252. Voafrine A and Voafrine B, New Dimeric Indole Alkaloids from Cell Suspension Cultures of *Voacanga africana* Stapf

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Summary

The structures of voafrine A and voafrine B, two novel dimeric indole alkaloids isolated from *Voacanga africana* Stapf cell suspension cultures, were established and the medium-dependent formation of both alkaloids was investigated.

Introduction. – Dimeric indole alkaloids have been a matter of intensive discussion since 1962, when the structure of the pharmacologically most important *Catharanthus* alkaloid vincaleucoblastine was established [1]. Up to now *ca.* 200 alkaloids formed by combination of two monoterpenoid indole alkaloid types have been detected in differentiated plants [2]. Although plant cell suspension cultures are an excellent source of monomeric monoterpenoid indole alkaloids (*ca.* 40 different indole alkaloids now have been isolated) the formation of dimeric or of bisindole alkaloids in cultured cells have not yet been established. The claim that vincaleucoblastine and vincristine are synthesized in *Catharanthus* cultures [3] cannot be considered because no structural proof of these alkaloids has been reported.

In this paper we describe for the first time the isolation, structure determination and 'production' of dimeric indole alkaloids synthesized in plant cell suspension cultures. Two novel indole alkaloids named voafrine A and voafrine B were detected in cultured cells of *Voacanga africana* Stapf.

Results and Discussion. – Thin layer chromatography (TLC) of an AcOEt-extract of *Voacanga* cells grown for 20 days under standard conditions in 4X-medium [4] indicated six alkaloids with a bright blue color after spraying with ceric ammonium sulfate (CAS). Of these compounds the known monomeric *Aspidosperma*-type alkaloids (-)-tabersonine, lochnericine and (-)-minovincinine have been identified as major alkaloids of the *Voacanga* culture [5]. Two of the unknown compounds formed only in trace amounts

(*ca.* 50 µg/l medium) showed a typical β-anilinoacrylate chromophore (UV: 328, 299 nm) but only a slight fluorescence at 366 nm, indicating that both compounds also might belong to the *Aspidosperma* group. Mass spectral analyses of these alkaloids revealed an identical fragmentation pattern with a peak of highest mass at *m/z* 670 indicating that the compounds were novel dimeric indole alkaloids. We have named the alkaloid exhibiting a $R_f = 0.85$ ($CHCl_3$/MeOH 100:0.5) voafrine A, the second compound with $R_f = 0.77$ voafrine B. To obtain sufficient material for an unambigous structure elucidation, the cellular production of the new compounds was optimized. As demonstrated in *Fig. 1* six standard media for plant cell suspension cultures can be successfully applied for the generation of cell material in substantial amounts. Whereas different medium composition does not strongly affect cell growth (dry weight/l medium only changes by a factor of *ca.* 2), the formation of alkaloids is drastically influenced. During a growth period of 12 days *Voacanga* cells synthesized per 1 medium (4X) 50 µg voafrine B and 0.1 mg tabersonine, respectively. However, when the cells were transferred into an alkaloid production medium (AP) [6], the alkaloid formation was enhanced about 400-fold in the case of voafrine A or voafrine B and *ca.* 10^3-fold for tabersonine. The latter culture conditions therefore allowed an efficient production of the original 'trace' alkaloids for a detailed investigation. From 2.5 kg of fresh cells (212 g dry weight) 17 mg voafrine A and 30 mg voafrine B were obtained by silica gel chromatography, and their structures were determined by spectroscopic methods.

HR-MS-measurement on the molecular peak at *m/z* 670 ($C_{42}H_{46}N_4O_4$) shows that voafrine A and voafrine B are isomers whose common IR bands at 2930 and 1670 cm^{-1} reveal that the alkaloids possess a N–H group and vinylogous amide unit reminiscent of *Aspidosperma* bases. The UV absorptions of both compounds λ_{max} 223 nm (log ε 4.27), 298 (4.27), 328 (4.38) can be interpreted as arising from the presence of two β-anilino-acrylic chromophores in the molecule. These data, coupled with the observation that the molecular formula corresponds to that of a dehydro-dimer of tabersonine, strongly

