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A Model for the Biosynthesis of Indole Alkaloids by Cell Suspensions of *Catharanthus roseus* G. Don

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ABSTRACT

The kinetics of alkaloid formation in a plant cell culture of *Catharanthus roseus* G. Don are shown to follow the differential equations:

$$\dot{P}_1(t) = a\dot{X}(t - \Delta t) - (b + c)P_1(t)$$

$$\dot{P}_2(t) = bP_1(t) - cP_2(t)$$

Where X, P_1, and P_2 are measures of the cell dry weight, intracellular ajmalicine, and serpentine, respectively. From the parameter estimates, it is evident that carry over of kinetin to the second-stage from the first-stage growth medium can have a major effect on alkaloid biosynthesis. This effect appears to be connected with the inhibition of the oxidation of ajmalicine to serpentine.

Index Entries: Biosynthesis of alkaloids; cell suspensions; *Catharanthus roseus*, a model for alkaloid biosynthesis; mathematical simulation of ajmalicine and serpentine production; alkaloid regulation by kinetin; differential equations.

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INTRODUCTION

Plant cell culture is presently receiving wide-spread attention as an alternative source of phytochemicals (*1–4*). Using more conventional agricultural cropping techniques, these substances are often in short supply and the quality of the product cannot always be guaranteed. This fact was highlighted ten years ago when the codeine crop failed, and has been more recently illustrated by the production of shikonin, using cells of *Lithospermum erythorhizon,* an herb that is fast becoming extinct in its native southeast Asia. However, as illustrated by the aforementioned example, when the yield has been increased to greater than 800-fold that of the plant, it is possible to increase the yield of the desired product, using suspension culture technology. Other examples may also be cited (*5*).

Catharanthus roseus is a source of the indole alkaloids, ajmalicine and serpentine, used in the treatment of circulatory disorders. This paper seeks to describe the formation of these alkaloids and to relate this to the growth of the cell culture. Such a model allows growth and product curves to be compared in a quantitative fashion with consequent improvements in reliability.

The data used in this exercise is that of Stafford et al. (*6*), together with some additional unpublished date (*7*). The paper describes two experiments. In the first, the inocula are prepared by routine subculture into Gamborg's B5 medium (*8*) at 14-d intervals and then transferred to an alkaloid induction medium, MZ, at various times after subculture. In the second experiment, the inocula, obtained from the same source culture as in the first experiment, are subcultured at intervals of either 7 or 14 d, respectively, in medium HB5; this medium lacks kinetin, but is otherwise identical to medium B5. In both experiments cell dry weight and the alkaloids, ajmalicine and serpentine, are monitored.

MATERIALS AND METHODS

The procedures are described in ref. (*6*). The compound identified as "ajmalicine," using similar criteria to the serpentine identification, cannot be distinguished from its stereoisomer tetrahydroalstonine (P. Morris and H. Woodhead, personal communication).

MODELING

(i) Growth Kinetics

From previous studies, it was discovered that growth (expressed as dry weight) could best be described by the Gompertz growth curve, previously used to describe the growth of tumors (*9*). This may be converted to a discrete time model, which belongs to the class of log–linear models.

A log–linear model has been previously applied to the growth and product formation of *C. roseus* cells and is reported elsewhere (*10*).

In the present study, the Gompertz curve was fitted to each growth curve, and the parameters were chosen to minimize the sum of the squares of the residuals, weighted by the standard errors of each measurement.

(ii) Product Kinetics

The alkaloids, ajmalicine and serpentine, are secondary metabolites of *C. roseus*. Thus, their production is often uncoupled from growth and primary metabolism (*11*) and follows the idiophase–trophophase type of relationship described for microbial secondary metabolism (*12*).

This paper reports how closely alkaloid biosynthesis may be predicted from models derived from primary metabolism.

The mechanisms assumed to operate are:

(a) Ajmalicine is assumed to be produced in direct proportion to dry weight gain.
(b) Ajmalicine is converted to serpentine, with a yield of 1.0, by an irreversible reaction.
(c) Both ajmalicine and serpentine "leak" from the cell mass to the medium. Such leakage may be the result of cell permeability and/or cell rupture. It is assumed that the alkaloid concentration within the cells is much greater than that of the medium and that the loss of alkaloid is directly proportional to the amount present.

