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The Synthesis of Some 3-Nitro- and 3-Amino-4-dialkylaminoalkylaminoquinoline Derivatives

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The nitration of 4-hydroxy-, 5-(and 7)-chloro-4-hydroxyquinolines with hot concentrated nitric acid to give the corresponding 3-nitro compounds is reported. The 4-hydroxy group was replaced with chlorine to yield the 4-chloro-3-nitroquinolines which in turn reacted with ammonia, aniline and 3-diethylamino-2-hydroxypropylamine to give the 4-amino-, 4-anilino- and 4-(3-diethylamino-2-hydroxypropylamino)-3-nitroquinoline derivatives. The corresponding 3-aminoquinolines were obtained by reduction of the nitro compounds. In addition, the condensation of 4,7-dichloro-3-nitroquinoline with several other diamines is also reported.

In continuation of our investigation of 4-dialkylaminoalkylaminoquinolines as possible antimalarial agents, we have prepared some 3-nitro and 3-amino derivatives Previous work indicated that in the 4-aminoquinoline series the presence of a halogen atom[1] in the 3-position of the quinoline nucleus resulted in a marked decrease in antimalarial activity when compared with 7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline.[2] The same effect results from the introduction of a nitro or amino group in the 3-position. Preliminary screening of the compounds tested against *Pl. lophurae* indicated that only 7-chloro-3-nitro-4-(3-diethylamino-2-hydroxypropylamino)-quinoline showed some suppressive activity during medication. Some of the nitro compounds, however, showed considerable antibacterial activity.[3]

The required intermediates for the present work were prepared from 4-hydroxy-, 5(and 7)-chloro-4-hydroxyquinoline. Nitration of these materials with hot concentrated nitric acid resulted in the formation of the corresponding 3-nitro compounds. Replacement of the 4-hydroxy group with chlorine yielded the 4-chloro-3-nitroquinolines, which reacted with ammonia, aniline and 3-diethylamino-2-hydroxypropylamine to give the 4-amino-, 4-anilino- and 4-(3-diethylamino-2-hydroxypropylamino)-3-nitroquinoline derivatives (Table I). The corresponding 3-aminoquinolines were obtained by reduction of the nitro compounds. In addition, 4,-7-dichloro-3-nitroquinoline was condensed with five primary–tertiary diamines to give the compounds listed in Table II.

After most of this work had been completed Bachman, *et al.*, reported the preparation of 3-nitro-4-(3-diethylaminopropylamino)-quinoline[4] from 4-chloro-3-nitroquinoline. These authors prepared 4-hydroxy-3-nitroquinoline from anthranilic and methazonic acids,[5,6] a synthesis which leaves no doubt as to the position of the nitro group. The melting point which they report for 4-chloro-3-nitroquinoline (121–122°)[7] and the one we obtained (120–120.5°) would indicate compounds of identical structure.

The nitration procedure which we have employed is essentially that described by Conrad and Limpach[8] for the preparation of 4-hydroxy-3-nitroquinaldine. This work was reinvestigated and confirmed by Halcrow and Kermack[9] who demonstrated that nitration with hot concentrated nitric acid gave 4-hydroxy-3-nitroquinaldine and concentrated sulfuric and nitric acids at 0–5° gave the 6-nitro isomer.

Gouley, Moersch and Mosher[10] have reported on the nitration of 4-hydroxyquinoline with concentrated sulfuric acid and fuming nitric acid at 0–5°. They obtained a mixture of isomers which was treated with phosphorus oxychloride. From the resulting mixture they isolated a 4-chloronitroquinoline melting at 145°.[11] Reductive cleavage of the halogen substituent yielded an aminoquinoline, m.p. 94°,[12,13] which these authors concluded was probably 3-aminoquinoline and, therefore, the product melting at 145° was 4-chloro-3-nitroquinoline. This is not in agreement[14] with our work or with that reported by Bachman, *et al.*[4]

In our proof of structure work, we have tried the nitration of 4-hydroxyquinoline with concentrated sulfuric and nitric acids at 0–5°. The 4-hydroxy-nitroquinoline which we obtained yielded a 4-chloronitroquinoline melting at 144–145°.[15] The 4-chloroaminoquinoline prepared from this material by catalytic hydrogenation melted at 186–187°.[16] Catalytic reduction and dehalogenation of the chloronitro compound (144–145°) yielded 6-aminoquinoline[13] (116–116.5°) which was characterized by its acetyl derivative (139–139.5°).[17] It would, therefore, appear that the product reported[10] melting at 145° is 4-chloro-6-nitroquinoline and the

(1) A. R. Surrey and R. A. Cutler, THIS JOURNAL, **68**, 2570 (1946).

