

Notes

Synthesis and Evaluation of Ureido- and Vinylureidopenicillins as Inhibitors of Intraruminal Lactic Acid Production¹

Robin D. Clark,* Joan M. Caroon, Ian T. Harrison, Arthur F. Kluge, Stefan H. Unger,

Institute of Organic Chemistry

Howard R. Spires,* and Thomas R. Mathews

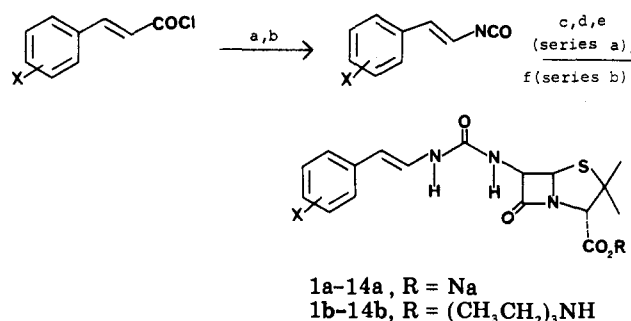
Institute of Agriscience, Syntex Research, Palo Alto, California 94304. Received April 13, 1981

A series of 14 vinylureidopenicillins and a series of 9 ureidopenicillins were prepared by reaction of 6-aminopenicillanic acid with vinyl isocyanates and isocyanates. These compounds were evaluated for their potential to protect ruminants against lactic acidosis. The compounds were tested for inhibition of *in vitro* ruminal lactic and propionic acid production, and six compounds inhibited lactic acid production to less than 10% of control at doses of 0.31 $\mu\text{g}/\text{mL}$ or lower, whereas they did not inhibit propionic acid production at doses greater than 10 $\mu\text{g}/\text{mL}$. The most active compounds also were screened for general antibacterial activity and were found to be weakly active against Gram-positive bacteria. The structure-activity relationships are discussed for both series. Triethylammonium 6-[3-[2-(4-*tert*-butylphenyl)vinyl]ureido]penicillanate (4) was chosen for evaluation as an inhibitor of intraruminal lactic acidosis *in vivo*.

Lactic acidosis is a nutrition-related metabolic disorder of cattle and sheep associated with overconsumption of rapidly fermentable carbohydrates. The volatile fatty acids (VFA) acetic, propionic, and butyric acids comprise the major end products of the normal rumen fermentation.² Lactic acid is produced in relatively small quantity in a normal fermentation and serves primarily as an extracellular intermediate during VFA production.³ Provided that animals are well adapted to a high-concentrate diet and maintain regular and frequent feeding intervals, a stable fermentation can be maintained. However, an interruption in the normal feeding pattern or a sudden increase in the soluble carbohydrate content of the diet (such as is encountered upon introduction to the feedlot) can result in a rapid increase in the intraruminal lactic acid concentration.⁴ Absorption of large quantities of lactic acid results in a systemic acidosis with clinical signs ranging from loss of appetite to death. In addition, animals which recover often develop laminitis, rumenitis, or liver abscesses which result in reduced weight gains and reduced efficiency of feed utilization.⁵ A prophylactic for the prevention of lactic acidosis could be of major importance to the feedlot industry, since no such agent is currently available commercially.

A number of studies have been conducted to evaluate chemical agents for the prevention of lactic acidosis.⁶⁻⁸ Microbiological evidence suggests that the initial rapid production of lactic acid is due to proliferation of the rumen bacterium *Streptococcus bovis*.⁶ A screen of a large

Scheme I^a



^a a = NaN₃, THF-H₂O; b = toluene, reflux; c = Me₃Si-6-APA; d = H₂O; e = sodium 2-ethylhexanoate; f = 6-APA, triethylamine, CH₂Cl₂.

number of antibiotics against *S. bovis* in culture identified two classes of effective inhibitors: penicillins (e.g., penicillin G) and the sulfur-containing peptide antibiotics (thiopeptin).⁶ Of these, only thiopeptin appears to offer selective protection against lactic acidosis *in vivo*,⁹ since penicillin G is active against a variety of ruminal bacteria¹⁰ and thereby inhibits normal rumen function.^{11,12}

