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The Use of *N*-Succinyl Derivatives in the Study of Amino Acids and Peptides by Mass Spectrometry

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Abstract—The terminal amino group of amino acids and peptides is blocked as the *N*-succinyl derivative by reaction with succinic anhydride. The product is then converted to the *N,O*-permethyl derivative in order to increase its volatility for use in mass spectrometry. The permethylated *N*-succinyl derivative retains the advantages of the permethylated *N*-acetyl derivative in regard to ease of preparation on a small scale, volatility and the presence of characteristic fragmentation patterns in their mass spectra. However, peaks in the high mass region are more abundant due to loss of $CH_3O\cdot$ from the *N*-succinyl carbomethoxyl group as well as from the *C*-terminal carbomethoxyl group. Ions characteristic of the sequence and of individual amino acids are observed, and molecular weight can be determined from the relatively abundant ion at $[M - CH_3O]^+$ and from the weak molecular ion.

Introduction

AS PART of an investigation of streptomycin resistance and ribosomal proteins, we have studied amino acids and small peptides obtained from tryptic digests of the *Str* protein S12.[1] Composition of the peptides is determined by an amino-acid analyzer and the sequence is ascertained by means of mass spectrometry. Since it was necessary to find a volatile derivative suitable for small quantities of peptides and which would give fragments in its mass spectrum related to the sequence of the amino acids, we tried a variety of possibilities, including those reported in the literature and new ones.

In 1969, Aplin *et al.*[2] showed that the *N*-acetyl derivative provided the best combination of volatility and abundance of important ions compared with a variety of other *N*-blocking groups. Since then, many other *N*-blocking groups have been studied, such as the 5-(*N,N*-dimethylamino)naphthalenesulfonyl group,[3] various Schiff bases,[3–6] and *N*-acyl substituents which contain 3-hydroxyl groups, or which are unsaturated.[7] These derivatives are used with or without permethylation to increase the volatility.

However, examination of actual applications of mass spectrometry to the determination of sequence indicates that the *N,O*-permethyl, *N*-acetyl derivatives are preferred, due to their ease of preparation on a small scale, volatility and characteristic fragmentations in the mass spectrometer.[8–10] Our experience has shown that this derivative is indeed convenient to use, but that, unfortunately, it does not give abundant ions in the high mass region, which is particularly frustrating if one is working with small amounts of samples which might not be clean. Therefore, we searched for a derivative which would retain the advantages of the *N*-acetyl, *N,O*-methyl derivative, but would have an improved appearance in the high mass region.

Attempts have been made to increase the abundance of ions in the high mass region by using *N*-blocking groups with aromatic rings which localize the charge.[2–6] However, we tried a different approach toward the same goal, the use of a derivative which would provide an abundant *fragment* ion of high mass, without, however, complicating the spectrum.

Experimental

PREPARATION OF THE DERIVATIVE

A solution of 1.1 μmol (110 μg) of succinic anhydride in 50 μl of dimethyl sulfoxide (DMSO) was added to 1 μmol of the peptide, and the mixture was heated at 80 °C for 30 min. After the solution was cooled to room temperature, about 5 times the required quantity of the carbanion of DMSO (*c.* 1 molar), prepared as reported,[11] was added. After 1 min, the permethylation was effected by the addition of 8 times the required quantity of methyl iodide. The reaction was quenched after 1 min by adding water.[12] The aqueous solution was immediately extracted with chloroform. Then, the chloroform layer was dried over anhydrous Na_2SO_4, followed by evaporation of the solvent.

Succinylation of N^δ-dimethylpyrimidylornithine, prepared from arginine by the reported procedure,[13] was carried out in aqueous solution at pH 7–8. The mass spectrum of the permethylated derivative of this product shows a molecular ion of 43% relative intensity.

If it is not possible to determine the quantity of peptide available, an apparent excess of succinic anhydride is used. Products from excess succinic anhydride are volatile and are removed along with residual DMSO by exposure to a high vacuum before introduction of the product into the mass spectrometer.

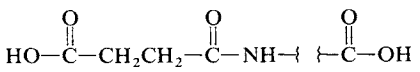
MASS SPECTRA

Mass spectra were obtained from an Hitachi Perkin–Elmer RMU-6D mass spectrometer. Ion source tem-

peratures for the spectra in the figures, taken directly from the dials, were: **2a** and **2b**, 50 °C; **3**, 110 °C; **4**, 120 °C; **8**, 185 °C.