(2) A. R. Surrey and H. F. Hammer, *ibid.*, **68**, 113 (1946).

(3) We are indebted to Dr. E. W. Dennis and the Chemotherapy Section for the antimalarial and antibacterial screening.

(4) G. B. Bachman, D. E. Welton, G. L. Jenkins and J. E. Christian, THIS JOURNAL, **69**, 365 (1947).

(5) German Patent 347,375, January 17, 1922.

(6) L. Musajo, *Gazz. chim. ital.*, **67**, 222 (1937).

(7) After the present work was completed K. Schofield and T. Swain, *J. Chem. Soc.*, 1367 (1949), reported the preparation of this compound (119–120°) from 4-hydroxy-3-nitroquinoline. The latter was prepared by nitration of 4-hydroxyquinoline with nitric acid at 95°.

(8) M. Conrad and L. Limpach, *Ber.*, **20**, 948 (1887).

(9) B. E. Halcrow and W. O. Kermack, *J. Chem. Soc.*, 415 (1945).

(10) R. W. Gouley, G. W. Moersch and H. S. Mosher, THIS JOURNAL, **69**, 303 (1947).

(11) R. H. Baker, G. R. Lappin, C. J. Albisetti and B. Riegel, *ibid.*, **68**, 1267 (1946), report a melting point of 141–141.5° for 4-chloro-6-nitroquinoline.

(12) W. A. Mills and W. H. Watson, *J. Chem. Soc.*, 741 (1910), reported two melting points for 3-aminoquinoline, 84° and 94° for the metastable and stable forms, respectively.

(13) W. La Coste, *Ber.*, **16**, 669 (1883), reported a melting point of 114° for 6-aminoquinoline.

(14) Schofield and Swain (ref. 7) and A. Adams and D. H. Hey, *J. Chem. Soc.*, 255 (1949), have recently shown that nitration of 4-hydroxyquinoline at 0–5° according to the conditions described by Gouley, Moersch and Mosher (ref. 10) gave the 6-nitro compound.

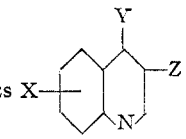
(15) The nitration was repeated with concentrated sulfuric acid and fuming nitric acid.[10] The same 4-chloronitroquinoline was obtained.

(16) Gouley, Moersch and Mosher[10] reduced the nitro compound with iron and acetic acid and obtained an amino compound melting at 170° (dec.).

(17) C. Knueppel, *Ann.*, **310**, 75 (1900), reported 6-acetylaminoquinoline as melting at 138°.

TABLE I

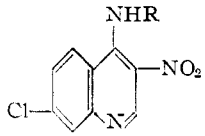
3-NITRO- AND 3-AMINOQUINOLINE DERIVATIVES



