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TRIMETHYLSILYLATION OF BIOGENIC INDOLEAMINES

PRACTICAL KINETIC AND ANALYTICAL ASPECTS RELATED TO TRYPTAMINE AND 5-HYDROXYTRYPTAMINE

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SUMMARY

An attempt is made to answer some practical kinetic and identification questions on the trimethylsilylated (TMS) derivatives of tryptamine (T) and 5-hydroxytryptamine (5HT). Considering the theoretically possible five and 11 TMS derivatives of T and 5HT, respectively, a gas chromatographic–mass spectrometric study of these reaction profiles established the identification of the five T derivatives and all of the six 5HT derivatives whose formation was considered kinetically feasible. Reaction parameters must be optimized to avoid a multiplicity of TMS derivatives in practical applications. A kinetic approach using different catalysts (trimethylsilylimidazole, trimethylchlorosilane, pyridine) indicates that the ease of silylation would follow the order $O^5 >$ 1st N^{ω} position $> N^1 >$ 2nd N^{ω} position. The best results were obtained by reaction with a N,O-bis(trimethylsilyl)trifluoroacetamide–trimethylsilylimidazole–pyridine mixture at 70° for 60 min, which enhances the formation of the fully silylated derivatives. The relative merits of the silyl *versus* the acyl derivatives of these amines are discussed in relation to practical applications.

INTRODUCTION

The chemical derivatization of physiologically significant biogenic indolealkylamines, such as tryptamine (T) and 5-hydroxytryptamine (5HT) (serotonin), for their gas chromatographic (GC) analysis has been described by various workers[1–12]. According to the literature, the determination of either of these two amines by GC usually involves the previous acylation[1,4,7] or silylation[2,3,5,6,9] of the active primary (N^{ω}) and secondary (N^1) amino groups of the alkyl side-chain and indole ring, respectively, and of the 5-hydroxyl group in 5-hydroxytryptamine. The biological applications of these derivatization procedures have often been aimed at the mass fragmentographic assay of their relatively low endogenous levels in living systems[11–15]. Thus, to achieve effectively the required detection limits, the reactions have to be optimized in relation to the selective synthesis of a suitable derivative with good response characteristics and high yield[15].

In this respect, while acylation is a well established and regularly used procedure in many of the laboratories concerned with the study of the biological role and function of these amines[13–17] and related indole metabolites[13–15,18–20], there is less information on the silylation of tryptamine and 5-hydroxytryptamine. Further, the results reported to date on their trimethylsilyl derivatives often lead to contradictory conclusions, as will be discussed below. For instance, the rate-limiting step in these trimethylsilylation reactions has been assigned to the indole ring nitrogen[21], although it has also been claimed that this nitrogen atom is more reactive than that in the side-chain[12]. Also, some of the structural assignments made for the partially silylated forms of tryptamine could be incorrect owing to possible misinterpretations of the corresponding mass spectrometric data.

On the other hand, although these reactions are usually manipulated so as to attain the substitution, if possible, of all active hydrogen atoms in the molecules to be derivatized, recently we have established the possible advantages of using the partially acylated[22] or mixed acyl-TMS forms[23] of these and related compounds. Likewise, the possible use of monosilylated tryptamine has also been reported[12]. For these reasons a study was undertaken on the structural, kinetic and practical chromatographic aspects of the trimethylsilylation of tryptamine and 5-hydroxytryptamine, for both the totally and possible partially silylated forms. The aim was to gather the necessary experimental data in order to establish the reaction conditions leading in each instance to the best derivative for practical qualitative and/or quantitative results. This requires in principle the selection of reagents with a high silylation potential, proper control of the reaction parameters and a study of the differentiating chromatographic and mass spectrometric features of each derivative thus obtained for easier and more reliable identification.

As it is known that these derivatives are prone to decomposition (decomposition meaning here any loss of derivative, regardless of cause) special emphasis was placed on the determination of possible structural changes either after the reaction or during their GC determination. For instance, it has been reported that on-column effects account for the loss of acyl groups during the GC of biogenic amines[24] and that the response of some amine-based drugs can also be seriously affected by this type of effect[25].

EXPERIMENTAL

Chemicals and reagents

The following silylation agents were obtained from Xpectrix (San Cugat, Barcelona, Spain): N,O-bis(trimethylsilyl)acetamide (BSA), N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), trimethylsilylimidazole (TMSI), dimethylchlorosilane (DMCS) and trimethylchlorosilane (TMCS). Methanol and pyridine (Merck, Darmstadt, G.F.R.) were used as solvents. The reference hydrocarbon standards (*n*-C_{16}, *n*-C_{20}, *n*-C_{22}, *n*-C_{24}, *n*-C_{26} and *n*-C_{28}) were obtained from Applied Science Labs. (State College, Pa., U.S.A.). Tryptamine hydrochloride and 5-hydroxytryptamine oxalate were purchased from Sigma (St. Louis, Mo., U.S.A.). Stock solutions of these two amines were prepared in distilled methanol at a concentration of 1 μg/μl.

Derivatization procedures

Aliquots of the stock solutions of the corresponding amine equivalent to 100–300 μg were evaporated to dryness under a stream of purified helium. The dry residues were derivatized according to the following procedures:

(I) Addition of 100 μl of either BSTFA, BSTFA–TMSI (100:1) or BSTFA–TMCS (100:1), and reaction at 110 or 60°[12,21].

(II) Addition of 100 μl of BSA and 20 μl of pyridine, and reaction at 60°[3].

(III) Addition of 100 μl of BSA–TMSI (100:1), and reaction at 60°.

(IV) Addition of 100 μl of BSA–TMSI (100:1) and 20 μl of pyridine, and reaction at 60°.

(V) Addition of 100 μl of BSTFA–TMSI (10:1) and 40 μl of pyridine, and reaction at 70°.

In all instances the reactions were carried out in a temperature-controlled oven. Aliquots of the reaction mixtures were injected at various time intervals into the gas chromatograph in order to follow the time course of the derivatizations.

Gas chromatography

The GC separations were carried out on a Perkin-Elmer Model 990 gas chromatograph, equipped with dual flame-ionization detectors. The glass columns (1.80 × 2 mm I.D.) were deactivated with DMCS in toluene, and packed with either 3% OV-17 or 5% OV-17 on Gas-Chrom Q (100–120 mesh). Glass columns were supplied by Xpectrix and the OV-17 by Applied Science Labs.

