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Note

Gas chromatographic resolution of the optical enantiomers of a homologous series of 3-alkyl-serines (2-amino-3-alkyl-3-hydroxy-butanoic acids)

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As part of work aimed at investigating the structure–activity relationship of threonine analogues towards the threonine dehydrogenase (EC 1.1.1.103) from *Trypanosoma brucei*[1], we have synthesized a series of homologous 3-alkyl-serines, of which serine and threonine are the first two members, and have examined the gas chromatographic (GC) properties of their N,O-heptafluorobutyryl-O-isobutyl and N,O-heptafluorobutyryl-O-L(—)-menthyl derivatives. We have shown that it is possible to resolve completely all the optical enantiomers for each homologue using SE-30 support-coated open tubular (SCOT) columns. Since these molecules contain two optically active centres at C-2 and C-3, two enantiomeric pairs will be produced (Fig. 1).
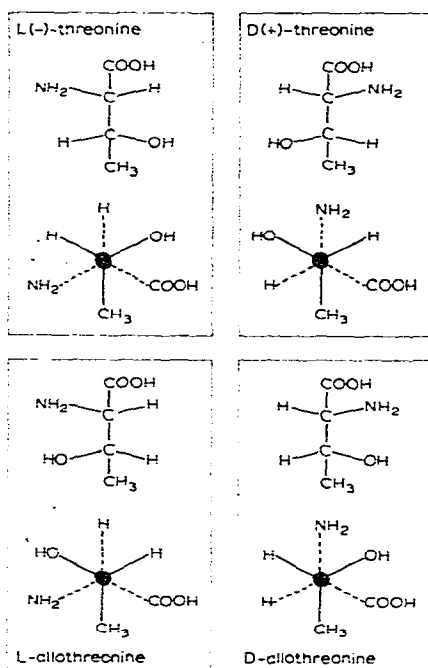

Fig. 1. Configurational isomers of threonine and its homologues.

The *allo* and *threo* forms are well separated using the isobutyl esters, whereas resolution into D- and L-*allo*, and D- and L-*threo* forms is obtained using the diastereoisomeric L(—)-menthyl esters.

We have also investigated the thermodynamic basis for the separation of the optical enantiomers, and have demonstrated that the separation of the D- and L-*threo* forms is entirely entropic in origin, whereas the D- and L-*allo* forms show a small enthalpic effect. The separation of the *allo–threo* pairs is characterized by a marked enthalpic contribution.

Of the chiral reagents available for the separation of racemic mixtures of amino acids as diastereoisomeric pairs[2], we chose to use a combination of the O-L(—)-menthyl ester[3] and the heptafluorobutyryl (HFB) protecting group for the amino and hydroxyl functions[4–6]. These derivatives are both easy to prepare and stable, and possess high sensitivity for electron-capture detection, an important consideration in the present work, as well as being suitable for use with flame-ionization detectors (FIDs).

EXPERIMENTAL

Threonine analogues were synthesized by condensing the appropriate aldehyde with copper glycinate under alkaline conditions[7]. Yields after recrystallization of the product from aqueous ethanol, ranged from 17 to 65% depending upon the homologue. The hydroxy amino acids ($<5~\mu$mole) were heated with 400 μl L(—)-menthol (or isobutanol) saturated with dry hydrogen chloride, in a sealed ampoule at 135 °C for 30 min. After removing excess alcohol under a stream of dry nitrogen at 60 °C, 50 μl HFB anhydride and 50 μl ethyl acetate were added, the ampoule resealed and the mixture heated again at 135 °C for 30 min. Excess reactants were removed under a stream of nitrogen, and the residue dissolved in 100 μl ethyl acetate for GC.

GC was carried out isothermally at either 120 or 150 °C on SE-30 SCOT columns (SGE, London, Great Britain), 0.50 mm $\times$ 25 m, using hydrogen as the carrier gas at a pressure of 210 mbar, giving a flow-rate of *ca.* 2 ml/min, and FIDs. HFB anhydride was obtained from either Serva (Heidelberg, G.F.R.), or from Pierce and Warriner (Chester, Great Britain). The isobutanol and ethyl acetate were analytical grade. L(—)-Menthol was obtained from Aldrich (Gillingham, Great Britain).

