ELSEVIER

Journal of Chromatography A, 873 (2000) 283–286

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Isolation of pure (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine from *Blighia sapida* (Akee)

Benedetto Natalini[a,*], Vita Capodiferro[a], Carlo De Luca[b], Romulo Espinal[c]

[a]*Istituto di Chimica e Tecnologia del Farmaco, Università degli Studi, Via del Liceo 1, 06123 Perugia, Italy*
[b]*C.S.E.C.F.I. (CNR), Via del Castro Laurenziano 7, 00161 Rome, Italy*
[c]*Dep. Farmacologia (C.I.M.B.U.C.), Universidad de Carabobo, Valencia, Venezuela*

Received 12 July 1999; received in revised form 1 December 1999; accepted 2 December 1999

Abstract

The isolation of (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (CCG I, **2**) from *Blighia sapida* (Akee) was achieved through column chromatography on deactivated silica gel followed by ion-exchange chromatography. A HPLC method has also been devised in order to assess the purity of the isolated product.

Keywords: Blighia sapida; Carboxycyclopropylglycine; Cyclopropaneamino acids; Amino acids

1. Introduction

Among the conformationally constrained glutamate analogs, carboxycyclopropylglycines constitute an excellent source of potent and selective ligands for the various members of the glutamate receptor family, including ionotropic receptors, metabotropic receptors, and uptake carrier proteins. In 1987 [1] it was reported that *cis*-(2*S*,1′*R*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CGA C, **1**) is a potent agonist of the *N*-methyl-D-aspartate (NMDA) site of the NMDA receptor complex [2]. Subsequently, *trans*-(2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (CCG I, **2**) was reported [3] to be a potent group II metabotropic glutamate receptors agonist (Fig. 1). A number of synthetic methods have been employed to prepare CCG I (**2**) [4–8]. The possibility of avoiding the expensive synthetic procedure is offered by a natural source. Since 1969 [9] this non proteinogenic amino acid has been known to be produced by *Blighia sapida* (Akee) along with other cyclopropyl amino acids. This tree grows in West Africa, the Caribbean, Central and South America, and South

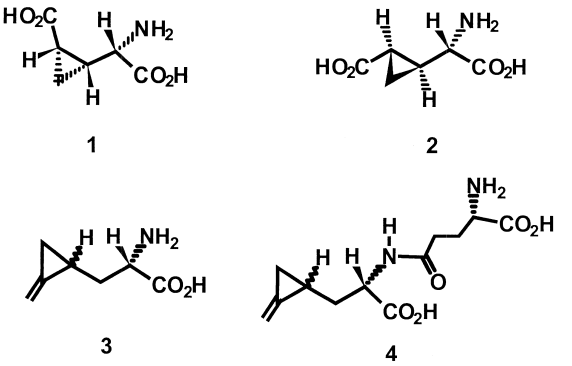

Fig. 1. CNS active amino acids: (**1**) (2*S*,1′*R*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (L-CGA C); (**2**) (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (CCG I); (**3**) α-(methylene cyclopropylmethyl)glycine (hypoglycin A, HG-A); (**4**) hypoglycin B (HG-B).

*Corresponding author.

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01281-9

Florida. Consumption of unripened fruit has been implicated as the cause of the condition referred to as Jamaican vomiting sickness (JVS) [10], characterized by severe hypoglycemia, vomiting, depletion of liver glycogen, accumulation of fat in the liver and increased plasma free fatty acid concentration, mainly attributed to the presence of hypoglycin A [α-(methylenecyclopropylmethyl)glycine, HG-A, **3**] and its less toxic γ-glutamyl peptide, hypoglycin B (HG-B, **4**) (Fig. 1). Moreover, it has been suggested that ingesting small amounts of HG-A over long periods may cause cellular injury with possible liver damage [11], profound central depression and convulsions [12].

