Synthesis of 2-[4′-(Ethylcarbamoyl)phenyl]-*N*-acetylglycine, the Proposed Structure for Giganticine

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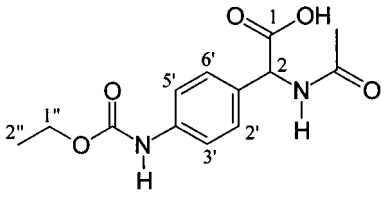
A compound (**1**) with the structure proposed for giganticine, an antifeedant principle isolated from the root bark of *Caloropis gigantea*, has been successfully synthesized by two independent methods. Comparison of physical properties and spectroscopic data of **1** with giganticine revealed that they are different compounds. All available evidence suggests that the proposed structure of giganticine is incorrect.

Giganticine is a nitrogen-containing compound isolated from the root bark of *Calotropis gigantea* L. collected in India.[1] It exhibited significant antifeedant activity against *Schistocerca gregaria* Froskal. The structure of giganticine was proposed to be 2-[4′-(ethylcarbamoyl)phenyl]-*N*-acetylglycine (**1**) based on spectroscopic evidence (IR, MS, ^{1}H NMR, ^{13}C NMR, and COSY experiments).[1] We were interested in synthesizing this simple amino acid and its derivatives in order to validate the proposed structure and also to study structure−antifeedant activity relationships. However, we have found that synthetic **1** showed very different physical properties and spectroscopic data from those of giganticine, details of which are discussed below.

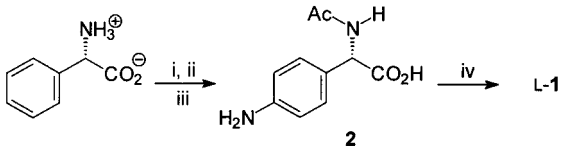
Although the stereochemistry of giganticine was not established, the L-enantiomer of phenylglycine was arbitrarily chosen as a suitable starting material for the synthesis of giganticine. Direct nitration of phenylglycines using a concentrated nitric acid−sulfuric acid mixture was reported to give predominantly the *meta*-nitrophenylglycine,[2] while nitration of *N*-acetylphenylglycine gave the *para*-isomer as the major product.[3] L-phenylglycine was thus converted to *N*-acetyl-L-(4-aminophenyl)glycine (**2**) by an acetylation−nitration−reduction sequence according to the literature procedure in 28% overall yield. Ethoxycarbonylation of **2** with ethyl chloroformate−K_2CO_3 in aqueous dioxane followed by acidification gave L-**1** as a white crystalline solid in 35% yield after recrystallization from ethyl acetate (Scheme 1).



1

Synthetic L-**1** gave a correct combustion analysis for $C_{13}H_{16}N_2O_5$ (C, H, N) and showed a correct mass at m/z = 280 (EI). However, it exhibited a different IR spectrum from that of giganticine, especially the absorption due to C=O stretching (1700 cm^{-1} for L-**1** and 1728 cm^{-1} for giganticine). The ^{1}H and ^{13}C NMR spectra of L-**1** (DMSO-d_6, 200 MHz) and giganticine ($CDCl_3$, 300 MHz) were also different, most notably at the position 2 (Table 1). The H-2 signal of L-**1** appeared at δ 5.20, while the H-2 signal of giganticine appeared at an unusually upfield position (δ 1.60). The C-2 signal L-**1** appeared at δ 56.3, which is more upfield than the corresponding C-2 signal in giganticine (δ 91.0). The position of ^{1}H and ^{13}C signals at the 2 position of L-**1** are in good agreement with the usual range for *N*-acylated α-substituted phenylglycine derivatives.[4] Furthermore, the multiplicity of the H-2 signal in L-**1** is a doublet (J = 7 Hz) due to the spin−spin coupling with the adjacent NH proton, which is in good agreement with the structure. In contrast, the same H-2 signal in giganticine is a singlet and also appeared in an unusual range. In

Scheme 1



i NaOH, Ac_2O, 0-5 °C, 15 min (64 %)
ii fuming HNO_3, conc H_2SO_4, 0 °C, 30 min (44 %)
iii cyclohexene, Pd/C (cat.), reflux, 2 h (99.5 %)
iv EtOCOCl, aq K_2CO_3-dioxane, rt, 15 min (35 %)

Table 1. Comparison of ^{1}H and ^{13}C NMR Spectra of Giganticine and L-**1**

	giganticine[a,b]		L-**1**[c,d]	
position	δ_H	δ_C	δ_H	δ_C
1	11.32 (1H, s)	170.5		173.0
2	1.60 (1H, s)	91.0	5.20 (1H, d, J = 7.5 Hz)	56.3
1′		128.8		131.6
2′,6′	7.50 (2H, d, J = 7 Hz)	128.2	7.26 (2H, d, J = 8 Hz)	128.8
3′,5′	7.24 (2H, d, J = 7 Hz)	121.9	7.46 (2H, d, J = 8 Hz)	118.8
4′		137.6		139.7
1″	4.25 (2H, q, J = 7 Hz)	60.4	4.10 (2H, q, J = 7 Hz)	60.6
2″	1.35 (3H, t, J = 7 Hz)	14.5	1.22 (3H, t, J = 7 Hz)	14.8
NH-2	11.24 (1H, s)		8.51 (1H, d, J = 7.5 Hz)	
NH-4′	5.57 (1H, brs)		9.64 (1H, s)	
Me*C*O		169.9		169.9
Et*O*CO		168.6		154.3
CO*Me*	2.36 (3H, s)	27.1	1.86 (3H, s)	22.6

