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Analysis of indole acid derivatives by gas chromatography using liquid phase OV-101

It has been reported that gas–liquid chromatography (GLC) is the most versatile procedure for the analysis of acidic indoles; however, to be useful the free indole acids must be converted to more volatile derivatives[1–5], such as methyl esters[1–3,5,6], trimethylsilyl derivatives[7] or trifluoroacetyl derivatives[8]. The most commonly used stationary phases for the analysis of indole derivatives are: Versamid 900 (refs. 3, 5 and 7), an ethylenediamine linoleic acid polyamide resin; SE-30 (refs. 1 and 5–8), a dimethylsiloxane polymer; and HI-EFF 8BP (refs. 5 and 7), a polycyclohexanedimethanol succinate. In a comparative study, using the above stationary phases, it has been reported that none of the substrates had a resolution of 95% or higher for ten closely related indole methyl esters[5]. At the 95% level of resolution, Versamid 900 and HI-EFF 8BP resolved five and SE-30 four indole methyl ester pairs. Therefore, in many biological studies two stationary phases have to be used for the analysis of indole compounds. The objective of the present report is to demonstrate that one stationary phase (OV-101), and two derivatives (methyl esters and trimethylsilyl derivatives) will resolve nine of ten closely related acidic indoles.

The GLC work was carried out on a F & M Model 402 gas chromatograph equipped with a flame ionization detector. The column temperature was 200° and the flash heater and flame detector temperatures were kept at 250°. A 1.80-m U-shaped glass column with a 6-mm I.D. was used. The column was packed with Anakrom ABS 80/90 mesh (Analabs, Hamden, Conn., U.S.A.) coated with 5% OV-101, a liquid dimethylsiloxane polymer (Supleco, Bellefonte, Pa., U.S.A.). The carrier gas was helium at a flow rate of 75 ml/min.

The indole methyl esters were formed as described previously[5]. The trimethylsilylated (TMS) indole derivatives were formed via two procedures. About 5 mg of the desired indole acid were dissolved in 0.4 ml acetonitrile and 0.2 ml bis(trimethylsilyl)acetamide (BSA) were added under anhydrous conditions and kept at 70° as described by KLEBE *et al.*[9]. Samples were removed at various times and injected directly into the gas chromatograph. In the second method, the TMS derivatives were formed by adding 0.2 ml of bis(trimethylsilyl)trifluoroacetamide (BSTFA) instead of BSA. Although the action of BSTFA as a silylating agent is similar to BSA, BSTFA is more volatile than BSA and is usually eluted from the GC column near the solvent front. Both trimethylsilylating agents produce ethers from alcohols, esters from acids and displace nitrogen-bound protons[9].

Our first aim was to study the silyl donor strength of BSA and BSTFA. Fig. 1 is a representative example using indole-3-butyric acid, which is one of the more difficult indole acids to silylate completely. Silylation of the carboxyl group was accomplished in less than 1 min; however, silylation of the indole nitrogen atom was more difficult. Indole acids with shorter aliphatic carboxylic acid groups were not as difficult to silylate with BSA, except for indole-2-carboxylic acid, which was not completely silylated even after 120 min. However, BSTFA completely silylated both indole-3-butyric and indole-2-carboxylic acid within 10 min. All other tested indole acids were completely silylated in less time.

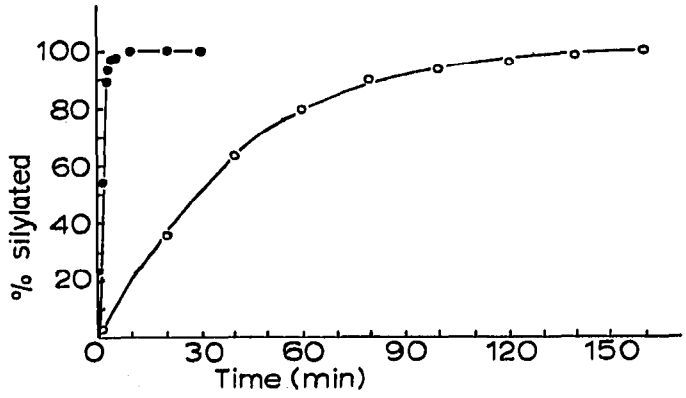

Fig. 1. Silyl donor strength of bis(trimethylsilyl)acetamide (BSA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) using indole-3-butyric acid as substrate. Reaction mixture: 5 mg indole-3-butyric acid dissolved in 0.4 ml acetonitrile and either 0.2 ml BSA or 0.2 ml BSTFA at 70° under anhydrous conditions. Reaction with BSA (○—○) and BSTFA (●—●).

