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BIOSYNTHESIS OF *N*-METHYLPHENETHYLAMINE IN *DOLICHOTHELE SPHAERICA*

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Abstract—Living *Dolichothele sphaerica* metabolized 1.91% of administered [2-^{14}C]phenylalanine to *N*-methylphenethylamine. Phenethylamine, the presumable intermediate in this biosynthetic conversion, was detected in an extract of the cactus in very low concentrations. The addition of carrier to the extract allowed the isolation of radiolabelled phenethylamine and the establishment of its probable involvement in *N*-methylphenethylamine formation. This is the first report of *N*-methylphenethylamine biosynthesis, of phenylalanine serving as an efficient precursor to cactus alkaloids, and of the occurrence of phenethylamine in the Cactaceae.

INTRODUCTION

N-Methylphenethylamine was first isolated in 1939 from *Arthrophytum leptocladum* Popov (Chenopodiaceae)[1] and has since been isolated from the legume genera *Acacia*[2–4], *Alhagi*[5], *Cassia*[4], *Dalea*[4] and *Gleditsia*[4]. *N*-Methylphenethylamine has also been isolated from species of the cactus genera *Gymnocactus*[6] and *Dolichothele*[7, 8]. The recent detection of *N*-methylphenethylamine in human urine suggests that it is a phenethylamine metabolite[9]. Phenethylamine has been identified in the human brain[10] and is suspected of playing an important role in the normal,[11, 12] as well as in the abnormal[13, 14], physiology of the central nervous system.

Dolichothele sphaerica (Dietr.) Br. and R. has an ethnobotanical relationship with the hallucinogenic *Lophophora williamsii* (Lem.) Coult. through the common name of peyote[15, 16]. The initial chemical investigation of this potentially psychoactive cactus resulted in the isolation of the unusual imidazole alkaloid dolichotheline (*N*-isovalerylhistamine)[17]. A subsequent phytochemical examination revealed the presence of substantial quantities of *N*-methylphenethylamine together with smaller amounts of synephrine, β-*O*-methylsynephrine and *N*-methyltyramine[7].

It has been postulated that *N*-methylphenethylamine biosynthesis in both plants[7] and animals[9] involves a decarboxylation of phenylalanine followed by an *N*-methylation of the resulting phenethylamine. The present study describes an examination of this postulate using the *N*-methylphenethylamine-rich *D. sphaerica* as the biological system.

RESULTS AND DISCUSSION

A group of four healthy *D. sphaerica* were injected at several above-ground sites with a 0.01 M hydrochloric acid solution of DL-[2-^{14}C]phenylalanine (1.82×10^8 dpm administered). After a 22 day incubation period, the cacti were extracted and processed[18] to give a nonphenolic alkaloid fraction. Because of the inherently small size of *D. sphaerica*, insufficient quantities of *N*-methylphenethylamine were present in the extract to allow the usual crystallizations and degradation. GC showed that the extract contained 53 mg *N*-methylphenethylamine before adding 477 mg carrier. During the GC assay, phenethylamine was detected at a concentration of less than 1 mg. To facilitate the isolation of this small quantity, 100 mg carrier phenethylamine was added to the cactus extract.

Preparative TLC of the carrier-enriched nonphenolic alkaloid mixture over Si gel (1 mm) with diethylether–acetone–methanol–18 M ammonium hydroxide (9 : 8 : 2 : 1) gave two bands. The collection and processing[19] of the upper band yielded 56 mg phenethylamine hydrochloride (3.51×10^4 dpm). The specific activity of the isolated phenethylamine hydrochloride remained constant after recrystallization. These data demonstrate the occurrence of phenethylamine in *D. sphaerica*, its formation from phenylalanine, and its probable role as a precursor to *N*-methylphenethylamine. The large carrier dilution effect resulted in the isolation of phenethylamine hydrochloride with such low radioactivity that chemical degradations were not feasible.

The lower band from the initial TLC contained both dolichotheline and *N*-methylphenethylamine. Rechromatography of this band extract over Si gel (1 mm) with diethyl ether–methanol–18 M ammonium hydroxide (17 : 2 : 1) gave a dolichotheline-containing band which was not radioactive and therefore was not collected. The other band was collected and afforded 291 mg of *N*-methylphenethylamine hydrochloride (1.50×10^6 dpm). Since this quantity of the hydro-

chloride salt represents 43% of the total recoverable free base, it was calculated that 1.91% of the administered phenylalanine was metabolized to give *N*-methylphenethylamine. In order to detect possible contamination and/or randomization, a portion of the radioactive *N*-methylphenethylamine hydrochloride was oxidized with potassium permanganate to unlabeled benzoic acid. Another portion of the labeled *N*-methylphenethylamine was converted to the methiodide followed by alkaline permanganate oxidation. The trimethylamine from this degradation was trapped as the hydrochloride and found to possess no radioactivity. Therefore, the radioactivity of the isolated *N*-methylphenethylamine was shown to be associated with the α-position.

These studies indicate that phenylalanine serves as a precursor to both phenethylamine and *N*-methylphenethylamine in *D. sphaerica*. Other than a report of an inefficient conversion of phenylalanine to mescaline in *Lophophora williamsii* [20], this work is the first description of this amino acid serving as a cactus alkaloid precursor. Although phenethylamine occurs widely in the plant kingdom [21], this is the first report of its occurrence in the Cactaceae. It appears that phenethylamine is *N*-methylated after it arises from a decarboxylation of phenylalanine. Even though this biosynthetic sequence is obviously straightforward and directly analogous to *N*-methyltyramine formation from tyrosine in various plants [22–24], this paper represents the first report of *N*-methylphenethylamine biosynthesis.

The phenolic fraction from the phenylalanine-treated *D. sphaerica* was not examined since *N*-methyltyramine, synephrine, and β-*O*-methylsynephrine are expected to be derived from tyrosine metabolism. However, it has recently been reported that in the rat, tyramine is formed from phenethylamine as well as by a decarboxylation of tyrosine [25]. This same situation may exist in *D. sphaerica* if tyramine is present. The oxidation of *N*-methylphenethylamine to *N*-methyltyramine is an analogous reaction that may also occur.

EXPERIMENTAL

Radiochemicals. DL-[2-^{14}C]Phenylalanine (sp. act. 1.05 mCi/mM) was purchased (ICN Pharmaceuticals).

Plant material and growing conditions. D. sphaerica was purchased from Abbey Garden. All cacti were watered at 2-week intervals and were maintained in a controlled environment on a diurnal cycle of 14 hr light (32°) and 10 hr dark (18°).

Quantitation of alkaloids. An int. standard (20 mg *N*, *N*-dimethyl-3,4-dimethoxyphenethylamine hydrochloride) was added to an EtOH homogenate of *D. sphaerica*. After extraction and partitioning, the nonphenolic alkaloid fraction was assayed quantitatively using GC as previously described [26].

Identification of isolated phenethylamines. The identity of the isolated phenethylamine and *N*-methylphenethylamine was established by cochromatography (GC and TLC) and by mp determinations on the hydrochloride derivatives. The hydrochlorides were crystallized ×3 in order to establish radiochemical purity.

Counting procedures. Triplicate samples dissolved in a scintillator consisting of 0.5% PPO and 0.05% dimethyl POPOP in toluene–*p*-dioxane (1:1) were counted in a liquid scintillation spectrometer. All samples were counted to an error of less than ±1%. The counter efficiency was determined for each sample by the int. standard method using [^{14}C]toluene. A blank value was obtained routinely to determine the magnitude of background radiation.

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