The present report describes the synthesis and evaluation of a series of penicillin derivatives that (1) have sufficient selectivity to inhibit *in vitro* intraruminal lactic acid but not VFA production and (2) have little or no activity toward potential human pathogenic bacteria. This latter

- (1) Contribution number 578 from the Institute of Organic Chemistry.
- (2) Hungate, R. E. "The Rumen and Its Microbes"; Academic Press: New York, 1966; pp 60-64, 454-457.
- (3) Jayasuriya, G. C. N.; Hungate, R. E. *Arch. Biochem. Biophys.* 1959, 82, 274.
- (4) Elem, C. J. *J. Anim. Sci.* 1976, 43, 898.
- (5) Brent, B. E. *J. Anim. Sci.* 1976, 43, 930.
- (6) Muir, L. A.; Barreto, A., Jr. *J. Anim. Sci.* 1979, 48, 468.
- (7) Chaplin, R. K.; Jones, G. A. Report on Conference on Rumen Function, Nov 30-Dec 1, 1977, Chicago, IL.
- (8) Muir, L. A.; Rickes, E. L.; Duquette, P. F.; Smith, G. E. *J. Anim. Sci.* 1980, 50, 547.

- (9) Muir, L. A.; Rickes, E. L.; Duquette, P. F.; Smith, G. E. *J. Anim. Sci.* 1980, 51, 1182.
- (10) Fulghum, R. S.; Baldwin, B. B.; Williams, P. P. *Appl. Microbiol.* 1968, 16, 301.
- (11) Spires, H. R., unpublished results. For example, in our *in vitro* test system, penicillin G at 50, 5, and 0.5 ppm inhibits propionate production to 22, 28 and 84% of control.
- (12) The development of apparent penicillinase activity upon long-term (several weeks) administration of penicillin G *in vivo* has been reported: Wiseman, R. F.; Jacobson, D. R.; Miller, W. M. *Appl. Microbiol.* 1960, 8, 76. Whether penicillinase activity would develop toward a more selective and generally weaker penicillin on short-term (several days) administration remains to be determined.

criteria was deemed necessary, since there is a growing reluctance by regulatory agencies to allow the use of antibiotics (e.g., chlorotetracyclin) in animals when such agents are also used in human therapeutics.¹³

Chemistry. Reaction of trimethylsilyl 6-aminopenicillinate with an isocyanate or a vinyl isocyanate, followed by in situ hydrolysis and treatment with sodium 2-ethylhexanoate, gave the sodium penicillates 1–23 (Scheme I). The isocyanates were commercially available and the vinyl isocyanates were prepared in situ by thermolysis of the vinyl acyl azide prepared from the corresponding cinnamoyl chloride. An alternative procedure, developed for the large-scale synthesis of the triethylammonium salt of 4, consisted of the reaction of 6-aminopenicillanic acid in methylene chloride–triethylamine with the vinyl isocyanate.¹⁴ This procedure was convenient and it provided the highly crystalline triethylammonium salt directly.

Biological Testing. Two in vitro screening models were used to examine the potential of the penicillin derivatives as selective inhibitors of intraruminal lactic acid production. For both models, fluid digesta obtained from a rumen-fistulated bovine was mixed with an equal volume of appropriate anaerobic buffer and was transferred under CO₂ to anaerobic incubation vessels.

A fermentation model designed to simulate the intraruminal environment under conditions conducive to lactic acid acidosis was used to test compounds for their potency in preventing lactic acid accumulation. The medium was high in glucose and low in buffering capacity; therefore, the system encouraged a high level of lactic acid production. Microscopic examination of control incubations after 5.5 h at 39 °C revealed a high concentration of Gram-positive cocci typical of a lactic acid fermentation in vivo.

A second fermentation model designed to simulate a normal rumen environment was used to determine the potency of compounds as inhibitors or propionic acid production. Buffer used in this model contained a moderate level of substrates and was buffered at normal intraruminal pH. This system encouraged production of volatile fatty acids with little or no accumulation of lactic acid.