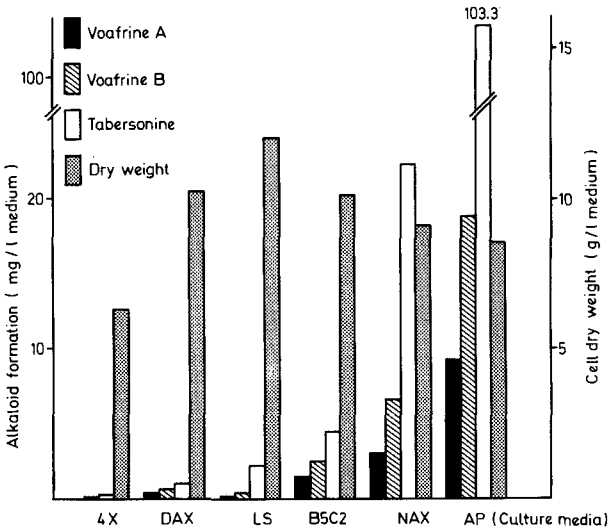
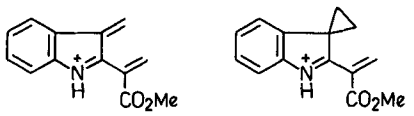


Fig. 1. *Optimization of alkaloid production in* Voacanga *cell suspension cultures*

suggest that both alkaloids consist of two tabersonine units. The exhibition of a peak at m/z 214 in the mass spectrum attributed to ion **A** [7], and of peaks at m/z 228 ($C_{14}H_{14}NO_2$, calc. 228.1024, found 228.1018) due to ion **B** and at m/z 168 (228–60) further supports the conclusion that the alkaloids possess the ABE-ring system of tabersonine.



A **B**

Table. *^{1}H-NMR Data of Voafrine A* (**1**)[a]) *and Voafrine B* (**2**)[b]) *in* $(CD_3)_2CO$

Protons	Voafrine A		Voafrine B	
H_β–C(3)	3.56	(*dd*, 15.6, 1.4)[c])	3.82	(br. *d*, 15.4)[c])
H_α–C(3)	3.08	(*dd*, 15.6, 2.0)	3.33	(br. *d*, 15.4, 2.0)
H_β–C(3′)			3.99	(br. *dt*, 4.6, 1.0, <1)
H_α–C(3′)	3.76	(*ddd*, 2.2, 1.5, <1)		
H_β–C(5)	3.02	(*ddd*, 8.5, 6.2, 1.4)	3.05	(*ddd*, 9.5, 7.0, 2.0)
H_β–C(5′)	3.03	(*ddd*, 8.5, 6.2, 1.4)	3.20	(*ddd*, 8.0, 8.0, 7.0)
H_α–C(5)	2.7[d])		2.88	(*ddd*, 9.5, 8.5, 5.2)
H_α–C(5′)			3.08	(*ddd*, 8.0, 8.0, 4.9)
H_β–C(6)	2.0[d])		2.05[e])	
H_β–C(6′)			2.07	(*ddd*, 12.0, 8.0, 8.0)
H_α–C(6)	1.70	(*dd*, 11.4, 4.2)	1.81	(*ddd*, 12.0, 5.2, 2.0)
H_α–C(6′)	1.74	(*ddd*, 11.4, 4.8, 1.4)	1.98	(*ddd*, 12.0, 7.0, 4.9)
H–C(9)	7.34	(*dd*, 7.6, 1.2)	7.36[f])	(*dd*, 7.6, 1.2)
H–C(9′)	7.35	(*dd*, 7.6, 1.2)	7.45	(*dd*, 7.6, 1.2)
H–C(10)	6.88	(*ddd*, 7.6, 7.6, 1.4)	6.86[f])	(*ddd*, 7.6, 7.6, 1.0)
H–C(10′)	6.88	(*ddd*, 7.6, 7.6, 1.4)	6.88	(*ddd*, 7.6, 7.6, 1.0)
H–C(11)	7.15	(*ddd*, 7.6, 7.6, 1.2)	7.13[f])	(*ddd*, 7.6, 7.6, 1.2)
H–C(11′)	7.16	(*ddd*, 7.6, 7.6, 1.2)	7.12	(*ddd*, 7.6, 7.6, 1.2)
H–C(12)	6.99	(*dd*, 7.6, 1.4)	7.03[f])	(*dd*, 7.6, 1.0)
H–C(12′)	7.00	(*dd*, 7.6, 1.4)	7.04	(*dd*, 7.6, 1.0)
H–C(14′)	5.54	(*dd*, 10.0, 1.5)	5.82	(*dd*, 10.2, 4.6)
H–C(15)	5.76	(*ddd*, 2.0, 1.4, <1)	5.71	(*ddd*, 2.0, 1.0, <1)
H–C(15′)	5.75	(*dd*, 10.0, 2.2)	5.93	(*dd*, 10.2, 1.0)
H_β–C(17)	2.49[g])	(*d*, 15.0)	2.40	(*d*, 14.9)
H_β–C(17′)	2.59[h])	(*d*, 15.0)	2.21	(*d*, 15.1)
H_α–C(17)	2.58[g])	(*dd*, 15.0, 1.8)	2.54	(*dd*, 14.9, 1.7)
H_α–C17′)	2.62[h])	(*dd*, 15.0, 1.2)	2.64	(*dd*, 15.1, 1.6)
H_3C(18)	0.66	(*t*, 7.5)	0.63	(*t*, 7.5)
H_3C(18′)	0.69	(*t*, 7.5)	0.71	(*t*, 7.5)
H_2C(19)	0.7–1.9 (*m*)		0.85–1.1 (*m*)	
H_2C(19′)				
H–C(21)	2.73[g])	(*d*, 1.8)	2.78	(br. *d*, 1.7)
H–C(21′)	2.83[h])	(*d*, 1.2)	3.17	(br. *d*, 1.6)
$COOCH_3$	3.70	(*s*)	3.67	(*s*)
	3.72	(*s*)	3.70	(*s*)
N–H	9.33	(*s*)	9.33	(*s*)
N′–H	9.34	(*s*)	9.41	(*s*)

