aminoethanesulfonanilide as needles from aqueous EtOH: mp 148–149 °C; 70% yield. Anal. ($C_{14}H_{15}N_3O_4S$) C, H, N, S. This side chain was coupled with 9-chloroacridine, and the resultant product was reduced with Fe/HCl to provide compound **674**.

Acknowledgment. The authors thank Priscilla Y. C. Jow for determination of log P values for some 9-anilinoacridines, Nicholas A. Dreyer for computational help, and Sam Fink for checking the calculated parameter values. We are particularly indebted to Ms. C. West and her assistants for carrying out the many biological tests. This work was supported by the Auckland Division of the Cancer Society of New Zealand (Inc.), by the Medical Research Council of New Zealand, and by Contract N01-CM-67062 and Grant CA-11110 from the National Cancer Institute. Part of the work was carried out while W.A.D. was a Visiting Research Professor at Pomona College.

Supplementary Material Available: Calculation of π values for representative side chains by the fragment constant method (15 pages). Ordering information is given on any current masthead page.

Quantitative Structure–Inhibitory Activity Relationships of Phenols and Fatty Acids for *Bacillus subtilis* Spore Germination

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Phenols and fatty acids were found to inhibit L-alanine-initiated germination of *Bacillus subtilis* spores without altering their heat resistance. Inhibitory effect was defined as the concentration necessary to cause 50% inhibition of the germination rate. The quantitative structure–inhibitory activity relationships for 39 phenols and 7 fatty acids were analyzed. The pH dependency of inhibition showed that the nonionized form of the molecule was responsible for inhibition. Hydrophobicity, which was expressed by the partition coefficient or the distribution coefficient of the compounds, was important for inhibition. In addition to hydrophobicity, the electronic effect, which was expressed by the dissociation constant, played a partial role in phenols. The correlation equation of the fatty acids was similar to those of the alcohols and other hydrophobic compounds, which had been reported earlier. That of the phenols, however, appeared to be different, indicating a different and more complex mechanism of inhibition. The type of inhibition by both compounds was mixed rather than competitive or noncompetitive.

Germination of bacterial spores is a sequential process, from a dormant state to a metabolically active vegetative form, in which a number of events take place shortly after exposure of spores to specific germinants. The spore sequentially loses its heat resistance as the earliest event, releases dipicolinic acid, acquires permeability to dyes, releases calcium ions, loses refractility, and shows a decrease in absorbance.[1]

To characterize the nature of the trigger reaction, several kinds of inhibitors of germination have been reported.[2] Our previous studies[3,4] had shown that alcohols and various kinds of hydrophobic compounds inhibited L-alanine-initiated germination of *B. subtilis* spores. The inhibitory activity of these compounds correlated quantitatively with their hydrophobicity, and the hydrophobic character near the L-alanine receptor site was demonstrated.

The present article describes the inhibition of germination of *B. subtilis* spores by some compounds, including phenols and fatty acids, which have an ionizable character in the molecule. Relationships between the chemical structure and inhibitory activity could contribute to a further understanding of the L-alanine receptor site and the mechanism of the trigger reaction of spore germination.

Results

Inhibition of Germination by Phenols at pH 7.2. The inhibitory effect of various concentrations of phenols on the germination rate in 0.1 mM L-alanine is shown in Figure 1. The molar concentration of a phenol necessary to cause 50% inhibition of the germination rate (I_{50}) was determined. The I_{50} values in Table I represent means of three determinations. For hydrophobic parameters, the partition coefficient (log P) according to Hansch and Leo[5] and the distribution coefficient (log D) according to Scherrer and Howard[6] are shown. For the electronic parameter the dissociation constant (pK_a) is shown. The regression analysis for 39 phenols in Table I, except salicylic acid, p-hydroxybenzoic acid, p-aminophenol, and L-tyrosine, is described below, where the figures in parentheses are the Student's t test values, n is the number of compounds submitted to the regression, r is the correlation coefficient, s is the standard deviation, and F is the overall statistical significance of the equation. Log P^0 and log D^0 are the ideal values of log P and log D, respectively, for parabolic dependence with the 95% confidence intervals in parentheses. The relationship between the inhi-

