PATENT SPECIFICATION

NO DRAWINGS

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901,643

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International Classification:—C07d. C09b.

COMPLETE SPECIFICATION

Phenanthridinium Salts and their preparation

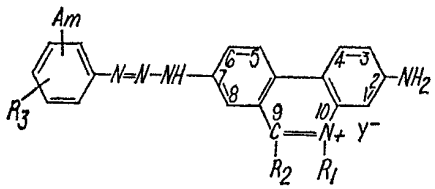
We, MAY & BAKER LIMITED, a British Company of Dagenham, Essex, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is for improvements in or relating to phenanthridinium salts and to processes for their production, and has for its object the provision of new and therapeutically useful substances.

While many phenanthridinium salts have heretofore been proposed for use as trypanocidal agents, only a few have been used to any substantial extent in the field. Not only degree of activity but also toxicity vary markedly with change in the number and nature of substituents and it is impossible at the present time to predict *a priori* the therapeutic properties (if any) of any new phenanthridine compound.

As the result of research and experimentation, the present Applicants have prepared new phenanthridinium salts which have a high activity against blood parasites, such as trypanosomes, and are surprisingly less toxic than known phenanthridinium salts possessing useful trypanocidal activity. In consequence, they exhibit a relatively high chemotherapeutic index.

The new compounds of the present invention are the amidinophenyldiazoaminophenanthridinium salts represented by the general formula:

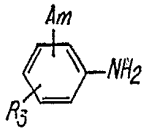


I

(wherein Am represents the amidino grouping $-C\langle^{NH}_{NH_2}$, R_1 represents a lower alkyl group, preferably an ethyl or methyl group, R_2 represents an aryl (preferably phenyl group, R_3 represents a lower alkoxy group or a halogen atom and Y represents a pharmaceutically acceptable anion, for example a chloride or bromide ion) including their acid addition salts, Insoluble salts, useful for certain applications, include those of amsonic acid (4,4¹-diaminostilbene-2,2¹-disulphonic acid), embonic acid (2,2¹ - dihydroxy - 1,1¹ - dinaphthylmethane-3,3¹ - dicarboxylic acid) and of suramin (symmetrical urea of *m* - aminobenzoyl - *p*-methyl - *m* - aminobenzoyl - 1 - aminonaphthalene - 4,6,8 - trisulphonic acid).

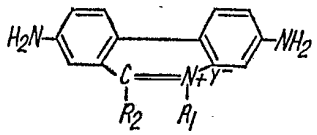
The word "lower" as used in this specification and in the appended claims with reference to alkyl and alkoxy groups denotes that the group contains not more than 6 carbon atoms.

According to a feature of the invention, the salts of formula I are prepared by diazotising an aniline of the formula:



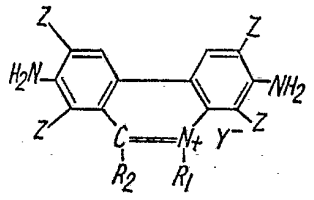
II

(wherein Am and R_3 are as hereinbefore defined) and coupling the resultant diazonium salt with an equimolecular proportion of a diaminophenanthridinium salt of the formula:



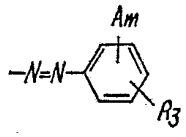
III

(wherein R_1, R_2 and Y are as hereinbefore defined). This process inevitably leads to a mixture of isomers, the mixture containing as products red salts of general formula I and isomeric purple salts of the formula:



IV

wherein one of the symbols Z represents the group:



V

(wherein Am and R_3 are as hereinbefore defined) and the others represent hydrogen atoms.

In an alternative procedure a salt of formula III may be diazotised and the resultant diazonium salt coupled with an aniline of formula II.

The salts conforming to formula I (products within the scope of the invention) may be separated from those of formula IV by conventional procedures, for example, fractional crystallisation from methanol. It is not essential to effect such a separation, however as the salts of formula IV are also therapeutically active.