[a]) At 300 MHz, 0.02 M solution. [b]) At 500 MHz, 0.02 M solution. [c]) Chemical shifts are in ppm from TMS. In Parentheses: multiplicity and apparent coupling constants. [d]) Multiplet centered at the indicated value. [e]) Overlapped with solvent signals. [f]) [g]) [h]) Correlated by double-resonance experiments.

The above data greatly facilitated the interpretation of the ^{1}H-NMR spectra of the voafrines by suggesting an early comparison with the spectrum of tabersonine [8] [9]. In spite of the large number of protons and of their similarity in terms of field position and multiplicity, diagnostically most valuable signals in the high-field spectra appear separated enough to allow a direct assignment which, in any case, has been corroborated by extensive decoupling experiments (Table).

The analysis permits not only the determination of the site of attachment of the two units, but also of the stereochemistry of the junction, leading to the formulation of voafrine A as 14-(3′β-tabersonyl)tabersonine (**1**) in (3′*R*)-configuration and voafrine B as 14-(3′α-tabersonyl)tabersonine (**2**) in (3′*S*)-configuration, respectively. In addition, taking advantage of the superb separation of signals in the 500-MHz spectrum *(Fig. 2)* of voafrine B and application of 400-MHz two-dimensional NMR techniques, a complete assignment of all aliphatic signals (with exception of those of the ethyl side-chains) was possible for this compound. Both ^{1}H-NMR spectra contain eight aromatic protons and two N–H signals excluding the involvement of the β-anilinoacrylic moieties in the coupling. On the other hand, there are only three olefinic H-atoms, two diastereotopic aminomethylene protons and one aminomethine proton in the 3.3–4.0 ppm region in which the H-atoms at C(3) of tabersonine resonate.

