A CONVENIENT SYNTHESIS OF 2 - [^{14}C] - HORDENINE.

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Received on September 13, 1971

SUMMARY

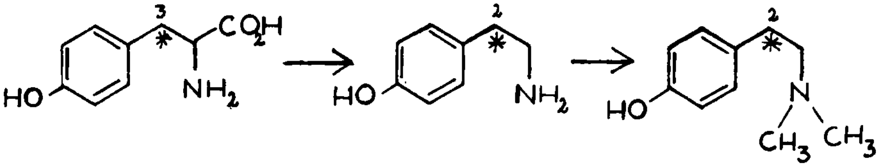
A simple and convenient laboratory procedure is described by which hordenine (III), labelled at the carbon residing on the benzene ring, can be synthesized by decarboxylation of 3-[^{14}C]-tyrosine (1) and subsequent reductive methylation of the resulting tyramine (II).

INTRODUCTION

Using phenethylalcohol as a starting material, Barger (1) obtained a base identical to natural hordenine. Rosenmund (2) condensed p-methoxybenzaldehyde with nitromethane to produce hordenine in low yield. Working with p-methoxyphenacyl chloride, Voswinckel (3) also obtained hordenine using tyrosol. Further syntheses of the alkaloid have been elaborated by Späth and Sobel (5) and Kindler (6). Raoul (7,8) obtained hordenine in 50% yield by methylating tyramine with formaldehyde and formic acid. In 1938 Buck, Baltzly, and Tde (9) reported a synthesis of hordenine starting with p-anisaldehyde. Cheng and coworkers (10), using a method they felt was more direct, reacted p-(β-hydroxyethyl)-anisole to yield hordenine.

Leete, Kirkwood, and Marion (11) synthesized 1-[^{14}C]-tyramine by a modification of the method of Koessler and Hanke (12). [^{14}C]-potassium cyanide was reacted with ethyl-p-chloromethylphenyl carbonate to give p-hydroxybenzylcyanide which in turn was reduced with hydrogen over Raney nickel in the presence of ammonia. The isolated tyramine was purified by sublimation and then converted to the water soluble hydrochloride (II). More recently Kanao and Suyama (13) have obtained hordenine hydrochloride in a 34.7% yield by decarboxylation of N,N-dimethyl-l-tyrosine.

In the course of our investigation of the biotransformation of the alkaloid hordenine in barley roots it was necessary to synthesize 2-[^{14}C]-hordenine (III). The present communication describes the procedure by which hordenine (III), labelled at the carbon adjacent to the benzene ring, can be synthesized by decarboxylation of 3-[^{14}C]-tyrosine (I) followed by reductive methylation of the resulting tyramine (II).



I II III

$* = {}^{14}C$

EXPERIMENTAL

Dl-3[^{14}C]-tyrosine was purchased from New England Nuclear Corporation, Boston, Massachusetts, U.S.A.

All activity measurements were made using a Packard Tricarb Scintillation Spectrometer Model 3375 with an efficiency of 75% and a background count of 30 cpm. The samples were counted in a liquid scintillation cocktail containing 2,5-diphenyloxazole (PPO) (4g), dimethyl-1,4-bis (2 (5-phenyloxazole)benzene) (POPOP) (0.1g), and Triton X-100 (370g) made up to 1 liter with distilled toluene. All cocktail chemicals were purchased from Packard Company, Downer's Grove, Illinois, U.S.A.

The diphenylamine was recrystallized from 95% v/v ethanol.

<u>The Decarboxylation of 3-[^{14}C]-Tyrosine to 2-[^{14}C]-Tyramine</u>

The procedure which was used is similar to that of Johnson and Daschavsky (14). In a typical experiment, dl-tyrosine (0.5g, 2.7mM) was mixed in a 50 ml round bottom flask with an aqueous solution of dl-3-[^{14}C]-tyrosine (1 ml, 0.013 mg) (8.1 X 10^5cpm/ml). The water was evaporated under reduced pressure in a flash evaporator at 35°C. The mixture was then dried overnight under high vacuum (0.5 mm Hg) at ambient room temperature. Freshly distilled diphenylmethane (3 ml) and diphenylamine (3g) were added to the dried residue. An air condenser was then attached to the flask and the mixture was heated at 260-265°C in a metal alloy bath for 40 minutes, or until the evolution of CO_2 stopped. The resulting clear yellow solution was allowed to cool to 60°C. Benzene (2.5 ml) was added and the contents of the flask thoroughly mixed. The reaction mixture was then allowed to stand for 1 hr in an ice bath. Tyramine, deposited as a fine yellow powder, was filtered and washed several times with warm benzene. The solid was then sublimed at 100° C (0.26 mm Hg) to produce a 72% yield of tyramine (0.274 g, 0.002 M) (2.15 X 10^3 cpm/mg). The identity of the product was verified by mixed melting points with authentic tyramine (mp 164-165°C) and IR spectra.

Only one spot (Rf = 0.65) could be detected with ninhydrin when a thin layer chromatogram was run on silica gel (0.25 mm thick) using chloroform-acetone-diethylamine (50:40:10) solvent. In this solvent mixture tyrosine had an Rf = 0.0. A radioautogram of the TLC plate showed only one radioactive spot other than that at the origin of the chromatogram.

Reductive Methylation of 2-[^{14}C]-Tyramine to 2-[^{14}C]-Hordenine

In methanol (30 ml) was dissolved 2-[^{14}C]-tyramine (0.2466g, 1.8mM). To this was added 40% formaldehyde (5 ml) and 10% palladium on charcoal (0.5g). The mixture was hydrogenated for 48 hr on a Parr hydrogenation apparatus.

The reaction mixture was filtered and washed with methanol. The filtrate and washings were combined and then reduced in volume to about 30 ml. The volume was brought up to 50 ml by adding a HCl solution (1.47 ml 10% HCl in 20 ml water). The mixture was heated in an Erlenmyer flask to reduce the volume to 30 ml, and water (20 ml) was again added. The procedure of evaporation and water addition was repeated four more times until a total of 100 ml of water was evaporated from the reaction mixture to remove paraformaldehyde, leaving the hordenine · HCl behind. The aqueous solution was made alkaline with concentrated ammonium hydroxide and extracted three times with equal volumes of ether. The ether solutions were combined and evaporated to dryness and the residue subsequently sublimed (125°C, 0.01 mm Hg). A 53.8% yield of hordenine (0.160g, 1.0mM) (2.7 X 10^5 cpm) was recovered.

Further purification of 2-[^{14}C]-hordenine was accomplished by thin layer chromatography on silica gel (0.50 mm) plates using chloroform-methanol-diethylamine (93:7:5). Under these conditions hordenine had an Rf value = 0.76 and tyramine Rf = 0.45. The hordenine was eluted from silica gel with 95% ethanol and the concentrated under vacuum. The identity of the so recovered hordenine was further established by nuclear magnetic resonance, by melting point (116-118°C), and mixed melting point (115-116°C) with authentic hordenine (generated from hordenine sulfate, Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A. and subsequently purified by sublimation). Also, when radioactive hordenine was chromatographed on Whatman No. 3 with n-butanol, saturated with water, according to the method of James and Butt (15) it exhibited Rf values (0.84) identical to authentic hordenine. Upon elution of the chromatogram with 95% ethanol the radioactivity was found to reside only in the hordenine spot.

ACKNOWLEDGMENT

This investigation was supported in part by a grant from the National Institute of Health, U.S. Public Health Service (5-R01AM12971-03 MCHA) and by a National Science Foundation undergraduate research grant (GY-7437).

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