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Synthesis and Antimicrobial Activity of Aliphatic Nitro Compounds

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A series of simple aliphatic nitro compounds has been synthesized and tested for antimicrobial activity *in vitro*. Some of the compounds inhibit the growth of a broad range of fungi and bacteria including species of *Pseudomonas*. The most active compounds are alcohols containing the grouping -CBrNO_2 . Replacement of bromine by hydrogen, chlorine, or alkyl groups diminishes the activity. Structure-activity relationships are discussed.

In the course of a synthetic program based on aliphatic nitro compounds we noted antimicrobial activity in a group of aliphatic halogenonitro alcohols. Low concentrations of these compounds inhibit the growth of a broad range of organisms including species of *Pseudomonas*. *Pseudomonads* are notoriously insensitive to many bacteriostatic agents, including some commonly used preservatives and antiseptics, and are of marked medical and economic importance. We have therefore explored, and now report on, the synthesis and antimicrobial activity of a series of these compounds. A preliminary communication describing 2-bromo-2-nitropropane-1,3-diol, one of the most active members of the series, has already appeared.¹

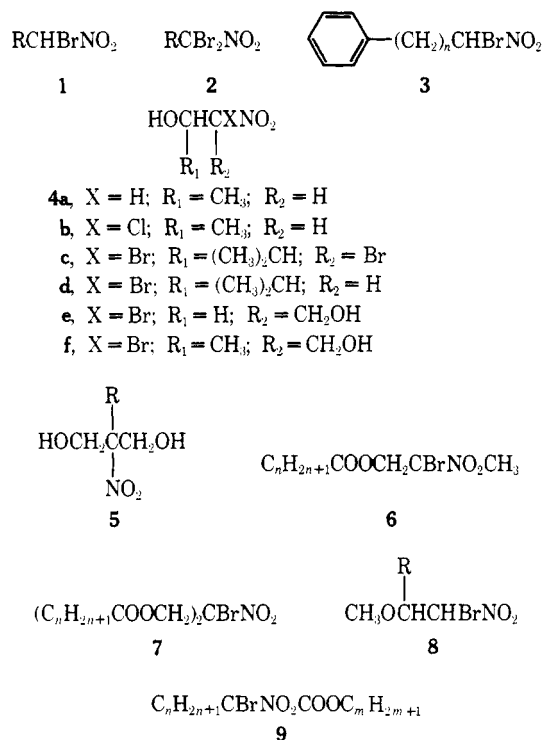
Results

The general formulas of the compounds studied are given in Chart I. The parent straight-chain 1-nitroalkanes ($\text{C}_n\text{H}_{2n+1}\text{NO}_2$, $n = 1-6$ and 8), at a concentration of 1000 $\mu\text{g/ml}$, did not inhibit the growth of any of the test organisms listed in Table I. The introduction of a bromine atom adjacent to the nitro group (1) enhanced the activity of these compounds somewhat but, with the exception of bromonitromethane (1, $\text{R} = \text{H}$), none of these derivatives was inhibitory to the bacteria at concentrations lower than 200 $\mu\text{g/ml}$.

Bromonitromethane, a volatile, highly irritant liquid, inhibited all the organisms at considerably lower concentrations but gave inconsistent results. This was attributed to the volatility of the compound, and it was not tested further. 1-Bromo-1-nitrooctane (1, $\text{R} = \text{C}_7\text{H}_{15}$), the next most active of this group, inhibited *Candida albicans* and the dermatophytes at 16.6 $\mu\text{g/ml}$ but was much less active against the other organisms.

The dibromo analog of this compound, 1,1-dibromo-1-nitrooctane (2, $\text{R} = \text{C}_7\text{H}_{15}$) was no more active although the other dibromonitroalkanes were somewhat more inhibitory than their monobromo analogs. Attachment of a benzene nucleus to the alkyl chain of these compounds did not enhance activity against the pseudomonads, although 1-bromo-1-nitro-2-phenylethane (3, $n = 1$) and 1-bromo-1-nitro-3-phenylpropane (3, $n = 2$) inhibited some of the other organisms at 16.6 $\mu\text{g/ml}$. Bromonitrophenyl-

Chart I



methane (3, $n = 0$) and 1-bromo-1-nitro-2,2-diphenylethane failed to inhibit any of the test organisms at concentrations less than 50 $\mu\text{g/ml}$.

