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Enantioselective gas chromatographic assay of 2-alkylamines using N-(trifluoroacetyl)prolyl derivatives and a chiral capillary column

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Abstract

The chromatographic properties of (*R*)-(+)-N-(trifluoroacetyl)prolyl and (*S*)-(−)-N-(trifluoroacetyl)prolyl derivatives on a chiral gas chromatography capillary column were assessed for the measurement of enantiomeric purities of 2-butylamine, 2-pentylamine, 2-hexylamine, 2-heptylamine and 2-octylamine and their N-methyl analogues, which are used as precursors in the synthesis of some selective, specific, irreversible monoamine oxidase-B inhibitors. Using a Chirasil-Val column it was possible to separate all four diastereomers of the primary amines, and three of the four isomers of the secondary amines. Quantitation of the enantiomers is facilitated even with enantiomerically impure reagent when compared to the use of an achiral phase.

Keywords: Enantiomer separation; 2-Alkylamines; N-(Trifluoroacetyl)prolyl derivatives

1. Introduction

Many biochemical processes at the molecular level are now well known to be either stereoselective or stereospecific. Thus, in the investigation of new chiral drugs, one must take into account the fact that each enantiomer may exhibit differing degrees of a particular biological activity.

During the development of a new series of N-(2-alkyl)-N-methyl-propargylamine monoamine oxidase-B inhibitors in this laboratory [1] it was found that the *R*-enantiomer had much greater activity than the *S*-enantiomer [2]. These drugs were synthesized from the *R*- and *S*-enantiomers of the corresponding 2-alkylamines by N-methylation followed by N-propargylation of the N-methylalkylamines. Verification of the enantiomeric purity of the starting 2-alkylamines, 2-butylamine, 2-pentylamine, 2-hexylamine, 2-heptylamine and 2-octylamine, was required before the subsequent steps were undertaken.

A high-performance liquid chromatographic (HPLC) method for the separation of (±)-2-heptylamine enantiomers using inductive adsorption chromatography has been reported recently [3]. However, the resolution of the method is not suitable for establishing enantiomeric purity to the required accuracy. Capillary gas chromatographic methods on the other hand are capable of a very high degree of resolution.

One gas chromatographic (GC) technique used for enantiomeric separations is to react the enantiomers with an optically pure chiral reagent, thus converting each to a corresponding diasteromeric derivative. These diastereomers may be separated on an achiral stationary phase using appropriate GC conditions. Perhaps the most popular chiral reagent for derivatizing amines is (*S*)-(−)-N-(trifluoroacetyl)prolyl chloride (STFAP-Cl) [4,5], due to its high resolving

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power with many amines [6] and its ready commercial availability. An early use of the reagent was for the separation of amphetamine enantiomers [6–8] and it is now used routinely for forensic purposes in the identification of the source of amphetamine and methamphetamine samples [9–12]. Many of these uses were recently reviewed by Cody [13]. STFAP-Cl has also been used for enantiomeric identification and quantification of a variety of other drugs such as fluoxetine [14], norfluoxetine [14], tranylcypromine [15] and methylenedioxyamphetamine [16].

A major problem in the use of STFAP with an achiral stationary phase for GC is the presence of small amounts of the *R*-enantiomer in the supposedly optically pure *S* reagent from commercial sources [17]. Unfortunately, the four possible diastereomers are not resolved, but instead elute as only two peaks. Consequently, the reagent must be "calibrated" with an optical isomer of known purity so that corrections can be made [18]. Use of a chiral stationary phase may overcome this problem, since it is frequently possible to separate all four diastereomers [18,19].

This work reports on the chromatographic properties of the *R*- and *S*-2-alkylamines, after derivatization with N-(trifluoroacetyl)prolyl chlorides, the separation of the diastereomers by a chiral capillary column and its use for determination of the enantiomeric purity of 2-alkylamines.

