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An amide of L-*threo*-γ-hydroxyglutamic acid from *Justicia ghiesbreghtiana*

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Abstract

A new amide of *threo*-γ-hydroxyglutamic acid, justiciamide, was isolated from *Justicia ghiesbreghtiana* and shown to be (−)-*N*-(2-hydroxy-4,5-dimethoxyphenyl)-(2 *S*,4 *S*)-γ-hydroxyglutamic acid. Justiciamide is the first amide of an uncommon amino acid found in this genus. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Justicia ghiesbreghtiana* Lem; Acanthaceae; γ-Hydroxyglutamic acid amide; Isolation

1. Introduction

The genus *Justicia* comprises about 600 species of perennial herbaceous plants and bushes (Mabberley, 1997). Lignans have been reported from this genus, some of which show biological activity (Chen, Hsin, & Huang, 1995; Rajasekhar, Subbaraju, Ravikumar, & Chandramohan, 1998; Ghosal, Banerjee, & Srivastava, 1979; Ghosal, Banerjee, & Jaiswal, 1980; Trujillo, Jorge, Navarro, & Boada, 1990; Asano, Chiba, Tada, & Yoshii, 1996; Olaniyi & Powell, 1980). The only aniline derivatives which had been found were 2-aminobenzyl alcohol and 2-(2-aminobenzylamino)benzyl alcohol from *J. gendarussa* (Chakravarty, Dastidar, & Pakrashi, 1982). Recently, however, we reported the isolation and synthesis of the α-malamic acid derivative 1 from the Mexican species *Justicia ghiesbreghtiana* Lem. (Ismail, Lorenz, & Stermitz, 1998). This plant, which seems to be known in the botanical garden literature under the synonym *J. spicigera* Schlechtend., has been used in traditional folk medicine as a medicinal herb and for dye purposes (Graham, 1998; Dominguez, Achenbach, González, & Ferré D'Amore, 1990; Euler & Alam, 1982). In the course of attempting to isolate additional 1 for further study, a new plant collection was made which yielded little or no 1, but a different, also novel amide.

2. Results and discussion

From 150 g of *J. ghiesbreghtiana*, 134 mg of a pure compound was isolated, which was shown by spectroscopic and chemical methods to be *N*-(2-hydroxy-4,5-dimethoxyphenyl)-(2 *S*,4 *S*)-γ-hydroxyglutamic acid (2), herein named justiciamide. The molecular formula for justiciamide (2) was established as $C_{13}H_{18}N_2O_7$ by HR-FAB mass spectral analysis. This formula, requiring six units of unsaturation, was fully supported by spectral data (Table 1). The noncrystalline solid had $[\alpha]_D$ −2.7° (*c* 0.07, H_2O) and showed on TLC a positive reaction with ninhydrin (NH_2–group). This observation, along with two carbonyl signals at δ 170.80 and 170.88 in the ^{13}C NMR spectrum as well as strong IR absorptions at 1554, 1630 and 1671 cm^{-1}, suggested that the unknown 2 was an amino acid amide. The presence of two OCH_3 groups was shown by the observation of singlets at δ 3.69 and 3.76 in the ^1H NMR spectrum. Furthermore, a bathochromic

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Table 1
NMR spectral data for compound 2 (DMSO-d_6)

No.	^{13}C, δ, CH_n	^{1}H, δ (*mult, J*, no. of H)	HMBC
1	–	9.24 (*bs*, 1H)	C2, C7, C8, C12
2	170.80 (C=O)	–	–
3	70.73 (CH)	4.14 (*dd*, 2.4, 11.2, 1H)	C2, C4, C5
4	35.34 (CH_2)	2.22 (*ddd*, 2.4, 14.0, 11.2, 1H) 1.74 (*ddd*, 3.2, 10.4, 14.0, 1H)	C2, C3, C5, C6
5	52.82 (CH)	3.52 (*dd*, 2.8, 10.4, 1H)	C3, C6
6	170.88 (C=O)	–	
7	118.17 (C)	–	–
8	142.87 (C)	–	–
9	100.62 (CH)	6.57 (*s*, 1H)	C7, C10, C11
10	142.92 (C)	–	–
11	140.42 (C)	–	–
12	106.01 (CH)	7.96 (*s*, 1H)	C7, C8, C9, C10, C11
13	56.64 (OCH_3)	3.69 (*s*, 3H)	C10
14	56.09 (OCH_3)	3.76 (*s*, 3H)	C11