No.	X	Y	Z	Yield, %	M.p., °C.	Empirical formula	Analyses, % Calcd.	Found
I	H	OH[a]	NO_2	43	360–361 d.[b]	$C_9H_6N_2O_3$	N, 14.74	14.76
II	5-Cl	OH	NO_2	35	370–372 d.[b]	$C_9H_5ClN_2O_3$	Cl, 15.79	15.80
III	7-Cl	OH	NO_2	24	370–372 d.[b]	$C_9H_5ClN_2O_3$	N, 12.47[c]	12.42
IV	H	Cl[d]	NO_2	90	120–120.5	$C_9H_5ClN_2O_2$	Cl, 17.00	16.85
V	5-Cl	Cl	NO_2	65	120.5–121	$C_9H_4Cl_2N_2O_2$	Cl, 29.18	28.73
VI	7-Cl	Cl	NO_2	85	151–152	$C_9H_4Cl_2N_2O_2$	Cl, 29.18	29.20
VII	H	NH_2[e]	NO_2		256–257	$C_9H_7N_3O_2$	N, 7.40[f]	7.35
VIII	5-Cl	NH_2	NO_2	86	190.5–191.5	$C_9H_6ClN_3O_2$	Cl, 15.86	15.56
IX	7-Cl	NH_2	NO_2	86	236–237	$C_9H_6ClN_3O_2$	Cl, 15.86[g]	15.67
X	H	NHC_6H_5	NO_2	95	134.5–135.5	$C_{15}H_{11}N_3O_2$	N, 15.85	15.90
XI	5-Cl	NHC_6H_5	NO_2	96	166–166.5	$C_{15}H_{10}ClN_3O_2$	NO_2, 15.35	14.91
XII	7-Cl	NHC_6H_5	NO_2	95	173–174	$C_{15}H_{10}ClN_3O_2$	N, 14.02	13.85
XIII	H	NHR[h]	NO_2	87	114–114.5	$C_{16}H_{22}N_4O_3$	N, 17.60[i]	17.72
XIV	5-Cl	NHR[h]	NO_2	90	105.5–106	$C_{16}H_{21}ClN_4O_3$	N, 15.88[j]	16.02
XV	7-Cl	NHR[h]	NO_2	90	136.5–137	$C_{16}H_{21}ClN_4O_3$	N, 15.88[k]	15.68
XVI	H	Cl	NH_2	78	149–149.5	$C_9H_7ClN_2$	N, 15.69	16.03
XVII	5-Cl	Cl	NH_2	25	192.5–193	$C_9H_6Cl_2N_2$	N, 13.15	13.30
XVIII	7-Cl	Cl	NH_2	60	170–171	$C_9H_6Cl_2N_2$	N, 13.15	12.86
XIX	5-Cl	NH_2	NH_2	63	158–159	$C_9H_8ClN_3$	N, 21.71	21.41
XX	7-Cl	NH_2	NH_2	75	191–192	$C_9H_8ClN_3$	N, 21.71	21.38
XXI	H	NHC_6H_5	NH_2	80	204–205	$C_{15}H_{13}N_3$	N, 17.86	17.76
XXII	5-Cl	NHC_6H_5	NH_2	32	184–184.5	$C_{15}H_{12}ClN_3$	N, 15.58	15.20
XXIII	7-Cl	NHC_6H_5	NH_2	31	179.5–180.5	$C_{15}H_{12}ClN_3$	N, 15.58	15.84
XXIV	H	NHR[h]	NH_2	46	80–81	$C_{16}H_{24}N_4O$	N, 19.43[l]	19.66
XXV	7-Cl	NHR[h]	NH_2	50	97–98	$C_{16}H_{23}ClN_4O$	N, 17.36[m]	17.26

[a] Previously prepared.[4,5,6,7] [b] Turns brown at about 350°. [c] Calcd.: Cl, 15.79. Found: Cl, 15.85. [d] Previously prepared.[4,7] [e] Prepared by A. Albert, D. J. Brown and H. Duewell, *J. Chem. Soc.*, 1284 (1948). [f] Titration of basic nitrogen by the G. Toennies and T. P. Callan method (*J. Biol. Chem.*, **125**, 259 (1938)). [g] Calcd. N, 18.80; NO_2, 20.57. Found: N, 18.97; NO_2, 20.48. [h] R equal to 3-diethylamino-2-hydroxypropyl. [i] Calcd.: C, 60.36; H, 6.96. Found: C, 60.45; H, 6.79. [j] Calcd.: C, 54.46; H, 6.00. Found: C, 54.55; H, 5.90. [k] Calcd.: C, 54.46; H, 6.00. Found: C, 54.73; H, 6.13. [l] Calcd.: C, 66.63; H, 8.39. Found: C, 66.83; H, 8.19. [m] Calcd.: C, 59.52; H, 7.18. Found: C, 59.59; H, 6.86.

TABLE II



No.	R	Side chain (moles)	Yield, %	M.p., °C.[a]	Chlorine, % Calcd.	Found	Nitrogen, % Calcd.	Found
XXVI	$CH_2CH_2CH_2CH_2N(C_2H_5)_2$	1.2	70	56.1–58.1[b]	10.11	9.83	15.97	15.49
XXVII	CH_2—$CH(C_6H_5)CH_2CH_2N(C_2H_5)_2$[c]	1.5	94	91.8–93.2	8.30	8.20	13.12	13.40
XXVIII	CH_2—$CH(4\text{-}ClC_6H_4)CH_2CH_2N(C_2H_5)_2$[c]	1.1	77	120.8–122.4	15.37	15.22	12.14	12.06
XXIX	CH_2—$CH(3,4\text{-}Cl_2C_6H_3)CH_2CH_2N(C_2H_5)_2$[c]	1.3	86	110.5–111.4	21.45	21.30	11.30	11.32
XXX	CH_3CH—$CH_2CH_2CH_2N(C_2H_5)CH_2CH_2OH$[d]	2	70	88–89.1	9.31	9.15	14.71	14.57

