8-Alkoxyquinolinium and 8-Alkoxy-*N*-alkylquinolinium Salts and Their Antibacterial Activity

By WILLIAM O. FOYE and JAMES R. MARSHALL*

A series of 8-alkoxyquinolinium hydrobromides and 8-alkoxy-*N*-alkylquinolinium quaternary iodides has been synthesized. Quaternization of the 8-alkoxy ethers was difficult or impossible with alkyl groups larger than ethyl. The quaternary salts showed characteristic antibacterial activities lower than that from 8-hydroxyquinoline itself. The lower ethers had antibacterial activities which showed more variation with liposolubility and may be attributed to metal ion complexation.

ALBERT'S PROOF that 8-hydroxyquinoline (oxine) exerts its antibacterial activity as a result of its metal-binding ability (1) may now be considered as classical, although several investigators have disagreed, at least in part, with this postulation (2*a*,*b*). One of the more convincing bits of evidence advanced by Albert in favor of a metal-binding mechanism was the lack of antibacterial activity of oxine derivatives unable to chelate metal ions, notably the *O*-methyl and the *N*-methyl derivatives. Other reports from the literature, however, state that 8-alkoxy derivatives and *N*-alkyl quaternary salts of oxine do possess antibacterial activity (3). To resolve this apparent discrepancy, a series of 8-alkoxy ethers and *N*-alkyl quaternary derivatives of oxine covering a range of liposolubilities has been synthesized and tested for activity against several microorganisms. It was believed that any activity found should vary widely with liposolubility if the derivatives were acting as metal-binding agents, as already shown by Albert (4) for oxine derivatives but would show only small changes with varying liposolubilities if the derivatives were behaving as quaternaries.

The formation of an 8-alkoxyquinoline was first reported in 1881 by Bedall and Fischer (5) by reaction of oxine with methyl iodide in alkaline solution. Other short chain 8-alkoxyquinolines have since been prepared by this procedure or by the Skraup synthesis (6), although Kuhn and Westphal (7) have reported 8-dodecoxyquinoline and its methyl methosulfate. The first reported quaternization of oxine was by Claus and Howitz (8), who prepared the *N*-methyl and *N*-methyl-8-methoxy iodides in a sealed tube at 100°. Vis (9) also reported the 8-ethoxy ether and its ethiodide at the same time. Dialkyl sulfates have also been used to prepare both the ether quaternary salts and the 8-alkoxy ethers (10). The quaternary derivatives of oxine reported have generally been the methyl, ethyl, or allyl, although Ioffe and Selezneva (11) recently reported the octyl and hexadecyl iodides of oxine from reaction in sealed tubes at 110°. However, the latter derivative was impure.

DISCUSSION

The 8-alkoxyquinolines reported here were prepared by refluxing the sodium salt of oxine and the appropriate alkyl halide, generally the iodide, in absolute ethanol. Exclusion of water from the reaction was necessary. After neutralization and distillation, the resulting oils were converted to stable hydrobromides. Crystallization of the hydrobromides was accomplished by a variety of methods, including repeated crystallization attempts from anhydrous ether or other anhydrous solvents, removal of moisture by azeotropic distillation, or freezing. Recrystallization could then be carried out in absolute ethanol.

Quaternization of oxine with alkyl iodides was attempted under pressure at 100°. The methyl and ethyl derivatives were obtained in quantitative yield, but the reaction failed with longer chain iodides. Albert (12) has claimed that quaternization of pyridine and quinoline occurs readily with methyl, allyl, or benzyl iodides but more reluctantly with ethyl or higher iodides. He stated further that difficult quaternizations may sometimes take place in refluxing nitrobenzene. When this solvent was utilized in the reaction with butyl iodide, a solid product was obtained which gradually decomposed over a 10-day period. The solid was found to be the hydroiodide of oxine by comparing the melting point and R_f value to a freshly prepared sample. Reaction of oxine and butyl iodide in a Parr bomb at temperatures up to 250° produced 8-butoxyquinoline hydroiodide which was characterized by conversion to the hydrobromide.

Further identification of the two possible derivatives, the ether and the quaternary salt, was made by treatment of each with silver oxide. The ethers were converted to the oils already identified, whereas the suspected quaternaries gave solid hygroscopic hydroxides. In addition, infrared absorption spectra in KBr wafer gave clear evidence of both *N*- and *O*-alkylation. A diffuse H-bonding absorption

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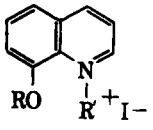
* Present address: Astra Pharmaceutical Products, Inc., Worcester, Mass.

