Table II—Blood Pressure Effects

Dose, mg/kg	Depression of Peak Systolic, %
10	15 ± 13
20	20 ± 31
40	29 ± 34

doses occurred; although a dose–response relationship is suggested, an analysis of variance revealed no significant differences in responses among the amount of drug given, the order of dose administration, and the responses of the individual animals to a given dose.

The suggestion of neuromuscular depressive activity prompts one to evaluate statistically muscle depression with control muscle contraction by comparison with the Student t test for significance of depression of all doses given ($0.2 < p < 0.3$). However, with the small number of animals used in this pilot study ($n = 3$) and the variability encountered, it is perhaps not surprising that statistical significance was not achieved. Attempts at reversal of these neuromuscular effects with calcium chloride administered after the nadir failed to alter the pattern of recovery (lincomycin block had been previously shown not to be reversed by calcium) (2).

Depression of peak systolic blood pressure was noted with all but one of the doses given. Mean responses with 1 *SD* (Table II) suggest that this blood pressure effect is dose related; however, an analysis of variance revealed the same lack of significant differences among responses to doses, dose order, and animal studied.

DISCUSSION

The manufacturer recommends that clindamycin phosphate be diluted (6 mg/ml) and administered slowly (not to exceed 1200 mg in any 1-hr period). The dose range is approximately 5–20 mg/kg (8). In this experiment, doses were in this range and also double the maximum recommended dose. Initial clinical studies with clindamycin used intravenously noted no acute neuromuscular depressive or cardiovascular effects (9–11). However, a summary of side effects in more than 1000 patients includes instances of hypertension, hypotension, and cardiac arrest, and the manufacturer warns of possible neuromuscular blocking properties (8). Nevertheless, even with the lack of dilution and the rapid use of up to twice the usual dose, this study revealed a lack of statistically significant dose-related neuromuscular depressive or blood pressure effects in the cat.

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Unexpected Sulfuration Reaction of 1-Substituted Azulenes

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Abstract □ Azulenes reacted unexpectedly and readily with thionyl chloride to give sulfonic acid chlorides and bisthioethers. The sulfonic acids but not the thioethers have antibacterial activity.

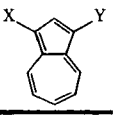
Keyphrases □ Azulenes, 1-substituted—reaction with thionyl chloride, antibacterial activity of resulting sulfonic acid derivatives and thioethers □ Antibacterial activity—sulfonic acid derivatives and thioether reaction products of 1-substituted azulenes with thionyl chloride □ Sulfuration reactions—1-substituted azulenes with thionyl chloride, antibacterial activity of resulting sulfonic acid derivatives and thioethers

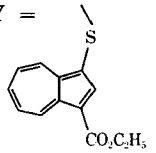
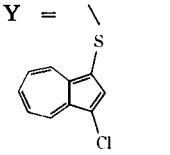
While preparing some derivatives of azulene *via* Friedel–Crafts acylation (1, 2) using acid chlorides prepared *in situ* with thionyl chloride, the unexpectedly facile reaction of 1-carboxyethylazulene and thionyl chloride was noted. Under the reaction conditions, the primary product was apparently the 3-sulfinic acid chloride, which rapidly disproportionated giving rise to the corresponding bisthioether and sulfonic acid chloride.

The corresponding sulfonic acid is relatively unstable and could not be completely characterized, but its identity was inferred by formation of the more stable amide derivative upon treatment of the anhydrous system with gaseous ammonia. In seeking to confirm the course of the reaction utilizing the more reactive unsubstituted parent compound, azulene, bis(3-chloroazulyl)thioether was separated with difficulty from polymerization products.

