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ALKALOID PRODUCTION IN *CATHARANTHUS ROSEUS* CELL CULTURES: ISOLATION AND CHARACTERIZATION OF ALKALOIDS FROM ONE CELL LINE*

JAMES P. KUTNEY,†§ LEWIS S. L. CHOI,† PAWEL KOLODZIEJCZYK,† STEPHEN K. SLEIGH,† KENNETH L. STUART,† BRIAN R. WORTH,† W. G. W. KURZ,‡ K. B. CHATSON‡ and F. CONSTABEL‡

† Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada, V6T 1Y6;

‡ Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada, S7N OW9

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Key Word Index—*Catharanthus roseus*; Apocynaceae; alkaloids; tissue culture; 943 cell line; ajmalicine; vallesiachotamine; hörhammerinine; hörhammericine; vindolinine; 19-*epi*-vindolinine; 19-acetoxy-11-methoxytabersonine; 19-acetoxy-11-hydroxytabersonine; 19-hydroxy-11-methoxytabersonine; yohimbine; isositsirikine.

Abstract—A detailed study of one cell line (coded as 943) of *Catharanthus roseus* cell cultures has revealed the presence of the following alkaloids: ajmalicine, vallesiachotamine, hörhammerinine, hörhammericine, vindolinine, 19-*epi*-vindolinine, 19-acetoxy-11-methoxytabersonine, 19-acetoxy-11-hydroxytabersonine, 19-hydroxy-11-methoxytabersonine, yohimbine and isositsirikine, together with dimethyltryptamine and a new strychnos-type alkaloid.

INTRODUCTION

The previous paper of this series [1] described the general initiation and subsequent growth of a large number of cell lines from *Catharanthus roseus* both in callus and suspension culture. The general observation of alkaloid production was also made and several of the more promising lines were chosen for more detailed study. The results obtained from our investigations with shake flask and bioreactor suspension cultures of one of these lines, coded as '943', form the subject of the present publication.

Although initial observations of alkaloids were made by TLC analysis [1], this was insufficient as a general method of analysing multicomponent alkaloid mixtures. For this purpose an extensive investigation was undertaken by HPLC and optimum conditions for separation of the mixtures determined.

RESULTS AND DISCUSSION

Initiation and propagation of the 943 cell line was performed as described earlier [1]. Cell samples were extracted using a standard, consistent, procedure of percolation with MeOH; separation of alkaloids into acidic medium and re-extraction with ethyl acetate. The crude alkaloid mixture was then further distinguished as CH_2Cl_2-soluble and MeOH-soluble fractions (see Experimental). The latter generally comprised basic, or water soluble, but non-alkaloidal material.

During the course of these studies, the '943' line of *C. roseus* was grown on numerous occasions to confirm reproducibility of alkaloid production. Suspension cultures grown in either shake flasks or in a bioreactor provided a complex mixture of alkaloids (Table 1).

Table 1. Alkaloid content of the '943' cell line grown under different conditions

Sample	Alkaloids as % of dry cell wt	Growth period (weeks)	Culture type
1	0.44	3	Microferm bioreactor
2*	0.11	3 + 1 day	Microferm bioreactor
3	0.19	2	Microferm bioreactor
4	0.39	3	Shake flasks
5	0.25	3	Shake flasks

* Tryptophan (100 mg/l.) was added to the same fermentation as sample 1 and harvesting occurred 1 day later.

* Part II in the series. For Part I see ref. [1].

§ To whom correspondence should be addressed.

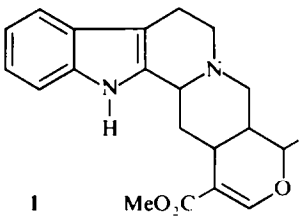
We have found that the alkaloid content is optimum if the fermentation is initiated by an inoculum in the logarithmic stage of growth and a high mitotic index. After inoculation the mitotic index initially decreases towards zero in 12–24 hr and then increases rapidly until one nutrient becomes limiting. At this stage the mitotic index decreases again until it reaches zero. During this latter stage alkaloid synthesis occurs. If any addition of nutrient, for example tryptophan (sample 2, Table 1), is made, alkaloid synthesis is interrupted and cell division starts again.

