Macromerine and Normacromerine Biosynthesis in *Coryphantha macromeris* var. *runyonii*

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Received April 17, 1978, from the *School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.* Accepted for publication June 22, 1978.

Abstract □ The biosynthetic conversion of epinephrine to normacromerine in *Coryphantha macromeris* (Engelm.) Br. and R. var. *runyonii* (Br. and R.) L. Benson (Cactaceae) has been studied. Metanephrine, which has been isolated from this cactus and is a normal metabolite of epinephrine in mammalian systems, appeared to be the likely intermediate between epinephrine and normacromerine. Normacromerine turnover studies suggested a 16-day interval between metanephrine administration and harvest of the cacti. During this incubation period, the cacti specifically converted 4.77% of the administered DL-7-^{3}H-metanephrine to normacromerine. Based on biochemical precedents, the postulated metabolic fate of normacromerine in the cactus was an enzymatic *N*-methylation to give macromerine. However, radiolabeled normacromerine was a very inefficient precursor to macromerine.

Keyphrases □ Epinephrine—biosynthetic conversion to normacromerine in *Coryphantha macromeris* cactus □ Normacromerine—biosynthetic conversion from epinephrine in *Coryphantha macromeris* cactus □ Macromerine—biosynthetic conversion from epinephrine in *Coryphantha macromeris* cactus □ *Coryphantha macromeris* cactus—biosynthetic conversion of epinephrine to normacromerine

The peyote cactus, *Lophophora williamsii* (Lem.) Coult., is a well-known hallucinogen because of its mescaline (3,4,5-trimethoxyphenethylamine) content (1, 2). The Dona Ana cactus, *Coryphantha macromeris* (Engelm.) Br. and R., is currently being promoted as a legal psychedelic agent and has about one-fifth the potency of peyote (3–7). By far the most abundant alkaloid in *C. macromeris* and its *runyonii* variety is the epinephrine analog normacromerine (*N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine) (8, 9). A recent study (10), using a battery of tests to evaluate the behavioral effects of various mind-altering substances in laboratory animals, showed normacromerine to be psychoactive. The behavioral effects produced by normacromerine correlated most closely with those associated with mescaline administration.

Keller *et al.* (9) initiated a preliminary study of the biosynthetic pathway leading to the formation of the psychoactive normacromerine in *C. macromeris* var. *runyonii*. A C_6–C_2 pathway, where tyrosine was decarboxylated and the resulting tyramine was converted stepwise to normacromerine, was established (9). Dopa and dopamine were not precursors of normacromerine. The natural occurrence of both epinephrine and norepinephrine in *C. macromeris* var. *runyonii* and the specific conversion of these catecholamines to normacromerine were demonstrated (11). The fact that norepinephrine was metabolized to normacromerine less efficiently than either tyramine or epinephrine indicated a branched biosynthetic pathway.

Metanephrine, a metabolite of epinephrine in mammals (12), was isolated from *C. macromeris* var. *runyonii* (8). Thus, metanephrine appears to be the logical intermediate during the biosynthetic conversion of epinephrine to normacromerine. Once formed in the cactus, normacromerine could undergo *N*-methylation to give macromerine. The present study used radiolabeled metanephrine and normacromerine to investigate this possible biochemical sequence in the cactus.

EXPERIMENTAL[1]

Plant Material—Living specimens of *C. macromeris* var. *runyonii* were obtained commercially[2]. Reference photos of the plants are on file, and live plants are being maintained in a controlled environment chamber[3]. The cacti were watered bimonthly and were maintained on a diurnal cycle of 14 hr of light and 10 hr of dark. The temperature was maintained at 32° during the light period and at 18° during the dark period.

Radioactivity Measurements—Organic samples were solubilized in 2 ml of 95% ethanol before the addition of 10 ml of the scintillation solvent. The solvent consisted of 0.5% 2,5-diphenyloxazole and 0.05% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene in a toluene–*p*-dioxane (1:1) mixture. Silica gel scrapings were extracted with 2 ml of 95% ethanol before addition of 10 ml of the scintillation solvent. Glass screw-capped liquid scintillation vials were used for all samples.

All radioactive samples were analyzed using a scintillation counter. Three samples were counted routinely, and the reported activities represent an average of these three values. Samples were counted to an error of less than ±1%. The counter efficiency was determined for each sample by the internal standard method using ^{14}C-toluene. A blank value was obtained routinely to determine the magnitude of background radiation.