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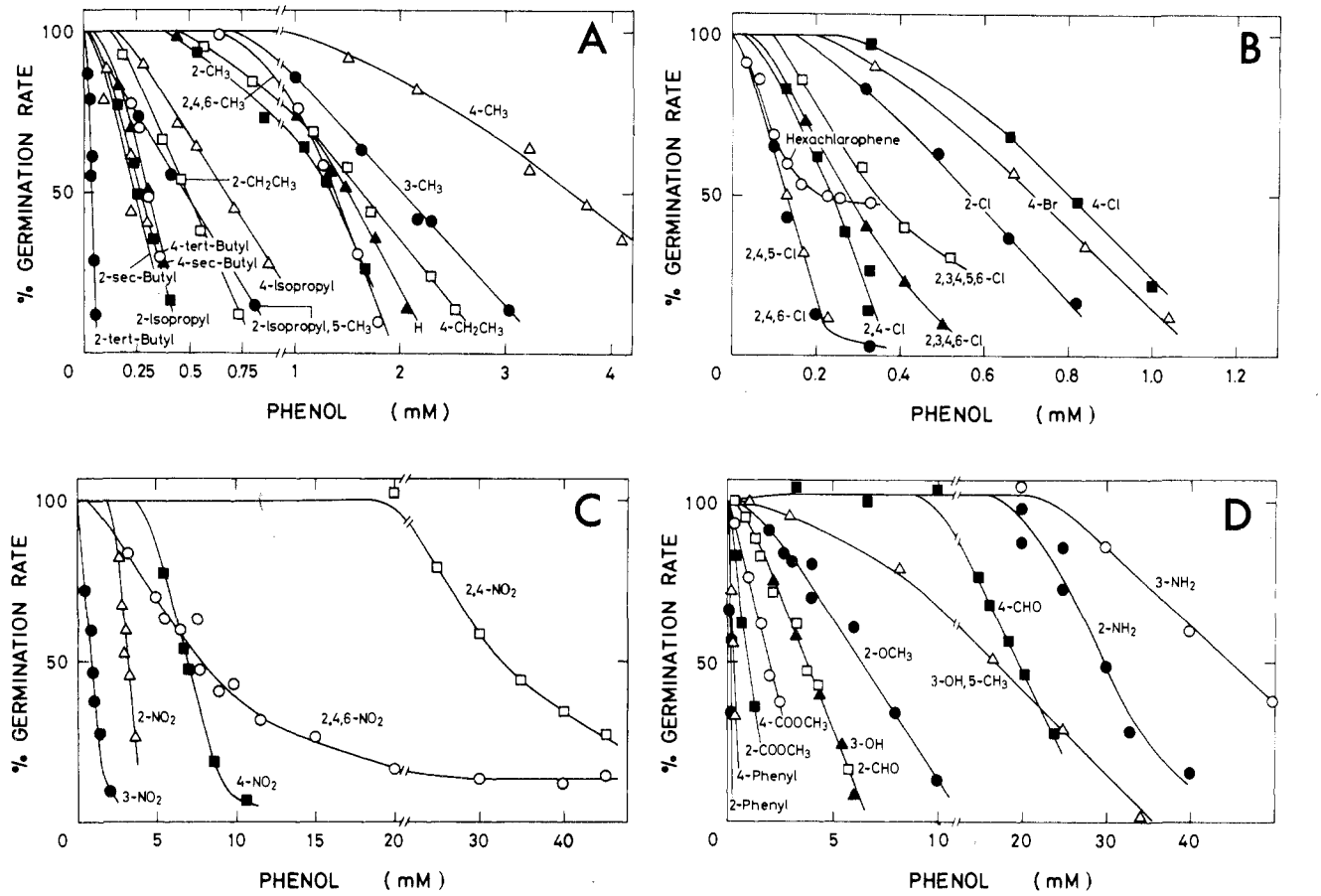

Figure 1. Effect of various concentrations of phenol derivatives on the germination of *B. subtilis* PCI219 spores: A, alkylphenols; B, holophenols; C, nitrophenols; D, other phenol derivatives. Data points of one of the three experiments are presented.

bitory activity of *B. subtilis* spore germination and the hydrophobicity and electronic nature of phenols can be demonstrated in eq 1–7. The equation with log D, log D^2,

$$\log 1/I_{50} = \underset{(6.64)}{0.35} \log D + 2.20 \quad (1)$$

$$n = 39,\ r = 0.737,\ s = 0.504,\ F_{1,37,0.05\%} = 44.0$$

$$\log 1/I_{50} = \underset{(6.83)}{0.43} \log P + 1.84 \quad (2)$$

$$n = 39,\ r = 0.747,\ s = 0.496,\ F_{1,37,0.05\%} = 46.6$$

$$\log 1/I_{50} = \underset{(6.95)}{0.40} \log D - \underset{(1.86)}{0.08}pK_a + 2.76 \quad (3)$$

$$n = 39,\ r = 0.764,\ s = 0.481,\ F_{2,36,0.05\%} = 25.2$$

$$\log 1/I_{50} = \underset{(8.77)}{0.49} \log P + \underset{(3.90)}{0.13}pK_a + 0.58 \quad (4)$$

$$n = 39,\ r = 0.830,\ s = 0.416,\ F_{2,36,0.05\%} = 39.9$$

$$\log 1/I_{50} = \underset{(5.88)}{0.30} \log D + \underset{(3.39)}{0.05} \log D^2 + 2.00 \quad (5)$$

$$n = 39,\ r = 0.809,\ s = 0.439,\ F_{2,36,0.05\%} = 34.0,$$
$$\log D^0 = -3.12\ (-8.91 \text{ to } -1.49)$$

$$\log 1/I_{50} = \underset{(8.48)}{1.13} \log P - \underset{(5.59)}{0.10} \log P^2 + 0.89 \quad (6)$$

