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QUATERNARY AMMONIUM SALTS

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5 Claims. (Cl. 260—397)

This invention relates to a series of quaternary ammonium compounds which contain as one of the radicals attached to the nitrogen atom a cholanyl or a mono-hydroxy or poly-hydroxy cholanyl group. These groups may be defined as being derived from synthetic or naturally occurring unconjugated bile acids (such as cholic, desoxycholic, hyodesoxycholic, lithocholic, apocholic, or cholanic acids) by the elimination of the carboxyl group therefrom, the point of attachment of these groups to the nitrogen atom of the quaternary ammonium salts being that at which the carboxyl group had been attached in the acid from which the group had been derived.

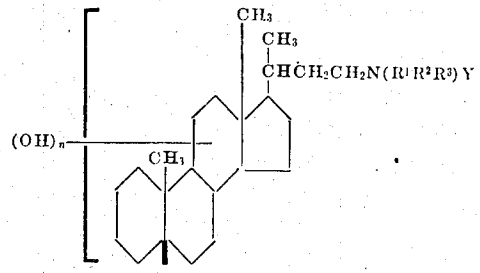
Quaternary ammonium compounds, as such, have long been known to organic chemists and many types of quaternary ammonium compounds derived from the common aliphatic and aromatic amines, and from the heterocyclic nitrogen bases, have been described. However, so far as we are aware, no quaternary ammonium salt has yet been prepared or described which contains any of the biologically significant cholanyl groups referred to and defined above. We have now found that such derivatives can be prepared containing the above-defined groups; we have further prepared a representative series of such compounds and have shown them to be useful in the arts, particularly as therapeutic agents and as agents for the lowering of the surface tension of water or of aqueous solutions. Since these compounds are all more or less soluble in water or dilute acid solutions, they also present a convenient manner of obtaining these groups in neutral or slightly acid aqueous solution, in contrast to the cholanic or cholic acids themselves, containing the same groups, which are water soluble only in strongly alkaline solutions.

We have discovered that all of the herein described series of compounds are antiseptics, some of them being of very great antiseptic efficiency. In the treatment of infectious diseases of the biliary tract, a need has been felt for an efficient antiseptic preparation which would be secreted or concentrated in this tract after administration by any conventional route. Methenamine (hexamethylenetetramine) is sometimes used as a biliary antiseptic but, in view of the fact that it is known to be an active antiseptic only in definitely acid media, there is some doubt whether it is truly effective in the biliary tract. Because of their close chemical structural relationship to the bile acids, which are normal constituents of bile, the highly antiseptic preparations of the series herein described are indicated in the treatment of such conditions.

A series of recent patents and other publications has shown that certain limited classes of quaternary ammonium salts exhibit a high degree of antiseptic potency. It has thus been established, for instance, that the simple quaternary ammonium salts must have an alkyl group containing at least six carbon atoms; that maximum efficacy is exhibited by those compounds which contain a carbon chain of sixteen atoms, there being a marked decrease in efficacy when the carbon chain is lengthened to eighteen carbon atoms or more; that the long carbon chain may be interrupted by an oxygen or sulfur atom at some point in the chain; that the nitrogen atom of the quaternary ammonium salt may be part of a heterocyclic nucleus provided that a relatively long carbon chain is also attached to that nitrogen atom. So far as we are aware, there is no published indication that a highly antiseptic preparation may be expected when, for the long carbon chain or alkyl group which is a prerequisite for antiseptic activity according to the teachings of the prior art, there is substituted a large, complex, carbocyclic group such as is found in the cholanyl and hydroxy cholanyl groups of the present invention. Though certain of the compounds contemplated within this invention do contain such relatively long alkyl groups as have been shown by the prior art to be effective in promoting antiseptic efficiency, we desire to point out that such long alkyl groups are in no way essential to the utility of the compounds of this invention, as antiseptics or otherwise, inasmuch as the same utility is shown by those compounds herein described which do not contain such long alkyl groups.

