neut. equiv. 115.0 (calcd. for $C_{12}H_{22}O_4$, 115.2). The anilide, prepared by the thionyl chloride–aniline procedure and recrystallized from 90% ethanol, had m. p. 172–173°.

(b) By **Ozonization of Ethyl Ester of Acid I**.—A solution of 5.94 g. of refractionated unsaturated ester in 100 ml. of ethyl chloride was ozonized at −20 to −30° until excess ozone was present, 10 ml. of water added, and the ethyl chloride allowed to boil off. The gelatinous ozonide was refluxed with 10 ml. of 30% hydrogen peroxide and a few mg. of palladium sponge for sixteen hours, 20 ml. of 5 *N* sodium hydroxide added, and refluxing continued an hour longer. Distillation of the alkaline solution gave no cyclohexanone. Acidification and ether extraction gave 3.70 g. of tan solid which after three recrystallizations from water yielded white crystals, m. p. 109.3–110.0°, neut. equiv., 123.9. The mixed melting point of this saturated keto-acid with the acid II produced by permanganate oxidation was 110.0–110.5°. The semicarbazone was prepared and its melting point and mixed melting point with the previously obtained acid II semicarbazone were the same, 167.0–167.5°.

Summary

The physical properties of a $C_{12}H_{20}O_2$ acid obtained by alkaline fusion of cyclohexanone or cyclohexanol are listed and the amide and anilide recorded.

From the hydrogenation and ozonolysis products of the pure ethyl ester and the oxidation product of the original acid, the original acid is shown to be 1-cyclohexene-1-caproic acid.

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[CONTRIBUTION FROM THE CHEMOTHERAPY SECTION, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Some Quaternary Ammonium Salts of Heterocyclic Bases. III. Bis-Quaternary Ammonium Salts[1]

BY JONATHAN L. HARTWELL AND MILTON A. POGORELSKIN

In continuation of the study of the effect of quaternary ammonium salts on tumors in animals,[2] it was desired to prepare several series of bis-quaternary ammonium salts for bioassay. Seven series of compounds were chosen for synthesis, namely, the bis-salts derived from the normal α,ω-alkylene dibromides C_2 to C_5, C_{10}, from glycerol-α,γ-dibromohydrin, and from 2,5-dibromohexane (see Table I). The bases used were the seven tertiary heterocyclic bases utilized in previous papers.

In the majority of cases, the reactions proceeded according to expectation and the alkylene dibromides added to two moles of the tertiary base to form the bis-quaternary ammonium salt (reaction 1). In those instances where the bromides were too hygroscopic for easy handling, analysis and the determination of melting points, they were converted into the corresponding perchlorates (in one case, the iodide).

$$Br(CH_2)_nBr + 2N \longrightarrow Br^- {}_+N-(CH_2)_n-N_+ + Br^- \qquad (1)$$

$$Br(CH_2)_nBr + N \longrightarrow H-N_+ Br^- \qquad (2)$$

$$BrCH_2CH_2Br + N \longrightarrow Br^- N^+-CH_2CH_2Br \qquad (3a)$$

$$BrCH_2CH(OH)CH_2Br + N \longrightarrow Br^- {}_+N-CH_2CH(OH)CH_2Br \qquad (3b)$$

There were several exceptions, however. The reaction of ethylene dibromide with 3-methylisoquinoline, and of 2,5-dibromohexane with quinoline and with 3-methylisoquinoline, gave the hydrobromide of the base; since the fate of the alkylene dibromide is not known, the unbalanced equation (2) may be written for the reaction.

The reaction of ethylene dibromide with quinoline yielded two different crystalline products, depending on conditions, neither one identical with the bis-compound expected.[3] One product, m. p. 255–257° cor., gave an analysis unsatisfactory for a probable compound. The other product, m. p. 203–204° cor., had analytical figures and chemical properties corresponding to 1-(β-bromoethyl)-quinolinium bromide (reaction 3a); the nitrogen–bromine ratio showed that 1 mole of ethylene dibromide had reacted with 1 mole of quinoline, and only one-half of the bromine was ionically bound.

An interesting exception was found in the reaction between glycerol-α,γ-dibromohydrin and the tertiary bases. In several cases, this dibromide also combined with only one mole of base to form products containing two atoms of bromine of which only one atom was ionic. These products are represented by reaction (3b), and were formed with α-picoline, quinoline and 3-methylisoquinoline. With α-picoline and 3-methylisoquinoline, further reaction yielded the bis-salts, but with quinoline the bis-salt could not be formed under any conditions tried.

The reactions reported as being exceptional to the general reaction for the formation of bis-salts occurred only with the bases α-picoline, quinoline and 3-methylisoquinoline. Only these, of the bases used, have a substituent or a ring α- to the nitrogen atom. It would thus appear that steric hindrance plays a part in the course of the

(1) Paper II, Hartwell and Kornberg, THIS JOURNAL, **68**, 1131 (1946).

