Table I—Standard Deviations and Relative Standard Deviations

	II		III		IV		V	
	s%	$2s$%	s%	$2s$%	s%	$2s$%	s%	$2s$%
Chromatographic variability	0.0075	5.83	0.0663	2.80	0.0083	1.27	0.0183	3.39
Standard variability	0.0040	1.79	0.0081	2.34	0.0051	6.58	0.0016	2.20
Sample variability	0.029	5.44	0.049	4.92	0.0002	8.11	0.0012	4.10
Procedural variability	0.023	3.68	0.044	4.47	0.00017	7.60	0.0008	2.77

Table II—Representative Samples [a]

Sample[b]	II, %	III, %	IV, %	V, %
Capsule 1	1.95	1.13	1.30	3.19
Capsule 2	1.08	1.08	0.05	0.16
Capsule 3	1.98	1.08	0.83	4.01
Capsule 4	2.07	1.05	0.06	0.22
Capsule 5	2.05	1.12	0.02	0.13
Powder 1	1.88	1.09	0.004	0.068
Powder 2	1.99	1.05	0.005	0.054

[a] Percentages are based on the labeled claim of tetracycline. [b] Hard, filled capsules and bulk powders were used.

Accuracy of the assay was determined by spike recoveries of impurities II–V from I. Tetracycline (I) was spiked with impurities at 100% of the target value. The percent recoveries were 96, 102, 104, and 85% for II, III, IV, and V, respectively.

Several tetracycline-containing products were analyzed for impurities (II–V) (Table II).

In summary, the reversed-phase HPLC method described in this paper provides a rapid, sensitive, simple, and quantitative method for the simultaneous determination of impurities (II–V) in tetracycline (I).

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Aminoalkanesulfonic Acids IV: Synthesis of Mitodepressive *N*-Nitrosoaminoalkanesulfonic Acids

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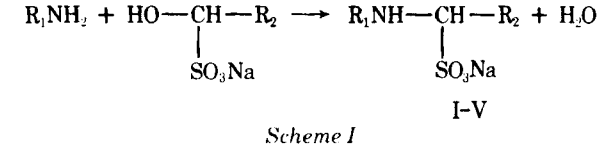
Abstract □ Sodium aminoalkanesulfonates were synthesized from substituted amines and aldehyde sodium bisulfites at 40–60°. Nitrosation yielded the corresponding *N*-nitroso derivatives. Mitodepressive activity on cresse (*Lepidium sativum* L.) was determined, and all compounds examined possessed growth inhibition activity. The *N*-nitroso derivatives were slightly less active but also showed less toxicity than the parent aminoalkanesulfonic acids.

Keyphrases □ Aminoalkanesulfonic acids—synthesis of *N*-nitroso derivatives, evaluation for mitodepressive activity □ Mitodepressive activity—evaluation of aminoalkanesulfonic acids and *N*-nitroso derivatives □ *N*-Nitrosoaminoalkanesulfonic acids—synthesis, evaluation for mitodepressive activity

Aminoalkanesulfonic acids are known to have antiviral (1, 2) and anticancer (3, 4) activities. Some *N*-nitroso compounds, such as *N*-nitrosomethylamine, *N*-nitrosodimethylamine, and *N*-nitrosodiethylamine, showed blastogenic action when applied on Hungarian hamsters (5). Some experiments (6) showed imbalances in DNA and histone synthesis during carcinogenesis induced by nitrosamines. On the other hand, some *N*-nitroso compounds, such as 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine), are useful in the treatment of certain malignant diseases (7).



Scheme I

These findings prompted the synthesis of some new derivatives in this series (8–10) to determine their mitodepressive activity.

This report describes the synthesis of some sodium aminoalkanesulfonates (I–V) in which *m*- and *p*-chloroanilines were used with the corresponding aldehyde sodium bisulfites (Scheme I and Table I). In some cases, the desired products were obtained by the addition of sodium metabisulfite to azomethine derivatives (Scheme II).

Nitrosation of I–V and sodium *p*-chloroanilinophenyl-

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Table I—Physical Properties and Reaction Conditions of Sodium Aminoalkanesulfonates

Compound	R_1	R_2	Reaction Conditions (Hours, Temperature, Method)	Yield, %	Melting Point	Recrystallization Solvent	Molecular Formula	Molecular Weight	Analysis, % Calc.	Found	IR ν_{max}, cm^{-1}
I	3-Chloro-phenyl	H	1, 60°, A	75	210–212°	Methanol	$C_7H_7ClNNaO_3S \cdot H_2O$	261.665	N 5.35	5.25	3600, 3320, 1210–1180, 1040
II	3-Chloro-phenyl	CH_3	2, 40°, A	40	123–125°	Ethanol	$C_8H_9ClNNaO_3S \cdot H_2O$	275.691	C 35.00 H 4.05 N 5.12	34.75 4.19 4.77	3535, 3400, 1210–1180, 1020
III	3-Chloro-phenyl	Phenyl	2, 65°, B and C	48	156–158°	Ethanol	$C_{13}H_{11}ClNNaO_3S \cdot H_2O$	355.843	C 43.88 H 4.03 N 4.38	43.90 4.33 4.18	3550, 3350, 1120–1180, 1140
IV	4-Chloro-phenyl	H	1, 60°, A	76	280–284°	Ethanol	$C_7H_7ClNNaO_3S \cdot H_2O$	261.665	N 5.35	5.45	3650, 3300, 1200, 1050
V	4-Chloro-phenyl	CH_3	1, 40–50°, A	45	117–121°	Ethanol	$C_8H_9ClNNaO_3S \cdot H_2O$	275.691	C 35.00 H 4.05 N 5.12	34.91 4.34 5.27	3535, 3400, 1210–1180, 1020
VI[a]	4-Chloro-phenyl	Phenyl	1, 40°, A and C	50, 70	144–147°	Ethanol	$C_{13}H_{11}ClNNaO_3S$	355.843	—	—	3600, 3350, 1215, 1040