The unhalogenated nitro alcohols (4, X = H), in which R_1 ranged from H to C_6H_{13} and R_2 from H to C_4H_9 , also possessed relatively low antimicrobial activity, generally requiring a concentration greater than 1000 $\mu\text{g/ml}$ for growth inhibition (e.g., 4a, Table I), while none of the chloro analogs (4, X = Cl) was inhibitory at less than 200 $\mu\text{g/ml}$ (e.g., 4b, Table I). However, when one or two bromine atoms were introduced adjacent to the nitro group, antimicrobial activity became much more pronounced. This is illustrated (Table II) by reference to the sensitivity

Table I. In Vitro Antimicrobial Activity of Some Aliphatic Nitro Alcohols, HOR₂CHCH₂CH₂XNO₂

Organism	Minimum inhibitory concentration, $\mu\text{g./ml.}$, after 18 hr at 37°						Chlorhexidine acetate (BPC)
	4a, X = H; R ₁ = CH ₃ ; R ₂ = H	4b, X = Cl; R ₁ = CH ₃ ; R ₂ = H	4c, X = Br; R ₁ = CH ₃ ; R ₂ = CH ₂ OH	4d, X = Br; R ₁ = CH ₃ ; R ₂ = H	4e, X = Br; R ₁ = H; R ₂ = CH ₂ OH	4f, X = Br; R ₁ = CH ₃ ; R ₂ = CH ₂ OH	
<i>Pseudomonas aeruginosa</i> (BCC 10S)	1000	200	16.6	16.6	16.6	16.6	200
<i>Escherichia coli</i> (BCC 36)	1000	200	16.6	16.6	16.6	16.6	50
<i>Aerobacter aerogenes</i> (NCTC 8172)	1000	1000	16.6	16.6	16.6	16.6	16.6
<i>Proteus vulgaris</i> (BCC A479)	1000	200	16.6	16.6	16.6	16.6	5.5
<i>Salmonella typhimurium</i> (NCTC 5710)	1000	1000	16.6	16.6	16.6	16.6	50
<i>Shigella sonnei</i> (NCTC 8221)	1000	200	16.6	16.6	16.6	16.6	5.5
<i>Bordetella parapertussis</i> (NCTC 5952)	200	200	16.6	16.6	16.6	16.6	5.5
<i>Staphylococcus aureus</i> (BCC 8452)	>1000	1000	16.6	16.6	16.6	16.6	5.5
<i>Streptococcus pyogenes</i> (NCTC 8326)	1000	200	16.6	16.6	16.6	16.6	1.8
<i>Corynebacterium pyogenes</i> (BCC VSD4)	1000	1000	16.6	16.6	16.6	16.6	5.5
<i>Bacillus subtilis</i> (NCTC 8236)	1000	1000	16.6	16.6	16.6	16.6	1.8
<i>Dermatophilus dermatomomus</i> (BCC VSD2)	1000	1000	50	50	50	50	16.6
<i>Candida albicans</i> (NCTC F1/51)	1000	1000	16.6	50	50	50	16.6
<i>Trichophyton rubrum</i> (BCC WB2)	1000	1000	5.5	50	16.6	16.6	NT ^a
<i>Trichophyton mentagrophytes</i> (A 280)	1000	200	16.6	100	16.6	16.6	NT ^a
<i>Penicillium roqueforti</i> (1070)	1000	1000	16.6	100	5.5	200	NT ^a
<i>Aspergillus niger</i> (H)	1000	1000	16.6	200	16.6	1000	NT ^a

^aNT = not tested.Table II. Activity against *Pseudomonas aeruginosa* of Some Nitro Alcohols and Derived Acetates

