98820-92-9; **44**, 71930-20-6; **45**, 71447-87-5; **46**, 71931-00-5; **47**, 71922-22-0; **48**, 71922-51-5; **49**, 71922-29-7; **50**, 76620-96-7; (L-Ala)$_3$-L-Ala(P), 65621-21-8; L-Ser(P), 94776-38-2; D-Ser(P), 90427-79-5; Cbz-L-AlaNHNH$_2$, 17350-66-2; $CH_3COP(O)(OMe)_2$, 17674-28-1; L-Arg-L-Ala(P), 76620-74-1; L-Cys-L-Ala(P), 76620-79-6; L-Met-L-Ala(P), 66449-66-9; L-Phe-L-Ala(P), 60668-55-5; L-Nva-L-Ala(P), 71447-87-5; Sar-L-Ala-L-Arg-L-Ala(P), 71930-49-9; Sar-L-Ala-L-Nva-L-Ala(P), 71930-65-9; *N*-(benzyloxycarbonyl)-L-alanine 2,4,5-trichlorophenyl ester, 7536-54-1; acetaldehyde, 75-07-0; dimethyl phosphite, 868-85-9; *N*-(benzyloxycarbonyl)-L-alanine, 1142-20-7; *N*-(carbobenzyloxy)glycine, 1138-80-3; benzaldehdye, 100-52-7; *N*-(benzyloxycarbonyl)-*N*-methyl-L-alanine, 21691-41-8; *N*-(benzyloxycarbonyl)-*N*-methyl-L-alanine *N*-succinimidyl ester, 71922-16-2; [L-1-[[*N*-(benzyloxycarbonyl)-*N*-methyl-L-alanyl]amino]ethyl]phosphonic acid monobenzyl amine salt, 98820-87-2; [L-1-[[*N*-(benzyloxycarbonyl)-*N*-methyl-L-alanyl-L-alanyl]amino]ethyl]phosphonic acid monobenzylamine salt, 98820-90-7; [L-]-[[*N*-(benzyloxycarbonyl)-*N*-methyl-L-alanyl-L-alanyl-L-alanyl]amino]ethyl]phosphonic acid, 71922-17-3; L-norvaline, 6600-40-4; *N*-(benzyloxycarbonyl)-L-norvaline, 21691-44-1; *N*-(benzyloxycarbonyl)-L-norvaline *N*-hydroxysuccinimide ester, 71447-85-3; [L-1-[(*N*-benzyloxycarbonyl-L-norvalyl)-amino]ethyl]phosphonic acid monobenzylamine salt, 71922-44-6; [L-1-[[*N*-(benzyloxycarbonyl)-L-norvalyl-L-norvalyl]amino]-ethyl]phosphonic acid monobenzylamine salt, 71930-97-7; [L-1-[(L-norvalyl-L-norvalyl)amino]ethyl]phosphonic acid, 71930-98-8; *N*-(benzyloxycarbonyl)sarcosine, 39608-31-6; *N*-(benzyloxycarbonyl)sarcosine *N*-hydroxysuccinimide ester, 53733-96-3; [L-1-[(*N*-(benzyloxycarbonyl)sarcosyl-L-norvalyl-L-norvalyl)-amino]ethyl]phosphonic acid, 71930-99-9.

Quantitative Structure–Activity Relationship of the Mutagenicity of Substituted *N*-Nitroso-*N*-benzylmethylamines: Possible Implications for Carcinogenicity†

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The relative mutagenicities of substituted *N*-nitroso-*N*-benzylmethylamines have been reexamined from a quantitative structure–activity relationship point of view. Most of the compounds were mutagenic toward *Salmonella typhimurium* TA 1535 with Aroclor-induced male hamster liver S9 activation. The dose–response data were subjected to a multiple linear regression equation calculated in a stepwise manner, which found that the differences in mutagenicities could be explained primarily by differences in the three-bond path molecular connectivity index, with smaller contributions from σ and π. Moreover, a polynomial regression analysis showed that the maximum mutagenicity could be explained by an optimal amount of electron withdrawal by the substituent which would cause a weakening, or activation, of the methylene C–H bond. The possible relevance of these observations to carcinogenesis is discussed.

In a recent study[1] we showed that the mutagenicity of para-substituted *N*-nitroso-*N*-benzylmethylamines toward *Salmonella typhimurium* TA 1535 varied as the substituent was changed. It appeared that there was an optimal amount of electron withdrawal necessary for maximum mutagenicity, but the number of data points available was not great enough to allow us to carry out definitive calculations or to reach any firm conclusions concerning the physicochemical parameters responsible for the mutagenic effects.