[a] Corrected melting points. [b] The monophosphate salt, m.p. 177.8–179.2° (cor.). Calcd. for $C_{17}H_{23}ClN_4O_3 \cdot H_3PO_4$: C, 45.49; H, 5.84; H_3PO_4, 21.83. Found: C, 45.49; H, 5.66; H_3PO_4, 21.30. [c] The side chain was prepared according to C. E. Kwartler and P. Lucas, THIS JOURNAL, **68**, 2395 (1946). [d] This side chain was prepared according to A. R. Surrey and H. F. Hammer, *ibid.*, **72**, 1814 (1950).

aminoquinoline (94°) obtained by these workers was probably an impure sample of 6-aminoquinoline.

The 4-chloro-3-nitroquinoline (121–121.5°) which we obtained by nitration of 4-hydroxyquinoline followed by treatment with phosphorus oxychloride, yielded on catalytic hydrogenation and dehalogenation 3-aminoquinoline (83.5–84.5°).[12] The structures of the remaining nitro derivatives were determined by replacement of the 3-amino group by iodine, *via* the diazo reaction, to yield 3-iodo-4,5-(and 4,7)-dichloroquinolines which were identified by a direct comparison with authentic samples[1] prepared in this laboratory.

Experimental[18]

4-Hydroxy-3-nitroquinolines (I, II and III).—Fifty grams of the 4-hydroxyquinoline was added to 500 ml. of nitric acid (d. 1.42) preheated to 85° and the resulting solution heated on the steam-bath for 45 minutes. In the

(18) All melting points are uncorrected unless otherwise specified.

preparation of I and III, the clear, dark red solution was poured cautiously into 1500 ml. of boiling water. The yellow fluffy solid which separated was filtered off hot, the filter cake was washed with water and then acetone until the washings were colorless. If the solution was allowed to cool appreciably before filtering or if the acetone washings were omitted, the resulting product was impure and treatment with phosphorus oxychloride resulted in a poor yield.

In the preparation of II, the desired product separated directly from the hot nitric acid mixture and was filtered off through a fritted glass funnel. The yellow product was washed with concentrated nitric acid, water and acetone as above to give practically pure II. If the reaction mixture were diluted with water as in the case of I and III or allowed to cool, impure product was obtained.

Analytical samples were prepared from the purified 4-chloro-3-nitroquinolines (IV, V and VI) by hydrolysis with dilute hydrochloric acid on the steam-bath for one-half hour.

4-Chloro-3-nitroquinolines (IV, V and VI).—One part by weight of the appropriate 4-hydroxy-3-nitroquinoline was refluxed with two to three volumes of phosphorus oxychloride until solution occurred (5–15 minutes for I and III; 10–12 hours for II). The reaction mixture was poured carefully with stirring into crushed ice, the solid which separated was filtered immediately[19] and dissolved in chloroform, and the solution was washed once with ice-cold sodium hydroxide solution, dried over Drierite and evaporated to give the 4-chloro compounds. The latter were sufficiently pure for reaction with the amino compounds.

An alternate procedure using anhydrous conditions was worked out for IV and VI. Equimolecular amounts of the 4-hydroxy-3-nitroquinoline and phosphorus oxychloride were mixed with 5 parts (based on the weight of the quinoline compound) of chlorobenzene and refluxed until solution occurred. Approximately one-half of the chlorobenzene was removed by distillation under reduced pressure and the residual solution brought up to the original volume with Skellysolve C. After filtering hot with charcoal, the product separated as long needles in practically pure form.

Analytical samples were prepared by recrystallization from Skellysolve B or C.

4-Amino-3-nitroquinolines (VII, VIII and IX).—Amination was carried out by passing anhydrous ammonia into a stirred solution of the 4-chloro-3-nitroquinoline in ten volumes of toluene (IV and V) or butanol (VI) and gradually warming the solution to 90°. After bubbling in the ammonia at this temperature for one hour, the reaction mixture was cooled, filtered and the filter cake slurried with water to remove ammonium chloride. The product was collected by filtration, washed with water, dried and recrystallized from ethanol (VII and IX) or benzene (VIII) to give yellow needles.

