where the difference between the two $pK_a'$ values is sufficient that they do not interfere with each other in the potentiometric determination. Thus the $pK_a'$ for *p*-cresol was found by the spectrophotometric method to be 10.14 and by potentiometric titration to be 10.08. These values correspond well with another recent determination of 10.10 (apparent) and 10.19 (thermodynamic).[2] The spectrophotometric and potentiometric phenolic $pK_a'$ values for *o*-tyramine were in good agreement, *i.e.*, 9.52 and 9.46, respectively. The $pK_a'$ for phenethylamine, 9.88 (average of 5 determinations), agrees well with the values reported earlier, *i.e.*, 9.86,[3] 9.83,[2] and 9.85.[26] The dissociation constants obtained from the spectrophotometric data have a relative accuracy of ±0.05 unit and the data from the titrations ±0.1 unit.

The dissociation constants for the amino groups in the phenolic amines were determined as follows; as an example the procedure used for *p*-tyramine is described. The dissociation constant (9.74) for the phenolic group was obtained from the spectrophotometric studies. At this pH it can be seen from the titration curve (Figure 5) that 67% neutralization had been effected. From this it is apparent that at half-neutralization of the phenolic group the amino group had been 17% neutralized as well. Because the titration curves for the individual groups should be symmetrical, it follows that half-neutralization of the amino group would be completed when 150 − 17% of the total 200% of alkali are added. The assumption that half-neutralization of the amino function occurs after addition of 133% of the alkali leads to $pK_a' = 10.52$. By using in this manner the spectrophotometrically determined $pK_a'$ for the phenolic group it was possible to estimate the per cent of the amino group ionized at the $pK'$ of the phenolic group for the different compounds. This ranged from zero or very small in the case of *o*-tyramine to 52% for **17** [N,N-dimethyl-β-(4-hydroxy-3-methoxyphenyl)-ethanolamine] and 55% for **14** [N,N-dimethyl-β-(4-hydroxyphenyl)ethanolamine].

(26) G. Bardinet and M. Metayer, *Compt. rend. soc. biol.*, **226**, 490 (1948).

# Antifungal Activity of a Series of Substituted [(α-Nitroalkyl)benzylthio]alkylamines[1]

ROBERT C. TWEIT, R. D. MUIR, SETH MIZUBA,

*Chemical and Biological Research Divisions, G. D. Searle & Co., Chicago, Illinois 60680*

AND WEBSTER R. CROWLEY, JR.

*The Morton Arboretum, Lisle, Illinois*

*Received November 9, 1964*

β-Nitrostyrenes, as well as compounds obtained from them by addition of thiols, are known to be antifungal agents and interest arose in preparing some water-soluble agents of this type. This was done by adding 2-aminoethanethiol hydrochloride to β-nitrostyrene (eq. 1). Strong electron-donating groups on the aryl ring prevented the addition, but a ring nitro group overcame this hindrance. Other substituents seem to have little effect. Addition is also more difficult when the amino group is replaced by dialkylamino. As antifungal agents the compounds inhibited the growth of *Trichophyton mentagrophytes*, *Candida albicans*, and *Ceratocystis ulmi in vitro*. In *in vivo* testing in mice, however, there was considerable toxicity at fungicidal levels. The compounds also showed activity *in vitro* against the bacteria *Bacillus subtilis*, *Escherichia coli*, *Diplococcus pneumoniae*, and *Erwinia carotovora;* the alga *Chlorella vulgaris;* and the protozoan *Tetrahymena geleii*. The most promising activity shown by these compounds has been against *Ceratocystis ulmi*, the organism responsible for Dutch elm disease. Several of the compounds caused inhibition of the growth of the fungus in trees, and field testing is currently in progress.

In recent years, a number of reports have been published on the antibacterial and antifungal activity of β-nitrostyrenes[2] and 2-nitro-1-phenyl-1-phenylthio(or alkylthio)alkanes[3] obtained by the addition of thiols to β-nitrostyrenes.[4] While many of these compounds have shown good antibiotic activity *in vitro*, Evans, *et al.*,[5] have shown that at least some of the nitrostyrenes are inactive in the mouse, possibly due to irreversible binding to sulfhydryl or amino groups present in blood.