The identification of alkaloidal components was made initially by isolation of pure compounds and spectral analysis. Due to the complexity of the mixtures, both normal column chromatography and TLC proved ineffective for this purpose. Thus an intermediate scale HPLC system was developed.* Here a 100 ml dead volume column of reverse phase packing could be employed for effective separations of samples of 0.01–1 g, over a period of 7–12 column volumes. Fractions obtained from this type of chromatography were further purified by 10–20 mg runs on analytical HPLC columns.

Subsequent recognition of alkaloid composition was facilitated by development of conditions for reproducible, quantitated, analytical HPLC separation of the alkaloid mixtures. The conditions were determined (see Experimental) as optimum after extensive trials with different packing materials, solvents and standard alkaloid mixtures* and showed that 13 identifiable peaks were present. The percentage of these various alkaloids found in the mixtures obtained from the shake flask and bioreactor cultures as shown in the HPLC analyses are given in Table 2. The alkaloids ajmalicine (**1**), vallesiachotamine (**2**), hörhammerinine (**3**), hörhammericine (**4**), vindolinine (**5**), 19-epivindolinine (**6**), yohimbine (**7**) and isositsirikine (**8**) were identified from spectral data comparison with that of authentic samples, and by co-injection on analytical HPLC.

In addition to these known alkaloids, four others were isolated. Three of these were assigned the structures 19-acetoxy-11-methoxytabersonine (**9**), 19-hydroxy-11-methoxytabersonine (or vandrikidine) (**10**) [2] and 19-acetoxy-11-hydroxytabersonine (**11**) on the basis of spectral data. The UV spectrum of each had a λ_{max} 328 nm, as was expected for an α-methylene-indoline type. Compound **11** also exhibited a hypsochromic shift on addition of base (λ_{max} 328 to λ_{max} 330 and 272 nm) consistent with a phenolic group. All three compounds displayed the same MS fragmentation pattern, though individual ions varied according to the different substituents (Fig. 1). The major ions **12** and **13** indicated that all three possessed oxygen substituents in the indole nucleus and in the side chain.

Analysis of the NMR spectrum of **9** showed the presence of an indole N—H (at δ 9.4: three proton singlets at δ 3.96, 3.90 (-CO_2Me and Ar-OMe) and δ 2.94 (-OCOMe). The



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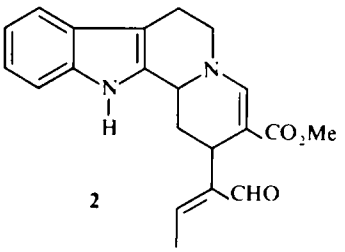

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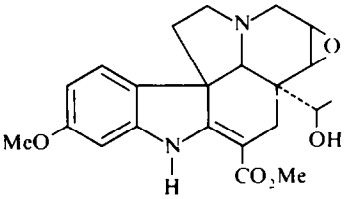

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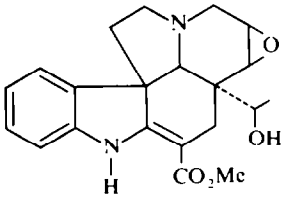

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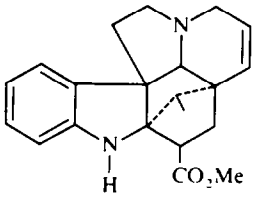

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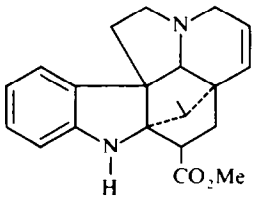

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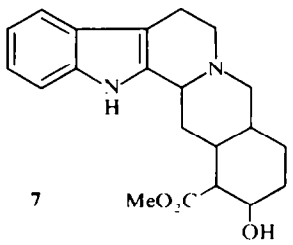

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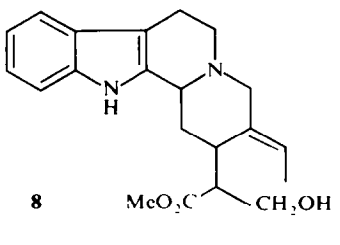

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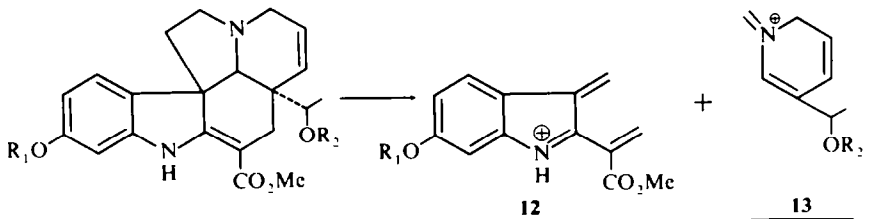
* Kutney, J. P., Choi, L. S. L., Sleigh, S. K. and Worth, B. R., unpublished results. A detailed description of this system will be published elsewhere.



