by *trans*-halogenation of the iodide in methanol solution with freshly prepared silver bromide and by bubbling anhydrous methyl bromide into a benzene solution of the basic ester. The salts were purified by recrystallization from methanol–ether.

BLOOMFIELD, NEW JERSEY

[CONTRIBUTION FROM THE SCHOOL OF PHARMACY OF THE UNIVERSITY OF CALIFORNIA]

The Synthesis of Some 6-N-Substituted Amido Derivatives of 4,6-Diaminoquinaldine and a Study of their *in vitro* Antibacterial Activity[1,2]

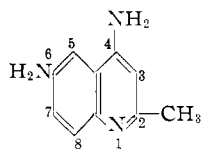
BY CHIN-TZU PENG AND T. C. DANIELS

RECEIVED JULY 21, 1955

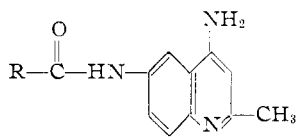
Two series of acylamino and N-substituted carbamylamino derivatives of 4,6-diaminoquinaldine were prepared and tested as antibacterials. Some correlation between the chemical structure and the bacteriostatic activity of these compounds was observed.

The antibacterial activity reported for Surfen (bis-4-amino-2-methyl-6-quinolylurea)[3] and its low toxicity for tissues prompted an interest in the preparation of related derivatives for study as antibacterial agents. According to recent biochemical studies on the mode of action of suramin,[4] Antrycide[5] and other trypanocidal agents,[4,5] the activity does not depend upon the symmetry or the bismolecular character of the active compound. The Surfen-type compounds[6] have a bis-molecular structure and it was of interest to ascertain whether the unsymmetrical analogs would have comparable activity.

The 4,6-diaminoquinaldine (I) was employed as the basic moiety for the synthesis of a wide variety of acyl and N-substituted carbamyl derivatives (II). The diamine base was condensed in acetic acid with acyl chlorides to form the 6-N-substituted amide derivatives and condensed in acetone with isocyanates or isothiocyanates to yield substituted ureas.



I R = alkyl, aralkyl, and aryl amino-



II

By analogy to the aminoquinolines,[7] 4-aminoquinaldine should be more basic than the isomeric 6-aminoquinaldine, nevertheless, acylation of the diamine base yielded exclusively the 6-N-substituted derivative. This confirms the findings of Pratt and Archer.[8] Our attempt to selectively benzoylate the 4-amino group of I by the Schotten–Baumann reaction was unsuccessful. However, both 4- and 6-amino groups were acylated when compound I was heated under reflux with acetic anhydride (or benzoyl chloride) and sodium acetate.

The substituted ureides were prepared by condensing I with isocyanates or isothiocyanates in dry acetone. When the condensation of *o*-nitrophenyl isocyanate with I was carried out in dry purified dioxane, two compounds were isolated. The close similarity of their absorption spectra in the region of 210–500 mμ suggested that the two compounds were positional isomers, *i.e.*, the 4- and 6-N-substituted derivatives of I. Based on structural considerations, particularly in relation to the resonance and basicity of these isomers, the compound having an absorption band at a longer wave length (265 mμ) and showing a greater solubility in dilute hydrochloric acid was tentatively designated as the 6-N-substituted derivative. This structure was confirmed by the following synthesis: (1) the condensation of *o*-nitrophenylcarbamyl chloride with I, and (2) the reaction of *o*-nitrophenyl isocyanate with the monohydrochloride of I in aqueous dioxane. Other methods for the preparation of this compound, such as the amination of 1-(*o*-nitrophenyl)-3-(4-chloro-6-quinaldyl)-urea and of 1-(*o*-nitrophenyl)-3-(4-methoxy-6-quinaldyl)-urea, led only to decomposition products. It is reported[9] that in acetone the condensation of *p*-nitrophenyl isocyanate with I yields the corresponding 6-N-substituted derivative; a repetition of the experiment using *o*-nitrophenyl isocyanate also gave exclusively 1-(*o*-nitrophenyl)-3-(4-amino-6-quinaldyl)-urea. The selective affinity of the substituent group for the 6-amino group of I when the condensation was carried out in acetone may be due to a dipole–dipole interaction of the solvent acetone molecules with the contributing quinonoid form of the 4-aminoquinaldine moiety, thereby leaving the 6-amino group more available for reaction.

The pK_a values of a number of the amides and ureas (Table III) were measured in order to determine the effect of the substituent groups on the basicity of the compounds. These data show that the compounds having an intact 4-amino group are more basic than those congeners with the 4-amino group either replaced or substituted.

(1) Abstracted from a thesis submitted by C. T. Peng in partial fulfillment of the requirements for Doctor of Philosophy in Pharmaceutical Chemistry, June, 1953.

(2) Presented in summary before the Division of Medicinal Chemistry at the 123rd National Meeting of American Chemical Society, Los Angeles, Calif., March, 1953.

(3) H. Jensch, *Angew. Chem.*, **50**, 891 (1937).

(4) E. D. Wills and A. Wormall, *Biochem. J.*, **47**, 158 (1950).

(5) W. E. Ormerod, *Brit. J. Pharmacol.*, **6**, 325, 334 (1951).

(6) German Patents 591,480, 606,495, 513,065; U. S. Patents 2,066,730, 2,118,224, 2,228,166.

(7) A. Albert and R. Goldacre, *Nature*, **153**, 467 (1944).

(8) M. G. Pratt and S. Archer, THIS JOURNAL, **70**, 4065 (1948).

(9) Report No. PB-981, Office of Publication Board, Department of Commerce, Washington, D. C. (July, 1945), p. 17.