Gas chromatography–mass spectrometry

The structural identifications of the various reaction products, observed as distinct peaks in the GC profiles, were based on their mass spectra, obtained on a Hitachi RMU-6H mass spectrometer coupled through a single-stage jet separator (Perkin-Elmer, Norwalk, Conn., U.S.A.) to a Perkin-Elmer Model 3920 gas chromatograph. The mass spectra were obtained at 70 eV and the accelerating voltage was set at 2400 or 1800 V, depending on the molecular weight of the derivative. The mass spectrometer is equipped with an accessory of our own design[26] for multiple ion detection (MID).

Determination of Kováts retention indices (I)

Retention indices were obtained at 180 ± 2° on OV-17 by co-injection of the appropriate hydrocarbon standards and calculated as described in a previous paper[23].

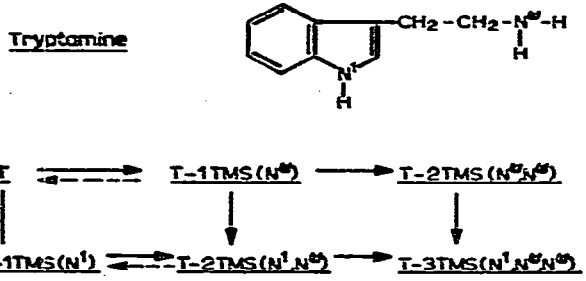
RESULTS AND DISCUSSION

As indicated previously[23], the derivatization of a given "problem substance" does not necessarily always produce a single and readily identifiable derivative. This is especially true when dealing with polyfunctional structures capable of incorporating more than one derivatizing group, as would happen with both of these amines. From a theoretical point of view, they could give several silylated products, as illustrated in Schemes I and II. For example, with tryptamine there would be five possible coexisting silylated species to consider: one fully silylated (T-3TMS), two bis-silylated (T-2TMS) and two monosilylated (T-1TMS).

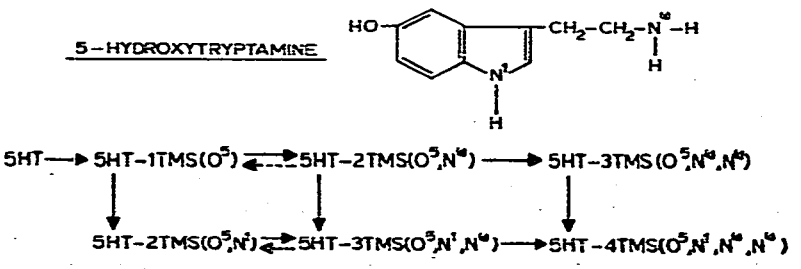
On the other hand, from a strictly theoretical point of view there would be eleven possible silylated species for 5-hydroxytryptamine, but we will consider only six of them: one fully derivatized (5HT-4TMS), two tris-silylated (5HT-3TMS), two bis-silylated (5HT-2TMS) and one monosilylated (5HT-1TMS). Such a simplification in the number of real possibilities to work with is based experimentally on the well known fact that a phenolic group, as the one in the O^5 position of 5HT, would always silylate first[27,28], relative to either the primary or secondary amino groups. In other words, whereas one could formulate the corresponding non-O^5-silylated counterpart for five of the six derivatives shown in Scheme II (the one corresponding to (O^5) 1TMS-5HT would be 5HT itself), none of these formulations could be considered as a kinetic possibility. However, aside from the kinetic considerations and according to criteria used for defining the suitability of a derivative for GC analysis[23], some of these reaction products might either be undetectable because they are produced in very small amounts or because their free amino groups could act as excessively active sites for good chromatographic behaviour, especially at very low levels.

Identification of reaction products

Representative GC profiles (on OV-17) corresponding to the silylation of tryptamine (T) and 5-hydroxytryptamine (5HT) (serotonin) are shown in Fig. 1. In both instances the derivatization was carried out under conditions inducing the synthesis of products primarily substituted on the primary (N^ω) amino group of the side-chain [BSA–pyridine (100:20) at 60°]. The possibility of obtaining more than one derivative in each instance is demonstrated by the appearance of various peaks in the



Scheme I.



Scheme II.

chromatograms, each silylated at the positions indicated, as shown by their corresponding mass spectral patterns and elution at the retention indices given below:

tryptamine derivatives:

$I = 2078$ N^1,N^ω-bis-TMS-tryptamine
$I = 2109$ N^ω-TMS-tryptamine
$I = 2335$ N^1,N^ω,N^ω-tris-TMS-tryptamine
$I = 2375$ N^ω,N^ω-bis-TMS-tryptamine

5-hydroxytryptamine derivatives:

$I = 2359$ O^5,N^1,N^ω-tris-TMS-5-hydroxytryptamine
$I = 2407$ O^5,N^ω-bis-TMS-5-hydroxytryptamine
$I = 2566$ $O^5,N^1,N^\omega,N^\omega$-tetrakis-TMS-5-hydroxytryptamine
$I = 2643$ O^5,N^ω,N^ω-tris-TMS-5-hydroxytryptamine

The GC peaks are labelled in Fig. 1 according to the positions that have accepted a silyl group (TMS), following the indications in Schemes I and II.

The interpretation of the mass spectral features of the last two peaks of each of the chromatograms in Fig. 1 is straightforward according to the data summarized in Table I, allowing for an unequivocal identification. In all instances the mass spectral patterns are characterized by common-origin fragment ions, each appearing on the spectra at the corresponding *m/e* values (Table I). The base peak, as would be expected from a process of β-cleavage in aliphatic amines[7,9] with retention of the positive charge

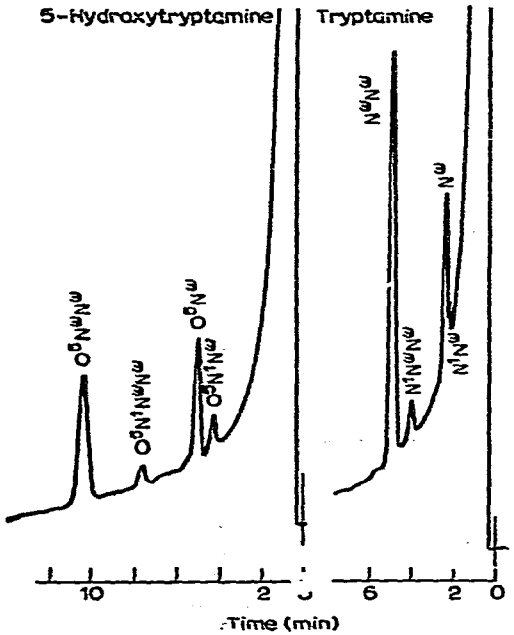


Fig. 1. GC separation of silylated derivatives of tryptamine and 5-hydroxytryptamine on a 3% OV-17 packed column at 240° (isothermal). The temperature of the injector and detector blocks was set at 250–260°. Both reactions were carried out using BSA–pyridine (100:20) at 60° for 1 h. About 200 μg of tryptamine and 300 μg of 5-hydroxytryptamine were derivatized. Injection of a 2-μl aliquot of the reaction mixture (120 μl). The positions silylated are indicated for each peak according to the nomenclature used in Schemes I and II. Their respective retention indices are given in the text.