	R_1	R_2	M⁺
9	Me	Ac	424
10	Me	H	382
11	H	Ac	410

12

R_1	*m/e*
Me	244
Me	244
H	214

13

R_2	*m/e*
Ac	193
H	151
Ac	193

Fig. 1. MS data on compounds **9–11**.

C-19 proton appeared as a quartet at δ 4.8 coupled to a three proton doublet at δ 0.97. The acetoxy group was therefore in the side chain and the methoxy group in the aromatic ring. The splitting of the aromatic protons was consistent with substitution at C-11. Finally two olefinic protons could be observed at δ 6.2 and 6.08. The NMR of **10** was very similar to **9** but lacked the presence of an acetoxy group, while that of **11** differed in that only one methoxy signal was present.

Since no direct comparison with authentic material was possible, their spectral data were compared with that of the known 19-hydroxytabersonine, prepared from vindolinine [3]. These comparisons confirmed the assigned structures.

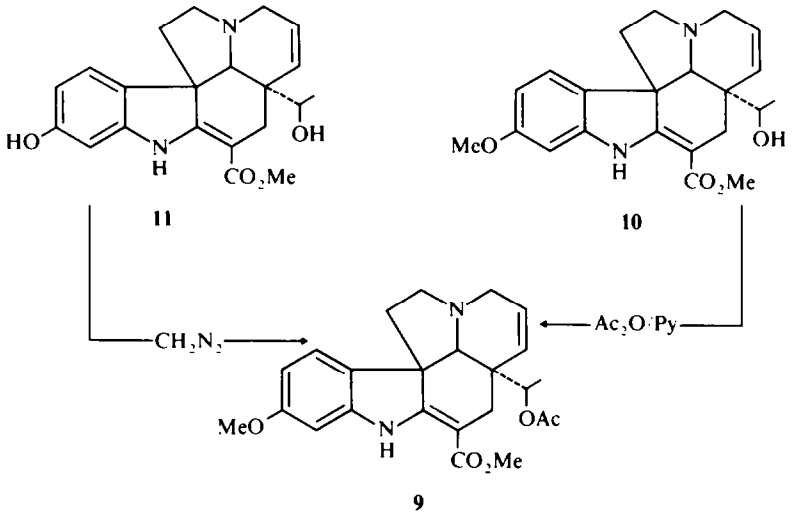


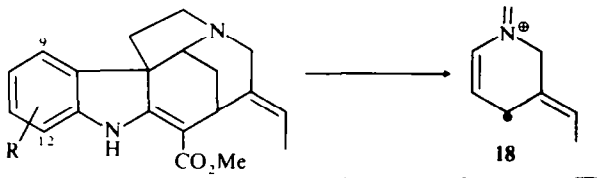
Table 2. Amounts of alkaloids in extracts separated by HPLC

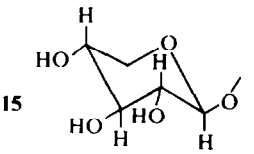
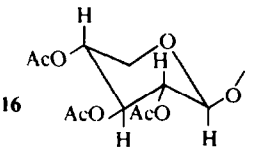
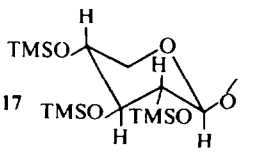
Peak No.	Alkaloid	Alkaloid in crude sample* (%)	
		Sample 5, Table 1	Sample 3, Table 1
1	Glycoside (**15**)	8.8	27.6
2	Unknown	5.2	7.5
3	Unknown	9.8	3.5
4	Yohimbine	—	4.6
5	Isositsirikine	—	4.5
6	Hörhammericine	5.06	3.0
7	Hörhammerinine	3.15	
8	19-*Epi*-vindolinine	2.7	1.5
9	Vindolinine	4.7	2.5
10	Unknown	3.0	
11	Dimethyltryptamine	--	4.11
12	Vallesiachotamine	0.12	0.52
13	Ajmalicine	18.94	7.74

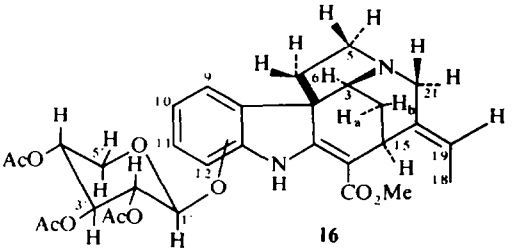
* Based on relative absorbance at 280 nm.