TABLE I

PROPERTIES OF 6-N-ACYL DERIVATIVES OF 4,6-DIAMINOQUINALDINE

No.	R = acyl	M.p., (cor.) °C.	Formula	Analyses, % Calcd. C	Calcd. H	Found C	Found H	Purification procedure[a]	Appearance
1	Propionyl	241.3–242.8	$C_{13}H_{15}N_3O$	68.10	6.59	68.44	6.81	A	Needles
2	Butyryl	244.4–245.4	$C_{14}H_{17}N_3O$	69.12	7.05	69.21	6.94	B	Thin plates
3	Isobutyryl	214–215.5	$C_{14}H_{17}N_3O$	69.12	7.05	69.21	7.04	B	Thin plates
4	Valeryl	202–203	$C_{15}H_{19}N_3O\cdot{}^1/_2H_2O$	67.64	7.57	67.82	7.61	B	Narrow prisms
5	Isovaleryl	216.4–218.4	$C_{15}H_{19}N_3O$	70.01	7.44	70.06	7.41	B	Prismatic leaflets
6	Caproyl	228.4–229.4	$C_{16}H_{21}N_3O$	70.82	7.80	71.21	7.87	B	Thin plates
7	Isocaproyl	232.5–233.5	$C_{16}H_{21}N_3O$	70.82	7.80	70.74	8.00	C	Thin needles
8	Sorbyl	275–276	$C_{16}H_{17}N_3O\cdot H_2O$[b]	67.34	6.71	67.74	6.73	D	Yellow plates
9	Heptoyl	230–231	$C_{17}H_{23}N_3O$	71.55	8.12	71.79	8.35	C	Hexagonal plate
10	Caprylyl	228.5–230	$C_{18}H_{25}N_3O$	72.20	8.42	71.93	8.36	D	Rectangular plates
11	Pelargonyl	218.8–219.8	$C_{19}H_{27}N_3O$	72.80	8.68	72.98	8.78	C	Needles
12	Capryl	199–210.5	$C_{20}H_{29}N_3O$	73.35	8.93	73.41	9.16	C	Small prisms
13	Lauroyl	174.3–175.3	$C_{22}H_{33}N_3O$	74.32	9.36	74.03	9.43	C	Thin plates
14	Myristoyl	145.7–147.2	$C_{24}N_{37}N_3O$	75.15	9.72	74.96	9.87	C	Rectangular plates
15	Palmitoyl	145.2–146.7	$C_{26}H_{41}N_3O$	75.86	10.04	75.91	10.13	E	Thin plates
16	Stearoyl	143.2–144.2	$C_{28}H_{45}N_3O$	76.48	10.32	76.35	10.16	C	Thin plates
17	α-Furoyl	246.4–246.9	$C_{15}H_{13}N_3O_2$	67.40	4.90	67.26	5.04	C	Needles
18	Nicotinyl	277.1–278.6	$C_{16}H_{14}N_4O$	N, 20.13		N, 19.73		I	Microscopic plates
19	*p*-Tosyl	287.8–289.8 d.	$C_{17}H_{17}N_3O_2S\cdot HCl\cdot{}^1/_2H_2O$	54.76	5.14	55.10	5.14	J	Needles
20	Benzoyl	251–252	$C_{17}H_{15}N_3O$	73.63	5.45	73.73	5.35	F	Thin needles
21	Phenylacetyl	250–251	$C_{18}H_{17}N_3O$	74.20	5.88	73.93	6.11	B	Needles
22	Phenoxyacetyl	249–250	$C_{18}H_{17}N_3O_2$	70.33	5.58	70.71	5.81	D	Thin needles
23	β-Phenylpropionyl	226.5–227.5	$C_{19}H_{19}N_3O$	74.73	6.27	74.58	6.40	I	Small needles
24	Cinnamoyl[c]	252–254	$C_{19}H_{17}N_3O$					D	Yellowish prisms
25	Phenylpropiolyl	262–263 d.	$C_{19}H_{15}N_3O$	75.73	5.02	75.97	5.13	H	Prisms
26	2-Chlorocinnamoyl	286.5–287.5 d.[d]	$C_{19}H_{16}N_3OCl$	67.55	4.78	67.63	5.04	K	Yellow hexagonal plates
27	4-Chlorocinnamoyl	290–291 d.	$C_{19}H_{16}N_3OCl$	67.55	4.78	67.40	4.97	K	Thin needles
28	4-Bromocinnamoyl	286–287 d.	$C_{19}H_{16}N_3OBr$	Br, 20.96		Br, 20.95		D	Yellowish needles
29	2,4-Dichlorocinnamoyl	295.5–297.5 d.	$C_{19}H_{15}N_3OCl_2$	Cl, 19.05		Cl, 18.73		D	Yellow needles
30	2-Ethoxycinnamoyl	158.2–159.2[e]	$C_{21}H_{21}N_3O_2\cdot{}^1/_2H_2O$[f]	70.76	6.22	70.36 71.13	6.35 6.38	G	Needles
31	4-Ethoxycinnamoyl	299–300 d.	$C_{21}H_{21}N_3O_2$	72.60	6.09	72.45	6.21	I	Prisms
32	β-(4-Bromobenzoyl)-acrylyl[g]	220.2–222.2	$C_{20}H_{16}N_3O_2Br\cdot{}^1/_2H_2O$[h]	57.29	4.09	57.24	4.27	I	Orange-red needles
33	β-(4-*n*-Octylbenzoyl)-acrylyl	182.8–184.3	$C_{28}H_{33}N_3O_2\cdot{}^1/_2H_2O$[h]	74.30	7.57	73.95	7.45	L	Orange needles
	Miscellaneous								
34	N^6-Furfurylidene-4,6-diaminoquinaldine[i]	240–241 d.	$C_{15}H_{13}N_3O$	71.70	5.21	71.82	5.33	D	Yellow prisms