All compounds were initially tested for their ability to inhibit lactic acid production at 50, 5, and 0.5 µg/mL of incubation contents. The most active compounds from this primary screen were retested at eight concentrations ranging from 10 to 0.08 µg/mL in both the lactic acid and volatile fatty acid fermentation models. Lack of activity toward inhibition of propionic acid is essential, since antibiotics of other classes (e.g., monensin) that promote high levels of propionic acid production are known to increase animal productivity.¹⁵

Results and Discussion

Results listed in Table I demonstrate that at 50 µg/mL all compounds reduced lactic acid production to less than 10% of control incubations which contained no compound. Ten of these compounds remained effective at 0.5 µg/mL. Minimum concentrations of these ten compounds necessary to inhibit lactic acid production to less than 10% of control and minimum concentrations which decreased propionic acid production to less than 95% of control were

then determined in the lactic acid and volatile fatty acid fermentation models, respectively.

The most potent inhibitor of lactic acid production was 22, which had a potency equal to that of thiopeptin. However, 22, along with 8 and 20, had the undesirable feature of being a more potent inhibitor of propionic acid production than the other six compounds, which inhibited lactic acid to less than 10% of control in the 0.008–0.31 µg/mL range. The two most effective compounds, in terms of combining maximal potency toward lactic acid production with minimal potency toward propionic acid production, were 4 and 11. Although less potent inhibitors of lactic acid production than thiopeptin, 4 and 11 did not inhibit propionic acid production at 10 µg/mL, whereas thiopeptin did show some inhibition at this concentration. Thus, the overall in vitro profile of these compounds appears to be comparable to that of thiopeptin.

On the basis of these data, some general structure–activity relationships can be established. In the vinylureido series, the most potent inhibitors of lactic acid contain a 4-substituted phenyl group (1, 3, 4, and, in part, 11) or a bulky, lipophilic group (8 and 9) at the terminus of the vinyl group. Substitution of chloro or bromo at the 3 or 4 position of the phenyl group led to less active compounds (2, 5, and 7). In the ureido series, the most potent inhibitors of lactic acid were those in which the urea was substituted with a large lipophilic group separated from the urea nitrogen by one or two methylenes (20, 22, and 23).

Inhibition of lactic acid does not appear to correlate with ΔR_M lipophilicities (Table I) alone, but the more active compounds appear to have greater length. For example, the 4-OEt and 4-H compounds 3 and 14 are close in lipophilicity, but the 4-OEt compound is considerably more active. The longer *t*-Bu analogue 4 is considerably more active than the isolipophilic 3,4-Cl₂ analogue 7. However, there are insufficient analogues to allow a definitive statistical analysis of these steric effects.

An interesting observation is the effect of saturation of the vinyl moiety on ΔR_M . Normally, addition of two hydrogens would lead to more lipophilic compounds by about 0.4 unit;¹⁶ however, comparing 14 with 20 and 9 with 23, we find that saturation leads to a decrease in lipophilicity. We attribute this to loss of conjugation with the ureido moiety. In the unconjugated molecule, the ureido moiety is more polar—is a stronger point source of partial charge—and is therefore more efficiently solvated by water.

Eight of the ten compounds found to be the most potent inhibitors of lactic acid production also were tested against 12 potentially pathogenic bacterial strains. The results against five of the strains are listed in Table II. None of the compounds demonstrated activity at less than 200 µg/mL toward seven other strains, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus morganii*, and *Proteus vulgaris*.

All vinylureido derivatives were less potent than the ureido derivatives toward the staphylococci and streptococci. Approximate correlations of ΔR_M with activity were observed for both *Staphylococcus* and the *Escherichia coli* strains for the vinylureido series, and for all but the *Staphylococcus aureus* (resistant) for the ureido series.

On the basis of its potency and selectivity toward inhibition of lactic acid as opposed to VFA production, its weak general antibacterial spectrum, and its ease of synthesis, 4b was chosen for a study to determine its efficacy

(13) Solomons, I. A. *J. Anim. Sci.* 1978, 46, 1360.

(14) Essentially the procedure of Perron, Y. G.; Minor, W. F.; Crast, L. B.; Cheney, L. C. *J. Org. Chem.* 1961, 26, 3365. The triethylammonium salts of 15–18 are reported in this paper.