2. Experimental

2.1. Reagents and materials

The following compounds were purchased from Aldrich (Milwaukee, WI, USA): *S*-(trifluoroacetyl)prolyl chloride, (*S*)-(−)-proline (L-proline), (*R*)-(+)-proline (D-proline), (2*R*,3*R*)-(+)-tartaric acid (L-tartaric acid), (2*S*,3*S*)-(−)-tartaric acid (D-tartaric acid), lithium aluminum hydride, thionyl chloride, ethyl chloroformate, *R*- and *S*-*sec*.-butylamine, *RS*-*sec*.-butylamine, 1-methylbutylamine, 2-aminoheptane, 1-methylheptylamine, 2-hexanone and trifluoroacetic anhydride. 2-Hexylamine was synthesized from 2-hexanone by reduction of the oxime with lithium aluminum hydride. Anhydrous diethylether, absolute methanol, benzene, hexane, ethyl acetate and dichloromethane were obtained from Caledon Laboratories (Georgetown, Canada).

2.2. Synthesis of (R)- and (S)-N-(trifluoroacetyl)-prolyl chlorides

The (*R*)- and (*S*)-N-(trifluoroacetyl)prolyl chlorides were synthesized according to the method of Lim et al. [20] (for the synthesis of (*S*)-N-(heptafluorobutyryl)prolyl chloride) from D- and L-proline, respectively. The residue was dissolved in dichloromethane to a concentration of about 0.1 *M*. The optical purity of each product was assessed by reaction with racemic *R*,*S*-2-heptylamine, as described below. The reagents prepared [STFAP-Cl and (*R*)-(+)-N-(trifluoroacetyl)prolyl chloride (RTFAP-Cl)] were routinely stored at −20°C.

2.3. Resolution and isolation of five racemic 2-alkylamines

Racemic 2-pentylamine, 2-hexylamine, 2-heptylamine and 2-octylamine were resolved as their tartrate salts according to the method of Mazur [21] for the resolution of 2-heptylamine. In this procedure, the racemic mixture of the free amines was converted to the L-tartrate (2*R*, 3*R*) salts of the *R*- and *S*-enantiomers of the amine. The precipitate (*R*-2-alkylamine-L-tartrate) was repeatedly recrystallized from absolute methanol until GC analysis of the (*S*)-(−)-N-(trifluoroacetyl)prolyl derivative showed an optical purity of >98% *R*. The number of recrystallizations required to achieve this purity increased with increasing chain length (five to eight recrystallizations). The original filtrate containing the L-tartrate of *S*-enriched 2-alkylamine was evaporated and the residue was converted to the free amine, as described by Mazur [21]. The distilled *S*-enriched amine was then converted to its D-tartrate salt and recrystallized until GC enantiomeric analysis indicated the desired optical purity of the *S*-enantiomer. For the isolation of resolved 2-pentylamine as free base (which is significantly soluble in aqueous solutions and which has a low boiling point), the solution of the tartrate was cooled in ice water during the basification with 50% sodium hydroxide and the wash with a potassium carbonate solution was omitted. The isolated free base amines were distilled

and converted to their hydrochloride- or sulfate salts. The optical purities of the resolved or purchased primary amines were found to be: *R*-2-Butylamine, 93.1% (Aldrich); *S*-2-butylamine, 95.1% (Aldrich); *R*-2-pentylamine, 99.0%; *S*-2-pentylamine, 98.5%; *R*-2-hexylamine, 99.1%; *S*-2-hexylamine, 98.5%; *R*-2-heptylamine, 99.2%; *S*-2-heptylamine, 98.7%; *R*-2-octylamine, 99.3% and *S*-2-octylamine, 99.3%. The amines were converted to their methylated analogues as described in Section 2.4.

2.4. Preparation of the N-methyl analogues of the R- and S-enantiomers of the 2-alkylamines

The optically pure free base of the primary amine was treated with ethyl chloroformate in dichloromethane and triethylamine and the resulting carbamate was reduced with lithium aluminum hydride. The isolated free base N-methyl amines were distilled and converted to their hydrochloride or sulfate salts.

