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Note

Thin-layer chromatographic determination of simple phenols in microbial extracts

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A study of the microbial metabolism of diverse chemical entities has commenced in our laboratories. The major purpose of this program is to define series of microorganisms that affect transformations identical to those occurring in mammals. It has been proposed that such systems could become a valuable tool in xenobiotic metabolism studies[1]. The hydroxylation of twelve simple aromatic compounds by eleven microorganisms has been studied[1] as an initial phase of this work. During these experiments, TLC systems were required for the qualitative determination of the potential phenolic metabolites of the substrates noted in Table I. More specifically, three solvent systems were sought for the metabolites of each substrate: for greatest possible discrimination[2], attempts were made to devise series containing acidic, basic and neutral solvent systems. The TLC systems were also required to distinguish between phenolic metabolites and co-extracted native components of the microorganisms employed. The literature describes numerous systems for the TLC analysis of simple phenols[3–10]. These reports were used as guides in developing TLC systems for determination of the compounds of interest in microbial extracts.

EXPERIMENTAL

TLC was carried out on 250-μm silica gel GF_{254} plates (Analtech, Newark, Del., U.S.A.) developed 10 cm in the following solvent systems: (A) benzene–methanol (95:5); (B) benzene–methanol (4:1); (C) benzene–ethyl acetate (9:1); (D) benzene–acetic acid (5:1); (E) benzene–acetic acid (5:2); (F) benzene–methanol–acetic acid (45:8:4); (G) chloroform–ethanol–acetic acid (18:1:1); (H) toluene–piperidine (5:2); (I) benzene–ethyl acetate–piperidine (6:3:1); (J) benzene–isopropanol–conc. ammonium hydroxide (5:4:1); (K) isopropanol–benzene–conc. ammonium hydroxide (3:1:1). Detection was via quenching of 254 nm-induced fluorescence and by diazotized sulfanilic acid[1].

RESULTS

Table I lists R_F values for the potential phenolic metabolites: in all systems, compounds developed as well consolidated spots, while developing times were usually

TABLE I

TLC OF PHENOLIC METABOLITES*

Compounds were chromatographed as such and as spiked ethyl acetate extracts (see ref. 1) of *Aspergillus niger* (ATCC 9142), *Penicillium chrysogenum* (ATCC 10002), *Cunninghamella blakesleeana* (ATCC 8688a), *Aspergillus ochraceous* (ATCC 1008), *Gliocladium deliquescens* (SP-WISC 1086), *Streptomyces* sp. (SP-WISC 1158w), *Rhizopus stolonifer* (NRRL 1477), *Curvularia lunata* (NRRL 2178), *Streptomyces rimosus* (ATCC 23955), *Cunninghamella bainieri* (ATCC 9244), and *Helicostylum piriforme* (QM 6945) cultures. ATCC= American Type Culture Collection, Rockville, Md.; NRRL= Northern Regional Research Laboratories, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill.; QM= Quartermaster Culture Collection, U.S. Army Laboratories, Natick, Mass.; SP-WISC= School of Pharmacy, University of Wisconsin, Madison, Wisc.

Substrate and phenolic metabolites	*Solvent systems and $R_F \times 100$ values*		
Acetanilide (AC)	*B*	*F*	*H*
2-Hydroxy AC	55	58	15
3-Hydroxy AC	47	47	34
4-Hydroxy AC	42	41	39
Aniline (AN)	*B*	*H*	*J*
2-Hydroxy AN	55	46	69
3-Hydroxy AN	49	40	61
4-Hydroxy AN	41	54	66
Anisole (AS)	*A*	*C*	*E*
2-Hydroxy AS	65	57	74
Phenol	48	48	68
4-Hydroxy AS	42	37	62
Hydroquinone	07	14	39
Benzoic acid (BA)	*D*	*G*	*K*
2-Hydroxy BA	60	65	63
3-Hydroxy BA	38	47	21
4-Hydroxy BA	38	40	34
3,4-Dihydroxy BA	21	26	05
Biphenyl (BP)	*A*	*D*	*I*
2-Hydroxy BP	69	64	52
4-Hydroxy BP	55	53	32
2,5-Dihydroxy BP	25	39	28
4,4'-Dihydroxy BP	15	27	18
Chlorobenzene (CB)	*A*	*D*	*H*
2-Hydroxy CB	59	63	21
3-Hydroxy CB	53	54	34
4-Hydroxy CB	48	52	41
Coumarin (CM)	*A*	*F*	*J*
4-Hydroxy CM	07	58	12
7-Hydroxy CM	18	55	30
o-Coumaric acid	03	51	04
Naphthalene (NA)	*A*	*D*	*H*
1-Hydroxy NA	50	56	55
2-Hydroxy NA	45	52	49

TABLE I (*continued*)

Substrate and phenolic metabolites	*Solvent systems and $R_F \times 100$ values*		
Nitrobenzene (NB)	*A*	*E*	*J*
2-Hydroxy NB	75	81	20
3-Hydroxy NB	43	60	52
4-Hydroxy NB	35	55	16
trans-Stilbene (ST)	*A*	*F*	*H*
4-Hydroxy ST	48	60	50
4,4'-Dihydroxy ST	16	53	38
Toluene (TL)	*A*	*D*	*I*
2-Hydroxy TL	51	58	49
3-Hydroxy TL	45	53	38
4-Hydroxy TL	45	50	40

* Reference materials were obtained commercially (see ref. 1) and used after homogeneity was established by TLC.

less than 20 min. Solvent systems A through K were also utilized to chromatograph ethyl acetate extracts of the aromatic hydroxylase-containing microorganisms (see footnote to Table I) spiked with the phenols of interest. In all instances, the TLC systems permitted differentiation of the phenolic metabolites from substrates and co-extracted microbial materials.

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REFERENCES

1 R. V. Smith and J. P. Rosazza, *Arch. Biochem. Biophys.*, 161 (1974) 551.
2 K. A. Connors, *Anal. Chem.*, 46 (1974) 53.
3 C. F. Van Sumere, G. Wolf, H. Teuchy and J. Kint, *J. Chromatogr.*, 20 (1965) 48.
4 L. S. Bark and R. J. T. Graham, *J. Chromatogr.*, 23 (1966) 120.
5 H. I. Joschek and S. I. Miller, *J. Amer. Chem. Soc.*, 88 (1966) 3273.
6 R. R. Scheline, *J. Pharm. Pharmacol.*, 18 (1966) 665.
7 A. N. Crabtree and A. E. J. McGill, *Mikrochim. Acta*, (1967) 85.
8 E. Sawicki, M. Guyer, R. Schumacher, W. C. Elbert and C. R. Engel, *Mikrochim. Acta*, (1968) 1025.
9 N. O. Jangaard, *J. Chromatogr.*, 50 (1970) 146.
10 J. E. Sinsheimer and R. V. Smith, *J. Pharm. Sci.*, 56 (1967) 1280.