Journal of Chromatography, 167 (1978) 77–90

CHROM. 11,161

MIXED PENTAFLUOROPROPIONYL–TRIMETHYLSILYL DERIVATIVES OF 5-HYDROXYTRYPTOPHAN FOR MASS FRAGMENTOGRAPHIC DETECTION. DEVELOPMENT OF A RETENTION INDEX MODEL FOR SUBSTITUTED INDOLES

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SUMMARY

The experimental conditions reported for the concurrent analysis of tryptophan and its metabolites usually discriminate against 5-hydroxytryptophan (5HTP), a difficulty that can obviated by the mixed pentafluoropropionyl–trimethylsilyl (PFP–TMS) derivatives described here. Direct perfluoroacylation of 5HTP followed by silylation gives a large and well resolved gas chromatographic peak on OV-17 at 200° with a Kováts retention index at 180° of 2237. Its mass spectrum suggests the structure of a TMS ester of 5-O-PFP-N^{1}-TMS,N^{ω}-PFP-hydroxytryptophan, detectable at the low picogram level by selected-ion monitoring of the prominent base peak at *m/e* 364. However, as these double reactions may give various related isomeric compounds with similar mass spectral patterns, a retention index model has been developed as an aid in the combined gas chromatographic–mass spectrometric identification of the different derivatives observed. The model, based on the individual ΔI values of the different substituent groups, takes into account the intramolecular interactions that may affect the expected retention index of a given derivative.

INTRODUCTION

The lack of reported values for endogenous 5-hydroxytryptophan (5HTP) in biological samples can be regarded a consequence of the unavailability of the necessary analytical techniques required for its detection at low levels in complex samples. In fact, only a few reports have appeared on the application of liquid column chromatographic techniques for 5HTP[1–6]. On the other hand, combined gas chromatographic and mass fragmentographic techniques, although not investigated in detail for this compound[7–10], would seem to be ideally suited, especially as they would allow the concurrent determination of other tryptophan (TP) metabolites[7,10,11]. However, the highly functional and non-volatile nature of 5HTP requires that it be previously derivatized in order to render it amenable to chromatography in the vapour phase. In our experience, this can pose technical difficulties as the experimental derivatization conditions that permit the concurrent gas chromatographic–mass spectrometric

(GC–MS) assay of various tryptophan metabolites[9,10] are often a compromise in favour of some of them at the expense of others, as observed with 5HTP. A possible solution to this problem involves a detailed study of various derivatization possibilities, reflected in the combined acylation–silylation method reported here. Unfortunately, this method introduces an additional degree of complexity to the overall analytical procedure in the sense that the "problem substance" plus the "derivatizing reagent" do not add up to a single, readily identifiable derivative in many instances. In other words, when the compound of interest is a polyfunctional structure capable of incorporating more than a single derivatizing group and if combined derivatization techniques are used, as in this instance, a large number of possible isomeric reaction products may appear in the chromatograms. Therefore, in accordance with the experimental observations, the development of the new double derivatization reaction for 5HTP, particularly under non-optimized conditions, may give rise to more than one product not only of 5HTP but also of the related tryptamine, 5-hydroxytryptamine and corresponding indoleacetic and 5-hydroxyindoleacetic acids. For instance, with five reactive hydrogen atoms available for substitution, 5HTP could give rise to 23 theoretically possible trimethylsilyl (TMS) by-products on silylation alone. Fortunately, not all of them are either really formed or detectable by GC.

Some of the isomeric TMS, pentafluoropropionyl (PFP) or mixed TMS–PFP derivatives obtained in several instances, although well resolved on the GC columns used, are not easily identifiable owing to the close similarities of their mass spectral patterns. Thus, with the aim of reducing the possible number of structural identifications suggested by the MS data, we have attempted to pursue an empirical approach to the prediction of the approximate Kováts retention indices (I) of all of the potential derivatives formed in a given reaction. The model developed for substituted indoles is based on a consideration of the individual ΔI contributions of the different substituent groups to the overall I value of a given member of this family of related compounds. The intramolecular interactions established between the carboxyl, the amino group and the N^1-substituted indole moieties, due to their proximity[12], became evident in the experimental data, precluding the direct use of the additivity principle for the prediction of retention values. Correction coefficients were therefore applied in order to take into account these interactions. Finally, although various approaches and models have been described for homologous series of simple aliphatic and alycyclic hydrocarbons, alcohols, *etc.*[12], there are no comparable data on such a body of biologically significant substances as these substituted indoles.

