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STEREOCHEMISTRY OF TROPANE ALKALOID FORMATION IN *DATURA*

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Key Word Index—*Datura innoxia*; Solanaceae; biosynthesis; tropane alkaloids; cuscohygrine; D(+) and L(−)-hygrine precursors.

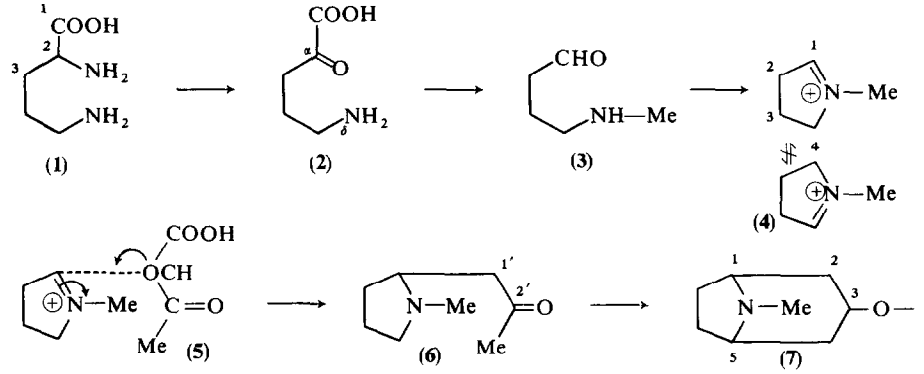
Abstract—Five-month-old *Datura innoxia* plants were fed via the roots with either D(+)-hygrine-[2′-^{14}C] or L(−)-hygrine-[2′-^{14}C]. After 7 days the root alkaloids 3α,6β-ditigloyloxytropane, 3α,6β-ditigloyloxytropan-7β-ol, hyoscine, hyoscyamine and cuscohygrine were isolated from both groups of plants. D(+) but not L(−)-hygrine acts as a precursor for the tropane alkaloids whereas both enantiomers appeared to serve equally well in the biosynthesis of cuscohygrine.

INTRODUCTION

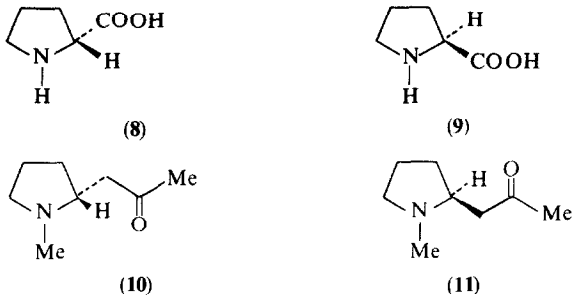
It has been established that carbons 1, 5, 6 and 7 of the tropane ring are derived from ornithine (**1**) which incorporates unsymmetrically, C(2) of ornithine giving C(1) of the tropane nucleus (**7**) [1–3] (Scheme 1). Experiments with α and δ-$^{15}NH_2$ labelled ornithines indicate that the δ-nitrogen is retained [4], transamination producing α-keto-δ-aminovalerate (**2**) [5]. Decarboxylation of the α-keto acid, perhaps preceded by methylation [6] gives rise to 4-*N*-methylaminobutanal (**3**) and the *N*-methyl-Δ^1-pyrrolinium salt (**4**). This cation is known not to undergo tautomerism *in vivo*, thus maintaining the non-symmetrical nature of the pathway [7]. The *N*-methyl-Δ^1-pyrrolinium salt (**4**) probably condenses with an acetoacetate anion (**5**) [8] producing hygrine (**6**) an established precursor of hyoscyamine and cuscohygrine [9]. Since there are two optical isomers of hygrine, both of which may be formed, depending upon which side of the *N*-methylpyrrolinium ring the acetoacetate approaches, we postulated that in nature they may have separate roles.

RESULTS AND DISCUSSION

Hygrine has been reported as a constituent of several plants, *Nicandra* and *Physalis* (Solanaceae), *Convolvulus* (Convolvulaceae), *Dendrobium* (Orchidaceae), *Cochlearia* (Cruciferae) and *Erythroxylum* (Erythroxylaceae) [10, 11]. When isolated, it appears to have no detectable optical activity and it has been shown that the small *laevo* rotation of the base isolated originally from coca leaves is due to contamination with traces of hygroline [12]. In our experience hygrine obtained from *Nicandra* also shows no optical rotation. However, hygrine is easily resolved by means of the D(+)-tartrate salt [13] and Lukeš [14] has correlated the absolute configuration of the (+) and (−) enantiomers (**10**) and (**11**) with D and L proline (**8**) and (**9**) respectively. The optically active base readily racemizes but the salts are stable and for this reason the tracers were administered as the tartrates to *Datura*. *Datura* exhibits a distinct preference for the (+) enantiomer in the cyclization process leading to the formation of the tropane ring (Table 1). D(+)-Hygrine (**13**) incorporates into the tigloyl (**19**) and tropoyl (**20**) series of esters indicated (Scheme. 3) entering the alkamines tropine, tropan-3α,6β-diol, teloidine and scopine (of alkaloids hyoscyamine (**16**), 3α,6β-ditigloyloxytropane (**18**), 3α,6β-ditigloyloxytropan-7β-ol (**17**) and hyoscine (**15**) respectively). Some radioactivity from the L(−)-hygrine (**14**) feed also enters these bases but this can easily be attributed to minor contamination with the *dextro* isomer. A single recrystallization of the less soluble D(+)-hygrine D(+)-tartrate was found to give *ca* 80% optical purity, but subsequent recrystallizations increased the optical



