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# MICROCALORIMETRIC STUDY ON THE BACTERIOSTATIC ACTIVITY OF ISOQUINOLINE ALKALOIDS

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The isoquinoline alkaloids were isolated from traditional Chinese drugs of Phellodendri Cortex, Radix Stephaniae Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana. The power–time curves of growth of *E. coli* at different concentrations of isoquinoline alkaloid at 37°C were determined by a 2277 Thermal Activity Monitor. The rate constant of bacteriostastic activity was calculated. The relationship between growth rate constant and concentration was established. The optimum bacteriostastic concentration was determined. Experimental results have indicated that all the isoquinoline alkaloids isolated from the four kinds of traditional Chinese drugs have bacteriostastic activity and the order is Phellodendri Cortex>Radix Stephaniae Tetrandrae>Corydalis Yanhusuo>Corydalis Bungeana.

***Keywords:*** *bacteriostastic activity, E. coli, growth rate constant, isoquinoline alkaloid, microcalorimetry*

## Introduction

Alkaloid is a very important bioactive substance. It is one of the most important classes of secondary metabolites due to their interesting physiological activities [1]. Isoquinoline is a significant heterocyclic template that is presented in a variety of natural products and pharmaceuticals [2, 3]. Isoquinoline alkaloid is widespread in alkaloid family and constitutes an important class of compounds in pharmaceuticals, agrochemicals and dyestuffs [4]. With the development of isolation technique and synthetic strategy, isoquinoline alkaloid chemistry has developed fast, and its remarkable bioactivity and medical value, such as anticancer, anti-infective and bacteriostastic property, have received much attention recently [5–11]. Moreover, the microcalorimetric method has been a very useful one to study the biological system, such as the growth metabolism of cell or bacterium, DNA mutation, enzyme catalysis reaction, drug metabolism, biological oscillation and so on [12–20].

In this paper, we isolated isoquinoline alkaloids from the traditional Chinese drugs of Phellodendri Cortex, Radix Stephaniae Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana and studied systematically the bacteriostastic activity of isoquinoline alkaloid by microcalorimetric method. The power–time curve of growth of *E. coli* was determined at different concentrations ($c$) of isoquinoline alkaloid. The rate constant ($\mu$) of bacteriostastic activity was calculated. The relationship of $\mu$ *vs*. $c$ was established. The optimum bacteriostastic concentration was determined.

## Theoretical model

Bacterial metabolic process was determined under isothermal and isochoric conditions; supply of nutrient matter and oxygen was limited, the growth process of *E. coli* was inhibited and the exponential model [21] could not be used in this process.

In the growth phase, theoretical model is in accordance with the following formula:

$$\frac{dN_t}{dt}=\mu N_t \pm \beta N_t^2 \tag{1}$$

where $\mu$ is the growth rate constant; $\beta$ the promotive or bacteriostastic rate constant; $N_t$ the bacterial number at time $t$; plus (+) represents the promotive process and minus (–) represents the bacteriostastic process. If the power produced by every bacterium is $P_0$, then

$$P_t=P_0N_t \tag{2}$$

and accordingly

$$\frac{dP_t}{dt}=\mu P_t \pm \left(\frac{\beta}{P_0}\right)P_t^2 \tag{3}$$

The integral of Eq. (3) is given by

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$$P_t^{-1} = \left( P_0^{-1} \mp \frac{\beta}{\mu P_0} \right) e^{-\mu t} \pm \frac{\beta}{\mu P_0} \qquad (4)$$

Through the experimental data of $P_t$ and $t$ obtained from the power–time curves, the growth rate constants (μ) were calculated according to Eq. (4).

## Experimental

### *Materials*

The *E. coli* was employed. A beef extract soluble medium (pH 7.2–7.4) was used, containing NaCl (1 g), peptone (2 g), beef extract (1 g) and different concentrations of isoquinoline alkaloids from Phellodendri Cortex, Radix Stephaniae Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana in each 200 mL. The isolated method of isoquinoline alkaloid was given in [22–24].

### *Method*

The complete cleaning and sterilization procedure for the flow tubing was as follows: sterilized distilled water, 0.10 mol $L^{-1}$ HCl, 0.10 mol $L^{-1}$ NaOH and 75% ethanol solution were pumped through the system for 30 min at a flow rate of 30 mL $h^{-1}$. Finally sterilized distilled water was pumped through the system for 30 min at a flow rate of 10 mL $h^{-1}$ and the baseline was determined. After a stable baseline was obtained, the bacterial sample, medium and isoquinoline alkaloid solution of different volume were pumped into the flow cell system, and the monitor began to record the power–time curves of continuous growth for *E. coli*. When the recording pen returned to the baseline, the process of *E. coli* growth was completed.

