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Separations of Enantiomeric Derivatives
of Amines and Amino Acids
by Adsorption Chromatography

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ABSTRACT

The gas-chromatographic behavior is described for three optically-active stationary phases at temperatures below the melting point. The high-temperature form of the solid ureide of L-valine isopropyl ester gave excellent enantiomer separations while the low-temperature form gave no detectable separations. The solid form of N-TFA-L-phenylalanine cyclohexyl ester did not separate enantiomers. N-TFA-L-valyl-L-valine cyclohexyl ester did not form a crystalline solid on the column wall but showed a very large, steady increase in α as the temperature was lowered. The ureide was also shown to have broader utility for the separation of the enantiomeric derivatives of amines and amino acids than the other commonly-used stationary phases. Faster separations of the enantiomers of amino acid derivatives were possible on the high-temperature form of the solid ureide than on the liquid valine dipeptide phase.

INTRODUCTION

The ureide of L-valine cyclohexyl ester has been shown by Feibush and Gil-Av (1) to be a useful liquid phase for the gas chromatographic resolution of enantiomers of a variety of secondary amine derivatives. Recently, it was shown that the solid ureide gave much larger α values and much faster separations than the liquid ureide phase while maintaining the same resolution (2). In addition, α increased at a rapid rate as the temperature of the solid ureide was lowered. One purpose of this work was to determine to what extent the separation could be improved, while maintaining a reasonable capacity ratio, by further lowering the temperature.

Since adsorption chromatography just below the melting point was superior to partition chromatography in the ureide system, the chromatographic behavior of other commonly-used, optically-active stationary phases was investigated at temperatures below their melting points. Both N-TFA-L-phenylalanine cyclohexyl ester (3) and N-TFA-L-valyl-L-valine cyclohexyl ester (4,5) have long been recognized as useful liquid phases for the separation of the enantiomers of amino acid derivatives, so they were chosen for investigation. Also, since it has been shown that the ureide phase was useful for the separation of the enantiomers of amino acid derivatives (2), the speed of analysis and the overall utility of the ureide phase were compared with the dipeptide stationary phase.

EXPERIMENTAL

Reagents. The ureide of L-valine isopropyl ester (m.p. 113-114 °C) and N-TFA-L-valyl-L-valine cyclohexyl ester (m.p. 95-98 °C) (both from Miles Laboratories, Elkhart, Indiana) were used as stationary phases and shall be referred to simply as the ureide and the dipeptide, respectively. The N-TFA-L-phenylalanine cyclohexyl ester (m.p. 62-65 °C) was also a Miles product. The DL-alanine was purchased from Matheson, Coleman and Bell, Norwood, Ohio. The D-alanine and L-alanine were obtained from Mann Research Laboratories, New York, N.Y. The trifluoroacetic anhydride was from Aldrich Chemical Company, Milwaukee, Wisconsin, while the pentafluoropropionic anhydride was from K & K Laboratories, Plainview, New York. The 2-amino-octane was purchased from Eastman Organic Chemicals, Rochester, N.Y.

The N-TFA-2-amino-octane and N-PFP-2-amino-octane derivatives were prepared by a previously described method (2). The N-TFA (trifluoroacetyl) and N-PFP (pentafluoropropionyl) D-, L-, and DL-alanine isopropyl ester derivatives were prepared in the following manner. Five grams of the solid amino acid were placed in 150 ml of dried (5A molecular sieve) isopropanol and HCl gas was bubbled in for 30 minutes to dissolve the amino acid. The reaction mixture was maintained at 60 °C for 12 hours with occasional stirring. The reaction mixture was then distilled to remove the isopropanol and HCl which produced a thick oil (I). The oil (I) was treated with 100 ml of dry ether to remove the remaining isopropanol, and the ether solution was discarded. The residue was treated with 100

