

# Planta medica

**Journal of  
Medicinal  
Plant Research**

**Editor - in - Chief**

E. Reinhard, Univ. Tübingen  
Pharmazeutisches Institut  
Auf der Morgenstelle  
7400 Tübingen

**Editorial Board**

H. P. T. Ammon, Tübingen  
W. Barz, Münster  
E. Reinhard, Tübingen  
O. Sticher, Zürich  
H. Wagner, München  
M. H. Zenk, Bochum

Hippokrates Verlag  
Stuttgart

---

## **Contents**

**Vol. 33/1978**

ADESINA, S. K., J. B., HARBORNE and J. D. PHILLIPSON, The Isolation of Quarternary Alkaloids from the Leaves of <i>Dioscoreophyllum cumminsii</i> (STAFF). DIELS	217
AVIV, D. and E. GALUN, Biotransformation of Monoterpenes by <i>Mentha</i> Cell Lines: Conversion of Pulegone to Isomenthone	70
BARZ, W. and E. MEYER, Degradation of Phenylethylamines in Plant Cell Suspension Cultures	336
BAUCH, H.-I. and E. LEISTNER, Aromatic Metabolites in Cell Suspension Cultures of <i>Galium mollugo</i> L.	105
BAUCH, H.-I. and E. LEISTNER, Attempts to Demonstrate Incorporation of Labelled Precursors into Aromatic Metabolites in Cell Suspension Cultures of <i>Calium mollugo</i> L.	124
BISHAY, D. W., W. H. SHELVER and S. K. WAHBA Khalil, Alkaloids of <i>Erythraea centaurium</i> PERS, Growing in Egypt. I. Isolation of Gentianine	422
CATALANO, S., A. MARSILI, J. MORELLI, L. PISTELLI and V. SCARTONI, Constituents of the Leaves of <i>Ilex aquifolium</i>	416
CAVÉ, A., H. GUINAUDEAU, M. LEOEUF, A. RAMAHATRA et. J. RAZAFINDRAZAKA, Alcaloides des Annonacées XVIII. Alcaloides des Ecorces de tronc du <i>Polyalthia suaveolens</i> ENGL. et DIELS. (Alkaloids of Annonaceae XVIII Alkaloids from Trunk-Barks of <i>Polyalthia suaveolens</i> ENGL. et DIELS)	243
DEMUTH, G., H. HINZ, O. SELIGMANN and H. WAGNER, Investigations on Anthrachinon-glycosides of <i>Rhamnus</i> Species, V. Emodin-8-O- $\beta$ -gentiobioside, a new O-glycoside from <i>Rhamnus frangula</i>	53
DROZDŽ, B. and E. BŁOSZYK, Selektive Detection of Sesquiterpene Lactones by TLC	379
EL-SAYYAD, S. and H. WAGNER, A Phytochemical Study of <i>Calligonum comosum</i> L. HENRY	262
FAIRBAIRN, J. W. and E. M. WILLIAMSON, Anatomical Studies on <i>Papaver bracteatum</i> (LINDLEY)	34
FAIRBAIRN, J. W. and E. M. WILLIAMSON, <i>Papaver bracteatum</i> (LINDLEY) Seedling Characters as a Rapid Aid to Identification	365
FISH, F., I. A. MESHAL and P. G. WATERMAN, Alkaloids of <i>Oricia suaveolens</i>	228
FRIEDRICH, H. und R. ENGELSHOWE, Monomere Gerbstoffvorstufen in <i>Juniperus communis</i> L. (Tannin Producing Monomeric Substances in <i>Juniperus communis</i> L.)	251
GONZALES, A. G., V. DARIAS, G. ALONSO, J. N. BOADA and M. FERIA, Cytostatic Activity of Sesquiterpene Lactones from Compositae of the Canary Islands	356
GRACZA, L. und W. SPAICH, Analytische und biopharmazeutische Untersuchung trans-isoasaronhaltiger Präparate (Analytical and Pharmaceutical Examination of Preparations containing trans-Isoasaron)	160
HARVALA, C. and P. I. HYLANDS, Saponins from <i>Cyclamen hederifolium</i> and <i>C. graecum</i>	180
HEGNAUER, R. und P. KOOIMAN, Die systematische Bedeutung von iridoiden Inhaltsstoffen im Rahmen von Wettstein's Tubiflora (The Taxonomic Significance of Iridoids of Tubiflorae sensu Wettstein)	1
HEINS, M., J. WAHL, H. LERCH, F. KAISER and E. REINHARD, Preparation of $\beta$ -Methyldigoxin by Hydroxylation of $\beta$ -Methyldigitoxin in Fermenter Cultures of <i>Digitalis lanata</i>	57
HELMBOLD, H., W. VOELTER and E. REINHARD, Sterols in Cell Cultures of <i>Digitalis lanata</i>	185
HIKINO, H., M. TAMADA and KUN-YING YEN, Mallorepine, Cyano- $\gamma$ -Pyridone <i>Mallotus repandus</i>	385
HORVATH, P., G. SZEPESI and A. KASSAI, An Improved Quantitative Thin-Layer Chromatographic Method Controlled by HPLC for the Determination of Ergotamine and Ergocristine in Crude Ergot	407
INOUE, H., Neuere Ergebnisse über die Biosynthese der Glucoside der Iridoidreihe (New Results on the Biosynthesis of Iridoidglucosides)	193