The presence in Akee of a selective mGluR2 agonist opens the way to further pharmacological studies aimed at assessing the potential involvement of this modulator of the central nervous system (CNS) excitatory pathways in some of the observed and so far unexplained neurological disorders which characterize this syndrome [13,14]. Isolation of pure HG-A was a major problem due to contamination by amino acids with similar solubility and chromatographic properties such as leucine and isoleucine [15]. Minor attention has been paid to the isolation of pure **2**. Only a long procedure reported by Fowden et al. in 1969 [9] describes extraction from fresh seeds followed by chromatographic purification using various ion exchange resins.

The methodology reported here for the purification of **2** from *Blighia sapida* (Akee) may be a useful alternative supply of this important pharmacological tool compared to the tedious and expensive synthetic procedure so far employed.

2. Materials and methods

2.1. Reagents and standards

All reagents were of analytical grade. HPLC-grade water was obtained from a tandem Milli-Ro/Milli-Q apparatus (Millipore, Bedford, MA, USA). HPLC-grade methanol was obtained from BDH (Milan, Italy). Column chromatography was performed on Merck (Darmstadt, Germany) silica gel (0.063–0.200 mm). Ion-exchange chromatography was performed on Dowex 50-X2-200. The size of the chromatographic bed are reported in parentheses. The ethanolic extract of *Blighia sapida* (Akee) was obtained as follows: fresh, unripened fruits (~5 kg) gathered in the neighborhood of Valencia (Venezuela) during February 1996 were finely triturated and macerated twice for 5 days with 95% ethanol at room temperature (25°C). Evaporation under reduced pressure and below 40°C gave a wet gummy solid (~80 g). The semi-solid ethanolic extract thus obtained was redissolved (15.5 g) in 500 ml methanol–water (95:5, v/v) under sonication: filtration and evaporation of the solvent yielded the dry methanolic extract (13.0 g). A pure sample of **2** was obtained by using a synthetic procedure already reported [5].

2.2. Instrumentation

The LC-Class 10 Shimadzu system used was composed of two LC-10 AD pumps, a SPD-10A UV–Vis detector (205 and 220 nm) and a CBM-10A computer driven control unit. The analytical column was a LiChrospher (Merck) RP-18 (250×4.0 mm, 5 μm); injections were made with a Rheodyne-type injector with a 20-μl loop.

2.3. Chromatographic conditions

All HPLC solvents were filtered through a 0.45-μm Millipore filter and degassed with 10 min sonication. The mobile phases were 25 m*M* NaH_2PO_4 (pH 2.10) and water containing 0.1% (v/v) trifluoroacetic acid (pH 2.20). Thin-layer chromatography (TLC) for controlling CCG I (**2**) consisted of *n*-butanol–water–acetic acid (70:15:15, v/v/v).

3. Results and discussion

Silica gel (400 g) was added to methanol (600 ml) and the resulting suspension was equilibrated overnight. The slurry thus obtained was poured into a column (28×5.6 cm) and washed with methanol until a clear eluate was obtained. The dry methanolic extract (0.80 g) obtained with the procedure described in the Materials and methods section was then chromatographed by eluting with methanol and collecting all the ninhydrin positive (TLC) fractions

(0.125 g) containing **2** contaminated by HG-A and trace amounts of HG-B [R_F 0.27 (**2**), 0.50 (HG-A)]. Ion-exchange chromatography (12×1 cm) on this product and elution with water (20 ml) and then with 0.3 *M* acetic acid afforded **2** (0.052 g). Analytical (RP-HPLC) and spectroscopic data confirmed the purity of the product obtained [m.p. 237–239°C; ^{1}H-NMR ($^{2}H_2O$) δ 1.15 and 1.65 (4H, 2m, cyclopropylic's), 3.25 (1H, d, J=9.5 Hz, 2-CH); ^{13}C-NMR ($^{2}H_2O$) δ 14.09, 19.10, 22.40, 57.31, 172.26,

