1,2-Dialkyl-4-pyrazolidinethiols as Potential Antiradiation Agents

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Abstract □ The reaction between 3-chloropropylene sulfide and the 1,2-dialkylhydrazines was employed to prepare a series of 1,2-dialkyl-4-pyrazolidinethiols. Evidence is presented to support the structure proposed for the product. These mercaptoheterocycles are related to the β-mercaptoethylamines and were prepared as potential radiation protective agents. No significant activity was observed.

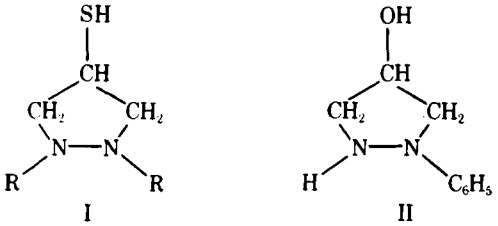
Keyphrases □ Dialkyl pyrazolidinethiols—synthesis, potential antiradiation agents, structure-activity relationships □ Antiradiation agents, potential—dialkyl pyrazolidinethiols, synthesis, structure-activity relationships □ Structure-activity relationships—dialkyl pyrazolidinethiols, as potential antiradiation agents

It has been firmly established that an important pharmacophoric grouping among radiation protective compounds is the 2-aminoethylmercaptan group or a closely related derivative (1, 2). In a program designed to synthesize molecular modifications of this grouping or a chemically similar group, a series of 4-pyrazolidinethiols (I) was investigated.

Examination of the parent structure (I) reveals the fundamental 2-aminoethylmercaptan moiety with the following modifications: (*a*) two such moieties are present owing to the presence of two nitrogen atoms, (*b*) these basic nitrogen atoms are present as a hydrazino group, and (*c*) the entire pharmacophore is contained in a rigid cyclic system. Conceivably, such a rigid system will show enhanced protective activity but lowered toxicity when compared with analogous open chain compounds. This report describes the preparation and antiradiation effects of a series of I compounds.

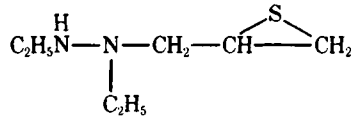
DISCUSSION

Chemistry—The preparation of an oxygen analog of I (II) was discovered by Gerhardt (3) in 1891. In that early work, treatment of epichlorohydrin with phenylhydrazine in ether afforded a very small yield of 1-phenyl-4-pyrazolidinol (II). A pathway to I was envisaged based on the anticipation that 3-chloropropylene sulfide would behave in a manner analogous to epichlorohydrin. Accordingly, the sulfur analog was treated with 1,2-diethylhydrazine in a 1:1.5 molar ratio in ether. The product isolated gave an elemental analysis consistent with either the desired

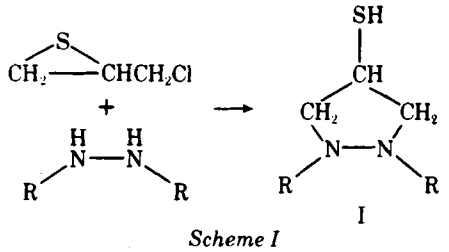


I

II



III



Scheme I

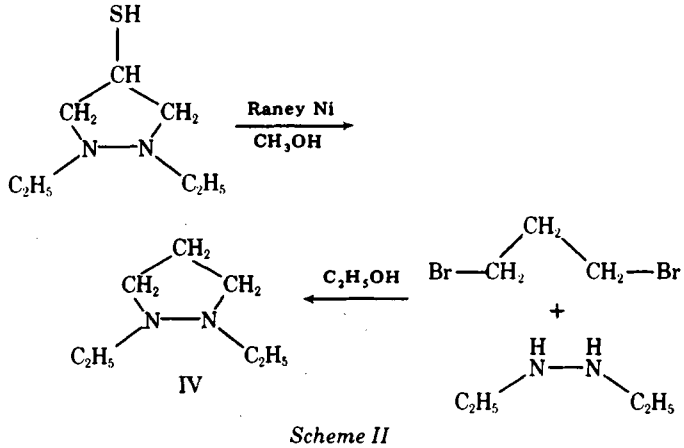
1,2-diethyl-4-pyrazolidinethiol (I*b*, R = C_2H_5) or the isomeric substance 1,2-epithio-4-ethyl-4,5-diazaheptane (III) (Scheme I).