We felt that the water-soluble compounds obtained by addition of 2-aminoethanethiol hydrochloride to β-nitrostyrenes might have advantages over the water-insoluble nitrostyrenes.[6] Accordingly, a series of these compounds was prepared and tested qualitatively for antibiotic activity against the following microorganisms: bacteria, *Bacillus subtilis*, *Escherichia coli*, *Diplococcus pneumoniae*, and *Erwinia carotovora;* fungi, *Candida albicans*, *Trichophyton mentagrophytes*, and *Ceratocystis ulmi;* alga, *Chlorella vulgaris;* and protozoan, *Tetrahymena geleii*.

All of the compounds displayed some degree of antibiotic action against most of the test organisms. *Candida albicans* was the most resistant organism, being affected by only 13 of the 34 compounds while *T. mentagrophytes* and *T. geleii* were universally susceptible.

(1) Presented before the Division of Medicinal Chemistry, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964. Abstracts, p. 23P.

(2) (a) M. Pianka, *J. Sci. Food Agr.*, **14**, 48 (1963), and ref. 4–17 cited therein; (b) M. Faguet, *Ann. inst. Pasteur*, **88**, 713 (1955); *Chem. Abstr.*, **49**, 12590*g* (1954); (c) F. Schönhöfer and M. Schoog, *Arzneimittel-Forsh.*, **8**, 374 (1958); (d) M. Koremura, *Takamine Kenkyusho Nempo*, **13**, 205, 212 (1961); (e) M. Koremura, H. Oku, H. Nakao, T. Shono, and Y. Morisawa, *ibid.*, **13**, 216 (1961); (f) M. Koremura and Z. Hattori, *ibid.*, **13**, 222 (1961); (g) M. Koremura and K. Tomita, *Nippon Nogeikagaku Kaishi*, **36**, 479 (1962); (h) M. Koremura, *ibid.*, **36**, 552, 557 (1962); (i) M. Koremura and M. Nagawa, *ibid.*, **36**, 629 (1962); ref. 2d–f appear in *Chem. Abstr.*, **57**, 16450*e* (1962).

(3) (a) E. B. Hodge, U. S. Patent 3,095,456 (June 25, 1963); (b) N. G. Clark, A. F. Hams, and B. E. Leggetter, *Nature*, **200**, 171 (1963).

(4) (a) R. L. Heath and A. Lambert, *J. Chem. Soc.*, 1477 (1947); (b) C. L. Arcus and P. A. Hallgarten, *ibid.*, 3407 (1957); (c) L. F. Cason and C. C. Wanser, *J. Am. Chem. Soc.*, **73**, 142 (1951); (d) A. Mustafa, A. H. E. Harhash, and M. Kamel, *ibid.*, **77**, 3860 (1955).

(5) E. E. Evans, R. F. Haines, A. C. Curtis, F. C. Bocobo, W. H. Block, and E. R. Harrell, *J. Invest. Dermatol.*, **28**, 43 (1957).

(6) Recently, I. Montanini, A. Martini, and S. Bersi [*Boll. Chim. Farm.*, **103**, 187 (1964); *Chem. Abstr.*, **61**, 4247*h* (1964)] have reported that compounds obtained by the addition of carboxymethanethiol and 2-carboxyethanethiol to β-nitrostyrenes are ineffective as bacteriostats.

No attempt was made to rate the compounds on the basis of the qualitative tests even though differences in degree of inhibition were apparent. Quantitative tests were run against the three fungi and the results are shown in Tables I and II. The general resistance of *C. albicans* to this type of compound was re-emphasized in the quantitative tests. *T. mentagrophytes* and *C. ulmi*, on the other hand, showed varying degrees of susceptibility to the different members of the series.

Unfortunately, when the most active compounds from the *in vitro* tests against *C. albicans* were tested *in vivo* in mice, considerable toxicity was evident at fungicidal levels. The activity against *C. ulmi*, however, has been apparent for several of the compounds in testing in elm trees (*Ulmus americana*) and field tests of **14** are currently in progress.

Dimond[7] has discussed the use of fungicides against Dutch elm disease and has suggested that the best effect should be obtained with a fungicide which would be distributed through the tree and adsorbed onto the wood. Possibly our compounds, which decompose slowly in aqueous solution to their precursors, are distributed through the tree in solution, with the insoluble β-nitrostyrenes being deposited on the wood.