ml of dry CHCl_3 in which the amino acid ester hydrochloride was soluble but the amino acid hydrochloride was not. CHCl_3 was removed under vacuum using a rotary evaporator, producing a clear oil (II). The oil (II) was dissolved in 35 ml of CH_2Cl_2 at 0°C and treated with a two-fold excess of anhydride. The solvent was removed under vacuum using a rotary evaporator which produced another clear oil (III). The oil (III) was treated with ice water to precipitate the solid derivative (yield 10%-20%). The precipitate was air-dried between two pieces of filter paper for 24 hrs. and placed in a desiccator over Drierite. As a check, the identity and purity of N-TFA-DL-alanine isopropyl ester were checked by NMR and mass spectrometry. The results showed that the material was the desired product and gave no evidence of any impurities.

Apparatus. The Aerograph 660 gas chromatograph, modified to minimize the dead volume in the system, was equipped with a Hamilton inlet splitter. The chromatograph was operated at a detector temperature of 180°C , an injection-port temperature of 200°C , and at different column temperatures. During studies of the effect of temperature change, fifteen minutes were allowed for equilibration after each new temperature had been reached, unless otherwise stated. A flow rate of approximately 2 ml/min of nitrogen, dried using 5A molecular sieve traps at ambient temperature, was used unless otherwise stated. An Esterline-Angus Speed Servo recorder was used to record the data.

Open tubular, Pyrex columns were used throughout this work. They were cleaned, drawn, coated, and conditioned as previously

described (2). The columns were 40 m by 0.25 mm and coated using 20% w/v solutions in dry ether (6). The dipeptide and amino acid derivative columns were submerged in a bath of liquid nitrogen, in some cases, in an attempt to hasten formation of a layer of solid stationary phase on the wall of the column.

DTA studies were done on an instrument consisting of an F&M Model 40 linear temperature programmer, an aluminum heating block wrapped with resistance wire, DuPont chromel-alumel thermocouples, L&N microvolt indicating amplifier, and an Esterline-Angus Speed Servo time-base recorder. The block was heated at a rate of 5.6 °C/min., starting at room temperature and continuing to at least 20 °C above the bulk melting point. The block was air cooled. The cooling rate depended on the difference between the block and air temperatures resulting in a cooling rate of approximately 2-3 °C/min in the range of 90 to 100 °C. Reported temperatures are those for the block and not for the sample.

Capacity ratios, k , were calculated from the formula:

$$k = \frac{t_r - t_m}{t_m}$$

where t_r was the retention time of the sample component, and t_m was the retention time of methane, which was assumed to be non-retained. Separation factors, α , were calculated from:

$$\alpha = \frac{k_2}{k_1} \quad (2)$$

The $\underline{k}$ and α values were reproducible to ± 0.003 . Resolution, $\underline{R}$, was calculated from:

$$\underline{R} = \frac{\underline{t}_{R2} - \underline{t}_{R1}}{1/2 (\underline{w}_{b1} + \underline{w}_{b2})} \quad (3)$$

were $\underline{t}_{R2}$ and $\underline{t}_{R1}$ were the adjusted retention times of the sample components and $\underline{w}_{b1}$ and $\underline{w}_{b2}$ were the base peak widths in units of time. Resolution-per-minute values were calculated by dividing the resolution, $\underline{R}$, by the retention time of the peak for the more strongly retained isomer.

RESULTS

A number of preliminary experiments, similar to those described previously (2), were done in order to make certain that a separation of enantiomers was indeed being achieved. The evidence clearly showed that an enantiomer separation was being achieved on both the ureide and dipeptide.

Forms of the Solid Ureide Phase. It has been shown previously that the solid ureide (m.p. 113-114 °C) was superior at 100 °C to the liquid ureide at 120 °C for the separation of the enantiomers of amine derivatives and an amino acid derivative (2). In addition, the separation on the solid ureide continued to improve down to 90 °C. In all cases, the α values were much larger on the solid than on the liquid ureide phase while the $\underline{k}$ values were much less. Separations of enantiomers of amino acid derivatives could be obtained approximately six times faster on the solid ureide than on the liquid while maintaining approximately the same resolution.

