NEW DIDEUTERO-PERFLUOROALKYLATED OLIGOPEPTIDE DERIVATIVES FOR PROTEIN-SEQUENCING BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Summary

New volatile peptide derivatives have been prepared by reduction of N-trifluoroacetyl, N-pentafluoropropionyl and N-heptafluorobutyryl oligopeptide methyl esters by lithium aluminum deuteride and subsequent O-trimethylsilylation. The resulting O-trimethylsilylated dideutero-perfluoroalkyl polyamino alcohols are shown to be the most volatile peptide derivatives hitherto known. Their mass spectra exhibit abundant and intensity-balanced sequence-determining ions as well as M-15 ions. These properties permit the determination of the sequence of oligopeptides in the extremely complex mixtures which result from the hydrolysis of polypeptides or proteins (as little as 1 nanomole of a particular peptide can be detected).

Mass spectrometry has proven to be a valuable alternative to the Protein Sequenator of Edman and Begg (1) for the determination of the amino acid sequence of polypeptides (for reviews see ref. 2, 3). A number of derivatization procedures have been developed to make peptides sufficiently volatile for analysis by mass spectrometry. The permethylation technique (4) has been the method of choice if a single peptide (5) or a very simple mixture of peptides was to be sequenced (6-8).

To sequence oligopeptides in more complex mixtures such as those derived by acid or enzymatic hydrolysis of polypeptides, more volatile derivatives have been prepared which can be separated by gas chromatography and thus permit complete mass spectrometric characterization of complex mixtures in a single experiment. The N-TFA (9) and PFP-oligopeptide methyl esters (10) had been used for this purpose, but we found that the O-TMS polyamino alcohols obtained by $LiAlD_4$-reduction and trimethylsilylation of N-Ac-oligopeptide methyl esters

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Abbreviations used: Ac, acetyl; TFA, trifluoroacetyl; PFP, pentafluoropropionyl; HFB, heptafluorobutyryl; TMSDEA, trimethylsilyl diethylamine; py, pyridine; GC-MS, gas chromatography-mass spectrometry.

are more volatile and have easily interpretable mass spectra (11-13).

We now report the preparation of even more volatile derivatives V, VI, VII in Scheme I by $LiAlD_4$-reduction and O-trimethylsilylation of the corresponding perfluoroacylated oligopeptide methyl esters (II, III, IV). Their gas chromatographic and mass spectrometric properties are discussed and compared to those of previously reported derivatives. All naturally occurring amino acid residues, including Arg, His, Trp, Gln, and Asn, can be derivatized by this technique without modification.

Materials and Methods

Methyl trifluoroacetate (Pfaltz and Bauer), PFP-anhydride (Pierce Chemical Co.), dimethoxyethane and pyridine (both "Distilled in Glass," Burdick and Jackson Lab.) were distilled before use. HFB-anhydride (Sequanal Grade) and TMSDEA (both from Pierce Chemical Co.) were used without further purification. The $LiAlD_4$ was obtained from Alpha-Ventron, the oligopeptides used from Cyclo Chemical, Sigma Chemical Co. and Miles Lab.

Esterification of the peptide mixtures was performed with 1 ml methanolic HCl for 45 min or, if cleavage of labile peptide bonds was to be avoided, with an ethereal solution of diazomethane. After evaporation of the reagents, the resulting oligopeptide methyl esters (I) were transformed into their TFA-derivatives by methyl trifluoroacetate (9) or into their PFP-(10) or HFB-(14) derivatives by PFP- or HFB-anhydride, respectively.

After completion of the acylation, the particular reaction mixture was transferred into a 3 ml capacity bulb blown from 30 cm of 9 mm o.d. pyrex tubing. The reagents were evaporated and 2 ml of a 1N $LiAlD_4$-solution in dimethoxyethane was added (ice cooling). The bulb was sealed, sonicated for several minutes (ice cooling) and then slowly heated under stirring to 90° and kept at that temperature for 24 hr. The contents of

the bulb were then transferred to a 50 ml flask, the excess of $LiAlD_4$ was quenched by the slow addition of methanol (ice cooling) and diluted with the same solvent to a volume of 15 ml. The aluminum salts were precipitated by the slow addition of 10 drops of water (vigorous stirring). After filtration, the residue was twice extracted with 10 ml hot methanol (sonication). The combined extracts were evaporated and the dry residue was three times extracted with 10 ml chloroform under extensive sonication. The extracts were evaporated and the dry residue was silylated by a mixture of 400 µl pyridine and 200 µl TMSDEA (55°, 1 hr.).

