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Note

Use of π-acceptors as spray reagents for the detection of penicillins on thin-layer plates

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Several methods for the separation and detection of natural and semisynthetic penicillins and their degradation products by thin-layer chromatography (TLC) have been reported. Beside bioautography, the spray reagents generally employed to detect these drugs are iodine[1,2], iodine–sodium azide[3–6], permanganate[7,8], ninhydrin[6,9–11], iodic acid[12] and isatin[13]. Phokas *et al.*[14] used a mixture of ninhydrin and collidine as spray reagent for ampicillin. McGilveray and Strickland[9] used a mixture of ferric chloride and potassium ferricyanide in 20% sulphuric acid for the identification of ten penicillins. This reagent, which has been widely used[11,15], requires up to 5 min for full colour development, depending on the nature of the penicillins. Manni *et al.*[16] visualized penicillin and its degradation products on thin-layer plates by spraying first with the ferricyanide reagent of McGilveray and Strickland, followed by exposure of the plates to iodine vapour which made visualization more sensitive. Cruceanu *et al.*[17] used a mixture of 10% $FeCl_3$, 2% potassium hexacyanoferrate(III) and HCl (1:2:6) for the visualization of penicillin spots. Sinsheimer *et al.*[18] used 9-isothiocyanatoacridine as fluorescent label for the detection of benzylpenicillin, phenoxymethylpenicillin and methicillin after hydrolysis of the sample with penicillinase. Walash and Hassan[19] used metal ions such as copper(II), cobalt(II), nickel(II), iron(III) and cerium(IV) for characterization of six semisynthetic penicillins.

Other spray reagents employed include arsenomolybdate[20] and chloroplatinate[21]. Some of the disadvantages associated with the use of spray reagents like ninhydrin (less sensitive)[6], iodine (colour fading with time)[6,22] and ferricyanide (requiring a further exposure to iodine)[16] limit their usefulness. The use of π-acceptors as spray reagents in the identification of pharmaceuticals on TLC plates has been reported[23–27]. Belal *et al.*[28] developed a spectrophotometric assay for penicillin G by reacting it with chloranil as the π-acceptor, forming a purple charge-transfer complex. Here, we report the use of several π-acceptors as effective spray reagents which produce highly stable colours for the detection of penicillins on thin-layer plates.

EXPERIMENTAL

Materials

Pure samples of ampicillin, nafcillin sodium, oxacillin sodium (all USP reference standards), carfecillin sodium, cloxacillin sodium, flucloxacillin sodium, meth-

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icillin sodium and phenethicillin sodium (Beecham Laboratories) were obtained from various sources. Some antibiotic dosage forms were obtained from the University Medical Centre. *p*-Chloranil, *p*-fluoranil and 2,5-dichloro-*p*-benzoquinone were from Pflatz and Baüer and were used as received. Solvents and other reagents were of reagent grade.

Spray reagents

The following spray reagents were freshly prepared: I, 0.5% *p*-chloranil in dioxane followed by dimethylformamide (DMF); II, 0.5% *p*-fluoranil in dioxane; III, 0.5% 2,5-dichloro-*p*-benzoquinone in dioxane.

Thin-layer chromatography

The drugs were dissolved in methanol. The sample was applied to silica gel G (0.2 mm) TLC plates and after development in methanol–ethyl acetate (80:20) the plates were air-dried and sprayed.

RESULTS

Table I gives the results of reactions of fifteen natural and semisynthetic penicillins on TLC plates with chloranil, fluoranil and 2,5-dichloro-*p*-benzoquinone as spray reagents. When 0.5% chloranil in dioxane alone was used as spray reagent the reaction between the π-acceptor and the penicillins was very poor, resulting in a blue spot for benzylpenicillin, faint pink spots for ampicillin and amoxycillin and a purple spot for becampicillin, while other penicillins remained invisible. Attempts were made to intensify the reaction with the aid of DMF as catalyst and this resulted in the immediate visualization of all spots. Most semisynthetic penicillins showed purple coloration while ampicillin and amoxycillin gave brown, becampicillin and carbenicillin orange and benzylpenicillin green spots. In a previous study[26] DMF was shown to exert a similar catalytic action on the reaction between the π-acceptor chloranil and alkaloids. The intensity of the colour was greatest with amoxycillin, benzylpenicillin and carfecillin and lowest with carbenicillin and ciclacillin. The spots are quite stable and can be seen even after the plates have been stored for several weeks.

With fluoranil (II) as the spray reagent there is an immediate development of a pink spot with most penicillins studied, except benzylpenicillin, phenoxymethylpenicillin and pivampicillin which gave purple spots. However, ciclacillin did not react with this reagent. The colours are quite prominent at the time of spraying but begin to fade after about 2 min. Overspraying with DMF was not helpful as it caused disappearance of all spots except those of becampicillin, benzylpenicillin and pivampicillin.

