

# Comparison Study of Alkyl Boronic Acids with Boric Acid

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The bacteriostatic activities of two alkyl boronic acids were compared with boric acid. Some primary irritation and chronic toxicity determinations of the compounds were also accomplished. The results indicated that nonyl boronic acid and dodecyl boronic acid possessed bacteriostatic activity superior to boric acid, and that very dilute concentrations of these compounds could be employed to achieve bacteriostasis. Toxicological testing showed the alkyl boronic acids employed to be only mildly irritating and apparently nontoxic when applied to animal skin.

**P**RELIMINARY REPORTS from the manufacturer indicated that some of the alkyl boronic acids possessed a considerable degree of antibacterial activity in quite dilute concentrations. Comparison studies with the activity of boric acid were necessary to determine their possible value as substitutes for boric acid in existing types of preparations. It was also necessary to ascertain within certain limitations the possibility of the development of toxic reactions from their use.

The compounds employed in this study were nonyl boronic acid [nonyl dihydroxy borane,  $C_9H_{19}B(OH)_2$ ] and dodecyl boronic acid [dodecyl dihydroxy borane,  $C_{12}H_{25}B(OH)_2$ ]. They are colorless liquids, having about the same consistency as glycerin. Both are completely miscible at room temperature with hexane, benzene, ethyl ether, and acetone. Nonyl boronic acid is slightly soluble in water (3% by weight) and very soluble in methanol (34% by weight). Dodecyl boronic acid is less than 1% soluble in water and 23% soluble in methanol. The surface tensions of the saturated aqueous solutions at 25° are 47.2 and 44.9 dynes/cm.<sup>2</sup>, respectively, for nonyl and dodecyl boronic acids. They are much weaker acids than boric acid (1).

## EXPERIMENTAL

**Determination of the Bacteriostatic Activity of the Alkyl Boronic Acids.**—Determination of the bacteriostatic activity of nonyl and dodecyl boronic acid was made by use of the standard serum-cup agar plate method. Dilutions of 1:1,000, 1:5,000, 1:25,000 and 1:50,000 were prepared for both compounds. Table I illustrates the bacteriostatic activity of the various dilutions of these compounds against *Micrococcus pyogenes* var. *aureus*, *Salmonella typhosa*, and *Bacillus subtilis*.

Since there must be a basis of comparison to the presently used boric acid, the same tests were made with boric acid itself. The dilutions and organisms employed were identical to those employed with the alkyl boronic acids. The results of these tests are also indicated in Table I. At the dilutions used with the alkyl boronic acids, boric acid exhibited no detectable bacteriostatic activity. To obtain more positive values, dilutions of boric acid more commonly in use were prepared. These results are also given in Table I.

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TABLE I.—BACTERIOSTATIC ACTIVITY

Compound	Dilution	Organism		
		<i>Micrococcus pyogenes</i> var. <i>aureus</i>	<i>Salmonella typhosa</i>	<i>Bacillus subtilis</i>
Nonyl boronic acid	1:1,000	4 <sup>a</sup>	4	4
	1:5,000	2	3	3
	1:25,000	2	3	2
	1:50,000	1	2	2
Dodecyl boronic acid	1:1,000	4	4	4
	1:5,000	3	4	4
	1:25,000	3	4	3
	1:50,000	2	2	2
Boric acid	1:1,000	0	0	0
	1:5,000	0	0	0
	1:25,000	0	0	0
	1:50,000	0	0	0
Boric acid	5%	2	1	1
	2%	1	0	0
	1%	0	0	0

<sup>a</sup> 4, Complete bacteriostasis (zone of inhibition 0.7 cm.); 3, bacteriostasis not complete (zone of inhibition 0.5–0.7 cm.); 2, small zone of inhibition (less than 0.5 cm.); 1, very slight activity (zone of inhibition less than 0.5 cm., bacterial overgrowth); 0, no zone of inhibition (bacterial overgrowth).

The data obtained from this phase of the study would seem to indicate that the alkyl boronic acids possess a degree of bacteriostatic activity considerably superior to that of boric acid. There is, however, some possibility that the greater zones of inhibition achieved with the alkyl boronic acids may have been due in part to the relatively low surface tensions of these compounds in aqueous solution, allowing greater diffusion of the solution being tested into the surrounding media.

