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Note

Highly specific and sensitive detection method for nitrofurans by thin-layer chromatography

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Several 5-substituted 2-nitrofurans (nitrofurans) have human and/or veterinary uses as antibacterial agents[1]. Nitrofurans have been detected on thin-layer chromatography (TLC) plates containing a fluorescent indicator[2,3] or by spraying chromatograms with a solution of fluorescein[4]. Methanolic potassium hydroxide has also been used as a detection reagent for some nitrofurans[4,5]. These detection methods lack sensitivity and are non-specific; for example, some nitrobenzamides used as antibacterial agents can also be detected with methanolic potassium hydroxide[5].

Methods have recently been reported[6–8] for the determination of furazolidone, a nitrofuran, in tissue, by measurement of the fluorescent intensity of an adduct or adducts of unknown structure with pyridine on a TLC plate. In these methods the fluorescent spot is produced by spraying a developed plate with pyridine followed by irradiation with longwave ultraviolet light. In a collaborative study recently reported by Heotis and coworkers[6], less than 1 ng of furazolidone was determined.

We have investigated the extension of the principle used for furazolidine to the detection of other nitrofurans. Several nitrofurans were examined including those with current medicinal applications. To demonstrate the specificity of the method, representative compounds of other classes of drugs used as antibacterial agents, including two nitroimidazoles and two nitrobenzene derivatives, were examined to see if fluorescent pyridine adducts could be detected.

EXPERIMENTAL

The sources of the compounds used were: Norwich-Eaton Pharmaceuticals (Norwich, NY, U.S.A.; furaltadone, nihydrazone, nitrofurazone, and furazolidone); Aldrich (Milwaukee, WI, U.S.A.; nitrofurantoin, 5-nitro-2-furoic acid, 5-nitro-2-furaldehyde diacetate, 2-furaldehyde, 2-furoic acid, and 5-nitro-2-thiophenemethanol diacetate), Salisbury Labs. [Charles City, IA, U.S.A.; aklomide (2-chloro-4-nitrobenzamide), dimetridazone (1,2-dimethyl-5-nitroimidazole), and 5-nitro-2-furaldehyde], Sigma [St. Louis, MO, U.S.A.; ipronidazole (2-isopropyl-1-methyl-5-nitroimidazole), nifuroxime (5-nitro-2-furaldoxime), chloramphenicol, chlortetracycline, and sulfathiazole], Upjohn (Kalamazoo, MI, U.S.A.; procaine penicillin), and American Cyanamid (Bound Brook, NJ, U.S.A.; sulfamethazine). All compounds and solvents were used as received. Reagent-grade pyridine (Baker, Phillipsburg, NJ, U.S.A.) was used without further purification.

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The compounds were dissolved in reagent-grade acetone to give solutions containing 0.5 ng/μl of the nitrofurans and 50 ng/μl of the other compounds. Aliquots of these solutions were applied with 1-, 2- or 10-μl micro-pipets to silica gel G pre-coated plates (2.5 × 10 cm) with 0.25-mm layers (Analtech). In the manner of Bortoletti and Perlotto[4], the nitrofurans were developed with dioxane–benzene (1:1), the other compounds were developed with ethyl acetate or with ethyl acetate–methanol (1:1). Using a procedure similar to the one reported by Heotis *et al.*[6], the plates were air dried, sprayed with pyridine, and placed under an ultraviolet lamp containing a 4-W, 366-nm tube (Blak-Ray UVL-21, Fisher Scientific Co.) for 10 min. Fluorescent spots were viewed in a chromatographic viewer under 366-nm light.

RESULTS AND DISCUSSION

All the nitrofurans examined gave fluorescent pyridine adducts and were detected in amounts ranging from less than 1 ng to 10 ng. The limits of detection, the color of the fluorescent spots, and the R_F values are listed in Table I. 5-Nitro-2-furaldehyde is a hydrolysis product of the nitrofuran drugs; 5-nitro-2-furoic acid is a metabolite of 5-nitro-2-furaldehyde[9], nihydrazone[10], and perhaps other nitrofurans. In order to learn more about the scope of the method, 5-nitro-2-furaldehyde diacetate was examined to determine if a nitrofuran with a saturated substituent could be detected.

TABLE I
TLC OF NITROFURANS: LIMITS OF DETECTION, COLOR OF SPOTS AND R_F VALUES
Solvent: dioxane–benzene (1:1).

Compound	*Limit of detection (ng)*	*Color of fluorescent spot*	R_F
Furaltadone	2	Green	0.41
Furazolidone	<1	Green	0.66
Nihydrazone	<1	Green	0.61
Nitrofurantoin	<1	Green	0.79
Nitrofurazone	<1	Yellow-green	0.44
Nifuroxime	3	Green	0.57
5-Nitro-2-furoic acid	1	Blue	0.21
5-Nitro-2-furaldehyde	3	Blue	0.87
5-Nitro-2-furaldehyde diacetate	10	Blue	0.88

We examined other compounds used as antibacterial agents by the same method. These compounds were: aklomide, chloramphenicol, dimetridazone, ipronidazole, chlortetracycline, procaine penicillin, sulfamethazine, and sulfathiazole. 2-Furaldehyde and 2-furoic acid were examined to determine if the nitro group was required for detection. All compounds were examined at 0.1, 1, and 5 μg. None of the compounds tested were detected when the plates were viewed under 366-nm light after treatment with pyridine and irradiation. In fact, of all the compounds examined, other than the nitrofurans, the only one detected by this method was an analogue of 5-nitro-2-furaldehyde diacetate (5-nitro-2-thiophenemethanol diacetate).

CONCLUSION

The formation of fluorescent pyridine adducts on silica gel TLC plates appears to be highly specific for nitrofurans. Procedures utilizing this method should be suitable for the detection of nitrofuran residues in tissue and biological fluids.

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