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Note

Identification of 6,7-dihydroxytetrahydroisoquinoline as an *in vitro* reaction product by gas–liquid radiochromatography

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Gas–liquid chromatography (GLC) in combination with radioactivity detection (gas–liquid radiochromatography, GLRC) is frequently employed in studies involving carbon-14 biosynthetically labeled compounds[1–3]. We have used this technique to demonstrate that ^{14}C-labeled N,N-dimethyltryptamine is the product of an *in vitro* methylation of N-methyltryptamine (NMT) by S-adenosyl-[Me-^{14}C]-methionine (SAM)[4], and that the cyclization product 2,3,4,9-tetrahydro-2-methyl-1*H*-pyrido[3,4-*b*]indole is formed by an *in vitro* reaction between NMT and methyltetrahydro[5-^{14}C]folic acid (MTHF)[5]. The latter compound has been reported to mediate the N-methylation of dopamine to epinine[6]. In view of our findings that MTHF does not act as an alternate to SAM in the formation of N-methylated tryptamines[5], studies on the identification of the reaction product of [5-^{14}C]MTHF with dopamine were carried out employing GLRC techniques.

EXPERIMENTAL

GLRC was carried out using a Barber-Colman Model 5000 instrument equipped with a hydrogen flame ionization detector and a radioactivity monitor (RAM) system consisting of a reactor tube (copper oxide and steel wool at 760°) connected via a magnesium perchlorate drying tube to a proportional-type counter (approx. 5 ml/min propane quench gas) for monitoring $^{14}CO_2$. The column effluent was split approx. 1:1 to allow simultaneous determination of mass and radioactivity. Three 6 ft. × 4 mm I.D. glass U-tube columns containing 1% OV-1 (205°), 1% OV-17 (235°) and 3% OV-210 (230°) packings, respectively, were employed (approx. 50 ml/min argon carrier gas).

Authentic epinine and 6,7-dihydroxytetrahydroisoquinoline (THIQ) were acetylated by reaction of the appropriate amine (200 μg) with 0.2 ml of acetic anhydride–pyridine (4:1) for 10 min at 60°. Evaporation to dryness (nitrogen stream) gave residues which were partitioned between water and ethyl acetate. Evaporation of the organic phases yielded the triacetates as demonstrated by combined GLC–mass spectrometry [molecular ions of *m/e* 293 (triacetyl epinine) and 291 (triacetyl THIQ)]. Both compounds exhibited very similar R_F values (triacetyl epinine, 0.69; triacetyl THIQ, 0.73) when subjected to thin-layer chromatography (TLC) on silica gel

(Analtech GF 250 μm; Analtech, Newark, Del., U.S.A.) using the solvent system chloroform–methanol–acetic acid (93:7:1).

An enzyme preparation from rat brain was obtained by homogenizing the tissue in three volumes of distilled water, centrifuging the homogenate at 75,000 *g* for 60 min at 0° and dialyzing the supernatant for 20 h at 4° against 300 volumes of 3–5 m*M* sodium phosphate buffer pH 6.7 containing 0.01 m*M* dithiothreitol. *In vitro* incubation reactions contained enzyme (6 mg protein/ml), 50 m*M* sodium phosphate buffer pH 6.7, 5 m*M* dopamine·HCl and 1–3 μCi [5-^{14}C]MTHF (specific activity, 60 μCi/μmole; Amersham/Searle, Great Britain). After incubation at 37° in the dark for 1–2½ h, epinine and THIQ (100 μg) were added as carriers (because of the minute amount of biosynthesized product expected) and the samples lyophilized. The lyophilized material was treated with 0.5 ml of acetic anhydride–pyridine (4:1) at 60° for 10 min, and the reaction mixture then reduced to dryness (nitrogen stream). The acetylated radioactive product was recovered by distribution between ethyl acetate and water. The extract was taken to dryness (nitrogen stream) and subjected to TLC as above. A large amount of radioactivity remained at the origin. The radioactivity found in the R_F 0.69–0.73 region was recovered from the silica gel by elution with methanol. GLRC was carried out on this fraction after recovery by distribution between ethyl acetate and water.