The sulfonic acid and sulfonamide show selective

Table I—Physical and Spectral Characteristics of New Azulene Derivatives



Substituents	Color and Form	Melting Point	Spectral Characteristics				Analysis[d], %	
			λ_{max}, nm[a]	log ε	IR, μ[b]	NMR, ppm[c]	Calc.	Found
X = $CO_2C_2H_5$ Y = SO_2NH_2	Red crystals	95–97°	Ethanol 229 251 (s) 284 (s) 289 301 468	 4.28 3.93 4.27 4.43 4.47 3.77	3.43 3.50 5.95 8.87 12.81 13.47	$CDCl_3$ 1.42 (t) 4.39 (q) 4.73 (s) 8.63 (s) 8.88 (d) 9.65 (d)	$C_{13}H_{13}NO_4S$: C 55.90 H 4.69 N 5.01 S 11.48	 54.07 4.91 4.88 11.02
X = $CO_2C_2H_5$ Y = SO_3H	Red crystals	70–72°	Ethanol 230 252 (s) 282 (s) 288 300 464	 3.72 3.35 3.69 3.81 3.90 3.19	3.02 3.43 5.96 8.27 9.60 12.82 13.43 15+	D_2O 1.40 (t) 4.30 (q) 8.43 (s) 8.49 (d)	Rapidly degrades at ambient temperature	
X = $CO_2C_2H_5$ Y = 	Green scales	127°	CCl 291[4] 302 (s) 318 (s) 373	 4.71 4.68 4.44 4.09	3.49 5.96 7.08 12.96 13.62	$CDCl_3$ 1.24 (t)[e] 4.23 (q) 8.08 (s) 8.68 (d)	$C_{26}H_{22}O_4S$: C 72.5 H 5.15 S 7.43	 72.67 4.83 7.43
X[f] = Cl Y = 	Green scales	57°	CCl_4 268 (s) 278 (s) 281 284 289 295 (s)	 4.33 4.45 4.47 4.48 4.49 4.48	3.48 12.72 13.63 14.51	9.36 (d)	$C_{20}H_{12}Cl_2S$: C 67.60 H 3.40 S 9.03	 67.64 3.21 8.91

[a] s = shoulder. [b] Major, correlated peaks only. [c] s = singlet, d = doublet, t = triplet, and q = quartet; complex patterns are not noted. [d] Elemental analyses were performed by Schwarzkopf Laboratories, New York, N.Y., and by Spang Microanalytical Laboratories, Ann Arbor, Mich. [e] A molecular ion of 430.12 amu was observed on the mass spectrum of this material; the spectrum was consistent with the identification of this compound as bis(3-carboxyethylazulyl)thioether. [f] Separated with difficulty from polymerization products.

antibacterial activity, but the highly insoluble thioethers do not.

EXPERIMENTAL

Materials—Azulene[1] was of 99+% purity; all other materials were of USP or reagent grade.

Physical Data—IR spectra[2] were obtained using potassium bromide disks. UV and visible spectra[3] were obtained using alcohol as solvent. NMR[4] spectra were obtained at ambient temperature using tetramethylsilane as the internal standard, and mass spectra[5] were obtained by direct probe methods. Melting points[6] are uncorrected.

General Chromatographic Procedure—Chromatographic separations were done on either alumina[7] or silica gel[8], utilizing the following procedure. Concentrated solutions of the reaction mixture, reaction mixture filtrates, or reaction mixture extracts were mixed with a small amount of adsorbent (5–15 g) and triturated until a dry, free-flowing powder was obtained. The adsorbate was packed above a 2 × 10-, 3 × 15-, or 4 × 40-cm column of the appropriate adsorbent and eluted with solvents of increasing polarity. The following solvents proved useful, either alone or in combination: petroleum ether, cyclohexane, carbon tetrachloride, toluene, chloroform, methylene chloride, *n*-butanol, 2-propanol, ethanol, and methanol.

[1] Henley and Co., New York, N.Y.
[2] Perkin-Elmer Infracord model 127 recording spectrophotometer.
[3] Cary model 15 recording spectrophotometer.
[4] Jeolco JNM-MH 6011 (60 MHz) recording spectrometer.
[5] Finnigan model 3000D mass spectrometer.
[6] Thomas–Hoover Unimelt, open capillary.
[7] Merck reagent aluminum oxide No. 71707.
[8] Mallinckrodt silicic acid powder No. 2847.

Chemistry—Physical and spectral data of these compounds are presented in Table I.

Thioethers—A solution of 5 mmoles of the appropriate azulene derivative and 3.57 g (30 mmoles) of thionyl chloride in 10 ml of benzene was refluxed for 1 hr and poured over crushed ice. The resultant biphasic system was made basic with ammonium hydroxide and stirred in an open vessel until the benzene evaporated spontaneously. Attempts to speed evaporation with heat drastically lowered yields. The solids were collected on a sintered-glass funnel and chromatographed on alumina using the general chromatographic method. Toluene eluted starting materials and several unidentified small bands; chloroform eluted the green thioethers. Typical yields averaged about 38% of theoretical.

3-Carboxyethylazulene Sulfonic Acid—A solution of 1.000 g (5 mmoles) of ethylazuloate and 3.57 g (30 mmoles) of thionyl chloride in 10 ml toluene was magnetically stirred for 24 hr in an open beaker contained in a sealed desiccator charged with solid sodium hydroxide. The reaction mixture was poured over crushed ice and made basic with ammonium hydroxide, and the phases were separated. The toluene phase was extracted once more with ammonium hydroxide.

The combined aqueous phases were made acidic with hydrochloric acid and extracted with *n*-butanol, and the *n*-butanol extract was subjected to chromatographic separation on silica gel. The material obtained from the major fraction eluted from the column by methanol was redissolved in water, made acidic with hydrochloric acid, and extracted into *n*-butanol. Evaporative concentration of the *n*-butanol yielded 3-carboxyethylazulene sulfonic acid as red crystals; yields averaged 12% of theoretical.