The reagents were evaporated, the residue was dissolved in 100 µl benzene and an aliquot of this solution (2-10 µl) was coinjected with three hydrocarbon standards into the gas chromatograph (Perkin-Elmer 990) which was directly coupled (by a fitted glass separator) to a low resolution mass spectrometer (Hitashi Perkin-Elmer RMU-6L). Glass columns (3') or stainless steel columns (5') filled with 3% or 10% OV-17 on Gas Chrom Q (Applied Science Lab.) were used in all experiments. The initial temperature was 80°; a linear temperature programming rate of 12°/min was used up to a final temperature of 330°. An IBM 1800 computer was used for the acquisition of the mass spectral data (15) and for the assignment of retention indices through automatic location of the coinjected hydrocarbon standards (16).

Results and Discussion

The derivatization procedures leading to the new dideutero-perfluoroalkylated peptide derivatives are outlined in Scheme I. The polyamino alcohols obtained by reduction of compounds of type II, III and IV are easily and efficiently isolated due to their perfluoroalkyl groups. We have found that as little as 1 nanomole of di-, tri- and tetrapeptides can be identified, even if they coelute with other components of the mixture. Thus, if 0.5 µmole of a polypeptide is hydrolyzed, oligopeptides generated during hydrolysis with only 0.2% yield can be identified.

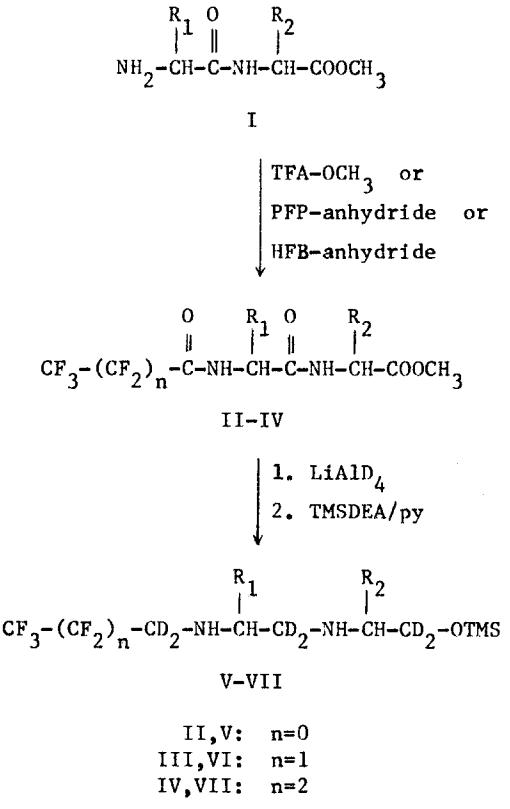

Figure 1 shows a comparison of the mass spectrum of the O-TMS polyamino alcohol of Ala-Ala derived from the Ac-dipeptide methyl ester (11, 13) with the mass spectra of the perfluoroalkylated O-TMS polyamino alcohols (type V, VI, VII); the structure of each Ala-Ala-derivative is shown in the upper right corner of the corresponding spectrum. The spectrum in Figure 1a is dominated, as is the case with most dipeptide derivatives of this type (11-13), by the A_1 ion indicating the N-terminal residue; the Z_1 ion (C-terminal residue) as well as the A_2 ion (N-terminal dipeptide) and the M-15 ion are of low intensity. In the mass spectrum of the trifluoromethylated polyamino alcohol (Figure 1b), the relative intensity of the A_1 ion is reduced by the electron-withdrawing CF_3-CD_2-group attached to the amino-terminal nitrogen, but this fragment still remains easily recognized and characteristic (and is substantiated by the A_1 +16 ion, m/e 144). As a consequence of the electron-withdrawing CF_3-CD_2-group,