Compd no.	R ₂ OCHCBrNO ₂		Min inhibitory concn, $\mu\text{g./ml.}$, after 18 hr at 37°	
	R ₁	R ₂	R ₃ =	
			H	CH ₃ CO
4d	H	H	200	
	CH ₃	H	16.6	50
	C ₂ H ₅	H	50	50
	C ₃ H ₇	H	16.6	50
	(CH ₃) ₂ CH	H	1000	
	C ₄ H ₉	H	200	50
	C ₅ H ₁₁	H	50	50
	C ₆ H ₁₃	H	16.6	
	C ₈ H ₁₇ CH ₂	H	16.6	
	H	CH ₃	50	200
	H	C ₂ H ₅	200	1000
	H	C ₃ H ₇	1000	
	CH ₃	CH ₃	1000	
	C ₂ H ₅	CH ₃	200	
4c	CH ₃	Br	50	50
	C ₂ H ₅	Br	200	50
	(CH ₃) ₂ CH	Br	5.5	

Table III. New Bromonitroalkanes RCXBrNO₂

R	X	Bp (mm), °C	Yield, %	Formula	Analyses
C ₄ H ₉	H	68 (2.0)	37	C ₄ H ₉ BrNO ₂	Br
C ₆ H ₅ CH ₂	H	106–107 (1.0)	68	C ₆ H ₅ BrNO ₂	Br, N
C ₆ H ₅ (CH ₂) ₂	H	108–109 (0.9)	51	C ₈ H ₉ BrNO ₂	Br
C ₄ H ₉	Br	70–71 (2.3)	70	C ₄ H ₈ Br ₂ NO ₂	Br
C ₅ H ₁₁	Br	84–85 (2.1)	67	C ₅ H ₁₀ Br ₂ NO ₂	Br
C ₇ H ₁₅	Br	85 (0.25)	25	C ₇ H ₁₄ Br ₂ NO ₂	Br

of *Pseudomonas aeruginosa* which may be taken as representative of that of the whole range of test organisms; the spectra of antimicrobial activities of some of the compounds are compared with that of 1,6-di(*N*-*p*-chlorophenyldiguanido)hexane diacetate (chlorhexidine acetate, BPC) in Table I. Unlike the alkane series, only one of the dibromo compounds (4c) was more active (Table II) than the monobromo analog 4d. Further, the introduction of an alkyl group onto the carbon atom attached to the bromine and nitro groups produced compounds (4, R₂ = alkyl) which generally exhibited less activity (Table II) than the lower homologs (4, R₂ = H). On the other hand, when R₂ = CH₂OH, highly active glycols (e.g., 4e,f) were obtained (Table I). Related glycols containing chlorine or no halogen (5, R = Cl, CH₃, or C₃H₇) were not inhibitory at concentrations less than 200 $\mu\text{g./ml.}$

Acylation of the nitro alcohols generally had little effect on the biological activity, and most acetyl derivatives exhibited activity similar to that of the parent alcohol (Table II). The activity of the homologous series of acyl derivatives of 2-bromo-2-nitropropanol (6, *n* = 1–7, 9, 11, 17) changed little until the higher molecular weight compounds (6, *n* = 9, 11, 17) were reached, when activity was diminished. By contrast, acylation of the glycol, 2-bromo-2-nitropropane-1,3-diol (4e), generally reduced its activity considerably. For example, the compounds (7, *n* = 1, 3, 4) were not inhibitory at concentrations less than 200 $\mu\text{g./ml.}$ and the related dibenzoate was inactive at 1000 $\mu\text{g./ml.}$ However, the dipropionyl derivative 7 (*n* = 2) was as active as the free glycol. Conversion of a few of the active al-

