Sprague-Dawley rats 50 days old induced breast tumors within 21–120 days of feeding. When testing against these tumors, 9-methoxyellipticine was found to be inactive at 30 mg./kg. i.p. and 60 mg./kg. *per os*. Furthermore, some weight loss and toxicity were evidenced in both regimens.

The biological data are summarized in Tables II and III.

SUMMARY

The oncolytic and CNS depressant activities first encountered in the authors' screening program with certain extracts from *Ochrosia maculata* Jacq. (*O. borbonica* Gmel.) have been found to be associated with 9-methoxyellipticine and reserpine, respectively. 9-Methoxyellipticine possesses a relatively broad spectrum of antitumor activity, 10 of 17 mouse neoplasms tested having responded. Both intraperitoneal and oral activity have been noted. Significant activity was seen against both the ascites and solid forms of the Walker rat carcinosarcoma 256.

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Keyphrases

Alkaloids—*Ochrosia maculata*
9-Methoxyellipticine—isolation, identity
Antitumor activity—9-methoxyellipticine
TLC—separation
X-ray crystallography—identity
UV spectrophotometry—identity
IR spectrophotometry—identity

Synthesis and Preliminary Evaluation of a New Quinuclidine Derivative as a Radioprotective Agent

By WARREN SHAPIRO, MARTIN F. TANSY, and SAMUEL ELKIN

7,10-Ethano-1-thia-4,7-diazaspiro [4.5] decane dihydrochloride was prepared by the condensation of 3-quinuclidone hydrochloride with 2-aminoethanethiol hydrochloride. The compound was shown to have potential radioprotective properties against a lethal dose of X-radiation in mice. Optimum protection was obtained in a group of mice pretreated intraperitoneally with 0.20 mg./g. of 7,10-ethano-1-thia-4,7-diazaspiro [4.5] decane dihydrochloride 15 min. prior to exposure, where 58.3 percent survived. A toxicity study showed the i.p. LD_{50} of the compound to be 295 mg./kg. with a dose range of 257–339 mg./kg. ($p < 0.05$).

INTEREST IN quinuclidine, 1-azabicyclo[2.2.2]-octane, and its derivatives has been fairly recent. Although this molecule was first synthesized in 1909 (1), it received little attention until World War II. At that time, because of the shortage of quinine and the need for substitutes, quinuclidine became an important tool in the chemistry of synthetic antimalarials.

The greatest amount of synthetic work has been done on 3-substituted quinuclidines. Pharmacologic activity reported to date has included spasmolytic (2, 3), central nervous system stimulation (4), and ganglionic blockade (5).

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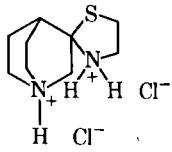
In 1951, Bacq and his co-workers, in Belgium, reported on the efficacy of a decarboxylation product of cysteine as a protective substance against X-radiation (6). The compound variously known as β-mercaptoethylamine, 2-aminoethanethiol, and 2-aminoethylmercaptan, is now commonly referred to as cysteamine. It is considerably more effective than cysteine on a weight basis, but it is also more toxic.

It appears there is a high degree of structural specificity with regard to the radioprotective activity of the compounds of the cysteine-cysteamine group. The sulfur atoms must be present as free sulfhydryl groups, disulfide groups, or derivatives which can undergo rapid intramolecular rearrangement under physiological conditions to give cysteamine derivatives possessing a free SH group (7).

Kaluszyner *et al.* (8) suggest that thiazolidines decompose *in vivo* to mercaptoamines and exert protection in this manner. It was therefore decided to synthesize a spiro thiazolidine derivative of quinuclidine as a potential precursor of free sulfhydryl groups. The following compound was prepared:



Such a compound could be an active radioprotectant without the toxicity of some of the aforementioned agents.

EXPERIMENTAL

All melting points are uncorrected. Analysis was performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

3-Quinuclidone Hydrochloride—Prepared by the method of Grob and Zergenyi (10).