It was of interest to see if the separation on the solid ureide could be further improved, while still maintaining reasonable k values, by going to lower temperatures. As shown in Table I, k and α depended on temperature in an unexpected way. Even though the ureide was clearly a solid at all temperatures below 105 °C, there was no indication of a separation of the enantiomers of N-TFA-DL alanine isopropyl ester at 70 °C. Also, N-TFA-2-aminooctane showed only a single peak at 70 °C.

The direction of temperature change was also quite significant. As the temperature was raised in 5 °C intervals from 70 °C to 100 °C, a separation of the enantiomers of N-TFA-2-aminooctane first appeared at approximately 90 °C. With ascending temperature, α values of 1.00, 1.39, 1.57, and 1.49 were observed at 85 °C, 90 °C, 95 °C and 100 °C respectively. However, as the temperature was lowered in the same manner, the separation persisted down to approximately 80 °C. With descending temperature, α values of 1.49, 1.58, 1.63, 1.74, 1.18 and 1.00 were observed at 100 °C, 95 °C, 90 °C, 85 °C, 80 °C and 75 °C, respectively.

Since visual inspection of the crystalline ureide under 10X magnification showed no change until the melting point was reached, a DTA study was done to determine if there was a change in the bulk ureide below its melting point. Two sharp endothermic peaks were observed at 92 °C and 113 °C respectively as the sample was heated. On cooling, the ureide sample produced two peaks at 81 °C and 107 °C. The two-peak behavior was reversible. A ureide sample which had been melted, returned to 40 °C, and reheated showed exactly the same behavior as a sample which had not previously been melted.

(Microanalytical data supplied by Miles Laboratories indicated that the observed changes with temperature were not due to impurities.) The DTA peak at 113°C corresponded to the melting point of the ureide. The peaks at 81°C and 92°C indicated that the transition shown by the chromatograms was indeed a characteristic of the solid ureide.

Therefore, the chromatographic and DTA data clearly indicated that there were two forms of the solid ureide. The high-temperature form produced excellent enantiomer separations while the low-temperature form was totally inactive. Also, once produced, the high-temperature form persisted down to 80°C for, at least, an hour.

Solid N-TFA-L-Phenylalanine Cyclohexyl Ester. The liquid form (m.p. $62-65^{\circ}\text{C}$) of this stationary phase has been shown to be useful for the separation of the enantiomers of amino acid derivatives (3). It was of interest to see if the solid form of this stationary phase behaved in the same manner as the ureide.

Visual inspection showed that cooling the column below its melting point after conditioning did not produce a solid coating. Also, a plot of $\ln k$ vs $1/T$ was a straight line through the melting point.

However, visual inspection showed that dipping the column in liquid nitrogen did produce a white, solid layer on the column. Also, a plot of $\ln k$ vs $1/T$ of N-TFA-DL-alanine ethyl ester showed that k went through a sharp increase in the vicinity of the melting point on being heated.

Unlike the ureide, the solid phase did not produce an enantiomer separation. N-TFA-DL-alanine ethyl ester produced a single peak at 40 °C with a k of 26.39 while, at 75 °C, the same compound produced two peaks with k values of 43.94 and 45.21 respectively, which represented an α value of 1.029.

A DTA study produced a single sharp endothermic peak at 65 °C on heating and no detectable peaks on cooling. The chromatographic and DTA data, therefore, indicated that this compound was not suitable for use as a solid stationary phase.

Behavior of N-TFA-L-Valyl-L-Valine Cyclohexyl Ester Below Its Melting Point. The liquid form (m.p. 95 °C-98 °C) of this stationary phase has long been recognized to be an excellent material for the separation of the enantiomers of amino acid derivatives (4,5). We were curious to see if the solid dipeptide could be used for amino acid derivatives as the ureide had for amine derivatives.