Table IV. New Nitro Esters

$\begin{array}{c} \text{X} \\ \\ \text{R}_1\text{CCOOR}_2 \\ \\ \text{NO}_2 \end{array}$						
R ₁	R ₂	X	Bp (mm), °C	Yield, %	Formula	Analyses
CH ₃	C ₅ H ₁₁	H	85 (1.0)	73 ^a	C ₈ H ₁₅ NO ₄	C, H, N
CH ₃	C ₁₂ H ₂₅	H	159–160 (1.5)	42 ^b	C ₁₅ H ₂₉ NO ₄	C, H, N
CH ₃	C ₆ H ₅	H	114.5 (1.5)	50	C ₉ H ₉ NO ₄	C, H
CH ₃	C ₆ H ₅ CH ₂	H	118–120 (1.0)	50	C ₁₀ H ₁₁ NO ₄	C, H, N
C ₆ H ₁₃	C ₂ H ₅	H	107–109 (2.3)	70	C ₁₀ H ₁₉ NO ₄	C, H, N
C ₄ H ₉	C ₂ H ₅	Br	99–100 (2.6)	67	C ₈ H ₁₄ BrNO ₄	Br
C ₅ H ₁₁	C ₂ H ₅	Br	104–105 (2.2)	51	C ₉ H ₁₆ BrNO ₄	Br
C ₆ H ₁₃	C ₂ H ₅	Br	115–116 (2.2)	39	C ₁₀ H ₁₈ BrNO ₄	Br
CH ₃	CH ₃	Br	47–48 (0.6)	67	C ₄ H ₈ BrNO ₄	Br
CH ₃	C ₂ H ₇	Br	65 (0.5)	69	C ₆ H ₁₀ BrNO ₄	Br
CH ₃	C ₄ H ₉	Br	78 (0.6)	65	C ₇ H ₁₂ BrNO ₄	Br
CH ₃	C ₅ H ₁₁	Br	95 (1.6)	54	C ₈ H ₁₄ BrNO ₄	Br
CH ₃	C ₆ H ₅ CH ₂	Br	142.5 (2.7)	40	C ₁₀ H ₁₆ BrNO ₄	Br

^aStarting material, pentyl α -bromopropionate, colorless liquid, bp 67–69° (2.2 mm). *Anal.* (C₈H₁₅BrO₂) Br. ^bStarting material, dodecyl α -bromopropionate, colorless oil, bp 118° (0.25 mm). *Anal.* (C₁₅H₂₉BrO₂) Br.

Table V. New Halogenonitro Alcohols

$\begin{array}{c} \text{R}_2 \\ \\ \text{R}_1\text{CH}-\text{CX} \\ \quad \\ \text{OH} \quad \text{NO}_2 \end{array}$						
R ₁	R ₂	X	Bp (mm) or mp, °C	Yield, %	Formula	Analyses
CCl ₃	CH ₃	Cl	103 (1.2)	59	C ₄ H ₅ Cl ₄ NO ₃	N
H	C ₃ H ₇	Cl	79 (1.3)	56	C ₅ H ₁₀ ClNO ₃	C, H, N
C ₃ H ₇	CH ₃	Cl	69 (1.4)	41	C ₆ H ₁₂ ClNO ₃	C, H, N
CH ₃	Cl	Cl	64 (2.0)	67	C ₃ H ₅ Cl ₂ NO ₃	Cl
(CH ₃) ₂ CH	H	Br	72 (0.8)	57	C ₃ H ₇ BrNO ₃	C, H, N
C ₄ H ₉	H	Br	80 (0.35)	70	C ₆ H ₁₂ BrNO ₃	Br
C ₅ H ₁₁	H	Br	108 (0.9)	65	C ₇ H ₁₄ BrNO ₃	Br
C ₆ H ₁₃	H	Br	129 (2.5)	52	C ₈ H ₁₆ BrNO ₃	N
CH ₃	CH ₃	Br	66 (1.3)	50	C ₄ H ₈ BrNO ₃	N, Br
C ₂ H ₅	CH ₃	Br	76 (1.2)	50	C ₅ H ₁₀ BrNO ₃	C, H, N
			94–95			
CCl ₃	CH ₃	Br	104 (0.7)	58	C ₄ H ₅ BrClNO ₃	N
H	C ₂ H ₅	Br	79 (1.7)	68	C ₄ H ₈ BrNO ₃	Br ^a
CH ₃	C ₂ H ₅	Br	99 (5)	54	C ₅ H ₁₀ BrNO ₃	N
CH ₃	Br	Br	80 (2.0)	75	C ₃ H ₅ Br ₂ NO ₃	Br
C ₂ H ₅	Br	Br	91 (2.0)	70	C ₄ H ₇ Br ₂ NO ₃	Br ^b
(CH ₃) ₂ CH	Br	Br	77 (0.2)	37	C ₃ H ₅ Br ₂ NO ₃	N