[a] Compound VI was synthesized by Neelekantan and Hartung (11).

Table II—Physical Properties and Reaction Conditions of Sodium *N*-Nitrosoaminoalkanesulfonates

Compound	R_1	R_2	Yield, %	Melting Point	Recrystallization Solvent	Molecular Formula	Molecular Weight	Analysis, % Calc.	Found	IR ν_{max}, cm^{-1}
VII	3-Chloro-phenyl	H	50	250–252°	Methanol–water	$C_7H_7ClN_2O_4S$	238.212	N 11.76	11.43	1500–1450, 1210–1180, 1040
VIII	3-Chloro-phenyl	CH_3	83	117–121°	Methanol–ethanol	$C_8H_8ClN_2NaO_4S$	286.687	N 9.77	9.42	1460, 1210–1180, 1040
IX	3-Chloro-phenyl	Phenyl	56	99–103°	Ethanol	$C_{13}H_{20}ClN_2NaO_4S$	348.753	N 8.03	8.17	1470, 1220–1180, 1040
X[a]	4-Chloro-phenyl	H	25	254–255°	Methanol–ethanol	$C_7H_7ClN_2O_4S$	238.213	N 11.76	11.38	1460, 1210, 1040
XI	4-Chloro-phenyl	CH_3	31	119–121°	Ethanol	$C_8H_8ClN_2NaO_4S$	286.687	N 9.77	9.38	1450, 1205–1190, 1040
XII[a]	4-Chloro-phenyl	Phenyl	31.5	250°	Methanol–ethanol	$C_{13}H_{10}ClN_2O_4S$	326.763	C 47.90 H 3.39 N 8.57	48.09 3.51 8.17	3550–3130, 1460, 1210–1180, 1040
XIII	4-Chloro-phenyl	Phenyl	58	119–122°	Ethanol	$C_{13}H_{10}ClNNaO_4S \cdot H_2O$	366.53	C 42.56 H 3.29 N 7.63	42.21 3.35 7.40	1460, 1210–1190, 1040

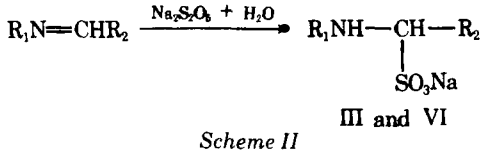
[a] Isolated in free acid form.

methane sulfonate[1] (VI) yielded the corresponding sodium *N*-nitrosoaminoalkanesulfonates (VII–XIII) (Scheme III and Table II). Compounds X and XII were isolated in the form of free sulfonic acids.

All products were tested for mitodepressive activity on cresse seeds (*Lepidium sativum* L.) by a literature method (12).

DISCUSSION

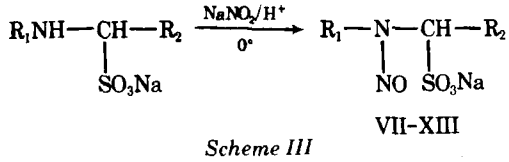
For the synthesis of the sodium salts of *m*- and *p*-chloroanilinoalkanesulfonic acids (I–VI), Method A was used at lower temperatures and shorter reaction times than those described by Neelekantan and Hartung



III and VI

Scheme II

[1] Compound VI was synthesized by Neelekantan and Hartung (11).



VII–XIII

Scheme III

(11). These conditions produced higher yields of both *m*- and *p*-chloroanilinoalkanesulfonic acids.

The method of Greco *et al.* (13) for nitrosation of α-aminocarboxylic acids was modified by employing a significantly shorter reaction time and a higher reaction pH to avoid extensive decomposition of the products.

In previous work (10), phytobiological experiments were carried out on a group of aminoalkanesulfonates in which 2-aminopyrimidine and 2-aminopyridine were incorporated. Significant inhibitory properties were observed.

The phytobiological experiments (Tables III and IV) showed that inhibitory properties of sodium alkanesulfonates (I–VI) varied between 50 and 80%. The R_1 and R_2 substituents did not influence inhibition greatly. Inhibition properties of *N*-nitroso derivatives (VII–XII) tended to be somewhat lower than those of the parent compounds. On the other hand, aminoalkanesulfonates were more toxic than the corresponding *N*-nitroso derivatives. Cresse seeds recovered their biological properties, such as growth, more readily when treated with *N*-nitroso compounds.

