

1

3,335,054

## ANTI-RADIATION AMINOETHANETHIOLS

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This application is a continuation-in-part of Fields et al. U.S. patent application Ser. No. 115,932, filed June 9, 1961, now abandoned.

This invention relates to chemical protection against ionizing radiations and in particular to a class of aminoethanethiols which show outstanding effectiveness as anti-radiation chemicals when administered to mammals.

It is known that certain aminoethanethiols, such as cysteine (2-amino-3-mercaptopropionic acid), cysteamine (2-aminoethanethiol), 2-mercaptoethylguanidine, and the like, confer some degree of protection against the harmful effects of ionizing radiations. It is believed that the biological effects of gamma rays, X-rays, etc., are due to the action of free radicals on important cell mechanisms, but there is no general agreement on the mechanism of action of anti-radiation agents.

In spite of the extensive investigation of chemicals for use against radiation, only a few materials are known to have any appreciable effectiveness. Cysteamine is among the best that have been described in the literature.

Chemicals which have been found effective as anti-radiation drugs have very often had to be administered with extreme care due to inherent toxicity. For instance, the administration of the drug might result in convulsions followed by death. Therefore, it has been desirable to find a drug which could be administered in quantities large enough to provide effective protection against ionizing radiation, without toxic reactions, such as convulsions or unwanted side effects, when administered in large quantities.

We have found that certain types of N-n-alkylaminoethanethiols can be used as anti-radiation drugs provided they have an alkyl chain of 8-10 carbon atoms.

One object of this invention is to provide anti-radiation drugs which will protect mammals against the effects of ionizing radiation.

Another object is to provide anti-radiation chemicals with markedly reduced propensity to induce convulsions.

A still further object is to provide anti-radiation drugs which have a dosage range large enough to provide adequate protection from ionizing radiation and at the same time are relatively free from toxicity and harmful side effects.

The N-n-alkylaminoethanethiols useful in this invention are those having a normal alkyl chain of 8-10 carbon atoms and comprise:



N-n-octyl aminoethanethiol



N-n-nonyl aminoethanethiol



N-n-decyl aminoethanethiol

These can be prepared by the synthesis described in the copending patent application Ser. No. 105,568, filed Apr. 26, 1961, in the name of Reynolds, now U.S. Patent 3,213,091.

Where the n-alkyl chain has less than 8 carbon atoms ( $\text{C}_8$ ) or more than about 10 carbon atoms ( $\text{C}_{10}$ ), the

2

compounds are relatively ineffective, the level of protection falling precipitously. Branched-chain alkyl group, e.g., iso-butyl, tert-octyl, tertiary nonyl, and the like, similarly depress the anti-radiation activity well below that of 2-aminoethanethiol. The reason for the unexpected improvement bestowed by the n-octyl, n-nonyl, and n-decyl substituents is not apparent. It may be related to the balance between lipid solubility and water solubility of the  $\text{n-C}_8\text{H}_{17}$  to  $\text{n-C}_{10}\text{H}_{21}$  compounds. It might be conjectured that any functional group showing some degree of protection against the harmful effects of ionizing radiation can be made more effective by conversion to a form having greater fat solubility. However, the multi-branched or poly-branched-chain alkyl compounds with fat-solubility characteristics similar to those of the  $\text{n-C}_8\text{H}_{17}$  to  $\text{n-C}_{10}\text{H}_{21}$ , are not effective. Neither are the normal alkyl compounds below  $\text{C}_8$  and above  $\text{C}_{10}$ .

A standard rodent test system for evaluating potential radiation protective agents has been used to determine the effectiveness of the compositions of our invention. About two-thirds of the maximum dose tolerated was used for the actual radiation test.

This test may be summarized as follows:

The test animal is a female albino mouse, Bagg Swiss strain of young age (7-day spread maximum), weighing 20-25 grams, and of closed-colony origin. The test animals must have a median survival of 10-11 days with  $\text{LD}_{100}$  radiation with no deaths sooner than 8 days. The term,  $\text{LD}_{100}$ , signifies the minimum dose of radiation required to kill 100% of an experimental group of organisms within a period of 30 days. Pretesting steps involve determining the maximum tolerated dose of the compound along with clinical manifestations and any resulting gross pathology.

