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Monitoring of optical isomers of some conformationally constrained amino acids with tetrahydroisoquinoline or tetraline ring structures

Antal Péter*[,a], Géza Tóth[b], Dirk Tourwé[c]

[a]*Department of Inorganic and Analytical Chemistry, Attila József University, P.O. Box 440, H-6701 Szeged, Hungary*
[b]*Hungarian Academy of Sciences, Biological Research Centre, Isotope Laboratory "B" Level, P.O. Box 521, H-6721 Szeged, Hungary*
[c]*Eenheid Organische Chemie, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium*

Abstract

Conformationally constrained amino acids were synthesized in optically pure or racemic forms: D- and L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, its *erythro*-D,L-4-methyl analogue, D- and L-1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid, D- and L-1,2,3,4-tetrahydro-7-hydroxy-6,8-dibromo- and -6,8-diiodo-isoquinoline-3-carboxylic acid and D,L-6-hydroxy-2-aminotetraline-2-carboxylic acid. A method was developed for the separation and identification of optical isomers using precolumn derivatization with chiral derivatization reagents: 1-fluoro-2,4-dinitrophenyl-4-L-alaninamide and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate. The effects of pH, eluent composition and different buffers on the separation were also investigated.

1. Introduction

Diastereomeric peptides, isomers in which one or more asymmetric centres have opposite configurations, may have similar or different physico-chemical or biological properties. The biological activities often exhibit antagonistic or agonistic properties which differ greatly from each other.

In the synthesis of receptor-selective peptides, unusual racemic amino acids are often used, or the originally pure amino acid can racemize to some extent in the course of protection and synthesis. It is therefore very important to develop effective chromatographic methods for the separation of racemic amino acids and for the characterization and identification of optically pure products.

Several papers and reviews have been published on the development of enantioselective separations. High-performance liquid chromatographic (HPLC) methods can be divided into three main groups: direct separation on chiral columns [1–3], separation on achiral columns with chiral mobile-phase additives [3–5] and separation of the diastereoisomers formed by precolumn derivatization with chiral reagents [3,6–17].

Some conformationally constrained aromatic amino acids have been synthesized in our laboratory for the design of receptor-selective opioid

* Corresponding author.

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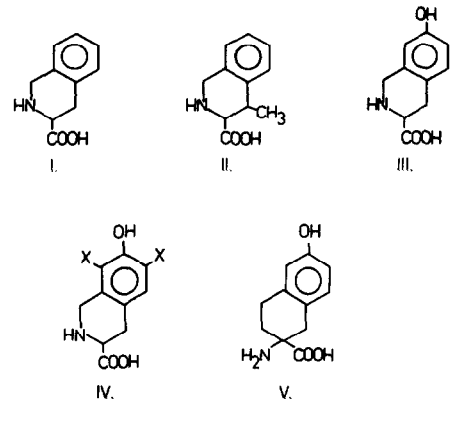

Fig. 1. I = Tic, D- and L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; II = β-Me-Tic [*erythro*, (*SS*, *RR*)], D,L-1,2,3,4-tetrahydro-4-methylisoquinoline-3-carboxylic acid; III = HOTic, D- and L-1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid; IVa = Br_2HOTic and IVb = I_2HOTic, D- and L-1,2,3,4-tetrahydro-7-hydroxy-6,8-dihaloisoquinoline-3-carboxylic acid; V = Hat, D,L-6-hydroxy-2-aminotetraline-2-carboxylic acid.

peptides (Fig. 1). This paper describes the separation of isomers of these unusual amino acids. For this purpose, an HPLC method was applied, using precolumn derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (FDAA, Marfey reagent) and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylisothiocyanate (GITC). The effects of the eluent composition and of the nature of the buffer on the separation of D- and L-isomers were investigated under isocratic conditions.

2. Experimental

2.1. Chemicals and reagents

The amino acids in Fig. 1 were prepared by literature methods: in optically pure form I [18], III [19] and IV [19], and in racemic form II [20] and V [21]. All compounds were checked by melting point determination, ^{1}H NMR spectrometry, fast atom bombardment MS and chiral TLC [22].

FDAA and L-amino acid oxidase were purchased from Sigma (St. Louis, MO, USA), GITC from Aldrich-Chemie (Steinhem, Germany) and trifluoroacetic acid, sodium acetate, potassium dihydrogenphosphate of analytical-reagent grade, HPLC-grade solvents (acetonitrile and methanol) and other reagents of analytical-reagent grade from Merck (Darmstadt, Germany). Buffers were prepared with doubly distilled water and further purified by pumping through a 0.45-μm CA filter (Paraplan, Budapest, Hungary). The pH was adjusted with phosphoric acid (phosphate buffer), acetic acid (acetate buffer) or sodium hydroxide.

