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THE MECHANISM OF ACETOACETATE COUPLING IN THE BIOSYNTHESIS OF HYGRINE

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Abstract: Sodium[3-^{14}C]- and [4-^{14}C]-acetoacetate were wick fed to 3-month-old *Nicandra physaloides* plants. The hygrine was isolated and by degradation it was shown that acetoacetate is a precursor. It was also shown that the C(2) carbon is the reactive site in its condensation with the N-methyl-Δ'-pyrrolinium cation.

Carbons (2), (3) and (4) of the tropane ring (V) were shown by Kaczkowski, et al.[1,2] to be derived from acetate. In separate feeds with [1-^{14}C]-acetate and [2-^{14}C]-acetate to *Datura metel* they showed that the C(1) carbon incorporated into the C(3) position of the isolated hyoscyamine (VII). The C(2) carbon of acetate incorporated into the C(2) and C(4) positions. Later work by O'Donovan and Keogh[3] showed that [1-^{14}C]-acetate incorporated, as expected, into the C(2') position of hygrine (III) in *Nicandra physaloides*. They showed that [2'-^{14}C]-hygrine (III), when fed to *Datura stramonium*, gave hyoscyamine (VII) labelled in the C(3) position. They suggested that hygrine (III) was formed by a Mannich condensation reaction between an ornithine derived[4] N-methyl-Δ'-pyrrolinium cation (I) and an acetoacetate anion (II); the latter being formed from two acetate units. That acetoacetate (II) is a closer precursor was demonstrated by Liebisch, et al.[5] in feeds of sodium[2-^{3}H]-acetate and sodium[3-^{14}C]-acetoacetate to *Datura metel*. The incorporation of the carbon label was approximately five times greater than that of the tritium in the isolated hyoscyamine (VII). It is thought[3,6], that in the condensation with the N-methyl-Δ'-pyrrolinium salt (I), it is the C(2) carbon of acetoacetate (IIa) that is the reactive site.

The molecule then undergoes decarboxylation to give hygrine (III). From a mechanistic point of view this is the most likely course. However, the C(4) carbon of the acetoacetate (II) must at some time be activated to couple with the N-methyl-pyrrolidine nucleus (IV) to give the tropane ring (V). It is possible, therefore, that the C(4) carbon of the acetoacetate (IIb) is the first to couple with the N-methyl-Δ'-pyrrolinium cation (I) to give hygrine (III). Having been formed, hygrine (III), is thought[6] to undergo a secondary dehydrogenation to the Δ^{4}-pyrroline derivative (IV) which then reacts with the anion produced by removal of a proton from the methyl terminus of the side chain, thus forming the tropane skeleton (V).

Firstly, it was thought important to establish, beyond doubt, that acetoacetate (II) is

