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THE ROLE OF HYGROLINE IN TROPANE ALKALOID BIOSYNTHESIS

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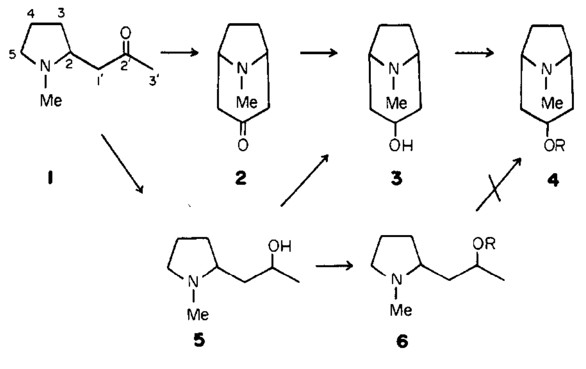
Key Word Index—*Datura*; Solanaceae; tropane alkaloids; biosynthesis; labelled hygroline.

Abstract—*Datura innoxia* Mill. were fed via the roots with [2′-^{3}H; 2′-^{14}C]hygroline. After 6 days the plants were harvested and the root alkaloids isolated. By ^{3}H/^{14}C label ratio it was shown that hygroline was not an immediate precursor of the tropane moiety.

INTRODUCTION

It is well-established that hygrine (**1**) is a precursor of the tropane alkaloids (**4**) [1,2]. The mechanism of this reaction is thought to involve an intra-molecular Mannich reaction between the quaternary Schiff's base on the pyrrolidine ring C-5 carbon and the C-3′ carbon of the side chain, giving tropinone (**2**) [3]. The tropinone undergoes stereospecific reduction [4] yielding tropine (**3**) which can then be esterified to give the various tropane ester alkaloids (**4**) commonly found in *Datura* [3, 5, 6].

Other routes from hygrine to the tropane alkaloids have been postulated [7] (Scheme 1). It was considered that reduction to hygroline (**5**) and even subsequent esterification with tiglic or tropic acid (**6**) may be precyclization events. When tigloyl hygroline (**6**), labelled in the acid and alkamine moieties, was fed to *Datura meteloides* the isolated alkaloids were hydrolysed and it was shown by changes in the label ratio between the esterifying acid and the alkamine that extensive hydrolysis of the precursor had occurred *in vivo* [7]. This excludes the possibility that it is a precursor for the tigloyl ester series of alkaloids (**4**). However, in the same experiment, [2′-^{14}C]hygrine (**1**) was fed as a control and it was found to be incorporated to a lesser extent than the pyrrolidine component produced by hydrolysis of tigloyl hygroline (**6**). This suggested that hygroline (**5**) may be the more immediate precursor of tropine (**3**). Possible support for this view has been lent by the identification of (−)-2*S*,2′*S*-hygroline (**5**) from *Cochlearia artica* (Cruciferae) [8] and *Erythroxylon coca* (Erythroxylaceae) [9]. Recently, [10] (−)-2*S*,2′*S*-hygrolin and (+)-2*R*,2′*S*-pseudohygroline have been found to be natural components of *Schizanthus hookeri* (Solanaceae).



R = Tigloyl or tropoyl moiety

Scheme 1. Possible routes to the tropane alkaloids from hygrine.

This paper reports on the results of feeding [2′-^{3}H; 2′-^{14}C]hygroline (**5**) to *Datura innoxia* plants to test whether the precursor is incorporated intact (an event which leads to the retention of the tritium) or whether it is first oxidized to hygrine (**1**), in which case loss of tritium may be anticipated.

RESULTS AND DISCUSSION

The data are presented in Table 1. It is clear that hygroline (**5**) is actively metabolized and incorporated into the tropane moiety (**4**). The label ratios, however, reveal that 90% of the tritium has been lost. Two possibilities were considered. Firstly, hygroline (**5**) could be the immediate precursor, but saturating the system with exogenously applied substrate caused the majority of it to be oxidized back to hygrine (**1**) and then incorporated via hygroline at a later date. This was thought to be unlikely because of the close agreement between the label ratios of the isolated alkaloids, whereas the specific incorporations of ^{14}C were all different.

Secondly, it was considered that hygroline (**5**) was not a direct precursor and that the small amount of tritium retained was not at the C-2′ carbon in the precursor. The simple synthetic procedure involved the reduction of hygrine with ^{3}H-labelled sodium borohydride and it is possible that under the alkaline conditions used some exchange of acidic protons at C-1′ or hygrine could have occurred. In testing this possibility [2′-^{3}H; 2′-^{14}C]hygroline was oxidized back to hygrine. This hygrine had a ^{3}H/^{14}C ratio of 115 (10.3% retention of tritium). Therefore, the second premise is correct and hygroline (**5**), although not a direct precursor of tropine (**3**), can be converted back to hygrine (**1**) in *Datura* and thus serve as a precursor.