(iii) Equations

The assumptions of (i) and (ii) result in the following differential equations:

$$\dot{X}(t) = \alpha X(t) \text{In } [\hat{X}/X(t]$$

$$\dot{P}_1(t) = a\dot{X}(t) - (b + c)P_1(t)$$

$$\dot{P}_2(t) = bP_1(t) - cP_2(t)$$

Where: X is the cell dry weight in g/L; P_1 is a measure of the concentration of intracellular ajmalicine/vol of culture (mg/L); P_2 is a measure of the concentration of intracellular serpentine/vol of culture (mg/L); a is a constant of specific productivity; b is the rate constant of the oxidation reaction; c is the rate of leakage of the alkaloids from the cell; and the dot ($\cdot$) signifies differentiation with respect to time.

It should be noted that the values of P_1 and P_2 do not represent the concentration of ajmalicine and serpentine within the cell, but an average value of the concentration of the intracellular alkaloids in the culture.

(iv) Parameter Estimation

The model equations were integrated, using a Runge–Kutta–Fehlberg variable step integrator (*13*), and the parameters were estimated by the method of weighted least squares, using the algorithm of Gill and Murray (*14*).

RESULTS AND DISCUSSION

(i) Parameter estimates obtained for the data of Expt. 1 are shown in Table 1.

Generally, the simulations based on all the inocula gave a much poorer prediction of alkaloid concentration than those based on the one single run.

Figures 1–3 show the measurements of dry weight, intracellular ajmalicine, intracellular serpentine, their errors, and the five model trajectories, with parameters chosen for each run.

(ii) Parameter estimates for the data of Expt. 2 are shown in Table 2.

Applying the same model to the inocula AV6 (14-d subculture) and AV7 (7-d subculture) resulted in negative estimates of the leakage parameter, *c*. The parameter was therefore set to zero under the assumption of no leakage of alkaloids from the cell. It is perhaps not surprising that "*c*" is effectively zero in Expt. 2, given that Expt. 2 is of shorter duration than Expt. 1 and that the "*c*" parameter estimates in Expt. 1 suggest cell permeability increases with cell age. One major factor still omitted from the model, but clearly apparent from the data, is the time lag between the

TABLE 1
Parameter Estimates for Cells Subcultured into Gamborg's B5 Medium at 14-d Intervals

Inoculum, days at transfer	Parameter estimates		
	a, mg/g	*b*, d^{-1}	*c*, d^{-1}
4	7.6	0.095	0.04
7	4.6	0.154	0.023
11	5.24	0.15	0.047
15	6.16	0.13	0.074
22	15.4	0.12	0.373[a]
All	6.7	0.118	0.09

[a]Of the estimates for the 22-d inoculum, the estimate "*b*" has been frozen at a value of 0.12 and the estimates *a* and *c* are optimal values given this choice. Without this procedure, parameters *a*, *b*, and *c* took values 100 or 1000 times greater than those of the other four inocula.

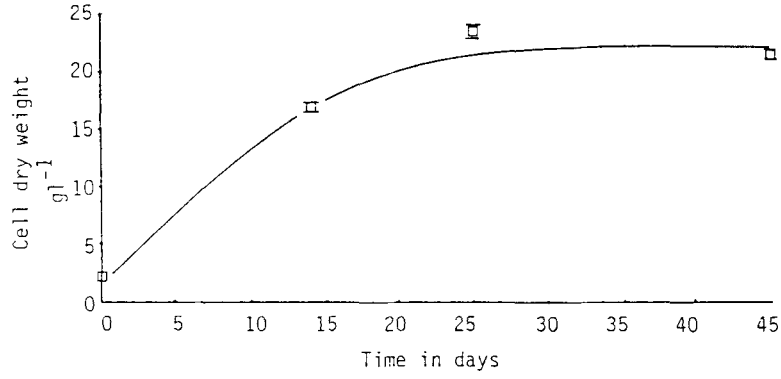

Fig. 1a. Observed and model dry weight concentration for cells transferred from B5 to MZ medium after 4 d. □, Experimentally determined dry weight; ═, errors for experimentally determined dry weight; continuous line represents the "model" dry weight concentration.

