Plant Cell Reports (1983) 2:105–107

Plant Cell Reports

Indole Alkaloids from Cell Suspension Cultures of *Tabernaemontana divaricata* and *Tabernanthe iboga*

K.-H. Pawelka and J. Stöckigt

Institut für Pharmazeutische Biologie, Universität München, D-8000 München 2, FRG

Received March 31, 1983

ABSTRACT

From cell suspension cultures of Tabernaemontana divaricata and Tabernanthe iboga grown under standard conditions, six monoterpenoid indole alkaloids have been isolated and identified. T. divaricata synthesized apparicine, catharanthine, coronaridine, conoflorine, tubotaiwine and vinervine, whereas T. iboga produced tubotaiwine and conoflorine. Both cultures are a reasonable source for conoflorine, which is expected to be a good candidate for studying the mechanism of Aspidosperma type alkaloid formation at the cell-free level.

INTRODUCTION

Plant cell suspension cultures of the family Apocynaceae represent an excellent tool for biogenetic investigations at cell-free level (Zenk 1980). They also are a good source of known and new indole alkaloids (Kohl et al. 1981, Stöckigt et al. 1981). During a screening program for the selection of cell cultures showing biogenetically interesting alkaloid compositions, we determined the indole alkaloid pattern in cultured cells of Tabernaemontana divaricata (syn. Ervatamia divaricata) and Tabernanthe iboga. T. divaricata synthesized six major indole alkaloids, vinervine (12-hydroxyakuammicine), tubotaiwine (dihydrocondylocarpine), apparicine (pericalline), coronaridine, catharanthine and conoflorine (voaphylline). In cell suspensions of T. iboga, only tubotaiwine and conoflorine were found as major alkaloids (Fig. 1).

EXPERIMENTAL AND RESULTS

Spectroscopic Determinations: UV spectra were recorded on a Perkin Elmer Spectrophotometer 551 S using methanol as solvent. Mass spectra (MS) were obtained on a Finnigan MAT 44 S instrument at 70 eV in EI-mode.
Plant Cell Cultures, Isolation And Identification Of Alkaloids, Cell-Free Experiments: Cell suspension of T. divaricata were grown for 18 days at 26°C in a modified B5 medium (Zenk et al. 1979). T. iboga cells were grown under the identical conditions in 4X medium (Rüffer et al. 1981). 5 kg T. divaricata cells (fresh weight) were stirred for two days with 5.5 l ethyl acetate containing 200 ml phosphate buffer (1M) and 20 ml 5% ammonia. The organic layer was removed and the cell material reextracted. The organic phases were combined, concentrated (200 ml) and the alkaloidal products extracted as described by Stöckigt and Soll (1980). 1.5 kg of the T. iboga cells were worked up in the same way. The resulting crude mixture of alkaloids was chromatographed on TLC (Si gel, Machery & Nagel). The solvent system used for the T. divaricata alkaloids was Xylol - n-hexane - ethylacetate - ether - diethylamine - acetone = 2: 2 : 1 : 3 : 2 : 2. Five major alkaloid fractions were observed and were eluted with

methanol (Td 1-5).
Td 1 was further purified in the solvent system chloroform - methanol - ammonia = 90 : 10 : 0.1 and showed an R_f value (0.32) identical with vinervine and gave a reddish purple colour after spraying with ceric ammonium sulfate (CAS). UV, MS data and chromatographic properties coincided with those of vinervine, indicating that Td 1 is vinervine. Td 2 was isolated after TLC purification (benzene - ethyl acetate - ether - methanol - diethylamine = 15 : 5 : 40 : 8 : 0.5, R_f=0.2, CAS blue) and was clearly identified as tubotaiwine by UV, MS and comparison with an authentic sample. Td 3 (R_f= 0.3, in same solvent system as used for Td 2, CAS grey) revealed identical spectroscopic data as found for apparicine. Fraction Td 4 was separated into Td 4.1 (CAS green) and Td 4.2 (CAS grey) with the TLC-system used for the purification of Td 2 (but in a ratio of 15 : 5 : 40 : 3 : 0.5). The compounds were identified as coronaridine (Td 4.1) and catharanthine (Td 4.2). Coronaridine is a typical constituent of Tabernaemontana species (Gorman et al. 1960, Achenbach and Raffelsberger 1980, van Beek et al. 1983).
Td 5 was purified with butanol - acetic acid - water = 1 : 1 : 0.1 and showed the same R_f (0.32), CAS-reaction (red) and spectroscopic properties as authentic conoflorine.
The alkaloid mixture of T. iboga was analyzed by TLC (ethyl acetate - methanol - diethylamine = 14 : 1 : 1) and two major alkaloids were observed and identified in the same way as described for the T. divaricata alkaloids. The TLC solvent system used was chloroform - methanol - ammonia = 90 : 10 : 0.02. Ti 1 (R_f=0.6, CAS blue) was determined to be tubotaiwine and Ti 2 (R_f=0.9, CAS red) was conoflorine. The amounts of the purified alkaloids of T. divaricata were found to be: Apparicine 2.5 µg, Conoflorine 3.9 µg, Coronaridine 10.4 µg, Tubotaiwine 10 µg, Vinervine 4.7 µg/l medium.

