ALKALOID PRODUCTION IN CATHARANTHUS ROSEUS CELL CULTURES. III.[1] CATHARANTHINE AND OTHER ALKALOIDS FROM THE 200GW CELL LINE.

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Abstract - The production of catharanthine by suspension cultures of the "200GW" cell line from *Catharanthus roseus* is reported. Other alkaloids isolated are vallesiachotamine isomers, ajmalicine, hörhammerinine, hörhammericine, vindolinine, 19-epivindolinine and strictosidine lactam.

The bisindole alkaloids vinblastine and vincristine are well known clinical agents in cancer chemotherapy. Due to their extremely low concentration in Catharanthus roseus their isolation is tedious and costly and furthermore the established toxicity observed during patient treatment with these agents also limits their use. Efforts to alleviate such problems have been directed toward partial or total syntheses in the hope that such studies would not only replace the plant isolation but, in addition, would afford novel synthetic bisindole analogues with broader spectra of activity and lower toxicity. A large number of publications from our and other laboratories[2] have shown that catharanthine (1) and its derivatives in the form of the corresponding N-oxide intermediate can be coupled with vindoline (2) or its derivatives to afford the requisite bisindole systems. Under appropriate conditions[3] reproducible high yields (>60%) of vinblastine analogues can be obtained thereby providing an efficient route from 1 and 2. Although the latter substances are available from C. roseus their isolation is also rather costly and we have turned our attention to the possibility of propagating these alkaloids in plant tissue cultures.

Various laboratories have made attempts to produce alkaloids from C. roseus tissue cultures and the earliest results date back to the 1960's[4-9]. Unfortunately none of the published results have recorded production of the target alkaloid catharanthine (1). Recently A.I. Scott[10] has informed us of his results in this area and we would now like to report our success in producing 1 in a cell line coded as "200GW".

During the past few years[1,11] several hundred serially cultured cell suspensions derived from 3 cultivars of C. roseus were established in our programme and subsequently shown to produce representative alkaloids of the Corynanthé, Strychnos and Aspidosperma families. Various individual cell lines proven stable over long (up to two years) time periods were grown in Gamborg's B5 medium[11] and then transferred to Zenk's alkaloid production medium[9] for large scale preparations. One of the most recently developed lines coded as "200GW" has proven to be our first producer of catharanthine (1).

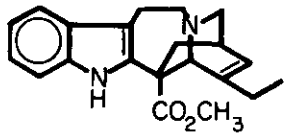
The "200GW" cell line was propagated and extracted under conditions described earlier[1,11] and gave its own "spectrum" of alkaloids (Table 1) including one of the target molecules, catharanthine (1). Here ca 140 g batches of freeze dried cells gave a crude mixture of alkaloids (520 mg, 0.37%) which was fractionated by HPLC[1] to give (1) (7 mg, 0.005% dry cell wt, identical with an authentic sample) together with the vallesiachotamine isomers (3) and (4), ajmalicine (5), hörhammericine (6), hörhammerinine (7), vindolinine (8), 19-epivindolinine (9), and strictosidine lactam (10).

TABLE I

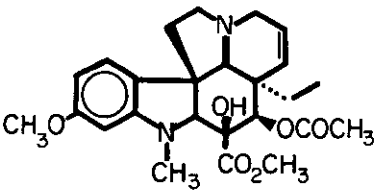
Alkaloids isolated from the 200GW Cell Line

Alkaloid	%† Yield from dry cell wt.	%† of crude alkaloid mixture
1	0.005	1.35
3	0.015	4.05
4	0.026	7.02
5	0.006	1.62
6	0.002	0.54
7	0.005	1.44
8	0.002	0.54
9	0.002	0.54
10	0.224	60.48

† % figures refer to isolated yields.



1



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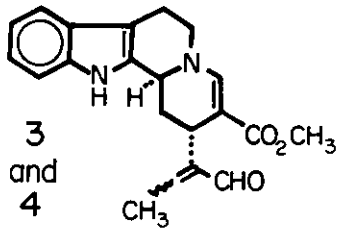
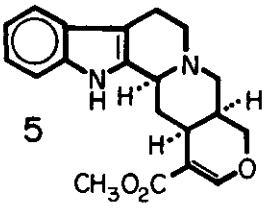


3 and 4



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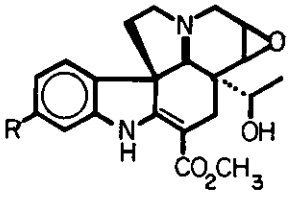
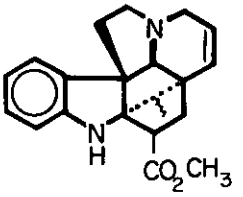
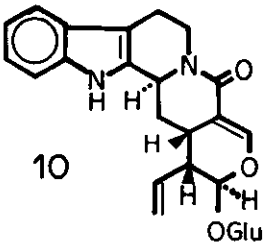


6 R=H
7 R=OCH$_3$



8 and 9



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The "200GW" cell line described here provides catharanthine (1) in amounts ca three times that obtainable from C. roseus plant material (generally ca 0.0017% yield from dried plant material). It appears that this line contains larger amounts of 1 than that recently developed by A.I. Scott and his group[10]. In this latter study suspension cultures initiated from callus tissue of C. roseus seedlings and maintained on SH medium were employed.

Vindoline (2), another major alkaloid in C. roseus was not detected although it is interesting to note that strictosidine lactam (10), a substance not normally found in significant amounts in this plant, is a predominant component in this cell line.

Experiments designed to optimize the production of 1 in this and several other cell lines are presently underway.

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