^aBr: calcd, 40.4; found, 40.9. ^bBr: calcd, 57.8; found, 58.3.

cohols to their methyl ethers was generally found to diminish their activity somewhat. For example, the compounds 8, R = CH₃ and C₂H₅, did not inhibit any organisms below a concentration of 200 μ g/ml.

In another class of bromonitro esters 9, the ethyl esters ($m = 2$) of a short homologous series ($n = 1$ –6) proved to be inactive at concentrations of 1000 μ g/ml against *Pseudomonas* species and less than 200 μ g/ml against the other organisms, while a similar series of esters of α -bromo- α -nitropropionic acid ($n = 1$; $m = 1, 3$ –5) was only inhibitory at 200 μ g/ml. None of the related unhalogenated esters was active at less than 1000 μ g/ml.

Discussion

The antimicrobial activity of the compounds described above is clearly associated with the structural feature –CBrNO₂–. Hodge, *et al.*,² while studying the antifungal activity of some aralkylnitro compounds, noted that the highest activity was associated with a grouping in which a chlorine or bromine atom (especially the latter) and a nitro group were attached to a common carbon atom. Zsolnai³ has also reported that, of a large number of alkyl

and aralkylnitro compounds tested against fungi, those embracing the above structural feature were highly inhibitory.

In general, the structural requirements for optimum antimicrobial activity in the halogenonitro alcohols are rather precise. Thus, the bromo compounds are more active than the chloro analogs, but the introduction of a second bromine atom only occasionally enhances the activity. In a series of nitropropanediols, Bowman and Stretton⁴ found that the bromo derivative was much more active than the chloro derivative. It is also desirable for the structural feature –CBrNO₂– to carry one hydrogen atom; homologs, in which this hydrogen atom is replaced by alkyl groups, are generally less active. Conversion of the free hydroxyl group to a simple ester generally has little effect on the antimicrobial activity, although diminution is occasionally experienced. On the other hand, conversion to the methyl ether generally weakens the activity. In the case of the glycols, however, acylation also causes a drop in activity.

On the assumption that the biological activity of the present halogen compounds, and many others, is due to