TABLE I.—OXINE ETHERS AND ETHER QUATERNARIES

A. 8-Alkoxyquinoline Hydrobromides

No.	R	M.p., °C.	Purification Procedure[a]	Yield, %	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found
1	CH_3	183(s)[b]	A	40	$C_{10}H_{10}BrNO$	50.02	49.52	4.20	4.74
2	C_2H_5	199(s)	A	48	$C_{11}H_{12}BrNO$	51.99	51.99	4.76	4.90
3	n-C_3H_7	200–202	A	54	$C_{12}H_{14}BrNO$	53.74	53.82	5.26	5.63
4	n-C_4H_9	178–180	B	60	$C_{13}H_{16}BrNO$	55.33	54.56	5.72	5.84
5	n-C_6H_{13}	137–139	B	75	$C_{15}H_{20}BrNO$	58.07	58.05	6.50	6.81
6	n-C_8H_{17}	105–107	B	71	$C_{17}H_{24}BrNO$	60.37	60.09	7.15	7.08
7	n-$C_{10}H_{21}$	70–72	C	68	$C_{19}H_{28}BrNO$	62.29	61.88	7.70	7.88

B. 8-Hydroxy- and 8-Alkoxy-N-alkylquinolinium Iodides



No.	R	R′	M.p., °C.	Yield, %	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found
8	H	CH_3	182–184[c]	100	$C_{10}H_{10}INO$	41.83	41.86	3.51	4.22
9	H	C_2H_5	134–136[d]	100	$C_{11}H_{12}INO$	43.88	44.22	4.03	4.23
10	CH_3	CH_3	166(s)[e]	100	$C_{11}H_{12}INO$	43.88	43.21	4.03	4.69
11	C_2H_5	C_2H_5	168–170[f]	100	$C_{13}H_{16}INO$	47.43	46.48	4.90	4.87
12	n-C_3H_7	n-C_3H_7	133–135	25	$C_{15}H_{20}INO$	50.43	51.55	5.64	5.61
13	n-C_4H_9	CH_3	165(s)	100	$C_{14}H_{18}INO$	48.99	47.94	5.29	5.01
14	n-C_6H_{13}	CH_3	137–139	100	$C_{16}H_{22}INO$	51.76	52.02	5.97	6.06
15	n-C_8H_{17}	CH_3	117–119	100	$C_{18}H_{26}INO$	54.16	53.36	6.56	6.38
16	n-$C_{10}H_{21}$	CH_3	119–121	100	$C_{20}H_{30}INO$	56.20	56.11	7.08	7.04

[a] A, repeated washing with hot anhydrous ether and cooling to 5°. B, repeated washing with hot ethyl acetate and cooling to 5°. C, azeotropic distillation with toluene and cooling to 5°. [b] s = sublimed. [c] A hydrate of this compound has been reported (8), m.p. 143° dec. [d] Reported (11) m.p. 129–132°. [e] Reported (8) m.p. 160° dec. [f] Reported (9) m.p. 168–169°

band at 3000 cm.$^{-1}$ was observed in both oxine hydrobromide and the *N*-methiodide of oxine but was absent in the methyl ether of oxine. Absorption due to N—CH_3 was found at 1350 cm.$^{-1}$ in the methiodide but was missing in both oxine hydrobromide and the methyl ether.

Since the longer chain quaternary salts were difficult or impossible to obtain in good yield or purity, a series of ether quaternaries of varying liposolubility was prepared. The length of the ether alkyl varied from methyl to decyl, while the quaternary alkyl was methyl in all cases. These compounds were easily obtained from the ethers described above. Attempts to obtain ether quaternaries with longer chain alkyls on the quaternary nitrogen gave the methyl methoxy iodide and the ethyl ethoxy iodide in quantitative yield but the propyl propoxy iodide in only 25% yield. Higher homologs of this type could not be obtained, probably because of steric hindrance. Construction of the Stuart-Briegleb molecular model of the 8-propoxy-*N*-propyl derivative showed that quaternization of an oxine ether with an alkyl chain longer than two carbon atoms was attended with considerable steric strain. The various ethers, quaternary salts, and ether quaternaries prepared are described in Table I.

Antimicrobial Activity—Generally, nonchelateable derivatives of oxine of the ether or quaternary type have shown antibacterial activity of a lower degree than oxine itself (3). A series of methyl methosulfates of 8-alkanoyl esters of oxine reported by Vogt (3*d*) particularly did not show a high degree of antibacterial activity *versus E. coli* or staphylococci. Dorier *et al.* (13) have stated that *N*-methyl-8-hydroxyquinolinium sulfate possessed no antibacterial activity, and Bahal *et al.* (3*e*) reported that the methyl ether of oxine was inactive.

