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Note

Separation of sequence isomeric dipeptides by high-resolution gas chromatography

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Polypeptide sequencing with dipeptidyl peptidases[1] is an excellent alternative to other methods using variations of the Edman degradation[2]. For this purpose, polypeptides are digested by dipeptidyl aminopeptidases or dipeptidyl carboxypeptidases into dipeptides[1]. Identification of all the dipeptides in the mixtures is required to determine the polypeptide primary structure. Separation and identification of dipeptides from dipeptidyl peptidase digestions are carried out by gas chromatography–mass spectrometry (GC–MS) after trimethylsilylation[1]. Underivatized dipeptides can also be separated by high-performance liquid chromatography[3].

Recently, we demonstrated the use of fused silica silica capillary columns for separation of trimethylsilyl (TMS) derivatives of dipeptides[4], as well as of dipeptide diastereomers[5]. In this paper, the separation of sequence isomeric dipeptides as their TMS derivatives by GC on a fused silica capillary column is reported.

EXPERIMENTAL*

Apparatus and materials were as previously described[5]. Dipeptides (all in L,L form) were purchased from Sigma (St. Louis, MO, U.S.A.). Separations were obtained on a fused silica capillary column (12 m × 0.2 mm I.D.) coated (WCOT) with SE-54 (5% phenyl-, 1% vinylmethyl silicone; siloxane deactivated; film thickness, 0.33 μm) (Hewlett-Packard, Avondale, PA, U.S.A.). The measured efficiency of the column was *ca.* 5200 theoretical plates per meter based on the pentadecane peak at 120°C (capacity factor, 6.43; linear volocity, 40 cm/sec). Helium was used as the carrier gas at an inlet pressure of 100 kPa. The split ratio was 20:1.

Trimethylsilylation of dipeptides was carried out according to the procedure given in ref. 5.

Mass spectra were taken at 70 eV using a Hewlett-Packard Model 5870A mass selective detector interfaced to a Hewlett-Packard Model 5880A gas chromatograph.

* Certain commercial equipment, instruments, or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

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RESULTS AND DISCUSSION

Fig. 1 shows the separation of sixteen pairs of sequence isomeric dipeptides after trimethylsilylation. Peak identification is given in Table I. Peaks not numbered in Fig. 1 correspond to some impurities in those dipeptides. The separated compounds were also analyzed by GC–MS to ensure their authenticity. Mass spectra were interpreted on the basis of previously published data[1].

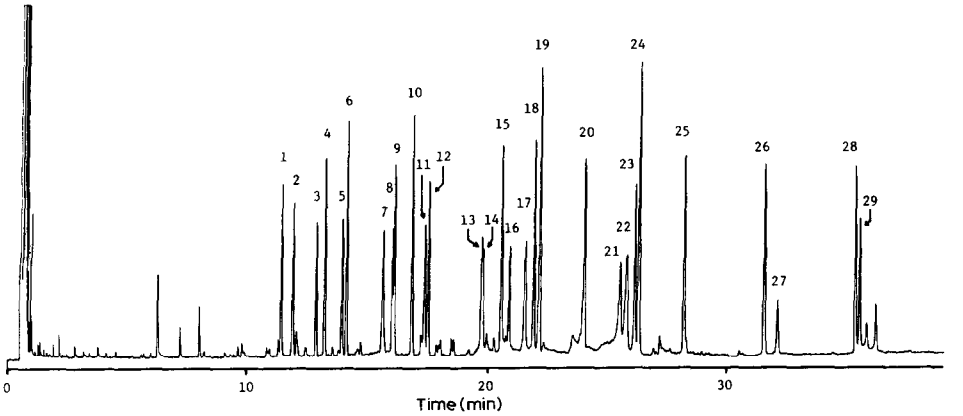

Fig. 1. Separation of TMS derivatives of some sequence isomeric dipeptides by high-resolution gas chromatography. Column, fused-silica capillary coated with SE-54, programmed at 4°C/min from 100 to 250°C. For other column details see Experimental. Peak identification is given in Table I. Identification was achieved by comparison of retention times of individually injected compounds and by GC–MS. Each peak corresponds to approximately 1 to 2 pmol of dipeptide.

TABLE I

IDENTIFICATION OF PEAKS IN FIG. 1

Peak No.	*Dipeptide*	*Peak No.*	*Dipeptide*
1	Ala–Leu	17	Pro–Met
2	Leu–Ala		Met–Pro
3	Ser–Ala	18	Phe–Val
4	Ala–Ser	19	Val–Phe
5	Val–Leu	20	Pro–Phe
6	Leu–Val		Phe–Pro
7	Pro–Leu	21	His–Ala
8	Ser–Leu	22	Ala–His
9	Leu–Pro	23	Ala–Tyr
10	Leu–Ser	24	Tyr–Ala
11	Ala–Met	25	Tyr–Val
12	Met–Ala		Val–Tyr
13	Ala–Phe	26	Ala–Trp
14	Phe–Ala	27	Trp–Ala
15	Met–Leu	28	Phe–Tyr
16	Leu–Met	29	Tyr–Phe

Sharp and symmetrical peaks were obtained for all dipeptides examined, except for those containing histidine. His–Ala (peak 21 in Fig. 1) and Ala–His (peak 22) gave broad leading peaks, although well resolved from each other. Of the sequence isomeric dipeptides examined here, three pairs, *i.e.* Pro–Met; Met-Pro (peak 17), Pro–Phe; Phe–Pro (peak 20) and Tyr–Val; Val–Tyr (peak 25) were not separable from each other. The other pairs were completely separated with the exception of Ala–Phe (peak 13) and Phe–Ala (peak 14), which were slightly resolved.

In conclusion, sequence isomers of dipeptides were shown to be separable by high-resolution GC as their TMS derivatives. This might be very important in sequencing of polypeptides using the dipeptidyl peptidase–GC–MS method[1].

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