Phytochemistry, 1969, Vol. 8, pp. 849 to 851. Pergamon Press. Printed in England

BIOSYNTHESIS OF CUSCOHYGRINE IN *ATROPA BELLADONNA* FROM SODIUM ACETATE-1-^{14}C*

FRANCISCO E. BARALLE and EDUARDO G. GROS†

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Perú 222, Buenos Aires, Argentina

(*Received* 11 *September* 1968)

Abstract—The administration of sodium acetate-1-^{14}C to a 2-yr-old *Atropa belladonna* plant yielded radioactive cuscohygrine. Degradation of the alkaloid clearly indicated that tracer was all located on the carbonyl carbon. This result strongly supports the postulated pathway to the alkaloid from *N*-methylpyrrolidine and acetoacetate.

THE BIOSYNTHESIS of cuscohygrine (I), one of the main alkaloids of *Atropa belladonna*,[1] has been the subject of speculative discussions since Robinson postulated his general theory of formation of pyrrolidine alkaloids.[2] The reaction mechanisms outlined were tentative and most of them were discussed along with the biosynthesis of tropane alkaloids.[3,4] Although the tropane alkaloids have been extensively studied with the aid of labeled compounds, as

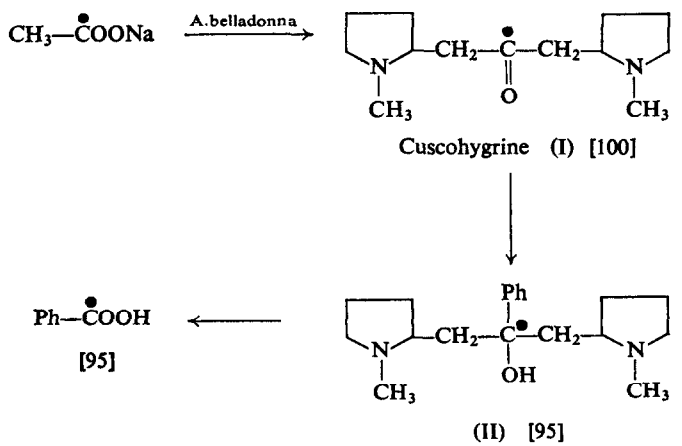

FIG. 1. DEGRADATION OF CUSCOHYGRINE DERIVED FROM SODIUM ACETATE-1-^{14}C.
Labeled carbon atoms are indicated with heavy dots. Figures in brackets represent relative specific activity.

* Part I in the series "Biosynthesis of Cuscohygrine in *Atropa belladonna*".
† Research member of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

[1] H. KING and L. L. WARE, *J. Chem. Soc.* 331 (1941); E. STEINEGGER and G. PHOKAS, *Pharm. Acta Helv.* **30**, 441 (1955); **31**, 284, 330 (1956).
[2] R. ROBINSON, *J. Chem. Soc.* 876 (1917).
[3] E. LEETE, Alkaloid biogenesis, in *Biogenesis of Natural Compounds* (edited by P. BERNFELD), p. 953, Pergamon Press, New York (1967).
[4] K. MOTHES and H. R. SCHÜTTE, *Angew. Chem. Int. Edit.* **2**, 341 (1963).

far as we know no tracer experiments have been done on the biosynthesis of cuscohygrine. The hypothetical pathway that leads to cuscohygrine (I) is closely related to the one for tropane alkaloids and probably involves a double Mannich reaction between *N*-methyl-Δ^1-pyrrolidine and acetoacetic acid;[5] if this hypothesis is correct it would be expected that acetate-1-^{14}C would label solely the carbonyl carbon of I, as has been found with hyoscyamine.[6]

We have fed sodium acetate-1-^{14}C, by the wick method, to one intact 2-yr-old *A. belladonna* specimen. The plant was harvested after 15 days and the alkaloids were extracted by the usual methods, separated by column chromatography on Celite and the fractions containing hyoscyamine, hyoscine, tropine and cuscohygrine were all found to be active.

The radioactive cuscohygrine was purified through its diperchlorate and this salt was then treated with phenyllithium in ether–benzene yielding the tertiary alcohol (II) which had essentially the same specific activity as the original alkaloid. Oxidation of II yielded benzoic acid which had almost the same specific activity as II. This result indicated that the alkaloid was only labeled on the carbonyl carbon and strongly suggested that it was derived directly from the carboxyl carbon of acetate, thus supporting, the biogenetic theory.

EXPERIMENTAL

M.ps were taken in a Fisher–Johns hot plate and are uncorrected. Samples were counted in a Packard Tri-Carb model 3305 liquid scintillation spectrophotometer in the usual scintillation solutions. NMR spectra were determined in a Varian A-60 spectrometer. Sodium acetate-1-^{14}C was purchased from the Comisión Nacional de Energia Atómica, Argentina. Microanalyses were performed by A. Bernhardt, Mülheim-Ruhr, Germany.

