Journal of Chromatography, 315 (1984) 421–424
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Note

Simple method for the separation of diastereomers of 4-fluoroglutamic acid by gas–liquid chromatography

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(Received July 5th, 1984)

Fluorinated analogues of many natural organic compounds have long attracted considerable attention due to their remarkable biological properties and to the problems inherent in their synthesis. The effect of substitution of hydrogen by fluorine on the biological properties is often unexpected; it is particularly striking in some amino acids which play a significant role in biological systems.

Many fluorinated amino acids exhibit properties quite different from those of the parent non-fluorinated compounds and some of them have already been used in clinical practice. The synthesis of monofluorinated amino acid derivatives has long been studied in this laboratory[1–4]. One of these compounds, 4-fluoroglutamic acid, was prepared first in 1961 by Hudlický[5]; however, this synthesis, as well as the synthetic procedures described later[2,6–9], yields only a mixture of both diastereomeric forms. Their separation is laborious[10] and a permanent analytical control is necessary. For routine analyses we successfully used gas–liquid chromatography (GLC).

Amino acid analysis by GLC has been extensively studied[12–18]. At present, novel methods of amino acid derivatization suitable for their quantitative GLC analysis continue to appear in the literature. In addition to the well known and tested procedures utilizing N-perfluoroacyl amino acid alkyl esters[12–15], some other techniques, *e.g.*, using (N,O)-heptafluorobutyryloxazolidinones[16], N(O,S)-isobutyloxycarbonyl methyl esters[17], etc., were successfully introduced. A simpler and faster derivatization of 4-fluoroglutamic acid to methyl 3-fluoro-2-pyrrolidone-5-carboxylate is described in this paper.

EXPERIMENTAL

Apparatus

A Varian gas chromatograph, Model 3700 (Varian, Palo Alto, CA, U.S.A.), equipped with a flame ionization detector, was used. Measurements were performed on glass columns (180 cm × 2 mm I.D.), packed with Varaport 30 (100–120 mesh) coated with 3% silicon phases OV-1, OV-17, OV-105, OV-210, OV-225, OV-275, OV-330 and XE-60 (Applied Science Labs., State College, PA, U.S.A.). Samples were analyzed isothermally at column temperatures of 190 and 230°C. The injector temperatures were 230 and 250°C, respectively. Nitrogen at flow-rates of 16 and 36

0021-9673/84/$03.00

ml/min served as carrier gas. Chromatograms were quantitatively evaluated using a Varian integrator, Model CDS 111.

Chemicals

4-Fluoroglutamic acid was synthesized and esterified in this laboratory[2]. Trifluoroacetic anhydride (TFAA) was from Pierce Eurochemie (Rotterdam, The Netherlands). Methanol and orther organic solvents were of analytical grade and were supplied by Lachema (Brno, Czechoslovakia).

Derivatization

Dimethyl N-trifluoroacetyl-4-fluoroglutamate (I) was prepared according to Darbre and Islam[11] by esterification with dry methanolic hydrochloric acid and acylation with TFAA. Methyl 3-fluoro-2-pyrrolidone-5-carboxylate (II) was prepared by thermal cyclization using the procedure previously described for the ethyl ester[2].

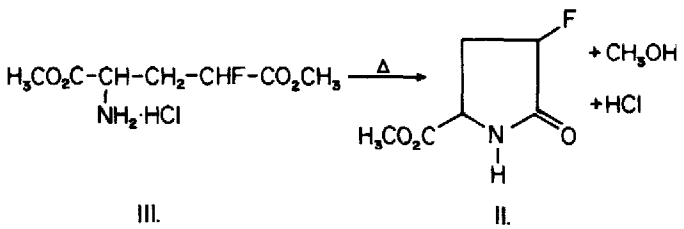
RESULTS AND DISCUSSION

When looking for analytical conditions suitable for the separation of 4-fluoroglutamic acid diastereomers we tried first the volatile derivative, dimethyl N-trifluoroacetyl-4-fluoroglutamate. The conversion into N-perfluoroacyl alkyl esters is known to be a suitable method for amino acid analysis.

The derivatized diastereomers were analyzed on columns packed with silicon stationary phases, 3% OV-225, 3% OV-210, 3% OV-330 and 3% XE-60, isothermally at 190 and 210°C. Under these conditions the diastereomers were only partially separated. A complete separation was achieved only on a column of OV-225 at lower temperatures which, however, result in long retention times and tailing peaks.

