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EFFECTS OF HISTIDINE DECARBOXYLASE INHIBITORS ON THE PRODUCTION OF AN ABERRANT ALKALOID IN *DOLICHOTHELE SPHAERICA*

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Key Word Index—*Dolichothele sphaerica*; Cactaceae; aberrant alkaloid; unnatural alkaloid; biosynthesis; histidine decarboxylase inhibitors.

Abstract—Attempts at regulating the production of the aberrant alkaloid 4(5)-[N-isovalerylaminomethyl]imidazole were initiated by pretreating *Dolichothele sphaerica* plants with known histidine decarboxylase inhibitors. Larger quantities of the unnatural precursor, 4(5)-aminomethylimidazole, were converted to the corresponding aberrant alkaloid when the cactus plants were pretreated with α-methylhistidine or α-hydrazinohistidine. These compounds appeared to be successful in inhibiting the formation of histamine from histidine, thereby limiting the availability of the natural precursor.

INTRODUCTION

The cactus *Dolichothele sphaerica* yields an imidazole alkaloid named dolichotheline [1, 2]. This amide appears to arise biosynthetically by means of a mechanism involving the condensation of histamine and isovaleryl-CoA [3]. Histamine is derived from histidine and the formation of isovaleryl-CoA follows the generally accepted pathway of leucine catabolism. In addition, the high incorporation of isovaleric acid into dolichotheline (8) suggests the presence of activating enzymes for the conversion of isovaleric acid to isovaleryl-CoA [3]. This enzyme system that is capable of forming the amide linkage between histamine and isovaleric acid is not sufficiently specific, so that molecules that are structurally closely related to the normal substrate molecules can react with the enzyme(s) and be converted to their corresponding aberrant alkaloids [4–6]. For example, the administration of 4(5)-aminomethylimidazole (7) to the cactus plant results in the formation of the aberrant alkaloid, 4(5)-[*N*-isovalerylaminomethyl]imidazole (9).

Such investigations with unnatural precursors point to the potential application of the procedures utilized in the preparation of biologically active natural products that are difficult to synthesize. However, for the technique to be of any practical value it must be elaborated to yield significantly larger quantities of the desired unnatural product. Our aim in the present study was to examine an approach to the production of greater yields of 4(5)-[*N*-isovalerylaminomethyl]imidazole.

If one assumes that the same enzyme is involved in the formation of the amide linkage in dolichotheline and the aberrant alkaloid, then, there must be competition

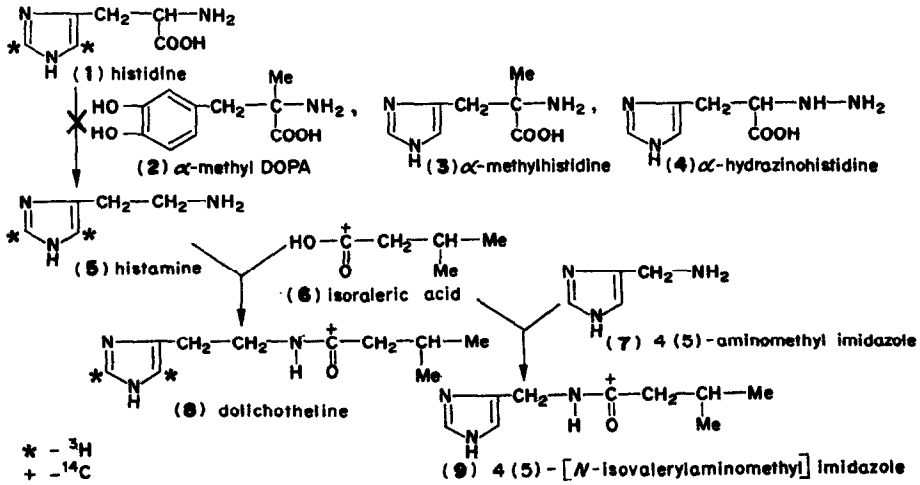

Table 1. Effects of histidine decarboxylase inhibitors on the production of *N*-isovalerylaminomethylimidazole

Experiment	% Injected activity recovered as dolichotheline		% Injected activity recovered as *N*-isovaleryl. aminomethylimidazole
	(^{14}C)	(^{3}H)	(^{14}C)
Histidine-^{3}H + Isovaleric-^{14}C	3·61	3·52	0
Histidine-^{3}H + Isovaleric-^{14}C + Aminomethylimidazole	0·39	0·47	0·73
Histidine-^{3}H + Isovaleric-^{14}C + Aminomethylimidazole + α-MethylDOPA	0·43	0·46	0·99
Histidine-^{3}H + Isovaleric-^{14}C + Aminomethylimidazole + α-Methylhistidine	0·20	0·18	1·46
Histidine-^{3}H + Isovaleric-^{14}C + Aminomethylimidazole + α-Hydrazinohistidine	0·13	0·11	2·07

Approximately 60 μCi (58 mCi/mM) of histidine-2,5-^{3}H and 60 μCi (52·4 mCi/mM) of isovaleric acid-1-^{14}C were injected into each of 5 groups of cactus plants. Three of these groups were pretreated with known histidine decarboxylase inhibitors. The plants were harvested 3 weeks after the injection of the labeled precursors.

