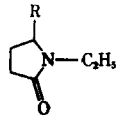
**Table III—1-Ethyl-5-alkyl-azacyclopentane-2-ones**



| Compound | R | Boiling Point/ 0.5 mm Hg | Yield, % | I.R. (C=O), $cm^{-1}$ | Formula | Analysis, % Calc. | Analysis, % Found | Tick Repellency at 0.44 $mg/cm^2$, % |
|---|---|---|---|---|---|---|---|---|
| IIIa | $C_6H_{13}$ | 110° | 89 | 1690 | $C_{12}H_{23}NO$ | C 73.04<br>H 11.75<br>N 7.10 | C 73.26<br>H 11.97<br>N 6.99 | 25 |
| IIIb | $C_8H_{17}$ | 120° | 94 | 1690 | $C_{14}H_{27}NO$ | C 74.61<br>H 12.08<br>N 6.21 | C 74.94<br>H 12.09<br>N 6.11 | 75[a] |
| IIIc | $C_{10}H_{21}$ | 154° | 90 | 1690 | $C_{16}H_{31}NO$ | C 75.83<br>H 12.33<br>N 5.33 | C 76.07<br>H 12.44<br>N 5.41 | 40 |

[a] 55% at 0.29 $mg/cm^2$.

cyclopentene series again indicates the importance of unsaturation as an enhancer of repellency toward the tick in this series of compounds.

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# Synthesis and Antileukemic Activity of 2-(2-Methylthio-2-aminovinyl)-1-methylquinolinium Iodides


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Received July 19, 1982, from the *Samuel M. Best Research Laboratory, Massachusetts College of Pharmacy and Allied Health Sciences, Boston, MA 02115.* Accepted for publication October 13, 1982. Present address: *U.E.C. Co., Ltd., Hanyang Building, Chung-ku, Seoul, Korea and ‡Department of Chemistry, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.



**Abstract** □ Reaction of 2-bis(2-methylthio)vinyl-1-methylquinolinium iodide with several heterocyclic aliphatic amines at 30–70° resulted in replacement of one methylthio group to give the title compounds. Reaction with pyrrolidine gave an unidentified product lacking sulfur. Antileukemic screening against P-388 lymphocytic leukemia showed positive activity only with the 6-methyl-morpholino derivative, whereas the 6-unsubstituted morpholino derivative was inactive. This result is in contrast to previous testing results with the 2-bis(2-methylthio)vinyl compounds where both 6-substituted and 6-unsubstituted derivatives showed activity.



**Keyphrases** □ 2-(2-Methyl-2-aminovinyl)-1-methylquinolinium iodides—synthesis, antileukemic activity in mice □ Synthesis—2-(2-methyl-2-aminovinyl)-1-methylquinolinium iodides, antileukemic activity in mice □ Antileukemia agents—potential, 2-(2-methyl-2-aminovinyl)-1-methylquinolinium iodides, synthesis, P-388 screen in mice


A series of 6-substituted-1-methylquinolinium-2-dithioacetic acid zwitterions (I) (1, 2) has shown appreciable antileukemic activity against P-388 lymphocytic leukemia in mice. An attempt to find compounds having better solubilities, in both water and organic solvents, led to the synthesis of the derived 2-bis(2-methylthio)vinyl-1-methylquinolinium iodides (II) (3). The latter compounds had comparable antileukemic activity, but at lower dose levels than found for the zwitterions. The 6-unsubstituted derivative, however, had activity equal to or better than that of the 6-substituted compounds. Compounds with electron-attracting and electron-releasing 6-substituents were equally active in this series.

Another type of derivative of the dithioacetic acid zwitterion structure (I), which should show improved solubility properties, is the 2-methylthio-2-aminovinyl compound (III) (3). Accordingly, a series of these compounds, using several heterocyclic amines, was prepared for anticancer screening. With the exception of the previously prepared morpholino derivative where a 6-methyl substituent was included, the 6-position was left unsubstituted.

Leukemia cell culture studies were carried out with the 6-methyl derivative of II, but no effects on cell cycle traverse were observed (4). This indicated that a metabolic conversion of the bis(methylthio)vinyl compounds, and most likely their dithioacetic acid precursors as well, is required for antileukemic activity. A study of DNA-binding specificity and RNA polymerase-inhibitory activity showed that the bis(methylthio)vinyl compounds (II), as well as the 2-methylthio-2-morpholinovinyl derivative (III, R = $CH_3$), had DNA-binding ability involving




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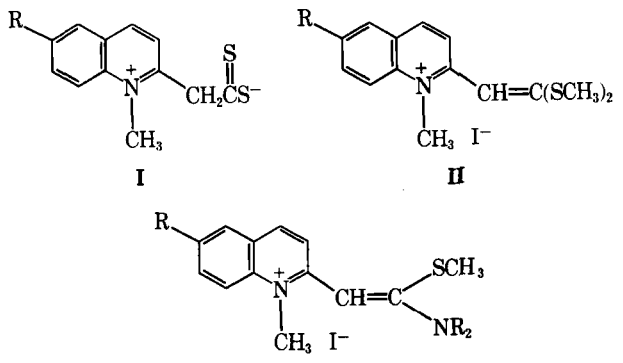
one binding function and inhibited RNA polymerase to a somewhat lower degree than was shown by chloroquine (5). It was believed that the function responsible for binding to DNA is the active methylene (V) resulting from loss of the bis(methylthio)methinyl moiety (4).

