Indole Alkaloids from Cell Suspension Cultures of *Stemmadenia tomentosa* and *Voacanga africana*

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Z. Naturforsch. **37 c**, 857–860 (1982); received June 3, 1982

Stemmadenia tomentosa var. *palmeri, Voacanga africana,* Apocynaceae, Cell Suspension Cultures, Indole Alkaloids

Cell suspension cultures of *Stemmadenia tomentosa* synthesized under normal growth condition the eight major indole alkaloids: (−)-tabersonine, (−)-minovincinine, (+)-conoflorine (voaphylline), condylocarpine, (+)-tubotaiwine (dihydrocondylocarpine), (−)-norfluorocurarine (vincanine), (−)-vinervine, and (−)-coronaridine. These alkaloids consist of the three different types, Aspidosperma, Strychnos and Iboga. In contrast, cultures of *Voacanga africana* produced mainly one alkaloid group (Aspidosperma-type) represented by (−)-tabersonine, lochnericine and (−)-minovincinine. Therefore this cell culture seems to be qualified for investigation concerning the biosynthesis of Aspidosperma alkaloids.

Introduction

Whereas the pattern of monoterpenoid indole alkaloids in differentiated *Stemmadenia* and *Voacanga* species have been widely studied [1–3], the formation of these secondary products in cell suspension cultures has not been described up to now. In the course of a phytochemical screening of cultured cells of the Apocynaceae family [4, 5], we determined here the alkaloid composition in *Stemmadenia tomentosa* var. *palmeri* and *Voacanga africana.*

Previous investigation of Apocynaceae cultures demonstrated the alkaloid pattern to be in general similar to that of the differentiated plant [4–7], although some alkaloids are formed in significantly higher amounts than in the intact species [4, 5] and on the other side the formation of bisindole alkaloids probably is not expressed in cultured cells [3, 5, 6]. Moreover, each of the cell cultures so far tested produced at least 4 types of indole alkaloids, which indicates a remarkable variability in alkaloid production. From the point of view of biogenetic investigations, however, cultured cells forming only one group of alkaloids are probably more useful in elucidating the biosynthetic pathway leading to this special alkaloid type.

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0341-0382/82/1000-0857 $ 01.30/0

Stemmadenia cells produced indole alkaloids of the Strychnos-, Iboga- and Aspidosperma-type, whereas only alkaloids of the Aspidosperma group have been isolated as major products from *Voacanga* cells in culture. In view of the alkaloid composition of *Stemmadenia* and *Voacanga* described here, it seems that both cultures represent a good tool for investigating the cell-free biosynthesis of alkaloids originating by a pathway other than the well known leading to the Corynanthe type [8, 9].

Materials and Methods

Cell cultures

Callus tissue was initiated from seeds of *Stemmadenia* and leaves of *Voacanga* and maintained for five years on solid medium with three weekly transfers.

Cell suspensions were obtained using a modified B 5 medium [10]. The cells were subcultured for three months. For the alkaloid isolation cells were grown for 14 days (at 26°) in 1L Erlenmeyer flasks containing 300 ml medium. The cell yields for *Stemmadenia* and *Voacanga* were 228 g fresh weight/l (12.1 g dry weight) and 126 g/l (9.2 g dry weight), respectively.

Alkaloid isolation

The procedure for the extraction and isolation of the alkaloids was identical to that described previously [4].

Thin-layer chromatography

For the separation and purification of the alkaloids the following solvent systems (A – E) were used:

A: Acetone/petroleum ether (40 – 60)/diethylamine (7:2:1);
B: benzene/ethylacetate/ether/methanol/diethylamine (15:5:40:8:0.5);
C: benzene/*n*-hexane/ethylacetate/ether (1.5:2:0.5:4);
D: chloroform/methanol/ammonia (90:10:0.02);
E: xylol/*n*-hexane/ethylacetate/ether (2:2:1:5).

Structure determination

UV spectra were measured on a Perkin Elmer Spectrophotometer 551 S using methanol Uvasol as solvent. Mass spectra (MS) were obtained on a Finnigan MAT 44 S and a Finnigan 4515 instrument at 70 eV in EI mode. When necessary CI mode (isobutane) was used for the determination of the quasi M^+ peak. Optical rotation was measured on a Perkin Elmer Polarimeter 241 using a 100 µl cell and chloroform as solvent.

Results and Discussion

Alkaloids of Stemmadenia cell cultures

The crude alkaloid residue obtained from 700 g *Stemmadenia* cells as reported in [4], could be separated on TLC (solvent A), yielding five crude alkaloid fractions (St 1 – 5).