When the compounds of general formula I are used for therapeutic purposes in the form of salts, it should be understood that only those such salts should in practice be employed as contain anions that are relatively innocuous to the animal organism when used in therapeutic doses so that the beneficial physiological properties inherent in the parent compound are not vitiated by side-effects ascribable to those anions. Suitable acid addition salts include, besides those named above, hydrohalides (for example hydrochlorides), phosphates, nitrates, sulphates, maleates, fumarates, citrates, tartrates, methane sulphonates and ethane disulphonates. These salts may be made by the methods heretofore used in the art for making acid addition salts. For example, the acid addition salts may be made (i) by passing a solution of the chloride hydrochloride in a suitable solvent down an ion-exchange column containing the required anion, and isolating the resultant salt after evaporation of part or all of the solvent or (ii) by treating an aqueous solution of the chloride hydrochloride with an aqueous solution of an equivalent quantity of the sodium salt of a non-toxic acid, and isolating the resultant salt by filtration. They may be purified by crystallisation or by any other method commonly used in the art.

The invention is illustrated by the following Examples.

Example I

A diazonium salt solution, prepared at 0—5°C. from 4-amino-3-bromobenzamidine monohydrochloride (6.3 g.), water (12.5 ml.), concentrated hydrochloric acid (5.65 ml.) and sodium nitrite (1.76 g.), was treated with sulphamic acid to remove excess of nitrous acid, and added, all at once, to a stirred solution of 2,7-diamino-10-ethyl-9-phenylphenanthridinium chloride (9.95 g.) in water (60 ml.) at 5—10°C. Anhydrous sodium acetate (14.25 g.) in water (45 ml.) was added, and the suspension was stirred for 1.5 hours, at 5—15°C. Sodium chloride (4.5 g.) in water (45 ml.) was added, and after stirring at 10—15°C. for 1 hour, the purple solid was filtered off, washed with brine, and dried over silica gel. Paper electrophoresis showed the presence of two main components—a red isomer, which was 7 - (4 - amidino 2 - bromophenyldiazoamino)-2 - amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride and a purple isomer, which was (4 - amidino - 2 - bromophenylazo) - 2,7 - diamino - 10 - ethyl - 9-phenylphenanthridinium chloride hydrochloride —which can be separated by fractional crystallisation from a suitable solvent. The purple isomer, (4 - amidino - 2 - bromophenylazo)-2,7 - diamino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride dihydrate was obtained as purple crystals, m.p. 282°C. (decomp.) by fractional crystallisation from methanol. The methanolic mother liquors contained substantially pure red isomer, 7 - (4-amidino - 2 - bromophenyldiazoamino) - 2-amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride, m.p. 295—297°C. (decomp.).

The 4-amino-3-bromobenzamidine monohydrochloride used as starting material, m.p. 262°C., was prepared from 4-amino-3-bromobenzonitrile (Crundwell, J. Chem. Soc. (1958), 371).

Example II

3 - Amino - 4 - chlorobenzamidine dihydrochloride (16.7 g.) in water (89 ml.) and concentrated hydrochloric acid (15 ml.) was diazotised at 5—8°C. with sodium nitrite (4.85 g.). After filtration from a little 3,3¹-diamidino - 6,6¹ - dichlorodiazoaminobenzene dihydrochloride, the diazonium salt solution was treated with sulphamic acid to remove excess of nitrous acid, and added, all at once, to a stirred solution of 2,7-diamino-10-ethyl-9-phenylphenanthridinium chloride (18.4 g.) in water (100 ml.) at 5—10°C. Anhydrous sodium acetate (27 g.) in water (81 ml.) was added, and the suspension was stirred for 1½

hours at 5—15°°C. The dark blue solid was filtered off, washed with brine, and dried over silica gel. Paper electrophoresis showed the presence of two main components, one red and the other purple. These mixed isomers—7 - (3 - amidino - 6 - chlorophenyldiazoamino)-2 - amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride and (3 - amidino - 6 - chlorophenylazo) - 2,7 - diamino-10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride — separated as purple microcrystals of a trihydrate, decomposing at 265—270°C., on addition of ether to a methanol solution of the crude mixture. The mixed isomers were separated by fractional crystallisation from methanol to give 7 - (3-amidino - 6 - chlorophenyldiazoamino) - 2-amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride trihydrate as red crystals, m.p. 278—280°C. (decomp.).

3 - Amino - 4 - chlorobenzamidine dihydrochloride, decomposing at 265°C., used as starting material, was prepared from 3-amino-4-chlorobenzonitrile, m.p. 93—94°C., obtained by the reduction of 4-chloro-3-nitrobenzonitrile (Le Fevre and Turner, J. Chem. Soc., (1927), 1118).

