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COMPARISON OF CANDICIDIN, LEVORIN AND TRICHOMYCIN BY MEANS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method for the separation of individual components of polyene macrolide antibiotics has been developed. The three heptaene macrolide antibiotics, candicidin, levorin and trichomycin, have been investigated and compared. In all instances these compounds proved to be complex mixtures. It is demonstrated that candicidin and levorin are identical, whereas trichomycin differs in composition from these two substances.

INTRODUCTION

Polyene macrolide antibiotics are divided into groups, primarily according to the number of conjugated double bonds present. Secondarily, heptaene macrolide antibiotics are grouped according to their content of other functional groups[1]. Of that group in which the compounds have a heptaene, carboxylic acid, mycosamine and *p*-aminoacetophenone function in common, candicidin, levorin, and trichomycin are the three most important.

The structure of these substances has not yet been completely elucidated; however, it is presumed by Hamilton-Miller[1] that, apart from containing an extra *p*-aminoacetophenone group it corresponds to that of amphotericin B (Fig. 1), which was determined by Ganis *et al.*[2] by means of X-ray crystallography.

So far, several attempts have been made to characterize these compounds more satisfactorily. Vining *et al.*[3] found no difference between candicidin and trichomycin when examined by paper chromatography, which finding was confirmed later by Bhate and Acharya[4]. By use of counter-current distribution Borowski *et al.*[5] and Kalász *et al.*[6] found that candicidin and the main fractions of levorin and trichomycin are identical. Bosshardt and Bickel[7] have also demonstrated identity between candicidin and levorin by using this technique. By use of pyrolysis gas chromatography

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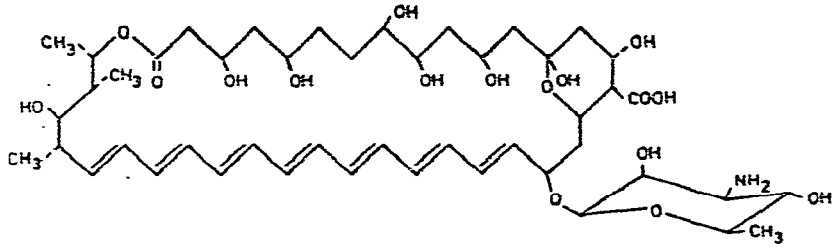

Fig. 1. Structure of amphotericin B.

Calam[8] found great similarity between candicidin and levorin, whereas trichomycin differs somewhat from them.

Recently, Mechlinski and Schaffner[9] have demonstrated by high-speed liquid chromatography that there is a difference between candicidin and trichomycin; they showed that these substances contain more than two components, but the resolution of the system was not adequate to make it possible to predict how many components each substance contains. The method presented here should provide a better solution to this problem.

EXPERIMENTAL

Apparatus

A DuPont Model 830 liquid chromatograph, equipped with a DuPont Model 837, spectrophotometer was used. Extinction at 380 nm was recorded by means of a Hewlett-Packard Model HP 3380 A integrator.

Column

This was a DuPont Permaphase ODS, 1 m long and 2.1 mm I.D., which is a reversed-phase partition column with octadecylsilane as stationary phase, chemically bonded to a controlled porous surface ["porosity beads" (20–37 μm)] by means of Si–O–Si bonds and contains about 1% of stationary phase. The pre-packed Permaphase ODS column has 600 theoretical plates, determined by isocratic elution of anthracene with methanol–water (6:4).

Mobile phase

Heptaene macrolide antibiotics are very sparingly soluble in non-polar liquids, and as it has been previously demonstrated that a certain degree of separation can be obtained by counter-current distribution[5–7], a reversed-phase system was applied.

All the antibiotics investigated are ampholytes, and therefore the pH of the eluent is of great importance. With increasing pH there is an increasing rate of elution, due to an increase in solubility. It was found to be an advantage to use an eluent with a buffer capacity between pH 7 and 9.5. A pH of higher than 9.5 should be avoided considering the nature of the packing material. Increasing ionic strength of the buffer also seems to increase the resolution.

With increasing temperature better resolution is also obtained because of the lower viscosity of the eluent and the increased mobility of the dissolved compounds.

