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Indirect Identification of 4,21-Dehydrocorynantheine Aldehyde as an Intermediate in the Biosynthesis of Ajmalicine and Related Alkaloids

J. Stöckigt*, M. Rueffer*, M. H. Zenk* and G.-A. Hoyer**

* Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum.
** Schering AG., Department für Spectrometrie und Quantenchemie, Berlin
Federal Republic of Germany

Key Word Index: Catharanthus roseus; Cell Suspension Cultures; Enzymatic Formations; Sitsirikine; 4,21-Dehydrocoryantheine Aldehyde; Heteroyohimbine Type Alkaloids.

Abstract

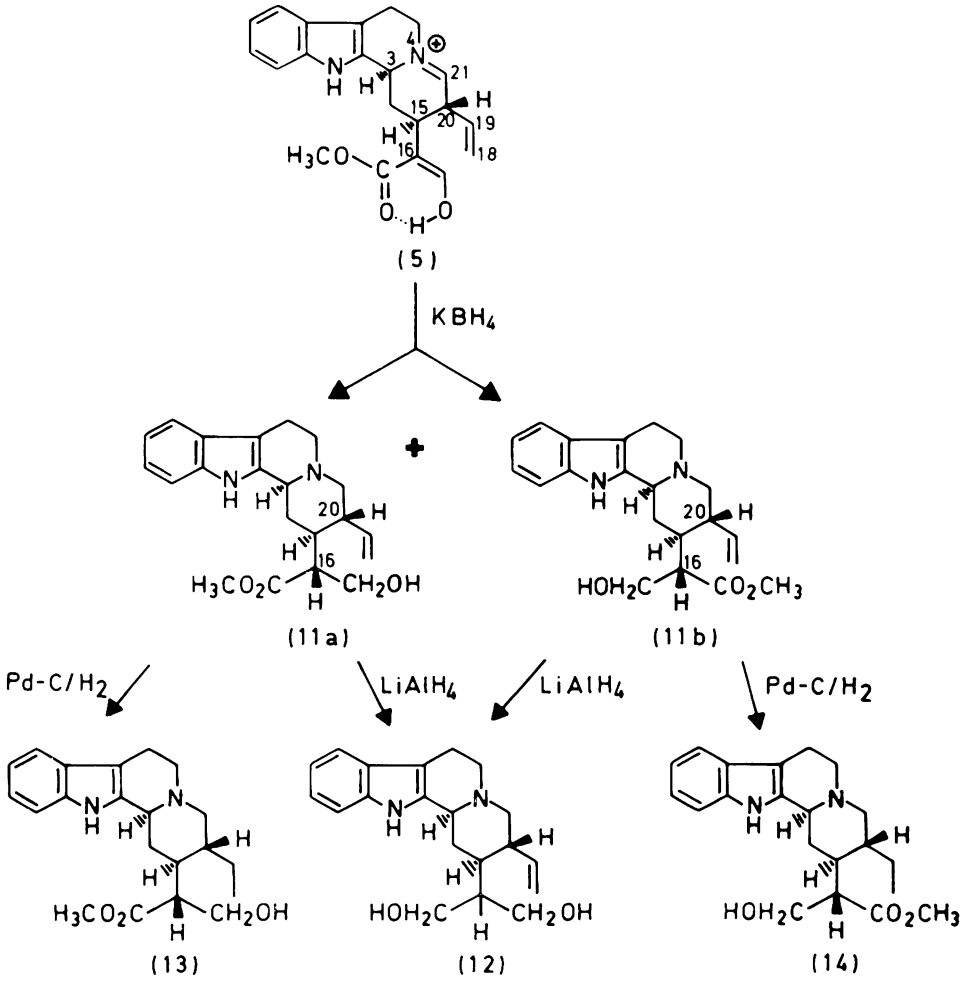
In the pesence of BH_4^- an enzyme preparation of *Catharanthus roseus* cell suspension cultures transformes strictosidine (*1*), to sitsirikine (*11a*) and 16-iso-sitsirikine (*11b*) which are derived from 4,21-dehydrocorynantheine aldehyde (*5*), an intermediate in the formation of heteroyohimbine type alkaloids.

