[CONTRIBUTION FROM THE LABORATORY OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF KANSAS SCHOOL OF PHARMACY]

Antimalarial Agents. VII.[1] The Synthesis of Certain Quinolylaminoquinolinols Based Upon the Schönhöfer Theory

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By means of the Mannich reaction, three isomeric quinolylamino quinolinols, analogs of Camoquin (IV), were synthesized in order to study the Schönhöfer theory of antimalarial action. It appears that a factor (*e.g.*, specificity of structure, ref. 3b, c and d) other than mere increased susceptibility to oxidation or quinone formation is important for high potency.

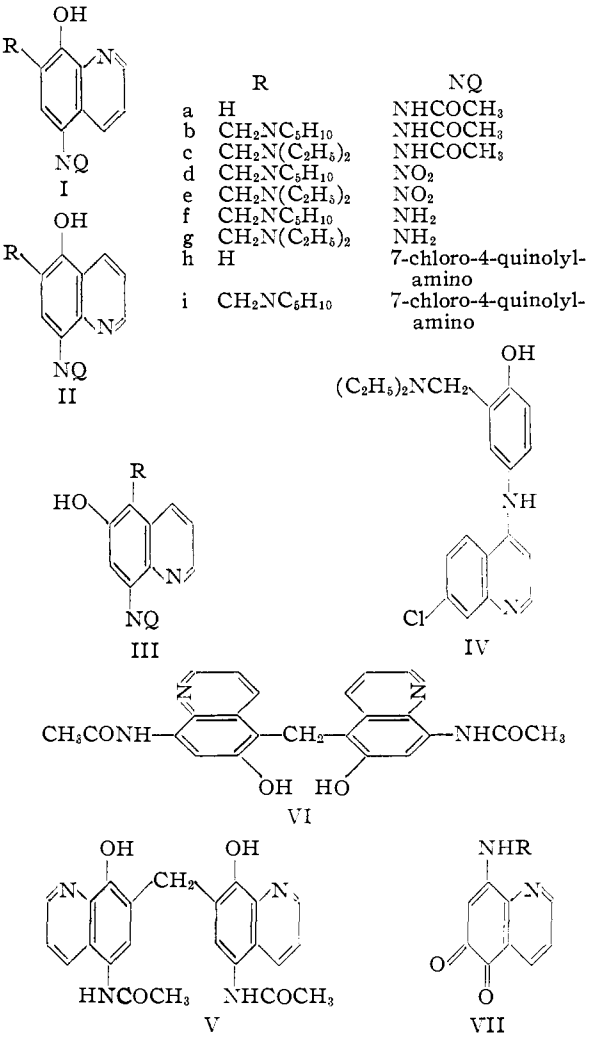
To explain the antimalarial activity of a number of basically substituted 4-, 6- and 8-aminoquinolines, Schönhöfer has suggested the importance of structural features which might be expected to allow tautomerism and a system of quinoid linkages.[2] Further, in the case of the 8-aminoquinolines, such as pamaquine and primaquine, *in vivo* oxidation of these drugs to quinonimines and quinones has been considered to account for their effectiveness.[2,3]

With Schönhöfer's theory in mind, it appeared that the activity of the drug Camoquin (IV)[4] might be enhanced by an alteration of its structure to that of Ii or IIi.[5] It was hoped that the latter, which is both a 4-amino- and 8-aminoquinoline, would combine the action of both types of drugs. IIIi, isomeric with Ii and IIi, was desired for comparative biological tests because it *fails* to meet the requirements of the Schönhöfer hypothesis.

Attempts to condense 5-amino-7-(1-piperidylmethyl)-8-quinolinol (If) with 4,7-dichloroquinoline to give Ii, by analogy with the synthesis of Camoquin (IV),[4] resulted in failure, and If was recovered. However, it was found that 5-amino-8-quinolinol would condense with 4,7-dichloroquinoline to give Ih in good yield. Then, base Ih, by treatment with paraformaldehyde and piperidine, was readily converted to the desired Ii.[6,7]

5-Acetamido-8-quinolinol (Ia), needed for the preparation of If, was obtained in small amounts directly from the catalytic hydrogenation of 5-nitroso-8-quinolinol in the presence of acetic acid and acetic anhydride. However, adequate quantities of the amide were better prepared by isolation of 5-amino-8-quinolinol dihydrochloride resulting from the stannous chloride reduction of the nitroso intermediate.[8] This salt was easily diacetylated to the O,N-diacetyl derivative, which gave 5-acetamido-8-quinolinol in excellent yield by simple trituration with ammonium hydroxide. With an N-acetyl serving as a protective grouping, the 7-dialkylaminomethyl substituent was introduced simply by means of formaldehyde and the appropriate secondary amine. Acid deacetylation of the 5-acetamido-7-dialkylaminomethyl-8-quinolinols readily gave If and Ig.