Figure 1 and Table I show that the k values decreased in a regular fashion as the temperature was raised from 70 °C to 130 °C. The lack of a sharp discontinuity in the $\ln k$ vs $1/T$ plot at the melting point and below indicated that the dipeptide layer did not solidify below its melting point. Dipping the column in liquid nitrogen also failed to produce a solid in this case.

However, Table I does show one very interesting point. The α values increased much more rapidly on the dipeptide phase than on the liquid ureide phase over a given temperature range. For example, the change in α on going from 120 °C to 130 °C was 0.018 units on the dipeptide phase while it was only 0.001 for the ureide.

Since the chromatographic data indicated that the stationary phase had not formed a crystalline solid, a brief study was undertaken to define the nature of the material above and below the melting point. Visual inspection of a film of the dipeptide phase showed it was quite viscous even at 120 °C. Also, a film of the liquid dipeptide on a cover slide produced either by deposition from ether solution or by melting of the solid did not crystallize as the temperature was lowered below the bulk melting point but continued to increase in viscosity until it produced a glass or very hard gum around 70 °C. A DTA study produced a single endothermic peak at 96 °C as the temperature was raised and no detectable peak as the temperature was lowered to room temperature.

The dipeptide phase was clearly a liquid at 100 °C. At 70 °C, the phase was a hard gum which should have severely restricted diffusion by the solute. Yet Figure 1 shows the $\ln k$ vs $1/T$ plot was a straight line between 100 °C and 70 °C indicating there was no change in the type of chromatographic interaction occurring over this temperature range. Therefore, the observed enantiomer separations were apparently due primarily to surface adsorption throughout that temperature range. In addition, the slight curvature in Figure 1 above 115 °C suggested that only above this temperature did penetration into the liquid film become a significant contributor to k .

At a given R value, faster separations of PFP amine derivatives over the corresponding TFA derivatives were reported on the liquid ureide phase (2). While working with the dipeptide phase, a brief study was done to determine if this behavior was also characteristic of amino acid derivatives. The results in Table II show that the behavior of PFP amino acid derivatives relative to TFA derivatives was analogous. It was possible to separate PFP amino acid derivatives significantly faster than the corresponding TFA derivatives on both the liquid ureide and dipeptide phases while maintaining the same values for R /min.

Relative Utility of the Ureide and Dipeptide Phases. Since the ureide phase was proved useful for both amino acid derivatives and amines, a study was done to see if the dipeptide was useful for the separation of the enantiomers of amine derivatives. Table II shows that, while amine derivatives were strongly retained by the dipeptide phase, there was little or no selective interaction toward the enantiomers.

In addition, since the ureide and dipeptide phases were both useful for separating the enantiomers of amino acid derivatives, it was of interest to compare the speeds with which the two phases would perform a given separation. Table II shows the R /min values on the high-temperature solid ureide phase were approximately four times those on the dipeptide phase for the separation of the enantiomers of *N*-TFA-DL-alanine isopropyl ester. These results clearly indicated that the high-temperature solid ureide was capable of separating the enantiomers of some amino acid derivatives much faster than the

dipeptide phase.

As noted previously (2), another characteristic of separations of both amine and amino acid derivatives on the solid ureide was much greater width of the peak for the more strongly retained enantiomer. Since this behavior was unexpected because of the great similarity of these two isomers, the greater width of the second peak might have been due to the presence of an unresolved component resulting from the selective decomposition of that enantiomer on the solid ureide (7).

Racemic mixtures of the enantiomers of N-TFA-2-aminooctane were placed on the column for additional 20- and 30-minute periods by shutting off the carrier gas flow. If a decomposition product was being formed, the longer exposure time should have formed more of that compound and should have caused the appearance of a separate peak or, at least, a change in the shape of the second peak. However, a third peak did not appear, nor did the shape or relative area of the second peak change. The only change was in the ratios of base peak-widths: 2.41, 2.37 and 2.24 for samples exposed to the solid ureide for an additional 0, 20 and 30 minutes, respectively. The decrease indicated that the first peak was spreading more rapidly than the second, as one would expect from a faster rate of diffusion for the less strongly retained isomer. Hence, the difference in peak widths was apparently due to accentuation of the differences in the interactions on sites of different energies on the surface of the solid.