We have recently detected strictosidine (*1*) [1, 2] and cathenamine [3] (*7*) as pivotal intermediates in the enzymatic formation of monoterpenoid indole alkaloids of the heteroyohimbine type (*8–10*) in cell-free extracts from *Catharanthus roseus* cell suspension cultures. The initial and final reactions are catalysed by strictosidine synthetase [2] and cathenamine reductase [3] respectively. The enzymatic step immediately beyond (*1*) should involve the action of a β-glucosidase hydrolysing the alkaloidal glucoside (*1*) to yield the unstable aglycone (*2*) which in turn opens to yield the dialdehyde [4] (*3*) with 3 α (S) configuration. This highly reactive (*3*) should undergo further transformations to yield (*7*) as precursor of (*8–10*). In an effort to intercept this sequence of reactions and to trap the precursor of (*7*) for identification, BH_4^- was included into the enzyme reaction mixture to reduce the expected dialdehyde generated from (*1*) and to prevent it from further conversion. A number of enzymes are known to be active in the presence of KBH_4 [5].

An enzyme preparation [6] (1600 ml, 1,93 g protein) was incubated at pH

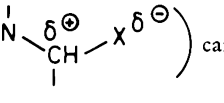
7.0 with (*1*) (474 μmol, 6 μCi 6-^{14}C) in the presence of KHB_4 (164 mmol) and 21 ml MeOH at 25° C for 120 min. Non-glucosidal material was extracted into EtOAc and subjected to TLC (acetone: pet. ether (40–60°): diethylamine = 20:70:10). Only two compounds were located (*11a*: R_f 0.46, and *11b*: R_f *0.56*) which were further purified and isolated (each 17 mg). In about twenty analytical and preparative isolations the material showed a 1:1 yield which was indicative of the compounds being stereoisomers. Spectroscopic analysis showed (*11a*): UV (MeOH λ_{max} (ε) 223 (26900), 273 (5490), 279 (5550), 282 (5550), 289 nm (4580). IR (KBr) ν 3410 (NH, OH), 2820, 2760 (Bohlmann bands), 1715 ($COOCH_3$), 1640 (C=C), 1050 (C–O), 1010, 1000, 925 (–CH = CH_2), 745 cm^{-1} (ar C–H). ^{1}H-NMR ($CDCl_3$) δ 3.59 (s, 3H, $-COOCH_3$), 3.94 (d, J = 6.0 Hz, 2H, $-CH_2OH$), 4.97 – 5.61 (m, 3H, $-CH=CH_2$), 7.00 – 7.52 (m, 4H, 4 ar H), 9.48 ppm (m,W $^1/_2$ = 14 Hz, 1H, NH). MS (70 eV) m/e 354 (100; M^+), 353 (76; M^+–H, 323 (14; M^+-CH_2OH), 251 (72; M^+-H_3COOC-CH-CH_2OH), 249 (35; 353 -H_3COC (OH)=$CHCH_2OH$), 223 (38), 221 (14), 184 (55), 170 (83), 169 (59), 156 (66), 144 (24), 129 (28), 115 (24). (*11b*): UV (MeOH) λ_{max} (ε) 223 (29400), 273 (5600), 279 (5630), 282 (5610), 289 nm (4680). IR (KBr) ν 3430 (NH, OH), 2820, 2760 (Bohlmann bands), 1725 ($COOCH_3$), 1635 (C=C), 1040 (C-O), 1010, 1005, 925 (-CH=CH_2), 745 cm^{-1} (ar C-H). ^{1}H-NMR ($CDCl_3$) δ 3.68 (d, J = 6.0 Hz, 2H, -CH_2OH), 3.73 (s, 3H -$COOCH_3$), 4.97 – 5.60 (m, 3H, -CH=CH_2), 7.00 – 7.53 (m, 4H, 4 ar H), 8.97 ppm (m, W $^1/_2$ = 14 Hz, 1H, NH). MS (70 eV) m/e 354 (83; M^+), 353 (61; M^+-H), 323 (13; M^+-CH_2OH), 251 (70; M^+ -$H_3COOCCH$-CH_2OH), 249 (39; 353 -H_3COC (OH) =CH-CH_2OH), 223 (39), 221 (17), 184 (57), 170 (100), 169 (74), 156 (83), 144 (35), 129 (39), 115 (39).