Drug preparation comprised dissolving soluble compounds in appropriate vehicles such as physiological salt solution, water or pH 7.4 phosphate buffer. Insoluble compounds are suspended in a vehicle such as 1% carboxymethylcellulose solution in water and optionally with 0.4% Tween-80, a non-ionic surfactant polyoxyalkylene derivative of sorbitan monooleate sold by Atlas Powder Co. The pH is adjusted to  $7.1 \pm 0.3$  with dilute hydrochloric acid or sodium hydroxide. Where pH changes are detrimental to the compound under test, unadjusted solutions or suspensions are utilized. The drug is administered into the mammalian tissue intraperitoneally. However, this method of injection is merely for convenience. Our preferred method of injection is intramuscularly. The usual range of dosages is given to determine the maximum mg./kg. tolerated by mice.

Five animals are used in each drug level. The 10-day observation period is employed to note clinical manifestations, mortality, and gross pathology of 10-day survivors.

The radiation source is a standard X-ray machine, a 250 kv. General Electric Maxitron having a 4.0 mm. copper shield and emitting 30 roentgens per minute. Forty mice are exposed at one time in a circular plywood or plastic chamber with a perforated Plexiglas cover.

The radiation level administered is  $\text{LD}_{100}$ , which for X-radiation of rodents is about 800 roentgens (r.). Thirty mice are given the drug 30 minutes prior to irradiation and ten are used as controls, receiving an equivalent volume of the vehicle used in administering the drug by an appropriate route of administration.

Eight mice are housed in each cage, divided equally as to group to minimize cage effect. Daily mortality is recorded and summarized as 30-day mortality. Test re-

sults are reported in terms of degree protection as given in the following chart:

| Degree protection: | 30-day survival, percent |
|--------------------|--------------------------|
| Good -----         | 80-100                   |
| Fairly good -----  | 60-79                    |
| Fair -----         | 35-59                    |
| Poor -----         | 6-34                     |
| None -----         | 0-5                      |

By use of the preceding standard test, the effect of alkyl substitution in the amine group of 2-aminoethanethiol on anti-radiation effectiveness has been determined. The results are given in Table I.

TABLE I.—EFFECT ON ANTI-RADIATION EFFECTIVENESS OF ALKYL SUBSTITUTION OF THE AMINE GROUP IN CYSTEAMINE

| Alkyl Group                                                                                 | No. of Carbon Atoms | Dose <sup>1</sup> given (mg. per kg. body wt.) | Degree of Protection |
|---------------------------------------------------------------------------------------------|---------------------|------------------------------------------------|----------------------|
| Control.....                                                                                |                     |                                                |                      |
| None (2-aminoethanethiol).....                                                              | 0                   | 125                                            | None.                |
| CH <sub>3</sub> .....                                                                       | 1                   | 50-150                                         | Good.                |
| CH <sub>3</sub> CH <sub>2</sub> .....                                                       | 2                   | 150-350                                        | Fair.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> .....                                       | 3                   | 60                                             | Do.                  |
| CH <sub>3</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ).....                                   | 4                   | 50                                             | None.                |
| (CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> .....                                     | 4                   | 50                                             | Do.                  |
| (CH <sub>3</sub> ) <sub>3</sub> C.....                                                      | 4                   | 100                                            | Do.                  |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub> .....                                       | 4                   | 100                                            | Poor.                |
| (CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )..... | 6                   | 10                                             | None.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>7</sub> .....                                       | 7                   | 20                                             | Do.                  |
| (CH <sub>3</sub> ) <sub>3</sub> CCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> .....     | 8                   | 40                                             | Good.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>8</sub> .....                                       | 8                   | 15                                             | None.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>9</sub> .....                                       | 9                   | 20                                             | Good.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>10</sub> .....                                      | 10                  | 25                                             | Do.                  |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>11</sub> .....                                      | 10                  | 125                                            | Poor.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>12</sub> .....                                      | 10                  | 30                                             | None.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>13</sub> .....                                      | 11                  | 20                                             | Fair.                |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>14</sub> .....                                      | 12                  | 75                                             | None.                |
| t-alkyl C <sub>15</sub> H <sub>31</sub> <sup>3</sup> .....                                  | 13                  | 35                                             | Do.                  |
| t-alkyl C <sub>16</sub> H <sub>33</sub> <sup>3</sup> .....                                  | 14                  | 100                                            | Do.                  |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>15</sub> .....                                      | 16                  | 120                                            | Do.                  |
| CH <sub>3</sub> (CH <sub>2</sub> ) <sub>17</sub> .....                                      | 18                  | 200                                            | Do.                  |
| t-alkyl C <sub>18</sub> -C <sub>27</sub> <sup>2</sup> .....                                 | 18-22               | 100                                            | Do.                  |

<sup>1</sup> 30 min. before irradiation.