DISCUSSION

The results obtained using the solid amino acid stationary phase and low-temperature solid ureide form clearly showed that the solid form of an optically active stationary phase is not necessarily better than the liquid form. Apparently, the solid phase must meet certain specific structural requirements, which are presently undefined, before a selective interaction can occur.

Since adsorption chromatography on the high-temperature solid ureide phase gave faster separations for the enantiomers of amino acid derivatives than the dipeptide phase, it should be preferred for many analytical applications. In addition, since the ureide phase can be used for separating the enantiomers of both amine and amino acid derivatives while the other commercially available phases cannot, it should be preferred for general use in many cases.

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Table I. Separations of the Enantiomers of N-TFA-DL-alanine

Isopropyl Ester as a Function of Temperature

Temperature	Ureide of L-Valine Isopropyl Ester					N-TFA-L-Valyl-L-Valine Cyclohexyl Ester				
$^{\circ}\text{C}$	k_1	k_2	α	R	R/min	k_1	k_2	α	R	R/min
130	10.125	10.545	1.041	0.80	0.03	3.610	3.892	1.078	0.92	0.08
125	11.699	12.188	1.042	0.90	0.03	4.220	4.583	1.086	1.04	0.07
120	14.050	14.642	1.042	0.94	0.03	4.711	5.163	1.096	1.12	0.07
100	0.470	0.634	1.349	0.72	0.20	10.828	12.052	1.113	1.36	0.04
95	0.504	0.707	1.403	0.75	0.19	13.930	15.700	1.125	1.53	0.04
90	0.568	0.826	1.454	0.75	0.19	17.129	19.442	1.135	1.67	0.03
70			0.764 ^a			48.287	56.390	1.168	1.74	0.01

methane

135 sec

150 sec

^a Single, badly-tailed peak

Table II. Comparison of the Separation of the Enantiomers
of Amine and Amino Acid Derivatives on the Ureide and
Dipeptide Stationary Phases

Compound	Ureide of L-Valine Isopropyl Ester										N-TFA-L-Valyl-L-Valine Cyclohexyl Ester									
	100 °C					120 °C					100 °C					120 °C				
	<u>k</u> ₁	<u>k</u> ₂	<u>α</u>	<u>R</u>	<u>R</u> /min	<u>k</u> ₁	<u>k</u> ₂	<u>α</u>	<u>R</u>	<u>R</u> /min	<u>k</u> ₁	<u>k</u> ₂	<u>α</u>	<u>R</u>	<u>R</u> /min	<u>k</u> ₁	<u>k</u> ₂	<u>α</u>	<u>R</u>	<u>R</u> /min
N-TFA-DL-alanine isopropyl ester	0.44	0.55	1.24	1.09	0.29	7.93	8.26	1.04	1.41	0.07	10.34	11.53	1.12	2.63	0.08	4.70	5.11	1.09	1.69	0.11
N-PFP-DL-alanine isopropyl ester	0.42	0.61	1.45	1.07	0.28	6.70	6.97	1.04	1.26	0.07	8.67	9.64	1.11	2.24	0.08	3.91	4.24	1.08	1.53	0.11
N-TFA-DL-2- aminooctane	1.97	2.72	1.38	2.03	0.24	23.72	25.01	1.05	1.53	0.03	25.95 ^a					10.75 ^b				
methane	133 sec					138 sec					147 sec					158 sec				

^a Single, flat-topped peak suggestive of a partial separation.

^b Single, broad peak.

FIGURE CAPTIONS

Figure 1. Plot of $\ln k$ vs $1/T$ for N-TFA-DL-alanine Isopropyl
Ester on an N-TFA-L-Valyl-L-Valine Cyclohexyl Ester Column.