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Short communication

Enantiomer separation of amino acids by capillary gas chromatography using cyclodextrin derivatives as chiral stationary phases

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Abstract

Amino acids were derivatized to N(O)-trifluoroacetyl methyl and isopropyl esters and studied with respect to the gas chromatographic separation of their enantiomers by using capillary columns coated with four types of cyclodextrin derivatives of 6-O-*tert.*-butyldimethylsilyl-2,3-di-O-acetyl or *n*-butyryl-β- and -γ-cyclodextrin. All amino acids could be separated into enantiomeric pairs except Trp. Methyl ester derivative showed the best separation on octakis(2,3-di-O-*n*-butyryl-6-O-*tert.*-butyldimethylsilyl)-γ-cyclodextrin and isopropyl esters on heptakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin. Pro showed complete separation as its N(O)-trifluoroacetyl isopropyl ester derivative, and the same derivative of Ala showed a very high separation factor of 1.808 at 100°C.

1. Introduction

Open-tubular capillary columns coated with chiral stationary phases are a powerful tool for the gas chromatographic (GC) determination of enantiomeric composition [1,2]. For amino acid enantiomers, Chirasil-Val is the most thoroughly studied capillary column and is capable of separating all protein amino acid enantiomeric mixtures almost completely in the form of N(O)-perfluoroacyl alkyl esters in a single run within 30 min [3]. This column has been applied to the determination of the optical purity of amino acids. However, it gives an unsatisfactory separation of the Pro enantiomeric pair, which is important in biomedical research [4,5]. Chiral recognition with Chirasil-Val can be attributed to diastereomeric associations formed mainly by hydrogen bonding between chiral solutes and the phase. Therefore, with Pro, the only amino acid with a pyrrolidine ring, and which possesses no amide hydrogen in its N-perfluoroacyl alkyl ester derivative, it is difficult to carry out an adequate interaction enantioselectively with this phase.

Recently, modified cyclodextrins have been introduced as chiral stationary phases in capillary GC and proved to be powerful tools in the enantioselective determination of volatile chiral compounds with different functional groups, including amino acids [6–10]. In this work, we

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investigated the GC separation of amino acid enantiomers as their N(O)-trifluoroacetyl (TFA) methyl and isopropyl ester derivatives on four types of cyclodextrin derivatives coated on capillaries.

2. Experimental

2.1. Synthesis of cyclodextrin derivatives

Four cyclodextrin derivatives were prepared: heptakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin (CD-1), octakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-γ-cyclodextrin (CD-2), heptakis(2,3-di-O-*n*-butyryl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin (CD-3) and octakis(2,3-di-O-*n*-butyryl-6-O-*tert.*-butyldimethylsilyl)-γ-cyclodextrin (CD-4). Samples of β- and γ-cyclodextrins were obtained form Wako (Osaka, Japan).

6-O-*tert.*-Butyldimethylsilyl-β- and γ-cyclodextrins were prepared according to the literature [11,12]. The raw products were purified by silica gel column chromatography using chloroform–methanol (3:1, v/v) as the eluent. The purified products were diacetylated with acetic anhydride in pyridine according to the cited references. The products were purified by chromatography on a silica gel column with toluene–ethanol (10:1, v/v) as the eluent. 2,3-Di-*n*-butyrylation was carried out in analogy with the procedure described in ref. [10]. The raw products were purified by silica gel column chromatography with toluene–ethyl acetate (5:1, v/v) as the eluent.

2.2. Preparation of glass capillary columns

Glass capillaries (0.8 mm O.D., 0.25 mm I.D.) were drawn from borosilicate glass tubing (Pyrex, 8 mm O.D., 2.5 mm I.D.) on a Shimadzu GDM-1B glass-drawing machine. The capillaries were leached with 6 *M* HCl, dehydrated and silylated with diphenyltetramethyldisilazane according to Grob [13]. The capillaries were coated with 10% of the cyclodextrin derivative dissolved in SE-30 (CD-1, CD-3, CD-4) or OV-1701 (CD-2) by a static method using a 0.3% solution of the stationary phases in CH_2Cl_2–*n*-C_5H_{12} (1:1, v/v).

