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BIOSYNTHESIS OF HYGRINE FROM [5-^{14}C]ORNITHINE VIA A SYMMETRICAL INTERMEDIATE IN *NICANDRA PHYSALOIDES**

EDWARD LEETE

Natural Products Laboratory†, Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, U S A

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Abstract—Radioactive hygrine (2 2% incorporation) was isolated from *Nicandra physaloides* plants which had been fed DL-[5-^{14}C]ornithine A systematic degradation of the hygrine yielded products whose activity was consistent with the pyrrolidine ring of this alkaloid being labeled equally at the C-2 and C-5 positions The result does not agree with the previous work of O'Donovan and Keogh, whose publication is critically examined

INTRODUCTION

The *N*-methyl-Δ^1-pyrrolinium salt (**6**) is considered to be the precursor of several alkaloids nicotine [1–3], the tropane moieties of hyoscyamine, scopolamine [4], cocaine [5] and hygrine [6] This iminium salt **6** is derived from ornithine (**1**) It has been established that there are two distinct biosynthetic pathways (illustrated in Fig 1) whereby this conversion takes place Route A proceeds via putrescine (**2**) and *N*-methylputrescine (**4**) with subsequent oxidation of the primary amino group of **4** to yield 4-methylaminobutanal which cyclizes to **6** Since this route involves a symmetrical intermediate (putrescine) the iminium salt **6** derived from [2-^{14}C] or [5-^{14}C]ornithine will be labeled equally at its C-2 and C-5 positions Route B proceeds via δ-*N*-methylornithine (**3**) with subsequent decarboxylation to *N*-methylputrescine The pyrrolidine rings in nicotine and cocaine are formed via Route A, while hyoscyamine and scopolamine are formed by Route B Hygrine (**5**) is a biosynthetic precursor of hyoscyamine in *Datura* species [7, 8], being formed by reaction of the iminium in **6** with acetoacetic acid (**7**) [6] Thus the formation of hygrine in these species presumably proceeds via Route B O'Donovan and Keogh [7] investigated the biosynthesis of hygrine in *Nicandra physaloides* The radioactive hygrine (0 1% incorporation) obtained after feeding DL-[2-^{14}C]ornithine was degraded and it was established that all its radioactivity was located at the C-2 position This result is consistent with Route B to the iminium salt Since this publication [7] contains several inexplicable errors (to be discussed later) the biosynthesis of hygrine in the same species was investigated using DL-[5-^{14}C]ornithine as a precursor Hygrine formed from this compound via Route B would be labeled only at its C-5 position

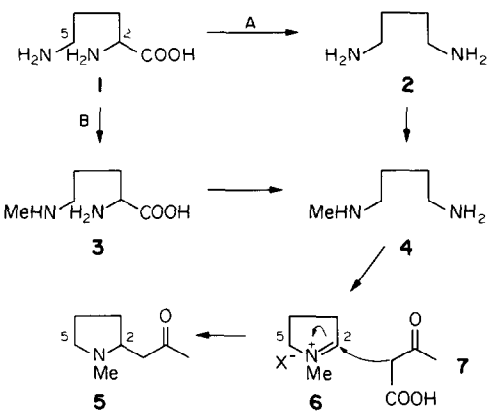

Fig 1 Biosynthetic routes from ornithine to the *N*-methyl-Δ^1-pyrrolinium salt and hygrine

RESULTS AND DISCUSSION

The hygrine isolated from the *Nicandra* plants which had been fed DL-[5-^{14}C]ornithine by the wick method was radioactive, the incorporation of activity being excellent (2 2% compared with 0 1% previously [7] obtained) The hygrine was crystallized to contrast specific activity as its picrate, and its oxime (**8**) was prepared to confirm its radiochemical purity The degradative scheme, illustrated in Fig 2, is essentially the same as one previously used [3] The specific activities of the various degradation products are also recorded in Fig 2 The final products of this degradation are the dimedone derivative of formaldehyde (**9**), arising from C-5 of hygrine, and the semicarbazone of hexanal (**10**) Within experimental error, these two compounds have the same specific activity, indicative of equal labeling at C-2 and C-5 of hygrine This result is thus consistent with the iminium salt being formed from [5-^{14}C]ornithine via Route A, i e via putrescine The [5-^{14}C]ornithine (Research Products International Corp) was obtained by the action of potassium [^{14}C]cyanide on α-benzamido-γ-butyrolactone followed by catalytic reduction and hydrolysis There is thus no ambiguity about the location of the ^{14}C label Since the