### *Instrument*

A 2277 Thermal Activity Monitor (Thermometric AB, Sweden) was used to determine the power–time curves of *E. coli* growth. With this instrument, reaction can be carried out between 10 and 90°C. It was maintained at a temperature within $\pm 2\cdot 10^{-4}$°C.

The detection limit was 0.15 μW and the baseline stability (over a period of 24 h) was 0.20 μW. The performance of this instrument and the details of its construction have been described previously [25, 26].

## Results and discussion

### *Determination of power–time curves*

The power–time curves of *E. coli* with different concentrations of isoquinoline alkaloid from Phellodendri Cortex, Radix Stephaniae Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana have been determined at 37°C, respectively. The parts of curves in exponential growth phase of *E. coli* were shown in Figs 1–4.

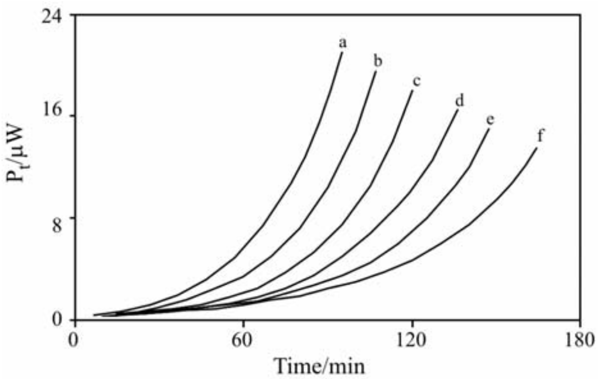


**Fig. 1** Power–time curves in exponential growth phase of *E. coli* with different concentrations of Phellodendri Cortex at 37°C; a – 0, b – $0.4475\cdot 10^{-3}$, c – $0.895\cdot 10^{-3}$, d – $1.790\cdot 10^{-3}$, e – $2.685\cdot 10^{-3}$ and f – $3.222\cdot 10^{-3}$ g $mL^{-1}$

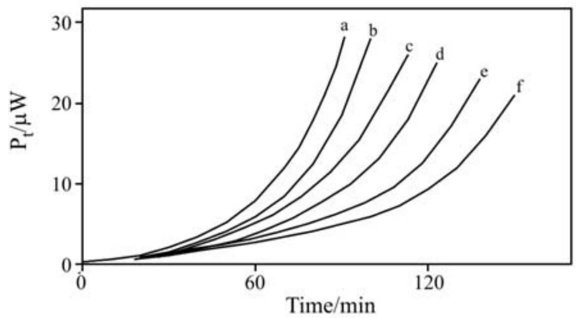


**Fig. 2** Power–time curves in exponential growth phase of *E. coli* with different concentrations of Radix Stephaniae Tetrandrae at 37°C; a – 0, b – $0.2312\cdot 10^{-3}$, c – $1.156\cdot 10^{-3}$, d – $2.312\cdot 10^{-3}$, e – $3.468\cdot 10^{-3}$ and f – $4.624\cdot 10^{-3}$ g $mL^{-1}$

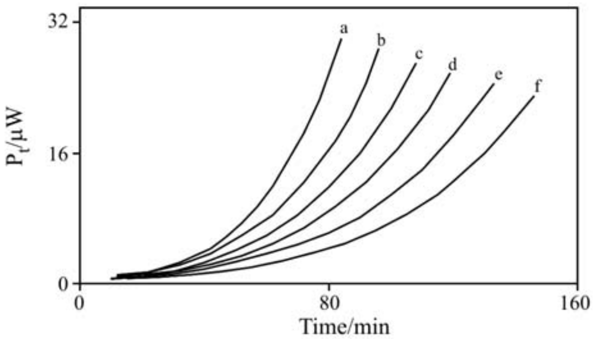


**Fig. 3** Power–time curves in exponential growth phase of *E. coli* with different concentrations of Corydalis Yanhusuo at 37°C; a – 0, b – $1.222\cdot 10^{-3}$, c – $2.443\cdot 10^{-3}$, d – $4.886\cdot 10^{-3}$, e – $7.329\cdot 10^{-3}$ and f – $9.772\cdot 10^{-3}$ g $mL^{-1}$

