

## CONCLUSIONS

1. The results of the assay of tinctures of *Adonis* by the pigeon emetic method parallel the results obtained by the one-hour frog and fatal cat methods.

2. Because the pigeon method possesses a definite end-point, is simple, economical and reasonably accurate, its use for the assay of *Adonis vernalis* appears worthy of consideration for adoption in the National Formulary as the official method.

3. Inasmuch as the standardization of the birds to a maximum and minimum dose

of ouabain seems important, the recommendation is made that this procedure be carried out prior to the assay of the drug. It is further suggested that a rest period of one month be allowed between successive injections.

## REFERENCES

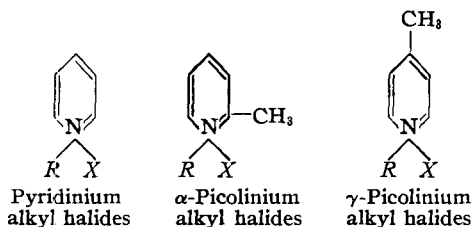
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## Germicidal Activity of Some Quaternary Ammonium Salts\*

By H. G. Kolloff, A. P. Wyss, R. E. Himelick and F. Mantele

Following the report of the germicidal activity of quaternary ammonium salts by Domagk in 1935 (1), considerable attention has been directed toward compounds of this type.

Since the introduction of a methyl group into the phenol molecule increases the germicidal activity (2, 3), it was thought desirable to compare the activity of certain alkyl pyridinium halides with the corresponding alpha- and gamma-picolinium compounds. The chemical relationship of these quaternary salts may be represented as follows:



in which  $R = C_{12}H_{25}$ ,  $C_{14}H_{29}$  or  $C_{16}H_{33}$ , and  $X =$  halogen.

The method used in the preparation of the 27 compounds herein reported was essentially that reported by Knight and Shaw (4).

Preliminary germicidal tests were made according to the F. D. A. method using *Staphylococcus aureus* at 37° C. (5). Each

test was accompanied by a special control to be certain that the dilution reported was germicidal. The preliminary results indicate that the introduction of a methyl group into the aromatic nucleus of these alkyl pyridinium halides does not in general increase the germicidal activity.

## EXPERIMENTAL

*Materials:*

*Pyridine*—Mallinckrodt, analytical reagent.

*α-Picoline*—Eastman Kodak Co., redistilled; b. p., 126–127.5° C.;  $n_D^{20}$ , 1.5009.

*γ-Picoline*—Pure *γ*-picoline was prepared by the procedure recently described by Kolloff and Hunter (6); b. p., 142° C.;  $n_D^{20}$ , 1.5052.

*Tetradecyl chloride* (b. p. 126–129° C. at 1.5 mm.;  $n_D^{20}$ , 1.4465) and

*Hexadecyl chloride* (b. p. 120–124° C. at 0.07 mm.;  $n_D^{20}$ , 1.4501) were prepared by the method of Norris and Taylor (7).

*Dodecyl iodide* (b. p. 142–144° C. at 5.0 mm.; yield, 32.6 Gm.) and

*Tetradecyl iodide* (b. p. 178–181° C. at 13.0 mm.; yield, 31.5 Gm.) were obtained from 25.0 Gm. quantities of the corresponding alcohols using the method of Levene and West (8).

All other alkyl halides were obtained from the Eastman Kodak Company and were used as supplied without further purification.

*Preparation of the Quaternary Ammonium Salts.*—The procedure was similar to that used by Knight and Shaw (4), and the following description for the preparation of dodecyl *α*-picolinium iodide serves as an illustration:

*Dodecyl α-Picolinium Iodide.*—Three grams of dodecyl iodide and 1.0 Gm. of *α*-picoline were mixed

\* From the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

in a flask equipped with an air condenser and heated on a steam bath over night. The solid product was treated with several portions of cold absolute ether to remove unreacted materials, filtered and air dried. Yield of crude product, 3.3 Gm.; m. p., 122–126° C. (corr.). The product was purified to constant melting point by repeated solution in absolute alcohol, treatment with charcoal and precipitation with absolute ether. Yield of pure product, 2.7 Gm.; m. p. 130–131° C. (corr.). Analysis: calculated for  $C_{18}H_{32}IN$ , 33.83% iodine; found, 33.90%.

As pointed out by Knight and Shaw (4), the alkyl chlorides required a temperature of 150° C. for at least eight hours. Even after this treatment, the yields were comparatively low. In general, the alkyl iodides gave the best yields and the bromides next best.

