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A modified McLafferty rearrangement in the electron impact mass spectra of dansylated amino-acid methyl esters[1]

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Abstract

Electron impact mass spectra of dansylated amino-acids and their methyl esters show a very intense peak at *m/z* 171. A systematic study failed to show the genesis and origin of this ion. The present work is devoted to the electron impact mass spectrometric (EIMS) analysis of the appearance of this ion *m/z* 171 in the spectra of dansylated amino-acid methyl esters and its diagnostic importance in the analysis of dansyl derivatives. A structural model for this study is offered by a series of *N*-alkylated aromatic sulphonamides. The EIMS spectra of these compounds indicate that the classic fragmentation is accompanied by hydrogen migration from aliphatic radicals to the aromatic ring in a manner analogous with dansylated amino-acids. A modified McLafferty rearrangement mechanism is proposed for this EI reaction. The metastable transitions and the deuterium-labelled compounds confirm this mechanism and the origin of the itinerant hydrogen.

Keywords: Dansylated amino-acids; Deuterium-labelled compounds; Mass spectra; McLafferty rearrangement; *N*-alkylated aromatic sulphonamides

1. Introduction

1 - Dimethylaminonaphthalene - 5 - sulphonyl (DANS) derivatives of amino-acids are widely used for the microscale identification of these compounds [1–3]. Since chromatographic procedures alone cannot give unambiguous identification, mass spectrometry of DANS-amino-acids is a necessary step for their identification.

There are few reports on the electron impact mass spectrometry (EIMS) of DANS-amino-acids [4,5] or their methyl esters [6]. From consideration of the principles of their fragmentation it was concluded that the fragmentation of these DANS derivatives under EI is very similar. The formation of the dimethylaminonaphthalene cation (*m/z* 171) is a general feature. Consequently, MS cannot distinguish with sufficient unambiguity the

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Table 1
Mass numbers (m/z) and relative intensities of molecular ions (M^+) and characteristic fragments m/z 170, 171[a] of DANS-amino-acid methyl esters

Dansylated amino-acid methyl ester	Molecular weight	M^+	m/z171	m/z170	Metastable transitions
α-Alanine	336	100	67	42	336→171
Asparagic (diester)	394	100	48	29	394→171
Glutamic (diester)	408	100	35	28	408→171
Glycine	322	100	96	58	322→171
Histidine	402	19	40	96	
Isoleucine	378	100	29	34	
Leucine	378	100	68	51	
Lysine (bis α,ε-DANS)	626	100	58	47	
Methionine	396	18	100	88	396→235,396→171
Phenylalanine	412	100	22	35	
Proline	362	95	89	100	
Serine	352	100	39	35	352→171
Threonine	366	100	63	83	
Tryptophan	451	48	16	26	
Tyrosine (*N*-DANS)	428	57	54	100	
Valine	364	100	42	56	

[a] The intensity of the peak m/z 171 was corrected for naturally occurring stable isotopes.

differences between DANS-amino-acids with the same weights.

The present paper discusses the correlation between the relative intensity of the peaks m/z 171 and m/z 170 and the chemical structures of DANS-amino-acid methyl esters with a veiw to establishing some criteria of analytical value for the identification of DANS-amino-acids. A possible rationalization of the ion m/z 171 is also suggested.

For the study of the formation of the peak m/z 171, a series of *N*-alkylated aryl sulphonamides, compounds exhibiting a strong structural analogy with DANS-amino-acid methyl esters, was used.

2. Materials and methods

L-DANS-amino-acids were obtained from Sigma Chemical Co. The DANS-amino-acid methyl esters were prepared with methanol and HCl under the usual conditions [6] and purified by thin-layer chromatography on silica gel G_3 using chloroform–methanol–acetic acid (15:14:1, v/v/v). The purified DANS-amino-acid methyl esters were extracted from the silica gel with methanol and the methanolic solution was evaporated to dryness in a gold vessel suitable for the direct inlet system of the mass spectrometer.

Aryl sulphonamides were obtained in the following way: 5 mg of the chloride of the aryl sulphonic acid was dissolved in 20 μl of anhydrous ether and 50 μl of the amine solution in ether was added. The solution was maintained at 0–4°C for 10 h and when the ether had been evaporated, 0.25 μl of water was added; this mixture was extracted twice, in each case with 10 μl of ether. After the evaporation of the combined ether solutions, the samples were dried with P_2O_5 in an exsiccator.

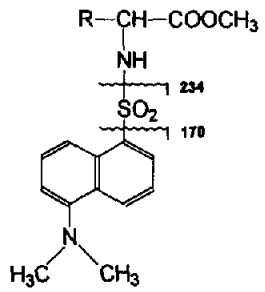

Fig. 1. The splitting of the C–S and N–S bonds in DANS-amino-acid methyl esters.