We have now prepared additional related compounds and have tested the entire set for mutagenicity against the TA 1535 strain. Multiple regression analyses of the data successfully showed several relationships between the mutagenicity data and physicochemical parameters. These observations may also be relevant to the carcinogenicity of nitrosamines.

Results

We knew from our previous study[1] that strain TA 1535 was the most sensitive of the Ames strains toward these compounds and thus this was the only strain we employed in this study. We also knew that activation was appreciably more effective with the S9 fraction from Aroclor-stimulated male hamster liver than from rat liver for these nitrosamines, as it is for most other nitrosamines.[2] The *p*-methyl, *p*-carboxyl, and the *p*-carboxymethyl derivatives were not mutagenic at any dose tested, and the *p*-methoxy derivative was not mutagenic in this experiment although it has shown moderate mutagenicity in previous trials (ref 1 and G. M. Singer and A. W. Andrews, unpublished data). These inactive compounds were not included in derivation of the quantitative structure–activity relationship (QSAR) equations. The detailed mutagenicity data from the dose–response assays are shown in Table I. As the index for mutagenic potency we chose the molar concentration of the nitrosamine which induced 50 revertants/plate. This point was on the linear portion of the dose–response curves for all of the active compounds (Figure 1). This index was chosen in preference to the initial slope of the dose–response curves because the length of the linear portion of the curve was different for each compound. For the more potent compounds, the linear portion was often only three points.

We analyzed the mutagenicity data using both a multiple linear regression model and also a polynomial regression model. The physicochemical parameters submitted to the linear regression analysis for each compound were the Hammett σ constant,[3] Hansch's π factor [log (P_X/P_H)], molar refractivity,[4] and two different molecular connectivity indices,[5] $^1\chi^v$ and $^3\chi_P^v$ (Table II). The π

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‡Program Resources Inc.

§Information Management Services.

(1) Singer, G. M.; Andrews, A. W. *J. Med. Chem.* **1983**, *26*, 309.
(2) Lijinsky, W.; Andrews, A. W. *Mutat. Res.* **1983**, *111*, 135.
(3) Hine, J. "Physical Organic Chemistry"; McGraw-Hill: New York, 1962; p 87.
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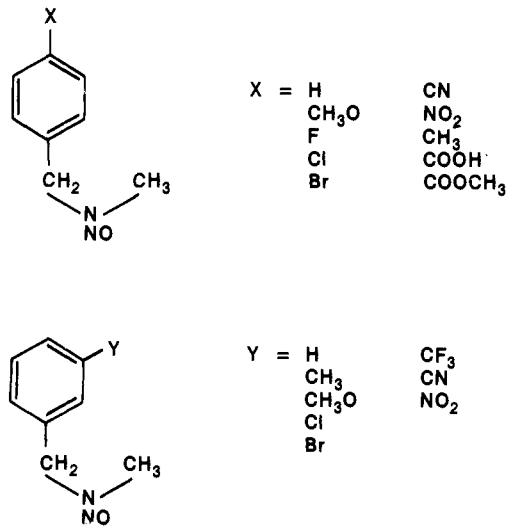

factors were calculated from partition coefficients measured as previously described[6] except that the quantitation of nitrosamine in the aqueous solution was by analytical HPLC.

In the stepwise regression, the strongest dependence was on $^3\chi_P^v$ (eq 1). The value of R^2 (0.544) indicates that about 54% of the total variation in the relative mutagenicity can be explained by $^3\chi_P^v$ alone.

$$\log (1/C) = -6.15 + 2.653\ (\pm 0.73)\ {}^3\chi_P^v \qquad (1)$$

$$n = 13,\ R^2 = 0.544,\ p = 0.004$$

The inclusion of σ improved the R^2 (0.645) (eq 2). The F statistic for the null hypothesis testing for all of the regression coefficients equal to zero yielded a p value of 0.006, indicating a good degree of significance.

$$\log (1/C) = -6.06 + 2.43\ (\pm 0.69)\ {}^3\chi_P^v + 0.71(\pm 0.42)\sigma \qquad (2)$$

$$n = 13,\ R^2 = 0.645,\ p = 0.006$$

By examining the relationship just between the relative mutagenicity and σ, we found a definite second-order dependence of log $(1/C)$ on σ (eq 3). The goodness-of-fit test yielded a p value of 0.45, showing that the equation is statistically valid.

