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Chiral Liquid Chromatography in analysis of the stereochemistry of marine natural compounds: contribution for Medicinal Chemistry

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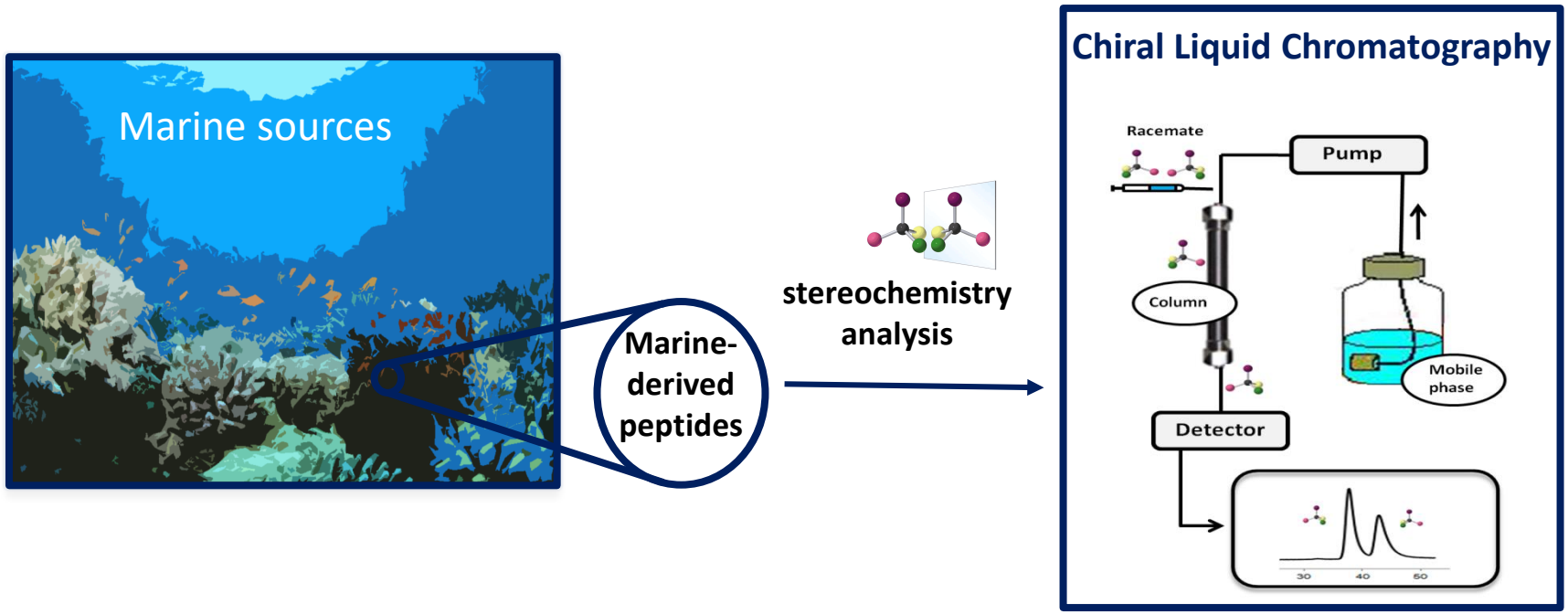
ciimar Centro Interdisciplinar de Investigação Marinha e Ambiental

Chiral Liquid Chromatography in analysis of the stereochemistry of marine natural compounds: contribution for Medicinal Chemistry

Graphical Abstract



Abstract:

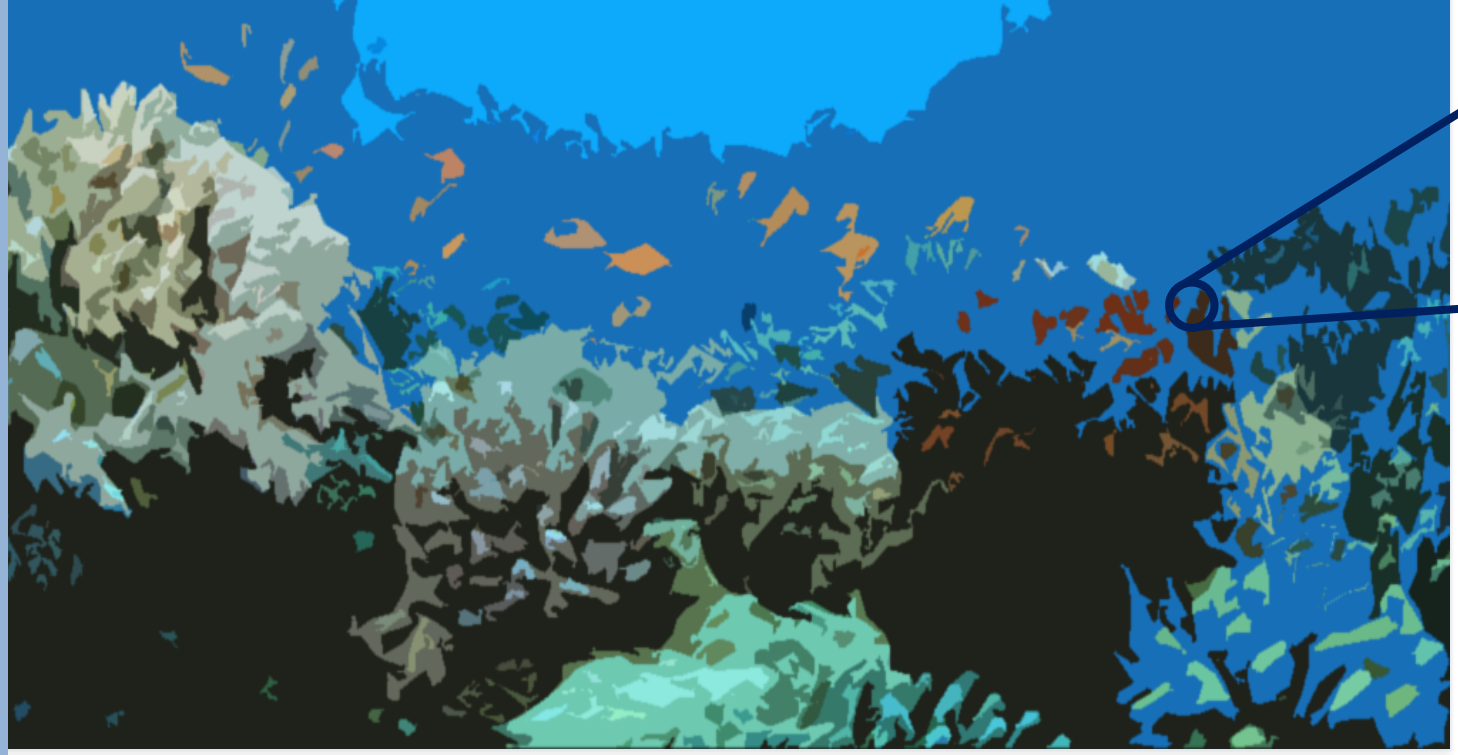
In Medicinal Chemistry many naturally occurring peptides have been used as pharmaceuticals or as models for drugs used in therapeutics. Thus, marine-derived peptides are certainly an interesting source for new drugs. Taking into account the mechanisms of molecular recognition and the influence of molecular three-dimensionality in this process, it is essential to define the amino acids components of the peptide fractions isolated from marine sources.
Herein, we describe the determination of the stereochemistry of the amino acid residues of three bioactive marine natural products, by chiral LC analysis of their acidic hydrolysates, using appropriate D and L amino acids standards. The enantioseparations of the amino acids were successfully performed on Chirobiotic T^{TM} column under reversed-phase elution conditions. Actually, the teicoplanin selector of this column has several characteristic features that make it suitable for amino acid analysis. The elution order of all the standards amino acids enantiomers was confirmed by injecting solutions of the racemic or enantiomeric mixtures and then each enantiomer separately.
Chiral LC technique demonstrated to be decisive leading to the unambiguous elucidation of the amino acid constituents of the three bioactive marine natural products.