$$n = 39,\ r = 0.874,\ s = 0.363,\ F_{2,36,0.05\%} = 58.0,$$
$$\log P^0 = 5.58\ (4.87 \text{ to } 6.93)$$

$$\log 1/I_{50} = \underset{(9.42)}{1.08} \log P - \underset{(5.51)}{0.09} \log P^2 + \underset{(3.84)}{0.10}pK_a + 0.07 \quad (7)$$

$$n = 39,\ r = 0.913,\ s = 0.305,\ F_{3,35,0.05\%} = 58.3,$$
$$\log P^0 = 6.17\ (5.31 \text{ to } 7.93)$$

and pK_a is not shown, since the pK_a term is negligible. These correlation equations indicated that the hydrophobic effect of the compound might be related to the inhibition, and the presence of the pK_a term seemed to be less important.

Dependency on pH of Germination Inhibition by Phenols. At pH 7.2 some of the phenols must be present partly in ionized and partly in nonionized forms. The good correlation with hydrophobicity as shown above indicates that the nonionized species might be the significant form at the spore site. To confirm this fact, the effect of pH on germination inhibition was examined. Phenol, with a pK_a of 10.0, was little affected throughout the range of pH studied, whereas compounds such as 3-nitro-, 2,4-dichloro-, 2-nitro-, and 2,4,6-trinitrophenol with pK_a's of 8.4, 7.9, 7.2, and 6.2, respectively, increased rapidly in inhibitory activity as the pH fell (Figure 2). The pH dependency curves of 2,4-dinitrophenol (45 mM) and 2,4,6-trinitrophenol (5 mM) were similar to those of 2,4,6-trichlorophenol (0.23 mM) and phenol (1.5 mM), respectively, in the pH range (data not shown).

In the case of 2,4,6-trichlorophenol, the pH dependency of the I_{50} value was determined (Figure 3, eq 8). The pH dependency of the inhibitory activity of *B. subtilis* spore germination of 2,4,6-trichlorophenol is related as shown in eq 8. The total I_{50} of the compound depended on the

$$\log 1/I_{50} = \underset{(18.30)}{-1.01}pH + 11.19 \quad (8)$$

$$n = 7,\ r = 0.993,\ s = 0.060,\ F_{1,5,0.05\%} = 335.3$$

pH, and the slope of the log $1/C$–pH plot was about −1, while the I_{50} based on the nonionized molecule was independent of the pH, indicating that ionization of the compound reduced its inhibitory action.

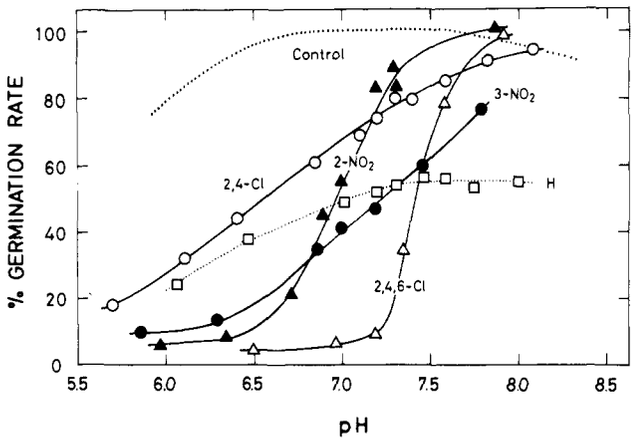

Figure 2. Effect of pH on the inhibition of germination of *B. subtilis* PCI219 spores by phenol derivatives: (○) 2,4-dichlorophenol, 0.17 mM; (□) phenol, 1.5 mM; (▲) 2-nitrophenol, 2.3 mM; (●) 3-nitrophenol, 1 mM; (△) 2,4,6-trichlorophenol, 0.23 mM. The percent germination rate was calculated as the percent of the control rate at pH 7.2. The control curve was determined without the inhibitor. The plots are shown in Figure 5.

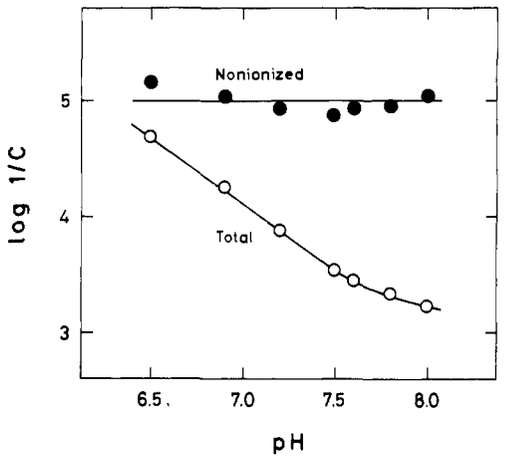

Figure 3. Effect of pH on the inhibition of germination of *B. subtilis* PCI219 spores by 2,4,6-trichlorophenol. *C* represents the total concentration (I_{50}, ○) for 50% inhibition of the germination rate or the nonionized concentration (●) calculated by $I_{50} \times [H^+]/([H^+] + K_a)$.