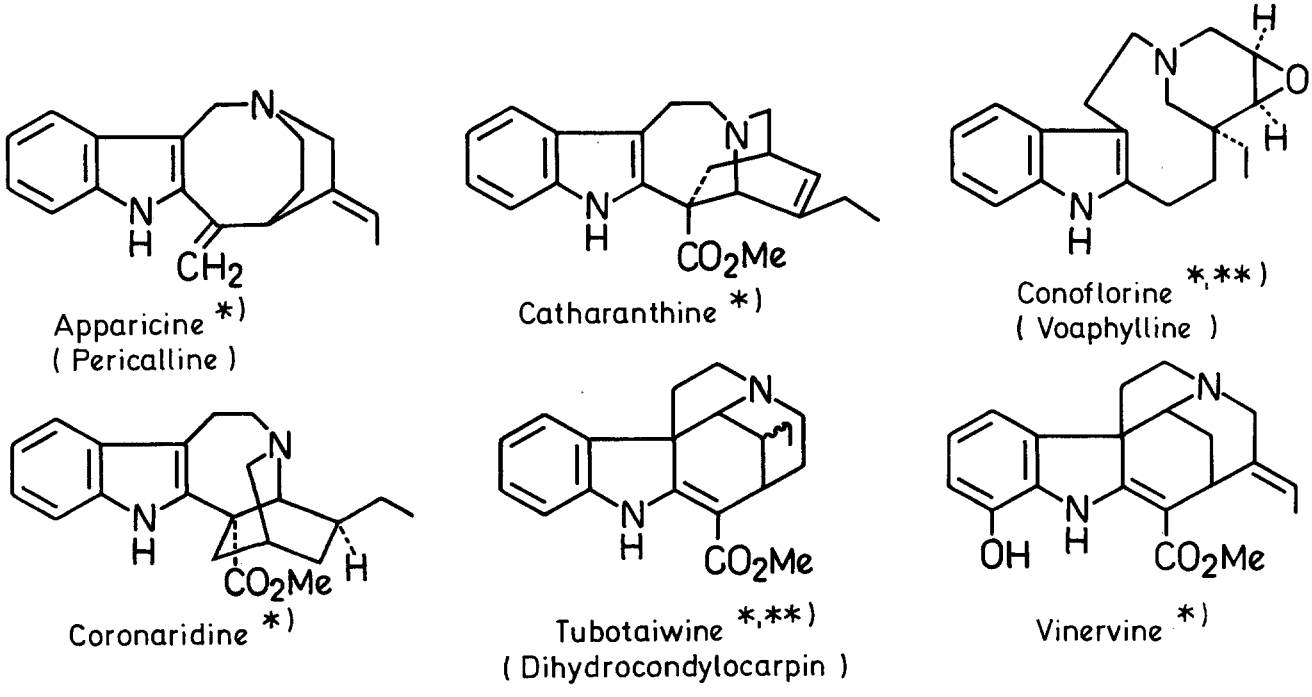

Fig 1. Indole alkaloids of T. divaricata *) and T. iboga **) cell suspension cultures

