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THE QUINOXALINOLS. I. CORRELATION BETWEEN ANTIBACTERIAL ACTIVITY AND CHELATING ABILITY

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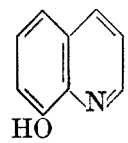
The efficacy of 8-quinolinol (I) as an antibacterial agent has been firmly established over a period of fifty years. It is believed that the powerful chelating ability of this substance is responsible for its antimicrobial properties (1, 2), and that it probably acts by interfering with particular metabolic processes essential for the growth of certain organisms by removing necessary metals from solution. Different trace-elements are required by different bacteria, *i.e.*, a small concentration of 8-quinolinol disturbs the glutamic acid metabolism of *Staphylococcus aureus*, apparently removing manganese from the cells.



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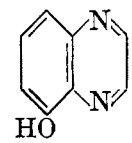

II

The present study was undertaken to determine the antibacterial activity of 5-quinoxalinol (II), and to correlate this property with the stability of its metal chelates. Merritt (3) has succinctly outlined the factors that might affect the formation and stability of chelate compounds. It is probable that the only difference between the stability of the complexes of 8-quinolinol and 5-quinoxalinol lies in the base strengths of the organic compounds themselves. It is known that the introduction of a second nitrogen into a six-membered ring already containing a nitrogen atom greatly reduces the basic strength of the compound (22). The data in Table I compare the basicities and acidities of 5-quinoxalinol and 8-quinolinol. It is seen that the base strengths differ by nearly 3 *p*K units and the acid strengths by only 0.7 *p*K units.

In the light of the theory relating antibacterial activity and chelating ability, one would expect 5-quinoxalinol to be a weaker antimicrobial agent than 8-quinolinol. This follows from the fact that the former should yield more unstable metal chelates than the latter, owing to a weaker bonding between metal and chelating nitrogen atoms, which, in turn, is due to the decreased basicity of the quinoxalinol molecule. The bacteriostatic potency, in a qualitative sense, would be inversely proportional to the amount of metal ions available to the bacteria in the presence of the bacteriostatic agent.

Previous reports concerning the correlation between chelation and antimicrobial action of compounds related to 8-quinolinol have appeared in the

[1] Abstracted from the dissertation submitted to the graduate faculty of the Polytechnic Institute of Brooklyn by Stanley K. Freeman (1950) in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

literature (4, 5, 6). Nearly all of the compounds examined contained a phenolic grouping *peri* to a heterocyclic tertiary nitrogen atom, but they differed from 8-quinolinol in molecular complexity. Since it is well known that the shape of a molecule often plays an important role in biological processes, a clearer picture of the problem would result by the comparison of this substance with 5-quinoxalinol.

For the present investigation, the metal chelates of 5-quinoxalinol and 8-quinolinol containing biologically important metals, *i.e.*, iron, copper, cobalt, calcium, magnesium, manganese and zinc, were studied with respect to their solubilities in aqueous medium. Measurements or indications of their solubility products would give information as to their stability. The compound MR_n will not be precipitated until $(M^{+n})\ (R^-)^n$ equals or exceeds S_{MR_n}, the solubility product. The solubility would be a measure of the stability of the substance in the sense of the equilibrium $MR_n \rightleftharpoons M^{+n} + nR^-$. The "sensitivities" of the two ligands, *viz.*, the smallest quantity of metal that will give a precipitate in a fixed volume of test solution under standard conditions, were determined following the procedure of Irving, Butler, and Ring (7). The results are recorded in

TABLE I

*p*K VALUES (*M*/70 DILUTION)

	—N=	—OH
8-Quinolinol[a]	4.99	9.42
5-Quinoxalinol	2.15	8.73

[a] These values are in good accord with other determinations in the literature (22).

Table II. In addition, a series of "insolubility" tests were carried out on the metal chelates themselves, and the data appear in Table III. These results were obtained by analyzing the filtrates of saturated aqueous solutions of the metal chelates for the metal ions. The authors prefer this latter method of finding relative stabilities of the complexes, for it does not depend upon the "visibility" of a precipitate. It is possible that a soluble chelate might exist at the low concentrations employed in the test, and the mere absence of a precipitate does not necessarily rule out the possibility of complex formation.