(2) Hartwell and Shear, *Cancer Research*, **7**, 716 (1947).

(3) Rhoussopoulos, *Ber.*, **16**, 879 (1883), reported ethylene-α,β-bisquinolinium bromide monohydrate on the basis of carbon and hydrogen analysis. No m. p. was given. Since the analytical values were almost equally valid for quinoline hydrobromide, and since the bis-salt was never obtained in the present work, it is considered doubtful that the bis-salt has ever been prepared.

TABLE I

Compound	Base used	X	Appearance[m]	M. p., °C., cor.	Yield, crude, %	Empirical formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Halogen Calcd.	Halogen Found
$\overset{+}{N}-(CH_2)_2-\overset{+}{N}\ 2X^-$												
1[a]	Pyridine	Br	Transp. prisms	>265	49	$C_{12}H_{14}Br_2N_2$	41.6	42.1	4.1	4.4	46.2	45.65
2[b]	α-Picoline	Br	Transp. prisms	>265	12	$C_{14}H_{18}Br_2N_2$	44.9	44.9	4.85	4.8	42.7	43.1
3	β-Picoline	Br	Needles	256.4–258.7 (dec.)	28	$C_{14}H_{18}Br_2N_2$	44.9	45.0	4.85	4.85	42.7	42.8
4	γ-Picoline	Br	Transp. plates	>265	27	$C_{14}H_{18}Br_2N_2$	44.9	45.05	4.85	5.1	42.7	42.7
5	Isoquinoline	Br	Tan transp. pr.	267.9–268.8 (dec.)	19	$C_{20}H_{18}Br_2N_2\cdot 1.5H_2O$	50.8	50.5	4.5	4.7	..	...[p]
$\overset{+}{N}-(CH_2)_3-\overset{+}{N}\ 2X^-$												
6[c]	Pyridine	Br	Transp. prisms	242.5–244.0	93	....	...	...	..	..	..	...
7	Pyridine	ClO₄	Transp. prisms	220.0–220.7	42[d]	$C_{13}H_{16}Cl_2N_2O_8$	39.1	39.2	4.0	3.85	17.8	17.8[e]
8	α-Picoline	Br	Transp. prisms	273.0–273.5 (↑)	26	$C_{15}H_{20}Br_2N_2$	46.4	46.6	5.2	5.3	41.2	41.1
9	β-Picoline	Br	Transp. prisms	214.0–216.0 (dark.)	51	....	...	...	..	..	..	...
10	β-Picoline	ClO₄	Prisms	144.8–145.8	67[d]	$C_{15}H_{20}Cl_2N_2O_8$	42.2	42.1	4.7	5.0	..	...
11	γ-Picoline	Br	Transp. prisms	70–240 (dark.)	45	....	...	...	..	..	..	...
12	γ-Picoline	ClO₄	Pale pink ne.	200.6–201.5 (dark.)	89[d]	$C_{15}H_{20}Cl_2N_2O_8$	42.2	42.1	4.7	4.7	..	...
13	Quinoline	Br	Ivory prisms	248.4–248.9 (sint.)	57	$C_{21}H_{20}Br_2N_2\cdot 2H_2O$	50.8	51.2	4.9	5.0	32.2	32.2
14	Isoquinoline	Br	Needles	244.0–244.8 (dark.)	76	$C_{21}H_{20}Br_2N_2$	54.8	54.7	4.4	4.6	34.7	34.0
15	3-Methylisoquinoline	Br	Needles	279.2–279.7 (dec.)	31	$C_{23}H_{24}Br_2N_2\cdot 1.5H_2O$	53.65	53.6	5.3	5.4	31.0	30.8
$\overset{+}{N}-(CH_2)_4-\overset{+}{N}\ 2X^-$												
16[f]	Pyridine	Br	Prisms	See text	89	$C_{14}H_{18}Br_2N_2\cdot 2H_2O$	41.0	41.2	5.4	5.6	..	...[p]
17	Pyridine	ClO₄	Needles	216.5–217.8	100[d]	$C_{14}H_{18}Cl_2N_2O_8$	40.7	40.7	4.4	4.4	..	...
18	α-Picoline	Br	Transp. prisms	257.1–258.6 (↑)	41	$C_{16}H_{22}Br_2N_2\cdot 2H_2O$	43.9	44.4	6.0	6.0	36.5	36.5
19	β-Picoline	Br	Transp. prisms	221.5–223.3	85	....	...	...	..	..	..	...
20	β-Picoline	ClO₄	Needles	129.3–130.4	85[d]	$C_{16}H_{22}Cl_2N_2O_8$	43.55	43.7	5.0	5.0	..	...
21	γ-Picoline	Br	Transp. prisms	See text	52	$C_{16}H_{22}Br_2N_2\cdot 2H_2O$	43.9	44.1	6.0	6.1	36.5	36.8
22	γ-Picoline	ClO₄	Transp. ne.	179.9–180.9	97[d]	$C_{16}H_{22}Cl_2N_2O_8$	43.55	43.8	5.0	5.05	..	...
23	Quinoline	Br	Pale gray flat ne.	262.6–263.1 (dec.)	27	$C_{22}H_{22}Br_2N_2\cdot 2H_2O$	51.8	52.2	5.1	5.3	31.3	31.3
