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Note

Polyamide thin-layer chromatographic separation of DOPA metabolites and related compounds

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The use of L-DOPA* in therapy of Parkinsonism[1] has stimulated interest in the metabolism of this compound in normal[2] and pathologic[3] states. Goodall and Alton[2] separated 35 DOPA metabolites from the urine of healthy subjects by GLC. Seventeen of these metabolites, including 11 acidic compounds, were unidentified. Recently, 3-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenyllactic acid, and 4-hydroxyphenyllactic acid were identified in the urine of patients receiving large oral doses of L-DOPA[4]. More recently, 3-hydroxyphenylacetic acid has been shown to be produced from DOPA by the intestinal microflora[5]. The substituted phenyllactic acids are products of a transamination pathway of metabolism enhanced by large doses of L-DOPA[4,6]. This pathway, a major route for the metabolism of L-3-O-methylDOPA[7], as well as other possible pathways, may have significance in Parkinson patients. The production of 3-methoxy-4-hydroxyphenyllactic acid by such patients receiving L-DOPA confirms earlier observations of this metabolite in the urine of patients with DOPA-secreting tumors[8–11]. The present study was prompted by the possibility that important DOPA metabolites may not yet have been identified and by the lack of a simple, readily-available method for the separation of potential DOPA metabolites differing only slightly in structure.

The advantages of polyamide thin layers for the separation of phenolic compounds has been recognized[12]. This report includes TLC data obtained with authentic samples of compounds not previously available for study.

MATERIALS AND METHODS

The compounds used in this study were obtained from the following sources: homovanillic acid, 3,4-dimethoxyphenylacetic acid (homoveratric acid), 3-hydroxyphenylacetic acid (Aldrich, Milwaukee, Wisc., U.S.A.); 3,4-dihydroxyphenylacetic acid, 3-methoxyphenylacetic acid (Nutritional Biochemicals, Cleveland, Ohio); the substituted phenyllactic acids and 2-(3-hydroxy-4-methoxyphenyl)ethanol were generously supplied by Professor Marvin Carmack, Department of Chemistry, Indiana University.

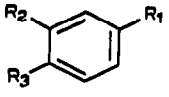
One-dimensional TLC was carried out on Bakerflex Polyamide 6 precoated sheets, 5×20 cm (J.T. Baker, Phillipsburgh, N.J., U.S.A.). Stock solutions (1%,

* DOPA = 3,4-Dihydroxyphenylalanine.

w/v) of each compound in 95% ethanol were applied to the sheets, 1 cm from the edge, 1.5 cm from the bottom, and 1 cm apart, by means of capillary pipettes (0.5–1.0 μl). Chromatograms were developed in a glass tank, $30 \times 10 \times 23$ cm, lined with Whatman No. 1 chromatography paper and filled with 100 ml of freshly prepared solvent mixture. 30 min were allowed for tank equilibration prior to chromatogram development. The following solvent systems were used: (A) chloroform–ethyl acetate–acetic acid (70:20:10); (B) benzene-2-butanone–methanol (60:20:20). All chromatograms were developed for an average time of 45 min with solvent system A and 30 min with solvent system B, corresponding to 15 cm of development. Chromatograms were air-dried and sprayed with a 0.2% solution of fluorescein in absolute ethanol, permitting the compounds to be observed as spots on a fluorescent background under UV light of 350 nm wavelength. All compounds were chromatographed at least four times (on different days) at 25°. Average hR_F values are presented in Table I.

TABLE I

hR_F VALUES OF DOPA METABOLITES AND RELATED COMPOUNDS ON POLYAMIDE



Compound		*Substituents*			*hR_F values*	
No.	*Name*	*R_1*	*R_2*	*R_3*	*Solvent systems*	
					A	*B*
1	3-Hydroxyphenylacetic acid	CH_2CO_2H	OH	H	30	59
2	3,4-Dihydroxyphenylacetic acid	CH_2CO_2H	OH	OH	12	37
3	Homovanillic acid	CH_2CO_2H	OCH_3	OH	63	66
4	3-Methoxyphenylacetic acid	CH_2CO_2H	OCH_3	H	80	79
5	3,4-Dimethoxyphenylacetic acid (homoveratric acid)	CH_2CO_2H	OCH_3	OCH_3	86	78
6	3-(3,4-Dihydroxyphenyl)lactic acid	$CH_2CH(OH)CO_2H$	OH	OH	3	20
7	3-(3-Hydroxy-4-methoxyphenyl)lactic acid	$CH_2CH(OH)CO_2H$	OH	OCH_3	35	34
8	3-(3-Metoxy-4-hydroxyphenyl)lactic acid	$CH_2CH(OH)CO_2H$	OCH_3	OH	36	48
9	3-(3,4-Dimethoxyphenyl)lactic acid	$CH_2CH(OH)CO_2H$	OCH_3	OCH_3	75	61
10	2-(3-Hydroxy-4-methoxyphenyl)ethanol	CH_2CH_2OH	OH	OCH_3	75	75

RESULTS AND DISCUSSION

The TLC characteristics of five metabolites of the antiparkinsonism drug, L-DOPA, were determined on polyamide in two solvent systems: (A) chloroform–ethyl acetate–acetic acid (70:20:10), and (B) benzene-2-butanone–methanol (60:20:20). Some related phenylacetic and phenyllactic acids were included in the study. Authentic samples of the four phenyllactic acids compared here were not syntheti-

cally available previously. The hR_F values of these compounds are presented in Table I.

Three of the five DOPA metabolites studied here are produced by pathways minor in healthy humans but significant in patients with Parkinsonism and DOPA-secreting tumors. Since several acidic metabolites of DOPA have not yet been identified[2], some phenylacetic and phenyllactic acids related to known minor metabolites were included. The data in Table I indicate that the known DOPA metabolites (1, 2, 3, 6 and 8) are well-separated on polyamide in both solvent systems. Comparable separation of phenolic acids has been obtained by paper chromatography[13] and TLC on cellulose[14] with appropriate solvent systems, but the length of time required for the development of these chromatograms is a major inconvenience. In general, comparable separation of phenolic acids of close structural similarity has not been obtained on silica gel G, and Egger has recognized the advantages of polyamide[12]. The separation of compounds 6–10 in solvent system B is a striking example of the separation of structurally similar compounds on polyamide. Some tailing of these compounds occurred in solvent system B; this could be reduced by the addition of a trace of acid to the system, but this modification of solvent system B reduced the separation of compounds 6–10 to that obtained with system A.

In conclusion, the present study indicates that L-DOPA metabolites and related compounds can be separated by TLC on polyamide, with effective separation of phenolic acids of very close structural similarity. These results should facilitate the study of the metabolism of L-DOPA and the search for DOPA metabolites that may be implicated in pathological processes.

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