Results and discussion

The derivative **1** which we use is related to succinamic acid. In the mass spectrum of the *N,O*-permethylated derivative of **1**, a molecular ion of low abundance is



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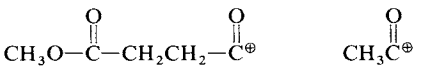
observed, followed by a relatively intense ion resulting from the loss of $CH_3O\cdot$ from the carbomethoxyl functions. An abundant ion characteristic of this *N*-blocking group is found at *m/e* 115, analogous to the peak at *m/e* 43 found in the mass spectra of *N*-acetyl

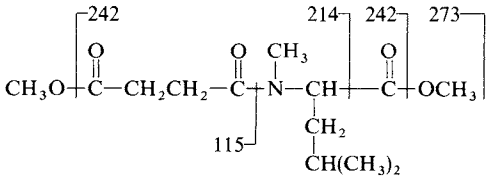


m/e 115 *m/e* 43

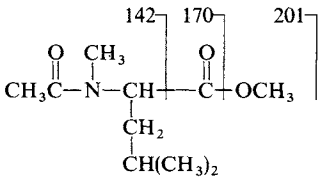
derivatives. Other fragmentations found in the mass spectra of the *N,O*-permethyl derivatives of **1** are also the same as those observed in the mass spectra of the corresponding derivatives of the *N*-acetylpeptides, which facilitates the interpretation of their spectra since the published spectra of the *N*-acetyl, *N,O*-methyl compounds can be used.

Thus, the advantages of this *N*-blocking group over the *N*-acetyl group are (1) the increased abundance of the peak at $[M - CH_3O]^+$ from which the molecular weight can be determined, along with the small molecular ion, and (2) added mass, which shifts the peaks due to the sequence to higher mass, away from the ions of lower mass characteristic of the individual amino acids making up the peptide. All the advantages of the use of the *N*-acetyl derivatives are retained, i.e. ease of preparation on a small scale, volatility and characteristic fragmentations.

To illustrate the fragmentations associated with this *N*-blocking group, the mass spectrum of the *N,O*-permethyl derivative (**2a**) of *N*-succinylleucine is given in Fig. 1(a). A small molecular ion is observed at *m/e* 273. A much more intense peak is observed at *m/e* 242, resulting from the elimination of $CH_3O\cdot$. Ions related to simple cleavages of the chain are explained in **2a**. In addition, two ions characteristic of leucine are

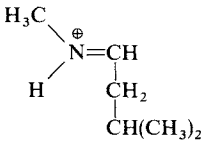


2a



2b

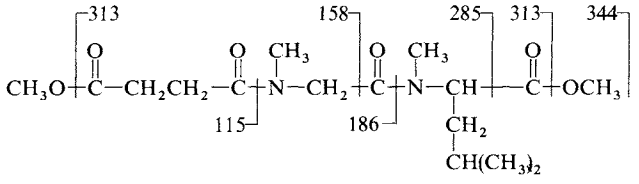
found, a small peak at *m/e* 217 (loss of C_4H_8 by a γ-hydrogen rearrangement) and the peak at *m/e* 100.



m/e 100

The same fragmentations are found in the mass spectrum, Fig. 1(b), of the *N,O*-permethyl derivative (**2b**) of *N*-acetylleucine. The peaks found at *m/e* 170 and 142 are characteristic of the sequence. The peak due to the loss of C_4H_8, *m/e* 145, and the peak at *m/e* 100 are characteristic of leucine. The major difference between the two spectra [Figs. 1(a) and 1(b)] is the much greater abundance of the peak due to the loss of $CH_3O\cdot$ found at *m/e* 242 in Fig. 1(a) and at *m/e* 170 in Fig. 1(b).

The mass spectra of the derivatives (**3** and **4**, respectively) of glycylleucine and serylglycine are given in Figs. 2 and 3. The peak at $[M - CH_3O]^+$ and the peaks of the sequence are prominent in both spectra.