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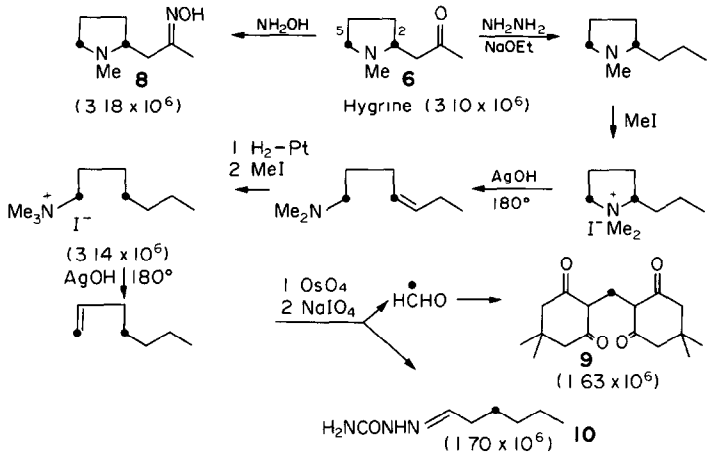

Fig 2 Degradations used to determine the distribution of radioactivity in hygrine derived from [5-^{14}C]ornithine ($^{14}C_\bullet$) Activities in parentheses are dpm/mmol

result reported in this paper does not agree with the one previously reported by O'Donovan and Keogh [7], it is now pertinent to examine critically their publication, which contains some serious errors

The first error concerns the incorporation of [2', *N-methyl*-^{14}C]hygrine into cuscohygrine (**11**), which is illustrated in Fig 3 The authors correctly state that the ratio of the specific activity of the *N*-methyl group to the activity in the C=O of cuscohygrine should be half that of the corresponding groups in the administered hygrine The cuscohygrine was degraded as illustrated in Fig 3 Reaction with phenylmagnesium bromide yielded 2-phenyldihydrocuscohygrine (**12**) which was oxidized to afford benzoic acid, representing the activity at the C=O group of cuscohygrine A Herzig–Meyer reaction on the

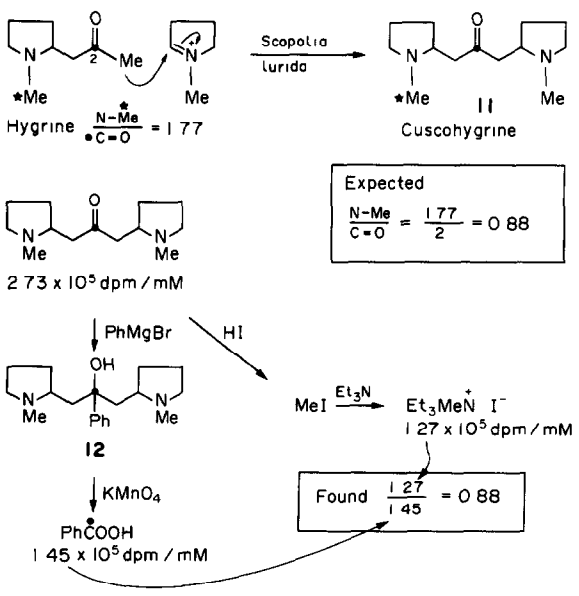

Fig 3 Results of O'Donovan and Keogh [7] on the biosynthesis of cuscohygrine

cuscohygrine yielded methyl iodide which was assayed as the quaternary salt triethylmethylammonium iodide Sure enough, the ratio of the specific activities of these two compounds was identical with the expected one However, the authors have overlooked the fact that two molecules of methyl iodide are generated from one of cuscohygrine Thus, the specific activity of the cuscohygrine = the specific activity of the benzoic acid + 2 × the specific activity of the triethylmethylammonium iodide Solving for a specific activity of 2 73 × 10^5 dpm/mmol for cuscohygrine, and a ratio of NMe/CO of 0 88 the theoretical activities of benzoic acid and the quaternary salt should be 9 89 × 10^4 and 8 70 × 10^4 dpm/mmol, respectively A second discrepancy relates to the physical properties of some of the degradation products The compound **12** was described as having mp 118–120°, whereas I [10] and others [11] have recorded an mp of 61–62° and 63–64°, respectively for this compound 3-Phenyltropine is reported as a compound which was recrystallized from ethanol having mp 177–179° In our hands [10] this compound had mp 162–163° and was very soluble in ethanol This substance was first described as having mp 158–159° [12]