<sup>2</sup> S-alkyl isomer.

<sup>3</sup> Mixture.

Derivatives formed from the N-n-alkyl-substituted aminoethanethiols by conversion of the thiol to related sulfur compounds, exhibit some protection to radiation, particularly those which are converted in vivo to the thiol. Substitutions of small alkyl groups or halogens, etc., may be made also without diminishing anti-radiation activity. However, the extensive branching reduces the anti-radiation effect.

One such group which is quite effective consists of the Bunte salts of the formula, RNHCH<sub>2</sub>CH<sub>2</sub>SSO<sub>3</sub>M, where R is an n-alkyl group of 8-10 carbon atoms and M is a sodium or potassium ion. These may be prepared by reaction of β-n-alkylaminoethyl halide and alkali thiosulfate. These compounds may be effective in oral administration to protect the alimentary canal from radiation effects. Their efficacy is probably due to their low oil/water partition co-efficient ratio and their slow rate of hydrolysis. This keeps them from being absorbed rapidly and therefore, they remain in the alimentary canal over a long period, gradually hydrolyzing to the free thiol and being absorbed. The slowness with which these derivatives pass from the alimentary canal into the bloodstream enables administering much larger quantities for action in the alimentary tract than could be tolerated if they were quickly absorbed. As a result, the Bunte salts of the longer N-n-alkyl substituted aminoethanethiols are particularly meritorious in cases where it is desired to protect the alimentary tract against the adverse effect of ionizing radiations, for example, ionizing radiations being used in cancer therapy, or the like.

The effectiveness of an anti-radiation chemical is usually stated as the ratio by which it will increase the LD<sub>50</sub>, that is, the dose of radiation required to kill 50% of an experimental group of organisms within a period of 30 days. For most mammals LD<sub>50</sub> for X-radiation is between 500 and 700 roentgens (r.). A chemical which

when injected 30 minutes before irradiation will increase the LD<sub>50</sub> from 500 to 1000 r. would be said to have a dose reduction factor (DRF) of 2. The DRF of 2-aminoethanethiol is between 1.4 and 1.7.

The DRF of the N-n-alkyl-substituted 2-aminoethanethiols of our invention is not appreciably different from that of 2-aminoethanethiol based on rodent tests. However, the alkyl-substituted agents possess an outstanding advantage over 2-aminoethanethiol in that the maximum safe dose of the alkyl-substituted agent is much higher than that required for anti-radiation activity. Introduction of the n-alkyl chain of 8-10 carbon atoms increases the ratio of the toxic dose to the therapeutic dose of the drug and, at the same time, decreases the therapeutic dose required for anti-radiation effectiveness. Normal C8, 9 and 10 alkyl derivatives are sufficiently effective in protecting mice against ionizing radiation injury that good protection also results following the administration of only 50% of the maximum tolerated dose.

The presence of the normal alkyl group of about 8-10 carbon atoms on the nitrogen of the aminoethanethiol produces a very striking reduction in the convulsive activity which is one of the most serious shortcomings encountered with 2-aminoethanethiol and its substituents with certain alkyl groups shorter than 8 carbons or longer than 10, e.g., methyl, etc. Even direct injection of the C8 and C10 aminoethanethiols intracerebrally in mice has produced no convulsive activity although comparable injection of aminoethanethiol brought on violent convulsions.