$$\log (1/C) = -2.92 + 5.36(\pm 1.37)\sigma - 6.18(\pm 1.84)\sigma^2 \qquad (3)$$

$$n = 13,\ R^2 = 0.625,\ p = 0.45$$

We then combined eq 2 and 3 by carrying out a multiple linear regression calculation using the parameters in Table II both as first-order and second-order terms (eq 4). The

$$\log (1/C) = -5.11 + 3.55(\pm 1.40)\sigma - 3.88(\pm 1.85)\sigma^2 + 1.62(\pm 0.71)\,{}^3\chi_P^v \qquad (4)$$

$$n = 13,\ R^2 = 0.761,\ p = 0.004$$

value of R^2 showed that about 76% of the total variation of log $(1/C)$ could be explained by electronic parameters (σ, σ^2) and the molecular connectivity index, $^3\chi_P^v$.

Discussion

The results described by eq 2–4 are intuitively satisfying. It has been apparent from the numerous studies of the mutagenicity and carcinogenicity of nitrosamines that the biological response depends on a combination of several factors that derive from each molecule's chemical structure. The degree to which each molecule's shape matches the activating enzyme's active site, whatever the nature of that site, is obviously important. One way to describe the shape of the molecule is by a molecular connectivity index,[5] in this case the three-bond path index, $^3\chi_P^v$.

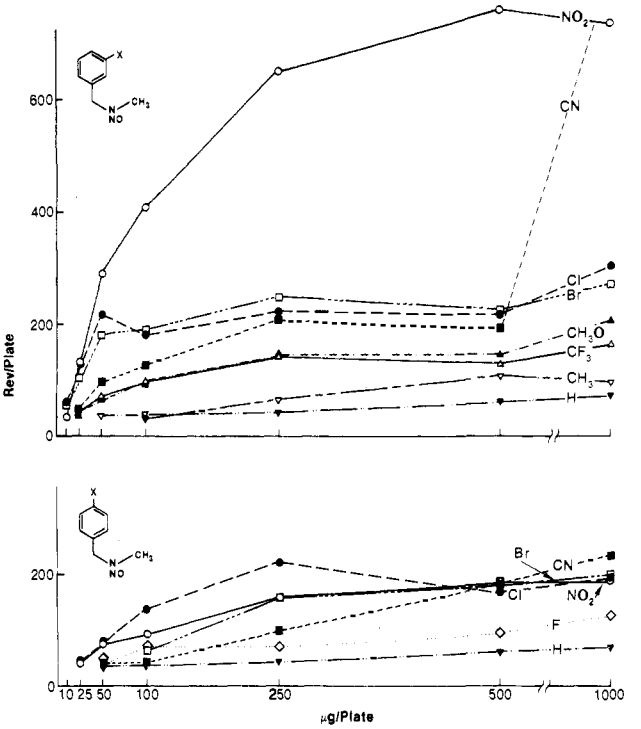

Figure 1. Dose–response of the mutagenicities of *N*-nitroso-*N*-benzylmethylamines in *Salmonella typhimurium* TA 1535 in the presence of hamster S9 mix. Top, meta-substituted compound; bottom, para-substituted compounds.

These indices are calculated from valence δ values which represent the number of valence electrons of each atom and have been shown to be correlated with atomic volumes. Appropriate combinations of these valence δ values thus give measures of molecular volumes, shapes, and branching. We looked first at the one-bond valence connectivity index, $^1\chi^v$, since it is representative of the lower order indices but found that the three-bond path valence index, $^3\chi_P^v$, was better correlated with the data. Both indices have been shown to be correlated with a variety of physical parameters and biological activities.

To be effective, the molecule must in some way facilitate its own reaction with an activating enzyme or other biomolecule. One measure of this ability is the Hammett σ constant, which is a measure of a substituent's effect on reactivity. The molecule must also be transported from the point of introduction into the cell or organism to the active site. Transportability is best described by Hansch's hydrophobicity parameter, π.

Equation 2 shows, however, that the relative mutagenicity of these substituted *N*-nitroso-*N*-benzylmethylamines is linearly dependent on a combination of only two of these parameters and that, at least for mutagenicity, the shape of the molecule is much more important than transport phenomena. The correlation parameter (R^2) shows that this linear equation can explain about 65% of the data variance. A cursory examination of these mutagenicity data, however, reveals maximum mutagenicity as the electron-withdrawing capacity of the substituent is varied. A polynomial regression analysis provided eq 3 (Figure 2),

(6) Singer, G. M.; Taylor, H. W.; Lijinsky, W. *Chem.-Biol. Interact.* **1977**, *19*, 133.