EXPERIMENTAL

Plant material. Datura innoxia Mill. plants were grown on open land in Leicester from seeds obtained from the Zentralinstitut für

Table 1. The specific activities (with reference to ^{14}C only) and ^{3}H/^{14}C label ratios of the major alkaloids of *Datura innoxia* following feeds with [2′-^{3}H; 2′-^{14}C]hygroline (^{3}H/^{14}C = 1113)

Alkaloid	Sp. act. × 10^{-5} (dpm/mmol)	% sp. incorporation (^{14}C only)*	^{3}H/^{14}C	%^{3}H retained
I	3.28	3.28	98.6	8.8
II	6.93	6.93	106.4	9.5
III	1.04	1.04	96.1	8.6
IV	0.22	0.22	115.0	10.3

*Calculated as [sp. act. base (dpm/mmol)]/[sp. act. precursor (dpm/mmol)] × 100.

I, hyoscine; II, hyoscyamine; III, 3α,6β-ditigloyloxytropane; IV, 3α,6β-ditigloyloxytropan-7β-ol.

Genetik und Kulturpflanzenforschung, Gatersleben, East Germany. The plants had all the characteristics previously described [11].

Counting procedures. Duplicate samples were counted in commercially available toluene or dioxane based POP–POPOP scintillators in a liquid scintillation spectrometer.

Tracer material. [3-^{14}C]-labelled Sodium acetoacetate and $NaB^{3}H_4$ were purchased from the Radiochemical Centre, Amersham, U.K.

Synthesis of (2-RS)-[2′-^{14}C] *hygrine.* This material (228.5 mg), of sp. act. 1 × 10^{7} dpm/mmol, was synthesized as previously reported [2, 12].

Synthesis of (2-RS,2′-RS)-[2′-^{3}H; 2′-^{14}C]*hygroline.* The above material was reduced by the addition of $NaB^{3}H_4$, 100 mCi (75 mg) in dry MeOH (8.9 ml) and refluxing overnight. TLC showed the presence of a single compound which co-chromatographed with hygroline prepared by catalytic reduction of hygrine [7]. The mixture was evaporated to dryness, the residue dissolved in 20% NaOH and extracted with $CHCl_3$ (5 × 20 ml). The $CHCl_3$ was bulked, dried (Na_2SO_4), filtered and evaporated to give [2′-^{3}H; 2′-^{14}C]hygroline (200 mg), picrate mp 128° (lit. [13] 128–129°). ^{1}H NMR (100 MHz, $CDCl_3$): δ 1.25 (3H, *d*, H-3′), 1.7 (4H, *m*, H-3, H-4), 2.1 (2H, *t*, H-1′), 2.4 (3H, *s*, N–Me), 2.6 (2H, *t*, H-1), 3.0 (1H, *m*, H-2), 4.0 (1H, *m*, H-2′) and 4.9 (1H, *s*, –OH).

Feeding expts. The labelled hygroline (50 mg) was dissolved in H_2O (5 ml) and distributed to five 5-month-old *Datura innoxia* plants that had been carefully uprooted and suspended in blackened beakers containing Phostrogen soln. After 6 days the plants were harvested and the roots and aerial parts separately dried at 60° for 24 hr.

Isolation of the alkaloids. The finely powdered roots (34 g) were extracted and the bases resolved on Pi partition columns at pH 6.8 and 5.6 as previously described [14, 15]. The bases were counted as their picrate salts: hyoscine picrate (7.0 mg) mp 186°, sp. act. (^{14}C only) 3.28 × 10^{5} dpm/mmol, ^{3}H/^{14}C = 98.6; hyoscyamine picrate (70.0 mg) mp 166°, sp. act. (^{14}C only) 6.93 × 10^{5} dpm/mmol, ^{3}H/^{14}C = 106.4; 3α,6β-ditigloyloxytropane picrate (10.0 mg) mp 150°, sp. act. (^{14}C only) 1.04 × 10^{5} dpm/mmol, ^{3}H/^{14}C = 96.1; 3α,6β-ditigloyloxytropan-7β-ol picrate (8.0 mg) mp 182°, sp. act. (^{14}C only) 0.22 × 10^{5} dpm/mmol, ^{3}H/^{14}C = 115.

Degradation of [2′-^{3}H; 2′-^{14}C]*hygroline.* The above labelled hygroline (50 mg) was refluxed with CrO_3 (50 mg) in 85% HOAc (5 ml) for 1 hr. The cooled mixture was basified (conc. NaOH) and extracted with $CHCl_3$ (5 × 10 ml). The $CHCl_3$ was dried (Na_2SO_4), filtered and evaporated under red. pres. Analysis of the residue by TLC [16] revealed the complete conversion of the starting material to hygrine. The hygrine was converted to its picrate which was recrystallized to a constant ^{3}H/^{14}C ratio of 115.1.

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