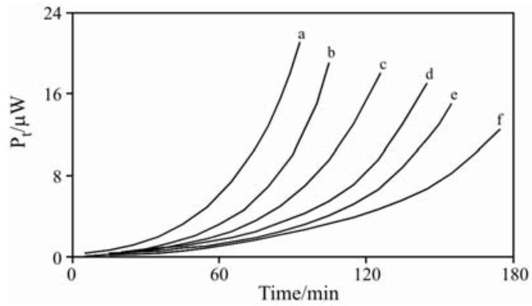


**Fig. 4** Power–time curves in exponential growth phase of *E. coli* with different concentrations of Corydalis Bungeana at 37°C; a – 0, b – $1.088\cdot10^{-3}$, c – $2.176\cdot10^{-3}$, d – $8.704\cdot10^{-3}$, e – $1.306\cdot10^{-2}$ and f – $1.741\cdot10^{-2}$ g $mL^{-1}$

*Determination of the growth rate constant* (μ)

From the data of Figs 1–4, the growth rate constants (μ) were calculated according to Eq. (4). The data were shown in Tables 1–4.

**Table 1** Growth rate constants (μ) at different concentrations of isoquinoline alkaloid isolated from Phellodendri Cortex

| $C/\cdot10^{-3}$ g $mL^{-1}$ | $\mu/min^{-1}$ | $r$* |
|---|---|---|
| 0 | 0.04305 | 0.9998 |
| 0.4475 | 0.03831 | 0.9997 |
| 0.8950 | 0.03421 | 0.9999 |
| 1.790 | 0.02972 | 0.9996 |
| 2.685 | 0.02567 | 0.9999 |
| 3.222 | 0.02315 | 0.9993 |

*correlation coefficient

**Table 2** Growth rate constants (μ) at different concentrations of isoquinoline alkaloid isolated from Radix Stephaniae Tetrandrae

| $C/\cdot10^{-3}$ g $mL^{-1}$ | $\mu/min^{-1}$ | $r$* |
|---|---|---|
| 0 | 0.04305 | 0.9998 |
| 0.2312 | 0.03700 | 0.9998 |
| 1.156 | 0.03118 | 0.9993 |
| 2.312 | 0.02829 | 0.9995 |
| 3.468 | 0.02605 | 0.9979 |
| 4.624 | 0.02399 | 0.9975 |

*correlation coefficient

**Table 3** Growth rate constants (μ) at different concentrations of isoquinoline alkaloid isolated from Corydalis Yanhusuo

| $C/\cdot10^{-3}$ g $mL^{-1}$ | $\mu/min^{-1}$ | $r$* |
|---|---|---|
| 0 | 0.04305 | 0.9991 |
| 1.222 | 0.03602 | 0.9994 |
| 2.443 | 0.03173 | 0.9985 |
| 4.886 | 0.02855 | 0.9991 |
| 7.329 | 0.02611 | 0.9995 |
| 9.772 | 0.02493 | 0.9993 |

*correlation coefficient

**Table 4** Growth rate constants (μ) at different concentrations of isoquinoline alkaloid isolated from Corydalis Bungeana

| $C/\cdot10^{-3}$ g $mL^{-1}$ | $\mu/min^{-1}$ | $r$* |
|---|---|---|
| 0 | 0.04305 | 0.9998 |
| 1.088 | 0.03736 | 0.9998 |
| 2.176 | 0.03136 | 0.9997 |
| 8.704 | 0.02681 | 0.9996 |
| 13.06 | 0.02340 | 0.9998 |
| 17.41 | 0.02147 | 0.9986 |

*correlation coefficient

*Relationship of* μ *vs. C*

The μ *vs. C* curves were shown in Figs 5–8 and the established corresponding equations were listed in Table 5, respectively.

According to the equation of, the optimum bacteriostatic concentrations of isoquinoline alkaloids from Phellodendri Cortex, Radix Stephaniae Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana were determined to be $7.023\cdot10^{-3}$, $1.617\cdot10^{-2}$, $3.586\cdot10^{-2}$ and $4.906\cdot10^{-2}$ g $mL^{-1}$, respectively.