Table 3. NMR data for the triacetate **16**

Proton	Chemical Shift (δ)	Multiplicity	Coupling constant J (Hz)
N-H	8.85	*br.s*	
H-3	4.13	*br.s*	
H-5α	4.38	*br.d*	14
H-5β	3.39	*d*	14
H-6α	2.68	*m*	
H-6β	4.37	*m*	
H-9 (H-12)	6.93	*dd*	6,2
H-10	6.94	*t*	6
H-11	7.26	*dd*	6,2
H-14a	1.47	*br.d*	14
H-14b	2.62	*br.d*	14
H-15	3.65	*br.s*	
C_{18}–H_3	1.69	*br.d*	7
H-19	5.69	*br.q*	7
H-21α	4.56	*dd*	13,4
H-21β	3.61	*dd*	13,2.5
OMe	3.87	*s*	
H-1′α	5.24	*d*	8
H-2′β	5.44	*dd*	8,6
H-3′α	5.18	*dd*	8,6
H-4′β	5.35	*br.m*	$W_{1/2} \sim 10$
H-5′β	4.78	*br.s*	$W_{1/2} \sim 6$
H-5′α	3.31	*dd*	10,6



	R	M^+	M^+ – sugar	*m/e*
14	H	322		121
15		470	338	121
16		596	338	121
17		686	338	121



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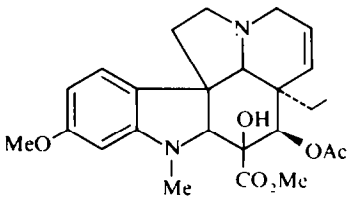
The interrelationships between these compounds were confirmed by chemical interconversion. Treatment of **10** with acetic anhydride/pyridine afforded **9**, as did methylation of **11** with diazomethane.

A fourth very polar compound was isolated from the methanol-soluble alkaloid fraction and assigned the β-glycoside structure **15**. Its UV spectrum with maxima at 290 and 327 nm, together with IR absorption bands at 1602 and 1665 cm^{-1} suggested the enamino-ester function noted above in the alkaloids **9–11** and in the strychnos alkaloid akuammicine (**14**). The MS of **15** and several of its derivatives (**16** and **17**) revealed an ion at *m/e* 121 (**18**) generally considered characteristic of the strychnos series and thus favored placing this new alkaloid within this group. The molecular ion of **15** occurred at *m/e* 470 and a significant ion at *m/e* 338 suggested loss of $C_5H_8O_4$, the latter being possibly attributed to a C_5-carbohydrate unit. Confirmation of the latter could be obtained from acetylation of **15** which provided a triacetate **16** (*m/e* 596 (M^+), $C_{31}H_{36}N_2O_{10}$) in which again loss of the acetylated carbohydrate unit (*m/e* 338, $M^+ - 258$) could be seen by MS. In similar fashion the trimethylsilyl ether derivative **17** revealed an ion at *m/e* 338 corresponding to the expected loss from the molecular ion.

Of particular importance were the very instructive NMR spectra (determined at 270 MHz) of **15** and its derivatives. The triacetate **16** revealed a series of well-separated proton signals and by appropriate decoupling experiments all the protons in the molecule could be assigned. Table 3 summarizes the NMR data and allows the assignment of structure **16** to the triacetate derivative. It was now clear that the carbohydrate unit attached to the aromatic ring was xylose with the only ambiguity being its attachment at C_9 or C_{12} since the NMR data did not allow a clear distinction between these alternatives. Unfortunately additional supplies of alkaloid were unavailable so that further studies to settle this point could not be undertaken.

