Biosynthesis of the Insect Antifeedant Steroid Nic-1: Origins of the Aromatic Ring-D

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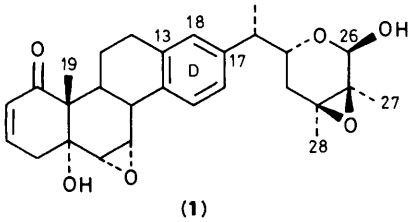
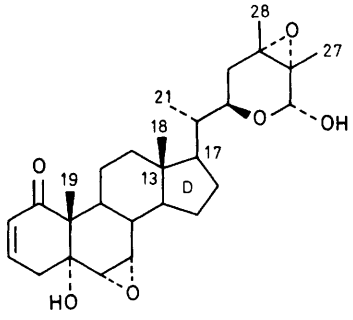
Isotope administration experiments with *Nicandra physaloides* plants using [3′-C^2H_3]- and [3′-$^{14}CH_3$]-mevalonic acid, analysed by ^2H n.m.r. and by degradation, respectively, show that the aromatic ring-D of Nic-1 (**1**) is formed by ring-D expansion in a steroid precursor with oxidative inclusion of the C/D angular methyl.

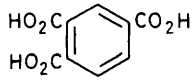
Nicandra physaloides (the Peruvian 'shoofly' plant) contains a group of highly oxidised 24-methylsteroids (nicandrenoids)[1] related to the withanolides.[2] The major metabolite Nic-1 (**1**) exhibits antifeedant properties towards the tobacco hornworm. A very unusual feature of the structure of Nic-1 is the aromatic D-ring, carrying a side chain displaced from its customary site in 'normal' steroids *e.g.* Nic-3 (**2**). The co-occurrence of Nic-1 and Nic-3 suggests late stage D-aromatisation. Two plausible biosynthetic hypotheses may be entertained (Scheme 1): in path (a) the C/D angular methyl is



(1)



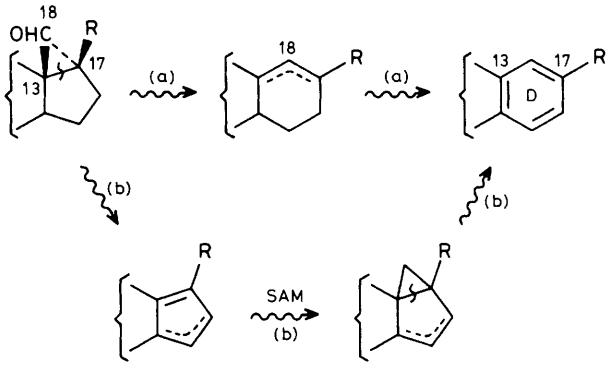
(2)



(3)

oxidised and incorporated into ring-D with subsequent aromatisation, while in path (b) oxidative elision of C-18 is envisaged (as, *e.g.* the removal of the C-14 methyl in lanosterol) followed by insertion of a C_1 unit from *S*-adenosylmethionine (SAM), and aromatisation. We report here experiments which distinguish these pathways and establish the origin of the aromatic ring. We thus prepared [3′-$^{14}CH_3$]-mevalonic acid (MVA), using a scaled-down version of the Macmillan–Scott procedure developed for [3′-$^{13}CH_3$]-MVA,[3] and administered a sample to *N. physaloides* plants, seven weeks old, using the wick method. [$^{14}CH_3$]-SAM was similarly administered. Nic-1 was isolated in each experiment and oxidised (potassium permanganate) to trimellitic acid (**3**). Table 1 shows the results after recrystallisation to constant activity. It is clear that although [$^{14}CH_3$]-SAM is incorporated into Nic-1, no activity appears in ring-D, but is presumably located only at C-28. However on incorporation of [3′-$^{14}CH_3$]-MVA, 26% of the activity appears in the trimellitic acid fragment: since four sites (C-19, C-18, C-21, and C-26 or C-27) in a steroid precursor such as (**2**) would be expected to be labelled, this experiment is consistent with retention of C-18 as part of the aromatic fragment.

To determine precisely the site in ring-D supplied by the MVA methyl we turned to deuterium labelling. Thus [3′-C^2H_3]-MVA was prepared from deuterioacetic acid, using the same procedure as above, and a large sample (408 mg) was applied to 23 plants. After 7 days metabolism, Nic-1 was isolated without carrier. A ^2H n.m.r. spectrum of this sample (160 mg, 12 680 scans, CH_2Cl_2–10% $CFCl_3$) showed a clear absorption at δ 7.01, (with other expected signals and no ^2H impurities); this signal corresponds to that of the 18-H (s) in the ^1H n.m.r.



Scheme 1. Possible pathways for ring-D aromatisation.

Table 1. Incorporation of ^{14}C-labelled species into (**1**) and (**3**) and subsequent specific activities.

	Absolute incorporation/ %	Specific activity/ d.p.m. mmol^{-1}	
	(**1**)	(**1**)[a]	(**3**)[b]
[3′-$^{14}CH_3$]-MVA	0.10	444×10^3	116×10^3
[$^{14}CH_3$]-SAM	0.002	241×10^3	0

[a] After dilution. [b] As trimethyl ester.

The evidence thus points to a biosynthetic pathway such as (a), Scheme 1, in which C-18 is oxidised and incorporated into ring-D, while retaining one hydrogen, at least in part. A possible mechanism would involve a fused cyclopropane intermediate and would initially parallel the C-19 transformation in the lanosterol–cycloartenol conversion,[4] but this remains to be determined.

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References

1 M. J. Begley, L. Crombie, P. J. Ham, and D. A. Whiting, *J. Chem. Soc., Perkin Trans. 1*, 1976, 296, 304.
2 E. Glotter, I. Kirson, D. Lavie, and A. Abraham in 'Bioorganic Chemistry,' ed. E. E. van Tamelen, Academic Press, New York, 1978, Vol. II, p. 57; I. Kirson and E. Glotter, *J. Natl. Prod.*, 1981, **44**, 633.
3 P. Lewer and J. MacMillan, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1417; E. Bardshiri, T. J. Simpson, A. I. Scott, and K. Shishido, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1765.
4 E. Caspi, *Tetrahedron*, 1986, **42**, 3, and references therein.