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STRICTOSIDINE, THE COMMON PRECURSOR FOR MONOTERPENOID INDOLE ALKALOIDS WITH 3 α and 3 ß CONFIGURATION

Martina Rueffer, Naotaka Nagakura and Meinhart H. Zenk*

(Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, D 4630 Bochum, W.-Germany

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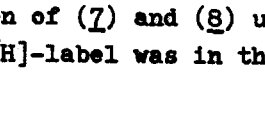
Recently we reported that strictosidine (1) is the key intermediate in the formation of the three classes (Aspidosperma, Iboga, Corynanthe) of monoterpenoid indole alkaloids in Catharanthus roseus and a variety of other plant species in cell culture using in vivo and in vitro techniques[1,2]. These results were independently confirmed in Manchester[3,4] and subsequently also Scott et al.[5] were able to confirm the precursor role of (1) using Catharanthus material. All these results are in accord with reports on the biosynthesis of an alkaloid of taxonomically distant origin, camptothecin, for which strictosidine lactam[6] was previously found to be a precursor, and recently[7] also (1).

The key intermediate in the biosynthesis of the majority of monoterpenoid alkaloids is therefore (1) with 3 α (S) configuration, rather than vincoside (2) with 3 ß (R) configuration as had previously been assumed[8]. However, a generalization of this precursor function of (1) may not be applicable to the alkaloid family with C-3 ß stereochemistry, especially if one takes into consideration biomimetic experiments[9], which were assumed to duplicate the in vivo process in respect that (1) is the precursor for 3 α alkaloids and (2) for 3 ß. To test the biological validity of these experiments and to gain clarity as to the assumed[2] universal role of (1) as a general precursor for monoterpenoid indole alkaloids, labelled (1) and (2) were fed separately to two plant species known to contain both 3 α as well as 3 ß alkaloids and belonging to taxonomically very different plant families: Rauwolfia canescens[10] concaining α-yohimbine (3, 3 α-H) and reserpiline (4, 3 ß-H) and Mitragyna speciosa[11] containing mitragynine (5, 3 α-H) and speciociliatine (6, 3 ß-H) To trace also the fate of the hydrogen atom at C-3, tritium label was introduced into this position in (1) as well as in (2). Synthesis of [$^{3}H/^{14}C$]-(1) and (2) was achieved either by condensation of [2-^{14}C]-

tryptamine (7) with [7-^{3}H]-secologanin[12] (8) in 1 M phosphate buffer, pH 4.0, and subsequent separation of the epimers[12], or by enzymatic condensation of (7) and (8) using strictosidine synthetase[1,2]. To prove that the [^{3}H]-label was in the desired position [^{3}H/^{14}C = 7.17 : 1]-(1)



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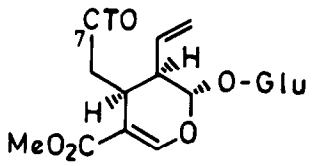

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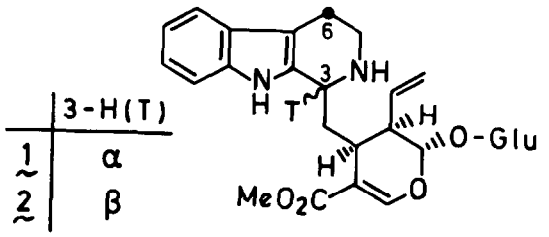
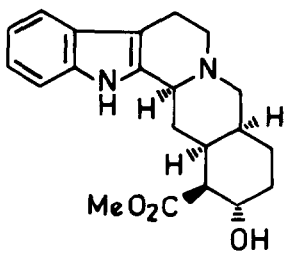


	3-H(T)
1	α
2	β



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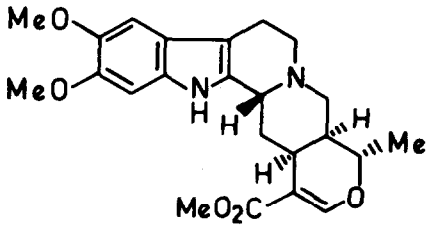

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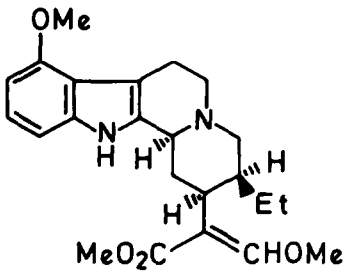

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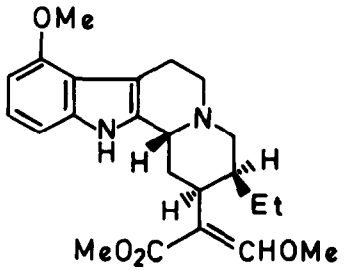

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was fed[2] to C. roseus seedlings. Ajmalicine was then isolated [$^3H/^{14}C$ = 7.08 : 1] and dehydrogenated with mercuric acetate[13] to yield dehydroajmalicine which was then reduced with borohydride. The recovered ajmalicine [$^3H/^{14}C$ = 0.41 : 1] carried 5.7 % of the original [^3H]-activity in agreement with the [^3H]-label being located at C-3.

The essential experimental data and results from feeding experiments using (1) with 3 α (S) stereochemistry are shown in the following Table.

Experimental Plant	Alkaloid Investigated	3-H Stereochem.	^{14}C Incorp. (%)	$^3H/^{14}C$ Ratio
R. canescens	α-Yohimbine (3)	α	0.70	7.37 : 1
(Apocynaceae)	Reserpiline (4)	β	0.34	0.10 : 1
M. speciosa	Mitragynine (5)	α	2.72	8.40 : 1
(Rubiaceae)	Speciociliatine (6)	β	0.53	0.10 : 1

[6-^{14}C, 3-^3H]-(1) [spec.act.: 18.49 x 10^6 dpm ^3H, 2.58 x 10^6 dpm ^{14}C/μmole; $^3H/^{14}C$ = 7.17 : 1] was administered in aqueous solution (ca. 5 % EtOH) to apical cuttings, which were maintained at 28° C under light for 24 hrs. Isolation and purification of alkaloids followed standard procedures[12].

Parallel feeding experiments were performed with [6-^{14}C, 3-^3H]-(2); no incorporation into (3), (4), (5), (6) or other alkaloids in these plants was observed (detection limit: < 0.001 %). These results demonstrate unequivocally that (1) is the common biosynthetic precursor for alkaloids with 3 α as well as 3 β configuration. This is contrary to the chemical conversions in which (1) is transformed to 3 α and (2) to 3 β heteroyohimbine alkaloids[9]. This fact shows the limitations of biomimetic experiments with respect to in vivo processes. The biosynthetic conversion of (1) to 3 β alkaloids proceeds with loss of hydrogen at C-3, while it is retained in the formation of the 3 α series. Thus it is unnecessary to assume special mechanisms[14,15] in the inversion of these precursors which would allow retention of hydrogen at C-3.

Furthermore, feeding of [6-^{14}C]-(1) resulted in heavily labelled alkaloids of the following plant species: Amsonia, Cinchona, Rhazia Stemmadenia, Uncaria and Vinca; in no single case incorporation of (2) was observed[12]. This proves that strictosidine (1) with 3 α (S) stereochemistry is the universal precursor for monoterpenoid indole alkaloids.

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