Stereochemistry of Hydroxylation in the Biosynthesis of Lycorine in *Clivia miniata* Regel

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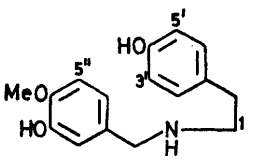
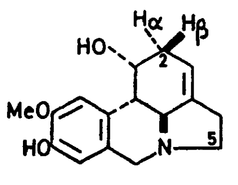
Summary In *Clivia miniata* Regel hydroxylation at C-2 of the intermediate leading to lycorine (**4**) occurs with retention of configuration.

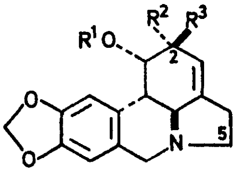
In Twink daffodils biological conversion of [3′,5′-$^3H_2$; 1-^{14}C]-*O*-methylnorbelladine (**1**) into lycorine (**4**) involves retention at C-2 of (**4**) of half the tritium originally present in the precursor, as the overall result of two stereospecific processes: (a) protonation to form the C-2 methylene of norpluviine (**2**) bearing the ^3H label in the β-configuration, and (b) hydroxylation of the next intermediate, caranine (**3**), with complete inversion, possibly through a multistage



(1)



(2)



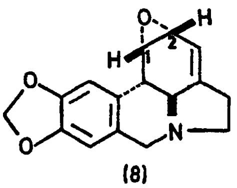
(3) R^{1} = R^{2} = R^{3} = H

(4) R^{1} = R^{2} = H; R^{3} = OH

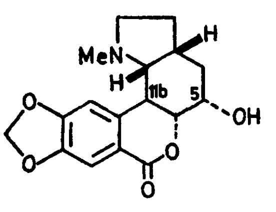
(5) R^{1} = Ac; R^{2}, R^{3} = O

(6) R^{1} = R^{3} = H; R^{2} = OH

(7) R^{1} = R^{3} = H; R^{2} = Cl



(8)



(9)

mechanism.[1,2] The same must at least partly occur in the conversion of singly labelled [2β-^{3}H]caranine (3) into [2-^{3}H]lycorine (4) in *Zephyranthes candida*.[3]

We now outline feeding experiments which establish that in *Clivia miniata* the conversion of *O*-methylnorbelladine (1) into lycorine (4) proceeds through the intermediacy of norpluviine (2) and caranine (3), but with almost complete loss of the tritium originally present *ortho* to the phenolic hydroxy-group of the C-6–2 fragment of (1). This is the consequence of a different stereochemical course of the hydroxylation process.

By chance, we observed incorporation of only ^{14}C activity into lycorine (4) (0·7% incorporation) and into clivonine (9), the ester component of clivimine[4] (0·01% incorporation), in feeding experiments with [3′,5′-^{3}H$_{2}$; *O*-Me-^{14}C]-*O*-methylnorbelladine (1). In order to clear up this disagreement with the previous results in daffodils and to check the specific incorporation of (1) into (4) and (9), [3′,5′,5″-^{3}H$_{3}$; 1-^{14}C]-*O*-methylnorbelladine (1) was synthesized as follows. Tritium was inserted *ortho* to the phenolic hydroxy-group of 3-benzyloxy-4-hydroxybenzaldehyde by base-catalysed exchange with tritiated water. The product was methylated, condensed with [3′,5′-^{3}H$_{2}$;1-^{14}C]tyramine and converted into (1). The relative ^{3}H activities for positions 3′, 5′, and 5″, determined by degradation, were 1 : 1 : 1.

Flowering *Clivia miniata* plants incorporated multiply labelled (1) into lycorine (4) and clivonine (9) with 37 and 32% tritium retention, respectively. The labelling pattern of the radioactive lycorine (4) was determined by degradation to acetyl-lycorin-2-one (5),[1,3] which retains nearly all the tritium, and by oxidation to hydrastic acid with retention of all the tritium activity. Similarly, positions 5 and 11b of clivonine (9) were devoid of ^{3}H, because hydrastic acid obtained from radioactive (9) holds all the starting ^{3}H activity. It follows that, if in *Clivia miniata* lycorine (4) is biosynthesized from (1) *via* norpluviine (2) and caranine (3) without the intermediacy of a 2-oxo-derivative, the remarkable tritium loss must be the consequence of a different steric course of the protonation and/or hydroxylation process.

Preliminary experiments with randomly ^{3}H-labelled (2) and (3) showed that they were efficiently converted into (4) in *Clivia miniata,* and we therefore devised experiments using (2) and (3) carrying stereospecific labelling at C-2 and ^{14}C as an internal reference.

[2α-^{3}H]Caranine (3) was synthesized by the procedure developed earlier for the synthesis of the β-isomer, which involves as the key step the stereospecific opening of 1,2α-lycorine epoxide (8) by treatment with hydride.[3] Thus, reduction (NaBT$_{4}$) of acetyl-lycorinone (5) gave, after hydrolysis, a separable mixture of [2-^{3}H]epilycorine (6) and [2-^{3}H]lycorine (4). From the latter, through the [2β-^{3}H]-2-chloro-derivative (7) and the [2-^{3}H]-1,2α-lycorine epoxide (8), the required [2α-^{3}H]caranine (3) was obtained.

[2β-^{3}H]Norpluviine (2) was isolated in feeding experiments with [3′,5′-^{3}H$_{2}$]-*O*-methylnorbelladine (1) in Twink daffodils. According to Bruce and Kirby,[2] in this plant, protonation to form the C-2 methylene of (2) is stereospecific and occurs from the α side of the molecule.

The two tritiated materials were mixed with the [5-^{14}C]-specimens extracted from Twink daffodil in feeding experiments with [1-^{14}C]-*O*-methylnorbelladine (1), and the doubly labelled precursors were crystallised to constant activity.

Clivia miniata incorporated [2α-^{3}H; 5-^{14}C]caranine (3) into lycorine (4) with 92% tritium retention (8% incorporation). The labelling pattern was determined on the radioactive lycorine (4) obtained in a larger feeding with singly labelled [2α-^{3}H]caranine (3). Because of the inactivity of the acetyl-lycorinone (5), the ^{3}H must be at C-2 of (4). Lycorine (4) derived in the same plant from [2β-^{3}H; 5-^{14}C]norpluviine (2) retains about 20% of the starting ^{3}H activity (3·4% incorporation). Unfortunately, the lack of radioactive material did not allow us to establish the position of the residual tritium in the molecule and, therefore, its biosynthetic significance. Clivonine (9) isolated in these experiments was inactive.

All this evidence points to the following conclusions. (a) Conversion of *O*-methylnorbelladine (1) into lycorine (4) in *Clivia miniata* occurs with the intermediacy of norpluviine (2) and caranine (3) without the participation of a 2-oxo-derivative. (b) Hydroxylation at C-2 takes place with removal of the hydrogen having the β-configuration (retention mechanism). (c) Protonation of the intermediate leading from (1) to (2) and (3) occurs from the α side of the molecule, as in daffodil, since lycorine (4) obtained from (1) can be devoid of tritium at C-2 only if in the intermediates (2) and (3) the ^{3}H originally present in (1) holds the β-configuration.

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