As a consequence, the choice of the attachment points is restricted between one olefinic C-atom (C(14) or C(15)) of the first half (unit I) and C(3′) of the second half (unit II), and

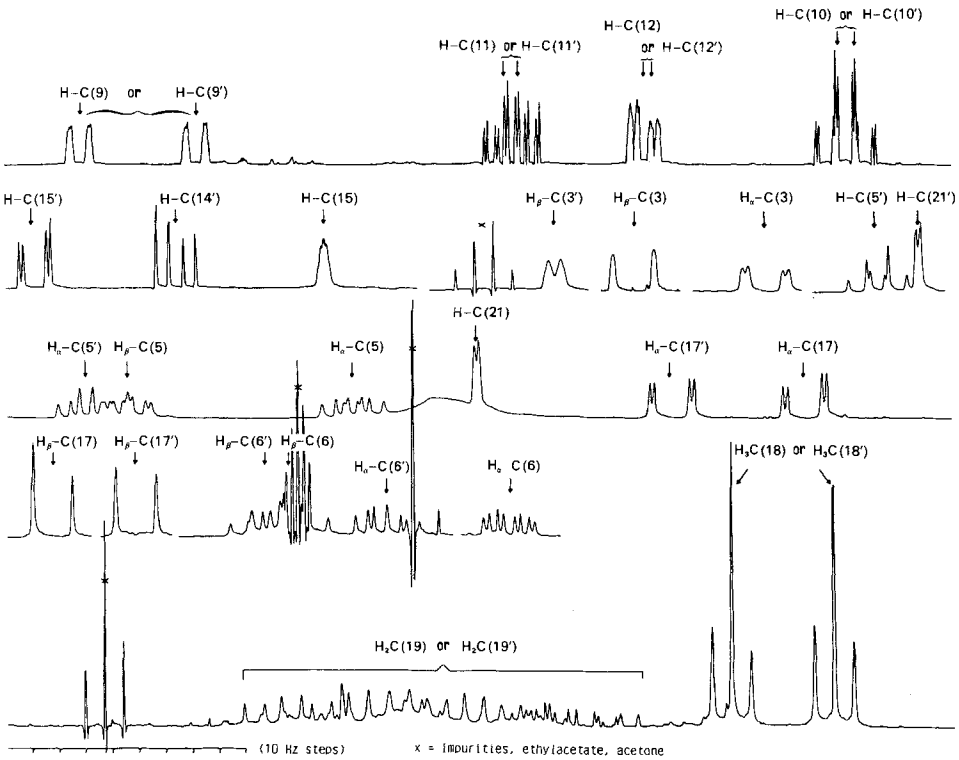
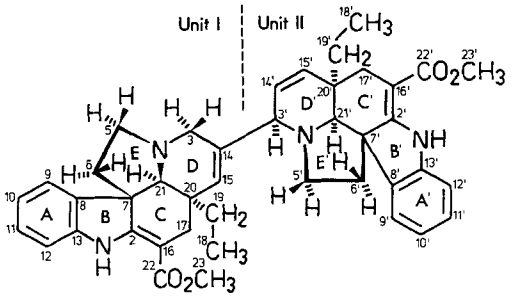
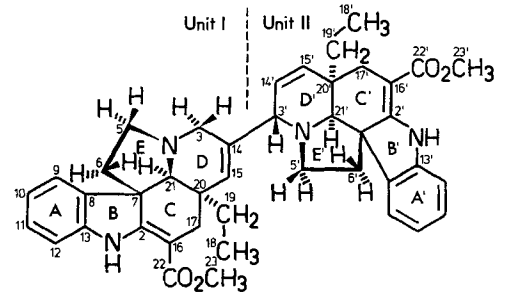


