# Cryptolepine Hydrochloride Effect on *Staphylococcus aureus*

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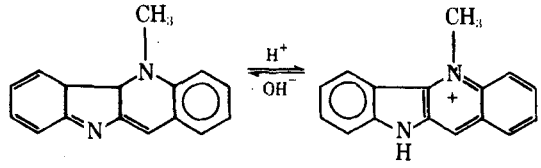
**Abstract** □ *Staphylococcus aureus* cultures treated with the hydrochloride salt of the indoquinoline alkaloid, cryptolepine, which was isolated from the roots of *Cryptolepis sanguinolenta*, showed biphasic steady-state generation curves at the same dose level. The apparent generation rate constant for the initial phase of action (phase I) was greater than that for phase II. The formal dependence of the apparent generation rate constant on drug concentration for the phase I action was linear at low drug concentrations but asymptotically approached zero at high concentrations, indicating saturation of the receptor sites engaged in microbial protein synthesis. The dependence for phase II action was linear in the entire concentration range and demonstrated a process that did not conform to a saturable receptor site model but resulted in a kill or lysis of the cells. Cryptolepine possesses bacteriostatic and bactericidal actions. Both phenomena occur in the initial stages of drug–bacteria reaction, but the bactericidal action predominates in subsequent stages. The effects of pH changes in broth media on generation inhibition of *S. aureus* by cryptolepine hydrochloride action indicated that the unprotonated drug fraction contributes to its activity, possibly because of its ready penetration through the cell membrane.

**Keyphrases** □ Cryptolepine hydrochloride—action on *Staphylococcus aureus*, microbial kinetics □ Antibacterial agents—cryptolepine hydrochloride, action on *Staphylococcus aureus*, microbial kinetics □ *Staphylococcus aureus*—effect of cryptolepine hydrochloride, microbial kinetics

Cryptolepine, an alkaloid, was first isolated in 1929 (1) from the roots of *Cryptolepis triangularis* N.E.Br. growing wild in the Belgian Congo. The alkaloid also has been isolated from another genus, *Cryptolepis sanguinolenta* (Lindl.) Schlecter, which is native to West Africa (2). It has the indoquinoline structure shown in Scheme I (3). Cryptolepis plants belong to the family *Asclepiadaceae* and grow as shrubs in tropical Africa. Extracts from these plants have been used in folk medicine and as a dye by the indigenous people (3). In Ghana[1], aqueous extracts from the roots of *C. sanguinolenta* have been used by herbalists to treat urinary tract infections and malaria.

Cryptolepine is reported to possess hypotensive properties and to produce a marked and prolonged fall in blood pressure as well as to lower body temperature (4, 5). Recent studies demonstrated that aqueous *C. sanguinolenta* extracts possess antimicrobial action against some urinary tract pathogens (6), while cryptolepine hydrochloride has a wide spectrum of activity against Gram-positive and Gram-negative bacteria as well as *Candida albicans* (7).

Microbial kinetic studies (8–11) were applied to evaluate the action of cryptolepine hydrochloride on *Staphylococcus aureus*, which is a susceptible organism. This paper discusses the functional dependency of kinetic parameters derived from growth inhibition of *S. aureus* cultures on cryptolepine hydrochloride concentrations, broth constituents, inoculum size, broth pH, and other conditions. A possible mechanism of cryptolepine action on *S. aureus* cells is postulated.



cryptolepine

*Scheme I*

## EXPERIMENTAL

**Materials and Methods**—*Staphylococcus aureus* (ATCC 6538) was used in all experiments. It was cultivated in broth medium[2] and was used in determining cryptolepine–bacteria reactions as described previously (8–11). Cryptolepine hydrochloride solutions were made by dissolving cryptolepine hydrochloride crystals[3] (mp 263–265°), referred to as cryptolepine in this paper, in aqueous 1% (v/v) polysorbate 80.

A particle-size counter[4] and the poured plate method with sandwiched agar plates were employed in determining the total and viable counts, respectively, on drug-free and drug-affected *S. aureus* cultures as described previously (8–11). Culture samples were withdrawn at 30-min intervals for both counts.

**Effect of 0.04% (v/v) Polysorbate 80 on Generation Rates**—Aliquots (2.0 ml) of sterile aqueous 1% (v/v) polysorbate 80 were added aseptically to replicate 48-ml volumes of *S. aureus* cultures growing at 37.5°. At this time, growth was in the logarithmic phase and the cell population was $2.0 \times 10^6$ organisms/ml. Culture samples were withdrawn every 30 min, and the cell population was determined by the total count method. Fifty-milliliter volumes of *S. aureus* cultures containing 0.04% (v/v) polysorbate 80 were used as controls in all experiments.

