Journal of Chromatography, 605 (1992) 103–107
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 175

Direct enantiomeric separation of phenylalanine, DOPA and their intermediates by supercritical fluid chromatography

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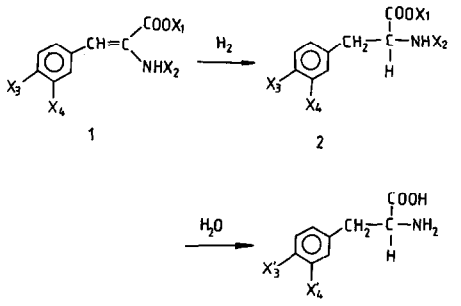
(First received December 6th, 1991; revised manuscript received March 12th, 1992)

ABSTRACT

Phenylalanine (Phe), 3-(3,4-dihydroxyphenyl)alanine (DOPA) and their corresponding intermediates [N-acyl alkyl esters of Phe, DOPA, 3-(3,4-dimethoxylphenyl)alanine, 3-(3-methoxyl-4-hydroxyphenyl)alanine and 3-(3,4-methylenedioxyphenyl)alanine] were enantiomerically separated by supercritical fluid chromatography with carbon dioxide as the mobile phase in a cross-linked polycyanoethyl vinyl siloxane, L-Val-*tert.*-butylamide fused-silica capillary column. The effects of substituents in the benzene ring and the acyl and alkyl groups of the intermediates or derivatives of Phe and DOPA on enantioselectivity were investigated. The optical purities of some intermediates of Phe and DOPA were determined.

INTRODUCTION

Phenylalanine (Phe) is an important amino acid and can be used as a nutriment and food additive [1–3]. Many of its substituted optically active isomers are of great importance in pharmacology; L-DOPA, for example, can be used to treat Parkinson's disease [4]. These optically active compounds can be obtained by asymmetric hydrogenation [5] and then normal hydrolysis [6], as shown below.



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The key step in the synthesis is the asymmetric hydrogenation of a prochiral alkene (**1**) to a specific optical isomer of Phe or a substituted Phe derivative (**2**) [7]. The selectivity of the key step can be determined by the enantiomeric excess of intermediate **2** [8]. The enantiomeric separation of compound **2** is of great significance in pharmacology, asymmetric synthesis and biochemistry. In intermediate, **2**, X_1 is usually a methyl or ethyl group and x_2 an acetyl (Ac) or benzoyl (Bz) group [9,10]. Most of the intermediates are of low volatility and are not suitable for separation by gas chromatography (GC). Supercritical fluid chromatography (SFC) with chiral stationary phases (CSPs) can be used to separate enantiomers of low volatility under mild conditions and with a higher enantioselectivity (α value) than GC [11,12].

The use of several CSPs in SFC with carbon dioxide as the mobile phase was reviewed by Macaudiere *et al.* [13]. Immobilized Chirasil-Dex [14] and Chirasil-Val [15] have been reported for the separation of enantiomers by SFC.

In this work, the SFC separation of Phe, DOPA

0021-9673/92/$05.00

and their corresponding intermediates with cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide was investigated.

EXPERIMENTAL

Chemicals and materials

Phe was obtained from Sigma, and DOPA and the N-acyl alkyl esters of (substituted) Phe isomers were kindly supplied by Dr. X. Liu. Blank fused-silica capillary tubes were from Yongnian Optical Fibre Manufacturer.

Chromatographic conditions

The cross-linked polycyanoethyl vinyl siloxane-L-V-*tert.*-butylamide fused-silica capillary column (10 m × 70 μm I.D.) was prepared as described previously [16]. The SFC experiments were carried out with a laboratory-made SFC chromatograph equipped with a flame ionization detector [17]. Phe and DOPA were derivatized as N-trifluoroacetyl (TFAc) isopropyl ester according to the method of McKenzie and Tenaschuk [18].

RESULTS AND DISCUSSION

In SFC with carbon dioxide as the mobile phase, the density of the mobile phase has no influence on the separation factors (α) of the solutes [11]. The capacity factors (k') and separation factors (α) of *N*-TFAc-Phe isopropyl ester and N-Ac-Phe isopropyl ester at different pressures are given in Table I.