TABLE I

MASS SPECTRA OF SILYLATED DERIVATIVES OF TRYPTAMINE AND 5-HYDROXYTRYPTAMINE

Substituted positions are indicated in parentheses (see Schemes I and II). See text for interpretation of the fragmentation patterns.

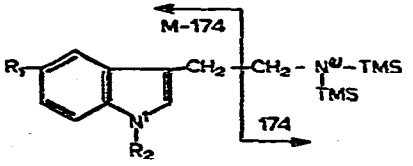
*Ion**	*5HT-4TMS* (O^5, N^1, N^ω, N^ω)		*5HT-3TMS* (O^5, N^ω, N^ω)		*T-3TMS* (N^1, N^ω, N^ω)		*T-2TMS* N^ω, N^ω	
	m/e	*Relative intensity*	*m/e*	*Relative intensity*	*m/e*	*Relative intensity*	*m/e*	*Relative intensity*
M	464	3.6	392	2.1	376	7.4	304	0.4
M − 15	449	7.2	377	11.9	361	37.7	289	5.4
M − 89	375	0.7	303	2.1	287	5.6	215	2.6
M − 103	361	2.2	289	3.5	273	22.2	201	3.7
M − 174***	290	13.8	218	9.0	202	25.3	130	15.2
(M − 174) − 72	218	2.2	146	4.2	130	17.9	58	N.P.**
174***		100		100		100		100
130		4.0		5.9		17.9		15.2
100		10.9		8.2		24.7		11.0
86		21.8		27.1		30.9		24.0
73		72.7		70.8		46.3		65.2

* M = molecular ion.

** N.P. = not present as there are no more TMS groups available in this instance after the loss of the 174 fragment.

*** See Scheme III.

on the nitrogen (N^{ω} in this instance), shows up at an *m/e* value of 174 (relative abundance 100% in Table I), corresponding to the fragment ion $CH_2 = N(TMS)_2^+$. There is also a much lower intensity M—174 fragment when the charge stays on the indolyl moiety. Both of these fragments are depicted in Scheme III. The M—174 ion seems to lose 72 mass units (TMS minus H), giving rise to ions at the indicated masses. In the high mass end of the spectra there are also small peaks at *m/e* values corresponding to M—89 and M—103. The M—89 ion would not be unexpected in silylated hydroxyl-bearing compounds[27] like the 5-hydroxytryptamine derivatives, although this would not be a likely explanation in this instance as this ion is also relatively abundant in the mass spectra of the non-hydroxylated tryptamine derivatives. A possible origin would be the loss of TMS+H from the M—15 ion. The M—89 fragment ion thus formed could then lose CH_2 to give the M—103 fragment.



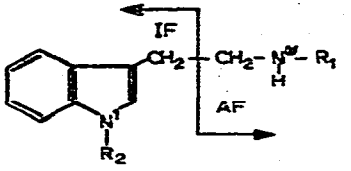
5-HT: R_1=OTMS, R_2=TMS or H
T: R_1=H, R_2=TMS or H

Scheme III.

However, the situation regarding the more volatile derivatives of both amines (*I* values of 2078, 2109, 2359 and 2407 in Fig. 1) is more complicated owing to their seemingly temperature-dependent GC behaviour. For instance, at 230–240° the first-eluting tryptamine derivative gives, on some OV-17 packed columns, an apparently clean and symmetrical peak, which at an elution temperature of 180° appears to open up into an unresolved profile made up by the appearance of a relatively gaussian peak ($I = 2109$) on the appreciable tail of another preceding peak ($I = 2078$), as illustrated by the chromatogram in Fig. 2 (bottom trace).

This peculiar behaviour led to a more detailed study of the nature of the components of this unresolved profile as a function of column temperature. For this purpose, the same reaction mixture was analysed by gas chromatography–mass spectrometry (GC–MS) on the same column. The results are shown in the upper three traces in Fig. 2. It can be seen that the total ion monitoring (TIM) profiles at 210°, 200° and 170° demonstrate that the single peak observed at 230° is accompanied at lower elution temperatures by a smaller unresolved component on its upward slope. Also, as the elution time (or column residence time) of these two closely eluting peaks increases at lower GC oven temperatures (from 7 to 19 min), this part of the gas chromatogram is progressively transformed into a badly tailing peak, as shown by the TIM profile obtained at a column temperature of 170° (Fig. 2).

The mass spectrometric study of this particular section of the gas chromatographic profile of the tryptamine TMS derivatives shows that the tailing peak at $I = 2078$ gives a mass spectrum consistent with the identity of an N^1,N^{ω}-bis-TMS derivative of tryptamine (Scheme IV). In other words, its molecular ion appears at



Derivative	R_1	R_2	*m/e* IF	*m/e* AF	M
N^1,N^ω-bis-TMS	TMS	TMS	202	102	304
N^1-TMS	H	TMS	202	30	232
N^ω-TMS	TMS	H	130	102	232

(IF = indolyl fragment ; AF = amine fragment)

Scheme IV.

m/e 304 and, in line with the process of β-cleavage characteristic of the alkylamine moiety, as indicated in Scheme III, the corresponding amine fragment (AF) appears at a mass of 102 while the mass of the indolyl fragment (IF) gives a peak at *m/e* 202.

On the other hand, the mass spectra of the second peak at $I = 2109$ (Fig. 2) shows ions at *m/e* 232, 217, 102 and 130, indicative of a monosubstituted N^ω-TMS derivative of tryptamine (see Scheme IV). This would be in line with the identification of N^ω-TMS-tryptamine in some of the reactions reported by Donike[21] and Vanden-Heuvel[3], who also pointed out the relative instability of this derivative.

