QUATERNARY AMMONIUM DERIVATIVES OF CHOLESTEROL

A. I. Lopushanskii and V. V. Udovitskaya

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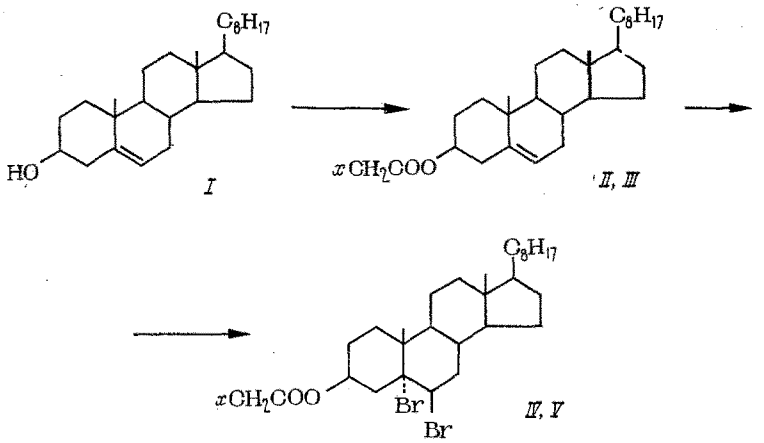
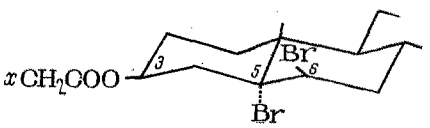
We have found substances with considerable antimicrobial activity among some quaternary ammonium derivatives of cyclohexanol [1]; this activity was heightened by modifying the structure of the substituents in the ring. It became of interest to ascertain how the activity varies for analogous compounds with the condensed polycyclic cholesterol system.

The starting cholesterol chloro- and bromoacetates (II, III) to be used for the synthesis of the quaternary ammonium salts were prepared by reacting the haloacetyl bromides with purified cholesterol [1, 2]. Dibromides (IV and V) were prepared by reacting them with bromine in glacial acetic acid.



The bromination of II and III proceeds considerably more slowly than the bromination of I which is evidently due to the inductive effect of the substituent in the 3β position which causes a reduction in the electron density at the double bond and hinders the electrophilic addition of bromine. The magnitudes of the increments of molecular rotation in the transition from dibromocholesterol to IV and V point to a 5α,6β-trans-diaxial configuration [3]:



The transition to the more stable trans-diequatorial 5β,6α-isomer takes place very slowly in solution because of the large dimensions of the substituents in the 3β position which results in its sterically interacting with the bromine atom on C_5. The configuration of the initial bromination product has also been confirmed by its IR spectra. The broad band at 565 cm^{-1} in the spectrum of IV coincides with the data for axial substitution in some dibromosteroids [4].

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TABLE 1. Cholesterol and Dibromocholesterol Haloacetates

Compound	Name of compound	Yield (in %)	Melting point (in deg)	$[M]_D$	% Halogen found	Empirical formula	% Halogen calculated
II	Δ^5-Cholestenol-3β chloroacetate	67	159-60	−162	7.42	$C_{29}H_{47}ClO_2$	7.65
III	Δ^5-Cholestenol-3β bromoacetate	59	146-8	−147	15.84	$C_{29}H_{47}BrO_2$	15.74
IV	5α,6β-Dibromocholestanol-3β chloroacetate	69	116-7	−280	0.48*	$C_{29}H_{47}Br_2ClO_2$	0.182*
V	5α,6β-Dibromocholestanol-3β-bromoacetate	75	119-21	−286	36.26	$C_{29}H_{47}Br_3O_2$	35.92

*The halogen content for IV is given in equiv. %.

The properties of the freshly prepared haloacetates are given in Table 1. They are white powders, highly soluble in ether, benzene, and chloroform and difficultly soluble in alcohol and acetone.