[a] A = crystallization from water; B = crystallization from 50% methanol; C = crystallization from ethyl acetate; D = crystallization from 50% ethanol; E = crystallization from ether; F = crystallization from methanol; G = crystallization from dilute ethanol; H = precipitation from an alcoholic solution with water; I = crystallization from dioxane; J = crystallization from methanol containing a drop of hydrochloric acid; K = crystallization from absolute ethanol; L = crystallization from benzene; M = precipitation from acetone with *n*-hexane. [b] *Anal.* Calcd. for monohydrate, $C_{16}H_{17}N_3O\cdot H_2O$: N, 14.73. Found: N, 14.89. [c] Listed under the German Patent 591,480 (1934). [d] The compound softened at 263–264° (uncor.); when inserted into melting-point bath at 269°, it liquified without decomposition. [e] The compound effervesced when melting; besides, it fused incipiently at 152° (uncor.). [f] Further purification failed to improve the analysis. [g] When the amide was recrystallized from ethanol, a white crystalline product formed which was insoluble in alcohol and melted at a higher temperature than the amide. [h] Slightly hygroscopic. [i] Prepared by refluxing equimolar quantity of α-furfural with 4,6-diaminoquinaldine in absolute ethanol for 30 minutes.

Experimental[10,11]

Preparation of the Acid Amides.—A method similar to that of Pratt and Archer[8] was used for the preparation of all amides. The hydrochloride of the amide in general showed good solubility in water; however, in a few instances warm dilute alcohol or 95% ethanol was necessary to bring about solution. The free base was precipitated by addition of ammonia, sodium hydroxide or sodium carbonate. The yield in most cases was nearly quantitative.

The analyses and physical data of these amides are recorded in Table I.

Preparation of Substituted Ureides.—All the substituted ureides were prepared according to the following procedure. To a warm solution of 3.5 g. (0.02 mole) of I in 150 ml. of dry purified acetone there was added an equimolar quantity of freshly distilled isocyanate or isothiocyanate in 50 to 100 ml. of dry acetone. The reaction mixture was heated under reflux for 30 minutes on a water-bath. For those ureas which failed to precipitate at this point, the acetone solution was concentrated to a small volume of about 100 ml. or less, diluted with *n*-hexane, petroleum ether or ether to slight turbidity and allowed to stand until crystallization

(10) All melting points are uncorrected unless otherwise indicated.

(11) Analyses are by the Microanalytical Division of the Department of Chemistry, University of California.

TABLE II

PROPERTIES OF 1-R-3-(4-AMINO-6-QUINALDYL) UREA

No.	R	M.p., (cor.) °C.	Formula	Nitrogen, % Calcd.	Found	Purif. procedure[a]	Appearance
35	H[b]	198.3–200.3	$C_{11}H_{12}N_4O \cdot C_2H_6O$	21.36	20.80	K	Needles
36	Phenyl	226.5–227.5	$C_{17}H_{16}N_4O$	19.17	18.96	F	Needles
37	*o*-Nitrophenyl	211.7–212.7 d.	$C_{17}H_{15}N_5O_3$	20.87	20.57	D	Yellow needles
38	*m*-Nitrophenyl	153–155[c]	$C_{17}H_{15}N_5O_3$	20.87	20.32	M	Yellow prisms
39	*p*-Nitrophenyl[d]	238–240 d.	$C_{17}H_{15}N_5O_3$			D	Yellow needles
40	*p*-Bromophenyl	281–282 d.	$C_{17}H_{15}N_4OBr$	15.09	14.89	D	Small needles
41	*m*-Tolyl	246–248 d.	$C_{18}H_{18}N_4O$	18.29	18.09	F	Needles
42	*p*-Tolyl	260.4–262.4 d.	$C_{18}H_{18}N_4O$	18.29	18.11	F	Needles
43	*o*-Methoxyphenyl	208.6–209.6 d.	$C_{18}H_{18}N_4O_2 \cdot C_4H_8O_2$[e]	13.65	13.28	I	Thin plates
44	*p*-Methoxyphenyl	263–264 d.	$C_{18}H_{18}N_4O_2$	17.38	17.52	F	Needles
45	*o*-Ethoxyphenyl	237.5–239.5 d.	$C_{19}H_{20}N_4O_2 \cdot {}^1/_2C_4H_8O_2$[f]	14.73	14.54	I[g]	Thin plates
46	*p*-Ethoxyphenyl	252–253 d.	$C_{19}H_{20}N_4O_2$	16.66	16.39	D	Clusters of spikes
47	α-Naphthyl	231.3–232.3 d.	$C_{21}H_{18}N_4O \cdot C_2H_6O$[h]	14.42	14.73	D	Needles
48	*o*-Biphenylyl	197.5–198.5	$C_{23}H_{20}N_4O$	15.21	15.17	M	Prisms
49	*p*-Biphenylyl	243.7–244.7 d.	$C_{23}H_{20}N_4O$	15.21	14.94	D	Needles
	Thioureas						
50	*p*-Methoxyphenyl	191.8–192.8 d.	$C_{18}H_{18}N_4OS$	16.56	16.43	F	Narrow plates
51	*p*-Ethoxyphenyl	152–154	$C_{19}H_{20}N_4OS$	15.90	15.73	K	Needles

[a] See Table I for symbols. [b] Prepared according to the method used for the synthesis of β-naphthylurea [W. J. Hickinbottom, "Reactions of Organic Compounds," 2nd Ed., Longmans, Green and Co., New York, N. Y., 1948, p. 299. [c] Effervesced when melting. [d] The compound was reported (reference 11) to have a melting point of 240–241°. [e] *Anal.* Calcd. for $C_{18}H_{18}N_4O_2 \cdot C_4H_8O_2$: C, 64.37; H, 6.38. Found: C, 64.28; H, 6.56. [f] *Anal.* Calcd. for $C_{19}H_{20}N_4O_2 \cdot {}^1/_2C_4H_8O_2$: C, 66.29; H, 6.36. Found: C, 66.00; H, 6.41. [g] The compound was digested with methyl ethyl ketone prior to crystallization from dioxane. [h] *Anal.* Calcd. for $C_{21}H_{18}N_4O \cdot C_2H_6O$: C, 71.11; H, 6.23. Found: C, 71.07; H, 6.23.

was complete. The ureas were generally recrystallized from 95% aldehyde-free alcohol, absolute ethanol or methanol, or dioxane. Most of the compounds once solubilized had the tendency to remain in solution, and crystallization was effected with difficulty. The yield of the crude product in most cases was approximately 90%.