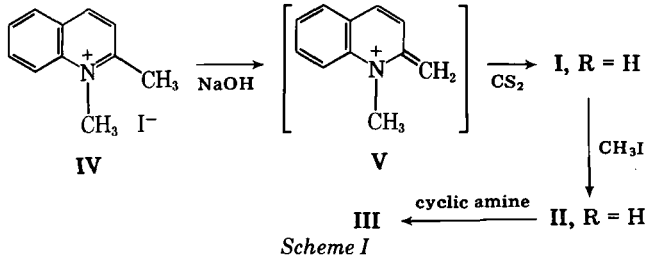


I II III

## DISCUSSION

**Chemistry**—The 2-methylthio-2-aminovinyl-1-methylquinolinium iodides (III) were prepared from the 2-bis(2-methylthio)vinyl-1-methylquinolinium iodides (II) by reaction with the appropriate amine at 30–70°, according to the previous procedure (3). The bis(methylthio)vinyl compounds were obtained from the reaction of the dithioacetic acid zwitterions (I) by reaction with methyl iodide (6, 7); the monomethyl esters of the dithioacetic acids were never isolated in these reactions. In actual practice, preparing a solution of the methylene base (V) in dry toluene, followed by reaction with carbon disulfide in toluene, and treatment with iodomethane in dimethyl formamide, was found to be superior to isolating the intermediate zwitterion (I). The synthetic route is indicated in Scheme I.

The reaction mixtures were examined for by-products, but only the original methiodide (IV) was found. Since the method of preparation precludes the carrying along of the methiodide because it is insoluble in toluene, and there was no chromatographic or other evidence that the products contained any of the active antileukemic compound II (R = H), any antileukemic activity found should be due to structure III.



IV V

*Scheme I*

The NMR spectra of III generally agreed with the spectrum of the morpholino derivative already described (3). The *S*-methyl protons appeared as a singlet at $\delta$ 2.6 ppm, and the *N*-methyl peak appeared as a singlet at $\delta$ 4.1 ppm. The vinyl proton, at $\delta$ 5.5–5.6 ppm, was shielded to a considerable extent, compared with its position at $\delta$ 6.76 ppm in the bis(methylthio) compound.

It has already been pointed out (3) that this shielding provided an indication for a *trans* arrangement of the amino function, with partial involvement of charge on the morpholino nitrogen. Calculations by the Tobey–Simon Rule (8) agreed with a *trans* structure.

The reduced heterocyclic amines employed in this synthesis gave variable yields of crystalline products. In the case of 2-methylpiperazine, only a 14% yield was realized, probably due to steric hindrance of the methyl. It is assumed that the nitrogen at the greater distance from the methyl was involved in replacing *S*-methyl, since less steric hindrance would result in the product. Reaction with pyrrolidine gave a product having no sulfur whose structure was not that of a nucleophilic replacement of the bis(methylthio)methinyl moiety by pyrrole. Nucleophilic replacement of the bis(methylthio)methinyl moiety by amines has been observed by Mizuyama *et al.* (9).

**Table I—Antileukemic Activity in Mice**[a]

| Structure III $NR_2$ | R | Dose, mg/kg[b] | Weight Difference[c], g | Median Survival Time, T/C%[d] Test 1 | Test 2 |
|---|---|---|---|---|---|
| —N O | $CH_3$ | 6.25 | −3.2, −3.4 | 141 | 149 |
| | | 3.13 | −1.7, −1.5 | 130 | 119 |
| —N O | H | 25.00 | −2.6, −2.2 | 121 | 110 |
| | | 12.50 | −2.1, −0.9 | 119 | 105 |
| —N S | H | 6.25 | −1.9 | 113 | — |
| | | 3.13 | −1.4 | 113 | — |
| —N | H | 1.56 | −2.8 | 116 | — |
| | | 0.78 | −0.5 | 109 | — |
| —N N—$CH_3$ | H | 25.00 | −0.9 | 102 | — |
| | | 12.50 | −1.1 | 98 | — |

[a] $CD_2F_1$ mice were inoculated with P-388 lymphocytic leukemia cells. [b] Drugs were administered intraperitoneally on days 1, 5, and 9. [c] Test group minus control group. [d] A T/C% value of ≥125 is considered a positive result.