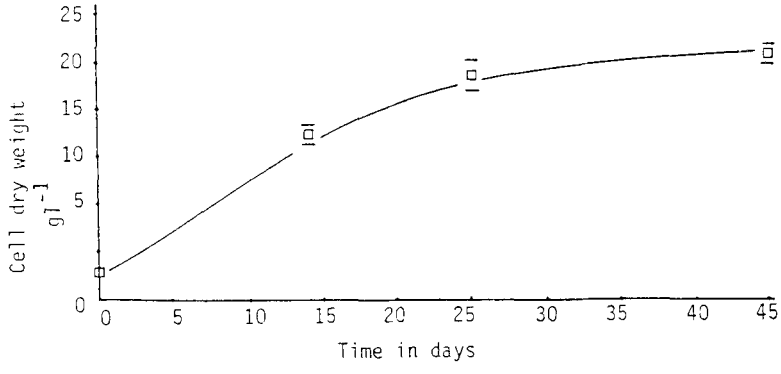

Fig. 1b. Observed and model dry weight concentration for cells transferred from B5 to MZ medium after 7 d. □, Experimentally determined dry weight; ═, errors for experimentally determined dry weight; continuous line represents the "model" dry weight concentration.

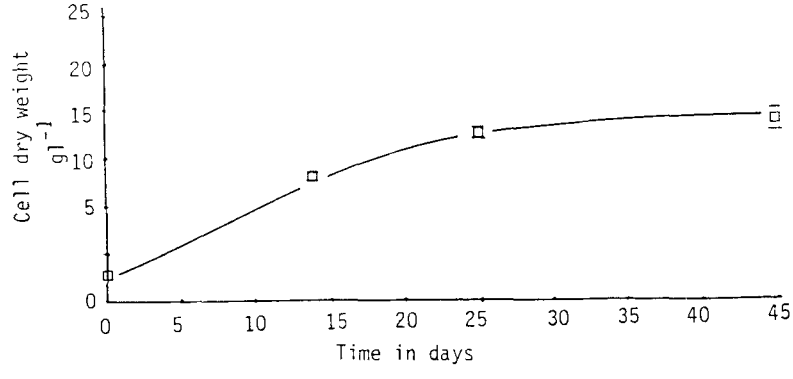

Fig. 1c. Observed and model dry weight concentration for cells transferred from B5 to MZ weight after 11 d. □, Experimentally determined dry weight; ═, errors for experimentally determined dry weight; continuous line represents the "model" dry weight concentration.

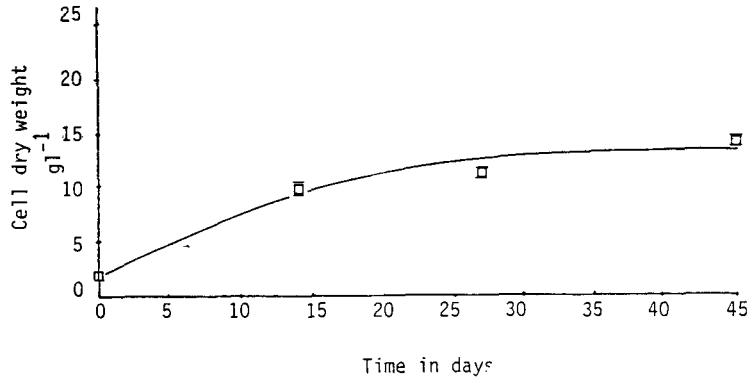

Fig. 1d. Observed and model dry weight concentration for cells transferred from B5 to MZ weight after 15 d. □, Experimentally determined dry weight; =, errors for experimentally determined dry weight; continuous line represents the "model" dry weight concentration.