[a] In $CDCl_3$ at 300 MHz (^{1}H). [b] Data obtained from ref 1. [c] In DMSO-d_6 at 200 MHz (^{1}H). [d] Assignment was made based on HMQC, HMBC, and NOESY experiments.

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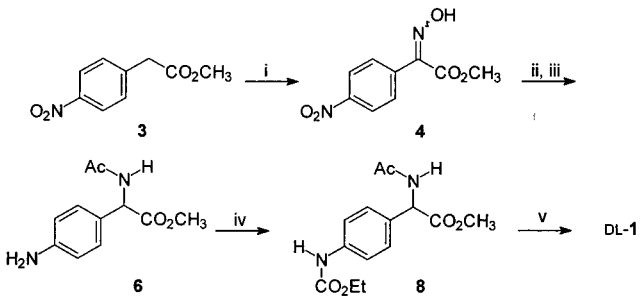
Scheme 2



i $^{i}C_5H_{11}ONO$, NaOMe, MeOH, 0 °C, 1 h (57 %, mixture of isomers)
ii Zn, HOAc, 5 °C, 1 h (quant)
iii Ac_2O, $^{i}Pr_2EtN$, CH_2Cl_2, rt, 30 min (46 %)
iv EtOCOCl, $^{i}Pr_2EtN$, CH_2Cl_2, rt, 30 min (82 %)
v aq NaOH, MeOH, rt, 15 min (80 %)

addition, the assigned ^{13}C resonance of the carbamoyl carbonyl carbon of giganticine appeared at an unusually downfield position (168.5 ppm), while most carbamoyl carbonyl carbons, including that of L-**1**, resonate at much higher field (154.3 ppm for L-**1**). These spectral differences are too much to be accounted for by the different solvents and the field strength of NMR spectrometer used.[5] Full assignments of the ^1H and ^{13}C NMR spectra of L-**1** were assisted by HMQC, HMBC, and NOESY experiments and are fully consistent with the proposed structure.

Giganticine is a relatively nonpolar compound (TLC on silica gel G, R_f 0.48, hexane−diethyl ether, 11:9; R_f 0.23, acetone−petroleum ether, 1:4), and it can be extracted from the $CHCl_3$-soluble crude extract of the root bark of *C. gigantea* using petroleum ether.[1] Synthetic L-**1** is a much more polar compound, and therefore it scarcely moved on TLC under the same conditions. Melting points of the two compounds are also different (giganticine, 159−162 °C; L-**1**, 209−210 °C). On the basis of the above differences in spectroscopic data and physical properties it is quite likely that the two compounds are different.

Another independent synthesis of **1** was also investigated (Scheme 2). Methyl 4-nitrophenylacetate (**3**) was treated with isopentyl nitrite in the presence of methanolic sodium methoxide[6] to give the crystalline hydroxyimino ester (**4**) in 57% yield. Reduction of both the nitro group and the hydroximino function was carried out in one step using zinc dust in acetic acid to give the diamino ester (**5**), which was further acetylated using a limited amount of acetic anhydride in the presence of *N*,*N*-diisopropylethylamine to give the N^2-acetylated amino ester (**6**) in 46% yield. Treatment of **6** with ethyl chloroformate in the presence of *N*,*N*-diisopropylethylamine gave the methyl ester (**8**) in 82% yield. Saponification gave DL-**1**. Synthetic DL-**1** and L-**1** obtained from two independent methods displayed identical ^1H and ^{13}C NMR spectra and other physical characteristics, except for melting point and optical rotation. These data are, however, distinctively different from those of the unspecified enantiomer of giganticine as reported by Pari et al.[1] It is therefore necessary to conclude that the structure of giganticine is unlikely to be **1**, and further work should be done in order to disclose the true identity of giganticine.[7]

Experimental Section

General Experimental Procedures. The optical rotation was determined on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Impact 410 spectrophotometer. Spectra of solid samples were recorded as KBr pellets. Routine ^1H NMR spectra were obtained on a Bruker ACF 200 operating at 200 MHz (^1H) and 50.28 MHz (^{13}C). 2D NMR experiments were performed on a JEOL JNM-A500 NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ) and are internally referenced to the residual protonated solvent peak. LREIMS spectra were obtained on a Fisons Instruments model Trio 2000 mass spectrometer at 70 eV. Elemental analyses were performed on a Perkin-Elmer elemental analyzer 2400 CHNS/O at the Research Equipment Centre, Chulalongkorn University. All chemicals and solvents were obtained from commercial suppliers (Aldrich, Fluka, and Merck) and were used as received.