**Antileukemia Test Results**—Antileukemia testing was performed at the National Cancer Institute using P-388 lymphocytic leukemia in mice according to their protocol (10). Details regarding dose and survival time are listed in Table I.

Positive activity was shown in this series only by the *N*-morpholino-6-methyl derivative (3). The *N*-morpholino derivative lacking the 6-methyl was inactive. This result is in contrast with testing data for the dithioacetic acid zwitterions (2) and the bis(methylthio)vinyl derivatives (3) where activity was found with both the 6-substituted and 6-unsubstituted derivatives. It is possible that in this (methylthioamino)vinyl series, the antileukemic activity does not depend on the formation of the active methylene compound (V) but on binding to nucleic acid or other macromolecules involving the morpholine moiety. Equilibrium binding constants to calf thymus DNA for several of the bis(methylthio)vinyl derivatives were lower than for the *N*-morpholino-6-methyl compound (5). Also, the (methylthioamino)vinyl group, a tautomeric form of a thioamide, should be more stable to hydrolysis than the ketene thioacetals.

## EXPERIMENTAL[1]

**1-Methyl-2-[2-methylthio-2-(1-morpholino)vinyl]-quinolinium Iodide**—To a suspension of 2-bis(2-methylthio)vinyl-1-methylquinolinium iodide (2.70 g, 0.007 mole) (2), in 50 ml of dimethylformamide was added an equimolar amount of morpholine (0.61 g, 0.007 mole). The mixture was stirred at 70° for 2 hr and then at 50° for 17 hr. The reaction was monitored by TLC (chloroform–methanol, 95:5). After it was cooled, the mixture was diluted with toluene (300 ml) and stored at 0°.

The precipitate was collected and dissolved in acetone, treated twice with charcoal, and the solvent was evaporated. The orange crystalline product was recrystallized from absolute ethanol to give 1.8 g (60%), m.p. 198–200°. $^1$H-NMR: $\delta$ 2.60 (s, 3, $SCH_3$), 3.70 (s, br, 8, morpholino), 4.09 (s, 3, $NCH_3$), 5.59 (s, 1, vinyl), and 7.6–8.2 ppm (m, 6, aromatic).

*Anal.*—Calc. for $C_{17}H_{21}IN_2OS$: C, 47.67; H, 4.94; N, 6.54; S, 7.48. Found: C, 47.52; H, 5.07; N, 6.43; S, 7.59.

**1-Methyl-2-[2-methylthio-2-(4-thiomorpholino)vinyl]-quinolinium Iodide**—To a suspension of II (R = H) (2.72 g, 0.007 mole) in 50 ml of dimethylformamide was added freshly distilled thiomorpholine (1.33 g, 0.013 mole), and the mixture was stirred at 35° for 4 days. The reaction was monitored by TLC (chloroform–methanol 9:1). It was diluted with anhydrous ether (200 ml) and stored at 0° for 2 hr.

The precipitate was collected and recrystallized twice from 1-propanol using charcoal, giving 2.0 g (69%) of orange crystals, m.p. 203–205°.

[1] Melting points were determined in capillaries with a Mel-Temp block and are uncorrected. $^1$H-NMR spectra were obtained with a Varian T-60 spectrometer using DMSO-$d_6$ as solvent and tetramethylsilane as internal standard. IR spectra were obtained with a Perkin–Elmer model 457A grating spectrophotometer using KBr pellets. Elemental analyses were done by F. B. Strauss, Oxford, England. TLC was carried out using silica gel plates, and products were detected by exposure to iodine vapor. Organic reagents were supplied by Aldrich Chemical Co. or Eastman Organic Chemicals.

$^1$H-NMR: δ 2.60 (s, 3), 2.9 (d, br, 4), 4.0 (d, br, 4), 4.10 (s, 3), 5.60 (s, 1), and 7.6–8.2 ppm (m, 6).

*Anal.*—Calc. for $C_{17}H_{21}IN_2S_2$: C, 45.94; H, 4.76; N, 6.30. Found: C, 45.59; H, 4.79; N, 6.32.

**1-Methyl-2 - [2-methylthio-2-(1-piperidino)vinyl] - quinolinium Iodide**—To a suspension of II (R = H) (2.72 g, 0.007 mole) in 25 ml of dimethyl sulfoxide was added freshly distilled piperidine (1.0 g, 0.012 mole), and the mixture was stirred at 30° for 5 days. It was treated as in the previous procedure, and the crude precipitate was dissolved in 1-propanol (300 ml), treated with charcoal, and filtered while hot. This procedure was repeated three times, and the combined filtrates were evaporated to dryness under reduced pressure. The residue was recrystallized from 2-propanol to give 2.70 g (90%) of orange crystals, m.p. 195–196°. $^1$H-NMR: δ 1.75 (s, 6), 2.63 (s, br, 3), 3.81 (s, br, 4), 4.10 (s, 3), 5.55 (s, 1), and 7.6–8.2 ppm (m, 6).

