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INDOLE ALKALOIDS FROM A CALLUS CULTURE OF *TABERNAEMONTANA ELEGANS**

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Key Word Index—*Tabernaemontana elegans*; Apocynaceae; callus culture; isolation; indole alkaloids.

Abstract—Fourteen indole alkaloids have been isolated from a callus culture of *Tabernaemontana elegans*. One of them is new, 3-oxo-isovoacangine, the others are the known isovoacangine, 3-*R*/*S*-hydroxy-isovoacangine, 3-*R*/*S*-hydroxy-coronaridine, isositsirikine, geissoschizol, tabernaemontanine, vobasine, vobasinol, apparicine, 16-hydroxy-16,22-dihydro-apparicine, tubotaiwine, 3-*R*/*S*-hydroxy-conodurine and monogagaine. The alkaloid content is similar to that of the whole plant, except for the absence of the tabernaelegantines, which were major components in the plant extract.

INTRODUCTION

In a previous report [1] we presented the isolation of 18 indole alkaloids from an extract of *Tabernaemontana elegans*. Several of these alkaloids have been shown to possess interesting biological activity [2, 3], e.g. antimicrobial effects. In order to study the biosynthesis of these alkaloids and to obtain a reliable source for these compounds we initiated callus and suspension cultures of a number of *Tabernaemontana* species. In the present study we report the isolation of 12 monomeric and two dimeric alkaloids from a callus culture of *T. elegans*.

RESULTS AND DISCUSSION

The callus material contained 0.016% total alkaloids (fr. wt). By means of prep. TLC a series of alkaloids was isolated and identified by their spectral data, the results are summarized in Table 1. Four iboga, five corynanthean, three aspidospermatan and two dimeric alkaloids were identified. One of the alkaloids has not yet been reported (see note added in proof). Its structure was determined as 3-oxo-isovoacangine (**2**) by means of UV, mass spectrometry and H NMR and further confirmed by reduction with $NaBH_4$, which yielded the known alkaloid isovoacangine (co-TLC, mass spectrum). This alkaloid could be an artefact due to the isolation method, as could the 3-hydroxy derivatives. However, the fact that none of these alkaloids was found in the whole plant extract which has been elaborated in the same way points to the possibility of the natural occurrence of these alkaloids. Apparicine was the major component in the callus culture material. It is one of the precursors of the alkaloid monogagaine (**6**) [4] also present in the callus material, this dimer not being identified in the plant extract. The biosynthetically less evolved alkaloids isositsirikine (**4**) and geissoschizol (**5**) were only found in the callus material; they were not isolated from the plant extract. The dimers of the tabernaelegantine type were only found in the plant extract. In the callus material they could not be detected, neither could their precursors dregaminol and tabernaemontaninol. The alkaloids vobasine and vobasinol are accompanied in the callus material by the dimer 3-hydroxy-conodurine, which has the latter alkaloid as one of its precursors.

Apparently, the plant (the plant from which the callus was initiated was grown from the same batch of seeds as the plant which was used for the identification of the alkaloids in the whole plant [1]) has similar but not completely the same pattern of alkaloids as occurs in the undifferentiated callus cells. On the other hand it is promising that the callus material is capable of producing highly complex secondary products as the dimeric alkaloids, as e.g. *Catharanthus roseus* has lost this capacity in tissue cultures.

EXPERIMENTAL

Plant material. Seed and samples of *T. elegans* stapf were collected in Mozambique in 1981. Voucher specimens are kept in the herbarium (No 81-3386 JKW 303) at Wageningen, The Netherlands.

Callus culture. Callus was induced on stem material of *T. elegans* placed on a Murashige–Skoog medium containing 1 ppm 2,4-D and 1 ppm zeatin. The callus obtained was subcultured every 4 weeks. Growth chamber conditions were temp. 28°, light: 12 hr dark–12 hr light (150 Lux from a Pope FT 65 W/33).

Isolation of alkaloids. Callus (1115 g fr. wt) was extracted with 96% EtOH using an Ultra Turrax. After filtration the alcohol was evapd *in vacuo*. The residue was partitioned between Et_2O and 2% HOAc. The acid layer was collected, brought to pH 9.5 with Na_2CO_3 and extracted with $CHCl_3$–*iso*-PrOH (9:1). The organic layer was collected and dried (Na_2SO_4) and evapd *in*

*Part 17 in the series "Pharmacognostical Studies of *Tabernaemontana Species*". For part 16 see ref. [1].