baseshift (UV_{max} 296 nm to 304 nm) and a broad IR absorption at 3126 cm^{-1} showed the presence of a phenolic OH-group. The ^{1}H NMR spectrum (Table 1) also revealed three spin systems: two methine units at δ 3.52 and 4.14 and one methylene unit at δ 1.74 and 2.22. Singlets at δ 6.57 and 7.96 showed the presence of a 1,2,4,5-tetrasubstituted benzene ring. Spectral similarities with the α-malamidic acid derivative 1, suggested the same aromatic substitution pattern for the unknown. Further NMR experiments involving HMQC, DEPT as well as ^{1}H decoupling led to the determination of the structure as 2 (stereochemistry unknown). Elemental analysis data suggested that justiciamide crystallized as a half-hydrate.

To determine the stereochemistry of 2, it was hydrolyzed with 6 N HCl to yield 2-hydroxy-3,4-dimethoxyaniline hydrochloride and 2 *S*,4 *S*-γ-hydroxyglutamic acid. The structure of the latter was shown by comparison to an authentic sample synthesized as follows. A mixture of *threo*- and *erythro*-γ-hydroxyglutamic acids (3) was prepared according to Kaneko, Lee, & Hanafusa (1962) (Fig. 1). The mixture in aqueous HCl was treated with HCl gas, from which solution lactone 4 crystallized. The lactone was converted back into *threo*-γ-hydroxyglutamic acid (3a) by stirring with 6 N aq. HCl. The ^{1}H- and ^{13}C-NMR spectra for 3a were identical with those of the amino acid isolated from the hydrolysis of 2. The *erythro*-acid 3b does not form a lactone and its hydrochloride was obtained from the mother liquor of the lactone-forming reaction. The 3b NMR spectra were different from those of 3a and the isolated hydrolysis product from 2. Thus, the final structure of justiciamide was shown to be 2.

2 *S*, 4 *S*-γ-Hydroxyglutamic acid (3a) has previously been found as a constituent of *Phlox* species (Polemoniaceae) (Bell, Meier, & Sørensen, 1981; Virtanen & Hietala, 1955), *Hemerocallis fulva* (Liliaceae) (Fowden & Steward, 1957) and *Linaria vulgaris* (Scrophulariaceae) (Hataneka, 1962). Two 3a amides of aliphatic amines were reported from

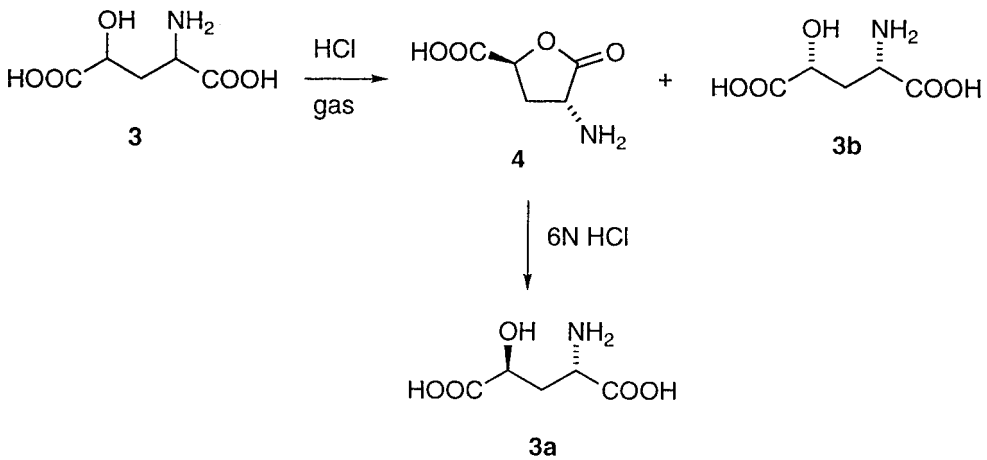
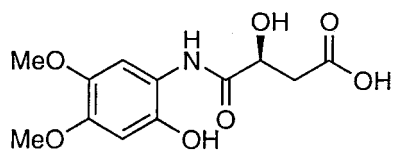


Fig. 1. Synthesis of *threo*- and *erythro*-γ-hydroxyglutamic acids.

