A Novel Insect Antifeedant Nonprotein Amino Acid from *Calotropis gigantea*

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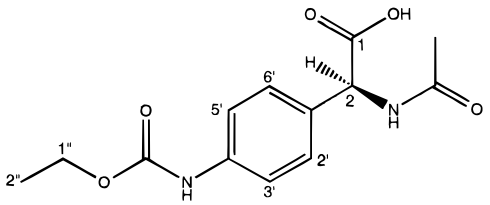
Giganticine (**1**), a novel nonprotein amino acid, has been isolated from a methanol extract of the root bark of *Calotropis gigantea* and its structure established by spectroscopic methods. It exhibited a significant antifeedant activity against nymphs of the desert locust *Schistocerca gregaria*.

The genus *Calotropis* R. Br. (Asclepiadaceae) is comprised of about six species of shrubs distributed in tropical and subtropical Africa and Asia. Two of the three species occurring in India, *Calotropis gigantea* and *Calotropis procera*, are of economic importance.[1]

C. gigantea L. grows widely throughout the Indian subcontinent. The latex of this plant is used in indigenous medicine as a purgative and a local irritant. Powdered flowers in small doses are useful in the treatment of colds, coughs, asthma, and indigestion. The root bark gives relief in dysentery. In small doses (0.2−0.6 g) it is a diaphoretic and expectorant and in large doses (2−4 g) an emetic. The root bark in the form of a paste is applied in the treatment of elephantiasis.[1]

A methanol extract of the root bark of *C. gigantea* has been shown to be a potent antifeedant for the desert locust.[2,3] Cardiac glycosides[4] and tetra- and pentacyclic triterpenoids[5−8] have also been reported from this plant. We report herein the bioassay-guided isolation of a new nonprotein amino acid, active as an insect antifeedant, to which we have assigned the trivial name giganticine (**1**).



Compound **1** was obtained pure by repeated crystallization from acetone−petroleum ether (3:1) and exhibited an elemental formula of $C_{13}H_{16}N_2O_5$. The EIMS of **1** showed a $[M]^+$ ion at *m/z* 280, a $[M + 2]^+$ ion at *m/z* 282, and prominent fragment ions at *m/z* 237, 156 (base peak), and 128. Thus, the fragmentation was characteristic for *N*-acetyl-α-amino acid derivatives.[9] Compound **1** showed an IR absorption band at 1728 cm^{-1}, indicating the presence of carbonyl functions, besides the absorption bands at 3368 cm^{-1} for a −NH group and 1578, 1538 cm^{-1} for an aromatic ring system. The UV spectrum exhibited two maxima at 290 and 260 nm.

The ^{1}H-NMR spectrum of **1** (see Table 1) displayed two downfield signals for aromatic protons at δ 7.50 (2H, d, J = 7 Hz) and 7.24 (2H, d, J = 7 Hz), and their multiplicity indicated the presence of a *para*-disubstituted benzene ring. Consequently, these signals were assigned to the H-2′, H-6′ and H-3′, H-5′ protons, respectively. Two downfield signals at δ 11.32 (1H) and 11.24 (1H) were assigned to the H-1 and amide −NH protons, respectively, of which the latter was thought to be chelated. A broad singlet at δ 5.57 (1H) was due to the −NH proton of an ethylcarbamoyl moiety. A quartet of two protons at δ 4.25 and a triplet of three protons at δ 1.35 were due to the ethyl group, while a signal at δ 2.36 (3H) was attributed to the acetyl group. A singlet at δ 1.60 (1H) was assigned to a proton at C-2.

The ^{1}H−^{1}H COSY experiment at 300 MHz clearly demonstrated two independent proton-coupling systems in **1**. The first was a three-proton triplet signal at δ 1.35 coupled with a two proton quartet signal at δ 4.25. This constituted part of the side chain, namely the ethylcarbamoyl unit that was attached to an aromatic ring system. The second was an AA′BB′ aromatic coupling system, which showed long-range coupling between the H-2′, H-6′ and H-3′, H-5′ protons, respectively. A methine singlet at δ 1.60 (H-2) did not show any coupling, thereby establishing the fact that is was affixed to both −COOH and −NH groups, as found in α-amino acids.[10]

The ^{13}C-NMR spectrum of **1** (see Table 1) exhibited three signals for three carbonyl carbons at δ 170.46, 169.89, and 168.55. Two signals at δ 14.47 and 27.07 were assigned to an $-OC_2H_5$ group. Characteristic signals at δ 121.89 (2Cs) and 128.79 (2Cs) were assigned to aromatic carbons. A signal at δ 90.96 was attributed to a methine carbon at C-2. Therefore, the structure of **1** [giganticine; 2-[(4-(ethylcarbamoyl)phenyl]-*N*-acetylglycine] was assigned on the basis of the interpretation of all these spectral data. This is the first report on the occurrence of a nonprotein amino acid in *C. gigantea*.