The GLC results for TMS and methyl ester derivatives of ten closely related indole acids, most likely found in biological material, are presented in Table I. All relative retention values (r) are given with respect to ethylindole-3-acetate, and their effective plate values (N) are calculated according to ETTRE[10]. The order of elution of the TMS and methyl ester derivatives was not the same. The total elution time of the TMS derivatives was about twice as long as that of the methyl esters, *viz.* 30 and 18 min, respectively. The effective plate values (N) on 5% OV-101 were about the

TABLE I

COMPARISON OF RELATIVE RETENTION (r), EFFECTIVE PLATE VALUE (N), AND RESOLUTION (R QF VARIOUS INDOLE TMS DERIVATIVES AND INDOLE METHYL ESTERS ON COLUMN SUBSTRATE OV-101

Column characteristics: column temperature, 200°; detector temperature, 250°; carrier gas, helium; column, 5% OV-101 on Analkrom ABS 80/90 mesh (for details see text); retention time ethylindole-3-acetate, 4.8 min.

Indole, TMS derivative	r	N	R	*Indole, methyl ester*	r	N	R
Indole-2-carboxylate	0.72	935		Indole-2-carboxylate	0.42	318	
			1.91				3.92
Indole-1-propionate	1.09	848		Indole-3-acetate	0.80	1063	
			2.30				0.18
Indole-3-acetate	1.49	905		Indole-5-carboxylate	0.82	653	
			1.69				0.52
Indole-3-carboxylate	1.85	1095		Indole-3-carboxylate	0.88	1290	
			1.98				0.30
Indole-3-propionate	2.32	1272		Indole-1-propionate	0.91	1373	
			0.20				1.87
Indole-5-carboxylate	2.37	1278		Indole-3-propionate	1.12	1110	
			3.92				3.28
Indole-3-butyrate	3.52	1765		Indole-3-butyrate	1.59	1745	
			0.61				0.38
Indole-3-lactate	3.73	1214		Indole-3-lactate	1.65	1628	
			2.19				4.73
Indole-3-glyoxylate	4.59	2490		Indole-3-glyoxylate	2.61	1834	
			3.79				1.42
Indole-3-acrylate	6.31	2190		Indole-3-acrylate	2.97	1695	

same for both derivatives; however, these N values were greater than those reported using Versamid 900 and HI-EFF 8BP as stationary phases[5].

For completeness of separation, or resolution (R), peak width at the base must be taken into account. The R values were calculated according to ETTRE[10]. Two adjacent peaks are 95% resolved when $R = 1$, and 99% resolved when $R = 1.5$. Only two pairs of the ten TMS derivatives were not resolved at the 99% level. Indole-3-propionate was not resolved from indole-5-carboxylate, and indole-3-butyrate was not resolved from indole-3-lactate. All other combinations could be resolved at the 99% level. Resolution of the methyl esters was not as good, only four adjacent indole pairs could be resolved at the 99% level; however, methylindole-3-propionate could be resolved from methylindole-5-carboxylate.

In summary, using TMS and methyl ester derivatives and the stationary phase OV-101, all indole acids could be resolved at the 99% level except indole-3-butyrate from indole-3-lactate. This makes stationary phase OV-101 superior to either Versamid 900, HI-EFF 8BP or SE-30 (ref. 5). It must be pointed out that the unresolved indole-3-butyrate indole-3-lactate pair can be resolved at the 99% level on either 3% HI-EFF 8PB or 3.5% Versamid 900 (ref. 5). It was found that BSTFA is more effective as a silyl donor than BSA and is, therefore, recommended for the formation of indole acid TMS derivatives.

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