IWU, M. M. and W. E. COURT, The Alkaloids of <i>Rauwolfia mombasiana</i> Leaves	232
IWU, M. M. and W. E. COURT, Leaf Alkaloids of <i>Rauwolfia cumminsii</i> STAFF.	360
JEWERS, K. and K. A. ZIRVI, The Coumarin Glycosides of <i>Daphne acuminata</i> : Use of <sup>13</sup> C-NMR Spectroscopy for their Identification	403
KAMAL, R. and S. C. JAIN, <i>Tephrosia falciformis</i> – a New Source of Rotenoids	418
KARTNIG, Th., A. HIERMANN und Ch. VRECER, Flavonoide in Herba Convallariae majalis	412
LOUNASMAA, M. Dérivés Phloroglucinoliques des Fougères du Genre <i>Dryopteris</i> . Analyse des Dérivés Phloroglucinoliques Caractéristiques par la Résonance Magnétique Nucléaire de <sup>13</sup> C. (Phloroglucinol Derivates of the Ferns of the Genus <i>Dryopteris</i> . Analysis of the Characteristic Phloroglucinol Derivatives by the <sup>13</sup> C Nuclear Magnetic Resonance)	173
MECHLER, E. and H. W. KOHLENBACH, Alkaloid Content in Leaves of Diploid and Haploid <i>Datura</i> Species.	350
MEHROTRA, P. K. and V. P. KAMBOJ, Hormonal Profile of Coronaridine Hydrochloride – an Antifertility Agent of Plant Origin	345
OGURA, M., K. KAZUHIRO, G. A. CORDELL and N. R. FARNSWORTH, Potential Anticancer Agents VIII. Constituents of <i>Baliospermum montanum</i> (Euphorbiacea)	128
OTSUKA, H., S. KOBAYASHI and S. SHIBATA, Separation and Determination of Saponins of Bupleuri Radix by Droplet Counter Current Chromatography	152
PREININGER, U., J. NOVÁK, V. ŠIMANEK and F. ŠANTAVÝ, Isolation and Chemistry of Alkaloids from Plants of the Papaveraceae LXXXIII: Isolation and Identification of Alkaloids from <i>Corydalis lutea</i> . (L) DC	396
PURI, H. S., C.-I. WIDÉN and H. K. WIDÉN, Phloroglucinol Derivatives in <i>Dryopteris marginata</i>	177
QUIGLEY, F. R., Diosgenin in West African <i>Dioscorea</i> Plants	414
RIMPLER, H., Strukturaufklärung von Iridoidglykosiden	313
SALEH, M. M. and H. KATING, Gas-Chromatographic Analysis of the Volatile Oil of <i>Perovskia abrotanoides</i> KAREL	85
SHELLARD, E. J., P. J. HOUGHTON and M. RESHA, The <i>Mitragyna</i> Species of Asia (Part XXX: Oxidation Products of Mitragynine and Speciociliatine)	223
SHELLARD, E. J. and P. K. LALA, The Alkaloids of <i>Mitragyna rubrostipulata</i> (SCHUM.) HAVIL	63
STÖCKIGT, J., M. RUEFFER, M. H. ZENK and G.-A. HOYER, Indirect Identification of 4,21-Dehydrocorynantheine Aldehyde as an Intermediate in the Biosynthesis of Ajmalicine and Related Alkaloids	188
TAKAHASHI, S., S. KITANAKA, M. TAKIDO, Y. EBIZUKA, U. SANKAWA, M. HOSON, M. KOBAYASHI and S. SHIBATA, Formation of Anthraquinones by the Tissue Culture of <i>Cassia obtusifolia</i>	389
TEMPLETON, I. F., R. C. S. AUDETTE, F. ZUNZA, H. R. GODAVARI and E. R. WAYGOOD, Characterisation of Ergosterol as the Major Sterol from <i>Euglena gracilis</i> Grown on Animal Waste	377
TILLEQUIN, F., M. PARIS, H. JACQUEMIN et R. R. PARIS, Flavonoïdes de <i>Piper marginatum</i> (Flavonoids from <i>Piper marginatum</i> )	46
ULUBELEN, A., S. DOGANCA and K. JEWERS, $\beta$ - Amyrin Acetate and Lupeyl Acetat from <i>Marsdenia erecta</i>	420
VAQUETTE, J., R. HOCQUEMILLER, J. L. POUSSET et A. CAVÉ, Alcaloides des feuilles de <i>Teclea boiviniana</i> (BAILLON) H. PERR. (Alkaloids from leaves of <i>Teclea boiviniana</i> (BAILLON) H. PEER.)	78
VERPOORTE, R., E. W. KODE, H. van DOORNE and A. BAERHEIM SVENDSEN, Antimicrobial Effect of the Alkaloids from <i>Strychnos afzelii</i> GILG.	237

