CHROM. 5760

Gas–liquid chromatography of 2,5-diketopiperazines as their trifluoroacetyl derivatives*

In connection with a study of the optical rotatory dispersion of 2,5-diketopiperazines (DKP's) it was necessary to check these compounds for racemization. Thin-layer chromatography has been used for this purpose[1,2] and gas–liquid chromatography (GLC) has also been applied to separate diastereoisomeric DKP's as the free compounds[2,3] and as the trimethylsilyl (TMS) derivatives. In this laboratory inconsistent results were obtained with these GLC methods which also did not resolve all of the *cis*- and *trans*-DKP's examined[2,3]. Accordingly a number of DKP's were converted to their trifluoroacetyl (TFA) derivatives and subjected to GLC on a packed silicone-coated column.

Apparatus

A Varian Aerograph Series 1520 gas chromatograph equipped with FID and a Servo/RiterII recorder (Texas Instruments, Inc.) was used. The glass column (5 ft. × 3 mm I.D.) was packed with 5 % SE-30 Ultraphase on Chromosorb W AW-DMCS, 60/80 mesh and was conditioned at 275° overnight with no gas flow. The inlet and detector were maintained at 250° and helium at 25 ml/min was used as carrier gas. A glass insert was used between the injector and the column.

Materials

The DKP's were prepared by hydrogenolysis of the corresponding benzyloxycarbonyl dipeptide methyl esters[4] or by boiling the formic acid salts of the dipeptide methyl esters in *sec.*-butanol–toluene (4:1) (ref. 1).

The TFA derivatives were prepared by heating 1–2 mg of the DKP's in 0.2 ml trifluoroacetic anhydride in a Reacti-Vial (Pierce Chemical Co.) at 90–100° for 5–30 min depending upon solubility.

The *trans-c*-ile-ile was prepared by racemization[3] of *c*-L-ile-L-ile.

Results

The DKP's examined, peak retention times and column temperatures are shown in Table I. The operating temperature of the column was adjusted to give sharp peaks and to achieve resolution of the diastereoisomeric pairs. The column temperature was kept constant where possible in order to determine the change of retention time with change in molecular weight and molecular geometry. Although baseline separation was not achieved for all of the *cis*- and *trans*-DKP's, resolution into distinct peaks was possible and is typified by *c*-ala-val and *c*-ser-phe (Fig. 1a and b), respectively.

Discussion

Treatment of DKP's with trifluoroacetic anhydride and chromatography on SE-30 Ultraphase permits the resolution of the diastereoisomeric pairs listed in Table I.

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TABLE I

COLUMN TEMPERATURES AND RETENTION TIMES OF TRIFLUORACETYL DIKETOPIPERAZINES

DKP	*T* (°C)	*t* (*min*)	*DKP*	*T* (°C)	*t* (*min*)
c-L-ala-L-ala	90	11.3	*c*-L-ala-L-phe	150 (140)	11.3 (19.0)
c-L-ala-D-ala	90	9.7	*c*-L-ala-D-phe	150 (140)	8.5 (13.2)
c-L-ala-L-val	100	9.8	*c*-L-val-L-phe	152	15.4
c-D-ala-L-val	100	9.2	*c*-L-val-D-phe	152	13.8
c-L-ala-L-ile	100	13.0	*c*-L-ile-L-phe	161	14.8
c-D-ala-L-ile	100	12.3	*c*-L-ile-D-phe	161	11.9
c-L-ala-L-leu	100	17.1	*c*-L-leu-L-phe	170	8.8
c-D-ala-L-leu	100	17.9	*c*-L-leu-D-phe	170	7.9
c-L-val-L-val	111	13.4	*c*-L-ala-L-tyr	155 (140)	14.3 (29.5)
c-L-val-D-val	111	14.7	*c*-L-ala-D-tyr	155 (140)	10.2 (19.3)
c-L-val-L-ile	130	8.5	*c*-L-ala-L-trp	182	14.2
c-D-val-L-ile	130	7.8	*c*-L-ala-D-trp	182	11.1
c-L-val-L-leu	130	9.4	*c*-L-phe-L-phe	180	8.8
c-D-val-L-leu	130	10.6	*c*-L-phe-D-phe	180	7.9
c-L-leu-L-leu	130	10.9			
c-L-leu-D-leu	130	12.7			
c-L-ile-L-ile	130	12.8	*c*-D-ala-D-phegly	150	7.7
c-L-ile-D-ile	130	14.2	*c*-L-ala-D-phegly	150	7.4
c-L-ile-L-leu	130	12.0	*c*-L-ser-L-phe	140	12.3
c-L-ile-D-leu	130	13.2	*c*-L-ser-D-phe	140	12.3, 18.2
			c-L-ser-L-phe[a]	140	12.7
			c-L-ser-D-phe[a]	140	18.5

[a] On-column injection, injector heater off.