24	Isoquinoline	Br	Light tan prisms	262.2–262.8 (↑)	79	$C_{22}H_{22}Br_2N_2\cdot 2H_2O$	51.8	51.7	5.1	5.2	31.3	31.1
25	3-Methylisoquinoline	Br	Prisms	275.5–276.0 (dark.)	53	$C_{24}H_{26}Br_2N_2\cdot 3.5H_2O$	51.1	50.8	5.9	5.9	28.3	26.9[g]
$\overset{+}{N}-(CH_2)_5-\overset{+}{N}\ 2X^-$												
26	Pyridine	Br	Plates	....	76	....	...	...	..	..	..	...
27	Pyridine	ClO₄	Plates	123.1–124.1	94[d]	$C_{15}H_{20}Cl_2N_2O_8$	42.2	42.4	4.7	4.8	..	...
28	α-Picoline	Br	Pale yell. prisms	....	49	....	...	...	..	..	..	...
29	α-Picoline	ClO₄	Prisms	118.1–119.4	85[d]	$C_{17}H_{24}Cl_2N_2O_8$	44.85	44.8	5.3	5.4	..	...
30	β-Picoline	Br	Transp. prisms	221.7–222.5	82	....	...	...	..	..	..	...
31	β-Picoline	ClO₄	Transp. prisms	127.5–128.5	93[d]	$C_{17}H_{24}Cl_2N_2O_8$	44.85	44.7	5.3	5.4	..	...
32	γ-Picoline	Br	Needles	215.5–217.5 (dark.)	84	....	...	...	..	..	..	...
33	γ-Picoline	ClO₄	Transp. prisms	143.1–144.2	88[h]	$C_{17}H_{24}Cl_2N_2O_8$	44.85	45.1	5.3	5.3	..	...
34[i]	Quinoline	Br	Lt. yell. prisms	See text	38	$C_{23}H_{24}Br_2N_2\cdot 2.5H_2O$	51.8	52.2	5.5	5.5	30.0	29.7
35	Quinoline	ClO₄	Needles	195.6–197.6 (dark.)	100[d]	$C_{23}H_{24}Cl_2N_2O_8$	52.4	52.5	4.6	4.8	..	...
36	Isoquinoline	Br	Cream needles	117.1–118.1	50	$C_{23}H_{24}Br_2N_2\cdot H_2O$	54.55	54.4	5.2	5.35	31.6	31.3
37	3-Methylisoquinoline	Br	Cream needles	266.5–267.1 (↑, dark)	52	$C_{25}H_{28}Br_2N_2\cdot 2.5H_2O$	53.6	53.5	5.9	6.2	28.5	28.3
$\overset{+}{N}-(CH_2)_{10}-\overset{+}{N}\ 2X^-$												
38	Pyridine	Br	Transp. prisms	196.5–198.1	82	$C_{20}H_{30}Br_2N_2$	52.4	52.3	6.6	6.6	34.9	35.0
39	α-Picoline	Br	Transp. prisms	See text	36	$C_{22}H_{34}Br_2N_2\cdot 2H_2O$	50.6	50.95	7.3	7.6	30.6	30.4
40	α-Picoline	ClO₄	Needles	116.0–116.5	98[d]	$C_{22}H_{34}Cl_2N_2O_8$	50.3	50.6	6.5	6.5	..	...
41	β-Picoline	ClO₄	Transp. prisms	79.3–80.0	58[h]	$C_{22}H_{34}Cl_2N_2O_8$	50.3	50.5	6.5	6.4	..	...
42	γ-Picoline	Br	Prisms	59.9–60.7	93	....	...	...	..	..	..	...
43	γ-Picoline	ClO₄	Needles	107.1–107.9	89[d]	$C_{22}H_{34}Cl_2N_2O_8$	50.3	50.3	6.5	6.6	..	...
44	Quinoline	Br	Buff needles	See text	22	$C_{28}H_{34}Br_2N_2\cdot 2.5H_2O$	55.7	55.8	6.5	6.1	26.5	26.5
45	Quinoline	ClO₄	Pale pink pl.	238.0–239.5 (dark.)	100[d]	$C_{28}H_{34}Cl_2N_2O_8$	56.3	56.2	5.7	5.8	..	...
46	Isoquinoline	Br	Lt. tan ne.	135.3–136.1	69	$C_{28}H_{34}Br_2N_2\cdot 2H_2O$	56.6	56.9	6.4	6.7	26.9	26.3
47	3-Methylisoquinoline	Br	Crystals	251.0–252.0 (dark.)	27	$C_{30}H_{38}Br_2N_2$	61.4	61.2	6.5	6.9	27.25	26.65[l]
$\overset{+}{N}-CH_2-CHOH-CH_2-\overset{+}{N}\ 2X^-$												
48	Pyridine	Br	Transp. prisms	215.2–215.8	11	$C_{13}H_{16}Br_2N_2O\cdot H_2O$	39.6	40.0	4.6	4.8	40.55	41.1
49	α-Picoline	Br	Prisms	291.2–292.0 (dec.)	20	$C_{15}H_{20}Br_2N_2O$	44.6	44.5	5.0	4.9	39.5	39.3
50	β-Picoline	Br	Transp. prisms	262.3–263.3 (dec.)	11	$C_{15}H_{20}Br_2N_2O$	44.6	44.1	5.0	5.2	39.5	39.1
51	γ-Picoline	ClO₄	Prisms	215.0–216.0	17[h]	$C_{15}H_{20}Cl_2N_2O_9.H_2O$	39.1	39.4	4.8	4.8	..	...
52	Isoquinoline	Br	Lt. buff ne.	66–185 (↑)	37	$C_{21}H_{20}Br_2N_2O\cdot 2H_2O$	49.2	49.4	4.7	4.7	31.2	31.5
53	3-Methylisoquinoline	Br	Buff prisms	292.4–292.9 (dec.)	22	$C_{23}H_{24}Br_2N_2O\cdot H_2O$	52.9	52.8	5.0	5.4	..	...[p]