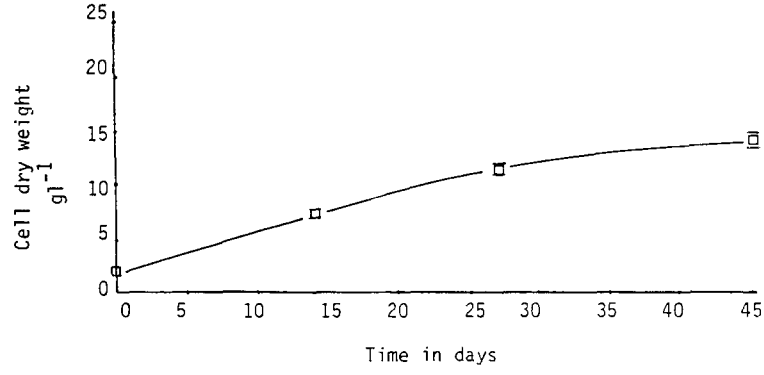

Fig. 1e. Observed and model dry weight concentration for cells transferred from B5 to MZ medium after 22 d. □, Experimentally determined dry weight; =, errors for experimentally determined dry weight; continuous line represents the "model" dry weight concentration.

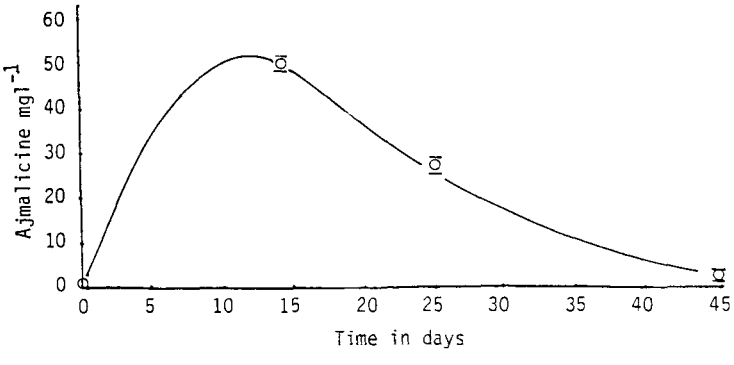

Fig. 2a. Observed and model ajmalicine concentration for cells transferred from B5 to MZ medium after 4 d. ○, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

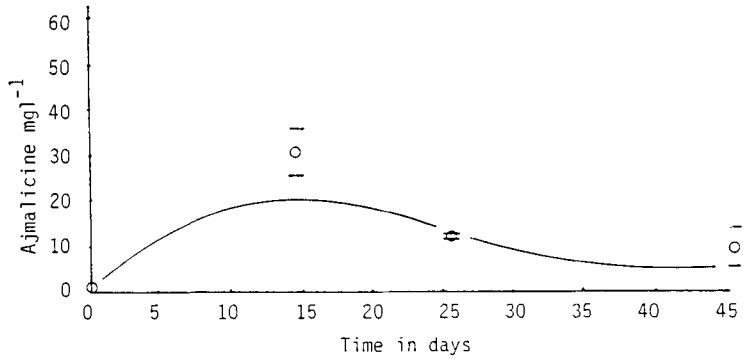

Fig. 2b. Observed and model ajmalicine concentration for cells transferred from B5 to MZ medium after 7 d. ○, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

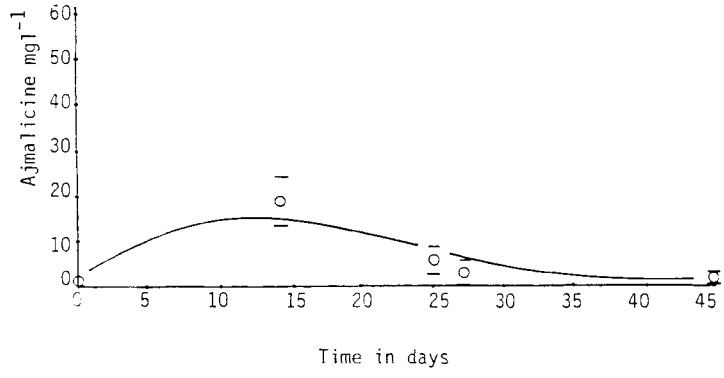

Fig. 2c. Observed and model ajmalicine concentration for cells transferred from B5 to MZ medium after 11 d. ○, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