*Anal.*—Calc. for $C_{18}H_{23}IN_2S$: C, 50.70; H, 5.43; N, 6.57. Found: C, 50.93; H, 5.23; N, 6.38.

**1-Methyl-2-[2-methylthio-2-(4-methyl-1-piperazino)vinyl]-quinolinium Iodide**—Following the previous procedure, II (R = H) (1.20 g, 0.0031 mole) in dimethyl sulfoxide (25 ml) was treated with 1-methylpiperazine (0.35 g, 0.0035 mole). The product was recrystallized from acetic acid and again from absolute ethanol to give 0.63 g (48%) of orange crystals, m.p. 188–191°. $^1$H-NMR: δ 2.58 (s, 3), 2.95 (s, 3), 3.40 (br, 4), 3.83 (br, 4), 4.10 (s, 3), 5.80 (s, 1), and 7.6–8.2 ppm (m, 6).

*Anal.*—Calc. for $C_{18}H_{24}IN_3S$: C, 48.98; H, 5.48; N, 9.52; S, 7.26. Found: C, 49.00; H, 5.49; N, 9.29; S, 7.30.

**1-Methyl-2-[2-methylthio-2-(3-methyl-1-piperazino)vinyl]-quinolinium Iodide**—Following the previous procedure, II (R = H) (2.72 g, 0.007 mole) in dimethyl sulfoxide (25 ml) was treated with 2-methylpiperazine (0.70 g, 0.007 mole). The thick, oily product crystallized on long storage in the refrigerator. It was recrystallized from absolute ethanol and ether to give 0.41 g (14%) of orange-brown crystals,m.p. 188–190°. $^1$H-NMR: δ 2.13 (s, 3), 2.58 (s, 3), 3.40–3.70 (br, 7), 4.10 (s, 3), 5.58 (s, 1), and 7.6–8.2 ppm (m, 6).

*Anal.*—Calc. for $C_{19}H_{24}IN_3S$: C, 48.98; H, 5.48; N, 9.52; S, 7.26. Found: C, 48.83; H, 5.58; N, 9.11; S, 7.08.

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# Quantitative Analysis of Ethchlorvynol in a Capsule Dosage Form by NMR Spectroscopy


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Received July 19, 1982, from the *College of Pharmacy, Rutgers University, Piscataway, NJ 08854.* Accepted for publication October 14, 1982.



**Abstract** □ A quantitative $^1$H-NMR procedure is described for measuring ethchlorvynol in capsules. Deuterochloroform is used as the solvent, and hexamethylenetetramine as the internal standard; the analysis is based on the comparison of the area of the AB peak system of ethchlorvynol with the area of the hexamethylenetetramine singlet. The $^1$H-NMR method yields results that are precise to within 1% and agree well with results of the more cumbersome and less specific USP titrimetric procedure.



**Keyphrases** □ Ethchlorvynol—NMR quantitative analysis, capsule dosage form □ NMR quantitative analysis—ethchlorvynol, capsule dosage form


Ethchlorvynol (I), a nonbarbiturate hypnotic, is a tertiary acetylenic carbinol (1-chloro-3-ethyl-1-penten-4-yn-3-ol). The official USP XX procedure for the analytical determination of this drug substance, both alone and in its pharmaceutical dosage form, is based on the reaction of I with excess silver nitrate, producing the silver acetylide and nitric acid (1, 2). The resultant acid is immediately titrated with ~0.05 *N* NaOH; however, end point determination with the methyl red–methylene blue indicator is hampered by the precipitation of the silver acetylide. According to the official procedure, capsules must be weighed, carefully opened, emptied, and reweighed, with the difference taken as the capsule contents. The difficult end point and sample manipulations often lead to poor results.

Although there are several procedures published for the determination of I in biological fluids (3–8), there are only two other published methods for its determination in the pharmaceutical dosage form. Davidson (9), proposed a GLC analysis, accepted by the AOAC (10), and that uses 1,3-dichloro-2-propanol as an internal standard; a standard deviation range of 1.3–2.9% on assays of known solutions and the 500-mg capsule dosage form is reported. Drawbacks inherent in this procedure include the need for column preparation and overnight conditioning and the necessity of injecting a I standard (purified by vacuum distillation and quantified by the USP XX titrimetric procedure) along with the unknown solutions. Rizk and associates (11) proposed a colorimetric method for the determination of certain monosubstituted acetylenic hypnotic drugs, including I. In this procedure, silver acetylide




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