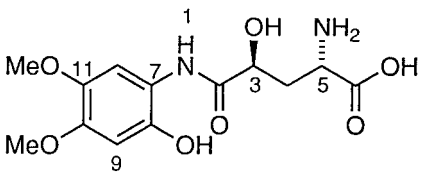
Staphylea pinnata L. (Staphyleaceae) (Grove, Weisleder, & Daxenbichler, 1973). Our finding of 2, in contrast to 1 in the previous work, is as yet inexplicable since collection was from the same plant source at approximately the same season, although five years later.

A more polar chromatographic fraction yielded 17.4 mg of *rac*.-allantoin (5), characterized by comparison of ^{1}H and ^{13}C NMR spectra with literature data and melting point (mp 229–230°C; lit. 230°C) (The Aldrich Library, 1997; Coxon, Fatiadi, Sniegoski, Hertz, & Schaffer, 1977). Compound 5 was previously reported from *Justicia spicigera* (Dominguez et al., 1990).

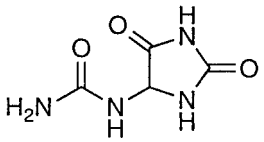
Justiciamide was inactive against gram-positive and gram-negative bacteria as well as against yeasts (*Candida albicans, Saccharomyces serevisiae*). Compounds 1 and 2 were not cytotoxic in the brine shrip test (*Artemia salina*) ($ID_{50} \approx 700$ ppm, after 24 h).



1



2



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3. Experimental

3.1. Plant material

Aerial plants of *J. ghiesbreghtiana* were collected in December 1997 in the Giza zoological garden, Cairo, Egypt. The plant was identified by Professor Dr Nabeil El Hadedi, Department of Botany. A voucher specimen was deposited in the Department of Pharmacognosy herbarium, Al-Azhar University.

3.2. Extraction and isolation

150 g of air dried and powdered aerial parts were extracted with 2:3 EtOAc–*n*-hexane. The marc was dried and extracted twice with 2 l MeOH (containing 10% H_2O). From the unified MeOH extracts the methanol was removed by rotaevaporation and the residual H_2O layer extracted with ether (3×300 ml). Evaporation of the H_2O from the water layer yielded an olive green foam (29.7 g), which was chromatographed on reverse phase silica gel (C_{18}), with water–MeOH (gradient: MeOH). Ten fractions were collected. Fractions 3 and 4 (water–MeOH 90:10 and 80:20) were unified and gave 1.70 g of a brown residue. The residue was rechromatographed on silica gel 60 (normal phase), solvent: $CHCl_3$–MeOH (gradient: MeOH). Seven fractions were collected. Fractions 6 and 7 (100% MeOH) yielded 0.42 g of almost pure 2. Final purification was achieved by preparative HPLC on an ODS RP (C_{18}) HPLC column (25 cm), solvent: 40% MeOH–H_2O (UV detector) to yield 87 mg of pure 2. Another fraction of the first RP silica gel chromatography yielded further pure 2 (47 mg). *Compound* 2. ^{1}H (400 MHz) and ^{13}C (300 MHz) NMR: Table 1. Mp. 185°C, $[a]_D^{25} -2.7°$ (*c* 0.07, H_2O). UV (H_2O) λ_{max} (log ϵ) 296 (5775), 244 nm (6220). IR (KBr) ν_{max} 3393, 3126, 1671, 1630, 1554, 1508, 1339, 1205, 1035, 907 cm^{-1}. HR FAB MS (MH^+) obsd. 315.1189 (calcd for $C_{13}H_{19}N_2O_7$, 315.1192). Anal. Calcd for $C_{13}H_{18}N_2O_7.0.5\ H_2O$: C, 48.30%, H, 5.92%, N, 8.66%. Found: C, 48.34%, H, 5.98%, N, 8.65%.

