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Note

Analysis of potassium penicillin G and its degradation products by thin-layer chromatography

The analysis of penicillin has been directed principally at methods of identifying and quantifying the intact molecule. However, recent interest in the pharmacokinetics of antibacterials and the possible role of penicillin degradation products in penicillin allergy requires that analytical methods be developed for both the intact penicillin molecule and degradation products. Thin-layer chromatography (TLC) has played an extensive role in the analysis of penicillins. McGILVERAY AND STRICKLAND[1] developed TLC procedures for the identification of ten natural and semi-synthetic penicillins. HELLBERG[2] has also developed a method for the separation of five penicillins. The TLC separation of ampicillin and cloxacillin in body fluids has been achieved[3], as has been the resolution of components in solutions of procaine penicillin G[4]. BIRNER[5] used a previously developed TLC system[1] to separate phenoxymethylpenicilloic acid and phenoxyethylpenicilloic acid from the parent penicillins; the penicilloic acids were then eluted and determined quantitatively by a colorimetric procedure. Recently, TLC was employed to separate the decomposition products of ampicillin from the parent molecule[6].

The emerging interest in thin-layer spectrodensitometry (TLS) for quantification of antibiotics[7,8] led us to apply this technique to the analysis of potassium penicillin G[9]. A prerequisite to the successful application of TLS is the development of TLC systems that will separate the analyte from other components of a mixture. From an operational standpoint the separations should occur quickly, and one system should be successful in simultaneously separating a number of possible interfering substances from the analyte. The purpose of this investigation was to develop a TLC system that would rapidly separate potassium penicillin G and its degradation products.

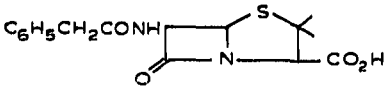
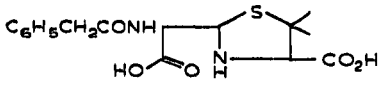
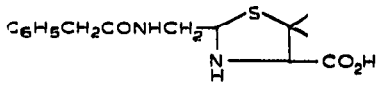
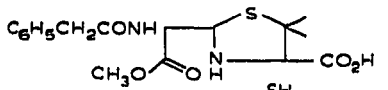
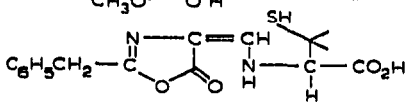
Experimental

Materials. Potassium penicillin G (Chas. Pfizer and Co., New York, N.Y.) and D-benzylpenicillenic acid (Sigma Chemical Co., St. Louis, Mo.) were obtained commercially. Monosodium D-benzylpenicilloate, D-benzylpenilloic acid, and α-methyl-D-α-benzylpenicilloate were synthesized by standard methods[10]. The spectroscopic properties of each product indicated that the major component was the desired compound contaminated with a small amount of penicillin G. This conclusion was confirmed by TLC. Further purification was not effected because a pure product was not required for this qualitative study.

Thin-layer chromatography. Silica gel plates (EM Laboratories, Inc., Elmsford, N.Y.) (20 × 20 cm) were spotted using a 10-μl. Hamilton syringe. Typically, 5 μg of each degradation product was spotted from an aqueous solution. Chromatograms were developed in each solvent system until the mobile phase had travelled 15 cm. Development occurred in chromatographic chambers (7 × 28 × 22 cm) lined with

TABLE I

STRUCTURE OF PENICILLIN G AND SOME DEGRADATION PRODUCTS

Name	*Structure*
Penicillin G	
Benzylpenicilloic acid	
Benzylpenilloic acid	
α-Methyl-D-benzylpenicilloate	
Benzylpenicillenic acid	

Whatman No. 1 filter paper and presaturated with vapors of the mobile phase. Chromatograms were visualized by first spraying with ferricyanide reagent[1] followed by exposing the plate to iodine vapor. The latter procedure made visualization more sensitive.

Results and discussion

Table I shows the structures of the penicillin degradation products studied. They were selected to represent the types of decomposition reactions which commonly occur in aqueous solution[11]. Penicilloic acid is produced during alkaline hydrolysis or in the presence of penicillinase. Penilloic acid is believed to arise from rearrangement of penicilloic acid in acidic media. Penicillenic acid forms via acidic decom-

TABLE II

SEPARATION OF PENICILLIN DEGRADATION PRODUCTS

Solvent systems: A = acetone–acetic acid (95:5); B = organic phase of amyl acetate–methanol–formic acid–water (65:20:5:10); C = chloroform–isopropanol–water (60:40:4); D = ethyl acetate–acetic acid–water (8:1:1); E = *n*-propanol–water (70:30).

Solvent system	*Reference*	*R_F value*			*Development time (h)*[a]
		Penicillin G	*Benzylpenicilloic acid*	*Benzylpenilloic acid*	
A	1, 4, 5	0.85	0.78	0.76	1.5
B	1, 4	0.62	0.45	0.42	2
C	2	0.65	0.56	0.55	3.5
D	3	0.90	0.74	0.66	2.5
E	6	0.65	0.56	0.55	4

[a] Time for solvent front to travel 15 cm.

position. α-Methyl-penicilloates represent a class of esters produced by decomposition of penicillins in the presence of alcohols.

The previously reported TLC methods were evaluated to determine if they could be employed for the separation of penicillin degradation products. As indicated by the data in Table II, these systems can separate intact penicillin from several degradation products but they cannot resolve two related decomposition products such as benzylpenicilloic acid and benzylpenilloic acid.

An acetone–chloroform–glacial acetic acid (50:45:5) solvent system was developed which is capable of resolving all of the degradation products studied in the presence of the parent penicillin. As indicated in Table III, each decomposition

TABLE III

SEPARATION OF PENICILLIN DEGRADATION PRODUCTS BY THE RECOMMENDED TLC SYSTEM[a]

Compound	*R_F value*
Penicillin G	0.33
Benzylpenicilloic acid	0.15
Benzylpenilloic acid	0.25
Benzylpenicillenic acid	0.38
α-Methyl-D-benzylpenicilloate	0.53

[a] Acetone–chloroform–glacial acetic acid (50:45:5).

product was separated from the parent penicillin. The decomposition products studied were also clearly separated from other decomposition products. In addition, development was relatively rapid requiring approximately 1 h for this system.

Consistent R_F values were obtained when mixtures containing two or three of the compounds in Table III were developed using this solvent system.

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