4,21-Dehydrogeissoschizine, an Intermediate in Heteroyohimbine Alkaloid Biosynthesis

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Summary 4,21-Dehydrogeissoschizine (**5**) was proven to be a metabolic intermediate in the enzymatic synthesis of cathenamine (**6**) and its transformation products ajmalicine (**7**), 19-epiajmalicine (**8**), and tetrahydroalstonine (**9**); the enzyme catalysing the synthesis of (**6**) from (**5**) was identified and named cathenamine synthase.

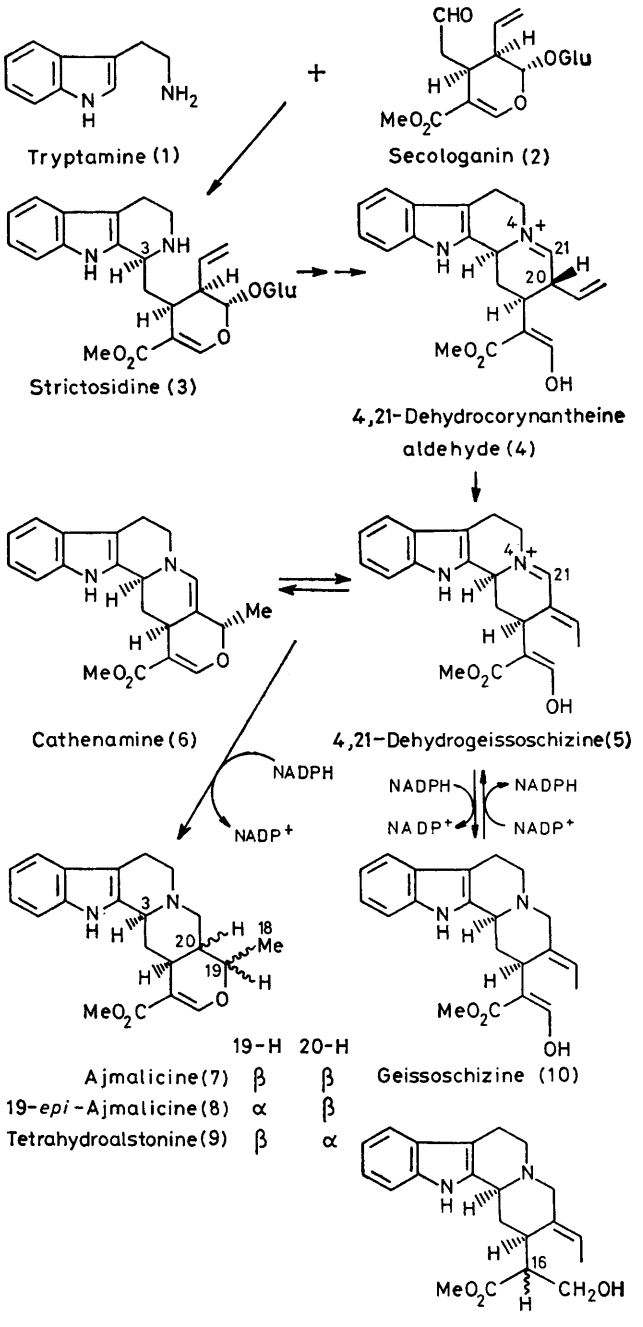
THE reaction sequence leading from tryptamine (**1**) and secologanin (**2**) to heteroyohimbine alkaloids has largely been elucidated using cell-free systems from *Catharanthus roseus*.[1–3] Besides strictosidine (**3**), a further intermediate in this pathway, cathenamine (**6**) (20,21-didehydroajmalicine) has been discovered.[4] Furthermore *Guettarda eximia* (*Rubiaceae*) proved to be a rich source for this natural

intermediate.[5] From the same plant material a second compound was isolated and its structure identified as 4,21-dehydrogeissoschizine[6](5). Chemical transformations[6] of this compound lead to either cathenamine (6) or geissoschizine (10). These biomimetic reactions reflect closely the postulated biosynthetic expectations of an intermediate located between 4,21-dehydrocorynantheine aldehyde (4) and cathenamine (6). 4,21-Dehydrogeissoschizine (5) was therefore tested as a substrate for heteroyohimbine alkaloid formation using enzyme preparations from *C. roseus* cell cultures in the presence of NADPH.[7] Under these conditions [0·2 μmol of (5), 1·0 μmol NADPH, 50 μmol KPO_4^{2-} buffer, pH 7·0, 1 mg protein, total vol. 0·5 ml, 30 °C, 120 min] transformation of this substrate (5) to (7) (25%), (8) (18%), and (9) (11%) was achieved. The products were analysed by quantitative t.l.c. followed by mass spectroscopy (each compound: *m/e* 352 M^+) as well as radioimmunoassay.[8] Omission of NADPH led to the formation of (6) in 74% yield. Cathenamine (6) was identified by mass spectroscopy (350 M^+) and quantified by conversion[5] into (9) using BH_4^- and subsequent radioimmunological determination.[8] Employing this assay system the conversion of (5) into (6) was further characterized. The reaction proceeded linearly with time, and was affected by pH (maximal conversion at pH 6·5), and protein (saturation at 3 mg ml^{-1}) and substrate concentration (K_m 0·6 mM). In the absence of enzyme no conversion of (5) into (6) occurred even after prolonged incubation up to 24 h.

In order to establish (5) as an obligatory intermediate in the biosynthetic sequence leading to the heteroyohimbine alkaloids, [1-^{14}C]-(1) and (2) were allowed to be transformed in the presence of a tenfold [with respect to (1)] excess of unlabelled (5), enzyme, and NADPH. The specific activity of the alkaloids (7), (8), and (9) formed was ten times less than that of the control experiment which was performed in the absence of (5). This proves that (1) is channelled through (5) in the course of the formation of (7), (8), and (9). Furthermore, short-term incubations of [1-^{14}C]-(1) and (2) in the presence of (5), led to the accumulation of radioactivity in (5). Reduction of labelled (5), in the presence of a carrier, by BH_4^- gave radioactive isositsirikine (11a) and its C-16 isomer (11b).[6] This experiment proves that the suspected intermediate is formed under cell-free conditions from distant precursors and establishes (5) as an obligatory intermediate in the formation of the heteroyohimbine alkaloids (7), (8), and (9). The new enzyme described here, which catalyses the formation of (6) from (5), is named cathenamine synthase.

Under NADPH regenerating conditions [isocitrate dehydrogenase system] (5) is transformed by *C. roseus* extracts in *ca.* 10% yield into geissoschizine (10) which is known *not* to be a direct precursor of (7) and its isomers[2] (8) and (9).

If the sequence of reactions from (1) and (2) to the heteroyohimbine derivatives (7), (8), and (9) is allowed to proceed in the presence of D_2O a total of two deuterium atoms is incorporated, one on to each of C-18 and C-20, respectively.[9] In contrast, if (5) is used as substrate for the NADPH-dependent reaction only one deuterium atom is incorporated into (7), (8), and (9). This result indicated that under these conditions no isomerisation takes place when (5) is reached which would lead to (4) or its 20, 21-isomer. Furthermore, the incorporation of only one deuterium atom during the conversion of (5) into (7), (8), and (9) supports the finding that (5) is the immediate precursor of (6) in the biosynthetic pathway as outlined in the Scheme.



SCHEME

We consider (5) to occupy a crucial position at the branch point in the biosynthesis of *Iboga*, *Aspidosperma*, and *Corynanthe* type alkaloids.

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