The mobile phase finally chosen was as follows: 0.05 *M* phosphate buffer (pH 7.0)–methanol (8:2), which in the course of 33 min becomes 100% methanol according to a concave exponential gradient: $c = kt^5$, where c is the percentage change during the run, t is the time fraction passed and k is a constant. The final composition (100% methanol) is maintained for 12 min, after which equilibrium of the column is obtained with the initial composition in the course of 5 min (Fig. 2). During chromatography, the column is operated at 55° ± 1°.

Test solutions and solvents

The heptaene macrolide antibiotics investigated by HPLC are as follows: Candicin, obtained from producers* A and B; levorin, obtained from the U.S.S.R.; and trichomycin, obtained from the U.K. The test solutions were prepared by dissolving the heptaene macrolides in 1 volume of dimethyl sulphoxide and subsequently diluting it with 9 volumes of methanol, making the concentration about 1.25 mg/ml; 5 µl were injected under pressure by means of a Hamilton Model HP 305 N high-pressure syringe. All the reagents used were of analytical grade and were obtained from E. Merck (Darmstadt, G.F.R.).

The following final system was chosen so as to avoid conversion of the heptaene macrolides during chromatography. Column: Permaphase ODS, 1 m × 2.1 mm; temperature: 55° ± 1°; mobile phase: A: 0.05 *M* phosphate buffer (pH 7.0)–methanol (8:2), B: methanol; gradient: 0 → 100% B at 3%/min, hold at limit, concave gradient $c = kt^5$; pressure: 1000 p.s.i.; flow-rate: 1.0 ml/min; detection wavelength: 380 nm; attenuation: 0.08 a.u.f.s.; and chart speed: 5 mm/min.

RESULTS AND DISCUSSION

The chromatograms of candicidin from the two different producers show great similarity (Fig. 2). The retention times for the individual peaks are identical, but small quantitative differences are to be seen, which can, however, be neglected as they do not affect their identities.

By varying the detection wavelength gradually between 425 and 325 nm, it has been demonstrated that the peaks eluted first are all heptaenes, whereas the final peak results from the influence of the gradient on the refractive index of the eluent (Fig. 2).

The chromatograms in Fig. 3 show that candicidin and levorin are identical, apart from some small quantitative differences corresponding to those which occur between producers. These differences may be due partly to differences in the purification procedures used and partly to incipient degradation.

Trichomycin is distinctly different in composition from candicidin and levorin. The main fraction of trichomycin consists of not less than two components, and the retention time of the first component corresponds to the first small peak of candicidin. Therefore, there is a possibility that candicidin (and levorin) may contain a small amount of trichomycin. The reverse may also be true but would be considerably more difficult to demonstrate by this method.

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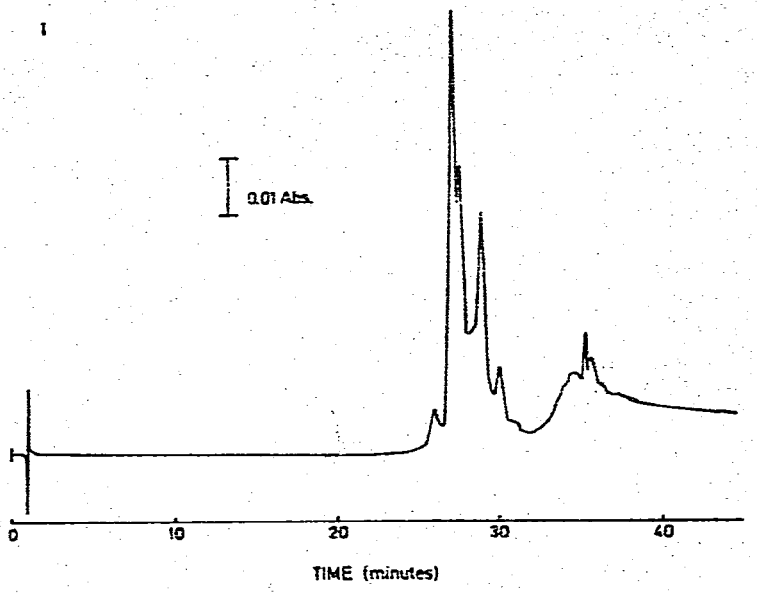
* A: Dumex (Copenhagen, Denmark), B: S. B. Penick (New York, U.S.A.).