**Chemistry.**—The compounds were prepared as shown in eq. 1, using pyrrolidine as a catalyst, where R and R′ are hydrogens or alkyl groups and Ar is a benzene, furan, or thiophene ring with or without substituents. The structures were assigned by analogy to the addition of aromatic thiols which Cason and Wanser[4c] have shown to add to the carbon atom β to the nitro group. The possible substitution on the aromatic ring was limited by the fact that nitrostyrenes bearing a strong electron-donating group such as dimethylamino, methoxyl, or hydroxyl did not give a crystalline adduct in our hands, although Cason and Wanser[4c] report the successful reaction of benzylmercaptan with 1-(4-methoxyphenyl)-2-nitroethene.

$$R_2NCH_2CH_2SH\cdot HCl + ArCH{=}C(R')NO_2 \longrightarrow ArCH(SCH_2CH_2NR_2\cdot HCl)CHR'NO_2 \quad (1)$$

The *p*-dimethylamino-β-nitrostyrene (I) definitely did not react, as the characteristic deep red color of a methanol solution was unchanged on standing in the presence of 2-aminoethanethiol hydrochloride (II) and pyrrolidine. Since an adduct forms readily from 1-(3,4-methylenedioxyphenyl)-2-nitroethene and since the *p*-methoxy compound which failed to react contained a methyl group on C-2 of the side chain, some *m*- or *p*-methoxy-β-nitrostyrenes may combine with II. Addition of a nitro group on the alkoxylated aryl rings produced compounds which formed adducts readily. Other groups such as halogen, alkyl, or cyano do not hinder the addition. Thus, these aminoalkylthiols can add readily to all β-nitrostyrenes, except those containing only the strongest electron-donating groups on the ring and appear to be more active in adding to nitrostyrenes than the aromatic amines whose addition has been studied by Worrall.[8,9]

An interesting comparison with our studies and those of Worrall,[8] is the work of Kamlet and Glover,[10] who found that barbituric acid, a relatively strong organic acid, added to β-nitrostyrenes in the absence of a catalyst and even formed an adduct with the *p*-dimethylamino compound I. They reported that I gave the slowest rate of reaction of the compounds examined while the 4-nitro compound had a rate 20 times as great. Thus the substituents which increase the rate of addition also shift the position of the equilibrium toward addition as well as producing a hypsochromic shift in the ultraviolet maximum.

We, too, see evidence of a mobile equilibrium when the ultraviolet spectra of our products are determined in methanol solution. The presence of up to 25% of 1-(2-chlorophenyl)-2-nitroethane (III) in a 1 mg./100 ml. solution of **14** is indicated by the peak at 300 mμ, characteristic of III. At higher concentrations the dissociation is somewhat reduced, suggesting a concentration-dependent equilibrium. We are investigating this phenomenon further. Steric factors in the thiol moiety seemed to have a strong influence on the reaction also, as a number of attempts to add the dimethyl- or diethylaminoethanethiols to various nitrostyrenes usually resulted in isolation of the disulfide derivative of the mercaptan and only two reactions gave the desired product in crystalline form.

The few heterocyclic-substituted nitroalkenes studied also combined readily with II. The one failure was with 1-(5-nitro-2-furyl)-2-nitroethene which decomposed when II was added.

## Experimental

The melting points and analyses of the addition compounds are listed in Tables I and II. Many of the compounds were analytically pure without recrystallization; others were recrystallized from methanol or acetonitrile–ether. Yields were usually in the 60–90% range. The method used to prepare them is illustrated by the preparation described below.

**2-[(α-Nitromethyl)-3,4-dichlorobenzylthio]ethylamine Hydrochloride.**—1-(3,4-Dichlorophenyl)-2-nitroethene (4.4 g., 0.02 mole) was mixed with 2.3 g. (0.02 mole) of 2-aminoethanethiol hydrochloride and 30 ml. of methanol. A few drops of pyrollidine was added, the temperature rose, and the solids dissolved. The solution was filtered through diatomaceous earth and when ether was added to the filtrate a solid formed which was separated by filtration to yield 5.0 g. of the title compound, m.p. 174–177° dec.

The nitrostyrenes were prepared by the method of Gairaud and Lappin.[11] Most of the compounds are known and references to their preparation are listed in Tables I and II. The new ones are listed in Table III, and the preparation described below is typical.

