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Note

Gas chromatographic resolution of substituted glutamic acid enantiomers

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The separation of N-perfluoracylated amino acid ester enantiomers was the first successful application of enantioselective gas chromatography (GC)[1] and was further developed on Chirasil-Val by Franck and co-workers[2–4] and on polysiloxane XE-60–(*S*)-valine–(*S*)- or (*R*)-phenylethylamide by König *et al.*[5,6]. N-Isopropyl or N-*tert.*-butylureido amino acid ester derivatives have also been suggested for enantiomer separations on an XE-60 column[7], but their use was limited by the low thermal stability of the stationary phase.

We describe here the use of an XE-60 column to separate the enantiomers of various substituted glutamic acids as their easily accessible N-trifluoracetyl diisopropyl ester derivatives.

EXPERIMENTAL

Materials

DL-2-Methylglutamic acid was supplied by Aldrich, DL-*threo*- and *erythro*-4-hydroxyglutamic acids[8,9], 4-methylglutamic acids[10,11] and 3-methylglutamic acids[12,13] were prepared and resolved as described previously and D- and L-*threo*- and *erythro*-4-fluoro-[10,14,15] and 3-fluoroglutamic acids[16] were obtained from Dr. M. Gaudry (Laboratoire de Chimie Organique Biologique, Université de Paris VI). With the 3-fluoro compounds, only the N-acetyl derivatives were available. DL-4-Methyleneglutamic acid was prepared by a modification of existing methods[17] and resolved as the N-acetyl derivative using porcine kidney acylase I (Sigma)[18].

Chromatography

GC analysis was performed on a Varian 3700 gas chromatograph equipped with a splitless injector (240°C), a flame-ionization detector (240°C) and a Chrompack fused-silica column (50 m × 0.25 mm I.D.) coated with polysiloxane XE-60–(*S*)-valine–(*S*)-phenylethylamide. Helium was used as the carrier gas with an inlet pressure of 1.5 bar, under isothermal conditions. The peak areas were computed with an electronic Icap5 Delsi integrator.

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Derivatization

Samples of 0.5–1 mg were derivatized in the usual way[2–5] using a 1.5 *N* solution of dry HCl in isopropanol for 30 min at 100°C. After drying with nitrogen, the residue was dissolved in 200 μl of dichloromethane and 50 μl of trifluoroacetic anhydride and left for 30 min at room temperature. The excess of reagent was evaporated in a stream of nitrogen and the residue dissolved in 0.5 ml of dichloromethane for injection. For the derivatization of N-acetyl compounds the second step was omitted.

RESULTS AND DISCUSSION

Analogues of glutamic acid have been extensively investigated in the search of inhibitors for glutamate decarboxylase[19–21] and other enzymes involved in neurotransmitter metabolism in the central nervous system[22,23]. More recently, some of these analogues have been included in substrate peptides for the study of vitamin K-dependent carboxylations of glutamic acid residues[14,24–26]. As most of these amino acids have been prepared in their racemic form by chemical synthesis, then resolved by chemical or enzymatic procedures, it was essential to have a simple analytical method for quantifying their optical purity. Table I illustrates the separation of some substituted glutamic acids as N-trifluoroacetyl O,O'-isopropyl diester derivatives on the polysiloxane XE-60–(*S*)-valine–(*S*)-phenylethylamide column described by König *et al.*[5] and commercialized by Chrompack. Except for the 2-substituted glutamate derivative, both enantiomers and most of the possible diastereoisomers of each compound were completely separated in one injection (Figs. 1–3) using temperatures that were within the thermal stability limits of the column, and it was possible to determine as little as 0.5% of a contaminating enantiomer. The 3-methylglutamic acid diaste-

TABLE I

SEPARATION FACTORS (α) AND ORDER OF ELUTION OF ENANTIOMERS OF N-TRIFLUOROACETYL DIISOPROPYL ESTER DERIVATIVES OF SUBSTITUTED GLUTAMIC ACIDS