The most polar fraction, St 1, showed after rechromatography in solvent system B (R_f 0.2) one main alkaloid, displaying a reddish purple colour with CAS (ceric ammonium sulfate/phosphoric acid), UV maxima at 235 (shoulder), 289 and 336 nm. A strong bathocromic shift with sodium hydroxide (λ_{max} 336 nm → 355 nm) indicating the presence of a phenolic OH-group, and main MS fragments at 121 *z/e* (100%) and 338 *z/e* (M^+) were characteristic for an hydroxylated akuammicine. By comparison of the spectroscopic and chromatographic data with those of authentic (–)12-hydroxyakuammicine (vinervine), St 1 has been clearly identified to be this Strychnos type alkaloid. Since (–)-vinervine is known to occur only in the genera *Vinca* [11] and *Catharanthus* [4, 12], its presence in *Stemmadenia* is reported here for the first time. Fraction St 2 and St 3 (R_f in system A: 0.25 and 0.42 resp.) contained only minor alkaloids and afforded insufficient amounts for a structure determination.

Fraction St 4 (R_f 0.55 in solvent system A) was rechromatographed in system B and yielded three alkaloids St 4.1, St 4.2, St 4.3 showing a blue CAS reaction. ST 4.1 had a UV spectrum characteristic of indole alkaloids but with an unusual long peak at 360 nm. The MS indicated a molecular weight of 292 (M^+); the predominant peak at *z/e* 121 implied an akuammicine type alkaloid. The MS fragmentation pattern did, however, not present the typical M^+-OCH_3 or M^+-CO_2CH_3 fragments, which are given by the akuammicines, but revealed a M^+-CHO peak (263 *z/e*). Due to this fact and in view of the UV data which were identical with the data of C-fluorocurarine (C-curarine III), we deduced the presence of an acrylic aldehyde function in St 4.1. Moreover, all the spectroscopic data were in absolute agreement with those reported for (–)-norfluorocurarine (vincanine) [13, 14]. So far, this Strychnos-type alkaloid has been isolated only from the genera *Dyplorrhynchus* [13] and *Vinca* [14], it is not a typical constituent of *Stemmadenia.* Both alkaloid fractions St 4.2 and St 4.3 resp. have the characteristic UV absorption of the α-methylene indolenine carboxylic ester chromophore (St 4.2: λ_{max} 228, 297, 326 nm; St 4.3: λ_{max} 228, 294, 328 nm). The MS fragmentation of the two compounds clearly indicated a carbomethoxy group St 4.2: M^+ (324 *z/e*), M^+-OCH_3 (293 *z/e*), M^+-CO_2CH_3 (265 *z/e*); St 4.3: M^+ (322 *z/e*), M^+-OCH_3 (291 *z/e*), M^+-CO_2CH_3 (263 *z/e*) and the overall patterns were identical with those of (+)-tubotaiwine (St 4.2) and condylocarpine (St 4.3). The former obviously is not widely distributed in *Stemmadenia,* because only one isolation of (+)-tubotaiwine has been described from leaves of *S. glabra* (Benth.) [15].

The crude fraction St 5 (R_f 0.68 in system A) could be separated into four alkaloids (St 5.1 – 5.4) using the solvent system C.

St 5.1 (CAS reaction blue) was again an α-methylene indolenine alkaloid (λ_{max}: 224, 298, 328 nm) but exhibits the typical mass fragmentation of Aspidosperma-type alkaloids [16]. St 5.1 had a M^+ of *z/e* 354 and identical MS data to that published for pandoline/epipandoline [17] as well as minovincinine/epiminovincinine [18]. To distinguish between both pairs of epimers, St 5.1 was acetylated,