**1-(2,6-Dichloro-3-nitrophenyl)-2-nitrobutene.**—2,6-Dichlorobenzaldehyde (25 g., 0.14 mole), 25 ml. (0.28 mole) of 1-nitropropane, 75 ml. of acetic acid, and 5 g. of ammonium carbonate were mixed, and the solution was refluxed for 6 hr. The next day the solution was poured onto ice and the aqueous layer was decanted from a heavy oil which was dissolved in ether and washed with water, $NaHSO_3$ solution, and water. The solution was dried and evaporated under nitrogen and, on cooling, the residue solidified to give 33 g. of 1-(2,6-dichlorophenyl)-2-nitro-

(7) A. E. Dimond, *Frontiers Plant Sci.*, **14**, No. 2, 4 (1962).

(8) (a) D. E. Worrall, *J. Am. Chem. Soc.*, **49**, 1598 (1927); (b) *ibid.*, **60**, 2841, 2845 (1938); (c) D. E. Worrall and F. Benington, *ibid.*, **60**, 2844 (1938); (d) *ibid.*, **62**, 493 (1940); (e) D. E. Worrall and J. Finkel, *ibid.*, **61**, 2969 (1939); (f) D. E. Worrall and H. T. Wolosinski, *ibid.*, **62**, 2449 (1940).

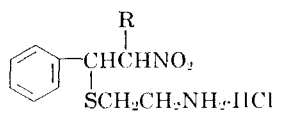
(9) M. Friedman, J. F. Cavins, and J. S. Wall (Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 41S) have shown that at comparable basicities, thiols are about 300 times as reactive as amines in adding to α,β-unsaturated compounds.

(10) (a) M. J. Kamlet, *J. Am. Chem. Soc.*, **77**, 4896 (1955); (b) M. J. Kamlet and D. J. Glover, *ibid.*, **77**, 5696 (1955); (c) *ibid.*, **78**, 4556 (1956).

(11) C. B. Gairaud and G. R. Lappin, *J. Org. Chem.*, **18**, 1 (1953).

## Table I

Physical Constants, Analytical Data, and Antifungal Activities of [(α-Nitroalkyl)benzylthio]alkylamines