Preparation of 7, 10-Ethano-1-thia-4,7-diazaspiro [4.5]decane Dihydrochloride–Into a 300-ml. round-bottom flask equipped with a stirrer, condenser, drying tube, and heating mantle was added 1.36 g. (0.02 mole) of 2-aminoethanethiol hydrochloride and 150 ml. of dry benzene. This mixture was heated until solution was complete and 1.88 g. (0.02 mole) of 3-quinuclidone hydrochloride was added. The mixture was refluxed with stirring for 20 hr. during which time a white precipitate formed. The mixture was filtered and filtrate discarded. The residue was recrystallized three times from dry methanol and anhydrous ether to yield a white crystalline product. Yield, 4.0 g. (77.8%); m.p. 250–255°.

Anal.—Calcd. for $C_9H_{18}Cl_2N_2S$: C, 42.02; H, 7.05; Cl, 27.57; N, 10.89; S, 12.47. Found: C, 42.00; H, 7.23; Cl, 27.51; N, 10.61; S, 12.42.

PHARMACOLOGY

Toxicity—The i.p. LD_{50} of 7,10-ethano-1-thia-4,7-diazaspiro [4.5] decane dihydrochloride was determined by the method of Litchfield and Wilcoxon (11). Six groups of 10 mice were used. The i.p. LD_{50} and dosage range in mg./kg. were determined by a rapid graphic method (see Table I and Fig. 1).

Radiation and Protection—Approximately 400 CBF male mice, 8–10 weeks of age at radiation time, arranged into groups of 12, weighing 17–27 g., were used for these experiments. The hybrid strain was obtained from the Cumberland View Farms Colony, Clinton, Tenn. They were kept in the laboratory for an acclimatization period of 7 days before use. The estimated X-ray LD_{100} for these animals is 825 r. For X-ray exposure, 12 mice were placed in perforated 50-ml. plastic tubes, radially positioned around a circular leucite turntable and rotated at 24 r.p.m. directly under the X-ray tube. Whole-body X-radiation was administered with a General Electric Maxitron 300 X-ray unit at 300 kVp and 20 ma. with 0.625 mm. Cu and 3.30 mm. Al added filtration and at a rate of 353 r/min. as measured in air with a Victoreen dosimeter. The target-to-animal distance was 50 cm. and the dose rate given is the value near the center of the mouse. The room temperature was maintained close to 25° throughout these experiments. After a single exposure to X-radiation, the mice were housed in suspended galvanized metal cages, two/cage, and they were observed over a

TABLE I—SOLUTION OF THE DOSE-EFFECT CURVE OF 7,10-ETHANO-1-THIA-4,7-DIAZASPIRO-[4.5]DECANE DIHYDROCHLORIDE

Dose, mg./kg.	Dead/Tested[a]	Observed % Dead[b]	Expected % Dead	Observed Minus Expected	Contribution to χ^2[c]
450	8/10	80	84	4	0.012
400	4/10	40	77	37	0.75
350	6/10	60	67	7	0.023
300	5/10	50	52	2	0.0016
250	4/10	40	31	9	0.035
200	0/10	0 (7.2%)	7.5	0.3	0.000

[a] Total animals = 60; No. of doses, $K = 6$; animals/dose = 60/6 = 10. [b] LD_{50} from graph (Fig. 1): LD_{84} = 450 mg./kg., LD_{50} = 295 mg./kg., LD_{16} = 220 mg./kg. Slope = $S = [(LD_{84}/LD_{50}) + (LD_{50}/LD_{16})]/2 = [(450/295) + (295/220)]/2 = 1.43$. $f LD_{50} = S^{2.77}/\sqrt{N'}$, $= S^{2.77}/\sqrt{50}$, $= 1.15$. N' = number of animals tested whose expected effects are between 16 and 84. $N' = 50$. LD_{50} range: $LD_{50} \times f LD_{50} = (295) \times (1.15) = 339$ mg./kg.; $LD_{50} \div f LD_{50} = (295)/(1.15) = 257$ mg./kg.; LD_{50} and 19/20 confidence limits: 295(257–339) mg./kg. [c] Total: 0.822. $\chi^2 = 0.822 \times 10 = 8.22$. df, $n = K - 2 = 4$. $(\chi)^2$ from Table II (12) for n of 4 = 9.49; 8.22 is less than 9.49, therefore the data are not significantly heterogeneous.