Compound **1** showed dose-dependent antifeedant activity in choice feeding assays on maize leaves against 5th instar hoppers of the desert locust *Schistocerca gregaria* Forskal (Table 2). Although meliantriol at 3 $\mu g/cm^2$ and azadirachtin at 1 ng/cm^2 gave absolute

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Table 1. ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$) Spectral Data of Compound 1

position	δ_H	δ_C
1	11.32 (1H, s)	170.46
2	1.60 (1H, s)	90.96
1′		128.79
2′, 6′	7.50 (2H, d, J = 7 Hz)	128.22
3′, 5′	7.24 (2H, d, J = 7 Hz)	121.89
4′		137.59
1″	4.25 (2H, q, J = 7 Hz)	60.44
2″	1.35 (3H, t, J = 7 Hz)	14.47
NH-2	11.24 (1H, s)	
NH-4′	5.57 (1H, brs)	
MeCO	2.36 (3H, s)	169.89
EtOCO		168.55
COMe		27.07

Table 2. Antifeedant Activity of Compound 1 against 5th Instar *Schistocerca gregaria* (Desert Locust)

concn (μg cm^{-2})	% feeding reduction[a]	concn (μg cm^{-2})	% feeding reduction[a]
1.6	55	5.0	80
3.2	68	6.6	90

[a] Each value is the mean of five separate feeding tests with each test consisting of 15 nymphs.

antifeedant effect against the same insect when feeding tests were conducted on filter paper,[11−13] compound **1** failed to exhibit the same effect even at 6.6 μg/cm^2 on maize leaves. Since the testing of the compound **1** was on natural food maize, a still higher concentration may be required for an absolute antifeedant effect. So far, nonprotein amino acids have been reported to be toxic to insects[14,15] but no information is available as to their antifeedant nature, hence, the importance of the present finding.

Experimental Section

General Experimental Procedures. The melting point was determined in an open capillary tube in a H_2SO_4 bath and was uncorrected. IR spectra were recorded on a Nicolet (Impact 400) FT-IR spectrometer and UV spectra on a Perkin-Elmer (Lambda 3B) spectrophotometer. ^{1}H-, ^{13}C-, and ^{1}H−^{1}H COSY-NMR spectra were obtained on Bruker AC 300F (300 MHz) NMR spectrometer, using $CDCl_3$ as solvent and TMS as the internal standard. Mass spectra were determined on JEOL JMS-DX 303 mass spectrometer at 70 eV. Silica gel G was used for TLC.

Plant Material. The roots of *C. gigantea* were collected in April 1995 from Kulahalli village, Bellary District, Karnataka, India. The plant was authenticated by Dr. P. R. Bhagwat of the Raw Materials Herbarium and Museum Delhi, National Institute of Science Communication, CSIR, New Delhi, and a reference specimen is on file in the herbarium (voucher no. 1798).

Extraction and Isolation. The root bark of *C. gigantea* was air-dried for 1 month and ground into a powder. The powder (4 kg) was repeatedly extracted with methanol (3LX2) in a Soxhlet apparatus. This extract (175 g) was partitioned between equal volumes (4 L) of MeOH and hexane. The hexane-soluble residue was a gum (45 g) and was separated from the MeOH solubles. The MeOH extract was concentrated under reduced pressure to give a gum-free residue (115 g). This residue was partitioned between equal volumes of $CHCl_3$ and H_2O (4 L). The $CHCl_3$-soluble fraction was separated from the aqueous layer, and the solvent was evaporated under reduced pressure to give a dry residue (50 g).

The dry residue (50 g) was repeatedly extracted with petroleum ether (3 ×700 mL), and fractions were combined, and the solvent was evaporated under reduced pressure to give a colorless amorphous residue (788 mg). On TLC it showed a single spot (R_f 0.48, hexane−diethyl ether, 11:9; R_f 0.23, acetone−petroleum ether, (1:4). Repeated crystallization of it from acetone−petroleum ether (3:1) gave crystals of **1** (41 mg).

Giganticine [2-[4-(ethylcarbamoyl)phenyl]-*N*-acetylglycine] (1): colorless crystals (acetone/petroleum ether); mp 159−162 °C; UV (EtOH) λ_{max} (log ϵ) 290 (4.20), 260 (6.16) nm; IR (KBr) ν_{max} 3368 (NH), 2917−2852, 1728 (C=O), 1578, 1538, 1472, 1301, 1229, 1084, 959, 834 cm^{-1}; ^{1}H- and ^{13}C-NMR spectral data, see Table 1; EIMS (solid probe, 70 eV) *m/z* 282 [M + 2]$^+$ (15), 280 [M]$^+$ (5), 156 (100), 128 (30), 127 (20), 112 (10), 84 (10), 67 (5); anal. C 55.46%, H 5.06%, N 9.85%, calcd for $C_{13}H_{16}N_2O_5$, C 54.93%, H 5.62%, N 9.85%.

Antifeedant Bioassay. Compound **1** was subjected to choice antifeedant assays at four doses (1.6, 3.2, 5.0, and 6.6 μg cm^{-2}) on maize leaves against 5th instar nymphs of the desert locust (*Schistocerca gregaria* Forskal). Percent feeding reduction (% FR) was determined by the equation % FR = (1 − treatment consumption/control consumption) × 100.[16] The antifeedant activity of **1** at different doses is presented in Table 2.

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