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SHORT COMMUNICATION

BIOSYNTHESIS OF THE TROPINE MOIETY OF HYOSCYAMINE FROM δ-*N*-METHYLORNITHINE

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Abstract—The hyoscyamine and hyoscine isolated from *Datura stramonium* plants which had been fed DL-α-*N*-methyl-^{14}C-ornithine-2-^{14}C were not radioactive. However, a significant incorporation of activity (0·63%) was found in hyoscyamine and hyoscine obtained from plants which had been fed DL-δ-*N*-methyl-^{14}C-ornithine-2-^{14}C. A systematic degradation of the hyoscyamine indicated that all the activity was located in the tropine base at the bridgehead carbon C-1 (having the (R)-configuration) and on the *N*-methyl group. Furthermore, the ratio of activity at these two positions indicated that the δ-*N*-methylornithine had been incorporated without any cleavage of its *N*-methyl group.

NEUMAN and Schröter,[1] studying the metabolism of α- and δ-*N*-methyl-^{14}C-ornithine in *Datura* spp., reported that the α-isomer yielded radioactive hyoscyamine and hyoscine which were labelled on their *N*-methyl groups. However, the δ-*N*-methylornithine was a much poorer precursor of these alkaloids, and the activity was not confined to the *N*-methyl groups. From these results the authors claimed that α-*N*-methylornithine is a direct precursor of the pyrrolidine ring of tropine. It would thus follow that the nitrogen of the tropine nucleus is derived from the α-amino group of ornithine. However, Schütte and co-workers have recently shown[2,3] that the tropine nitrogen is derived from the δ-amino group of ornithine by carrying out feeding experiments with ^{15}N-labelled ornithine. Similar conflicting results were reported on the biosynthesis of the pyrrolidine ring of nicotine.[4,5] By the use of doubly labelled methylornithines we showed[6] that δ-*N*-methylornithine and not α-*N*-methylornithine was a specific precursor of the pyrrolidine ring of nicotine. We considered that the methylornithines used by Neuman and Schroter were not authentic since they were prepared by an ambiguous method.[7]

We have now tested α- and δ-*N*-methyl-^{14}C-ornithine-2-^{14}C, prepared by unequivocal methods,[6] as precursors of tropine. The labelled amino acids were added to the nutrient solution in which *Datura stramonium* plants were growing hydroponically. Neither isomer was absorbed rapidly from the nutrient solution; however, the δ-*N*-methylornithine was absorbed more quickly than the α-isomer. The alkaloids were isolated as previously described,[8] inactive hyoscyamine and hyoscine being added to facilitate separation and purification. The alkaloids obtained from plants which had been fed the α-*N*-methylornithine

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were inactive. The hyoscyamine and hyoscine obtained from the plants fed δ-*N*-methylornithine were both radioactive and the incorporation of activity (0·63%) was significantly greater than the incorporation of ornithine-2-^{14}C administered by the same methods to plants of a similar age (0·085%).[9] The hyoscyamine was degraded systematically by the previously described stereospecific method which gave information on the activity at C-1 having the (R)-configuration.[8,10] Activity at the *N*-methyl group of tropine was determined by the method of Brown and Byerrum.[11] Activities of the degradation products are recorded in Table 1. It was established that all the activity was located on the *N*-methyl group and at C-1. Furthermore the ratio of activity at C-1 to that of the *N*-methyl group of tropine was almost the same as the ratio of activity at C-2 to that of the *N*-methyl group in the administered δ-*N*-methyl-^{14}C-ornithine-2-^{14}C, indicating that the methylated amino acid was being incorporated intact without cleavage of the *N*-methyl group. Baralle and Gros,[12] comparing α- and δ-*N*-methyl-^{3}H-ornithine, found that the δ-*N*-methylornithine was a much superior precursor of the *N*-methyl groups of cuscohygrine and hyoscyamine in *Atropa belladonna*.

In tobacco, δ-*N*-methylornithine, although serving as a precursor of the *N*-methylpyrrolidine of nicotine, is not a "normal" biosynthetic intermediate between ornithine and

TABLE 1. HYOSCYAMINE AND ITS DEGRADATION PRODUCTS

	Activity (dis/min/mM × 10^{-6})	Relative activity
Hyoscyamine hydrochloride	1·8	100
(±)-Tropidine methiodide	1·8	100
(+)-α-Methyltropidine dibenzoyl-*d*-tartrate	1·8	100
Cycloheptanone	1·55	86
1-Phenylcycloheptanol	1·53	85
Benzoic acid [C-1]	1·47	82
Triethylmethylammonium iodide [*N*-methyl]	0·15	8·3

Activity at C-1/Activity at *N*-methyl = 9·8.

nicotine. Thus ornithine-2-^{14}C labels the pyrrolidine ring equally at C-2′ and C-5′, whereas δ-*N*-methylornithine-2-^{14}C labels only C-2′. These results were rationalized[6] by postulating that the tobacco plant is able to convert δ-*N*-methylornithine to *N*-methylputrescine with a non-specific decarboxylase.

