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# FORMATION OF CATHARANTHINE, AKUAMMICINE AND VINDOLINE IN *CATHARANTHUS ROSEUS* SUSPENSION CELLS

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**Key Word Index**—*Catharanthus roseus*; Apocynaceae; plant tissue culture; biosynthesis; indole alkaloids; catharanthine; akuammicine; vindoline.

**Abstract**—Catharanthine and akuammicine, together with ajmalicine and strictosidine, were isolated from a culture strain of *Catharanthus roseus* suspension cells. The biosynthetic capability of the cultured cells to produce akuammicine, catharanthine and vindoline was confirmed by feeding experiments with DL-tryptophan-[3-$^{14}$C] to yield the radioactive alkaloids.

## INTRODUCTION

During the past few years important information about the biosynthesis of *Corynanthé*-type alkaloids has been obtained with cell-free systems of *Catharanthus roseus* cultured cells [1, 2]. However, the hypothetical pathway [3] for the biosynthesis of *Iboga*-, *Aspidosperma*- and *Strychnos*-type alkaloids remains untested since proof for presence of any indole alkaloids beyond the *Corynanthé*-type in the cultured cells is not yet rigorous. There is preliminary evidence for akuammicine (**1**) [4] and vindoline (**2**) [5] in *C. roseus* callus based on TLC data, but a note has also appeared to the effect that catharanthine (**3**), vindoline (**2**) and dimeric alkaloids are not found in the callus [6]. In this paper we describe evidence for the production of catharanthine (**3**) (*Iboga*), akuammicine (**1**) (*Strychnos*) and vindoline (**2**) (*Aspidosperma*) by a strain of *C. roseus* suspension cultures.

## RESULTS AND DISCUSSION

Callus tissues derived from *C. roseus* seedlings were maintained for more than 1 year on SH medium [7] in Petri dishes. Distinguishable suspension cultures initiated from callus tissues produced in different Petri dishes have been maintained for a further year as culture strains before screening their alkaloid compositions. One strain (CRW) was found to have an enriched alkaloid composition, including akuammicine (**1**) and catharanthine (**3**), as well as ajmalicine (**4**) and strictosidine (**5**).

These alkaloids were isolated and identified by means of chromatographic, physicochemical and spectroscopic methods. The content of catharanthine (**3**) (2 mg from 5.1 kg wet cells) in this strain is similar to that of ajmalicine (**4**) (1.9 mg from 5.1 kg wet cells). A small amount of akuammicine (**1**) was also isolated. However, vindoline (**2**), which is another main indole alkaloid of *C. roseus* intact plants, could not be isolated from any of the culture strains, although a feeding of tryptophan-[$^{14}$C] to the suspension cultures indicated the production of this alkaloid. Since tryptophan is a precursor of the indole alkaloids [8–10], DL-tryptophan-[3-$^{13}$C] was fed to the suspension cultures of the callus, leading to a positive incorporation of the tracer into akuammicine (**1**), vindoline (**2**), catharanthine (**3**) and ajmalicine (**4**), as shown in Table 1.

Table 1. Incorporation of DL-tryptophan-[3-$^{14}$C] into some indole alkaloids by suspension cultures of *C. roseus* cells

| Feeding time (hr) | 24 | 48 |
|---|---|---|
| Fresh wt of cells (g) | 17.8 | 21.7 |
| Tryptophan fed (dpm) | $5.02\times10^{7}$ | $5.02\times10^{7}$ |
| Tryptophan recovered (dpm) | $5.17\times10^{6}$ | $4.71\times10^{6}$ |
| Total radioactivity of alkaloids fr. (dpm) [% Incorp.]* | $3.92\times10^{6}$ [7.8] | $4.07\times10^{6}$ [8.1] |
| Akuammicine (**1**) isolated (dpm) [% Incorp.]* | $9.49\times10^{3}$ [0.019] | $1.05\times10^{4}$ [0.021] |
| Vindoline (**2**) isolated (dpm) [% Incorp.]* | $8.02\times10^{2}$ [0.0016] | $9.14\times10^{2}$ [0.0018] |
| Catharanthine (**3**) isolated (dpm) [% Incorp.]* | $7.51\times10^{4}$ [0.15] | $1.02\times10^{5}$ [0.20] |
| Ajmalicine (**4**) isolated (dpm) [% Incorp.]* | $9.52\times10^{4}$ [0.19] | $1.33\times104$[0.27] |

* No adjustment made for recovered precursor.

