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AN APPROACH TO THE DIFFERENTIATION OF LEUCINE AND ISOLEUCINE RESIDUES IN EI MASS SPECTRA OF PEPTIDES(1)

R. Waern and H. Falter(2)

Department of Chemistry, Laurentian University,
Sudbury, Ontario. Canada. P3E 2C6

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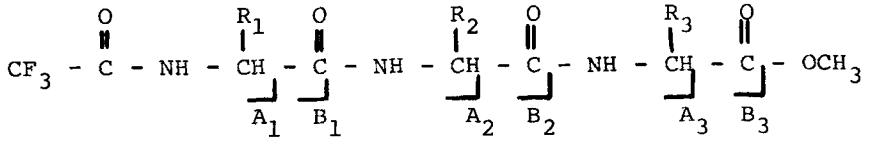
SUMMARY

Residues of leucine and isoleucine cannot generally be distinguished in the electron impact (EI) generated mass spectra of N-acylated peptide esters. We have obtained the mass spectra of model peptide esters containing leucine or isoleucine in various positions and trifluoroacetyl perdeutero leucine as the N-terminal blocking group. The mass spectra of the peptide derivatives show a pair of peaks as a result of the elimination from the M^+ ion of neutral fragment of perdeuterated isobutene (M^+-64) from the leucine side chain of the N-terminal blocking group and isobutene or butene (M^+-56) from leucine or isoleucine residues of the peptide. The ratios of the intensities of the peaks M^+-56/M^+-64 show considerable variation with the position of leucine or isoleucine in the peptide chain and the length of the peptide, but for peptides which are identical except for the fact that one contains leucine and the other isoleucine in a given position the ratio is always smaller for the isoleucine containing peptide. The differences are sufficient to distinguish the isomeric residues if comparison spectra are available.

Mass spectrometry is gaining increasing acceptance as a tool for the determination of the amino acid sequence in peptides and proteins.(3) It is well established that the amino acid sequence can be deduced from the electron impact (EI) generated mass spectra of N-acylated peptide esters largely on the basis of "sequence ions" which arise from the fragmentation of the peptide backbone in positions A and B and retention of the positive charge on the N-terminal fragment.



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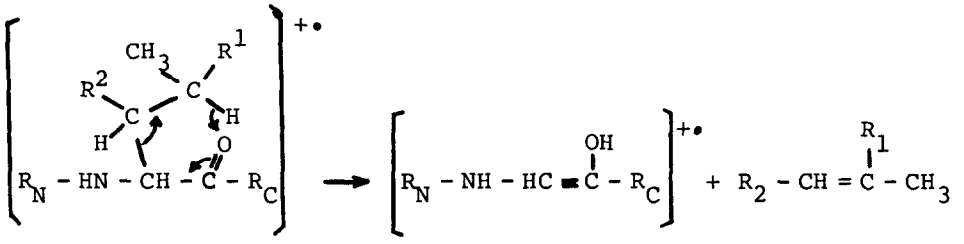
Essential for the deduction of the sequence from the mass spectrum is a fact that all the common twenty amino acids, with the exception of the isomeric amino acids leucine and isoleucine, differ from each other in the mass of the side chain R.

In order to distinguish leucine and isoleucine additional ions must be considered which arise from the fragmentation of the side chains of these amino acids. The work of Weygand et al(4) indicated that leucine and isoleucine can in some cases be distinguished on the basis the further fragmentation of ions of type A by the preferential elimination of a propyl radical from the leucine residue and a methyl or ethyl radical from an isoleucine residue. Unfortunately these ions do not occur in sufficient intensity to provide a general basis for distinguishing these isomers.

The most important fragmentation of the side chains of leucine and isoleucine involves the elimination of a neutral molecule of either isobutene or butene in what is now commonly called the McLaffery rearrangement(5).



I II

R_1 = CH_3, R_2 = H for leucine

R_1 = H, R_2 = CH_3 for isoleucine

The rearrangement occurs via a six membered transition state involving the abstraction of a tertiary carbon atom in the case of leucine and a secondary carbon atom in the case of isoleucine. Because of the greater ease with which a tertiary

hydrogen atom can be abstracted it is expected that ion (II) formed from a leucine residue would be more intense than the corresponding ion from an isoleucine residue. Similar reasoning has been used to explain the differences observed in the spectra of O-trimethylsilylated trifluoro-dideuteroethyl polyamino alcohols derived from peptides containing leucine and isoleucine, particularly when these residues occur in C-terminal positions (6). In these polyamino alcohol derivatives differences are observed in the spectra of leucine and isoleucine containing peptides which depend on the elimination of the entire side chain as a radical of mass 57, where the isoleucine side chain is more readily eliminated than the leucine side chain. The elimination of the entire side chain as a radical is generally much less important in N-acylated peptides esters than the elimination of butene or isobutene via the McLafferty rearrangement.

The nature of the N-terminal blocking group has a strong effect on the relative intensity of various sequence peaks as well as such non-sequence peaks which arise from the McLafferty rearrangement.(7) On the basis of earlier work with the model peptide valylisoleucylalanine we selected the trifluoroacetyl group for this study because this N-terminal blocking group appears to affect favorably the occurrence of the McLafferty rearrangement.

While the EI spectra of leucine and isoleucine containing peptides are generally very similar it has been shown that they can be distinguished on the basis of their metastable ion (MI) or collisional activation (CA) spectra (8). However, special techniques and equipment are required to obtain MI and CA spectra.