EXAMPLE III

A diazonium salt solution, prepared at 0—5°C. from 3-amino-4-methoxybenzamidine dihydrochloride (6.4 g.), water (24.2 ml.), concentrated hydrochloric acid (6 ml.), and sodium nitrite (1.9 g.), was treated with sulphamic acid to remove excess of nitrous acid, and added, all at once, to a stirred solution of 2,7-diamino - 10 - ethyl - 9 - phenylphenanthridinium chloride (10.8 g.) in water (6.5 ml.) at 5—10°C. Anhydrous sodium acetate (15.4 g.) in water (48.4 ml.) was added, and the red solution was stirred for 2 hours at 10—15°C. Sodium chloride (40 g.) was added, and the tar which separated was ground with brine until it solidified. The purple solid was filtered off, washed with brine and dried over silica gel. Paper electrophoresis showed the presence of one major component—a red compound—which was separated by fractional crystallisation from methanol. This red isomer, 7 - (3 - amidino - 6 - methoxyphenyldiazoamino) - 2 - amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride m.p. 264°C. (decomp.), was obtained as needles of the hydrate from aqueous ethanol.

3 - Amino - 4 - methoxybenzamidine dihydrochloride, decomposing at 277°C., used as starting material was prepared from 3 - amino- 4 - methoxybenzonitrile (Blanksma and Petri, Rec. trav. chim., (1947), *66*, 365).

EXAMPLE IV

Proceeding as described in Example I, a diazonium salt solution, prepared from 4-amino-3-methoxybenzamidine monohydrochloride hydrochloric acid and sodium nitrite, was reacted with 2,7-diamino-10-methyl-9-phenylphenanthridinium chloride. The crude product was isolated as described in Example I and the predominant red isomer was separated by fractional crystallisation of this crude product from methanol. After recrystallisation from aqueous ethanol 7 - (4 - amidino - 2-methoxyphenyldiazoamino) - 2 - amino - 10-methyl - 9 - phenylphenanthridinium chloride hydrochloride monohydrate was obtained as red needles m.p. 247—248°C. (decomp.).

The present invention further includes within its scope pharmaceutical compositions which comprise one or more compounds of the invention or their acid addition salts as aforesaid together with a significant amount of a pharmaceutical carrier. The invention includes especially such compositions made up for parenteral administration, which is preferred in clinical practice.

Preparations according to the invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or suspending media are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. These compositions may also contain adjuvants such as wetting, emulsifying and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporation in the compositions of sterilizing agents, or by irradiation. They may also be manufactured in the form of sterile solid compositions, which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

Solid compositions for oral administration include compressed tablets, pills, dispersible powders, and granules. In such solid compositions one or more of the active compounds of the invention is or are admixed with at least one inert diluent such as calcium carbonate, potato starch, alginic acid, or lactose. The compositions may also comprise, as is normal practice, additional substances other than inert diluents, e.g. lubricating agents, such as magnesium stearate.

Liquid compositions for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs containing inert diluents commonly used in the art, such as water and liquid paraffin. Besides inert diluents such compositions may also comprise adjuvants, such as wetting and suspending agents, and sweetening and flavouring agents.

The compositions according to the invention, for oral administration, also include capsules of absorbable material such as gelatin containing one or more of the active substances of the invention with or without the addition of diluents or excipients.

The percentage of active ingredient in the compositions of the invention may be varied, it being necessary that it should constitute a proportion such that a suitable dosage shall be obtained. Obviously several unit dosage

forms may be administered at about the same time. In general, the preparations of the present invention should normally contain at least 0.025% by weight of active substance in the case of injectable solutions and at least 0.1% by weight of such substance in the case of oral preparations.

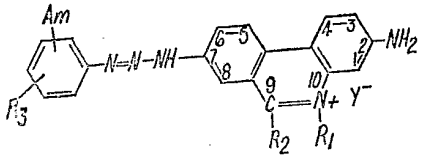
In clinical practice the compounds of the invention are normally administered as a 2% aqueous solution which may be prepared in accordance with the following Example.

EXAMPLE V

7 - (4 - Amidino - 2 - bromophenyldiazoamino) - 2 - amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride (2 g.) is dissolved in distilled water and the solution is made up to 100 ml. After filtration through a bacteria-retaining filter, the solution is filled into ampoules which are then sealed.