2.5. Derivatization of the enantiomers of the 2-alkylamines

Amounts of the amine salts, either the racemic salt or each of the enantiomers separately (1.5 mg), were weighed and dissolved in 1.0 ml of ethanol. Two methods were used to prepare the N-(trifluoroacetyl)prolylamide derivatives.

The first was adapted from that of Torok-Both et al. [14]. Aliquots of the ethanol solutions (0.1 ml) were transferred (Socorex air interface pipette) to conical reaction vials (Reactivials, Pierce) and the ethanol was removed under a stream of nitrogen at about 30–40°C. The reagent, 50 or 100 μl in dichloromethane, was added, the vials were sealed and the mixture was heated at 50°C for 20 min. The vial was then cooled to room temperature, 100 μl of hexane were added, followed by 200 μl of phosphate buffer, pH 8. After vortex-mixing, the vial was cooled in a dry ice–isopropanol bath until the aqueous layer froze. The organic layer was then transferred to a new vial and an aliquot was analysed by either GC–flame ionization detection (FID) or GC–MS.

A second method was based on that of Lim et al. [20]. Aliquots (1 ml) of 5% (w/v) $NaHCO_3$–Na_2CO_3 (7:3) buffer, pH 7.43, were placed in 100×16 mm test tubes and 100-μl volumes of the ethanolic amine solutions were added. The reagent, 0.1 *M* in dichloromethane, was added in amounts of 50 to 100 μl and the tubes were gently vortex-mixed, periodically, over a 20-min period. The N-(trifluoroacetyl)prolylamides were extracted with 1 or 2×2 ml of hexane, transferred to a new test tube and taken to dryness under a nitrogen stream. The residue was then dissolved in 200 μl of hexane and 1 μl was injected into the GC system.

2.6. Nomenclature

For convenience, the four diastereomers for each amine were given acronyms following the system used previously by Liu and Ku [18]. Four diastereomers result, i.e. *RR*, *SR*, *RS* and *SS*, with the first letter indicating the chirality of the amine and the second letter indicating the chirality of the N-(trifluoroacetyl)prolyl moiety.

2.7. Determination of the relative concentrations of STFAP-Cl and RTFAP-Cl

Equal volumes (50 μl) of STFAP-Cl and RTFAP-Cl solutions were premixed and then used to derivatize racemic 2-heptylamine. The ratios of the peak areas of *RR*/*RS* and *SR*/*SS* were averaged to calculate the relative concentration of RTFAP-Cl to STFAP-Cl.

2.8. Determination of the order of elution of the (R/S)-N-(trifluoroacetyl)prolyl-(R/S)-amine diastereomers

Initially the GC retention times were established by reacting pure *R*- or *S*-amine with pure RTFAP-Cl or STFAP-Cl. Various combinations of *R*- and *S*-amine and *R*- and *S*-reagent were used to clarify any ambiguities in relative retention times. To further verify these retention times and to be able to compare them in one chromatogram, a 0.1-ml volume of the stock solution of the racemic amine and a 0.1-ml volume of the *R*-amine, to give a ratio *S*/*R*= 1:3, was dried in the Reactivial. This was reacted with a 1:2 mixture of RTFAP-Cl and STFAP-Cl. The resulting peak areas would thus be theoretically in

the ratio *SR–SS–RR–RS*=1:2:3:6 and could be identified on the chromatograms, as shown in Fig. 1.

2.9. Stability of the RTFAP-Cl and STFAP-Cl reagents

Since we had observed increasing amounts of RSTFAP-Cl in commercial STFAP-Cl, especially after storage, a sample of each was left at room temperature for about one week. The ratio of the enantiomers was measured using (*R*,*S*)-2-heptylamine as the substrate.