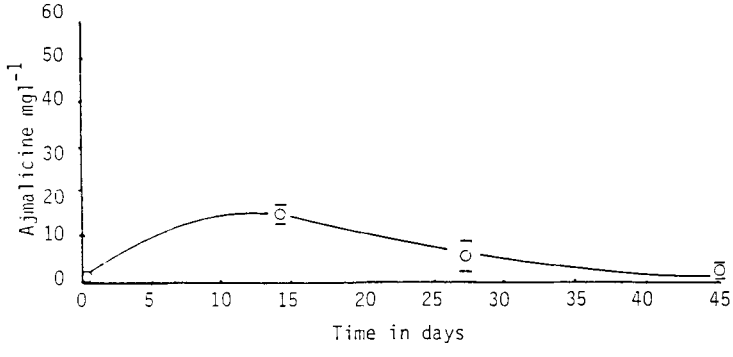

Fig. 2d. Observed and model ajmalicine concentration for cells transferred from B5 to MZ medium after 15 d. ○, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

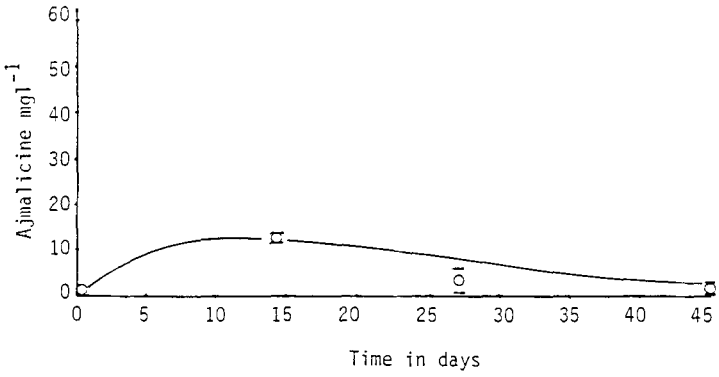

Fig. 2e. Observed and model ajmalicine concentration for cells transferred from B5 to MZ medium after 22 d. ○, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

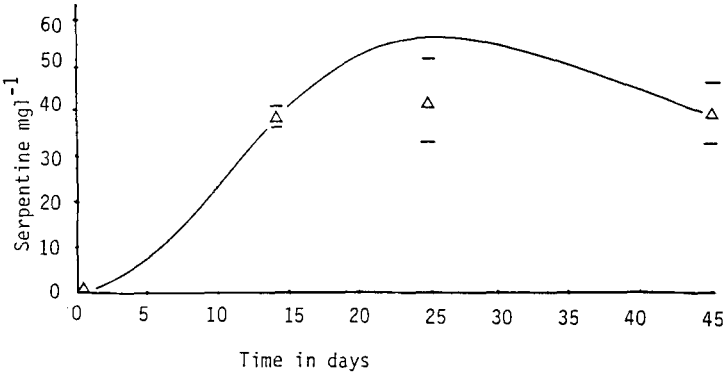

Fig. 3a. Observed and model serpentine concentration for cells transferred from B5 to MZ medium after 4 d. △, Experimentally determined serpentine concentration; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

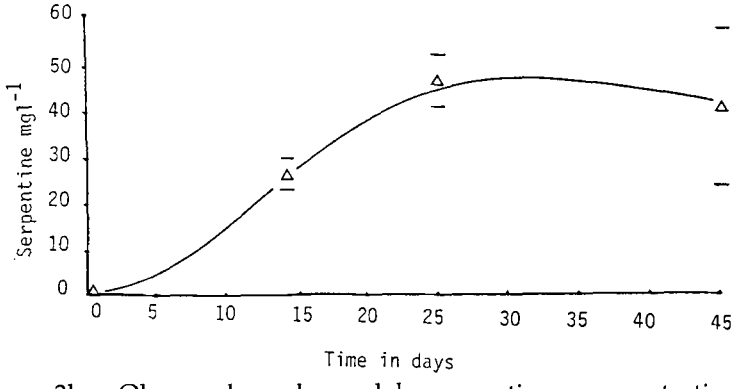

Fig. 3b. Observed and model serpentine concentration for cells transferred from B5 to MZ medium after 7 d. △, Experimentally determined serpentine concentration; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

