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QUATERNARY AMMONIUM SALTS OF METHENAMINE

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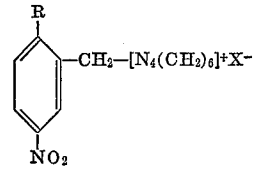
ABSTRACT OF THE DISCLOSURE

Quaternary ammonium salts of the formula

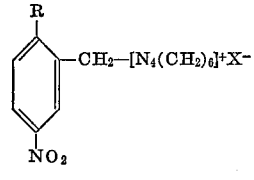


wherein R is hydroxy or lower alkoxy, and X^- is the anion of a physiologically tolerated acid at least as strong as phosphoric acid have been found to have antimicrobial effects on many types of pathogenic microorganisms which cause infections of the intestinal and urinary tract.

This invention relates to compounds and compositions effective in controlling pathogenic microorganisms, and particularly to derivatives of methenamine.

The preferred known chemotherapeutic agents against many infections of the intestinal tract are 5-chloro-7-iodo-8-quinolinol and sulfaguanidine. The first mentioned compound is not well tolerated by persons allergic to iodine. The latter is relatively weak in its antimicrobial effects. Methenamine, mandelic acid, and the salt of methenamine with mandelic acid have been used as disinfectants for the urinary tract.

It has now been found that quaternary ammonium salts of the formula



in which R is lower alkoxy, preferably methoxy, or hydroxy, and X^- is the anion of an acid at least as strong as phosphoric acid, have much stronger bacteriostatic effects in vitro against a wide variety of microorganisms including those which are not readily controlled by tolerable doses of the known chemotherapeutic agents, and that the quaternary ammonium salts are relatively nontoxic.

Limited tests on human patients indicate that the compounds of the invention are useful in combating intestinal infections and are well tolerated in effective amounts.

The results of comparison tests between typical compounds of this invention and known chemotherapeutic agents are tabulated in Table 1.

TABLE 1.—DIAMETER OF INHIBITED GROWTH ZONE, MM.

Microorganism	Compound A	B	C	D
Escherichia coli	12	9	14	11
Klebsiella	28	22	9	11
Pseudomonas aeruginosa	13	16	9	11
Proteus mirabilis	25	22	9	10
Proteus vulgaris	22	11	9	11
Salmonella typhi Ty 2	23	22	9	9
Salm. paratyphi:				
A	21	13	9	12
B	17	24	9	9
C	25	24	9	9
Salmonella typhi murium	21	14	9	9
Shigella No. 108	30	25	15	11
Shigella shigae No. 401	34	33	15	10
Vibrio cholerae El Tor No. 1418	30	28	18	11
Vibrio cholerae 569 B	40	35	17	14
Staphylococcus aureus	26	16	15	10
Staphylococcus wood 46	24	22	12	9

NOTE.—The tested compounds are identified in the table by capital letters as follows: A=Methenamine 2-,ethoxy-5-nitrobenzyl chloride; B=Methenamine 2-hydroxy-5-nitrobenzyl chloride; C=5-chloro-7-iodo-8-quinolinol; D=Sulfaguanidine.

In testing the compounds, discs of filter paper having a diameter of 9 millimeters were sterilized in an autoclave and placed on cultures of the microorganisms on agar plates. 10% solutions or suspensions of each tested compound containing 2.5 mg. of the compound were applied to the discs, and the cultures carrying the discs were incubated at 37° C. Table 1 lists the diameters of the zones in which the compounds were effective in inhibiting microbial growth. A diameter of 9 mm. indicates no practical bacteriostatic action, and a large diameter value is indicative of high bacteriostatic potency.

Additional comparison tests were made with methenamine and Compounds A and B of this invention. In these tests, solid nutrient mixtures (blood plates) containing 25, 50, and 100 mg./100 ml. of Compounds A and B, and 100 mg./100 ml. methenamine (Compound E) were inoculated with the listed microorganisms, and the growth of the cultures after incubation at 37° C. was evaluated qualitatively. The symbols employed have the following meaning in Table 2:

— No growth of the inoculum
(±) Very little growth
± Weak growth
+ Normal growth

All microorganisms showed good, normal growth under the test conditions on control plates not containing Compounds A, B, or E.

Compounds A and B of this invention are shown in the tables to be clearly superior to the known Compounds C, D, and E in their effect in vitro against a wide spectrum of pathogenic microorganisms while having relatively little effect on *E. coli*, the principal microorganism in a normal intestinal flora.