The classical method of derivatization of Phe for separation by GC and SFC is their conversion into N-perfluoroacyl alkyl esters [19]. Replacing TFAc by pentafluoropropionyl or heptafluorobutyryl has little effect on the α and k' values in GC [20]. Other acylation reagents have not yet been fully investigated.

The α and k' values of the solutes tested in SFC are given in Table II.

For Phe, the α value of *N*-TFAc-Phe isopropyl ester is much greater than N-Bz-Phe isopropyl ester but much lower than N-Ac-Phe isopropyl ester; that is, the α values of Phe are greatly changed by replacing TFAc with Ac or Bz. The k' value of N-TFAc-Phe isopropyl ester increased significantly

TABLE I

α AND k' VALUES OF N-TFAc-Phe ISOPROPYL ESTER AND N-Ac-Phe ISOPROPYL ESTER AT DIFFERENT PRESSURES IN SFC (60°C)

Solute	13.0 MPa		14.0 MPa		15.0 MPa	
	α	k'	α	k'	α	k'
N-TFAc-Phe isopropyl ester	1.17	0.48	1.17	0.11	–	–
N-Ac-Phe isopropyl ester	–	–	1.21	0.40	1.22	0.13

TABLE II

α AND k' VALUES OF N-ACYL-(SUBSTITUTED) Phe ALKYL ESTERS IN SFC (65°C)

Solute	α	k'	Pressure (MPa)
N-TFAc-Phe isopropyl ester	1.16	0.23	14.0
N-Ac-Phe isopropyl ester	1.19	0.56	14.0
N-Ac-Phe methyl ester	1.16	0.67	14.0
N-Bz-Phe methyl ester	1.09	0.88	16.0
N-Ac-3-(3,4-dimethoxyphenyl)alanine ethyl ester	1.17	0.31	16.0
N-Bz-3-(3,4-dimethoxyphenyl)alanine isopropyl ester	1.14	0.77	16.0
N-Bz-3-(3,4-dimethoxyphenyl)alanine methyl ester	1.11	0.89	16.0
N-Bz-3-(3,4-methylenedioxyphenyl)alanine methyl ester	1.09	0.96	16.0
N-Bz-3-(3-methoxy-4-hydroxyphenyl)alanine methyl ester	1.12	1.17	16.0
N-TFAc-DOPA isopropyl ester	1.16	0.32	14.0

TABLE III

ENANTIOMERIC EXCESS OF SOME SYNTHESIZED N-ACYL-(SUBSTITUTED) Phe ALKYL ESTERS DETERMINED BY SFC

	PNNP[a] (%)	PMEO[b] (%)
N-Ac-Phe-methyl ester	–	78
N-Ac-3-(3,4-dimethoxyphenyl)alanine ethyl ester	71	75
N-Bz-Phe-methyl ester	55	–
N-Bz-3-(3,4-dimethoxyphenyl)alanine methyl ester	–	92
N-Bz-3-(3,4-methylenedioxyphenyl)alanine methyl ester	67	–
N-Bz-3-(3-methoxy-4-hydroxyphenyl)alanine methyl ester	71	–

[a] Enantiomeric excess of D-enantiomers with PNNP [N,N′-bis(*S*-1-phenylethyl)-N,N′-bis-(diphenylphosphino)ethylenediamine] as the chiral ligand of rhodium catalyst.

[b] Enantiomeric excess of L-enantiomers with PMEO [N,N′-bis(*R*-1-(*p*-methoxyphenyl)ethyl-N,N′-bis(diphenylphosphino)ethylenediamine] as the chiral ligand of rhodium catalyst.

when N-TFAc was replaced with N-Ac and increased further when replaced with N-Bz.