Fig. 2. *Part of the 500-MHz ^{1}H-NMR spectrum of voafrine B in $(CD_3)_2CO$*

voafrine A and B are C(3′)-epimers of each other. It is known [8] that ring D of tabersonine exists in a slightly modified half-chair form with H_β–C(3) and H_α–C(3) in a quasi-equatorial and in a quasi-axial orientation, respectively. In agreement with this conformation, the vicinal coupling of H–C(14) with H_β–C(3) is large (5.0 Hz), whereas the coupling with H_α–C(3) is small (2.0 Hz). The allylic couplings of H–C(15) are of the same order of magnitude, 2.0 Hz with H_β–C(3) and *ca.* 1 Hz with H_α–C(3). Comparison of the spectra of both voafrines shows that H_β–C(3) and H_α–C(3) exhibit very similar splitting patterns with non-geminal couplings less than 2 Hz. This is consistent with the presence of only one H at C(15) and makes C(14) the candidate for the point of attachment of unit I to unit II. The remaining olefinic H-atoms at C(14′) and C(15′) are part of an *ABX*-system in which H–C(3′) is species X. The vicinal coupling of H–C(14′) and H–C(3′) was found to be 1.5 Hz in voafrine A and 4.6 Hz in voafrine B, whereas allylic coupling constants of H–C(15′) and H–C(3′) are 2.2 and 1.0 Hz, respectively. In view of the well-known dependence of vicinal and allylic coupling on dihedral angle and considering the ^{1}H–NMR data of tabersonine, these values are expected if H–C(3′) is placed nearly perpendicular to the plane of the double bond (α-orientation) in voafrine A, and nearly coplanar to the double bond plane (β-orientation) in voafrine B. H_β–C(3′) and H_α–C(3′) show an additional small but significant allylic coupling with H–C(15), indicating a quasi-perpendicular orientation with respect to the double bond of unit I. These data lead to the formulation for the voafrines of the structures depicted in formula **1** and **2**, which also represent the absolute stereochemistry of the alkaloids owing to the similarity of chiroptical characteristics with those of (-)-tabersonine *(vide infra)*.



(1) (3′*R*)-Voafrine A



(2) (3′*S*)-Voafrine B

Further assignment of aliphatic signals in the spectrum of voafrine B (**2**) results from the observation of a very small coupling among H_β–C(3′) and H_β–C(3) and the broad doublets at 3.17 and 2.78 ppm, which are thus attributed to H–C(21′) and H–C(21), respectively. H–C(21′) is additionally coupled through four σ-bonds in a periplanar 'W'-arrangement with signals at 3.08 and 1.98 ppm, due to H_α–C(5′), and H_α–C(6′). The last mentioned signals are then correlated to the ddd signals at 3.20 and 2.07 ppm, which are attributed to H_β–C(5′) and H_β–C(6′). Similar observations indicate coupling of H–C(21) to signals at 2.88 and 1.81 ppm (H_α–C(5) and H_α–C(6)) and these, in turn, to 3.05 and 2.05 ppm (H_β–C(5) and H_β–C(6)).

Examination of molecular models of the two epimeric alkaloids reveal that the two tabersonine halves have very different relative geometries. In voafrine A (**1**), the pseudo-equatorially oriented unit I points away from unit II, resulting in limited or nor spatial interaction between the tabersonine halves which also possess a similar conformation, close to that of tabersonine itself. As a consequence, signals of corresponding protons in the two units show very similar shifts and coupling constants, similar to those of tabersonine. In voafrine B (**2**), the pseudo-axially oriented substituent at C(3′) strongly modifies the conformation of unit II, giving rise to different shifts and coupling constants for corresponding protons. This is particularly evident for $H_2C(5')$ and $H_2C(6')$ (with respect to $H_2C(5)$ and $H_2C(6)$) where the data indicate a significant modification of the conformation of ring C′. Other differences are presumably due to steric compression or intramolecular dipolar and magnetic interactions (*e.g.* H–C(9) and H–C(9′), $H_3C(18)$ and $H_3C(18')$).

Additional evidence for the voafrine B structure derives from inspection of the ^{13}C-NMR spectrum (see *Exper. Part*) in comparison with that of tabersonine [10]. The lowest field saturated C-atoms at 71.0 and 66.0 ppm are assigned to C(21) and/or C(21′). One of these appears to be slightly downfield with respect to tabersonine (69.9 ppm) and can be attributed to C(21), whereas the second is consistently upfield shifted and must be involved in a strong steric interaction. This is the case for C(21′) if substitution at C(3′) takes place at α-side, the H-atom at position 21′ being involved in a 1,3-type dipolar interaction.

Finally, the CD spectra of voafrine A and B at long wavelengths are the summation of spectra of two (-)-tabersonine units [11], supporting the absolute configuration as shown before. However, CD curves strongly differ at short wavelengths below 220 nm. By analogy with other dimeric alkaloids having opposite absolute stereochemistry of the chiral center joining the constituent moieties [12], we interpret these differences in terms of the appearance of optical rotation arising from dipolar coupling of the chromophores. Although the polarization of the electronic moments of the β-anilinoacrylic moiety is not known, molecular models reveal that any conformation which retains the long-range coupling between H–C(3′) and H–C(15), results in a chiral exiton coupling between the two chromophores with the appearance of split *Cotton* effects. On the other hand, the larger distance of the chromophores in voafrine A seem to prevent a similar interaction and even at short wavelengths the CD spectrum is the same in character as the ones of tabersonine.