Ig was also prepared starting with 5-nitroso-8-quinolinol by nitric acid oxidation to 5-nitro-8-quinolinol,[9] which in turn was converted by the Mannich Reaction to 7-diethylaminomethyl-5-



(1) Prior publications, THIS JOURNAL, **65**, 2012 (1943); **68**, 1894 (1946); **70**, 1363 (1948); **72**, 1024 (1950); **74**, 271 (1952); *J. Am. Pharm. Assoc.*, **38**, 658 (1949).

(2) F. Schönhöfer, *Z. physiol. Chem.*, **274**, 1 (1942).

(3) (a) K. C. Blanchard and L. H. Schmidt, in F. Y. Wiselogle, 'Survey of Antimalarial Drugs," Vol. I, Edwards Bros., Ann Arbor, Mich., 1946, p. 134; (b) N. L. Drake and Y. T. Pratt, THIS JOURNAL, **73**, 544 (1951); (c) E. S. Josephson, J. Greenberg, D. T. Taylor and H. L. Bami, *J. Pharmacol. Exptl. Therap.*, **103**, 7 (1951); (d) E. S. Josephson, D. T. Taylor, J. Greenberg and A. P. Ray, *Proc. Soc. Exp. Biol. Med.*, **76**, 700 (1951); (e) A. Burger and G. T. Fitchett, THIS JOURNAL, **75**, 1359 (1953).

(4) J. H. Burckhalter, *et. al.*, *ibid.*, **70**, 1363 (1948).

(5) This speculation was based upon the fact that 5-amino-7-diethylaminomethyl-8-quinolinol trihydrochloride (Ig), the side chain amine of Ii, darkens much more rapidly upon standing in the air than the analogous side chain of Camoquin.[4]

(6) Piperidine was used instead of diethylamine because such derivatives of piperidine appear to crystallize more readily.

(7) Camoquin has been synthesized by an analogous procedure. *Cf.* J. H. Burckhalter, H. A. DeWald and F. H. Tendick, THIS JOURNAL, **72**, 1024 (1950).

(8) S. von Kostanecki, *Ber.*, **24**, 150 (1891).

(9) T. N. Ghosh, A. C. Roy and S. Banerjee, *J. Indian Chem. Soc.* **22**, 221 (1945).

nitro-8-quinolinol (Ie). The nitro compound was reduced to Ig by use of Raney nickel in acetic acid.

In the light of the Schönhöfer hypothesis, intermediates If and Ig appeared in their own right to possess the structures of potential antimalarial agents, and early biological results with If indicated an unusual kind of activity against malarial parasites.[10]

During the course of preparation of 5-acetamido-7-diethylaminomethyl-8-quinolinol (Ic), a small amount of high-melting insoluble by-product, 7,7'-methylene-bis-(5-acetamido)-8-quinolinol (V), was obtained. Structural assignment of V was based upon two different preparative methods. It was prepared in 87% yield by condensation of 5-acetamido-8-quinolinol (Ia) with paraformaldehyde in acetic acid solution, and in 48% yield by condensation of Ia with 5-acetamido-7-(1-piperidylmethyl)-8-quinolinol (Ib) in acetic acid.

8-Nitro-5-quinolinol, required in the synthesis of IIi, was obtained by means of the procedure of Fuson.[11] When this method involving the nitration of 3-chloroacetanilide to give 2-nitro-5-chloroacetanilide is used on a larger scale, the cold mixture must be allowed to come to room temperature slowly and with stirring in order to prevent an explosion. Also, heretofore, the Skraup reaction using 2-nitro-5-chloroaniline has been the method of choice for the preparation of 5-chloro-8-nitroquinoline, but in our experience neither the method of Fourneau[12] nor the modification of Lutz[13] proved to be satisfactory. However, by a careful application of the Manske modification[14] of the Skraup reaction, which employs an acetanilide instead of an aniline, 2-nitro-5-chloroacetanilide was converted to 5-chloro-8-nitroquinoline in yields of 60 to 70%. Hydrolysis of the latter compound then gave 8-nitro-5-quinolinol, which was reduced by stannous chloride to 8-amino-5-quinolinol,[15] using the method of Kostanecki.[8] Condensation of this amine with 4,7-dichloroquinoline gave 8-(7-chloro-4-quinolylamino)-5-quinolinol (IIh), which was converted by the Mannich reaction to IIi. It may be noted that these two compounds are isomeric respectively, with Ih and Ii.

8-Nitro-5-quinolinol also was used for the synthesis of 8-nitro-6-(1-piperidylmethyl)-5-quinolinol (IId), but the latter compound was not needed in the preparation of IIi because of the success, meanwhile, of the foregoing approach.