The monoquaternary ammonium salts VI to XII (Table 2) were obtained by reacting haloacetates II and III with the tertiary amines in benzene. Trimethylamine, triethylamine, dimethylaniline, diethylaniline, Novocaine, pyridine, 4-methylpyridine, and quinoline were used as the amines. The reaction proceeded with the formation of crystalline products in all cases except for diethylaniline. Attempts to obtain the quaternary salt by reacting III with diethylaniline did not yield positive results probably because of the low nucleophilicity of the alkylarylamine or steric hindrances.

Under the same conditions, II to V react with ethylene-, hexa-, hepta-, and decamethylene bis-dimethylamines at both nitrogen atoms forming the bisquaternary ammonium salts XIII to XIX (Table 3). The reaction was carried out with component ratios of 2 : 1. The bisquaternary salts (XIII and XVIII) are formed when chloroacetates II and IV react with ethylene-1,2-bis-dimethylamine at this ratio regardless of the medium (ether or benzene). Bromoacetates (III and V) form the monoquaternary ammonium salts XXI and XXIII (Table 4) under these conditions. The monoquaternary derivatives of ethylene- and hexamethylene-bis-dimethylamine XX and XXII (see Table 4) are formed with a relative increase in the diamine taken for the reaction.

The series of unsymmetrical ammonium salts XXIV to XXXVII (Table 5) was obtained by reacting XXII with a slight excess of the monochloroacetic acid esters with prolonged heating in an alcohol—acetone solution. Compound XX does not form the bisquaternary derivatives under the same conditions.

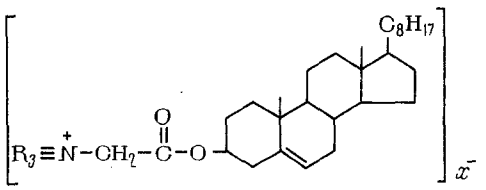
The salts that were synthesized are white crystalline substances soluble in water and alcohol. Their IR spectra in the area where the stretching vibrations of the C=O group occur showed that there is no interaction between the ammonium cation and the carbonyl of the ester group (compare [5]). The absorption band of the carbonyl group in the spectra of haloacetates (II to V) in the same region is shifted by 15 to 20 cm^{-1} toward a higher frequency compared to the corresponding band in the spectrum of cholesterol acetate [6].

According to the data of G. A. Troyan and G. K. Palii, the majority of the compounds described have practically no antimicrobial activity toward golden staphyloccus and Escherichia coli. Only the unsymmetrical bisquaternary ammonium salts (XXIV to XXXIII) were noted to have some bacteriostatic activity which increases with an increase in the alkyl chain R.

EXPERIMENTAL

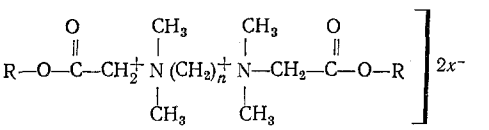
The IR spectra were run as KBr pellets on a UR-10 spectrophotometer equipped with the following prisms in the ranges: 500 to 700 cm^{-1} KBr, 700 to 2000 cm^{-1} NaCl, and 2000 to 3800 cm^{-1} LiF.

TABLE 2. Monoquaternary Salts



Compound	R_3N	X	Melting point (in deg)	Yield (in %)	Found (in %) N	Found (in %) Br or Cl	Empirical formula	Calculated (in %) N	Calculated (in %) Br or Cl
IV	$(CH_3)_3N$	Cl	226—7	63	2,73	6,69	$C_{32}H_{56}ClNO_2$	2,63	6,79
VII	$(C_2H_5)_3N$	Cl	204	81	2,27	6,18	$C_{35}H_{62}ClNO_2$	2,48	6,28
VIII	$C_6H_5N(CH_3)_2$	Br	160—2	36	2,16	12,94	$C_{37}H_{58}BrNO_2$	2,23	12,71
IX	$NH_2C_6H_4COOC_2H_4N(C_2H_5)_2$	Br	182—4	62	3,92	11,03	$C_{42}H_{67}BrN_2O_4$	3,77	10,74
X	C_5H_5N	Br	188—9	78	2,47	13,30	$C_{34}H_{52}BrNO_2$	2,34	13,35
XI	$CH_3C_5H_4N$	Br	198	83	2,43	13,20	$C_{35}H_{54}BrNO_2$	2,29	13,04
XII	C_9H_7N	Br	208	81	2,22	12,58	$C_{38}H_{51}BrNO_2$	2,20	12,53