It occurred to us that it might be possible to distinguish leucine and isoleucine on the basis of EI mass spectra if a suitable internal standard were provided. Since peptides generally must be derivatized in order to yield interpretable mass spectra we decided to incorporate perdeuterated leucine into the N-terminal blocking group of a number of synthetic peptides containing either leucine or isoleucine.

Methods:

Perdeuterated L-leucine ([^{2}H] L-Leu) of minimum isotopic purity 98% (Merck, Sharp and Dome) was derivatized to trifluoroacetyl L-leucine (TFA [^{2}H] L-Leu) by reaction with a slight excess of trifluoroacetic anhydride in trifluoroacetic acid (9). The excess anhydride was hydrolyzed by the addition of water and the product was then lyophilized and dissolved in dioxane. TFA [^{2}H] L-Leu was coupled to various amino acid and peptide esters by the intermediate conversion to the N-hydroxy succinimide ester employing N,N-dicyclohexylcarbodiimide essentially by the method of Anderson et al(10). The insoluble dicyclohexylurea was filtered off and the peptide derivative was extracted with aliquots of ethyl ether. The ether extract was dried and transferred without further purification to a glass capillary tube used in the solid inlet system of the mass spectrometer.

Mass spectra were obtained with a Dupont 21-491 mass spectrometer with a resolution of approximately 1000 (10% valley definition). The sample vial was heated slowly and the spectra were recorded repeatedly. The temperature range over which the sample was recorded is indicated in the appropriate position. The source temperature was maintained somewhat higher than the probe temperature. All spectra were obtained with an ionization potential of 70 eV.

Table I

Relative Intensities of Selected Peaks of Mass Spectra of Peptide Derivatives

No.	Peptide Derivative	Ratio $\frac{M^+-56}{M^+-64}$ (McLafferty ratio)	Normalized Ratio*	Probe Temperature
1	TFA [^{2}H]-L-Leu-L-Leu-OCH_3	.78	100%	170-190
2	TFA [^{2}H]-L-Leu-L-Ile-OCH_3	.47	60%	150-170
3	TFA [^{2}H]-L-Leu-L-Leu-Gly-OCH_3	8.1	100%	170-190
4	TFA [^{2}H]-L-Leu-L-Ile-Gly-OCH_3	3.9	48%	150-170
5	TFA [^{2}H]-L-Leu-Gly-L-Leu-OCH_3	3.5	100%	150-190
6	TFA [^{2}H]-L-Leu-Gly-L-Ile-OCH_3	.35	10%	180-200
7	TFA [^{2}H]-L-Leu-L-Leu-Gly-Gly-OCH_3	6.3	100%	230-250
8	TFA [^{2}H]-L-Leu-L-Ile-Gly-Gly-OCH_3	.82	13%	170-220
9	TFA [^{2}H]-L-Leu-Gly-L-Leu-Gly-OCH_3	12.3	100%	210-240
10	TFA [^{2}H]-L-Leu-Gly-L-Ile-Gly-OCH_3	9.1	74%	210-240
11	TFA [^{2}H]-L-Leu-Gly-Gly-L-Leu-OCH_3	.98	100%	210-240
12	TFA [^{2}H]-L-Leu-Gly-Gly-L-Ile-OCH_3	.57	58%	190-210

* See text

Results and Discussion:

Table I shows various amino acid and peptide methyl ester derivatives for which mass spectra have been obtained. All of these derivatives contain the TFA [^{2}H] L-Leu blocking group and a residue of leucine or isoleucine in various positions in the peptide chain. As a result of the McLafferty rearrangement all of these spectra show peaks corresponding to $M^+ - 64$ from the elimination of perdeuteroisobutene from the common N-terminal blocking group and $M^+ - 56$ due to the loss of isobutene or butene from the side chain of leucine or isoleucine, respectively. The ratio of the peaks $\frac{M^+-56}{M^+-64}$ (McLafferty ratio) is recorded for

each derivative. Despite the limited number of derivatives investigated and the restricted number of amino acid residues involved some tentative conclusion may be drawn from Table 1.
1. The ratio of relative intensity of the two peaks which result from the McLafferty rearrangement (McLafferty ratio) varies with the length of the peptide and for a given length with the position of leucine or isoleucine in the peptide chain.
2. For a pair of peptide derivatives which are identical except that one contains leucine and the other isoleucine the McLafferty ratio is always lower for the isoleucine containing peptide.

The lower McLafferty ratio for isoleucine clearly indicates the greater ease with which the leucine side chain is eliminated via the McLafferty rearrangement. To emphasize this point we have set the McLafferty ratio equal to 100% for each leucine containing derivative of a given pair and expressed the isoleucine containing derivative as a fraction of this ratio. It can be seen that this normalized ratio for the isoleucine containing derivatives varies between 10% and 74% of that of the leucine containing derivatives.

For the limited number of compounds investigated the McLafferty ratio M^+-56/M^+-64 would serve to distinguish leucine and isoleucine containing peptides provided both spectra are available. It remains to be seen whether this relationship will hold for other peptide derivatives which contain the usual twenty amino acid residues. Some limitations are, however, apparent even from the restricted number compounds investigated. The total ion current resulting from the McLafferty ions decreases as the derivatives become larger which may ultimately lead to an inability to measure these at all or with sufficient

accuracy. For application in peptide sequencing generally only one peptide is available which contains either leucine or isoleucine. It would be impractical to require the corresponding peptide for a definite identification. A more practical method might rely on the use of two N-terminal blocking groups, one of which contained labelled leucine and the other labelled isoleucine or a similar substance. Studies in this direction are now in progress.

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