2.10. Gas chromatographic assays

Samples were analysed using a HP5710A gas chromatograph equipped with a flame ionization detector. The GC column was a Chirasil-Val, 25 m×0.25 mm, 0.16 μm film (Alltech, Heliflex; purchased from Mandel Scientific, Guelph, Canada). The carrier gas was helium with an inlet pressure of 15 p.s.i. (100 kPa) and nitrogen was added as the make-up gas (30 ml/min) for the flame ionization detector. The solvent front (t_a) eluted at about 1.6 min. Separation conditions were as follows: Initially, to establish the elution temperature, the oven temperature was set at 80°C for 4 min, then was increased to 220°C at a rate of 8°C/min and then held for 8 min. Once the elution temperatures had been established, a different program was used for the experiments in which the resolution was to be measured. The initial temperature was adjusted for each compound (to the nearest degree) so that the last diastereomer eluted between 14 and 16 min, to maintain the capacity factor as close to a value of ten as was feasible with the equipment. Thus, for the primary amine derivatives, the initial temperatures were 132°C for 2-butylamine, increasing by 10°C for each additional carbon up to 172°C for 2-octylamine, and for the N-methylamines were from 145°C for N-methyl-2-butyl, again increasing by 10°C for each carbon up to 185°C for N-methyl-2-octylamine. A 1-μl volume of each sample was injected. Chromatograms were recorded using a HP3380 or a Spectrophysics SP 4270 integrator or on a Houston B5000 paper chart recorder (Figs. 1 and 2).

GC–MS analyses were performed for confirmation of the chemical identities of the four GC peaks (to ensure that the designated peaks were not due to impurities of reagents, solvents or by-products) with a second system consisting of a HP5700 GC interfaced to a VG7070F mass spectrometer through an open split interface. A Chirasil-Val column or a J&W DB-5-MS, 30 m×0.32 mm I.D., 0.5 μm film capillary was used. The capillary was connected to the GC inlet by a 1–2 m length of deactivated 0.53 mm I.D. retention gap, into which the samples were injected directly, at 200°C, without splitting. The outlet was connected (Supelco Glasseal connector) to a (0.2 m length, 0.15 mm I.D.) silica capillary inlet of the open split interface, which was connected into the mass spectrometer ion source by a further 0.7 m length of 0.15 mm I.D. silica capillary. The interface was maintained at 250°C and the mass spectrometer ion source was maintained at 180°C. Full spectra were recorded at a mass spectrometer resolution of 1000.

2.11. Calculations

The following values were used to assess the resolving power of the column towards the diastereomers: Selectivity factor, alpha, $\alpha = t'_{r(x+1)}/t'_{r(x)}$ and column resolution $R_s = 1.177\ [t'_{(x+1)} - t'_{(x)})/(W_{1/2\,(x+1)} + W_{1/2(x)}]$ where $t'_{r(x+1)}$ and $t'_{r(x)}$ are the adjusted retention times of peaks x and $x+1$, respectively, and $W_{1/2}$ is the width at half height [22].

3. Results and discussion

3.1. Chromatography of the R- and S-(trifluoroacetyl)prolyl derivatives of 2-alkylamines

The four diastereomers of the derivatives of each of the 2-alkylamines (ranging from C4, 2-butylamine, to C8, 2-octylamine) are clearly separated by the Chirasil-Val column, as shown in Fig. 1. Perhaps the most interesting observation is that the order of elution changes as the carbon chain length increases. Thus, for the C4 compound, the order is *SR*, *RR*, *RS* and *SS*, whereas, for the C8 compound it is *SR*, *RS*, *RR*, *SS*, with the two centre peaks *RR* and *RS* changing places in the elution order at C6. The interaction of the *S*-amine with the column is very dependent upon the chirality of the (trifluoroacetyl)prolyl moiety. For the *R*-amine, there is less