These data clearly confirm the depicted structures for voafrine A and voafrine B, which have not been described before in the literature. An alkaloid isolated from differentiated plants of *Criocera dipladeniiflorus* [13] was found to be a dimeric

tabersonine (ditabersonine), which showed nearly the same mass spectrum as voafrine A and B. The published spectroscopic data did, however, not allow a detailed structure elucidation of this particular alkaloid. It was argued [2], that coupling of one tabersonine unit takes place between C(3) or C(21) yielding ditabersonine, but the evidence taken from ^{1}H-NMR and MS data was not mentioned in the original report. Therefore at this point, we cannot clearly exclude the identity of ditabersonine with one of the isolated voafrines.

Because of the instability of tabersonine, it would be interesting to ask, whether a spontaneous or an enzyme-catalyzed dimerization of tabersonine could lead to voafrine A and B. When a solution of tabersonine in AcOEt was stirred under O_2, a dimeric tabersonine was observed (yield $< 0.1\%$) which revealed the same R_f-value as voafrine B. We therefore examined, whether both voafrines are artifical alkaloids formed from tabersonine during their isolation. When [aryl-^{3}H]-tabersonine or the unlabelled alkaloid was added to the cell tissue before the isolation of voafrine A and B, the obtained dimers were not labelled, nor was the quantity of alkaloid A and B changed. Therefore, we can conclude, that voafrine A and voafrine B are most probably biosynthesized, although the involved enzyme(s) are still unknown. Sometimes cell suspension cultures might be contaminated by slowly growing microorganisms. To exclude a microbial dimerization of tabersonine before the isolation, which is well-known in the case of vindoline [14], *Voacanga* cells were plated on different media. After incubation at 35 °C for up to 30 days, microbial infections of the plant cells were not detected, which demonstrates that indeed the *Voacanga* cells synthesize voafrine A and B. The facts, that some cultures produce only minor amounts of tabersonine, but preferentially the dimeric alkaloids A and B and that cell strains can be obtained, yielding quite different ratios of both voafrines (*e.g.* A/B = 1:1 or A/B = 1:2.5) strongly point to an enzyme-catalyzed process.

The appropriate biogenetic investigations concerning the enzymatic biosynthesis of voafrine A and voafrine B are in progress.

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Experimental Part

General. For TLC 0.25-mm layers of silica gel (*Polygram, Sil G/UV$_{254}$, Marcherey & Nagel*) were used. The plates were developed with solvent systems a ($CHCl_3$/MeOH 100:0.5), b (hexane/Et_2O 1:1), c (xylene/hexane/Et_2O 1:1:1) and the alkaloids were visualized with ceric ammonium sulfate/H_3PO_4 (CAS). All solvents were of analytical reagent grade. High-pressure liquid chromatography (HPLC) was performed using a microprocessor-based *Spectra-Physics* HPLC system, equipped with a variable wavelength detector, automatic sampler *ASI 120 (Ismatec)* and a reversed phase column *(Hibar LiChrocart®, Merck, LiChrosorb® RP 18)*. HPLC solvent system was CH_3CN/H_2O 85:15, gradient to 99:1 within 10 min, 20 min 99:1, flowrate 1 ml/min. Optical rotation was determined using a *Perkin-Elmer 241* polarimeter. UV spectra were measured on a *Perkin-Elmer 551 S* instrument using MeOH as solvent, absorption in nm ($\log \varepsilon$). CD spectra were obtained on a *Jasco 500 A* instrument. IR spectra were obtained in KBr with a *Perkin-Elmer 257* spectrophotometer, wave number (cm^{-1}). ^{1}H-NMR spectra were determined at 500, 400 and 300 MHz with *Bruker WM 500, AM 400* and *AM 300* spectrometers, respecitvely. ^{13}C-NMR data were obtained with a *WM 400* at 100.6 MHz and with a *WP 200*

at 50.3 MHz. Chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz. Signal multiplicities are abbreviated as: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. Mass spectra (electron impact, EI/MS 70 eV; direct chemical ionization, DCI/MS 190 eV, with i-C_4H_{10}) were measured on a *Finnigan MAT44S* instrument. HR–MS were performed on a *Finnigan MAT 212* equipped with a *SS 200* data system and on a *VG-ZAB* instrument using the peak-matching technique.