Chromatography—Analytical silica gel GF-254 TLC plates were prepared in the usual manner, developed with benzene–chloroform–methanol–28% ammonium hydroxide (8:6:5:1), and visualized under shortwave UV light.

Preparative TLC was conducted as previously described (8, 9). Resolution of the nonphenolic alkaloid mixture was accomplished on 1-mm layers of silica gel PF-254 with the previously mentioned solvent system. From 12 to 16 plates were necessary for each isolation of normacromerine and macromerine. After the alkaloids were eluted from the silica gel with methanol, the eluates were filtered, combined, and concentrated under reduced pressure.

Labeled Compounds—DL-7-^{3}H-Metanephrine (label in the β-position) was assayed for chromatographic homogeneity as suggested by the supplier[4], and its radioactivity was determined immediately prior to administration to the cacti.

Generous quantities of (−)-7-^{14}C-normacromerine (label in the β-position) were available from a previous study (11). Chromatographic homogeneity and exact radioactivity were determined immediately before use.

Administration of Precursors—Administration of the precursors was accomplished by injecting aqueous solutions into selected specimens at several aboveground sites. Groups of five plants were used for the normacromerine and macromerine turnover studies while groups of four plants were employed to obtain percent incorporation data. After the precursor solutions were slowly injected, the needle was left in place for a few seconds to allow dispersion. The plants were watered and returned to the growth chamber for incubation immediately after administration of the radioactive compounds.

[1] Melting points were determined using a Fisher model 355 digital melting-point analyzer and are uncorrected. Scintillation counting was conducted using a Beckman model LS-230.

[2] Abbey Garden, Carpenteria, Calif.

[3] Scientific Systems, Baton Rouge, La.

[4] New England Nuclear Corp., Boston, Mass.

0022-3549/79/0100-0085$01.00/0

Table I—Specific Activity of Labeled Normacromerine Hydrochloride, Formaldemethone, and Veratraldehyde Semicarbazone from Plants Injected with DL-7-^{3}H-Metanephrine (9.276 mCi/mmole; 540 μCi Administered)

Compound	Crystallization	Yield, mg	Melting Point	Literature Melting Point	Specific Activity, dpm/mmole
Normacromerine	1	460	130–131°	132–133° (14)	3.07×10^7
	2	405	131–132°	—	3.05×10^7
	3	351	131–132°	—	3.04×10^7
Veratraldehyde semicarbazone	1	56	175–176°	177° (15)	2.92×10^7
	2	29	175–176°	—	2.91×10^7
Formaldemethone	1	46	192–193°	193–194° (16)	1.72×10^3

Extraction of Alkaloids—Following the incubation period, the plants were freed from adhering soil, weighed, and processed as previously described (8).

Determination of Optimum Harvesttime—To determine an ideal incubation period, normacromerine turnover was studied after the administration of DL-7-^{3}H-metanephrine. Macromerine turnover also was examined after 7-^{14}C-normacromerine administration.

In each case, identical volumes of an aqueous solution of precursors were injected into each specimen of *C. macromeris* var. *runyonii*. The plants were watered and returned to the controlled environment chamber for incubation. A harvest interval of 4 days was maintained for the metanephrine-treated cacti, with the sacrifice of one plant constituting a harvest. Individual normacromerine-injected plants were harvested after 9, 18, 36, 54, and 72 days. The harvested plant was extracted to yield its nonphenolic alkaloids. A modification of the procedure suggested by Fleming and Clark (13) was used to quantitate the radioactivity associated with the macromerine and/or normacromerine after analytical TLC. Details of this method were described previously (9).

Isolation and Identification of Macromerine and Normacromerine—In all cases where macromerine and normacromerine were isolated from the nonphenolic fraction, preparative TLC was used. The identity of the isolated alkaloid was established by cochromatography and melting-point determinations on the hydrochloride derivative. The normacromerine hydrochloride was crystallized three times to establish radiochemical purity; the radioactivity of 5-mg portions was determined in triplicate after each crystallization. Macromerine hydrochloride was crystallized one time, and its radioactivity was determined in triplicate following this single crystallization.