Keywords: marine peptides; chiral liquid chromatography; stereochemistry; amino acids

INTRODUCTION

MARINE-DERIVED PEPTIDES

In Medicinal Chemistry many naturally occurring peptides have been used as pharmaceuticals or as models for drugs used in therapeutics.



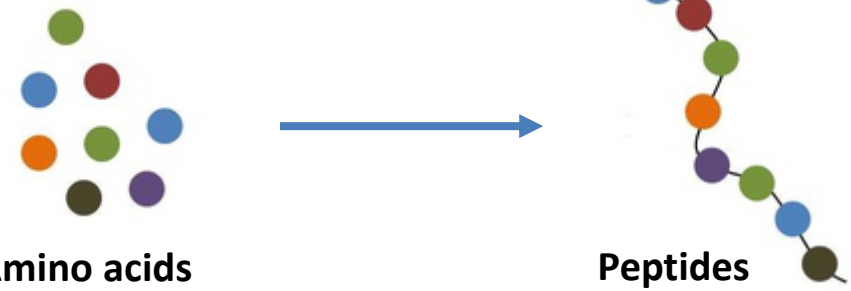
Marine-derived peptides

interesting source for new drugs

Saleem, M.; Ali, M.S.; Hussain, S.; Jabbar, A.; Ashraf, M.; Lee, Y.S. Marine natural products of fungal origin. Nat. Prod. Rep. 24 (2007) 1142–1152.

INTRODUCTION

PEPTIDES – CHIRAL MOLECULES



Taking into account:

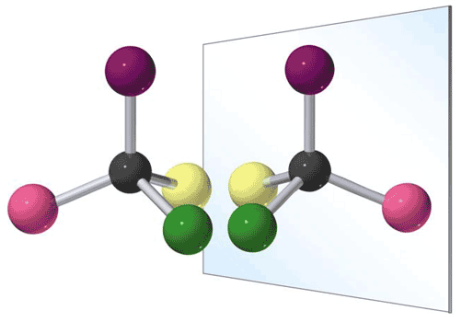
- the mechanisms of molecular recognition
- the influence of molecular three-dimensionality

in this process

It is essential

to define the amino acids of the peptide fractions isolated from marine sources

CHIRAL MOLECULES

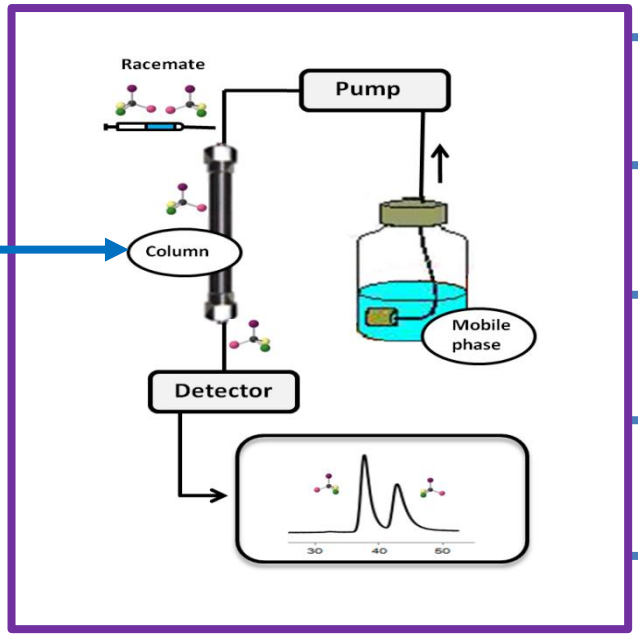


INTRODUCTION

CHIRAL LIQUID CHROMATOGRAPHY

LIQUID CHROMATOGRAPHY

CHIRAL



Very helpful and highly applicable method for:

- Preparative resolution of racemates
- Determination of the enantiomeric purity
- Monitoring enantiomeric reactions
- Analysis of the stereochemistry of natural compounds
- Other applications