In further work we made use of the fact that esters of 4-fluoroglutamic acid are quantitatively cyclized to pyrrolidone derivatives at high temperatures[2]. It was found that for the GLC analysis this conversion need not be performed separately since it occurs directly during the injection of 4-fluoroglutamic acid dimethyl ester hydrochloride (III) in the injection space. The thermal cyclization is illustrated in Fig. 1.

The pyrrolidone (II) produced is a stable compound with a boiling point of 113–117°C at 0.5 mmHg. Its formation at the above injector temperature was verified with a synthetically prepared internal standard. Sharp, non-tailing peaks were obtained when using the direct sampling of the ester III and the diastereomers were completely separated.



III. II.

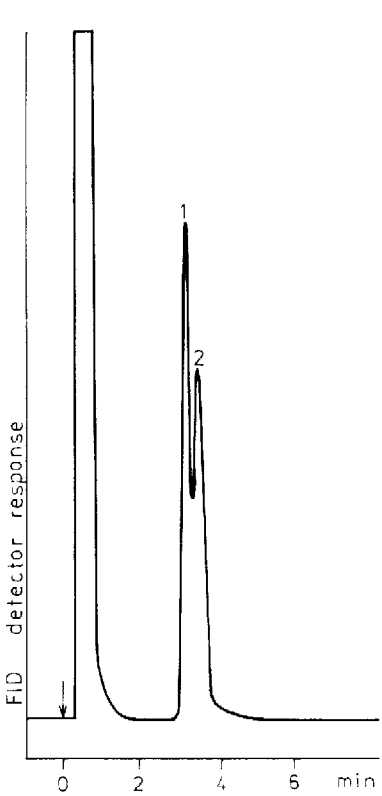
Fig. 1. Thermal cyclization of 4-fluoroglutamic acid dimethyl ester hydrochloride (III) to methyl 3-fluoro-2-pyrrolidone-5-carboxylate (II).

TABLE I

RETENTION INDICES, *I*, OF DIASTEREOMERS OF 4-FLUOROGLUTAMIC ACID AFTER CONVERSION INTO PYRROLIDONE DERIVATIVE

α = Separation factor.

Stationary phase	*Temperature (°C)*	*I*		α
		Erythro form	*Threo form*	
OV-1	190	1405	1460	1.21
OV-105	190	1529	1567	1.28
OV-17	190	2952	2125	1.29
OV-210	190	2234	2305	1.30
OV-330	230	2348	2422	1.28
OV-275	230	2635	3520	1.38



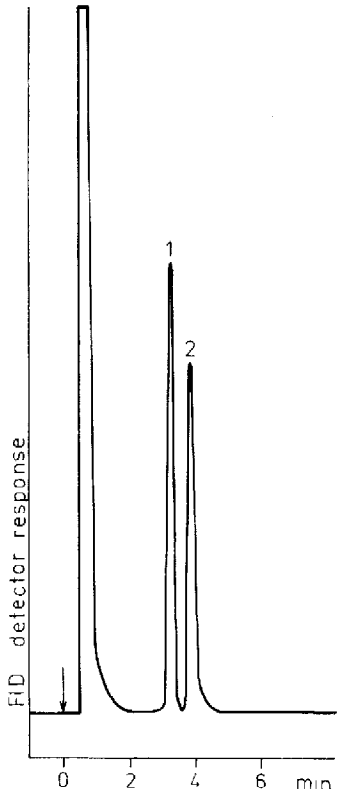


Fig. 2. Separation of diastereomers of 4-fluoroglutamic acid in the form of N-TFA methyl esters. Conditions: column, 180 cm × 2 mm I.D., glass, filled with 3% OV-330; temperature 230°C; nitrogen flow-rate 16 ml/min; flame ionization detector. Peaks: 1 = *erythro* form; 2 = *threo* form.

Fig. 3. Separation of diastereomers of 4-fluoroglutamic acid in the form of cyclic pyrrolidones. Details as in Fig. 2.

The compound was analyzed on several columns with different silicon phases at column temperatures of 190 and 230°C. The best separation of diastereomers was achieved on the phases OV-330 and OV-17. Retention indices were calculated using the customary method with non-branched hydrocarbons as reference compounds. The values on different stationary phases at different temperatures are summarized in Table I. Chromatograms obtained after both types of pre-column derivatization are illustrated in Figs. 2 and 3.

It is concluded that the latter of the two procedures of pre-column derivatization, *i.e.*, the conversion to cyclic pyrrolidone II, is substantially faster and simpler. In addition, the diastereomers are much better separated.

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