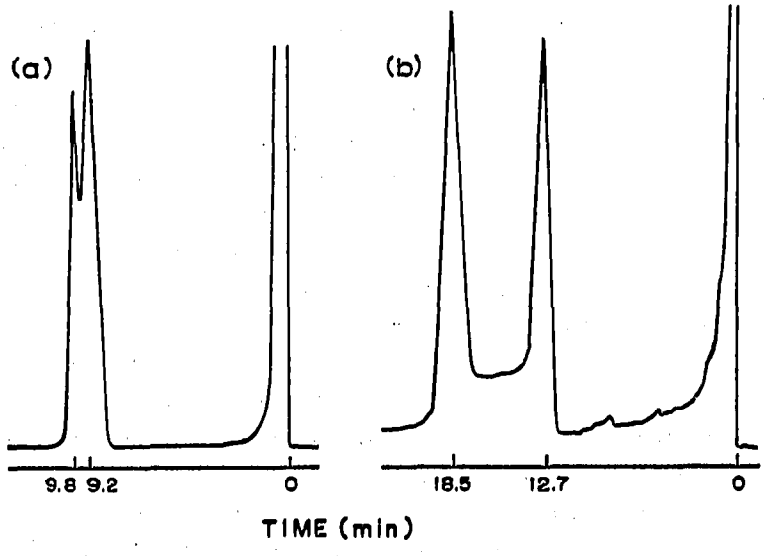

Fig. 1. Chromatogram of (a) *c*-ala-val and (b) *c*-ser-phe.

This method has obvious advantages over the previously reported techniques[2,3], whereby the diastereoisomers of *c*-ala-ala[2], *c*-ala-val[2], *c*-leu-leu[2], *c*-ala-leu[3], and *c*-leu-phe[3] could not be resolved. The column temperatures (90–182°) used in the present study are similar to those employed for GLC of the TMS derivatives (115–165°)[3] but

are appreciably lower than those required for GLC of the untreated DKP's (196–240°)[2,3]. The retention times for the TFA and TMS[3] DKP's are less than the retention times of the parent DKP's[2,3].

Though the column temperature was not held constant it is apparent from Table I that retention time increases with increasing molecular weight. Table I also indicates that in some cases (in the aliphatic series, left-hand column) the *cis*-diastereoisomer is eluted first and in other cases it is eluted second. In the aromatic–aliphatic series (right-hand column) the *cis*-diastereoisomer is always eluted second, except in the case of *c*-ser-phe, when the *cis*-compound is eluted first. The *cis*-DKP's have only hydrogen atoms projecting from one side of the DKP ring and would be expected to interact with stationary phase in preference to the *trans*-DKP's, which have substituents on each side of the ring. The longer retention times of the *cis*-DKP's have been explained on this basis[2] but the explanation failed for *c*-ala-ala and the proline-containing DKP's. The results in Table I show that in the lower-molecular-weight DKP's the *cis*-diastereoisomer has the longer retention time but as the molecular weight increases the *cis*-isomer has the shorter retention time. The space requirements of the alkyl substituents of the DKP's also increase with increasing molecular weight. This may indicate that the decrease in retention time of the *cis*-DKP's (relative to the *trans*-DKP's) is due to puckering of the DKP ring as a result of steric repulsion. The *trans*-DKP's are not subject to steric repulsion and the molecule as a whole may be flatter than the corresponding *cis*-compound and be preferentially absorbed. This explanation does not hold for all of the compounds studied and it is not readily apparent why *cis*-ala-ile is eluted after the *trans*-isomer, but *cis*-ala-leu is eluted before *trans*-ala-leu. The effect of leucine and isoleucine, however, appears to be consistent as the same relationship is evident for *c*-val-ile and *c*-val-leu. Molecular models indicate that steric repulsion is greater in leucyl-containing DKP's than in isoleucyl-containing DKP's and is in keeping with the aforementioned explanation of the relative retention times.

In the aliphatic–aromatic DKP's (right-hand column, Table I) all of the *cis*-compounds have longer retention times than the corresponding *trans*-compounds. Nuclear magnetic resonance studies[5,6] show that a number of these compounds, in solution, exist with the aromatic ring held preferentially over the DKP ring, which is planar except in the case of *c*-L-val-L-tyr[6]. Thus the *cis*-DKP's would have one side of a planar, unhindered DKP ring available for interaction with the stationary phase, whereas this interaction would be reduced in the *trans*-compounds.

An exception is *cis*-ser-phe, which has a shorter retention time than the *trans*-isomer. Extension of the alanylmethyl to hydroxymethyl introduces steric repulsion between the substituents at positions 3 and 6 of the DKP ring and may account for the reversal of relative retention times of the *cis*- and *trans*-diastereoisomers.

Racemization due to trifluoroacetylation and/or reaction in the gas chromatograph was observed only in the case of *c*-L-ser-D-phe. Introducing a sample of the trifluoroacetylated compound in the usual way resulted in two peaks (Fig. 1b), the first of which corresponded to *c*-L-ser-D-phe. However, on-column injections of *c*-L-ser-D-phe, with the injector heater off, gave only a single peak.

The effect of increasing the number of TFA groups is shown by comparison of the retention times of *c*-ala-phe, *c*-ala-tyr, and *c*-ser-phe. A TFA group in the *para*-position of the phenylalanyl ring causes an increase in the retention time whereas

a TFA group attached to the alanylmethyl group leads to a reduction in retention time in addition to a reversal of the order of elution of the *cis*-DKP relative to the *trans*-DKP.

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