The α values of isopropyl esters of N-acyl-(substituted)-Phe are greater than their corresponding methyl esters as reported in separation by GC [21]. The retention behaviour of methyl esters of N-acyl-(substituted)-Phe and their corresponding isopropyl esters in GC and SFC was also compared. In GC, methyl esters usually elute before their corresponding isopropyl esters [21], whereas in SFC the elution order of the tested samples is changed, *i.e.* methyl esters elute after their corresponding isopropyl, esters (Table III and Fig. 1). According to Matire and Boehm [22], the solute capacity factor (k value) in SFC can be represented by

$$\ln k = \ln k^0 + F(T_R, \rho_R) \quad (1)$$

where T_R and ρ_R are the reduced temperature and reduced density of the mobile phase and K^0 is the capacity factor corresponding to ideal GC. The change of elution order of the tested methyl and isopropyl esters of N-acyl-(substituted)-Phe is probably due to their difference in solubility in carbon dioxide. When the benzene ring of Phe is substituted with 3,4-dimethoxyl or 3-methoxy-4-hydroxy or 3,4-methylenedioxy, the α values were slightly increased.

From these results, the following conclusions can be made. (1) Both the acyl and alkyl groups of N-acyl-(substituted)-Phe isopropyl esters have considerable effects on the α and k' values. Replacing TFAc with the Ac or Bz group, the changes in α values are large; (2) The substituents in the benzene ring of Phe have some effect on the α values. The substituted isomers tested do not have α values lower than their corresponding N-acyl-Phe alkyl esters.

The enantiomeric excesses of some synthesized N-acyl-(substituted)-Phe alkyl esters determined by SFC are given in Table III.

Fig. 2 is a chromatogram of the enantiomeric

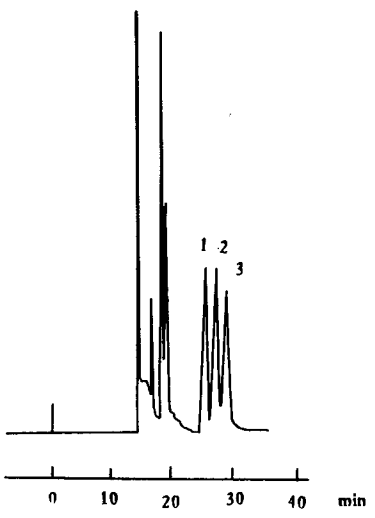

Fig. 1. Chromatogram of D,L-N-Ac-Phe isopropyl ester and L-excess D,L-N-Ac-Phe methyl ester by SFC. Column, cross-linked polycyanoethyl vinyl siloxane-L-Val-*tert.*-butylamide (10 m × 70 μm); temperature, 60°C; mobile phase, carbon dioxide, 13.5 MPa; detector, flame ionization. Peaks: 1 = D-N-Ac-Phe isopropyl ester; 2 = L-N-Ac-Phe isopropyl ester + D-N-Ac-Phe methyl ester; 3 = L-N-Ac-Phe methyl ester.