The analyses and some physical characteristics of the compounds are given in Table II.

Preparation of the Intermediates. (a) **The Acyl Chlorides.**—The acyl chlorides were obtained commercially or synthesized according to the methods available in literature. Among the compounds prepared, the physical properties of both 2,4-dichlorocinnamoyl chloride[12] and β-(4-*n*-octylbenzoyl)-acrylyl chloride[13] have not been reported previously. These compounds were used as intermediates and were not analyzed.

(b) **Isocyanates and Isothiocyanates.**—All the isocyanates used were Eastman Kodak Co. white label products. They were either recrystallized or fractionated *in vacuo* prior to use.

Both 4-methoxy- and 4-ethoxyphenyl isothiocyanates were prepared using the procedure described by Vogel[14] for obtaining phenyl isothiocyanate from aniline.

Preparation of 4,6-Diaminoquinaldine (I).—The diamine base was prepared according to the method of Pratt and Archer.[8] The intermediate 6-acetamido-4-methoxyquinaldine, on recrystallization from alcohol, melted at 232–234° (cor.); the reported value was 217–219°.

The absorption characteristics of I in acetone: $\lambda\lambda_{max}$: 233.5, 257, 296, 350 mμ; log ε 4.53, 4.46, 3.91, 3.64; $\lambda\lambda_{min}$: 246, 279, 321 mμ; log ε 4.40, 3.78, 3.24. In dioxane: $\lambda\lambda_{max}$: 236.5, 256.5, 295, 350 mμ; log ε 4.57, 4.53, 3.83, 3.65; $\lambda\lambda_{min}$: 245, 277, 318.5 mμ; log ε 4.47, 3.75, 3.22.

Reaction of *o*-Nitrophenyl Isocyanate (III) and I in Dioxane.—To a warm solution of 4.3 g. (0.025 mole) of I in 70 ml. of dry purified dioxane there was added 4.1 g. (0.025 mole) of III. The reaction mixture was heated for 30 minutes on a water-bath and then allowed to cool. The contents of the flask were filtered to yield 4.8 g. of a yellowish orange-colored crystalline product melting at 183–187°. An additional 3.2 g. of the product, m.p. 181–185°, was obtained from dilution of the dioxane solution with water. Recrystallization of the crude product from 95% ethanol followed by allowing the solution to cool slowly, gave on standing two kinds of differently colored crystals, one red and the other yellow. These crystals were separated manually. The red variety melted at 206–207° while the yellow one melted at 195–197°. Because of their difference in solubility in hot acetone, the former was freed of the latter by digestion with the solvent. This process of separation was continued until following recrystallization from alcohol the crystals appeared to be uniform and the melting point of the red-colored product remained unchanged. The red species was apparently an alcoholate since on drying at 100° *in vacuo*, the crystals disintegrated with an attendant conversion of the red to an orange color, m.p. 211.7–212.7° (cor.) dec.; $\lambda\lambda_{max}^{EtOH}$: 264–265, 345 mμ; log ε 4.72, 3.90; λ_{min}^{EtOH}: 324 mμ; log ε 3.84; shoulder: 231–245 mμ; log ε 4.49–4.52. This compound was later identified as 1-(*o*-nitrophenyl)-3-(4-amino-6-quinaldyl)-urea.

Anal. Calcd. for $C_{17}H_{15}N_5O_3$: N, 20.87. Found: N, 20.57.

The acetone solution containing the yellow colored product was concentrated to a small volume. On standing, a yellow precipitate was formed which was mixed with some brown crystals, which then were separated manually. The yellow colored product was repeatedly recrystallized from acetone and then ethanol. It melted at 200.5–201.5° (cor.) dec. and was tentatively designated as 1-(*o*-nitrophenyl)-3-(6-amino-4-quinaldyl)-urea; $\lambda\lambda_{max}^{EtOH}$: 257, 348 mμ; log ε 4.61, 3.96. λ_{min}^{EtOH}: 318 mμ; log ε 3.78; shoulder: 233–242 mμ; log ε 4.46–4.50.

Anal. Calcd. for $C_{17}H_{15}N_5O_3$: N, 20.87. Found: N, 20.50.

The red species of the product was observed to have a greater solubility in dilute hydrochloric acid than the yellow one.

1-(*o*-Nitrophenyl)-3-(4-amino-6-quinaldyl)-urea. IV. (1) Attempted Synthesis. (a) **1-(*o*-Nitrophenyl-3-(4-chloro-6-quinaldyl)-urea** was prepared by refluxing 1.9 g. of 6-amino-4-chloroquinaldine (obtained from 6-acetamide-

(12) B.p. 157–159° (5 mm.), m.p. 81.5–82.5° from petroleum ether.

(13) Prepared from the acid (F. K. Kirchner, J. H. Bailey and C. J. Cavallito, THIS JOURNAL, **71**, 1210 (1949)) by treating with phosphorus pentachloride (*cf.* R. E. Lutz and W. P. Boyer, *ibid.*, **63**, 3191 (1941)). The acid chloride melted at 13° and was not distillable at 0.5 mm. It was very soluble in benzene, petroleum ether and *n*-hexane.