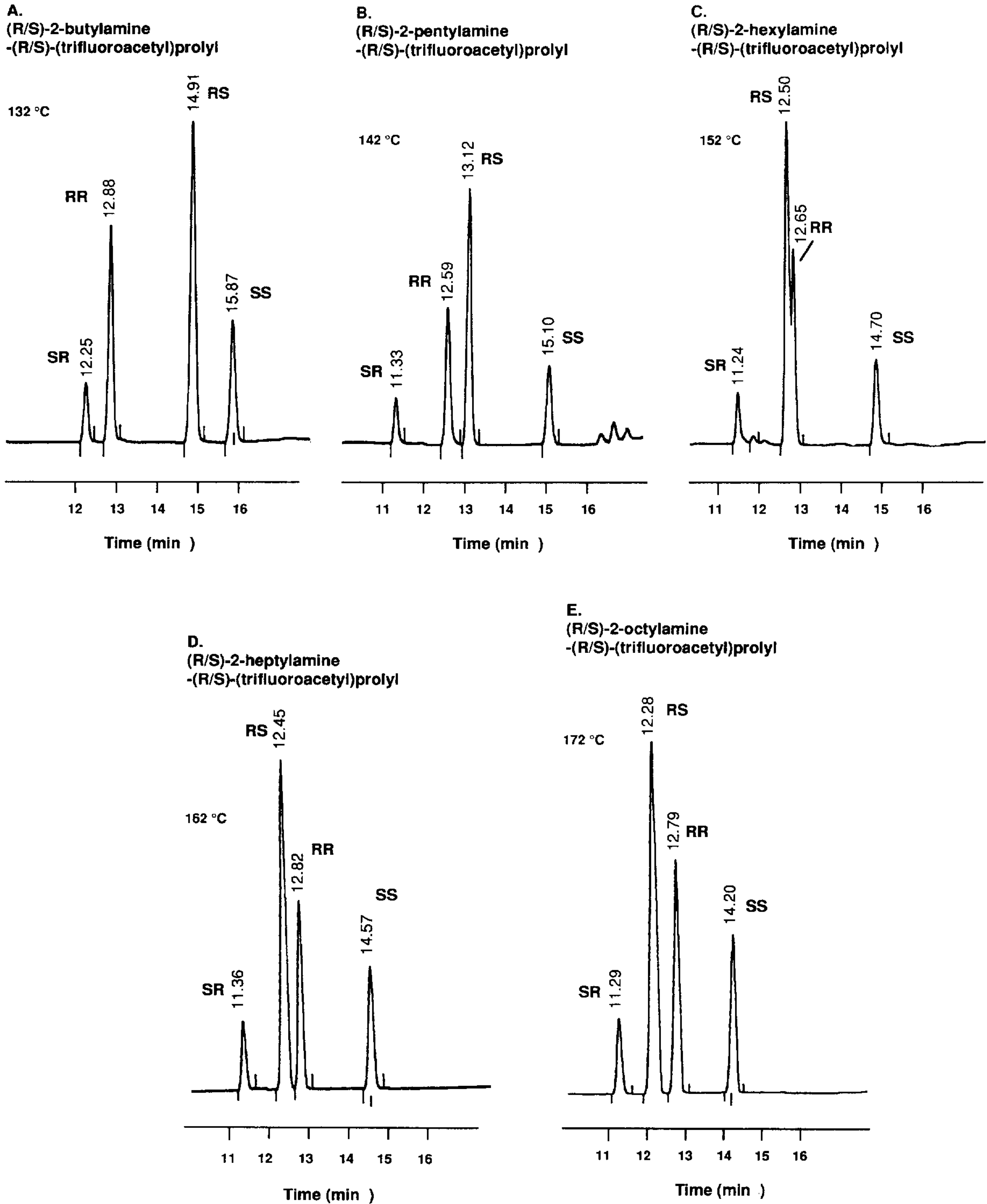


Fig. 1. Isothermal chromatograms of the N-(trifluoroacetyl)prolyl derivatives of 2-alkyl amines: (A)=2-butylamine; (B)=2-pentylamine; (C)=2-hexylamine; (D)=2-heptylamine and (E)=2-octylamine. The molar quantities of the peaks were in the ratio *SR*–*SS*–*RR*–*RS*= 1:2:3:6.

