Phytochemistry, 1980, Vol. 19, pp. 413–414. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0301-0413 $02.00/0

N-METHYLTYRAMINE: FORMATION IN *OPUNTIA CLAVATA* AND METABOLISM IN *CORYPHANTHA MACROMERIS* VAR. *RUNYONII*

WILLIAM J. KELLER

School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209, U.S.A.

(*Received* 4 *July* 1979)

Key Word Index—*Opuntia clavata*; *Coryphantha macromeris* var. *runyonii*; Cactaceae; biosynthesis; phenethylamines; normacromerine; *N*-methyltyramine.

Abstract—Administration of tyramine-[1-^{14}C] to *Opuntia clavata* resulted in the formation of labeled *N*-methyltyramine. This procedure established the biosynthetic origin of the major alkaloid in this cactus as well as providing a radiolabeled chemical that was not commercially available. The *N*-methyltyramine-[1-^{14}C] was in turn administered to *Coryphantha macromeris* var. *runyonii* to determine its metabolic role in the biosynthesis of the psychoactive cactus alkaloid normacromerine (*N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine). This feeding experiment established *N*-methyltyramine as a precursor to normacromerine.

INTRODUCTION

Of the 6 β-hydroxylated phenethylamines known in *Coryphantha macromeris* var. *runyonii*, normacromerine (*N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine) is by far the most abundant [1]. This methylated epinephrine derivative has been shown to be psychoactive in rats with behavioral effects correlating most closely with those associated with mescaline administration [2]. A preliminary biosynthetic investigation demonstrated a C_6–C_2 pathway where tyrosine is decarboxylated and the resulting tyramine is converted to normacromerine in *C. macromeris* var. *runyonii* [3]. Later work demonstrated the operation of catecholamine metabolism in the cactus when both epinephrine and norepinephrine were found to be precursors to normacromerine [4]. Very recently metanephrine was shown to be the immediate precursor to the psychoactive normacromerine [5].

This laboratory is presently concerned with determining the biosynthetic reaction sequence that exists between tyramine and the catechol derivatives. Since dopamine is not involved in normacromerine biosynthesis [3], it has been assumed that catechol formation is a relatively late biosynthetic reaction. The other two possible metabolites of tyramine are octopamine and *N*-methyltyramine. Since *N*-methyltyramine occurs in *C. macromeris* var. *runyonii* and while octopamine does not [1], it appeared logical to first investigate *N*-methyltyramine as a potential normacromerine precursor.

RESULTS AND DISCUSSION

Opuntia clavata has been reported to accumulate large quantities of *N*-methyltyramine with just traces of tyramine and hordenine [6]. In an effort to secure radiolabeled *N*-methyltyramine, 3 living specimens of this cactus were injected at several above-ground sites with an aqueous solution of tyramine-[1-^{14}C]HCl (5.544×10^8 dpm administered). After a 25 day incubation period, the cacti were extracted and processed [1] to give an alkaloid fraction. PLC of the alkaloid mixture over Si gel (1 mm) with Et_2O–Me_2CO–MeOH–18 M NH_4OH (9:8:2:1) gave 177 mg of *N*-methyltyramine HCl (5.240×10^6 dpm). Based on the total activity of tyramine-[1-^{14}C] administered to the cacti, 0.95% was recovered as crystalline *N*-methyltyramine HCl. Leete *et al.* [7] were the first to demonstrate the *N*-methylation of tyramine in barley while Wheaton and Stewart [8] found the same biosynthetic reaction to occur in *Citrus* species. This paper represents the first report of the biosynthetic conversion of tyramine to *N*-methyltyramine in the Cactaceae.

A portion (80 mg) of the radioactive *N*-methyltyramine HCl from *O. clavata* was dissolved in 2 ml of distilled water and injected into three large, healthy *C. macromeris* var. *runyonii* specimens (2.37×10^6 dpm administered). Following a 31 day incubation period, the cacti were extracted and processed in the usual manner [4, 5] to give 993 mg of normacromerine HCl (2.77×10^4 dpm). Therefore 1.17% of the radioactivity of the administered *N*-methyltyramine was associated with the isolated normacromerine. In order to detect possible contamination and/or randomization, the radioactive normacromerine HCl was oxidized with sodium periodate as previously described [5]. The formaldemethone from this degradation was found to contain over 97% of the original radioactivity associated with normacromerine HCl while the veratraldehyde semicarbazone contained less than 3%. This chemical degradation demonstrated that the ^{14}C label

was specifically associated with the 1 position of the precursor *N*-methyltyramine and the α position of the end product normacromerine.

The work presented in this paper indicates that *N*-methyltyramine serves as a precursor to normacromerine in *C. macromeris* var. *runyonii*. Synephrine has also been isolated from this plant [1] and this β-hydroxylated phenethylamine appears to be a likely biosynthetic intermediate between *N*-methyltyramine and normacromerine. The β-hydroxylation of *N*-methyltyramine to give synephrine has been established in *Citrus* species [8]. Experiments are now in progress in this laboratory to determine if the same reaction is operational during the biosynthesis of normacromerine in *C. macromeris* var. *runyonii*.

EXPERIMENTAL

Radiochemicals. Tyramine-[1-^{14}C] (sp. act. 58.76 mCi/mM) was purchased (New England Nuclear Corp.). *N*-Methyltyramine-[1-^{14}C] (sp. act. 2.50 μCi/mM) was produced by *O. clavata* after administration of radioactive tyramine.

Plant material and growing conditions. Cacti were purchased (*O. clavata* from New Mexico Cactus Research and *C. macromeris* var. *runyonii* from Abbey Garden). They were watered bimonthly and were maintained in a controlled environment chamber (Scientific Systems) on a diurnal cycle of 14 hr light and 10 hr dark. The temp. was maintained at 32° during the light period and at 18° during the dark period.

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%. Counter efficiency was determined for each sample by the int. standard method using toluene-[^{14}C]. A blank value was obtained routinely to determine the magnitude of background radiation.

Alkaloid identification was established by co-chromatography and mp determinations on the HCl derivatives. These derivatives were crystallized ×3 in order to establish radiochemical purity.

Acknowledgements—This research was supported by grants from NIDA (1 RO1 DA 02074-01) and from the Cactus and Succulent Society of America.

REFERENCES

1. Keller, W. J., McLaughlin, J. L. and Brady, L. R. (1973) *J. Pharm. Sci.* **62,** 408.
2. Bourn, W. M., Keller, W. J. and Bonfiglio, J. F. (1978) *Life Sci.* **23,** 1175.
3. Keller, W. J., Spitznagle, L. A., Brady, L. R. and McLaughlin, J. L. (1973) *Lloydia* **36,** 397.
4. Keller, W. J. (1978) *Lloydia* **41,** 37.
5. Keller, W. J. (1979) *J. Pharm. Sci.* **68,** 85.
6. Vanderveen, R. L., West, L. G. and McLaughlin, J. L. (1974) *Phytochemistry* **13,** 886.
7. Leete, E., Kirkwood, S. and Marion, L. (1952) *Can. J. Chem.* **30,** 749.
8. Wheaton, T. A. and Stewart, I. (1969) *Phytochemistry* **8,** 85.