Effective Derivatization of Indole Compounds in Negative Ion Chemical Ionization Mass Spectrometry

Tokishi Hayashi*, Hiroshi Naruse*, Satoru Kamada*, Yoshio Iida**, Shigeki Daishima**, Masayuki Kuzuya***, and Takachiyo Okuda***

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Hunt et al. went on to show considerable increases in negative ion currents relative to positive ion currents for compounds with high electron affinity. In order to use the negative ion chemical ionization (NICI) mass spectrometry for metabolic studies of tryptophan and its metabolites, basic investigation were carried out to examine the usefulness of TFA- or PFP-derivatization in NICI mass spectrometry. As a result, these derivatization was confirmed to be very effective with respect to enhancing sensitivity, favorable fragmentation and other practical characteristics.

1. Introduction

The discovery that certain psychoactive indoles occur naturally in man has intensified research into causative agent of psychotic disease, especially schizophrenia. A number of methods have been established for the determination of them in human plasma,¹⁾ urine²⁾ and cerebrospinal fluid.³⁾ Although some differences have been found between control and psychiatric populations, the findings are not yet conclusive.

Recently, many investigators have reported that gas chromatography/mass spectrometry (GC/MS) using a stable isotope labeled compound as a tracer was a pow-

erful tool for metabolic investigation in clinical medicine^{4,5)}. This method was used to investigate the kinetics of tryptophan to its metabolites by Curtius et al.^{6,7)} Their methods, however, were limited in its usefulness mainly because of the insufficient sensitivity of GC/MS.

It was reported that negative ion chemical ionization (NICI) mass spectrometry could provide sample ion current 100–1000 times greater than that available from positive ion methodology for compounds which exhibit high electron affinity.⁸⁾ However, it is unlikely that biological indole compounds show high electron affinity. In a previous paper,⁹⁾ it was

*National Center for Nervous, Mental and Muscular Disorders, 2620 Ogawa-Higashi-Machi, Kodaira, Tokyo 187, Japan

**Department of Engineering, Seikei University, Kichijoji-Kita-Machi, Musashino, Tokyo 180, Japan

***Gifu College of Pharmacy, 6-1 Mitahara-Higashi 5-Chome, Gifu 502, Japan

predicted from the results of molecular orbital calculations using the MINDO-3 method that the electron affinity of indole compounds could be markedly increased by the introduction of a trifluoroacetyl (TFA) group to the nitrogen atom in an indole ring.

This paper describes detectability enhancement of some indole compounds by the introduction of TFA or pentafluoropropionyl (PFP) in NICI mass spectrometry and their fragmentation in the ion-source.

2. Materials and Methods

2.1. Apparatus

All experiments were carried out using a Finnigan 4023 GC/MS equipped with a pulsed positive ion negative ion chemical ionization (PPINICI) accessory and interfaced to an Incos data system. Methane used as the GC carrier gas also served as the chemical ionization reagent gas. The ion-source pressure, ion-source temperature, electroin accelerating energy and emission current were maintained at 0.20 Torr, 250°, 70 eV and 300 μ A, respectively. The apparatus was adjusted so that anthraquinone provided N/P 20. A 2 m glass column (2 mm I. D.) packed with 3 % OV-17 on Gas Chrom Q was used throughout the study.

2.2. Reagents

Trifluoroacetic anhydride (TFAA) and pentafluoropropionic anhydride (PFPA) were purchased from Merck Japan Ltd. Indole, indole-3-acetic acid, 5-hydroxyindole-3-acetic acid, tryptamine hydrochloride, serotonin creatinine sulfate and L-tryptophan were obtained Wako Pure Chemical In-

dustries Ltd. Serotonin- $\alpha,\alpha,\beta,\beta$ -d₄ creatinine sulfate was purchased from Merck Frosst Canada, Inc. L-Tryptophan-2', 4', 5', 6', 7'-d₅ was synthesized by chemical exchange according to the method reported by Matthews et al.¹⁰⁾ Indole-3-carboxylic acid was supplied by Tokyo Chemical Industries Co. Ltd. The other reagents and solvents used in this work were all reagent grade. The solution of diazomethane in ether was prepared from nitrosomethylurea using the ordinary method.

2.3. Derivatization

TFA- or PFP-derivatives of indole, indole-3-acetonitrile, tryptamine or serotonin were prepared in the following manner: 100 μ g of the compound was dissolved in 50 μ l of acetonitrile and 50 μ l of TFAA or PFPA was added. The mixture was then allowed to stand for 30 min at room temperature.