Plant Cell Cultures. Cell suspensions were maintained under standard conditions on a *Gyratory* shaker (100 rpm) in 300-ml *Erlenmeyer* flasks at 25° (rel. humidity 65%) in 4X-medium [4]. Cells were transferred to fresh medium every 7 days. For optimization of alkaloid synthesis equal cell amounts (3.5 g fresh weight) were used as inoculum for 70 ml of 4X [4], LS [15], modified B5 (here called *NAX*) [16], modified B5 (named *DAX*) [16] and of alkaloid production medium (abbreviated AP) [6] in 300-ml *Erlenmeyer* flasks. After 12 days cells were harvested, frozen with liquid N_2 and extracted twice with AcOEt (1 ml/g). The extracts then were subjected to HPLC analysis for quantitation of alkaloids. Callus tissue was maintained on solidified 4X medium (0.7% agar) and transferred every 4 weeks to fresh medium.

For a 'large scale' production of alkaloids *Voacanga* suspensions were grown for 22 days in 1 l flasks (AP medium). Cells were harvested by suction filtration, frozen with liquid N_2 and stored at $-20°$. To prove the cultured cells to be free of slowly growing microorganisms, cells were plated on 3 different solidified media and incubated at 35°.

Alkaloid Ratios in Different Voacanga *Callus and Cell Suspension Strains.* From plated *Voacanga* cells two cell strains (Va 1 and Va 2) were obtained, grown under identical standard conditions on agar medium and as cell suspensions in 4X medium. HPLC analyses of callus extracts of Va 1 (2 g cells) revealed alkaloid ratios; tabersonine/voafrine A/voafrine B 1 : 0.3 : 0.5. The ratio found in extracts of Va 1 cell suspension was determined to be tabersonine/voafrine A/voafrine B 1 : 0.5 : 0.7. Alkaloid ratio in Va 2 callus was tabersonine/voafrine A/voafrine B 1 : 1.7 : 4.4. In cell suspensions of Va 2 a similar ratio was determined.

Isolation of Alkaloids. Cell material (fresh weight, 2.5 kg), was stirred in AcOEt (5 l) for 24 h at 4°. After filtration the cells were re-extracted by the same procedure. Org. phases were then combined, evaporated and the alkaloidal residue submitted to a silica gel column (60 g silica gel *60*, 0.063 mm, *Merck*; column 60 × 2.5 cm). The column was eluted with hexane/Et_2O 1 : 1 with a flow rate of 0.7 ml/min; 7-ml fractions were collected and analyzed for the alkaloid content. Fractions 21–27 and 27–32 contained voafrine A and voafrine B, respectively. Final purification was achieved by TLC (pre-washed plates) with solvent system b yielding 17 mg voafrine A and 30 mg voafrine B. TLC: voafrine A (system a, R_f 0.85; system b, R_f 0.6; system c, R_f 0.47); voafrine B (system a, R_f 0.77; system b, R_f 0.5; system c, R_f 0.35).

Examination of Artificial Formation of Voafrine A and Voafrine B during their Isolation. – Time-Dependent Extraction of Tissue. Fresh or frozen tissue (3 g, stored at $-20°$) was extracted with MeOH or AcOEt (3 ml for periods of 1–24 h). The extracted alkaloids were quantitatively determined by HPLC. The found ratios for tabersonine/voafrine A/voafrine B were constant.

Isotope Dilution Analysis. To 45 g of frozen cells 1.5 μCi [aryl-^{3}H]-tabersonine (spec. activity 34.8 μCi/μmol) was added and the tissue extracted with AcOEt for 1 h. Isolated voafrine A and voafrine B were not labelled.