M.E. Sousa, M.E. Tiritan, K.R.A. Belaz, M. Pedro, M.S.J. Nascimento, Q.B. Cass, M.M.M. Pinto, J. Chromatogr. A, 1120 (2006) 75-81.
B. Silva, C. Fernandes, M.E. Tiritan, M.M.M. Pinto, M.J. Valente, M. Carvalho, P.G. de Pinho, F. Remião, Forensic Toxicol., (2016) 1-14.
C. Fernandes, P. Brandão, A. Santos, M.E. Tiritan, C. Afonso, Q.B. Cass, M.M. Pinto, J. Chromatogr. A, 1269 (2012) 143-153.
C. Prompanya, C. Fernandes, S. Cravo, M.M.M. Pinto, T. Dethoup, A.M.S. Silva, A. Kijjoa, Mar. Drugs, 13 (2015) 1432-1450.

INTRODUCTION

CHIRAL STATIONARY PHASES

Pirkle-type

Polysaccharide-based

Macrocyclic antibiotics-based

Cyclodextrin-based

Crown ether-based

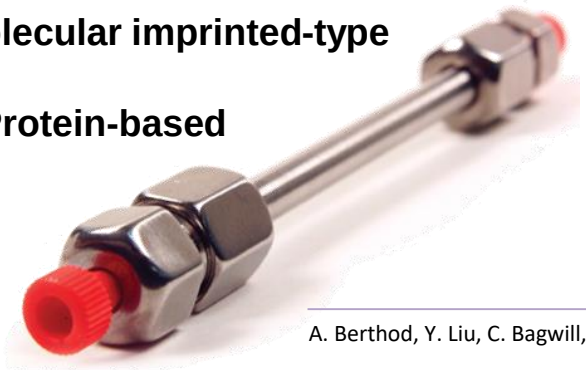
Ion- and ligand-exchange-type

Synthetic polymer-based

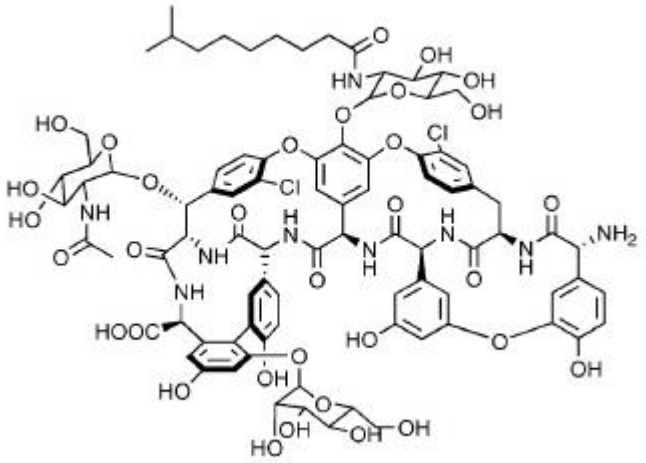
Cyclofructan-based

Molecular imprinted-type

Protein-based



Teicoplanin-based CSP
(Chirobiotic T™)



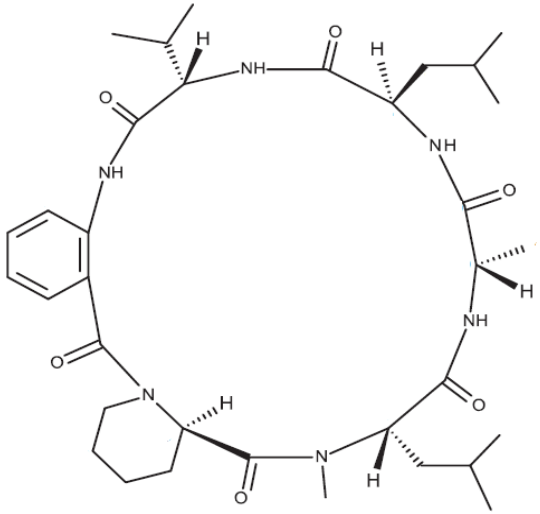
Teicoplanin selector has several distinctive features that make it **suitable for amino acid analysis**.