It is seen from Table II that the apparent solubility products for the complexes of 8-quinolinol are lower in every instance than those for 5-quinoxalinol. The data from Table III confirm this, for the concentrations of metal ions in saturated solutions of 5-quinoxalinol chelates are greater than for the corresponding 8-quinolinol complexes.

The results of bacteriological studies, employing *Staph. aureus* as the test organism, show clearly that 8-quinolinol is a more effective antimicrobial agent than 5-quinoxalinol. The former compound exhibited bacteriostatic potency in a concentration as low as 10 gamma/ml., whereas the latter displayed no activity below 120 gamma/ml. It should be mentioned that the two other isomeric quinoxalinols (the 2- and 6-hydroxy compounds) were not bacteriostatic when

tested in concentrations as high as 1000 gamma/ml. This is probably due to the inability of these substances to chelate with metals. With respect to bactericidal action, 8-quinolinol was found to be germicidal in 1% concentration, while 5-quinoxalinol was ineffective in solutions of 2.5% concentration.

TABLE II

"SENSITIVITY" TESTS[a]

METAL	5-QUINOXALINOL			8-QUINOLINOL		
	(A)[b]	(B)[c]	(C)[d]	(A)	(B)	(C)
Cu	4.0	4.0	NP	0.6	0.6	1.0
Fe	NP[e]	NP	NP	2.0	1.8	NP
Zn	6.5	6.5	NP	1.0	1.0	1.0
Mn	NP	5.5	700	90	2.5	40
Co	3.0	4.0	NP	0.3	2.0	4.0
Al	NP	NP	NP	4.0	4.0	NP
Ca	NP	NP	NP	NP	NP	NP

[a] Values given in gammas/ml. of metal in solution to yield a precipitate with the reagents. [b] Solution *p*H 5. [c] Solution *p*H 8. [d] Solution *p*H 12. [e] No precipitate observed with metal concentration of 1000 gamma/ml.

TABLE III

"INSOLUBILITY" TESTS[a]

METAL	5-QUINOXALINOL	8-QUINOLINOL
Cu	0.4	0.08
Zn	0.6	.08
Fe	2.3	.10
Mg	2.4	.11
Mn	0.95	.10

[a] Values given in gammas/ml. of metal in solution.

EXPERIMENTAL[2]

2,3-Diaminophenol. The reduction of 18.4 g. (0.1 mole) of 2,3-dinitrophenol (9) was carried out in the presence of Raney nickel catalyst in ethanol at an initial gage pressure of 100 atm. and 46°. A quantitative yield of a crude brown solid was obtained after filtering off the catalyst and removing the alcohol by a water pump. Two recrystallizations from ethanol gave a nearly white product melting at 169–170°. This compound was also prepared by reduction of the nitrophenol over 10% palladium-charcoal catalyst at 37° and 50 atm. This product has been prepared by Mailhe and Murat (10) by the reduction of the same nitrophenol with sodium hypophosphite in the presence of copper.

Anal. Calc'd for $C_6H_8N_2O$: N, 22.57. Found: N, 22.48.

3,4-Diaminophenol. The reduction of 3,4-dinitrophenol (11) was carried out as described above, yielding a compound melting at 170–172°. Heinemann (12) synthesized the aminophenol by reducing the nitrophenol with selenious acid.

Anal. Calc'd for $C_6H_8N_2O$: N, 22.57. Found: N, 22.49.

[2] All melting points are corrected.

5-Quinoxalinol.[3] To a solution of 12.4 g. (0.1 mole) of 2,3-diaminophenol in 200 cc. of 2 *M* acetic acid was added 125 cc. of 4 *M* sodium acetate solution. The mixture was heated to 60° and poured rapidly into a solution of 30 g. (0.105 mole) of sodium glyoxal bisulfite in 750 cc. of water, previously heated to 60°. After stirring the solution for one hour it was brought to *p*H 8. The alkaline solution was extracted several times with ether, and 11.7 g. (80% yield) of a tan solid was obtained. The material melted at 98–100° and distilled at 184° at 7 mm. to give a light yellow solid, m.p. 101–102°.