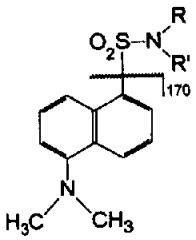

Fig. 2. The splitting of the C–S bond in deuterium-labelled DAN-sulfonamides.

Deuterium-labelled compounds of aryl sulphonamides were obtained as described above, using deuterated amines (Carlo Erba, vials of 1 ml ether solution with 10% amine and a deuteration greater than 99%).

Deuterium-labelled compounds, 2-*d*-alanine and 3,3,3-*d*-alanine, were prepared from 2,2-*d*-propionic acid and 3,3,3-*d*-propionic acid (Roth Lab. Chem.) respectively, according to Refs. [7,8]. 2-*d*-Alanine and 3,3,3-*d*-alanine were then dansylated with DANS-Cl in acetone–water solution to give DANS-2-*d*-alanine and DANS-3,3,3-*d*-alanine respectively. The corresponding methyl esters were obtained in a similar manner as unlabelled compounds. *N*-*d*-DANS-alanine methyl ester was prepared from the unlabelled compound by the action of *O*-*d*-methanol/D_2O directly in the gold vessel suitable for the direct inlet system.

The mass spectra were prepared with the CH7 mass spectrometer (Varian MAT, Bremen, Germany) at 70 eV, the temperature of the electron source was 250°C. Metastable peaks were directly recorded and the corresponding transitions were calculated.

Table 2
Mass numbers (m/z) and relative intensities of molecular ions (M^+) and characteristic fragments m/z 170, 171[a], 172[a] of DANS-sulphonamides (Fig. 3)

R	R'	I_{170}(%)	I_{171}(%)	I_{172}(%)
H	H	5	5	–
H	CH_3	20	30	–
H	C_2H_5	24	68	–
D	D	5	5	1
D	CH_3	20	30	2
H	CD_3	16	6	30
D	C_2H_5	25	70	3
H	CD_2CH_3	16	18	65
H	CH_2CD_3	29	82	4

[a] The intensities of the peaks m/z 171, 172 were corrected for naturally occurring stable isotopes.

Table 3
Mass numbers (m/z) and relative intensities of molecular ions (M^+) and characteristic fragments m/z 170, 171[a], 172[a] of deuterium-labelled DANS-alanine methyl esters

Deuterium-labelled compounds	Molecular weight	M^+	m/z 172	m/z 171	m/z 170
DANS-*N*-*d*-alanine methyl ester	337	60	2	63	41
DANS-2-*d*-alanine methyl ester	337	100	65	1	43
DANS-3,3,3-*d*-alanine methyl ester	339	100	1	61	44

[a] The intensities of the peaks m/z 171, 172 were corrected for naturally occuring stable isotopes.

3. Results and discussion

According to the data previously reported [4–6] all DANS-amino-acids methyl esters examined (Table 1) show as characteristic fragmentation the splitting of the C–S bond with or without the transposition of one hydrogen atom towards the rest of dimethylaminonaphthalene (DAN) as well as the formation of the ions m/z 171 and m/z 170 respectively (Fig. 1).

The abundance of these ions is, in all cases, over 20%; m/z 170 represents the basic peak for DANS-proline and DANS-tyrosine whereas m/z 171 is the basic peak for DANS-methionine.

The ion m/z 171 from the DANS derivatives is formed by the rearrangement of one hydrogen atom from the amino-acid towards the naphthalene nucleus. The metastable transitions $M^+ \rightarrow m/z$ 171 noticed in the case of some amino-acid methyl esters (Table 1) indicate that the transposition ions m/z 171 are formed directly from the molecular ions.

In order to establish the position of the itinerant hydrogen atom within this rearrangement, it would be advisable for the mass spectra of the deuterated *d*-amino-acid methyl esters to be con-

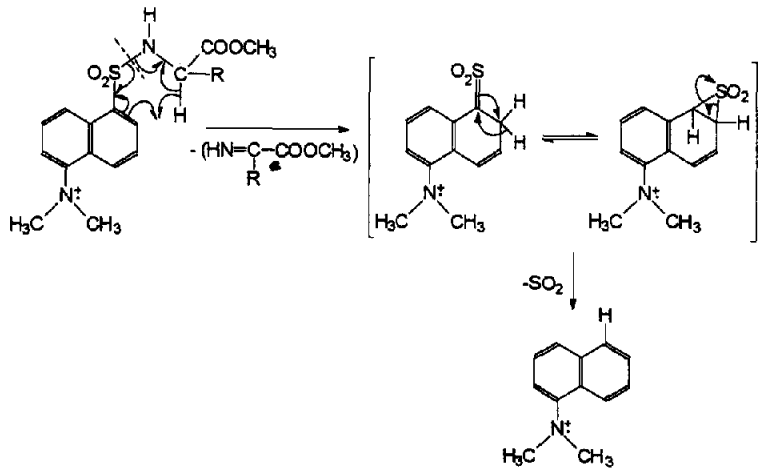

Fig. 3. The formation of the ion m/z 171 by the McLafferty rearrangement in DANS-derivatives.