Table I. Dose–Responses of *N*-Nitroso-*N*-(substituted benzyl)methylamines in Strain TA 1535 with (+) and without (–) Hamster S9 (3 mg/plate)

	number of revertants per plate[a], dose																					
	cells only		Me_2SO		1 μg		2.5 μg		5 μg		10 μg		25 μg		50 μg		100 μg		250 μg		500 μg		1000 μg	
X	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+
H	19	17	13	13	16	18	14	12	13	23	13	23	13	19	14	*36*	16	*38*	12	*42*	17	*60*	17	*70*
m-CH_3O	25	14	16	20	20	14	18	25	19	24	15	24	10	*46*	13	*66*	16	*98*	7	*145*	18	*145*	15	*205*
m-CH_3	16	20	17	14	19	22	13	21	21	13	13	19	15	27	27	28	16	*35*	15	*64*	14	*106*	15	*96*
m-Cl	17	15	18	15	23	20	12	26	17	22	15	*62*	13	*128*	21	*217*	40	*180*	20	*221*	15	*215*	13	*302*
m-Br	11	24	15	13	13	17	13	26	15	30	11	*54*	16	*105*	15	*179*	15	*191*	31	*249*	11	*223*	8	*269*
m-CF_3	21	21	15	21	16	17	17	17	17	24	17	20	22	*41*	15	*65*	16	*96*	13	*140*	18	*127*	10	*163*
m-CN	14	24	14	17	20	22	18	20	22	25	11	30	15	*48*	15	*98*	*84*	*127*	*2132*	*209*	*3053*	*185*	*3111*	*987*
p-CH_3O	12	16	11	20	21	17	16	25	17	20	16	23	13	20	25	22	14	*50*	14	25	15	24	19	29
p-CH_3	19	18	9	18	18	21	14	17	15	9	16	17	16	19	20	23	14	17	11	31	19	*36*	22	30
p-F	15	19	8	24	18	15	8	15	21	21	17	22	20	27	16	*39*	22	*71*	11	*71*	19	*94*	15	*126*
p-Cl	22	23	19	22	17	18	16	19	14	21	18	24	21	*42*	19	*77*	19	*136*	19	*221*	24	*166*	17	*192*
p-Br	12	17	14	13	14	12	13	12	21	15	18	14	14	21	10	32	16	*64*	11	*155*	13	*183*	15	*201*
p-CN	15	19	17	21	12	21	15	18	13	16	12	23	16	28	12	*37*	16	*42*	15	*98*	12	*180*	16	*234*
p-NO_2	19	23	16	19	28	24	20	20	17	22	19	22	16	*40*	19	*74*	16	*93*	14	*159*	23	*176*	12	*187*
p-COOH	13	19	18	19	21	19	16	18	11	21	17	15	21	15	10	20	16	18	21	21	21	15	11	17
p-$COOCH_3$	14	20	16	16	15	18	9	10	13	11	12	11	15	19	11	19	16	20	12	12	15	18	15	17

[a] Mean values based on duplicate plates. Italic numbers indicate that the number of revertants is significant (greater than twice the current control mean). The means (N = 18) were as follows. Cells only: without S9, 17 ± 4; with S9, 19 ± 3; Me_2SO; without S9, 15 ± 3; with S9, 18 ± 4.

Table II. Physicochemical Parameters of *N*-Nitroso-*N*-benzylmethylamines

				log (1/*C*)	
substit	σ	$^3\chi_P^v$	π	obsd[a]	calcd[b]
H	0.000	1.201	0.000[c]	–3.416	–3.168
m-CH_3	–0.069	1.454	0.410	–3.039	–3.022
m-CH_3O	0.120	1.505	0.0465	–1.886	–2.307
p-F	0.060	1.180	0.119	–2.969	–3.003
p-Cl	0.230	1.507	0.644	–1.933	–2.063
m-Cl	0.370	1.517	–0.0941	–1.512	–1.876
p-Br	0.230	1.767	0.0150	–1.998	–1.642
m-Br	0.390	1.760	0.105	–1.552	–1.471
m-CF_3	0.430	1.492	0.420	–1.961	–1.890
p-CN	0.660	1.401	–0.560	–2.780	–2.194
m-CN	0.560	1.437	–0.628	–2.139	–2.017
p-NO_2	0.760	1.434	–0.732	–2.202	–2.337
m-NO_2	0.710	1.462	–0.378	–1.796	–2.184
Nonmutagenic Compounds					
p-CH_3	–0.170	1.229	0.589		–3.838
p-CH_3O	–0.270	1.477	0.0690		–3.962
p-COOH	0.406	1.367			–2.099
p-$COOCH_3$	0.45	1.400	–0.341		–2.036

[a] C = concentration in nanomoles/plate to induce 50 revertants/plate. [b] Calculated by eq 4. [c] P_H = 35.18 (octanol/water partition constant of the unsubstituted compound).