Inhibition of Germination by Fatty Acids at pH 7.2. As *n*-octanoic acid was shown to be the only inhibitor among the carboxylic acids tested earlier,[4] several fatty acids were examined at various concentrations (Figure 4, Table II). Equations 9 and 10 were derived from the regression analyses of seven fatty acids with the composition C7 to C13. The relationship between the inhibitory activity of *B. subtilis* spore germination and the hydrophobicity of fatty acids can be expressed as eq 9 and 10.

$$\log 1/I_{50} = \underset{(9.79)}{0.72 \log P} - 0.74 \qquad (9)$$

$$n = 7,\ r = 0.975,\ s = 0.164,\ F_{1,5,0.05\%} = 95.8$$

$$\log 1/I_{50} = \underset{(9.79)}{0.72 \log D} + 0.91 \qquad (10)$$

$$n = 7,\ r = 0.975,\ s = 0.164,\ F_{1,5,0.05\%} = 95.9$$

A pK_a term could not be entered at a significant level, since the pK_a values of the compounds in the analysis did not vary in a broad range.

The concentrations of fatty acid required to inhibit germination decreased as the chain length of the compound increased. Fatty acids with longer chains (C14 to C18) showed an inhibitory effect even at 0.2 mM (Table II). The I_{50} values could not be determined because of their poor solubility. A compound with a more acidic group than carboxyl, such as *n*-undecanesulfonic acid, did not inhibit germination.

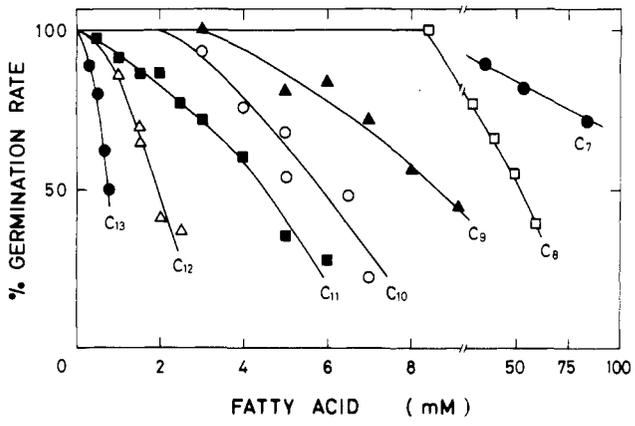

Figure 4. Effect of various concentrations of fatty acids on the germination of *B. subtilis* PCI219 spores. Straight-chain fatty acids are indicated with their carbon number. Data points of one of the three experiments are presented.

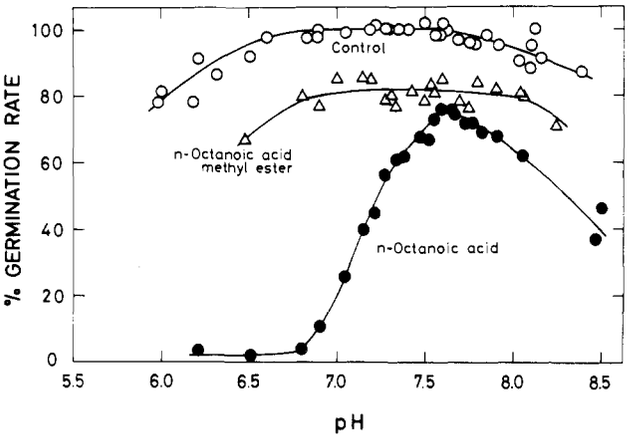

Figure 5. Effect of pH on the inhibition of germination of *B. subtilis* PCI219 spores by *n*-octanoic acid and its methyl ester: (●) *n*-octanoic acid, 40 mM; (△) *n*-octanoic acid methyl ester, 0.33 mM; (○) control. See also the legend to Figure 2.

Dependency on pH of Germination Inhibition by Fatty Acid. In Figure 5 the pH dependency of the inhibition of *n*-octanoic acid with a pK_a of 4.9 and the pH independency of its methyl ester derivative which seems to be present only in a nonionized form are shown. Therefore, we may conclude that the nonionized molecule was responsible for germination inhibition.

Nature of the Inhibition by Phenols and Fatty Acids. Heat resistance and stainability of spores were not affected by the phenols and the fatty acids at the experimental concentrations, and a normal germination rate was observed after removal of the inhibitor.

Preliminary experiments of inhibition by phenol and *n*-octanoic acid with various concentrations of L-alanine showed that the type of inhibition exhibited by both compounds was mixed rather than competitive or noncompetitive (Figure 6).