EXPERIMENTAL

Chemicals and reagents

The following standards were used: D,L-5-hydroxytryptophan (5HTP), L-tryptophan (TP), serotonine oxalate (5HT) and 5-hydroxyindole-3-acetic acid (5HIAA) from Regis (Morton Grove, Ill., U.S.A.), tryptamine hydrochloride (T) and indole-3-acetic acid (IAA) from Sigma (St. Louis, Mo., U.S.A.) and 3-methylindole (skatole) (SK) from Aldrich Europe (Beerse, Belgium). All of these compounds were kept refrigerated at $+4°$ under anhydrous conditions, except for the acids, which were stored at $-10°$. The hydrocarbon standards (n-C_{16}, n-C_{20}, n-C_{22}, n-C_{24} and n-C_{28}) were supplied by Applied Science Labs. (State College, Pa., U.S.A.). N,O-Bis(tri-

methylsilyl)trifluoroacetamide (BSTFA), trimethylsilylimidazole (TMSI), pentafluoropropionic anhydride (PFPA), hexamethyldisilazane and the boron trichloride–methanol solution were supplied by Xpectrix International (Sant Cugat, Barcelona, Spain). Benzene and ethyl acetate (per cromatografia grade) were obtained from Carlo Erba (Milan, Italy).

Mixed PFP–TMS 5HTP derivatives

An aliquot from a stock solution of 5HTP in 0.01 *N* hydrochloric acid, equivalent to 300 μg, was evaporated to dryness under a stream of helium. The dry residue was derivatized with 100 μl of PFPA in 100 μl of ethyl acetate for 15 min at 60°. After removing the excess of reagent, the residue was silylated at 70° for 35 min with 100 μl of BSTFA and 1% of TMSI. *n*-Octocosane was used as an internal GC reference. No prior methylation of the carboxyl group was needed.

Perfluoroacetylated or silylated derivatives

The PFP derivatives of IAA, 5HIAA, T, 5HT, TP and 5HTP were synthesized by reaction with PFPA in ethyl acetate, prior methylation of the carboxyl group of acids and amino acids, according to the procedure of Gelpí *et al.*[9]. The corresponding silyl derivatives of the same substances were obtained by direct reaction with BSTFA containing 1% of TMSI[13]. In certain instances the reaction was interrupted by sudden cooling of the vial in order to be able to detect final and intermediate products simultaneously. It must be noted that the reaction conditions for compounds with a number of possible substitution sites, such as these TP metabolites, have to be controlled carefully[14], depending on the degree of substitution sought.

Gas chromatography

The GC separations were carried out on a Perkin-Elmer Model 900 gas chromatograph, equipped with dual flame-ionization detectors. The 1.80 m × 2.5 mm I.D. glass column, deactivated with hexamethyldisilazane in toluene, was packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh). Measured efficiencies were of the order of 2000 plates/m. Purified helium was used as the carrier gas at a flow-rate of 25 ml/min. The manifold and injector block temperatures were maintained at 40° above the column temperature.

Gas chromatography–mass spectrometry

The structural assignments established by combined GC–MS analysis of the reaction products were based on the mass spectral patterns obtained on a Hitachi RMU-6H mass spectrometer coupled through a single-stage gold-lined jet separator to a Perkin-Elmer Model 3920 gas chromatograph. The GC column temperature varied between 180 and 240°. Spectra were taken at 70 eV and different accelerating voltages, depending on the molecular weight of the derivative being analysed. The mass spectrometer is equipped with an accessory for multiple ion detection (MID) of our own design[15], which allows the simultaneous recording of up to four selected ions.

Determination of Kováts retention indices (I)

Retention indices were calculated at 180 ± 2° by co-injection of the appropriate hydrocarbon standards (*n*-C_{16}, *n*-C_{20}, *n*-C_{22} and *n*-C_{24}). Dead times were calculated from the expression[16]

$$t_m = \frac{t_{n+i} \cdot t_{n-i} - t_n^2}{t_{n+i} + t_{n-i} - 2t_n}$$

where $n = 20$ and $i = 4$, thus giving the corrected retention times (t'_R) of the four hydrocarbons. Substitution of the t'_R values on the straight line defined by $\log t'_R = a\,I + b$ gives the values of a and b with a calculated correlation coefficient $r^2 \geqslant 0.9999$. With both of these constants known and with an experimentally determined t'_R value, the corresponding I values can be readily calculated. This has been defined as the most precise method for calculating retention indices[17].