RESULTS AND DISCUSSION

The use of the N,O-heptafluorobutyryl isobutyl esters provided good resolution of the *allo* and *threo* forms for all the members of the homologous series studied, *i.e.* serine through to 3-pentyl-serine. The N,O-heptafluorobutyryl-O-L(—)-menthyl derivatives permitted further resolution into both D and L enantiomeric pairs as well as separating the *allo* and *threo* forms (Figs. 2–4), thus achieving the complete separation of all four configurational isomers in a single run. Under the conditions used, resolution and separation factors[8] between L- and D-threonine were 1.06 and 1.043, and between L- and D-*allo*-threonine 0.70 and 1.037, respectively, compared to values of 1.75 and 1.057 for the L- and D-*threo* forms and 2.90 and 1.099 for the L- and D-*allo* forms of 3-butyl-serine.

MacKenzie and Tenaschuk[5] have reported two partially resolved peaks for the
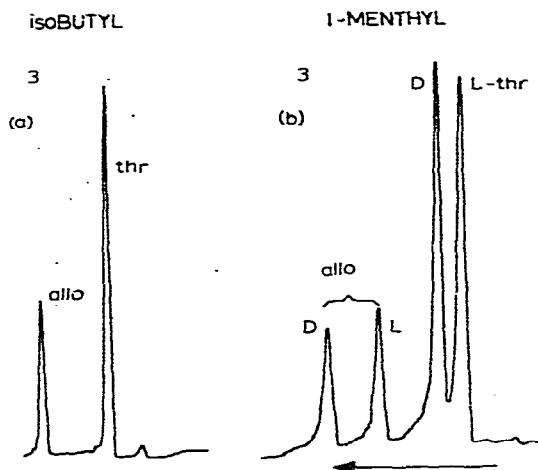
isoBUTYL   l-MENTHYL

Fig. 2. GC traces of (a) HFB-isobutyl derivative and (b) HFB-L(−)-menthyl derivative of 3-butyl-serine. SE-30 SCOT columns run isothermally at either 120 °C (isobutyl) or 150 °C (menthyl).
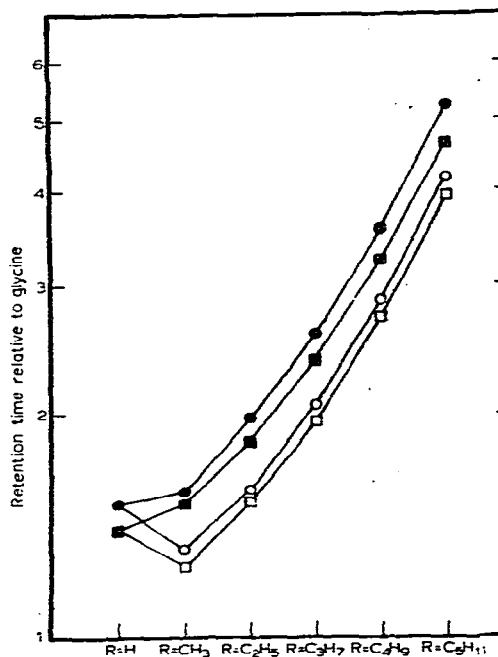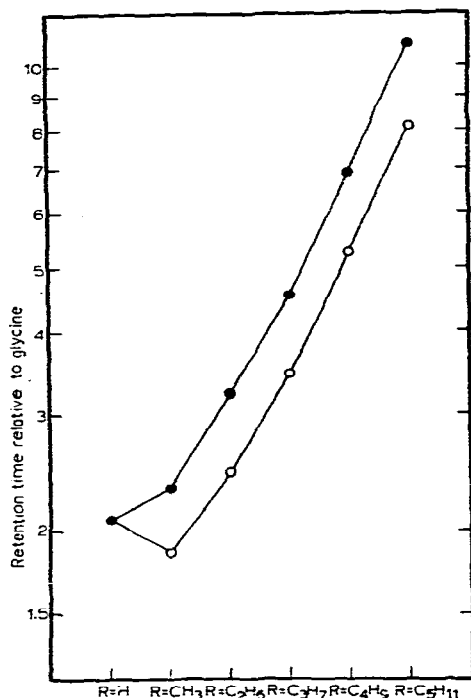