**Effect of Cryptolepine Hydrochloride Concentrations on Generation Rates**—Two-milliliter aliquots of cryptolepine hydrochloride solutions were added to replicate 48-ml culture samples to yield the required drug concentrations. Drug was added when the cultures were in the exponential growth phase at 37.5° and the cell population was $2.0 \times 10^6$ cells/ml. Generation curves were obtained by the total count method for cultures affected by cryptolepine concentrations within the 0–30-μg/ml range. Generation curves of the cultures affected with 0, 5, 10, 20, and 30 μg of cryptolepine/ml also were obtained by the total and viable count methods.

**Nutrient Concentration Effect on Drug-Affected Generation Rates**—Antibiotic Medium 3 was prepared to contain half, single, and double the concentrations of ingredients specified by the manufacturer, with pH adjustment to 7.05 ± 0.05 where necessary. The three media were used in determining generation curves of cultures treated with 0, 2, 10, 18, and 26 μg of cryptolepine/ml at 37.5°. The cell population at the time of drug addition was $2.0 \times 10^6$ cells/ml. Total counts were obtained on culture samples withdrawn at 30-min intervals.

**Organism Population Effect on Drug-Affected Generation Rates**—Generation curves of replicate cultures treated with 0, 2, 10, 18, and 26 μg of cryptolepine/ml at three bacterial population levels ($2.0 \times 10^5$, $2.0 \times 10^6$, and $1.0 \times 10^7$ cells/ml) were determined. Total counts were obtained on samples withdrawn every 30 min.

**pH Effect on Drug-Affected Generation Rates**—Broth media were adjusted with 2.0 *N* HCl and 2.0 *N* NaOH to pH 6.5, 7.0, and 7.5. Replicates of each broth were inoculated with the test organism and incubated at 37.5°. Aliquots (2.0 ml) of cryptolepine solutions were added to replicate cultures to yield 0 and 14 μg of cryptolepine/ml. The cell population

[1] Dr. Oku-Ampofo, Centre for Plant Medicine Research, Mampong-Akwapim, Ghana, personal communication.

[2] Antibiotic medium 3, Difco Laboratories, Detroit, Mich.

[3] Prepared in the Department of Pharmaceutical Chemistry, University of Science and Technology, Kumasi, Ghana.

[4] Coulter counter, model ZBI, Coulter Electronics Co., Hialeah, Fla.



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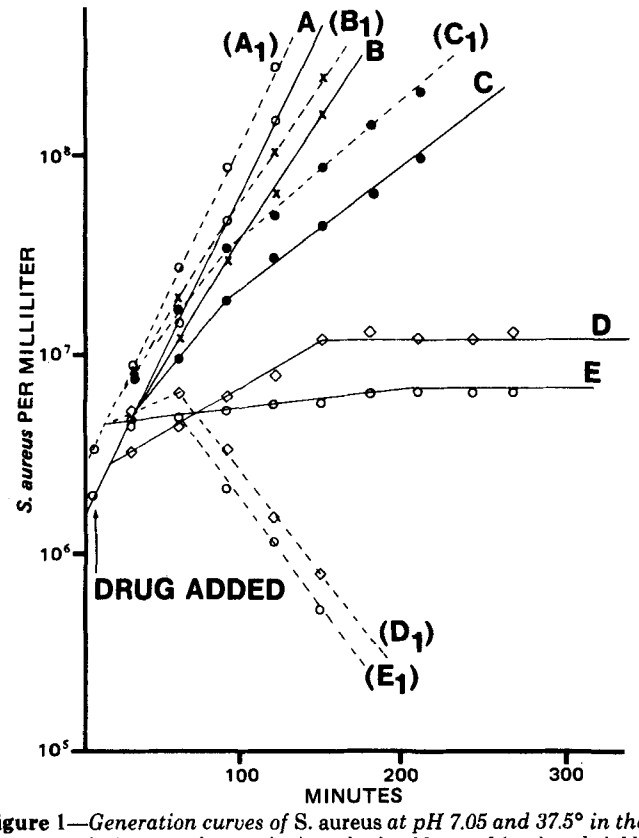



**Figure 1**—*Generation curves of* S. aureus *at pH 7.05 and 37.5° in the absence and presence of cryptolepine, obtained by total (—) and viable (---) counts. The curves and respective drug concentrations (micrograms per milliliter) were: A, $A_1$ = 0; B, $B_1$ = 5; C, $C_1$ = 10; D, $D_1$ = 20; and E, $E_1$ = 30.*