Likewise, the mass spectrometric study of the tailing peak obtained at 170° (Fig. 2) indicates that it is a derivative without TMS groups on the aliphatic amine (N^ω) but with one TMS group of the ring N^1 (IF at *m/e* 202). Accordingly, the molecular weight is also 232. However, as the mass spectrum of this GC peak also shows traces of ions at *m/e* 130 (unsubstituted indole ring) and 102 (mono-TMS-amine fragment) (see Scheme IV), there may be a small amount remaining of the isomeric N^ω-TMS derivative which also elutes in this region.

The formation of the N^1,N^ω-bis-TMS derivative, barely visible in the profile in Fig. 1 and practically not resolved from the peak of the N^ω-TMS derivative, is further demonstrated on the chromatogram in Fig. 3. This chromatogram was obtained with a higher resolution 5% OV-17 packed column, which at 230° was able to separate clearly the N^1,N^ω from the N^ω silylated tryptamine derivative. At lower elution temperatures, as indicated above, the N^1,N^ω-bis-TMS derivative seems to be degraded to the N^1-TMS form while the N^ω-TMS apparently decomposes to tryptamine. Traces of free tryptamine (molecular weight 160) have also been detected mass spectrometrically under the peak at $I = 2109$ (Fig. 2).

The formation of free tryptamine by on-column degradation is indicated by the fact that when injected alone, as shown in Fig. 3, it practically coelutes with its N^1,N^ω-bis-TMS derivative. However, a detailed mass spectrometric screening of the zone of elution of the two unresolved N^1,N^ω and N^ω derivatives has verified the appearance of the molecular ion of tryptamine at *m/e* 160 together with its characteristic *m/e* 30 ion on the downward slope of the N^ω derivative. Further, of the possible desilylations of either the N^1 or the N^ω derivatives (see Scheme I), it seems that this "delayed" tryptamine comes from the N^ω-TMS derivative. It appears preferentially

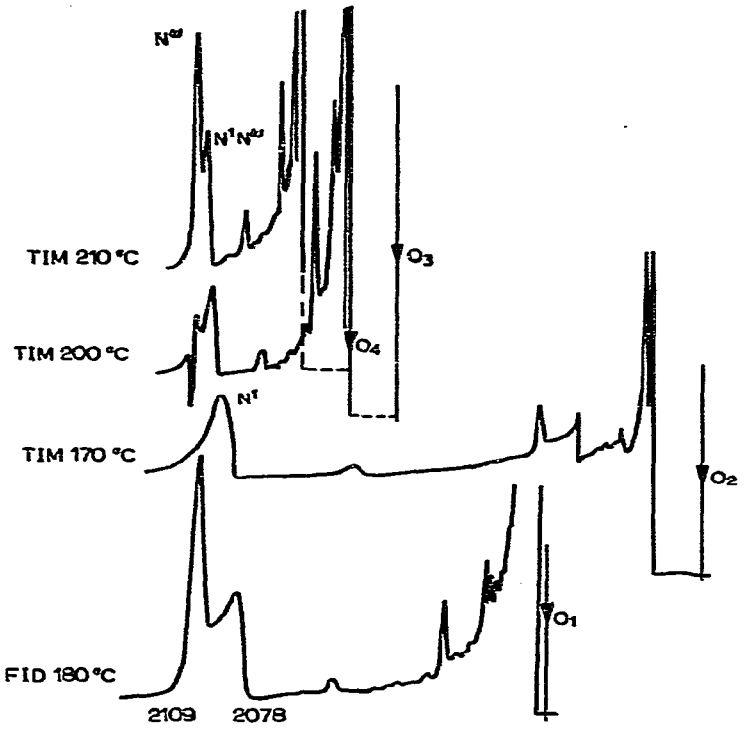


Fig. 2. GC profiles of the first part of the chromatogram corresponding to the silylated tryptamine derivatives shown in Fig. 1. The study of this section was made with the same GC column at different temperatures, as indicated. The injection points are indicated by 0; 0_1, injection on the Perkin-Elmer 990 and FID detection at 180; 0_2, 0_3, 0_4, injection on the Perkin-Elmer 3920 coupled to the mass spectrometer at the temperatures indicated on the TIM traces. Ionizing voltage, 70 eV. Accelerating voltage, 1800 V. Injector and GC manifold temperature, 200. Same reaction conditions as in Fig. 1 except for the reaction time, which was 40 min.

in the reactions in which the N^{ω} derivative predominates over the N^1,N^{ω} species (*e.g.*, Fig. 1).

Similar behaviour was observed for the first two peaks in the profile of the TMS derivatives of 5-hydroxytryptamine. For example, the FID trace in Fig. 4, obtained at 200°, shows a minimum of four badly resolved components.

As indicated in Fig. 1, the first-eluting peak in the GC profile of the 5-hydroxytryptamine TMS derivatives ($I = 2359$) gives a mass spectrum corresponding to the O^5,N^1,N^{ω}-tris-TMS-5-hydroxytryptamine derivative, while the second peak ($I = 2407$) gives a mass spectrum corresponding to the O^5,N^{ω}-bis-TMS-5-hydroxytryptamine derivative.

However, when the same sample is eluted at 200°, this part of the chromatogram also opens up into the broad and unresolved pattern shown in Fig. 4, resembling the effect observed for tryptamine. The mass spectrometric study of this section of the chromatographic profile verifies the coexistence of the O^5,N^1,N^{ω}-tris-TMS and O^5,N^1-bis-TMS derivatives of 5-hydroxytryptamine in the first unresolved broad peak on the lower trace in Fig. 4. Likewise, the following two broad bands give mass spectrometric patterns confirming the detection of the O^5,N^{ω}-bis-TMS derivative of

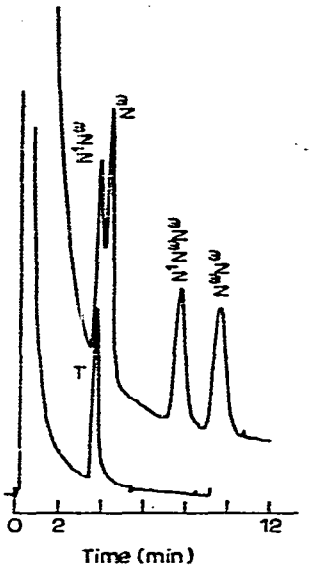


Fig. 3. Gas chromatogram illustrating the separation achieved at 230 on a column packed with 5% OV-17 on Gas-Chrom Q (100–120 mesh). With this column it becomes possible to separate all of the T derivatives in 10 min with better resolution of the first two peaks (compare with Fig. 1). The lower trace represents a direct injection of underivatized tryptamine, which elutes together with the first eluting N^1,N^ω-bis-TMS derivative at about 210 sec.

