

## On the Trifluoroacetylation of Nitrogen in Amide Bonds

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In the mass spectrometric analysis of natural products it is often necessary to increase the volatility of a compound studied through the preparation of suitable derivatives. Lederer *et al.*<sup>1</sup> have demonstrated that methylation of peptide bonds increases the volatility of peptides by reducing hydrogen bonding.

Weygand *et al.*<sup>2</sup> showed that trifluoroacetic anhydride not only reacts with primary amino groups but also with nitrogen in an amide bond. As the work of Weygand *et al.* was carried out before mass spectrometry became available as a tool in the structure investigation of peptides, it was of interest to make a mass spectrometric study of the products obtained by trifluoroacetylation of peptide bonds.

In order to obtain information about the fragmentation of the trifluoroacetyl derivative of a single peptide bond, *N*-dodecyl-*N*-TFA-dodecanamide was studied. The mass spectrum of *N*-dodecyl-*N*-TFA-dodecan-

amide is compared with that of the unsubstituted compound in Fig. 1. The significant molecule-ion peaks are observed at *m/e* 463 and *m/e* 367, respectively. The increased volatility due to trifluoroacetylation made it possible to keep the direct inlet probe at a temperature 50°C lower than in the case of the compound without the *N*-TFA group. A characteristic peak in the fragmentation pattern of the *N*-TFA compound is the acylium ion of *m/e* 183. The corresponding peak is hardly significant in the spectrum of the unsubstituted compound, where the base peak is at *m/e* 227, which corresponds to a rearranged fragment  $\text{CH}_3 - (\text{CH}_2)_{11} - \text{NH} - \text{C} = \text{CH}_2$  (cf.



Gilpin *et al.*).<sup>3,4</sup> The long-chain nature of the unsubstituted compound is indicated by the long series of peaks of moderate intensity spaced 14 mass units apart. This type of fragmentation is suppressed in the *N*-TFA substituted compound. The characteristic peak at *m/e* 394 is due to loss of the  $\text{CF}_3$  group. The "amine" fragment of *m/e* 30 is practically absent in the *N*-TFA compound.

The *N*-TFA derivatives of the *N*-acetylated methyl esters of glycine and phenylalanine have also been studied. The *N*-acetyl methyl esters of these amino

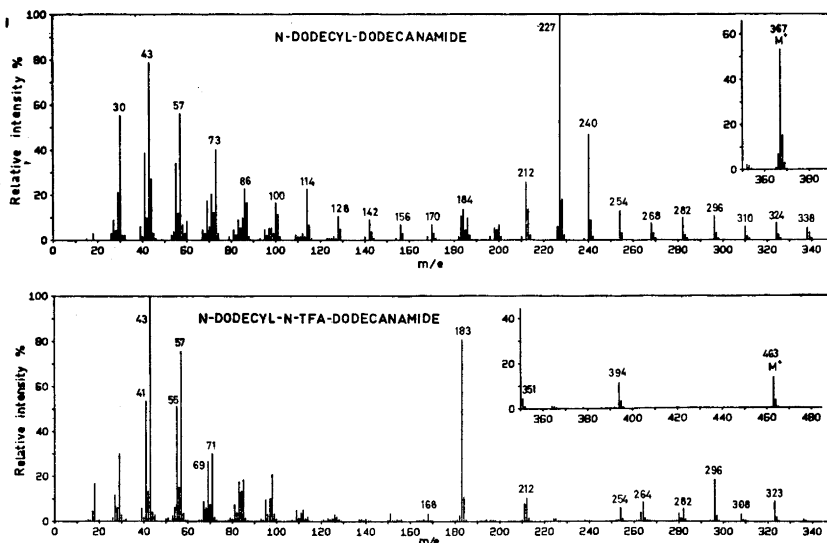


Fig. 1. Mass spectrum of (a) *N*-dodecyl-dodecanamide; (b) *N*-dodecyl-*N*-TFA-dodecanamide.

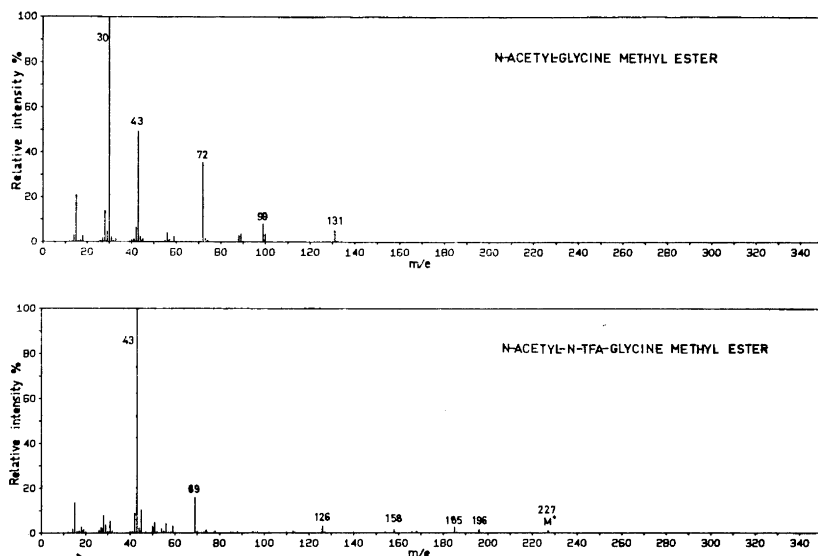


Fig. 2. Mass spectrum of (a) *N*-acetyl-glycine methyl ester; (b) *N*-acetyl-*N*-TFA-glycine methyl ester.