In *Datura* the present results are consistent with δ-*N*-methylornithine being a real intermediate between ornithine and tropine, since it has previously been established that ornithine-2-^{14}C labels tropine specifically at C-1. *N*-Methylputrescine[13] and hygrine,[14,15] have also been shown to be precursors of tropine. We thus favor the biosynthetic scheme illustrated in Fig. 1. 4-Methylaminobutanal-^{14}C has been detected in *Datura* plants which were fed ornithine-2-^{14}C.[16]

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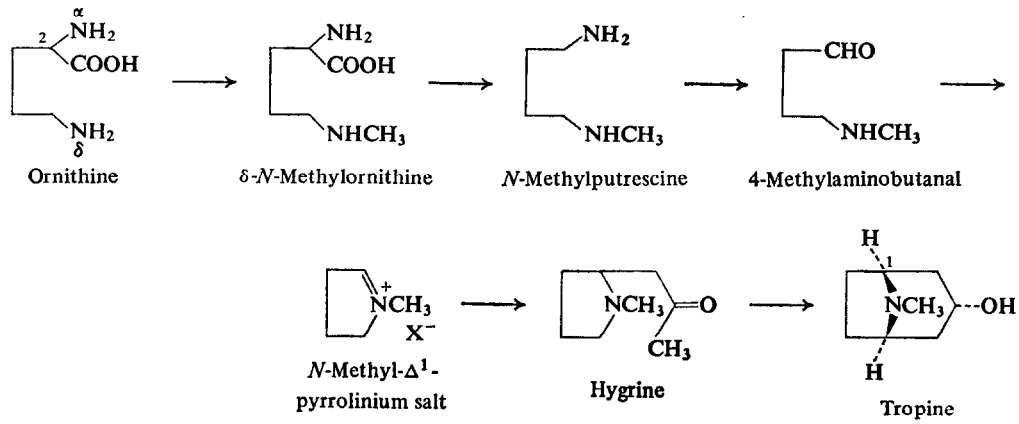

FIG. 1. BIOSYNTHETIC SCHEME FOR TROPINE.

EXPERIMENTAL

General Methods

Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation system, Model 724, using as solvents either toluene or dioxane, with the usual scintillators.[17]

α- *And* δ-N-*methyl-*^{14}*C-ornithine-2-*^{14}*C*

These *N*-methylornithines were prepared as previously described.[6] The actual weights and activities fed were:

α-N-*Methylornithine.* DL-α-*N*-methyl-^{14}C-ornithine hydrochloride (135 mg), $4{\cdot}1 \times 10^6$ dis/min. DL-α-*N*-methylornithine-2-^{14}C hydrochloride (18 mg), $3{\cdot}4 \times 10^7$ dis/min. Activity at C-2/Activity at *N*-methyl = 8·3.

δ-N-*Methylornithine.* DL-δ-*N*-methyl-^{14}C-ornithine hydrochloride (14·5 mg), $3{\cdot}0 \times 10^7$ dis/min. DL-δ-*N*-methylornithine-2-^{14}C hydrochloride (138·5 mg), $3{\cdot}15 \times 10^8$ dis/min. Activity at C-2/Activity at *N*-methyl = 10·5.

Feeding of the Tracers and Isolation of the Alkaloids

The *Datura* plants were 5 months old and growing in hydroponics at the time of feeding (December). The residual radioactivity in the nutrient solution to which the α-*N*-methyl-^{14}C-ornithine-2-^{14}C had been added was: 80% 1 week after feeding, 78% after 2 weeks, 38% after 3 weeks. Residual activity in the nutrient solution to which the δ-*N*-methyl-^{14}C-ornithine-2-^{14}C had been added was 78%, 47% and 1% after the same times. The plants were harvested 3 weeks after administering the tracers and worked up as previously described,[8] inactive hyoscyamine hydrochloride (200 mg) and hyoscine (200 mg) being added as carriers. The alkaloids obtained from the plants which had been fed the α-*N*-methylornithine had negligible activity. From the plants which had been fed the δ-*N*-methylornithine, hyoscyamine hydrochloride (198 mg, $1{\cdot}8 \times 10^6$ dis/min/mM) and hyoscine hydrochloride (188 mg, $1{\cdot}8 \times 10^6$ dis/min/mM) were obtained. These activities indicate an absolute incorporation of radioactivity into the alkaloids of at least 0·63%.

The degradation of the hyoscyamine was carried out as previously described.[8] Pyrolysis of the alkaloid yielded (±)-tropidine which was converted to its methiodide and subjected to a Hofmann degradation. The resultant (±)-α-methyltropidine was resolved with dibenzoyl-*d*-tartaric acid affording the (+)-isomer. Acid treatment followed by hydrogenation in the presence of Pd/C yielded cycloheptanone. Reaction with PhLi afforded 1-phenylcycloheptanol which was oxidized with $KMnO_4$ yielding benzoic acid. The hyoscyamine was demethylated with HI,[12] the resultant MeI being collected in an ethanolic solution of NEt_3 yielding $MeNEt_3I$. The activities of these degradation products are recorded in Table 1.

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