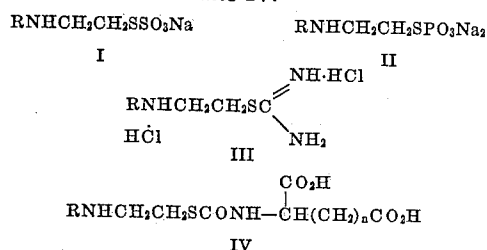
The chemical toxicity was observed by administering the drugs at a series of levels between 10 and 500 milligrams per kilogram of body weight. The test animals were observed for manifestations of acute toxicity in the period immediately after administration of the drug and again after 1, 2 and 10 days. The maximum tolerable drug level selected for use in the anti-radiation tests was chosen at about two-thirds the maximum level which produced no toxicity.

Anti-radiation chemicals can be used for other purposes than disclosed above. For instance, they are useful in cancer therapy by judicious injection in the healthy tissue surrounding the cancerous area, so that radiation treatment can be used to irradiate the diseased area without materially damaging the injected healthy tissue. An example of this use would be the protection of rectal mucosa during ionizing radiation therapy of cancer of the cervix, wherein the anti-radiation chemicals are kept away from the malignancy but in the presence of the normal cells.

It should be realized that the effectiveness of the drug decreases with time so that the drug to be effective, must be administered shortly before exposure to the ionizing radiation, preferably within an hour.

It will be appreciated that there can be co-action between these drugs and other drugs which may be desirable to administer, including other anti-radiation chemicals. Moreover, it may be possible to obtain a synergistic effect by the proper combination of these drugs.

The aminoethanethiols (RNHCH<sub>2</sub>CH<sub>2</sub>SH) may be rendered more soluble by preparing the S-substituted derivatives which are known to be cleaved to yield the parent thiol. Examples of such derivatives are the Bunte salts I, the thiophosphate salts II, the isothiuronium salts III, and the thiocarbamates IV.



The preparation of compounds of classes I, II, and III are well known in the art. Thiocarbamates may be obtained by the reaction of isocyanates with the aminoethanethiol hydrochloride. Of particular interest are those prepared from the isocyanate esters which may be hydrolyzed to yield IV.

Soluble complexes such as the chelates might also be useful.

The aminoethanethiols can be administered as their heavy metal complex salts, thereby providing a method of slow gradual release within the body. Another method of obtaining this effect is to administer them as the sparingly soluble salts of such acids as 4,4'-methylenebis(3-hydroxy-2-naphthoic acid). Salts of other organic or inorganic acids can also be used to vary solubility and toxicity properties.

The aminoethanethiols may also serve as intermediates for the preparation of carbon disulfide addition products which may have antifugicidal activity.

It will be appreciated that the dosage of the drug will depend upon the animal being treated and will also vary depending upon the form in which the drug is administered. For instance, if the drug is administered in an olive oil emulsion or as a salt, it might be absorbed into the system at a much slower rate permitting a much larger dosage than if administered directly.

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention as described hereinabove and as defined in the appended claims.

We claim:

1. A process of reducing the harmful biological effects of ionizing radiation for mammalian tissue comprising infiltrating the tissue with at least one N-n-alkylaminoethanethiol in which the n-alkyl group contains 8-10 carbon atoms.

2. A process of administering anti-radiation protecting N-n-alkylaminoethanethiols in which the n-alkyl group

contains 8-10 carbon atoms comprising injecting the N-n-alkylaminoethanethiol intramuscularly.

3. A process of reducing the harmful biological effects of ionizing radiation for mammalian tissue comprising infiltrating the tissue with at least one water-soluble S derivative of an N-n-alkylaminoethanethiol in which the n-alkyl group contains 8-10 carbon atoms.

4. A process of reducing the harmful biological effects of ionizing radiation for mammalian tissue comprising infiltrating the tissue with at least one N-n-alkylaminoethanethiol in which the n-alkyl group contains 8-10 carbon atoms, said alkylaminoethanethiol(s) being administered in the form of an aqueous suspension.

5. A process of reducing the harmful biological effects of ionizing radiation for mammalian tissue comprising injecting intramuscularly at least one water-soluble S derivative of an N-n-alkylaminoethanethiol in which the n-alkyl group contains 8-10 carbon atoms, said alkylaminoethanethiol(s) being administered in the form of an aqueous solution.

6. The process of claim 3 comprising injecting the drug intramuscularly.

7. The process of claim 4 comprising injecting the drug intramuscularly.

8. The process of claim 5 comprising injecting the drug intramuscularly.

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