RESULTS AND DISCUSSION

If transmethylation of dopamine were to pertain, then dopamine would be converted to epinine; if a cyclization occurred with insertion of a methylene group (and concomitant loss of two hydrogen atoms), the product would be THIQ. GLRC was chosen as the method to characterize the radioactive product from the *in vitro* reaction. Because both of the potential products are very polar species, it was decided to employ carriers and to carry out a derivatization step prior to any chromatographic separations. Acetylation was chosen over trimethylsilylation because of the greater stability of the N-acetyl moiety. Three stationary phases of widely different partitioning properties were chosen for the GLRC, *viz.* OV-1 (dimethylpolysiloxane), OV-17 (methylphenylpolysiloxane) and OV-210 (methylfluoroalkylpolysiloxane), so as to distinguish unequivocally between the acetyl derivatives of epinine and THIQ and to demonstrate that the radioactive product was indeed one of these two compounds. As is clear from the retention data in Table I, the two acetyl derivatives are well separated by each of the stationary phases. GLRC of the isolates demonstrated

TABLE I

RETENTION BEHAVIOR OF TRIACETYL EPININE AND TRIACETYL THIQ

The column conditions are given in Experimental.

Compound	*Retention time (min)*		
	OV-1	*OV-17*	*OV-210*
Triacetyl epinine	2.5	3.0	4.7
Triacetyl THIQ	4.6	6.2	9.0

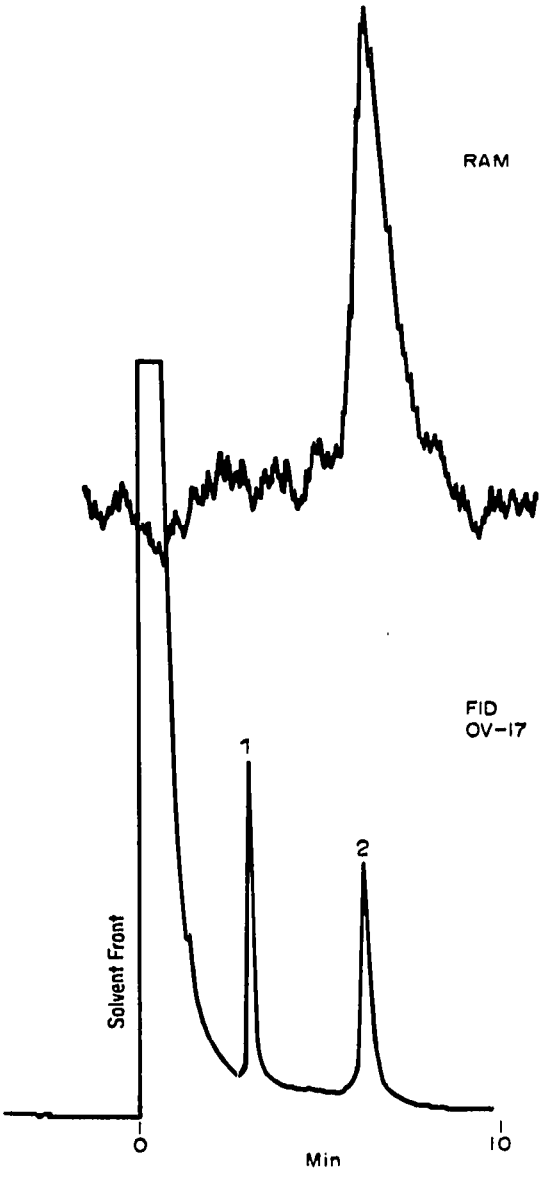

Fig. 1. GLRC of the acetylated product from the *in vitro* reaction of dopamine with [5-^{14}C]MTHF. Upper chromatogram, radioactivity monitor. Lower chromatogram, flame ionization detection; 1 = triacetyl epinine; 2 = triacetyl THIQ. The column conditions (OV-17) are given in Experimental.

that with each stationary phase the radioactivity was associated with the triacetyl derivative of THIQ (see Fig. 1).

While these studies were in progress*, similar results were published by Meller *et al.*[7]. Using several TLC systems, these workers demonstrated that THIQ has chro-

* A preliminary report of our findings was presented recently[8].

matographic characteristics very similar to that of epinine and that the radioactive product from this reaction was indeed the former. They further speculated that the TLC similarities probably account for the original failure to identify the radioactive product correctly. It is clear from our report that use of GLRC is a superior approach, at least in this case, to TLC for the identification of ^{14}C-labeled compounds.

In summary, the data described in this communication demonstrate that MTHF reacts with dopamine to form THIQ and not epinine. Although we have not examined the mechanism for the formation of THIQ, other workers[7,9] have shown that MTHF is enzymatically converted to formaldehyde which may react non-enzymatically with dopamine to form THIQ in a Pictet–Spengler condensation[10]. The significance of the THIQ formation *in vitro* remains to be established.

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