Chiral TLC was performed on Chiralplates (Macherey–Nagel, Düren, Germany) with detection using 0.1% ninhydrin spray reagent.

2.2. Apparatus

The HPLC system consisted of an L-6000 liquid chromatographic pump (Merck–Hitachi, Tokyo, Japan), a Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA), a variable-wavelength UV 308 spectrophotometric detector (Labor MIM, Budapest, Hungary) and an HP 3395 integrator (Hewlett-Packard, Waldbroun, Germany). The columns used were Vydac 218 TP 104 C_{18} (250 × 4.6 mm I.D., 10 μm particle size) (Separations Group, Hesperia, CA, USA) and Nucleosil 10C_{18} (250 × 4.6 mm I.D., 10 μm particle size) (Macherey–Nagel).

^{1}H NMR spectroscopy was performed on an AM 400 spectrometer (Brucker, Zug, Switzerland).

2.3. Derivatization of amino acids

Amino acids (0.5–1 mg) were derivatized with FDAA or GITC by the method of Marfey [12] or Nimura *et al.* [9].

2.4. Enzymatic digestion of *erythro*-D,L-β-MeTic

erythro-D,L-β-MeTic (0.3 mg) was dissolved in 0.1 *M* Tris buffer (pH 7.2) in a test-tube and 0.3 mg of L-amino acid oxidase was added. The test-tube was filled with oxygen, tightly closed and incubated for 24 h at 37°C. The reaction mixture was used for derivatization reactions.

The configuration of D,L-Hat could not be determined directly with L-amino acid oxidase because of the lack of an α-hydrogen. The sequence of isomers was presumed by analogy with other amino acids.

3. Results and discussion

3.1. Separation of FDAA derivatives

The HPLC separation of the derivatized amino acids was carried out in the three different aqueous buffer systems containing acetonitrile as organic modifier: 0.1% trifluoroacetic acid, 0.01 *M* potassium dihydrogenphosphate (pH 3) and 0.01 *M* sodium acetate (pH 3); pH 3 was chosen on the basis of preliminary experiments and our earlier work on D,L-β-methylphenylalanine separations [23]. The k' values at low buffer pH were very high, probably because of the total protonation of the amino acids. At higher pH, the R_s values decreased.

It is interesting to compare the effects of the organic modifier content of the eluent and of different buffers on the separation of the isomers. With a decrease in the organic content of the mobile phase from 50% to 40–35%, k' increased and the separation of the L- and D-forms improved. In some instances an acceptable separation was attained (Table 1). A further decrease in the acetonitrile concentration to 30% improved the resolution of the L- and D-forms of I, II and III (Figs. 2–4), whereas only a partial resolution of V was observed even at 20% acetonitrile. IVa and b exhibited large values of k' (>30) at 30% acetonitrile, and the use of a high concentration of organic modifier is therefore favourable (Fig. 5).

Investigation of the effects of the buffers on the separation showed that the TFA systems have high k' values compared with the phosphate and acetate systems, probably because the pH of the eluent was lower than 3. In the sodium acetate system, the k' values (except that for II) were lower and the resolution and peak shape also seemed better (Figs. 2–5).

Table 1
Dependence of capacity factor (k'), separation factor (α) and resolution (R_s) of FDAA derivatives on eluent composition

Amino acid	Eluent[a]	k'_L	k'_D	α	R_s
I	TFA	11.00	18.00	1.64	3.88
	KH_2PO_4	9.55	15.53	1.61	2.15
	NaOAc	5.50	8.50	1.54	3.33
II	TFA	20.66	25.53	1.23	1.60
	KH_2PO_4	17.17	22.50	1.31	2.34
	NaOAc	22.66	28.22	1.24	3.64
III	TFA	5.86	9.00	1.54	2.24
	KH_2PO_4	5.20	8.00	1.54	2.15
	NaOAc	3.82	5.66	1.48	2.36
IVa	TFA (40:60)	15.05	17.50	1.16	1.02
	KH_2PO_4 (35:65)	11.00	14.96	1.30	1.23
	NaOAc (35:65)	8.86	12.50	1.41	1.30
IVb	TFA (35:65)	21.13	22.61	1.07	0.98
	KH_2PO_4 (35:65)	15.66	17.53	1.12	1.07
	NaOAc (35:65)	15.11	21.20	1.40	2.20
V	TFA (20:80)	8.33	8.91	1.07	0.43
	KH_2PO_4 (20:80)	6.67	7.27	1.09	0.42
	NaOAc (20:80)	6.27	6.77	1.08	0.49

Column, Vydac 218 TP 104 C_{18} (250 × 4.6 mm I.D.); flow-rate, 1 ml/min.
[a] CH_3CN–buffer (30:70). Buffer: TFA = 0.1% trifluoroacetic acid; KH_2PO_4 = 0.01 *M* KH_2PO_4 (pH 3); NaOAc = 0.01 *M* CH_3COONa (pH 3).