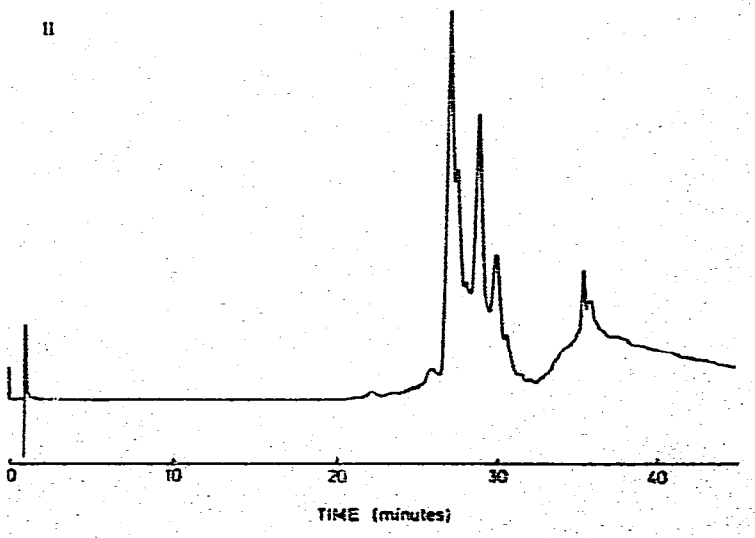


Fig. 2.

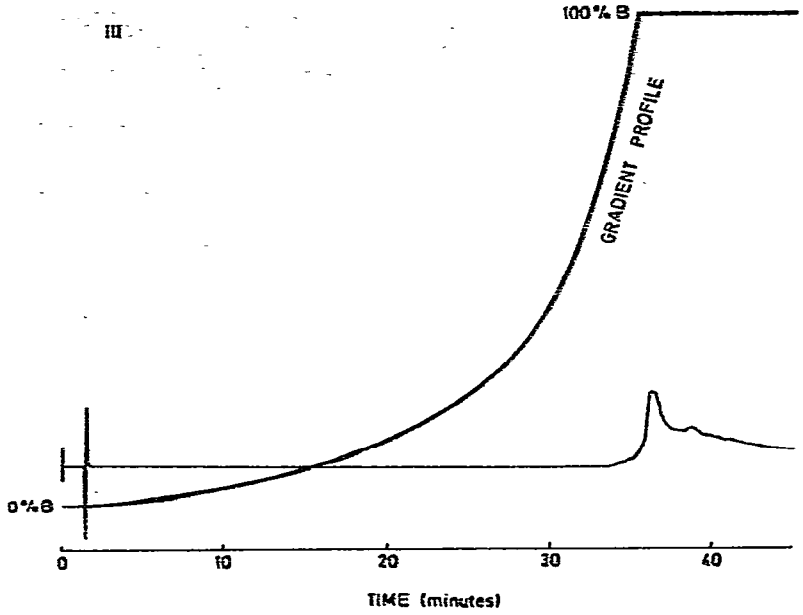


Fig. 2. High-performance liquid chromatograms of candicidin from two different producers. I, producer A; II, producer B; III, a blank and the shape of the gradient.