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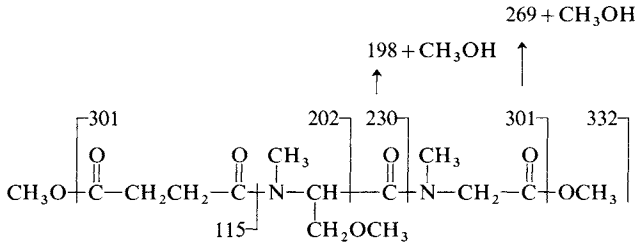

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In the spectrum of **3**, the loss of the leucyl side chain as C_4H_8 via the γ-hydrogen rearrangement can be observed in the formation of the peaks at *m/e* 288 $[M - C_4H_8]^+$ and 229 $[288 - CH_3OCO]^+$. The presence of glycine in **3** gives rise to the peak at *m/e* 44. Once again, we find that the peak at *m/e* 313 is much more abundant than the corresponding peak in the

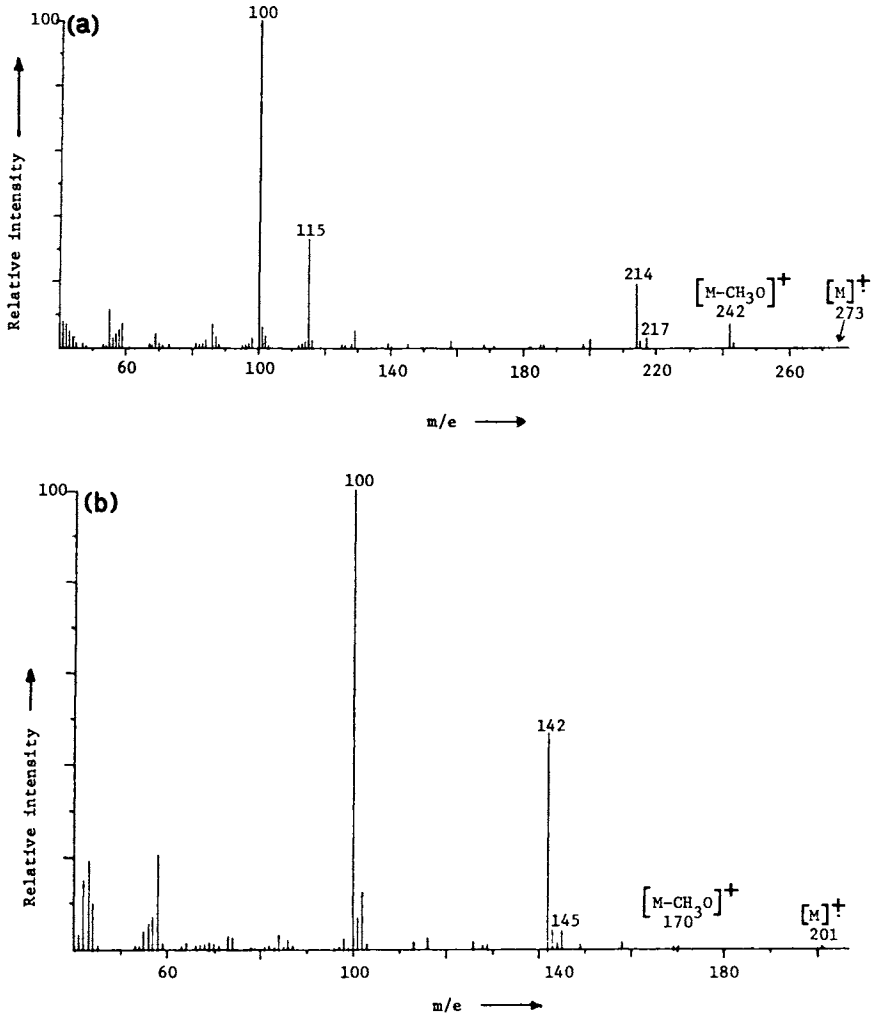


FIG. 1. (a) Mass spectrum (70 eV) of the *N,O*-methyl derivative (**2a**) of *N*-succinylleucine. (b) Mass spectrum (70 eV) of the *N,O*-methyl derivative (**2b**) of *N*-acetylleucine.