TABLE 2.—BACTERIOSTATIC EFFECT

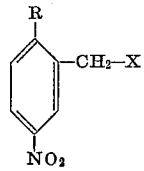
Microorganism	Compound B, mg./dl. 25	50	100	Compound A, mg./dl. 25	50	100	Compound E
Escherichia coli	±	(±)	—	(±)	(±)	—	+
Klebsiella	±	------	—	(±)	—	—	—
Pseud.aeruginosa	(±)	—	—	(±)	—	—	+
Salm.typhi Ty 2/728	(±)	—	—	—	—	—	—
S.paratyphi A 105	—	—	—	—	—	—	(±)
S.paratyphi B 223	—	—	—	—	—	—	—
S.paratyphi C 502	—	—	—	—	—	—	—
S.typhi murium	(±)	—	—	±	—	—	±
Salm.enteritidis	±	—	—	±	—	—	±
Shigella No 108	—	—	—	—	—	—	—
Sh.shigae No 401	—	—	—	—	—	—	—
Sh.shigae No 406	—	—	—	—	—	—	------
Sh.flexneri 102 Typ. IB	—	—	—	—	—	—	(±)
Staphyloc. aureus	±	—	—	±	—	—	±
Staphyloc. wood 46	—	—	—	(±)	—	—	—
Vibrio cholera 1418	—	—	—	—	—	—	—
Vibrio cholera 38383	—	—	—	—	—	—	—
Vibrio cholera 34580	—	—	—	—	—	—	—
(*Neiss.gonorrhoe*)	(±)	—	—	—	—	—	(±)

The compounds of the invention are practically nontoxic. The median lethal dosis DL_{50} in oral application to mice is above 8000 mg. per kg. It could not be determined with any precision since dosage rates higher than 8000 mg./kg. cannot be applied. No unusual side effects were observed with the large doses that were given. The value of DL_{50} in mice for 5-chloro-7-iodo-8-quinolinol (Compound C) is 7200 mg./kg. under the same conditions under which Compounds A and B were tested.

When ten rats were fed daily doses of 100 mg./kg. of Compounds A and B for thirty days by means of an esophagus tube in addition to their normal food and water, they did not show any noticeable differences in behavior as compared to a control group of ten rats not receiving the compounds of the invention. The weight changes in both groups and the results of blood tests performed three times on each animal during the tests were normal and no significant differences could be found between the two groups. All rats were killed at the end of the test period, and their vital organs were subjected to histological examination. No pathological changes were found in the livers, lungs, spleens, thymus, kidneys, ovaries, testes, or blood vessels of the rats that had received the compounds of the invention.

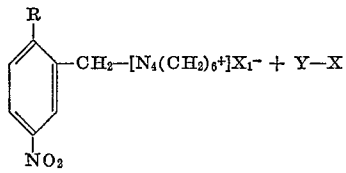
Limited clinical data available at this time indicate that a dosage of 1.5 to 2 g. of the compounds of the invention is effective in adult patients suffering from enteritis and enterocolitis. No side effects have been observed when the active agents of the invention were applied in dosage units of 250 mg. in the form of dragées. Two dragées given three to four times a day caused rapid relief of symptoms, such as diarrhea, in most cases.

The compounds of the invention are prepared by reacting methenamine with an equimolecular amount of a 2-hydroxy- or 2-alkoxy-5-nitrobenzyl ester of the formula

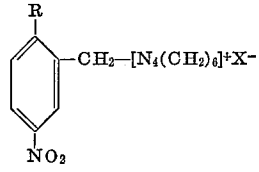


wherein R is hydroxy or lower alkoxy, and X is the anionic radical of a physiologically tolerated acid, at least as strong as phosphoric acid. The 2-hydroxy- and 2-methoxy-5-nitrobenzyl halides are most readily available and are preferred. The reaction occurs readily in a liquid medium, such as an inert solvent, at any temperature at which the reaction mixture is liquid, a reaction temperature of 20° to 50° C. being most convenient.

The several compounds of the invention can also be converted into each other by a double reaction of the type.



wherein R is hydroxy or lower alkoxy, X_1^- and X^- are anions of an acid at least as strong as phosphoric acid, and Y is a cation whose salt with X_1 is much less soluble than the desired, antimicrobial compound



in any convenient solvent. The starting materials are mixed in stoichiometrically equivalent amounts, and the reaction products are then separated by filtration, decantation, centrifuging, or the like. The reaction is rapid in solvent in which the reactants ionize, but is not limited to such solvents.

The following examples further illustrate the manner of making and using the compounds of the invention.

EXAMPLE 1

9.38 g. 2-hydroxy-5-nitrobenzyl chloride (0.05 mole) of high purity were dissolved in 250 ml. chloroform, and the solution was added in a thin stream with stirring to a solution of 7.01 g. pure methenamine (0.05 mole) in 120 ml. chloroform. A slightly exothermic reaction started at once, and yellow methenamine 2-hydroxy-5-nitrobenzyl chloride started precipitating at once. The reaction mixture was further stirred for 18 hours at ambient temperature whereupon the precipitate was filtered off with suction and dried in a vacuum at room temperature. It weighed 16.4 g. (100% yield) and melted with decomposition at 195° C.

It was identified by elementary microanalysis and by its equivalent weight as $C_{13}H_{18}ClN_5O_3$.

Calculated (percent): C, 47.65; N, 21.38; Cl, 10.82. Found (percent): C, 47.44; N, 21.13; Cl, 10.55.

The equivalent weight, as determined with $HClO_4$ in glacial acetic acid in the presence of mercury acetate was 329.8 as compared to a calculated value of 327.69.