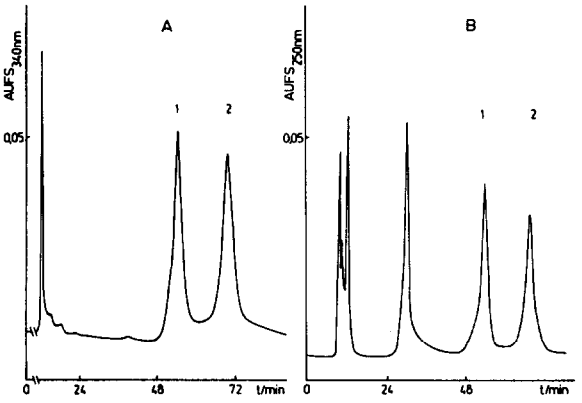

Fig. 2. Chromatograms of Tic derivatives. Column: Vydac 218 TP 104 C_{18} (250 × 4.6 mm I.D.); flow-rate, 1 ml/min; eluent, acetonitrile–0.01 *M* sodium acetate (pH 3) (30:70); derivatization reagent, (A) FDAA and (B) GITC. Peaks: 1 = L-Tic; 2 = D-Tic.

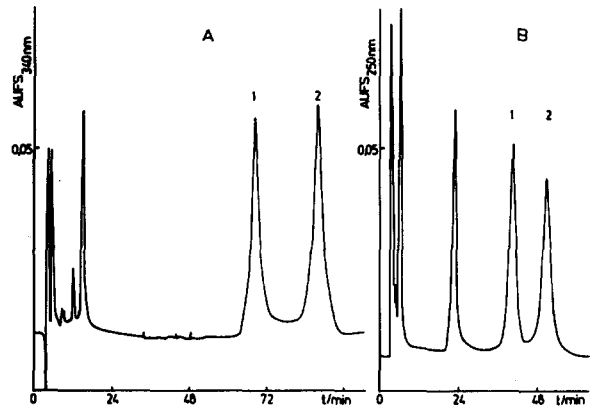

Fig. 3. Chromatograms of β-Me-Tic derivatives. Conditions as in Fig. 2. Peaks: 1 = L-β-MeTic; 2 = D-β-MeTic.

3.2. Separation of GITC derivatives

The separations were carried out with the same eluent systems and the results are summarized in Table 2. The GITC derivatives of amino acids had lower k' values than those of the FDAA derivatives. Good separations were achieved for I, II and III at 30% acetonitrile (Figs. 2–4). IVa and b had lower k' values than those of the FDAA derivatives and the peaks remained somewhat overlapped. Surprisingly, the high k' values for V were not accompanied by good resolution. When the organic modifier was changed from acetonitrile to methanol, a good resolution with low k' values was observed with the sodium acetate buffer system (Fig. 6).

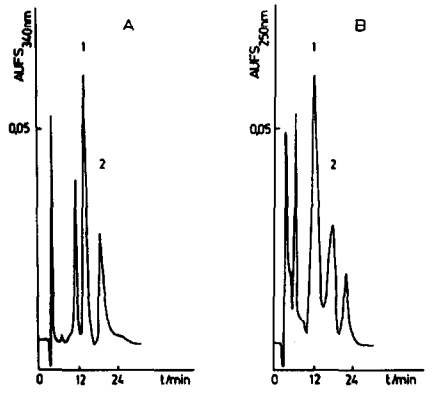

Fig. 4. Chromatograms of HOTic derivatives. Conditions as in Fig. 2. Peaks: 1 = L-HOTic; 2 = D-HOTic.