Racemate	*Temperature (°C)*	*First enantiomer eluted [retention time (min) from the solvent peak*]*	α
Glutamic acid	165	*R* (11.7)	1.082
2-Methylglutamic acid	160	– (7.8)	1.000
	165	– (6.5)	1.000
4-Methyleneglutamic acid	165	*R* (9.9)	1.086
threo-4-Hydroxyglutamic acid	165	2*R*,4*R* (14.3)	1.058
erythro-4-Hydroxyglutamic acid	165	2*R*,4*S* (12.7)	1.071
threo-3-Methylglutamic acid	160	2*R*,3*S* (10.9)	1.092
erythro-3-Methylglutamic acid	160	2*R*,3*R* (10.9)	1.073
threo-4-Methylglutamic acid	160	2*R*,4*R* (11.8)	1.076
erythro-4-Methylglutamic acid	160	2*R*,4*S* (12.4)	1.105
threo-3-Fluoroglutamic acid**	175	2*S*,3*S* (30.2)	1.065
erythro-3-Fluoroglutamic acid**	175	2*S*,3*R* (40.0)	1.050
threo-4-Fluoroglutamic acid	165	2*R*,4*R* (15.9)	1.063
erythro-4-Fluoroglutamic acid	165	2*R*,4*S* (15.0)	1.053

* Retention time of the solvent peak: 5.0 min.