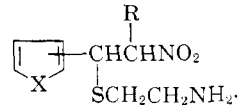



| Compd. | Substituent Aryl | Substituent R | M.p., °C. | Formula | Chlorine (ionic), % Calcd. | Chlorine (ionic), % Found | Nitrogen, % Calcd. | Nitrogen, % Found | Ref. to nitrostyrene | Lowest level of inhibition, γ/ml. *C. albicans* | Lowest level of inhibition, γ/ml. *T. mentagrophytes* | Lowest level of inhibition, γ/ml. *C. ulmi* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H | H | 152.5–154.5 | $C_{10}H_{14}N_2O_2S\cdot HCl$ | 13.50 | 13.86 | [a] | [a] | [b] | 1000 | 15 | 50 |
| 2 | 4-$CH_3$ | H | 155–157 | $C_{11}H_{16}N_2O_2S\cdot HCl$ | 12.81[c] | 12.90[c] | | | [d] | >1000 | 13 | 10 |
| 3 | 2,4-$(CH_3)_2$ | H | 130–137 dec. | $C_{12}H_{18}N_2O_2S\cdot HCl$ | 12.19[c] | 12.49[c] | | | New | >1000 | 100 | 50 |
| 4 | 4-$CH(CH_3)_2$ | H | 135–138 | $C_{13}H_{20}N_2O_2S\cdot HCl$ | 11.63[c] | 12.01[c] | | | [e] | >1000 | 100 | 50 |
| 5 | 4-$CH_3$, 3-$NO_2$ | H | 157–161 dec. | $C_{11}H_{15}N_3O_4S\cdot HCl$ | 11.02 | 11.32 | | | [d] | >1000 | 40 | 50 |
| 6 | 3-$NO_2$ | H | 129–135 | $C_{10}H_{13}N_3O_4S\cdot HCl$ | 11.52 | 11.43 | 13.65 | 13.44 | [b] | >1000 | 1000 | 50 |
| 7 | 4-$NO_2$ | H | 155–156 dec. | $C_{10}H_{13}N_3O_4S\cdot HCl$ | 11.52[c] | 11.45[c] | 13.65 | 13.37 | [b] | >1000 | 40 | 10 |
| 8 | 4-CN | H | 166–169 | $C_{11}H_{13}N_3O_2S\cdot HCl$ | | | 14.60 | 14.64 | [f] | 1000 | 15 | 1000 |
| 9 | 3,4-$OCH_2O$ | H | 148–150 dec. | $C_{11}H_{15}N_2O_4S\cdot HCl$ | 11.56 | 11.77 | 9.13 | 8.94 | [b] | >1000 | 20 | 100 |
| 10 | 2-$OCH_3$, 5-$NO_2$ | H | 163–166 dec. | $C_{11}H_{15}N_3O_5S\cdot HCl$ | 10.50 | 10.56 | | | [g] | >1000 | 100 | 50 |
| 11 | 4-Cl | H | 139–142 | $C_{10}H_{13}ClN_2O_2S\cdot HCl$ | 23.86[c] | 23.68[c] | 9.43 | 9.36 | [d] | 1000 | 40 | 50 |
| 12 | 4-Cl, 3-$NO_2$ | H | 187–188 dec. | $C_{10}H_{12}ClN_3O_4S\cdot HCl$ | 20.72[c] | 20.88[c] | | | [d] | >1000 | 40 | 10 |
| 13 | 4-Cl | $CH_3$ | 136–138 | $C_{11}H_{15}ClN_2O_2S\cdot HCl$ | 11.39 | 11.40 | [h] | [h] | [i] | >1000 | 40 | 1000 |
| 14 | 2-Cl | H | 133–135 | $C_{10}H_{13}ClN_2O_2S\cdot HCl$ | 23.89 | 23.95 | | | [d] | >1000 | 40 | 10 |
| 15 | 2-Cl, 5-$NO_2$ | H | 180–182 | $C_{10}H_{12}ClN_3O_4S\cdot HCl$ | | | 12.28 | 12.00 | [j] | 1000 | 1000 | 1000 |
| 16 | 2-Cl, 5-$NO_2$ | $CH_3$ | 179–180 dec. | $C_{11}H_{14}ClN_3O_4S\cdot HCl$ | 19.91[c] | 19.92[c] | | | New | >1000 | 40 | 100 |
| 17 | 2-Cl, 5-$NO_2$ | $C_2H_5$ | 177–179 | $C_{12}H_{16}ClN_3O_4S\cdot HCl$ | | | 11.35 | 11.74, 11.53 | New | 100 | 10 | 1000 |
| 18 | 3-Cl | H | 117.5–120 | $C_{10}H_{13}ClN_2O_2S\cdot HCl$ | 11.93 | 12.26 | | | [d] | 100 | 100 | 50 |
| 19 | 2,4-$Cl_2$ | H | 113.5–117 | $C_{10}H_{12}Cl_2N_2O_2S\cdot HCl$ | 10.69 | 11.05 | | | [k] | 1000 | 100 | 10 |
| 20 | 3,4-$Cl_2$ | H | 174–177 dec. | $C_{10}H_{12}Cl_2N_2O_2S\cdot HCl$ | 10.69 | 10.97 | | | [k] | >1000 | 100 | 10 |
| 21 | 2,6-$Cl_2$ | $C_2H_5$ | 130–133 | $C_{12}H_{16}Cl_2N_2O_2S\cdot HCl$ | | | 7.79 | 7.53 | New | >1000 | 40 | >1000 |
| 22 | 2,6-$Cl_2$, 3-$NO_2$ | $C_2H_5$ | 196–198 dec. | $C_{12}H_{15}Cl_2N_3O_4S\cdot HCl$ | | | 10.38 | 10.00 | New | >1000 | 1000 | >1000 |
| 23 | 4-Br | H | 179–180 dec. | $C_{10}H_{13}BrN_2O_2S\cdot HCl$ | 10.38[c] | 10.64[c] | [l] | [l] | [d] | >1000 | 1000 | 10 |
| 24 | 2-Br, 5-$NO_2$ | H | 185–187 dec. | $C_{10}H_{12}BrN_3O_4S\cdot HCl$ | | | 10.86 | 10.71 | [j] | 1000 | 40 | 10 |
| 25 | 4-F | H | 163–166 | $C_{10}H_{13}FN_2O_2S\cdot HCl$ | 12.63[c] | 12.89[c] | | | [m] | 1000 | 1000 | 250 |
| 26 | 2-F | H | 117–119 | $C_{10}H_{13}FN_2O_2S\cdot HCl$ | 12.63[c] | 12.98[c] | | | [n] | >1000 | 100 | 250 |
| 27 | 2-F, 5-$NO_2$ | H | 143–146 | $C_{10}H_{12}FN_3O_4S\cdot HCl$ | | | 12.90 | 12.65 | [n] | 1000 | 1000 | 1000 |
| 28[o] | 2-Cl, 5-$NO_2$ | $C_2H_5$ | 143–146 dec. | $C_{14}H_{20}ClN_3O_4S\cdot HCl$ | 8.91 | 8.85 | [p] | [p] | New | 1000 | 40 | 1000 |
| 29[q] | 3-$NO_2$ | H | 133–134.5 | $C_{14}H_{21}N_3O_4S\cdot HCl$ | 9.74 | 9.75 | | | [b] | >1000 | 10 | 50 |

