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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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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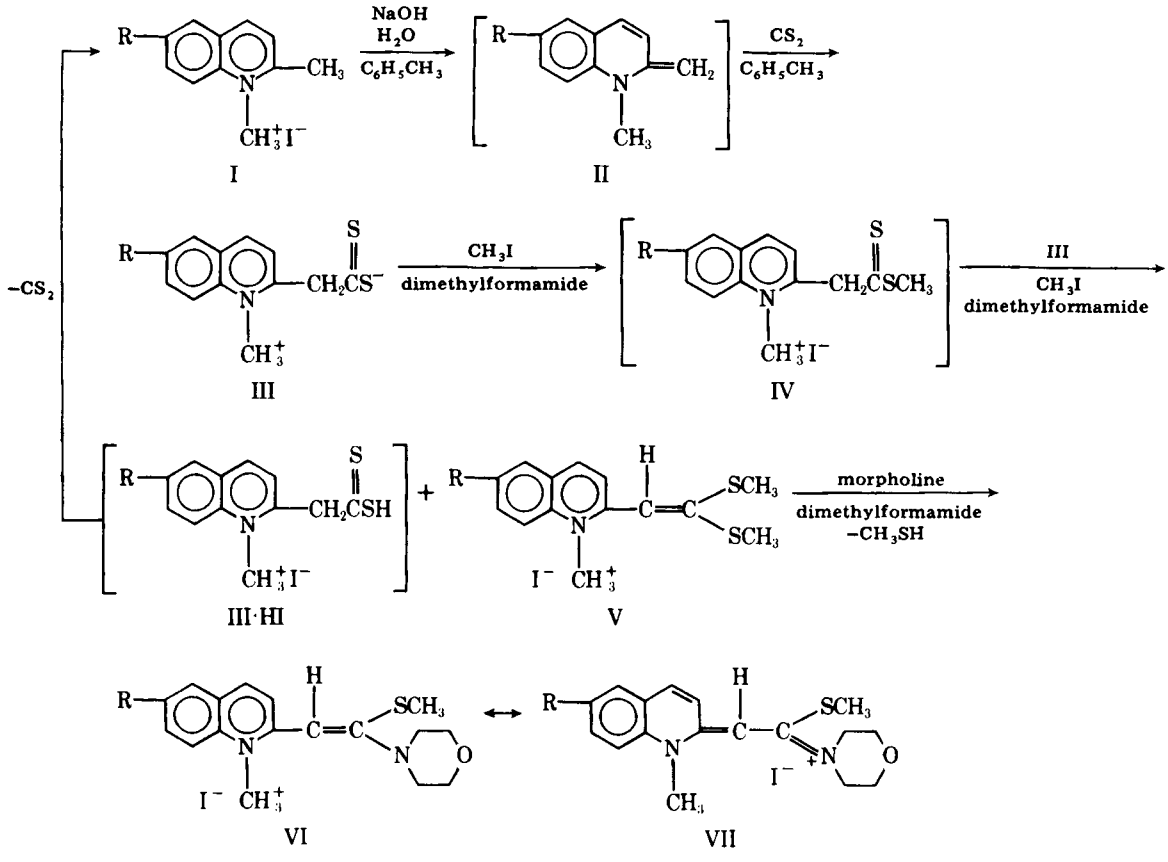


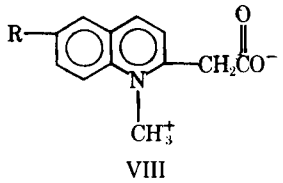
a: R = H
b: R = CH_3
c: R = OCH_3
d: R = Br

Scheme I

nolinium iodide. It is unlikely that this compound was unreacted starting material since it would not be soluble in the toluene extraction of the methylene base (II*b*). Instead, abstraction of one of the methylene protons of the mono(methylthio) derivative (IV*b*) by the intermediate zwitterion (III*b*) may have taken place to form a free dithioacetic acid (III*b*-HI), which lost carbon disulfide to give I*b*. The mono(methylthio) derivative then reacted with a second mole of iodomethane to give the bis(methylthio) compound (V*b*). The ease of abstraction of a methylene proton from quinolinium-2-acetate esters has been recorded (6).