4-Anilino-3-nitroquinolines (X, XI and XII).—One part of the appropriate 4-chloro-3-nitroquinoline was added to a stirred solution of one part of aniline dissolved in five parts of glacial acetic acid. After the initial exothermic reaction, the solution was heated for 5–15 minutes on a steam-bath, poured into twenty volumes of water and the product which separated collected by filtration. Analytical samples were prepared by recrystallization of the dried product from Skellysolve C. The reaction may also be run without solvent but for large amounts the reaction is liable to become uncontrollable. Chloroform also serves well as a suitable solvent.

4-(3-Diethylamino-2-hydroxypropylamino)-3-nitroquinolines (XIII, XIV and XV).—To a stirred solution of one mole of the 4-chloro-3-nitroquinoline in five volumes of chloroform was added dropwise two moles of 3-diethylamino-2-hydroxypropylamine. After the initial exothermic reaction, the mixture was refluxed for one-half hour on the steam-bath, cooled and extracted with dilute hydrochloric acid. The combined acid extracts were treated with ammonium hydroxide and the yellow solid which separated (in the case of XIII and XV) filtered off and washed with water. After drying, the bases were recrystallized from benzene–Skellysolve C mixture (XIII) or from ethanol (XV). In the case of XIV, an oil was obtained which was taken up in chloroform, the solution dried and evaporated. The residue was crystallized from Skellysolve C. The condensation was also carried out in glacial acetic acid or in the absence of solvent. In the latter instance extreme caution is required to prevent a violent reaction.

4-Dialkylaminoalkylamino-7-chloro-3-nitroquinolines (Table II).—These compounds were prepared by essentially the same method described above. In most cases the amount of side chain (as indicated in Table II) was less than two moles. In addition, for compounds XXVI and XXIX, ethylene dichloride was used as the solvent.

3-Amino-4-chloroquinolines (XVI, XVII and XVIII).—The 4-chloro-3-nitroquinolines[20] were catalytically reduced in absolute alcohol with Raney nickel at room temperature. After removal of the catalyst and evaporation of the solvent, the residue was taken up in dilute hydrochloric acid (in the case of XVI and XVII), filtered with charcoal and the base reprecipitated with ammonium hydroxide. The hydrochloride of XVIII was too insoluble for this treatment. Each of the 3-amino-4-chloroquinolines was recrystallized from Skellysolve C to give practically white needles.

3,4-Diaminoquinolines (XIX and XX).—A mixture of one part by weight of 5-(or 7)-chloro-4-amino-3-nitroquinoline, 4 parts of iron filings, 0.1 part of glacial acetic acid, 12 parts of ethanol and 6 parts of water was refluxed with stirring for six hours. Solid potassium carbonate was added to alkalinity, the reaction mixture filtered hot, the filter cake washed with hot alcohol and the solvent removed from the filtrate under reduced pressure. The residue was extracted with dilute hydrochloric acid, the extract filtered with charcoal and the diamine liberated with ammonium hydroxide solution. The crude, dried material was recrystallized from benzene (XIX) or xylene (XX) to give a white product in each case.

3-Amino-4-anilinoquinolines (XXI, XXII and XXIII).—Compounds XXI and XXII were prepared by reduction of the corresponding nitro compounds according to the procedure described above for the preparation of 3,4-diaminoquinolines. After evaporation of the ethanol–water solution, the crude residue was recrystallized twice from benzene (XXI) or a large volume of Skellysolve E followed by a second recrystallization from a large amount of Skellysolve C (XXII) to give small white crystals and light fawn needles, respectively.

Compound XXIII was prepared by reduction of XII in alcohol in the presence of Raney nickel. After removal of the catalyst by filtration, the solvent was removed by distillation, the product taken up in a large volume of warm dilute hydrochloric acid, the acid solution filtered with charcoal, and the base liberated with ammonium hydroxide. The product was removed by filtration, dried and recrystallized from a large volume of Skellysolve C to give white needles.