A. Berthod, Y. Liu, C. Bagwill, D.W. Armstrong, J. Chromatogr. A, 731 (1996) 123-137.

RESULTS AND DISCUSSION

MARINE-DERIVED CYCLOPEPTIDES

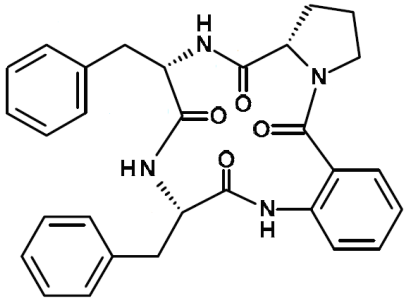
Cyclohexapeptide



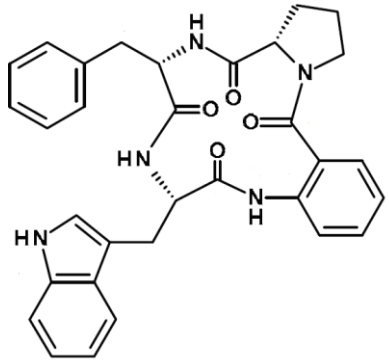
1

Isolated from marine sponge-associated fungus *Aspergillus similanensis* KUFA 0013

Cyclotetrapeptides



2



3

Isolated from marine sponge-associated fungus *Neosartorya glabra* KUFA 0702

C. Prompanya, C. Fernandes, S. Cravo, M.M.M. Pinto, T. Dethoup, A.M.S. Silva, A. Kijjoa, Mar. Drugs, 13 (2015) 1432-1450.
W.W.M. Zin, S. Buttachon, T. Dethoup, C. Fernandes, S. Cravo, M.M.M. Pinto, L. Gales, J.A. Pereira, A.M.S. Silva, N. Sekeroglu, A. Kijjoa, Mar. Drugs, 14 (2016).

STEREOCHEMISTRY OF AMINO ACIDS

The stereochemistry of the amino acids was determined by chiral HPLC analysis of the acidic hydrolysate from cyclopeptides (**1**, **2** and **3**).

Acid Hydrolysis

- Dissolution of cyclopeptide in 6 N HCl
- Heating at 110°C in a furnace (24 h)
- Cooling to room temperature
- Drying under N_2
- Addition of MeOH HPLC grade
- Filtration through Syringe Filter (0.2 µm pore size)

Chiral HPLC analysis

Chiral HPLC analysis

Chromatographic conditions

Chiral column: Chirobiotic T^{TM} (15 cm × 4.6 mm I.D., 5 µm particle size)

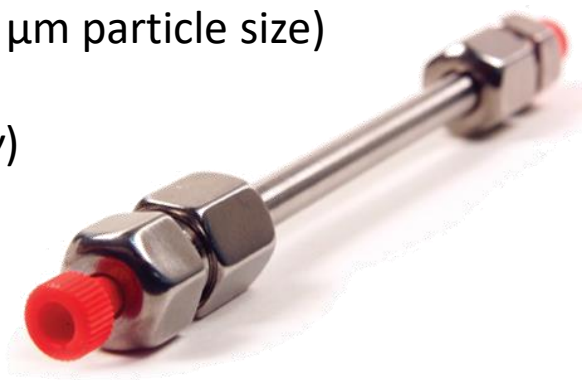
Mobile phase: MeOH:H_2O:CH_3CO_2H (70:30:0.02, *v/v/v*)
or MeOH:H_2O (80:20 *v/v*)

