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BIOSYNTHESIS OF AZETIDINE-2-CARBOXYLIC ACID FROM METHIONINE IN *NICOTIANA TABACUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; azetidine-2-carboxylic acid; methionine; biosynthesis.

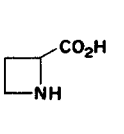
Abstract—The administration of DL-methionine-[1-^{14}C] to *Nicotiana tabacum* resulted in the formation of radioactive azetidine-2-carboxylic acid (isolated by dilution) which was specifically labelled on its carboxyl group. This result and other evidence strongly indicates that this imino acid is a normal component of tobacco.

INTRODUCTION

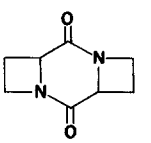
More than 200 non-protein amino acids have now been isolated from plants [1]. Fowden [2] has made the novel suggestion that many of these individual amino acids which currently have only been detected in relatively few species, could well be synthesized in more types of plants, or even by all plants, but only in minute amounts. A notable result in support of this concept was the isolation of azetidine-2-carboxylic acid (**1**, abbreviated A-2-C) from sugar beets (*Beta vulgaris*) [2]. This imino acid was present in the nitrogenous fraction arising as a byproduct of sugar refining from beets. Its concentration was about 1/50th that of proline and normal chromatographic procedures applied to beet extract failed to detect it. One method of revealing the presence of minute amounts of a natural product is by isotopic dilution. We have used this technique to establish the presence of A-2-C in tobacco (*Nicotiana tabacum*). The biosynthesis of this compound has been investigated in *Convallaria majalis* [3,4] and *Delonix regia* [5]. It is derived from methionine in both species, however details of this transformation are open to question [3]. If A-2-C were present in tobacco it seems reasonable to expect that it would also be formed from methionine in this species.

RESULTS AND DISCUSSION

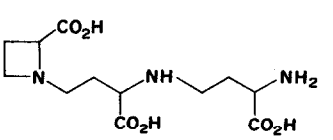
Accordingly DL-methionine-[1-^{14}C] was fed to a *N. tabacum* plant. After 7 days the plant was harvested and non-radioactive A-2-C added to the plant extract as a carrier. It was separated from the other amino acids by chromatography, and after several crystallizations had constant and significant radioactivity (0·2% incorporation). To confirm the radiochemical purity of the reisolated A-2-C it was converted to its anhydride (**2**) [6]. The specific activity of this derivative was as expected, and not changed after extensive purification. Decarboxylation of the A-2-C with ninhydrin [7] afforded carbon dioxide collected as barium carbonate which had essentially the same specific activity as the A-2-C, indicating that all the activity was located on its carboxyl group.



(**1**) Azetidine 2-carboxylic acid



(**2**)



(**3**) Nicotianamine

* Contribution No. 137 from this Laboratory.

Aspartic and glutamic acids were also isolated from the amino acid fraction of the tobacco which had been fed methionine-[1-^{14}C] and found to have significant activity. This observation is similar to the previous results [3] obtained on the metabolism of methionine in *Convallaria majalis*.

Whilst this work was in progress a new amino acid, nicotianamine was isolated from *N. tabacum* [8] and this compound has recently been shown to have the structure **3** [9]. Nicotianamine can be prepared non-enzymatically from A-2-C [9], and its presence in tobacco is strong circumstantial evidence that A-2-C also is a normal component of tobacco.

EXPERIMENTAL

General methods. A Nuclear Chicago Mark II Liquid Scintillation Counter was used for assay of the radioactive compounds using dioxane-EtOH with the usual scintillators [10].

Administration of DL-*Methionine-*[1-^{14}*C*] *to* N. tabacum *and isolation of A-2-C.* DL-Methionine-[1-^{14}C] (Radiochemical Centre, Amersham) (50 μCi, 62 mCi/mM) dissolved in H_2O (2 ml) was administered to one 4-month-old *N. tabacum* plant growing in soil in a greenhouse by the wick method. After 7 days the whole plant was harvested (fr. wt 220 g) and macerated with 70% EtOH. L-Azetidine-2-carboxylic acid (Calbiochem, 400 mg) was added to this initial extract which was then processed by the procedure used for the isolation of A-2-C from *Convallaria majalis* [11]. The recovered A-2-C (190 mg) was purified by sublimation (200°, 10^{-3} mm) and crystallization from EtOH–H_2O. Material having a constant sp. act. of $6{\cdot}0 \times 10^4$ dpm/mM was finally obtained. Decarboxylation of this A-2-C with ninhydrin [7] afforded CO_2 collected and assayed as $BaCO_3$ ($5{\cdot}8 \times 10^4$ dpm/mM). Aspartic acid (7·8 mg, $2{\cdot}2 \times 10^5$ dpm/mM) and glutamic acid (28 mg, $1{\cdot}5 \times 10^5$dpm/mM) were isolated from the amino acid fraction of the tobacco.

L-*Azetidine-2-carboxylic acid anhydride* (**2**). Following the established method [6] the recovered L-A-2-C was converted to its anhydride (sp. act. $1{\cdot}17 \times 10^5$ dpm/mM) which had mp 210–211°. The reported mp for the anhydride of DL-A-2-C is 171–172° [6]. High resolution MS (AEI-MS-30 double beam instrument) indicated a *m/e* of 166·0742. $C_8H_{10}N_2O_2$ requires: 166·0742. IR: γ_{max}^{KBr} 1660 cm^{-1} (C=O).

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