On the basis of the spectroscopic data and comparison (TLC) with the authentic compound (*11a*) proved to be sitsirikine [7]. The vinyl group in sitsirikine is α oriented. (*11a*) and (*11b*) show the same chemical shift (4.97 – 5.60 ppm) for the vinyl group. An axial vinyl group should show a 19-H signal which is strongly paramagnetically shifted due to the lone electron pair at the tertiary N. This shift is not observed, however, and consequently (*11b*) possesses the same configuration at C-20 as (*11a*) (equatorial (α) oriented vinyl group). The isomery between (*11a*) and (*11b*) must therefore be located at the C-16 center. This is indeed the case since (*11a*) and (*11b*) if reduced to a diol by $LiAlH_4$ gave an unseparable (TLC) and identical compound [7] (*12*); (*11b*) is therefore 16-iso-sitsirikine. (*11a*) hydrogenated over Pd-C [7] gave (*13*): MS (70 eV) m/e 356 (100; M^+), 355 (85; M^+-H), 253 (49; M^+-$C_4H_7O_3$), 251 (12; 355-$C_4H_8O_3$), 184 (11), 170 (22), 169 (17), 156 (15) in every respect identical to 18,19-dihydrositsirikine [7]; (*11b*) gave (*14*): MS (70 eV) m/e 356 (100; M^+), 355 (85; M^+-H), 253 (44; M^+-$C_4H_7O_3$), 251 (12; 355-$C_4H_8O_3$), 184 (11), 170 (22), 169 (17), 156 (13), consistent with 18, 19-dihydro-16-isositsirikine. On the basis



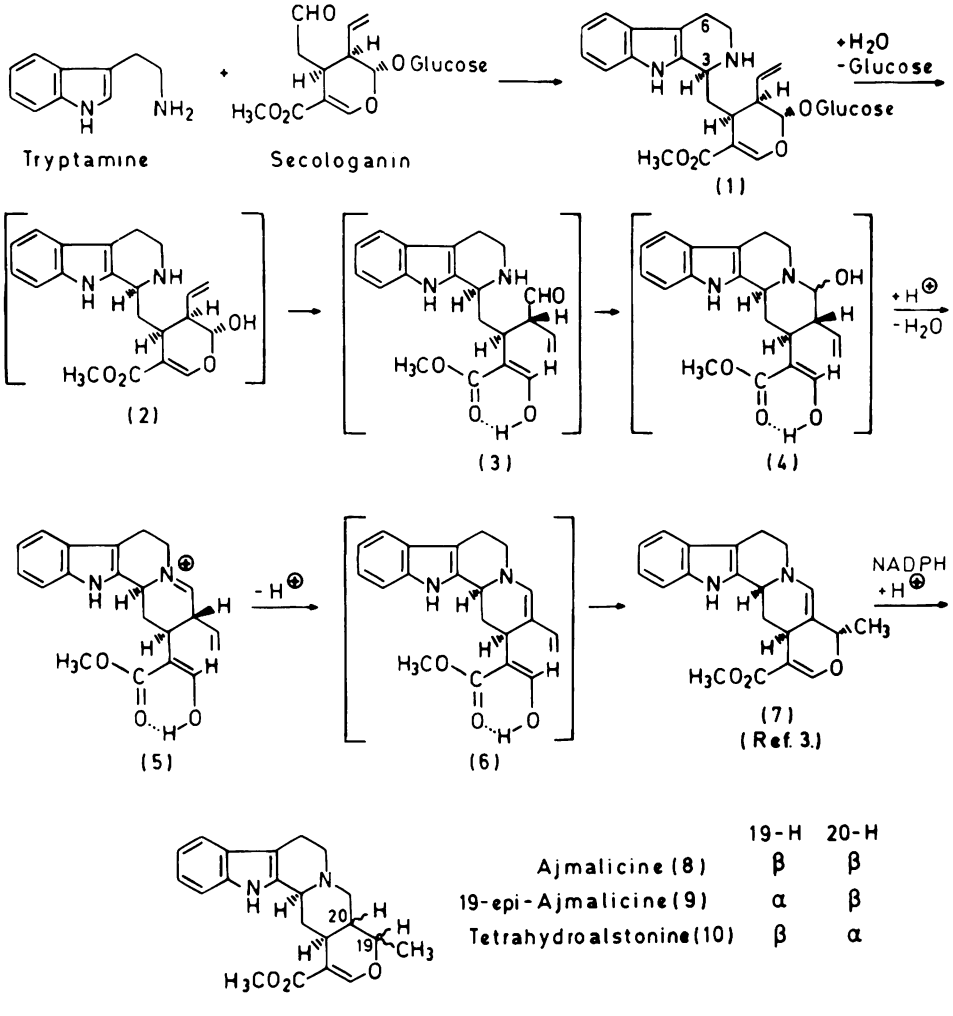
of the NMR spectrum (position of $-CH_2OH$) it can be assumed, that $-CH_2OH$ and the vinyl group are spatially neighbouring in (*11a*). The occurrence of this pair of isomers (*11a, b*) under the above reaction condition demonstrates that the dialdehyde (*3*) is so highly reactive that it cannot be trapped by BH_4^- but rather undergoes intramolecular closure of ring D to give the immonium ion (*5*) or its polarised form*.