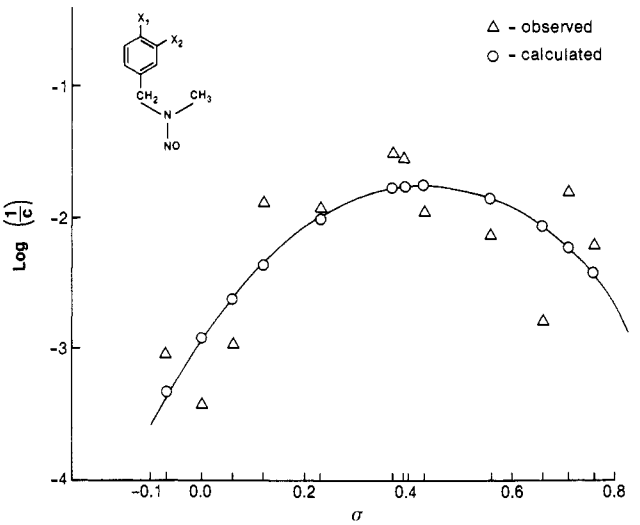

Figure 2. Second-order relationship between log (1/*C*) and σ.

which describes this second-order relationship and which by itself accounts for more than 62% (R^2) of the variation in the relative mutagenicity. This equation indicates that maximal mutagenicity results from the *p*- and *m*-chloro and -bromo derivatives.

All of these correlations were combined by performing a multiple linear regression calculation in a stepwise manner in which all of the parameters were submitted as first-, second-, and third-order terms. In this instance, the term describing transport (π) became insignificant and the resulting equation retained only the parabolic dependence on σ and a linear dependence on $^3\chi_P^v$. The correlation parameter (R^2) showed that the resulting equation accounted for 76% of the variation in the data.

A number of studies have focused attention on activation of nitrosamines by hydroxylation at the α-carbon and subsequent conversion to a more reactive species which is believed to be responsible for the biological activity. Regardless of the mechanistic details of that hydroxylation, i.e., via carbonium ion, free radical, etc., at some point a C–H bond must be broken. The relationship described by eq 2 indicates that substituents which are electron withdrawing and, therefore, tend to polarize and weaken the methylene C–H bond result in greater mutagenicity. Equations 3 and 4, however, indicate that there is an optimum amount of electron withdrawal to maximize mutagenicity. This suggests that excessive electron withdrawal may, in fact, lead to decomposition of the test compound rather than activation.

In this study and our previous one[1] we have examined a series of *N*-nitroso-*N*-benzylmethylamines in which aromatic substituents were varied systematically. A similar study of substituted *N*-nitroso-*N*-methylanilines[7] did not find any obvious relationship between mutagenicity and the nature of the substituent, but the authors did not examine their data in this manner. A very recent reexamination of the *N*-nitroso-*N*-methylaniline data in this way did, in fact, find comparable results.[8]

It is reassuring that the calculated log (1/*C*) values for the *p*-methyl and *p*-methoxy derivatives are appreciably more negative than for any of the active compounds, in accord with the lack of mutagenicity of these compounds. On the other hand, the calculated values of log (1/*C*) for

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(8) Jinno, K. *Mutat. Res.* 1984, *141*, 141.

the *p*-carboxyl and ester derivatives are within the range of bioactivity. Obviously, other factors, such as the polarity of the substituents, must be of overriding importance in these cases.

Similar studies of this large a group of compounds for relative carcinogenic effects is obviously not feasible, but a comparison of these relative mutagenicities with what little is known of the relative carcinogenicities is interesting. The unsubstituted *N*-nitroso-*N*-benzylmethylamine has been tested for carcinogenicity numerous times and has been found to be a potent esophageal carcinogen in rats.[9] The *m*-methyl and *p*-methyl derivatives are slightly less carcinogenic[10] in general agreement with our mutagenicity results. Schweinsberg et al.[11] have also shown that the *m*- and *p*-methyl derivatives are readily converted to the corresponding carboxylic acids and that these are excreted. This oxidative detoxification procedure may account for the observed nonmutagenicity of the *p*-methyl, *p*-carboxyl, and *p*-carboxymethyl derivatives. Unfortunately, none of the other derivatives have been tested for carcinogenicity.

