Relative Stereochemistry of Protonation and Hydroxylation in the Biosynthesis of Lycorenine and Haemanthidine from Protocatechualdehyde

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Summary Tracer experiments show that in the biosynthesis of lycorenine and haemanthidine the hydrogen introduced in the incorporation of protocatechualdehyde into the aromatic C_6–C_1 unit of *O*-methylnorbelladine is the one removed in the hydroxylation.

THE biological conversion of protocatechualdehyde (1) into the aromatic C_6–C_1 unit of the Amaryllidaceae alkaloids lycorenine (8) and haemanthidine (4)[1] proceeds with the intermediacy of *O*-methylnorbelladine (2) and, respectively, norpluviine (5) and haemanthamine (3)[2] on the biosynthetic pathway.[3] Incorporation of (1) into (4) and (8) requires the formal addition of a hydrogen atom to form the C-1′ methylene group of (2) that will later become the benzylic methylene of (3) and (5), followed by hydrogen removal in the benzylic hydroxylation.

We report on feeding experiments which establish the relative stereochemistry of the two biological processes.

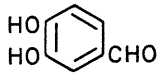
[1′-^{3}H; 1-^{14}C]-*O*-methylnorbelladine (2) was converted by daffodil plants ("Tresamble" and "Inglescombe") into pluviine (6) and lycorenine (8), but *Sprekelia formosissima* converted it into haemanthamine (3) and haemanthidine (4). The tritium retentions are listed in the Table. The

^{3}H *Relative molar activities and incorporations*

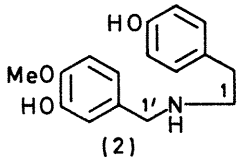
Substance	[1′-^{3}H, 1 ^{14}C]*O* methylnorbelladine (2) (inc %)	Biosynthetic [^{3}H, ^{14}C]norpluviine (5) (inc %)	Biosynthetic [^{3}H, ^{14}C]haemanthamine (3) (inc %)
Pluviine (6)	105 (0 7)	97 (3 2)	—
Lycorenine (8)	45	102 (1 9)	—
Homolycorine (9)	no ^{3}H	no ^{3}H	—
Lactam (7)	no ^{3}H	no ^{3}H	—
Haemanthamine (3)	98	—	104[a]
Haemanthidine (4)	53	—	97 (2 3)

[a] About 5% recovery after two weeks.

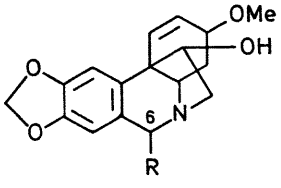
labelling pattern of the radioactive (6) and (8) is based on their conversion into (7)[4] and (9)[5] with almost complete



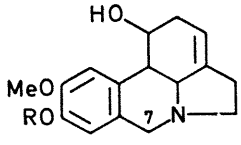
(1)



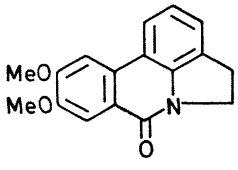
(2)



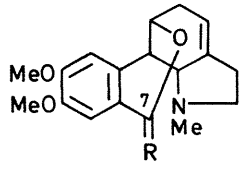
(3) R = H
(4) R = OH



(5) R = H
(6) R = Me



(7)



(8) R = H,OH
(9) R = O

trıtium loss Therefore, incorporation of *O*-methylnorbelladine (2) into pluviine (6) and haemanthamine (3) proceeds without hydrogen removal from the position α to the nitrogen, and into lycorenine (8) and haemanthidine (4) with loss of *ca* half the trıtium

This result pointed to the next experiment which was to study the biosynthesis of [7-^{3}H]norpulviine (5) and [6-^{3}H] haemanthamine (3) from [*formyl*-^{3}H]protocatechualdehyde (I) The latter alkaloids were mixed with identical, ^{14}C-labelled, material obtained in the same plants ("Twink" and "Texas" daffodil) in a separate feeding with [1-^{14}C]-*O*-methyl norbelladine (2), and the ^{3}H,^{14}C-labelled samples were crystallised to constant activity Incorporation of doubly labelled (2) and (5) into (4) and (8) proceeds in the above mentioned plants with almost complete trıtium retention The labelling pattern of the injected norpluviine (5) and of the biosynthesized lycorenine (8) was determined as before, as expected, the site of activity was position 7 in (5) and (8)

The evidence therefore suggests that both hydrogen addition and hydrogen removal occuring in the biological conversion of protocatechualdehyde (I) into lycorenine (8) and haemanthidine (4) are two stereospecific processes The last feature is indeed not new in Amaryllidaceae alkaloid biosynthesis [6,7] Also, the hydrogen introduced at C-1′ in the biosynthesis of *O*-methylnorbelladine (2) from protocatechualdehyde (1) is that removed by hydroxylation in the conversion into lycorenine (8) and haemanthidine (4)

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