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Note

Gas chromatographic separation of enantiomers of some dipeptides on an optically active stationary phase

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The advantage of gas chromatographic (GC) separation of diastereomeric dipeptides has been demonstrated by Weygand *et al.*[1] in their study on racemization occurring in peptide syntheses. Halpern and Westley[2] subsequently resolved many amino acids as N-trifluoroacetyl (TFA)-L-prolyl peptide methyl esters. Westley *et al.*[3] have also resolved a series of cyclic dipeptides (diketopiperazines) for the determination of optical purity and absolute configuration of diastereoisomers.

As the GC separation in these studies was achieved with ordinary optically inactive stationary phases, the enantiomers of dipeptides were not resolved. Therefore it was necessary to hydrolyse the dipeptides and determine the configuration of the resulting amino acids in order to distinguish DL from LD or DD from LL diastereoisomers.

In this paper we report the direct GC separation of enantiomers of some linear and cyclic diastereomeric dipeptides with an optically active stationary phase.

EXPERIMENTAL

The dipeptides alanylalanine (I), valylvaline (II), prolylalanine (III) and prolylvaline (IV), were prepared from the N-TFA- or N-pentafluoropropionyl (PFP)-amino acid chlorides by treatment with the corresponding amino acid esters. Cyclic alanylalanine (V) was kindly provided by Dr. Y. Yamamoto of Kyoto University, Kyoto, Japan.

GC was carried out with a Shimadzu GC-7A gas chromatograph equipped with a flame ionization detector. Chromatographic conditions used for the separation of optical isomers are summarized in Table I. A thermostable optically active stationary phase, N,N'-[2,4-(6-ethoxy-1,3,5-triazine)diyl]bis(L-valyl-L-valyl-L-valine isopropyl ester) (OA-300) was prepared as described previously[4].

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TABLE I

GAS CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS OF DIPEPTIDES*

	Dipeptide	*Column temp. (°C)*	*Retention time** (min)*		*Separation factor, α (DL/LD)*	*Retention time** (min)*		*Separation factor, α (LL/DD)*
			LD	*DL*		*DD*	*LL*	
I	DL-Alanyl-DL-alanine***	180	37.1	38.5	1.038	39.7	45.4	1.144
II	DL-Valyl-DL-valine§	180	34.6	37.2	1.075	37.2	38.6	1.038
III	DL-Prolyl-DL-alanine***	180	34.2	32.8	0.959	43.4	50.1	1.154
IV	DL-Prolyl-DL-valine***	180	41.1	39.5	0.961	49.3	55.9	1.134
V	Cyclo-DL-alanyl-DL-alanine	185	183.4		—	166.0	170.8	1.029

* Chromatographed on a glass capillary column (40 m × 0.25 mm I.D.) coated with OA-300; carrier gas, helium at a flow-rate of 0.5–0.7 ml/min.

** Time from solvent peak.

*** Resolved in the form of N-TFA-isopropyl ester.

§ Resolved in the form of N-PFP-isopropyl ester.

RESULTS AND DISCUSSION

The results of the GC separation of optical isomers of dipeptides are given in Table I. The LD, DL, DD and LL isomers of I, III and V were resolved as their N-TFA-dipeptide isopropyl esters. A typical chromatogram is shown in Fig. 1. The peaks of DL and DD isomers of II were superimposed under these conditions. It is notable that the LD and DL, and DD and LL isomers which have not been separated on ordinary optically inactive stationary phases, are resolved with good separation factors (1.038–1.154).

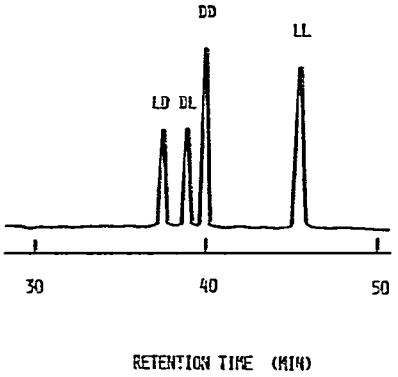

Fig. 1. Gas chromatogram of N-TFA-DL-alanyl-DL-alanine isopropyl ester. Conditions: see Table I.

Peak identifications were made by chromatographing successively racemic and some synthetic mixtures of enantiomers of dipeptides with known configuration; DD isomers eluted prior to LL isomers in every instance. This indicates that there is a greater interaction between LL isomers and the optically active stationary phase with

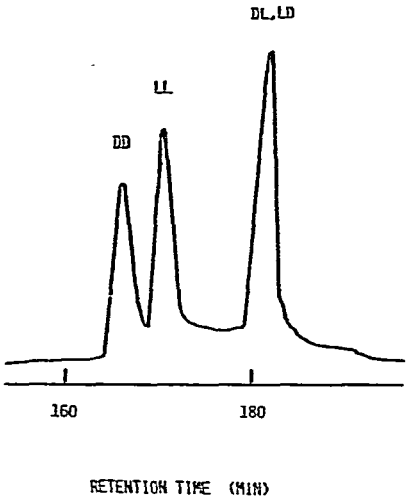

Fig. 2. Gas chromatogram of cyclo-DL-alanyl-DL-alanine. Conditions: see Table I.

an L-amino acid moiety than is the case for DD isomers. These findings are supported by the fact that D-isomers of both N-acylamino acid esters and amides have shorter retention times than L isomers on the same chiral stationary phase, OA-300.

The order of elution of the four isomers of I and II was LD > DL > DD > LL, and for III and IV was DL > LD > DD > LL. It is interesting that the elution sequence of the LD and DL isomers is reversed. It is considered that the alanine ester part of I, the valine ester part of II, and the proline amide part of III and IV make a greater contribution to the separation, and the enantiomers with the L configuration in these moieties are more retained in the column. This view is compatible with the findings that the separation factor of N-TFA-alanine amide is smaller than that of N-TFA-alanine ester, and that of N-TFA-proline amide is much larger than those of N-TFA-alanine and valine esters[5].

Cyclic alanylalanine (2,5-dimethyldiketopiperazine) was resolved into three peaks (Fig. 2). As the DL- and LD-isomers are identical in this dipeptide, their peaks are superimposed, and the ratio of the three peak areas is 1:1:2. The DD isomer eluted again before the LL isomer.

In conclusion, we have succeeded in the direct resolution of the enantiomers of some linear and cyclic dipeptides by use of an optically active stationary phase. This technique is suitable for the determination of optical purity and for the configurational assignment of dipeptides.

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