An attempt was made to prepare IIIb by way of IIIa. 8-Acetamido-6-quinolinol (IIIa) was prepared by acetylation of 8-amino-6-quinolinol, which had been obtained either by reduction of 8-nitro-6-quinolinol[16] or by hydrogen bromide demethylation of 8-amino-6-methoxyquinoline. IIIa was then heated at reflux temperature with piperidine and paraformaldehyde, under the usual conditions of the Mannich reaction. But, instead of IIIb, the bis compound VI was isolated. However, when a warm alcoholic solution of paraformaldehyde and piperidine[17] was added to IIIa without further heating, IIIb was obtained in 82% yield. The fact that heating an alcoholic solution of desired intermediate, IIIb, results in its essentially quantitative conversion to the bis compound VI explains why the customary conditions of the Mannich reaction can give only VI.[17]

The great lability of intermediate IIIb thus made difficult the approach to IIIi *via* IIIb and IIIf, and other routes were then examined.

Another unsuccessful approach to IIIi was made through the intermediate 8-nitro-5-(1-piperidylmethyl)-6-quinolinol (IIId). Attempts to reduce IIId using Raney nickel in methanolic ammonia, platinum oxide in glacial acetic acid and acetic anhydride, or iron and acetic acid resulted in a failure to obtain a well-defined product. Attempts to condense 4,7-dichloroquinoline with the hydrochloride of the unisolated product of reduction gave only a water-insoluble high-melting powder.

A successful approach to IIIi was made through the intermediate IIIh. 8-Nitro-6-quinolinol, obtained by demethylation of 6-methoxy-8-nitroquinoline, was reduced to 8-amino-6-quinolinol, which, in the form of its dihydrochloride, was condensed with 4,7-dichloroquinoline to yield IIIh. Initial attempts to convert IIIh to IIIi by means of the customary conditions of the Mannich reaction—*i.e.*, simultaneous heating together of all the reactants—had given only an insoluble high-melting yellow solid. However, similar to the successful synthesis of IIIb, IIIi was obtained by the addition of IIIh to preformed Mannich reagent under mild conditions.

Pharmacological Results.—Dr. Paul E. Thompson, of the Research Department, Parke, Davis and Co., Detroit, Mich., tested compounds Ii, IIi and IIIi against *Plasmodium lophurae* in the chick. Ii was very active at a 0.125% level in the diet, inactive at 0.00625% and well tolerated at 0.2%, the highest level tested. IIi was active at 0.2% or the maximum tolerated dose, and inactive at 0.1%. IIIi was inactive at 0.4%, which is near the M.T.D. The isomers were assigned respective quinine equivalents[18] of 3.2, 0.15 and <0.1.

Since Ii and IIi are considerably less effective than the analogous Camoquin (IV) when they might be considered to be more susceptible to oxidation and, therefore, more active than Camoquin, susceptibility to oxidation to quinonimines or quinones of non-specific structure does not of itself appear to be an important factor in determining the potency of aminoquinolines. Also, the low effectiveness of If and Ig, *p*-aminophenolic substances quite unstable to atmospheric oxidation even as the pure hydrochlorides, might also point to the greater importance of specificity of structure.

Recent evidence[3b,c,d] strongly points to the quinoline-5,6-quinones (VII) as the biologically

(10) Dr. Paul E. Thompson, Parke, Davis and Co., reported that *Plasmodia lophurae* in chicks were completely annihilated. Nevertheless, despite this interesting observation, quantitative studies of If resulted in the assignment of a quinine equivalent of only 0.5.

(11) R. C. Fuson, E. W. Howard, Jr., and E. N. Marvell, *J. Org. Chem.*, **12**, 804 (1947).

(12) E. Fourneau, J. Tréfouel, Mme. J. Tréfouel and A. Wancolle, *Bull. soc. chim.*, [4] **47**, 738 (1930).

(13) R. E. Lutz, *et al.*, THIS JOURNAL, **68**, 1324 (1946).

(14) R. H. F. Manske, F. Leger and G. Gallagher, *Can. J. Research*, **19B**, 318 (1941).

(15) L. Gattermann, *Ber.*, **27**, 1940 (1894).

(16) M. S. Morgan and R. S. Tipton, THIS JOURNAL, **68**, 1570 (1946).

(17) For analogous results, see J. H. Burckhalter, *et al.*, *ibid.*, **76**, 4904 (1954).

(18) E. K. Marshall, Jr., in F. Y. Wiselogle, ref. 3a, p. 62.

active degradation products of the 8-aminoquinoline antimalarials. If structure VII be rather specific for intrinsic activity, it appears that the most favorable structure of the three isomeric compounds is IIi, since it is an 8-aminoquinoline. Its low antimalarial activity may be explained by noting that it contains a carbon-to-carbon bonded substituent in position 6, and biological oxidation would not be expected to convert it to a quinoline-5,6-quinone.

IIIh, a 6-hydroxy-8-aminoquinoline, might be expected to be oxidized *in vivo* to the quinoline-5,6-quinone, which would possess the requirements for intrinsic activity. However, IIIh was found to be ineffective[19] presumably because it lacks a basic side chain for proper absorption and distribution. The comparatively low activity of Ii and IIi, as well as the inactivity of IIIi, might also be explained on the basis that their side chains are structurally less suitable than that of another 4-aminoquinoline, Camoquin, for proper absorption and distribution.