---

WACKER, A. und W. HILBIG, Virushemmung mit <i>Echinacea purpurea</i> (Virus-Inhibition by <i>Echinacea purpurea</i> )	89
WAGNER, H., S. M. EL-SAYYAD, O. SELIGMANN and V. M. CHARI, Chemical Constituents of <i>Cassia siamea</i> LAM., I. 2-Methyl-5-acetonyl-7-hydroxychromone (Cassiachromone)	258
WAGNER, H., G. WENZEL and V. M. CHARI, The Turpethinic Acids of <i>Ipomoea turpethum</i> L.	144
WAHBI, A. M. and B. UNTERHALT, Spectrofluorometric Determination of Sennosides in Tablets	393
WENIGER, B., M. HAAG-BERRURIER, M. ROHMER and R. ANTON, Some Constituents of <i>Casearia ilicifolia</i> VENT	170
WEST, L. G., K. TEMPLETON and J. L. McLAUGHLIN, Analysis of Cactus Pentacyclic Triterpenes by Reversed Phase High Performance Liquid Chromatography	371
Abstracts of the International Meeting on Medicinal Plant Research 1978	265
In Memoriam Prof. Debelmas	105

# 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-Dehydrocorynantheine Aldehyde; Heteroyohimbine Type Alkaloids.

## Abstract

In the presence of  $\text{BH}_4^-$  an enzyme preparation of *Catharanthus roseus* cell suspension cultures transforms 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 monoterpene 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 synthe-