Structure III was ruled out because there was no absorption in the N–H region of the IR spectrum of the product. Further support for the cyclic Structure I*b* was obtained by desulfurizing the product with Raney nickel and isolating 1,2-diethylpyrazolidine (IV). The structure of the desulfurized product was established by its unequivocal synthesis from 1,2-diethylhydrazine and trimethylene bromide (Scheme II). The IR spectrum of the picrate of the product was superimposable upon that of the picrate of the desulfurized product. Furthermore, no depression was obtained when a mixed melting point was determined.

In a similar manner, four other 1,2-dialkyl-4-pyrazolidinethiols were prepared in 12–32% yields. Because N–H absorption bands were absent in the IR spectra of all products, they were assigned the 4-pyrazolidinethiol structure (I). All of the compounds were oils and possessed the distinctive disagreeable mercaptan odor. They were characterized as their hydrochloride salts or as their 3,5-dinitrothiolbenzoates.

Attempts were made to prepare 4-pyrazolidinethiol (I, R = H) through hydrazine addition to 3-chloropropylenesulfide. However, this reaction produced polymeric material only. Table I records relevant data for the 1,2-dialkyl-4-pyrazolidinethiols.

Antiradiation Activity—The 4-pyrazolidinethiols were tested[1] for antiradiation activity as reported previously (4). The compounds were administered to mice intraperitoneally 15–30 min before whole body lethal radiation. Compounds I*a*, I*c*, and I*e* at doses of 48, 200, and 500 mg/kg, respectively, all produced 0% 30-day survivors. Compounds I*b* and I*d* were not tested. An explanation for the observed lack of activity



Scheme II

[1] At the Walter Reed Army Institute of Research.

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Table I—Physical Properties of 1,2-Dialkyl-4-pyrazolidinethiols

Compound	R	Reaction Time, days	Boiling Point (torr) or Melting Point	n_D	Yield, %	Recrystallization Solvent[a]	Formula	Analysis, %	Calc.	Found
Ia	CH_3	11[b]	69.5° (33)	1.5031 (24.5°)	12	—	$C_5H_{12}N_2S$	C	45.42	45.57
								H	9.15	9.38
								N	21.19	20.81
								S	24.25	23.80
Ia HCl	—	—	88–90°	—	—	A	$C_5H_{12}N_2S$·HCl	C	35.60	35.19
								H	7.77	8.18
								Cl	21.02	20.59
								N	16.61	16.39
								S	19.01	18.79
Ib	CH_3CH_2	7	82° (9)	1.4911 (22.5°)	31	—	$C_7H_{16}N_2S$	C	52.45	52.60
								H	10.06	9.96
								N	17.48	17.41
								S	20.01	19.80
Ib HCl	—	—	82–84°	—	—	A	$C_7H_{16}N_2S$·HCl	C	42.73	42.52
								H	8.71	8.97
								Cl	18.02	18.00
								N	14.24	14.20
								S	16.30	16.28
Ic	$CH_3(CH_2)_2$	4	108° (10)	1.4835 (24.5°)	29	—	$C_9H_{20}N_2S$	C	57.39	57.00
								H	10.70	10.67
								N	14.88	15.28
								S	17.03	17.05
Ic HCl	—	—	91.5–92.5°	—	—	A	$C_9H_{20}N_2S$·HCl	C	48.08	48.36
								H	9.42	9.83
								Cl	15.77	15.38
								N	12.46	12.25
								S	14.26	14.20
Id	$(CH_3)_2CH$	27	34° (0.03)	1.4845 (22°)	26	—	$C_9H_{20}N_2S$[c]		—	—
Id HCl	—	—	105.5–107.5°	—	—	B	$C_9H_{20}N_2S$·HCl	C	48.08	47.98
								H	9.42	9.02
								Cl	15.77	15.98
								N	12.46	12.60
								S	14.26	14.58
Ie	$(CH_3)_2CHCH_2$	20	64° (0.12)	1.4740 (31°)	32	—	$C_{11}H_{24}N_2S$	C	61.05	61.06
								H	11.18	11.23
								N	12.95	13.15
								S	14.82	14.85
Ie[d]	—	—	104.5–105°	—	—	C	$C_{18}H_{26}N_4SO_5$	C	52.66	52.52
								H	6.38	6.52
								N	13.65	13.44
								S	7.81	7.90

[a] A = ethanol–diethyl ether, B = ethyl acetate, and C = 95% ethanol. [b] 4°. [c] Not analyzed. [d] 3,5-Dinitrothiolbenzoate.

is not readily apparent; however, nitrogen alkylation has generally resulted in diminished activity (5).