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Experimental[20]

5-Acetamido-8-quinolinol (Ia).—A solution of 15 g. (0.06 mole) of 5-amino-8-quinolinol dihydrochloride,[8] in as little water as possible, was heated with efficient stirring to 50°. A solution of 17.4 g. (0.12 mole) of crystalline sodium acetate in water, together with 13.8 g. (0.12 mole) of acetic anhydride, was quickly added with continued stirring. After heating at 50–55° for two hours, a yellow mass separated. The cooled diacetylated derivative was collected on a filter, m.p. 207–208°. Upon trituration with excess ammonium hydroxide, it gave 12.0 g. (92% yield) of grey 5-acetamido-8-quinolinol, m.p. 214–215°, which was satisfactory as a synthetic intermediate. When recrystallized from alcohol, it melted at 219–220° (lit.[15] 221–222°).

5-Acetamido-7-(1-piperidylmethyl)-8-quinolinol (Ib).—A solution of 7.8 g. (0.039 mole) of 5-acetamido-8-quinolinol, 1.3 g. (0.041 mole) of paraformaldehyde and 9.0 ml. of piperidine in 150 ml. of alcohol was heated at reflux for one hour. By distillation, the volume was reduced to about 40 ml., whereupon a small amount of insoluble by-product V was removed by filtration. The filtrate was evaporated to dryness and the residue triturated with isopropyl alcohol. The solid produced was collected on a funnel and washed with Skelly solvent A to give 10.6 g. (92% yield) of product, m.p. 171° dec. White crystals were obtained from an alcohol–isopropyl alcohol mixture, m.p. 197–198° dec.

Anal. Calcd. for $C_{17}H_{21}N_3O_2$: C, 68.20; H, 7.07. Found: C, 68.05; H, 6.94.

7,7'-Methylene-bis-5-acetamido-8-quinolinol (V). Method A.—A solution of 9 g. (0.044 mole) of 5-acetamido-8-quinolinol (Ia) and 0.67 g. (0.022 mole) of paraformaldehyde in glacial acetic acid was heated at reflux for two hours. After filtration and washing of the product with acetone, 8 g. (87% yield) of tan solid V was recovered, m.p. >300°.

Method B.—A solution of 3 g. (0.01 mole) of 5-acetamido-7-(1-piperidylmethyl)-8-quinolinol (Ib) and 2 g. (0.01 mole) of 5-acetamido-8-quinolinol (Ia) in hot glacial acetic acid was heated at reflux for five hours. The addition of 35 ml. of water precipitated 2 g. (48% yield) of V. Purification was accomplished by recrystallization of a small portion from a large volume of quinoline, m.p. about 375° (block determination).

Anal. Calcd. for $C_{23}H_{20}N_4O_4$: C, 66.33; H, 4.84. Found: C, 66.33; H, 4.84.

V is very insoluble in the common organic solvents. It may be dissolved in alkali but dilution causes precipitation.

5-Acetamido-7-diethylaminomethyl-8-quinolinol Dihydrochloride (Ic).—A solution of 20 g. (0.1 mole) of 5-acetamido-8-quinolinol, 3 g. (0.1 mole) of paraformaldehyde, 21.5 ml. (0.21 mole) of diethylamine and one liter of alcohol was heated at reflux for 90 minutes. A small amount of by-product V was removed by filtration. The solvent was distilled *in vacuo* to leave a thick sirup which was dissolved in ether. Passage of an excessive amount of dry hydrogen chloride into the ether solution precipitated a thick sirup. Decantation of the ether and vigorous trituration under acetone gave 26 g. (74% yield) of yellow product, m.p. 198° dec. Recrystallized from alcohol with difficulty, a small sample melted at 202–204° dec.

Anal. Calcd. for $C_{16}H_{21}O_3 \cdot 2HCl$: C, 53.34; H, 6.43. Found: C, 53.34; H, 6.56.

7-Diethylaminomethyl-5-nitro-8-quinolinol (Ie).—A solution of 5 g. (0.026 mole) of 5-nitro-8-quinolinol, 0.8 g. (0.026 mole) of paraformaldehyde, 3 ml. (0.029 mole) of diethylamine and 400 ml. of alcohol was heated at reflux for 90 minutes. Cooling produced 6 g. (83% yield) of yellow crystals, m.p. 205–206° dec. Concentration of the filtrate brought the yield to nearly quantitative. Recrystallization from butanol failed to change the melting point.

Anal. Calcd. for $C_{14}H_{17}N_3O_3$: C, 61.07; H, 6.23. Found: C, 61.16; H, 6.25.