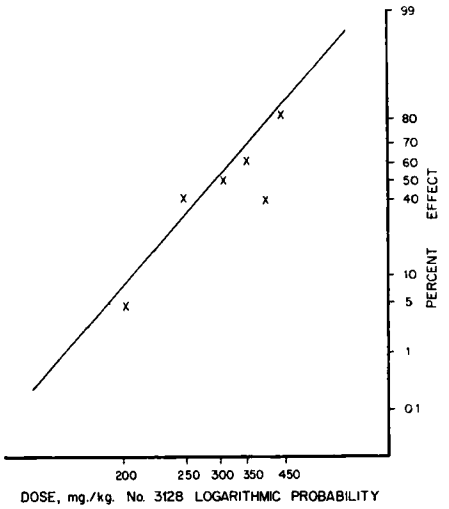


Fig. 1—Dose effect line of 7,10-ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride.

period of 1 month. The pellet form of Purina laboratory chow and tap water was available *ad libitum*.

In the first series of experiments each of a group of 12 mice was injected i.p. with 0.02 mg./g. of 7,10-ethano-1-thia-4,7-diazaspiro [4.5]decane dihydrochloride (dissolved in isotonic saline; final at pH 3.5) while a second group received 0.2 mg./g. of the test compound.

As already indicated these doses of the test compound were below the threshold of toxic, *i.e.*, lethal, dose for mice, administered by this injection route. A third group was injected intraperitoneally with an equivalent volume (0.2 ml.) of saline only. A fourth group was injected *via* the same route with cysteamine hydrochloride (0.24 mg./g.). The latter served as reference. A fifth group of control mice was kept at all times to determine what percentage, if any, of the radiation mortality could be attributed to the well-known "cage effect." Twenty-four hours later, the first four groups were exposed together to an X-ray dose of 825 r. This experimental series was then repeated with four additional groups of mice of comparable number injected intraperitoneally with the same test compound in identical concentrations, the same reference, and physiological saline just 15 min. before they were exposed to the 825 r of X-rays. In the third series of experiments the protective action of a single postradiation intraperitoneal injection of the test compound against radiation lethality was determined. The next three series of experiments were patterned after the first, except that these mice received the test substance, reference, and vehicle by oral intubation.

RESULTS AND DISCUSSION

7,10-Ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride was prepared by the condensation of 3-quinuclidone hydrochloride with 2-aminoethanethiol[1] in dry benzene.

The IR spectra of carbonyl groups of saturated aliphatic six-membered ring ketones usually show an absorption band between 1725 cm.$^{-1}$ and 1705 cm.$^{-1}$ (9). The spectrum of 3-quinuclidone hydrochloride shows this characteristic carbonyl absorbance at 1725 cm.$^{-1}$. The absence of this band in the spectrum of 7,10-ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride is indicative of structural change. The structure was confirmed by elemental analysis.

The toxicity study showed the i.p. LD_{50} of 7,10-ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride to be 295 mg./kg. with a dose range of 257–339 mg./kg. ($p < 0.05$) in mice.

In the first series of radiation experiments optimum protection was obtained in the group of mice pretreated with 0.20 mg./g. of 7,10-ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride administered intraperitoneally 15 min. before the beginning of exposure to 825 r where 58.3% survived (see Table II and Fig. 2). However, in the group of mice pretreated with 0.02 mg./g. of the test compound 15 min. prior to exposure, there was no significant increase in survival. At the doses of 0.20 mg./g. and 0.02 mg./g., 7,10-ethano-1-thia-4,7-diazaspiro-[4.5]decane dihydrochloride was not effective in increasing survival when it was administered intraperitoneally 24 hr. before or immediately after exposure of mice to 825 r. All of the saline-treated control mice died within 18 days of exposure to 825 r. The entire group of untreated control mice survived 30 days indicating that none of the deaths in the exposed groups of mice were due to the "cage effect."