veniently registered. The shortcoming of this method, which seems to be the most exact, is the laborious synthesis of the deuterate derivatives. This is why another series, more easily obtained, was chosen for preliminary study, namely *N*-alkylated sulphonamides: benzyl-sulphonamides, naphthyl-sulphonamides, and DAN-sulphonamides. The mass spectra indicate a C–S splitting with the occurrence, in all cases, of a peak characteristic to the rearrangement of one hydrogen atom. By comparing the intensities of the C–S splitting peak, it can be seen that DAN-sulphonamides present the most intense peak. Further deuterated DANS-sulphonamides (Fig. 2) in a convenient position were synthesized in order to analyze the position from where hydrogen migration proceeded. From the results it can be seen that a peak m/z 172 with as great an intensity occurs only when deuterium is linked to the carbon adjacent to the nitrogen (Table 2).

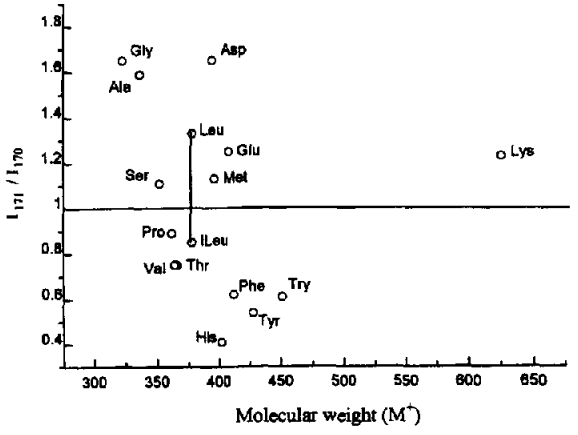

Fig. 4. The distribution of the ratio I_{171}/I_{170} in relation to the nature of DANS-amino-acid methyl esters arranged by their moleuclar weights.

To confirm that this rearrangement is also valid in the case of DANS amino-acid methyl esters, DANS-alanine methyl ester was synthesized specifically labelled with deuterium at the carbon atoms 2 and 3 or alternatively at nitrogen. The mass spectra of deuterium-labelled DANS-alanine methyl ester (Table 3) provide evidence that the intensities of the peaks m/z 171 and m/z 170 remain practically the same as in unlabelled DANS-alanine methyl ester if the deuterium atom is attached to the carbon atom 3 or nitrogen. However, the peak m/z 172 with an intensity of 2% occurs. If deuterium is linked to carbon 2, the peak m/z 171 practically disappears and the peak m/z 172 with an intensity of 61% occurs in exchange. These data indicate that the hydrogen atom rearranged from the dimethylaminonaphthalene cation comes almost exclusively from the carbon atom 2 of alanine.

The specific rearrangement of hydrogen from the carbon atom 2 with the formation of the ion m/z 171 observed in the case of DANS-alanine methyl ester is very likely to be general in the series of DANS-amino-acids. It could be rationalized as proceeding via a McLafferty cyclic mecha-

nism in two stages (Fig. 3). In the former stage the less stable ion m/z 235 is formed whereas in the latter stage this ion is stabilized by SO_2 splitting with the reconstruction of the aromatic ring. Actually, the ion m/z 235 is present in the spectra of all DANS-amino acids. Its formation directly from the molecular ion is supported in the case of DANS-methionine methyl ester by the presence of the metastable transition M(396) → 235, although other manners of formation are not excluded.

By examining the ratio of the intensities of these peaks (I_{171}/I_{170}) in the studied series it is observed that this ratio is more than one in some cases, wereas in other cases it is subunitary (Table 2). It is obvious that the nature and especially the volume of the R group can exert a great influence on the McLafferty rearrangement, a bulky R substituent preventing the hydrogen from approaching the carbon to which it is transferred. Indeed, the ratio I_{171}/I_{170} is subunitary in the case of dansylated derivatives of histidine, phenylalanine, tyrosine and tryptophan (Fig. 4), which means that the McLafferty rearrangement with the formation of molecular ion m/z 171 is made more difficult, with the predominance of C–S splitting followed by the formation of the ion m/z 170. In contrast, in the case of amino-acids with a less bulky R group such as glycine, alanine and aspartic acid, the transposition prevails as the ratio I_{171}/I_{170} is greater than unity. The influence is so great that it can differentiate leucine [$R = CH_2-CH(CH_3)_2$] from isoleucine [$R = CH(CH_3)-CH_2-CH_3$]. It is worth noting that this ratio is 1.33 for DANS-leucine and subunitary (0.85) for DANS-isoleucine isomer. If it is taken into account that the mass spectra of both isomers are very similar in terms of other characteristics, this ratio enables the identification of each isomer.

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