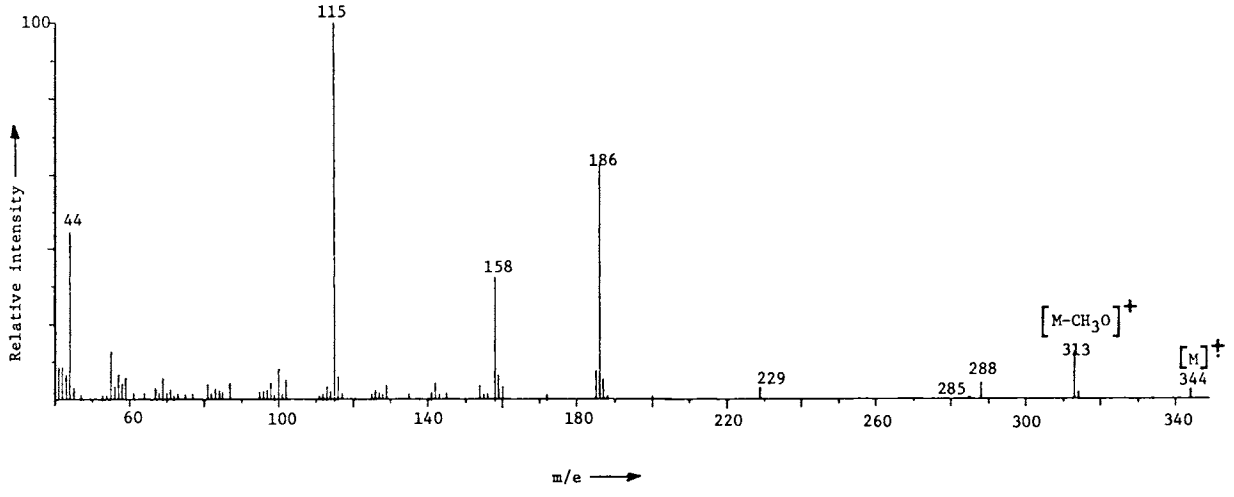


FIG. 2. Mass spectrum (70 eV) of the *N,O*-methyl derivative (**3**) of *N*-syccinylglycylleucine.

spectrum of the permethylated *N*-acetyl derivative. In the mass spectrum (Fig. 3) of **4**, the peak at $[M - CH_3O]^+$ is relatively abundant, and there is a minor peak at *m/e* 269 due to elimination of the CH_3OH from this fragment ion. The peak at *m/e* 88 is characteristic of serine; the presence of a metastable ion indicates that it can form from the ion of *m/e* 202. Again, the relative sensitivity of the method is better with this derivative than with the *N*-acetyl derivative.

In order to obtain a general idea of the relative

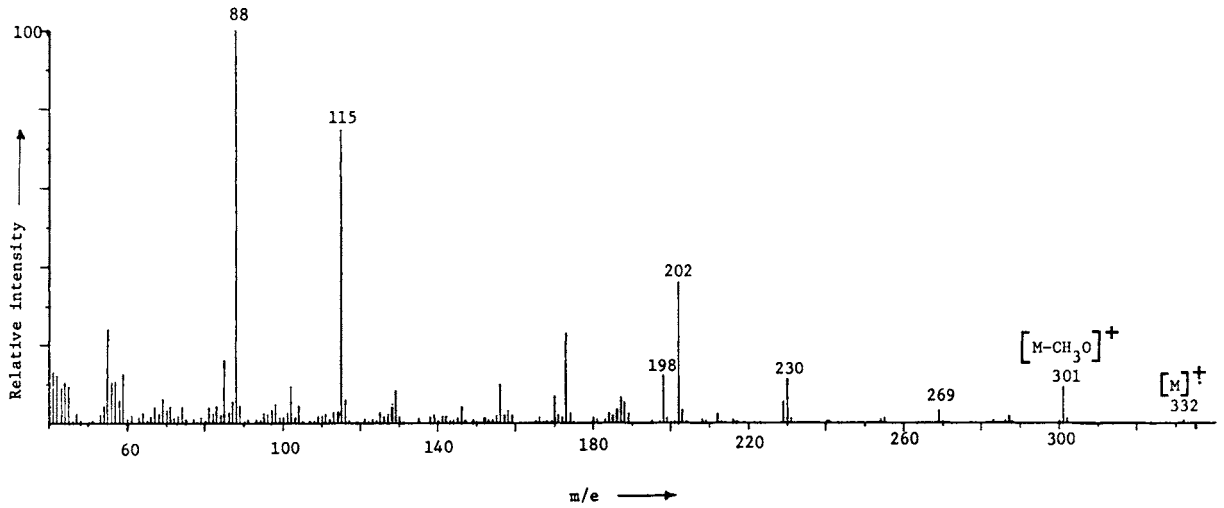
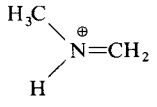
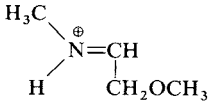


FIG. 3. Mass spectrum (70 eV) of the *N,O*-methyl derivative (**4**) of *N*-succinylserylglycine.