Fig. 3. Logarithm of the relative retention time with respect to glycine for the isomers of the homologous 3-alkyl-serines as the N,O-heptafluorobutyryl-O-isobutyl derivatives on SE-30 at 120 °C. ○ = *threo* series; ● = *allo* series.

Fig. 4. Logarithm of the relative retention time with respect to glycine for the isomers of the homologous 3-alkyl-serines as the N,O-heptafluorobutyryl-L(−)-menthyl derivatives on SE-30 at 150 °C. □ = L-enantiomers of the *threo* series, ■ = L-enantiomers of the *allo* series, ○ = D-enantiomers of the *threo* compounds, ● = D-enantiomers of the *allo* compounds.

HFB-isobutyl ester of hydroxylysine, and have suggested that this was due to the separation of the DL and DL-*allo* isomers.

In order to investigate the basis for the separation between isomers, an Arrhenius plot of the relative retention times for the isomeric 3-butyl-serines was constructed between 130 and 180 °C. The separation between any two isomeric pairs can be characterized by an enthalpy of separation, ΔH_{sep}, and an entropy of separation, ΔS_{sep}. The logarithm of the separation factor for the four possible pairs is shown plotted against the reciprocal of the absolute temperature (Fig. 5). The separation between the L- and D-*threo* forms shows no temperature dependence and is, therefore, totally entropic. The L- and D-*allo* pair, however, shows a small but significant enthalpy of approximately 150 cal (628 J).
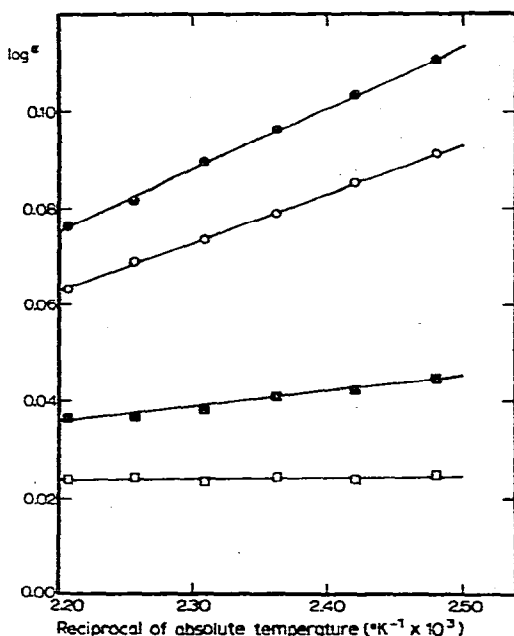

Fig. 5. Log α (α = separation factor) against reciprocal of the absolute temperature for pairs of the isomers of N,O-heptafluorobutyryl-O-L(—)-menthyl esters of the 3-butyl-serines. ● = D-*allo*–D-*threo*; ○ = L-*allo*–L-*threo*; ◼ = D-*allo*–L-*allo*; □ = D-*threo*–L-*threo*; where α is the ratio of the retention times for the two isomers.

The temperature dependence for the separation of the isomeric pairs resulting from inversion at the 3-carbon, *i.e.* L-*allo*–L-*threo* and D-*allo*–D-*threo*, is characterized by higher enthalpic contributions of between 450 and 600 cal (1883 and 2510 J) approaching the value of kT per molecule. By comparison, the temperature dependence for the relative retention times of glycine and the 3-butyl-serines show very much higher enthalpies of 15.6 kcal (65.3 kJ) and 18.4 kcal (77 kJ), respectively.

The predominantly entropic basis for separation of the configurational isomers, suggesting a mechanism involving solute–solvent ordering–disordering phenomena, ha analogies with the low temperature coefficients found for many partition processes[9–12].

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