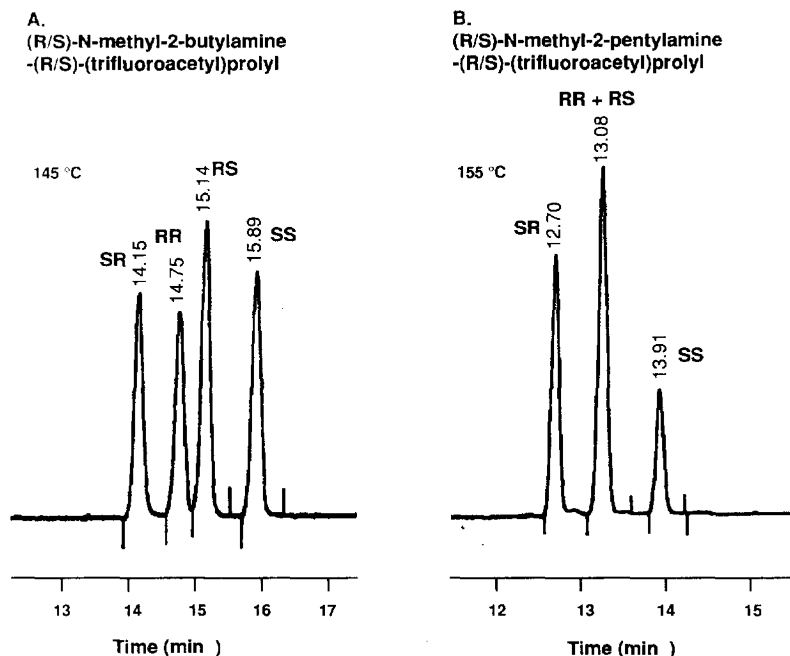


Fig. 2. Isothermal chromatograms of the N-(trifluoroacetyl)prolyl derivatives of (A) N-methyl-2-butylamine with molar quantities of the peaks approximately in the ratio *SR–SS–RR–RS*=1:1:1:1 and (B) N-methyl-2-pentylamine with molar quantities of the peaks approximately in the ratio *SR–SS–RR–RS*=2:2:1:1.

dependence on the chirality of the (trifluoroacetyl)prolyl. Initially, it is the effect of the (trifluoroacetyl)prolyl moiety that interacts with the column (C4 and C5) with the *R*-configuration providing the greater interaction and hence the larger capacity factor, as shown by the α values in Fig. 3A. For the larger molecules C6 to C8, this effect becomes smaller and it is the overall configuration that predominates. From an analytical stand point, the peaks for C4, C5, C7 and C8 are very well resolved and thus an impurity of RSTFAP-Cl in STFAP-Cl does not affect the accuracy of quantitation, as would be the case using an achiral column in which a correction is required [18]. Quantitation of the enantiomeric purity of the amine e.g. *R*% in the total can be calculated two ways. Either from the ratio of the *RS*/(*RS*+*SS*) peaks or from the ratio of (*RS*+*RR*)/[(*RS*+*RR*)+(*SR*+*SS*)], i.e., areas of peaks (2+3)/all four peaks. Quantitation of *R*-2-heptylamine in *S*-2-heptylamine using the *RS* and *SS* peaks gave a linear calibration (r^2=0.997) over the range 0 to 10% *R* in *S*. The purity of the newly synthesized STFAP-Cl, (>99.9%) was such that the *SR* and *RR* peaks were not measurable.