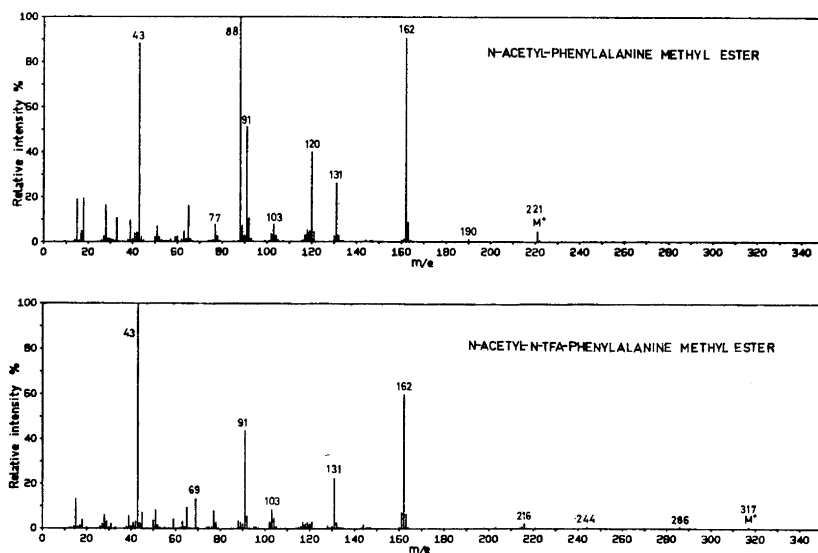


Fig. 3. Mass spectrum of (a) *N*-acetyl-phenylalanine methyl ester; (b) *N*-acetyl-*N*-TFA-phenylalanine methyl ester.

Table 1. Measured and calculated  $m/e$  values of some ions in the mass spectra of  $N$ -acyl and  $N,N$ -diacyl methyl esters of glycine and phenylalanine.

| Methyl ester of                     | $m/e$ | measured value | calculated value | empirical formula |
|-------------------------------------|-------|----------------|------------------|-------------------|
| $N$ -Acetyl-glycine                 | 88    | 88.0403        | 88.0399          | $C_3H_4NO_2$      |
| $N$ -Acetyl- $N$ -TFA-glycine       | 88    | 88.0402        | 88.0399          | $C_3H_4NO_2$      |
| $N$ -Acetyl-phenylalanine           | 88    | 88.0402        | 88.0399          | $C_3H_4NO_2$      |
| $N$ -Acetyl- $N$ -TFA-phenylalanine | 88    | 88.0405        | 88.0399          | $C_3H_4NO_2$      |
| $N$ -Acetyl-phenylalanine           | 131   | 131.0500       | 131.0497         | $C_9H_7O$         |
| $N$ -TFA-Phenylalanine              | 131   | 131.0495       | 131.0497         | $C_9H_7O$         |
| $N$ -Acetyl- $N$ -TFA-phenylalanine | 131   | 131.0493       | 131.0497         | $C_9H_7O$         |
| $N$ -Acetyl-phenylalanine           | 162   | 162.0675       | 162.0681         | $C_{10}H_{10}O_2$ |
| $N$ -TFA-Phenylalanine              | 162   | 162.0677       | 162.0681         | $C_{10}H_{10}O_2$ |
| $N$ -Acetyl- $N$ -TFA-phenylalanine | 162   | 162.0678       | 162.0681         | $C_{10}H_{10}O_2$ |

acids have previously been investigated by Andersson *et al.*<sup>5</sup> The spectra were run on an MS 902 instrument in order not to allow instrumental differences to influence comparison with the  $N$ -TFA compounds. It was found that the mass spectra tallied very well with those obtained by a single focussing 60° instrument. The mass spectra are reproduced in Figs. 2 and 3. The most characteristic feature is the complete lack of the "amine" ions at  $m/e$  30 and  $m/e$  120, respectively. A characteristic peak of  $m/e$  88 is found in most  $N$ -acetyl methyl esters of amino acids. The composition of this fragment has been found by peak-matching in all cases to correspond to  $C_3H_4NO_2$  (see Table 1). The previous suggestion that it was due to the fragment  $[H_2N - \dot{C}H - CO - OCH_3]^+$  is therefore correct. This fragment is absent in the  $N$ -TFA derivative. In both types of derivatives there are characteristic peaks due to the loss of 42 mass units (ketene). The mass spectra of  $N$ -acetyl-phenylalanine,  $N$ -acetyl- $N$ -TFA-phenylalanine, and  $N$ -TFA-phenylalanine methyl esters have dominant peaks at  $m/e$  162 and  $m/e$  131 (Table 1). The composition of these fragments is  $C_{10}H_{10}O_2$  and  $C_9H_7O$ , respectively. The previous assumption<sup>3</sup> that  $m/e$  162 was due to the loss of the methoxy-carbonyl group is therefore not correct. The 59 mass units must be made

up by the  $CH_3CONH_2$  group. This agrees with the findings of Grützmacher *et al.*<sup>6</sup> and Manhas *et al.*<sup>7</sup>

We have also tried to trifluoroacetylate the peptide bond in di- and tripeptides, but very complex mass spectra were obtained.<sup>8</sup>

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