Biosynthesis of the Nicandrenoids: Stages in the Oxidative Elaboration of the Side Chain and the Fate of the Diastereotopic 25-Methyl Groups of 24-Methylenecholesterol

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Experiments with *Nicandra physaloides* plants show that the insect antifeedant steroid Nic-1 (**1**) is formed from 24-methylenecholesterol: in double bond isomerisation to 24-methylcholesta-5,24-dien-3β-ol, the 25-(*pro-S*) methyl in (**2**) becomes the 25-(*pro-Z*) methyl, in (**3**); further oxidations lead to triol (**6**) and hence to lactol (**1**) with partial retention of 26-hydrogen.

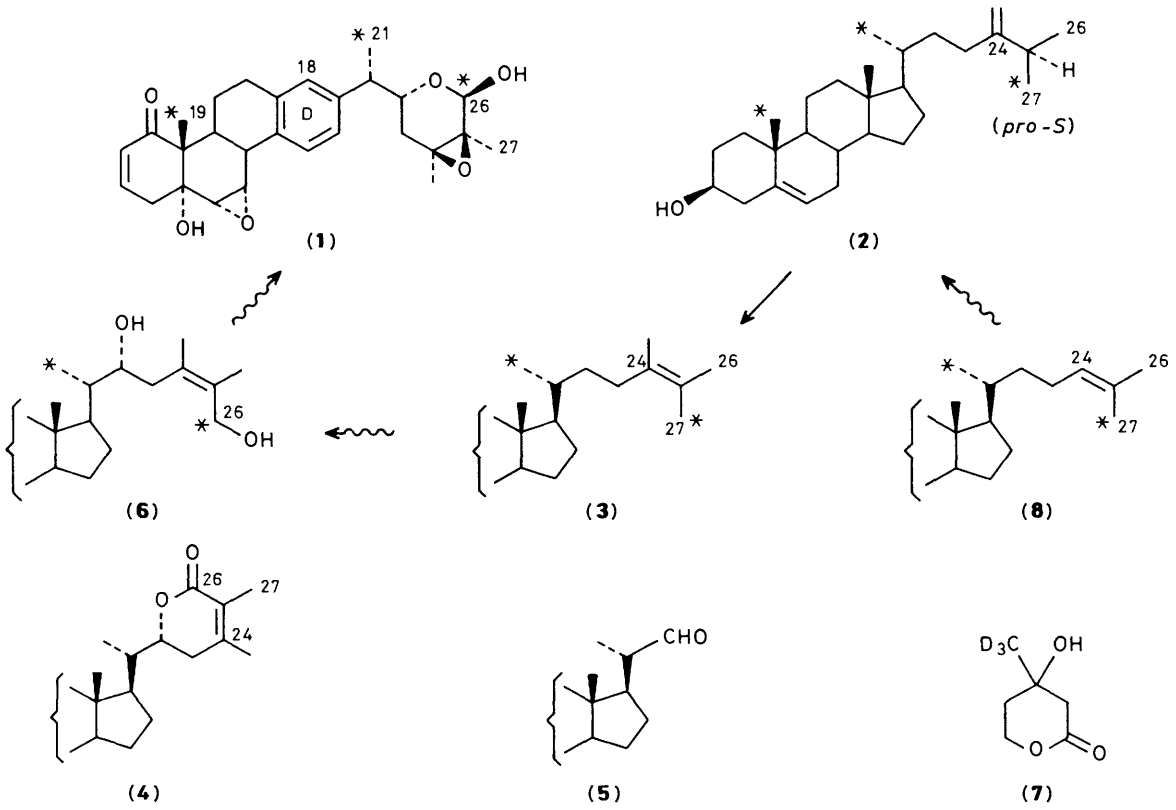
The withanolides,[1] a large group of 24-methyl plant steroids occurring in the Solanaceae, are characterised by extensive oxidative modifications. The insect antifeedant Nic-1 (**1**), belonging to a distinctive sub-set isolated from *Nicandra physaloides*,[2] contains, as well as the unique aromatic ring-D,[3] an unusual epoxylactol side chain. This feature also appears in Nic-3 and, in modified form, in other nicandrenoids. Structural comparisons within the group[1,4] suggest that side chain modification may either precede ring A/B modification or be partly independent of the A/B system (as in a metabolic grid with enzymes of low substrate specificity). We report here experiments which confirm this view and outline stages in the oxidative development of Nic-1: selectivity in the oxidation of the diastereotopic C-25 methyl groups in 24-methylenecholesterol (**2**) is revealed in the sequence.