Administration of the Tracer to Atropa belladonna *and Isolation of Cuscohygrine*

One 2-yr-old healthy specimen of *A. belladonna* growing in soil was fed by the wick method in six different points with sodium acetate-1-^{14}C (231 mg; $2{\cdot}33 \times 10^9$ dpm/mM). The plant was harvested after 15 days and was macerated in a Waring blendor with $CHCl_3$–ammonia. After 2 days the mixture was filtered through cloth and the cake was extracted again in the same manner. The combined filtrates were acidified with 10% HCl and the organic solvent was removed. The remained acid aqueous solution was extracted twice with $CHCl_3$–ether (1:1), the organic extract being shaken with 10% HCl and discarded. The aqueous solutions were combined, made basic with 50% ammonia and extracted ten times with $CHCl_3$–ether (1:1). The mixture of crude alkaloids, obtained from evaporation of this dried extract, was separated on an acid-treated Celite column.[7] Elution with $CHCl_3$ gave fractions (125 ml each) containing hyoscyamine, hyoscine and tropine; when elution was carried out with ammonia-saturated $CHCl_3$ cuscohygrine and an unidentified alkaloid were collected in separate fractions. The different fractions were monitored by paper chromatography (Whatman No. 1, *n*-butanol:acetic acid:water (4:1:5)). The fraction containing cuscohygrine was evaporated after being made acidic with perchloric acid; the residue which had a specific activity of 2950 dpm/mg ($1{\cdot}25 \times 10^6$ dpm/mM, as the diperchlorate; specific incorporation 0·053 per cent) was diluted with inactive cuscohygrine diperchlorate (150 mg) and recrystallized from ethanol–ether to constant activity (142 mg; $1{\cdot}15 \times 10^4$ dpm/mM). Cuscohygrine diperchlorate had m.p. 212–213°, i.r. and NMR spectra were in agreement with its structure. Found: C, 36·90; H, 6·28; N, 6·51; Cl, 16·59. $C_{13}H_{26}O_9N_2Cl_2$ required: C, 36·71; H, 6·16; N, 6·59; Cl, 16·68 per cent.

Treatment of Cuscohygrine Diperchlorate with Phenyllithium

Radioactive cuscohygrine diperchlorate (74 mg; $1{\cdot}15 \times 10^4$ dpm/mM) was suspended in benzene (10 ml), treated with phenyllithium in ether (10 ml) and the mixture was refluxed for 3 hr in N_2. The cold mixture was then hydrolyzed by addition of water and 2 N HCl. The acid aqueous solution was extracted with ether (the extracts being discarded after showing negative alkaloid reaction). The remaining aqueous solution was made basic with 50% ammonia and extracted again with ether. The crystalline residue obtained from the dried extract by removal of the solvent was recrystallized from ethanol–water yielding 49 mg of 1,3-di-(2-

[5] E. LEETE, *Ann. Rev. Plant. Physiol.* **18**, 178 (1967).
[6] J. KACZKOWSKI, H. R. SCHÜTTE and K. MOTHES, *Biochim. Biophys. Acta* **46**, 588 (1961).
[7] E. LEETE, *J. Am. Chem. Soc.* **82**, 612 (1960).

(*N*-methylpyrrolidine))-2-phenyl-propan-2-ol (II) with an activity of $1{\cdot}10 \times 10^4$ dpm/mM. This was diluted with 50·8 mg of inactive product and recrystallized from the same solvent until constant activity (70·5 mg; $4{\cdot}71 \times 10^3$ dpm/mM). 1,3-Di-(2-(*N*-methylpyrrolidine))-2-phenyl-propan-2-ol (II) had m.p. 63–64°, its i.r. and NMR spectra being in accordance with the proposed structure. Found: C, 75·67; H, 10·09; N, 9·24. $C_{19}H_{30}ON_2$ required: C, 75·45; H, 10·00; N, 9·26 per cent.

Oxidation of the 1,3-*di*-(2-(N-*Methyl Pyrrolidine*))-2-*Phenyl-Propan*-2-*ol*

Compound II (70 mg; $4{\cdot}71 \times 10^3$ dpm/mM) was treated with CrO_3 (3 g) in 10% H_2SO_4 (10 ml) and the solution was distilled during 3 hr, keeping the volume of the reaction solution by addition of water and the distillate being collected over 5% KOH. The alkaline distillate was extracted with ether, the extract being discarded; the aqueous solution was then acidified with 2 N HCl and extracted with ether. The crystalline benzoic acid obtained on evaporation was sublimed twice (40–45°, 10^{-3} mm); it had m.p. 119–120° and its i.r. spectrum was identical to one obtained from an authentic sample. The benzoic acid had an activity of $4{\cdot}63 \times 10^3$ dpm/mM.

Acknowledgements—We thank Ing. Agr. A. M. Collura (INTA, Castelar) for making available the *A. belladonna* plant and the Consejo Nacional de Investigaciones Científicas y Técnicas (Buenos Aires) and FORGE (New York) for financial support.