[a] Sulfur: calcd., 12.20; found, 12.38. [b] H. B. Hass and E. F. Riley, *Chem. Rev.*, **32**, 373 (1943). [c] Total Cl. [d] W. S. Emerson, *Chem. Rev.*, **45**, 347 (1949). [e] Ref. 4c. [f] A. Vecchi and G. Melone, *J. Org. Chem.*, **22**, 1637 (1957). [g] Ref. 8c names the compound incorrectly as 2-methoxy-4,β-dinitrostyrene. [h] Sulfur: calcd., 10.30; found, 9.97. [i] Ref. 2d. [j] Ref. 8e. [k] O. Schales and H. A. Graefe, *J. Am. Chem. Soc.*, **74**, 4486 (1952). [l] Bromine: calcd., 23.39; found, 23.31. [m] Z. Eckstein and J. Plenkiewicz, *Roczniki Chem.*, **37**, 907 (1963). [n] Ref. 8f. [o] Amine group is dimethyl. [p] Sulfur: calcd., 8.05; found, 8.26. [q] Amine group is diethyl.

TABLE II

PHYSICAL CONSTANTS, ANALYTICAL DATA, AND ANTIFUNGAL ACTIVITIES OF HETEROCYCLIC DERIVATIVES

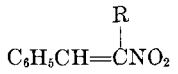


| Side chain position | Substituents X | Substituents Ring | Substituents R | M.p., °C. | Formula | Chlorine, % Calcd. | Chlorine, % Found | Ref. to nitrostyrene | Lowest level of inhibition, γ/ml. *C. albicans* | *T. mentagrophytes* | *C. ulmi* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | S | 5-$C_2H_5$ | H | 131–132 | $C_{10}H_{16}N_2O_2S_2 \cdot HCl$ | 11.95 | 12.47, 12.38 | *a* | 1000 | 40 | 250 |
| 2 | S | None | $CH_3$ | 125–128 | $C_9H_{14}N_2O_2S_2 \cdot HCl$ | 12.54[b] | 12.77[b] | *a* | >1000 | 10 | 1000 |
| 2 | S | 5-Cl | H | 157–161 dec. | $C_8H_{11}ClN_2O_2S_2 \cdot HCl$ | 23.38 | 23.49 | *a* | >1000 | 40 | 50 |
| 3 | S | None | H | 141–142 | $C_8H_{12}N_2O_2S_2 \cdot HCl$ | 13.19 | 13.48 | *c* | >1000 | 1000 | 100 |
| 2 | O | None | H | 139–140 | $C_8H_{12}N_2O_3S \cdot HCl$ | *d* | *d* | *e* | 1000 | 40 | 1000 |

[a] W. J. King and F. F. Nord, *J. Org. Chem.*, **14**, 405 (1949). [b] Ionic Cl. [c] E. E. Campaigne and W. C. McCarthy, *J. Am. Chem. Soc.*, **76**, 4466 (1954). [d] Sulfur: calcd., 12.69; found, 12.54. [e] J. Thiele and H. Landers, *Ann.*, **369**, 300 (1909).