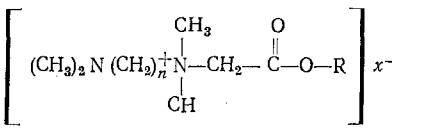
TABLE 3. Bisquaternary Salts



Compound	R	n	X	Melting point (in deg)	Yield (in %)	Found (in %) N	Found (in %) Br or Cl	Empirical formula	Calculated (in %) N	Calculated (in %) Br or Cl
XIII	Δ^5-Cholestenyl-3β	2	Cl	211—2	71	2,70	7,07	$C_{64}H_{110}Cl_2N_2O_4$	2,69	6,80
XIV	The same	6	Cl	217—8	88	2,48	6,82	$C_{68}H_{118}Cl_2N_2O_4$	2,55	2,45
XV	" "	6	Br	226—7	94	2,52	13,70	$C_{68}H_{118}Br_2N_2O_4$	2,36	13,46
XVI	" "	7	Cl	218—20	73	2,80	6,59	$C_{69}H_{120}Cl_2N_2O_4$	2,52	6,37
XVII	" "	10	Cl	210—11	75	2,47	6,25	$C_{72}H_{126}Cl_2N_2O_4$	2,43	6,14
XVIII	5α, 6β-Dibromocholestyl-3β	2	Cl	206—8	32	1,98	0,436[1]	$C_{64}H_{110}Br_4Cl_2N_2O_4$	2,06	0,440*
XIX	The same	6	Cl	200—2	48	2,20	0,426[1]	$C_{68}H_{118}Br_4Cl_2N_2O_4$	1,98	0,423*

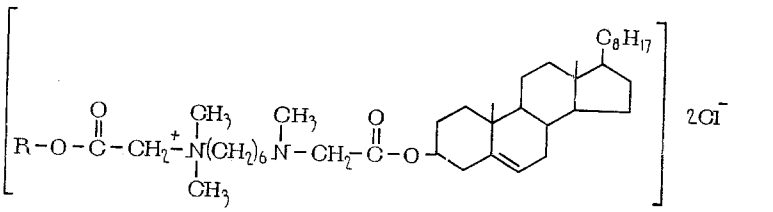
* The halogen content for XVIII and XIX is given in equiv. %.

TABLE 4. Monoquaternary Salts



Compound	R	n	X	Melting point (in deg)	Yield (in %)	Found (in %) N	Found (in %) Br or Cl	Empirical formula	Calculated (in %) N	Calculated (in %) Br or Cl
XX	Δ^5-Cholestenyl-3β	2	Cl	191	92	5,00	6,36	$C_{35}H_{63}ClN_2O_2$	4,84	6,12
XXI	The same	2	Br	219—20	98	4,13	13,02	$C_{35}H_{63}BrN_2O_2$	4,49	12,81
XXII	" "	6	Cl	176—7	86	4,51	5,27	$C_{39}H_{71}ClN_2O_2$	4,41	5,58
XXIII	5α, 6β-Dibromocholestyl-3β	2	Br	226—8	75	3,69	30—81	$C_{35}H_{63}Br_3N_2O_2$	3,58	30,59

