aureus, *Streptococcus faecalis*, *Streptococcus haemolyticus*, *Escherichia coli*, *Pasteurella pseudotuberculosis*, *Shigella paradysenteriae*, *Mycobacterium ranae*, and *Mycobacterium tuberculosis*. Test levels in phenol red broth base or Long's medium were 0.1, 1.0, 10, 25, 50 and 100 μg./ml.

Acknowledgments.—We are indebted to the following members of this laboratory: to Dr. S. P. Lingo for infrared spectra, to Mr. M. C. Bachman for bacteriological data, to Mr. H. G. Payne for pharmacological data, and to Mr. D. F. Aldrich for formulation. We also thank Dr. C. D. Hurd for his critical review of the manuscript.

Summary

Reactions of benzylpenicillinic anhydride with simple amino compounds such as ammonia, butylamine, diethylamine, piperidine, *p*-aminobenzoic acid and sulfanilamide yielded the corresponding amides of benzylpenicillin in good yields.

Under comparable conditions hydrazine and hydroxylamine yielded disubstituted hydrazine and hydroxylamine derivatives, respectively.

None of the compounds described had appreciable penicillin activity. Apparently these carboxy derivatives were not hydrolyzed to free penicillin in the blood or in the dog's intestinal tract. Some evidence indicated that protein binding was responsible for the apparent inactivity of penicillinamide.

All of the compounds were appreciably less active than sodium benzylpenicillinate against a representative group of organisms. Benzylpenicillinamide, benzylpenicillinopiperidide, and bis-(benzylpenicillino)-hydroxamic acid exhibited partial inhibition of the growth of *M. tuberculosis*, *in vitro*.

TERRE HAUTE, INDIANA RECEIVED APRIL 27, 1950

[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

The Spectrophotometric Determination of the Approximate Dissociation Constants of the Monofluoroquinolines

BY WILLIAM K. MILLER WITH SAMUEL B. KNIGHT AND ARTHUR ROE

The optical method of Stenström and Goldsmith[1] for the determination of dissociation constants was applied to the fluoroquinolines. These determinations were carried out not only to test the method when applied to weak bases but also to compare the effects of substituting fluorine in the various positions of quinoline on its ionization. The constants of quinoline and 6-chloroquinoline were measured for comparison. Dissociation constants of the above compounds were also determined by measuring the *p*H of solutions of the hydrochlorides in order to test the spectrophotometric method. Since the quinoline derivatives are insoluble in water, all of the above measurements were made in 10% ethanol by weight.

According to the derivations of Stenström and Goldsmith,[1] the graphical determination of the *p*H at which the extinction coefficient is halfway between the values in alkaline and in neutral solution gives pK_a. Analogous considerations show that $pK_b = p\text{OH}$ when the extinction coefficient is midway between that in neutral and that in acid solution. The method has been applied by Stone and Friedman[2] and Phillips and Merritt[3] to the determination of the acid ionization constant of 8-hydroxyquinoline, and the latter authors also used the method to determine basic dissociation constants of 8-hydroxyquinoline and some of its derivatives. The values obtained by the two authors did not agree, but this discrepancy could probably be traced to the *p*H measurements rather than to the method.

Duplicate determinations at 25° of the dissociation constants of quinoline, the fluoroquinolines, and 6-chloroquinoline were made at each of two wave lengths. The wave lengths of the measurements were predetermined from the spectra of the compounds in neutral and acid solutions[4]; wave lengths at which the ion and molecule absorbed most differently were chosen. Sample plots of *p*H *vs.* extinction coefficient, from which the dissociation constants were determined graphically, are shown in Fig. 1. It was not found necessary to buffer the solutions which were measured since all of the critical values were in the distinctly acid region where absorption of carbon dioxide was not likely.