(14) A. I. Vogel, "A Textbook of Practical Organic Chemistry," Longmans, Green and Co., New York, N. Y., 1948, p. 615.

4-hydroxyquinaldine[15]) with 1.64 g. of III in 20 ml. of dry purified dioxane, yield 3.0 g. (85%). The compound crystallized from ethanol as yellow plates, m.p. 248.5–249.5° (cor.) dec.; $\lambda\lambda_{max}^{EtOH}$: 256–257; 352–353 mμ; log ε 4.78, 3.98; λ_{min}^{EtOH}: 311 mμ; log ε 3.63.

Anal. Calcd. for $C_{17}H_{13}N_4O_3Cl$: N, 15.70. Found: N, 15.67.

Amination of the ureide was carried out according to the method of Backberg and Marais[16] for converting 4-chloroquinaldine to 4-aminoquinaldine. The ureide decomposed under these conditions to give *o*-nitroaniline which was isolated in almost quantitative yield.

(b) **1-(*o*-Nitrophenyl)-3-(4-methoxy-6-quinaldyl)-urea** was prepared by adding 3.3 g. of III to a warm solution of 6-amino-4-methoxyquinaldine[17] in 35 ml. of purified dioxane. The resulting solution was refluxed for 30 minutes to permit complete precipitation of the crystalline orange-red ureide, yield 5.0 g. (83%). The ureide was recrystallized from absolute ethanol as yellow plates. When heated, the yellow color of the compound deepened to orange-red around 80°, m.p. 215–216° (cor.) dec.; $\lambda\lambda_{max}^{EtOH}$: 252–253, 283–284, 337 mμ; log ε 4.76, 4.22, 3.81; $\lambda\lambda_{min}^{EtOH}$: 279, 313 mμ: log ε 4.21, 3.71; shoulder: 229–233 mμ; log ε 4.46.

Anal. Calcd. for $C_{18}H_{16}N_4O_4$: N, 15.90. Found: N, 15.90.

Amination of the ureide was carried out by heating a mixture of 4 g. of the above ureide and 16.5 g. of ammonium acetate at 135–140° for 3 hr. A complete solution was observed after the mixture had been maintained at that temperature range for 2 hr. The contents of the flask, after cooling, were poured into water. The orange-yellow crystalline product thus formed was filtered and recrystallized from dilute alcohol, m.p. and mixed m.p. with *o*-nitroaniline, 71–72°.

When the solution from which the *o*-nitroaniline had been removed was rendered alkaline with 35% sodium hydroxide, a colorless, crystalline substance precipitated which after crystallization from acetone melted above 260°. The compound was not identified.

(2) **Synthesis.**—(a) The N-(*o*-nitrophenyl)-carbamyl chloride was obtained according to Gatewood's method[18] of preparing chloroformanilide. The carbamyl chloride was treated with 3.46 g. of I in glacial acetic acid and the mixture allowed to stand overnight at room temperature. The yellow precipitate was filtered and successively washed with acetic acid and ether before dissolving in water. The material insoluble in water was separated by filtration and then recrystallized from 95% ethanol to yield 1,3-bis-(*o*-nitrophenyl)-urea, m.p. and mixed m.p. with an authentic sample 224–225°, yield 2.0 g.

The aqueous filtrate was basified with ammonium hydroxide to form some flocculent precipitate. Recrystallization of the compound from ethanol gave an orange-colored crystalline product, m.p. and mixed m.p. with IV obtained by condensation of I with III in dioxane, 206–207°. The identity was further verified by the absorption spectra in both ultraviolet and visible regions.

The alkaline aqueous solution after standing overnight at room temperature gave a precipitate consisting of a mixture of colorless flakes and yellow needles, which were found to be I (2.0 g.) and *o*-nitroaniline, respectively.

(b) To a solution containing 1 g. of I in 30 ml. of 0.2 *N* hydrochloric acid and 30 ml. of purified dioxane there was added slowly with stirring 0.95 g. of III in 40 ml. of dioxane. Upon completion of the addition, the reaction mixture was heated on a water-bath for an hour and then allowed to cool. Neutralization with ammonia yielded a substance melting at 190–193° which was raised to 205–206° upon recrystallization from ethanol. The mixed melting point with IV was not depressed.

Acylation Reactions of the 4-Amino Group of I. (a) **Schotten–Baumann Reaction.**—To a mixture of 3.5 g. (0.02 mole) of I suspended in 90 ml. of water containing 2.0 g. (0.05 mole) of sodium hydroxide was added, slowly with stirring, a solution of 2.8 g. (0.02 mole) of benzoyl chloride in 45 ml. of purified dioxane. The addition of the acid chloride required 1 hr. and the reaction mixture was then stirred for 6 hr. longer. The crude amide was recrystallized from 50% ethanol to give a 69% over-all yield. The purified product melted at 251–252° (cor.) and did not depress a mixed m.p. with an authentic sample of 6-benzamido-4-aminoquinaldine.

When 6-acetamido-4-aminoquinaldine was similarly treated with benzoyl chloride in an alkaline medium as described above, it was recovered unchanged.

(b) **4,6-Diacetamidoquinaldine.**—This compound was prepared by refluxing 3.5 g. of I with 1.0 g. of freshly fused sodium acetate and 15 ml. of acetic anhydride for 30 minutes. The yellow solution was poured into 100 ml. of ice-water and treated with ammonia to precipitate the diamide, yield 5.0 g. (96%). After several crystallizations from a small amount of 95% aldehyde-free alcohol, the product appeared as rhombic plates with a m.p. 259.5–261.0° (cor.).

Anal. Calcd. for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.88. Found: C, 65.48; H, 5.88.