5-Amino-7-(1-piperidylmethyl)-8-quinolinol Trihydrochloride (If).—A solution of 9.2 g. (0.031 mole) of 5-acetamido-7-(1-piperidylmethyl)-8-quinolinol (Ib) in an excess of alcoholic hydrogen chloride was heated at reflux until yellow crystals separated. The product was removed by filtration and washed with acetone to yield 11.2 g. (98% yield) of If, m.p. 234° dec. Several recrystallizations from absolute alcohol raised the m.p. only to 236–237° dec.

Anal. Calcd. for $C_{15}H_{19}N_3O \cdot 3HCl$: C, 49.13; H, 6.05. Found: C, 49.50; H, 6.10.

5-Amino-7-diethylaminomethyl-8-quinolinol Trihydrochloride (Ig).[21] **Method A.**—A mixture of 9 g. (0.045 mole) of 5-acetamido-8-quinolinol (Ia), 1.5 g. (0.05 mole) of paraformaldehyde, 5.2 ml. (0.05 mole) of diethylamine and 400 ml. of absolute alcohol was heated at reflux for three hours. After the addition of 100 ml. of a saturated solution of alcoholic hydrogen chloride, the reflux period was continued for an hour. A small amount of insoluble product, possibly the hydrochloride of V, was removed by filtration, and the filtrate was reduced in volume by distillation to about 50 ml. Cooling produced 10 g. (64% yield) of red solid, m.p. 209–211° dec. Several recrystallizations were made from methyl alcohol, m.p. 218–219° dec.

Anal. Calcd. for $C_{14}H_{19}N_3O \cdot 3HCl$: C, 47.40; H, 6.25. Found: C, 47.64; H, 6.61.

Method B.—A solution of 9 g. (0.047 mole) of 7-diethylaminomethyl-5-nitro-8-quinolinol (Ie) in glacial acetic acid was reduced with Raney nickel in a low pressure hydrogenation apparatus. The theoretical amount of hydrogen was rapidly absorbed, whereupon the mixture was filtered into an excess of concentrated hydrochloric acid. The resulting red solution was concentrated *in vacuo* to a low volume. After slight dilution with alcohol, the addition of ether precipitated a red sirup which solidified after extensive stirring to give 9.5 g. (82% yield) of brown solid, m.p. 205–206° dec. Purification as in method A gave a light brown solid, m.p. 219–220° dec. There was no depression of melting by mixture with a sample from method A.

Anal. Found: C, 47.02; H, 6.14.

5-(7-Chloro-4-quinolylamino)-8-quinolinol Dihydrochloride (Ih).—A mixture of 13.5 g. (0.058 mole) of 5-amino-8-quinolinol dihydrochloride,[8,15] 11.5 g. (0.058 mole) of 4,7-dichloroquinoline and 400 ml. of alcohol was heated at reflux for three hours. After 12 hours, a dull green solid which had separated was collected on a funnel and washed with hot alcohol. There was obtained 19 g. (81% yield) of product, m.p. >300°. For analysis a sample was dissolved in boiling methyl alcohol. Ethyl alcohol was added and the methyl alcohol allowed to boil off to cause recrystallization.

Anal. Calcd for $C_{18}H_{12}ClN_3O \cdot 2HCl \cdot {}^1/_2H_2O$: C, 53.55; H, 3.75. Found: C, 53.43; H, 3.73.

5-(7-Chloro-4-quinolylamino)-7-(1-piperidylmethyl)-8-quinolinol (Ii).—The free base of 5-(7-chloro-4-quinolyl-

(19) By Dr. Paul E. Thompson.

(20) Microanalyses by Mr. C. W. Beazley, Skokie, Illinois.

(21) This compound was first prepared by J. H. Burckhalter and M. Darwish, unpublished results.

amino)-8-quinolinol dihydrochloride (Ih) was prepared by dissolving the salt in water and precipitating the yellow base with ammonium hydroxide, m.p. 142–143°. A mixture of 1.2 g. (0.0036 mole) of the free base, 0.11 g. of paraformaldehyde, 0.33 g. of piperidine and 100 ml. of alcohol was heated at reflux for two hours. The volatile materials were then removed *in vacuo* until a white solid began to appear in the warm solution. Cooling produced 1.4 g. (93% yield) of reddish colored solid, m.p. 203–204°. Several recrystallizations from acetone gave white crystals, m.p. 211–212°, which are soluble in 5% hydrochloric acid but insoluble in 5% sodium hydroxide solution.

Anal. Calcd. for $C_{24}H_{23}ClN_4O \cdot {}^1/_4H_2O$: C, 68.07; H, 5.71. Found: C, 68.08. 68.12; H, 5.65, 5.68.

5-Chloro-2-nitroacetanilide.—The procedure of Fuson[11] was followed. However, considering the fact that 445 g. of *m*-chloroacetanilide was nitrated, it was found to be very important to allow such large nitration mixtures to come to room temperature slowly and *with stirring* in order to prevent explosions. Also, for the proper drying of the large bulk of crude product, several successive portions of phosphorus pentoxide were needed. Further, continuous benzene extraction was advantageous.