3.3. Hydrolysis of 2

A solution of 400 mg of 2 in 5 ml 6N HCl was stirred with a magnetic stirrer bar in a capped scintillation vial at 90°C for 12 h. The crude brown hydrolysate was filtered over Celite (rinsed with H_2O) and the HCl–H_2O removed by rotaevaporation. The residue was partially purified by vacuum liquid chromatography (VLC) on C_{18}-RP silica (solvent MeOH–water; gradient MeOH). A second VLC of the first fraction (352 mg) on silica gel (solvent: $CHCl_3$–MeOH; gradi-

ent MeOH), yielded 49 mg of 4-hydroxyglutamic acid hydrochloride, $[\alpha]_D^{25}+12$ (*c* 0.98, 5N HCl), lit. +4.5 (Lee & Kaneko, 1973). The ^1H and ^{13}C NMR data were essentially identical with those of a synthetic sample of *threo*-γ-hydroxyglutamic acid hydrochloride (3a), prepared as follows.

3.4. Synthesis of threo- and erythro-γ-hydroxyglutamic acid hydrochloride (3a, 3b)

Tetraethyl-1-acetamido-3-chloropropane-1,1′,3,3′-tetracarboxylate (mp 90–92°C; $mp._{lit.}$ = 91–92°C), prepd according to Kaneko et al. (1962) was hydrolyzed with conc HCl (37%) to give an *erythro*/*threo* mixture, 3. Treatment of the mixture in acid with HCl gas yielded a crystalline *threo*-lactone hydrochloride (4) while the *erythro*-acid (3b), which does not form a lactone, stayed in solution (Fig. 1) (Kaneko et al., 1962; Lee & Kaneko, 1973). The *threo*-γ-hydroxyglutamic acid lactone hydrochloride (4) was transformed to 3a hydrochloride by stirring with 6 N HCl (90°C, 6 h). Evaporation of the mother liquor from the lactone formation and recrystallization of the residue yielded 3b HCl.

3.5. Threo-γ-hydroxyglutamic acid lactone HCl (4)

(mp 239–240°C, dec; mp_{lit} 230–232°C); ^1H NMR (DMSO-d_6): δ 2.30 (*dd*, 1H, *J* = 11.7 Hz, CH_2), 2.87 (*ddd*, 1H, *J* = 6.6, 9.0, 12.3 Hz, CH_2), 4.45 (*dd*, 1H, *J* = 9.0, 11.7 Hz, CH), 5.04 (*dd*, 1H, *J* = 6.6, 10.8 Hz, CH), 9.04 (*bs*, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ 30.19 (CH_2), 48.26 (CH), 73.49 (CH), 169.29 (C═O), 171.93 (C═O); anal. C, 33.19%, H, 4.51%, N, 7.46%, calcd for $C_5H_8NO_4Cl$, C 33.07%, H, 4.44%, N, 7.71%.

3.6. Threo-γ-hydroxyglutamic acid HCl (3a)

^1H-NMR (DMSO-d_6): δ 2.03 (*dd*, 1H, *J* = 3.6, 10.5 Hz, CH_2), 2.15 (*dd*, 1H, *J* = 3.6, 9.3 Hz, CH_2), 3.92 (*m*, 1H, CH), 4.45 (*m*, 1H, CH); ^{13}C NMR (DMSO-d_6): δ 34.21 (CH_2), 49.47 (CH), 66.00 (CH), 170.73 (C═O), 174.48 (C═O).

3.7. Erythro-γ-hydroxyglutamic acid HCl (3b)

^1H NMR (DMSO-d_6): δ 1.97 (*ddd*, 1H, *J* = 3.6, 5.1, 10.5 Hz, CH_2), 2.20 (*ddd*, 1H, *J* = 3.3, 7.5, 10.8 Hz, CH_2), 3.95 (*m*, 1H, CH), 4.24 (*dd*, 1H, *J* = 3.3, 10.5 Hz, CH); ^{13}C NMR (DMSO-d_6): δ 34.21 (CH_2), 49.73 (CH), 66.72 (CH), 170.55 (C═O), 174.63 (C═O).

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