The diacetamidoquinaldine could also be obtained by refluxing 6-acetamido-4-aminoquinaldine with sodium acetate and acetic anhydride.

(c) **6-Acetamido-4-benzamidoquinaldine.**—A mixture of 2.2 g. (0.01 mole) of 6-acetamido-4-aminoquinaldine, 1.7 g. (0.012 mole) of benzoyl chloride and 1.4 g. (0.01 mole) of freshly fused sodium acetate in 50 ml. of purified dioxane was refluxed for 30 minutes. The diamide was isolated in a yield of 1.7 g. (53%) by treating the reaction mixture with ice-water and ammonia. Several recrystallizations from 50% aqueous ethanol afforded the product as colorless needles, m.p. 283–284° (cor.) with sintering at 276°.

Anal. Calcd. for $C_{19}H_{17}N_3O_2$: C, 71.45; H, 5.37. Found: C, 71.24; H, 5.41.

Measurement of Absorption Spectra.[19]—All spectra were determined with a Cary recording spectrophotometer, model II. The spectrum of I in both acetone and dioxane was measured using a concentration of 1.000 g. per liter in a 0.01-cm. cell which was obtained by introducing a 0.990-cm. quartz insert into a 1.000-cm. cuvette. The use of the 0.01-cm. cell was necessary to reduce the absorption of the solvent sufficiently to make the measurements possible. The quartz cells were corrected for their difference in absorption by matching with pure solvent at all wave lengths, and the correction thus obtained was applied for resetting the spectrum.

Determination of pK_a Values.—The determination was carried out by means of a Beckman *p*H meter, model G. Aliquots of the solution of bases containing 0.1–0.2 millimole in 50 ml. of solvent were titrated to 40, 50 and 60% neutralization with 0.1 *N* aqueous hydrochloric acid at 24–25°. Because of the difference in solubility, the acid amides were dissolved in 50% ethanol and the ureas, 67% methanol.[20] The *p*H readings obtained from these stages of neutralization were utilized to calculate the pK_a of the compound. The pK_a values are recorded in Table III and represent averages of 8 to 12 determinations. Standard deviations for these values are also given.

The whole of the *p*H scale of the Beckman *p*H meter was calibrated with various standard buffer solutions as described in the literature.[21] The solvent used was carbon dioxide free distilled water.

Antibacterial Tests.[22]—The compounds were screened as growth inhibitors of *Micrococcus pyrogenes* var. *aureus* (NRRL B 313), *Bacillus megatherium* (ATTC 9885),

(15) H. Jensch, German Patent 591,480 (1934); W. O. Kermack and A. P. Weatherhead, *J. Chem. Soc.*, 563 (1939); and C. E. Kaslow, J. D. Genzer and J. C. Goodspeed, *Proc. Indiana Acad. Sci.*, **59**, 134 (1950).

(16) O. G. Backberg and J. L. C. Marais, *J. Chem. Soc.*, 381 (1942).

(17) Obtained from 6-acetamido-4-methoxyquinaldine by acid hydrolysis. The crude product was recrystallized from benzene and then from ether, m.p. 158–159°. The free base absorbed hydrogen chloride freely and was converted into the yellow hydrochloride, m.p. 225–227° (effervesced).

(18) E. S. Gatewood, THIS JOURNAL, **47**, 110 (1925).

(19) All the absorption spectra were measured in Spectrographic Laboratory with the assistance of M. K. Hrenoff.

(20) According to Albert and Goldacre (*J. Chem. Soc.*, 454 (1943)), the pK_a values obtained from these two solvents were practically identical, although averaged 0.5 unit smaller than the corresponding values in water.

(21) D. I. Hitchcock and A. C. Taylor, THIS JOURNAL, **59**, 1812 (1937); G. G. Manov, N. J. DeLollis, P. W. Lindvall and S. T. Acree, *J. Research Natl. Bur. Standards*, **36**, 543 (1946).

(22) The tests were carried out with the technical assistance of Miss Grace Gardner.

TABLE III

IONIZATION CONSTANTS OF QUINALDINE DERIVATIVES AT 25°

Name	pK_a[a]	Dilution (1/M)
6-Acylamino-4-aminoquinaldine		
Acyl = acetyl	8.78 ± 0.04	250
n-Butyryl	8.86 ± .05	250
n-Caproyl	8.80 ± .03	330
n-Capryl	8.74 ± .03	500
α-Furoyl	8.59 ± .06	330
Benzoyl	8.61 ± .03	330
Phenoxyacetyl	8.61 ± .02	330
Cinnamoyl	8.56 ± .06	330
β-Phenylpropionyl	8.58 ± .06	330
4,6-Diacetamidoquinaldine	4.99 ± .08	250
6-Acetamido-4-benzamidoquinaldine	4.80	500
1-(R)-3-(4-amino-6-quinaldyl)-urea		
R = phenyl	8.72 ± .03	500
p-Tolyl	8.61 ± .03	500
p-Methoxyphenyl	8.75 ± .02	500
p-Ethoxyphenyl	8.74 ± .03	330
o-Nitrophenyl	8.74	500
1-(*p*-Methoxyphenyl)-3-(4-amino-6-quinaldyl)-thiourea	8.53 ± .06	500
1-(*p*-Ethoxyphenyl)-3-(4-amino-6-quinaldyl)-thiourea	8.62 ± .04	330
1-(*o*-Nitrophenyl)-3-(4-chloro-6-quinaldyl)-urea	3.77[b]	1000
1-(*o*-Nitrophenyl)-3-(4-methoxy-6-quinaldyl)-urea	6.04 ± .02[b]	500

[a] Using the same technique of measurement, 9-aminoacridine was found to have a pK_a of 9.28 in 50% ethanol (reported value 9.38; A. Albert and R. Goldacre, *J. Chem. Soc.*, 454 (1943)), pyridine was 5.13 in water (reported value 5.23; *ibid.*, 2240 (1948)) and 4.50 in 50% ethanol, and diethylaniline was 5.85 in 50% ethanol (reported value 5.85; Davies and Addis, *ibid.*, 1622 (1937)). [b] These pK_a values correspond to those obtained for 4-chloro- and 4-methoxyquinoline (2.59 and 5.35, respectively) by J. R. Keneford, J. S. Morley, J. C. E. Simpson and P. H. Wright, *ibid.*, 1356 (1949).