5-hydroxytryptamine plus the O^5-mono-TMS derivative which accounts for the larger part of the middle broad tailing peak.

Identifications are based on the fragment and molecular ions observed at the masses indicated in Table II. However, because of the difficulties in obtaining clean mass spectra of each derivative under these chromatographic conditions, relative intensities are not included in this table.

Also with regard to GC identifications the *I* values of all of the silylated species shown in the GC profiles in Fig. 1 are in agreement with the previously calculated and experimentally determined values, according to the recently developed retention index model for indole substituted compounds[23]. It must be noted, however, that the *I* value of 2109 given before for the N^1,N^ω-bis-TMS derivative of tryptamine has been revised in light of the new data, to the more accurate value of 2078 (a ΔI of 31 units), the former value corresponding to the retention index of N^ω-TMS tryptamine. Nevertheless, although the retention index data now appear to be well supported by the corresponding mass spectrometric identifications, in the course of this work we came across some contradictory data in relation to relative retention times. It is known that irrespective of the silylation potential of the reagents used by different workers, once a given derivative has been synthesized it should always have a perfectly defined retention time in a given GC system. Surprisingly, this does not seem to be the case for the N^ω-TMS and N^ω,N^ω-bis-TMS derivatives of tryptamine. According to Donike *et al.*[11], N^ω,N^ω-bis-TMS-tryptamine is eluted on OV-17 at 180° a few seconds after the peak of N^ω-TMS-tryptamine and thus ahead of N^1,N^ω,N^ω-tris-TMS-tryptamine. This fact is difficult to reconcile with our own data,

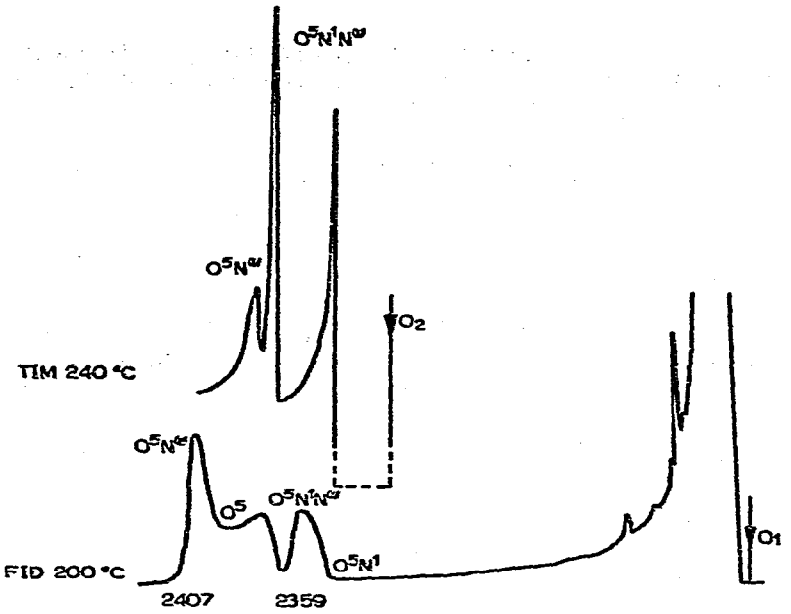


Fig. 4. GC profiles of the first part of the chromatogram of the silylated derivatives of 5-hydroxytryptamine. 3% OV-17 at 240° for the TIM trace and at 200° for the FID. In this instance the reaction was carried out with BSA–pyridine (100:20) at 60° for 40 min.

TABLE II

MASS VALUES OF CHARACTERISTIC IONS OBSERVED IN THE MASS SPECTRA OF PARTIALLY SILYLATED DERIVATIVES OF 5-HYDROXYTRYPTAMINE

*Ion**	O^5, N^1, N^ω	O^5, N^1	O^5, N^ω	O^5
M	392	320	320	248
M − 15	377	305	315	233
M − AF	290	290	218	218
(M − AF) − 72	218	218	146	
AF	102	30	102	30

* AF = amine fragment. The M − AF fragment is the base peak in all instances.

as we have always detected the N^ω,N^ω-bis-TMS derivative after N^1,N^ω,N^ω-tris-TMS-tryptamine on OV-17.

Similarly, we cannot explain the major difference in the retention time of tryptamine when compared with its reported elution 3 min after the tris-silylated tryptamine derivative[12]. These deviations in retention time are summarized in Table III.

In relation to the primary or possibly secondary origin of all of the derivatives silylated at the positions indicated on the chromatograms in Fig. 1, probably these were synthesized during the derivatization and thus were already present as primary reaction products in the aliquots injected into the gas chromatograph. However, the chromatographic data would indicate that with regard to the additional species

TABLE III

ORDER OF RELATIVE RETENTIONS OF TRYPTAMINE AND ITS SILYLATED DERIVATIVES ON 3% OV-17 ON GAS-CHROM Q AS REPORTED BY DIFFERENT WORKERS

Reference	*Order*[*]
Donike *et al.*[11]	(N^{ω})–$(N^{\omega}, N^{\omega}) > (N^{1}, N^{\omega}, N^{\omega})$
Narashimachari and Leiner[12]	(N^{1}, N^{ω})–$(N^{1}) > (N^{1}, N^{\omega}, N^{\omega}) > (T)$
This work	(T)–(N^{1}, N^{ω})–(N^{1})–$(N^{\omega}) > (N^{1}, N^{\omega}, N^{\omega}) > (N^{\omega}, N^{\omega})$

[*] Order of elution from left to right; compounds not baseline resolved are joined by a dash. Derivatives with unexplained relative retention differences are italicized.