Since so few of these substituted aromatic nitrosamines have been tested for carcinogenicity, the effect of activation of α-methylene protons by substituents is not known. In aliphatic compounds, substituent effects are generally not transmitted effectively through more than one bond, but a rough correlation of nitrosamine α-hydrogen acidity with carcinogenicity has been demonstrated.[12] The relationship of carcinogenicity of a restricted series of nitrosoheterocycles with a transport parameter has also been demonstrated.[6]

The nitrosamine mutagenicity data described here have clearly been shown to be dependent almost entirely on two physicochemical parameters associated with the individual compounds. The activity is dependent on an optimal amount of activation of the α-hydrogens and linearly on a descriptor of the overall shape and volume of the molecule but not to any significant extent on the hydrophobicity of the compound. To the extent that comparisons have been possible, it appears that the same factors may also affect carcinogenicity.

Experimental Section

Chemicals. Analytical HPLC was performed on a μ-Bondapak C_{18} column from Waters Associates installed in a Waters Associates Model 440 HPLC. Preparative HPLC was done with two silica gel cartridges in a Waters Associates Prep 500 LC. IR spectra were recorded as films or KBr pellets on a Perkin-Elmer Model 297 IR spectrometer.

Several of the *N*-nitroso-*N*-(*p*-substituted benzyl)methylamines were available from the previous study.[1] The remainder were prepared by the procedure described below.

***N*-Nitroso-*N*-(4-bromobenzyl)methylamine.** *p*-Bromobenzyl bromide (8.83 g, 35.3 mmol) was boiled under reflux in ethanol (50 mL) with 33% ethanolic CH_3NH_2 (50 mL) for 4 h. An additional portion of CH_3NH_2 (25 mL) was added and boiling continued for 2 h. The solution was cooled and concentrated, and the residue was dissolved in a mixture of 2 N HCl (100 mL) and ether (50 mL). The layers were separated, and the aqueous was extracted with additional ether (2 × 50 mL). The aqueous solution was chilled to 0 °C, basified to pH 11 with NaOH and extracted with CH_2Cl_2 (3 × 50 mL), and the extracts were dried ($NaCO_3$) and evaporated to yield crude *N*-(4-bromobenzyl)-methylamine: 4.27 g (61%). The amine (2.0 g, 10 mmol) was nitrosated by stirring at room temperature in 30% acetic acid (30 mL) and 6 N HCl (1.7 mL) with $NaNO_2$ (2.8 g, 40 mmol). The nitrosamine was isolated by neutralization to pH 9 and extraction with CH_2Cl_2 (3 × 10 mL). Thin-layer chromatography (1% EtOAc/CH_2Cl_2) showed an impurity and thus the oil obtained after evaporaing the solvent was chromatographed on silica gel (40 g). Elution by 1% EtOAc/CH_2Cl_2 gave the pure nitrosamine as a yellow oil: NMR (300 MHz, $CDCl_3$) 2.93 ppm (s, NCH_3 of major rotamer [74%], 5.26 (s, major rotamer methylene), 3.70 (s, NCH_3), 4.74 (s, NCH_2), 7.0–7.6 (m, aromatic); MS, *m/z* (relative intensity) 230 (M^+, 19) 228 (M^+, 20), 171 (100), 118 (9), 90 (20). Anal. ($C_8H_9BrN_2O$) C, H, N.

***N*-Nitroso-*N*-(4-carbomethoxybenzyl)methylamine.** The ester was prepared in quantitative yield as a yellow oil by treating an ethereal solution of the carboxylic acid[1] with diazomethane. The compound was analytically pure after removal of the ether: MS, *m/z* (relative intensity) 208 (M^+, 39), 178 (9), 177 (11), 149 (100), 121 (20), 119 (18), 90 (11), 89 (9), 77 (9). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

***N*-Nitroso-*N*-(3-chlorobenzyl)methylamine.** *N*-(3-Chlorobenzyl)methylamine was prepared in 91% yield by the above described procedure.