TABLE 4. Bisquaternary Salts



Compound	R	Melting point (in deg)	Yield (in %)	Found (in %)		Empirical formula	Calculated (in %)	
				N	Cl		N	Cl
XXIV	CH_3	195	54	3,97	9,58	$C_{42}H_{76}Cl_2N_2O_4$	3,77	9,53
XXV	C_2H_5	203—4	61	3,45	9,18	$C_{43}H_{78}Cl_2N_2O_4$	3,70	9,35
XXVI	n-C_3H_7	191—2	59	3,70	8,86	$C_{44}H_{80}Cl_2N_2O_4$	3,63	9,18
XXVII	iso-C_3H_7	194—5	63	3,40	9,35	$C_{44}H_{80}Cl_2N_2O_4$	3,63	9,18
XXVIII	n-C_4H_9	180—1	56	3,53	9,20	$C_{45}H_{82}Cl_2N_2O_4$	3,56	9,02
XXIX	iso-C_4H_9	185	64	3,41	9,00	$C_{45}H_{82}Cl_2N_2O_4$	3,56	9,02
XXX	n-C_5H_{11}	185—6	69	3,57	8,68	$C_{46}H_{84}Cl_2N_2O_4$	3,50	8,86
XXXI	iso-C_5H_{11}	192—4	63	3,30	9,15	$C_{46}H_{84}Cl_2N_2O_4$	3,50	8,86
XXXII	C_7H_{13}	191—3	42	3,66	8,62	$C_{48}H_{88}Cl_2N_2O_4$	3,38	8,56
XXXIII	C_8H_{17}	189	55	3,25	8,60	$C_{49}H_{90}Cl_2N_2O_4$	3,33	8,42
XXXIV	C_9H_{19}	187	57	3,10	8,67	$C_{50}H_{92}Cl_2N_2O_4$	3,27	8,28
XXXV	$C_{10}H_{21}$	183	52	3,14	8,42	$C_{51}H_{94}Cl_2N_2O_4$	3,22	8,15
XXXVI	$C_6H_5CH_2$	194—6	54	3,34	8,69	$C_{18}H_{80}Cl_2N_2O_4$	3,41	8,65
XXXVII	$CH_3C_6H_6C_3H_7$	178—9	53	3,47	8,31	$C_{51}H_{92}Cl_2N_2O_4$	3,23	8,17

The ditertiary amines based on 1,2-ethylene-, 1,6-hexamethylene-, 1,7-heptamethylene-, and 1,10-decamethylenediamines were prepared by the Eschweiler-Clarke reaction [7] in which the diamines listed above are reacted with formaldehyde and formic acid.

The alkylhaloacetates were prepared by Conrad's method [8], which was proposed to synthesize ethylchloroacetate.

Δ^5-Cholestenol-3β chloroacetate (II). This substance was prepared by reacting 0.25 mole of monochloroacetyl bromide with 0.2 mole of cholesterol in 150 ml of chloroform (or benzene). The mixture was boiled for 4 h, cooled, and treated with a saturated sodium carbonate solution; 100 ml of ether was added and the ether solution was first washed with water, then with a saturated sodium chloride solution, and evaporated in air. The impure product was chromatographed on aluminum oxide columns capable of handling 0.5 to 1 g of substance per 1 cm^2 of column cross section. Gradient elution was used: carbon tetrachloride–benzene—ethyl ether.

5α,6β-Dibromocholestanol-3β chloroacetate (IV). To 0.05 mole of II in 400 ml of ether was gradually added a solution of 10 g of bromine in 100 ml of glacial acetic acid. Needle-like crystals appeared in the solution after a lapse of 15 to 20 min with constant stirring. A finely crystalline product, which was recrystallized from a boiling acetone solution, was isolated after 24 h from the solution which was cooled to 0°C.

Mono- and bisquaternary ammonium salts. These were prepared by reacting tertiary and ditertiary amines with the cholesterol and dibromocholesterol haloacetates in absolute benzene with heating for $\frac{1}{2}$ to 2 h. They were recrystallized from an alcohol—ether solution.

Ethylene-1-dimethylamino-2-dimethylcarbo-Δ^5-cholestenoxymethylammonium monochloride (XX). To 0.004 mole of ethylene-1,2-bis-dimethylamine was added in small portions 0.001 mole of II as a 10% solution in benzene. The precipitate which formed was filtered off, washed with a large amount of ether, and recrystallized from hot acetone.

The synthesis of the unsymmetric bisquaternary derivatives of hexamethylenediamine was accomplished by reacting 0.002 mole of XXII with 0.003 mole of the appropriate alkylchloroacetate in 10 ml of an absolute alcohol—acetone (2 : 1) mixture. The reaction mixture was heated for 12 to 18 h after which the bisquaternary salt was precipitated with acetone.

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