Biosynthesis of Cuscohygrine and Hyosciamine in *Atropa belladonna* from DL-α-*N*-Methyl-[^{3}H]ornithine and DL-δ-*N*-Methyl-[^{3}H]ornithine†

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Summary DL-δ-*N*-Methyl-[^{3}H]ornithine is a good precursor of cuscohygrine and hyosciamine in *A. belladonna* plants while DL-α-*N*-methyl-[^{3}H]ornithine is not.

IT has been reported[2] that *Nicotiana tabacum* was able to utilize δ-*N*-methylornithine for the synthesis of the *N*-methylpyrrolidine ring of nicotine, but that α-*N*-methylornithine was a much poorer precursor of nicotine, suggesting that it was demethylated in the plant prior to its incorporation into the alkaloid. This result was in agreement with previous findings using α- and δ-^{15}N-labelled ornithine[3] but in disagreement with the results from Schröter and Neumann[4] who suggested a reverse situation.

The latter authors also reported[5] that administration of α-*N*-methyl-[^{14}C]ornithine to *Datura metel* and *D. stramonium* led to the formation of labelled tropane alkaloids (atropine, tropine, scopolamine, and scopoline) while δ-*N*-methyl-[^{14}C]ornithine was a poorer precursor of these alkaloids.

We report that when *Atropa belladonna* plants were inoculated with α-*N*-methyl-[^{3}H]ornithine and δ-*N*-methyl-[^{3}H]ornithine in separate experiments, the latter compound was a good precursor of cuscohygrine and hyosciamine whilst the former was hardly incorporated at all (see Table).

The values shown in the Table are in conflict with the results reported by the above mentioned authors;[5] however, they are in good accordance with recent findings by Schütte *et al.*[9,10] who proved, by the use of ^{15}N-labelled ornithine, that the δ-nitrogen of the amino-acid was incorporated into hyosciamine and cuscohygrine.

As ornithine is incorporated into tropine stereospecifically,[11] our experiments strongly suggest that ornithine is methylated on the δ-amino-group leading to δ-*N*-methylornithine, which is incorporated into the tropine moiety of hyosciamine and into the *N*-methylpyrrolidine rings of cuscohygrine with retention of the methyl group. A similar biosynthetic pathway was also suggested by O'Donovan and Keogh.[12]

Experiment 1. *Feeding of* α-N-*methyl*-[^{3}H]*ornithine hydrochloride*[a]

(m.p. 222—228°, $2{\cdot}50 \times 10^8$ d.p.m./mmole)[b]

Product isolated	Specific activity d.p.m./mmole	Specific incorporation %	Activity of *N*-methyl group[c] d.p.m./mmole
Cuscohygrine[d]	$2{\cdot}55 \times 10^2$	0·0001	—
Hyosciamine[d]	$2{\cdot}45 \times 10^3$	0·0009	—

Experiment 2. *Feeding of* δ-N-*methyl*-[^{3}H]*ornithine hydrochloride*[e]

(m.p. 231—234°, $1{\cdot}15 \times 10^9$ d.p.m./mmole)

Product isolated	Specific activity d.p.m./mmole	Specific incorporation %	Activity of *N*-methyl group[c]
Cuscohygrine	$5{\cdot}19 \times 10^6$	0·45	$2{\cdot}68 \times 10^6$
Hyosciamine	$1{\cdot}00 \times 10^6$	0·09	$0{\cdot}84 \times 10^6$

[a] Ref. 6; [b] radioactivities were determined in a Packard Model 3305 liquid scintillation spectrometer; [c] demethylation of the alkaloids was carried out as already described[7]; [d] for the isolations of the alkaloids, see ref. 1; [e] ref. 8.

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† For previous paper in this series, see ref. 1.

[1] F. E. Baralle and E. G. Gros, *Phytochemistry*, 1969, **8**, 853.
[2] T. J. Gilbertson and E. Leete, *J. Amer. Chem. Soc.*, 1967, **89**, 7085.
[3] E. Leete, E. G. Gros, and T. J. Gilbertson, *Tetrahedron Letters*, 1964, 587.
[4] H. B. Schröter and D. Neumann, *Tetrahedron Letters*, 1966, 1279.
[5] D. Neumann and H. B. Schröter, *Tetrahedron Letters*, 1966, 1273.
[6] H. Streib, *Z. physiol. Chem.*, 1926, **155**, 279; see also ref. 2.
[7] S. S. Brown and R. U. Byerrum, *J. Amer. Chem. Soc.*, 1952, **74**, 1523.
[8] K. Thomas, J. Kapfhammer, and B. Flaschenträger, *Z. physiol. Chem.*, 1922, **124**, 75; see also ref. 2.
[9] H. W. Liebisch and H. R. Schütte, *Z. Pflanzenphysiol.*, 1967, **57**, 434.
[10] H. W. Liebisch, A. S. Radwan, and H. R. Schütte, *Annalen*, 1969, **721**, 163.
[11] E. Leete, *Tetrahedron Letters*, 1964, 1619.
[12] D. G. O'Donovan and M. F. Keogh, *J. Chem. Soc.* (*C*), 1969, 223.