

1

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2-N - METHYLAMMONIUMETHYLTHIOSULFURIC ACID AND ITS USE AS A RADIOPROTECTIVE AGENT

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No Drawing. Filed Dec. 22, 1961, Ser. No. 161,370
3 Claims. (Cl. 167-65)

This invention relates to radioprotective agents and more particularly, to a novel compound which is especially useful as a protective agent against the deleterious effects of ionizing radiation.

In recent years there has been a growing interest in the development of chemical compounds capable of being injected into or ingested by biological organisms to provide a degree of protection against the effects of ionizing radiation. Such radioprotective agents are useful in a number of different fields. For example, in connection with the current space exploration program it is contemplated that various forms of animal life will be projected through areas of concentrated radiation wherein either external or internal protection will be required. It is evident that to the extent that chemical radioprotective agents are available, the external shielding provided in the space vehicle and hence its weight can be materially reduced. Therefore, the development of such radioprotective agents is important to the space program.

In the event of atomic war large numbers of people and animals will be exposed to lethal and near lethal doses of radiation. The utility under such circumstances of chemical radioprotective agents capable of being taken internally to increase the dose of radiation that an individual can tolerate is obvious.

It has been known for many years that radiation is capable of destroying diseased body tissue in the treatment of cancer. However, the effectiveness of this technique has been limited by the fact that radiation also damages healthy tissue, and hence the radiation intensity employed for the treatment of the diseased tissue must be limited to a value which will not produce excessive damage of adjacent healthy tissue. Chemical radioprotective agents provide the radiotherapist with means for increasing the dosage tolerance of healthy tissue surrounding a diseased area, thereby making possible the use of higher and hence more effective radiation levels for treatment of the diseased tissue.

A considerable number of compounds have been previously proposed as radioprotective agents. As early as 1950 Dr. Harvey Patt at Argonne National Laboratory observed that cysteine protected mice against otherwise lethal doses of radiation. In 1952 Dr. Z. M. Bacq found that beta-mercaptoethyl amine worked even better than cysteine. Dr. David Doherty of Oak Ridge National Laboratory found that 2-mercapto-ethylguanidine was also effective in protecting mice against the effects of radiation. In 1957 Drs. B. G. Crouch and R. R. Overman demonstrated that mercapto-ethylguanidine was also effective in protecting monkeys against radiation. In 1959 Dr. David P. Jacobus reported that chemical compounds from the families of nitriles, thiocarbamates, phenones, thiosugars and mercaptoamines and mercapto-guanidines having alkaline nuclei showed promise as radioprotective agents.

Although these previously proposed compounds confer a measure of protection against radiation, they are subject to the disadvantage that they exhibit excessive chemical toxicity. For example, mercapto-ethylguanidine, which has been shown to be effective in protecting mice, is so toxic that no protection was observed at radiation levels

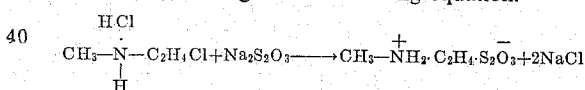
2

that can be tolerated by large animals. Less potent compounds, when administered in the large doses required for protection, still proved to be toxic. A further problem arose out of the fact that many of the previous compounds are not soluble in water. Water solubility is important, since the protective compound must be propagated through the body and produce mercaptans or disulfides *in vivo* before its effects will be manifest.

It is accordingly an object of the present invention to provide a novel chemical radioprotective agent which substantially increases the amount of radiation that animal organisms can safely tolerate, and which is substantially non-toxic at the dosages which provide such protection. It is another object of the invention to provide a radioprotective agent that is water-soluble and more effective at safe dosages than similar previously proposed compounds. It is still another object of the invention to provide an agent of this character that can be easily and cheaply made from readily available raw materials. Other objects of the invention will be in part obvious and in part pointed out hereafter.

The objects and advantages of the present invention can be achieved by employing as a radioprotective agent 2-N-methylammoniummethylthiosulfuric acid. It has been found that this compound is unexpectedly superior in its protective action to other compounds of closely related chemical structure that might be expected to exhibit a comparable protective effect. Moreover, this compound is substantially less toxic than certain previously proposed protective compounds and this reduction in toxicity is achieved without any significant diminution in protective effect. Data in support of the foregoing statements are given in Table I below.

The 2-N-methylammoniummethylthiosulfuric acid, which is believed to be a new compound, can be conveniently prepared by reaction of nitrogen mustard (N-methyl-beta-chloroethyl amine hydrochloride) with an alkali metal thiosulfate according to the following equation.



As indicated by this equation, the product obtained is an internal salt of the organothiosulfuric acid, commonly known as a zwitterion. While the equation has been written in terms of sodium thiosulfate, other alkali metal thiosulfates can be used. In order to illustrate the method of preparation of this compound, two exemplary procedures which have been found satisfactory are given herein.

Example 1

A one-liter flask equipped with a reflux condenser, mechanical stirrer and heating mantle was charged with 0.55 mol (137 grams) of sodium thiosulfate pentahydrate and 137 grams of ethylene glycol solvent, and the mixture in the flask was heated and agitated until the thiosulfate dissolved. Thereafter, 0.5 mol (65 grams) of N-methyl-beta-chloroethylamine hydrochloride was added to the solution in the flask with continued heat and agitation. A 10% molar excess of the thiosulfate was used.

The reaction mixture was held at pH 6 and refluxed at a temperature ranging from 110° to 113° C. for a period of 3.5 hours. Consumption of thiosulfate was followed by iodine titration of aliquots withdrawn from the reaction mixture at various time intervals. During the terminal portion of the reaction period, no further consumption of thiosulfate was noted.