TABLE I (*Continued*)

Compound	Base used	X	Appearance[m]	M. p., °C., cor.	Yield, crude, %	Empirical formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Halogen Calcd.	Halogen Found
				$CH_3—CH(N^+)—(CH_2)_2—CH(N^+)—CH_3\ 2X^-$								
54	Pyridine	Br	Aggregates	....	39	....	...	...	..	..	..	...
55	Pyridine	ClO_4	Plates, dil. alc.	180.7–181.1	58	$C_{16}H_{22}Cl_2N_2O_8$	43.5	43.6	5.0	5.1[j]	..	...
56	α-Picoline	Br	Needles	260.0–265.0	2.2	$C_{18}H_{26}Br_2N_2·2H_2O$	46.4	46.75	6.5	6.8	34.3	33.6
57	α-Picoline	ClO_4	Needles, water	>300 (dec.)	1.5[h]	$C_{18}H_{26}Cl_2N_2O_8$	46.0	46.6	5.6	5.8[k]	..	...
58	β-Picoline	Br	Prisms	109.4–110.4	25	$C_{18}H_{26}Br_2N_2·2H_2O$	46.4	46.4	6.5	6.2	34.3	34.6[l]
59	γ-Picoline	Br	Buff transp. pr.	243.6–244.6 (shr. *ca.* 90)	40	....	...	...	..	..	..	...
60	γ-Picoline	I	Pale yell. pr. and ne.	221.5–223.5 (dark.)	45[d]	$C_{18}H_{26}I_2N_2·2H_2O$	38.6	38.4	5.4	5.3	45.3	45.7
61	Isoquinoline	Br	Lt. gray pr.	252.8–253.8 (↑, dark.)	30	$C_{24}H_{26}Br_2N_2·2H_2O$	53.5	53.7	5.6	5.6	29.7	29.4

[a] Davidson, *Ann.*, 121 254 (1862), and *J. Chem. Soc.*, 14, 162 (1862), reported silky plates from alcohol, analysis but no m. p.; Baer and Prescott, THIS JOURNAL, 18, 989 (1896), reported m. p. 295° (dec.) but no analysis; Schmidt, *Arch. pharm.*, **251**, 201 (1913), reported crystals from alcohol, analysis for ionic Br, m. p. >260°. [b] Davidson, *J. Chem. Soc.*, **14**, 162 (1862), reported a crystalline product from "picoline" but gave no m. p. or analysis; the picoline was undoubtedly a mixture of isomers. [c] Flintermann and Prescott, THIS JOURNAL, **18**, 33 (1896); m. p. 225–226° (partial dec.); anal. for Br and N. [d] Based on the bromide. [e] Also: Calcd. for N, 7.0. Found: N (Dumas), 6.8. [f] Gautier and Renault, *Compt. rend.*, **225**, 682 (1947), reported crystals m. p. 239° (dec.) but gave no analysis; product may have been anhydrous. [g] The analyst reports "Compound difficult to dissolve in water; accuracy of the bromine determination questionable." [h] Based on the alkylene bromide; quaternary bromide not isolated. [i] Reported by v. Braun, *Ber.*, **41**, 2164 (1908), as a yellowish-red crystalline powder, m. p. 200° (sinters); nitrogen analysis given. [j] Also: Calcd. for N, 6.3. Found: 5.8. [k] Also: Calcd. for N, 6.0. Found, 5.7. [l] Determined by Parr bomb method. [m] Colorless unless color given. [n] Purified by recrystallization from alcohol. [o] Purified by recrystallization from alcohol–ether. [p] Attempts by different methods to secure suitable halogen analyses gave variable and discordant results.