In order to test the values obtained by the spectrophotometric method, duplicate determinations of the dissociation constants of the bases were made by the approximate method of hydrolysis. The *p*H of solutions of the hydrochlorides of the compounds were measured, and dissociation constants were calculated from the expression

$$[H^+]^2/C - [H^+] = K_w/K_b$$

where C is the concentration of the salt, and $[H^+]$, the hydrogen ion concentration is obtained from the measured *p*H. Since the determinations were made in 10% ethanol, it was necessary to know K_w, the ion product of water, in 10% ethanol. This value was not directly

(1) Stenström and Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).
(2) Stone and Friedman, THIS JOURNAL, **69**, 209 (1947).
(3) Phillips and Merritt, *ibid.*, **70**, 410 (1948).
(4) Miller with Knight and Roe, *ibid.*, **72**, 1629 (1950).

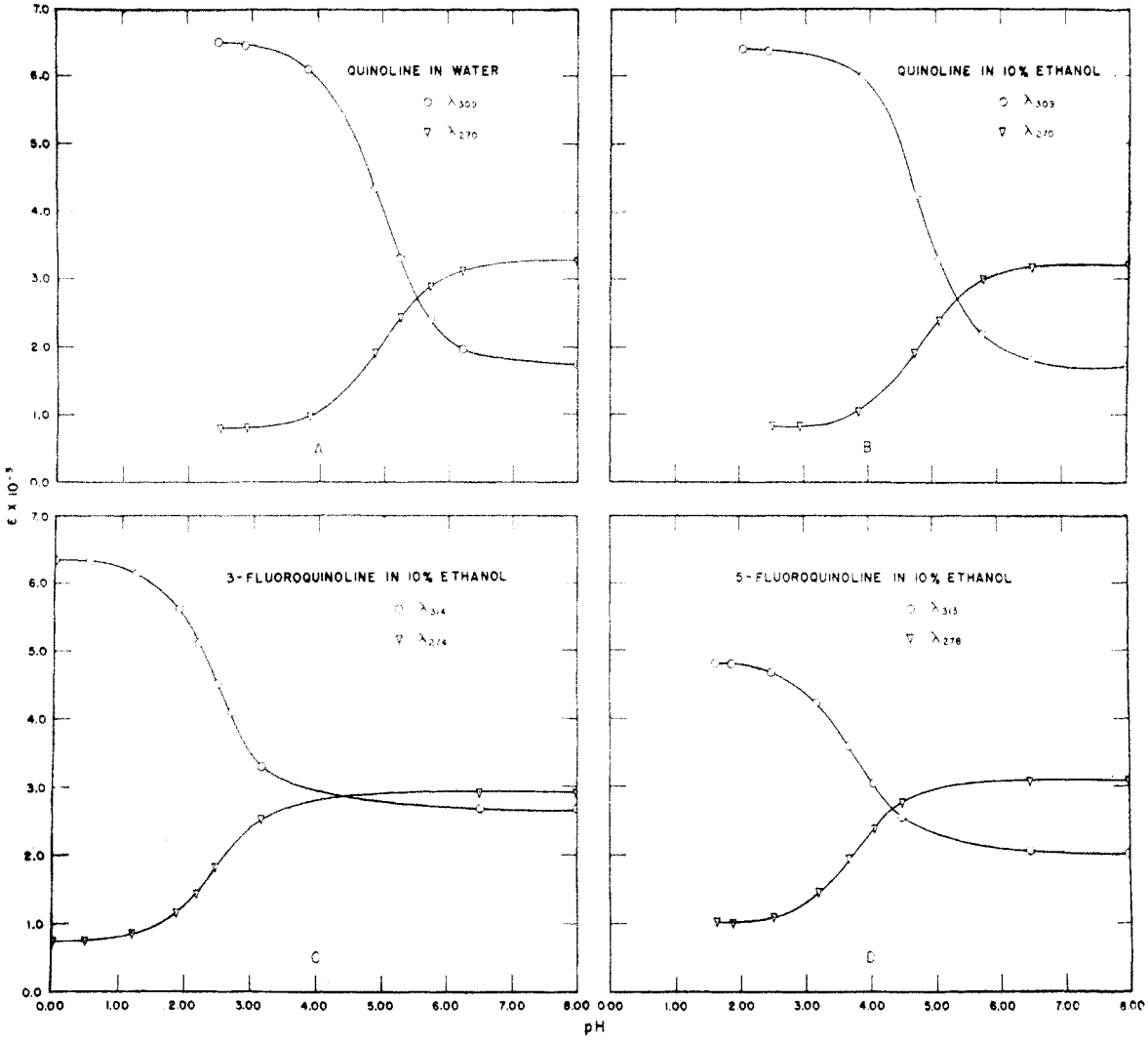

Fig. 1.—*p*H *vs.* extinction coefficient of the fluoroquinolines at two wave lengths.

available in the literature, but by plotting the pK's determined by Kilpi and Warsila[5] for a series of mixtures of ethanol and water against the per cent. ethanol by weight, a smooth curve was obtained, and from this graph pK_w in 10% ethanol was calculated to be 14.10 and K_w to be 0.79×10^{-14}. This value of pK_w was also necessary in the calculations of the spectrophotometric method of determining dissociation constants since *p*H was measured, and *p*OH must necessarily be calculated from the expression: $pOH = pK_w - pH$.

The four values of the dissociation constants of quinoline, the fluoroquinolines and 6-chloroquinoline in 10% ethanol are listed in Table I and compared with duplicate determinations by the hydrolysis method. The checks between any two values by the same method are excellent, while the two methods compare favorably although the precision between the two decreases with decreasing dissociation constant. It is believed that the spectrophotometric method is the more accurate.

