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δ-*N*-METHYLORNITHINE: A NATURAL CONSTITUENT OF *ATROPA BELLADONNA*

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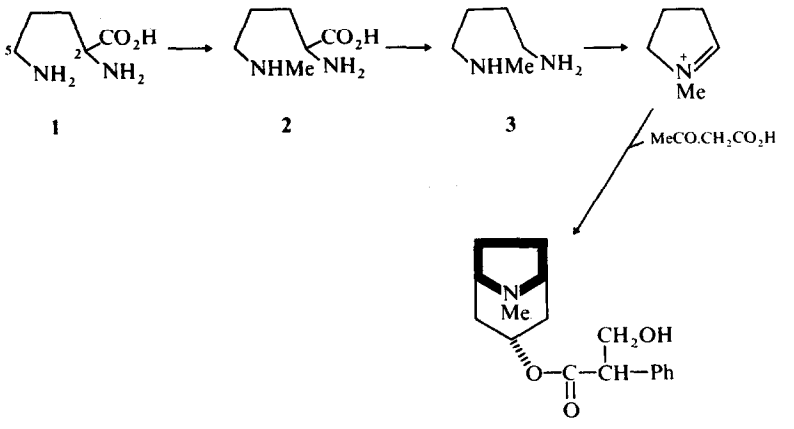
Key Word Index—*Atropa belladonna*; Solanaceae; δ-*N*-methylornithine; tropane alkaloid biosynthesis.

Abstract—δ-*N*-Methylornithine, a tropane alkaloid precursor, is shown for the first time to be a natural plant constituent; it was isolated in radioactive form after feeding [5-^{14}C]- and [5-^{3}H]ornithine to *Atropa belladonna*. This finding supports the deduced role of δ-*N*-methylornithine in tropane alkaloid biosynthesis.

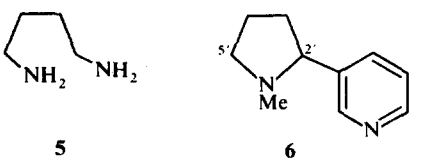
Tropane alkaloids, e.g. hyoscyamine (**4**), are formed, in part, from the α-amino acid, ornithine (**1**) [thickened bonds in **4**]. Label from C-2 of **1** appears at one of the bridgehead carbon atoms, and one only [1,2]. It follows from this that the steps which lie between **1** and **4** cannot involve symmetrical intermediates, such as putrescine (**5**), because this would result in label, from C-2 of **1**, being located on both bridgehead carbon atoms of **4**. In order to avoid symmetrical intermediates, it is believed that biosynthesis proceeds through methylation of **1** to give δ-*N*-methylornithine (**2**) as a first step with ensuing steps as illustrated in Scheme 1 [2]. This pathway is strongly supported by the intact incorporation into, for example, **4** of labelled δ-*N*-methylornithine (**2**) [3,4] and *N*-methylputrescine (**3**) [5,6]. However, **2** is also a precursor for nicotine (**6**) [7]. It cannot be a normal intermediate in the biosynthesis of **6** because this alkaloid is formed in a symmetrical manner from ornithine (**1**); label from C-2 of **1** appears equally divided between C-2′ and C-5′. In the light of this finding for nicotine, it is clear that more evidence is needed to establish whether, or not, δ-*N*-methylornithine (**2**) is *normally* implicated in tropane alkaloid biosynthesis.

Essential preliminary evidence relating to this involves discovering if **2** is a natural plant constituent formed, like the tropane alkaloids, from ornithine; **2** has not previously been reported as present in a plant. D,L-[5-^{14}C]- and D,L-[5-^{3}H]-ornithine were administered on separate occasions (August 1979 and June 1980) to *Atropa belladonna*, a plant which produces tropane alkaloids. At the end of the experiments the plant alkaloids were isolated; inactive D,L-δ-*N*-methylornithine (**2**) was added as diluent for any radioactive **2** present and this amino acid was then extensively purified. Significant



1 2 3 4

Scheme 1.



5 6

amounts of radioactivity (0.37 and 0.02% incorporation) were present in pure **2** establishing that **2** is present in *A. belladonna* and is formed from the same precursor (ornithine) as tropane alkaloids, thus strengthening the conclusion that **2** is normally involved in tropane alkaloid biosynthesis.

Unfortunately, in these experiments, neither the hyoscyamine (**4**) nor the cuscohygrine present was found to be radioactive. This may be because of the large amount of total alkaloid that was isolated. However, this meant that preliminary correlation of the biosynthesis of **2** with that of alkaloid biosynthesis was not possible; further experiments are in hand.

EXPERIMENTAL

All PCs were developed in the descending mode on Whatman No. 1 (analytical) or 3MM (preparative) at constant temp., after equilibration with solvent vapour. Chromatograms containing alkaloids were visualized with Dragendorff's reagent, those containing amino acids, with ninhydrin. Radioactive samples were recrystallized to constant activity. Mps were obtained on a hot stage.

