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Note

Gas–liquid chromatography of methyl esters of natural penicillins

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During a study on the different types of penicillins produced by several mutants of *Penicillium chrysogenum*, a rapid analytical method was needed. Chromatography on water-saturated, buffered paper strips with diethyl ether as the mobile phase has been used extensively for the separation of natural penicillins in culture broths[1–3]; however, this technique is time consuming and gives very variable results. Although a few workers have described the gas chromatographic separation of esters of semi-synthetic penicillins[4–6], a similar method for naturally occurring penicillins is not available. In this paper we describe the separation of methyl esters of benzylpenicillin, phenoxymethylpenicillin and six penicillins with aliphatic side-chains by gas–liquid chromatography (GLC), and its application to the analysis of culture broths.

EXPERIMENTAL

Benzyl- and phenoxymethylpenicillin were obtained from R.I.T. (Genval, Belgium). Methyl-, *n*-propyl-, *n*-pentyl-, *n*-heptyl- and *n*-nonylpenicillin were prepared by condensing the appropriate acid chloride with 6-aminopenicillanic acid. The penicillins were extracted into diethyl ether from aqueous solutions at pH 2 and esterified with a slight excess of diazomethane at 0–2°; the ether solutions of the methyl esters were evaporated to dryness and the residues were dissolved in acetone. Samples of 1–3 μl were analyzed on a Pye 104 gas chromatograph with 150-cm columns of 3% OV-1 or 3% OV-17. Temperature programmes for both columns were used; after 10 min at 150° (OV-1 column) or 180° (OV-17 column), the temperature was increased at the rate of 4°/min for 10 min and than held constant to the end of the run.

RESULTS AND DISCUSSION

Chromatograms of a synthetic mixture of the penicillin methyl esters studied are shown in Fig. 1a for the OV-1 column and in Fig. 1b for the OV-17 column. Good separations were obtained on both columns. The retention times of the aliphatic penicillins increased with the length of the side-chain; also, benzylpenicillin was eluted before phenoxymethylpenicillin. However, the elution patterns on the two columns were not identical: the separation of *n*-heptyl- and benzylpenicillin was better on the

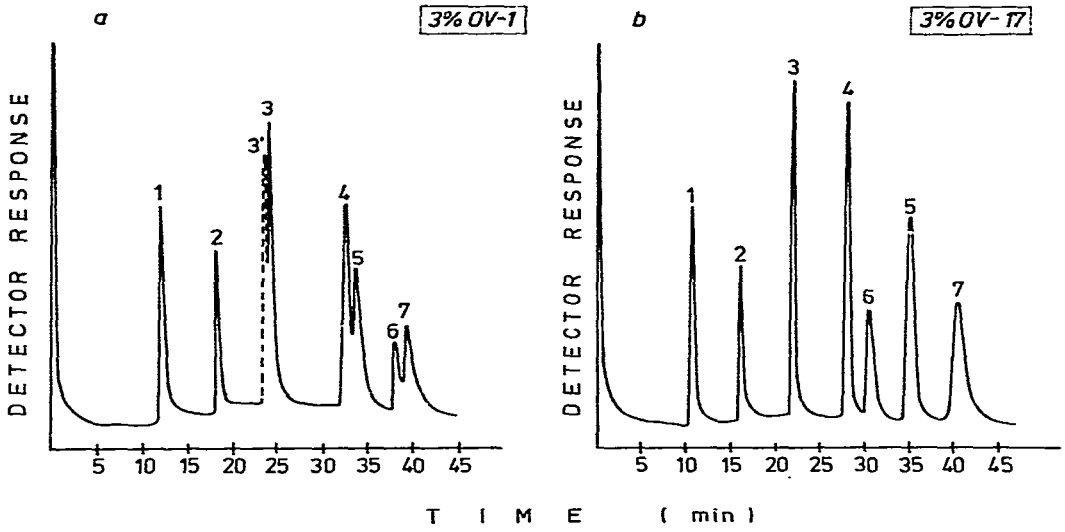

Fig. 1. Gas chromatograms of a synthetic mixture of methyl esters of naturally occurring penicillins. Coiled glass columns, 150 cm × 4 mm I.D., packed with (a) 3% OV-1 or (b) 3% OV-17 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs., State College, Pa., U.S.A.) were used. The carrier gas was nitrogen at a flow-rate of 60 ml/min; details of the temperature programme are given in the text. Peaks: 1 = methylpenicillin; 2 = *n*-propylpenicillin; 3 = *n*-pentylpenicillin; 3′ = *n*-2-pentenylpenicillin; 4 = *n*-heptylpenicillin; 5 = benzylpenicillin; 6 = *n*-nonylpenicillin; 7 = phenoxymethylpenicillin.

OV-17 column, and *n*-nonylpenicillin was eluted before benzylpenicillin on the OV-17 column and just before phenoxymethylpenicillin on the OV-1 column.

The method was used to identify the different penicillins produced by several mutants of *P. chrysogenum*. Culture filtrates of *P. chrysogenum* ATCC 26818, a high-producing strain, grown in the medium of Jarvis and Johnson[7] without added side-chain precursor, were saturated with ammonium sulphate and extracted with ethyl acetate at pH 2; the extracted penicillins were esterified with diazomethane and analysed by GLC. On the OV-17 column, *n*-pentyl- and *n*-heptylpenicillin could be detected. However, on the OV-1 column, a third penicillin was eluted just before *n*-pentylpenicillin and was identified by mass spectrometry as *n*-2-pentenylpenicillin (m/e^+ 326), which is known to occur in *Penicillium* broths[2]. To confirm that these compounds were penicillins, a portion of the culture filtrate was first treated with penicillinase; after extraction and esterification, the penicillin methyl esters were no longer detected.

For analysis of low-yielding *Penicillium* mutants producing not more than 5 μg/ml and grown in a rich medium[8], the penicillinase test was very useful for the differentiation of penicillins and impurities in the extract. Ethyl acetate extracts of culture filtrates from a leu–met–cys auxotroph of *P. chrysogenum* Wis. 49-2105 were analyzed by GLC, before and after treatment with penicillinase. Although grown in the presence of phenylacetic acid, this strain produced relatively high levels of *n*-propyl-, *n*-pentyl-, *n*-2-pentenyl- and *n*-heptylpenicillin, in addition to penicillin G. In addition, an unknown penicillin was eluted between *n*-pentyl- and *n*-heptylpenicillin on the OV-1 column, but coincided with the latter on the OV-17 column. Further studies on the identification of this penicillin are in progress.

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