In addition to the measurements made in 10% ethanol, the dissociation constant of quinoline was determined in water so that a comparison between ionization in the two solvents could be made. It was necessary to use a small amount of ethanol to dissolve the compound, but the quantity was considered negligible, and the value of $pK_w = 14.00$ was used in the calculations. Hahn and Klockman[6] determined the dissociation constant of quinoline in water at room temperature by potentiometric titration with the quinhydrone and hydrogen electrodes and obtained values of 7.1 and 7.3×10^{-10} with no temperature or carbon dioxide control. Careful measurements at 20° gave them a value of 5.96×10^{-10}, but no value at 25° was reported. However, dissociation increases with increasing

(5) Kilpi and Warsila, *Z. physik. Chem.*, **A177**, 427 (1936).

(6) Hahn and Klockman, *ibid.*, **A146**, 373 (1930).

temperature; hence the value of 8.9×10^{-10}, which was obtained by the spectrophotometric method, must be reasonably accurate. Comparison of this value with that of 4.9×10^{-10} in 10% ethanol shows that a marked decrease of ionization is apparent in passing from water to 10% ethanol as the solvent.

Since the halogens are negative groups, they should decrease the basicity of quinoline when substituted in various positions. It would be reasonable to predict that the dissociation constant of a haloquinoline would be smaller when the halogen was closer to the nitrogen atom. Table I illustrates that this order is closely followed, 3-fluoroquinoline being the weakest base of these measured and the 6- and 7-isomers being the strongest of the fluoroquinolines. The dissociation constant of 2-fluoroquinoline could not be measured because the compound hydrolyzed in acid solution, but it is believed that, disregarding hydrolysis, the dissociation constant of the 2-isomer would be too small to be measured. The hydrolysis of 2-fluoroquinoline will be discussed in a subsequent paper.

Since the halogens decrease in electronegativity in passing from fluorine through iodine, it might be predicted that a certain fluoroquinoline would be less basic than the corresponding chloroquinoline. This supposition was tested by measuring the dissociation constants of both the 6-fluoro and the 6-chloro derivatives of quinoline. It is evident from Table I that the result is the reverse of what was expected.