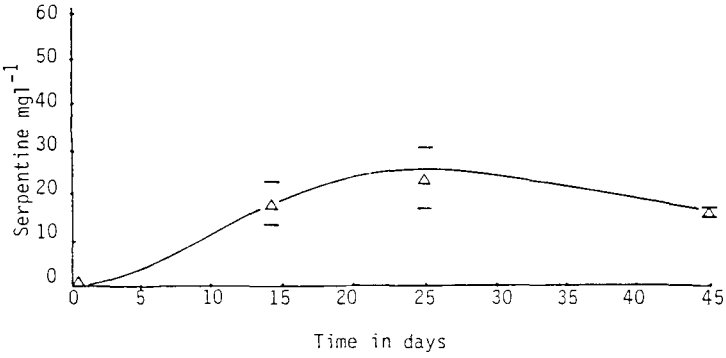

Fig. 3c. Observed and model serpentine concentration for cells transferred from B5 to MZ medium after 11 d. △, Experimentally determined serpentine concentration; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

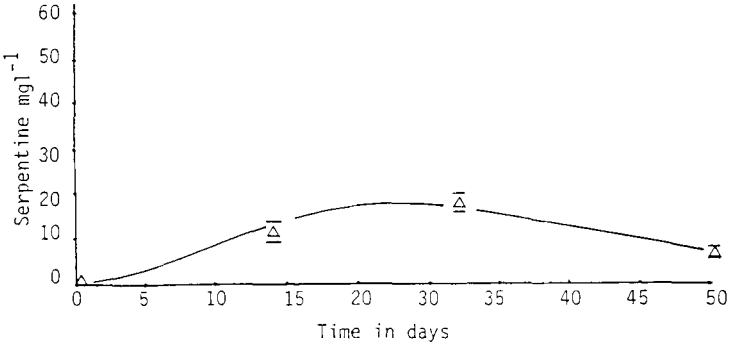

Fig. 3d. Observed and model serpentine concentration for cells transferred from B5 to MZ medium after 15 d. △, Experimentally determined serpentine concentration; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

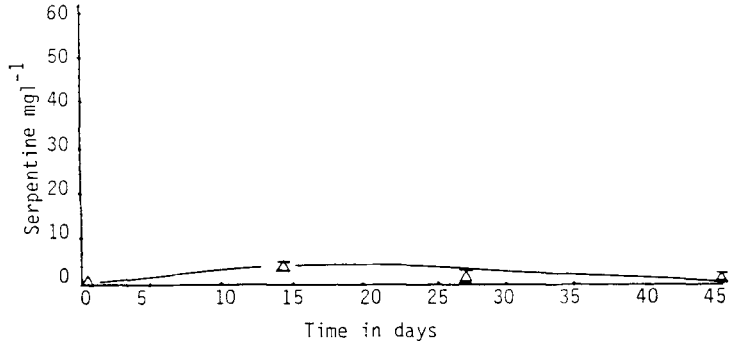

Fig. 3e. Observed and model serpentine concentration for cells transferred from B5 to MZ medium after 22 d. △, Experimentally determined serpentine concentration ; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

TABLE 2
Parameter Estimates for Cells Subcultured into Kinetin-Free HB5 Medium at either 14- or 7-d Intervals

	Parameter estimates			
	a, mg/g	b, d^{-1}	c, d^{-1}	Δt, d
AV6	6.2	0.7	0	2.8
AV7	8.0	0.7	0	2.8

inoculation of the production medium and the production of ajmalicine. This factor is included in the model as follows:

$$\dot{P}_1(t) = a\dot{X}(t - \Delta t) - (b + c)P_1(t)$$

Such a time lag has been used to describe the delay in penicillin biosynthesis (*15*).

As a result of this time lag, parameters were chosen by "trial and error" to describe the general form of the alkaloid curves. Figures 4–6 show the data and model trajectories for this experiment.