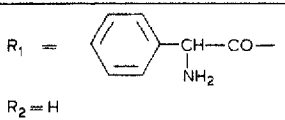
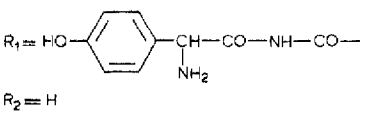
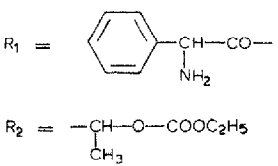
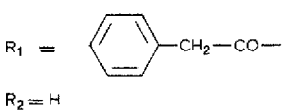
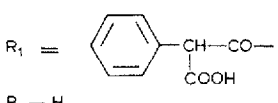
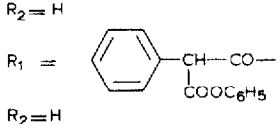
2,5-Dichloro-*p*-benzoquinone (III) generally gave a purple spot with the penicillins studied, except for ampicillin, amoxycillin, becampicillin, pivampicillin (all pink) and nafcillin (brown). Here too, overspraying of the plate with DMF did not result in any improvement.

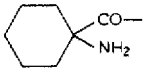
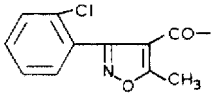
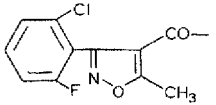
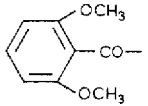
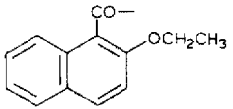
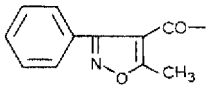
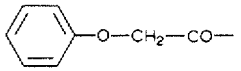
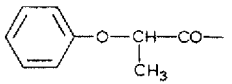
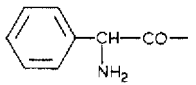
Other π-acceptors such as chloranilic acid or 2,3-dichloro-5,6-dicyano-*p*-benzoquinone alone or in combination with DMF did not give any colour reaction with penicillins. Salts of benzylpenicillin such as benethamine penicillin, benzathine penicillin, clemizole penicillin and procaine penicillin all reacted with chloranil–DMF (I)

TABLE I

REACTIONS OF PENICILLINS WITH SPRAY REAGENTS

Order of increasing response: +, + +, + + +.

Compound	*Structure*	*Chloranil + DMF*		*Fluoranil*		*2,5-Dichloro-p-benzoquinone*	
		Colour (response)	*Detection limit (μg)*	*Colour (response)*	*Detection limit (μg)*	*Colour (response)*	*Detection limit (μg)*
Ampicillin		Brown (+)	2.0	Pink (+)	2.0	Pink (+)	3.0
Amoxycillin		Brown (+ + +)	1.0	Pink (+)	2.0	Pink (+)	3.0
Becampicillin		Orange (+ +)	1.5	Pink (+)	2.0	Pink (+ +)	2.0
Benzylpenicillin		Green (+ + +)	1.0	Purple (+)	2.0	Pink (+ +)	2.0
Carbenicillin		Orange (+)	3.0	Pink (+)	2.0	Purple (+)	4.0
Carfecillin		Purple (+ + +)	1.0	Pink (+)	2.0	Purple (+)	4.0

Ciclacillin	R_1 =  R_2 = H		Purple (+)	3.0	–		Purple (+)	4.0
Cloxacillin	R_1 =  R_2 = H		Purple (++)	1.5	Pink (+)	2.0	Purple (+)	4.0
Flucloxacillin	R_1 =  R_2 = H		Purple (++)	1.5	Pink (+)	2.0	Purple (+)	4.0
Methicillin	R_1 =  R_2 = H		Purple (+)	2.0	Pink (+)	2.0	Purple (+)	4.0
Nafcillin	R_1 =  R_2 = H		Purple (+)	2.0	Pink (+)	2.0	Brown (+)	4.0
Oxacillin	R_1 =  R_2 = H		Purple (+)	2.0	Pink (+)	2.0	Purple (+)	4.0
Phenoxymethyl-penicillin	R_1 =  R_2 = H		Purple (++)	1.0	Purple (++)	2.0	Purple (+)	4.0
Phenethicillin	R_1 =  R_2 = H		Purple (++)	1.0	Pink (+)	2.0	Purple (+)	4.0
Pivampicillin	R_1 =  R_2 = $-CH_2-OCO-C(CH_3)_3$		Purple (+)	3.0	Purple (++)	2.0	Pink (+)	4.0

as spray reagent to give purple spots, except for benzathine penicillin which produced a yellow spot. Cephalexin, a cephalosporin antibiotic, was also reactive giving prominent brown spots with chloranil–DMF (I) or 2,5-dichloro-*p*-benzoquinone (III) and a pink spot with fluoranil (II).

In conclusion, chloranil–DMF (I) is recommended as a spray reagent for the detection of penicillins on silica gel G plates as (i) it reacts immediately giving highly coloured spots (ii) it is quite sensitive and (iii) the spots are stable for several weeks.

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