5-Chloro-8-nitroquinoline.—To a homogeneous mixture of 15 g. (0.07 mole) of 5-chloro-2-nitroacetanilide, 10.6 g. of arsenic pentoxide and 51 ml. of previously dehydrated glycerol, 28.7 ml. of concentrated sulfuric acid was added. Heat was applied by a luminous flame until a vigorous reaction ensued. When the reaction had subsided, the mixture was allowed to stand for two hours whereupon it was poured into ice water. After filtration through porous plate, excess ammonium hydroxide was added to the filtrate to precipitate 9.8 g. (68% yield) of brown solid, m.p. 123–124°. It was recrystallized from 50% acetic acid, m.p. 135–136° (lit.[12] gives 135–136°).

8-Nitro-5-quinolinol.—5-Chloro-8-nitroquinoline was hydrolyzed to this compound by means of potassium hydroxide, following the procedure of Fuson.[11] The yield of quinolinol was increased to 86% through precipitation of the potassium salt from the reaction mixture by means of ether. The method was unsatisfactory when applied to more than 4 g.

8-Nitro-6-(1-piperidylmethyl)-5-quinolinol Hydrochloride (IId).—A heated mixture of 0.4 g. of paraformaldehyde, 1.5 ml. of piperidine and a few ml. of alcohol was added to 2.5 g. (0.013 mole) of 8-nitro-5-quinolinol in 1500 ml. of absolute alcohol. Concentration of the solution to about 50 ml. gave upon cooling 2.5 g. (83% yield) of yellow solid, m.p. 124°. The base was converted to the hydrochloride, which was recrystallized as yellow crystals, m.p. 193–194° dec.

Anal. Calcd. for $C_{15}H_{17}N_3O_3 \cdot HCl$: Cl, 10.92. Found: Cl, 11.10.

8-Amino-5-quinolinol Dihydrochloride.—Using the method of Kostanecki,[8] 11.8 g. of 8-nitro-5-quinolinol was reduced with 47.4 g. of stannous chloride and 36 ml. of concentrated hydrochloric acid to give 10.5 g. (73% yield) of yellow crystalline 8-amino-5-quinolinol dihydrochloride, m.p. 252–255° dec.

Anal. Calcd. for $C_9H_8N_2O \cdot 2HCl$: C, 46.40; H, 4.32. Found: C, 45.30; H, 4.11.

8-(7-Chloro-4-quinolylamino)-5-quinolinol Dihydrochloride (IIh).—A solution of 2.5 g. (0.011 mole) of 8-amino-5-quinolinol dihydrochloride and 2.1 g. (0.011 mole) of 4,7-dichloroquinoline in alcohol was heated at reflux for two hours before 3.7 g. (87% yield) of yellow product had separated, m.p. 265–270° dec. It was recrystallized twice from alcohol, m.p. 281–282° dec.

Anal. Calcd. for $C_{18}H_{12}ClN_3O \cdot 2HCl \cdot {}^1/_2H_2O$: C, 53.55; H, 3.75. Found: C, 53.27; H, 4.30.

8-(7-Chloro-4-quinolylamino)-6-(1-piperidylmethyl)-5-quinolinol (IIi).—A preheated solution of 0.39 g. (0.015 mole) of paraformaldehyde and 4.5 ml. (0.045 mole) of piperidine in alcohol was added to a solution of 6 g. (0.015 mole) of 8-(7-chloro-4-quinolylamino)-5-quinolinol dihydrochloride in 400 ml. of alcohol. After a reflux period of 30 minutes, the volatile materials were removed *in vacuo*. The yellow residue was extracted with ether in a Soxhlet. After drying the solution over sodium sulfate, the ether was evaporated under a stream of dry air to leave 4.4 g. (69% yield) of yellow crystalline product IIi, m.p. 182–183°. It may be recrystallized from alcohol, m.p. 186°. IIi is soluble in 5% hydrochloric acid and insoluble in 5% sodium hydroxide.

Anal. Calcd. for $C_{24}H_{23}ClN_4O$: C, 68.81; H, 5.53. Found: C, 69.25; H, 5.73.

8-Amino-6-quinolinol Dihydrobromide.—A mixture of 20 g. (0.081 mole) of 6-methoxy-8-aminoquinoline dihydrochloride[22] and 150 ml. of 48% hydrobromic acid was heated at reflux for two hours. The mixture was then distilled until crystals appeared. After standing overnight, the product was collected on a funnel and recrystallized from 100 ml. of water. There was obtained 18.5 g. (88% yield) of golden yellow crystals, m.p. 240–242° dec.

Anal. Calcd. for $C_9H_8N_2O \cdot HBr \cdot H_2O$: C, 41.72; H, 4.28. Found: C, 42.31; H, 4.27.