EXPERIMENTAL[2]

Hydrazines—1,2-Dimethylhydrazine (6), 1,2-diethylhydrazine (7), 1,2-dipropylhydrazine (7), and 1,2-diisopropylhydrazine (7) were prepared by literature methods. 1,2-Diisobutylhydrazine was obtained by the lithium aluminum hydride reduction of isobutylideneazine (8) following the method of Renaud and Leitch (7). A 71% yield of a colorless product was obtained, bp 64° (11 torr), n_D^{24} 1.4312 [lit. (9) bp 169.5–170°, 70.5° (16 torr) and 63.5° (10 torr)].

1,2-Dialkyl-4-pyrazolidinethiols (Ia–Ie)—A typical reaction is described, that for the preparation of 1,2-diethyl-4-pyrazolidinethiol (Ib). Table I lists the physical and analytical data.

To a solution of 20.0 g (0.227 mole) of 1,2-diethylhydrazine in 100 ml of anhydrous ether was added 16.4 g (0.152 mole) of 3-chloropropylene sulfide (10, 11). The stoppered flask was kept at room temperature for 1 week. The precipitated hydrochloride salt was filtered, and the ether was evaporated under reduced pressure. The residue was distilled and gave 7.2 g (31%) of a colorless liquid, bp 82° (9 torr), $n_D^{22.5}$ 1.4911. A substantial amount of residue, which was undoubtedly a polymerization product of 3-chloropropylene sulfide, always remained in the distillation flask.

A hydrochloride was prepared and recrystallized from alcohol–ether, mp 82–84°.

[2] Melting points and boiling points are uncorrected. Analyses were performed by Dr. Kurt Eder, Geneva, Switzerland, and Drs. Weiler and Strauss, Oxford, England. IR spectra were determined on a Beckman IR-4 spectrophotometer using sodium chloride optics.

1,2-Diethylpyrazolidine (IV)—*By Desulfurization of 1,2-Diethyl-4-pyrazolidinethiol (Ib)*—To a stirred suspension of 2 spoons of W-2 Raney nickel in 250 ml of methanol was added a solution of 7.30 g (0.0456 mole) of Ib in 50 ml of methanol over 45 min. After 2.5 hr, another spoonful of W-2 Raney nickel was added. One hour later, an additional 2 spoonfuls were added. A total of 5 spoonfuls (~15 g) was added.

After an additional hour (total stirring time of 5.25 hr), the mixture was filtered. The dark-brown filtrate was distilled at atmospheric pressure through a 20-cm column packed with glass helixes to remove the methanol. The residue was distilled and yielded 0.3 g (5.1%) of a liquid, bp 62° (46 torr), n_D^{24} 1.4382 [lit. (12) bp 138°, $n_D^{27.8}$ 1.4409].

A picrate was prepared and recrystallized from absolute ethanol, mp 149.5–150°.

Anal.—Calc. for $C_{13}H_{19}N_5O_7$: C, 43.69; H, 5.36; N, 19.60. Found: C, 44.20; H, 5.31; N, 19.70.

By Alkylation of 1,2-Diethylhydrazine with Trimethylene Bromide—A solution of 34.5 g (0.392 mole) of 1,2-diethylhydrazine and 45.9 g (0.227 mole) of trimethylene bromide in 250 ml of 95% ethanol was added dropwise over 5 hr to 750 ml of refluxing 95% ethanol. The mixture was refluxed for an additional 10 hr, cooled, and neutralized with 28 g (0.50 mole) of solid potassium hydroxide.

The precipitated salts were removed by filtration, and the filtrate was concentrated at atmospheric pressure through a 20-cm column packed with glass helixes. After distillation of the ethanol, the fraction boiling at 132–150° (760 torr) was collected. It was redistilled through the same column to yield 1.7 g (5.8%) of product, bp 140–144°, n_D^{22} 1.4409 [lit. (12) bp 138°, $n_D^{27.8}$ 1.4409].