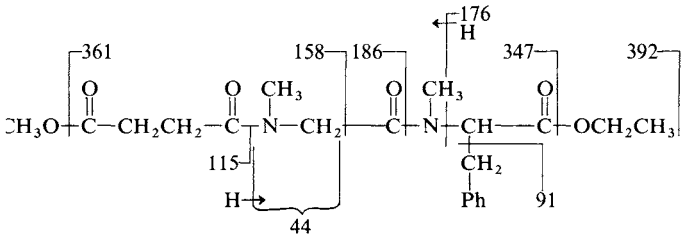


m/e 44

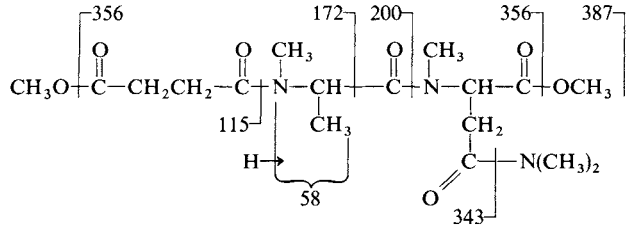


m/e 88

amounts of cleavage of the two ester bonds, we examined the mass spectrum of the derivative (**5**) of the



ethyl ester of glycylphenylalanine. The ion of *m/e* 361 is approximately twice as abundant as the ion of *m/e* 347. Thus, substantial amounts of cleavage occur from each side of the molecule. Additionally, in the mass spectrum of the derivative **6** prepared from alanyl-asparagine, $(CH_3)_2N\cdot$ is eliminated as well as $CH_3O\cdot$, in the ratio of approximately 1 : 3.



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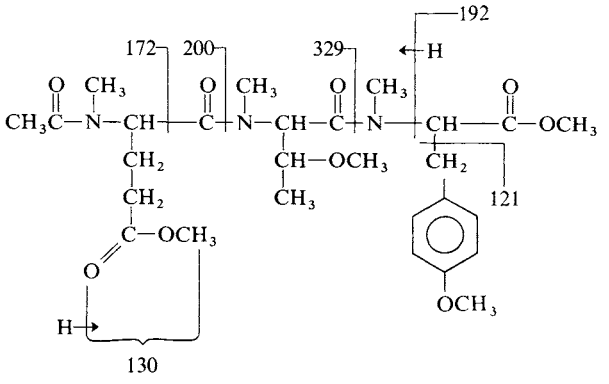
As a further example of the use of this derivative, in the mass spectrum of the *N,O*-permethyl derivative (**7**) of *N*-acetylglutamylthreonyltyrosine, a tripeptide, we found no relatively abundant ions higher than the one at *m/e* 329. Major ions are summarized in **7**. On the



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other hand, in the mass spectrum of the corresponding *N*-succinyl derivative (**8**, Fig. 4), a relatively abundant ion formed from the loss of $CH_3O\cdot$ is observed, even though the molecular ion is not present. Other ions characteristic of the sequence and of individual amino acids are abundant, as can be seen in Fig. 4 and in **8**.

The *N*-succinyl derivative is prepared from the amino acid or peptide and succinic anhydride in dimethyl sulfoxide (DMSO), Eqn (1). The *N*-succinyl derivative is then *N,O*-methylated by addition of the carbanion of DMSO directly to the solution resulting from the reaction. In the preparation of the *N*-acetyl derivative from acetic anhydride in methanol, the solvent must be removed and subsequently, DMSO is added to the residue. This manipulation is unnecessary in the preparation of **1**; the carbanion of DMSO is added directly to the solution formed in Eqn (1), without prior removal of DMSO or of excess succinic anhydride. Excess succinic anhydride, and/or products resulting