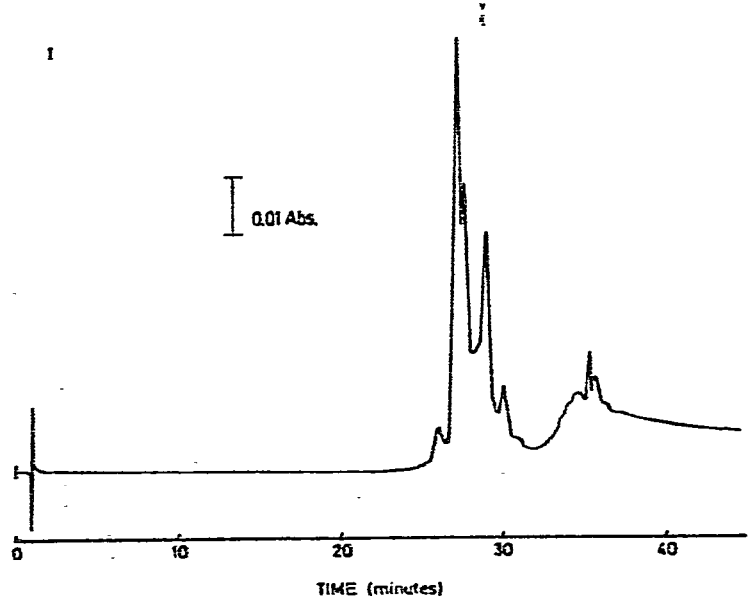


Fig. 3.

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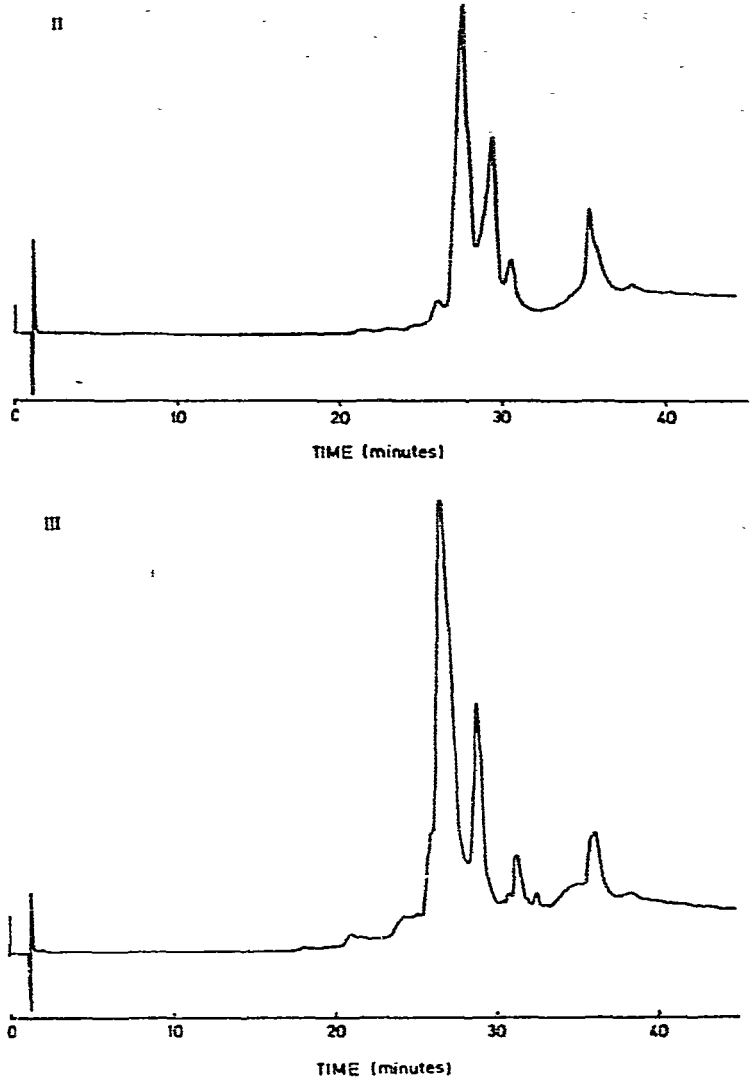

Fig. 3. High-performance liquid chromatograms of I, candicidin; II, levorin; III, trichomycin.

The method proposed here makes it possible to observe even small differences in the composition of the individual heptaene macrolides. The excellent resolution is especially due to the use of gradient elution.

CONCLUSION

A HPLC method has been developed for the characterization and comparison of heptaene macrolide antibiotics. This method is shown to be superior to other methods, *e.g.*, paper and thin-layer chromatography and counter-current distribution, both as regards resolution and the time required for analysis.

Candicidin, from two different producers, and levorin have proved to be identical, and to contain seven heptaene components. Trichomycin is different in composition from these two substances.

The method evolved is also shown to be applicable to other polyene macrolide antibiotics such as nystatin and amphotericin B.

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