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Note

Comparative gas–liquid chromatography of biologically important indoles, and their benzo[*b*]thiophene and 1-methylindole analogs

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The indole nucleus is found in a number of biologically important compounds and the literature on their gas chromatographic properties is extensive. Horning *et al.*[1-3] reported on the gas chromatography of several indole compounds derivatized with hexamethyldisilazane (HMDS) and subsequently used N,O-bis(trimethylsilyl)-acetamide (BSA)/HMDS, trimethylchlorosilane (TMCS)/HMDS, and diazomethane/HMDS for the derivatization of indole alcohols and acids[4,5]. The derivatization and gas chromatography of tryptophan, using several different derivatizing agents, has been extensively studied by Gehrke and coworkers[6-9].

One of the major problems encountered in the derivatization and chromatography of indole compounds has been the formation of multiple derivatives. Vanden-Heuvel[10] reported the formation of multiple derivatives of tryptamine using BSA and Coward and Smith[11] found multiple derivatives of urinary indole acids with BSA. Albro and Fishbein[12] successfully obtained single derivatives of sixteen indole compounds using bis(trimethylsilyl)trifluoroacetamide (b-TMSTFA)–trimethylsilyldiethylamine (TMSDEA)–TMCS–pyridine (99:30:1:100) as a silylating reagent mixture.

Recent efforts in this area have focused on the preparation of derivatives which are suitable for electron capture detection. Trifluoroacetic acid has been used to derivatize indole acids[13] while derivatives of indole amines have been prepared using heptafluorobutyrylimidazole (HFBI)[14,15] and pentafluoropropionic anhydride (PFPA)[16,17].

Our study of the chemical pharmacology of the sulfur and 1-methyl analogs of biologically active indole derivatives has necessitated the development of a rapid, simple method for their separation and identification. This report describes the gas–liquid chromatographic (GLC) properties of biologically active indoles along with their benzo[*b*]thiophene and 1-methylindole analogs.

MATERIALS AND METHODS

BSA and PFPA were purchased from Pierce (Rockford, Ill., U.S.A.). All of the indole derivatives were purchased from Regis (Chicago, Ill., U.S.A.). The benzo-[*b*]thiophene and 1-methylindole analogs were synthesized in the laboratories of Dr. Ernest Campaigne, Chemistry Department, Indiana University, Bloomington, Ind., U.S.A. The internal standards fluoranthene and triphenylmethane were purchased

from Chemical Service, Media, Pa., U.S.A. The OV-17 and the Gas-Chrom Q were purchased from Applied Science Labs. (State College, Pa., U.S.A.). All other reagents and chemicals were reagent grade or better.

The GLC analysis was performed using a Varian-Aerograph Model 1840 gas chromatograph equipped with either a flame ionization detector (FID) (TMS derivatives) or an 8 mCi ^{63}Ni electron capture detector (ECD) (PFPA derivatives) operated in the d.c. mode. A Varian Model 20 recorder was used to record the chromatograms. A 6 ft. × 1/8 in. O.D. silanized glass column packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q was used in all the studies.

Nitrogen, at a flow-rate of 30 ml/min, was used as a carrier gas. Hydrogen, at 30 ml/min, and compressed air, at 300 ml/min, were used in the operation of the FID. A column oven temperature of 170° was used for the chromatography of the TMS derivatives and 150° was used for the PFPA derivatives. The injector ports were operated at 195°, the FID at 195° and the ECD at 225°. Attenuation settings of 4×10^{-10} A/mV were used with both detectors. Injection of 1 μl of the TMS derivative or 0.1 μl of the PFPA derivative was used for each analysis.

Derivative formation

BSA. A methanolic solution containing 30 μg of the compound chromatographed along with 20 μg of each of the internal standards was transferred to a thick-walled 13-ml centrifuge tube having a PTFE-lined screw cap. This solution was blown to dryness under nitrogen. 50 μl of BSA was added, and the tubes were flushed with nitrogen and mixed on a Vortex mixer for 20 sec.

PFPA. Derivatives were formed according to the method of Cattabeni *et al.*[17] 30 ng of a methanolic solution of the compound to be chromatographed along with 30 ng of a solution of tryptamine were added to a centrifuge tube. The solution was blown to dryness under nitrogen. PFPA (100 μl) and freshly distilled ethyl acetate (20 μl) were added, and the tubes were heated at 60° for 3 h. The excess reagent was blown to dryness under nitrogen and the derivative was redissolved in 50 μl of ethyl acetate.

RESULTS AND DISCUSSION

Relative retention times of TMS derivatives of indole, benzo[b]thiophene, and 1-methylindole compounds

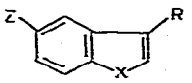
The formation of multiple derivatives of indoles using BSA as a silylating reagent has been reported previously[10,11]. We have found that either silylation with a pyridine–BSA mixture or the chromatography of some BSA derivatives on stainless-steel columns promoted multiple derivative formation. Using BSA (neat) and silylated glass columns, single, stable derivatives of the compounds listed in Table I were obtained. Optimal silylation conditions and stability were determined by measuring the peak height ratio of the TMS derivative relative to triphenylmethane. All derivatives were found to be stable for at least 12 h at room temperature.