Discussion

Phenols have several biological activities, which are generally nonspecific and somehow involve membrane functions, such as antimicrobial activity,[7,8] uncoupling

Table I. Inhibitory Activity of Phenols on Germination of *B. subtilis* PCI219 Spores

no.	phenol substituent	log P [a]	pK_a [b]	log D [c] (pH 7.2)	I_{50}, [d] M	log $1/I_{50}$, 1/M obsd	calcd [e]	calcd [f]	calcd [g]
1	H	1.48	10.0	1.48	$1.50 \pm 0.15 \times 10^{-3}$	2.82	2.72	2.48	2.44
				Alkylphenols					
2	2-CH_3	1.98	10.3	1.98	$1.30 \pm 0.05 \times 10^{-3}$	2.89	2.90	2.70	2.86
3	3-CH_3	1.97	10.1	1.97	$2.01 \pm 0.05 \times 10^{-3}$	2.70	2.89	2.69	2.83
4	4-CH_3	1.94	10.3	1.94	$3.64 \pm 0.16 \times 10^{-3}$	2.44	2.88	2.68	2.83
5	2-CH_2CH_3	2.47	10.2	2.47	$4.93 \pm 0.07 \times 10^{-4}$	3.31	3.07	2.91	3.19
6	4-CH_2CH_3	2.26	10.0	2.26	$1.60 \pm 0.09 \times 10^{-3}$	2.80	3.00	2.82	3.03
7	2-$CH(CH_3)_2$	2.88	10.4	2.88	$2.68 \pm 0.11 \times 10^{-4}$	3.57	3.22	3.09	3.45
8	4-$CH(CH_3)_2$	2.86	10.1	2.86	$6.74 \pm 0.71 \times 10^{-4}$	3.17	3.21	3.08	3.41
9	2-$CH_2CH(CH_3)_2$	3.27	10.5	3.27	$2.42 \pm 0.30 \times 10^{-4}$	3.62	3.35	3.26	3.68
10	4-$CH_2CH(CH_3)_2$	3.40	10.2	3.40	$3.02 \pm 0.21 \times 10^{-4}$	3.52	3.40	3.31	3.71
11	2-$C(CH_3)_3$	3.31	11.3	3.31	$3.45 \pm 0.12 \times 10^{-5}$	4.46	3.37	3.27	3.77
12	4-$C(CH_3)_3$	3.12	10.1	3.12	$3.02 \pm 0.12 \times 10^{-4}$	3.52	3.30	3.19	3.56
13	2-$CH(CH_3)_2$, 5-CH_3	3.30	10.7	3.30	$4.94 \pm 0.64 \times 10^{-4}$	3.31	3.37	3.27	3.71
14	2,4,6-$(CH_3)_3$	2.86	10.9	2.86	$1.40 \pm 0.10 \times 10^{-3}$	2.85	3.21	3.08	3.49
				Halophenols					
15	4-Br	2.55	9.3	2.55	$7.17 \pm 0.53 \times 10^{-4}$	3.14	3.10	2.94	3.15
16	2-Cl	2.15	8.5	2.13	$5.62 \pm 1.40 \times 10^{-4}$	3.25	2.95	2.77	2.81
17	4-Cl	2.39	9.4	2.39	$7.97 \pm 0.71 \times 10^{-4}$	3.10	3.04	2.87	3.06
18	2,4-Cl_2	3.14	7.9	3.06	$2.42 \pm 0.46 \times 10^{-4}$	3.62	3.28	3.20	3.36
19	2,4,5-Cl_3	3.72	7.0	3.31	$1.28 \pm 0.57 \times 10^{-4}$	3.89	3.37	3.45	3.55
20	2,4,6-Cl_3	3.78	6.2	2.74	$1.30 \pm 0.61 \times 10^{-4}$	3.89	3.17	3.48	3.50
21	2,3,4,6-Cl_4	4.10	5.7	2.59	$2.69 \pm 1.42 \times 10^{-4}$	3.57	3.11	3.62	3.57
22	2,3,4,5,6-Cl_5	5.07	5.0	3.07	$3.46 \pm 0.95 \times 10^{-4}$	3.46	3.28	4.04	3.77
23	(hexachlorophene)	7.54	5.0	5.54	$1.98 \pm 0.17 \times 10^{-4}$	3.70	4.16	5.11	3.72
				Nitrophenols					
24	2-NO_2	1.77	7.2	1.47	$3.15 \pm 0.44 \times 10^{-3}$	2.50	2.72	2.60	2.40
25	3-NO_2	2.00	8.4	1.97	$9.01 \pm 0.92 \times 10^{-4}$	3.05	2.89	2.70	2.69
26	4-NO_2	1.96	7.1	1.61	$6.83 \pm 0.60 \times 10^{-3}$	2.17	2.77	2.69	2.53
27	2,5-$(NO_2)_2$	1.51	4.1	−1.59	$3.34 \pm 0.36 \times 10^{-2}$	1.48	1.63	2.49	1.90
28	2,4,6-$(NO_2)_3$	2.03	0.8	−4.37	$8.03 \pm 2.72 \times 10^{-3}$	2.10	0.65	2.72	1.98
				Others					
29	2-C_6H_5	3.09	9.9	3.09	$2.80 \pm 0.32 \times 10^{-4}$	3.55	3.29	3.18	3.52
30	4-C_6H_5	3.20	9.5	3.20	$1.29 \pm 0.25 \times 10^{-4}$	3.89	3.33	3.23	3.54
31	3-OH	0.78	9.4	0.78	$3.83 \pm 0.21 \times 10^{-3}$	2.42	2.47	2.17	1.77
32	2-OCH_3	1.33	9.9	1.33	$6.31 \pm 0.18 \times 10^{-3}$	2.20	2.67	2.41	2.31
33	3-OH, 5-CH_3	1.27	9.3	1.27	$1.68 \pm 0.25 \times 10^{-2}$	1.77	2.65	2.39	2.20
34	2-CHO	1.66	8.3	1.63	$3.78 \pm 0.43 \times 10^{-3}$	2.42	2.77	2.56	2.42
35	4-CHO	1.37	7.6	1.22	$2.00 \pm 0.31 \times 10^{-2}$	1.70	2.63	2.43	2.12
36	2-$COOCH_3$	2.46	10.2	2.46	$1.03 \pm 0.05 \times 10^{-3}$	3.00	3.07	2.90	3.18
37	4-$COOCH_3$	1.92	8.5	1.90	$2.01 \pm 0.46 \times 10^{-3}$	2.70	2.87	2.67	2.64
38	2-COOH	2.24	3.0, 14.0	−1.96	*h*				
39	4-COOH	1.57	4.6, 9.4	−1.03	*h*				
40	2-NH_2	0.57	9.7	0.57	$2.99 \pm 0.60 \times 10^{-2}$	1.52	2.40	2.08	1.59
41	3-NH_2	0.16	9.9	0.16	$4.47 \pm 0.39 \times 10^{-2}$	1.35	2.25	1.90	1.20
42	4-NH_2	0.04	10.3	0.04	*h*				
43	4-$CH_2CH(NH_2)COOH$	−2.26	2.2, 10.1	−7.26	*i*				