The compounds of the present invention are further useful in the lowering of the surface tension of water. This effect, though a property of all of the compounds herein described, is particularly marked in those in which there is either no hydroxyl group, or a single hydroxyl group substituted upon the cholanyl radical. It is made manifest in the property of these compounds to produce a persistent and stable foam, and in their ability to stabilize emulsions and dispersions of water-insoluble solids or liquids.

The quaternary ammonium salts contemplated within this invention may all be represented by the formula.



wherein n represents an integer from zero through three, (the hydroxyl groups being locat-

ed on the ring system in the same position in which they are located in the bile acids from which these quaternary ammonium salts have been derived), and wherein R^1 and R^2 are unsubstituted alkyl groups containing not more than three carbon atoms, and wherein R^3 is a benzyl group or an unsubstituted alkyl group containing not more than eighteen carbon atoms, and wherein Y is the anion of an inorganic acid. In the case of the quaternary ammonium salts derived from apocholic acid there is somewhere in the ring system a double bond, concerning the exact location of which there is some disagreement among chemists. We have found that these compounds are best prepared from the corresponding primary amino derivatives of the cholanyl or substituted cholanyl group. These primary amino derivatives may in turn be prepared by application of the methods described by Caldwell (Journal of the American Chemical Society, vol 60, page 991) to the appropriate cholanic or hydroxycholanic acid ester. The following specific examples will illustrate the methods of preparing the new quaternary ammonium salts contemplated within this invention.

Example 1.—A convenient quantity of cholanamine (prepared by application of the procedures described by Caldwell to the methyl ester of cholanic acid) is boiled with approximately 2½ equivalents of formaldehyde and an excess of formic acid until evolution of carbon dioxide has ceased. The solution is diluted somewhat with water, made alkaline with caustic soda, and the insoluble cholanyl dimethyl amine filtered, washed with water and dried. This is dissolved in absolute ethyl acetate, and, after filtering to remove any unreacted primary amine, is treated with about 1½ molecular equivalents of methyl iodide and allowed to stand at room temperature for about 24 hours. The insoluble quaternary ammonium salt is filtered and dried. The cholanyl trimethyl ammonium iodide thus obtained is sparingly soluble in water, forming a solution of approximately neutral reaction.

Example 2.—The substitution of equivalent amounts of methyl chloride or methyl bromide for the methyl iodide in Example 1, and allowing the resulting reaction mixture to stand at 55° centigrade for 24 hours instead of at room temperature, results in the formation of the corresponding chloride or bromide salt. The properties of these compounds are very similar to that described in Example 1.

Example 3.—A convenient quantity of cholanamine is suspended in a warm dilute solution of sodium hydroxide and treated with about three molecular equivalents of diethyl sulfate to form cholanyl triethyl ammonium ethylsulfate. The mixture is neutralized, evaporated to dryness, and the product separated from inorganic salts by extraction with alcohol.

Example 4.—The substitution of an equivalent amount of di-n-propyl sulfate (Organic Syntheses, vol. 19, page 28) for the diethyl sulfate in Example 3 results in the formation of cholanyl tripropyl ammonium propylsulfate as an end product.

Example 5.—A convenient quantity of lithocholamine (from the methyl ester of lithocholic acid by the reactions of Caldwell) is di-methylated by the use of formaldehyde and formic acid as described in Example 1. The lithocholyl dimethyl amine thus obtained is treated in ethyl acetate solution with a small excess of benzyl chloride at 55° centigrade for at least 24 hours, and the insoluble quaternary ammonium salt filtered. This product is lithocholyl benzyl dimethyl ammonium chloride.

Example 6.—In place of the lithocholamine of Example 5, desoxycholamine or hyodesoxycholamine or apocholamine (prepared from the methyl esters of desoxycholic, hyodesoxycholic, or of apocholic acids, respectively, by the reactions of Caldwell) is used as a starting material. These result in the ultimate formation of desoxycholyl, hyodesoxycholyl, and apocholyl benzyl dimethyl ammonium chlorides, respectively.