RESULTS AND DISCUSSION

Gas chromatographic evaluation

A typical gas chromatogram of the reaction products obtained by stepwise derivatization of 5HTP with PFPA in ethyl acetate and 1% TMSI in BSTFA, as described above, is shown in Fig. 1. The response *versus* time course of this reaction at 70° is illustrated in Fig. 2. The main product (peak I) reaches its maximum yield in about 35 min under these conditions, whereas the minor by-product (peak II) does not increase above the 5% relative height level. It must be noted, however, that depending on the quality and activity of the reagent batch that is used, occasionally it may take longer to attain the maximum yield. The kinetic curve illustrated here

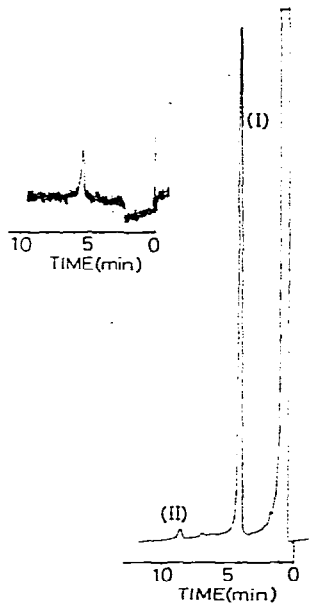


Fig. 1. GC separation of the products obtained by derivatization of 5HTP with PFPA and 1% TMSI in BSTFA. The reaction was carried out at 70° for 20 min. Column, 3% OV-17 on Gas-Chrom Q (100–120 mesh) operated at 230° and a flow-rate of 25 ml/min. Injector and detector temperatures, 270°. Inset: selective-ion trace of peak I obtained by focusing on *m/e* 364. The response shown corresponds to an absolute amount of 700 pg. Column temperature, 220°.

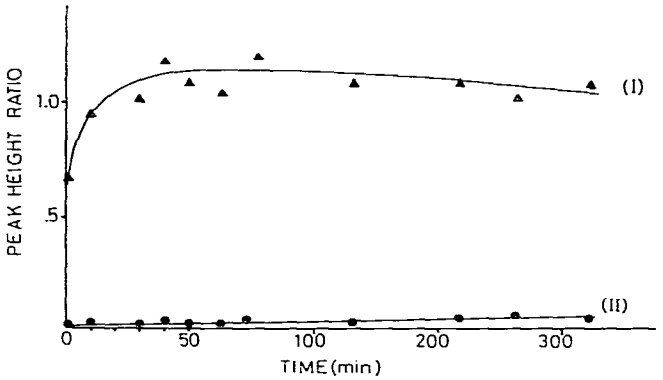


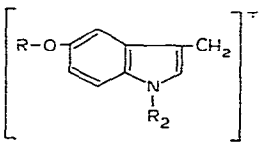
Fig. 2. GC response *versus* time of reaction curves of the two derivatives corresponding to peaks I and II in Fig. 1. Each point illustrated represents the ratio of the peak height of the derivative to that of the internal standard (n-C_{28}).

would be representative of the type of results one could obtain with controlled quality acylating and silylation reagents.

Mass spectrometric identification

The MS patterns of both peaks (I and II), shown in Fig. 3, contribute to the identification of the major GC peak appearing in Fig. 1 at approximately 4 min as the TMS ester of 5-O-PFP,N^1-TMS,N^ω-PFP-hydroxytryptophan and the second minor peak at *ca.* 8–9 min as the TMS ester of 5-O-TMS-N^1-TMS,N^ω-PFP-hydroxytryptophan.

In agreement with work previously described[7,9], the major ionic species would originate from β-cleavage with charge transfer to the indole nucleus:



This ion is observed as the base peak in all instances reported to date, regardless of the nature of both R and R_2 ($R = R_2$ = TMS[7,8] or $R = R_2$ = PFP[9,18]). As illustrated in Fig. 3A, the mass spectrum of the second minor GC peak shows a base peak at *m/e* 290 corresponding to a structure where $R = R_2$ = TMS, while the same fragment is correctly observed at *m/e* 364, as would be expected from a mixed ($R \neq R_2$) TMS, PFP-indolyl moiety (Fig. 3B). Other structurally significant ions are indicated in Fig. 3.

These data demonstrate the synthesis of a new mixed bis-PFP,bis-TMS derivative of 5HTP with a characteristic fragment at *m/e* 364 with an intensity and a mass that are very suitable for selective-ion detection (SIM), as shown in Fig. 1 (inset). The linearity of the SIM response at this mass is good in the range assayed between 10 ng and 350 pg, with a detection limit of the order of 350 pg at a signal-to-noise ratio of 2:1.