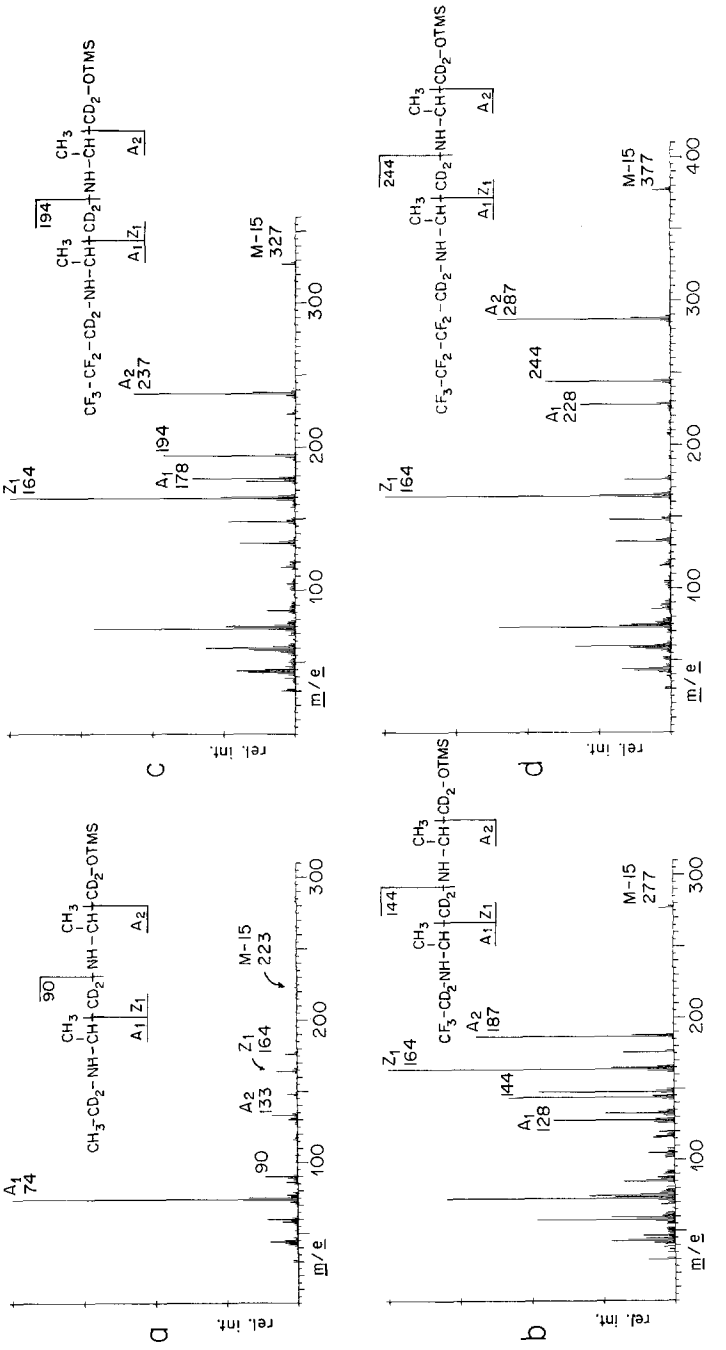

Figure 1 Mass spectra of derivatives of Ala-Ala, obtained by $LiAlD_4$-reduction and O-trimethylsilylation of the Ac-(a), TFA-(b), PFP-(c) and HFB-(d) dipeptide methyl esters.