Table VI. New Acylated Halogenonitro Alcohols

$ \begin{array}{c} R_3 \\ \\ R_1CH-CX \\ \quad \\ R_2COO \quad NO_2 \end{array} $				Bp (mm) or mp, °C	Yield, %	Formula	Analyses
R ₁	R ₂	R ₃	X				
C ₃ H ₇	CH ₃	H	Cl	81 (1.5)	86	C ₇ H ₁₂ ClNO ₄	C, H, N
H	CH ₃	CH ₃	Cl	61 (0.9)	82	C ₅ H ₈ ClNO ₄	C, H, N
H	CH ₃	C ₂ H ₅	Cl	68 (1.0)	75	C ₆ H ₁₀ ClNO ₄	C, H, N
CH ₃	CH ₃	Cl	Cl	65 (2.8)	88	C ₅ H ₇ Cl ₂ NO ₄	Cl
CH ₃	C ₆ H ₅	Cl	Cl	106 (0.1)	42	C ₁₀ H ₉ Cl ₂ NO ₄	C, H, N
C ₄ H ₉	CH ₃	H	Br	98 (0.6)	61	C ₈ H ₁₄ BrNO ₄	Br
C ₅ H ₁₁	CH ₃	H	Br	128 (3.5)	62	C ₉ H ₁₆ BrNO ₄	Br
CH ₃	CH ₃	Br	Br	86 (1.2)	71	C ₅ H ₇ Br ₂ NO ₄	Br ^a
C ₂ H ₅	CH ₃	Br	Br	95 (0.8)	67	C ₆ H ₉ Br ₂ NO ₄	Br ^b
H	CH ₃	CH ₃	Br	75 (1.4)	81	C ₅ H ₈ BrNO ₄	C, H, N
H	C ₂ H ₅	CH ₃	Br	68 (0.6)	59	C ₆ H ₁₀ BrNO ₄	C, H, N
H	C ₄ H ₉	CH ₃	Br	100 (3.0)	30	C ₈ H ₁₄ BrNO ₄	C, H, N
H	C ₅ H ₁₁	CH ₃	Br	114 (1.5)	81	C ₉ H ₁₆ BrNO ₄	C, H, N
H	C ₆ H ₁₃	CH ₃	Br	126 (2.0)	68	C ₁₀ H ₁₈ BrNO ₄	C, H, N
H	C ₇ H ₁₅	CH ₃	Br	135 (1.5)	30	C ₁₁ H ₂₀ BrNO ₄	C, H, N
H	C ₈ H ₁₇	CH ₃	Br	163 (1.5)	37	C ₁₃ H ₂₄ BrNO ₄	Br
H	C ₁₁ H ₂₃	CH ₃	Br	155 (0.6)	88	C ₁₅ H ₂₈ BrNO ₄	C, H, N
H	C ₁₇ H ₃₅	CH ₃	Br	42-43	53	C ₂₁ H ₄₀ BrNO ₄	H, N, C ^c
H	CH ₃	C ₂ H ₅	Br	95 (2.5)	74	C ₆ H ₁₀ BrNO ₄	Br
H	CH ₃	CH ₂ OOCH ₂	Br	109 (1.0)	79	C ₇ H ₁₀ BrNO ₆	Br
H	C ₂ H ₅	CH ₂ OOCH ₂	Br	112 (0.7)	77	C ₈ H ₁₄ BrNO ₆	Br
H	C ₃ H ₇	CH ₂ OOCH ₂	Br	126 (0.05)	50	C ₁₁ H ₁₈ BrNO ₆	C, H
H	C ₄ H ₉	CH ₂ OOCH ₂	Br	134 (0.4)	54	C ₁₃ H ₂₂ BrNO ₆	C, H, N

^aBr: calcd, 52.5; found, 52.0. ^bBr: calcd, 50.2; found, 49.6. ^cC: calcd, 56.0; found, 56.5.

the activating influence of an adjacent electrophilic group (cf. Zsolnai³), a series of esters (9), in which the activating influence of the nitro group is supplemented by that of an alkoxy carbonyl group, was synthesized. Unfortunately, all these compounds are devoid of any noteworthy antimicrobial activity.

The most active compounds, the bromonitro alcohols (4, X = Br) and closely related glycols, are of particular interest because they inhibit the growth of a broad range of bacteria including *P. aeruginosa*. The compounds probably react at relatively nonspecific sites common to all the organisms and, perhaps, near the surfaces of the cells. Zsolnai³ suggested that the halogenonitro compounds can react with sulfhydryl groups which are widely distributed in essential enzyme systems, and this is supported by the finding¹ that sulfhydryl compounds, such as cysteine, antagonize one of the more active bromonitro glycols, 2-bromo-2-nitropropane-1,3-diol (4e). A series of halonitro compounds shows decreased antibacterial activity on the addition of thiol-containing compounds and it has been suggested that their mode of action is by the oxidation of sensitive thiol groups to disulfides.⁴ A more detailed biochemical study of 2-bromo-2-nitropropane-1,3-diol has given results consistent with this hypothesis.⁵

Although inhibiting a wide range of bacteria and fungi some of the more active compounds described are neither irritant nor highly toxic to animals. Because of its relative stability in aqueous solution 2-bromo-2-nitropropane-1,3-diol (4e) was selected for further investigation.¹ The compound has found application as a preservative for shampoos⁶ and other cosmetic and pharmaceutical preparations⁷ and to control blackarm disease of cotton, caused by *Xanthomonas malvacearum*,⁸ under the British Approved Name bronopol.