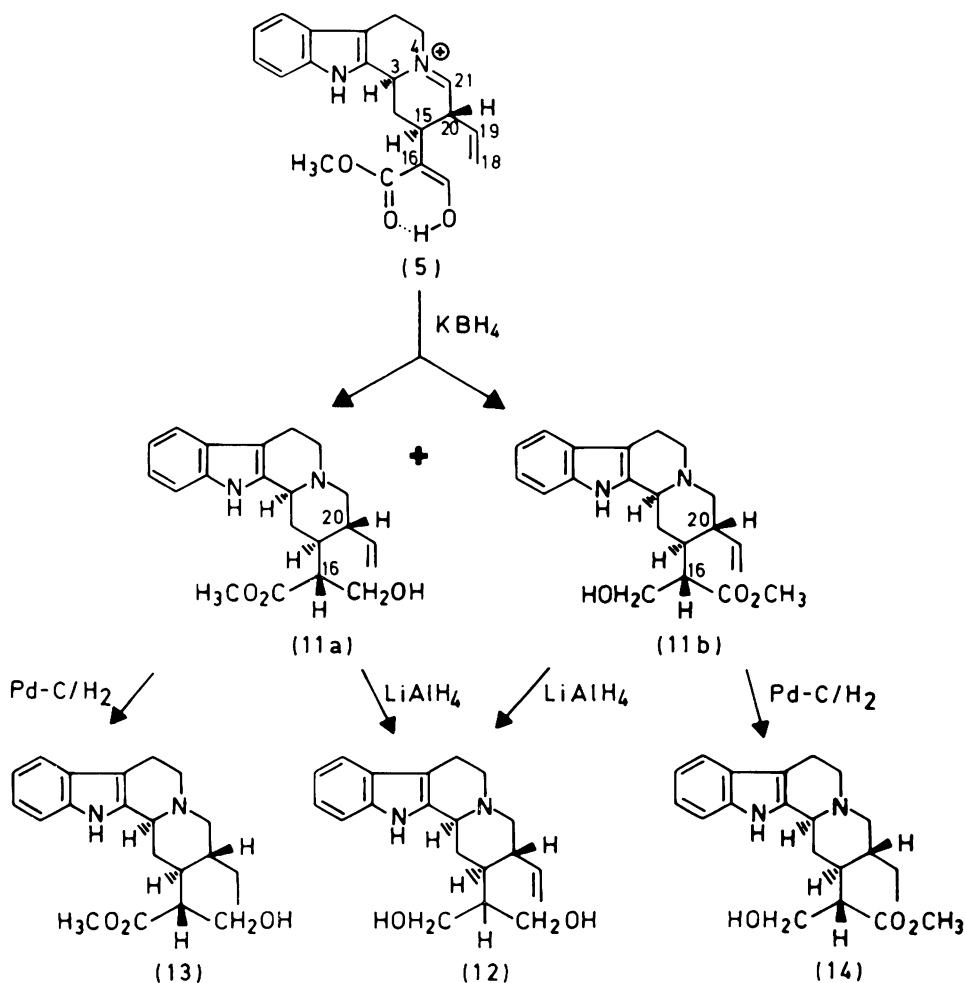
tase [2] and cathenamine reductase [3] respectively. The enzymatic step immediately beyond (1) should involve the action of a  $\beta$ -glucosidase hydrolysing the alkaloidal glucoside (1) to yield the unstable aglycone (2) which in turn opens to yield the dialdehyde [4] (3) with 3  $\alpha$  (S) configuration. 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,  $\text{BH}_4^-$  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  $\text{KBH}_4$  [5].

