Biosynthesis of (16*R*)-Isositsirikine from Geissoschizine with a Cell-Free Extract of *Catharanthus roseus* Callus

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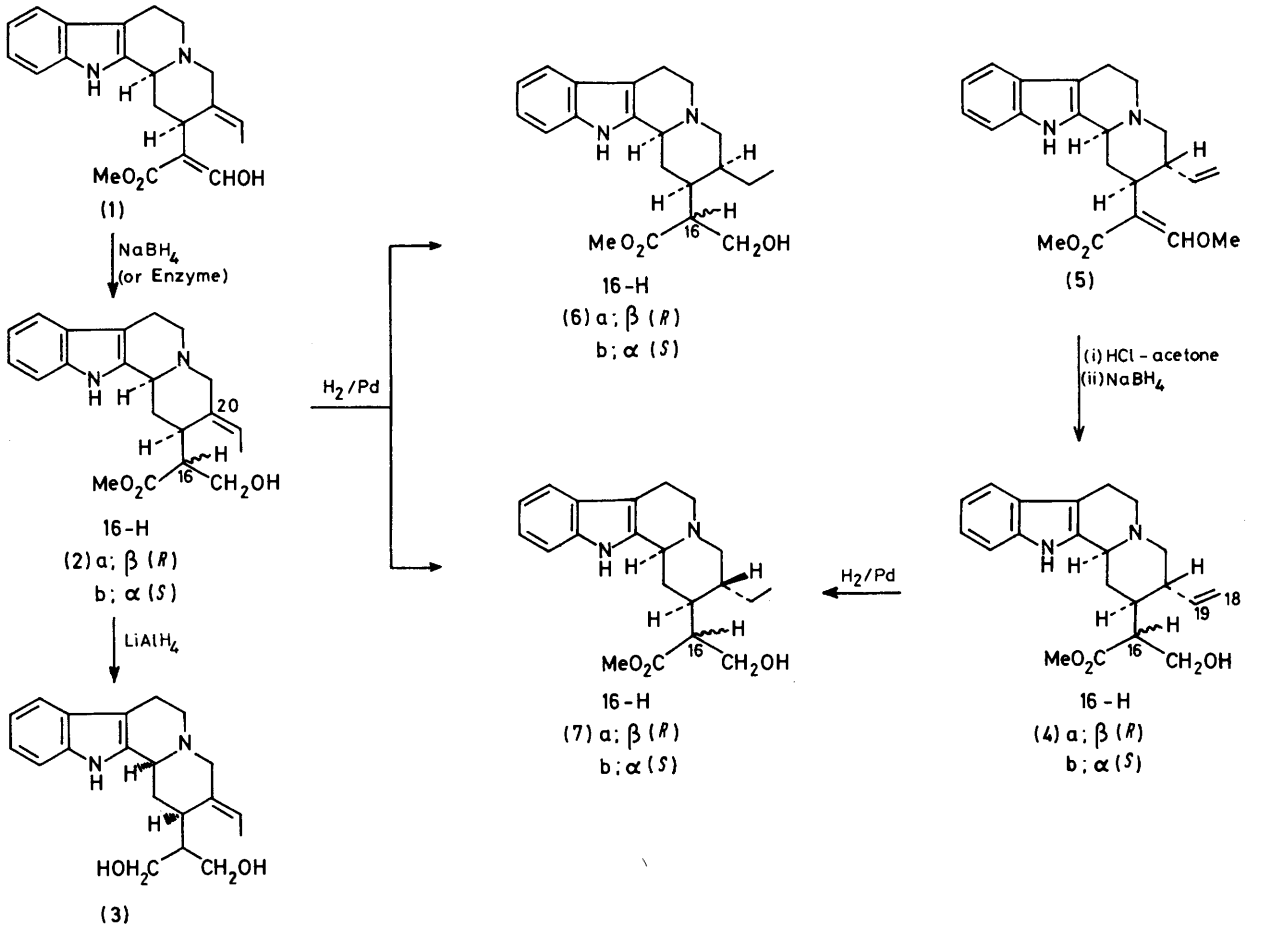
Summary The absolute configuration of isositsirikine (**2a**), an alkaloid of *C. roseus*, has been determined by enzymatic conversion from geissoschizine (**1**) and comparison with authentic samples of known chirality.

THE isolation of a cell-free system from *Catharanthus roseus* callus[1] led to the discovery of new intermediates and clarification of some problems in indole alkaloid biosynthesis, as well as the rapid development of enzymatic

investigations of *Corynanthé*-type alkaloids.[2–4] As part of these studies, it was found that geissoschizine (**1**) was converted into one of the C-16 isomers of isositsirikine (**2a**,**b**) in good yield by a cell-free extract of *C. roseus* callus. The absolute configuration at C-16 of isositsirikine has remained unsettled, although by analogy with sitsirikine[5,6] and diploceline[7] it has been suggested that the C-16 configuration of naturally occurring isositsirikine is (16*R*)-(**2a**) in *Aspidosperma cuspa* and (16*S*)-(**2b**) in *C. roseus*, respectively.[8,9] In this paper we describe the results of the enzymatic reduction of geissoschizine (**1**) and evidence for the absolute configuration (16*R*)-(**2a**) of isositsirikine in *C. roseus*, thus reversing the previous assignments.