(15) Richardson, L. F.; Raun, A. P.; Potter, E. L.; Cooley, C. O.; Rathmacher, R. P. *J. Anim. Sci.* 1976, 43, 657.

(16) Leo, A.; Jow, P. Y. C.; Silipo, C.; Hansch, C. J. *J. Med. Chem.* 1975, 18, 865.

Table I. Vinylureidopenicillins and Inhibition of Lactic and Propionic Acid Production

no.	R ₁	R ₂	yield, ^a %	dec pt, ^b °C	formula	anal. ^c	ΔR _M ^d	lactic acid inhibn, ^e % of control			effective inhib concn, μg/mL	
								50 ppm	5 ppm	0.5 ppm	lactic acid ^f	propionic acid ^g
1	4-CH ₃ C ₆ H ₄	H	87	215-217	C ₁₈ H ₂₀ NaN ₃ O ₄ S·2.5H ₂ O	C, H, N	0.50	2	2	0	0.31	>10
2	4-ClC ₆ H ₄	H	24	214-216	C ₁₇ H ₁₇ ClNaN ₃ O ₄ ·2H ₂ O	C, H; N ⁱ	0.58	10	2	0	1.25	>10
3	4-CH ₃ CH ₂ OC ₆ H ₄	H	80	215-220	C ₁₉ H ₂₂ NaN ₃ O ₄ S·2.5H ₂ O	C, H, N	0.43	1	1	0	0.31	>10
4a	4-(CH ₃) ₃ CC ₆ H ₄	H	69	228-230	C ₂₁ H ₂₆ NaN ₃ O ₄ S·4H ₂ O	C, H, N	1.31	0	0	0	0.16	>10
4b ^h			72	182-184	C ₂₇ H ₄₂ N ₄ O ₄ S	C, H, N, S						
5	3-BrC ₆ H ₄	H	52	214-216	C ₁₇ H ₁₇ BrNaN ₃ O ₄ S·H ₂ O	C, H; N ^j	0.69	3	0	142		
6	3,4,5-OCH ₃ C ₆ H ₂	H	33	205-206	C ₂₀ H ₂₄ NaN ₃ O ₄ S·2.5H ₂ O	C, H, N	-0.45	8	2	105		
7	3,4-ClC ₆ H ₃	H	18	207-210	C ₁₇ H ₁₆ Cl ₂ NaN ₃ O ₄ S·H ₂ O	H, N; C ^k	1.44	1	106	107		
8	cyclohexyl	H	77	210-213	C ₁₆ H ₂₄ NaN ₃ O ₄ S·1.5H ₂ O	C, H, N	0.77	2	2	0	0.31	2.5
9	2-naphthyl	H	60	218-221	C ₂₁ H ₂₀ NaN ₃ O ₄ S·1.5H ₂ O	C, H, N	1.05	8	2	1	0.31	>10
10	1-naphthyl	H	80	206-208	C ₂₁ H ₂₀ NaN ₃ O ₄ S·H ₂ O	C, H, N	0.66	3	0	111		
11	3,4-(OCH ₂ O)C ₆ H ₃	H	83	208-210	C ₁₈ H ₁₈ NaN ₃ O ₆ S·1.5H ₂ O	C, H, N	-0.06	4	0	0	0.16	>10
12	2-thienyl	H	62	220-222	C ₁₅ H ₁₆ NaN ₃ O ₄ S ₂ ·3H ₂ O	C, H, N	-0.20	0	52	102		
13	2-furanyl	H	10	230-233	C ₁₅ H ₁₆ NaN ₃ O ₄ S·2.5H ₂ O	C, H; N ^l	-0.26	3	89	101		
14	C ₆ H ₅	CH ₃	25	210-215	<i>m</i>		0.30	0	5	87		
15	cyclohexyl		50	235-238	C ₁₅ H ₂₂ NaN ₃ O ₄ S·2H ₂ O	C, H, N	-0.62	2	0	99		
16	(CH ₃) ₂ CH		69	225-230	C ₁₂ H ₁₈ NaN ₃ O ₄ S·1.5H ₂ O	C, H, N	-2.0	7	119	108		
17	C ₆ H ₅		81	218-221	C ₁₅ H ₁₆ NaN ₃ O ₄ S·H ₂ O	C, H, N	-0.75	3	110	96		
18	4-ClC ₆ H ₄		20	214-216	C ₁₅ H ₁₅ ClNaN ₃ O ₄ S·1.5H ₂ O	C, H, N	-0.01	2	112	102		
19	4-CH ₃ CH ₂ O ₂ CC ₆ H ₄		35	208-211	C ₁₈ H ₂₀ NaN ₃ O ₆ S·H ₂ O	C, H, N		3	95	101		
20	C ₆ H ₅ CH ₂ CH ₂		42	193-195	C ₁₇ H ₂₀ NaN ₃ O ₄ S·H ₂ O	C, H, N	-0.60	9	6	4	0.31	5
21	C ₆ H ₅ -c-Pr		87	225-227	C ₁₈ H ₂₀ NaN ₃ O ₄ S·1.25H ₂ O	C, H, N	-0.29	8	4	104		
22	2-naphthyl-CH ₂		53	195-197	C ₂₀ H ₂₀ NaN ₃ O ₄ S·1.25H ₂ O	C, H, N	0.0	9	4	4	0.08	5
23	2-naphthyl-CH ₂ CH ₂		33	182-185	C ₂₁ H ₂₂ NaN ₃ O ₄ S·H ₂ O	C, H, N	0.89	9	7	2	0.31	>10
	thiopeptin ⁿ										0.08	10