Experimental Section

Microbiology. The compounds were tested for antimicrobial activity *in vitro* by the agar dilution technique. In initial tests solutions of compounds in water or acetone were diluted with melt-

ed nutrient agar (% w/v: glucose 0.1; yeast extract 0.3; peptone 1.5; NaCl 0.5; agar 2.0) or Sabouraud dextrose agar to give concentrations of 1000, 200, and 50 µg/ml. Further tests of the more active compounds were carried out using a threefold dilution series from 50 µg/ml. The agar plates were surface-inoculated with 0.01-ml amounts of overnight broth cultures of bacteria or of fungal spore suspensions using a multipoint inoculator.⁹ Horse blood was added to the *Corynebacterium pyogenes* and *Streptococcus pyogenes* inocula. The lowest concentrations of compounds completely inhibiting growth were noted after incubating plates inoculated with bacteria and *Candida* for 18 hr at 37° and those inoculated with fungi for 4 days at 26°.

Chemistry. Most of the halogenonitro compounds are irritant, and suitable precautions were taken in handling them. Further, all crude nitro compounds were treated with a few drops of glacial acetic acid before distillation in order to destroy any traces of their explosive sodium salts; distillations were always performed behind protective screens. When pure, all the new compounds were colorless liquids or solids.

Nitroalkane Derivatives. Monobromination of the nitroalkanes^{10,11} was not achieved satisfactorily in aqueous solution as suggested by Worstell.¹² However, the desired products were obtained by adding bromine to a suspension of the dry sodium salt of the nitroalkane in ether.¹³ Dibromination presented no difficulty in cold aqueous methanolic solution. New compounds are listed in Table III.

Nitro Ester Derivatives. With the exception of ethyl nitroacetate, the α-nitro esters were obtained by the action of sodium nitrite in dimethylformamide¹⁴ on the corresponding α-bromo esters. Bromination of the α-nitro esters occurred satisfactorily in the presence of either ethanolic potassium hydroxide or a solution of sodium alkoxide in the alcohol from which the nitro ester was derived. A typical example is ethyl α-bromo-α-nitropentanoate. A solution of potassium hydroxide (4.7 g) in ethanol (85 ml) was stirred and held below 0° while ethyl α-nitropentanoate (14.7 g) was added. The resulting solution was then treated dropwise with bromine (4.2 ml) and diluted with water. The precipitated oil was isolated by ether extraction and purified by fractional distillation: colorless, pungent liquid; bp 92.5° (3.5 mm); yield, 10.8 g (50%). Anal. (C₇H₁₂BrNO₄) Br. Related new compounds are listed in Table IV.

Halogenonitro Alcohols. The nitro alcohols, all known compounds derived from aldehydes or ketones and nitroalkanes in the presence of bases,¹⁵ were brominated by the method¹³ used for the monobromonitroalkanes. Chloro derivatives were obtained similarly by passing excess chlorine into the sodium salt suspen-

sion. In some cases, however, the chloronitro alcohols were prepared by addition of aldehydes to 1-chloro-1-nitroethane (commercially available) in the presence of potassium carbonate. A typical example is **3-chloro-3-nitrobutan-2-ol**. An aqueous solution of acetaldehyde (10 g in 20 ml) was added to a mixture of 1-chloro-1-nitroethane (21.9 g), water (20 ml), and potassium carbonate (1 g). The homogeneous mixture became warm, the temperature rising spontaneously to 50°. After standing overnight, the reactants were acidified with hydrochloric acid, and the product was isolated by ether extraction and purified by fractional distillation: colorless oil; bp 56° (1.2 mm); yield, 18.5 g (60%). *Anal.* ($C_4H_8ClNO_3$) C, H, N. Related new compounds are listed in Table V.