Periodate Degradation of Normacromerine—The isolated normacromerine from the DL-7-^{3}H-metanephrine-treated cacti was degraded with sodium periodate to locate the position of the tritium label in the molecule to determine the degree of label randomization and/or the presence of radioactive contaminants. A portion of the isolated normacromerine hydrochloride (200 mg) was dissolved in 10 ml of 0.1 *M* phosphate buffer (pH 6.2), and 220 mg of sodium periodate was added to this solution. After stirring at room temperature for 15 min, the pH of the reaction mixture was adjusted to 4.0 (pH paper) with 10% acetic acid, followed by the immediate addition of 40 ml of 0.5% dimedone (5,5-dimethyl-1,3-cyclohexanedione). After 3 hr of refrigeration, the dimedone derivative of the liberated formaldehyde (formaldemethone) was filtered and recrystallized from ethanol–water.

The filtrate was extracted with two 50-ml volumes of ether, and the ether extract was evaporated *in vacuo*. After the residue was dissolved in 10 ml of an ethanol–water mixture, 100 mg of semicarbazide hydrochloride and 150 mg of sodium acetate were added, followed by 1 min of boiling. Overnight refrigeration induced the crystallization of veratraldehyde semicarbazone. This derivative was filtered and recrystallized from an ethanol–water mixture. The radioactivity associated with each degradation product was measured as previously described.

RESULTS AND DISCUSSION

Keller (11) observed that the radioactivity associated with normacromerine reached a maximum between 20 and 24 days after administration of DL-7-^{14}C-epinephrine. When considering that metanephrine is only one biosynthetic step from normacromerine, an incubation period of 20–24 days may not be ideal for the suspected precursor. To investigate this possibility, normacromerine turnover was examined in the living cacti after the administration of DL-7-^{3}H-metanephrine. Maximum incorporation of radioactivity into normacromerine occurred within 16 days after precursor administration. Consequently, in the subsequent experiment, the plants were harvested and processed 16 days after administration of labeled metanephrine.

Table II—Percent Distribution of Activity per Millimole after Administration of (−)-7-^{14}C-Normacromerine

Harvest Day	Normacromerine	Macromerine
9	99	1
18	97	3
36	96	4
54	98	2
72	100	0

To evaluate the significance of metanephrine in normacromerine biosynthesis, four cacti were injected with aqueous DL-7-^{3}H-metanephrine. These plants yielded 460 mg of normacromerine hydrochloride after the first crystallization. After the hydrochloride was recrystallized twice from absolute ethanol–ether, the specific activity remained relatively constant. Based on the total activity of DL-7-^{3}H-metanephrine administered to the cacti, 4.77% was recovered as crystalline normacromerine hydrochloride. The periodate degradation revealed that 96% of the activity in the labeled normacromerine hydrochloride was associated with the β-position (Table I).

Once formed in the cactus, the most logical metabolic fate of normacromerine appears to be *N*-methylation to give macromerine. To examine this possibility, four plants were injected with an aqueous solution of (−)-7-^{14}C-normacromerine hydrochloride (2.82 μCi/mmole; 0.57 μCi administered). After an 18-day incubation, these plants were harvested and extracted as usual. The nonphenolic fraction yielded 75 mg of macromerine hydrochloride. The radioactivity associated with this material was barely above background values. Based on the total activity of (−)-7-^{14}C-normacromerine hydrochloride administered to the cacti, only 0.0005% was recovered as crystalline macromerine hydrochloride. Because of this very low activity, the isolated macromerine hydrochloride was not chemically degraded.

One possible explanation for the poor incorporation of normacromerine into macromerine might be that the *N*-methylation of normacromerine is a very slow process in the cactus. This possibility was explored by measuring macromerine turnover after administration of (−)-7-^{14}C-normacromerine. The cacti were harvested at selected intervals over 72 days. The radioactivity associated with normacromerine also was determined for each harvest. Table II presents the percent of the total macromerine–normacromerine activity associated with the individual compounds from each harvest.

The results of this investigation indicate metanephrine to be the immediate precursor to normacromerine in *C. macromeris* var. *runyonii*. Since very little normacromerine was converted to macromerine over 72 days, *N*-methylation of normacromerine appears not to be a major biosynthetic route. Macromerine may be biosynthetically derived from *N*-methylmetanephrine, a compound known to occur in the cactus (8). Alternatively, macromerine may be demethylated to form normacromerine. Experiments designed to answer these questions are in progress.

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ACKNOWLEDGMENTS

Supported by grants from the Cactus and Succulent Society of America and the Northeast Louisiana University Research Council.

