hours to a series of 500-ml Erlenmeyer flasks containing 100 ml of production medium having the following composition: glucose, 0.5%; soluble starch, 2.0%; casamino acids, 1.0%; soybean meal, 1.5%; $MgSO_4 \cdot 7H_2O$, 0.05% and $CaCO_3$ 0.5%. The pH was adjusted to 7.0 prior to sterilization. Incubation was carried out for two days at 28°C

Fig. 1. (a) IR spectrum of holomycin.

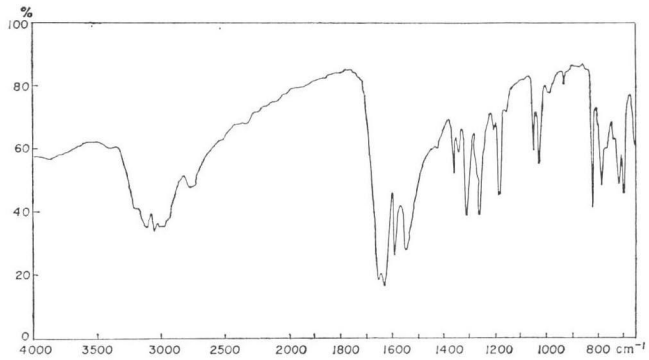


Fig. 1. (b) IR spectrum of N-propionylholothin.

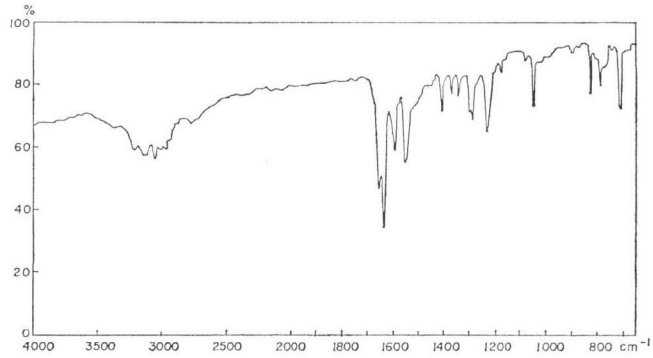


Fig. 2. Mass spectra of holomycin and N-propionylholothin.

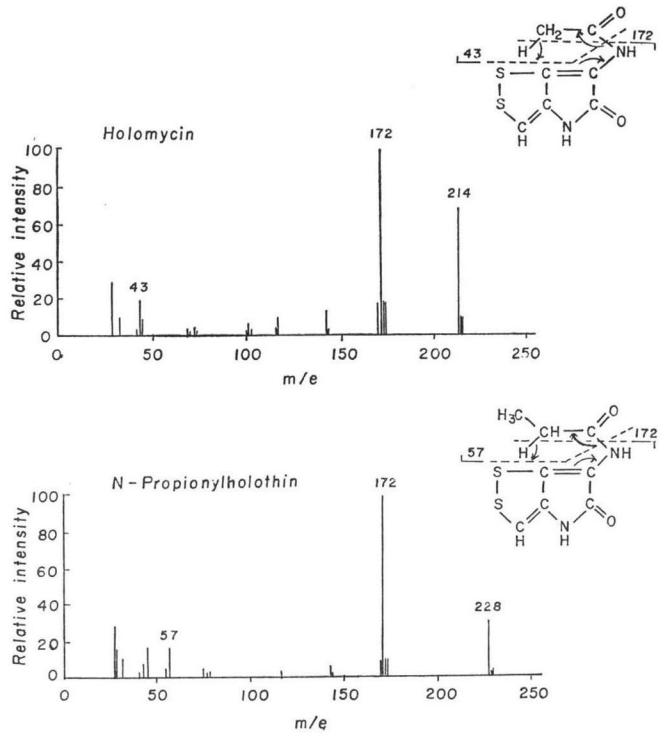
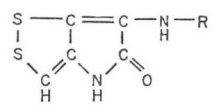


Fig. 3. Structure of N-acylholothins.



R: CH_3CO Holomycin
CH_3CH_2CO N-Propionylholothin

on a rotary shaker (200 rpm, eccentricity 70 mm).

The mycelium was removed by filtration. Five liters of broth filtrate were extracted twice with two liters of *n*-butanol and the extract was concentrated to dryness *in vacuo* to give a yellow powder. The yellow powder was dissolved in a small amount of methanol and subjected to Sephadex LH-20 column chromatography. After elution with methanol the yellow colored fractions were collected and concentrated to dryness to yield yellow crystals.

When a methanol solution of the yellow crystals was developed on a TLC plate (Merck Silicagel 60 F_{254}) with benzene - acetone (1: 1), two yellow spots were recognized and designated factor 1 (Rf 0.31) and factor 2 (Rf 0.25) respectively. After preparative thin-layer chromatography and recrystallization in methanol, 3.0 mg of factor 1 was obtained and 25 mg of factor 2. Factor 2 was in the form of orange yellow prisms and was identified as holomycin on the basis of the following data.

Mp: >300°C (lit.[2] 264~271°C), UV spectrum: $\lambda_{max.}^{MeOH}$ nm (log ε); 246 (3.81), 302 (3.49), 388 (4.05). PMR (100 MHz, DMSO-d_6) δ: 2.03 (3H, s, CH_3CO–), 7.05 (1H, s, >C=CH–), 9.86 (1H, s, –CONH–), 10.68 (1H, s, –CONH–). Mass spectrum: *m/e* 214 (M^+), 172 (M^+–CH_2CO, base peak) and 43 (CH_3CO^+). High resolution mass spectrum: *m/e* 213.9877 (M^+, 213.9870 calcd. for $C_7H_6N_2O_2S_2$). Factor 1 was also in the form of orange yellow prism and identified as N-propionylholothin on the basis of the following data. Mp: 255~264°C (lit.[2] 250~260°C). UV spectrum: $\lambda_{max.}^{MeOH}$ nm (log ε); 246 (3.89), 302 (3.63), 388 (4.16). Mass spectrum: *m/e* 228 (M^+), 172 ($M^+-C_2H_4CO$, base peak), 57 ($C_2H_5CO^+$). High resolution mass spectrum: *m/e* 228.0022 (M^+, 228.0027 calcd. for $C_8H_8N_2O_2S_2$). IR spectra are shown in Fig. 1. Mass spectra and the mode of fragmentation of factors 1 and 2 are illustrated in Fig. 2 and the structure of

holomycin and N-propionylholothin are shown in Fig. 3. N-Propionylholothin had been synthesized chemically but not reported before as the microbial product. The mutant 7N49 still produces about half the amount of cephamycin C as is produced by the parent strain which does not produce the holothins.

It is known that cephalosporin C is biologically synthesized from α-aminoadipic acid, cysteine, valine and acetate[3] and the nucleus of the antibiotic is derived from cysteine and valine.[4] From their structure it is considered likely that biosynthesis of the holothin also involves cysteine which decreases the pool available for cephamycin C biosynthesis and thus diminishes the level of cephamycin C produced.

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