Journal of Chromatography, 77 (1973) 458–460

CHROM. 6528

Note

Detection of penicillins with chloroplatinic acid on thin-layer chromatoplates

There are several chemical reagents that can be used for the detection of penicillins on thin-layer chromatographic (TLC) plates. However, most of them are neither specific nor sensitive enough (iodine vapour, sulphuric acid, potassium permanganate, iodine–potassium iodide–ninhydrin), while the others are more specific but not sensitive and it is not easy and simple to work with them (iodine–azide, nitroprusside, chlorine–toluidine, iodine–starch). Therefore, it seemed of interest to seek a reagent that would be both sufficiently specific and sensitive for penicillins.

Chloroplatinic acid[1], the so-called platinum reagent, is very often used in amino acid biochemistry as a specific and highly sensitive reagent for the chromatographic detection of thioether amino acids. Recently, it has been found that it also can be used for the detection of many organosulphur compounds[2,3]. Since the penicillins are also thioethers, we considered chloroplatinic acid as a possible reagent for their chromatographic detection. This paper describes the use of chloroplatinic acid[1], in a slightly modified form, as a highly sensitive and a specific reagent for the rapid and simple detection of all penicillins and penicillin-related substances on TLC plates.

Experimental

The compounds used were obtained commercially or synthesized in our laboratory by standard procedures[4]. Amoxicillin, fluoxacillin and propenyl penicillins were kindly given by Dr. U. VALCAVI, I.B.I., Milan, Italy.

Solutions of the tested compounds were freshly prepared by dissolving them in water, buffer or organic solvents in a concentration of 10 or 100 μg/ml. Aliquots of 1–10 μl were spotted quantitatively on TLC plates coated with either Silica Gel G (Merck) or cellulose (Merck) in standard manner. The TLC plates were developed one-dimensionally with one of the following solvent systems: (A) *n*-butanol–ethanol–water (12:8:2); (B) *n*-butanol–ethanol–acetic acid–water (50:15:15:20); (C) *n*-butanol–acetic acid–water (60:15:25). The solvent front was allowed to travel 10 cm from the starting point. The developed plates were thoroughly dried and the spots were detected by spraying with a chloroplatinic reagent comprising 1 ml of 0.2% $PtCl_4$ solution, 0.1 ml 20% KI solution, 0.1 ml of 3–4% HCl and 20 ml of acetone (p.a. grade). It is convenient to combine the prepared solutions of $PtCl_4$, HCl and acetone and to add the KI solution just before spraying; these two solutions stored in refrigerator are stable for several weeks if kept separately, but the final combined reagent is stable for only 1 day.

Results

The chloroplatinic reagent used in this work is qualitatively the same as that proposed for paper chromatography[1], except for the concentration of $PtCl_4$, which

TABLE I

LIMITING SENSITIVITIES AND R_F VALUES OBTAINED WITH THE CHLOROPLATINIC REAGENT FOR PENICILLIN COMPOUNDS

TLC plates: Silica Gel G. Solvent system: *n*-butanol–acetic acid–water (60:15:25).

Compound	*Limit of detection*[a] *(μg)*	*R_F value* × *100*
6-Aminopenicillanic acid	0.05	46
Penicilloic acid	0.1	36
Penilloic acid	0.05	43
8-Hydroxypenicillic acid	0.05	65
Penicillamine	0.3	18
Benzylpenicillin	0.05	85
Benzylpenicilloic acid	0.05	65
Benzylpenicillenic acid	0.3	82
Benzylpenillic acid	0.1	85
Benzylpenicillinic acid	0.1	55
Ampicillin	0.05	58
N-BOC-Ampicillin	0.1	95
Amoxicillin	0.1	58
Hetacillin	0.1	58
Epicillin	0.1	55
Thenoilpenicillin	0.1	88
Tritilpenicillin	0.3	90
Meticillin	0.3	78
Oxacillin	0.8	83
Cloxacillin	1.3	85
Dicloxacillin	1.4	85
Fluocloxacillin	0.8	95
Phenoxymethylpenicillin	0.1	82
1-Hydroxy-2-methyl-3-phenyl-2-propenylpenicillin	1.1	90
2-Methyl-3-phenylpropenylpenicillin	2.0	95
2-Methyl-2-phenylpropenylpenicillin	1.6	83
6-Aminopenicillanic acid sulphoxide	0.5	10
Benzylpenicillin sulphoxide	0.7	12
Phenoxymethylpenicillin sulphoxide	0.6	12
Tritilpenicillin sulphoxide	1.6	8
Benzylpenicillin benzyl ester	1.2	95
Benzylpenicillin ethyl ester	1.0	95
Phenoxymethylpenicillin benzyl ester	1.2	98
Phenoxymethylpenicillin sulphone	n.d.	n.d.
Benzylpenicillin sulphone	n.d.	n.d.
Tritilpenicillin sulphone	n.d.	n.d.

[a] Average values from three chromatoplates.

n.d. = not detected over 20 μg.

is four times higher. In this way, a better contrast on the TLC plates between the spots and the background is obtained. The contrast is also improved by exposing the sprayed chromatoplates to HCl vapour. After spraying, penicillins and related compounds at once give a white spot on a red–purple background, sulphoxides give a yellow to yellow–blue spot after several minutes, while sulphones do not give a colour reaction at all. The spots are stable for at least 24 h, provided that the ambient air is free from alkali (even cigarette smoke) and phenolic compounds.

The results obtained with many penicillinic compounds (Table I) unambiguously indicate that the chloroplatinic reagent can be used successfully for the detection of all penicillins and practically all penicillin intermediates and biological metabolites. We have not found any differences in sensitivity and behaviour between

silica gel and cellulose plates and the different solvent systems used. From Table I, it can be seen that the limit of detection for penicillins depends on the nature of the acylamino side-chain. The chloroplatinic reagent is highly sensitive for several common penicillins (penicillin G, penicillin V, ampicillin, epicillin, amoxicillin and hetacillin) and their metabolites; the limit of detection varies from 0.05 to 0.3 μg; the sensitivity is the same as that obtained by microbiological procedures. The method is less sensitive, but still adequate, for isoxazolinic and propenyl penicillins, penicillin esters and sulphoxides.

Since this reagent is sensitive, specific and general for all penicillinic substances, it is convenient for chromatographic use in biochemical, pharmacological and chemical studies.

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Received October 23rd, 1972