The models defined represent a simple description of the processes affecting the alkaloid content of the cells. It is recognized that the effect of medium and intracellular pH has not been considered, although this has been shown to play a significant part in the alkaloid accumulation mechanism (*16*). Furthermore, very little account has been taken of cell age, and no explicit dependence on medium composition has been considered. However, the model shares many common features of the experimental observations and it offers a small set of descriptive parameters that may be used to compare the alkaloid production of different media.

Comparison of the parameter estimates in Expts. 1 and 2 indicate that the main effect of kinetin in the growth medium (on product synthesis in the production medium) is to inhibit the oxidation of ajmalicine to serpentine. The rate constant of this reaction is increased by a factor of

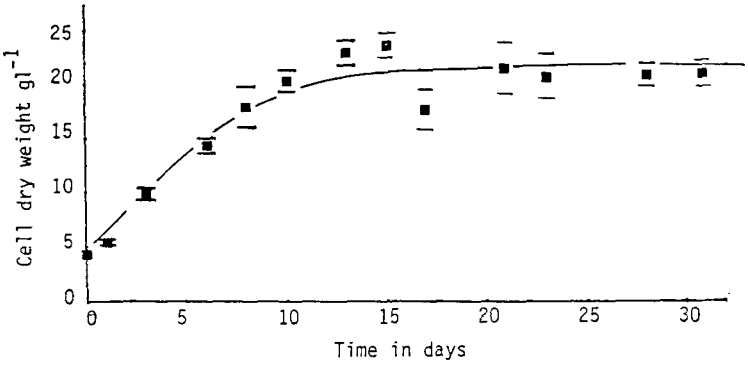

Fig. 4a. Observed and model dry weight concentration for inoculum AV7. ■, Experimentally determined dry-weight concentration; =, errors for experimentally determined dry weight; continuous line represents "model" dry weight.

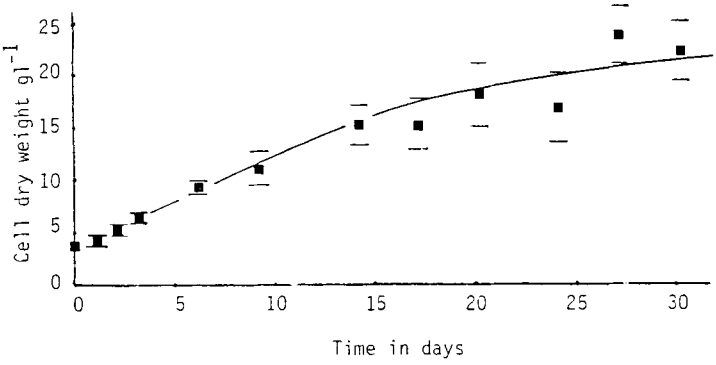

Fig. 4b. Observed and model dry weight concentration forr inoculum AV6. ■, Experimentally determined dry-weight concentration; =, errors for experimentally determined dry weight; continuous line represents "model" dry weight.

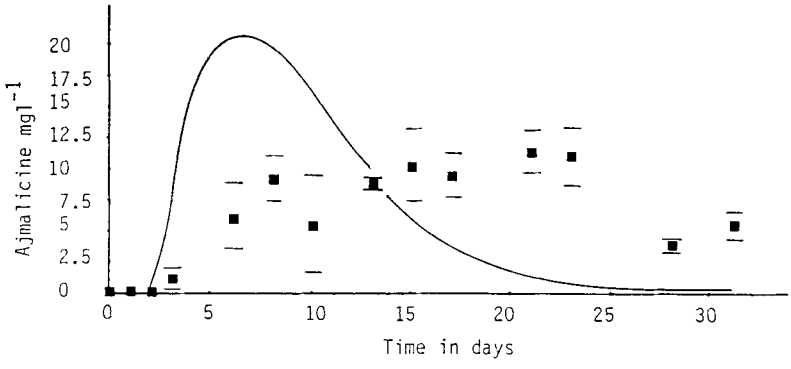

Fig. 5a. Observed and model ajmalicine concentration for inoculum AV7. ■, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