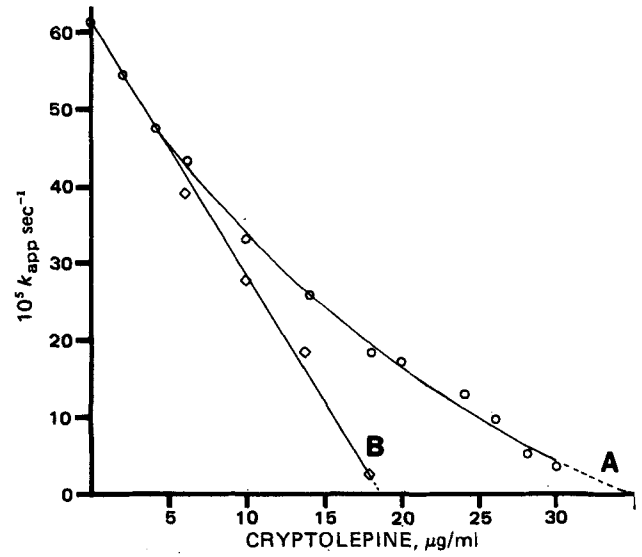



**Figure 2**—*Functional dependence of apparent generation rate constant,* $k_{app}$ *in seconds*$^{-1}$*, for drug-affected* S. aureus *on cryptolepine concentrations (micrograms per milliliter) at pH 7.05 and 37.5°. Curve A (○) is for dependence in phase I, and curve B (□) is for dependence in phase II.*

at the time of drug addition was $2.0 \times 10^6$/ml, and total counts were determined on samples withdrawn every 30 min.

**Size Frequency Distribution of Drug-Free and Drug-Treated Cultures**—Size frequency distribution curves of a control drug-free culture and test culture treated with 14 μg of cryptolepine/ml were generated from a Channelyzer[5] calibrated with a threshold factor of 0.034, at base channel threshold 5 and window width 100. The curves were recorded with an *x–y* recorder II[5].

## RESULTS

**Effect of 0.04% (v/v) Polysorbate 80 on Generation of *S. aureus* Cultures**—*S. aureus* growing in the absence or presence of 0.04% (v/v) polysorbate 80 showed an exponential generation phase:

$$\ln N = \ln N_0 + k_0 t \qquad \text{(Eq. 1)}$$

where $N$ is the number of organisms per unit volume at a time $t$; $N_0$ is the number of organisms per unit volume at some initial time, 0; and $k_0$ is the apparent generation rate constant. The apparent generation rate constant, $k_0$ in seconds$^{-1}$, obtained from the slopes of ln $N$ *versus* $t$ plots, was $40.93 \times 10^{-5}$ sec$^{-1}$ in the absence of polysorbate 80 but was $61.17 \times 10^{-5}$ sec$^{-1}$ in the presence of 0.04% (v/v) polysorbate 80. The mean generation time (mgt) for *S. aureus* was calculated in accordance with:

$$\text{mgt} = 0.693/k_0 \qquad \text{(Eq. 2)}$$

The mgt of the test organism decreased by ~30% in the presence of 0.04% (v/v) polysorbate 80 to 18.88 min (8). The increased rate of cell growth and division of *S. aureus* in the presence of polysorbate 80 may be attributed to: (*a*) the surfactant effect of polysorbate 80 enhancing the nutrient material permeation and/or oxygen availability into the cells, or (*b*) the polysorbate 80 enriching the broth medium as a nutrient source (12–16).

[5] Coulter Electronics Co., Hialeah, Fla.

**Generation Curves of Cryptolepine-Treated *S. aureus* Cultures**—Semilogarithmic plots of the total count *versus* time are shown in Fig. 1. The addition of graded cryptolepine concentrations to balanced *S. aureus* cultures decreased the generation rate after a lag phase of ~10 min. The initial steady-state generation phase (phase I) was followed by another steady-state generation phase (phase II) after one to four successive generations of the cultures affected by high drug concentrations. Apparent generation rate constants ($k_{app}$ in seconds$^{-1}$) of drug-affected cultures were obtained from linear portions of ln $N$ *versus* $t$ plots in accordance with:

$$\ln N = \ln N_0 + k_{app} t \qquad \text{(Eq. 3)}$$

The generation rate constants, $k_{app_I}$, for phase I generation were greater than those, $k_{app_{II}}$, for phase II generations. These observations suggest that cryptolepine has more than one mechanism of action on *S. aureus*.

**Comparison of Generation Curves of Drug-Treated Cultures by Total and Viable Count Methods**—Semilogarithmic plots of the total and viable counts *versus* time for *S. aureus* cultures treated with 0, 5, 10, 20, and 30 μg of cryptolepine/ml, also are shown in Fig. 1. Parallel curves were obtained for plots of the total and viable counts for cultures affected with low cryptolepine concentrations, 0–10 μg/ml, in all growth phases.

Cultures affected with higher drug concentrations, 20 and 30 μg/ml, yielded curves that were parallel and coincident, respectively, for 55 min in phase I growth. Thereafter, the viable count dropped rapidly with time, which may indicate a bactericidal or lytic action superimposed on generation inhibition at the higher concentration range. Where parallel curves were obtained, the total count was 50–60% of the viable count.

