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Antibacterial Properties of Some Positive Univalent Iodine Compounds.

JACOB KLEINBERG, MILAN NOVAK AND VIVIAN GERBER.

From the Department of Chemistry, University of Illinois, College of Pharmacy, and the Department of Bacteriology and Public Health, University of Illinois, College of Medicine.

The antibacterial behavior of iodine and many of its compounds is well known. The present report represents the results of experiments on a type of compound not previously tested, which because of its chemical structure and properties, purports to be an efficient germicidal agent.

Carlsohn[1] first prepared salts which are derivatives of the very labile base IOH, stabilized by co-ordination of the iodine with pyridine. The compounds were prepared by the action of iodine on a silver or mercurous salt in the presence of pyridine or a non-aqueous solution of pyridine. The chemical reactions may be summarized by representative equations.

$$AgX^* + 2\ py^* + I_2 \xrightarrow{CHCl_3} IpyX + AgI$$

$$Hg_2X_2 + 6\ py + 3I_2 \xrightarrow[\text{solvent}]{\text{inert}} 2\ (HgI_2\ 2\ py) + 2IpyX$$

The iodine in these compounds acts chemically as a positive ion. Upon the electrolysis of these substances in pyridine, iodine is liberated at the cathode; hydrolysis or reduction yields free iodine.

Compounds in which the acid radicals were the succinate, benzoate, phthalate and p-nitrobenzoate were prepared and tested for their antibacterial activity. Since preliminary tests revealed that the p-nitrobenzoate compound was most stable, the data in this report is chiefly concerned with this compound. Evidence is presented to show that monopyridine iodine (I) p-nitrobenzoate is bactericidal against *Staphylococcus aureus, Eberthella typhosa* and *Clostridium tetani* and that the growth of *Aspergillus niger* is inhibited by this compound. Tests evaluating the p-nitrobenzoate as a skin bactericide are also described.

Experimental. Broth Inhibition Tests. One cc of a 10^{-2} dilution of a 24-hr broth culture of *Staphylococcus aureus* (F.D.A. 209) was added to 9 cc of phenol coefficient broth.[2] 0.1 g of the p-nitrobenzoate compound was placed in the tube and after shaking, 1 cc quantities were withdrawn at 2 min intervals and plated in phenol coefficient agar. A total of 10 samples were cultured. After incubation for 48 hr, no growth was observed on plates inoculated with the test culture exposed 4 min or longer to the germicide. The number of colonies developing in control plates exceeded 1000.

The above experiment was carried out with *Eberthella typhosa* (Hopkins) except that 0.05 g of compound and one loopful (4 mm) of a 24 hr culture was used. At the end of 4 min exposure, no bacterial growth occurred in the subcultures, while the number of control colonies was 1000+.

Agar and Serum Agar Inhibition Tests. The inhibitory action of monopyridine iodine (I) p-nitrobenzoate against various bacteria in nutrient agar and serum agar was determined as described by Ruehle and Brewer[2] with one slight modification. Ten per cent human serum was used in place of horse serum. The results of these tests are shown in Table I.

Bactericidal Tests In Vitro. From the zones of inhibition in the experiments described above (with the exception of those plates containing *Aspergillus niger*) a 4 mm loopful of

[1] Carlsohn, H., *Über eine Neue Klasse von Verbindungen des Positiv Einwertigen Iods*, 1932, Verlag von S. Hirzel, Leipzig.

* X signifies a univalent acid radical; py refers to pyridine.

[2] Ruehle, G. L. A., and Brewer, C. M., Circular No. 198, 1931, United States Department of Agriculture. *United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants.*

TABLE I.
Agar and Serum Agar Inhibition Tests.

Organism	Zone of inhibition in cm† Serum agar 24	Agar 24	Agar 72	Agar 96	Agar 168 hr
S. aureus (F.D.A. 209)	0.9	1.2	1.5	—	—
E. typhosa (Hopkins)	1.3	1.6	1.7	—	—
Cl. tetani (American type culture)	—	—	—	1.9	—
A. niger	—	—	—	—	1.6

† The data given are the averages of 5 plates.

TABLE II.
Monopyridine Iodine (I) p-Nitrobenzoate-Bactericidal Tests *in vivo*.

	No. of mice	No. dead within 48 hr
Surgical controls	5‡	0
Hemolytic streptococci	10	10
Compound and streptococci	10‡	2
Compound alone	10‡	0

‡ Experiment observed for 16 days. No deaths occurred after 48 hours.

agar was withdrawn, mashed and placed into 10 cc of phenol coefficient broth and incubated for 5 days. In the case of *Clostridium tetani* the phenol coefficient broth contained glucose. In each instance, the absence of growth after 5 days incubation showed that monopyridine iodine (I) p-nitrobenzoate is bactericidal towards the organisms named.

Bactericidal Tests In Vivo. These experiments were performed according to the method of Sarber.[3] Pieces of abdominal skin coated respectively with *Hemolytic streptococci* (strain 1048M) alone, the compound alone, and streptococci plus compound were sealed into the peritoneum of mice and the animals were observed for a number of days. The results are tabulated in Table II.

Experiments on Hydrolysis Products. When compounds of the type IpyX hydrolyze, pyridinium iodate and the pyridinium salt of the acid radical are among the products formed. To determine whether these products of hydrolysis might contribute to the germicidal action, solutions of pyridinium iodate and pyridinium phthalate were tested against *Staphylococcus aureus* (F.D.A. 209) in 24 hr broth cultures. Since the bactericidal action of these compounds was negligible at the end of 30 min, it was concluded that these substances made little or no contribution to the antibacterial behavior of the original positive univalent iodine compounds.

Value as Skin Antiseptic. To test the local toxicity of one application of the p-nitrobenzoate, the following experiment was performed. Ten guinea pigs were anesthetized and their abdomens were shaven clean, washed, and dried. Two 1 cm square areas were scraped on each side of the abdomen until a serous fluid issued from the wounds. Into one area p-nitrobenzoate was rubbed, while saline solution was placed on the control area. The guinea pigs were examined each day and the length of time for the test areas and controls to heal was recorded. The time of healing for the wounds containing the compound on 9 of the animals was identical with that required by the control area. On none of the animals did the skin show signs of necrosis.

In order to evaluate the ability of monopyridine iodine (I) p-nitrobenzoate to act as a cutaneous germicide, tests were performed as described by Novak and Hall.[4] An area on the flexor side of the forearm of a human subject was first swabbed with sterile saline and then a fine coating of powdered p-nitrobenzoate was applied in a similar manner. After 30 sec, a sterile watch glass filled with blood agar was inverted over the area and held there 5 min, after which it was removed and incubated. After 48 hr, the colonies on the impression plates were counted. In all cases where no growth occurred, *Staphylococcus aureus* (F.D.A. 209) was streaked over the blood agar and after 24 hr incubation, the plates were examined again. In each instance growth occurred, indicating that an insufficient amount of the compound was removed from the skin surface to act as a bacteriostatic agent in the blood agar medium. The flexor surface of the other arm was used as a control. Data on 15 subjects showed the compound to be destructive to approximately 94% of the bacteria on the skin. No signs of local irritation were observed in these subjects.

Summary. From these experiments, one can conclude that monopyridine iodine (I) p-nitrobenzoate rapidly destroys common pathogenic bacteria and that it is relatively non-toxic and non-irritating.

[3] Sarber, R. W., *J. Bact.*, 1942, **43**, 50.

[4] Novak, M., and Hall, H., *Surgery*, 1939, **5**, 560.