Scheme 1. The formation of tropane alkaloids from ornithine.



Scheme 2. The configurational relationships between D and L proline, and (+) and (−)hygrine

rotation only marginally. Similarly, after crystallizing out as much D(+)-hygrine D(+)-tartrate as possible, some was still left in the mother liquor.

The hyoscyamine (**16**) labelled from the D(+)-hygrine feed, was diluted with carrier, hydrolysed and the resultant tropine was oxidized to troponone with chromium trioxide in acetic acid. The tripinone was then coupled with phenyl magnesium bromide to yield 3-phenyltropan-3-ol. The latter compound when refluxed with aqueous permanganate gave benzoic acid which contained all the radioactivity of the original base (C(3) of the tropane ring), thus confirming the non-random incorporation of the precursor [8, 9].

Table 1. The specific activities of the root alkaloids isolated from *Datura innoxia* plants after feeding with D(+) and L(−)-hygrine-[2′-^{14}C]

Precursor	D(+)-Hygrine-[2′-^{14}C] 1×10^7 dpm/mmol		L(−)-Hygrine-[2′-^{14}C] 1×10^7 dpm/mmol	
Alkaloid	Sp. act. dpm/mmol $\times 10^{-5}$	%Sp. incorporation*	Sp. act. dpm/mmol $\times 10^{-5}$	%Sp. incorporation*
I	1.34	1.34	0.36	0 36
II	2.29	2.29	0.42	0.42
III	2.09	2.09	0.22	0.22
IV	2.25	2.25	0.21	0.21
V	3.44	3.44	2.03	2.03

I—Hyoscine; II—hyoscyamine; III—3α,6β-ditigloyloxytropane; IV—3α,6β-ditigloyloxytropan-7β-ol; V—cuscohygrine. Calculated as [sp. act. base (dpm/mmol)]/[sp. act. precursor (dpm/mmol)] × 100.

The pathway to cuscohygrine displays little stereochemical preference for either isomer, both are apparently able to condense with a second *N*-methyl-Δ^1-pyrrolinium (**4**) cation. Even so, the *dextro* isomer incorporates nearly twice as well as the *laevo*. Natural cuscohygrine (**12**) is known to exist as the *meso* form and the bridgehead proton of the second *N*-methylpyrrolidine is arranged in biosynthesis *cis* with respect to the first bridgehead proton.

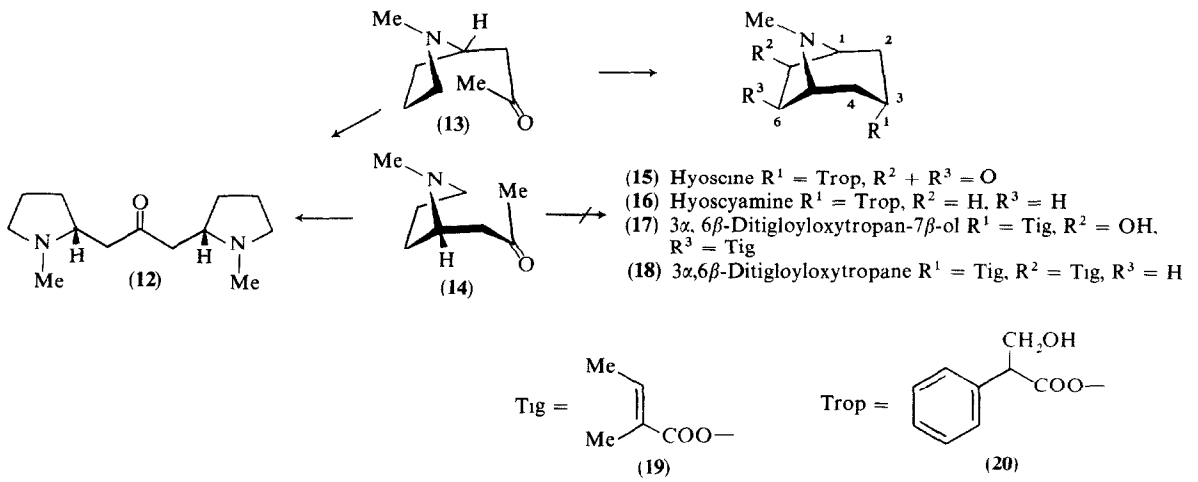
EXPERIMENTAL

Datura innoxia plants were grown on open land in Leicester from seeds obtained from the Zentralinstitut für Genetic und Kulturpflanzenforschung, Gatersleben, D.D.R. The plants had all the characters previously described [15].