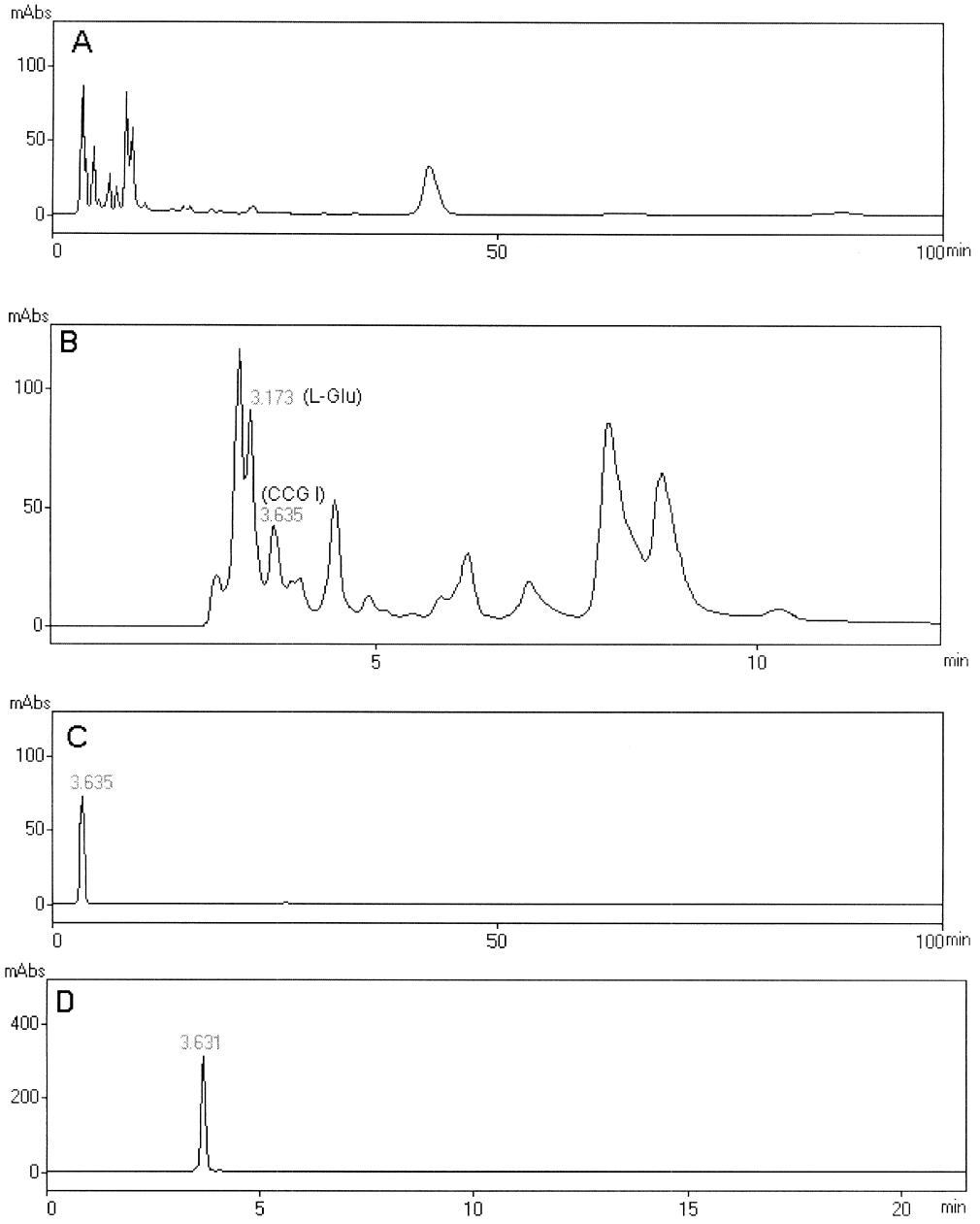

Fig. 2. Chromatograms of (A) methanolic extract of *Blighia sapida* (Akee); (B) expansion of the first 10 min of the above chromatogram showing the peaks attributed to L-Glu and CCG I (**2**); (C) CCG I (**2**) isolated from *Blighia sapida* (Akee); (D) synthetic CCG I (**2**).