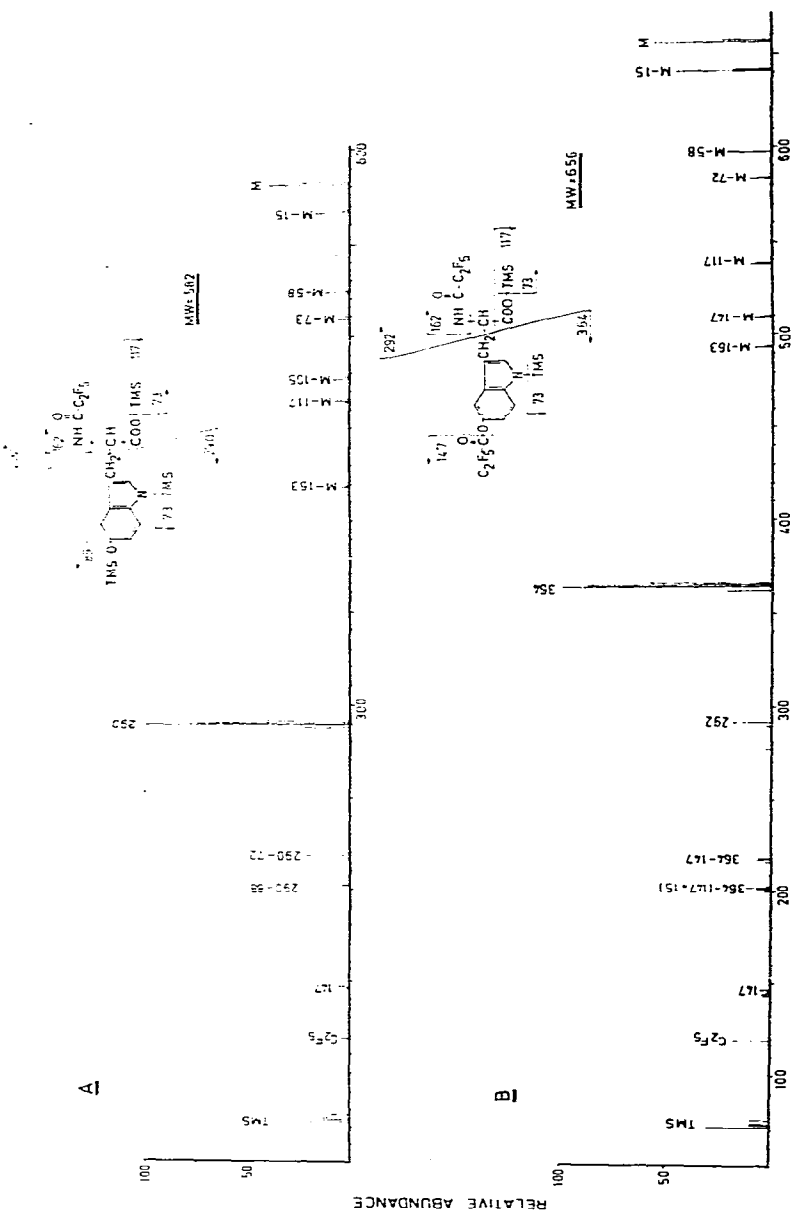


Fig. 3. (A) Mass spectrum of the minor peak (II) shown in Fig. 1 and identified as the TMS ester of 5-O-TMS-N^{1}-TMS, N$^{\omega}$-PFP-hydroxytryptophan. (B) Mass spectrum of the major peak (I) shown in Fig. 1 and identified as the TMS ester of 5-O-PFP-N^{1}-TMS, N$^{\omega}$-PFP-hydroxytryptophan.

TABLE I

DETECTION OF 5HTP BY SELECTED ION MONITORING: COMPARISON OF PARAMETERS FOR VARIOUS DERIVATIVES

Parameter	*Derivative*			
	TMS	*Me/2PFP*	*Me/3PFP*	*TMS/PFP*
m/e	290[7,8]	292[18]	438[9]	364
Detection limit	N.R.*	500 pg	>1 ng	350 pg
GC peak shape	Good	Bad	Good	Good
Reaction kinetics	N.R. 3 h at 60°	2 min at R.T.**	180 min at 100°	35 min at 70°

* N.R. = not reported in the literature.
** R.T. = room temperature.

Characteristic features of the mixed 5HTP derivative

Among the most important properties of this mixed derivative for potential biological applications are its relatively low limit of detection, good GC peak shape, rapid reaction kinetics and high specific mass for SIM. A comparison of these properties with those of other derivatives is given in Table I. Although we have previously studied both the methyl-bis-PFP and methyl-tris-PFP derivatives of 5HTP[9,19], none gave satisfactory results in the sense that, although in principle the ion at *m/e* 438 would be a better specific mass for biological applications than the fragment at *m/e* 292 (Table I), the sensitivity attained with the fully acylated derivative is relatively poor (> 1 ng) and, while the partially acylated form gives a relatively much better SIM response[19], the peak suffers from a high degree of tailing due to the free functional group. Likewise, kinetically one is forced to work in a region of the response *versus* time curve where the peak heights of the 5HTP-Me-2PFP derivative are rapidly decreasing with time in favour of the fully acylated 5HTP-Me-3PFP derivative, so that the reproducibility may not be as good as when working on the plateau of the curve (*e.g.*, Fig. 2). This would be a situation similar to that experienced with tryptamine 1PFP and tryptamine 2PFP[9]. However, it must also be noted that as kinetics is prone to vary with decreasing concentrations of the compound being derivatized, the yields obtained at lower concentrations may adversly raise the 500 pg limit of detection of the mixed 5HTP derivative if reaction conditions are not properly adjusted. In any case, an accurate quantitative analysis of biological samples would require the use of a deuterated analogue.