An attempt was made to hydrolyze the 6-unsubstituted zwitterion (III*a*) with 3 *N* HCl. Hydrogen sulfide was evolved slowly at 25–35°, and the reaction still was incomplete after 10 days. Refluxing produced elemental sulfur. Evaporation of the resulting neutralized solution gave no organic products. A possible hydrolysis product, 1-methylquinolinium-2-acetate (VIII), was not found in the literature, and the ready decarboxylation of quinoline-2-acetic acids has been noted (7). Thus, with the synthesis of the quinoliniumdithioacetic acid zwitterions, it was possible to prepare stable sulfur analogs of acids that are not stable in the oxygen series. In addition, a recent attempt to prepare quinoline-2-acetic acid by carbonation of quinaldinyl lithium gave no identifiable acid (8).

As an indication of the reactivity of the bis(methylthio)vinyl compounds with amines, the 6-methyl derivative (III*b*) was reacted with morpholine in dimethylformamide at 35°. A 91% yield of the mono(methylthio)morpholino compound (VI*b*) was obtained over 5 days, with evolution of methanethiol. The 6-bromo analog (VI*d*) gave a comparable conversion to the mono(methylthio)morpholine derivative in a comparable period. Although these conditions were not physiological, the temperature was comparable; the mixture remained nearly neutral throughout the reaction. Evidently, the bis(methylthio)vinyl compounds may react readily with cellular amines.



VIII

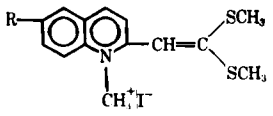
The NMR spectrum of VI*b* showed the remaining *S*-methyl protons as a singlet at δ 2.51 ppm. This finding allows the assignment of the *S*-methyl protons in the bis(methylthio) derivatives since the morpholine is assumed to be *trans* to the quinoline ring. The eight morpholine protons appeared as a broad singlet at δ 3.71 ppm. This observation can be explained by the existence of a partial positive charge on the morpholino nitrogen. The *N*-methyl peak at δ 4.09 ppm was shifted upfield from 4.41 ppm in the starting material, indicating decreased positive charge on the quinoline nitrogen. The vinyl proton, at δ 5.59 ppm, was shielded drastically compared with its position at δ 6.76 ppm in the bis(methylthio) compound. These facts, as well as the green color of the compound, indicate the contribution of resonance form VII to the structure and reinforce the assumption of the *trans* morpholine configuration.

Antileukemia Test Results—Antileukemia testing[2] of the bis(methylthio) compounds was performed in mice. The NCI protocol (9) and P-388 lymphocytic leukemia were used. Details regarding the dose and survival times of the treated animals are listed in Table I. No weight loss was found at the highest dose used, except with the 6-bromo derivative, which gave T/C (test/control) values of −0.3, −1.2, and −2.7 at 6.25, 12.5, and 25.0 mg/kg, respectively.

All tested compounds showed positive activity (a T/C × 100 ratio of 125 or better). Similar testing of the corresponding dithioacetic acid zwitterions (2) showed that the 6-unsubstituted derivative gave slightly less activity than the 6-substituted compounds. With the bis(methyl-

[2] Results were reported through the courtesy of Dr. Ralph G. Child, Lederle Laboratories; the 6-bromo derivative was tested at the National Cancer Institute (NCI).