**Generation Rate Dependency on Drug Concentrations**—A plot of $k_{app}$ *versus* concentration is shown in Fig. 2. The extent of generation inhibition was directly proportional to the cryptolepine concentration in the ranges of 0–4.25 μg/ml for phase I action and 0–18.55 μg/ml for phase II action. The $k_{app}$ dependence on cryptolepine concentration ($D$) is expressed:

$$k_{app} = k_0 - k_d D \qquad \text{(Eq. 4)}$$

where $k_d$ is the specific inhibitory rate constant. At a >4.25-μg/ml concentration in phase I, $k_{app}$ was not a linear function of increasing drug concentration but asymptotically approached zero.

**Applicability of Saturation Kinetics to Cryptolepine Action on *S. aureus***—Figure 3 shows a plot of $D/(k_0 - k_{app})$ *versus* $D$ in accordance with a previously derived (12) saturable receptor site model:

$$D/(k_0 - k_{app}) = D(k_b/k_a) + 1/k_a \qquad \text{(Eq. 5)}$$

**Table I—Derived [a] Kinetic Parameters from Generation Curves of Cryptolepine-Treated *S. aureus* Cultures**

| Kinetic Parameter | Phase I | Phase II |
|---|---|---|
| $10^5\ k_d$, ml/μg sec[b] | 3.33 | 3.26 |
| $10^5\ k_A$, ml/μg sec[c] | 3.77 | — |
| $10^5\ k_a^1$, ml/μg sec[d] | — | 3.30 |
| $10^5\ k_a$, ml/μg sec[e] | 0.47 | — |
| $10^5\ k_b$, ml/μg[c] | 3460.00 | — |
| $10^5\ k_a k_b^1$, $sec^{-1}$ | 13.58 | — |
| $(k_a/k_b)/k_0$ | 0.22 | — |
| MIC, μg/ml[f] | 37.07 | 18.54 |

[a] Derived from Fig. 2 data. [b] Calculated from the slope of the plot of $k_{app}$ *versus* concentration from 0–4.25 μg of cryptolepine/ml in phase I and from 0–18 μg of cryptolepine/ml in phase II. [c] The $k_A$ and $k_b$ values were estimated from the slope and intercept of the plot of $D/(k_0 - k_{app})$ *versus* $D$ from Eq. 5 for cryptolepine concentrations greater than 4.25 μg/ml in phase I. [d] Derived from the slope of the plot of $1/(k_0 - k_{app})$ *versus* $1/D$ from Eq. 4. [e] The difference between the $k_A$ and $k_a^1$ values from Eq. 17*a*. [f] Calculated from Eqs. 6 and 8 for phases I and II, respectively, when cryptolepine is at the minimal inhibitory concentration.

where $k_a$ and $k_b$ are proportionality constants related to drug availability in the biophase and drug affinity for receptor or binding sites. Adherence to Eq. 5 is observed for linear plots obtained for cryptolepine concentrations greater than 4.25 μg/ml in phase I. Deviation of the plot from linearity occurs at concentrations less than 4.25 μg/ml, indicating nonadherence to a saturable process at lower concentration ranges in phase I. There was no adherence to the model for phase II of cryptolepine action in the concentration range studied. These findings demonstrate a different mechanism of action for phases I and II. The $k_a$ and $k_b$ values are given in Table I. The minimum inhibitory concentration (MIC) of cryptolepine during phase I was calculated from the expression (8):

$$D_{m_I} = k_0/(k_a - k_0 k_b) \qquad \text{(Eq. 6)}$$

where $D_{m_I}$ is the minimum inhibitory concentration of phase I. The minimum inhibitory concentration during phase II, $D_{m_{II}}$, is derived from:

$$k_{app_{II}} = k_0 - k_a^1 D \qquad \text{(Eq. 7)}$$

where $k_a^1 = k_d$ in Eq. 4 during phase II. At $D_{m_{II}}$, where $k_{app_{II}} = 0$, Eq. 4 simplifies to:

$$D_{m_{II}} = k_0/k_a^1 \qquad \text{(Eq. 8)}$$

The calculated $D_{m_I}$ and $D_{m_{II}}$ values are given in Table I.

**Medium Composition Effect on Drug-Affected Generation Rates**—Apparent generation rate constants, $k_{app}$, of drug-free and drug-treated *S. aureus* in Antibiotic Medium 3 at three concentrations are given in Table II. The generation rate constants varied significantly when the medium concentration was varied, which may indicate interference with drug action by medium constituents.

**Cell Population Effect at Time of Drug Addition on Generation Rates**—Table II also gives apparent generation rate constants of drug-free and drug-affected *S. aureus* in single strength Antibiotic Medium 3 with three different organism populations at the time of drug addition. There were no significant variations of the generation rate constants with organism populations at any of the drug concentrations.

**pH Effect on Generation Rates**—The generation rate constants of drug-free and drug-affected *S. aureus* in broth adjusted to pH 6.5, 7.0, and 7.5 are given in Table III. The generation rate constant, $k_0$, of drug-free cultures was invariant with pH, and that of drug-treated cultures varied significantly with changes in pH. A pH increase reduced the generation rate constant, indicating an increase in drug action on *S. aureus* cultures.

