

Conclusions.* 1. The intrinsic factor which is present in desiccated hog stomach and normal gastric juice may be salted out from a solution saturated with ammonium sulfate. 2. The intrinsic factor after salting out with ammonium sulfate is not dialyzable.

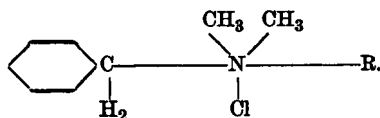
9684

Fungicidal Properties of Alkyl-Dimethyl-Benzyl Ammonium Chlorides.*

CECIL G. DUNN. (Introduced by J. W. Williams.)

From the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge.

The organic compound, alkyl-dimethyl-benzyl ammonium chlorides, is a new type of germicide having the following formula:



R represents a mixture of the alkyl radicals C_8H_{17} , $\text{C}_{10}\text{H}_{21}$, $\text{C}_{12}\text{H}_{25}$, $\text{C}_{14}\text{H}_{29}$, $\text{C}_{16}\text{H}_{33}$, and $\text{C}_{18}\text{H}_{37}$ as derived from the fatty acids of coconut oil.

Some of the germicidal, antiseptic, physical and chemical properties of this rather unusual germicide have been reported by Domagk,¹ Heineman,² and Dunn.^{3, 4}

The test organisms used in the present investigation were secured from the following sources: *Actinomyces gedanensis* and *Cryptococcus hominis* from the American Type Culture Collection, and the others from stock collections maintained by the Department of Biology and Public Health.

The fungi were grown in Sabouraud's dextrose media and subcultured in Sabouraud's dextrose broth, with the exception of *Micro-*

* The conclusions obtained are in agreement with those of Helmer and Fouts³ who published a similar report during the preparation of this paper.

³ Helmer, O. M., and Fouts, P. J., *Am. J. M. Sc.*, 1937, **194**, 399.

*Contribution No. 112 from the Department of Biology and Public Health. Research aided by a grant from the Alba Pharmaceutical Company, Inc.

¹ Domagk, G., *Deut. med. Wochenschr.*, 1935, **21**, 829.

² Heineman, P. G., *J. Am. Pharm. Assn.*, 1937, **26**, 711.

³ Dunn, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 427.

⁴ Dunn, C. G., *Am. J. Hyg.*, 1937, **26**, 46.

sporon audouini, which was grown and subcultured in Sabouraud's maltose broth principally, and *Act. gedanensis*, which was grown and subcultured in standard Reddish broth. The fungi were incubated at 30°C., with the exception of *Monilia albicans*, which was grown at 37°C.

Suspensions of the molds were prepared from the broth cultures by transferring the contents of one or more tubes† to a sterile glass bottle containing sterile glass beads, or broken glass, and shaking the bottle vigorously by hand for 5 minutes. A few suspensions were prepared from cultures grown on solid media, as described by the author previously.⁵ The suspension thus obtained was placed in a medication tube and cooled or heated to the temperature of the test. It was used as prescribed by F. D. A. Methods,⁶ generally after standing for 15 minutes, or less.

A 1/1,000 tincture of alkyl-dimethyl-benzyl ammonium chlorides was employed in the fungicidal tests. This tincture contained 50 cc. of ethyl alcohol (95%), 10 cc. of reagent acetone, the ammonium compound (0.1 gm.), and distilled water, q.s. 100 cc.

Results of fungicidal tests are given in Table I. Each value rep-

TABLE I.
Action of a 1/1000 Tincture* of Alkyl-Dimethyl-Benzyl Ammonium Chlorides
Against Some Fungi

Test organisms	Average phenol coefficients		Aver. highest dilution destroying test organism in 10 but not 5 min.‡			
			Ammonium Phenol compound		Ammonium Phenol compound	
	20°C.	37°C.	20°C.	37°C.	20°C.	37°C.
<i>Achorion schoenleinii</i>	.2—	.36†	1/87	1/17	1/140	1/50
<i>Actinomyces gedanensis</i>	1.0	1.3	1/130	1/130	1/205	1/275
<i>Cryptococcus hominis</i> ‡	.21	.4	—	1/24	—	1/70
<i>Epidermophyton inguinale</i>	.36	.44	1/123	1/45	1/167	1/73
<i>Microsporon audouini</i>	.074	.12±	1/175	1/13	1/320±	1/35
<i>Microsporon lanosum</i>	.089†	—	1/90	1/8	—	—
<i>Monilia albicans</i>	.17	.60	1/77	1/13	1/93	1/55
<i>Saccharomyces cerevisiae</i>	.09	.15	1/100	1/9	1/135	1/21
<i>Trichophyton interdigitale</i> , No. 1	.1—	.28†	1/60	1/6	1/90	1/25
<i>Trichophyton interdigitale</i> , No. 2	.12	.28†	1/63	1/7	1/90	1/25

*Alcohol-acetone—aqueous solution.

†One test only.

‡Figures based on 1/1000 aqueous solution of the ammonium compound.

§Dilutions of the 1/1000 solution.

† Bacterial culture tubes.

⁵ Dunn, C. G., *J. Inf. Dis.*, 1937, **61**, 31.

⁶ Food and Drug Administration Methods of Testing Disinfectants and Antiseptics, Circular No. 198, U. S. D. A., 1931.

resents the average of from 2 to 5 individual tests, unless otherwise specified.

An examination of the table shows that *Act. gedanensis*, *E. inguinale*, *C. hominis*, and *Ach. schoenleinii* were most readily destroyed by the ammonium compound at both 20°C. and 37°C. The two strains of *T. interdigitale* were the most resistant of the pathogenic molds both to the ammonium compound and to phenol. Dilutions of the 1/1,000 tincture of the ammonium compound were effective in destroying all of the pathogenic fungi examined under the conditions of the tests.

In the presence of 20% horse serum, *E. inguinale*, *Ach. schoenleinii*, and *Act. gedanensis* were readily destroyed by dilutions of the 1/1,000 tincture of the ammonium compound.

Cup-plate tests were carried out as suggested by F. D. A. Methods, with the exception that 0.2 cc. of germicide was added to each cup instead of the 6 drops advocated. This modification was adopted in order to insure greater accuracy and uniformity of results.

The 1/1,000 tincture of alkyl-dimethyl-benzyl ammonium chlorides was used in the tests. Fairly good results were obtained against *Ach. schoenleinii* and *C. hominis*, but the action against *Monilia albicans*, though effective, was of low order.

Other solvents for the ammonium compound were used in an effort to improve its penetration in agar. The use of 50% tertiary butanol as the solvent for the ammonium compound increased the areas of the clear zones in every case. When alkyl-dimethyl-benzyl ammonium chlorides were dissolved in full-strength tertiary butanol to form a 1/1,000 dilution of the ammonium compound, the results obtained with *C. hominis* and *Monilia albicans* were superior to those secured by using the 50% tertiary butanol. Thus tertiary butanol was shown to be a better solvent for alkyl-dimethyl-benzyl ammonium chlorides than the alcohol-acetone solution where cup-plate tests were concerned.