In the case of 2-hexylamine (C6), the *RS* and *RR* peaks are not as well resolved, although the value of column resolution, R_s, measured with equimolar amounts, was 1.45 and the valley was about 6%. In the quantitation of small amounts of *R*-2-hexylamine in *S*-2-hexylamine, with small amounts of RSTFAP-Cl in STFAP-Cl, the contribution of the *RR* peak to the *RS* peak area is negligible and could be corrected for by the *SR*/*SS* peak-area ratio. The ability to measure all four peaks in one chromatogram obviates the requirement to quantitate the reagent purity with a control compound, as would be required if an achiral column was used.

3.2. Chromatography of the R- and S-(trifluoroacetyl)prolyl derivatives of N-methyl-2-alkylamines

Separation of the four diastereomers of the derivatives of N-methyl-2-butylamine and of N-methyl-2-

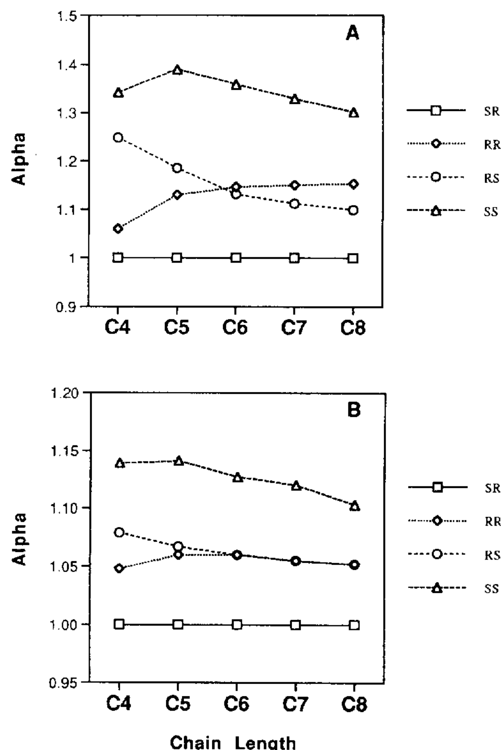


Fig. 3. Selectivity factor α of the four diastereomers of (A) N-(trifluoroacetyl)prolyl derivatives of 2-alkylamines and (B) N-(trifluoroacetyl)prolyl derivatives of N-methyl-2-alkylamines versus carbon chain length.

pentylamine is shown in Fig. 2. The chromatograms of 2-hexylamine, 2-heptylamine and 2-octylamine were similar to that of 2-pentylamine. For this group of compounds, the first eluting peak was the *SR* diastereomer, followed by the *RR*, *RS* and the *SS* diastereomers. Again it is the *S*-isomer of the amine that is affected in a major way by the chirality of the (trifluoroacetyl)prolyl moiety. In the case of the butyl compound, all four isomers elute separately, but the *RR* and *RS* isomers coelute in the C5–C8 compounds, as is demonstrated by the values of α in Fig. 3B. It would appear that loss of the free hydrogen on the amine reduces the difference in the interactions of the *RR* and *RS* compounds with the Chirasil-Val stationary phase. Quantitation of minor amounts of *R*-amine in *S*-amine is easily accomplished using the ratio of peaks 1 and 3 to peaks 1–3.