^a Yield is based on 6-aminopenicillanic acid. ^b All decomposition points are uncorrected. The decomposition point refers to the temperature at which complete decomposition occurred. In some cases shrinking and darkening started below the final decomposition point. ^c Elements shown, unless otherwise indicated, analyzed correctly to ±0.4% of calculated values. ^d Lipophilicities were estimated on reversed phase TLC, relative to compound 22, which was run on each plate. Silica gel G plates were coated by submerging in a bath of 20 vol % Dow Corning 200cS silicone oil in hexane, removing and allowing to dry. Each compound was run on two to six plates and the average ΔR_M was determined. Mobile phase was 30 vol % MeOH in pH 7.0 buffer. ^e Numbers are lactic acid production expressed as a percentage of control incubations containing no experimental compound. ^f Numbers are maximum concentration of experimental compound required for reduction of lactic acid production to less than 10% of control. ^g Numbers are maximum concentration of experimental compound required for reduction of propionic acid production to less than 95% of control. ^h 4a refers to the sodium salt. 4b refers to the triethylammonium salt. No differences in activity between the two salt forms was noted. Data refer to sodium salt. ⁱ N: calcd, 9.23; found, 8.24. ^j N: calcd, 8.75; found, 7.28. ^k C: calcd, 43.50; found, 44.57. ^l N: calcd, 10.05; found, 9.40. ^m Purity determined by NMR spectroscopy and found to be >95%. ⁿ Obtained from the Merck Institute for Therapeutic Research, Rahway, NJ.

Table II. In Vitro Antibacterial Activities of Penicillin Derivatives

compd	min inhibitory concn, ^a $\mu\text{g/mL}$				
	S.a. (S) ^b	S.a. (R) ^c	S.f. ^d	S.p. ^e	E.c. ^f
2	6.25	50	>200	3.12	>200
4	3.12	100	>200	1.60	>200
8	0.40	50	50	0.20	>200
9	3.12	100	200	3.12	>200
11	6.25	50	200	1.60	100
20	0.10	50	3.12	0.10	100
22	0.05	50	3.12	0.05	>200
23	0.10	25	6.25	0.05	>200
penicillin G ^g	0.05	50	0.40	0.01	25

^a See Experimental Section for procedures used.

^b *Staphylococcus aureus* ATCC-6538P (penicillin G sensitive). ^c *Staphylococcus aureus* ATCC-14154 (penicillin G resistant). ^d *Streptococcus faecium* ATCC-8043-2.