Routine nitrosation provided the nitrosamine in 88% yield as a yellow oil: MS, *m/z* (relative intensity) 186 (M^+, 7), 184 (M^+, 22), 154 (21), 139 (13), 127 (55), 125 (100), 118 (14), 111 (16), 99 (13), 91 (20), 89 (48), 77 (19), 75 (22). Anal. ($C_8H_9ClN_2O$) C, H, N.

***N*-Nitroso-*N*-(3-methoxybenzyl)methylamine.** *N*-(3-Methoxybenzyl)methylamine was prepared in 91% yield as described above. Nitrosation provided the nitrosamine as a yellow oil in 87% yield after elution from a silica gel column by 2% acetone/methylene chloride: MS, *m/z* (relative intensity) 180 (M^+, 4), 150 (36), 135 (9), 125 (10), 121 (100), 91 (34), 78 (14), 77 (20), 63 (13), 42 (17). Anal. ($C_9H_{12}N_2O_2$) C, H, N.

***N*-Nitroso-*N*-(3-bromobenzyl)methylamine.** *N*-(3-Bromobenzyl)methylamine was prepared in 87% yield as described above. The crude nitrosation product was purified on a short silica gel column and eluted by 2% acetone/methylene chloride as a light orange oil in 82% yield: MS, *m/z* (relative intensity) 230 (M^+, 38), 228 (M^+, 39), 200 (10), 198 (13), 171 (98), 169 (100), 157 (6), 155 (6), 91 (25), 90 (57), 89 (39), 43 (51), 42 (72). Anal. ($C_8H_9BrN_2O$) C, H, N.

***N*-Nitroso-*N*-(3-nitrobenzyl)methylamine.** The corresponding amine was prepared from 3-nitrobenzyl bromide as described above in 88% yield and subsequent nitrosation afforded the nitrosamine as an orange oil in 91% yield. The nitrosamine was purified by silica gel chromatography (2% acetone/methylene chloride): MS, *m/z* (relative intensity) 195 (M^+, 4), 165 (48), 136 (100), 119 (36), 118 (35), 91 (23), 90 (90), 89 (40), 43 (41), 42 (37). Anal. ($C_8H_9N_3O_3$) C, H, N.

***N*-Nitroso-*N*-(3-methylbenzyl)methylamine.** α-Bromo-*m*-xylene was converted to the amine as described above in 71% yield. Nitrosation afforded the nitrosamine as a yellow oil which was purified by chromatography over a short silica gel column with elution by 2% acetone/methylene chloride (70%): MS, *m/z* (relative intensity) 164 (M^+, 30), 134 (20), 105 (100), 55 (39). Anal. ($C_9H_{12}N_2O$) C, H, N.

***N*-Nitroso-*N*-(3-cyanobenzyl)methylamine.** The *N*-(3-cyanobenzyl)methylamine was prepared from 3-cyanobenzyl bromide (10.0 g, 51 mmol) as described above. The amine was nitrosated by stirring at room temperature in a solution of 30% acetic acid (50 mL) and 6 N HCl (6.7 mL) with sodium nitrite (14 g, 0.2 mol) for 5 h. The mixture was basified with Na_2CO_3 and NaOH to pH 10 and extracted with $CHCl_3$ (4 × 50 mL). The $CHCl_3$ solution was washed with 6 N HCl (4 × 50 mL), dried (Na_2CO_3), and evaporated to a yellow oil, 5.6 g (63% from the benzyl bromide). GC (DB-5 capillary) detected no impurities after silica gel chromatography with elution by 2% acetone/methylene chloride. MS, *m/z* (relative intensity) 175 (M^+, 36), 116 (100), 102 (10), 89 (14), 60 (18). Anal. ($C_9H_9N_3O$) C, H, N.

The acid wash solution was basified and extracted with $CHCl_3$ (3 × 50 mL), and the extracts were dried ($NaCO_3$) and evaporated to give *N,N*-bis(3-chlorobenzyl)methylamine, 2.26 g (17%).

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Mutagenicity Studies. The mutagenicity of the nitrosamines was assessed with *Salmonella* tester strain TA 1535, which detects base-pair substitution mutations, and hamster Aroclor 1254 induced S9 mix instead of rat S9, since nitroso compounds have been reported to be more sensitive to hamster S9 activation.[2] The protein content of the added S9 was 3 mg/plate. The dose-response study used duplicate plates over a 10-point range of 1-1000 g/plate. The plate incorporation test was performed as recommended by Ames et al.[13] with the modifications of Andrews et al.[14] Plates were incubated at 37 °C for 48 h and counted by using a hand-held tally. A compound was considered to be mutagenic only if there were two consecutive doses which showed revertant numbers that were greater than twice the mean of the current controls.