Escherichia coli and *Pseudomonas aeruginosa* in nutrient broth by the serial dilution method. The compounds were dissolved in 1.5 equivalents of hydrochloric acid and warmed at 40–50° until solution occurred. One-half milliliter of a 1:250 dilution in broth of an 18-hr. culture of the test organism was introduced as an inoculum to each tube containing a total volume of 10 ml., thereby achieving a final concentration of 1:5000. The seeded tubes were incubated at 37° for 18 hr. and then observed for a bacteriostic endpoint which was taken as the highest dilution that completely prevented the visible growth of the organism. Surfen was used as a reference compound for the series of tests conducted on different days. Some results of the more active compounds are presented in Table IV.

TABLE IV

INHIBITORY CONCENTRATIONS OF SOME 6-N-SUBSTITUTED 4,6-DIAMINOQUINALDINES IN MICROGRAMS/ML.

Compound	*E. coli*	*M. pyogenes* var. *aureus*	*B. megatherium*	*Ps. aeruginosa*
11	5	2.5	<1.25	100
12	5	5	1.25	>200
20	>200	>200	100	>200
23	100	100	20	100
24	5	5	1.25	20
25	20	20	<10	100
26	5	5	<2.5	>100
27	5	5	<2.5	>100
30	20	10	<5	>100
31	5	5	<2.5	>100
37	10	10	<5	10
40	2.5	2.5	0.625	5
45	20	20	<5	20
46	10	10	<5	10
47	20	10	<5	20

Structure and Antibacterial Activity Relationship.—The results of the antibacterial tests indicate that a structure–activity relationship exists for some of the series of compounds studied. The bacteriostatic potency of the fatty acid amide derivatives (Table II, 1–16) increases with increasing number of carbon atoms in the acid chain, and this relationship is represented by the plot in Fig. 1. As with

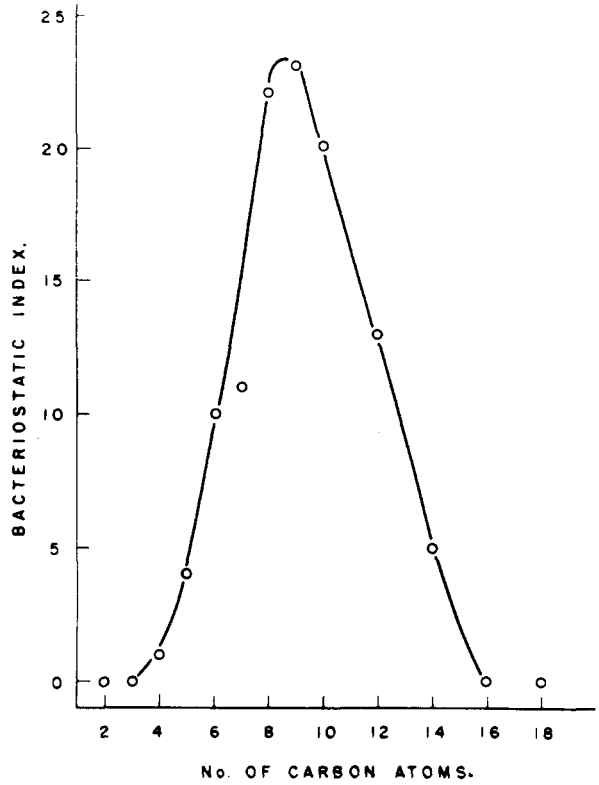


Fig. 1.—Relationship between bacteriostatic index[a] and number of carbon atoms in the fatty acid chain of 6-acylamino-4-aminoquinaldines.

[a] The bacteriostatic index is assigned in a manner similar to that described by A. Albert, *et al.* (*Brit. J. exp. Path.*, 26, 160 (1945)).

other homologous series, where there is a similar dependence of biological activity on chemical structure, a parabolic curve results and is best explained on the basis of the Ferguson principle.[23] The aralkyl carboxamides (20–23) with the exception of cinnamoyl and nuclear substituted cinnamoyl derivatives, exhibit comparable bacteriostatic activity as the fatty acid amides. The phenomenal increase in the bacteriostatic property of the cinnamoyl derivative and its congeners suggests a new factor may be involved. This may relate to the coplanarity of the molecule. Introduction of hetero atoms as in compounds 17–19 causes a diminution of the bacteriostatic activity. However, in the substituted ureas or ureides (35–51) the presence of the ureylene linkage enhances the activity; nevertheless, on account of the limited number of compounds investigated, no definite trend was observed which might suggest further favorable substitutions. In general, the ureides are more active than the acid amides and most of them show a higher level of activity than the bis-molecular Surfen.

(23) J. Ferguson, *Proc. Roy. Soc.* (*London*), **127B**, 387 (1939).

The 4-aminoquinaldines appear to function as cationic bacteriostatic agents. This is in accord with the observation of Ormerod[5] on Antrycide in which it was shown that this compound acts as a cationic trypanocide. The unsubstituted 4-aminoquinaldines are strong bases and correlation of the bacteriostatic activity with the cationic concentration would be expected, providing the basic strength of the various derivatives showed significant differences. The compounds measured (Table III) exhibit the same order of basicity and, therefore, this property does not correlate with the differences observed for the bacteriostatic activity. When the 4-amino group was replaced or substituted, complete inactivity resulted. The 4-amino group of the quinaldine derivatives is responsible for the strong basic properties and appears to be essential for the bacteriostatic activity. This view is supported by our inhibition-reversal studies.[24] The inhibition of growth of test organisms by compound 40 could be quantitatively abolished with an anionic detergent, Aerosol OT (dioctyl sodiosulfosuccinate).