[a] Mean log P values in an octanol-water system are from the list of Hansch and Leo,[5] and the following values were calculated:[5] 4-isopropylphenol = phenol + $\pi_{i\text{-Pr}}$ + para position = 1.48 + 1.53 + (−0.15) + 2.86; 4-*sec*-butylphenol = phenol + $\pi_{s\text{-Bu}}$ + para position = 1.48 + 2.04 + (−0.12) = 3.40; orcinol = resorcinol + π_{Me} + meta position = 0.78 + 0.56 + (−0.07) = 1.27. [b] Dissociation constant was from ref 24 and 25 or was determined spectrophotometrically[26] in 2- and 4-isopropylphenol, 2- and 4-*sec*-butylphenol, 2- and 4-*tert*-butylphenol, and thymol. [c] Distribution coefficient according to Scherrer and Howard.[6] [d] Molar inhibitory concentration necessary to cause 50% germination rate determined by the experiments shown in Figure 1. [e] Calculated from eq 1. [f] Calculated from eq 2. [g] Calculated from eq 7. [h] No inhibition at 5×10^{-2} M. [i] No inhibition at 2.5×10^{-3} M.

activity with oxidative phosphorylation,[9-17] and inhibition of membrane permeability.[18,19]

Fujita[20] has analyzed several data concerning the effects of phenols and emphasized that whatever the true active form might be in the ionized or nonionized form, the hydrophobic effects (log P), as well as the electronic effects

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Table II. Inhibitory Activity of Fatty Acids on Germination of *B. subtilis* PCI219 Spores

no.	fatty acid	C no.	log P [a]	pK_a [b]	log D [c] (pH 7.2)	I_{50}, [d] M	log $1/I_{50}$, 1/M obsd	log $1/I_{50}$, 1/M calcd [e]
1	*n*-heptanoic	C7	2.42	4.9	0.12	$1.51 \pm 0.18 \times 10^{-1}$	0.82	1.00
2	*n*-octanoic	C8	2.92	4.9	0.62	$5.35 \pm 0.07 \times 10^{-2}$	1.27	1.36
3	*n*-nonanoic	C9	3.42	4.9	1.12	$8.95 \pm 0.18 \times 10^{-3}$	2.05	1.72
4	*n*-decanoic	C10	3.92	4.9	1.62	$6.03 \pm 0.71 \times 10^{-3}$	2.22	2.08
5	*n*-undecanoic	C11	4.42	4.9	2.12	$4.47 \pm 0.45 \times 10^{-3}$	2.35	2.44
6	*n*-dodecanoic	C12	4.92	4.9	2.62	$1.98 \pm 0.14 \times 10^{-3}$	2.70	2.79
7	*n*-tridecanoic	C13	5.42	4.9	3.12	$7.50 \pm 1.81 \times 10^{-4}$	3.12	3.15
						% inhibn at 2×10^{-4} M [f]		
8	myristic	C14				21.0 ± 7.9		
9	palmitic	C16				17.3 ± 5.1		
10	stearic	C18				14.2 ± 9.9		
11	oleic	C18				30.2 ± 4.4		
12	linoleic	C18				34.6 ± 1.9		
13	linolenic	C18				32.0 ± 2.8		

[a] Partition coefficient in an octanol–water system from Table III of ref 6. [b] Dissociation constant from ref 6. [c] Distribution coefficient.[6] [d] Molar inhibitory concentration necessary to cause 50% germination rate determined by the experiments shown in Figure 4. The value for *n*-heptanoic acid was extrapolated. [e] Calculated from eq 9 or 10. [f] I_{50} could not be determined because of its low solubility.