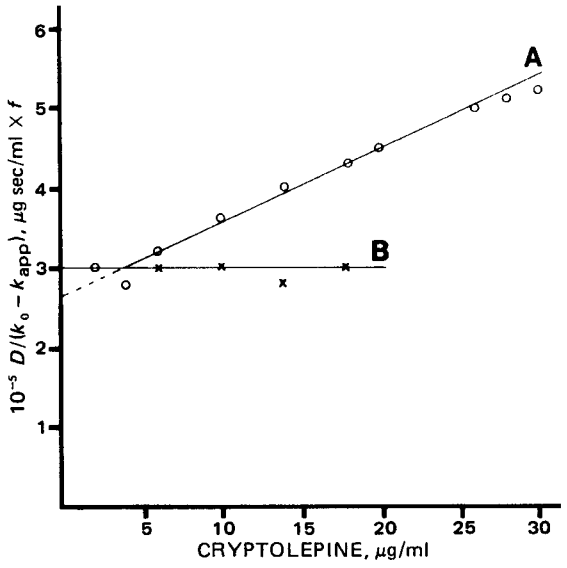



**Figure 3**—*Applicability of saturation kinetic model to the dependency of apparent growth rate constants,* $k_{app}$, *of drug-affected* S. aureus *on higher cryptolepine concentrations at pH 7.05 and 37.5°. Curve A is for* S. aureus*-affected culture in phase I generation, and curve B is for* S. aureus*-affected culture in phase II generation. The actual values of* $D/(k_0 - k_{app})$ *are multiplied by a factor,* f, *where* f = 0.001.

**Cryptolepine Effect on Size Frequency Distribution Curves of *S. aureus***—The size frequency distributions of drug-free and drug-affected *S. aureus* cultures are given in Fig. 4. Table IV shows the predominance of two population types with mean cell diameters of 0.769 and 1.549 μm. The drug-free cultures demonstrated a relative percentage increase of cell diameter throughout the experiment. The drug-treated cultures showed an initial cell diameter increase in 20% of the population, but this increase was followed by cessation of growth.

## DISCUSSION

*S. aureus* cultures treated with cryptolepine showed biphasic steady-state generation curves (Fig. 1). The generation rate constant in phase I was greater than that in phase II, indicating an enhanced generation inhibition in the latter phase. Figure 2 shows that the dependency of the generation rate constant on cryptolepine concentration for phase I was linear at low drug concentrations, 0–4.25 μg of cryptolepine/ml, but gave an asymptotic curve at concentrations greater than 4.25 μg/ml. The phase II dependency was linear in the entire concentration range.

The plot of $D/(k_0 - k_{app})$ *versus* $D$ (Fig. 3) reveals that the phase I cryptolepine action in the high concentration range adhered to a saturable receptor site model, except that deviations occurred in the low concentration range (17). The phase II cryptolepine action in the entire concentration range did not follow saturation kinetics, which may imply that few receptors interacted with cryptolepine molecules to produce generation inhibition. The inference is that the mechanism of cryptolepine in phase II is different from phase I.

Organism population at the time of drug addition did not affect the generation rate (Table II), demonstrating that cryptolepine was not metabolized by *S. aureus*. Moreover, the drug was not depleted in the medium as a result of adsorption to cellular components, and the drug

**Table II—Effect of Variation in Broth Composition and Inoculum Size on Phase I and II Generation Rates of Cryptolepine-Treated *S. aureus***

| Cryptolepine, μg/ml | Phase I | | | Phase II | | | Phase I | | | Phase II | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $B_1$[a] | $B_2$[b] | $B_3$[c] | $B_1$ | $B_2$ | $B_3$ | $I_1$[d] | $I_2$[e] | $I_3$[f] | $I_1$ | $I_2$ | $I_3$ |
| 0 | 61.91 | 60.40 | 63.61 | — | — | — | 60.12 | 62.10 | 61.81 | — | — | — |
| 2 | 48.19 | 55.01 | 63.18 | — | — | — | 55.54 | 56.21 | 57.00 | — | — | — |
| 10 | 19.17 | 34.50 | 57.76 | 7.0 | 27.0 | 38.78 | 34.63 | 35.28 | 36.94 | 27.57 | 28.86 | 25.53 |
| 18 | 9.61 | 18.25 | 46.55 | 0 | 2.50 | 23.56 | 15.85 | 16.51 | 16.08 | 2.49 | 3.18 | 2.71 |
| 26 | 5.77 | 9.5 | 23.70 | 0 | 0 | 7.74 | 9.27 | 9.10 | 8.20 | 0 | 0 | 0 |

[a] Half-strength broth ($B_1$). [b] Normal-strength broth ($B_2$). [c] Double-strength broth ($B_3$). [d] Inoculum of $2.0 \times 10^5$ cells/ml ($I_1$). [e] Inoculum of $2.0 \times 10^6$ cells/ml ($I_2$). [f] Inoculum of $1.0 \times 10^7$ cells/ml ($I_3$).