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

7.0 with (*1*) (474  $\mu\text{mol}$ , 6  $\mu\text{Ci}$   $6\text{-}^{14}\text{C}$ ) in the presence of  $\text{KHB}_4$  (164 mmol) and 21 ml MeOH at  $25^\circ\text{C}$  for 120 min. Non-glucosidal material was extracted into EtOAc and subjected to TLC (acetone: pet. ether ( $40\text{--}60^\circ$ ): 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)  $\lambda_{\text{max}}$  ( $\epsilon$ ) 223 (26900), 273 (5490), 279 (5550), 282 (5550), 289 nm (4580). IR (KBr)  $\nu$  3410 (NH, OH), 2820, 2760 (Bohlmann bands), 1715 ( $\text{COOCH}_3$ ), 1640 ( $\text{C}=\text{C}$ ), 1050 ( $\text{C}-\text{O}$ ), 1010, 1000, 925 ( $-\text{CH}=\text{CH}_2$ ),  $745\text{ cm}^{-1}$  (ar  $\text{C}-\text{H}$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  3.59 (s, 3H,  $-\text{COOCH}_3$ ), 3.94 (d,  $J = 6.0\text{ Hz}$ , 2H,  $-\text{CH}_2\text{OH}$ ), 4.97 – 5.61 (m, 3H,  $-\text{CH}=\text{CH}_2$ ), 7.00 – 7.52 (m, 4H, 4 ar H), 9.48 ppm (m,  $W^{1/2} = 14\text{ Hz}$ , 1H, NH). MS (70 eV)  $m/e$  354 (100;  $\text{M}^+$ ), 353 (76;  $\text{M}^+-\text{H}$ ), 323 (14;  $\text{M}^+-\text{CH}_2\text{OH}$ ), 251 (72;  $\text{M}^+-\text{H}_3\text{COOC}-\text{CH}-\text{CH}_2\text{OH}$ ), 249 (35;  $353-\text{H}_3\text{COC}(\text{OH})=\text{CHCH}_2\text{OH}$ ), 223 (38), 221 (14), 184 (55), 170 (83), 169 (59), 156 (66), 144 (24), 129 (28), 115 (24). (*11b*): UV (MeOH)  $\lambda_{\text{max}}$  ( $\epsilon$ ) 223 (29400), 273 (5600), 279 (5630), 282 (5610), 289 nm (4680). IR (KBr)  $\nu$  3430 (NH, OH), 2820, 2760 (Bohlmann bands), 1725 ( $\text{COOCH}_3$ ), 1635 ( $\text{C}=\text{C}$ ), 1040 ( $\text{C}-\text{O}$ ), 1010, 1005, 925 ( $-\text{CH}=\text{CH}_2$ ),  $745\text{ cm}^{-1}$  (ar  $\text{C}-\text{H}$ ).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$  3.68 (d,  $J = 6.0\text{ Hz}$ , 2H,  $-\text{CH}_2\text{OH}$ ), 3.73 (s, 3H  $-\text{COOCH}_3$ ), 4.97 – 5.60 (m, 3H,

$-\text{CH}=\text{CH}_2$ ), 7.00 – 7.53 (m, 4H, 4 ar H), 8.97 ppm (m,  $W^{1/2} = 14\text{ Hz}$ , 1H, NH). MS (70 eV)  $m/e$  354 (83;  $\text{M}^+$ ), 353 (61;  $\text{M}^+-\text{H}$ ), 323 (13;  $\text{M}^+-\text{CH}_2\text{OH}$ ), 251 (70;  $\text{M}^+-\text{H}_3\text{COOC}-\text{CH}-\text{CH}_2\text{OH}$ ), 249 (39;  $353-\text{H}_3\text{COC}(\text{OH})=\text{CH}-\text{CH}_2\text{OH}$ ), 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  $\alpha$  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 ( $\alpha$ ) 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  $\text{LiAlH}_4$  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;  $\text{M}^+$ ), 355 (85;  $\text{M}^+-\text{H}$ ), 253 (49;  $\text{M}^+-\text{C}_4\text{H}_7\text{O}_3$ ), 251 (12;  $355-\text{C}_4\text{H}_8\text{O}_3$ ), 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;  $\text{M}^+$ ), 355 (85;  $\text{M}^+-\text{H}$ ), 253 (44;  $\text{M}^+-\text{C}_4\text{H}_7\text{O}_3$ ), 251 (12;  $355-\text{C}_4\text{H}_8\text{O}_3$ ), 184 (11), 170 (22), 169 (17), 156 (13), consistent with 18,19-dihydro-16-isositsirikine. On the basis



of the NMR spectrum (position of  $-\text{CH}_2\text{OH}$ ) it can be assumed, that  $-\text{CH}_2\text{OH}$  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  $\text{BH}_4^-$  but rather undergoes in-

tramolecular closure of ring D to give the immonium ion (5) or its polarised form\*.