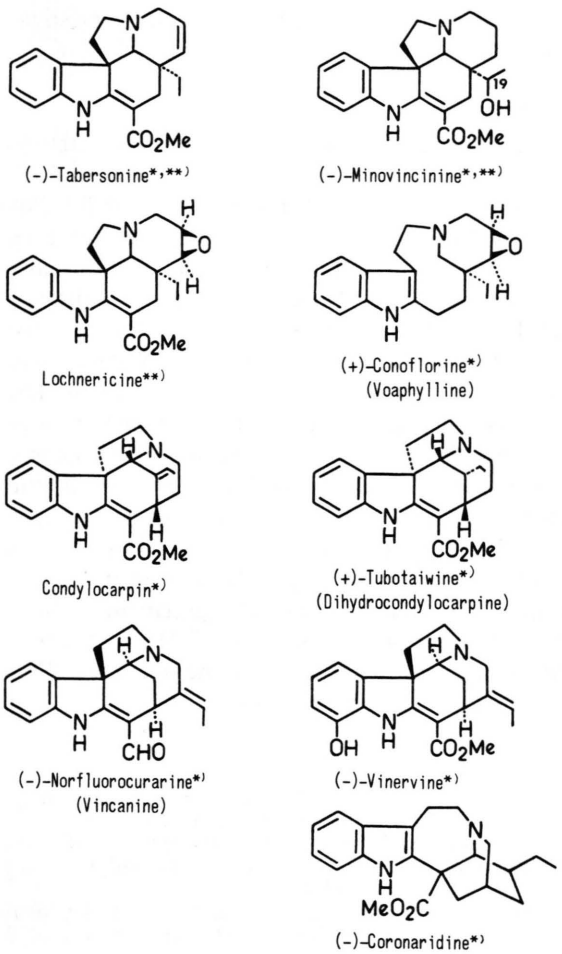

Fig. 1. Indole alkaloids identified in cell suspension cultures of *Stemmadenia tomentosa* var. *palmeri** and *Voacanga africana***.

yielding a monoacetate (M^+ 396 *z/e*). The pandolines, however, can be ruled out because St 5.1 exhibits different TLC behaviour and because they possess a tertiary OH group that could not be acetylated. We conclude from the spectroscopic data and TLC comparison with an authentic sample that St 5.1 is (−)-minovincinine.

St 5.2, 5.3, 5.4 (CAS green, violet, blue) were the main alkaloids of the *Stemmadenia* cells. The three alkaloids could be identified by comparison of the UV and MS spectra as well as the chromatographic behaviour with those of authentic compounds. St 5.2 showed identity with (−)-coronaridine, St 5.3 was found to be (+)-conoflorine (voaphylline) and St 5.4 has been determined as being (−)-tabersonine. Corresponding to (−)-coronaridine and (−)-tabersonine resp., which are described to occur in several *Stemmadenia* species, (+)-conoflorine is also reported to be present in several species of the Apocynaceae family, *e.g. Conopharyngia longiflora, Voacanga africana* and *Hedranthera barteri* [19−21]. (+)-Conoflorine exhibits the basic ring system of stemmadenine, which is assumed to be a key intermediate in the biosynthesis of Aspidosperma and Iboga alkaloids [22, 23]. Even with more "careful" isolation procedures − extraction of the cell material at low temperature (4 °C) − stemmadenine, the typical *Stemmadenia* alkaloid, could not be detected in our cultures grown under the conditions indicated.

Alkaloids of Voacanga cell cultures

The crude alkaloid extract from 900 g (fresh weight) of *Voacanga* cells has been separated on TLC in solvent system D, to yield besides two minor alkaloids (Va 1, Va 2) the major fraction Va 3.

Va 1 and Va 2 were found to be indole alkaloids (CAS reaction was orange), but only insufficient material was available for a definite characterisation. Therefore, at the present time work is in progress to enhance the production of both compounds for an unequivocal identification. The selection of optimal growth and production media is expected to be one hopeful avenue of investigation.

The alkaloid mixture Va 3 consisted of three indole alkaloids (solvent system E; Va 3.1: R_f 0.4; Va 3.2: R_f 0.7; Va 3.3: R_f 0.8). The UV and mass spectral characteristics suggested these alkaloids to be Aspidosperma-types with an β-anilinoacrylate chromophore. Correlation of spectroscopic (UV, MS) and chromatographic properties (five TLC systems) with the characteristics of authentic alkaloids of the Aspidosperma group clearly identified Va 3.2 to be lochnericine and Va 3.3 (−)-tabersonine. The data of compound Va 3.1 were in full conformity with those found for St 5.1. We therefore conclude, that Va 3.1 is (−)-minovincinine.

The isolation of these three Aspidosperma alkaloids is interesting, because they are not common constituents of *Voacanga* plants. In contrast, the Iboga alkaloids, which could not be detected in our cultured cells, are major compounds of the differentiated plant. Recently, however, (−)-tabersonine was isolated from seeds of *Voacanga africana* in a yield

of about 0.05% [24]. The presence of this alkaloid as a main product in the cell suspension therefore confirms our earlier observation that cell cultures are in a stage representing a seedling, This might offer the possibility of producing alkaloids which usually do not exist in mature plants in substantial amounts. Moreover, there are some indications that of the typical *Voacanga* alkaloids at least one 2-acylindole secosarpagine derivative is produced by the cell suspensions under the employed growth conditions, whereas the known bisindole alkaloids are not.

If it can be shown that cell cultures of other Apocynaceae synthezise exclusively Iboga alkaloids as reported for some differentiated plants (*e.g. Tabernaemontana quadrangularis*) [25], we would expect those cultures and our *Voacanga* cell suspensions to be very usefull in the exploration of the cell free biosynthesis of the Iboga and the Aspidosperma skeleta. Preliminary experiments with a crude cell free extract from *Voacanga* cells demonstrate, that the common precursor of monoterpenoid indole alkaloids – strictosidine – is completely metabolized. The structure determination of the cell free formed alkaloids is presently under investigation.

