suspensions of the compounds including phenytoin were prepared. None of the test compounds exhibited any protection against maximal electroshock seizures at 40 mg/kg ip (suspension, footnote *a*, Table I). Compounds II*e* and I*b* produced definite signs of neurological toxicity (33 and 83%, respectively) as measured on the rotorod at 400 mg/kg (Table I). No CNS toxicity was observed with any other test compound.

The anticonvulsant evaluation indicated that compounds possessing a nitrile group at the 3-position (I*a*–I*c*) were more potent compared to compounds possessing a carbamyl group at that position (II*a*–II*i*). Introduction of the more polar carbamyl group (13) in II*a*–II*i* resulted in decreased lipid solubility compared to I*a*–I*c*, which contain the nitrile group. Decreased lipid solubility probably retards the passage of these compounds into the CNS.

The effect of the administration route and solubilization method on the most potent compound (I*b*) was examined further (Table II). Suspensions of I*b* were administered intraperitoneally due to solubility limitations. Suspensions then were compared to the anticonvulsant effects produced by propylene glycol solutions of I*b* when it was administered intraperitoneally or intravenously.

In the electroshock studies, 30 mg of I*b*/kg was chosen since phenytoin produced 100% protection against maximal electroshock seizures when it was administered as an intraperitoneal suspension. Variation in neither the injection route (intraperitoneal *versus* intravenous) nor the means of solubilization (suspension *versus* solution) was effective in producing anticonvulsant activity.

Compound I*b*, 75 mg/kg, was lethal following intravenous administration. Gross observation of the animals following this dose indicated that death was probably due to cardiovascular toxicity. Therefore, 50 mg/kg iv was selected as the maximum allowable dose for comparative purposes. Unfortunately, at 50 mg of I*b*/kg, variation in the administration route (intravenous *versus* intraperitoneal) was ineffective in producing protection against pentylenetetrazol-induced seizures.

Since 400 mg of I*b*/kg ip (suspension) afforded 67% protection in the pentylenetetrazol seizure test, 400 mg/kg ip of I*b* then was administered as a solution in propylene glycol for comparison (Table II). The completely dissolved solution of I*b* appeared to be slightly more effective than the intraperitoneally injected suspension of I*b* (83 *versus* 67% protection, respectively). However, the extent of rotorod toxicity appeared equivalent (83%). Therefore, complete solubilization of I*b* was concluded to be unnecessary for anticonvulsant activity.

Drugs that are useful in petit mal seizures are effective in elevating the threshold of electroshock- and drug-induced convulsions (14). Drugs used for grand mal epilepsy do not significantly affect the threshold of electrically induced seizures. None of the test compounds exhibited anticonvulsant activity against maximal electroshock but did block pentylenetetrazol-induced seizures. The active pyrrolopyrimidinediones and intermediates probably exert their activity through an elevation of the convulsive threshold, similar to trimethadione (14).

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High-Pressure Liquid Chromatographic Analysis of Pramoxine Hydrochloride in High Lipoid Aerosol Foam Dosage Form

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Abstract □ A rapid and quantitative method for the determination of pramoxine hydrochloride by high-pressure liquid chromatography is presented. The drug is extracted as the salt from a preparation with a high lipoid composition by partitioning it to the aqueous phase of an ether–methanol–water–acetic acid system. The extract is chromatographed on an octadecylsilane bonded packing with a methanol–water–acetic acid–methanesulfonic acid mobile phase. The time required for each separation is ~6 min. Analytical recoveries of 100.4 ± 1.5% were obtained.

Keyphrases □ Pramoxine hydrochloride—analysis, high-pressure liquid chromatography, high lipoid aerosol foam dosage form □ High-pressure liquid chromatography—analysis, pramoxine hydrochloride, high lipoid aerosol foam dosage form □ Anesthetics, topical—pramoxine hydrochloride, high-pressure liquid chromatographic analysis, high lipoid aerosol foam dosage form

Pramoxine hydrochloride, a widely used topical anesthetic, can present analytical difficulties due to its surfactant behavior. Its hydrophilic and lipophilic properties result in substantial matrix effects from common pharmaceutical excipients, particularly when the drug is incorporated into a high lipoid content base.

The conventional analytical method described in NF XIV (1) is based on nonspecific, nonaqueous titrimetry and spectrophotometric determinations. TLC[1] was used for qualitative analysis. Mario and Meehan (2) used the drug as an internal standard for a GLC assay of cough–cold preparations. Analysis of the high lipoid composition by GLC in this laboratory resulted in lengthy sample preparation and 30-min separations.