NOTE ADDED IN PROOF.—Since this paper was submitted for publication, we have seen the paper by Barlow and Ing, *Brit. J. Pharmacol.*, **3**, 298 (1948). They report three compounds (two of them new) listed in the table; trimethylene bis-quinolinium bromide, m. p. 247° uncor. (from ethanol); pentamethylene bis-quinolinium bromide dihydrate, m. p. 195° uncor. (from ethanol); and decamethylene bis-quinolinium bromide dihydrate, m. p. 113° uncor. (from ethanol–ether).

reactions. Consistent with this view is the fact that only the alkylene dibromides which showed a relatively lower activity in adding to non-hindered bases (ethylene dibromide, glycerol-α,γ-dibromohydrin and 2,5-dibromohexane; compare yields, Table I) took part in the "exceptional" reactions. It is assumed that resistance to forming bis-salts with the hindered bases was too great and the reaction took other courses.

Experimental[4,7]

Intermediates.—The alkylene dibromides and the bases were obtained from commercial sources and used as received; all were good grades except α-picoline, β-picoline, isoquinoline and 3-methylisoquinoline which were "practical" grades. The quinoline was synthetic.

General Procedure for Preparation of Quaternary Bromides.—A mixture of 0.1 mole of the dibromide (except ethylene dibromide) with 0.24 mole of the base and 50 cc. of absolute alcohol was refluxed for six and one-half hours. The crystalline products usually separated out either (a) after cooling to room temperature or below, sometimes only after scratching the sides of the vessel (Table I, Nos. 6, 8, 9, 11, 14, 15, 16, 18, 19, 21, 23, 24, 25, 26, 28, 30, 32, 37, 38, 46, 47; Table II, No. 3) or (b) after adding ether to the reaction mixture to decrease solubility (Table I, Nos. 13, 34, 36, 39, 42, 44, 48, 50, 52; Table II, Nos. 2 and 4). In several cases crystallization was slow, requiring from one to 14 days (Table I, Nos. 19, 21, 26, 32; Table II, No. 3). The crude products were purified by recrystallization from alcohol (Table I, Nos. 6, 8, 9, 13, 14, 15, 16, 18, 21, 23, 24, 25, 37, 38, 47, 50, 52; Table II, Nos. 2 and 3) or absolute alcohol plus absolute ether (Table I, Nos. 11, 19, 26, 28, 30, 32, 34, 36, 39, 42, 44, 46; Table II, No. 4), using Norite where necessary; they were usually but not always colorless. Deviations from this general procedure are detailed below.

General Procedure for Preparation of Perchlorates.—To a concentrated solution of a sample of the bromide in water (No. 7) or alcohol (Nos. 10, 12, 17, 20, 22, 27, 29, 31, 33, 35, 40, 41, 43, 45, 51, 55, 57) was added a 50% excess of a concentrated perchloric acid solution. The white crystalline products usually separated out rapidly, except Nos. 43 and 51 which required several days. The perchlorates were recrystallized from water (Nos. 7 and 57), dilute alcohol (No. 55) or alcohol. All perchlorates gave a negative or negligible test for halogen with silver nitrate solution, and exploded considerably above their m. p.'s when heated slowly on platinum foil.

Preparation of Bis-salts from Ethylene Dibromide.—Ethylene dibromide (0.1 mole) was heated on the steam-bath with 0.24 mole of base. With pyridine, the mixture was solid within twenty minutes. With α-picoline, the mixture was heated for four days. With β- and γ-picoline the mixtures were heated for forty-three hours. On cooling, the mixtures went solid or deposited crystals. With isoquinoline, a vigorous exothermic reaction set in and material was lost. With quinoline and 3-methylisoquinoline, a vigorous exothermic reaction set in at about 150° when heated on a hot plate. The reactions with the last three bases were re-run, modifying the vigor with 50 cc. of absolute alcohol per mole of bromide and refluxing for twenty-three hours. After cooling, the reaction mixtures were worked up in the usual manner except in the case of quinoline.