It is clear that the '943' cell line of *C. roseus* provides a rather broad spectrum of alkaloids with representatives from the corynanthe, strychnos and aspidosperma types of compound. Members of the iboga family were, however, not observed during these studies. The possibility that such systems are present cannot be excluded since a number of very minor components in the mixture remain unidentified.

Each batch of cells was also monitored both by HPLC and radioimmunoassay for the presence of vindoline (**19**), the major component of the leaves of *C. roseus*, but no indication of its presence was found.



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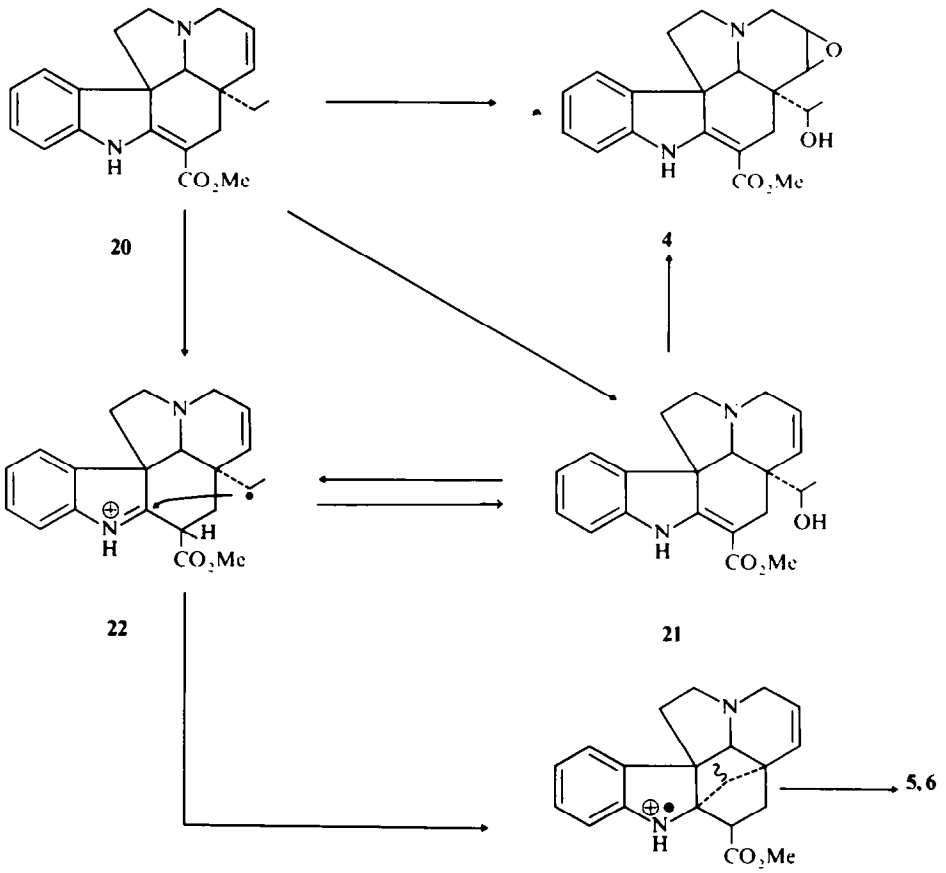

Fig. 2.

From a biosynthetic standpoint a possible interrelation between the aspidosperma-type alkaloids found in the '943' cell line is suggested in Fig. 2. Here activation of the saturated side chain of tabersonine (**20**), perhaps by enzyme mediated hydrogen abstraction, could provide the intermediate **22** which in turn cyclizes to **23** and finally to vindolinine (**5**) and 19-*epi*-vindolinine (**6**). It is also feasible to consider that the free radical intermediate **22** undergoes oxygenation to a peroxide species which on reduction would yield the hydroxyethyl side chain observed in one of isolated alkaloids (**4**) or that some alternative enzyme hydroxylation reaction occurs to yield **21**. It is plausible to consider that **21** then undergoes epoxidation to **4**. Such biogenetic considerations imply that formation of the isolated alkaloids **4**, **5** and **6**, via enzymic activation of the ethyl side chain in **20**, consumes the necessary tabersonine system required for elaboration to vindoline (**19**), the latter bearing additional oxygen functionality in ring C. On this basis it would appear that the '943' cell line lacks the required enzymes for ring C functionalization but yet maintains enzymatic activity associated with side chain functionalization.