It is this intermediate (*5*) the involvement of which in the biosynthesis of monoterpenoid alkaloids (*8–10*) is thus demonstrated by reduction with BH_4^- to (*11a, b*). In the absence of BH_4^- (*1*) is transformed to (*7*) [3, 6]. (*1*) is hydro-

* an equilibrium between (*5*) and  can easily be visualised.

lysed by β-glucosidase from sweet almonds or by acid treatment gives rise to vallesiachotamine [8, 9] while under cell-free conditions of alkaloid biosynthesis (*5*) is the crucial intermediate; this indicates that specific enzymes are involved in the metabolic sequence from (*1*) to (*5*).

Compound (*5*), among others, has previously already been considered [4] on theoretical grounds to be involved in monoterpenoid indole alkaloid biosynthesis. A highly interesting hydride trapping experiment using $NaBH_3CN$ in the conversion of (*1*) to heteroyohimbine alkaloids under *non* physiological conditions has recently been conducted by BROWN et al. [9]. This experiment, however, did not give evidence for the naturally occurring intermediate (*5*) reported here. The proposed biosynthetic pathway leading to the *Corynanthe*-type alkaloids (*8–10*) with hypotensive activity is shown below.



	19-H	20-H
Ajmalicine (8)	β	β
19-epi-Ajmalicine (9)	α	β
Tetrahydroalstonine (10)	β	α

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References

1. Stöckigt, J. and M. H. Zenk: FEBS Letters *79*, 233 (1977).
2. Stöckigt, J. and M. H. Zenk: J. C. S., Chem. Comm. 646 (1977).
3. Stöckigt, J., H. P. Husson, C. Kan-Fan and M. H. Zenk: J. C. S. Chem. Comm. 164 (1977).
4. Battersby, A. R., A. R. Burnett and P. G. Parsons: J. Chem. Soc. (C) 1193 (1969).
5. e. g. Mahler, H. R., S. J. Wakil and R. M. Bock: J. Biol. Chem. *204*, 453 (1953).
6. Stöckigt, J., J. Treimer and M. H. Zenk: FEBS Letters *70*, 267 (1976).
7. Kutney, J. P. and R. T. Brown: Tetrahedron *22*, 321 (1966).
8. De Silva, K. T. D., G. N. Smith and K. E. H. Warren: Chem. Comm. 905 (1971).
9. Brown, R. T., J. Leonard and S. K. Sleigh: J. C. S. Chem. Comm. 636 (1977).

Address: Prof. Dr. M. H. Zenk,
Lehrstuhl für Pflanzenphysiologie,
Postfach 2148,
D-4630 Bochum-Querenburg
Federal Republic of Germany