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L-3-(3′-CARBOXY-4′-HYDROXYPHENYL)ALANINE (3-CARBOXYTYROSINE) IN SEEDS OF *NEONOTONIA WIGHTII* (*GLYCINE WIGHTII*) AND ITS POSSIBLE SYSTEMATIC SIGNIFICANCE

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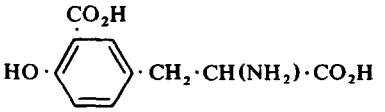
Key Word Index—*Neonotonia wightii* (*Glycine wightii*); Glycineae; Leguminosae; L-3-(3′-carboxy-4′-hydroxyphenyl)alanine (3-carboxytyrosine); non-protein amino acids; chemotaxonomy.

Abstract—L-3-(3′-Carboxy-4′-hydroxyphenyl)alanine (3-carboxytyrosine) constitutes 3% of the seeds of *Neonotonia wightii* (*Glycine wightii*); it has also been detected in the seeds of 3 species belonging to 2 other genera of the Glycineae. The systematic significance of these findings is discussed.

INTRODUCTION

The presence of high concentrations of an unidentified non-protein amino acid (designated GWI) in seeds of the legume *N. wightii* (*Glycine wightii*) has been reported [1]. The present paper describes the isolation of this amino acid and its identification as L-3-(3′-carboxy-4′-hydroxyphenyl)alanine (**1**), an amino acid previously found together with L-3-(3′-carboxyphenyl)alanine, L-2-(3′-carboxyphenylglycine) and L-2-(3′-carboxy-4′-hydroxyphenyl)glycine in *Reseda* (Resedaceae) species [2]. A survey of the distribution of this and other non-protein amino acids occurring in the seeds of species belonging to 11 genera of the Glycineae [3] has been made in an attempt to improve our understanding of inter-specific relationships, particularly those of *N. wightii* within this tribe of the Leguminosae.



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RESULTS AND DISCUSSION

The distribution of non-protein amino acids in seeds of 28 species of Glycineae is given in Table 1. The principal acidic amino acid in seeds of *N. wightii* is L-3-(3′-carboxy-4′-hydroxyphenyl)alanine. This amino acid has also been identified in seeds of *Pseudoeriosema andongense* which is the type species of the genus and of *P. borionii* formerly known as *Glycine schliebenii*. In the seeds of *N. wightii*, L-3-(3′-carboxy-4′-hydroxyphenyl)-alanine is accompanied by major concentrations of canavanine. Canavanine is absent from the seeds of the two *Pseudoeriosema* species and *O. radicosa*. In these 3 species it is replaced by a second basic non-protein amino acid tentatively identified (from ionic mobility and colour reactions) as 4-hydroxyarginine.

The species, formerly wrongly called *Glycine javanica* (i.e. *G. wightii*) and now separated as *N. wightii*, has been referred to *Glycine* since Bentham and Hooker [4] where *Johnia* Wight & Arn. (non DC.) was reduced to *Glycine*. More recent studies have shown, however, that this species differs from other species of that genus in respect to morphology [5], cytology [6], the composition of its seed protein [7] and in its ability to synthesize and accumulate the non-protein amino acid canavanine [8].

Table 1. Distribution of canavanine and L-3-(3′-carboxy-4′-hydroxyphenyl)alanine in the Glycineae

	Canavanine	L-3-(3′-Carboxy-4′-hydroxy-phenyl)alanine
Amphicarpa bracteata L.	–	–
Centrosema plumieri (Pers.) Benth.	+	–
Clitoria biflora Dalz.	–	–
C. mariana L.	–	–
C. racemosa Benth.	–	–
C. rubiginosa Pers.	–	–
C. ternatea L.	–	–
Dumasia truncata Sieb. & Zucc.	–	–
Glycine clandestina Wendl.	–	–
G. formsana Hosokawa	–	–
G. max (L.) Merr.	–	–
G. soja Sieb. & Zucc.	–	–
G. tomentella Hayata	–	–
Hardenbergia comptonia (Andr.) Benth.	+	–
H. violacea (Schneev.) Stearn	+	–
Kennedia coccinea Vent.	+	–
K. macrophylla (Meisn.) Benth.	+	–
K. nigricans Lindl.	+	–
K. prorepens F. Muell.	+	–
K. prostrata R.Br.	+	–
K. retusa F. Muell.	+	–
K. rubicunda Vent.	+	–
Neonotonia wightii (*G. wightii*, *G. javanica*)	+	+
Ophrestia hedysaroides (Willd.) Verdc.	–	–
O. radicosa (A. Rich.) Verdc. var. Schliebenni (Harms) Verdc.	–*	+
Pseudoeriosema andongense (Bak.) Hauman	–*	+
P. borianii (G. borianii) (Schweinf.) Hauman	–*	+
Pueraria phaseoloides (Roxb.) Benth.	–	–

* Accumulate an amino acid tentatively identified as 4-hydroxyarginine as a major component of free amino acid pool.

Verdcourt [5] gave it subgeneric status within *Glycine* while Lackey [9] considered it sufficiently different to merit a separate genus and renamed it *Neonotonia wightii*.

