The Role of Hydroxygeraniol and Hydroxynerol in the Biosynthesis of Loganin and Indole Alkaloids

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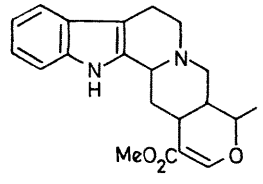
Summary Loganin and indole alkaloids are efficiently biosynthesised in *Vinca rosea* from 10-hydroxygeraniol (**16**) and 10-hydroxynerol (**18**) with considerable randomisation of C-9 and C-10.

PREVIOUS work[1] on the biosynthesis of the three main types of indole alkaloids, exemplified by ajmalicine (**1**), vindoline (**2**), and catharanthine (**3**) has revealed that upon feeding of sodium (±)-[2-^{14}C]mevalonate the methoxycarbonyl group of the alkaloids was labelled to the extent of *ca.* 25%. Similar results have been reported for the formation of biogenetically related monoterpenes and monoterpene glucosides.[2] These findings have been interpreted as the consequence of a process which at some stage in the biosynthesis equilibrates the C-2 and the C-3′ derived atoms in one of the participating C_5 units. We now report on further work aimed at the elucidation of this randomisation process.

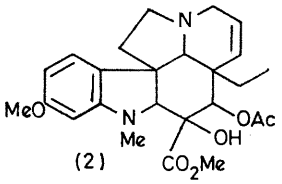
The postulated loss of identity was first verified in experiments with [3′-^{14}C]mevalonate (**4**), prepared by controlled oxidation (CrO_3–acetic acid) of the triol (**5**), which in turn is available from ozonolysis of 4-methylhepta-1,6-dien-4-ol,[3] followed by reductive work-up with $NaBH_4$.† The radioactive alkaloids (**1**)—(**3**) obtained upon feeding

† A similar preparation of mevalolactone employing Ag_2CO_3 as the oxidizing agent has been reported.[4]

the sodium salt of (**4**) to young shoots of *Vinca rosea* L. were pyrolysed as their hydrochlorides to give CO_2 from the methoxycarbonyl group. The observed amount of label (17·2%, 20·8%, and 19·5% of the total, respectively) was in each case only slightly less than that expected for complete equilibration between C-2 and C-3′ in the terminal unit.



(1)



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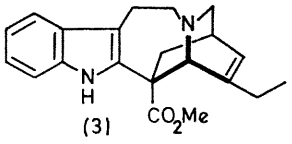

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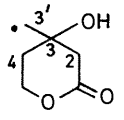

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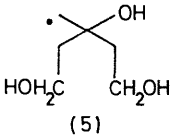

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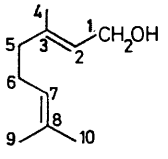

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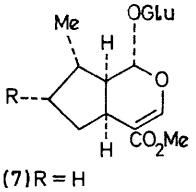
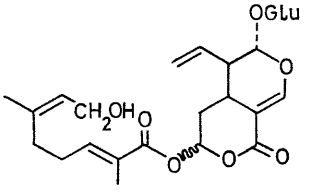


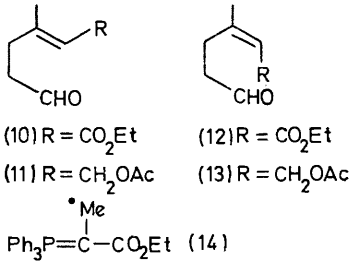
(7) R = H
(8) R = OH



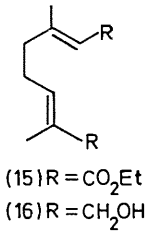
(9)

skeleton of (**7**) and (**8**). In order to check whether randomisation in the terminal unit could occur beyond the geraniol stage [9-^{14}C]-labelled specimens of the 10-hydroxy-compounds (**16**), (**18**), (**20**), and (**22**) were tested as possible precursors.

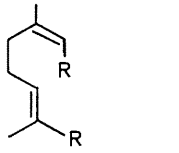
A mixture of aldehydo-esters was obtained from the dimethylacetal of levulinic aldehyde through a sequence



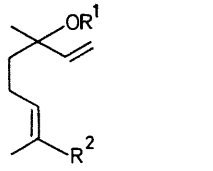
(10) R = CO_2Et
(11) R = CH_2OAc
(12) R = CO_2Et
(13) R = CH_2OAc
(14)



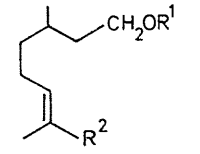
(15) R = CO_2Et
(16) R = CH_2OH



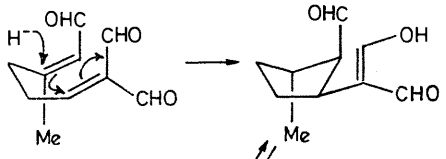
(17) R = CO_2Et
(18) R = CH_2OH



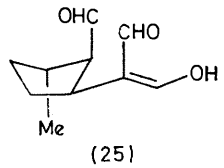
(19) R^1 = Ac, R^2 = Me
(20) R^1 = H, R^2 = CH_2OH



(21) R^1 = Ac, R^2 = Me
(22) R^1 = H, R^2 = CH_2OH



(23) (24)