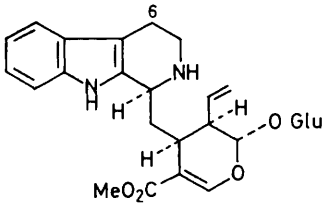


Incubation of geissoschizine (**1**) with a cell-free extract of *C. roseus* callus in the presence of NADPH caused the accumulation of the NADPH-dependent compound (**2**) {$[\alpha]_D^{25}$ — 190° (*c* 0·20, $CHCl_3$); M^+ 354} in *ca.* 10% yield. It was found that the product was one of the C-16 isomers of isositsirikine, because $NaBH_4$-reduction of geissoschizine (**1**) afforded the identical compound (**2a**), together with a larger amount (67%) of its C-16 isomer (**2b**) {$[\alpha]_D^{25}$ — 181° (*c* 0·21, $CHCl_3$); M^+ 354}. Fragmentation patterns [*m/e* 354 (M^+), 353 (M — 1), 336 (M — H_2O), 323 (M — CH_2OH), 295, 251 (base), 184, 170, 169, and 156] of the mass spectra of (**2a**) and (**2b**) were identical and $LiAlH_4$-reduction of both compounds gave a common diol (**3**), showing that the compounds differ only in the configuration at C-16. The C-16 absolute configurations of (**2a**) and (**2b**) were established by correlation with sitsirikine (**4a**) and 16-episitsirikine (**4b**), the configurations of which have been recently established.[6] Authentic sitsirikine (**4a**) (16*R*-configuration) and its C-16 isomer (16*S*)-(**4b**) were synthesized from corynantheine (**5**) by acid-catalysed cleavage of the methyl ether and subsequent $NaBH_4$-reduction. The 18,19-dihydro derivatives of (**4a**) and (**4b**) were prepared by catalytic hydrogenation (Pd–C). Catalytic hydrogenation (Pd–C) of (**2a**) afforded two C-20 isomers, (**6a**) {m.p. 116—118 °C; $[\alpha]_D^{25}$ — 27·8° (*c* 0·15, MeOH); M^+ 356} and (**7a**) {m.p. 213—214 °C; $[\alpha]_D^{25}$ — 52·1° (*c* 0·06, MeOH): M^+ 356} in 87:13 ratio. Compound (**7a**) was identical with the 18,19-dihydro derivative (16*R*-configuration) of sitsirikine (**4a**). Similarly, (**2b**) gave two dihydro compounds, (**6b**) {m.p. 117—119 °C; $[\alpha]_D^{25}$ — 31·5° (*c* 0·25, MeOH); M^+ 356} and (**7b**) {m.p. 212—214 °C; $[\alpha]_D^{25}$ — 12·3° (*c* 0·26, MeOH); M^+ 356} in 38:62 ratio by catalytic hydrogenation, (**7b**) being identical with dihydro derivative (16*S*) of 16-episitsirikine (**4b**). The identities of these compounds were confirmed by direct comparison [mixed m.p., co-T.L.C. (three different solvent systems), i.r. u.v., n.m.r., and mass spectroscopy]. These correlations indicate that the chirality at C-16 is *R* in enzymatically synthesized isositsirikine (the natural alkaloid of *C. roseus*) as shown in (**2a**), whereas

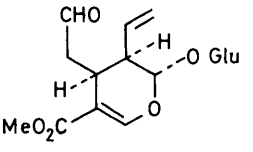
TABLE. Incorporation of radioactive tracers, geissoschizine (**1**), tryptamine, and strictosidine (**8**), into (16*R*)-isositsirikine (**2a**) by a cell-free extract of callus and by intact plants of *C. roseus*.[a]