TABLE I

DISSOCIATION CONSTANTS OF SOME HALOQUINOLINES AT 25°

Compound	Dissociation constants: Spectrophotometric method	Hydrolysis method
Quinoline in water	$8.9, 8.9, 8.9, 8.7 \times 10^{-10}$	$8.3, 8.6 \times 10^{-10}$
Quinoline[a]	$4.9, 4.9, 4.9, 5.0 \times 10^{-10}$	$4.6, 4.5 \times 10^{-10}$
3-Fluoroquinoline	$2.3, 2.3, 2.3, 2.4 \times 10^{-12}$	$3.0, 3.0 \times 10^{-12}$
5-Fluoroquinoline	$4.8, 4.8, 4.8, 4.8 \times 10^{-11}$	$5.0, 4.9 \times 10^{-11}$
6-Fluoroquinoline	$1.0, 1.0, 1.0, 1.1 \times 10^{-10}$	$9.8, 9.8 \times 10^{-11}$
7-Fluoroquinoline	$1.1, 1.0, 1.1, 1.2 \times 10^{-10}$	No result[b]
8-Fluoroquinoline	$1.2, 1.2, 1.2, 1.2 \times 10^{-11}$	$1.4, 1.4 \times 10^{-11}$
6-Chloroquinoline	$5.4, 5.4, 5.4, 5.3 \times 10^{-11}$	$6.8, 7.0 \times 10^{-11}$

[a] Unless otherwise designated the solvent is 10% ethanol. [b] Not enough 7-fluoroquinoline was available to determine the dissociation constant by hydrolysis measurements.

Experimental

Absorption Measurements.—The same instrument and techniques were used in the absorption measurements as previously described[4]; the solvents were also the same.

Materials.—The bases were obtained and purified as previously described[4]; solutions of hydrochlorides of the bases were prepared by adding a calculated amount of standard hydrochloric acid to a weighed quantity of the base.

***p*H Measurements.**—All *p*H measurements were made with a Beckman Glass Electrode *p*H Meter, Model M, Serial No. 17082, with a saturated calomel electrode as reference electrode. Each solution was placed in a 25° constant temperature bath for at least an hour before measuring its *p*H.

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Summary

1. The dissociation constants of quinoline, the fluoroquinolines and 6-chloroquinoline in 10% ethanol have been measured spectrophotometrically by the method of Stenström and Goldsmith and compared with values obtained by measurement of the *p*H of the salts of the bases.

2. The dissociation constant of a fluoroquinoline is weaker when the halogen is closer to the nitrogen atom.

3. 6-Chloroquinoline is a weaker base than the 6-fluoro derivative.

CHAPEL HILL, NORTH CAROLINA

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The Hydrolysis of 2-Fluoroquinoline in Acid Solution

BY WILLIAM K. MILLER WITH SAMUEL B. KNIGHT AND ARTHUR ROE

In a previous paper[1] the spectra of 2-fluoroquinoline in both neutral and acid solutions were reported. The spectrum of the compound in 0.01 *M* HCl exhibited two distinct maxima as compared with a single maximum in the spectra of its isomers in acid solution. Roe and Hawkins[2] reported that the 2-fluoro derivatives of both quinoline and pyridine are insoluble in dilute hydrochloric acid, indicating that they do not form hydrochlorides. However, it was assumed that the shift in absorption must be due to salt formation, and an attempt was made to measure the dissociation constant of 2-fluoroquinoline by the method of Stenström and Goldsmith.[3]

A plot of *p*H *vs.* extinction coefficient at two wave lengths resulted in the curves illustrated in Fig. 1. It is readily seen that instead of the usual leveling of extinction, a maximum was reached, and in more acid solutions a decrease in extinction was evident. These results suggested decomposition, and the most logical decomposition was hydrolysis to carbostyril (2-hydroxyquinoline). Comparison of the spec-

(1) Miller with Knight and Roe, THIS JOURNAL, **72**, 1629 (1950).

(2) Roe and Hawkins, *ibid.*, **69**, 2443 (1947); **71**, 1785 (1949).

(3) Stenström and Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1926).