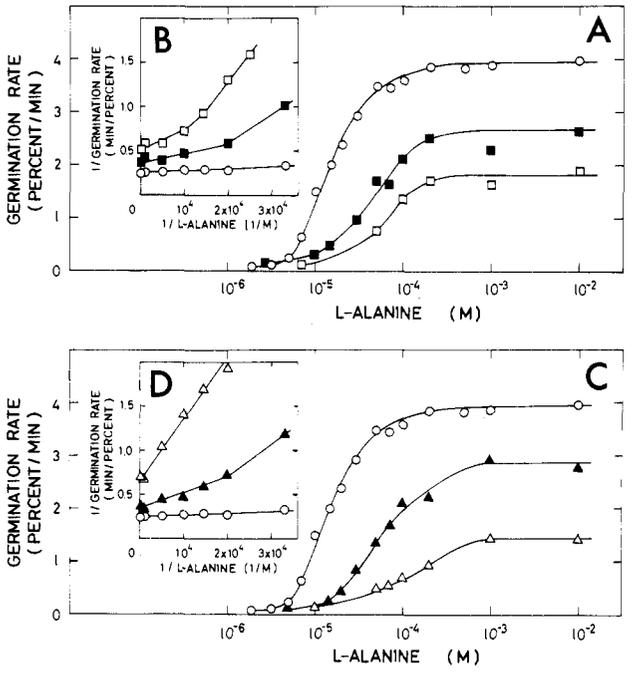

Figure 6. Effect of various concentrations of L-alanine on the inhibition of germination of *B. subtilis* PCI219 spores by phenol (A, B) and *n*-octanoic acid (C, D): (○) control, L-alanine only; (■) L-alanine + phenol, 1.5 mM; (□) L-alanine + phenol, 2.0 mM; (▲) L-alanine + *n*-octanoic acid, 50 mM; (△) L-alanine + *n*-octanoic acid, 66 mM. Double-reciprocal plots are shown in B and D.

(pK_a), would be important for their physiological activities.

Scherrer and Howard[6] have proposed for ionizable compounds the use of the distribution coefficient, which is related to the amount of the compound at the site or in a membrane, and they have illustrated good correlations with log D and pK_a in various cases, including phenols and fatty acids.

Inhibition of germination of *B. subtilis* spore by phenols seems to be due to the nonionized molecule, and the inhibitory activity is correlated mainly with the hydrophobicity and partly with the electronic effects of the compound. Analyses using log P and log D gave similar results. For all active 39 phenols the correlation with log P, however, was a little better than that with log D, and the pK_a term entered to a less degree in the latter case, since it had already included the pK_a value.

Among alkylphenols, the introduction of methyl group(s) into the phenol ring, e.g., 2-, 3-, and 4-methylphenol and 2,4,6-trimethylphenol, did not cause a marked effect on inhibition. But as alkyl chains were lengthened, then the inhibitory activity was increased (Figure 1A). The introduction of halogen group(s) into the phenol ring increased their inhibitory effects. The retardation of the inhibitory curves of pentachlorophenol and hexachlorophene at the higher concentrations seemed to be due to their poor solubility (Figure 1B). The introduction of a nitro group(s), however, mostly decreased the apparent inhibitory effect (Figure 1C). The introduction of a bulky phenyl group increased it, and aldehyde, hydroxyl, methoxyl, and amino groups were less effective than phenol (Figure 1D). Carboxyl derivatives of phenol, i.e., salicylic acid and *p*-hydroxybenzoic acid, were not effective even at 0.05 M, whereas the methyl esters were effective as had been reported for the esters of benzoic acid and *n*-octanoic acid.[4] The ortho position of the substituents was compared with the para one to determine whether the ortho effect of the intramolecular hydrogen bond formation between a hydroxyl group and the substitutent might be concerned (eq 11–14). The relationship between the inhibitory activity of *B. subtilis* spore germination and hydrophobicity of 2-phenols is defined in eq 11 and 12. That of 4-phenols

$$\log 1/I_{50} = \underset{(10.41)}{0.89 \log P + 1.02} \quad (11)$$

$$n = 12,\ r = 0.957,\ s = 0.217,\ F_{1,10,0.05\%} = 108.5$$

$$\log 1/I_{50} = \underset{(10.28)}{0.87 \log D + 1.09} \quad (12)$$

$$n = 12,\ r = 0.956,\ s = 0.220,\ F_{1,10,0.05\%} = 105.6$$

is defined in eq 13 and 14. The Student's t test to determine the difference of the mean values for the estimated log $1/I_{50}$ values showed no significant differences between both phenols. Among 43 phenols in Table I, 2-*tert*-butylphenol was the most effective inhibitor. The concen-

