

SHORT COMMUNICATION

AZETIDINE-2-CARBOXYLIC ACID FROM THE LEGUME *DELONIX REGIA*

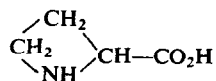
MAY-LIN SUNG and L. FOWDEN

Department of Botany and Microbiology, University College, London, W.C.1

(Received 14 May 1969)

Abstract—L-Azetidine-2-carboxylic acid (I) has been isolated from leaves of *Delonix regia*. The imino acid could not be detected in dry seed of the plant, but it was produced rapidly during germination, being present in all parts of the plant.

Delonix regia seed (Leguminosae) was used for the first isolation of *trans*-3-hydroxy-L-proline from a plant source.¹ This imino acid formed the predominant component of the soluble-nitrogen pool of the dry seed, and further quantities were synthesized during the early stages of growth of *Delonix* seedlings. Germination was associated also with the production of large amounts of γ -methyleneglutamic acid and γ -methyleneglutamine and smaller quantities of γ -methyl- and γ -hydroxy- γ -methyl-glutamic acids, four compounds not detected in the dry seed. Another compound, giving a brown ninhydrin chromophore but not immediately recognized as azetidine-2-carboxylic acid, was synthesized in substantial quantities by the seedlings, although it too was absent from dry seed. Our failure to recognize this compound



(I) Azetidine-2-carboxylic acid

initially as azetidine-2-carboxylic acid (I) undoubtedly was due to the belief that this unique imino acid was confined to some members of the Liliaceae and the allied family Amaryllidaceae.^{2,3}

Unlike the group of branched-chain C₆ dicarboxylic amino acids, whose concentration decreased markedly as *Delonix* plants produced their first mature leaves, azetidine-2-carboxylic acid continued to form a major component of the free amino-acid pool present in tissues of plants collected during their first year of growth. In fact the imino acid still represented one of the principal ninhydrin-reactive constituents of leaves collected from mature *Delonix* trees.

Unequivocal identification as azetidine-2-carboxylic acid was effected on material isolated from 4.5 kg of dried *Delonix* leaves, which contained about 0.6–0.7 g of the imino acid per kilogram.

¹ M.-L. SUNG and L. FOWDEN, *Phytochem.* **7**, 2061 (1968).

² L. FOWDEN, *Biochem. J.* **64**, 323 (1956).

³ L. FOWDEN and F. C. STEWARD, *Ann. Bot. N.S.* **21**, 53 (1957).

During a limited survey of the amino-acid complement of some other legume seedlings related to *Delonix*, i.e. those placed in the same group of the subfamily Caesalpinioideae by Hutchinson,⁴ azetidine-2-carboxylic acid was detected in only one other species, namely *Peltophorum pterocarpum*. Like *Delonix*, these seedlings also contained γ -methyleneglutamine in high concentration and γ -methylene-, γ -methyl- and γ -hydroxy- γ -methyl-glutamic acids in smaller amounts. This complex of branched C₆ amino acids was encountered more widely, being present in seedlings of all four species of *Caesalpinia* examined and of *Gymnocladus dioica*. It is interesting to note that these compounds are present in a small proportion of species from the Liliaceae,³ but no liliaceous species examined contained both azetidine-2-carboxylic acid and the compounds based on γ -methyleneglutamic acid.

Azetidine-2-carboxylic acid (\pm) is known to strongly inhibit the growth of seedlings of species unable to synthesize the imino acid,⁵ but concentrations of azetidine-2-carboxylic acid, lethal to mung bean seedlings, were without effect upon the growth rate of *Delonix* seedlings.

EXTRACTION AND CHARACTERIZATION OF AZETIDINE-2-CARBOXYLIC ACID

Dried *Delonix* leaves (4.5 kg) were finely ground and twice extracted with 35 l. of 75% (v/v) ethanol. The extract was reduced *in vacuo* to 6 l., decolorized and adjusted to pH 4, before fractionation of the amino-acid complex by standard procedures using a series of cation-exchange resin columns. Eventually azetidine-2-carboxylic acid (3 g) was crystallized, which was twice recrystallized to yield 0.7 g pure imino acid. $[\alpha]_D^{20} - 102^\circ$ (c, 1 in 5 N HCl) and -110 (c, 2 in water), c.f. literature value² for L-azetidine-2-carboxylic acid -108° (c, 3.6 in water). The i.r. spectra determined on nujol mulls of the isolated material and of L-azetidine-2-carboxylic acid were identical in every detail.

When treated with 6 N HCl for 18 hr at 100°, the isolated material was destroyed completely with the production of homoserine, γ -amino- α -chlorobutyric acid and homoserine lactone, a pattern of breakdown established for azetidine-2-carboxylic acid.²

Acknowledgement—We wish to thank Professor L. B. Throver (University of Hong Kong) for supplying the dried *Delonix* leaves. M.-L. Sung is presently a Commonwealth scholar.

⁴ J. HUTCHINSON, *The Genera of Flowering Plants*, Clarendon Press, Oxford (1964).

⁵ L. FOWDEN, *J. Exptl. Botany* **14**, 387 (1963).