Treatment of the toluene phase as described under *Thioethers* affords small quantities of bis(3-carboxyethylazulyl)thioether and of 3-chloroethyl azuloate.

3-Carboxyethylazulene Sulfonamide—A solution of 1.000 g (5 mmoles) of ethyl azuloate and 3.57 g (30 mmoles) of thionyl chlo-

ride in 10 ml of toluene was magnetically stirred for 24 hr in an open beaker contained in a sealed desiccator charged with solid sodium hydroxide. The toluene solution was saturated with dry ammonia, allowed to stand in a closed vessel for an additional 72 hr, and poured over crushed ice. The resultant biphasic system was made alkaline with ammonium hydroxide and allowed to stand with stirring in an open vessel until the toluene had spontaneously evaporated. The aqueous suspension was then filtered through a sintered-glass funnel.

The filtrate was made acidic with hydrochloric acid and extracted with chloroform, and the chloroform extract was subjected to chromatographic separation on an alumina column. The material obtained from the major fraction eluted from the column with 2-propanol was redissolved in water, made acidic with hydrochloric acid, and extracted into chloroform. Evaporative concentration of the chloroform yielded 3-carboxyethylazulene sulfonamide as red crystals; yields averaged 16% of theoretical.

Chromatographic separation of the residue on the filter yielded small quantities of 3-chloroethyl azuloate and bis(3-carboxyethylazulyl)thioether.

Microbiological Testing—Preliminary screening of the compounds 3-carboxyethylazulene sulfonic acid, 3-carboxyethylazulene sulfonamide, bis(3-carboxyethylazulyl)thioether, and bis(3-chloroazulyl)thioether was accomplished by a simple bioautographic method (3). The preliminary screening was performed in duplicate using *Escherichia coli*[9] and *Staphylococcus aureus* (ATCC 6538) as test organisms. Erythromycin stearate[10] was employed as a positive control.

Because 3-carboxyethylazulene sulfonamide was more active, stable, and water soluble than the other test compounds, it was selected for additional screening. A sensitivity disk method (4) was used against eight microorganisms: *Corynebacterium xerosis* (ATCC 373), *Staph. aureus* (ATCC 6538), *E. coli, Pseudomonas aeruginosa* (ATCC 8308), *Proteus vulgaris, Streptococcus pyogenes* (ATCC 8668), *Candida albicans* (ATCC 1950), and *Mycobacterium fortuitum*[9]. All tests were performed in duplicate.

An aqueous solution of sodium bicarbonate was utilized as a negative control, and commercially available disks impregnated with sodium ampicillin[10] were utilized as a positive control. The equimolar stock solutions were sterilized prior to use by passage through sterile Swinney filters. The nutrient medium was brain–heart infusion agar[11]. Cultures were incubated for 24 and 48 hr, and the diameters of zones of inhibition were measured to the nearest millimeter.

RESULTS AND DISCUSSION

The facile route to the preparation of azulene sulfonic acids, the corresponding sulfonamides, and symmetrical azulylthioethers employing thionyl chloride is reported. Few methods for the sulfuration of the azulene nucleus have been reported (5), and all reported methods employ sulfur trioxide as the primary agent.

The proportions of thioether and presumed intermediate sulfonyl chloride formed in the reaction are sensitive to reaction conditions. The thioether predominates after reaction at 80° and above, while the sulfonyl chloride is the major product after reaction for an extended period at ambient temperature.

The physical and spectral properties and elemental analytical data are presented in Table I.

The free sulfonic acid and sulfonamide inhibited the growth of *Staph. aureus*, as evidenced by clear zones of inhibition in seeded culture medium overlaying the bands of these compounds on the thin-layer bioautographic plate. The thioethers, which are highly water insoluble, were inactive against this organism. No azulene compound showed activity against *E. coli*.

In the study employing the sensitivity disk method, 3-carboxyethylazulene sulfonamide produced zones of 11 and 12 mm on the *S. pyogenes* culture and zones of 13 and 14 mm on the *M. fortuitum* culture after 24 hr.

Further work is underway to obtain 50% inhibitory concentration values as well as kinetic growth curves for these and other members of several series of azulenoid derivatives possessing antibacterial activity.

Because these sulfonic acid derivatives do not possess amino functions, it appears that they exert their antibacterial properties independently of the folate synthetase system.

[9] The identity of cultures of *E. coli, P. vulgaris,* and *M. fortuitum* was verified by a combination of staining reactions, morphological features, and biochemical reactions.

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[11] Difco Laboratories, Detroit, Mich.

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