3.3. Mass spectrometry of the (trifluoroacetyl)-prolyl derivatives

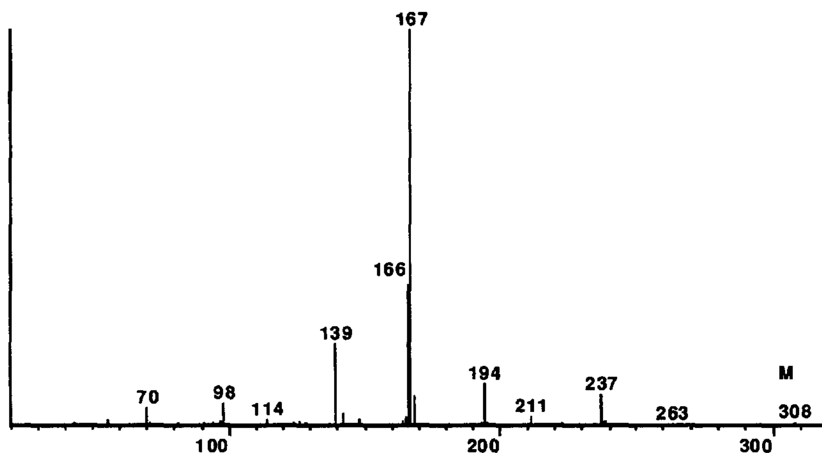
Two mass spectra of the (trifluoroacetyl)prolyl derivatives of 2-heptylamine and N-methyl-2-heptylamine are shown, as examples, in Fig. 4. The spectra demonstrate characteristics similar to those previously reported for amphetamine [19]. The base peak, either *m/z* 166 or 167 and *m/z* 194 are due to (trifluoroacetyl)prolyl fragmentation. The main difference between these spectra is the presence of the molecular ion. In the amphetamine spectra, the molecular ion is absent due to the high stability of the *m/z* 91 fragment. In these spectra, the loss of the alkyl chain fragment (*m/z* 71, C_5H_{11}) is less favourable. Thus, a molecular ion may be used for identification. We did not observe discernible differences between the spectra of the four diastereomers.

3.4. Stability of STFAP-Cl and RTFAP-Cl

Freshly prepared reagents were found to be more than 99.9% enantiomerically pure. After one week at room temperature, the amount of *R* in STFAP-Cl had increased to 3.3% and the amount of *S* in RTFAP-Cl to 2.2%. These measurements were made using the anhydrous derivatization and the use of a catalyst such as triethylamine was avoided to prevent possible racemization during the reaction [23]. Thus, it appears that racemization of the prolyl moiety is catalysed by the reagent itself or perhaps by small amounts of acid formed by hydrolysis by atmospheric moisture.

4. Conclusion

This paper demonstrates that the diastereomeric N-trifluoroacetylprolyl derivatives of 2-alkylamines and of N-methyl-2-alkylamines are readily separated by a chiral capillary column and facilitate quantitation of the *R*- and *S*-enantiomers of the amines. Because of the order of separation of the enantiomers, it is possible to determine the enantiomeric purity of the 2-alkylamine or N-methyl-2-alkylamine, even with stereochemically impure reagent. The reagents, STFAP-Cl and RTFAP-Cl, are



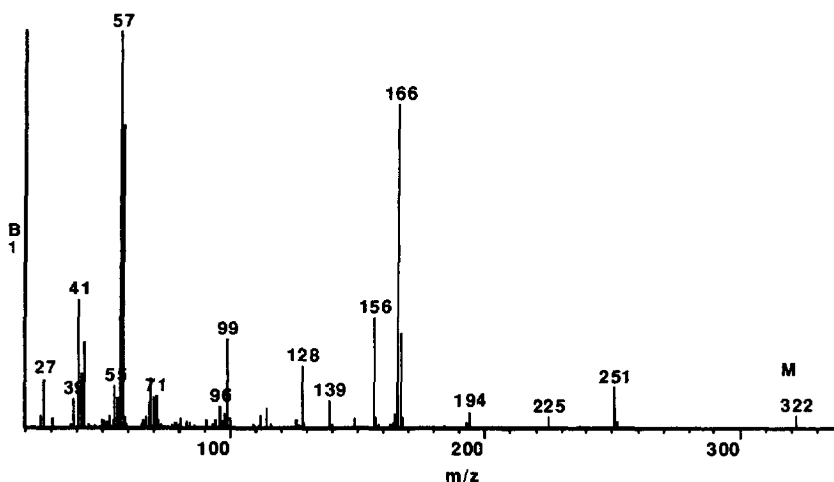


Fig. 4. Quantitation of small amounts of (*R*)-2-heptylamine in (*S*)-2-heptylamine using the *RS* and *SS* peaks.

stable at −20°C, but racemize at a rate of 2–3% per week at room temperature.

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