Table III—Inhibitory Properties of Sodium Aminoalkanesulfonates

Compound	R_1	R_2	Inhibition, % 2 mg/ml	1 mg/ml
I	3-Chlorophenyl	H	56	38
II	3-Chlorophenyl	CH_3	78	75
III	3-Chlorophenyl	Phenyl	70	61
IV	4-Chlorophenyl	H	48	38
V	4-Chlorophenyl	CH_3	80	73
VI[a]	4-Chlorophenyl	Phenyl	70	53

[a] Synthesized by Neelekantan and Hartung (11).

Table IV—Inhibitory Properties of Sodium *N*-Nitrosoaminoalkanesulfonates

Compound	R_1	R_2	Inhibition, % 2 mg/ml	1 mg/ml
VII	3-Chlorophenyl	H	41	40
VIII	3-Chlorophenyl	CH_3	60	52
IX	3-Chlorophenyl	Phenyl	52	41
X[a]	4-Chlorophenyl	H	40	30
XI	4-Chlorophenyl	CH_3	46	28
XIII	4-Chlorophenyl	Phenyl	75	67

[a] Isolated in free acid form.

Mitodepressive effects of *N*-nitroso compounds probably result from a combination of the inhibitory effect of the parent sulfonic acids and the growth-stimulating activity of the incorporated *N*-nitroso group in VII–XII. Preliminary experiments with carcinoma Hep-2 cells of the nasopharynx showed that the parent compounds (I–VI) were predominantly cytotoxic while the related *N*-nitroso derivatives (VII–XIII) had mainly cytostatic activity.

EXPERIMENTAL[2]

Preparation of Sodium *m*- and *p*-Chloroanilinoalkanesulfonates—*Method A*—*m*- or *p*-Chloroaniline (6.3 g, 50 mmoles) and formaldehyde, acetaldehyde, or benzaldehyde sodium bisulfite (5.2 mmoles) were mixed in water (10 ml) for 1–2 hr at 40–50°. The reaction mixture was cooled to room temperature, and the crystals that formed were separated and recrystallized.

Method B—Benzaldehyde (5.3 g, 50 mmoles) was stirred with a solution of sodium metabisulfite (5.3 g, 2.8 mmoles) in 30 ml of water at room temperature for 1 hr. *m*- or *p*-Chloroaniline (6.3 g, 50 mmoles) was added to the reaction mixture, and the mixture was stirred and warmed to 60° for 1 hr. After cooling, the resulting crystals were removed by suction, dried in air at room temperature, and recrystallized.

Method C—Benzylidenechloroaniline (2.2 g, 10 mmoles) was warmed in a solution of sodium metabisulfite (2.7 g, 14 mmoles) in 10 ml of water for 1 hr at 40–50°. On cooling, the crystals that formed were isolated and recrystallized.

[2] Melting points were determined using a Buchi (Totoli) melting-point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 457 grating instrument using potassium bromide wafers. TLC was carried out mainly on silica gel F-254 with butanol–concentrated ammonia (8:2) or benzene–methanol–acetic acid (7:2:1) as the solvent system.

Synthesis of Sodium *N*-Nitrosoaminoalkanesulfonates—A solution of sodium nitrite (7 mmoles) in 1 ml of water was added to a suspension (5 mmoles) of sodium chlorosulfonic acids (I–VI) in 10 ml of water at 0°. Dilute (5%) hydrochloric acid was added with mixing at 0–5° until pH 4 was reached. Stirring was continued for an additional 20 min at room temperature, and the solvent was removed at 30°. Colored crystals were obtained and purified by dissolution in methanol and precipitation in ether at room temperature.

The purity of all products was established chromatographically.

Phytobiological Experiments—Chromatographically pure products were tested for mitodepressive activity on germinating seeds of cresse[3] (*L. sativum* L.) in concentrations of 1 and 2 mg/ml. The values for inhibitory properties are calculated according to:

$$\% I = \frac{L_B - L_X}{L_B} \times 100 \qquad \text{(Eq. 1)}$$

where L_B is the mean value of the length of the cresse radicles formed by germination at 25 ± 1° in water and L_X is the mean value of cresse radicles formed in aqueous solutions of the tested compounds.

The experiments were carried out in the following manner. Twenty to 30 cresse seeds were placed on filter paper in a petri dish and covered with 15 ml of water. After the seeds were incubated for 24 hr at 25 ± 1°, water was decanted and an aqueous solution of the experimental compounds was added. One seed sample left in the water was used as a blank.

After further incubation for 24 hr at the same temperature, test solutions were decanted. The seeds were washed several times with water and fixed with a solution containing 2 g of salicylic acid, 2 g of zinc sulfate, and 15 drops of freshly prepared concentrated sodium phenoxide in 100 ml of distilled water. After fixation, radicles from 20 seeds were measured by using millimetric paper and a lens. A mean value of the measured radicle length was found for each sample, and the inhibitory properties were calculated (Tables III and IV).

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