8-Acetamido-6-quinolinol (IIIa).—To a vigorously stirred solution of 10 g. (0.032 mole) of 8-amino-6-quinolinol dihydrobromide in 200 ml. of water, 20 ml. of acetic anhydride and a saturated solution of 50 g. of sodium acetate trihydrate were added. After a few minutes, the solid which had formed was collected on a funnel. It was suspended in water and dissolved in excess 10% sodium hydroxide solution. The resulting solution was filtered and from it the product was reprecipitated at *p*H 6 by means of dilute acetic acid. After collecting and air drying, the material was recrystallized from alcohol as light yellow needles; 4.6 g. (71% yield), m.p. 240–242° dec.

Anal. Calcd. for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.95. Found: C, 65.40; H, 5.14.

8-Acetamido-5-(1-piperidylmethyl)-6-quinolinol (IIIb).—To a suspension of 8.1 g. (0.04 mole) of 8-acetamido-6-quinolinol (IIIa) in 100 ml. of alcohol, a hot solution of 1.2 g. (0.04 mole) of paraformaldehyde and 4.5 g. (0.04 mole) of piperidine in 10 ml. of alcohol was added.[23] The mixture was shaken without further heating for 15 minutes when complete solution occurred. Cooling overnight gave 11.7 g. (82% yield) of white crystalline product, which decomposed at 139–140° to give a high-melting, alcohol-insoluble substance. Attempted recrystallization from alcohol also gave a high-melting product. Note the following description of preparation of VI.

Anal. Calcd. for $C_{17}H_{21}N_3O_2$: C, 68.23; H, 7.03. Found: C, 68.29; H, 7.07.

5,5'-Methylene-bis-8-acetamido-6-quinolinol (VI).—A suspension of 5 g. of 8-acetamido-5-(1-piperidylmethyl)-6-quinolinol (IIIb) in 120 ml. of absolute alcohol was heated at reflux for about 36 hours to give 3.5 g. (nearly quantitative yield) of white solid, m.p. >300°. Because of bumping, it was necessary at intervals to remove the product by filtration.

Anal. Calcd. for $C_{23}H_{20}N_4O_4$: C, 66.33; H, 4.84. Found: C, 65.89; H, 5.14.

8-Nitro-5-(1-piperidylmethyl)-6-quinolinol (IIId).—To a warm solution of 5 g. (0.026 mole) of 8-nitro-6-quinolinol[16] in 500 ml. of alcohol, there was added a warm solution of 2.8 g. (0.033 mole) of piperidine and 0.8 g. (0.026 mole) of paraformaldehyde in 100 ml. of alcohol. After a reflux period of an hour, more than 500 ml. of solvent was removed by distillation. The warm solution was filtered and cooled to precipitate yellow crystals. Recrystallization from alcohol gave 6.5 g. (80% yield) of product, m.p. 157–158° dec.

IIId Dihydrochloride, made by addition of dry hydrogen chloride to an ether solution of the base, was recrystallized from alcohol, m.p. 195–196° dec.

Anal. Calcd. for $C_{15}H_{17}N_3O_3 \cdot 2HCl$: Cl, 19.58. Found: Cl, 19.70.

5-Diethylaminomethyl-8-nitro-6-quinolinol (IIIe).—A mixture of 5 g. (0.026 mole) of 8-nitro-6-quinolinol,[16] 0.8 g. (0.026 mole) of paraformaldehyde, 3 ml. (0.026 mole) of diethylamine and 400 ml. of alcohol was heated at reflux for two hours. A small amount of dark solid was removed by filtration. The volume of the solution was reduced by distillation until solid (about one gram) began to separate. The remainder of the base was obtained by passing in an excess of dry hydrogen chloride and dilution of the mixture

(22) Made by passing hydrogen chloride into an ether-alcohol solution of the base as a means of purifying the free base. Of course, the base may also be used in this procedure.

(23) Under the customary conditions of the Mannich reaction, bis compound VI was obtained as the only product.

with ether. The hydrochloride thus isolated was dissolved in water and neutralized with dilute sodium hydroxide to precipitate the base. Recrystallization from acetone gave 4.5 g. (68% yield) of yellow crystalline product, m.p. 141–142° dec.

Anal. Calcd. for $C_{14}H_{17}N_3O_3$: C, 61.07; H, 6.23. Found: C, 61.12; H, 5.95.

8-(7-Chloro-4-quinolylamino)-6-quinolinol Dihydrochloride (IIIh).—A solution of 2.3 g. (0.01 mole) of 8-amino-6-quinolinol dihydrochloride and 2 g. (0.01 mole) of 4,7-dichloroquinoline in 100 ml. of alcohol was heated at reflux for 15 minutes, whereupon 2.9 g. (71% yield) of reddish yellow crystals precipitated, m.p. 283–285° dec. The product was recrystallized from alcohol, m.p. 295° dec.