Flow rate: 0.5 mL/min or 1.0 mL/min

Detection: UV at 210 nm

Room temperature

Isocratic mode



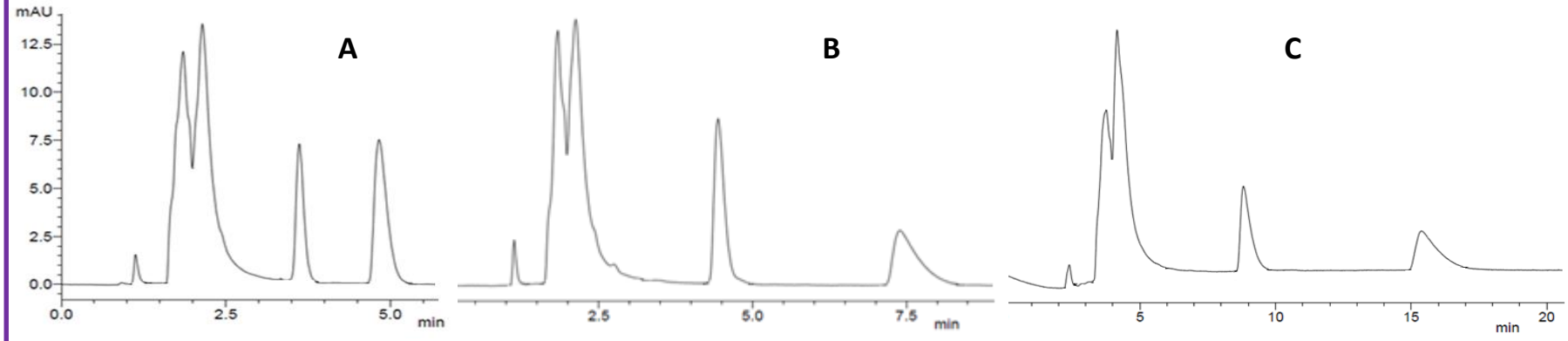
HPLC system consisted of Shimadzu LC-20AD pump, equipped with a Shimadzu DGV-20A5 degasser, a Rheodyne 7725i injector fitted with a 20 µL loop, and a SPD-M20A DAD detector (Kyoto, Japan). Data acquisition was performed using Shimadzu LCMS Lab Solutions software, version 3.50 SP2.

Enantioseparation of standards amino acids

Single enantiomeric amino acids: solutions of 1 mg/mL in MeOH (10 µL sample injection)

Enantiomeric mixtures: mix equal aliquots of each enantiomer (20 µL sample injection)

Examples



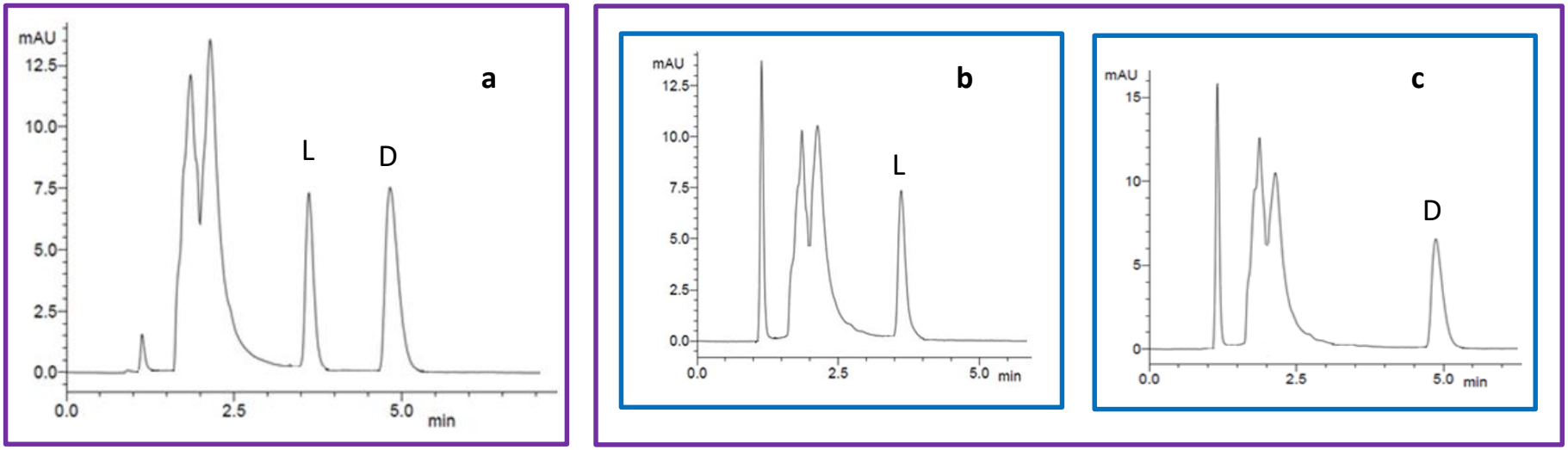
Chromatograms of enantiomeric mixture of DL-alanine **(A)**, DL-pipecolic acid **(B)** and DL-*N*-methyl-valine **(C)**.