At the end of the reaction period the water of crystallization of the thiosulfate and a portion of the glycol solvent were removed by evaporation in a roto-dryer

and the residue was allowed to cool to room temperature. The white crystalline NaCl powder that had formed during the course of the reaction was filtered off. The filtrate was chilled to crystallize out the methylammoniumethylthiosulfuric acid. A first crop of white crystalline material formed which, after separation from the mother liquor by filtration, was washed in cold methanol and dried. The mother liquor was recrystallized and a second crop of crystalline material formed which was washed and dried in the same manner as the first crop. A total net weight of 26 grams of crystalline methylammoniumethylthiosulfuric acid was obtained having a molecular weight by non-aqueous titration of 177 as compared with a theoretical M.W. of 171. The yield of the product was 27.3% and its melting point 160° to 162° C. Microchemical analysis showed that the product contained 20.96% carbon, 5.38% hydrogen, 8.25% nitrogen, and 37.16% sulfur as compared with a theoretical content of these elements of 21.20% C, 5.28% H, 8.20% N and 37.4% S. Ignition of a sample of the crystals over an open flame left no inorganic residue.

Example 2

Two reaction bombs were each charged with 0.74 mol (96 grams) of nitrogen mustard, 0.81 mol (129 grams—10% excess) of anhydrous sodium thiosulfate and 600 ml. of a mixture of equal volumes of ethanol and water. The bombs were placed in a 120° C. oven for four hours. The contents of the bombs were then combined and filtered. The filtrate was concentrated on a roto-dryer to remove most of the solvent, and then filtered hot to separate the salt precipitated during concentration.

On cooling, the filtrate separated into two layers, one of which contained crystals of the methylammoniumethylthiosulfuric acid which were separated by filtration. The product was recrystallized from ammoniacal 95% ethanol to yield 181 grams of impure product, which corresponds to a yield of 73%. After further recrystallization a product was obtained having a purity similar to that of the product of Example 1.

A quantity of 2-N-methylammoniumethylthiosulfuric acid as prepared in Example 1 was tested for toxicity and radioprotective activity. In making the toxicity tests, adult male mice weighing between 20 and 25 grams were employed. Both the control and experimental animals were picked at random from the same lot in order that their age and weight would be comparable. Groups of five mice were injected intra-peritoneally with an aqueous saline solution at a pH of about 7. The quantity of the solution used in the toxicity tests (and also in the radiation tests) was somewhat less than 2% of the body weight of the animals. Dosage levels of 250 to 500 milligrams per kilogram of body weight were administered to different groups of animals and mortality was reported for a period of 10 days. It was found that no mortality occurred at dosages of 300 mg./kg., and this drug level was used as a maximum in the radiation tests.

To evaluate the radioprotective activity of the methylammoniumethylthiosulfuric acid, groups of 10 mice were injected with a solution of the type described above. Drug levels of 300, 150, 75 and 0 mg./kg. were administered to different groups of mice. The animals were then inserted in individual 50 ml. plastic centrifuge tubes which were placed radially on a rotating turntable so that each animal received an equal exposure of 800 roentgens of whole body X-radiation. The X-ray apparatus was operated at 250 kv., 15 ms.; the radiation was filtered by 0.25 mm. of copper and 1.0 mm. of aluminum; and the target-skin distance was 75 cm. Dose rate was 40 r. to 43 r. per minute.

Observations on mortality and weight loss of both

the control and treated irradiated groups were made daily for a period of 30 days after radiation exposure. It was found that X-ray doses of 800 r. uniformly caused 100% mortality among the control mice within a period of about two weeks. Typical results of the tests are given in Table I wherein drug level is given in mg./kg. of body weight of the mice and ST 50 is the median survival time in days of the animals that died within 30 days. The last column of Table I gives the percentage of animals surviving at the end of 30 days.

In order to bring out the superiority of the product here claimed, there are included in Table I data reported by Kaluszyn, Czerniak and Bergmann (Radiation Research 14, 23-28, 1961) for a number of compounds that are chemically related to the present product but are substantially less effective than the present product. The Kaluszyn et al. data appeared to have been obtained on essentially the same basis as those for the present product, except that a lower radiation level (725 r.) was used.

TABLE I

Compound	Dose	ST-50	30 day Survival
Controls (no radioprotective agent).....	0	9	0
N-methylammoniumethylthiosulfuric acid.....	300	20	70
Do.....	150	11	30
Do.....	75	10	0
Do.....	0	11	0
Ammoniumethylthiosulfuric acid.....	300	-----	46±5
Ammoniumpropylthiosulfuric acid.....	300	-----	34±7
Dimethylammoniumethylthiosulfuric acid.....	250	-----	57±5

From the foregoing description it should be apparent that the present invention provides a novel radioprotective compound that is markedly superior to various closely related chemical compounds. Thus the data of Table I show that the monomethyl derivative of ammoniumthiosulfuric acid claimed herein is superior to the unsubstituted ammoniumethylthiosulfuric acid and is also superior to the dimethyl substituted ammoniumethylthiosulfuric acid. Moreover, the product of the present invention is substantially less toxic than for example the mercapto compounds previously proposed as radioprotective agents.

I claim:

1. 2-N-methylammoniumethylthiosulfuric acid.
2. As a radioprotective agent, an aqueous solution of 2-N-methylammoniumethylthiosulfuric acid.
3. A radioprotective agent as in claim 2 and wherein said solution is a substantially neutral saline solution.

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