# Stereochemistry of Protonation and Hydroxylation in the Biosynthesis of Norpluviine and Lycorine

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BIOLOGICAL conversion of the multiply labelled *O*-methylnorbelladine (I) into norpluviine [as (II)] has been shown[1] to involve loss of 2 of the 4 tritium atoms, the derived alkaloid being labelled with tritium equally at C-2 and C-8. We now report experiments with "Twink" and "Deanna Durbin" daffodils which establish the relative stereochemistry of biological protonation and hydroxylation at C-2 in the lycorine (III; $R^1$=OH, $R^2$=H) group of alkaloids.

DL-[$\alpha$-$^{14}$C; 3,5-$^3H_2$]Tyrosine was incorporated into norpluviine (II; 0·09% incorporation of $^{14}$C) and into lycorine (III; $R^1$=OH; $R^2$=H; 0·17%) with the usual[2] efficiency. As expected, incorporation of tyrosine into norpluviine involved loss of half the tritium (see Table). More importantly,

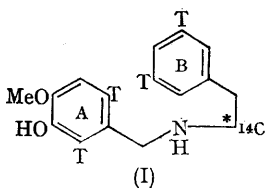
TABLE

*Tracer experiments in daffodils*

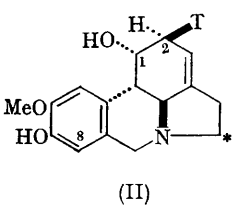
| Precursor | $^3$H:$^{14}$C ratio | Plant | Norpluviine $^3$H:$^4$C (incorp. %) | Lycorine $^3$H:$^{14}$C (incorp. %) |
|---|---|---|---|---|
| DL-[$\alpha$-$^{14}$C; 3,5-$^3H_2$]Tyrosine .. .. | 16·5 | Twink | 8·3 (0·09) | 8·3 (0·17) |
| L-[3,5-$^3H_2$]- and DL-[$\alpha$-$^{14}$C]Tyrosine .. | 15·4 | Twink | 6·1 (0·10) | 6·3 (0·16) |
| D-[3,5-$^3H_2$]- and DL-[$\alpha$-$^{14}$C]Tyrosine .. | 14·9 | Twink | 6·1 (0·11) | 6·2 (0·16) |
| [$^{14}$C,$^3$H]-*O*-Methylnorbelladine .. .. | 13·8 | Twink | 7·0 (1·5) | 6·7 (0·93) |
| [$^3$H]-*O*-Methylnorbelladine .. .. .. | — | Deanna Durbin | — (0·72)* | — (0·46)* |

* Allowing for loss of half the tritium during incorporation.

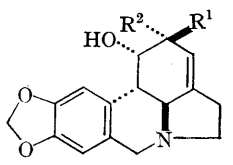
the biosynthetic lycorine had the same tritium content as the norpluviine. Conversion[2] of norpluviine into lycorine cannot therefore involve an intermediate 2-keto-derivative, and any alternative pathway from tyrosine to lycorine *via* a tetraoxygenated aromatic intermediate is excluded. The lycorine was converted[3] into *O*-acetyl-lycorinone [acetyl derivative of (III; $R^1R^2$=O)] with retention (98%) of $^{14}C$ activity and essentially



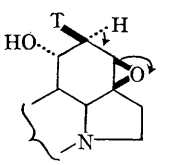

(I)




(II)




(III)




(IV)

complete loss (95%) of tritium. Unambiguous degradation of lycorine derived from [α-$^{14}C$]tyrosine has been reported earlier.[2] Similarly, mixtures of L-[3,5-$^3H_2$]tyrosine with DL-[α-$^{14}C$]-tyrosine and D-[3,5-$^3H_2$]tyrosine with DL-[α-$^{14}C$] tyrosine were fed to daffodils. Similar incorporations into norpluviine and lycorine were observed for the two mixtures. Also the $^3H$:$^{14}C$ ratios in the alkaloids were again approximately half those in the corresponding precursors. Thus, in these plants, L and D-tyrosine are metabolised with closely comparable efficiency. Finally, *O*-methylnorbelladine (I), labelled with $^{14}C$ in the methoxyl group and with $^3H$ *ortho* to the phenolic hydroxyl group in ring B, was fed into the plants. Incorporation into norpluviine (1·5%) and into lycorine (0·93%) was found, as expected, to involve loss of half the original tritium. Hydrolysis of the lycorine gave formaldehyde, originating from the methylenedioxy-group, containing 94% of the $^{14}C$ activity and Herzig–Meyer demethylation of the norpluviine showed that 97% of the $^{14}C$ resided in the methoxyl group. Similarly, *O*-methylnorbelladine labelled with tritium in ring B gave [$^3H$]lycorine (0·46% incorporation). Degradation of the alkaloid to *O*-acetyl-lycorinone removed essentially all (99%) the tritium.

Comparison of the $^3H$:$^{14}C$ ratios in norpluviine and lycorine derived from the double-labelling experiments shows (Table) that no significant loss of tritium had occurred during biological hydroxylation. This strongly suggests that both protonation, to form the C-2 methylene group in norpluviine, and hydroxylation, to produce lycorine, are stereospecific.† Also, the hydrogen introduced at C-2 during the biosynthesis of norpluviine is the one removed by hydroxylation.

Wildman and Heimer[4] have recently observed the biological conversion of caranine (III; $R^1$=$R^2$=H) into lycorine in *Zephyranthes candida*. Caranine, singly labelled with tritium as shown (III; $R^1$=T, $R^2$=H) gave [2-$^3H$]lycorine. Thus hydroxylation had occurred with at least partial inversion of configuration at C-2. In so far as results from experiments with different plants can be combined, the present work and that of Wildman and Heimer indicates that hydroxylation involves complete inversion. In contrast, biological hydroxylation at methylene groups in steroids is known (see citations in ref. 4) to involve retention of configuration. Wildman and Heimer have suggested that the hydroxylation of caranine might involve an allylic carbonium ion or the related 1,2-α-epoxide (steroid notation). An alternative route *via* the 3,3a-β-epoxide (IV) might be considered. Enzyme-catalysed ring opening [as in (IV)], followed by allylic rearrangement of the resulting alcohol, would give lycorine (III; $R^1$=OH; $R^2$ = T) with the required inversion of configuration at C-2.

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† Rigorous proof that hydroxylation is stereospecific would require a feeding experiment with norpluviine or caranine labelled with tritium α (steroid notation) at C-2 and with a reference label elsewhere in the molecule.

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[2] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J. Chem. Soc.*, 1964, 1595.
[3] Y. Nakagawa and S. Uyeo, *J. Chem. Soc.*, 1959, 3736.
[4] W. C. Wildman and N. E. Heimer, *J. Amer. Chem. Soc.*, 1967, **89**, 5265.