D,L-*δ*-N-*Methylornithine*. This compound, **2**, was prepared as described previously [7], but the intermediate, *α*-*N*-benzoyl-*δ*-*N*-*p*-toluenesulphonylornithine, was hydrolysed to give D,L-*δ*-*N*-methylornithine (**2**) by a different method [8]. The monohydrochloride of **2**, from aq. EtOH had mp 242–243°, lit. 236–237° [7]. (C, 39.25; H, 8.2; N, 15.25; Cl, 19.15. Calc. for $C_6H_{14}N_2O_2$. HCl: C, 39.45; H, 8.25; N, 15.35; Cl, 19.45%).

Administration of radioactive ornithine to Atropa belladonna. D,L-[5-^{14}C]Ornithine (80 μCi) was assimilated, over *ca* 2 days, into two *A. belladonna* plants through wicks in the lower parts of the stems. After 15 days the plants were uprooted and processed for alkaloid and *δ*-*N*-methylornithine (August 1979). A similar procedure was used for the assimilation of D,L-[5-^{3}H]ornithine (500 μCi) (June 1980); 3 flowering plants were used.

Isolation and purification of alkaloids and δ-N-*methylornithine*. (Procedure for [5-^{14}C]ornithine feeding). The *A. belladonna* plants (*ca* 570 g) were macerated with H_2O (1 l.) and allowed to stand for successive periods of 16 hr with H_2O (1 l.), aq. EtOH (50%, 1 l.) and EtOH (1 l.). The combined extracts were concd *in vacuo* to *ca* 100 ml and the solid was removed by centrifugation. The solid was then extracted with hot H_2O (20 ml) and the combined solns were washed with toluene (3 × 100 ml). The pH of the soln was adjusted to 7 (aq. NH_3) and the ppt. removed by centrifugation.

Inactive D,L-*δ*-*N*-methylornithine (10 mg) was added to the above soln which was then passed down an ion-exchange column (Amberlite IRC-50, analytical grade, acetate form, 20 × 4 cm; cf. ref. [9]). Neutral and acidic amino acids were eluted with H_2O (200 ml). Aq. NH_3 (3 M, 500 ml) was used to elute basic amino acids (including **2**) and alkaloids.

Alkaloids were extracted from the ammoniacal eluate with $CHCl_3$ (6 × 200 ml). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evapd *in vacuo* to leave a light brown oil (0.31 g). Extraction of the first aq. eluates from the column gave further alkaloidal material of similar composition (0.27 g). (−)-Hyoscyamine was the major component of both extract residues; a small amount of cuscohygrine was also present. The alkaloids were separated on Celite 545 impregnated with 2 M HCl [10]. Hyoscyamine (0.49 g) was eluted with $CHCl_3$; picrate, mp 164–166° (from EtOH) undepressed on mmp with authentic material. Cuscohygrine (30 mg) was eluted with $CHCl_3$ satd with conc. aq. NH_3; dipicrate, mp 214–216° (from aq. EtOH) undepressed on mmp with authentic material; diperchlorate, mp 206–208° (from MeOH–Et_2O). In both feeding expts the isolated alkaloids were devoid of detectable radioactivity.

The aq. ammoniacal column eluate above, after extraction of alkaloids, was evapd to dryness *in vacuo* leaving a light brown solid (100 mg). This material was dissolved in H_2O (15 ml) and passed down an ion-exchange column (Dowex 50W-X8, 100–200 mesh, H^+ form, 12 × 2 cm). The column was washed with H_2O (150 ml) and then eluted with aq. NH_3 (3 M, 200 ml). The latter eluate, on evapn, gave a light brown solid (40 mg). This material was taken up in a small vol. of H_2O and applied to two sheets of prewashed 3MM paper. The chromatograms were developed with *n*-BuOH–HOAc–H_2O (2:1:1) and 3 ninhydrin-positive bands were observed (R_f 0.3, 0.51 and 0.62). The slowest moving band corresponded to *δ*-*N*-methylornithine and this band was eluted (H_2O) and the chromatography repeated. After elution of this band a second time the eluate was evapd to dryness. HCl (2 M, 10 ml) was added and the soln obtained evapd to dryness. PC showed that the *δ*-*N*-methylornithine thus obtained was pure and of identical R_f to authentic material. Inactive *δ*-*N*-methylornithine monohydrochloride (11 mg) was added and the material recrystallized to constant activity from aq. EtOH.

In the expt with [5-^{3}H]ornithine, the *δ*-*N*-methylornithine was further purified by PC (*m*-cresol–PhOH–borate buffer (0.1 M, pH 9.4), 4:1:satd; R_f 0.8) [11]. Material eluted from the paper was adsorbed on Dowex 50W-X8 and eluted with aq. NH_3. Dilution and recrystallization of the *δ*-*N*-methylornithine monohydrochloride was carried out as described above.

Incorporations: from [5-^{14}C]ornithine: 0.37%; from [5-^{3}H]ornithine: 0.02%.

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