It is also noteworthy that none of the alkaloids isolated of the aspidosperma type contain an *N*-methyl function present in vindoline and thus the '943' cell line appears to lack this type of methylating enzyme. Attempts to overcome these enzymatic deficiencies are currently under study in our laboratories.

EXPERIMENTAL

General. All mps are uncorr. ^{1}H NMR data are presented in δ units (ppm) downfield from internal TMS. MS were determined at 70 eV. Analytical HPLC was carried out on a Waters Associates ALC 100 modified to incorporate a 440 UV detector, and Data Module using dual channel detection at 254 and 280 nm. Separation was achieved using a Waters Radial Compression Module fitted with a reverse phase pack, at a flow rate of 4 ml/min. Known alkaloids were identified by comparison of spectral data (MS, NMR, IR, UV) with that of authentic samples, by mmp and by TLC and co-injection on analytical HPLC.

Procedures for culture growth. The two procedures described below are typical conditions employed for the large scale propagation of cultures in bioreactor and shake flasks. The details on initiation of callus and and cell suspension cultures are given under Experimental in ref. [1].

Growth in bioreactor (samples 1 and 2, Table 1). The inoculum was grown up in 1B5 medium in shake flasks over a period of *ca* 4 days. To 5 l. of Zenk's alkaloid production medium [4] contained in a 7.5 l. Microferm bioreactor, 500 ml of inoculum was added. Under agitation at 200 rpm and aeration of 35 ml/l./min at 26° the growth of the cultures was allowed to proceed for 3 weeks. Samples were withdrawn at various intervals and analysed for mitotic index (MI), dry cell wt and pH. The results of these analyses are given in Table 4.

Sample 3 (Table 1). Employing the procedure described above another 500 ml of inoculum was introduced into a 7.5 l. bioreactor and the growth was allowed to proceed for a 2 week period. As before the sample analyses were taken and the data follow in Table 5.

Harvesting of the cells after the 2 week period provided 145 g of freeze-dried cells.

Growth in shake flasks (sample 4, Table 1). A small piece of callus was added to a 250 ml Erlenmeyer flask containing 100 ml of 1B5 medium and shaken for 5 days on a gyratory shaker (130 rpm) at 27° in continuous light. After this period the resulting cell suspension was transferred to a 500 ml Erlenmeyer flask containing 250 ml of Zenk's alkaloid production medium and agitation continued for a further 3 weeks. Harvesting provided 95 g of freeze-dried cells.

Table 4.

Day	MI (%)	Dry wt (mg/10 ml culture)	pH
0	0.1	12.6	
1	0	13.5	
2	0	14.3	6.04
3	0	15.4	6.0
6	1.8	25.4	6.16
8	1.3	30.6	5.87
10	1.5	30.0	6.07
13	1.2	78.1	5.89
17	1.1	113.0	6.03
20*	0	116.0	6.38
21	0	122.4	6.28

* At this point, L-tryptophan (500 mg) and sucrose (5 g) were fed continuously over a 24 hr period. After 1 day (day 21) the culture was harvested to provide 114 g of freeze-dried cells.

Standard extraction procedure. A typical extraction of freeze-dried cells was carried out as follows: Freeze-dried cells (7.8 g, sample 1, Table 1) were suspended in MeOH (100 ml) and extracted using an ultrasonic bath for 4 hr. The suspension was filtered and the solid re-extracted (2 × 100 ml). The extracts were combined and concd *in vacuo*. The residue was suspended in 1 N HCl (30 ml) and washed with EtOAc (4 × 30 ml). The aq. soln was neutralized ($NaHCO_3$), the pH adjusted to *ca* 9.5 (1 N NaOH) and extracted with EtOAc (4 × 30 ml). The combined extracts were dried and concd to afford the crude alkaloids (35 mg). This mixture was further fractionated by trituration to give 25 mg of CH_2Cl_2-soluble material. The residue (soluble in MeOH) contained very few detectable, and only in one case isolable alkaloids.

Alkaloid isolation from bioreactor samples. A typical procedure is as follows: The crude CH_2Cl_2-soluble fraction (180 mg, sample 3, Table 1) was chromatographed over Porasil B (150 g, Waters Associates) in a stainless steel column (30 × 2.5 cm) with H_2O–MeCN (68:32) containing 0.1% Et_3N modifier, at a flow rate 18 ml/min. A total of 40 × 25 ml fractions were collected and the column then eluted with MeCN (400 ml, fraction 41). The 41 fractions were analysed by HPLC [reverse phase packing, H_2O–MeCN (62:38) containing 0.1% Et_3N at 4 ml/min, detection at both 254 and 280 nm].