**Table III—Effect of Broth pH on Phase I and II Generation Rates of *S. aureus* Cultures Treated with 14 μg of Cryptolepine/ml**

| pH | $k_{app} \times 10^{-5}$ sec$^{-1}$ | | | | |
|---|---|---|---|---|---|
| | $k_0$ | $k_{appI}$ | $k_{appII}$ | $k_0 - k_{appI}$ | $k_0 - k_{appII}$ |
| 6.5 | 61.37 | 40.08 | 26.97 | 21.29 | 34.40 |
| 7.0 | 60.92 | 24.09 | 13.36 | 36.84 | 47.57 |
| 7.5 | 61.09 | 15.66 | 3.01 | 45.43 | 58.00 |

was not inactivated either by cellular excretions or by other interactions as a function of organism numbers.

Variation in the concentration of medium constituents significantly altered the generation rate constant (Table II). This variation is attributed to the fact that the drug either is bound to broth constituents or is inactivated by metabolites in the broth medium that competitively bind to the drug receptor site in the biophase. However, the curve for $k_{app}$ dependency on cryptolepine concentration (Fig. 2) does not yield an initial lag phase that is typical of drug binding to broth constituents or of drug complexation with metal ions in the broth medium. Therefore, the latter assumption holds.

The viable count determinations (Fig. 1) showed that, during phase I action, there was a generation inhibition of *S. aureus* by low cryptolepine concentrations, 0–20 μg/ml, as demonstrated by parallelism of the curves for both total and viable counts. At 30 μg of cryptolepine/ml, dynamic bacteriostasis (18) is superimposed on generation inhibition, evidenced by coincident curves for both total and viable counts. During phase II, however, generation inhibition was observed only in cryptolepine concentrations of <20 μg/ml. At 20–30 μg of cryptolepine/ml, the viable count fell rapidly ~55 min after drug addition, which indicates cell death and/or lysis.

The size frequency analysis in Table IV reveals two predominant population types with mean cell diameters of 0.769 and 1.549 μm for the drug-free culture. The former diameter agrees with the reported (19) minimal cell diameter for *S. aureus*. The latter diameter is double the minimal diameter and is the maximum cell diameter attained by a majority of the cell population before cell division. About 20% of the cell population in the cultures treated with cryptolepine (14 μg/ml) showed a cell diameter increase equal to that of the drug-free culture at 5 min. This increase occurred during the first 30 min after drug addition, when the drug was being equilibrated in the biophase. Thereafter, the cells maintained the minimal cell diameter with a concomitant cell number decrease.

These observations support the hypothesis that cryptolepine possesses bactericidal action that is superimposed on normal generation inhibition. The bactericidal action predominates in the later stages of the drug–

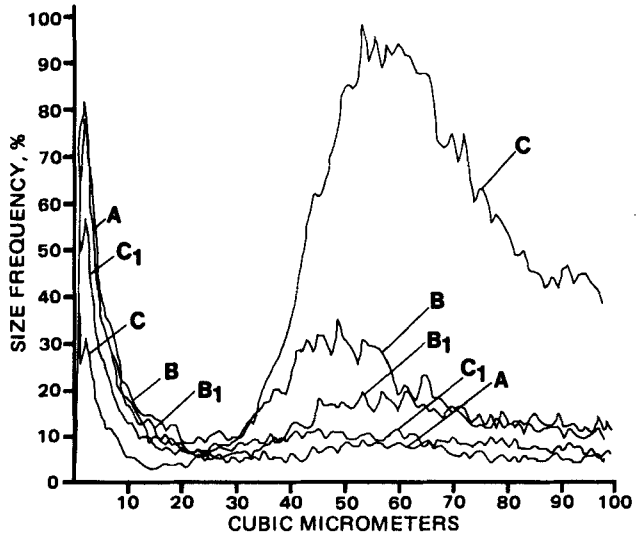
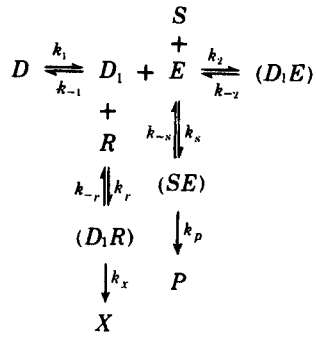



**Figure 4**—*Size frequency distribution curves of drug-free and drug-affected* S. aureus *cultures. Curve A is for the drug-free culture 5 min before cryptolepine addition (14 μg/ml); curves B and $B_1$ are for drug-free and drug-affected cultures, respectively, 30 min after drug addition; and curves C and $C_1$ are for drug-free and drug-affected cultures, respectively, 120 min after drug addition.*