3-Amino-4-(3-diethylamino-2-hydroxypropylamino)-quinolines (XXIV and XXV).—The nitro compounds, XIII and XV, were reduced with iron and acetic acid according to the procedure described for the 3,4-diaminoquinolines above. After removal of the iron filings by filtration, two equivalents of hydrochloric acid was added and the alcohol removed under reduced pressure. Sodium hydroxide (35%) was added to the residual aqueous solution, the liberated base extracted with a large volume of ether and the latter dried over anhydrous potassium carbonate. Filtration with charcoal at this point gave a pale yellow solution which yielded a pale yellow oil (XXIV) or solid (XXV) upon removal of the ether by distillation. Compound XXIV was obtained as a solid by slurrying the oil with three volumes of ether. The solid bases were further purified by recrystallization from a mixture of benzene and Skellysolve B.

Proof of Structure, Conversion of XVII and XVIII to 4,5- and 4,7-Dichloro-3-iodoquinolines, Respectively.—The aminoquinoline (0.5 g. of XVII; 1 g. of XVIII) was dissolved in dilute sulfuric acid, cooled to 0° and the pasty mass treated with sodium nitrite solution. The excess diazotizing agent was destroyed with a little urea, a solution of potassium iodide and a little copper powder added and the mixture allowed to stand overnight at room temperature. The brick red precipitate which formed was removed by filtration, treated with warm 10% sodium hydroxide solution and filtered with charcoal. Addition of acetic acid to

(19) The 4-chloro-3-nitroquinolines are easily hydrolyzed to the 4-hydroxy compounds by water especially in the presence of acids. They vary in the ease with which they undergo hydrolysis in the following order: V > IV > VI.

(20) All of the nitro compounds which were to be reduced catalytically were carefully purified beforehand.

the filtrate liberated the 4-hydroxy-3-iodoquinoline. The latter was removed by filtration, dried and treated with 3 volumes of phosphorus oxychloride to yield the 4,5- and 4,7-dichloro-3-iodoquinolines according to the procedure originally used in their preparation.[1] By this method was obtained 0.06 g. of 4,5-dichloro-3-iodoquinoline (m.p. 109–110.5° after recrystallization from a mixture of Skellysolve A and B followed by recrystallization from methanol) from XVII and 0.35 g. of 4,7-dichloro-3-iodoquinoline (m.p. 111–112° after recrystallization from Skellysolve B) from XVIII. Mixed melting points with authentic samples were not depressed in either case.

Proof of Structure. Preparation of 3-Aminoquinoline from 4-Chloro-3-nitroquinoline.—Three grams of 4-chloro-3-nitroquinoline were reduced in methanol in the presence of Raney nickel at room temperature. After the hydrogen uptake had indicated the complete reduction of the nitro group, 2 g. of solid potassium hydroxide and a little fresh catalyst were added and the reduction continued at 50°. After filtering off the catalyst, the methanol was removed by distillation and the residue taken up in dilute hydrochloric acid, filtered with charcoal, the filtrate treated with excess sodium hydroxide solution and the aqueous solution extracted four times with ether. The ether solution, after drying with Drierite and filtering with charcoal, was evaporated to give a pale yellow oil which solidified on standing. This was crystallized from 200 ml. of Skellysolve B to give 0.5 g. of small white needles; m.p. 83.5–84.5°. The acetyl derivative, prepared according to the procedure of Mills and Watson[12] melted at 168–168.5°. These workers report this derivative as melting at 166–167°.

3-Amino-7-chloroquinoline.—A solution of 4,7-dichloro-3-nitroquinoline in methanol was reduced catalytically in the presence of Raney nickel as described in the preceding paragraph. The crude product was recrystallized from Skellysolve C followed by recrystallization from alcohol and water to give long silky white needles, m.p., 143–143.5°.

Anal. Calcd. for $C_9H_7ClN_2$: Cl, 19.85. Found: Cl, 19.74.

4-Chloro-6-nitroquinoline.—The nitration procedure was adapted from that used by Halcrow and Kermack[9] for the preparation of 6-nitro-4-hydroxyquinaldine.

A mixture of 10 ml. of concentrated sulfuric and 10 ml. of concentrated nitric acids was dropped into a solution of 20 g. of 4-hydroxyquinoline in 100 ml. of concentrated sulfuric acid maintaining the temperature at 0–5°. After addition was complete the mixture was allowed to warm to room temperature and stand for two hours. The solution was poured into 500 ml. of ice and water, allowed to stand overnight and the separated solid filtered off, washed with water and dried to give 18.3 g. (70%) of material which was largely 4-hydroxy-6-nitroquinoline. The product may be recrystallized from thirty volumes of glacial acetic acid but this purification is unnecessary if the product is to be chlorinated.