(4) All m. p.'s are corrected. Determinations were made with the Hershberg apparatus[5] containing Aroclor 1248.[6]

(5) Hershberg, *Ind. Eng. Chem. Anal. Ed.*, **8**, 312 (1936).

(6) Hartwell, *Anal. Chem.*, **20**, 374 (1948).

(7) Analyses were carried out by the Microanalytical Laboratory of the National Institutes of Health (Mr. C. A. Kinser, Mr. W. C. Alford, Mrs. M. M. Ledyard, and Mrs. E. Peake). The halogen of the quaternary halides was determined as ionic halide by precipitation with silver nitrate, except in the two instances noted. The chlorine in the only perchlorate analyzed for halogen (compound 7) was determined by catalytic combustion.

TABLE II

Compound	Base used	X	Appearance	M. p., °C., cor.	Crude yield, %	Empirical formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Bromine (total, ionic) Calcd.	Bromine (total, ionic) Found
						$BrCH_2CH_2—N_+ X^-$						
1[a]	Quinoline	Br	Nearly white pl.	203.0–204.0	41[e]	$C_{11}H_{11}Br_2N$	41.7	41.9	3.5	3.6	50.4, 25.2	49.2, 24.8[b]
						$BrCH_2CH(OH)CH_2—N_+ X^-$						
2	α-Picoline	Br	Pale gray transp. ne.	160.1–160.7	14	$C_9H_{13}Br_2NO$	34.8	34.9	4.2	4.3	51.4, 25.7	51.7, 25.8[c]
3	Quinoline	Br	Transp. pr.	213.0–214.5 (↑, dark.)	40	$C_{12}H_{13}Br_2NO$	41.5	41.7	3.8	3.7	46.0, 23.1	46.2, 23.0
4	3-Methyliso-quinoline	Br	Buff. transp. ne.	185.8–187.8 (↑, dark	14	$C_{13}H_{15}Br_2NO$	43.3	43.5	4.2	4.4	44.3, 22.1	42.8, 22.4[d]

[a] Berend, *Ber.*, 14, 1349 (1881); yellowish needles from alcohol; analyses for C, H and Br, but no m. p. reported. [b] Also: Calcd. for N, 4.4. Found, 4.8. [c] Also: Calcd. for N, 4.5. Found, 4.2. [d] Also: Calcd. for N, 3.9. Found, 3.9. [e] Purified also by recrystallization from alcohol.

Since the quinoline reaction mixture deposited no crystals, it was steam-distilled. The residual red oil was dissolved in alcohol. Crystals slowly appeared until after several days there was a large crop (2.1 g. from 0.09 mole of ethylene dibromide). Repeated crystallization from alcohol gave deep yellow aggregates of small prisms, m. p. 255.2–256.7° (shrinks 140°) dec.

Anal. Found: C, 55.85, 56.05; H, 5.19, 5.25; N (Dumas), 6.04; Br (ionic, catalytic combustion, Parr bomb), 25.35, 25.14, 24.77. For these figures, no reasonable formula could be calculated; the figures correspond to $C_{29.6}H_{33}Br_2N_{2.7}O_3$.

Because ethylene-α,β-bis-quinolinium bromide had been previously reported,[3] an attempt was made to duplicate the earlier conditions as nearly as possible (molecular proportion of dibromide to quinoline of 1:2, no solvent, temperature of 38–43°). From a run of 0.1 mole of ethylene dibromide, a total of 13.0 g. (41% yield) of crystals was obtained in several crops which appeared during several weeks. After crystallization from alcohol (Norite), nearly white small plates were obtained. Analytical values are found in Table II. The product had the analysis and chemical properties of 1-(β-bromoethyl)-quinolinium bromide.

The product from the 3-methylisoquinoline reaction mixture consisted of pink prisms, m. p. 250.0–252.5° (dec.). It proved to be identical by analysis and by mixed m. p. with 3-methylisoquinoline hydrobromide. *Anal.* Calcd. for $C_{10}H_{10}NBr$: C, 53.6; H, 4.5; N, 6.25; Br, 35.7. Found: C, 53.6; H, 4.6; N, 6.3; Br, 35.2. The authentic specimen (which has not hitherto been reported) was prepared by adding the theoretical amount of hydrobromic acid to a solution of the base in alcohol, and recrystallizing the product that separated on cooling; the nearly white needles had a m. p. 257.0–257.6°.