177.19; $[\alpha]_D^{20} = +107$ (c 0.5, H_2O)]. RP-HPLC of the methanolic extract of *Blighia sapida* and of **2** was obtained with a phosphate buffer as the mobile phase. Fig. 2 shows the chromatograms at 1 ml/min and 205 nm of the crude extract (A) together with an expanded zone (B) on which L-glutamic acid (L-Glu) and **2** are indicated. The chromatograms of **2** isolated from *Blighia sapida* (C) and synthetic (D) are also reported. Individual spiking showed that peaks at 3.17 min and 3.63 min were L-Glu and **2**, respectively, with a separation factor of 2.62. An analogous result was obtained when the phosphate buffer was replaced with water containing 0.1% trifluoroacetic acid. Under these conditions (0.6 ml/min, 205 nm) the two peaks were eluted at 4.99 (L-Glu) and 6.97 min (**2**) with a separation factor of 2.29. A baseline resolution of **2** was then obtained with column lengthening. When a second, similar column (125 mm) was fitted to the previous one and the same chromatographic conditions were used, a higher retention time for **2** was observed (10.86 min) with a separation factor of 2.56. The procedure described here for the purification of **2** from *Blighia sapida* (Akee) is simple and very efficient. It allows a rapid preparative scale isolation of this compound from the fresh material. Moreover, the use of an easy removable mobile phase such as water containing trifluoroacetic acid together with the improved separation obtained with column lengthening provide the possibility of a preparative HPLC separation of **2** directly from the methanolic extract.

Acknowledgements

Financial support from Ministero della Università e della Ricerca Scientifica e Tecnologica (MURST) is gratefully acknowledged.

References

[1] R. Pellicciari, B. Natalini, J.B. Monahan, T.H. Lanthorn, D. Pilipauskas, J.P. Snyder, in: Abstracts of 6th Camerino-Noordwjikerhout Symposium on Recent Advances in Receptor Chemistry, Camerino (MC), 1987, pp. 73–74.
[2] R. Pellicciari, B. Natalini, M. Marinozzi, L. Selvi, C. Chiorri, J.B. Monahan, T.H. Lanthorn, J.P. Snyder, in: E.A. Cavalheiro, J. Lehman, L. Turski (Eds.), Frontiers in Excitatory Amino Acid Research, Vol. 46, Alan R. Liss, New York, 1988, pp. 67–70.
[3] H. Shinozaki, M. Ishida, K. Shimamoto, Y. Ohfune, Br. J. Pharmacol. 98 (1989) 1213–1224.
[4] S.R. Landor, P.D. Landor, M. Kalli, J. Chem. Soc., Perkin Trans. I (1983) 2921–2925.
[5] R. Pellicciari, M. Curini, B. Natalini, P. Ceccherelli, in: 9th International Symposium on Medicinal Chemistry, Berlin, 1986, p. 118.
[6] K. Yamanoi, Y. Ohfune, K. Watanabe, P. Novales Li, H. Takeuchi, Tetrahedron Lett. 29 (1988) 1181–1184.
[7] K. Shimamoto, M. Ishida, H. Shinozaki, Y. Ohfune, J. Org. Chem. 56 (1991) 4167–4176.
[8] D. Ma, Z. Ma, Tetrahedron Lett. 38 (1997) 7599–7602.
[9] L. Fowden, A. Smith, D.S. Millington, R.C. Sheppard, Phytochemistry 8 (1969) 437–443.
[10] P.C. Feng, West Ind. Med. J. 18 (1969) 238–243.
[11] K. Tanaka, K.J. Isselbacher, V. Shih, Science 175 (1972) 69–71.
[12] K.L. Stuart, in: E.A. Kean (Ed.), Hypoglycin, Academic Press, New York, 1975, pp. 39–44.
[13] J.T. Addae, G.N. Melville, West Ind. Med. J. 37 (1988) 6–8.
[14] E.A. Kean, West Ind. Med. J. 37 (1988) 139–142.
[15] C. McGowan, V.A. Wiley, R.P. Bates, BioChromatography 4 (1989) 161–164.