** As N-acetyl O,O'-diisopropyl ester.

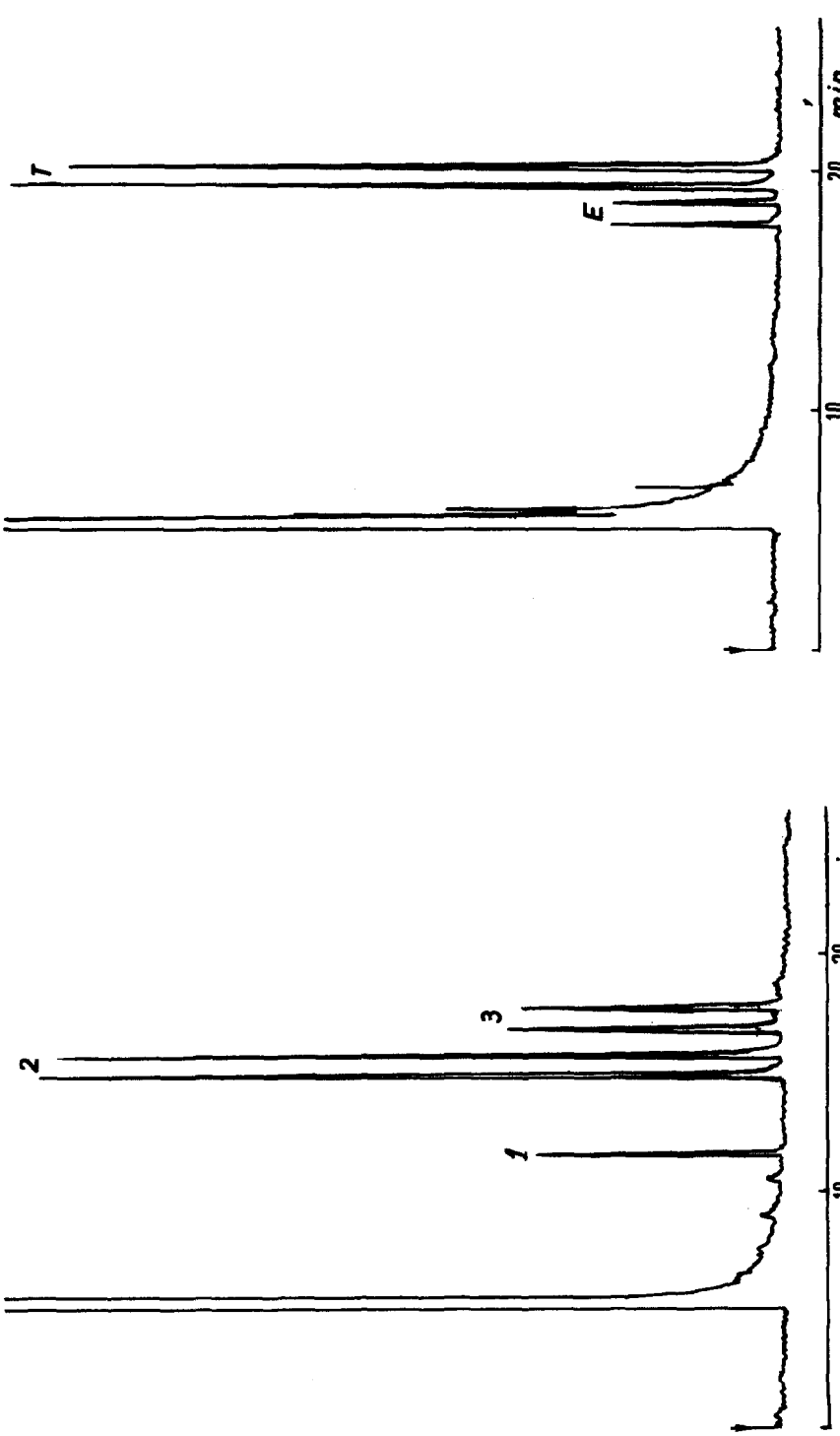

Fig. 1. Separation of enantiomers of N-TFA O,O'-diisopropyl esters of (1) DL-2-methylglutamic acid, (2) DL-4-methyleneglutamic acid and (3) DL-glutamic acid. Column temperature: 165°C.

Fig. 2. Separation of enantiomers of N-TFA O,O'-diisopropyl esters of (T) *threo*- and (E) *erythro*-4-hydroxyglutamic acids. Column temperature: 165°C.