(25)

The intermediacy of geraniol (**6**), deoxyloganin (**7**), and loganin (**8**) in the biosynthetic process leading to the alkaloids is well documented.[5–7] Retention of biogenetic identity in the isopropylidene unit of (**6**) is made highly probable (i) by the results of a study[8] on the stereochemistry of label incorporation from [4-^{3}H,2-^{14}C](3*R*,4*R*)-mevalonate into *Vinca* alkaloids and (ii) by the observation that this identity is preserved in the corresponding segment of the triterpene ursolic acid which is simultaneously produced in the same plant.[9] On the other hand, both mechanistic considerations and the occurrence of foliamenthin (**9**) and related glucosides in *Menyanthes trifoliata*[10] suggest that oxidation of the isopropylidene group is a prerequisite for the biological conversion of (**6**) into the cyclic carbon

involving Reformatsky reaction with bromoacetic ester, dehydration with $POCl_3$ in pyridine, and removal of the acetal group. Separation by preparative g.l.c. gave the pure *trans*- and *cis*-isomers (**10**) and (**12**),[11] which displayed characteristic n.m.r. signals for their methyl groups at δ 2·16 and 1·88, respectively. Condensation of (**10**) with the labelled phosphorane (**14**), obtained from the lower homologue by addition of $^{14}CH_3I$ and subsequent treatment with aqueous NaOH gave the *trans,trans*-diester (**15**) (new olefinic proton at δ 6·70), shown by g.l.c. to contain less than 5% of the *cis*-Δ^7-isomer. Reduction of (**15**) with $LiAlH_4$ in the presence of $AlCl_3$ produced the desired 10-hydroxygeraniol (**16**). 10-Hydroxynerol (**18**) contaminated with less than 5% of the 9-hydroxy-isomer was similarly prepared by the sequence (**12**) → (**17**) → (**18**).

The stereochemistry assigned to the Δ^2-double bond of (**16**) and (**18**) was verified by an independent synthesis of the two compounds using as a key step the addition of the phosphorane (**18**) to the trisnor-aldehydes (**11**) and (**13**), derived from the controlled ozonolysis[12] of pure geraniol acetate and nerol acetate. The latter procedure could also be used for converting (**19**) and (**21**) into (±)-10-hydroxylinalool (**21**) and (±)-10-hydroxycitronellol (**22**).

a detail in the structure of foliamenthin (**9**). Methanolysis of the penta-*O*-acetyl derivative of (**9**) followed by reduction with $LiAlH_4$ gave a product identical (by n.m.r.) with (**18**) and quite different from (**16**). Foliamenthin therefore belongs to the nerol rather than to the geraniol set of compounds. This, together with the good incorporation of (**18**), suggests that the immediate precursor of iridoid compounds has a *cis*- rather than a *trans*-Δ^2-double bond.

TABLE 1

Tracer experiments in V. rosea

Expt. No.	Substrate	Incorporation (%) Loganin (**8**)	Ajmalicine (**1**)	Vindoline (**2**)	Catharanthine (**3**)
1.	[9-^{14}C]-10-Hydroxygeraniol (**16**)	0·09	0·17	0·72	0·5
2.	[9-^{14}C]-10-Hydroxynerol (**18**)	0·16	0·14	1·2	0·9
3.	[9-^{14}C]-10-Hydroxylinalool (**20**)	—	0·0004	0·003	0·002
4.	[9-^{14}C]-10-Hydroxycitronellol (**22**)	0·0002	—	0·001	0·002
5.	[1-^{3}H]Citronellol	0·0002	0·0004	0·0008	0·0004

The [9-^{14}C]-labelled substrates were emulsified in water by the addition of Tween and fed hydroponically to young shoots of *V. rosea*. Data for the incorporation into loganin and the alkaloids are collected in Table 1 together with the results of an additional feeding experiment with [1-^{3}H]-citronellol. The efficiency of 10-hydroxygeraniol (**16**) and 10-hydroxynerol (**18**) as precursors of loganin and the alkaloids compares very favourably with that previously reported for geraniol,[5] whereas the low figures observed with the other substrates can hardly be considered significant.

To detect the amount of label located in the methoxycarbonyl groups, loganin and the alkaloids from experiments 1 and 2 were submitted to the usual degradation. From the results summarised in Table 2 it is clear that incorporation of (**16**) and (**18**) has occurred with extensive, if not complete, randomisation in the terminal unit. It follows that equilibration of the two atoms corresponding to C-9 and C-10 of geraniol (**6**) requires introduction of oxygen at both centres.

The availability of (**16**) and (**18**) has enabled us to refine The available evidence is best accommodated by the scheme (**23**) → (**24**) → (**25**).

TABLE 2

Amount of label in the methoxycarbonyl groups (% *of total*)

	Expt. No. 1	Expt. No. 2
Ajmalicine (**1**)	39·4	48
Vindoline (**2**)	38·8	39·8
Catharanthine (**3**)	43·0	43·3
Loganin (**8**)	39·8	38·5

Independent evidence for the participation of (**16**) and (**18**) in the biosynthesis of loganin and indole alkaloids is presented by Battersby and his co-workers in the accompanying communication.

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