**Preliminary Tests for Germicidal Action.**—In general, 1:1000 stock solutions of the salts were prepared in water or, in cases of too low solubility, 50% alcohol. Suitable aqueous dilutions of the stock solution were subjected to the F. D. A. method for testing germicidal activity (5) using *Staphylococcus aureus* at 37° C. Phenol controls were made with each determination as a check on the viability of the test organism. The approximate dilutions of the quaternary ammonium salts which were capable of killing the test organism in ten minutes, but not in five minutes, are reported in Table IV. In order to be certain that the dilution reported was germicidal, the following control was performed:

One-half cc. of the suspension of *Staphylococcus aureus* test organism was thoroughly mixed with 5 cc. of distilled water. One standard loopful of this mixture was transferred to a tube of 10 cc. of sterile nutrient broth, to which was added one standard loopful of the dilution of the compound being tested. This mixture was incubated for 48 hours after which positive or negative growth indicated the absence or presence of bacteriostatic activity under the conditions of the experiment.

The results of such tests performed with the compounds described in this paper show that each of the dilutions reported in Table IV is definitely germicidal.

Table I—Pyridinium Alkyl Halides

Pyridine plus:	Melting Points, ° C. (Corr.)		Per Cent Halogen	
	First	Second	Calcd.	Found
$C_{12}H_{25}Cl$	78–80 <sup>a</sup>	147–148	12.50	12.51
$C_{12}H_{25}Br$	44.5–45.5 <sup>b</sup>	125.5	24.35	24.08
$C_{12}H_{25}I$	88–89 <sup>c</sup>	93	33.83	33.90
$C_{14}H_{29}Cl$	77–78 <sup>d</sup>	200–201	11.38	11.41
$C_{14}H_{29}Br$	54.5–55.5	198	22.44	22.16
$C_{14}H_{29}I$	95.5–96 <sup>e</sup>	161	31.48	31.31
$C_{16}H_{33}Cl$	82–83 <sup>f, h</sup>	217	10.44	10.08
$C_{16}H_{33}Br$	60.5–61.5	217	20.80	20.60
$C_{16}H_{33}I$	89–90 <sup>g</sup>	211	29.43	29.30

Knight and Shaw (4) reported: <sup>a</sup> (71°, 145°); <sup>b</sup> (89–90°, 125°); <sup>c</sup> (88–89°, 93°); <sup>d</sup> (77°, 205°); <sup>e</sup> (94°, 155°); <sup>f</sup> (83°, 217°); <sup>g</sup> (98°, 205°).

<sup>h</sup> French patent (9); no constants given.

Table II— $\alpha$ -Picolinium Alkyl Halides

$\alpha$ -Picoline plus:	Melting Points, ° C. (Corr.)		Per Cent Calcd.	Halogen Found
	First	Second		
$C_{12}H_{25}Cl$	94.5–95.5 <sup>a, b</sup>	141	11.91	11.67
$C_{12}H_{25}Br$	123–124	...	23.35	23.62
$C_{12}H_{25}I$	130–131	...	32.61	32.30
$C_{14}H_{29}Cl$	...	...	10.89	10.11 <sup>d</sup>
$C_{14}H_{29}Br$	125–126	192.5	21.59	21.29
$C_{14}H_{29}I$	130–131	146.5	30.42	30.60
$C_{16}H_{33}Cl$	...	...	10.02	9.80
$C_{16}H_{33}Br$	123.5–124.5	214	20.07	19.92
$C_{16}H_{33}I$	119–120	201	28.51	28.33

<sup>a</sup> French patent (9); no constants given.

<sup>b</sup> Hygroscopic; dried in vacuum desiccator, filled m. p. tube, again dried in vacuum desiccator, sealed in flame, and determined m. p. as usual.

<sup>c</sup> Extremely hygroscopic; liquefied in air so rapidly that m. p. tube could not be charged to follow the procedure described under "b."

<sup>d</sup> Low yield and, with the very hygroscopic nature, this was the best analysis obtained.

Table III— $\gamma$ -Picolinium Alkyl Halides

$\gamma$ -Picoline plus:	Melting Points, ° C. (Corr.)		Per Cent Calcd.	Halogen Found
	First	Second		
$C_{12}H_{25}Cl$	61–63 <sup>a</sup>	...	11.91	11.75
$C_{12}H_{25}Br$	66–67	...	23.35	23.08
$C_{12}H_{25}I$	61.5–62	...	32.61	32.38
$C_{14}H_{29}Cl$	73–74 <sup>a</sup>	...	10.89	11.06
$C_{14}H_{29}Br$	79–80	...	21.59	21.51
$C_{14}H_{29}I$	74–75	...	30.42	30.30
$C_{16}H_{33}Cl$	81–82	104–105	10.02	9.82
$C_{16}H_{33}Br$	84.5–85.5	110	20.07	20.07
$C_{16}H_{33}I$	67–68	110–110.5	28.51	28.41

<sup>a</sup> Hygroscopic; dried in vacuum desiccator, filled m. p. tube, again dried in vacuum desiccator, sealed in flame, and determined m. p. as usual.