It is apparent that seeds of *N. wightii* differ in free amino acid content from those of all other species examined. This difference supports the views of Verdcourt and Lackey that this species cannot be readily classed with those species of *Glycine* which are listed in Table 1.

The presence of 4-hydroxyarginine and L-3-(3′-carboxy-4′-hydroxyphenyl)alanine in seeds of both species of *Pseudoeriosema* also emphasizes that this genus is biochemically distinct from *Glycine*, while the presence of the same two compounds in *Ophrestia radicosa*, but not *O. hedysaroides*, suggests that *O. radicosa* may have stronger biochemical affinities with *Pseudoeriosema* than has *O. hedysaroides*. It is of interest that both Hermann [10] and Lackey [11] have commented on a close morphological affinity between *O. radicosa* (named *Paraglycine radicosa* by Hermann) and species of *Pseudoeriosema*. Discussing this affinity Lackey states "*Pseudoeriosema* is probably little more than a specialized *Ophrestia*, the level of distinction between the two genera being less than the range of variation in the latter genus".

Nothing is yet known of the significance of L-3-(3′-carboxy-4′-hydroxyphenyl)alanine in *N. wightii* but it has been established that the amino acid is liberated in high concentrations from the seeds of *N. wightii* during imbibition [1].

EXPERIMENTAL

Isolation of L-3-(3′-*carboxy*-4′-*hydroxyphenyl*)*alanine.* Ground seed (1 kg) of *N. wightii* was extracted with Me_2CO for 24 hr to remove lipids. The dry, de-fatted seed powder was shaken ×4 for 48 hr with 75% EtOH (10 l.). After filtration the bulked extracts were applied to a column (10 × 60 cm) of cation exchange resin (Dowex 50W × 8) in the H^+ form. The acidic and neutral amino acids were displaced from the column with 1% aq. C_5H_5N. The soln of amino acids was concd to 50 ml (pH 6) in a rotary evaporator and applied to an anion exchange column (2.5 × 50 cm; Amberlite IR-45) in the $MeCOO^-$ form. The column was washed with H_2O (5 l.) and the amino acids eluted with 0.5 M HOAc (5 l.). The eluate was collected in 5 ml fractions. Fractions 552–603 containing the acidic 'unknown' were bulked and reduced to 20 ml on a rotary evaporator. Me_2CO was added until the soln became cloudy. Crystals separated on standing at 4°; these were recrystallized from 50% EtOH. Yield 2 g. (Found: C, 37.41; H, 3.69; N, 4.50. Calc. for $C_{10}H_{11}O_5N$: C, 36.82; H, 3.71; N, 4.29%). IR (solid nujol mull) showed a medium vibration at 835 cm^{-1} ascribed to two adjacent H atoms of an aromatic ring, strong vibrations at 1590 and 1691 cm^{-1} ascribed to the carboxylic acid moieties and a broad vibration around 3400 cm^{-1} indicating the presence of an amino group. 360 MHz ^1H NMR revealed 3 aromatic protons, two adjacent at 6.94 and 7.35 ppm, ($J_{5',6'}$ = 8 Hz) respectively and the third as a singlet at 7.69 ppm. The proton ascribed to the methine group of the alanine side chain gave rise to a triplet at 3.96 ppm ($J_{2,3}$ = 6.5 Hz) with hyperfine splitting. The two protons ascribed to the methylene group are asymmetric and therefore give rise to two doublets at 3.26 and 3.06 ppm with further splitting into two pairs of doublets due to the aromatic protons ($J_{3,1'}$ = 7.3 Hz). A mixture of the isolated material and authentic L-3-(3′-carboxy-4′-hydroxyphenyl)alanine gave an identical spectrum.

Quantitative determination of L-3-(3′-*carboxy*-4′-*hydroxyphenyl*)*alanine.* Finely ground seed of *N. wightii* (200 mg) was extracted with EtOH (1 ml) for 48 hr. After partial purification by ion exchange chromatography the amino acids were resolved with an LKB Model 4101 automatic amino acid analyser using the methods and Li buffer system previously described [12]. L-3-(3′-Carboxy-4′-hydroxyphenyl)alanine constituted 3% of the dry wt of the seed, a standard of the pure amino acid being used for comparison.

High voltage electrophoresis. HVE was carried out on paper using buffers of pH 1.9 and 3.6 as described [13]. The isolated compound moved identically with an authentic sample of L-3-(3′-carboxy-4′-hydroxyphenyl)alanine. Both the isolated and authentic compound gave a blue fluorescence on paper under UV light, a grey-blue with ninhydrin and a brown with Pauli's reagent. Location reagents were prepared according to Smith [14].

Analysis of seed extracts. Finely ground seed (200 mg) was shaken with 75% EtOH (1 ml) for 24 hr. The supernatant was subjected to analysis by HVE as described.

Identification of canavanine. Canavanine was identified by co-chromatography and spraying electrophoresis papers with pentacyanoammonioferrate reagent [15].

Tentative identification of 4-*hydroxyarginine.* 4-Hydroxyarginine was identified by co-electrophoresis with the authentic compound and by its scarlet reaction with Sakaguchi's reagent [16].

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