DISCUSSION

Successful investigations regarding the cell-free biosynthesis of indole alkaloids of the heteroyohimbine type (Zenk 1980) and the sarpagine/ajmaline group (Pfitzner and Stöckigt 1983) were based on plant cell suspension cultures capable of synthesizing the appropriate alkaloids in substantial amounts. Probably the most important result obtained during the investigations on heteroyohimbines with Catharanthus roseus cultures was the detection of strictosidine which is the central biogenetic precursor for monoterpenoid indole alkaloids (Stöckigt and Zenk 1977, Nagakura et al. 1979). This precursor is now easily synthesized by the immobilized strictosidine synthase (Pfitzner and Zenk 1982) and therefore strictosidine is readily available for cell-free investigations of alkaloid pathways involving this compound. A further prerequisite for the elucidation of biogenetic pathways leading to one of the different indole alkaloid groups would be a cell culture that synthesized this alkaloid group preferentially. In this case the incubation of strictosidine or another plausible precursor with crude cell-free preparations obtained from such a culture would preclude the interference of different pathways and prevent the different enzymes from competing for the chosen precursor. From this point of view the more complex alkaloid pattern found in T. divaricata cells would make this culture less valuable for cell-free experiments, since this culture produces 4 alkaloid groups, Apparicine- (apparicine), Iboga- (catharanthine, coronaridine), Stychnos- (tubotaiwine, vinervine) and the Quebrachamine-group (conoflorine). In contrast T. iboga cells synthesize, under standard growth conditions, only two major indole alkaloids, tubotaiwine (Strychnos-type) and conoflorine (Quebrachamine-type). It might be expected that the latter culture would be more useful for biosynthetic studies. Both cultures grown under not improved conditions for alkaloid production synthesize alkaloids in the lower µg-range/l medium. They can be used especially after optimisation of alkaloid synthesis as a good source of conoflorine which might be a model compound to investigate the cell-free formation of the Aspidosperma skeleton. When conoflorine was incubated in presence of various enzyme preparations (e.g. isolated from Stemmadenia or T. iboga cells) and cofactors, a complete conversion of conoflorine into three major compounds was observed. The structural determination of these products is presently under investigation.

ACKNOWLEDGEMENTS

We thank Mrs. B. Krausch for excellent technical assistance and the members of our cell culture laboratory for providing us with cell suspensions. We are grateful to Prof. H. Achenbach (Erlangen), Prof. G.N. Smith (Manchester), Prof. R. Verpoorte (Leiden), Prof. M.S. Yunusov (Taschkent) for generous gifts of reference alkaloids and to Prof.R.L. Mansell (Tampa) for checking the english version of the manuscript. Our thanks are due to the "Deutsche Forschungsgemeinschaft" for a Finnigan MAT 44 S instrument and for financial support (SFB 145).

REFERENCES

Achenbach H, Raffelsberger B (1980) Z.Naturforsch. 35b: 219-225.

van Beek TA, Verpoorte R, Baerheim Svendsen A (1983) Planta med. 47: 83-86.

Gorman M, Neuss N, Cone NJ, Deyrup JA (1960) J.Am.Chem.Soc. 82: 1142-1145.

Kohl W, Witte B, Höfle G (1981) Z.Naturforsch. 36b: 1153-1162.

Nagakura N, Rüffer M, Zenk MH (1979) J.Chem. Soc.Perkin Trans. 1: 2308-2312.

Pfitzner A, Stöckigt J (1983) Tetrahedron Letters, in press.

Pfitzner A, Stöckigt J (1983) J.Chem.Soc. Chem.Commun., in press.

Pfitzner U, Zenk MH (1982) Planta med. 46: 10-14.

Rüffer M, El-Shagi H, Nagakura N, Zenk MH (1981) FEBS Letters 129: 5-9.

Stöckigt J, Soll HJ (1980) Planta med. 40: 22-30.

Stöckigt J, Zenk MH (1977) J.Chem.Soc.Chem. Commun. 646-648.

Stöckigt J, Pfitzner A, Firl J (1981) Plant Cell Reports 1: 36-39.

Zenk MH (1980) J.Nat.Products 43: 438-451.

Zenk MH, El-Shagi H, Schulte U (1979) Planta med. Suppl. 79-101.