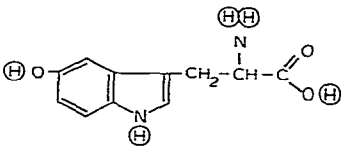
With regard to the specific mass to be selected for SIM, it is generally acknowledged from practical experience with biological extracts that the higher the mass monitored the lower is the number of potentially interfering substances and thus the higher is the ultimate specificity attained. In view of these considerations, the mixed derivative described here clearly shows interesting advantages in terms of the four experimental parameters cited in Table I. The only better property shown by the Me-3PFP derivative lies in its specific mass at *m/e* 438. However, this would be more than offset by the other characteristics of the mixed TMS–PFP derivative.

In our work on the derivatization of indolic compounds, such as tryptophan and its various metabolites, we have observed that reactions have to be adequatedly controlled in order either to drive them to the formation of a single major product[9,19] or to enhance the most favoured structural possibility from a GC view point. This

implies that not all kinetically possible by-products of a reaction would necessarily be amenable to GC analysis, as indicated in Table II. Even if they were, they might not be readily identifiable by MS for lack of sufficient GC resolution. This applies, for instance, to the tris-TMS and tetrakis-TMS derivatives of 5HTP on SE-30. On the other hand, a reaction may give different derivatives of relatively high abundance well separated by the GC column used, as illustrated in Fig. 4, whose closely related structures may not be definitedly established from the MS data alone. For instance, the mass spectra of components 2, 3, 4 and 6 in Fig. 4 do not yield sufficient structural information for a definite identification of these products. Likewise from the MS pattern alone, the assignment of the first PFP group on the indole nucleus to either the 5-0- or N^1-position is not straightforward, though we also[18] favour the assumption that it goes onto the hydroxyl group giving the corresponding 5-0-PFP moiety[9,19].

TABLE II

POSSIBLE AND OBSERVED DERIVATIVES OF 5-HYDROXYTRYPTOPHAN



Derivative	*No. of theoretically possible products**	*No. of products structurally suitable for GC analysis***	*Derivatives observed*	*Ref.*
Methyl-PFP	7	4	2PFP	9
			3PFP	9
TMS	23	6	3TMS	7
			4TMS	
Mixed TMS–PFP	48	23	2TMS–2PFP	
			3TMS–PFP	
			4TMS	
			+ others not conclusively identified (see Fig. 4)	

* The theoretically possible products can be calculated considering a total of five hydrogens that can be substituted by a TMS or PFP group. (In methyl-PFP derivatives the carboxyl hydrogen is always substituted by a methyl group). All of these five positions are illustrated by the circled hydrogen atoms above. For the substitution of the two equivalent hydrogen atoms on the primary amino group the following five cases were considered: (1) substitution by two TMS groups, (2) substitution by one TMS group, (3) substitution by one PFP group, (4) no substitution by one TMS and one PFP group and (5) no substitution by two PFP groups. These criteria are based on experimental observations on the substitution of the αNH_2 in 5-hydroxytryptamine and tryptamine.

** Based on the criterion that regardless of the rest of the structural arrangement both active 5-hydroxy and carboxyl hydrogen atoms have been replaced by the derivatizing reagent.

Retention index model for substituted indoles

On the basis of the above facts and the availability of chromatographic data obtained in our laboratory for individual PFP and TMS derivatives of these indolic compounds, we have attempted to develop a simple model to relate the structure of the compound synthesized in a given derivatization reaction to the corresponding chromatographic behaviour.

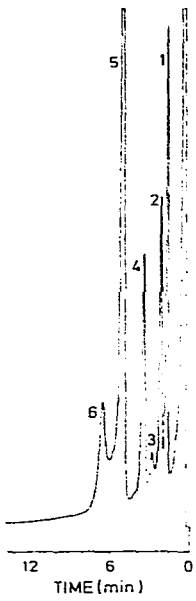


Fig. 4. GC separation of the various derivatives obtained with a mixture of BSTFA and PFPA (10:1) at 100° for 1 h. Glass column, 1.20 × 2 mm I.D. packed with 3% OV-17 on Gas Chrom Q (100–120 mesh). Oven temperature, 230°. Helium flow-rate, 25 ml/min. The amount of 5-HTP derivatized was equivalent to approximately 4 μg.