**Table IV—Size Frequency Distribution of *S. aureus* Cultures Treated with 0 and 14 μg of Cryptolepine/ml**

| Minutes | Drug-Free Cells | | Drug-Treated Cells | |
|---|---|---|---|---|
| | Mean Cell Diameter, μm | Relative Frequency, % | Mean Cell Diameter, μm | Relative Frequency, % |
| 5 | 0.769 | 78 | — | — |
| 30 | 0.769 | 69.3 | 0.769 | 80.40 |
| | 1.521 | 30.7 | 1.589 | 19.60 |
| 60 | 0.769 | 52 | 0.769 | 86.00 |
| | 1.537 | 48 | | |
| 120 | 0.769 | 23.85 | 0.769 | 57.00 |
| | 1.555 | 76.15 | | |
| 180 | 1.547 | 99.00 | 0.769 | 52.00 |

bacteria reaction. A kinetic model that may define the mechanism of cryptolepine action on *S. aureus* is shown in Scheme II:




*Scheme II*

In this model, $D$ is the cryptolepine concentration in the broth medium, which is equilibrated with cryptolepine concentration in the biophase $D_1$, which is reversibly bound with enzyme $E$ to form a drug–enzyme complex $D_1E$; $S$ is a metabolite in the broth medium that is used as a substrate by the enzyme $E$ in the synthesis of protein product $P$ from the substrate–enzyme complex, $SE$. Moreover, $D_1$ reacts with a receptor site, $R$, to form an injured site or toxic product, $X$, from a reaction complex, $D_1X$. The receptor site may be a vital target site that is a sensitive cell membrane component, a protoplasmic or nuclear material, or a transport agent essential for organism survival (20–23).

By assuming that the microbial generation rate is proportional to: (*a*) the difference between the protein synthesis rate, $dP/dt$, which causes a microbial number increase from cell growth and division, and the rate of injury or formation of toxic product, $dX/dt$, which causes a microbial number decrease from kill or lysis of the cells, and (*b*) the number of viable organisms, $N$, present, the following apply:

$$dN/dt = k(dP/dt - dX/dt)N = k_{app}N \quad \text{(Eq. 9)}$$

where $k$ is a proportionality constant and $k_{app}$ is the apparent generation rate constant of the drug-treated culture. Therefore:

$$k_{app} = k(dP/dt - dX/dt) \quad \text{(Eq. 10)}$$

$$k_{app} = k[\{[k_p k_s(S)(E)]/(k_p + k_{-s})\} - \{[k_x k_r(D_1)(R)]/(k_x + k_{-r})\}] \quad \text{(Eq. 11)}$$

$$k_{app} = k_p^1 S(1 - \theta)E_T - k^1(D_1)(R) \quad \text{(Eq. 12)}$$

where $\theta = (D_1E)/E_T$ is the fraction of total enzyme bound to drug, $k_p^1 = kk_pk_s/(k_p + k_{-s})$, and $k^1 = k_xk_r/(k_x + k_{-r})$.

Expansion of Eq. 12 yields:

$$k_{app} = k_p^1 SE_T - k_p^1 SE_T\theta - k^1(D_1)(R) \quad \text{(Eq. 13)}$$

In the absence of drug, $D_1 = 0$, $\theta = 0$, and Eq. 13 simplifies to:

$$k_{app} = k_p^1 SE_T = k_0 \quad \text{(Eq. 14)}$$

where $S$ and $E_T$ are assumed to be available in constant amounts during bacteria–drug reaction and $k_0$ is the generation rate constant for drug-free cultures. Substituting Eq. 14 in Eq. 13 gives:

$$k_{app} = k_0 - k_0\theta - k^1(D_1)(R) \quad \text{(Eq. 15)}$$

$\theta$ is expressed (17, 24) as $K_1K_2D/1 + K_1K_2D$, where $K_1$ is the drug par-

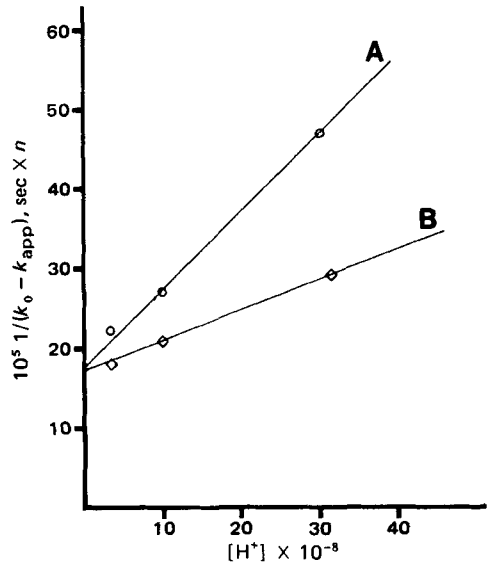



**Figure 5**—*Dependence of apparent generation rate constant,* $k_{app}$, *for* S. aureus *on broth medium pH. Curve A is for dependence of* $k_{app}$ *on pH during phase I generation, and curve B is for dependence of* $k_{app}$ *on pH during phase II generation. The actual values of* $1/(k_0 - k_{app})$ *are multiplied by a factor,* n, *where* n = 0.001.