The degradation used by O'Donovan and Keogh is unequivocal, indeed it is based on one developed by me 30 years ago for the degradation of stachydrine [13] and later used by others [14] In conclusion, I propose that the senior author (O'Donovan) has been the victim of what I choose to call the 'ESP-syndrome' This is an acronym for Eager to Satisfy the Preconceived ideas of one's research advisor

EXPERIMENTAL

General Radioactive materials were assayed in duplicate (yielding sp acts which were within 5% of each other) in a Tracor Analytic Mark III liquid scintillation counter using dioxane–EtOH as a solvent with the usual scintillators [15] Seeds of *Nicandra physaloides* were obtained from the Institut fur Pharmazeutische Biologie und Phytochemie through the courtesy of Professor H Friedrich

Administration of DL-[5-^{14}C]*ornithine to* Nicandra physaloides *plants and isolation of the hygrine* DL-[5-^{14}C]Ornithine (2 73

$\times 10^8$ dpm, 11 5 mCi/mmol) dissolved in H_2O was fed to 10 *Nicandra physaloides* plants (2 months old), growing in soil in a greenhouse, by means of cotton wicks inserted into the stems After 9 days the plants (fr wt 1450 g) were harvested and chopped up in a Waring blender with a mixture of CH_2Cl_2 (41) and conc NH_3 (300 ml) Non-radioactive hygrine (2 mmol) was added to the mixture at this stage After filtering, the organic layer was evapd in the presence of 2 N HCl (200 ml) The residual aq soln was filtered from some tar, then made basic with NaOH, and extracted with $CHCl_3$ The residue obtained on evaporation of this dried ($MgSO_4$) extract was distilled (60°, 10^{-3} mm) into a U-tube cooled in dry ice The crude hygrine (285 mg) was converted to its picrate which was crystallized from EtOH to yield material with a constant sp act ($3\ 10 \times 10^6$ dpm/mmol) TLC of the crude alkaloids from the plant (on SiO_2 developing with a mixture of $CHCl_3$–EtOH–conc NH_3, 60 40 5) indicated that 77% of the radioactivity was located at a spot, R_f 0 6, coincident with hygrine

Degradation of the hygrine Details of the degradation have been previously described [3] except for the oxidation of the 1-heptene, which was carried out as follows 1-Heptene (98 mg, 1 mmol), obtained from the Hofmann degradation of 1-dimethylaminoheptane methiodide, dissolved in Et_2O, was allowed to react with OsO_4 (250 mg) in the presence of one drop of pyridine After 18 hr the brown reaction mixture was evapd and the residue refluxed in 50% MeOH (40 ml) with sodium sulphite (1 g) for 1 hr The filtered solution was evapd to small bulk and the residual aq soln extracted with Et_2O in a continuous extractor for 18 hr The residue obtained on evaporation of the Et_2O was dissolved in H_2O (10 ml), and $NaIO_4$ (0 4 g) added After 30 min the solution was extracted with Et_2O (3×20 ml) The Et_2O extract was evapd in the presence of semicarbazide hydrochloride (0 5 g) and NaOAc (0 5 g) in H_2O (10 ml) After standing for 2 days, this soln was extracted with Et_2O Evaporation of this extract (without drying) yielded hexanal semicarbazide (38 mg) which was obtained as colourless plates from aq MeOH, mp 114–115° The aq soln from the cleavage of heptane-1,2-diol with $NaIO_4$ was distilled into a soln of dimedone (200 mg) in H_2O (100 ml) The formaldehyde dimedone which separated (70 mg) was crystallized from MeOH

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