Preparation of Bis-salts from Trimethylene Bromide.—Of these salts, the pyridinium, β-picolinium, and γ-picolinium bromides were hygroscopic. The preparation of the iodides, by metathesis with potassium iodide in aqueous solution, was unsuccessful. The perchlorates were readily prepared in the regular way. The conditions described (above and below) for the formation of the bis-bromides are not by any means to be considered the optimum conditions. In other runs carried out to prepare larger amounts of the β- and γ-picolinium bromides, the reaction mixtures were refluxed eighteen hours instead of six and one-half hours, with resulting yields of 70% in both cases instead of 51% and 45%, respectively.

Preparation of Bis-salts from Tetramethylene Bromide.—The pyridinium bromide melted to a slush at 78°, gave off vapors and solidified at 140–145°, and remelted at 240.4–242.4°; the β-picolinium bromide was very hygroscopic; the γ-picolinium bromide melted to a slush at 98°, gave off vapor at 145–173° (darkened), partly solidified, and remelted at 226.5–229.5°. These three bromides were converted into their perchlorates in the usual way.

Preparation of Bis-salts from Pentamethylene Bromide.—The pyridinium bromide and the three picolinium bromides were very hygroscopic, while the quinolinium bromide, although not hygroscopic, had an indefinite m. p. (softened at 78°, melted at 166–201°). To prepare well-defined derivatives for analysis, the iodides and perchlorates were studied. The pyridinium bromide and the α- and γ-picolinium bromides did not yield corresponding iodides in alcohol solution by metathesis with potassium iodide or hydriodic acid. All the bromides mentioned, however, readily gave perchlorates in the usual manner.

Additional quantities of the pyridinium, α-picolinium and γ-picolinium bromides were prepared in a manner similar to that described, but refluxing for fifteen and one-half hours instead of six and one-half. The yields were 90, 78 and nearly 100%, respectively, instead of 76, 49 and 84%.

Preparation of Bis-salts from Decamethylene Bromide.—The α-picolinium bromide melted over a range of 91–197°; the γ-picolinium bromide was very hygroscopic; and the quinolinium bromide melted over a range of 136–180°, giving off vapor at 144–162°. These bromides were readily converted into the corresponding perchlorates by the general procedure.

No crystalline β-picolinium bromide could be isolated. In order to obtain a β-picolinium salt for analysis and bioassay, the bromide reaction mixture was treated with an excess of ether. The oil that separated was washed by decantation twice with ether and the last traces of solvent finally removed by warming under reduced pressure. The pink oil so obtained (107% of theory for the bromide) yielded a perchlorate by the usual treatment.

The Reaction of Glycerol-α,γ-dibromohydrin with Heterocyclic Bases.—All the reaction mixtures yielded crystalline products except the γ-picoline which yielded an oil. The latter, after washing with ether, was converted into a crystalline perchlorate by the usual procedure. Analysis for carbon, hydrogen and ionic bromine (except, of course, in the case of the γ-picolinium perchlorate where no halogen determination was made) revealed (see Tables I and II) that while the pyridinium, β- and γ-picolinium, and isoquinolinium salts were the expected bis-salts, the α-picolinium, quinolinium, and 3-methylisoquinolinium bromides were different. The latter three bromides had only one-half the expected ionic bromine, although analysis for total halogen proved the presence of another (non-ionic) bromine atom. These results, and the nitrogen–bromine ratios, proved that only one mole of these bases had reacted with one mole of the dibromide.

More vigorous conditions were used in an attempt to force a second mole of base to react with the dibromide. A mixture of the dibromide and 2.4 moles of each of the three bases under consideration was heated on the steam-bath, without solvent, for seven hours. On adding alcohol to the mixtures and cooling, crystalline products were obtained in each case. After purification by recrystallization from alcohol, the products (except from quinoline) gave the correct analytical values for the expected bis-bromides. From the mother liquor of the α-picolinium bis-salt was obtained a yield of 11% of the mono-salt previously described. From the mother liquor of the 3-methylisoquinolinium bis-salt was obtained a small yield of buff prisms with m. p. identical to that of the mono-salt; the mixed m. p., however, showed a large depression. Nothing further was done with the new product. In the case of the quinoline reaction, only the mono-salt was isolated (in 40% yield). A mixture of bromohydrin and 2.4 moles of α-picoline, heated without solvent on the steam-bath for twenty-three hours, gave a 20% yield of bis-salt and no recoverable mono-salt.

Preparation of Bis-salts from 2,5-Dibromohexane.—It was anticipated that the yields of bis-salts with this secondary bromide would be less than with the previously used primary bromides and this was generally realized. At the end of the usual period of refluxing, there was little evidence of reaction as judged by adding ether and chilling, except in the case of 3-methylisoquinoline. With this exception, the reaction mixtures were freed of their volatile solvents by boiling, and heated on the steam-bath without solvent for seventeen hours. The γ-picoline mixture crystallized on cooling. On the addition of alcohol, and with cooling, the isoquinoline mixture crystallized. After adding alcohol and ether, all the other mixtures crystallized except the α-picoline mixture which deposited an oil requiring several months to crystallize; the γ-picoline mixture also yielded most of its product after treatment with alcohol and ether. The 3-methylisoquinoline (and the quinoline) reaction mixture evolved a strong diene odor during the six and one-half hours of refluxing. After cooling and adding ether, nearly white crystals deposited.