TFA(PFP)-methyl-derivative of indole-3-acetic acid, 5-hydroxyindole-3-acetic acid or indole-3-carboxylic acid was prepared in the following manner: 100 μ g of the compound was dissolved in 50 μ l of methanol and 0.5 ml of a solution of diazomethane in ether was added to it. Excess diazomethane was removed immediately after methylation for 2 – 3 min. TFA derivatization was then carried out in the same manner as described above.

TFA-methyl-derivative of L-tryptophan was prepared by TFA derivatization and methylation as described above. Finally, the reaction product was dissolved in a mixture of 50 μ l each of acetonitrile and TFAA.

One micro-liter of the reaction mixture

was injected to measure the methane PPI-NICI mass spectrum, and N/P was measured after diluting the reaction mixture with a mixture of TFAA and acetonitrile.

3. Results and Discussion

Table I shows the methane PPINICI mass spectra of indole, indole-3-acetonitrile and their TFA- or PFP-derivatives. The N/P is a ratio of the sample ion current of the most abundant negative ion to that of positive ion. As expected, the introduction of the TFA group to indole resulted in a large increase in the N/P ratio. Also, the TFA-derivatives showed useful prominent negative fragment ions which contained the moiety of the original compound to be analyzed. Such fragment ions permit the utilization of certain techniques using stable isotope labeled compounds. These data seem to support the prediction described in the previous paper⁹⁾ that TFA derivatization of indole compounds would be very effective with respect to both the

sensitivity and nature of the fragmentation.

Next, it was investigated whether or not the biological indole compounds would show similar properties. Table II shows methane PPINICI mass spectra of some derivatives of tryptophan and its metabolites. The N/P values listed in Table II are the ratios of the sample ion current of each negative ion ($M-HF$)⁻ to that of each positive base peak. The compounds in Table II also provided abundant negative ions which contained the moiety in the molecule to be analyzed. Moreover, their ion currents were very large compared with that of each positive base peak. The large N/P ratios found in the TFA- or PFP-derivatives might be due to increased electron affinity and a drop in the ionization potential resulting from the introduction of the TFA or PFP group into the indole compound as discussed in the previous paper.⁹⁾ These derivatizations using TFAA or PFPA have been widely employed for GC or GC/MS analysis (positive mode) of indole

Table I. Methane PPINICI Mass Spectra of Indole, Indole-3-acetonitrile and Their TFA- or PFP-Derivatives

Compound	MW	N/P	<i>m/z</i> of negative ion peak (r.i.)	<i>m/z</i> of positive ion peak (r.i.)
117	0.03	116(100)	117(34), 118(100), 119(13) 146(35)
213	17	212(100)	144(49), 214(100), 242(24) 254(11)
156	0.26	125(80), 129(80), 155(100)	130(100)
252	290	154(30), 232(100), 252(60)	226(100), 253(60)
302	220	147(50), 154(100), 282(35)	276(100), 303(60)

Table II. Methane PPINICI Mass Spectra of Derivatives of Tryptophan and Its Metabolites

Compound	MW	N/P	m/z of negative ion peak (r.i.)	m/z of positive ion peak (i.e.)
410	200	183(100), 390(31)	184(16), 228(25), 298(33), 351(60) 379(63), 411(75), 412(20)
352	340	332(100), 333(15)	240(95), 241(13), 268(10), 353(100) 354(16)
452	110	225(11), 257(25), 302(23), 372(35) 412(60), 432(100), 433(18), 451(22)	290(60), 453(100), 454(17)
464	170	291(13), 367(57), 404(20), 424(12) 444(100), 445(18)	352(35), 465(100), 466(17)
285	10	187(15), 265(100), 266(16), 285(27)	226(100), 227(17), 238(17), 254(27) 266(10), 285(20), 286(82)
335	20	147(100), 148(14), 187(15), 315(53) 334(10)	276(100), 277(16), 304(28), 316(20) 335(21), 336(92), 364(12)
397	70	300(100), 301(17), 324(12), 377(37) 397(48)	338(100), 339(15), 366(20), 398(98) 399(15)

compounds because of their excellent practical properties regarding derivatization, including good reactivity, ease of handling, good volatility of the derivative, and so on. It can therefore be expected that the use of NICI mass spectrometry may lead to a substantial practical enhancement in the sensitivity of these derivatives.

No marked difference in N/P value was found between TFA- and PFP-derivatives. This phenomenon could be effectively used to avoid overlap between of the monitoring ion signal and background signals derived from different kinds of sources, which must be very important for highly sensitive analysis.