The serial dilution procedure of McKee (14) was followed in determining the minimum inhibitory dilutions of the oxine derivatives prepared using five common microorganisms. These included Gram-positive cocci, a Gram-positive rod, Gram-variable rod, and Gram-negative rod. As shown in Table II, the oxine derivatives were less effective than oxine itself. The best activity among the ethers was shown by the ethyl derivative, and the best activity among the quaternaries was shown by the *N*-methyl-8-octoxy derivative. Increase in alkyl chain length, either on the oxygen or nitrogen, generally appeared to lower the activity, except for the hexyl and octyl ethers and the *N*-methyl 8-octoxy quaternary salt. A greater variation in activity with chain length is evident among the ethers, compared to the quaternary salts, and may be expected where a metal-binding mechanism of antibacterial activity is involved.

The activity of the quaternary salts may be due to the cationic group, known to be antibacterial in a variety of compounds. The activity of the ether derivatives cannot immediately be explained as

TABLE II.—ANTIBACTERIAL ACTIVITIES OF THE OXINE DERIVATIVES

Compd.	Min. Inhibitory Concn., 1/M: *S. faecalis*	*B. subtilis*	*B. stearothermophile*	*B. circulans*	*S. aureus*
Oxine·HBr	42,500	42,500	42,500	42,500	42,500
1	2,125	2,125	1,063	1,063	1,063
2	8,500	8,500	4,250	4,250	2,833
3	850	850	850	850	850
4	850	850	850	850	850
5	2,833	2,125	2,125	2,125	1,063
6	2,833	2,833	4,250	2,833	1,214
7	850	850	850	850	850
8	850	850	850	850	850
9	850	850	850	850	850
10	850	850	850	850	850
11	1,417	1,417	1,417	1,417	1,063
12	850	850	850	850	850
13	1,063	1,063	1,063	1,063	1,063
14	1,063	1,063	1,063	1,063	1,063
15	8,500	8,500	8,500	8,500	8,500
16	850	850	850	850	850

due to either metal ion chelation or cationic activity. Pershchin and Vichkanova (*3b*) claimed that their higher 8-alkoxyquinolines possessed antibacterial activity because of the membrane-disrupting effect of substances with long aliphatic chains. However, the methoxy and ethoxy ethers hardly qualify for this type of action, and a more likely explanation may be derived from a statement of Thilo and Demant (15). They claimed that residual valences were present in metal-oxine chelates, as shown by their ability to add hydrogen chloride. A corollary to this postulation is that the oxine ethers may undergo weak metal binding analogous to oxonium salt formation.

Accordingly, the ethers were tested with ferric chloride test solution, and a weak positive test for complexation was given in each case. In addition, 1% ferric chloride solution was added to 0.1 *N* sodium hydroxide solution saturated with 8-ethoxyquinoline and compared to its reaction with 0.1 *N* sodium hydroxide solution alone. The latter solution gave a copious precipitate of ferric hydroxide, whereas the former produced a dark, blue-green solution. Acidification discharged the color. This evidence may indicate that the lower oxine ethers may still undergo metal binding or complexation of a weak nature, and their antibacterial activity may be associated with this ability.

The conclusion is that the antibacterial activities exerted by the oxine ether quaternaries are due to the positively charged nitrogen rather than to a metal-binding effect. However, the latter mechanism is a possibility with the oxine ethers because of their demonstrated ability to prevent precipitation of ferric hydroxide from solutions of ferric ion and their variation in antibacterial activity with variation in alkyl chain length.

EXPERIMENTAL

Melting points were taken on a Fisher-Johns or Mel-Temp block and are uncorrected. Carbon-hydrogen analyses were done by Weiler and Strauss, Oxford, England.

Sodium 8-Quinolate.—One mole (145.15 Gm.) of 8-hydroxyquinoline was dissolved in 500 ml. of acetone. While the solution was stirred, a hot solution of 40.01 Gm. (1.0 mole) of sodium hydroxide in 50 ml. of water was added slowly. Bright yellow crystals of sodium 8-quinolate precipitated immediately and were filtered, washed with acetone, and recrystallized from hot water. A yield of 160.2 Gm. (90%) was obtained, m.p. above 300°.