tition constant through cell membranes and $K_2$ is the drug affinity constant for the enzyme $E$. Therefore:

$$k_{app} = k_0 - k_0(K_1K_2D/1 + K_1K_2D) - k^1(K_1D)(R) \quad \text{(Eq. 16)}$$

Equation 16 may be simplified to:

$$k_{app} = k_0 - [k_aD/(1 + k_bD) + k_a^1D] \quad \text{(Eq. 17}a\text{)}$$

where $k_a = k_0K_1K_2$, $k_b = K_1K_2$, $k_d = kK_1R$, and $R$ is assumed to be present in an infinitely large amount relative to drug molecules.

At low cryptolepine concentrations when few receptors are interacted with drug, $k_bD \ll 1$ and Eq. 17$a$ reduces to:

$$k_{app} = k_0 - (k_aD + k_a^1D) = k_0 - k_AD \quad \text{(Eq. 17}b\text{)}$$

where $k_A = k_a + k_a^1$. Equation 17$b$ is Eq. 4, where $k_A = k_d$, and describes the functional dependency of $k_{app}$ on drug concentrations of 0–42.5 μg/ml for cryptolepine-affected *S. aureus* in phase I (Fig. 2).

Equation 17$a$ may be expanded to:

$$k_{app} = k_0 - \{(k_a + k_a^1)D + k_bk_a^1D^2\}/1 + k_bD \quad \text{(Eq. 18)}$$

At high drug concentrations in phase I, where the cryptolepine bactericidal action is negligible and $(k_a + k_a^1)D \gg k_bk_a^1D^2$, Eq. 18 simplifies to:

$$k_{app} = k_0 - (k_a + k_a^1)D/(1 + k_bD) = k_0 - k_AD/(1 + k_bD) \quad \text{(Eq. 19)}$$

Rearrangement of Eq. 19 yields Eq. 5, where $k_b/k_A = k_b/(k_a + k_a^1)(k_b/k_a)\,1/k_0$. At the minimal inhibitory concentration in phase I when $k_{app} = 0$, Eq. 19 simplifies to Eq. 6.

In phase II, where cryptolepine bactericidal action predominates over its growth inhibitory action, $k_{app}$ becomes solely dependent on the rate of cell death or lysis. Therefore, Eq. 17$a$ approaches the expression $k_{app} = k_0 - k_a^1D$, which is Eq. 4, where $k_a^1 = k_d$. At the minimal inhibitory concentration where $k_{app} = 0$, Eq. 4 simplifies to Eq. 8. The minimal inhibitory concentration values for phases I and II of cryptolepine on *S. aureus* calculated from derived kinetic parameters in Eqs. 6 and 8, respectively (Table I), agree with the experimental minimal inhibitory concentration values (Fig. 2).

Changes in broth medium pH influenced the cryptolepine effect on *S. aureus*. The extent of generation inhibition $(k_0 - k_{app})$ increased as some function of the broth medium pH (Table III).

If $f$ is the fraction of the total cryptolepine salt concentration $(D_T)$ that is unprotonated, then for a weak basic compound like cryptolepine:

$$f = K_a/[H^+] + K_a \quad \text{(Eq. 20)}$$

and, therefore:

$$D^1 = D_TK_a/[H^+] + K_a \quad \text{(Eq. 21)}$$

where $K_a$ is the dissociation constant, $[H^+]$ is the hydrogen-ion concentration, and $D^1$ is the unprotonated drug concentration at a specified pH. By assuming that the unprotonated drug concentration contributes to cryptolepine activity and then substituting Eq. 21 in Eq. 19 and simplifying, the $k_{app}$ dependency on pH in phase I may be expressed as:

$$1/(k_0 - k_{app}) = [H^+]/k_aK_aD_T + 1/k_aD_T(1 + k_bD_T) \quad \text{(Eq. 22)}$$

Equation 22 is an expression for the linear dependency of the plot of $1/(k_0 - k_{app})$ *versus* $[H^+]$ in Fig. 5. Likewise, the $k_{app}$ dependency on pH during phase II cryptolepine action is described by an expression derived from Eqs. 21 and 4:

$$1/(k_0 - k_{app}) = [H^+]/k_a^1K_aD_T + 1/k_a^1D_T \quad \text{(Eq. 23)}$$

where $K_a$ was calculated from the slope and intercept obtained from the linear plot of $1/(k_0 - k_{app})$ *versus* $[H^+]$ in phase II (Fig. 5). The pKa of cryptolepine was 6.34. The linear plots (Fig. 5) obtained from experimental data in accordance with Eqs. 22 and 23 confirm the assumption that the unprotonated cryptolepine molecules contribute to the activity because of their possible ready penetration through cell membranes.

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