Relative retention times of PFPA derivatives of indole, benzo[b]thiophene, and 1-methylindole compounds

The PFPA derivatives used in this study were prepared as described in Me-

TABLE I

RELATIVE RETENTION TIMES OF TMS DERIVATIVES OF INDOLE COMPOUNDS AND THEIR BENZO[*b*]THIOPHENE AND 1-METHYLINDOLE ANALOGS



No.	*Compound*	*Substituent group*			*Relative retention times*	
		X	*R*	*Z*	*Triphenylmethane*	*Fluoranthene*
1	Tryptophan*	NH	$CH_2CHCOOH$ NH_2	H	1.41	1.15
2	Tryptophan-S	S	$CH_2CHCOOH$ NH_2	H	0.88	0.72
3	Tryptophan-1-Me	NCH_3	$CH_2CHCOOH$ NH_2	H	1.18	0.96
4	Tryptamine**	NH	$CH_2CH_2NH_2$	H	0.54	0.44
5	Tryptamine-S	S	$CH_2CH_2NH_2$	H	0.31	0.26
6	Tryptamine-1-Me	NCH_3	$CH_2CH_2NH_2$	H	0.41	0.33
7	Monomethyl-tryptamine	NH	$CH_2CH_2NHCH_3$	H	0.72	0.60
8	Monomethyl-tryptamine-S	S	$CH_2CH_2NHCH_3$	H	0.36	0.30
9	Monomethyl-tryptamine-1-Me	NCH_3	$CH_2CH_2NHCH_3$	H	0.54	0.44
10	Dimethyl-tryptamine	NH	$CH_2CH_2N(CH_3)_2$	H	0.41	0.33
11	Dimethyl-tryptamine-S***	S	$CH_2CH_2N(CH_3)_2$	H	0.21	0.18
12	Dimethyl-tryptamine-1-Me***	NCH_3	$CH_2CH_2N(CH_3)_2$	H	0.32	0.26
13	Tryptophol	NH	CH_2CH_2OH	H	0.41	0.33
14	Tryptophol-S	S	CH_2CH_2OH	H	0.23	0.18
15	Tryptophol-1-Me	NCH_3	CH_2CH_2OH	H	0.44	0.36
16	Indole-3-acetic acid	NH	CH_2COOH	H	0.73	0.59
17	Indole-3-acetic acid-S	S	CH_2COOH	H	0.34	0.28
18	Indole-3-acetic acid-1-Me	NCH_3	CH_2COOH	H	0.54	0.44
19	5-Hydroxy-tryptophan§	NH	$CH_2CHCOOH$ NH_2	OH	3.57	2.92
20	5-Hydroxy-tryptophan§	S	$CH_2CHCOOH$ NH_2	OH	2.17	1.99

TABLE I *(continued)*

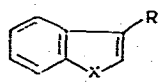
No.	*Compound*	*Substituent group*			*Relative retention time*	
		X	*R*	*Z*	*Triphenylmethane*	*Fluoranthene*
21	5-Hydroxy-tryptamine	NH	$CH_2CH_2NH_2$	OH	1.78	1.46
22	5-Hydroxy-tryptamine-S	S	$CH_2CH_2NH_2$	OH	0.91	0.75
23	5-Methoxy-N-acetyltryptamine§§	NH	$CH_2CH_2NCCH_3$ (H, O)	CH_3O	—	2.20
24	5-Methoxy-N-acetyltryptamine-S§§	S	$CH_2CH_2NCCH_3$ (H, O)	CH_3O	—	1.70
25	5-Methoxy-N-acetyltryptamine-1-Me§§	NCH_3	$CH_2CH_2NCCH_3$ (H, O)	CH_3O	—	2.20

* Heated at 100° for 5 h.
** Complete derivatization, room temperature, for 2 h.
*** Underivatized.
§ Heated at 100° for 3 h.
§§ Column oven temperature of 200°.

thods and their retention times were measured relative to the PFPA derivative of tryptamine. Table II presents the relative retention time data for PFPA derivatives of indole amines and alcohols and their benzo[*b*]thiophene and 1-methylindole isosteres.

TABLE II

RELATIVE RETENTION TIMES OF PFPA DERIVATIVES OF INDOLE COMPOUNDS AND THEIR BENZO[*b*]THIOPHENE AND 1-METHYLINDOLE ANALOGS



No.	*Compound*	*Substituent group*		*Relative retention time (PFPA tryptamine)*
		X	*R*	
1	Tryptamine-S	S	$CH_2CH_2NH_2$	1.62
2	Tryptamine-1-Me	NCH_3	$CH_2CH_2NH_2$	1.83
3	Monomethyltryptamine	NH	$CH_2CH_2NHCH_3$	1.12
4	Monomethyltryptamine-S	S	$CH_2CH_2NHCH_3$	1.90
5	Monomethyltryptamine-1-Me	NCH_3	$CH_2CH_2NHCH_3$	2.68
6	Tryptophol	NH	CH_2CH_2OH	0.25
7	Tryptophol-S	S	CH_2CH_2OH	0.50
8	Tryptophol-1-Me	NCH_3	CH_2CH_2OH	1.04

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