The Biosynthesis of Narciclasine

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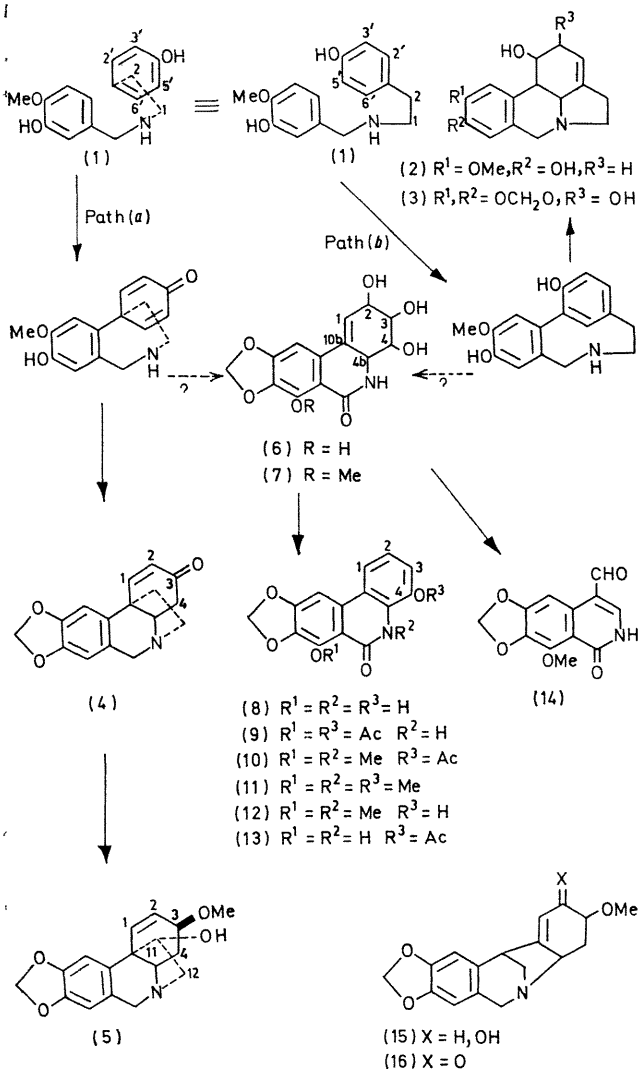
Summary Tracer experiments prove that the antimitotic agent narciclasine (6) is biosynthesised from *O*-methylnorbelladine (1) by *para–para* coupling followed by late elimination of two carbon atoms.

MANY Amaryllidaceae plants contain the lactam narciclasine[1] (6), a substance having marked antimitotic activity, in addition to the well known alkaloids *e.g.* haemanthamine



(5), norpluviine (2), and lycorine (3). The alkaloids are biosynthesised[2,3] from *O*-methylnorbelladine (1) by phenol-coupling *via* paths (*a*) or (*b*) (see arrows from 1) and it seemed probable that narciclasine could be formed by a variant of the same biosynthetic processes. This could involve late degradation of the skeleton produced by either path (*a*) or path (*b*). We now outline experiments which support this view and further, establish the operation of path (*a*).

O-Methyl[3′,5′-$^3H_2$; O-*methyl*-^{14}C]norbelladine (1) was prepared by condensation of [3′,5′-$^3H_2$]tyramine (from base-catalysed exchange[4] of tyramine with tritiated water) with [O-*methyl*-^{14}C]isovanillin, followed by reduction. The labelling pattern was confirmed by degradation of (1) to anisic acid which retained all the tritium but which yielded the radio-inactive 3,5-dibromo-derivative. ^{3}H-Labels were inserted by exchange *ortho* to the phenolic hydroxy-group of mono-*O*-benzylhydroquinone and the hydroxy-group was then reductively removed *via* the *N*-phenyltetrazole derivative.[5] The product was converted into [2′,6′-$^3H_2$]tyramine by standard methods and *O*-methyl[2′,6′-$^3H_2$; O-*methyl*-^{14}C]norbelladine (1) was derived from it by the foregoing sequence.