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Table 1. Alkaloids isolated from a *T. elegans* callus culture

Alkaloid	Biosynthetic group*	Identification	Relative abundance†	
			Callus	Whole plant (1)
Isovoacangine	I	UV, MS, co-TLC	+ +	+ +
3-*R*/*S*-Hydroxy-isovoacangine **1**	I	UV, MS, ^{1}H NMR, ch. corr.‡	+ +	−
3-Oxo-isovoacangine **2**	I	UV, MS, ^{1}H NMR, ch. corr.	+	−
3-*R*/*S*-Hydroxy-coronaridine **3**	I	UV, MS, ^{1}H NMR, ch. corr.	+ +	−
Isositsirikine **4**	C	UV, MS, ^{1}H NMR, co-TLC	+ +	−
Geissoschizol **5**	C	UV, MS, ^{1}H NMR, co-TLC	+	−
Tabernaemontanine	C	UV, MS, ^{1}H NMR, co-TLC	+ + +	+ + + +
3-*R*/*S*-Hydroxy-conodurine	C-I	UV, ^{1}H NMR, co-TLC	+ +	+ +
Vobasinol	C	UV, MS, ^{1}H NMR	+ +	+
Apparicine	A	UV, MS, ^{1}H NMR, co-TLC	+ + + +	+ + +
16-Hydroxy-16,22-dihydro-apparicine	A	UV, MS, ^{1}H NMR, co-TLC	+	+
Tubotaiwine	A	UV, MS, ^{1}H NMR, co-TLC	+ +	+
Vobasine	C	UV, MS, ^{1}H NMR, co-TLC	+ + +	+ + + +
Monogagaine **6**	C-A	UV, MS, ^{1}H NMR, co-TLC	+	−
Dregamine	C		−	+ + + +
Dregaminol	C		−	+ +
Tabernaemontaninol	C		−	+ +
Tabernaelegantine A	C-I		−	+ + +
Tabernaelegantine B	C-I		−	+ + +
Tabernaelegantine C	C-I		−	+ +
Tabernaelegantine D	C-I		−	+ +
Dregaminol-methylether	C		−	+
3-*R*/*S*-Hydroxy-tabernaelegantine B	C-I		−	+ +
3-Methoxy-tabernaelegantine C	C-I		−	+ +

*I, Iboga; C, corynanthean; A, aspidospermatan.
† + + + +, Main components; + + +, major components; + +, minor components; +, trace; −, not isolated.
‡ch. corr., Chemical correlation.

vacuo to dryness. Yield: 0.18 g (0.016% fr. wt). The extract was separated by means of prep. TLC on home-made silica plates using solvents S1-S4. S1: cyclohexane–$CHCl_3$–Et_2NH (6:3:1), S2: toluene–EtOH containing 1.74% g/v NH_3 (19:1); prior to development the plates were stored for 20 min in an atmosphere of NH_3, S3: $CHCl_3$–MeOH (9:1), S4: EtOAC–*iso*-PrOH–26% g/v NH_3 (17:2:1). For co-TLC; all on silica gel F254 'fertig platte' Merck in saturated chambers. The spray reagents used were: A, 1% ceric sulphate in 10% H_2SO_4; B, 0.2 M $FeCl_3$ in 35% $HClO_4$, followed by heating with hot air; C, iodoplatinate spray; D, Dragendorff's spray.

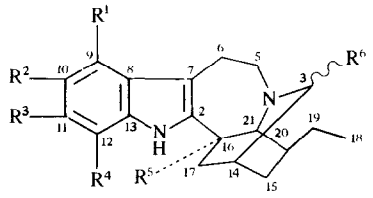
UV spectra were recorded in MeOH. 300 MHz ^{1}H NMR spectra were recorded in $CDCl_3$ and CD_3OD. Shifts are represented in δ values relative to TMS. MS were obtained using EI, 42 eV, single focus, heating direct inlet system and at 70 eV, double focus, direct inlet.

Characterization of alkaloids. Isovoacangine, tabernaemontanine, vobasine, vobasinol, apparicine, 16-hydroxy-16,22-dihydro-apparicine, tubotaiwine, 3-*R*/*S*-hydroxyconodurine see ref. [1].

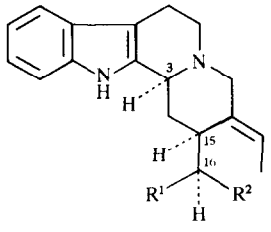
3-R/S-*Hydroxy-isovoacangine* (**1**). Mixture 3*R*: 3*S* = 1:2. R_f-values and chromogenic reactions see ref. [7]; UV, MS and ^{1}H NMR data see ref. [3]. Reduction with $NaBH_4$ gave one product which was identical (co-TLC, MS) with isovoacangine.