Table I—Antileukemic Activities in Mice[a]



R	Dose, mg/kg[b]	Median Survival Time, T/C, %[c] Test 1	Test 2
H	25.0	115	
	12.5	120	130
	6.25	120	140
	3.12		125
	1.56		120
	0.78		110
CH_3	25.0	85	
	12.5	110	130
	6.25	120	130
	3.12		130
	1.56		120
	0.78		110
OCH_3	25.0	45	
	12.5	125	130
	6.25	125	110
	3.12		115
	1.56		110
	0.78		110
Br	25.0	107	
	12.5	125	127
	6.25	121	117
	3.12		110

[a] BDF_1 mice were inoculated with P-388 lymphocytic leukemia. [b] Drugs were administered intraperitoneally on Days 1, 5, and 9. [c] A T/C % value of ≥125 is considered a positive result.

thio)vinyl derivatives, the 6-unsubstituted compound was slightly more active than the 6-substituted derivatives, but the differences in both cases were small. Apparently, a 6-substituent is not required for antileukemic activity of the bis(methylthio)vinyl compounds.

Leukemic cell culture studies also were performed using the 6-methyl derivative[3]. Human leukemic lymphoblasts in culture were incubated with 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} *M* test compound for 24 hr. Then the cells were harvested, stained with propidium iodide, and analyzed by flow cytometry. The flow cytometry data showed no accumulation of cells in any particular phase of the cell cycle at any drug concentration used. This negative result *in vitro* may indicate that a metabolic conversion of the bis(methylthio)vinyl compounds as well as their dithioacetic acid precursors, possibly to the reactive methylene intermediates (II), may be necessary for antileukemic activity.

EXPERIMENTAL[4]

2-Bis(2-methylthio)vinyl-1,6-dimethylquinolinium Iodide—A suspension of 22.44 g (0.075 mole) of 1,2,6-trimethylquinolinium iodide (1) and 100 ml of water was covered with 450 ml of toluene and stirred while 22 g of 50% NaOH solution was added quickly. Stirring was continued for 1 hr, and the toluene layer was decanted, dried with potassium carbonate for 5 min, decanted, and treated with 6.77 ml (0.1125 mole) of carbon disulfide. The mixture was swirled, capped, and allowed to stand overnight at 25°. The precipitated zwitterion was filtered, washed with toluene, suspended in 75 ml of dimethylformamide, and treated with 14.0 ml (0.225 mole) of iodomethane.

The mixture was kept overnight at 25°, and the yellow-green powder was filtered, washed with 50 ml of acetone, and dried at 80° (30 torr) for 4 hr. Recrystallization from water, with cooling only to 25°, gave 8.3 g (27% yield) of bronze needles, mp 205–207° dec.; NMR: δ 2.50 (s, 3H, SCH_3), 2.60 (s, 3H, 6-CH_3), 2.70 (s, 3H, SCH_3), 4.41 (s, 3H, NCH_3), 6.76 (s, 1H, vinyl), and 7.95–9.00 (m, 5H, aromatic) ppm.

Anal.—Calc. for $C_{15}H_{18}INS_2$: C, 44.66; H, 4.50; N, 3.47; S, 15.90. Found: C, 44.60; H, 4.46; N, 3.39; S, 16.10.

6-Methoxy-1-methyl-2-bis(2-methylthio)vinylquinolinium Iodide—Sodium hydride (2.1 g, 0.05 mole) (57% dispersion in oil)[5] was allowed to react with 150 ml of 2-propanol at 27–37°, and the flask then was packed in ice. 1,2-Dimethyl-6-methoxyquinolinium iodide (2) (15.76 g, 0.05 mole) was added below 10°, with stirring for 15 min after the addition. Carbon disulfide (11.7 g, 0.15 mole) was added dropwise over 60 min, and stirring was maintained for another 60 min. The resulting red zwitterion was filtered and washed with ether. The solid was suspended quickly in 50 ml of dimethylformamide and treated with iodomethane (21.29 g, 0.15 mole).