Anal. Calcd. for $C_{18}H_{13}ClN_3O\cdot 2HCl\cdot H_2O$: C, 52.25; H, 3.90. Found: C, 52.33; H, 4.12.

8-(7-Chloro-4-quinolylamino)-5-(1-piperidylmethyl)-6-quinolinol Trihydrochloride (IIIi).—A warm solution of 2.6 g. (0.03 mole) of piperidine and 0.3 g. (0.01 mole) of paraformaldehyde in 25 ml. of alcohol was added to 4.1 g. (0.01 mole) of 8-(7-chloro-4-quinolylamino)-6-quinolinol dihydrochloride (IIIh) suspended in 150 ml. of absolute alcohol. The mixture was shaken until complete solution was almost obtained. The trace of insoluble material, considered to be a bis compound similar to VI, was removed by filtration. The filtrate was poured into 40 ml. of saturated alcoholic hydrogen chloride solution. Cooled by ice, the solution gave 5 g. (94% yield) of yellow crystalline product, m.p. 210–212° dec.

Because of the effect of heat in causing the formation of bis compounds, the product was not recrystallized for analysis.

Anal. Calcd. for $C_{24}H_{23}ClN_4O\cdot 3HCl\cdot 3H_2O$: C, 49.48; H, 5.49. Found: C, 49.57; H, 5.73.

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An Estimation of Pathways of Glucose Catabolism in Yeast[1,2]

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A procedure has been devised for the estimation of the relative extents of participation of the Embden–Meyerhof and shunt pathways in glucose catabolism. It depends on the assumptions: (a) that these pathways are essentially the only ones involved; and (b) that trioses, or 3-carbon compounds derived therefrom, formed *via* the E.M. process arise equally from glucose carbons 1 to 3 and 4 to 6, whereas those derived *via* pentose pathways arise only from carbons 4 to 6. The procedure consists in carrying out simultaneously, with the same tissue or cell preparation, experiments with glucose-1-C^{14} and uniformly labeled glucose. A 3-carbon compound such as lactate or pyruvate, or a 2-carbon compound derived therefrom, *e.g.*, acetate (or acetoacetate), ethanol, etc., is isolated from each, and is purified and assayed for radioactivity. When an appropriate intermediate does not accumulate, one can be "trapped" by the addition of the substance in question. The data from glucose-1-C^{14} provide an indication of the relative incorporation of this carbon in the intermediate, and the data from uniformly labeled glucose provide a correction factor for the endogenous metabolism, whereby "true" values may be calculated for the specific activities of compounds derived from glucose-1-carbon. Under aerobic conditions in *S. cerevisiae* the extent of the shunt process ranged from zero to 30%, and in *T. utilis* it ranged from 30 to 50%. Anaerobically, at least 95% of the glucose was catabolized *via* the Embden–Meyerhof pathway.

It is now recognized that glucose may be dissimilated in certain cells by at least one route which differs from the classical glycolytic pathway of Embden and Meyerhof,[4–6] namely, the hexose monophosphate or oxidative shunt. As presently conceived, this process involves, in successive reactions, the oxidation of glucose-6-phosphate to 6-phosphogluconic acid, decarboxylation of the latter to CO_2 and ribose-5-phosphate, isomerization of the pentose ester to ribulose-5-phosphate, and cleavage of the ketopentose phosphate to triose phosphate and an as yet incompletely identified "diose" closely related to glycolaldehyde.[7] Thus far, most of the evidence for the occurrence of the shunt is based either on the presence of the enzymes involved, or on indirect calculations based on rates of liberation of CO_2 from different positions in the glucose chain. As yet, no direct information is available concerning the relative magnitude of each process in intact, living cells. In the present report a procedure is described, using glucose, variously labeled with C^{14}, which we believe yields a reliable approximation of the extents to which the Embden–Meyerhof (E.M.) and the hexose monophosphate shunt pathways participate in the catabolism of glucose by living cells or tissues. In addition, results are reported of its application to several strains of yeast. Data with regard to other microörganisms and animal tissues will be reported separately.

Basis of Method.—The method is based on the fact that, as shown in Fig. 1, the "symmetrical" cleavage of the glucose carbon chain *via* the Embden–Meyerhof pathway yields two trioses, and ultimately two C2 units, one of which is derived from glucose carbons 1 and 2 and the other from carbons 5 and 6. Thus, any acetate derived from C-1-labeled glucose should have one of four carbons labeled, and its specific activity will therefore be one-fourth that of the labeled glucose-1-carbon, or 1.5 times that of the over-all specific activity of the glucose-1-C^{14}. On the other hand, operation of the shunt should lead to unlabeled acetate, since in this process carbon 1 of glucose is lost by decarboxylation of 6-phosphogluconate. If these two pathways account for essentially all of the glucose catabolized, the specific activity of any acetate produced should fall between 0 and 1.5 times that

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