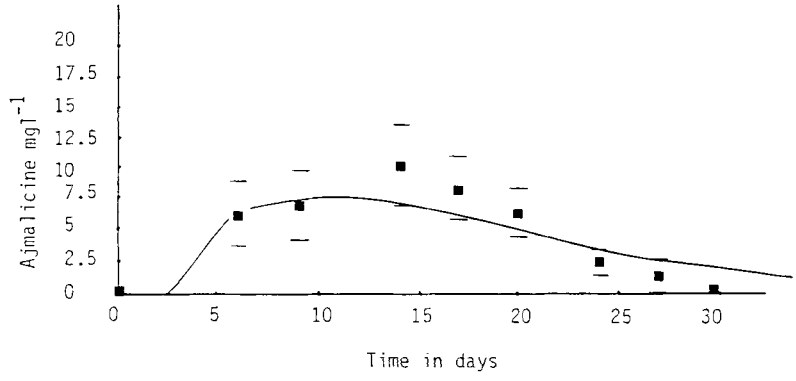

Fig. 5b. Observed and model ajmalicine concentration for inoculum AV6. ■, Experimentally determined ajmalicine concentration; =, errors for experimentally determined ajmalicine; continuous line represents the "model" ajmalicine concentration.

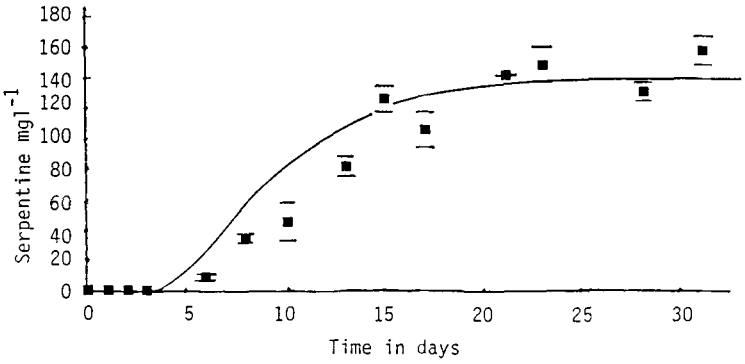

Fig. 6a. Observed and model serpentine concentration for inoculum AV7. ■, Experimentally determined serpentine concentration; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

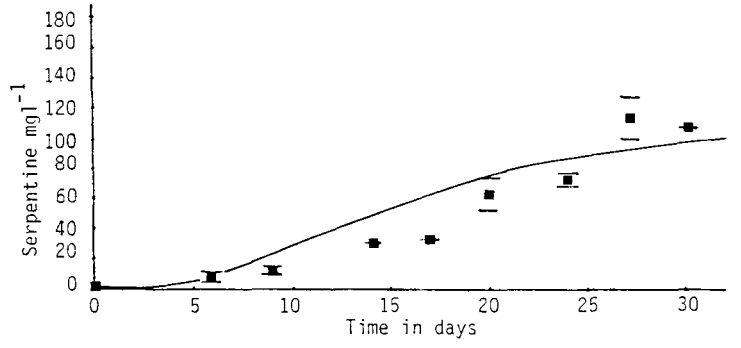

Fig. 6b. Observed and model serpentine concentration for inoculum AV6. ■, Experimentally determined serpentine concentration; =, errors for experimentally determined serpentine; continuous line represents the "model" serpentine concentration.

five when kinetin-free growth medium is used. It is possible that this may be a result of reduced enzyme synthesis, since Simpkins and Street (*17*) have noted that kinetin inhibits oxygen uptake, growth, and protein synthesis (but not amino acid synthesis) in *Acer pseudoplatanus*.

The omission of a time lag in the results of Expt. 1 may be attributed to the incomplete record of alkaloid accumulation during the initial 15 d.

CONCLUSION

A simple model of alkaloid synthesis has been produced. This has been applied to two sets of experimental results. Comparison of the parameter estimates has suggested possible mechanisms, relating changes in the inoculum medium to changes in product synthesis.

ACKNOWLEDGMENTS

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