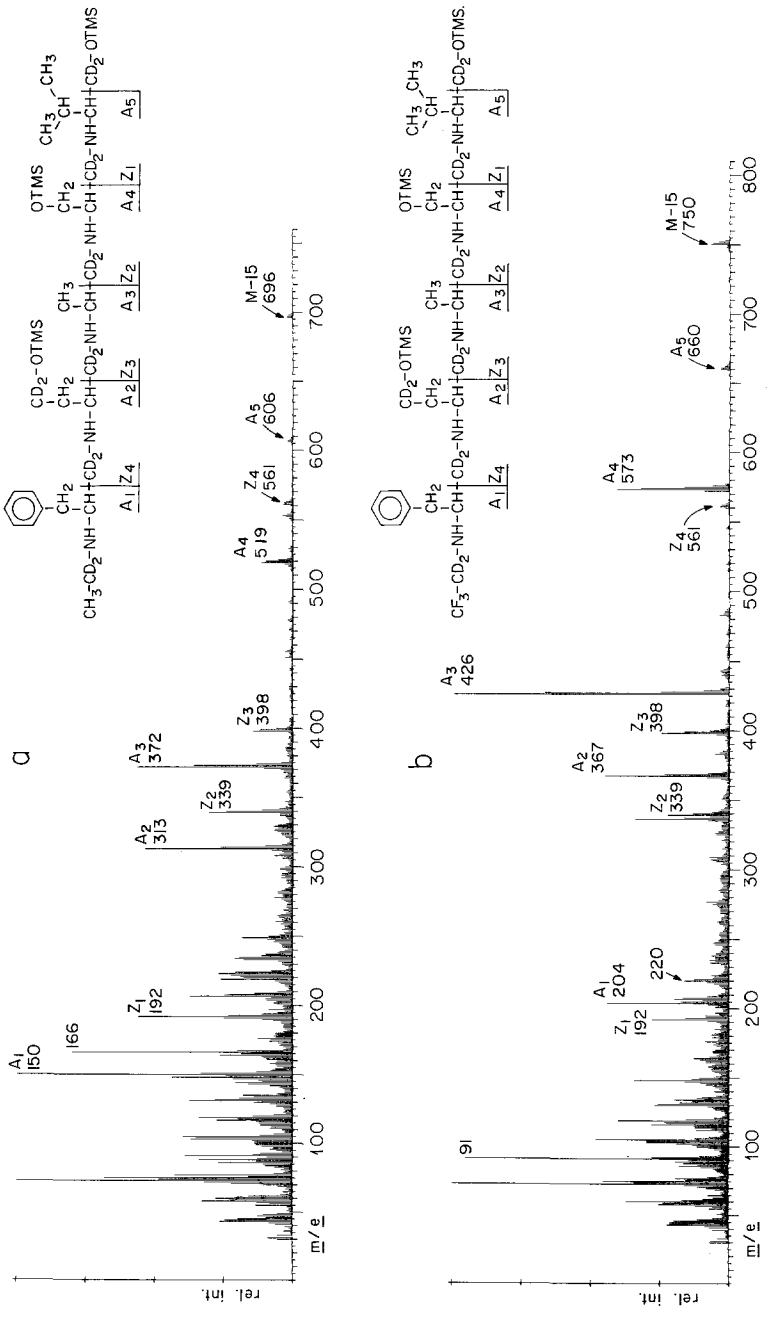

Figure 2 Mass spectra of derivatives of Phe-Asp-Ala-Ser-Val obtained by $LiAlD_4$-reduction and O-trimethylsilylation of the Ac-(a) and TFA-(b) pentapeptide methyl esters.

the relative abundance of the other sequence-determining ions are much increased (including that of the M-15 ion) and a mass spectrum with balanced intensities of all sequence ions is obtained. The same fragmentation pattern appears for the derivatives with a CF_3-CF_2-CD_2-(Figure 1c) and CF_3-CF_2-CF_2-CD_2-(Figure 1d) group on the N-terminal nitrogen. While the ions derived from the C-terminal portion of the molecule remain the same, those derived from the N-terminus are shifted 50 AMU toward higher mass for each added CF_2 group.

Figure 2 shows a comparison of the mass spectrum of a pentapeptide derivative obtained by reduction and trimethylsilylation of the Ac-peptide methyl ester (11-13) with that of the derivative of type V of the same pentapeptide. Both spectra contain intense sequence ions; in the mass spectrum of the latter, however, the relative higher abundance of the A_3, A_4 and M-15 ions (and the lower abundance of the A_1 ion) is apparent.

An important characteristic of peptide derivatives which are to be amenable to gas chromatography is their volatility, in particular the volatility of larger peptides (penta- and hexapeptides) and of those containing more complex and polar residues (Trp, Arg, His). We have already noted (13) that O-TMS polyamino alcohols are considerably more volatile than compounds of type II or the permethylated peptides (17). The new derivatives described, however, are even more volatile. The data in Table I show that the major effect is the significant decrease of the retention indices of the more complex derivatives; the retention indices of the smaller derivatives are not significantly decreased which again is desirable.

We have determined the retention indices of a large number of the fluorinated O-TMS polyamino alcohols of type V, VI and VII and found that their retention indices may be related to their structure. A detailed discussion of the predictability of the gas chromatographic retention behavior of these compounds - the number of residues in the molecule, their relative position and the size of the molecule are important parameters - will be the subject of a future paper. The simplicity of the mass spectra of these compounds together with the

Table I

Retention Indices (OV-17) of Peptide Derivatives
(indicated by their substituents on the terminal amino group;
the Roman numerals denote the structures
of the derivatives shown in Scheme I).

Peptide	Derivative			
		V	VI	VII
	CH_3-CD_2-	CF_3-CD_2-	CF_3-CF_2-CD_2-	CF_3-CF_2-CF_2-CD_2-
Ala-Ala	1330	1305	1285	1284
Asp-Phe	2313	2207	2138	2127
Tyr-Lys	2870	2635	2515	2503
Phe-Gly-Gly-Phe	3480	3309	3283	3185
Phe-Asp-Ala-Ser-Val	3508	3362	3304	3230

predictability of their retention indices are of advantage in the computer assignment of peptide sequences.

Thus, because of their highest volatility, the compounds of type VII, obtained by reduction and trimethylsilylation of HFB-peptide methyl esters, are now the peptide derivatives of choice. Derivatives of type V may be preferred if a compromise between volatility and reduced mass range is desired.

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