The presence of strongly polar functional groups in positions relatively close to a resonating nucleus, such as that of indole, in principle precludes the simple additive approach that was initially attempted, making necessary the introduction of correction coefficients in order to obtain a better fit of the experimental data.

The model described below attempts to relate structure to chromatographic behaviour based on the following hypotheses:

(1) The retention index (I) on a given stationary phase and at a particular temperature (in this instance OV-17 at 180°) can be expressed as a linear combination of the different retention index increments (ΔI) assigned to the various structurally significant components of the molecule under study.

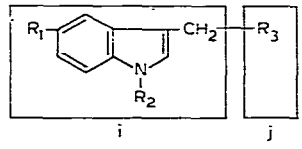
(2) The nucleus, with or without substituents R_1 and R_2 (Fig. 5), and the functionalysed moiety R_3 will be considered in this model as the two main structural components.

(3) Substitution in position N^1 of the indole nucleus plays a major role in any of the interactions established with the various compounds studied.

(4) The coefficients assigned to the linear combination mentioned above are a measure of the degree of the interaction established between the different structural components and, as such, can be considered a function of the nature of both the N^1 substituent (R_2) and the functional group R_3.

(5) If the nucleus is not substituted, there is no interaction between it and the functional moiety R_3.

(6) The retention indices of 3-methylindole (SK) and corresponding TMS or PFP derivatives will be considered as base values for the nucleus.



R_1: -H, -OH, -OPFP, -OTMS

R_2: -H, -PFP, -TMS

R_3: $-CO_2Me$, $-CO_2TMS$, $-CH_2NHPFP$, $-CH_2NHTMS$, $-CH_2N(TMS)_2$, $-CH(NHPFP)-CO_2Me$, $-CH(NHTMS)-CO_2TMS$, $-CH(NHPFP)-CO_2TMS$

Fig. 5. Scheme of structural assignments.

The various structural assignments are indicated in Fig. 5.

Table III shows the coefficients assigned in each instance for all of the derivatives considered. According to the stated hypotheses, the model rationale is as follows. The experimentally determined retention index of the methyl ester of IAA (IAA-Me) is 2148 and that of SK is 1650. As there is no N^1 substitution, the coefficients of both structural components of IAA-Me (the SK nucleus plus $R_3 = CO_2Me$ according to Fig. 5) are assigned a value of 1, which permits the calculation of $\Delta I_{CO_2Me} = 2148 - 1650 = 498$. The same reasoning would apply to the other derivatives included in the table, provided that there is no N^1 substitution (5HIAA-Me, 5HIAA-Me-PFP, T-1PFP, TP-Me-1PFP, IAA-TMS, 5HIAA-2TMS, T-2TMS, and 5HT-2TMS).

However, with IAA-Me-1PFP the pentafluoroacylation of the indolic N^1 atom introduces an interaction effect between the nucleus and R_3 that dictates the application of a coefficient α_1 so that $I = \Delta I_{SK-PFP} + \alpha_1 \cdot \Delta I_{CO_2Me} = 1469 + \alpha_1 \cdot 498 = 1841$, and $\alpha_1 = 0.75$. In the same way we can calculate the values of α_2 for T-2PFP, α_3 for TP-Me-2PFP, α_4 for IAA-2TMS and 5HIAA-3TMS, α_5 for TP-3TMS and α_6 for T-2TMS*.

However, in the case of TP-3TMS, where the retention index would be given by $I = \Delta I_{CO_2TMS-CHNHTMS} + \alpha_7 \Delta I_{SK-TMS}$ the value of coefficient α_7 cannot be calculated directly as there are two unknown parameters in this equation; coefficient α_7 and the ΔI of the CO_2TMS-CHNHTMS group in the amino acid. This is a consequence of the failure of synthesizing and/or identifying, if formed upon derivatization of TP, the corresponding TP-2TMS derivative with its N^1 position free.

Nevertheless, this difficulty can be obviated by considering that the ratio of the correction coefficient of TP-3TMS (α_7) to that of the corresponding PFP form, TP-Me-2PFP (α_3 in Table III) will be similar to the ratio found for the silylated acids (IAA-TMS, 5HIAA-3TMS) and the corresponding PFP forms IAA-Me-PFP and 5HIAA-Me-2PFP (α_4 and α_1).