Sixteen grams of the above crude product was heated with 30 ml. of phosphorus oxychloride until solution occurred (5–10 minutes), the mixture poured into ice-water, warmed briefly to 40° and filtered with charcoal. The clear yellow solution was treated with sodium hydroxide solution, the free base taken up in ether, the latter solution dried over Drierite and the solvent distilled off to give 10.5 g. (60%) of pale yellow solid, m.p. 140–142°. Recrystallization from methanol gave long fluffy yellow tinted needles, m.p. 144–145°.[11]

Nitration of 4-hydroxyquinoline according to the procedure of Gouley, Moersch and Mosher[10] followed by treatment with phosphorus oxychloride and purification of the product gave the same compound, melting at 144–145°.

6-Aminoquinoline.—Three grams of 4-chloro-6-nitroquinoline, twice recrystallized from methanol, was reduced stepwise by the procedure described above for the preparation of 3-aminoquinoline. The residue, after evaporation of the methanol, was taken up in dilute hydrochloric acid, the solution adjusted with ammonium hydroxide until just basic to litmus and filtered with charcoal. The filtrate was made strongly basic with sodium hydroxide solution and the alkaline solution extracted three times with ether. Evaporation of the dried ether extracts gave 0.93 g. of a pale yellow solid, m.p. 115–116°. Recrystallization from Skellysolve C gave large white leaflets, m.p. 116–116.5°.[13] The acetyl derivative melted at 138.5–139°.[17]

6-Amino-4-chloroquinoline.—Three grams of purified 4-chloro-6-nitroquinoline was reduced catalytically to give 1.95 g. of crude material, m.p. 184–186°. Recrystallization from 70 ml. of methanol gave pale yellow needles, m.p. 186–187°.[16]

Anal. Calcd. for $C_9H_7ClN_2$: Cl, 19.85; N, 15.69. Found: Cl, 19.60; N (Kjeldahl), 15.30.

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Analysis of the Products Formed on Hydrolysis of Hyaluronic Acid by Testicular Hyaluronidase[1,2]

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A scheme for the analysis of enzymic hydrolysates of hyaluronic acid has been devised based upon adsorption on charcoal (Darco G-60) followed by fractional elution with 100-ml. volumes of a succession of solvents: water, 5% ethanol, 15% ethanol, 0.1% pyridine, 0.5, 1.0, 1.5, 2.0, and finally 5.0% pyridine. The effluent is collected in 10-ml. fractions which are analyzed for reducing sugar, uronic acid and acetylglucosamine equivalents. Analysis of the data so obtained shows the chromatographic technique employed to be capable of separating fractions of different molecular size but similar chemical composition. Although the technique does not yield definitive separations, its application to hydrolysates obtained after various time intervals of enzymic action has led to the following points being confirmed or established with regard to hyaluronic acid structure and the mechanism of *testicular hyaluronidase* action: (1) Hyaluronic acid is composed of a uniform structure of alternating acetylglucosamine and glucuronic acid residues. There is no evidence of the presence of a core of polyacetylhexosaminides or polyuronides. (2) The enzyme attacks the substrate close to the center of the polysaccharide chains breaking only the glucosidic bond formed by the oxygen on carbon-1 of the acetylglucosamine residue. (3) The enzymic hydrolysis does not proceed down to the liberation of monosaccharides.

Introduction

Hyaluronic acid is a very high molecular weight polysaccharide acid of widespread occurrence. It has been isolated from vitreous humor, synovial fluid, umbilical cord, skin, tumor fluids and hemolytic streptococci in the mucoid phase. It is a substance of considerable importance in such diverse problems as bacterial invasiveness, permeability of ground substance and the so-called "spreading reaction," fertilization, the etiology of rheumatic disease, and related fields in physiology and medicine.[3]

The detailed structure of hyaluronic acid is not known, although analyses of carefully purified preparations show that it is composed of equimolar

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(2) Presented at the 118th Meeting of the American Chemical Society, Chicago, Illinois, September, 1950.

(3) *Ann. N. Y. Acad. Sci.*, **52**, 943 (1950).