Daffodil plants ("Twink" and "Deanna Durbin") incorporated the two samples of labelled (1) into the substances shown in the Table. Barton and Kirby[3] have proved that

Incorporation and ^{3}H-*retention values*

Substance	Expt. 1 with [3′,5′-$^3H_2$]-(8). (Incorp. %)[a] % ^{3}H-Retained[b]	Expt. 2 with [2′,6′-$^3H_2$]-(8). (Incorp. %)[a] % ^{3}H-Retained[b]
Haemanthamine (5) ..	(0·11)	(0·09)
	100	102
Norpluviine (2) ..	(0·73)	(1·1)
	49	85
Lycorine (3)	(0·09)	—
	49	—
(15)	106	—
(16)	57	—
Narciclasine[c] (6) ..	(0·35)	(0·5)
	75	103
(9)	50	51
(10)	47	—
(11)	50	—
(14)	<5	100
(13)	—	48
$^2H_2$-(12)	—	<5

[a] Based on ^{14}C activity.
[b] The reported values hold to ±8%; assay for ^{3}H was complicated by fluorescence of several of the substances.
[c] Assayed as tetra-*O*-acetate.

the *O*-methyl group of (1) is retained as it is incorporated into the methylenedioxy-group of (5); we could thus use the ^{14}C-methyl of (1) as a standard against which to measure retention of the ^{3}H-labels. The reliability of this approach was supported by the results from Expt. 1 for norpluviine (2) and lycorine (3) which, in confirmation of Kirby's findings,[4] show a ^{3}H:^{14}C ratio *ca.* half that of (1). There was in contrast no significant change in ratio for haemanthamine (5) and ^{3}H-labelling was expected at positions 2 and 4. This was supported by rearrangement[6] of (5) to iso-

haemanthamine (15) which caused no loss of tritium but oxidation to the ketone (16) eliminated half of the ^{3}H-activity.

Narciclasine (6) showed a lower ^{3}H:^{14}C ratio than haemanthamine though much higher than those of norpluviine (2) and lycorine (3). This indicated incorporation of *O*-methylnorbelladine (1) into narciclasine by path (*a*) rather than (*b*), to label positions 2 and 4 of (6). Confirmation was obtained by dehydration of (6), using acidified deuterium oxide, to form narciprimine[1] (8) when the ^{3}H content [measured as the di-*O*-acetate (9)] fell to half that in the precursor (1) and in haemanthamine (5); there was no ^{2}H-uptake. Partial loss of ^{3}H from C-4 has evidently occurred at some stage(s) during the biosynthesis of narciclasine (6). This aspect requires further study; it does not affect the present argument. The ^{3}H:^{14}C ratio in (8) was confirmed by conversion into (10) and (11) without significant change in the ratio. Finally, periodate oxidation of *O*-methylnarciclasine (7) gave the aldehyde (14) which was essentially devoid of tritium; there was no uptake of ^{2}H into (14) from deuterium oxide in the medium.

The results from Expt. 2 clearly show that positions 1 and 4b of narciclasine (6) are labelled in this case. Loss of 50% of the ^{3}H-activity on conversion of (6) into (8) [measured as the *O*-acetate (13)] is in agreement. When (12) derived from (13) was treated with triethylamine in deuterium oxide, virtually complete loss of ^{3}H-activity occurred with concomitant uptake of ^{2}H (91% D_2, 8% D_1 and 0% D_0 species by mass spectrometry). The site of ^{3}H-labelling is thus at positions 1 and/or 3 of (8); because of the activity of the aldehyde (14), it follows that position 1 of (8) carries all the ^{3}H-activity.

Attention is called to the formation of haemanthamine (5) from (1) in Expt. 2 without appreciable change in ^{3}H content whereas norpluviine (2) loses a significant proportion of the label.[7]

Decisive confirmation of path (*a*) to narciclasine was obtained by feeding ^{3}H-oxocrinine (4) to the plants; the label was introduced by heating the ketone with triethylamine and tritiated water. A parallel experiment with deuterium oxide showed that D_3 and D_2 species are formed and that partial ^{2}H-loss occurs during chromatography and recrystallisation of (4) (probably from C-4, see below). The incorporation of ^{3}H-oxocrinine (4) into haemanthamine (5) was 2·7% and into narciclasine (6) was 0·75%. Conversion of the former into (15) occurred with 94% retention of ^{3}H, but (16) prepared from it contained 19% of the original ^{3}H. These values can be compared with that obtained when the ^{3}H-narciclasine (6) was converted into (8); loss of 26% of the original ^{3}H occurred.

When ^{3}H-norpluviine (2) was fed to "King Alfred" plants, there was no significant incorporation into narciclasine (6).

The foregoing evidence establishes that narciclasine is biosynthesised from *O*-methylnorbelladine (1) *via* path (*a*) with the intermediates being related to the oxocrinine (4) and haemanthamine (5) skeletons. The nature of these intermediates and the steric course of the reactions are being investigated (by C.F.).

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[7] The formation of norpluviine and lycorine was accompanied by a similar small loss when (±)-[2′,6′-$^3H_2$; 2-^{14}C]tyrosine was used as precursor, I. T. Bruce and G. W. Kirby, personal communication.