Table IV—Approximate Germicidal Dilutions

Alkyl Halides	Pyridine	$\alpha$ -Picoline	$\gamma$ -Picoline
$C_{12}H_{25}Cl$	20,000	25,000	20,000
$C_{12}H_{25}Br$	20,000	15,000	15,000
$C_{12}H_{25}I$	10,000	10,000 <sup>a</sup>	15,000
$C_{14}H_{29}Cl$	50,000	45,000 <sup>b</sup>	50,000
$C_{14}H_{29}Br$	45,000	50,000	40,000
$C_{14}H_{29}I$	50,000 <sup>a</sup>	40,000 <sup>a</sup>	40,000 <sup>a</sup>
$C_{16}H_{33}Cl$	50,000	50,000	50,000
$C_{16}H_{33}Br$	50,000 <sup>a</sup>	50,000	50,000 <sup>a</sup>
$C_{16}H_{33}I$	20,000 <sup>a</sup>	20,000 <sup>a</sup>	20,000 <sup>a</sup>

<sup>a</sup> Used 50% alcohol as solvent for stock solution.

<sup>b</sup> Low halogen analysis, cf. Table II.

## SUMMARY

A group of 27 alkyl pyridinium- and alkyl picolinium-halides were synthesized and preliminary germicidal tests made to discover any increase in activity due to the introduction of a methyl radical into the aromatic nucleus of these quaternary compounds. Although each of the products in this group showed definite germicidal activity, in general the introduction of the methyl group was not accompanied by any significant increase in such activity.

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## The Influence of Aloe and Podophyllum on the Flow of Hepatic Bile in the Dog\*

By Lloyd W. Hazleton†

Recent investigations (1, 2) indicate that acute fistula methods for studying the flow of hepatic bile give reliable results if conditions are properly controlled. The work of Kocour and Ivy (3) establishes the uniform production of bile by the liver in dogs with chronic biliary fistulas in which the gall

bladder is excluded and the bile collected under suction at all times.

The following investigations on the choleretic properties of Extract of Aloe (N. F. VI) and of Resin of Podophyllum (U. S. P. XI) employ the acute technique of Co Tui (2) with certain modifications.

Table I.—The Effect of Extract of Aloe and Resin of Podophyllum on the Flow of Hepatic Bile and Blood Pressure of Dogs

Dog No.	Drug <sup>a</sup>	Choleretic Dose, Mg.	Route	Bile, Average per Period, Cc.		Blood Pressure, Mm. Hg	
				Control	Exptl.	Control	Exptl.
3	C	...	..	0.7	0.5 <sup>b</sup>	...	...
4	C	...	..	1.5	1.5	...	...
13	C	...	..	1.0	1.4	80-166	68-104
24	C	...	..	0.8	1.4	96-137	146
32	C	...	..	1.8	2.8	108-160	108-116
Total							
2	P	100	Tube	0.7	1.2	...	...
5	P	100	Tube	1.0	1.1	...	...
6	P	100	Tube	1.7	1.4	...	...
7	P	100	Tube	2.0	2.2	...	...
26	P + A	50 Each	Tube	1.4	0.8	140-174	112-160
28	P + A	50 Each	Tube	1.7	2.7	106-135	110-124
per Kg.							
9	A	5	Vein	0.7	1.4	...	...
11	A	5	Vein	1.5	2.0	138	138-150
14	A	5	Vein	0.6	0.9	160	150
15	A	5	Vein	2.1	2.4	160-170	160-172
18	P	5	Vein	1.0	2.0	144-188	140-166
20	P	5	Vein	1.2	2.2	148-166	150-200
34	P	2	Vein	3.5	5.5	98-114	108-138
36	P	2	Vein	1.6	3.2	148	156-165
38	P	2	Vein	1.6	3.2	132-154	134-164
62	R	2	Vein	1.6	1.8	130	135
68	R	5	Vein	1.3	1.6	145	158

<sup>a</sup> C = control, no drug; P = resin of podophyllum; A = extract of aloe; R = rosin.

<sup>b</sup> Experimental interval in control animals corresponds to interval following administration of drug to treated animals.

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### EXPERIMENTAL

Adult dogs, fasted over night and anesthetized with 30 mg. per Kg. of sodium pentobarbital administered intravenously, were used in these ex-