Partition Coefficients. The octanol/water partition coefficients were measured by the shake procedure as previously described.[6] Quantitation of the nitrosamine in the aqueous layer was determined by HPLC.

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Dipole Moment in Relation to H_2 Receptor Histamine Antagonist Activity for Cimetidine Analogues

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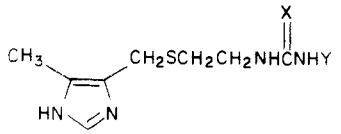
The activities of a series of H_2 receptor histamine antagonists structurally related to cimetidine (1) have been compared to investigate the effect of replacing the cyanoguanidine moiety by other neutral, dipolar groups. Antagonist activity, as measured in vitro on the histamine-stimulated guinea pig right atrium, was found to be very sensitive to relatively minor structural changes. Differences in H_2 antagonist activity are accounted for by dipole moment orientation and lipophilicity and are rationalized in terms of an optimum requirement for alignment of a hydrogen-bonding moiety in the antagonist with respect to the receptor and desolvation effects at the receptor. The most active compound in the series is the 2-amino-3-nitropyrrole derivative **5**, which combines a near-optimal dipole orientation with high lipophilicity.

The success of cimetidine (1) in the treatment of peptic ulcer disease has promoted a great deal of research interest in H_2 receptor antagonists as clinically effective agents.

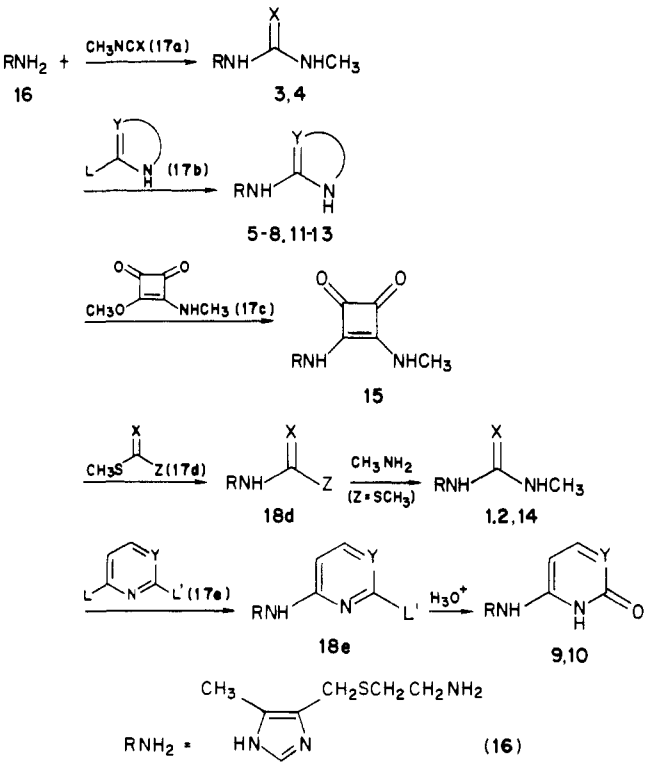
The search for H_2 antagonists of increased potency by the structural modification of cimetidine has led to the discovery of several new compounds, notable amongst which are ranitidine[1] and tiotidine,[2] in which imidazole has been replaced by the highly effective [(dimethylamino)-methyl]furan and guanidinothiazole moieties, respectively. At the opposite end of the cimetidine molecule, suitable replacement of the cyanoguanidine group has provided antagonists of equivalent or increased potency, including the 5-substituted aminopyrimidinone derivative, oxmetidine.[3]

In a recent investigation,[4,5] a series of 12 cimetidine analogues were compared in order to analyze differences in antagonist activity on replacement of the cyanoguanidine group. For most compounds, a reasonable relationship was found between in vitro H_2 antagonist activity and a lipophilicity parameter (octanol/water log P of a cyanoguanidine or corresponding alternative model compound). A notable anomaly, however, was the nitrodiaminoethene analogue (**2**), whose activity was only poorly predicted, and it was concluded that some other property was making a marked contribution to activity.



1, X = NCN; Y = CH_3
2, X = $CHNO_2$; Y = CH_3

Scheme I



One property which cyanoguanidine shares with other neutral moieties found in active analogues of cimetidine is its polarity. Simple derivatives of urea, thiourea,

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