4,6-Diaminoquinaldine has a basic strength of the same order of magnitude as the 6-substituted derivatives, but shows no bacteriostatic activity. Only through an appropriate increase of the molecular weight above a necessary threshold value[25] is an active compound obtained. These compounds appear to act by virtue of being cations and as such they assume a coplanar configuration. Structural modifications which may lead to an increase in the coplanarity of the molecule as in the case of cinnamoyl derivatives enhances the observed bacteriostatic activity.

Acknowledgment.—The authors wish to thank Professors W. D. Kumler and L. A. Strait for some valuable discussions.

(24) C. T. Peng and T. C. Daniels, unpublished data.

(25) *Cf.* T. S. Work, *J. Chem. Soc.*, 1315 (1940); J. R. Keneford, *et al.*, *ibid.*, 2595 (1952).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BUCKNELL UNIVERSITY]

Intramolecular Substitution Reactions. VIII. The Formation of 2-Oxazolines from N-2-Bromoethylbenzamides[1]

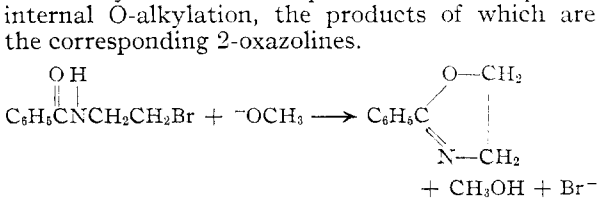
BY HAROLD W. HEINE

RECEIVED MARCH 1, 1956

The reaction rates of some N-2-bromoethylbenzamides with methoxide ion have been studied. It was found that the kinetics was first order with respect to the N-2-bromoethylbenzamide and first order with respect to the methoxide ion and that the products were 2-oxazolines. Introduction of electron-withdrawing groups in the benzene ring enhanced the reactivity of the N-2-bromoethylbenzamides. Mechanisms are suggested to explain these results. The syntheses of a new N-2-bromoethylbenzamide and a new 2-oxazoline are reported.

A recent kinetic study of the alkaline methanolysis of some N-aryl-4-bromobutanamides[2] revealed that the reaction was first order with respect to the bromoamide and first-order with respect to methoxide ion and that N-arylpyrrolidones were formed in high yields. Furthermore, the rate of displacement of bromine was increased by the introduction of electron-withdrawing groups in the aryl system. To explain these kinetic results as well as the solvolytic products a two-step mechanism was proposed in which the first step was postulated to be a rapid-reversible transfer of a proton between the N–H group of the amide and the methoxide ion followed by a displacement of the bromine by the formed amido ion. An alternate possibility would be a concerted mechanism involving the removal of a proton from the nitrogen by the base simultaneously with displacement of the halogen by nitrogen.

We have now extended our studies to the kinetics of the alkaline methanolysis of some N-2-bromoethylbenzamides. In contrast to the N-aryl-4-bromobutanamides where internal N-alkylation takes place, the alkaline methanolysis of the 2-bromoethylbenzamides represents an example of internal O-alkylation, the products of which are the corresponding 2-oxazolines.



(1) Presented at the American Chemical Society Meeting-in-Miniature at Philadelphia, Penna., February 16, 1956.

(2) H. W. Heine, P. Love and J. L. Bove, THIS JOURNAL, **77**, 5420 (1955).

The kinetics of reaction as followed by release of bromide ion is first order with respect to methoxide ion and first order with respect to the N-2-bromoethylbenzamides. The benzamides selected for study indicate that the rate of formation of the 2-oxazolines depends in large part on the ease of removal of the proton from the nitrogen.

Experimental

Method of Rate Measurement.—The procedure for following the rate of release of bromide ion from the N-2-bromoethylbenzamides was the same as the method employed in measuring the rates of pyrrolidone formation.[1] In the case of N-2-bromoethylbenzamide and to a lesser extent of N-2-bromoethyl-*p*-chlorobenzamide the measurement of the second-order process was complicated because of a concurrent first-order solvolytic process also taking place. In order to evaluate the true second order rate constants for these two compounds the equation for a simultaneous first- and second-order reaction was integrated,

$$d[Br^-]/dt = k_1(b - x) + k_2(a - x)(b - x) \quad (1)$$

$$\frac{t[k_1 + k_2(a - b)]}{2.303} = \log \frac{b[k_1 + k_2(a - x)]}{(b - x)(k_1 + k_2a)} \quad (2)$$

and the first-order solvolytic constants determined experimentally. The first-order constants for N-2-bromoethylbenzamide and N-2-bromoethyl-*p*-chlorobenzamide were 2.36 and 1.52 $\times 10^{-5}$ sec.$^{-1}$, respectively. Various values of k_2 were then assumed until one was found which equated the two sides of equation 2. This is essentially the same method employed by Chadwick and Pacsu[3] for determining the second-order constants for the alkaline hydrolysis of 2-bromopropanoic acid.

Typical rate data for the alkaline methanolysis of N-2-bromoethyl-*p*-nitrobenzamide, which is uncontaminated by the first-order process and also N-2-bromoethylbenzamide are presented in Table I. The constants listed in Table I for N-2-bromoethylbenzamide were calculated by the use of equation 2. Table II is a summary of the kinetic studies for all the N-2-bromoethylbenzamides investigated. In cal-

(3) A. F. Chadwick and E. Pacsu, *ibid.*, **65**, 392 (1943).