^e *Streptococcus pyogenes* ATCC-8668. ^f *Escherichia coli* ATCC-25922. ^g Penicillin G also had activity (<200 $\mu\text{g/mL}$) against *Proteus mirabilis* ATCC-2110 and *Klebsiella pneumonia* ATCC-39645.

as an inhibitor of lactic acidosis in vivo.

Experimental Section

Melting points are uncorrected; infrared spectra were recorded with a Perkin-Elmer 237B spectrometer; NMR spectra were obtained with Varian A-60 and HA-100 instruments. Chemical shifts are in parts per million and coupling constants (J) in hertz. Microanalyses were performed by Atlantic Microlab, Atlanta, GA.

4-*tert*-Butylcinnamoyl Azide. A solution of 4-*tert*-butylcinnamoyl chloride (107.8 g, 0.485 mol) in THF (750 mL) was cooled in an ice bath while a solution of sodium azide (39.0 g, 0.60 mol) in water (125 mL) was added dropwise. After stirring for 1 h at ice-bath temperature, the mixture was extracted with ether, and the ether extract was washed with water, aqueous sodium hydroxide, and brine and dried over Na_2SO_4 . Evaporation gave 108.0 g (97.3%) of an analytically pure, pale yellow powder: mp 98–99 °C dec; IR (KBr) 2120, 1680, 1620 cm^{-1} ; NMR (CDCl_3) δ 1.35 (s, 9 H), 6.43 (d, $J = 15$ Hz, 1 H), 7.49 (s, 4 H), 7.77 (d, $J = 15$ Hz, 1 H). Anal. ($\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}$) C, H, N.

Triethylammonium 6-[3-[2-(4-*tert*-Butylphenyl)vinyl]ureido]penicillanate (4b). 4-*tert*-Butylcinnamoyl azide (37.8 g, 165 mol) in toluene (150 mL) was refluxed for 0.5 h, and the solution was then cooled to room temperature. Triethylamine (38 mL) and 6-aminopenicillanic acid (32.4 g, 0.15 mol) in CH_2Cl_2 (250 mL) were stirred for 1.5 h, and the resulting solution was filtered and cooled in an ice bath. The above toluene solution was added at once, and the mixture was stirred for 18 h at room temperature, after which time a precipitate had formed. Ether (1400 mL) was added, and the mixture was cooled and filtered to afford 56.0 g (72%) of pure 4b as a white powder: mp 182–184 °C dec; $[\alpha]_D^{25}$ 185.5° (c 0.02, EtOH); IR (KBr) 3400–3100, 2950, 1770, 1680, 1640, 1600, 1545 cm^{-1} ; NMR (CDCl_3) δ 1.21 (t, $J = 7$ Hz, 9 H), 1.25 (s, 9 H), 1.67 (s, 6 H), 3.03 (q, $J = 7$ Hz, 6 H), 4.36 (s, 1 H), 5.60 (m, 2 H), 5.76 (d, $J = 15$ Hz, 1 H), 7.26 (s, 4 H), 7.36 (d, $J = 15$ Hz, 1 H).

Sodium 6-[3-[2-(2-Naphthyl)vinyl]ureido]penicillanate (9). The acyl azide from 2-(2-naphthyl)acryloyl chloride (1.8 g, 9.2 mmol), prepared as above, was refluxed in toluene (15 mL) for 1 h, and the solution was cooled to room temperature. Bis(trimethylsilyl)acetamide (0.84 g, 4.1 mmol) and 6-aminopenicillanic acid (1.08 g, 5 mmol) in acetonitrile (25 mL) were stirred for 3 h, the above toluene solution was then added, and the stirring was continued for 18 h. The mixture was cooled to 0 °C and water (0.4 mL) was added. After 0.5 h, the solution was dried over MgSO_4 , filtered, and added to a solution of sodium 2-ethyl-

hexanoate (0.8 g, 4.8 mmol) in ether (50 mL)–THF (20 mL). The precipitate was washed quickly with ether and acetone and dried in a vacuum oven at 60 °C to afford 1.21 g (56%) of 9 as a tan powder: mp 218–221 °C dec; IR (KBr) 1770, 1680, 1640 cm^{-1} ; NMR ($\text{Me}_2\text{SO}-d_6$) δ 1.57 (s, 3 H), 1.63 (s, 3 H), 4.05 (s, 1 H), 5.58 (m, 2 H), 6.16 (d, $J = 15$ Hz, 1 H), 7.25–8.00 (m, 7 H), 8.10 (d, $J = 15$ Hz, 1 H).