Anal. Calc'd for $C_8H_6N_2O$: N, 19.17. Found: N, 19.16.

Hydrochloride. This derivative was prepared by reacting equimolar proportions of the quinoxalinol with hydrochloric acid, in ethanol solution. One recrystallization from ethanol yielded a yellowish orange product.

Anal. Calc'd for $C_8H_7ClN_2O$: Cl, 19.42. Found: Cl, 19.33.

2-Quinoxalinol. The preparation of this substance followed the method of Gowenlock, *et al.* (13), and was effected by condensing ethyl glyoxalate (14) with *o*-phenylenediamine (8). A white material was obtained melting at 271–272°.

6-Quinoxalinol. The condensation of 3,4-diaminophenol and sodium glyoxal bisulfite yielded a pale orange material melting at 248–250° (15).

"Sensitivity" tests. The procedure followed to determine the lowest concentration of metal ion necessary to give a visible precipitate with the two ligands under investigation was that of Irving, Butler, and Ring (7). Instead of recording results after one hour of mixing, a waiting period of 24 hours was chosen.

"Insolubility" tests. The metal complexes were precipitated by the addition of pure ligand solution to a solution of the metal. The resultant solid was filtered off and washed 15 to 20 times with water, and then allowed to remain in contact with water for over a week with occasional stirring. The material was washed again with numerous small portions of water. Finally, the pure complex was resuspended in water and, after one to two weeks, filtered off and the filtrate analyzed for the presence of metal. In all instances, the determinations were carried out colorimetrically by visual comparison with standard solutions. Distilled water was used for all washings and tests. Copper and zinc were determined with dithizone (16, 17), magnesium with Titan Yellow (18), manganese by oxidation to permanganate (19), and iron with 2,2′-dipyridyl (20).

The zinc and copper chelates of 5-quinoxalinol were analyzed for metal content with the following results.

Zinc-5-quinoxalinate. Calc'd for $(C_8H_5N_2O)_2$ Zn: Zn, 18.38. Found: Zn, 18.32.

Copper-5-quinoxalinate. Calc'd for $(C_8H_5N_2O)_2Cu$: Cu, 17.96. Found: Cu, 18.04.

Bacteriostatic and bactericidal studies were carried out using *Staph. aureus* as the test organism, following the F.D.A. procedure (21). Both the Agar Cup Plate and the Beef Broth test methods yielded identical results.

It is of interest to note that the concentration of iron-5-quinoxalinate was determined spectrophotometrically. A Beckman spectrophotometer Model DU was used and Beer's law was followed in the concentration range examined. From the values obtained for the quinoxalinol by this method and for iron by the dithizone procedure, the formula of the chelate appears to be $(C_8H_6N_2O)_3Fe$.

SUMMARY

1. 5-Quinoxalinol was prepared by the condensation of 2,3-diaminophenol with sodium glyoxal bisulfite.

2. Bacteriological investigations have shown that 8-quinolinol is a more powerful antibacterial agent than 5-quinoxalinol. Both 2- and 6-quinoxalinol were ineffective antimicrobial agents.

[3] This compound had been synthesized prior to the publication of King, *et al.*, *J. Chem. Soc.*, 3012 (1949). Their preparation consisted of the demethylation of 5-methoxyquinoxaline.

3. The metal chelates of 5-quinoxalinol and 8-quinoxalinol containing cobalt, copper, iron, magnesium, manganese, and zinc were studied with respect to their solubilities in aqueous medium. In all instances, complexes of the latter ligand were more insoluble, and therefore more stable than those of the former.

4. The lower antibacterial activity of 5-quinoxalinol as compared with 8-quinolinol may be explained on the basis of weaker chelating ability of the former, due to a weaker bonding between metal and chelating nitrogen atoms.

5. The copper- and zinc-5-quinoxalinates were found to contain the expected 2:1 ratio of organic residue to metal respectively.

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