Column, Chirobiotic T; mobile phase, MeOH:H_2O (80:20 *v*/*v*); flow rate, 1.0 mL/min (A and B) or 0.5 mL/min (C); detection, 210 nm.

Elution order of standards amino acids

The elution order of the enantiomers of all the standards amino acids was confirmed by injecting the solutions of enantiomeric mixtures, and then each enantiomer separately.

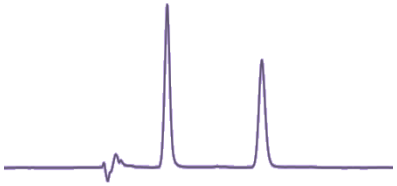
Example



Chromatograms of enantiomeric mixture of DL-alanine **(a)**, L-alanine **(b)**, and D-alanine **(c)**.

Column, Chirobiotic T; mobile phase, MeOH:H_2O (80:20 *v*/*v*); flow rate, 1 mL/min; detection, 210 nm.

Chiral HPLC analysis of the acidic hydrolysates of 1, 2 and 3 by co-injection with amino acids standards



	Retention time (min)		Retention time (min)
anthranilic acid (A)	1.92	**D- tryptophan** (A)	5.20
L-valine (B)	6.60	**Acidic hydrolysate of 1** (B)	6.59, 7.20, 8.09, 8.83, 9.67, 10.57, 14.69
D-valine (B)	8.32	**Acidic hydrolysate of 1 + DL-valine (co-injection)** (B)	6.61, 7.31, 8.30, 8.10, 8.84, 9.70, 10.50, 14.95
L-alanine (B)	7.16	**Acidic hydrolysate of 1 + DL-alanine (co-injection)** (B)	6.59, 7.19, 8.04, 8.81, 9.37, 9.70, 10.50, 14.90
D-alanine (B)	9.36	**Acidic hydrolysate of 1 + DL-leucine (co-injection)** (B)	6.60, 6.76, 7.26, 8.04, 8.83, 9.67, 10.54, 15.02
L-leucine (B)	6.78	**Acidic hydrolysate of 1 + DL-pipecolic acid (co-injection)** (B)	6.58, 7.20, 8.09, 8.64, 8.84, 9.77, 10.64, 14.64
D-leucine (B)	9.67	**Acidic hydrolysate of 1 + *N*-methyl-L-leucine (co-injection)** (B)	6.59, 7.20, 8.09, 8.83, 9.67, 10.57, 14.69
L-pipecolic acid (B)	8.68	**Acidic hydrolysate of 2** (A)	1.91, 2.55, 2.86, 3.49, 3,89, 6.79
D-pipecolic acid (B)	14.67	**Acidic hydrolysate of 2 + DL-phenylalanine (co-injection)** (A)	1.87, 2.50, 2.89, 3.68, 5.01, 6.82
***N*-methyl-L-leucine**	8.09	**Acidic hydrolysate of 2 + DL-proline (co-injection)** (A)	1.96, 2.60, 2.96, 3.52, 3,92, 6.70, 21.09
L-phenylalanine (A)	3.81	**Acidic hydrolysate of 3** (A)	1.93, 3.07, 3,80, 4.29, 4.60, 6.62
D- phenylalanine (A)	5.00	**Acidic hydrolysate of 3 + DL-phenylalanine (co-injection)** (A)	1.90, 3.10, 3,78, 4.39, 5.04, 6.70
L-proline (A)	6.72	**Acidic hydrolysate of 3 + DL-proline (co-injection)** (A)	2.04, 3.02, 3,72, 4.30, 4.60, 6.66, 19.40
D-proline (A)	20.10	**Acidic hydrolysate of 3 + DL-tryptophan (co-injection)** (A)	1.93, 2.99, 3,70, 4.29, 4.60, 5.07, 6.33
L- tryptophan (A)	4.51		