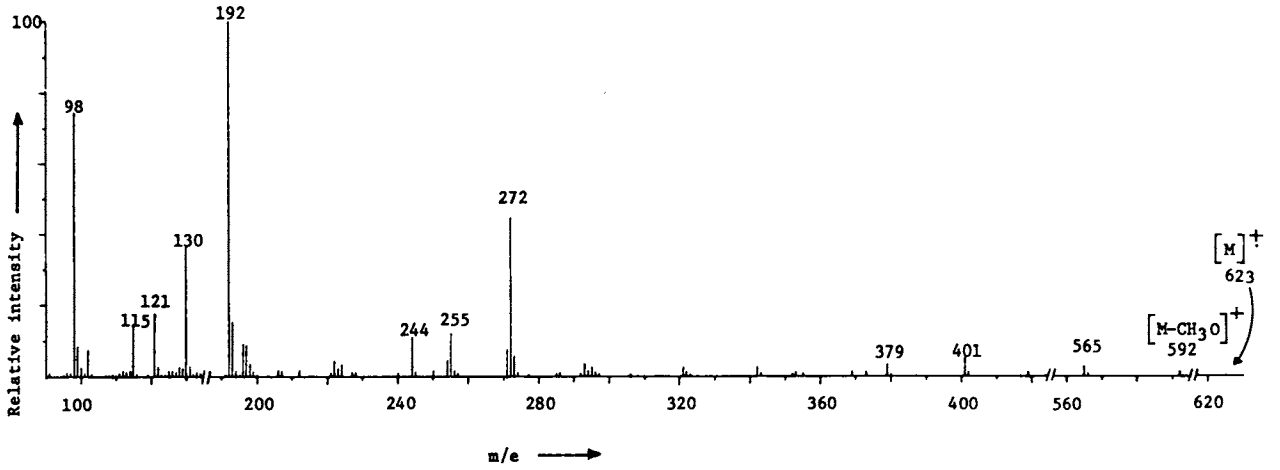
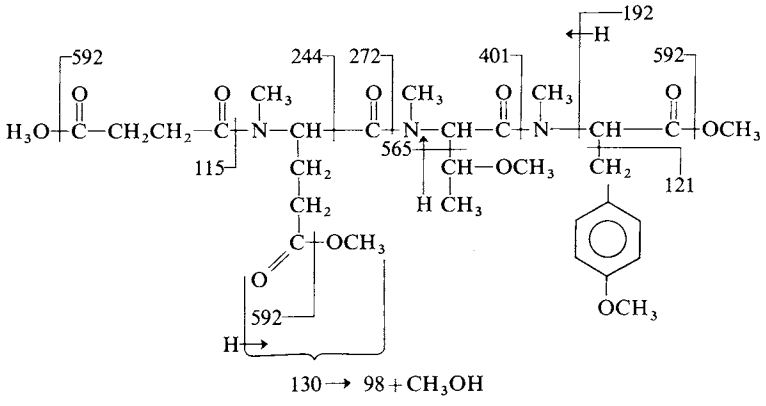
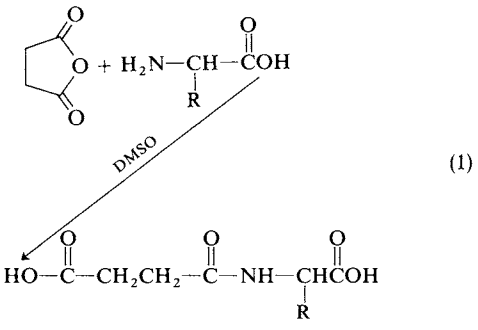


FIG. 4. Mass spectrum (70 eV) of the *N,O*-methyl derivative (**8**) of *N*-succinylglutamylthreonyltyrosine.



8



(1)

from it, are volatile and removed along with the solvent before introduction into the mass spectrometer. The fact that there is one manipulation less constitutes an advantage over the preparation of the *N*-acetyl derivative.

In the mass spectrum[14] of dimethyl succinate there is no molecular ion and the base peak is found at *m/e* 115 from the loss of $CH_3O\cdot$; this ion is stable and little fragmentation is observed from it. This leads us to propose that those ions in the mass spectra of the *N,O*-methyl derivatives of **1** which lose $CH_3O\cdot$ from the succinyl portion of the molecule are stable, giving rise to a peak at $[M - CH_3O]^+$ of enhanced relative abundance compared with the corresponding peak found in the mass spectra of the *N*-acetyl derivative. Our experience has been that this derivative is easy to work with and gives mass spectra which are readily interpreted in terms of molecular weight, sequence and identification of the constituent amino acids.

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