The modified capability of this strain to produce complex indole alkaloids has been retained for at least 4 months (8 generations). The present study shows that production of *Strychnos*-, *Aspidosperma*- and *Iboga*-type alkaloids can be retained (or induced) in some cultured cells. The genetic basis of such a cellular variation for alkaloid biosynthesis remains unclear. However, it is of interest to note that the isolation of this culture strain should lead to the elucidation of the biosynthesis of *Strychnos*-, *Aspidosperma*- and *Iboga*-type alkaloids in cell-free systems and that it may be possible to obtain variant cell lines producing dimeric alkaloids of the vinblastine type by further selection in the near future.

## EXPERIMENTAL

*Plant material and culture method.* Callus tissues were induced from seedlings of *C. roseus* (L.) G. Don on SH medium [7] containing 0.8% agar. Subcultures have been maintained on the same medium for more than a year. Suspension cultures were initiated by inoculating callus tissues into liquid SH medium and subcultured in the same medium. Suspension cells were incubated on a rotary shaker (*ca* 100 rpm) under dim light at *ca* 27°. For isolation of alkaloids the cultured cells were grown at room temp. in a 14 l. fermentor (New Brunswick Microferm).

*Extraction and isolation of alkaloids.* After 14 days culture, suspension cells (5.1 kg wet wt) were harvested and extracted with MeOH. After removal of solvent, the residue was diluted with $K_2CO_3$ soln and then extracted with $CHCl_3$ to give an oily mass (271 mg). Crude oil was subjected to TLC on Si gel plates (1 mm) using 4 different solvent systems [(i) $CHCl_3$–MeOH, 17:3, (ii) petrol–$Me_2CO$–diethylamine, 7:2:1, (iii) petrol–$CHCl_3$–$Me_2CO$, 8:5:4 and (iv) $Et_2O$] and HPLC (Waters equipment) using a $\mu C_{18}$-Bondapak column eluted with MeOH–10 mM K-Pi (pH 7.2) (3:2) at a flow rate of 2 ml/min, to afford catharanthine (**3**) (2 mg) [UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$): 291 (3.86), 283 (3.91), 224 (4.55); CD (*c* $9.6\times10^{-4}$, EtOH) $[\theta]_{273}+12\,000$, $[\theta]_{239}-26\,200$; MS *m/e* (rel. int.): 336 (70, $M^+$), 135 (100); $^1$H NMR($CDCl_3$): $\delta$1.13 (*t*, 3H, $J=7$ Hz), 3.77 (*s*, 3H), 4.79 (*s*, 1H), 6.26 (*ed*, 1H, $J=6$ Hz), 7.93 (*bs*, 1H)] and akuammicine (**1**) (125 $\mu$g) [UV $\lambda_{max}^{MeOH}$: 328, 296, 225; $[\alpha]_D^{25}-700°$ (*c* 0.01, MeOH); MS *m/e* (rel. int.) 322 (10, $M^+$), 121 (100); $^1$H NMR ($CDCl_3$): $\delta$ 1.57 (*d*, 3H, $J=6$ Hz), 3.73 (*s*, 3H), 5.30 (*q*, 1H, $J=6$ Hz)]. In addition, ajmalicine (**4**) (1.9 mg) [identified with direct comparison of mmp, co-TLC, co-HPLC, UV and IR] and strictosidine (**5**) (4.5 mg) [direct comparison of its co-TLC, co-HPLC and IR] were also isolated.

*Feeding of* DL-*tryptophan*-[3-$^{14}$C] *into* C. roseus *cells.* Labeled tryptophan (0.44 mol, $5.02\times10^7$ dpm) dissolved in $H_2O$ was fed to suspension cells cultured for 1 week prior to feeding. After incubation for 1 or 2 days (see Table 1) at 27° under dim light, the callus was extracted with MeOH, then re-extracted with $CHCl_3$ in the same manner as above to afford crude alkaloids. After addition of carrier materials (each 10 mg), the crude alkaloids were subjected to 2-D TLC [Si gel; solvent (i) $CHCl_3$–MeOH, 17:3 and (ii) $Et_2O$] to afford catharanthine, akuammicine, ajmalicine and vindoline, which were recrystallized repeatedly to constant sp. act. Results are summarized in Table 1.

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