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BIOSYNTHESIS OF CUSCOHYGRINE IN *ATROPA BELLADONNA* FROM SODIUM ACETATE-2-^{14}C*

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Abstract—The administration of sodium acetate-2-^{14}C to a 2-yr-old *Atropa belladonna* plant yielded, among other alkaloids, radioactive cuscohygrine. Degradation of the alkaloid clearly showed that tracer was all located on carbon-atoms 1 and 3.

IN OUR first paper on the biosynthesis of cuscohygrine[1] sodium acetate-1-^{14}C was administered to an intact *Atropa belladonna* plant resulting in the formation of radioactive cuscohygrine which was labeled specifically at the carbonyl carbon-atom. Recently, this result has been confirmed by O'Donovan and Keogh[2] and by Schütte[3] using plants of the genus *Datura*. The mentioned authors claimed that acetate feeding experiments confirm the proposed pathway[4] for the biosynthesis of cuscohygrine.

We wish now to report on extension of our previous work[1] confirming the hypothesis that the three-carbons chain between the two pyrrolidine rings is derived from acetoacetic acid.

Sodium acetate-2-^{14}C was fed by the wick method to a 2-yr old *A. belladonna* plant and after 15 days the plant was harvested. Cuscohygrine was isolated as described previously.[1] The radioactive alkaloid was oxidized by known methods[5] and the hygric acid (II) thus obtained was decarboxylated, by heating with calcium oxide;[6] CO_2 was collected as $BaCO_3$. This had, within experimental error, half the specific activity of the alkaloid, as it occurred with hygric acid.

This result indicated conclusively that cuscohygrine was labeled solely at carbon-atoms 1 and 3 (indicated with heavy dots in I) sustaining in this way the previously conceived hypothesis on the biogenesis of cuscohygrine.

* Part II in the series "Biosynthesis of cusgohygrine in *Atropa belladonna*" for Part I, see reference 1.
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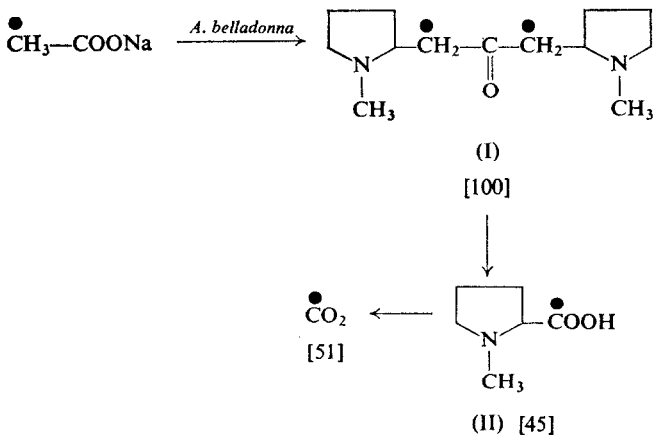

SCHEME. 1. DEGRADATION OF CUSCOHYGRINE DERIVED FROM SODIUM ACETATE-2-^{14}C. Labeled carbon-atoms are indicated with heavy dots. Figures in brackets represent relative specific activity.

EXPERIMENTAL

M.ps were determined with a Fisher–Johns block and are uncorrected. I.r. spectra were recorded with a Perkin-Elmer Infracord spectrophotometer. Samples were counted in a Packard Tri-Carb model 3305 liquid scintillation spectrometer in the usual scintillation solutions; $BaCO_3$ was counted by decomposing with H_2SO_4 in an evacuated and closed system, collecting the liberated CO_2 in methanolic hydroxide of Hyamine 10 X which was then diluted with the scintillation solution. Sodium acetate-2-^{14}C was purchased from New England Nuclear Corp., Boston, Mass. Solvents were removed under diminished pressure below 50°.

Administration of the Tracer and Isolation of Cuscohygrine (I)

Sodium acetate-2-^{14}C (41 mg; $4{\cdot}44 \times 10^9$ dpm/mM) was administered to one intact 2-yr old *Atropa belladonna* plant by means of wicks inserted into the stems of the plant in four different points. The plant was growing out of doors in soil. The plant was harvested after 15 days and the alkaloids were extracted by methods previously described.[1] The cuscohygrine was purified through its diperchlorate; it had a specific activity of $2{\cdot}42 \times 10^5$ dpm/mM (specific incorporation: 0·0055 per cent). It was diluted with inactive cuscohygrine diperchlorate (330 mg) and recrystallized from ethanol:ether to constant activity (287 mg, m.p. 212–213°, $4{\cdot}04 \times 10^3$ dpm/mM).

Oxidation of Cuscohygrine to Hygric Acid (II)

Cuscohygrine (free base, 140 mg, $4{\cdot}04 \times 10^3$ dpm/mM) was oxidized with CrO_3 (300 mg) in water (10 ml) and conc H_2SO_4 (1·5 ml) at 70–75° for 3 hr. The CrO_3 in excess was destroyed by addition of formaldehyde and the solution was then made alkaline with hot solution of $Ba(OH)_2$. The excess of hydroxide was eliminated by passing CO_2 through the solution, the solid was filtered off, and the filtrate was evaporated. The residue was dissolved in the minimum amount of water, copper carbonate (150 mg) was added and the mixture was boiled for 2 min. The solid was filtered off, and the filtrate was evaporated. The solid residue was extracted with $CHCl_3$ until the last two extracts came out colorless, the extracts being combined. The $CHCl_3$ solution was filtered through a small alumina column (Fluka 507 C, neutral, grade I), the column being washed with $CHCl_3$ until there was no more blue color in the eluate. The solution was evaporated and the blue, solid residue (58 mg) was dissolved in water (4 ml) and H_2S was bubbled through. The solid was filtered off and the aqueous solution was lyophilized. The yellow residue was recrystallized from ethanol:ether yielding hygric acid (II) (30 mg), m.p. 160–172°, its i.r. spectrum being identical to one from authentic sample. Activity $1{\cdot}81 \times 10^3$ dpm/mM.

Decarboxylation of Hygric Acid

A mixture of compound II (23 mg) and calcium oxide (100 mg) was heated at 300° under N_2 for 1 hr. The *N*-methylpyrrolidine which distilled off, was not collected. The remained solid residue was acidified with 50% H_2SO_4 and the evolved CO_2 was absorbed in $Ba(OH)_2$ to yield $BaCO_3$ (18 mg); this had an activity of $2{\cdot}05 \times 10^3$ dpm/mM.

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