detected at lower elution temperatures (N^{1}-TMS-tryptamine, tryptamine, O^{5},N^{1}-bis-TMS and O^{5}-TMS derivatives of 5-hydroxytryptamine) these could be considered as secondary decomposition forms of the higher silylated parent derivatives. In other words the partial or even total decomposition of a given silyl derivative by loss of a reactive TMS group (*e.g.*, loss of a TMS group from N^{1},N^{ω}-bis-TMS tryptamine) at any point between derivatization and its GC detection, is not a problem of the inherent chemical stability of the derivative initially synthesized as much as it is due to irreversible reactions with active (hydroxyl) sites located within the walls and packing of the column (on column effects[24]) or with the hydrated surfaces of the glassware that come in contact with the primary derivatization products. This effect is difficult to control and irreproducible enough to warrant a word of caution in the quantitative analysis of indoleamines as their corresponding TMS derivatives. For instance, Fig. 5 shows the FID profiles at 170° corresponding to a sample of tryptamine silylated with BSA-pyridine under conditions that would enhance the formation of the partially derivatized N^{1},N^{ω} and N^{ω},N^{ω} species. The remarkable lack of linearity of response for the unresolved peak of the N^{1},N^{ω} (with traces of N^{1}) and N^{ω}derivatives relative to that of the N^{ω},N^{ω}-bis-TMS derivative is evident on the superimposed GC traces of three consecutive injections. In a sense, this effect is not unexpected for polar compounds with free functional groups as most chromatographic systems have a certain degree of residual activity. In view of these results, it can be stated that the derivatives monosilylated on the terminal amine are more prone to decomposition during chromatography, depending on the elution temperature and residence time in the columns as well as on the residual activity of the GC system. It is also important to note that in following the time course of the silylation reactions and in consecutive injections there is the possibility of obtaining different relative responses for the various reaction products, depending on the absolute amounts injected into the column. These considerations demonstrate the need to establish reaction conditions leading to the formation of the more stable fully silylated derivatives. In consequence, work was undertaken along this line, as discussed below.

Kinetic aspects

The silylation of tryptamine with BSTFA (a strong silyl donor under different conditions of acidic (1% TMCS) or basic (1% TMSI) catalysis[27] indicates that the addition of a second silyl group on the primary amino group of N^{1},N^{ω}-TMS-tryptamine to obtain the N^{1},N^{ω},N^{ω} species is a slow process (a relatively large amount of N^{1},N^{ω},N^{ω}-tris-TMS-tryptamine is obtained only after 290 min at 110° with BSTFA–TMSI), so that this could be considered as a limiting step in the silylation

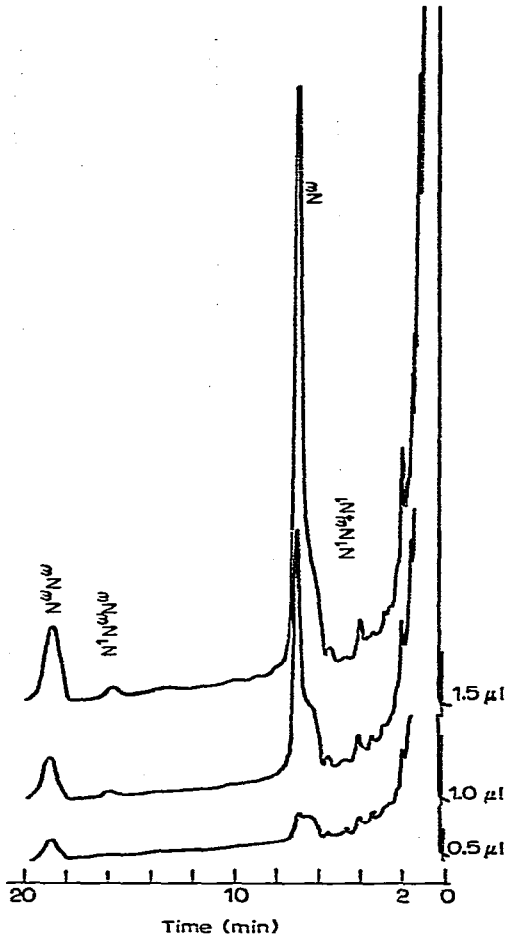


Fig. 5. Responses obtained from different injections of the same reaction mixture (T derivatized with BSA–pyridine at 60° for 40 min) on a 1 m × 1 mm I.D. glass column filled with 3% OV-17 on Gas-Chrom Q, at a column temperature of 180°. Injector and manifold at 250°. Volumes injected: 0.5, 1, 1.5 µl.

of these indolealkylamines. It is known that the introduction of one TMS group in primary amines hinders the access of a second[28,29]. Also, the indole nucleous remains intact when the reaction is catalised by a small amount of TMSI but occasionally may give a relatively high yield of an unidentified degradation product if carried out without TMSI or with TMCS catalysis[30].

Along these lines it was shown that pyridine, which also provides a basic medium for the reaction, enhances substitution at the N^{ω} position, giving predominantly the N^{ω},N^{ω}-bis-TMS-tryptamine derivative. However, in accordance with the data reported by Donike[21], TMSI favours the silylation of the indolic N^{1} position, giving a higher yield of the N^{1},N^{ω}-bis-TMS and N^{1},N^{ω},N^{ω}-tris-TMS derivatives, and by combining the effect of both pyridine and TMSI with BSA the reaction can be driven towards the formation of the fully silylated N^{1},N^{ω},N^{ω} species. The time

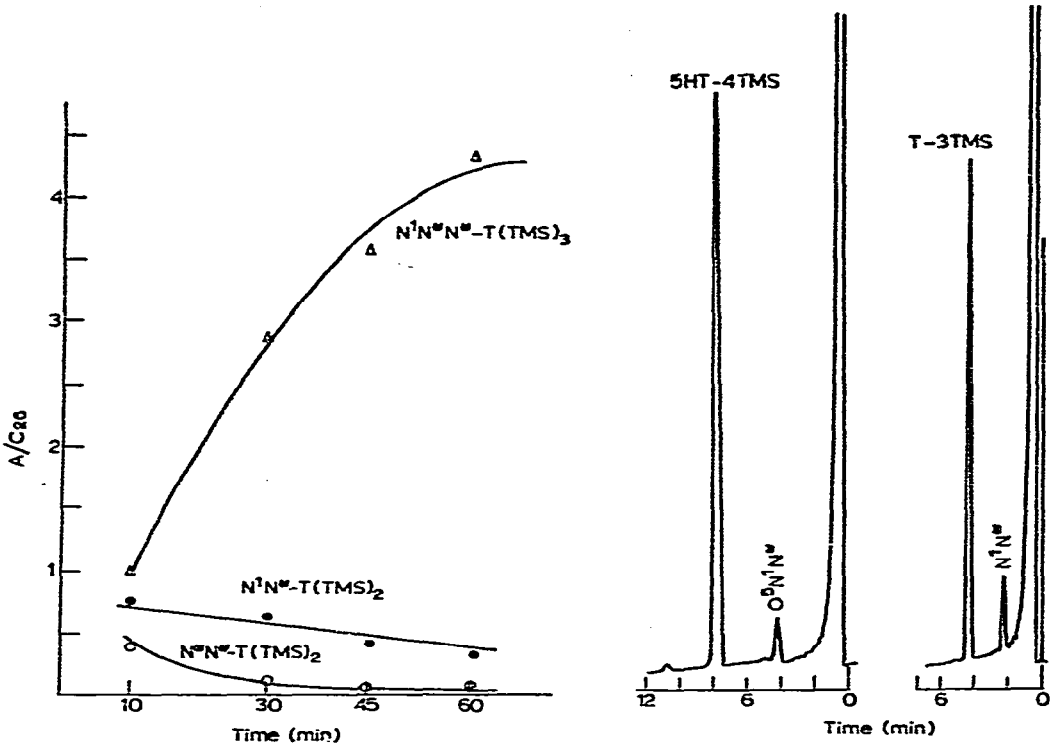


Fig. 6. Plot of the time course of the reaction of tryptamine with BSTFA–TMSI–pyridine (100:10:4) at 70°. *n*-C_{26} used as internal reference standard.

