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Note

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Comparative thin-layer chromatography of indole-containing metabolites of tryptophan and their sulfur isosteres

The metabolism of tryptophan in man may occur by several different pathways which may involve either the retention or destruction of the indole nucleus[1]. Several reports have appeared in the literature describing the application of thin-layer chromatography (TLC) to the separation of these metabolites[2–5]. Our study[6] of the chemical pharmacology of the sulfur isostere of tryptophan and its metabolites has necessitated the development of a rapid, simple method for their separation and identification. This report describes the comparative TLC characteristics of tryptophan metabolites possessing an indole nucleus and their sulfur isosteres.

Material and methods

The indole-containing metabolites of tryptophan used in this study were purchased from Regis Chemical Company, Chicago, Ill. The corresponding sulfur isosteres, benzo[*b*]thiophenes, have been prepared in our laboratory, as reported elsewhere[6,7].

One-dimensional TLC was carried out on Analtech Silica Gel G precoated plates (Analtech, Inc., Newark, Del.). Glass plates of 20 × 20 cm and a thickness of 250 μ were activated at 110° for 30 min and stored in a desiccator prior to use. Stock solutions (1 %, w/v) of each compound were prepared in methanol–water (1:1) and were applied to the plate, 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). When necessary, the minimum amount of 0.1 *N* hydrochloric acid was added to dissolve the compounds insoluble in methanol–water. A glass tank, 30 × 10 × 23 cm, lined with Whatman No. 1 chromatography paper and filled with 100 ml of freshly prepared solvent, 30 min prior, was used for plate development. The method of SANKOFF AND SOURKES[8] was employed in order to achieve reproducible tank saturation. All plates were developed 12 cm and air-dried 30 min prior to drying in an oven at 100° for 2 min. The compounds were visualized using one or more of the following reagents[9]: Van Urk's reagent, alkaline potassium permanganate, ninhydrin, and iodine vapor. Fluorescent spots were visualized with UV light, 254 and 350 mm. All compounds were run a minimum of six times at room temperatures of 22–24°, and on different days. Average hR_F values were calculated and appear in Table I.

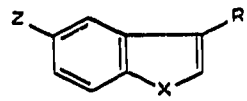
Results and discussion

The TLC characteristics of fourteen indole-containing metabolites of tryptophan and their sulfur isosteres have been determined on Silica Gel G using basic solvent system A (methyl acetate–2-propanol–ammonia, 45:35:20) and acidic solvent system B (chloroform–methanol–acetic acid, 60:35:5) and the data appear in Table I. In order to facilitate discussion, the compounds will be considered as amino acids, basic, and acidic metabolites of tryptophan.

TABLE I

hR$_F$ VALUES OF INDOLE-CONTAINING METABOLITES OF TRYPTOPHAN AND THEIR SULFUR ISOSTERES ON SILICA GEL G

Solvent systems: (A) methyl acetate–2-propanol–conc. ammonia (45:35:20); (B) chloroform–methanol–acetic acid (60:35:5).



No.	Compound name	Substituent groups			*hR*$_F$ values	
		X	*R*	*Z*	*Solvent A*	*Solvent B*
1	Tryptophan	NH	CH_2-CH-COOH | NH_2	H	34	28
2	Tryptophan (S)[a]	S			55	43
3	N-Acetyltryptophan	NH	CH_2-CH-COOH | NH | C=O | CH_3	H	50	80
4	N-Acetyltryptophan (S)	S			70	82
5	5-Hydroxytryptophan	NH	CH_2-CH-COOH | NH_2	HO	24	17
6	5-Hydroxytryptophan (S)	S			46	35
7	Tryptamine	NH	CH_2-CH_2-NH_2	H	77	50
8	Tryptamine (S)	S			82	59
9	5-Hydroxytryptamine	NH		HO	67	30
10	5-Hydroxytryptamine (S)	S			76	50
11	5-Methoxytryptamine	NH		CH_3O	80	43
12	5-Methoxytryptamine (S)	S			84	57
13	5-Methoxy-N-acetyltryptamine (Melatonin)	NH	CH_2-CH_2-NH | C=O | CH_3	CH_3O	93	88
14	5-Methoxy-N-acetyltryptamine (S)	S			92	92
15	N,N-Dimethyltryptamine	NH	CH_2-CH_2-$N(CH_3)_2$	H	88	25
16	N,N-Dimethyltryptamine (S)	S			92	42
17	5-Hydroxy-N,N-dimethyltryptamine (Bufotenine)	NH		HO	83	18
18	5-Hydroxy-N,N-dimethyltryptamine (S)	S			85	40
19	5-Methoxy-N,N-dimethyltryptamine	NH		CH_3O	88	28
20	5-Methoxy-N,N-dimethyltryptamine (S)	S			93	46
21	Indole-3-acetic acid	NH	CH_2-COOH	H	47	90
22	Indole-3-acetic acid (S)	S			68	91
23	5-Hydroxyindole-3-acetic acid	NH		HO	35	85
24	5-Hydroxyindole-3-acetic acid (S)	S			57	88
25	5-Methoxyindole-3-acetic acid	NH		CH_3O	48	89
26	5-Methoxyindole-3-acetic acid (S)	S			70	91
27	5-Hydroxytryptophol	NH	CH_2-CH_2-OH	HO	88	84
28	5-Hydroxytryptophol (S)	S			88	87

[a] (S) designates the replacement of the indole nucleus by benzo[*b*]thiophene.

The amino acids, compounds 1, 2, 5 and 6 are readily seperable by use of either solvent system A or B. Tryptophan (1) can be separated from both its sulfur isostere (2) and 5-hydroxytryptophan (5) by use of either solvent system. The greater lipophilicity of the sulfur isosteres is reflected in both solvent systems by larger hR_F values.

The basic metabolites of tryptophan and their sulfur isosteres are separable by use of solvent system B and possess minimal differences in hR_F value in solvent system A. Addition of a 5-hydroxy group to tryptamine (7) to give serotonin (9) or dimethylation to give dimethyltryptamine (15) increases the polarity of the tryptamine molecule as reflected by the decrease in hR_F values. 5-Methoxylation of 7 and 15 produces a very small change in hR_F value. Similar effects are observed with the corresponding sulfur isosteres. In any pair of basic tryptophan metabolites and their sulfur isosteres, the sulfur compounds are less polar and exhibit larger hR_F values.

The acidic metabolites of tryptophan and their sulfur isosteres can be readily separated by use of solvent system A and show minimal differences in hR_F value in solvent system B. The effect of 5-hydroxylation or 5-methoxylation of indole-3-acetic acid (21) is similar to those observed with tryptamine (7). In each pair of acidic metabolites the sulfur isostere possesses the greater hR_F value.

In summary, the comparative TLC of indole-containing metabolites of tryptophan and their sulfur isosteres has been described on Silica Gel G layers, using an acidic and a basic solvent system. The amino acids were separated by both solvent systems, the basic metabolites by the acidic solvent system, and the acidic metabolites by the basic solvent system. In all instances the indole compounds demonstrated a greater polarity than their sulfur isosteres, which was reflected in the lower hR_F values for a given pair of compounds.

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Department of Pharmacology,
Medical Sciences Program,
Indiana University, Bloomington, Ind. 47401 (U.S.A.)

T. R. BOSIN
C. WEHLER*

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