**Determination of the Toxicity of the Alkyl Boronic Acids.**—A primary irritation study was first accomplished according to the procedure outlined by Draize (2) and co-workers. This procedure involved the application of the compounds being tested to the intact and abraded skin of albino rabbits. A given compound was applied so that there were three applications to intact skin and three to abraded skin. At the end of a 24-hour exposure the resulting reactions were evaluated for erythema and edema formation. Evaluations were also made after 72 hours to determine the duration and intensity of any resulting irritation.

Table II illustrates the results of the irritation study as well as the method used to score the results. Averages of the 24- and 72-hour scores for all dilutions were calculated. The combined average figure obtained by this method is usually referred to as the primary irritation index.

The results of this phase of the study indicated that although the alkyl boronic acids were several

TABLE II.—SCORES FOR PRIMARY IRRITATION

Compound	Rabbit Skin				Combined Average
	Intact		Abraded		
	24 hr.	72 hr.	24 hr.	72 hr.	
Nonyl boronic acid 1:1,000	3 <sup>a</sup>	1	3	2	
	1	0	2	0	
	2	1	2	1	
Average		1.33		1.67	1.50
Nonyl boronic acid 1:5,000	2	0	2	0	
	0	0	1	1	
	0	0	1	0	
Average		0.33		0.83	0.58
Nonyl boronic acid 1:25,000	0	1	1	1	
	0	0	0	1	
	0	0	0	0	
Average		0.17		0.50	0.33
Dodecyl boronic acid 1:1,000	4	2	6	3	
	2	3	4	4	
	2	0	2	1	
Average		2.16		3.33	2.74
Dodecyl boronic acid 1:5,000	2	1	3	2	
	1	1	2	1	
	1	0	3	2	
Average		1.0		2.17	1.58
Dodecyl boronic acid 1:25,000	0	0	1	0	
	1	0	2	1	
	0	1	2	0	
Average		0.33		1.0	0.67
Boric acid, 5%	0	0	0	0	
	0	0	1	0	
	1	0	1	0	
Average		0.17		0.33	0.25
Boric acid, 2%	0	0	1	0	
	0	0	0	0	
	0	0	0	0	
Average		0.0		0.17	0.08

<sup>a</sup> Evaluation of skin reactions:

## Erythema formation

- 1, very slight
- 2, well defined
- 3, moderate to severe
- 4, severe

Total possible erythema score 4

## Edema formation

- 1, very slight
- 2, slight
- 3, moderate
- 4, severe

Total possible edema score 4

Total possible score for primary irritation 8.

times more irritating than boric acid, they would still be classified as only mildly to moderately irritating to the skin.

A 20-day subacute toxicity determination was also performed with these compounds using the procedure outlined by Draize (3). The compounds and dilutions employed were the same as those used in the primary irritation study. In addition, a control was employed using normal saline solution. Quantities of 10 ml. per Kg. of body weight were applied daily to the clipped skins of albino rabbits. Each application covered approximately 10% of the body surface of the animal. The test animals were observed daily for visible symptoms of boron intoxication, and their body weights and food consumption were also checked daily. Weekly urine samples were collected and tested for boron content. The animals were also observed for a 2-week period following the last application for visible symptoms (4).

None of the animals died during the testing period. The two animals exposed to the lowest dilutions (1:1,000) of nonyl and dodecyl boronic acid and the control animal were sacrificed at the end of the observation period to be examined for pathological findings. Examination revealed no gross or microscopic evidence of boron poisoning in liver, kidney, or other tissues.

The results obtained by this study, while en-

couraging, should be extended to consider acute toxicity of these compounds by injection or oral ingestion.

## SUMMARY

A comparison of the bacteriostatic activity, toxicity, and primary irritation of nonyl and dodecyl boronic acids with boric acid has been made.

The conclusions drawn from this study indicate that nonyl and dodecyl boronic acids possess bacteriostatic activity superior to the activity of the free form of boric acid. Very dilute concentrations of the alkyl boronic acids can be employed to achieve bacteriostasis.

Toxicological testing indicates that the alkyl boronic acids employed are only mildly irritating and apparently nontoxic when applied to rabbit skin. These results suggest that the compounds might be used as substitutes for ordinary boric acid in presently used topical preparations. *In vivo* testing of a more comprehensive nature is needed to establish the toxicity levels of these compounds by accidental ingestion or injection.

## REFERENCES

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