Fractions 23–28 were combined and concd to give a yellow foam (20.5 mg). Purification of the three major components was

Table 5.

Day	MI (%)	Dry wt (mg/10 ml culture)	pH
0	0.6	14.9	5.01
3	0.5	22.0	6.28
7	0.5	49.9	6.17
10	0.1	74.3	
14	0	81.3	6.35

achieved by HPLC (10 × 2 mg injections; Radial Compression Pak A, H_2O MeCN (62:38) with 0.1% Et_3N, at 4 ml/min, monitored at 254 and 280 nm) to give: a compound (2 mg, peak No. 4, Table 2) identical with an authentic sample of yohimbine (**7**), a sample (3 mg, peak No. 5) identical with isositsirikine (**8**) and 5 mg of *N,N*-dimethyltryptamine (peak No. 11) identical with an authentic sample.

Fractions 29-33 gave 6.5 mg of material which on purification by HPLC (3 × 2.1 mg injections) yielded 2.0 mg of material (peak No. 6) identical with an authentic sample of hörhammericine (**4**) and 2.5 mg of material (peak No. 7) identified as hörhammerinine (**3**) on the basis of the following data: mp 208–210° (d), [lit. [5,6] 209.5–211° (d)]; UV λ_{max}^{MeOH} nm: 325, 300 and 242; ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.87 (1H, *s*, NH), 3.81 (3H, *s*, OMe), 3.79 (3H, *s*, OMe), 1.12 (3H, *d*, J = 7 Hz, Me): MS *m/e* 398 (M^+), 380, 368, 244, 184, 155, 154 (bp). (Found: M^+ 398.1859, $C_{22}H_{26}N_2O_5$ requires: 398.1842). *Fractions* 34–39 gave 20.5 mg of material which was further purified by HPLC (5 × 4 mg injections) to provide: 6 mg of 19-*epi*-vindolinine (**6**) (peak No. 8) and 8.5 mg of vindolinine (**5**) (peak No. 9) identical with respective authentic samples. *Fraction* 41 (45 mg) was triturated with MeOH to give ajmalicine (**1**) (20 mg), mp 251–252° (lit. [7] 253–254°) identical with an authentic sample. The remainder was concd and fractionated by HPLC (5 × 5 mg injections, as before using H_2O–MeCN, 2:3) to give: 1 mg of vallesiachotamine (**2**, peak No. 12) identical with an authentic sample; 19-acetoxy-11-methoxytabersonine (**9**) (2 mg); UV λ_{max}^{MeOH} nm: 328 and 240; ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.4 (1H, *s*, NH), 7.20 (1H, *d*, J = 8 Hz, C-9), 6.40 (1H, *br. s*, C-12), 6.38 (1H, *br. d*, J = 8 Hz, C-10), 5.90 (1H, *dd*, J = 10 and 5 Hz, C-14), 5.82 (1H, *d*, J = 10 Hz, C-15), 4.8 (1H, *q*, J = 7 Hz, C-19), 3.80 (3H, *s*, OMe), 3.76 (3H, *s*, OMe), 1.94 (3H, *s*, OAc), 0.97 (3H, *d*, J = 7 Hz, C-18); MS *m/e* 424 (M^+), 382, 259, 244, 193, 151, 138. (Found: 424.1989, $C_{24}H_{28}N_2O_5$ requires: 424.1990); 19-hydroxy-11-methoxytabersonine (**10**) (2.5 mg) UV λ_{max}^{MeOH} nm: 328 and 240; ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.86 (1H, *s*, NH), 7.20 (1H, *d*, J = 8 Hz, C-9), 6.40 (1H, *dd*, J = 8 and 2 Hz, C_{10}), 6.38 (1H, *d*, J = 2 Hz, C-12), 5.90 (1H, *dd*, J = 10 and 5 Hz, C-14), 5.82 (1H, *d*, J = 10 Hz, C-15), 3.77 (3H, *s*, OMe), 3.76 (3H, *s*, OMe), 3.35 (1H, *q*, J = 7 Hz, C-19), 0.90 (3H, *d*, J = 7 Hz, C-18); MS *m/e* 382 (M^+), 381, 258, 244, 198, 191, 189, 151, 149, 147, 121, 119 and 117; M* 175. (Found: 382.1870, $C_{22}H_{26}N_2O_4$ requires 382.1872); 19-acetoxy-11-hydroxytabersonine (**11**) (0.5 mg) UV λ_{max}^{MeOH} nm: 328 and 240; $\lambda_{max}^{MeOH+1NNaOH}$ nm: 330 and 280; ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.0 (1H, *br. s*, NH), 3.68 (3H, *s*, OMe), 1.90 (3H, *s*, OAc), 0.95 (3H, *d*, J = 7 Hz, C-18). MS *m/e* 410 (M^-), 409, 296, 278, 263, 245, 244, 184, 148, 144, 143. (Found: 410.1879, $C_{23}H_{26}N_2O_5$ requires: 410.1882). Acetylation of **10** using Ac_2O–pyridine gave a sample identical with **9**. Methylation of **11** with CH_2N_2 in Et_2O also gave **9** identical with that isolated above.