Column, Chirobiotic T; mobile phase, methanol:water:acetic acid (70:30:0.02 *v/v/v*) (A) or MeOH:H_2O (80:20 *v/v*) (B); flow rate, 1 mL/min (A) or 0.5 mL/min (B); detection, 210 nm.

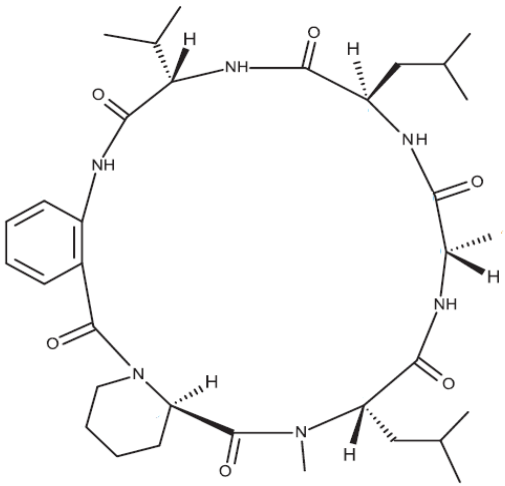
CONCLUSIONS

- The D-enantiomer was always more strongly retained than the corresponding L-enantiomer on Chirobiotic T^{TM} column.

- Mix HPLC analyses of the acidic hydrolysates with standard amino acids (co-injection) confirmed the stereochemistry of the amino acids of cyclopeptides **1**, **2** and **3**.

Cyclopeptide 1

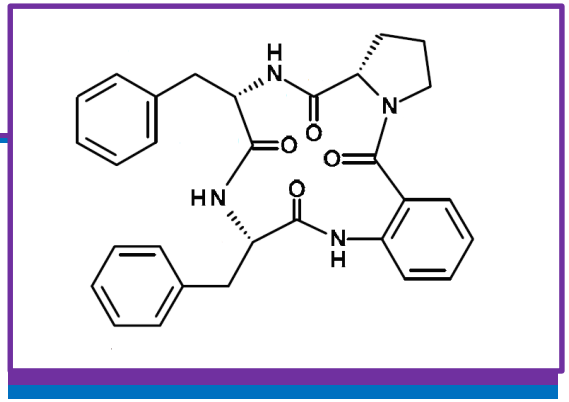
Elucidated unambiguously as
cyclo (anthranilic acid-L-Val-D-Leu-
L-Ala-*N*-methyl-L-Leu-D-pipecolic acid)



CONCLUSIONS

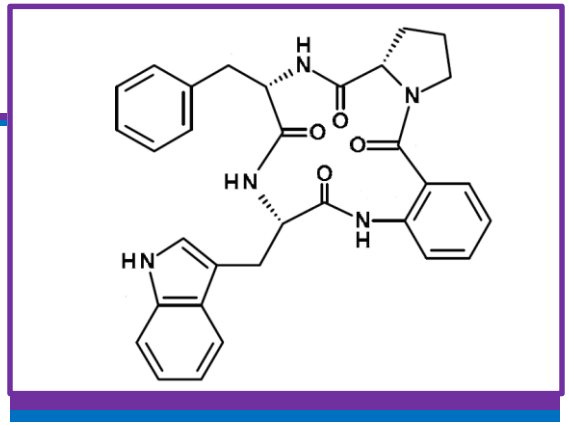
Cyclopeptide 2

Elucidated unambiguously as cyclo (anthranilic acid-L-Phe-L-Phe-L-Pro)



Cyclopeptide 3

Elucidated unambiguously as cyclo (anthranilic acid-L-Trp-L-Phe-L-Pro)



Acknowledgments

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