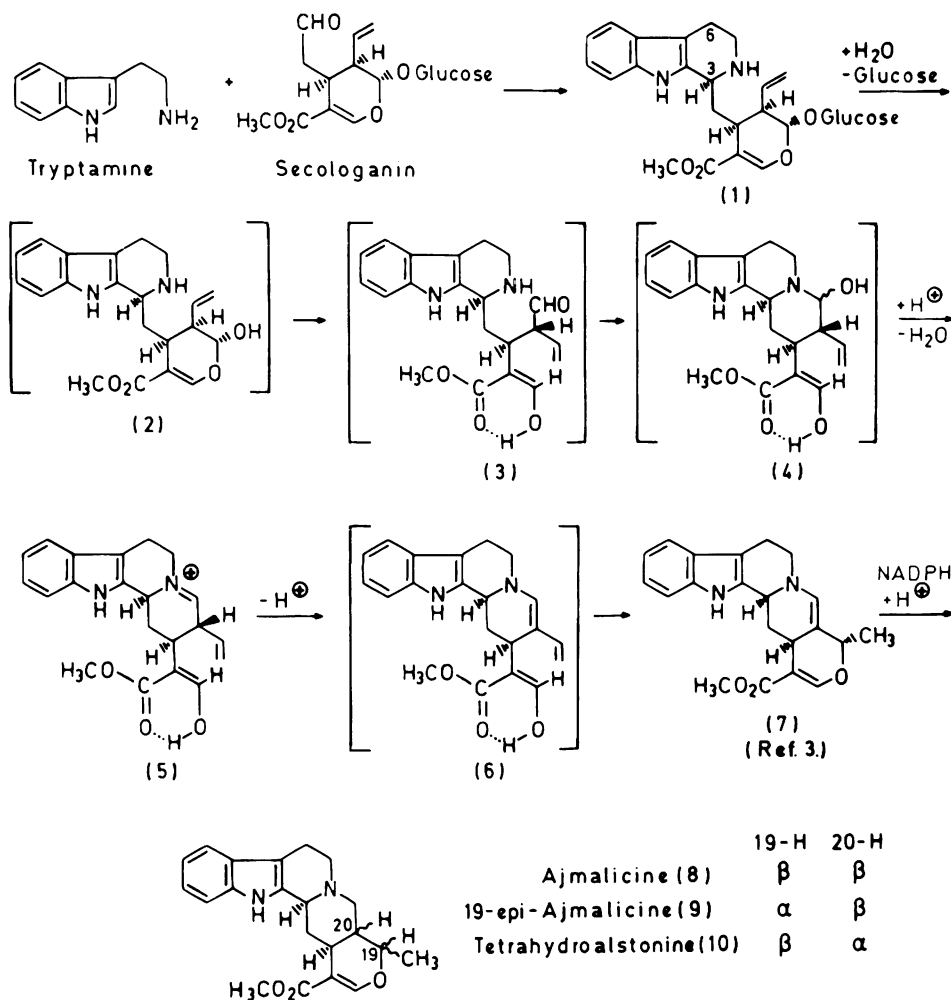
It is this intermediate (5) the involvement of which in the biosynthesis of monoterpene alkaloids (8-10) is thus demonstrated by reduction with  $\text{BH}_4^-$  to (11a, b). In the absence of  $\text{BH}_4^-$  (1) is transformed to (7) [3, 6]. (1) is hydro-

\* an equilibrium between (5) and  $\left( \begin{array}{c} \delta^+ \\ | \\ -\text{N} \\ | \\ \text{CH} \\ | \\ \delta^- \end{array} \text{X} \right)$  can easily be visualised.

lysed by  $\beta$ -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  $\text{NaBH}_3\text{CN}$  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.





## Acknowledgements

This research was supported by the Ministry of "Forschung und Technologie", Bonn, and a research fellowship (USA) of the "Deutsche Forschungsgemeinschaft" to J. S. We thank Eli-Lilly Co. for a gift of authentic sitsirikine.

## 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. Sleight: 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*