Esters and Ethers of Halogenonitro Alcohols. The acyl derivatives were all obtained by heating the free alcohol or glycol in chloroform solution with a moderate excess of the appropriate acyl chloride (*cf.* Tindall¹⁶). See Table VI.

The ethers were prepared by treating an α -nitroalkene with sodium methoxide; this gave an intermediate sodium salt of the methoxynitroalkane which, on treatment with chlorine or bromine, yielded the desired halogenonitro ether. A typical example is **1-chloro-2-methoxy-1-nitrobutane**. 1-Nitrobut-1-ene (20 g) was added slowly to a stirred mixture of methanol (50 ml) and a methanolic sodium methoxide solution (50 ml of 4.4 *N*) cooled to 0°. The resulting solution was diluted with ice-water (300 ml) and chlorine bubbled into the liquid until no more oil was precipitated. This oil was isolated by ether extraction and purified by fractional distillation: colorless oil; bp 51–52° (2.0 mm); yield, 18.1 g (54%). *Anal.* ($C_5H_{10}ClNO_3$) N.

The following compounds were prepared similarly by adding a slight excess of bromine in place of the stream of chlorine. **1-Bromo-3,3,3-trichloro-2-methoxy-1-nitropropane**: colorless oil; bp 105° (1.6 mm); yield, 34%. *Anal.* ($C_4H_5BrCl_3NO_3$) N. **1,1-Dibromo-2-methoxy-1-nitrobutane**: Colorless prisms; mp 26°; yield, 75%. *Anal.* ($C_5H_9Br_2NO_3$) N, Br.

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Reinvestigation of a "Nonadditive" Quantitative Structure-Activity Relationship

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The toxicities toward white mice for a series of disubstituted benzenes have earlier been reported as being correlated by an interaction model of the form $\log A_{XY} = b_X + b_Y + e_X e_Y$ where b_X and b_Y are the toxicities of the corresponding monosubstituted benzenes and e_X and e_Y are interaction parameters. It is shown in this report that these compounds can also have their toxicities correlated using the Free and Wilson additive model approach. All of the regression models developed for the correlation of biological activities are therefore interrelated.

Additive and linear multiple regression models used for the derivation of quantitative structure-activity relationships have been shown, in many instances, to be theoretically,¹ statistically,^{2,3} and practically^{1,2,4-6} equivalent so long as the derived parameters are defined relative to a structurally similar parent nucleus. An apparent exception to this generalization would seem to be found in the work of Boček, Kopecký, and coworkers.⁷⁻⁹ In this instance the toxicities of meta- and para-substituted benzenes toward white mice are reported as best correlated by an interaction model

$$\log A_{XY} = b_X + b_Y + e_X e_Y \quad (1)$$

where A_{XY} is the LD_{50} for a disubstituted benzene derivative relative to that of benzene, b_X and b_Y (or $\log A_{XH}$ and $\log A_{HY}$) are the corresponding measures of toxicity for monosubstituted benzenes, and e_X and e_Y are parameters characterizing the effect on the LD_{50} due to substituent interaction in the disubstituted benzenes.

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Singer and Purcell¹⁰ have made an effort to assess the implications of eq 1 in relation to the additive and linear multiple regression models. Their conclusion was that substituent interactions lead to a breakdown of the additive model, giving rise to a parabolic form of the linear multiple regression model. This conclusion is valid so long as, in taking an additive approach to obtaining structure-activity relationships, additivity means replicating the observed activity for a multisubstituted compound in terms of the activities for the corresponding monosubstituted derivatives. However, additivity in the Free and Wilson¹¹ sense, which is the additive model Singer and Purcell sought to relate to the linear multiple regression model, is not defined in this manner. Rather, additivity in the Free and Wilson approach means that for two or more compounds in a series, mono- and multisubstituted, corresponding substituents at equivalent positions of a molecular nucleus affect the observed biological activity, on average, in an identical manner. This is the criterion of additivity used by Cammarata¹ in his assessment of the relation between the additive and the linear multiple regression models.