Papaver bracteatum Lindl.: Quantitative Extraction and Determination of Thebaine

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Abstract □ An interlaboratory procedure for the quantitative extraction and analysis of thebaine from different tissues of *Papaver bracteatum* Lindl. is presented. Each step was evaluated for the yield of thebaine by use of 1-^{3}H-thebaine and GLC. The method of drying and milling of tissue and the size of resultant particles were important factors in the quantitative recovery of thebaine.

Keyphrases □ *Papaver bracteatum*—various plant tissues, extraction and analysis of thebaine □ Thebaine—extraction and analysis from various plant tissues of *Papaver bracteatum* □ Alkaloids—thebaine, extraction and analysis from various plant tissues of *Papaver bracteatum* □ Narcotics—thebaine, extraction and analysis from various plant tissues of *Papaver bracteatum*

Papaver bracteatum Lindl. is being evaluated as a source of the alkaloid thebaine from which codeine may be derived (1, 2). Methods for the analysis of thebaine include quantitative TLC followed by spectrophotometry (3), colorimetry (4), GLC (5, 6), and high-speed liquid chromatography (7, 8).

A United Nations Working Group on *P. bracteatum* (9) recommended a general method for thebaine analysis. In this study, each step of the method is presented for the analysis of thebaine in roots, leaves, and capsules of *P. bracteatum*. The purposes of this investigation were to maximize the yield of thebaine and to provide a basis for comparison of interlaboratory results of analysis of thebaine in such tissues.

EXPERIMENTAL

Plant Culture—The seed source and plant culture were previously described[1] (6).

Preparation of Tritiated Thebaine—1-^{3}H-Thebaine was prepared by the acid-catalyzed tritiation of salutaridine, followed by borohydride reduction to the epimeric salutaridinols and subsequent conversion to thebaine. The entire sequence was first performed on the deuterated analogs, which permitted unambiguous assignment of the label to the 1-position by NMR and mass spectrometry. The specific activity was 6.17 μCi/mg.

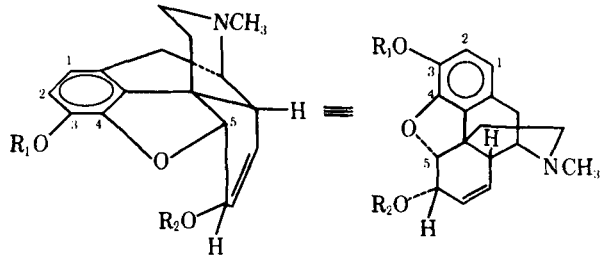
Sample Preparation—The effects on thebaine yield of oven drying (60°) *versus* freeze drying of plant tissue were compared. The effect of ball[2] or blade[3] milling was evaluated, as was the influence of particle size on thebaine recovery. The residual water content of samples before analyses was determined. Thebaine extractions with 5% aqueous acid and methanol–ammonium hydroxide (98:2) were compared. The efficiencies of other extracting solvents (5) are also discussed.

Assay—Thebaine was determined as previously described (6).

RESULTS AND DISCUSSION

(−)-Thebaine (I) (except for the position of the double bonds), (−)-codeine (II), and (−)-morphine (III) have the same skeletal structure and absolute configuration.

Apparently, thebaine was not stable at 60°. Thebaine, present at time of harvest, may have been transformed during heat drying into other compounds, as evidenced by the appearance of unidentified "alkaloidal" substances on thin-layer chromatograms following drying and by the appearance of several radioactive substances following addition of 1-^{3}H-thebaine to plant tissues before drying. The mechanism and nature of the additional substances derived from thebaine during oven drying have not been studied adequately; however, enzymatic and/or physical factors undoubtedly are involved in the transformation processes. No additional products from thebaine were observed when tissues were freeze dried, as measured by TLC of tissue extracts to which 1-^{3}H-thebaine had been added.



I: $R_1 = R_2 = CH_3$ (double bonds between C6—7 and C8—14)
II: $R_1 = CH_3$, $R_2 = H$
III: $R_1 = R_2 = H$

[1] The plant material was identified as *Papaver bracteatum* Lindl. by Dr. P. G. Vincent and M. L. Stiff, U.S. Department of Agriculture, Beltsville, Md. A voucher (preserved and living) specimen (see Ref. 6 for numbers) representing material collected for this investigation is available for inspection at the U.S. Department of Agriculture, Beltsville, Md.

[2] Dangoumau quantitative microgrinder; Prolabo, Microbroyeur Quantitatif Dangoumau, Appareil No. 07-449.02, France.

[3] Wiley.

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