

## Notes

HEXAENE H-85, A HEXAENE  
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The hexaene macrolide antibiotics constitute the smallest group of the polyenes (12~15). Mediocidin, endomycin, hexaene H-85 and hexafungin each have a significant tetraene spectrum in addition to a hexaene spectrum (hexaene spectrum being predominant) and show high activity against fungi and moderate against Gram-positive bacteria. It was not possible to isolate the separate tetraene and hexaene components from the complex<sup>1</sup>.

The antibiotic producing strain earlier named *Actinomyces* sp. 468 was identified as a strain of *Streptomyces hygroscopicus*<sup>2</sup>. The strain has been deposited in the culture collection of the Faculty of Chemistry, Belgrade. The strain was isolated from a soil sample from Vojvodina, Yugoslavia.

The fermentation was carried out in a fermenter (model Churchill, L. H. Engineering Co., Ltd., England) of 20 liters capacity, filled with 10 liters of the production medium. The fermenter was inoculated with 200 ml inoculum (2%). The fermentation was run at 28°C under agitation of 1 v/v/minute.

The antibiotic was isolated from the fermentation broth by extraction with 1-butanol and purified

by Sephadex LH-20. The active principle, named hexaene H-85, is yellow powder which gradually decomposes on heating over 130°C. An elemental analysis gave the following values: C 58.83, H 8.14, N 3.91, O (Calcd) 29.12 which corresponds to an empirical formula  $(C_{18}H_{29}NO_6)_n$ .

On the basis of pH-chromatography in 1-butanol saturated with water<sup>3</sup>, hexaene H-85 is an amphoteric substance. No sugars were found in acid hydrolysates<sup>4</sup>. No aromatic moiety was found in alkaline hydrolysates<sup>4</sup>.

Hexaene H-85 showed a single spot on silica gel (F<sub>254</sub>, 0.25 mm, Merck, Darmstadt, FRG) with several kinds of solvent systems (BuOH-EtOH-H<sub>2</sub>O, 4:1:5, Rf 0.57; BuOH-AcOH-H<sub>2</sub>O, 6:2:2, Rf 0.64; BuOH-MeOH-H<sub>2</sub>O, 4:1:5, Rf 0.41) but

Fig. 1. UV spectrum of the compounds of hexaene H-85 in MeOH.

(A) —, (B) ---.

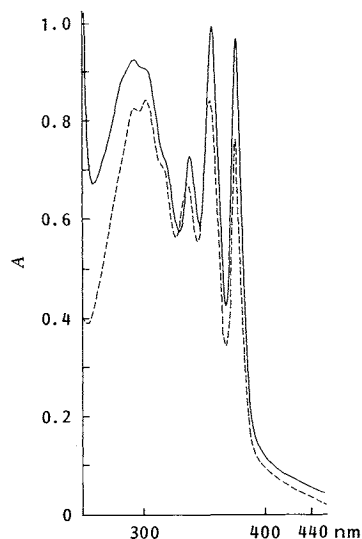


Fig. 2. IR spectrum of the compounds of hexaene H-85 in KBr.

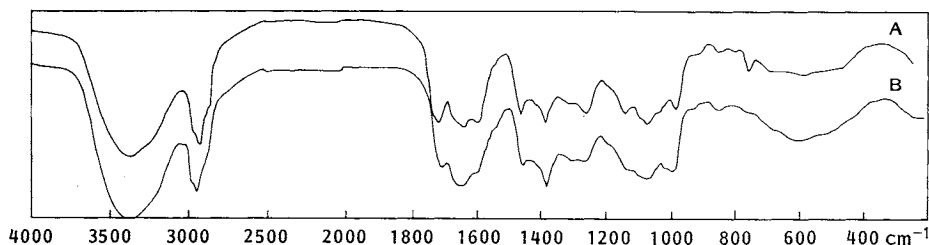


Table 1. MICs of hexaene H-85 against various microorganisms.

Strain	MIC ( $\mu\text{g/ml}$ )
<i>Candida albicans</i>	3.13
<i>C. stelatoidea</i>	3.13
<i>Cryptococcus neoformans</i>	1.56
<i>Scopulariopsis brevicaulis</i>	6.25
<i>Trichophyton mentagrophytes</i>	1.56
<i>T. rubrum</i>	1.56
<i>Epidermophyton floccosum</i>	1.56
<i>Microsporum gypseum</i>	3.13
<i>Aspergillus niger</i>	6.25
<i>Cladosporium werneckii</i>	0.78
<i>Fusarium oxisporum</i>	0.78
<i>Botrytis cinerea</i>	0.39
<i>Streptococcus faecalis</i>	50.00
<i>Escherichia coli</i>	100.00

two spots in solvent systems recommended by CIFTCI *et al.*<sup>1)</sup> Rf values obtained by the solvent system  $\text{CHCl}_3$ -MeOH-NH<sub>4</sub>OH, 4:10:1 were 0.54 and 0.75 and by system  $\text{CHCl}_3$ -MeOH-H<sub>2</sub>O, 6:10:1 were 0.84 and 0.90. Both of those spots were active similarly to complex hexaene H-85 (agar diffusion assay). No significant differences in the UV and IR spectrum of the compounds and the complex hexaene H-85 (Figs. 1 and 2).

The UV spectrum in methanol shows a characteristic polyene spectrum with maxima at 295, 303, 344, 364 and 384 nm, corresponding to a polyene antibiotic which has a significant tetraene spectrum in addition to a dominant hexaene spectrum.

The IR spectrum showed the presence of several hydroxyl groups (bands at 3400 and 1070  $\text{cm}^{-1}$ ); a band at 1715  $\text{cm}^{-1}$  (possibly lactone carbonyl) is

indicative of the macrolide nature of the antibiotic, and band at 1640  $\text{cm}^{-1}$  is indicative of polyene system.

MICs of hexaene H-85 against yeast, filamentous fungi and bacteria are given in Table 1.

On the basis of the spectral characteristics of the two components of the hexaene H-85 complex, it appears that this antibiotic complex belongs to the group of hexaene macrolides represented by hexafungin, mediocidin, endomycin and hexaene H-80<sup>1)</sup>. It is very likely that we are talking about the presence of very similar, possibly identical active principles that are variously combined in the previously isolated hexaene complexes. The lack of sugars on hydrolysis and the greatly reduced antifungal activity suggest that these two hexaene components of hexaene complex H-85 may be aglycones.

#### References

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