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

Synthesis of DL-hygrine-[2′-^{14}C]. [13]. Ethyl acetoacetate-[3-^{14}C] (10 g, 350 μCi) was stirred at room temp for 24 hr with 2.5% KOH (300 ml) and then added to a soln which had been prepared by reducing freshly dist. *N*-methylpyrrolidone (4 g) in dry Et_2O (8 ml) by the dropwise addition of $LiAlH_4$ (0.98 g) in dry Et_2O over a 1 hr period. The mixed solns were adjusted to pH 7 with HCl (0 1 N), stirred at room temp. for a further 40 hr. and then reduced to a vol. of *ca* 60 ml under red. pres. The soln was basified (NaOH) and continuously extracted with Et_2O for 24 hr. Evapn of the dried (Na_2SO_4) Et_2O extract gave DL-hygrine-[2′-^{14}C], 3 g, IR (film) identical to authentic compound, sp. act. 1×10^7 dpm/mmol.

Resolution of DL-*hygrine*-[2′-^{14}C]. DL-Hygrine-[2′-^{14}C] (2 g) in dry MeOH (10 ml) was treated with D(+)-tartaric acid (2.2 g) in dry MeOH (20 ml). On standing, the cooled (−10)



(**15**) Hyoscine R^1 = Trop, $R^2 + R^3 = O$
(**16**) Hyoscyamine R^1 = Trop, R^2 = H, R^3 = H
(**17**) 3α, 6β-Ditigloyloxytropan-7β-ol R^1 = Tig, R^2 = OH, R^3 = Tig
(**18**) 3α,6β-Ditigloyloxytropane R^1 = Tig, R^2 = Tig, R^3 = H

Scheme 3. The metabolism of D(+) and L(−)-hygrine in *Datura*.

soln deposited rosettes of D(+)-hygrine D(+)-tartrate which was recrystallized from dry MeOH, yield 1.4 g, mp 129–130°, $[\alpha]_D^{18} = +27.5°$ (lit. [13] $[\alpha]_D^{18} = +28.7°$). The conc mother liquor, when treated with a small vol. of aq. MeOH gave the other diastereoisomer as long needles, recrystallization from MeOH giving 1.3 g, $[\alpha]_D^{18} = +4°$ (lit. [13] $[\alpha]_D^{18} = -1.8°$).

Feeding experiments. D(+)-Hygrine-[2′-^{14}C] D(+)-tartrate (100 mg) was dissolved in 100 ml H_2O and distributed to 5 × 5-month-old *D. innoxia* plants which had been carefully uprooted and suspended in blackened beakers containing Phostrogen soln. Five similar plants were fed via the roots with L(−)-hygrine D(+)-tartrate. After 7 days the plants were harvested and the roots and aerial parts separately dried at 60° for 18 hr. The finely powdered roots (26 g in each case) were extracted and the bases resolved on Pi partition columns at pH 6.8 and 5.6 as in refs [16, 17].

Degradation of hyoscyamine from D(+)-hygrine-[2′-^{14}C]D(+)-tartrate feeds. The active dil. hyoscyamine sp. act. 2.04×10^3 dpm/mmol was recovered from its picrate (400 mg) with NH_4OH–$CHCl_3$ and refluxed with 10% NaOH soln (10 ml) for 30 min. Extraction of the cooled hydrolysate with $CHCl_3$ afforded tropine which was immediately oxidized by refluxing with Cr_2O_3 (40 mg) in 85% HOAc (15 ml) for 3 hr when the resultant tropinone was extracted from the basified (10% NaOH) reaction mixture with $CHCl_3$ and converted to the picrate mp 218–219°. The reextracted and dried (over P_2O_5) tropinone was dissolved in dry Et_2O and reacted with a twofold excess of PhMgBr in dry Et_2O for 24 hr. Addition of 5% HCl (15 ml) hydrolysed the Mg complex and after washing several times with Et_2O the acid layer was basified with 10% NaOH and the 3-phenyltropan-3-ol extracted into $CHCl_3$. Examination of the $CHCl_3$ extract by TLC (Merck Al_2O_3, Et_2O–EtOH, 19:1, located by Dragendorff's reagent) showed that no tropinone remained, and after evapn of the solvent the residue was refluxed with $KMnO_4$ (200 mg) in H_2O (15 ml) for 12 hr. A few drops of EtOH were added to the cooled soln which was filtered, acidified with 10% HCl and extracted with Et_2O. After drying (Na_2SO_4), the Et_2O was evapd and the residue sublimed to yield benzoic acid (0.43 mg) sp. act. 2.01×10^3 dpm/mmol.

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