As potential precursors to Nic-1 we have prepared (i) [28-^{14}C]-24-methylenecholesterol (**2**), from 24-oxocholesterol;[5] (ii) [28-^{14}C]-24-methylcholesta-5,24-dien-3β-ol (**3**), by isomerisation of (**2**);[6] (iii) the [23,28-$^{3}H_2$]-(22*R*)-lactone (**4**), synthesised from aldehyde (**5**)[7] using methods similar to those of Glotter and coworkers,[8] and labelled by tritium exchange ($^{3}H_2O$–1,5-diazabicyclo[4.3.0]non-5-ene); and (iv) the [23,28-$^{3}H_2$]-(22*R*),(24*Z*)-diol (**6**), by $LiAlH_4$ reduction of lactone (**4**). The four sterols were then administered to seven week old *Nicandra physaloides* plants in Tween-20–water–2-methoxyethanol, through cut stems. After 4 days metabolism, Nic-1 was isolated and recrystallised to constant activity; the incorporations, based on sterol uptake, are shown in Table 1.

The results indicate that both 24-methylenecholesterol, the first product from *S*-adenosylmethionine methylenation of

Table 1. Absolute (%) and specific (%) incorporations of labelled steroids into Nic-1 in *N. physaloides* plants.

	Absolute incorporation	Specific incorporation
(**2**)	0.23	0.056
(**3**)	0.16	0.068
(**4**)	0.17	0.015
(**6**)	0.50	0.057



cholesterol, and its 24(25)-double bond isomer (**3**) are incorporated, at levels expected in such experiments: these two sterols are probably adjacent in the biosynthetic sequence. The triol (**6**) shows a higher absolute incorporation, as appropriate to a later stage precursor and is a distinctly better utilised precursor than the lactone (**4**). This suggests that the predominant pathway from alcohol (**6**) to the hemi-acetal (**1**) proceeds by way of a C-26 aldehyde, in preference to reduction of the lactone (**4**) (although the latter route may constitute a minor pathway). This view is reinforced by the outcome of administration of [3′-C^2H_3]-mevalonolactone (**7**) to *N. physaloides*. Examination of the ^2H n.m.r. spectrum of the resulting Nic-1 shows signals arising from (i) the methyl groups (C-19, C-21), as expected; (ii) 18-H, see ref. 3, and (iii) 26-H (δ 4.9). This indicates that C-26 in Nic-1 is derived from the mevalonic acid 3′-methyl with a degree of retention of hydrogen, thus excluding lactone (**4**), or a related compound with C-26 fully oxidised, from the predominant pathway.

A further inference may be made from this experiment. It has been shown[9] that, in the biosynthesis of 24-methylenecholesterol in a closely related solanaceous plant, *Physalis peruviana*, C-27 of lanosterol† (**8**) becomes C-27 [the 25-(*pro-S*)-methyl] of 24-methylenecholesterol (**2**). Since C-26 of Nic-1 must derive from C-27 of sterol (**3**) then it follows that this latter carbon must correlate with C-27 of sterol (**2**), so that the *net* methylation of lanosterol† (**8**) to sterol (**3**) proceeds with retention of double bond geometry.

† A closely related alternative precursor may be involved.

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