This study was undertaken to develop a rapid and reliable method for the determination of pramoxine hydrochloride in high lipoid preparations. The method was re-

[1] T. E. Rusch, Abbott Laboratories, Chicago, Ill., personal communication.

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Table I—Recovery of Pramoxine Hydrochloride from a High Lipoid Composition at Different Potency Levels

Potency Level, %	Amount Added, mg	Amount Recovered, mg	Recovery, %	*RSD*	*n*[a]
75[b]	165.0	164.8	99.9	0.5	4
100	220.0	220.9	100.4	1.5	14
125[b]	275.0	276.4	100.5	2.0	4

[a] Number of assays. [b] An expanded standard curve (0.7–1.2 mg/ml) was used to bracket the expected range of potencies.

Table II—Analysis of the Commercial High Lipoid Preparation

Batch	Pramoxine Hydrochloride, mg/can[a]	Mean	*RSD*
7071	228, 230, 214, 225, 224, 219	223	2.4
8811	224, 229, 217, 211, 227, 222	222	2.6
8041	225, 223, 223, 223	224	0.4

[a] Single-can assays; theoretical amount = 220 mg/can.

quired to handle relatively large samples to accommodate a total contents assay for the aerosol dosage form. A practical requirement was that the technique not utilize large quantities of solvents or reagents.

EXPERIMENTAL[2]

Reagents and Chemicals—A commercial pramoxine hydrochloride sample[3] was used as the reference standard. Absolute methanol[4], ether[4], acetic acid[4], and deionized water were used for the extraction. The mobile phase contained 50% methanol[5], 48.9% deionized water, 1% acetic acid[4], and 0.1% methanesulfonic acid[6]. After mixing, the mobile phase was filtered through a 0.45-μm filter[7]. The solution was prepared fresh daily and was degassed prior to use.

Standard Solutions for Calibration Curves—Standard solutions of pramoxine hydrochloride (0.8, 0.9, and 1.0 mg/ml) were prepared in 50% methanol. The three standards were analyzed, and the results were subjected to regression analysis.

Sample Preparation—The contents of each aerosol unit were dispensed into a 500-ml separator. After expulsion of the foam, the unit was opened and the remainder of the contents was transferred to the separator with 100 ml of ether–methanol (5:1). The sample was extracted twice with 100- and 75-ml aliquots of 20% acetic acid, respectively. The aqueous extracts then were combined in a 250-ml volumetric flask and brought to volume with water.

Chromatographic Conditions—Octadecyltrichlorosilane was permanently bonded to 10-μm porous silica particles and packed into a 25-cm × 4-mm i.d. stainless steel column[8]. The mobile phase was pumped through the column at 2 ml/min at room temperature until a stable baseline was obtained. The variable-wavelength detector was set at 286 nm. A range of 0.1 aufs was used.

Replicate 15-μl injections were made using an autosampler[9]. The output of the spectrophotometer was monitored with a 10-mv strip-chart recorder[10]. Peak height measurements, determined electronically[11], were used for all calculations.

RESULTS AND DISCUSSION

The pharmaceutical aerosol foam dosage form presents a unique problem; it is difficult to analyze a portion of the aerosol content with precision because of the solubility of the propellant in the foam base. To circumvent this feature, the contents of an entire aerosol unit may be assayed, with the results reported in milligrams per can. The technique fits in well with the commercial production of pharmaceutical aerosols.