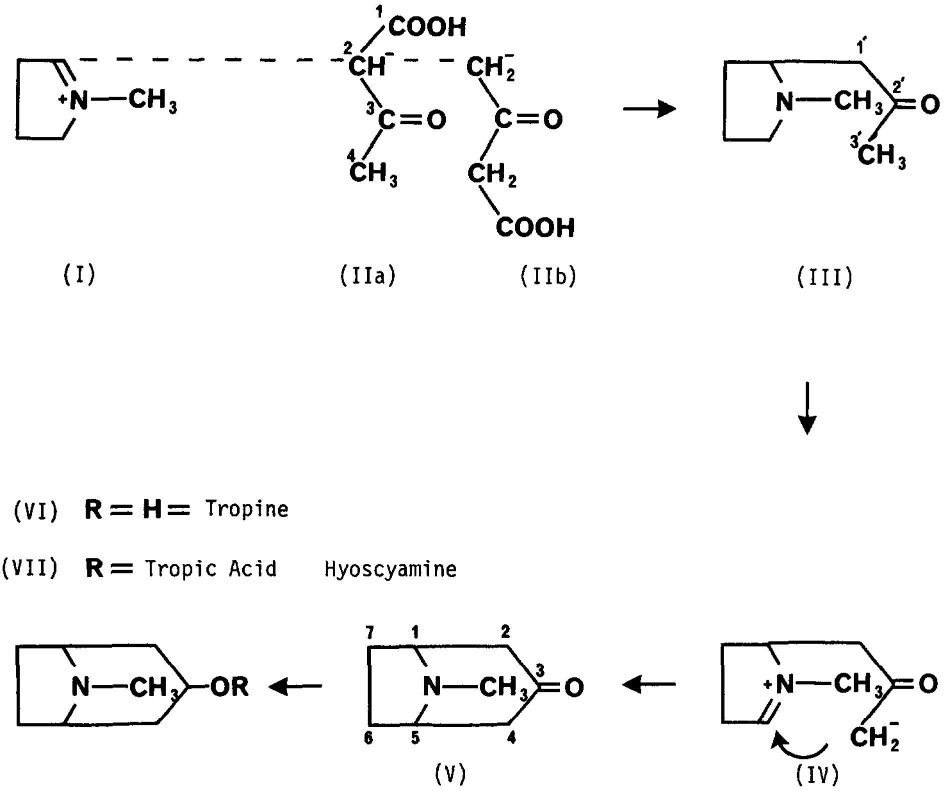

Fig. 1. The Biosynthesis of the Tropane Alkaloids

a precursor of the side chain of hygrine (III). Sodium$[3\text{-}^{14}C]$-acetoacetate was prepared[7] and wick fed to 10 x 3-month-old *Nicandra physaloides* plants (of which hygrine is the major alkaloid[8]). The plants were harvested after 2 weeks and the alkaloids were separated by partition column chromatography on kieselguhr at pH 6.8[9,10]. Hygrine (III) was eluted from the column in the chloroform fraction. The hygrine (III) was diluted and treated with an excess of phenyl magnesium bromide. The resulting carbinol (VIII) was oxidized with potassium permanganate to give benzoic acid (IX). From Table 1 it can be seen that the benzoic

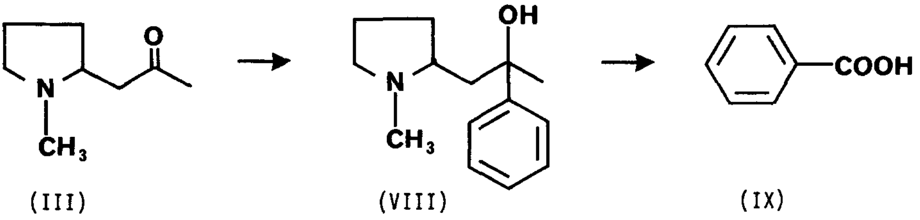

Fig. 2. The Degradation of Hygrine to Benzoic Acid

acid (IX) had 104% of the activity of the original hygrine confirming that the C(3) carbon of acetoacetate (II) gives rise to the C(2') carbon of the hygrine. Acetoacetate (II), therefore, is a direct precursor of the hygrine side chain. To investigate the coupling mechanism, sodium[4-^{14}C]-acetoacetate was prepared[7] and was wick fed to 8 x 3-month-old *Nicandra* plants. If the C(2) carbon of acetoacetate (IIa) is the reactive site in the formation of hygrine (III), then the C(3') position of the isolated hygrine would be labelled. On the other hand, if it is the C(4) carbon of acetoacetate (IIb) that is involved, the activity would reside in the C(1') carbon of the hygrine (III).

Table 1. Specific Activities of Hygrine and Benzoic Acid

Sp. act. of isolated hygrine dpm/mmol x 10^{-5}	Sp. act. of diluted hygrine dpm/mmol x 10^{-3}	Sp. act. of benzoic acid dpm/mmol x 10^{-3}	% Recovery of label in benzoic acid
1.13	2.48	2.50	100.8%

The isolated hygrine (III) was oxidized with chromium trioxide and sulphuric acid[3,11] to yield acetic (X) and hygrinic (XI) acids. The acetic acid (X) was found to contain 95.4% of the label of the original hygrine, confirming the mechanistically based view that the C(2) carbon of acetoacetate is the site of reaction in the formation of hygrine and probably, therefore, the first to react in the formation of the tropane moiety.

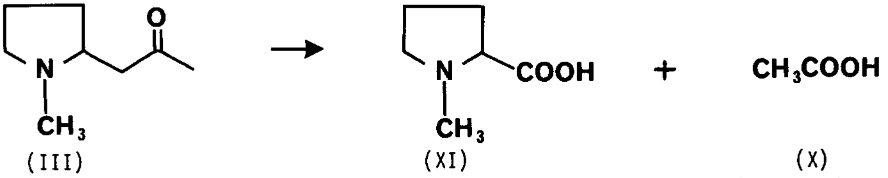

Fig. 3. The Degradation of Hygrine to Acetic and Hygrinic Acids

Table 2. Specific Activities of Hygrine and Acetic and Hygrinic Acids

Sp. act. of isolated hygrine dpm/mmol x 10^{-4}	Sp. act. of diluted hygrine dpm/mmol x 10^{-3}	Sp. act. of acetic acid dpm/mmol x 10^{-3}	Sp. act. of hygrinic acid dpm/mmol	% Recovery of label in acetic acid
9.72	2.40	2.29	0	95.4

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