Ruminal Fluid Incubations. Compounds were prepared for initial testing by dissolving them in distilled water at 2.5 mg/mL and serially diluting tenfold to 0.025 mg/mL. The most active compounds chosen for retesting were dissolved at 0.5 mg/mL and serially diluted to 0.004 mg/mL; 200- μL aliquots of each dilution were pipetted into 18 \times 150 mm incubation tubes. Duplicate incubations for the lactic acid fermentation model and triplicate incubations for the volatile fatty acid fermentation model were conducted for each level of compound tested.

Rumen fluid obtained from a rumen-fistulated bovine was strained through cheesecloth, and the fluid was mixed with an equal volume of CO_2 -saturated buffer. Buffer used in the lactic acid fermentation model had the following composition in grams per liter: glucose, 27; trypticase, 1.50; Na_2HPO_4 , 4.26; NaH_2PO_4 , 8.28; NaCl, 0.43; KCl, 0.43; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.12; K_2SO_4 , 0.15; CaCl_2 , 0.05; urea, 0.60. Buffer for the volatile fatty acid fermentation model had the following composition in grams per liter: glucose, 1.08; cellobiose, 4.10; maltose, 2.39; soluble starch, 5.38; amino acids, 4.68; NaHCO_3 , 9.80; Na_2HPO_4 , 3.69; NaCl, 0.43; KCl, 0.43; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.12; CaCl_2 , 0.05; K_2SO_4 , 0.15; urea, 0.12.

Appropriate rumen fluid–buffer solution was pipetted into each incubation tube to bring the volume to 10 mL. All tubes were fitted with gas-release stoppers and were incubated in a shaking water bath at 39 °C. Fermentation was terminated at 5.5 h for the lactic acid fermentations and at 9 h for the volatile fatty acid fermentations by addition of 2 mL of 25% metaphosphoric acid to the incubation tubes. Aliquots were centrifuged at 3000g for 10 min, and the resulting supernate was analyzed for *l*(+)-lactic acid or volatile fatty acids.

l(+)-Lactic acid was analyzed via a specific enzymatic assay.¹⁷ Volatile fatty acids were analyzed via gas chromatography. The instrument used was a Perkin-Elmer 900B equipped with a hydrogen flame ionization detector. The acids were separated on a stainless-steel column [0.125 in. \times 6 ft (0.32 \times 182.9 cm)] packed with 10% SP-1200/1% H_3PO_4 on Chromosorb-WAW, 80–100 mesh.¹⁸ The following additional chromatographic conditions were employed: column temperature, 145 °C; injector temperature, 190 °C; detector temperature, 190 °C; carrier gas flow, 45 mL/min; injection volume, 1 μL .

Antibacterial Assays. Minimum inhibitory concentrations were determined using an Ames Handititer 2 to make serial twofold dilutions from 200 to 0.0008 $\mu\text{g/mL}$. Medium (brain–heart infusion broth containing 0.066 g/L of tetrazolium chloride dye) was dispensed in 0.05-mL volumes into cups in the dilution tray. Eight 0.05-mL diluting loops transferred fluid for serial dilution. Each cup was inoculated with 1 μL of inoculum produced by a 1:10 dilution of bacterial culture which had been grown for 18 h at 37 °C. Growth was detected by formation of a red precipitate following incubation for 16 h at 37 °C. Triplicate trays were used for each test compound against each bacteria.

Acknowledgment. We thank Tony Feuerman for assistance with the TLC lipophilicity measurements and the Syntex Analytical Department for analytical support. The technical assistance of Bonita Koller and Vicki Blondeau in determining the VFA and lactic acid levels also is gratefully acknowledged.

(17) Sigma Technical Bulletin No. 826-UV, Sigma Chemical Co., St. Louis, MO 63178.

(18) Supelco Inc., Bellefonte, PA 16823.