The crude MeOH-solubles (76 mg) (sample 3, Table 1) was purified by HPLC (Radial compression Pak A, H_2O–MeCN (68:32) with 0.1% Et_3N as before) to give the glycoside (**15**) (20 mg): UV λ_{max}^{MeOH} nm: 327 and 290; $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–3200, 1665 and 1601; ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.29 (1H, *br. s*, NH), 6.99 (1H, *d*, J = 8.1 Hz, Ar-H), 6.94 (1H, *d*, J = 8.1 Hz, Ar-H), 6.80 (1H, *t*, J = 8.1 Hz, Ar-H), 5.37 (1H, *br. q*, J = 6 Hz, H-19), 4.75 (1H, *d*, J = 9 Hz, H'-1' of sugar), 3.78 (3H, *s*, CO_2Me), 1.6 (3H, *d*, J = 6 Hz, C-18), 1.3 (1H, *br. d*, H-14); MS *m/e* 470 (M^+ + 1), 470 (M^+), 338, 169, 168, 149, 143, 130, 121, 107 and 92 (Found: 470.2050, $C_{25}H_{30}N_2O_7$ requires: 470.2047). The glycoside (8 mg) was acetylated with Ac_2O-pyridine in the normal manner to afford, on work-up, the triacetate (**16**) (6 mg); UV λ_{max}^{MeOH} nm: 328 and 289; $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1675, 1650; ^{1}H NMR (270 MHz, $CDCl_3$): see Table 3; MS *m/e* 597 (M^+ + 1), 596 (M^-), 581, 565, 537, 496, 380, 338, 279, 259, 231, 199, 167, 157, 149, 139, 121, 107, 97, 92 (Found: 596.2379, $C_{31}H_{36}N_2O_{10}$ requires 596.2388). The TMSi derivative of the glycoside was prepared in the usual manner: MS *m/e* 686 (M^+), 671, 655, 622, 609, 596, 549, 535, 437, 414, 338, 256, 213, 185, 149, 130, 121, 107 and 92.

Alkaloid isolation from shake flask samples. The procedure was described earlier for the bioreactor samples. Separation of the crude CH_2Cl_2-soluble fraction (313 mg, sample 5 Table 1) by prep. HPLC again resulted in 41 fractions. Yohimbine (**7**) (2 mg) and isositsirikine (**8**) (3 mg) were isolated from fractions 21–25. Fractions 26–30 gave hörhammericine (**4**) (3 mg) and hörhammerinine (**3**) (2.5 mg). While fractions 35–40 afforded 19-*epi*-vindolinine (**6**) (1 mg) and vindolinine (**5**) (5 mg). The final fraction contained a quarter of the alkaloids by weight (85 mg) of which the major component was found to be ajmalicine (**1**) (50 mg). Of the minor components, vallesiachotamine (**2**) (0.5 mg), 19-acetoxy-11-methoxytabersonine (**9**) (2 mg), 19-hydroxy-11-methoxytabersonine (**10**) (1 mg) and 19-acetoxy-11-hydroxytabersonine (**11**) (0.5 mg) were isolated in the same manner as described above. Chromatography of the MeOH-soluble fraction (89 mg) afforded the akuammicine derivative (**15**) (5 mg).

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