At doses of 0.20 mg./g. and 0.02/mg./g., 7,10-ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride was not effective in increasing survival when it was administered by oral intubation 15 min. before, 24 hr. before, or immediately following expo-

TABLE II—COMPARATIVE EFFECTIVENESS OF ORAL AND PARENTERAL DOSES OF 7,10-ETHANO-1-THIA-4,7-DIAZASPIRO[4.5]DECANE DIHYDROCHLORIDE WITH MEA AGAINST A LETHAL DOSE OF X-RADIATION (12 MICE/GROUP)

	i.p.		Intubation	
	Days Surviving	No. Mice	Days Surviving	No. Mice
Saline, radiated	15	1	17	0
	18	0		
Saline, not radiated	30	12	30	12
MEA	30	11	30	6
0.2 mg./g., postradiation	30	1	22	0
0.02 mg./g., postradiation	15	0	19	0
0.2 mg./g., 15 min. before radiation	30	7	21	0
0.02 mg./g., 15 min. before radiation	13	0	20	0
0.2 mg./g., 24 hr. before radiation	15	0	17	0
0.02 mg./g., 24 hr. before radiation	15	0	21	0

[1] Obtained commercially from the Aldrich Chemical Co. Inc., Milwaukee, Wisc.

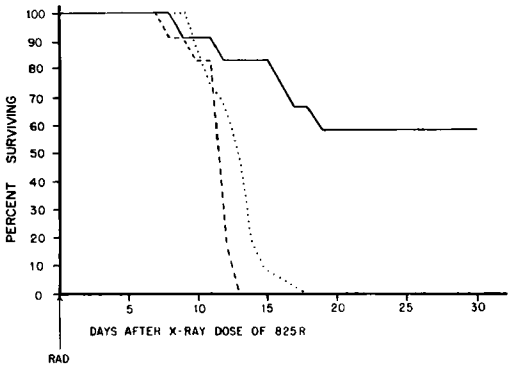


Fig. 2—Radioprotection by 7,10-ethano-1-thia-4,7-diazaspiro [4.5] decane dihydrochloride injected i.p. in mice 15 min. before X-radiation. Key: —, 0.2 mg./g. drug; · · ·, control; - - -, 0.02 mg./g. drug.

sure of mice to 825 r. Since the absorption and fate of the test compound has not yet been established, it is not known whether the failure to protect was due to the route of administration or the lack of absorption of the compound in the form of the dihydrochloride salt.

The mechanism(s) by which 7,10-ethano-1-thia-4,7-diazaspiro [4.5] decane dihydrochloride enhances the radiation resistance of rodents is not clear. However, the fact that the agent provides protection when it is administered intraperitoneally 15 min. before radiation exposure, places it in the same category as the classical chemical radioprotectors such as cysteine, cysteamine, or AET which are only effective when administered immediately before radiation.

The synthesis and radioprotective action of several analogs of 7,10-ethano-1-thia-4,7-diazaspiro[4.5]-decane dihydrochloride will be reported in a later publication.

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Keyphrases

Radioprotective agent—quinuclidine derivative
7,10-Ethano-1-thia-4,7-diazaspiro[4.5]decane dihydrochloride—synthesis
IR spectrophotometry—structure
Antiradiation activity—quinuclidine derivative

Preliminary Pharmacology of Ellagic Acid from *Juglans nigra* (Black Walnut)

By U. C. BHARGAVA, B. A. WESTFALL, and D. J. SIEHR*

A crystalline compound was isolated from *Juglans nigra* which by comparison with the known compound was found to be ellagic acid. The ellagic acid injected (i.p.) produced significant sedation, ataxia, potentiated sodium pentobarbital sleeping time, and protected mice from death after electroconvulsive shock. Intravenous injection of ellagic acid caused a fall in blood pressure and an elevation of the T wave, whereas the heart and respiration rate initially increased followed by a decrease. The ellagic acid produced no significant effect on isolated duodenal and uterus segments of the rat.

DURING THE isolation of sedative principles from *Juglans nigra*, a crystalline compound was obtained and characterized as ellagic acid by comparing it with the known compound. Ellagic acid is a polyphenolic constituent of many plants such as the pericarp tissue of some castor varieties, cashew nut shell, and some walnut species. *Juglans nigra* is a naturally grown plant in eastern and central North America.

Westfall *et al.* (1) observed that the ether ex-

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