2.3. Instrumentation

A Shimadzu Model 7AG gas chromatograph equipped with a flame ionization detector was used throughout. The carrier gas was helium and split-mode injection was used. The output signal was processed by a Shimadzu C-R1A digital integrator.

3. Results and discussion

Table 1 gives the separation factors of N(O)-TFA methyl esters of fifteen amino acid enantiomers on four different types of cyclodextrin derivatives. Similarly, Table 2 gives the separation factors of N(O)-TFA isopropyl esters of amino acid enantiomers. The highest separation factor for each amino acid is given in italics. The differences in the enantioselectivities of these four cyclodextrin derivatives towards amino acid derivatives can be easily established from these results.

With N(O)-TFA methyl esters, CD-4 showed the best enantioselectivity. High values of the separation factor could be obtained from Pro (1.563, 100°C) and Asp (1.406, 120°C); however, Thr, Phe, and Trp could not be separated. The highest values of the separation factor were observed for Thr on CD-3 (1.348, 120°C) and Phe on CD-1 (1.084, 130°C). None of these four phases could separate Trp enantiomers.

For N(O)-TFA isopropyl esters, CD-1 was the most efficient. Ala showed a very high separation factor of 1.808 at 100°C. The separation factors of Thr and Ser were 1.694 (110°C) and 1.292 (120°C), respectively. However, the other amino acids showed lower values of the separation factor than with the N(O)-TFA methyl ester derivatives. Especially Pro and Asp showed very low values, and there was no separation from Trp on the four phases. Notwithstanding, Pro could be separated completely on CD-4 with separation factor of 1.236 (100°C).

Table 1
Separation factors for N(O)-TFA methyl esters of amino acid enantiomers

Amino acid	Separation factor[a]				Column temperature (°C)
	CD-1	CD-2	CD-3	CD-4	
Ala	1.128	1.125	1.102	1.124	100
Val	1.102	1.146	1.042	1.243	100
Ile	1.112	1.131	1.013	1.288	100
Leu	1.137	1.109	1.000	1.229	100
Pro	1.198	1.398	1.315	1.563	100
Thr	1.290	1.168	1.348	1.000	120
Asp	1.310	1.300	1.000	1.406	120
Ser	1.347	1.369	1.108	1.158	130
Glu	1.103	1.169	1.000	1.262	130
Phe	1.084	1.009	1.017	1.000	130
Met	1.017	1.049	1.040	1.138	140
Tyr	1.021	1.022	1.070	1.029	160
Orn	1.088	1.026	1.077	1.098	170
Lys	1.054	1.020	1.021	1.071	170
Trp	1.000	1.000	1.000	1.000	170

The highest separation factor for each amino acid is underlined.
[a] Stationary phases: CD-1 = heptakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin; CD-2 = octakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-γ-cyclodextrin; CD-3 = heptakis(2,3-di-O-*n*-butyryl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin; CD-4 = octakis(2,3-di-O-*n*-butyryl-6-O-*tert.*-butyldimethylsilyl)-γ-cyclodextrin.

Table 2
Separation factors for N(O)-TFA isopropyl esters of amino acid enantiomers

Amino acid	Separation factor[a]				Column temperature (°C)
	CD-1	CD-2	CD-3	CD-4	
Ala	1.808	1.156	1.117	1.233	100
Val	1.078	1.012	1.039	1.000	100
Ile	1.020	1.011	1.000	1.000	100
Leu	1.061	1.021	1.025	1.000	100
Pro	1.016	1.034	1.017	1.236	100
Thr	1.694	1.114	1.453	1.042	110
Ser	1.292	1.075	1.000	1.000	120
Asp	1.016	1.000	1.000	1.000	130
Glu	1.029	1.000	1.000	1.000	130
Met	1.048	1.018	1.030	1.000	140
Phe	1.052	1.013	1.000	1.000	140
Tyr	1.047	1.049	1.000	1.000	160
Orn	1.137	1.069	1.069	1.000	180
Lys	1.073	1.022	1.025	1.000	180
Trp	1.000	1.000	1.000	1.000	180

The highest separation factor for each amino acid is underlined.
[a] See Table 1.