TABLE III

NEW NITROSTYRENES



| Substituents Ring | Substituents R | M.p., °C. | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|
| 2-Cl, 5-$NO_2$ | $CH_3$ | 64–67 | 14.62 | 14.58 |
| 2-Cl, 5-$NO_2$ | $C_2H_5$ | 40–52 | 10.92 | 10.72 |
| 2,6-$Cl_2$ | $C_2H_5$ | Oil | 5.69 | 5.39 |
| 2,6-$Cl_2$, 3-$NO_2$ | $C_2H_5$ | 102–106 | 9.63 | 9.76 |
| 2,4-$(CH_3)_2$ | H | 119–126[a] | 7.91 | 7.62 |

[a] Boiling point (0.2 mm.).

butene which was added to 100 ml. of fuming $HNO_3$ with stirring. The temperature of the reaction rose to 40–45°. Stirring was continued for 3 hr. and then the solution was poured onto ice. The mixture was extracted with $CH_2Cl_2$ and the extracts were dried and concentrated. When methanol was added to the residue, a solid formed which was separated by filtration to yield 15.9 g. of the title compound, m.p. 102–106°.

**Testing.**—Qualitative tests with the bacteria, the fungi, and the alga were run by placing about 5 mg. of each compound directly on the surface-inoculated agar in 100 × 20 mm. Petri dishes. These were incubated at room temperature or 37°, depending on the requirements of the test organism, until growth was satisfactory. Antibiotic activity was indicated by the presence of a clear zone surrounding a compound due to failure of the organism to grow in this area. Nutrient agar was used for *B. subtilis* and *E. coli*, blood agar base containing 5% defibrinated rabbit blood for *D. pneumoniae*, and beef extract agar for *E. carotovara*. The fungi were cultivated on Sabouraud dextrose agar and *C. vulgaris* on modified Bristol agar. The latter organism was grown under continuous fluorescent illumination. *Tetrahymena geleii* was cultured in proteose peptone–sucrose broth for 24 hr. at room temperature and then transferred aseptically in 0.5-ml. quantities to sterile 13 × 100 mm. test tubes to which about 5 mg. of the individual compounds had been added. Incubation was continued for 24 hr., and the degree of growth was determined by microscopic examination of the cultures.

The quantitative tests with *C. albicans*, *T. mentagrophytes*, and *C. ulmi* were run in Sabouraud dextrose agar. The test compounds were dissolved in the hot agar and then diluted serially in test tubes. These were permitted to cool in a vertical position and the test organisms were inoculated onto the surface of the agar. Following a suitable incubation period, the presence or absence of growth was determined by visual inspection.

**Acknowledgment.**—The authors thank A. R. Zigman, Richard Salzmann, and Russell Reuter, and especially Ivar Laos for the preparation of initial or larger amounts of a number of these compounds.

# Phosphorylated Benzenesulfonamides as Animal Systemic Insecticides[1]

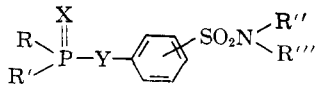
FRANK A. WAGNER, JR., RONALD W. BAER, AND GERALD BERKELHAMMER

*Chemical Research and Development Laboratories, Agricultural Division, American Cyanamid Company, Princeton, New Jersey*

*Received November 14, 1964*

A number of phosphorylated benzenesulfonamides (I) are active as anthelmintics and animal systemic insecticides, as well as plant insecticides. Animal systemic activity (mouse–mosquito test) and mouse toxicity data are presented and structure–activity relationships are discussed. The most active compounds are phosphates and phosphorothionates with *para*-positioned sulfamoyl groups, the sulfamoyl group being substituted by hydrogen, lower alkyl, or acyl groups.

A number of phosphorylated benzenesulfonamides have been prepared of general structure I, where R and



R′ are alkoxy, alkyl, alkylamino, methylthio, phenyl, chloro, or *p*-sulfamoylphenoxy; R″ and R‴ are hydrogen, alkyl, aryl, heterocyclic, or acyl; X is O or S; and Y is O, S, NH, or $SCH_2$. Members of this series exhibit activity as plant insecticides[2] and animal systemic insecticides and anthelmintics. A number of these compounds have particular utility in the animal systemic area since they combine high insecticidal activity

(1) Presented in part at the 140th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1961; Abstracts of Papers, p. 27-O.

(2) R. G. Dent and L. P. Ditman, *J. Econ. Entomol.*, **57**, 177 (1964); G. Guyer and A. Wells, Proceedings of the Entomological Society of America North Central Branch, **18**, 49 (1963); R. Redfern, M. Cleveland, and D. Hamilton, *ibid.*, **18**, 88 (1963).