Example 7.—A convenient quantity of cholamine (prepared from the methyl ester of cholic acid by the method of Caldwell) is di-methylated by the use of formaldehyde and formic acid as described in Example 1. The cholyl dimethyl amine thus obtained is treated in absolute ethyl acetate solution with about 1½ equivalents of lauryl bromide at 55° centigrade for about two weeks. The insoluble quaternary salt—cholyl dimethyl lauryl ammonium bromide—is filtered, powdered and dried.

Example 8.—In place of lauryl bromide in Example 7, other long chain alkyl halides may be used. Hexyl bromide, thus, will yield cholyl dimethyl hexyl ammonium bromide; cetyl bromide will yield cholyl cetyl dimethyl ammonium bromide. These long chain halides need not be chemically pure entities, but may be mixtures of halides such as may be readily prepared from natural oils by well-known methods.

The above examples teach the preparation of the quaternary ammonium chlorides, bromides and iodides. Other salts with inorganic acids may be prepared by utilizing the conventional reactions of inorganic chemistry. For instance, the sulfate or nitrate may be prepared from the halide by treatment of an aqueous solution of the halide with an exactly equivalent quantity of silver nitrate or sulfate in aqueous solution. The precipitate of silver halide is filtered off, leaving the quaternary ammonium sulfate or nitrate in solution. Or, as a more general procedure, the quaternary ammonium hydroxide may be prepared by any of the conventional methods of organic chemistry, and the salt with any desired acid formed from that hydroxide by neutralization with that acid. These techniques may be applied to any of the above examples or to any of the other combinations referred to in the following paragraph.

The above examples are in no way intended to limit this invention, but are merely for the purpose of demonstrating how these compounds may be prepared. By such minor variations of conditions as are illustrated in these examples, quaternary ammonium salts containing the cholanyl or hydroxycholanyl groups described in the first paragraph of this specification together with a great variety of different combinations of alkyl groups have been and may be prepared, so that the invention is to be limited only by the scope of the herein-appended claims.

The quaternary ammonium salts described herein are all soluble in water to a greater or less degree. Those containing a non-hydroxylated cholanyl radical are but sparingly soluble in water. In view of their great power as dispersing agents, it is difficult to determine what is dissolved and what suspended, rendering an exact determination of solubility impractical.

Quaternary ammonium salts containing a mono-hydroxylated cholanyl group are but slightly soluble in water, but are definitely more

so than those containing the non-hydroxylated radical. Quaternary ammonium salts containing a di-hydroxylated cholanyl radical are moderately soluble in water, while those containing the tri-hydroxylated cholanyl radical are very readily soluble in water. The less soluble compounds of this invention are relatively tasteless, while the more soluble ones have an intense bitter taste. These quaternary ammonium salts are also soluble in ethyl alcohol to a greater or less degree, depending upon the exact constitution of the compound at hand. The solubility of those which are more difficultly soluble in water may generally be improved by the use of some ethyl alcohol in the solution, or by previously dissolving the dry salt in 95% ethyl alcohol and adding this solution to the water.

Testing according to standard techniques has shown all the salts of the series herein described to be bactericidal, some of them to a very high degree. The following table sets forth the results of some representative determinations, the figures quoted being the greatest dilution which will kill the organism under standard test conditions.