The pyridine product was hygroscopic. An attempt to prepare the iodide by metathesis with potassium iodide in aqueous solution failed. The perchlorate was readily prepared in the usual way.

The α-picoline product could not be obtained constant melting, the m. p. sometimes rising and sometimes lowering after recrystallization. The perchlorate was made in the usual manner but using the crude bromide obtained as an oil from the reaction mixture by adding an excess of ether.

The β-picoline and isoquinoline products behaved normally, were not hygroscopic, and could be purified by crystallization from alcohol–ether and alcohol, respectively.

The γ-picoline product was hygroscopic. The iodide was prepared by metathesis with potassium iodide in concentrated aqueous solution; the desired iodide, only, crystallized out.

The quinoline product was obtained, after recrystallization from alcohol–ether, as hygroscopic, colorless, transparent prisms, melting indefinitely under 100°. A perchlorate was prepared in the usual manner; it melted at 130.2–131.0°. This perchlorate was identical, by analysis and mixed m. p., with an authentic sample of quinoline perchlorate prepared similarly from the components. *Anal.* Calcd. for $C_9H_8ClNO_4$: C, 47.1; H, 3.5; N, 6.10. Found: C, 47.2; H, 3.6; N, 5.9. Quinoline perchlorate has not hitherto been characterized in the literature.[8] The original quinoline product was therefore quinoline hydrobromide. From 0.03 mole of dibromide, 5.45 g. (0.026 mole, if anhydrous) of quinoline hydrobromide was obtained; this would represent a yield of 43% if both bromine atoms were removed from the dibromide.

The 3-methylisoquinoline product proved, by analysis and mixed m. p., to be identical with 3-methylisoquinoline hydrobromide. From 0.03 mole of dibromide, 4.54 g. (0.020 mole) of hydrobromide was obtained.

Summary

1. The synthesis and properties of sixty-one new quaternary ammonium salts, prepared in the course of studies in the chemotherapy of cancer, are reported.

2. The salts were formed by adding alkylene dibromides to heterocyclic bases. In most cases, bis-salts were formed. With glycerol-α,γ-bromohydrin and sterically-hindered bases, mono-salts were formed. In several instances, no addition occurred but the alkylene dibromide lost hydrogen bromide which was isolated as the hydrobromide of the base used.

3. The analysis and m. p. of quinoline perchlorate and of 3-methylisoquinoline hydrobromide are reported.

(8) Lundsgaard, English Patent 163,946 (1921) (*Chem. Zentr.*, **92**, IV, 727 (1921), and Cordier, *Monatsh.*, **43**, 525 (1923) (*Chem. Zentr.*, **94**, II, 948 (1923)) mention the compound but give no properties or analyses.

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The Isolation of Blood Group A-Substance from Hog Gastric Mucin by Ethanol Fractionation and Electrodecantation[1]

BY GEORGE HOLZMAN[2] AND CARL NIEMANN

In a previous communication,[3] wherein it was shown that a modification of the ethanol fractionation procedure of Landsteiner and Harte[4] was preferable to other procedures for the concentration of A-substance from commercial (Wilson) hog gastric mucin, it was noted that further concentration could be expected by a process which was called electrodialysis[3,5,6] but which is now recognized as electrodecantation.[7–9] A method involving both ethanol fractionation and electrodecantation has now been devised and the principal features of its application to the fractionation of hog gastric mucin are given in Fig. 1.

The relative activities of the various fractions, assigning the starting material unit activity, were evaluated on the basis of inhibition of hemolysis[3,10] and inhibition of isoagglutination,[11] the latter test being used primarily to disclose

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(3) D. H. Brown, E. L. Bennett, G. Holzman and C. Niemann, *Arch. Biochem.*, **13**, 421 (1947).

(4) K. Landsteiner and R. A. Harte, *J. Expll. Med.*, **71**, 551 (1940).

(5) G. Holzman and C. Niemann, *J. Biol. Chem.*, **174**, 305 (1948).

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(9) P. Stamberger, *J. Colloid Science*, **1**, 93 (1946).

(10) D. H. Brown, E. L. Bennett and C. Niemann, *J. Immunol.*, **56**, 1 (1947).

(11) G. Holzman, E. L. Bennett, D. H. Brown and C. Niemann *Arch. Biochem.*, **11**, 415 (1946).