All of the TFA- or PFP-derivatives listed in Table II provided a characteristic negative fragment ion $(M-HF)^-$. It is important for the design of a useful tracer compound to investigate which hydrogen is eliminated as HF in the ion-source, because

researchers are obliged to use deuterium labeled compounds as tracers in most tracer experiments due to mainly economic reasons. The TFA- or PFP- derivative of indole-3-acetonitrile also yielded a corresponding negative fragment ion $(M-HF)^-$, but the TFA- or PFP-derivative of indole did not. Fig. 1 shows a methane NICI mass spectrum of the TFA-methyl-derivatives of L-tryptophan-2',4',5',6',7'-d₅. The negative ion in question was observed at m/z 395. The peaks at 1 and 2 lower m/z can be considered to be derived from tryptophan-d₄ or -d₃, which were contained as impurities in the L-tryptophan-2',4',5',6',7'-d₅ sample, because similar peaks were observed on the lower mass side of the quasi-molecular ion peak $(M+H)^+$ in a methane PICI mass spectrum of the TFA-methyl-derivative of L-tryptophan-2',4',5',6',7'-d₅. These data suggest that a hydrogen atom in the side-chain might be eliminated

as HF. Fig. 2 shows a methane NCI mass spectrum of the TFA-methyl-derivatives of indole-3-carboxylic acid, in which two prominent peaks were found at m/z 174 and 271. These two peaks could be clearly assigned to $(M-TFA)^-$ and $(M)^-$. From these results, it seems quite probable that the hydrogen atom eliminated as HF is the one at α -carbon in the case of TFA-methyl-derivatives of indole-3-acetic acid and 5-hydroxyindole-3-acetic acid. The TFA-derivative of serotonin- $\alpha, \alpha, \beta, \beta, d_4$, however, yielded an $(M-HF)^-$ peak, and not an $(M-DF)^-$ peak, as shown in Fig. 3. Therefore, in the case of TFA-derivative of serotonin or tryptamine, a hydrogen atom might be eliminated from $-NH-TFA$ as HF to give the fragment ion $(M-HF)^-$. The above discussion about the fragment ion $(M-HF)^-$ can be summarized as follows: if the 1-TFA- or PFP-derivatives of indole compounds have an active hydrogen atom in the molecule, the active hydrogen atom may be eliminated as HF so that the characteristic fragment ion $(M-HF)^-$ may be produced in the ion source. Also, it can be considered that the TFA (PFP)-derivatives which yielded a prominent negative ion corresponding to $(M-HF)^-$ might tend to capture a thermal electron with the TFA-group at 1-position of indole in the ion-

Fig. 1. Methane NCI mass spectrum of TFA-methyl-derivative of L-tryptophan-2',4',5',6',7'- d_5 .

Fig. 2. Methane NCI mass spectrum of TFA-methyl-derivative of Indole-3-carboxylic acid.

source.

Among the TFA-derivatives containing active hydrogen atoms, TFA-methyl-derivatives of L-tryptophan and 5-hydroxyindole-3-acetic acid provided a base peak other

than that corresponding to $(M-HF)^-$ in their methane NICI mass spectra. The former compound yielded a base peak at m/z 183 as shown in Table II. Moreover, as shown in Fig. 1, the derivatives of tryptophan- d_5 in which hydrogen atoms on the indole ring were exchanged for deuterium atoms also provided a base peak at the same m/z . These results clearly suggest that the base peak could be assigned to $(M-H- \text{indole ring})^-$.

This estimation may be consistent with the fact that no peaks were observed on the lower mass side as observed on the lower mass side of the peak corresponding to $(M-HF)^-$. In the methane NICI mass spectra, a base peak which could be assigned to $(M-TFA)^-$ was found. A similar fragment peak was also found in the NICI mass spectrum of the TFA-derivative of serotonin, although it was not the base peak. The $(M-TFA)^-$ peak might be characteristic for TFA-derivative of indole compounds containing a hydroxyl group on the indole ring. However, we could not reach a conclusion as to which the TFA group in the TFA-methyl-derivative of 5-hydroxyindole-3-acetic acid or the TFA-derivative of serotonin was eliminated in the ion-source.

4. Conclusions

Recently, the reports dealing with sensitive analysis using NICI mass spectrometry has increased rapidly and the question of detectability enhancement has received

Fig. 3. Methane NICI mass spectrum of TFA-derivative of serotonin- $\alpha, \alpha, \beta, \beta$ - d_4 .

a great deal attention. In this work, we carried out basic investigations with a view to applying NICI mass spectrometry to metabolic studies of tryptophan and its metabolites. Also, TFA or PFP derivatization was confirmed to be a very effective method with respect to enhancing sensitivity, favorable fragmentation and other practical characteristics. The results obtained in this work could be effectively used not only for the ultra-micro determination of tryptophan and its metabolites but also for the metabolic investigation of these substances using labeled compounds with stable isotopes.

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