Voafrine A. Colorless, amorphous. $[\alpha]_D^{20} = -8° \pm 2°$ ($c = 0.62$, MeOH). UV: max. 328, 298, 223; min. 308, 260. CD (MeOH, $c = 0.0705$): −24.2 (321), 0 (298), +6.33 (289), +6.45 (286), +1.15 (262), +24.5 (235), +12.0 (217), +47.8 (202). IR: 2930, 1730, 1670, 1610, 1470, 1440, 1380, 1240, 1190, 1170, 1115, 745 cm^{-1}. ^{1}H-NMR (see the *Table*). EI/MS: 670 (4, M^+), 349 (6), 335 (8), 229 (16), 228 (60), 227 (15), 214 (11), 213 (8), 212 (4), 203 (4), 197 (12), 196 (23), 170 (10), 169 (34), 168 (100), 167 (39), 154 (20), 141 (7), 122 (9), 121 (8), 108 (6). DCI/MS: 727 (0.5, $[M+C_4H_9]^+$), 672 (2), 671 (4, $[M+H]^+$), 670 (2), 443 (3), 442 (11), 441 (6), 440 (1), 399 (9), 233 (6), 232 (23), 231 (27), 230 (80), 229 (50), 228 (12), 213 (13), 212 (7), 199 (19), 198 (100), 197 (54), 196 (8), 173 (8), 172 (30), 171 (16). HR-MS: $C_{42}H_{46}N_4O_4$, calc. 670.3519 found 670.3513.

Voafrine B. $[\alpha]_D^{20} = -265°$ (MeOH, $c = 0.83$). UV: max. 328 (4.38), 298 (4.27), 223 (4.27); min 308 (4.20), 260 (3.30). CD (MeOH, $c = 0.0978$): −23.2 (327), 0 (302), +7.8 (289), +1.8 (262), +16.2 (240), 0 (220), −4.35 (214), 0 (207), +2.3 (205), 0 (202). IR: 2930, 1723, 1670, 1605, 1465, 1432, 1380, 1240, 1200, 1185, 1165, 1100, 745 cm^{-1}. ^{1}H-NMR: see the *Table*. ^{13}C-NMR ($CDCl_3$)[1]: 166.7, 165.9 (C(2,2′)); 59.7 (C(3′)); 52.9[a] (C(3)); 50.9[a], 50.9[a] (C(5,5′)); 43.8, 45.4 (C(6,6′)); 56.8, 56.3 (C(7,7′)); 139.9, 139.1 (C(8,8′)); 122.9, 122.4 (C(9,9′)); 121.5, 121.3 (C(10,10′)); 128.4, 128.4 (C(11,11′)); 110.4, 110.4 (C(12,12′)); 144.6, 144.6 (C(13,13′)); 136.8 (C(14)); 133.9[b] (C(14′)); 130.8[b], 126.6 (C(15,15′)); 92.2, 91.1 (C(16,16′)); 28.9, 28.6 (C(17,17′)); 8.3, 8.0 (C(18,18′)); 31.9, 31.1 (C(19,19′)); 41.1, 38.6 (C(20,20′)); 71.0 (C(21)); 66.0 (C(21′)); 168.6, 168.6 (C(22,22′)); 52.0[a], 51.9[a] (C(23,23′)). EI/MS: 670 (2, M^+), 349 (7), 335 (6), 229 (14), 228 (59), 227 (9), 214 (10), 213 (9), 212 (5), 203 (7), 197 (12), 196 (28),

[1]) a, b: exchangable.

170 (12), 169 (19), 168 (100), 167 (31), 154 (12), 141 (6), 122 (9), 121 (9), 108 (7). DCI/MS: 727 (1, $[M+C_4H_9]^+$), 672 (2), 671 (4, $[M+H]^+$), 670 (2), 443 (8), 442 (25), 441 (10), 233 (3), 232 (17), 231 (28), 230 (100), 229 (80), 228 (10), 214 (4), 213 (23), 212 (14), 199 (4), 198 (29), 197 (19), 172 (5), 171 (2). HR-MS: $C_{42}H_{46}N_4O_4$: calc. 670.3519, found 670.3524; $C_{14}H_{14}NO_2$: calc. 228.1024, found 228.1018.

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