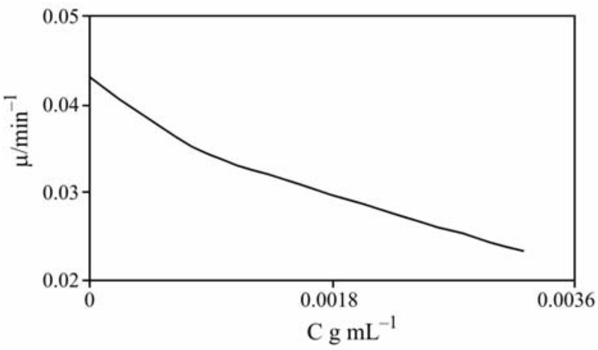


**Fig. 5** μ *vs. C* curve of isoquinoline alkaloid of Phellodendri Cortex

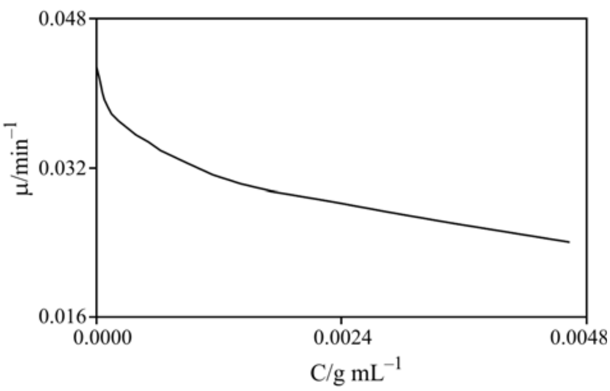


**Fig. 6** μ *vs. C* curve of isoquinoline alkaloid of Radi Stephaniae Tetrandrae

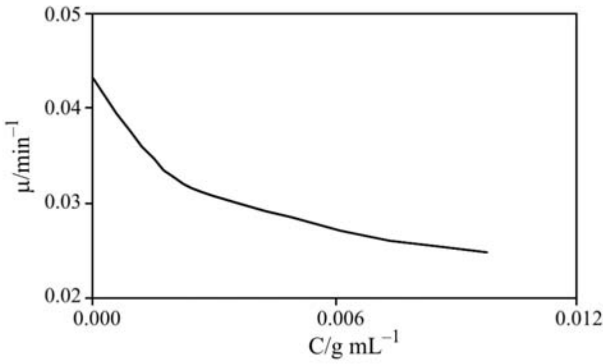


**Fig. 7** μ *vs*. *C* curve of isoquinoline alkaloid of Corydalis Yanhusuo

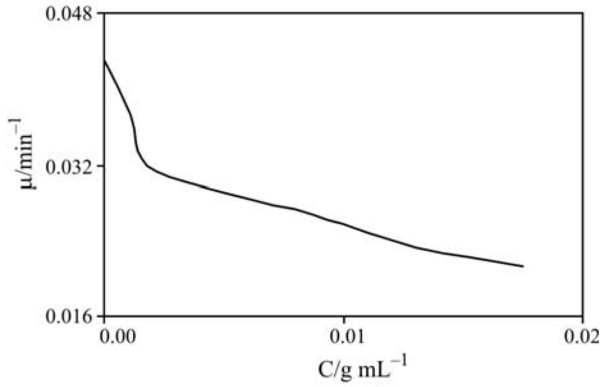


**Fig. 8** μ *vs*. *C* curve of isoquinoline alkaloid of Corydalis Bungeana

**Table 5** Relationship of μ *vs*. *C* of isoquinoline alkaloid

| Traditional Chinese drugs | Equation of μ–*C* | *r* |
|---|---|---|
| Phellodendri Cortex | $\mu=-5.8663C+0.0412$ | 0.9856 |
| Radix Stephaniae Tetrandrae | $\mu=-2.0597C+0.0333$ | 0.9968 |
| Corydalis Yanhusuo | $\mu=-0.9341C+0.0335$ | 0.9815 |
| Corydalis Bungeana | $\mu=-0.6645C+0.0326$ | 0.9955 |

Comparing the results of bacteriostatic action of the four kinds of isoquinoline alkaloids, we found that all the isoquinoline alkaloids have bacteriostatic activity and it is clear that the order of bacteriostatic activity is Phellodendri Cortex>Radix Stephaniae Tetrandrae>Corydalis Yanhusuo>Corydalis Bungeana.

## Conclusions

Isoquinoline alkaloids were isolated from four kinds of traditional Chinese drugs. The power–time curves of *E. coli* at different concentrations of isoquinoline alkaloids from Phellodendri Cortex, Sephania Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana were determined using a 2277 Thermal Activity Monitor. On the basis of the experimental results and theoretical model, the growth rate constant and the optimum bacteriostatic concentration were calculated. The results have indicated that isoquinoline alkaloids isolated from Phellodendri Cortex, Radix Stephaniae Tetrandrae, Corydalis Yanhusuo and Corydalis Bungeana possess bacteriostatic activity and the order of bacteriostatic activity is Phellodendri Cortex>Radix Stephaniae Tetrandrae>Corydalis Yanhusuo>Corydalis Bungeana. The results of the present study are significant for the drug screening and the synthesis of specific medicine.

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