Compound	*Staphylococcus aureus*	*Escherichia coli*
Cholanyl trimethyl ammonium chloride	1:47000	1:23400
Lithocholyl trimethyl ammonium chloride	1:55200	1:5800
Desoxycholyl trimethyl ammonium chloride		1:900
Hyodesoxycholyl trimethyl ammonium chloride.	1:4450	
Cholyl trimethyl ammonium chloride	1:4600	
Apocholyl trimethyl ammonium chloride	1:6300	
Cholanyl benzyl dimethyl ammonium chloride	1:85300	1:24700
Lithocholyl benzyl dimethyl ammonium chloride.	1:85100	1:10200
Cholyl benzyl dimethyl ammonium chloride	1:8300	
Cholyl lauryl dimethyl ammonium bromide	1:112000	1:24500
Cholyl cetyl dimethyl ammonium bromide	1:18400	1:16100

With respect to bactericidal efficiency, we have found that change in the anion of the inorganic acid portion of these molecules results in relatively little effect on the properties of the compounds.

We claim:

1. Quaternary ammonium salts whose composition is expressed by the formula

$$(Chol)N(R^1R^2R^3)Y$$

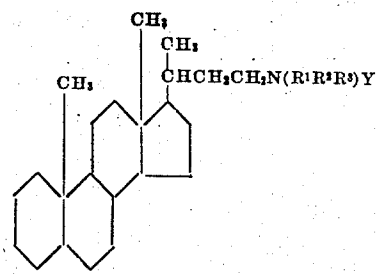
wherein "Chol" stands for the radical obtained by the elimination of the carboxyl group from an unconjugated bile acid; R^1 and R^2 are unsubstituted alkyl groups containing not more than three carbon atoms; R^3 is chosen from the group consisting of the benzyl group and unsubstituted alkyl groups containing not more than eighteen carbon atoms; and Y is the anion of an inorganic acid.

2. Quaternary ammonium salts whose composition is expressed by the formula

$$(Chol)N(R^1R^2R^3)Y$$

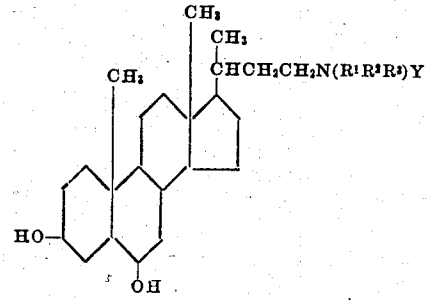
wherein "Chol" stands for the radical obtained by the elimination of the carboxyl group from one of the following unconjugated bile acids: cholanic, lithocholic, apocholic, desoxycholic, hyodesoxycholic, and cholic acids; and wherein R^1 and R^2 are unsubstituted alkyl groups containing not more than three carbon atoms; R^3 is chosen from the group consisting of the benzyl group and unsubstituted alkyl groups containing not more than eighteen carbon atoms; and Y is the anion of an inorganic acid.

3. Quaternary ammonium salts whose composition is expressed by the formula



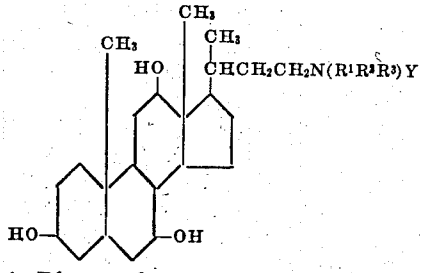
wherein R^1 and R^2 are unsubstituted alkyl groups containing not more than three carbon atoms; R^3 is chosen from the group consisting of the benzyl group and unsubstituted alkyl groups containing not more than eighteen carbon atoms; and Y is the anion of an inorganic acid.

4. Quaternary ammonium salts whose composition is expressed by the formula



wherein R^1 and R^2 are unsubstituted alkyl groups containing not more than three carbon atoms; R^3 is chosen from the group consisting of the benzyl group and unsubstituted alkyl groups containing not more than eighteen carbon atoms; and Y is the anion of an inorganic acid.

5. Quaternary ammonium salts whose composition is expressed by the formula



wherein R^1 and R^2 are unsubstituted alkyl groups containing not more than three carbon atoms; R^3 is chosen from the group consisting of the benzyl group and unsubstituted alkyl groups containing not more than eighteen carbon atoms; and Y is the anion of an inorganic acid.

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