Fig. 7. GC profiles of the reaction products of tryptamine and 5-hydroxytryptamine under the conditions indicated in Fig. 6 with a reaction time of 1 h. Separation on 3% OV-17 column at 240° with injector and detector temperatures set at 250°.

course of the latter reaction is plotted in Fig. 6. As shown, the maximum yield of the tris-TMS derivative would be obtained at a reaction time of 60 min at 70°, while amounts of the other two partial derivatives can be kept to a minimum by changing from BSA to BSTFA (a stronger silyl donor[28]) and adjusting the proportions of BSTFA, TMSI and pyridine (increasing the proportions of TMSI and pyridine).

Likewise, it was found that 5HT behaves very much like T, also giving a predominant response for the fully silylated O^5,N^1,N^ω,N^ω-tetrakis-TMS derivative, as shown in Fig. 7.

In 1968, VandenHeuvel[3] made some comments on the relative trimethylsilylation rates of the nitrogen atoms on the side-chain (N^ω) and on the indole ring (N^1), indicating that the N^1 silylation is more difficult to achieve. More recently, Donike[21] showed the influence of acidic and basic catalysts such as TMCS, pyridine and TMSI on the rate of N^1 silylation, concluding that the indolic N–H proton exchange is a very slow process which is kinetically and not thermodynamically controlled and is accelerated by base catalysis (TMSI). This is essentially in agreement with our own observations.

From these experimental data, it could be concluded that pyridine somehow

accelerates the rate of introduction of the second TMS group on the N^{ω} position while N^1 substitution can be more readily achieved via TMSI catalysis. Also, the relative ease of silylation of these groups would seem to follow the order $O^5 > N^{\omega} > N^1 > N^{\omega}$ (second substitution). However, on contrast with this and other data on relatives rates of silylation of these indole-related products[3,21], there is a report[12] on a predominant TMS substitution at the N^1 position with BSTFA–TMCS in 15 min at 100°.

This apparent discrepancy was studied and the results shown in Fig. 8 were obtained. For this purpose, a total of five reactions were run for 15 min at a temperature of 100°, each with a different reagent mixture (see Fig. 8). The GC separation of the reaction products was performed on a 5% OV-17 column at 230° and the chromatograms were recorded on purpose at twice the normally used recorder chart speed so as to facilitate the uncovering of small retention time differences and also to enhance the appearance of shoulders or any other hidden features. As stated above, the reaction carried out with BSA-pyridine leads to the formation of derivatives preferentially substituted on the N^{ω} position. In any case, there is an appreciable shoulder in front of the peak of N^{ω}-TMS-tryptamine and in the position corresponding to N^1-substituted forms. The addition of TMSI to this BSA-pyridine reagent mixture, as expected from the patterns obtained with longer reaction times, induces substitution at N^1 so that now the relative amount of the $N^1,N^{\omega},N^{\omega}$-tris-TMS-tryptamine derivative increases significantly, while there is a clear predominance over the N^{ω} derivative of what in the previous profile was just a shoulder. This section of the GC trace is very much enhanced by the reaction with BSTFA alone, whereas in Fig. 2, two peaks are

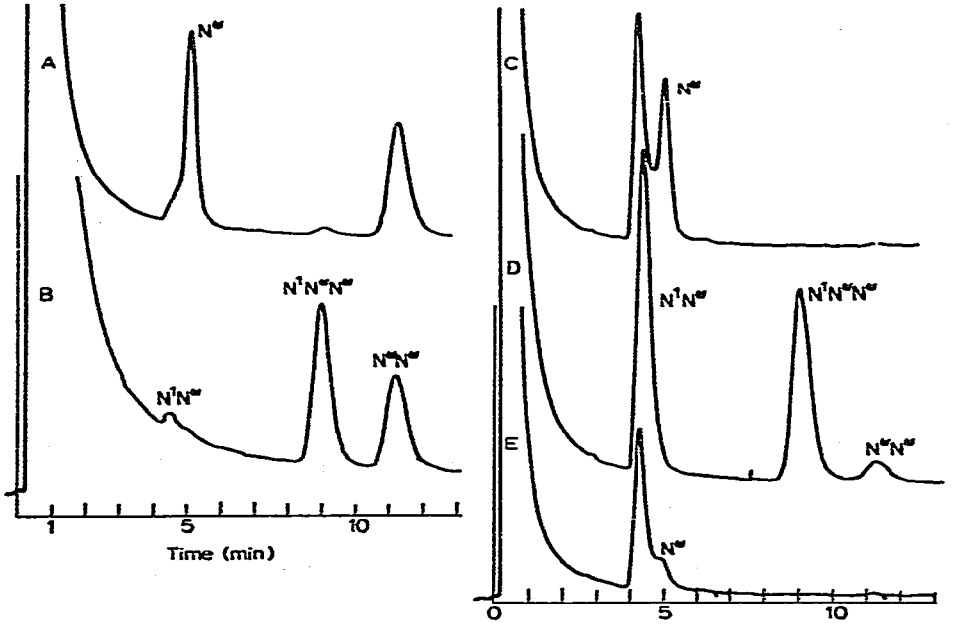


Fig. 8. GC separation of the reaction products obtained by derivatization of tryptamine at 100° for 15 min with (A) BSA–pyridine (100:20), (B) BSA–TMSI–pyridine (100:1:20), (C) BSTFA, (D) BSTFA–1% TMSI and (E) BSTFA–1% TMCS. Products separated at 230° on a 5% OV-17 column. Helium flow-rate slightly lower than in Fig. 3.

distinctively separated. As indicated by the mass spectrometric data, the first of these two peaks would represent the N^1,N^ω derivative, which practically co-elutes with the corresponding N^1-TMS derivative. The addition of TMSI favours the N^1 substitution with a substantial relative predominance of the first peak (N^1,N^ω-TMS-tryptamine). Finally, the BSTFA–TMCS reaction gives a GC profile almost exclusively dominated by the peak of the co-eluting N^1-TMS and N^1,N^ω-bis-TMS species, followed by a smaller smount of the N^ω-TMS derivative. The appearance of the first peak in this instance would be in line with the data reported by Narasimhachari and Leiner[12]. However, on packed OV-17 columns wee have found that in some instances it might be difficult to assign the structure of this compound exclusively to the N^1-TMS derivative of tryptamine, especially as even mass spectrometrically there might be the possibility of confusion with the N^1,N^ω-bis-TMS derivative.