Methenamine 2-hydroxy-5-nitrobenzyl chloride is only sparingly soluble in cold water and practically insoluble in the usual organic solvents. Methanol or ethanol may therefore be used instead of chloroform in the aforedescribed procedure.

EXAMPLE 2

70 g. 2-methoxy-5-nitrobenzyl chloride (0.348 mole) of high purity were dissolved in 400 ml. chloroform, and 48.7 g. pure methenamine (0.348 mole) were gradually added with stirring and dissolved almost completely. Methenamine 2-methoxy-5-nitrobenzyl chloride started precipitating after about two hours at ambient temperature, and stirring was continued for three days.

The precipitate was then recovered by suction filtration and dried in a vacuum. It weighed 102.7 g. (86.3% yield) and melted with decomposition at 184° C. The compound was identified as $C_{14}H_{20}ClN_5O_3$ by elementary microanalysis and by its equivalent weight which was determined as in Example 1.

Calculated: Eq. wt. 341.8; C, 49.19%; N, 20.49%; Cl, 10.37%. Found: Eq. wt. 339.8; C, 49.10%; N, 20.23%; Cl, 10.24%.

The compound dissolves very readily in water and methanol, is soluble in glacial acetic acid, slightly soluble in ethanol, and only sparingly soluble in other common organic solvents.

EXAMPLE 3

Methenamine 2-methoxy-5-nitrobenzyl bromide was obtained in the same manner as in Examples 1 and 2 by reacting 8.55 g. 2-methoxy-5-nitrobenzyl bromide with 4.87 g. methenamine in 40 ml. chloroform. The compound decomposes and melts at about 200° C. Similarly, 2-hydroxy-5-nitrobenzyl bromide yielded methenamine 2-hydroxy-5-nitrobenzyl bromide.

EXAMPLE 4

5 g. methenamine 2-methoxy-5-nitrobenzyl chloride (0.0146 mole) were reacted in 30 ml. water with 2.3 g. powdered silver sulfate (0.0073 mole). The mixture was shaken at ambient temperature for two hours. The silver chloride formed was removed by filtration, and the filtrate was evaporated to dryness in a vacuum at ambient temperature.

The residue consisted of 4.48 g. methenamine 2-methoxy-5-nitrobenzyl sulfate (86% yield) having a melting point of 90°–91° C. The compound was identified by elementary microanalysis:

Calculated for $C_{28}H_{40}N_{10}O_{10}S$ (percent): C, 47.45; N, 19.75; S, 4.53. Found (percent): C, 47.62; N, 19.49; S, 4.6.

It dissolves readily in water, methanol, and warm ethanol, but is only sparingly soluble in chloroform and low-boiling petroleum hydrocarbons.

EXAMPLE 5

10 g. methenamine 2-methoxy-5-nitrobenzyl chloride (0.0292 mole) and 5 g. silver nitrate (0.0292 mole) were stirred in 50 ml. water and 8.5 g. methenamine 2-methoxy-5-nitrobenzyl nitrate were recovered from the reaction mixture as described in Example 4.

The nitrate sinters at 167° C. and melts at 168°–168.5° C. It is readily soluble in water, but insoluble or only sparingly soluble in common organic solvents. It was identified by elementary microanalysis:

Calculated for $C_{14}H_{20}N_6O_6$ (percent): C, 45.65; N, 22.82. Found (percent): C, 45.51; N, 22.56.

Salts of methenamine 2-hydroxy-5-nitrobenzyl hydroxide and of its lower alkyl ethers with all strong acids are readily prepared from methenamine and the corresponding 2-hydroxy-5-nitrobenzyl esters or 2-lower-alkoxy-5-nitrobenzyl esters, as illustrated in Examples 1 to 3 or by double reaction of salts as illustrated in Examples 4 and 5. The salts with acids weaker than phosphoric acid are not stable enough and have not been isolated successfully.

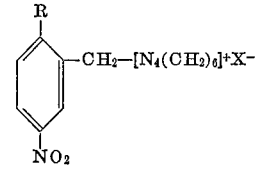
The quaternary ammonium salts of the invention are compounded with suitable carriers, excipients, and compatible other physiologically active agents in the usual manner to convert them to dosage units, as illustrated in the following example.

EXAMPLE 6

2.5 kg. methenamine 2-methoxy-5-nitrobenzyl chloride were granulated with starch and gelatine as an inert excipient in the usual manner, and 100,000 tablets were formed from the granulate on a press. The tablets were coated with sugar syrup and further with a conventional enteric coating to prevent dissolution in the gastric fluid.

What is claimed is:

1. A compound of the formula



wherein R is hydroxy or lower alkoxy, and X^- is the anion of a physiologically tolerated acid at least as strong as phosphoric acid.

2. A compound as set forth in claim 1, wherein R is hydrogen and X is chlorine.

3. A compound as set forth in claim 1, wherein R is methyl and X is chlorine.

References Cited

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JOHN M. FORD, Primary Examiner

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