between the normal substrate, histamine, and the unnatural precursor, 4(5)-aminomethylimidazole (**7**) (Scheme 1). The rate of formation of dolichotheline and the unnatural alkaloid is then governed primarily by the affinities of histamine and **7** for the enzyme, and by the concentrations of these reactants. Although there is little that can be done to promote the affinity of **7** for the condensing enzyme, it should be possible to inhibit the decarboxylation of histidine to histamine, thus, limiting the availability of the latter, and thereby favoring the incorporation of **7** into its corresponding aberrant alkaloid. With this goal, three compounds: α-methylDOPA (**2**), α-methylhistidine (**3**), and α-hydrazinohistidine (**4**), all known inhibitors of mammalian histidine decarboxylase, were tested for their ability to limit the availability of histamine within the plant.

RESULTS AND DISCUSSION

The results obtained from the present *in vivo* study are summarized in Table 1. Calculations of % injected activity recovered as dolichotheline are based on yields as determined by isotope dilution and those of 4(5)-[*N*-isovalerylaminomethyl]imidazole are based on 250 mg of the non-labeled compound added as carrier.

The results indicate that when tritiated histidine and ^{14}C-labeled isovaleric acid are injected into the plants, both of these labeled precursors, as might be expected, are incorporated into the natural alkaloid, dolichotheline. The addition of 20 mg 4(5)-aminomethylimidazole (**7**) causes competition between histamine and the unnatural precursor, with the result that there is a decrease in the incorporation of the labeled precursors into dolichotheline, while at the same time there is significant incorporation of **7** into the aberrant alkaloid. When α-methylDOPA is added to the plants, there is no further decrease in the incorporation of histidine into dolichotheline, while there is significant reduction when either α-methylhistidine or α-hydrazinohistidine is present. α-Hydrazinohistidine appears to have a greater inhibitory influence on the decarboxylation of histidine to histamine than α-methylhistidine. As a result, a greater amount of **7** is converted to the aberrant alkaloid when the plants are pretreated with α-hydrazinohistidine.

It is interesting to note that α-methylDOPA has no inhibitory influence on the decarboxylation of histidine to histamine in *Dolichothele sphaerica*. One possible explanation may be that this compound is an inhibitor of mammalian "non-specific" histidine decarboxylase whereas the other two are inhibitors of "specific" histidine decarboxylase [7]. Mammalian "non-specific" histidine decarboxylase has a low affinity for histidine and may be thought of as a non-specific aromatic amino acid decarboxylase [7]. Perhaps this enzyme is not functional, or in fact, may not be present in *D. sphaerica*.

The present investigation does indicate that it is possible to significantly increase the yields of unnatural alkaloids by successfully inhibiting the formation of natural precursors. Such an approach limits the availability of the natural precursor and thus larger amounts of the unnatural precursor may be converted to the unnatural alkaloid.

EXPERIMENTAL

Administration of test compounds. Isovaleric acid—[1-^{14}C], L-histidine-[2,5-^{3}H], DL-α-methylhistidine diHCl, α-methyl-L-DOPA hydrate, and D-α-hydrazinohistidine were purchased from commercial sources. 4(5)-Aminomethylimidazole and 4(5)-[*N*-isovalerylaminomethyl]imidazole were prepared by the method previously described [4]. Living plants of *D. sphaerica* were maintained in a greenhouse. The method employed for the introduction of test compounds in the cactus was previously reported [4]. Twenty plants, all of equal size, were divided into 5 groups, each containing 4 plants. Group 3 received 75 μmol α-methylDOPA while groups 4 and 5 received like amounts of α-methylhistidine and α-hydrazinohistidine, respectively. Three days after the injection of the histidine decarboxylase inhibitors, groups 2, 3, 4, and 5 each received 20 mg of 4(5)-aminomethylimidazole, 60 μCi (58 mCi/mM) of histidine labeled with ^{3}H in the 2 and 5 position of the imidazole ring, and 60 μCi (52·4 mCi/mM) of isovaleric acid labeled with ^{14}C in the carboxyl carbon. Group 1 received the allotted amounts of the labeled precursors only. After the injections, the plants were repotted, watered and returned to the greenhouse.

Isolation procedure. Three weeks after the injection of the labeled precursors, all groups of plants were harvested, dried, ground and 250 mg of non-radioactive 4(5)-[*N*-isovalerylaminomethyl]imidazole was added as carrier. The ground material was subsequently defatted, basified, and extracted with $CHCl_3$. The resulting $CHCl_3$ extract from each group was divided into two equal vol, to one of which 100 mg of dolichotheline was added. In each case, dolichotheline and the aberrant alkaloid were then isolated and separated from each other by preparative TLC by procedures reported previously [4].

Radioactive assay. The method employed for the preparation of samples for scintillation counting was described previously [8]. All samples were counted in a Beckman LS-230 liquid scintillation system at a preset 2σ statistical counting error of 1%. The external standard ratio method was used to determine losses due to quenching. All counts were corrected for counter efficiency.

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