3-*Oxo-isovoacangine* (**2**) R_f-value in TLC system S2 0.12. Colour with spray reagent B after heating grey. UV λ_{max}^{MeOH} nm: 275 (sh), 288 (sh), 295, EIMS (70 eV) *m/z* (rel. int.): 383 $[M+1]^+$ (11), 382 $[M]^+$ (40), 368 (5), 354 (6), 353 (10), 352 (28), 324 (5), 323 (5), 293 (5), 255 (8), 247 (5), 246 (5), 245 (5), 227 (14), 197 (12), 182 (20), 181 (18), 180 (100), 179 (21), 168 (15), 154 (12), 124 (40). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (1H, *br* s, NH) 7.33 (1H, *d*, J = 8 Hz, H-12), 6.75 (2H, *m*, H-9 and H-10), 4.49 (1H, *s*, H-21) 4.53–4.41 (1H, *m*, H-5a), 3.82 (3H, *s*, ArOMe), 3.64 (3H, *s*, COOMe), 3.22–3.13 (3H, *m*, H-6a,b, H-5b), 2.65 (1H, *s*, H-14), 2.64 (1H, *dd*, J = 7 and 1Hz, H-17a), 2.31 (1H, *ddd*, J = 14, 7 and 3Hz, H-17b), 2.00 (1H, *ddd*, J = 12, 10 and 3.8 Hz, H-15a), 1.60–1.30 (4H, *m*, H-15b, H-19, H-20), 1.01 (3H, *t*, J = 7 Hz, H-18). These data were compared with those of 3-oxo-coronaridine [5]. The difference in spectra could be explained by the presence or absence of 11-methoxysubstitution. Reduction with $NaBH_4$ gave a product which was identical with isovoacangine.

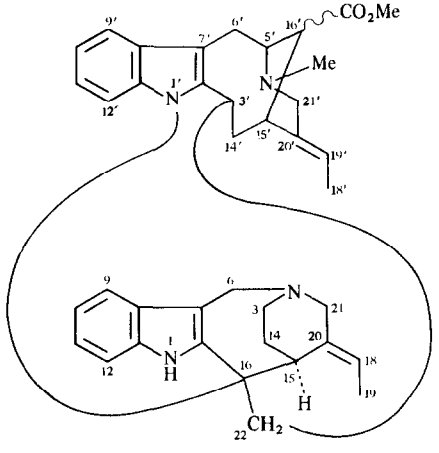
3-R/S-*Hydroxy-coronaridine* (**3**). Mixture 3*R*:3*S* = 3:2. R_f-values in TLC system S2 0.46, S3 0.73. Colour with spray reagent B brown grey after heating. UV λ_{max}^{MeOH} nm: 228, 285 and 292. EIMS 70 eV *m/z* (rel. int.): 354 $[M]^+$ (1), 353 $[M-H]^+$ (4), 352 $[M-2H]^+$ (20), 338 $[M-16]^+$ (16), 337 $[M-17]^+$ (27), 336 $[M-18]^+$ (100), 335 (15), 307 (12), 292 (7), 277 (10), 253 (10), 238 (15), 194 (12), 180 (11), 169 (25), 168 (20), 167 (21), 154 (13), 143 (18), 124 (18). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (1H, *br* s, NH), 7.51 (1H, *dd*, H-12), 7.28 (1H, *dd*, H-9), 7.18 (2H, *m*, H-10 and H-11), 4.40 (1H, *br d*, H-3, *S*), 4.01 (1H, *d*, H-3, *R*), 3.83 (1H, *br s*, H-21, *S*), 3.78 (1H, *br s*, H-21, *R*), 3.72 (3H, *s*, CO_2Me, *R*), 3.70 (3H, *s*, CO_2Me, *S*), 3.30 (4H, *m*, H-5a, b, *RS*), 3.11 (4H, *m*, H-6a, b, *RS*), 2.75 (2H, *m*, H-17a, *RS*), 1.95 (2H, *m*, H-14, *R*, H-17b, *R*), 1.55–1.20 (5H, H-15, H-19, H-20), 0.90 (3H, *t*, J = 7 Hz, H-18). These data are in close agreement with those reported in refs [5, 6]. Reduction with $NaBH_4$ gave one product which was identical (co-TLC, MS) with coronaridine.



1 $R^1 = R^2 = R^4 = H$, $R^3 = OMe$, $R^5 = CO_2Me$, $R^6 = OH$
2 $R^1 = R^2 = R^4 = H$, $R^3 = OMe$, $R^5 = CO_2Me$, $R^6 = O$
3 $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = CO_2Me$, $R^6 = OH$



4 Isositsirikine $R^1 = CH_2OH$, $R^2 = CO_2Me$
5 Geissochizol $R^1 = H$, $R^2 = CH_2OH$



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Isositsirikine (**4**). R_f-values and chromogenic reactions see ref. [7] UV, MS and ^{1}H NMR data see ref. [8].

Geissoschizol (**5**). R_f-values in TLC system S2 0.03. Colour with spray reagent **B** yellow-brown-grey after heating. UV λ_{max}^{MeOH} nm: 223, 275 (sh), 282, 289 (sh). MS 70 eV *m/z* (rel. int.): 296 $[M]^+$ (30), 295 $[M-H]^+$ (28), 279 (5), 265 (5), 251 (9), 249 (6), 223 (6), 169 (18), 156 (8), 129 (5). ^{1}H NMR see ref. [9].

Monogagaine (**6**). TLC, UV, MS and NMR data see ref. [4].

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NOTE ADDED IN PROOF

Panas *et al.* (*Phytochemistry*, 1975, **14**, 1120) reported the alkaloid M, which was given the structure 6-hydroxy-3-oxo-isovoacangine, based on comparison of spectral data with eglandulosine. As the structure of the latter alkaloid has recently been revised, alkaloid M was possibly 3-oxo-isovoacangine.