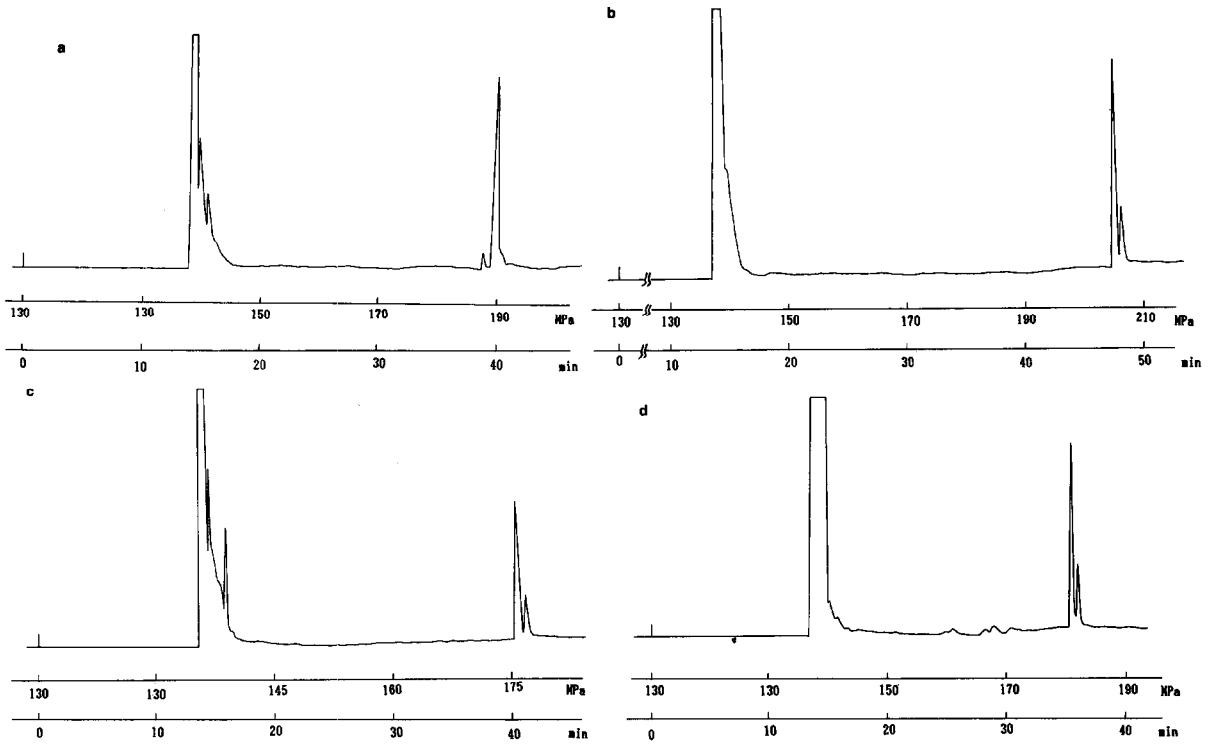

Fig. 2. Enantiomeric separation of some intermediates of Phe and DOPA by SFC. Chromatographic conditions as in Fig. 1. (a) N-Bz-3-(3,4-dimethoxyphenyl)alanine methyl ester; (b) N-Bz-3-(3-methyoxy-4-hydroxyphenyl)alanine methyl ester; (c) N-Bz-3-(3,4-methylenedioxyphenyl)alanine methyl ester; and (d) N-Bz-Phe methyl ester. D-enantiomers eluted first.

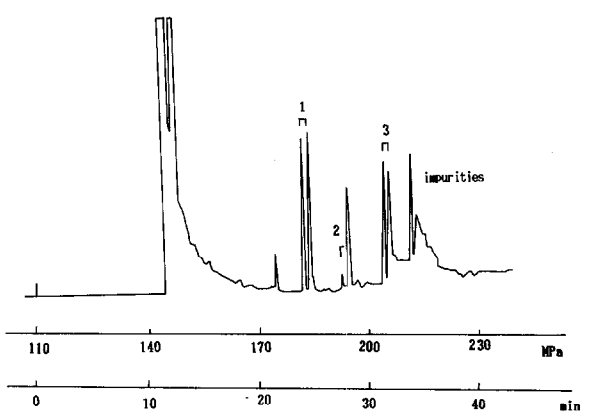

Fig. 3. Chromatogram of some N-acyl-(substituted)-Phe alkyl ester enantiomers by SFC. Chromatographic conditions as in Fig. 1 with mobile phase carbon dioxide (11.0 MPa) increased at 0.3 MPa/min. Peaks: 1 = D,L-N-TFAc-Phe isopropyl ester; 2 = D,L-N-Ac-Phe methyl ester; 3 = D,L-N-Ac-3-(3,4-dimethoxyphenyl)alanine methyl ester. D-enantiomers eluted first.

separation of some intermediates by SFC with pressure programming.

The enantiomeric separation of some N-acyl-(substituted)-Phe alkyl esters with pressure programming by SFC is shown in Fig. 3.

CONCLUSIONS

The acyl groups, alkyl groups and substituents in the benzene ring of N-acyl-(substituted) Phe alkyl esters have considerable effects on both α and k' values. When TFAc is replaced with Ac or Bz, the α values of the solutes tested were greatly changed.

ACKNOWLEDGEMENTS

We thank Professors Daoqian Zhu, Qinghai Wang and Yafong Guang for technical help and valuable discussions. This work was supported by the National Natural Science Foundation of China

and Youth Science Foundation of Dalian Institute of Chemical Physics.

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