Acknowledgement

Our thanks are due to Dr. N. Kunesch (Chatenay-Malabry), Prof. H. Achenbach (Freiburg), Prof. G. N. Smith (Manchester), Prof. G. A. Cordell (Chicago), and Prof. R. Verpoorte (Leiden) for generous gifts of reference alkaloids. We would like to thank Mrs. B. Krausch for excellent technical assistance and the members of our cell culture laboratory for providing us with cell suspensions of *Stemmadenia tomentosa* and *Voacanga africana.* We thank Dr. Ott, Finnigan GmbH (Munich) for running some of the MS spectra and Dr. Stenz and Dr. Böhme, Perkin Elmer (Überlingen) for their help in the determination of optical rotation values. We also thank Prof. J. M. Edwards (Storrs, USA) for his help in preparing the English version of the manuscript. The Deutsche Forschungsgemeinschaft is acknowledged for a Finnigan MAT 44 S instrument. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 145).

[1] M. Hesse, Indolalkaloide in Tabellen, Springer-Verlag, Berlin-Göttingen-Heidelberg 1964, 1968.
[2] W. I. Taylor, in R. H. F. Manske (ed.): The Alkaloids 1965, **Vol. VIII,** p. 203, 1968, **Vol. XI,** p. 79, and B. Gilbert ibid. p. 335, 205.
[3] D. W. Thomas and K. Biemann, Lloydia **31,** 1 (1968).
[4] J. Stöckigt and H. J. Soll, Planta Med. **40,** 22 (1980).
[5] J. Stöckigt, A. Pfitzner, and J. Firl, Plant Cell Reports **1,** 36 (1981).
[6] W. G. W. Kurz, K. B. Chatson, F. Constabel, J. P. Kutney, L. S. L. Choi, P. Kolodziejczyk, S. K. Sleigh, K. L. Stuart, and B. R. Worth, Helv. Chim. Acta **63,** 1981 (1980).
[7] W. Kohl, B. Witte, and G. Höfle, Z. Naturforsch. **36b,** 1153 (1981).
[8] M. H. Zenk, J. Nat. Prod. **43,** 438 (1980).
[9] J. Stöckigt, in: Indole and Biogenetically Related Alkaloids (J. D. Phillipson and M. H. Zenk, eds.) p. 113, Academic Press Inc. London, New York 1980.
[10] M. Rüffer, H. El-Shagi, N. Nagakura, and M. H. Zenk, FEBS Letters **129,** 5 (1981).
[11] M. R. Yagudaey, V. M. Malikov, and S. Y. Yunusov, Khim. Prir. Soedin. **8,** 260 (1972).
[12] A. I. Scott, P. B. Reichardt, M. B. Slaytor, and J. G. Sweeny, Bioorganic Chem. **1,** 157 (1971).
[13] D. Stauffacher, Helv. Chim. Acta **44,** 2006 (1961).
[14] B. Pyuskyulev, I. Ognyanov, and P. Panov, THL, 4559 (1967).
[15] J. F. Ciccio, C. H. Herrera, V. H. Castro, and M. Ralitsch, Rev. Latinoam. Quim. **1979,** 67.
[16] M. Hesse, Indolalkaloide, in: Fortschritte der Massenspektrometrie (H. Budzikiewiez, ed.) **Vol. 1,** Verlag Chemie Weinheim 1974.
[17] M. Zeches, M.-M. Debray, G. Ledouble, L. Le Men-Olivier, and J. Le Men, Phytochemistry **14,** 1122 (1975).
[18] M. Plat, J. Le Men, M.-M. Janot, H. Budzikiewiez, J. M. Wilson, L. J. Durham, and C. Djerassi, Bull. Soc. Chim. France **1962,** 2237.
[19] J. J. Dugan, M. Hesse, U. Renner, and H. Schmid, Helv. Chim. Acta **50,** 60 (1967).
[20] N. Kunesch, B. C. Das, and J. Poisson, Bull. Soc. Chim. France **1967,** 2155.
[21] V. Agwada, M. B. Patel, M. Hesse, and H. Schmid, Helv. Chim. Acta **53,** 1567 (1970).
[22] G. A. Cordell, Lloydia **37,** 219 (1974), and lit. cited therein.
[23] G. A. Cordell, Introduction to Alkaloids, J. Wiley and Sons, New York-Chichester-Brisbane-Toronto 1981.
[24] Urbanizaciones y Construcciones de Menorca S. A. Span. 467, 763 (Cl. Co7D), C. A. **92,** P 125291 b (1980).
[25] H. Achenbach and B. Raffelsberger, Z. Naturforsch. **35b,** 219 (1980).