Thus

$$\alpha_7 = \alpha_3 \frac{\alpha_4}{\alpha_1} = 0.78 \frac{0.87}{0.75} = 0.90$$

TABLE III

RETENTION INDEX INCREMENTS (*AI*) CORRESPONDING TO VARIOUS FUNCTIONAL GROUPS ASSOCIATED WITH THE INDOLE NUCLEUS AND COEFFICIENTS ASSIGNED TO EACH FUNCTIONAL OR STRUCTURAL GROUP

All experimental *I* values were calculated according to the method described under Experimental. The structural assignments of all of the derivatives, in accordance with their respective MS and GC data are as follows. IAA-Me = methyl ester of indole-3-acetic acid; IAA-Me-PFP = methyl ester of N^1-PFP-indole-3-acetic acid; 5HIAA-Me = methyl ester of 5-hydroxyindole-3-acetic acid; 5HIAA-Me-PFP = methyl ester of 5-0-PFP-hydroxyindole-3-acetic acid; 5HIAA-Me-2PFP = methyl ester of 5-0-PFP-N^1-PFP-hydroxyindole-3-acetic acid; T-PFP = N^ω-PFP-tryptamine; T-2PFP = N^1,N^ω-bis-PFP-tryptamine; TP-Me-PFP = methyl ester of N^ω-PFP-tryptophan; TP-Me-2PFP = methyl ester of N^1,N^ω-bis-PFP-tryptophan; IAA-TMS = TMS ester of indole-3-acetic acid; IAA-2TMS = TMS ester of N^1-TMS-indole-3-acetic acid; 5HIAA-2TMS = TMS ester of 5-0-TMS-hydroxy-indole-3-acetic acid; 5HIAA-3TMS = TMS ester of 5-0-TMS-N^1-TMS-hydroxy-indole-3-acetic acid; T-2TMS = N^ω-bis-TMS-tryptamine; T-3TMS = N^1,N^ω-tris-TMS-tryptamine; 5HT-2TMS = 5-0-TMS-N^ω-TMS-serotonine; T-2TMS* = N^1,N^ω-bis-TMS-tryptamine; TP-3TMS = TMS ester of N^1,N^ω-bis-TMS-tryptophan.

Derivative	*Functional moieties and substituted groups*																
	SK	*SK-PFP*	*SK-TMS*	*HOSK*	*PFPOSK*	*PFPOSK-PFP*	*TMSOSK*	*TMSOSK-TMS*	*CO_2Me*	*CO_2TMS*	*CH_2NHPFP*	*CH_2NHTMS*	*CH_2NTMS_2*	*CHNHPFP — CO_2Me*	*CHNHTMS — CO_2TMS*	$I_{exp.}$
IAA-Me	1								1							2148
IAA-Me-PFP		1							α_1							1841
5HIAA-Me				1					1							2562*
5HIAA-Me-PFP					1				1							2239
5HIAA-Me-2PFP						1			α_1							1808
T-1PFP	1										1					2157
T-2PFP		1									α_2					1925
TP-Me-PFP	1													1		2319
TP-Me-2PFP		1												α_3		1989
IAA-TMS	1									1						2184
IAA-2TMS			1							α_4						2168
5HIAA-2TMS							1			1						2494
5HIAA-3TMS								1		α_4						2410
T-2TMS	1												1			2375
T-3TMS			1										α_5			2335
5HT-2TMS								1				1				2407
T-2TMS*			1									α_6				2109
TP-3TMS			1												α_7	2360
AI	1650	1469	1705	2064	1741	1435	1960	1945	498	534	507	447	725	669	728	
α									0.75	0.87	0.90	0.90	0.87	0.78	0.90	

* Data from ref. 9.

which, substituted in the above equation allows the calculation of

$$\Delta I_{CO_2TMS-CHNHTMS} = 728$$

A consideration of the individual ΔI contributions to the value of ΔI_{R_3} for the amino acids where $R_3 = CO_2R_4\text{–}CH\text{–}NHR_5$ leads to the introduction of a coefficient β to account for the specific interactions within R_3 (R_4 = methyl or TMS; R_5 = PFP or TMS):

$$\Delta I_{CO_2R_4-CH-NHR_5} = \Delta I_{CO_2R_4} + \beta_1 \cdot \Delta I_{CHNHR_5}$$

$$\Delta I_{CO_2Me-CH_2NHPFP} = 498 + \beta_1 \cdot 507 = 669$$

$$\Delta I_{CO_2TMS-CH_2NHTMS} = 534 + \beta_2 \cdot 447 = 728$$

giving $\beta_1 = 0.34$ and $\beta_2 = 0.43$.