8-Alkoxyquinoline Hydrobromides.—The following procedure is representative. A solution of sodium 8-quinolate (50.1 Gm., 0.3 mole), absolute ethanol (200 ml.), and iodomethane (42.6 Gm., 0.3 mole) was stirred and refluxed for 6 hours. The alcohol was then removed by distillation through a short Vigreux column. The oily residue was treated with 20 ml. of 10% sodium hydroxide solution, and the resultant two-phase system was extracted with diethyl ether for 6 hours using a liquid-liquid continuous extractor. The ether extract was distilled *in vacuo* to remove the solvent, and the remaining viscous yellow oil was distilled *in vacuo* with an acetone–dry ice bath surrounding the receiver. The clear yellow oil was redissolved in diethyl ether and dried over anhydrous sodium sulfate for 24 hours. Anhydrous hydrogen bromide was passed into the filtered solution cooled in an ice-salt bath, and an ether-insoluble oil was isolated. The oil was crystallized by three washings with anhydrous diethyl ether and cooling to 5° and recrystallized from absolute ethanol. The yield of yellow-green solid was 28.8 Gm. (40%), m.p. 183° with sublimation.

Anal.—Calcd. for $C_{10}H_{10}BrNO$: C, 50.02; H, 4.20. Found: C, 49.52; H, 4.74.

8-Hydroxy- and 8-Alkoxy-N-alkylquinolinium Iodides.—The following procedure is representative. 8-Hydroxyquinoline (14.5 Gm., 0.1 mole) and 21.3 Gm. (0.15 mole) of iodomethane were placed in a Parr bomb, capped and heated in a water bath at 100° for 1 hour. After the contents were cooled, precipitation of golden crystals occurred and was increased by the addition of 100 ml. of diethyl ether. The crystals were isolated, extracted with diethyl ether in a Soxhlet extractor for 2 hours, and recrystallized from hot acetone-ethanol solution. A quantitative yield (28.5 Gm.) of product was obtained, m.p. 182–184°.

Anal.—Calcd. for $C_{10}H_{10}INO$: C, 41.83; H, 3.51. Found: C, 41.86; H, 4.22.

Antimicrobial Testing

Each compound was placed in a separate sterile 100-ml. volumetric flask, and sterile nutrient broth

was added to the mark, giving $^1/_{850}$ M solutions. A 10.0-ml. portion of this solution was placed in each of five sterile test tubes inoculated with 0.5 ml. each of a 24-hour nutrient broth culture of the following organisms: *Streptococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus stearothermophile*, and *Bacillus circulans*. The seeded test tubes were incubated for 24 hours at 37°. If growth was observed at the initial concentration of compound, no dilutions were made. If growth was not observed at this concentration, additional dilutions were tested by taking appropriate quantities of the stock solution of inhibiting compounds and adding sterile broth in the required amount. The tubes were examined visually for growth. Minimum inhibitory concentrations, expressed as 1/molarity are recorded in Table II.

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Effect of Procainesterase Levels on Duration of Procaine Local Anesthesia

By H. B. DANIELL*, A. E. WADE, and F. FORD MILLIKAN

This study originated with the observation that rabbit serums consistently exhibited low levels of cholinesterase activity while having variable levels of procainesterase activity. This finding enabled the authors to determine the effects of procainesterase on the duration of procaine anesthesia using a single species. On conducting double blind duration studies, an inverse correlation between serum procainesterase activity and duration of conduction anesthesia existed which was highly significant ($p < .001$). Atropinesterase activity was present in the serums of those rabbits that contained intermediate or high procainesterase activity but was not evident in the serums of rabbits exhibiting low procainesterase levels. This, coupled with the fact that atropine inhibited procaine hydrolysis, suggested that in rabbit serum procaine and atropine are hydrolyzed by the same enzyme.

WHILE THE LIVER is the chief site of enzymatic detoxication of drugs, other tissues in the body are capable of drug inactivation. Human blood serum contains an enzyme capable of hydrolyzing acetylcholine and certain other choline esters (1). This enzyme is also responsible for the hydrolysis of the local anesthetic procaine (2) and other noncholine esters (3). Serums of other species appear to contain esterases that differ from those in human serum (4–8). Sawyer (9) reported the presence of an esterase in guinea pig and rabbit liver which hydrolyzes benzoylcholine but is not concerned with acetylcholine hydrolysis. A similar enzyme has been found in the plasma of rabbits (6).

The serum of certain rabbits exhibits atropinesterase activity, while that of others does not (10). This enzyme is also capable of deacetylating some derivatives of morphine (11) and hydrolyzing several alkyl and aryl esters (12). It appears to be different from cholinesterase (12, 13).

Kalow (2) suggested that an inverse correlation existed between the duration of action of certain local anesthetics and their speed of hydrolysis *in vitro* by human serum cholinesterase. It was the purpose of this investigation to determine the correlation between serum procainesterase activity *in vitro* and duration of procaine local anesthesia *in vivo* and to determine if any relationship exists between the activities of procainesterase, acetylcholinesterase, and atropinesterase in the serum of rabbits.

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* Present address: Department of Pharmacology, Medical College of South Carolina, Charleston.