Preparation of L-2-[4′-(Ethylcarbamoyl)phenyl]-*N*-acetylglycine (L-1). *N*-Acetyl-L-(4′-aminophenyl)glycine (**2**)[3] (0.651 g, 3.39 mmol) was dissolved in a solution of potassium carbonate (0.430 g, 3.12 mmol) in 1:1 aqueous dioxane. Ethyl chloroformate (0.35 mL, 3.67 mmol) was added dropwise with stirring at room temperature. After 15 min, TLC indicated completed reaction. Water (10 mL) was added, and the reaction mixture was extracted twice with ether. Acidification with 5% HCl followed by extraction with ethyl acetate gave a yellow oil, which was crystallized from ethyl acetate to give a white crystalline solid (0.332 g, 35% yield): mp 209−210 °C; $[\alpha]_D^{22}$ +153.6° (*c* 0.5, DMF); IR (KBr) ν_{max} 3500 (OH, NH), 3300 (NH), 1700 (C=O), 1600, 1500, 1230 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS (solid probe, 70 eV) *m*/*z* 280 [M^+], 262, 236, 193, 165, 147. *anal.* C 55.22%; H 5.91%; N 9.72%, calcd for $C_{13}H_{16}N_2O_5$, C 54.93%, H 5.62%, N 9.85%.

Preparation of DL-2-[4′-(Ethylcarbamoyl)phenyl]-*N*-acetylglycine (DL-1). A suspension of methyl hydroxyimino-4-nitrophenylacetate (**4**)[6] (1.00 g, 4.46 mmol) in 50% aqueous acetic acid was treated with excess zinc dust at 5 °C for 1 h. After the reaction was judged complete by TLC, the insoluble matter was removed by filtration, and the solution was adjusted to pH 8 with concentrated aqueous ammonia solution followed by extraction with dichloromethane. The solvent was evaporated to give the diamino ester (**5**) as a brown oily residue. This was taken up in dichloromethane and treated with acetic anhydride (0.28 mL, 2.94 mmol) in the presence of excess *N*,*N*-diisopropylethylamine. After 30 min at room temperature, the solvent was removed, and the residue was dissolved in 10% aqueous HCl and extracted several times with ethyl acetate to remove the diacetylated product (**7**). The aqueous phase was made basic by addition of solid $NaHCO_3$ and extracted with ethyl acetate. Evaporation of the solvent gave the N^2-monoacetylated product (**6**) as an oil (300 mg, 46% 2 steps). Treatment of **6** (220 mg, 1.35 mmol) with ethyl chloroformate (0.14 mL, 1.50 mmol) and *N*,*N*-diisopropylethylamine (0.25 mL, 1.50 mmol) in dichloromethane for 30 min at room temperature followed by chromatography on silica gel eluting with 60−80% ethyl acetate−hexane gave methyl ester **8** as a light yellow solid (0.327 g, 82% yield): ^1H NMR (DMSO-d_6) 1.22 (3H, t, J = 7 Hz, H-2″), 1.87 (3H, s, CH_3CO), 3.59 (3H, s, CH_3 ester), 4.07 (2H, q, J = 7 Hz, H-1″), 5.27 (1H, d, J = 7 Hz, H-2), 7.25 (2H, d, J = 8 Hz, H-2′ and H-6′), 7.43 (2H, d, J = 8 Hz, H-3′ and H-5′), 8.60 (1H, d, J = 7 Hz, NH-2), 9.67 (1H, s, NH-4′).

Aqueous NaOH (5%, 2.0 mL) was added to a solution of the methyl ester **8** (0.258 g, 0.88 mmol) in methanol (5 mL) and the reaction stirred at room temperature for 15 min. After evaporation of the solvent, the residue was dissolved in H_2O and acidified to pH 2 with concentrated HCl. DL-**1** precipitated as a light yellow crystalline solid, which was collected by filtration and dried under vacuum (0.196 g, 80%): mp 180−182 °C; ^1H NMR (DMSO-d_6) 1.22 (3H, t, J = 7 Hz, H-2″), 1.86 (3H, s, CH_3CO), 4.10 (2H, q, J = 7 Hz, H-1″), 5.21 (1H, d, J = 7.5 Hz, H-2), 7.26 (2H, d, J = 8 Hz, H-2′ and H-6′), 7.42 (2H, d, J = 8 Hz, H-3′ and H-5′), 8.50 (1H, d, J = 7.5 Hz, NH-2), 9.64 (1H, s, NH-4′); ^{13}C NMR (DMSO-d_6) 14.8, 22.6, 56.3, 60.6, 118.8, 128.7,131.6, 139.7, 154.3, 169.9,172.9.

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(5) L-**1** is insoluble in $CDCl_3$; therefore direct comparison of the spectra in the same solvent cannot be made.
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(7) Attempts to isolate giganticine from the root bark of *C. gigantea* collected in Thailand were unsuccessful since ^{1}H NMR analysis of the crude and partially purified extracts according to the method described by Pari et al (ref 1) showed no sign of the compound.

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