The mixture was kept overnight at 30°, diluted with 100 ml of ether, allowed to stand at 25° for 3 hr, and filtered. The crude product was washed with ether, dried at 20° (1 torr) for 16 hr, and recrystallized from water (cooling only to 20°) to give 2.12 g (10% yield) of brown needles, mp 222–225° dec.; NMR: δ 2.40 (s, 3H, SCH_3), 2.60 (s, 3H, SCH_3), 3.90 (s, 3H, OCH_3), 4.35 (s, 3H, NCH_3), 6.63 (s, 1H, vinyl), and 7.6–9.1 (m, 5H, aromatic) ppm.

Anal.—Calc. for $C_{15}H_{18}INOS_2$: C, 42.96; H, 4.33; N, 3.34. Found: C, 43.04; H, 4.26; N, 3.29.

Isolation of 1,2,6-Trimethylquinolinium Iodide in Preparation of 2-Bis(2-methylthio)vinyl-1,6-dimethylquinolinium Iodide—The aqueous mother liquor from which V*b* was crystallized deposited no solid on prolonged cooling at 1°. The liquid, 700 ml, was treated with 0.5 g of activated charcoal[6] at 20°, and water was removed with a rotary evaporator until a solid appeared. The yield was 0.3 g, mp 240–244° dec. Further evaporation and three recrystallizations from 95% ethanol gave 1.9 g, mp 252–252.5° dec.; mixed with a sample of I*b*, the melting point was 248–250° dec. The NMR spectrum was identical to that of I*b*.

1,6-Dimethyl-2-[2-methylthio -2- (1-morpholino)vinyl]quinolinium Iodide—A flask was charged with 7.26 g (0.018 mole) of 2-bis(2-methylthio)vinyl-1,6-dimethylquinolinium iodide, 80 ml of dimethylformamide, and 1.74 g (0.020 mole) of morpholine (redistilled after drying with a 3-Å molecular sieve). A drying tube containing 20 g of indicating calcium sulfate[7] was attached, and the reaction was stirred magnetically at 35° for 5.5 days. The reaction could be followed by the progression of brown coloration on the calcium sulfate and the detection of the odor of methanethiol. Toluene, 80 ml, was added, and the mixture was stored at 25° for 4 hr.

Following filtration, the filtrate was diluted with 350 ml of toluene and kept at −20° to give a first crop of 6.78 g (85% yield), mp 198–199° dec. A second crop was obtained by heating the filtrate to 90°, diluting with 250 ml of toluene, and cooling to −20°; 0.44 g was obtained (6% yield), mp 197–199° dec.; NMR: δ 2.51 (s, 3H, SCH_3), 2.57 (s, 3H, 6-CH_3), 3.71 (s, br, 8H, morpholine), 4.09 (s, 3H, NCH_3), 5.59 (s, 1H, vinyl), and 7.65–8.20 (m, 5H, aromatic) ppm.

Anal.—Calc. for $C_{18}H_{23}IN_2OS$: C, 48.87; H, 5.24; N, 6.33. Found: C, 48.77; H, 5.04; N, 5.93.

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[3] Testing was performed by Dr. Ram Ganapathi, University of Miami School of Medicine, Miami, Fla.

[4] Melting points were determined in capillaries with a Mel-Temp block and are uncorrected. PMR spectra were obtained with a Varian T-60 spectrometer using dimethyl sulfoxide-d_6 as the solvent and tetramethylsilane as the internal standard. Elemental analyses were done by F. B. Strauss, Oxford, England, and by Instranal Laboratory, Rensselaer, N.Y. TLC was carried out using silica gel plates, and the products were detected by exposure to iodine vapor. Organic reagents were supplied by Aldrich Chemical Co. and Eastman Organic Chemicals.

[5] Alfa-Ventron.

[6] Aqua Nuchar A.

[7] Drierite.

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The authors are indebted to Dr. Ralph G. Child, Lederle Laboratories, and to the Division of Cancer Treatment, National Cancer Institute, for the antileukemia test results, and to Dr. Ram Ganapathi, University of Miami School of Medicine, for carrying out the cell culture studies.