$$\log 1/I_{50} = \underset{(8.57)}{0.95 \log P + 0.59} \quad (13)$$

$$n = 11,\ r = 0.944,\ s = 0.203,\ F_{1,9,0.05\%} = 73.4$$

$$\log 1/I_{50} = \underset{(10.60)}{0.89 \log D + 0.79} \quad (14)$$

$$n = 11,\ r = 0.962,\ s = 0.167,\ F_{1,9,0.05\%} = 112.4$$

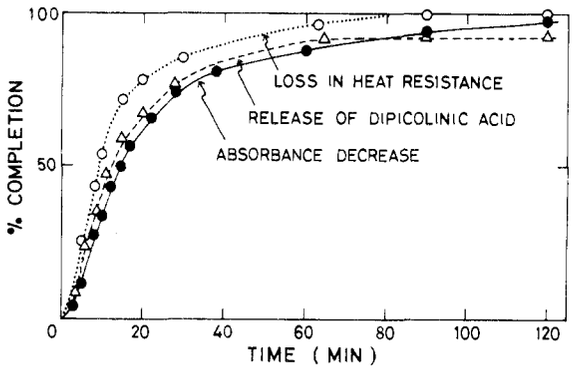

Figure 7. Time course of germination events in *B. subtilis* PCI219 spores.

tration for 50% inhibition of the germination rate was 3.45 ± 0.12 × 10^{-5} M in 10^{-4} M L-alanine. Some structural specificity to the site may be required, as well as the hydrophobic and electronic effects of the inhibitor. Moreover, the pH dependency curves (Figure 2) on germination inhibition by phenols showed that the inflection points were not always around the pK_a value of the compound, indicating the occurrence of complex interactions of the compound with the spore.

For fatty acids the good correlation between the inhibitory effect and the log P or log D suggested that hydrophobicity at the site was important. The nonionized form might be an inhibitory molecule, but the pH dependency curve (Figure 5) indicated more complex interactions with the spore. The slope of eq 9 and 10 were quite close to those of alcohols and various hydrophobic compounds which had been determined earlier.[4] The action of these compounds seems to be similar. However, the slope of eq 1 and 2 of 39 phenols was much smaller than that of the fatty acids, indicating that a different type of interaction was involved.

Morris et al.[21] have suggested in the studies of *S*-nitrosothiol derivatives on *B. cereus* spores that the inhibitory effectiveness of outgrowth was dependent on the electronic character and, conversely, that of germination was independent of it.

Experimental Section

The method for preparing spores of *B. subtilis* PCI219 was previously described.[4] Spores were not heated before germination. In Figure 7 the sigmoidal response of some events in L-alanine as a germinant is shown. Heat resistance was determined by counting surviving colonies on nutrient agar (Eiken Chemical Co.) after heating at 70 °C for 30 min. Dipicolinic acid was determined by the method of Janssen et al.[22] Absorbance at 650 nm was measured with a Shimadzu–Bausch & Lomb Spectronic 20 A spectrophotometer. In this strain, spore suspensions had lost 70% of their original absorbance when germination had been completed.[23] In the present study, the rate of germination was determined by the absorbance decrease as the slope of the most rapid linear portion of the curve. Experiments were carried out at 37 °C with spore suspensions containing about 2.2 × 10^8 cells per mL, 0.1 mM L-alanine, and 0.05 M phosphate buffer at pH 7.2, unless otherwise stated. To determine the pH dependency of germination inhibition, 0.05 M phosphate buffers at pH 6.0 to 8.0 were used. The values of log P (partition coefficient in an octanol–water system) were from the list of Hansch and Leo.[5] The value of log D (distribution coefficient) at pH 7.2 was calculated according to Scherrer and Howard,[6] who defined it as the ratio of the equilibrium concentration of the compound in an organic phase to the total concentration in an aqueous phase at a given pH. The pK_a's of the compounds were from the literature[6,24,25] or were determined spectrophotometrically[26] with a Hitachi recording spectrophotometer, Type 323. The regression analyses were made by the least-squares method with a Hitachi computer, HITAC 20. Phenols were purchased from Tokyo Kasei Kogyo Co. Fatty acids were obtained in their free acid forms from the Katayama Chemical Co. All compounds were dissolved in 0.05 M phosphate buffer and adjusted to pH 7.2.

Acknowledgment. The authors acknowledge Dr. Roy H. Doi, Department of Biochemistry and Biophysics, University of California, Davis, and Dr. Robert A. Scherrer, Riker Laboratories, Inc., St. Paul, for helpful suggestions and critical readings of the manuscript. The valuable discussions by Dr. Tomohiro Matsuda, Department of Pharmacology, the computer analyses by Dr. Yasuhiro Hasegawa, Department of Physiology, and the technical assistance of Mrs. Michiko Murase, Department of Microbiology, Nagoya City University Medical School, are also appreciated.

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