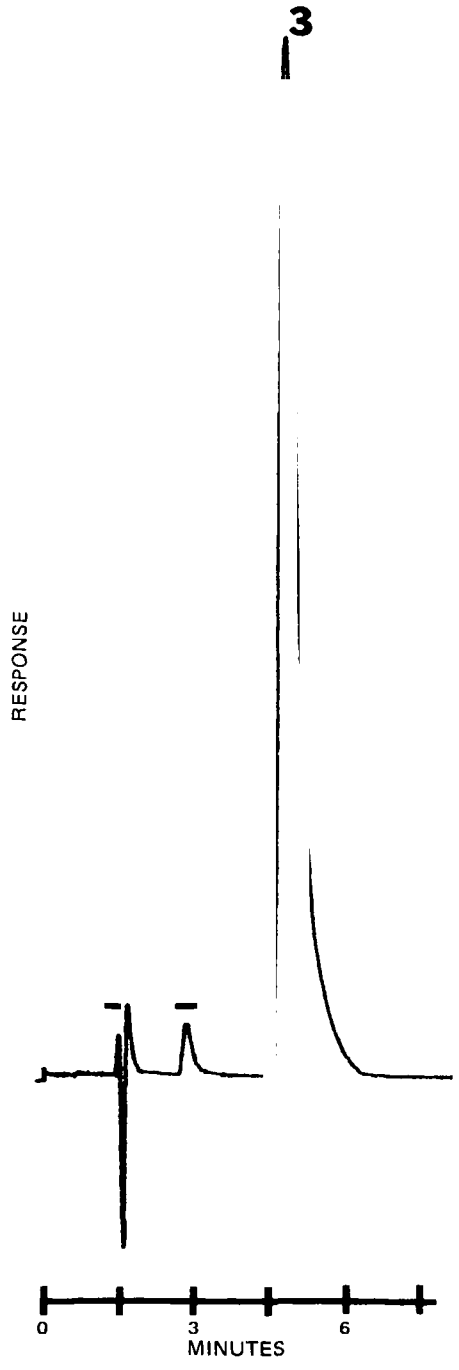

Figure 1—*Chromatogram of high lipoid extract obtained under the assay conditions. Key: 1 and 2, methylparaben; and 3, pramoxine hydrochloride.*

Information concerning potency, net content, and uniformity can be discerned simultaneously from the analytical data.

In this context, it became necessary to analyze 20–25-g high lipoid samples containing pramoxine hydrochloride, and several approaches were followed. Direct extraction with chloroform yielded substantial chromatographic interference. Dissolution of the sample required almost 1 liter of methanol–tetrahydrofuran (50:50).

The described extraction procedure required only 100 ml of ether–methanol and 175 ml of 20% acetic acid. The method had the advantage of partitioning the drug directly into the aqueous phase, which allowed determination by reversed-phase liquid chromatography without further manipulation.

To determine the accuracy and precision of the method, aerosol units were prepared quantitatively at three levels of potency, and these units were assayed. The average recovery at the 100% potency level was 100.4 ± 1.5 (*SD*)% (Table I). Recoveries at other potency levels were compa-

[2] A Waters Associates liquid chromatograph equipped with an M6000A pump and a Schoeffel variable-wavelength UV-visible detector were used.
[3] Abbott Laboratories, Chicago, Ill.
[4] Reagent grade.
[5] Burdick & Jackson, Muskegon, Mich.
[6] Pfaltz and Bauer, Stamford, Conn.
[7] FHUPO4700, Millipore Corp., Bedford, Mass.
[8] μBondapak C_{18}, Waters Associates, Milford, Mass.
[9] WISP, Waters Associates, Milford, Mass.
[10] Linear Instruments, Irvine, Calif.
[11] Minigrator, Spectra-Physics, Santa Clara, Calif.

rable. Analysis of the commercial preparation[12] gave results (Table II) that were consistent with the potency and the amount of the aerosol concentrate contained in each unit. Typical correlation coefficients for the standard curve were usually ≥0.999. Therefore, with the accuracy of the system established, the external standardization was considered satisfactory. A placebo preparation gave no interference.

The amount and concentration of the acetic acid solution were critical to the success of the extraction. If less than the prescribed amounts were used, difficulties with emulsification and incomplete recoveries resulted. A stronger acid such as hydrochloric acid could have been used at lower concentrations; but since the system was to be automated, a milder acid was preferred to preserve the instruments.

Prior to introduction into the high-pressure liquid chromatograph, good chromatographic practice generally requires filtering the analytical solutions through a fractional micrometer filter. This practice caused a 1–2% increase in recovery above that expected in the analytically prepared samples. Because of this feature, filtering samples is not recommended. The speed and accuracy of the method make occasional chromatograph filter and frit changes worthwhile.

A typical chromatogram of the high lipoid extract is shown in Fig. 1. The only excipient carried through the extraction and appearing on the chromatogram was methylparaben ($k' = 1$). Pramoxine hydrochloride ($k' = 2.2$) eluted in ~6 min.

The described method has general applicability to most pharmaceutical dosage forms containing pramoxine hydrochloride. In many cases, the drug can be extracted directly with the mobile phase or the entire composition can be dissolved. The high lipoid aerosol foam dosage form analysis was described because of the restrictions imposed on the analytical method.

[12] Non-Steroid Proctofoam, Reed & Carnrick, Kenilworth, N.J.

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Antileukemic Activity of 2-Bis(2-methylthio)vinyl-1-methylquinolinium Iodides

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Received September 13, 1979, from the *Samuel M. Best Research Laboratory, Massachusetts College of Pharmacy, Boston, MA 02115.* Accepted for publication November 16, 1979.