Now, in a case such as that of the mixed 5HTP derivative reported here, where $R_3 = CO_2TMS\text{–}CH_2NHPFP$, we have

$$\Delta I_{R_3} = 534 + 0.34 \cdot 507 = 706$$

This figure, together with the retention index of the TMS ester of 5-O-PFP-N^1-TMS, N^{ω}-PFP-hydroxytryptophan, which was experimentally determined as 2237, permits the calculation of the ΔI corresponding to the PFPO-SK-TMS moiety:

$$\Delta I_{bis\text{-}TMS-bis\text{-}PFP-5HTP} = \Delta I_{PFPO-SK-TMS} + \alpha_7 \cdot \Delta I_{R_3}$$

By substitution of the appropriate values, we obtain

$$\Delta I_{PFPO-SK-TMS} = 1602.$$

As a practical application of this model, the theoretical retention indices for various derivatives calculated from the ΔI values reported in Table III can be compared with the experimentally determined values as shown in Table IV. Accordingly, the retention indices of the three 5HTP derivatives shown in Table IV can be predicted to within 20 retention index units. The model can also be applied to 5HT, although the difference between the calculated and experimental I values is somewhat larger in this instance (10–48 index units).

However, to a first approximation and considering the complexity of these molecules together with the multiplicity of potential intramolecular structural interrelationships, these results indicate that the model could be very useful in practice to help locating in a chromatogram the various possible by-products of these types of complex derivatization reactions, serving also as an aid to their MS identification in cases where the MS patterns are not specific enough to differentiate between closely related isomeric derivatives.

TABLE IV

CALCULATED AND EXPERIMENTAL RETENTION INDICES FOR SUBSTITUTED INDOLIC COMPOUNDS

The structural assignments of these derivatives according to their respective MS and GC data are as follows: 5HT-2PFP = 5-0-PFP-N^{ω}-PFP serotonine; 5HT-3PFP = 5-0-PFP-N^{1},N^{ω}-bis-PFP serotonine; 5HT-3TMS = 5-0-TMS, N^{ω}-bis-TMS serotonine; 5HT-3TMS* = 5-0-TMS, N^{1},N^{ω}-bis-TMS serotonine; 5HT-4TMS = 5-0-TMS-N^{1},N^{ω}-tris-TMS-serotonine; 5HTP-4TMS = TMS ester of 5-0-TMS-N^{1},N^{ω}-bis-TMS-hydroxytryptophan; 5HTP-Me-2PFP = Methyl ester of 5-0-PFP-N^{ω}-PFP-hydroxytryptophan; 5HTP-Me-3PFP = Methyl ester of 5-0-PFP-N^{1},N^{ω}-bis-PFP-hydroxytryptophan; 5HT-TMS-2PFP** = 5-0-PFP-N^{1}-TMS, N^{ω}-PFP-serotonine. To be more consistent with the general nomenclature used throughout the text in the case of the amino acids, tryptophan and 5-hydroxytryptophan, the α-amino group is designated here, in line with the nomenclature used for the rest of the indoleamines, as N^{ω}.

Derivative	*Retention index*			
	*Calculated**		*Experimental*	
	$\Delta I_i + \Delta I_j \times \alpha_j^i = I_{calc.}$		$I_{exp.}$	$I_{calc.} - I_{exp.}$
5HT-2PFP	1741 + 507	= 2248	2296	−48
5HT-3PFP	1435 + 507 × 0.90	= 1891	1920	−29
5HT-3TMS	1960 + 725	= 2685	2643	+42
5HT-3TMS*	1945 + 447 × 0.90	= 2347	2359	−12
5HT-4TMS	1945 + 725 × 0.87	= 2576	2566	+10
5HTP-4TMS	1945 + 728 × 0.90	= 2600	2580	+20
5HTP-Me-2PFP	1741 + 669	= 2410	2399	+11
5HTP-Me-3PFP	1435 + 669 × 0.78	= 1957	1944	+13
5HT-2PFP-TMS	1602 + 507 × 0.90	= 2058	2100	−42

* The calculated retention index, $I_{calc.}$ is derived from the sum of the individual retention increments of the indole moiety (ΔI_i) and the retention increments (ΔI_j) of the indole side chain (R_3) at position 3 (Fig. 5) adjusted by the corresponding correction coefficients (α_j^i) needed to compensate the interaction between the i and j structural moieties (Fig. 5).

** This derivative, used as a test of the retention index model for the case of mixed PFP–TMS indoleamine derivatives was identified mass spectrometrically, after being prepared according to a procedure similar to that reported here for the 5-0-PFP-N^{1}-TMS, N^{ω}-PFP-hydroxytryptophan.

ACKNOWLEDGEMENTS

We are grateful to Dr. G. Firpo of the Instituto Químico de Sarriá (Barcelona) for helpful discussions on the retention index model and to Professor G. Guiochon for his constructive criticism of the manuscript. This work was supported by a Research Grant from the Comisión Asesora de Investigación Científica y Técnica awarded to Research Project No. 1287/1976.

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