Exp. No.	Enzyme system[b] and co-factor[c]	Precursor (d.p.m.)[d]	Geissoschizine recovered; d.p.m.	Isositsirikine d.p.m. (% incorporation)[e]
1	Cell-free extract	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$1{\cdot}91 \times 10^6$	$4{\cdot}88 \times 10^4$(1·1)
2	Cell-free extract + NADPH	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$2{\cdot}44 \times 10^6$	$4{\cdot}51 \times 10^5$(10·2)
3	Extract treated with Sephadex G-15[f]	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$2{\cdot}81 \times 10^6$	$3{\cdot}98 \times 10^2$(0·009)
4	Extract treated with Sephadex G-15[f] + NADPH	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$2{\cdot}34 \times 10^6$	$5{\cdot}51 \times 10^5$(12·4)
5	Extract treated with active charcoal[f]	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$2{\cdot}30 \times 10^6$	$4{\cdot}40 \times 10^3$(0·10)
6	Extract treated with active charcoal[f] + NADPH	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$2{\cdot}12 \times 10^6$	$4{\cdot}75 \times 10^5$(10·7)
7	Cell-free extract boiled[g]	^{3}H-Geiss($4{\cdot}44 \times 10^6$)	$3{\cdot}86 \times 10^6$	$5{\cdot}11 \times 10$(0·001)
8	Cell-free extract + NADPH	^{14}C-Tryp($2{\cdot}22 \times 10^6$) + secologanin[k]	—[h]	$4{\cdot}00 \times 10^4$(1·8)
9	Extract treated with Sephadex G-15[f] + NADPH	^{14}C-Strict($2{\cdot}22 \times 10^5$)[j]	—[h]	$6{\cdot}22 \times 10^3$(2·8)
10	Intact plant (feeding)[i]	^{14}C-Strict($2{\cdot}61 \times 10^6$)[j]	—[h]	$2{\cdot}58 \times 10^3$(0·099)

[a] Incubations were carried out at 37 °C for 2 h by enzyme preparations (3 ml; 1·0—1·5 mg of protein ml^{-1}) in 0·05 M citrate–phosphate buffered solution (pH 7·2) containing 10 mM mercaptoethanol. [b] Cell-free extracts were prepared essentially as described in ref. 1. [c] The concentration of NADPH is 2 mM. [d] ^{3}H-Geiss, ^{14}C-Tryp, and ^{14}C-Strict denote [arom-^{3}H]geissoschizine (**1**) (specific activity 700 μCi μmol^{-1}), [2-^{14}C]tryptamine (50 μCi μmol^{-1}), and [6-^{14}C]strictosidine (**8**) (10 μCi mol^{-1}), respectively. [e] No adjustment was made for recovered precursor. [f] In order to remove low-molecular materials, the crude cell-free extract was treated with Sephadex G-15 or active charcoal. [g] Extract boiled for 5 min prior to incubation. [h] Effectively zero. [i] Cut stems of intact plant of *C. roseus* (1 month old; 10 cm length) were fed for 30 h by the hydroponic method. [j] [6-^{14}C]Strictosidine (**8**) was prepared from [2-^{14}C]tryptamine and secologanin (**9**) by the method of Battersby *et al.* (see A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc.* (*C*), 1969, 1193; the stereochemistry at C-3 was subsequently revised to 3α-H for strictosidine and 3β-H for vincoside, see O. Kennard, P. J. Roberts, N. W. Isaacs, F. H. Allen, W. D. S. Motherwell, K. H. Gibson, and A. R. Battersby, *Chem. Comm.*, 1971, 899; K.T.D. De Silva, G. N. Smith, and K. E. H. Warren, *ibid.*, p. 905; W. P. Blackstock, R. T. Brown, and G. K. Lee, *ibid.*, p. 910). [k] As substrate, 0·02 μmol of secologanin (**9**) was added.



(8)



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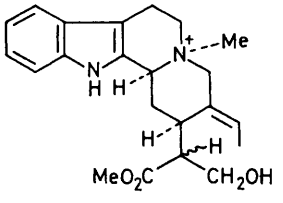

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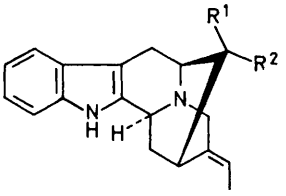

(11): $R^1 = CH_2OH$, $R^2 = CO_2Me$
(12): $R^1 = CO_2Me$, $R^2 = CH_2OH$

the major product (corresponding to the alkaloid of *A. cuspa*) of the $NaBH_4$-reduction of geissoschizine (**1**) is (**2b**).

The enzymatic conversion of geissoschizine (**1**) into isositsirikine (**2a**) was further investigated using radioactive tracers. [Arom-^{3}H]geissoschizine (**1**), [2-^{14}C]tryptamine, and [6-^{14}C]strictosidine (**8**) were incubated with the cell-free extract under various conditions. The precursors were incorporated into (**2a**) in good yield, as shown in the Table. The results suggest that geissoschizine is a direct intermediate in the biosynthesis of isositsirikine (**2a**) and that NADPH is required in the enzymatic reaction. Intact plants also synthesize isositsirikine (**2a**), although the incorporation is very low (exp. 10; Table). However, no 16-epi-isositsirikine (**2b**) was detected in the cell-free incubations or in feeding experiments with intact plants of *C. roseus*, showing that only the (16*R*-isomer (**2a**) is biosynthesized in *C. roseus* callus and the intact plant, and that the enzyme system is stereospecific. It is of interest to note that (**2a**) also may serve as one of the intermediates in the biosynthesis of several alkaloids such as diploceline (**10**),[7] polyneuridine (**11**),[10] and akuammidine (**12**),[11] thus lending further evidence for the important role of geissoschizine[1,2,12] in the metabolism of *C. roseus*.

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