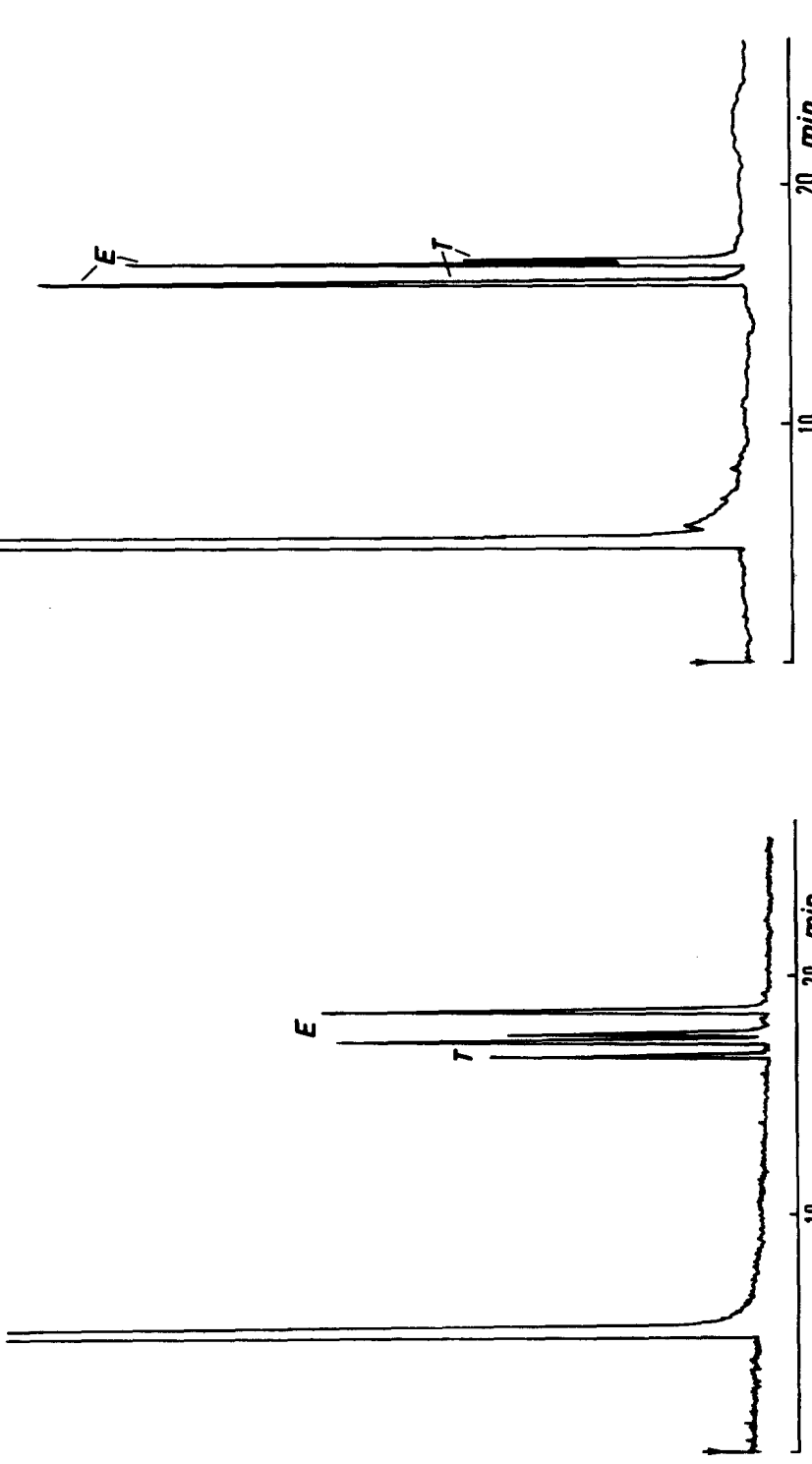

Fig. 3. Separation of enantiomers of N-TFA O,O'-diisopropyl esters of (T) *threo*- and (E) *erythro*-4-methylglutamic acids. Column temperature: 160°C.

Fig. 4. Separation of enantiomers of N-TFA O,O'-diisopropyl esters of (T) *threo*- and (E) *erythro*-3-methylglutamic acids. Column temperature: 160°C.

reoisomers were difficult to separate and their determination could be achieved only on the L-enantiomers (Fig. 4). With the 3-fluoroglutamic acids, for which only the N-acetyl derivatives were available, the separation was possible at the temperature limit of the column (Fig. 5).

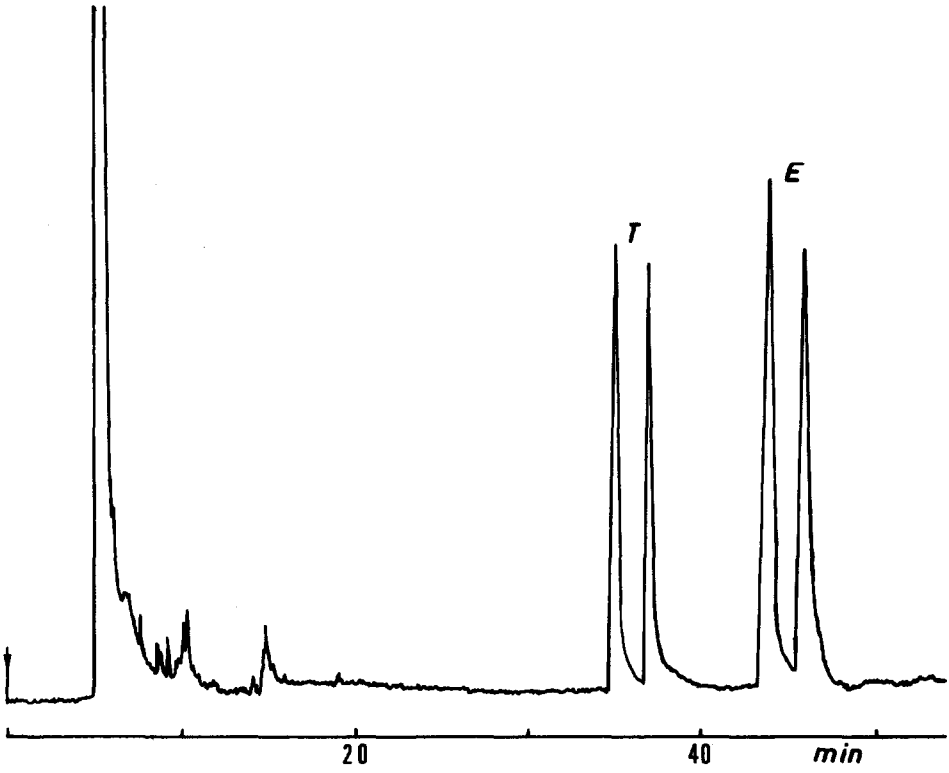

Fig. 5. Separation of enantiomers of N-acetyl O,O'-diisopropyl esters of (T) *threo*- and (E) *erythro*-3-fluoroglutamic acids. Column temperature: 175°C.

As generally noted for the common amino acids[2–6], the L-isomers were more retained on this column [the 2*R* configuration of 3-fluoroglutamic acids corresponds to the L-(2*S*) configuration of the common amino acids]. No particular rule could be deduced from the comparative behaviour of the diastereoisomeric substituted glutamates.

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