WHAT WE CLAIM IS:—

1. Phenanthridinium salts of the formula:



(wherein Am represents the amidino grouping $-C\langle^{NH}_{NH_2}$, R_1 represents a lower alkyl group, R_2 represents an aryl group, R_3 represents a lower alkoxy group or a halogen atom and Y represents a pharmaceutically acceptable anion) including their acid addition salts.

2. Phenanthridinium salts as claimed in claim 1 in which R_1 represents a methyl or ethyl group and R_2 represents a phenyl group.

3. A salt according to claim 1 containing the 7 - (4 - amidino - 2 - bromophenyldiazoamino) - 2 - amino - 10 - ethyl - 9 - phenylphenanthridinium cation.

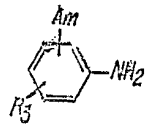
4. A salt according to claim 1 containing the 7 - (3 - amidino - 6 - chlorophenyldiazoamino) - 2 - amino - 10 - ethyl - 9 - phenylphenanthridinium cation.

5. A salt according to claim 1 containing the 7 - (3 - amidino - 6 - methoxyphenyldiazoamino) - 2 - amino - 10 - ethyl - 9 - phenylphenanthridinium cation.

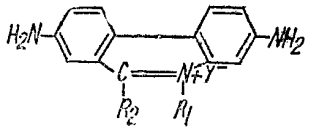
6. A salt according to claim 1 containing the 7 - (4 - amidino - 2 - methoxyphenyldiazoamino) - 2 - amino - 10 - methyl - 9 - phenylphenanthridinium cation.

7. An acid addition salt of a compound as claimed in any one of claims 3 to 7.

8. Process for the preparation of a mixture of isomeric compounds including a phenanthridinium salt as defined in any one of claims 1 to 6 which comprises diazotising an aniline of the formula:



(wherein Am and R_3 are as defined in claim 1) and coupling the resulting diazonium salt with an equimolecular proportion of a phenanthridinium salt of the general formula:



(wherein R_1, R_2 and Y are as defined in claim 1).

9. Process according to claim 8 which comprises the further step of isolating from the mixture of isomers the phenanthridinium salt as defined in any one of claims 1 to 6.

10. Process as claimed in claim 8 or 9 when carried out substantially as described in any one of Examples I to IV.

11. Phenanthridinium salts as claimed in claim 1 when prepared by the process claimed in any of claims 8 to 10.

12. A mixture of isomeric compounds including a compound as defined in any of claims 1 to 6 when prepared by the process of claim 8.

13. Pharmaceutical compositions comprising at least one phenanthridinium salt as claimed in any one of claims 1 to 7 and 12 in association with a significant amount of a pharmaceutical carrier.

14. Pharmaceutical compositions as claimed in claim 13 substantially as described in Example V.

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PROVISIONAL SPECIFICATION

Phenanthridinium Salts and their preparation

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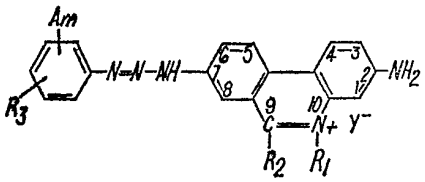
This invention is for improvements in or relating to phenanthridinium salts and to processes for their production, and has for its object the provision of new and therapeutically useful substances.

While many phenanthridine compounds, in

the form of their quaternary salts, have heretofore been proposed for use as trypanocidal agents, only a few have been used to any substantial extent in the field. Not only degree of activity but also toxicity vary markedly with change in the number and nature of substituents and it is impossible at the present time to predict *a priori* the therapeutic properties (if any) of any new phenanthridine compound.

As the result of research and experimentation, the present Applicants have prepared new phenanthridinium salts which have a high activity against blood parasites, such as trypanosomes, are surprisingly less toxic than known phenanthridinium salts possessing useful trypanocidal activity and, in consequence, exhibit an exceptionally high chemotherapeutic index.