Abstract □ Reaction of 1-methylquinolinium-2-dithioacetic acid zwitterions with excess methyl iodide in dimethylformamide gave the corresponding bis(2-methylthio)vinyl derivatives. These compounds were more soluble in both aqueous and organic media than the dithioacetic acid zwitterions but showed comparable antileukemic activity in mice. Reaction with morpholine converted a bis(2-methylthio)vinyl derivative almost quantitatively to the 2-mono(methylthio)-2-morpholino derivative. Leukemia cell culture studies of the 6-methyl derivative showed no effect on cell cycle processes.

Keyphrases □ Antileukemic activity—2-bis(2-methylthio)vinyl-1-methylquinolinium iodides, comparative testing in cell culture and mice □ 2-Bis(2-methylthio)vinyl-1-methylquinolinium iodides—NMR analysis and synthesis of bis(methylthio)vinyl derivatives, comparative testing for antileukemic activity in cell culture and mice □ NMR spectroscopy—analysis, synthesized bis(methylthio)vinyl derivatives

1,6-Dimethylquinolinium-2-dithioacetic acid zwitterion (III*b*) was found to have appreciable activity against P-388 lymphocytic leukemia in mice (1) and against CD_8F_1 mammary tumor in mice[1]. Other 6-substituted 1-methylquinolinium-2-dithioacetic acid zwitterions showed comparable antileukemic activity in mice (2), regardless of the electron-donating or electron-releasing ability of the 6-substituent. Since these zwitterions were amorphous and poorly soluble in both aqueous and organic media, more soluble derivatives were desired having potential chemical reactivity similar to that of the dithioacetic acid function. Accordingly, the bis(methylthio)vinyl derivatives were prepared (Scheme I), and their antileukemic activities were determined. These derivatives are crystalline and more soluble in both water and organic solvents.

[1] Screening results were obtained from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.

DISCUSSION

Chemistry—Gompper *et al.* (3) reported the formation of the 2-bis(2-methylthio)vinyl compound (V*a*) by reaction of iodomethane with the dithioacetic acid zwitterion (III*a*). No reaction temperature or yield was stated. Mizuyama *et al.* (4) also reported the formation of a mono(methylthio) compound from the corresponding 1,2-dihydroquinoline analog and iodomethane, but no physical constants or experimental data were given. The procedure of Rosenhauer (5), in which the intermediate zwitterion (III*a*) was not isolated but was treated with excess iodomethane, was employed accordingly. The 6-unsubstituted bis(methylthio)vinyl compound and the corresponding 6-bromo derivative were reported previously (2).

Dilute sodium hydroxide generally sufficed for removal of the 2-methyl proton prior to condensation with carbon disulfide, but sodium hydride in 2-propanol was required for the 6-methoxy derivative. To obtain the bis(methylthio)vinyl derivatives, the methiodides (I) were treated with aqueous base, and the methylene derivatives (II) were extracted into toluene. The toluene solutions were treated with carbon disulfide, and the precipitated zwitterions (III) were taken up in dimethylformamide and were allowed to react with excess iodomethane. The 6-bromo and 6-unsubstituted analogs (3) were prepared by suspending the zwitterions (III) in dimethylformamide and adding iodomethane.

In the preparation of the methiodides (I), use of colored quinaldines gave impure methiodides, which could not be purified by recrystallization. Therefore, freshly distilled quinaldine was used. The methiodides were prepared with excess iodomethane without solvent. The quinaldines were prepared as described previously (2). The prepared bis(methylthio)vinyl compounds gave NMR spectra that agreed with the proposed structure (V). The *N*-methyl protons appeared at δ 4.4 ppm, and the aromatic protons appeared at δ 7.5–9.4 ppm. The 2-methyl protons of the methiodides at δ 3.1 ppm were absent in the bis(methylthio)vinyl derivatives; a methine proton appeared as a singlet at δ 6.6–6.8 ppm. The *S*-methyl protons were found as separate peaks at δ 2.5 and 2.7 ppm. The 6-methyl protons of V*b* appeared at δ 2.6 ppm, and the 6-methoxy protons (V*c*) were found at δ 4.0 ppm.

With the possible exception of the 6-bromo analog, none of the bis(methylthio)vinyl compounds was obtained in yields over 50%. The recrystallization liquors of the 6-methyl derivative were examined, and only one definable material was found, the original 1,2,6-trimethylqui-

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