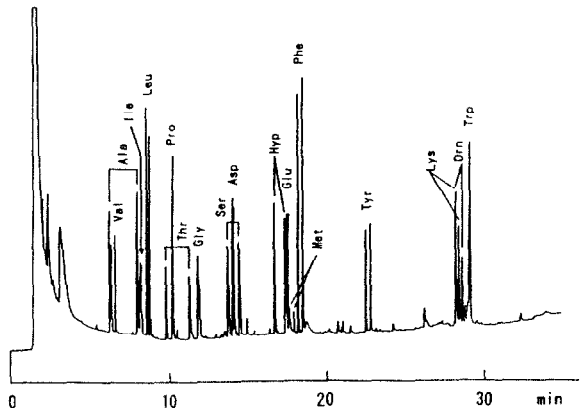

Fig. 1. Gas chromatogram of N(O)-TFA isopropyl esters of amino acid enantiomeric mixture. Column, glass capillary (20 m × 0.25 mm I.D.) coated with heptakis(2,3-di-O-acetyl-6-O-*tert.*-butyldimethylsilyl)-β-cyclodextrin–SE-30 (1:9, w/w). Column temperature, 100°C, held for 4 min, then programmed at 4°C/min to 220°C. For each amino acid enantiomeric pair, the D-enantiomer eluted faster.

From the data in Tables 1 and 2, the cyclodextrin derivative with the largest cavity and with a large substituent (*n*-butyryl) at the 2- and 3-positions (CD-4) gave the best results for the separation of N(O)-TFA methyl esters of amino acid enantiomers. On the other hand, the amino acid derivatives esterified with larger-sized alcohol of N(O)-TFA isopropyl esters could be separated best on the cyclodextrin derivative with smallest cavity size acylated with a smaller substituent (acetyl) at the 2- and 3-positions. The reasons for these unexpected results are not yet understood. Konig *et al.* [10] reported that N(O)-TFA methyl esters of amino acid enantiomers can be separated almost completely on octakis(3-O-*n*-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin. Taking into account the simultaneous use with Chirasil-Val in practical applications, the choice of the N(O)-TFA isopropyl ester derivatives is considered to be more versatile. Fig. 1 shows a representative gas chromatogram of N(O)-TFA isopropyl esters of an amino acid enantiomeric mixture on CD-1. Pro enantiomers can hardly be separated. Nevertheless, the same derivative of Pro enantiomers could be separated completely on CD-4 without interference from other amino acid peaks (Fig. 2).

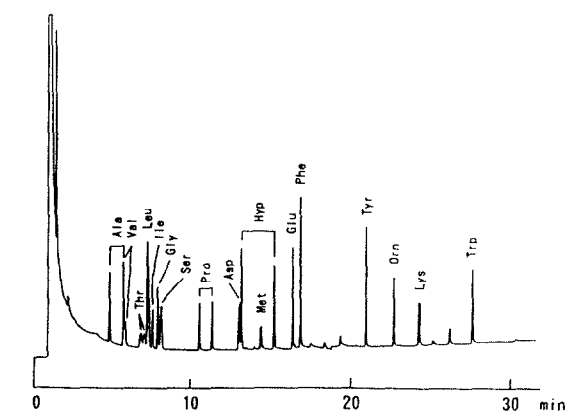

Fig. 2. Gas chromatogram of N(O)-TFA isopropyl esters of amino acid enantiomeric mixture. Column, glass capillary coated with octakis(2,3-di-O-*n*-butyryl-6-O-*tert.*-butyldimethylsilyl)-γ-cyclodextrin–SE-30 (1:9, w/w). Column temperature, 90°C, held for 4 min, then programmed at 4°C/min to 200°C. For other conditions, see Fig. 1 and text.

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