The new compounds of the present invention are the amidinophenyldiazoaminophenanthridinium salts represented by the general formula:

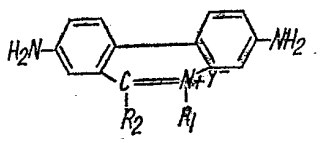


I

wherein the symbol Am represents the amidino grouping $-C\leqslant^{NH}_{NH_2}$, R_1 represents a lower alkyl group, preferably an ethyl or methyl group, R_2 represents an aryl (preferably phenyl) group, R_3 represents an alkoxy group or a halogen atom and Y represents a pharmaceutically acceptable anion, for example, a chlorine or bromine ion. Insoluble salts, useful for certain applications, include those of amsonic acid (4:4¹-diamino-stilbene-2:2¹-disulphonic acid), embonic acid (2:2¹-dihydroxy-1:1¹-dinaphthyl-methane-3:3¹-dicarboxylic acid) and of suramin (symmetrical urea of *m*-aminobenzoyl-*p*-methyl-*m*-aminobenzoyl-1-amino-naphthalene-4:6:8-trisulphonic acid).

The term "lower alkyl" as used in this specification denotes that the group in question contains not more than 6 carbon atoms.

According to a feature of the present invention, the said new salts may be prepared by diazotising the appropriate substituted aniline and coupling the resultant diazonium salt with an aminophenanthridinium salt of the formula:



II

in which the various symbols are as hereinbefore defined. The process inevitably leads to a mixture of isomers, but since both isomers are therapeutically active it is not essential to effect separation. Such separation can, however be achieved by conventional procedures as illustrated in the Example which follows.

In an alternative procedure a salt of formula II is diazotised and the resultant diazonium salt is coupled with an appropriately substituted aniline.

The present invention includes within its scope pharmaceutical preparations having trypanocidal activity and comprising a salt of general formula I in association with a pharmaceutical carrier, which may be either a solid material or a liquid. In actual practice the compounds of the present invention will normally be administered parenterally, and consequently they will normally take the form of sterile solutions or suspensions in water or other liquids, with or without the addition of soluble or insoluble diluents and/or solid or liquid excipients.

Preparations for oral ingestion can be liquids or solids or any combination of these forms, such as solutions, suspensions, syrups, elixirs, emulsions, powders or tablets. Pharmaceutical preparations for administration of the active therapeutic agents in unit dose form can take the form of compressed powders (or tablets) or of a powder enclosed in a suitable capsule of absorbable material such as gelatin. These compressed powders (or tablets) can take the form of the active materials admixed with suitable excipients and/or diluents such as starch, lactose, stearic acid, magnesium-stearate or dextrin.

In a further embodiment, the active material may, as such or in the form of a diluted composition, be put up in powder packets and employed as such.

The percentage of active ingredient in the composition of the invention may be varied, it being necessary that it should constitute a proportion such that a suitable dosage shall be obtained. Obviously, several unit dosage forms may be administered at about the same time. In general, the preparations of the present invention should normally contain at least 0.025% by weight of active substance in the case of injectable solutions and at least 0.1% by weight of such substance in the case of oral preparations.

The present invention is illustrated by the following Example.

EXAMPLE

A diazonium salt solution prepared at 0—5°C. from 4-amino-3-bromobenzamidine monohydrochloride (6.3 g.), water (12.5 ml.), concentrated hydrochloric acid (5.65 ml.) and sodium nitrite (1.76 g.) was treated with sulphamic acid to remove excess of nitrous acid, and added, all at once, to a stirred solution of 2:7-diamino-10-ethyl-9-phenylphenanthridinium chloride (9.95 g.) in water (60 ml.) at 5—10°C. Sodium acetate anhydrous (14.25 g.) in water (45 ml.) was added, and the suspension was stirred for 1.5 hours, at 5—15°C. Sodium chloride (4.5 g.) in water (45 ml.) was added, and after stirring at 10—15°C. for 1 hour, the purple solid was filtered off, washed with brine, and dried over silica gel. Paper electrophoresis showed the presence of two main components, which can be separated by fractional crystallisation from a suitable solvent. The blue isomer, 7(2) - (*p*-amidino - *o* - bromophenyldiazoamino) - 2(7)-amino - 10 - ethyl - 9 - phenylphenanthridinium chloride hydrochloride dihydrate was obtained as purple crystals, m.p. 282°C., (decomp.), by fractional crystallisation from methanol.

The 4 - amino - 3 - bromobenzamidine monohydrochloride used as starting material, m.p. 262°C., was prepared from 4-amino-3-bromobenzonitrile (Crundwell, J. Chem. Soc., (1958), 371).

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