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Note

Gas–liquid and thin-layer chromatographic determinations of xylenols in microbial extracts

ROBERT V. SMITH

Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Texas 78712 (U.S.A.)

and

JOHN P. ROSAZZA, KENNETH O. ENGEL and DAVID W. HUMPHREY

Division of Medicinal Chemistry and Natural Products, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242 (U.S.A.)

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Recent studies in these laboratories indicate that series of microorganisms can be defined that mimic mammalian aromatic hydroxylations[1–3]. It has been suggested that these so-called "microbial models of mammalian metabolism" be utilized in xenobiotic metabolism studies for the preparative synthesis of metabolites formed in parallel mammalian and microbial experiments[1,3].

The mammalian phenolic metabolites of *o*-, *m*- and *p*-xylenes (see Table I) have been characterized[4]. In order to enhance the predictive value of our previously described microbial models of mammalian aromatic hydroxylation, a study of the microbial aromatic hydroxylation of the isomeric xylenes was proposed. In this connection, gas–liquid chromatographic (GLC) and thin-layer chromatographic (TLC) systems were sought that would permit analyses of isomeric xylenols in the presence of native components co-extracted from the organisms under study.

The literature contains a number of potentially useful GLC and TLC methods for the xylenols indicated in Table I[1,4–10]. These reports were used as guides in devising chromatographic systems for determinations in microbial extracts.

EXPERIMENTAL

Materials

All reference compounds were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). 2,4-Dimethylphenol and *o*-, *m*- and *p*-xylenes were purified by distillation *in vacuo*; 2,3-, 2,5-, 2,6- and 3,4-dimethylphenols were recrystallized from *n*-hexane. Prior to use, homogeneity was established by TLC (xylenols) and GLC (xylenes) with the systems indicated below. All other solvents and reagents were reagent grade.

Thin-layer chromatography

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–ethyl acetate (9:1), and (C) benzene–ethyl acetate–

piperidine (18:1:3). Detection was via quenching of 254-mm induced fluorescence and by diazotized sulfanilic acid[1].

Gas–liquid chromatography

GLC was performed with a Hewlett-Packard Model 5700 gas chromatograph equipped with dual flame ionization detectors, on-column injection and 6-ft. × $\frac{1}{8}$-in.-I.D. 3% OV-17 on 100–120 mesh Gas-Chrom Q columns obtained from Applied Science Labs. (State College, Pa., U.S.A.). Operating conditions were: column and injection port temperatures, 100°; detector temperature, 150°; carrier gas (helium) flow-rate, 60 ml/min; range, 10^2; attenuation, 32 to 2.

Recovery experiments

Using previously described conditions, 24-h Stage II cultures of the following microorganisms were produced: *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*.

4-ml aliquots of cultures spiked with 25–125 μg/ml of the xylenols indicated in Table I were acidified with three drops of 5 *N* hydrochloric acid and extracted in 12-ml glass-stoppered centrifuge tubes with single 1-ml portions of ethyl acetate. Emulsions were broken by centrifugation at 2000 × *g* for 10–15 min. Blank extracts were prepared with microbial culture aliquots devoid of xylenols. TLC was performed with 30- to 50-μl aliquots of the ethyl acetate extracts while 2- to 5-μl portions of these extracts (or those concentrated tenfold) were submitted to GLC analysis.

RESULTS AND DISCUSSION

The TLC systems indicated in Table I were found to affect development of compounds as well consolidated spots and provided satisfactory separation of the isomeric phenolic metabolites of *o*- and *m*-xylene. In addition, the TLC systems noted permitted differentiation of the xylenol metabolites from substrates and co-extracted microbial materials. Parallel results were achieved with the GLC procedure devised. As indicated in Table II, adequate separation of the "critical pairs" of *o*- and *m*-xylene metabolites was observed. Standard curves for the xylenols were linear over the range of 0.5 to 10.0 μg with correlation coefficients consistently greater than 0.999. Preliminary experiments indicated that ethyl acetate extraction could be used to recover quantitatively xylenols from acidified aqueous solutions. A mean recovery of 101.6 (range, 90–107%, R.S.D. = 5.36, $N = 14$) was obtained for the xylenols extracted with ethyl acetate from spiked microbial cultures (see Experimental). With one of the

* 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, Natrick, Mass.; SP-WISC = School of Pharmacy, University of Wisconsin, Madison, Wisc.

TABLE I

TLC OF XYLENOLS

For solvent systems A, B and C, see text.

Substrate	*Xylenol metabolite*	$R_F \times 100$ *values**		
		A	*B*	*C*
o-Xylene	2,3-Dimethylphenol	53	57	61
	3,4-Dimethylphenol	42	48	56
m-Xylene	2,4-Dimethylphenol	49	53	65
	2,6-Dimethylphenol	64	60	69
p-Xylene	2,5-Dimethylphenol	56	50	65

* Averages of four to seven determinations.

TABLE II

GLC OF XYLENOLS

Substrate	*Xylenol metabolite*	*Retention time relative to 2,3-dimethyphenol**
o-Xylene	2,3-Dimethylphenol	1.00
	3,4-Dimethylphenol	1.12
m-Xylene	2,4-Dimethylphenol	0.78
	2,6-Dimethylphenol	0.61
p-Xylene	2,5-Dimethylphenol	0.78

* Retention time (relative to solvent front) = 3.57 min (22 determinations).

organisms studied, *A. ochraceous* (ATCC 1008), a co-extracted component with the same retention time as 2,4- and 2,5-dimethylphenol was detected. Further studies are planned to eliminate this interference.

The GLC and TLC methods described above provide rapid, selective and precise determinations of xylenols in microbial extracts. It is believed that these methods should be readily applicable to similar compounds in microbiological systems.

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