As a conclusion to these kinetic considerations, it could be added that the direct co-injection of 2 μl of a solution of tryptamine in pyridine (4 μg/μl) plus 2 μl of BSTFA, with the injector block at 250°, produces in a qualitative sense a GC profile almost identical in shape with that in Fig. 1, although of course there is a larger relative amount of underivatized tryptamine eluting together with the first peak. This instantaneous reaction on the injector of the gas chromatograph throws some doubt on the studies related to the silylation kinetics with these compounds. In any case, whatever happens in the injector would probably be an additive effect in the sense that it would increase the apparent reaction yield, although in some instances it might change the relative proportions of the various silylated by-products of a given reaction.

Analytical implications

The results obtained largely confirm the theoretical derivatization schemes drawn up for tryptamine (Scheme I) and 5-hydroxytryptamine (Scheme II) in relation to the various silylated species detected by GC. Thus, all of the derivatives of tryptamine have been identified by their mass spectra and retention indices[23]. In a similar manner, it has been possible to detect and identify all of the different silylated species of 5-hydroxytryptamine with a TMS group at the O^5-position. Likewise, the reported identifications of final and intermediate derivatives reported here rest on the corresponding spectrometric and GC retention data[23].

On the other hand, in accordance with what the GC behaviour of some of the partially silylated derivatives identified in both instances seems to indicate, namely for tryptamine and its N^1-monosilylated derivative plus the O^5,N^1-bis-TMS and O^5-mono-TMS forms of 5-hydroxytryptamine (Figs. 2 and 4), these may not be primary derivatization by-products but the corresponding GC decomposition products of N^ω-TMS-tryptamine, N^1,N^ω-bis-TMS tryptamine, O^5,N^1,N^ω-tris-TMS and O^5,N^1-TMS-5 hydroxytryptamine, respectively. This reaction reversibility has been indicated in Schemes I and II by reverse discontinuous arrows, indicating that the N^ω-mono-TMS group is a reactive group which can be lost due to interactions with active sites, as discussed above.

While the use of the N^1-TMS derivative of tryptamine has been suggested for the quantitation of this amine in biological samples[12], our results would be at variance with this suggestion, as demonstrated by the lack of linearity of response of the partially silylated forms of tryptamine at different injection loads (Fig. 5). On the

other hand, it has been claimed that this N^1-TMS derivative of tryptamine[12] has excellent GC characteristics, but in our experience the only cases where the synthesized derivatives give relatively stable and good GC peaks are those in which the primary amino (N^ω) group is completely silylated (N^1,N^ω,N^ω and N^ω,N^ω in Fig. 1). This was also confirmed for the 5-hydroxytryptamine derivatives (O^5,N^ω,N^ω and O^5,N^1,N^ω, N^ω in Fig. 1).

Finally, in the light of these results, a few selected comparisons would be useful regarding the relative merits and practical applications of these silylated derivatives *versus* their acylated counterparts, for which more data and practical experience are available[4,7,13–18].

Briefly, all of the information gathered to date on these indole-related compounds would support the following considerations:

(1) Both the acylation reagents and the acylated products are more stable to the usual laboratory conditions than are the corresponding silylating agents or silylated products.

(2) The number of possible partial derivatives is lower in the acylation reactions. The N^ω position only accepts one acyl residue[7].

(3) Both procedures give comparable detection limits in our GC–MS system. This has been established, as illustrated in Fig. 9, by parallel GC–MS MID analyses of equal aliquots of a tryptamine standard, one of them acylated with pentafluoropropionic anhydride (PFPA) according to our standard procedure[7,15], and the other silylated as described herein. The ions selected for monitoring purposes are the indolyl (IF) fragments at *m/e* 276 and 202 for the acyl and silyl derivatives, respectively, and the amine fragment (AF) at *m/e* 174 for the silylated tryptamine. In the mass spectra of pentafluoroacylated tryptamine the corresponding AF fragment is of low intensity[7]. The response obtained for the *m/e* 276 and 174 iosn in Fig. 9 corresponds to an absolute amount injected of 405–500 pg and, as illustrated, it would be comparable in the two instances. However, it must be taken into account that the fragment at *m/e* 276 would be truly characteristic of the N^1-PFP acylated indole

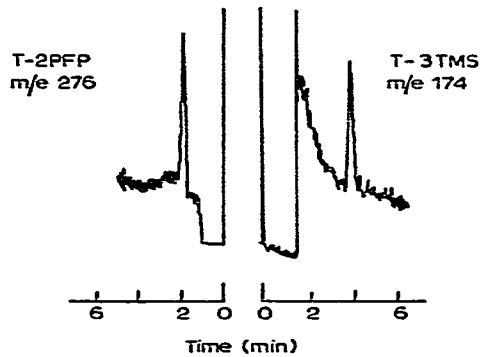


Fig. 9. Selected ion monitoring of characteristic ions in the mass spectra of pentafluoropropionated tryptamine (T-2PFP) (*m/e* 276) and silylated tryptamine (T-3TMS) (*m/e* 174) on 3% OV-17 at 240°. The derivatizations were carried out as follows. Acylation with PFPA–acetonitrile (1:1) at 60° for 1 h. Dry residue dissolved in 200 μl of benzene containing 3% of PFPA. Amount injected, 405 pg (see refs. 7 and 15 for more details). Silylation with BSTFA–TMSI–pyridine (100:10:40) at 70° for 1 h. Amount injected, 540 pg.

nucleus, while the fragment at *m/e* 174 would be characteristic of all N^{ω},N^{ω}-bis-silylated amines and with possibilities for β-cleavage, and thus not specific for indoleamines. In consequence, although the ion at *m/e* 174 would be helpful for functional group mass spectrometry, as proposed by Abramson *et al.*[9], the more specific mass at *m/e* 276 would be more suitable for GC–MS profiling of biogenic indoleamines and related indole metabolites, as shown in earlier papers[15,19].

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