COMMUNICATIONS

Hepatic Extraction of Endogenous Inhibitors of Plasma Protein Binding

Keyphrases □ Drug binding—plasma protein, hepatic extraction of endogenous inhibitors □ Pharmacokinetics—plasma protein binding, hepatic extraction of endogenous inhibitors □ Plasma protein binding—hepatic extraction of endogenous inhibitors

To the Editor:

Determination of the systemic intrinsic clearance of free (unbound) drug requires measurement of the drug's free fraction in blood, plasma, or serum. For drugs whose total clearance from the blood is much lower than the blood flow through the eliminating organ(s), the intrinsic clearance of the free drug is equal to the total clearance divided by the free fraction (1, 2). The use of free fraction values obtained in the usual manner (*i.e.*, by *in vitro* determinations of protein binding in plasma from peripheral venous blood) for intrinsic clearance calculations is based on the assumption that drug protein binding does not change as the blood passes through the liver or another drug-eliminating organ.

The free fraction value of many drugs in blood or plasma is reasonably constant over a wide drug concentration range. Consequently, it will not be affected by modest drug concentration changes during a single pass of blood through the liver. If, however, protein binding of the drug is affected by endogenous or exogenous (other drugs) inhibitors of binding, then the organ extraction ratio of these inhibitors must be considered. The potential consequences of hepatic extraction of binding inhibitors on the pharmacokinetics and pharmacological activity of a restrictively cleared drug can be appreciated by considering the following limiting cases.

If the extraction ratio [(concentration in inflowing blood − concentration in outflowing blood)/concentration in inflowing blood] of inhibitors of protein binding is close to zero (*i.e.*, there is very little extraction), then the inhibitors will cause an increase in the free fraction, a decrease in the steady-state total concentration, and *no change* in the steady-state free concentration of drug in peripheral blood or plasma (3). Since the pharmacological activity of a drug is usually a function of its free concentration (4), the intensity of pharmacological activity at steady state should not be affected by poorly extracted inhibitors of binding unless they have pharmacological effects of their own.

Conversely, if an inhibitor of protein binding is very rapidly and completely extracted from blood by the liver during a single pass (extraction ratio is near unity and the time for extraction of inhibitor from blood is much shorter than the transit time of that blood through the liver), then the inhibitor will have little or no effect on the steady-state concentration of total (free plus bound) drug, but it will cause the free fraction and the steady-state concentration of free drug in extrahepatic blood or plasma to be *increased*. This result would come about for two reasons:

1. Protein binding of the drug in blood is normal (rather than reduced) during most of the residence time of any one small portion of blood in the liver.
2. Protein binding of the drug leaving the liver is soon reduced as that blood mixes with inhibitor-containing blood in the vena cava, causing the free fraction and concentration of free drug to rise again.

Drug binding inhibitors with hepatic extraction ratios near unity may be cleared quite rapidly from the body. However, their concentration in blood can be quite stable if they are continuously formed endogenously, are continuously administered (other drugs or dietary components), or have a large apparent volume of distribution. Such inhibitors can be expected to increase the pharmacological activity of drugs that they displace by increasing the steady-state free concentration of these drug in extrahepatic blood. Since the steady-state concentration of total drug may be almost unchanged, the increased pharmacological activity may be unanticipated.

It is relatively easy to determine the hepatic extraction characteristics of drugs that act as displacers of other drugs from plasma protein binding sites; such a determination can be done by measuring the hepatic clearance of the displacer drugs. However, this approach is not possible (at least at this time) in the case of endogenous inhibitors associated with certain changes in the pathophysiological status of humans and animals. The hepatic extraction characteristics of binding inhibitors associated with pregnancy (5) and renal failure (6) are presently being investigated directly. To describe the method used for this investigation and to demonstrate in principle that certain endogenous inhibitors are indeed subject to considerable hepatic extraction, data on heparin-induced inhibitors of protein binding will be reported here. Since the composi-

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