A picrate was prepared and recrystallized from absolute ethanol, mp 149.5–150.5°.

Anal.—Calc. for $C_{13}H_{19}N_5O_7$: C, 43.69; H, 5.36; N, 19.60. Found: C, 44.15; H, 5.65; N, 19.50.

A mixed melting point taken with the picrate obtained by desulfuri-

zation of 1,2-diethyl-4-pyrazolidinethiol was not depressed. The IR spectra of the two picrates were superimposable.

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Colorimetric Determination of Penicillins and Related Compounds in Intravenous Solutions by Nickel(II)-Catalyzed Hydroxamic Acid Formation

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Abstract □ Solutions of ampicillin, carbenicillin, methicillin, oxacillin, penicillin G, and cephalothin in 5% dextrose were analyzed by nickel(II)-catalyzed hydroxylaminolysis. The reactions of these antibiotics were complete within 20 min at room temperature. Under the analytical conditions, molar absorptivities of the ferric–hydroxamate complexes ranged from 830 to 1005 liters/mole/cm. Coefficients of variation for the analysis of these antibiotics in 5% dextrose were typically <3% at concentrations of 1 mg/ml. Oxacillin was analyzed by the same method in normal saline and/or lactated Ringer solutions. The method also was applied to the analysis of chloramphenicol in aqueous solutions. Only ampicillin showed a significant decrease in concentration in 48 hr.

Keyphrases □ Penicillins—analysis, colorimetry, intravenous solutions, dextrose, nickel(II)-catalyzed hydroxamic acid formation □ Antibiotics—analysis, colorimetry, ampicillin, carbenicillin, methicillin, oxacillin, penicillin G, cephalothin, intravenous solutions, dextrose □ Colorimetry—analysis, penicillins in intravenous solutions, dextrose, nickel(II)-catalyzed hydroxamic acid formation

Hydroxamic acid formation has been employed in the analysis of various carboxylic acid derivatives. Typically, the substrate is reacted with an alkaline hydroxylamine solution, acidified, and then complexed with ferric iron to form a reddish-violet complex. This complex absorbs in the 515–545-nm range with molar absorptivities near 10^3 liters/mole/cm. Esters (1–3), amides (4–6), acid chlorides (7), acid anhydrides (8), lactones (9), and imides (10) have been determined by the alkaline hydroxylaminolysis reaction.

Compounds containing the β-lactam moiety, *e.g.*, penicillins, also have been analyzed by the alkaline reaction (11). However, these compounds also react satisfactorily with hydroxylamine at neutral pH (12, 13). Nickel(II)-catalyzed hydroxylaminolysis has been applied to the analysis of carboxylic acids and acid hydrazides (14, 15). This method also has been applied to cephalosporins that contain the β-lactam group (16).

Table I—Stability of the Ferric–Hydroxamate Complex in Methicillin–Dextrose 5% Solutions

	Neutral Hydroxylamine Method (12)		Nickel(II)-Hydroxylamine Method	
Time[a]	In Water, absorbance[b]	In 5% Dextrose, absorbance[b]	In Water, absorbance[c]	In 5% Dextrose, absorbance[c]
2	0.465	0.455	0.485	0.475
5	0.450	0.400	0.480	0.475
10	0.440	0.330	0.477	0.475
15	0.425	0.165	0.470	0.455
30	—	—	0.463	0.455

[a] Time after mixing ferric iron reagent with the reaction mixture. [b] Absorbance at 490 nm in 1-cm cell. [c] Absorbance at 520 nm in 1-cm cell.

In this study, the stability of some penicillins and cephalothin was examined in solutions for intravenous administration. In the presence of relatively high dextrose concentrations (5% w/v), the neutral hydroxylamine method produced low color yields and color instability. Since the drugs were present in low concentrations, it was not feasible to dilute the samples to reduce the dextrose concentration. Consequently, the nickel(II)-catalyzed hydroxylaminolysis method was applied to these systems.

This paper reports the results on the application of the nickel(II)-catalyzed method to the analysis of ampicillin, carbenicillin, methicillin, oxacillin, penicillin G, and

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