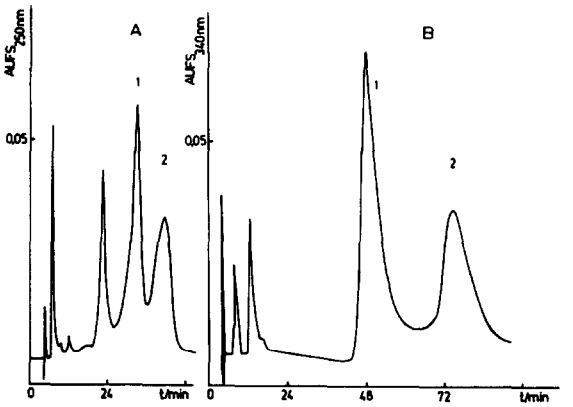

Fig. 5. Chromatograms of Br_2HOTic and I_2HOTic derivatives. Column, Vydac 218 TP 104 C_{18} (250 × 4.6 mm I.D.); flow-rate, 1.0 ml/min; eluent, (A) acetonitrile–0.01 *M* sodium acetate (pH 3.0) (30:70) and (B) acetonitrile–0.01 *M* sodium acetate (pH 3.0) (35:65). (A) Br_2HOTic + GITC; (B) I_2HOTic + FDAA. Peaks: 1 = L-isomer; 2 = D-isomer.

4. Conclusions

The described procedures can be applied to the separation and identification of conformationally constrained unusual aromatic amino acids. The acetonitrile–sodium acetate systems are generally more efficient than TFA or phosphate systems in the separation of isoquinoline

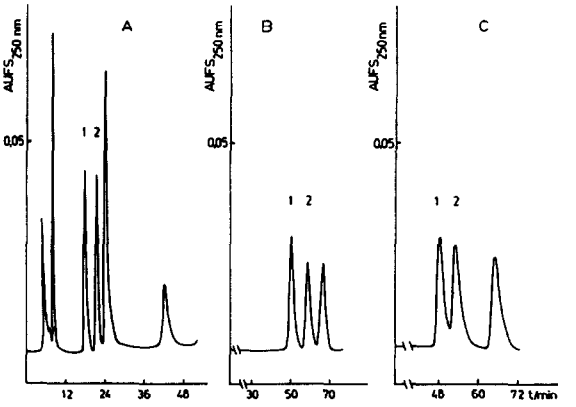

Fig. 6. Chromatograms of Hat derivatives. Column: Vydac 218 TP 104 C_{18} (250 × 4.6 mm I.D.); flow-rate, 1.0 ml/min; eluent, (A) methanol–0.01 *M* sodium acetate (pH 3) (40:60), (B) methanol–0.01 *M* potassium dihydrogenphosphate (pH 3) (35:65) and (C) methanol–0.1% trifluoroacetic acid (35:65); derivatization reagent, GITC. Peaks 1 = L-Hat; 2 = D-Hat.

Table 2
Dependence of capacity factor (k'), separation factor (α) and resolution (R_s) of GITC derivatives on eluent composition

Amino acid	Eluent[a]	k'_L	k'_D	α	R_s
I	TFA	11.48	15.53	1.35	1.83
	KH_2PO_4	9.56	12.33	1.29	2.50
	NaOAc	6.78	10.00	1.59	4.11
II	TFA	17.20	2.08	1.33	1.66
	KH_2PO_4	15.47	20.11	1.30	3.10
	NaOAc	12.74	16.23	1.27	2.18
III	TFA	4.07	6.27	1.53	1.16
	KH_2PO_4	4.01	6.22	1.55	1.34
	NaOAc	3.08	4.66	1.52	1.32
IVa	TFA	12.11	14.77	1.22	0.92
	KH_2PO_4	14.23	17.08	1.20	1.19
	NaOAc	10.00	12.77	1.28	1.32
IVb	TFA (35:65)	9.44	10.95	1.16	0.89
	KH_2PO_4 (35:65)	11.27	13.41	1.19	1.08
	NaOAc (35:65)	6.86	8.37	1.22	1.33
V	TFA (20:80)	23.00	25.33	1.11	0.96
	KH_2PO_4 (20:80)	22.33	25.33	1.13	0.85
	NaOAc (20:80)	14.80	16.86	1.14	0.73
V[b]	TFA (35:65)	15.33	16.94	1.10	1.12
	KH_2PO_4 (35:65)	15.83	18.43	1.16	1.49
	NaOAc (35:65)	4.72	5.88	1.25	1.78

Column, Vydac 218 TP 104 C_{18} (250 × 4.6 mm I.D.).
[a] CH_3CN–buffer (30:70). Buffer: TFA = 0.1% trifluoroacetic acid; KH_2PO_4 = 0.01 *M* KH_2PO_4 (pH 3); NaOAc = 0.01 *M* CH_3COONa (pH 3).
[b] Organic modifier: CH_3OH.

ring-containing amino acids. GITC derivatives have lower k' values than those of FDAA derivatives. Dibromo- and diiodohydroxy-Tic exhibit large k' values with characteristic band broadening. The optical isomers of hydroxy-aminotetralincarboxylic acid could only be analysed in the methanol-containing system.

5. Acknowledgement

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