α and β, 4.72, about 13 aromatic protons. *Anal.* ($C_{23}H_{21}O_7N_3 \cdot H_2O$) H, N; C: calcd, 58.84; found, 59.28.

Hydrolysis Procedure. Exactly 0.4 mmol of the ester was dissolved in 200 ml of acetone contained in a 250-ml volumetric flask. The solution was diluted to the mark with H_2O and thoroughly mixed to give a 0.0016 *M* solution of the ester. Aliquots (25 ml) were transferred *via* a volumetric pipet to 50-ml erlenmeyer flasks, the flasks were stoppered, and the contents were equilibrated in a bath maintained at 40 ± 0.2°. Into each aliquot was rapidly pipetted exactly 5 ml of 0.100 *N* NaOH. This gave an initial concentration of the ester of 0.00133 *M*. The mixture was stirred magnetically, and at time intervals the hydrolysis was stopped by rapidly pipetting exactly 5 ml of 0.100 *N* HCl into the flask. The contents were cooled to room temperature, a few drops of phenolphthalein solution were added, and the mixture was titrated to the end point with 0.100 *N* NaOH. The amount required to reach the end point, minus the blank value, was equivalent to that consumed during the hydrolysis. Blank values were determined by pipetting 5 ml of the standard 0.1 *N* NaOH into 25-ml aliquots of the acetone–H_2O solvent, pipetting into this mixture 5 ml of the standard 0.1 *N* HCl, and titrating this mixture to the phenolphthalein end point with the standard 0.1 *N* NaOH. The same pipets were used for the blanks that were used for the hydrolysis.

Molar concentrations of the ester at time intervals during the hydrolysis were calculated from the hydrolysis data, and the $-\log C$ values were plotted as a function of time as shown in Scheme I. The hydrolysis constant k was calculated from the relationship $k = 2.3 \log C/t$.

Determination of Partition for the Palmitate Ester 2. ^{14}C-Palm-*O*-*ara*-C (**2**) (19.2 μCi/mg, labeled in the 2 position of the pyrmidine ring) was added to a stoppered erlenmeyer flask containing 15 ml each of 1-octanol and sodium phosphate aqueous buffer (pH 7.0, 0.035 *M*, ionic strength 0.1). After vigorous shaking (37°, 1 hr) and subsequent phase separation, radioactivity in 1.0-ml aliquots of each phase was determined using a liquid scintillation spectrometer. The aqueous phase was removed and replaced with fresh buffer. The shaking procedure was repeated with fresh buffer until a constant partition coefficient was obtained. The value obtained was 127.3 (octanol–water) and the octanol concentration of Palm-*O*-*ara*-C (**2**) in octanol at equilibrium was 1.5 μg/ml.

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Linear Regression Analysis of Inhibitory Potency of Organic Disulfides against *Histoplasma capsulatum*†

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The Free–Wilson equations are derived for the case of symmetrical substitution and are applied, in four modifications, to *in vitro* inhibitory activity of 77 organic disulfides against *Histoplasma capsulatum*. Substituent constants are listed to aid in the design of new inhibitory agents against this human pathogen (and perhaps other fungal organisms).

As part of a search for improved inhibitory agents against *Histoplasma capsulatum*, the causative organism of histoplasmosis, a regression analysis of the *in vitro* activity against *H. capsulatum* was carried out for 77 organic disulfides. There are two main approaches to the problem of correlating biological activity with chemical structure. The one, due to Hansch,[1] correlates biological activity with other physical parameters, especially the partition ratio between octanol and water. The other, by Free and Wilson,[2] estimates biological activity from empirically fitted substituent constants. Craig[3] gives a readable comparison of the two. A recent chapter by Cammarata and Rogers[4] reviews applications of these methods and contains a useful discussion of the physical basis for the mathematical models.

Since the compounds to be considered here are all disulfides with varying substituents, and since we have no knowledge of the details of the drug action against *H. capsulatum* and cannot reasonably postulate a correlation with any particular physical parameter, the Free–Wilson approach seemed the more applicable.

Mathematical Background. Free and Wilson defined their activity parameters relative to the average activity of the set of compounds studied. This is only one of several equivalent methods. To see how these arise from a linear

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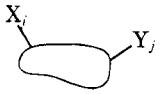
dependence between the parameters being determined and, more importantly, to show the modifications necessary in the case of symmetric substitution, consider the molecule indicated by I which can have any one of the groups X_i (i =



I

1, 2, . . . , N) at the first position and any one of the Y_j (j = 1, 2, . . . , M) at the second. Depending upon the symmetry of the molecule, substituting, say $-CH_3$ at the first position and –Cl at the second, may or may not give a result identical with substituting –Cl at the first and $-CH_3$ at the second. For the moment assume these two possibilities are not identical. If the effect of substituents X and Y on some biological activity, f, is additive for a set of K molecules of type I

$$f_k = \mu + \sum_{i=1}^{N} n_{ki}x_i + \sum_{j=1}^{M} m_{kj}y_j \qquad k = 1, 2, \ldots, K \tag{1}$$

where f_k is the activity of the kth molecule, x_i is the contribution of substituent X_i and n_{ki} is the number of times (0 or 1) this substituent appears in the kth molecule, and m_{kj} and y_j are defined similarly for substituent Y_j. The constant μ may be thought of as the contribution of the remainder of the molecule to the activity. It appears that there are $N + M + 1$ constants to be determined (N values of x plus M values of y plus the value of μ). However, since each molecule must contain exactly one X substituent and one Y substituent

$$n_{k1} + n_{k2} + \ldots + n_{kN} = 1 \text{ and}$$

$$m_{k1} + m_{k2} + \ldots + m_{kM} = 1 \tag{2}$$

Solving for n_{k1} and m_{k1}, substituting into eq 1, and collecting coefficients of the independent variables $n_{k2} \ldots m_{kM}$ gives

$$f_k = (\mu + x_1 + y_1) + \sum_{i=2}^{N} (x_i - x_1)n_{ki} + \sum_{j=2}^{M} (y_j - y_1)m_{kj} = \mu' + \sum_{i=2}^{N} n_{ki}x_i' + \sum_{j=2}^{M} m_{kj}y_j' \tag{3}$$

Unlike the parameters in eq 1, the $N + M - 1$ parameters (μ', x_2', . . . , y_M') in eq 3 are all independent and, hence, can be determined experimentally. Equation 2 implies an arbitrariness in the $N + M + 1$ parameters of eq 3. One might choose to set $x_1 = y_1 = 0$. This would be analogous to the Hammett treatment of substituent constants, where parameter values are measured relative to the standard hydrogen substituent. Or one might impose the conditions used implicitly by Free and Wilson[2]

$$x_1 + x_2 + \ldots + x_N = 0 \text{ and}$$

$$y_1 + y_2 + \ldots + y_M = 0 \tag{4}$$

which lead to the result that μ equals the average activity of the K molecules. All choices, of course, lead to identical predicted molecular activities.

A set of ($N + M - 1$) of the K values of f_k could be used to give $N + M - 1$ equations of the form of eq 2, and these could be solved to give the ($N + M - 1$) independent parameters. However, it is better to use the entire set of f_k's, and minimize eq 5

$$\sum_{k=1}^{K}[f_k - (\mu' + x_2'n_{k2} + \ldots + y_M'm_{kM})]^2 \tag{5}$$

with respect to μ', x_2', . . . , and y_M' to give the least-squares equations

$$\mu'K + x_2'\sum_k n_{k2} + \ldots + y_M'\sum_k m_{kM} = \sum_k f_k$$

$$\mu'\sum_k n_{k2} + x_2'\sum_k n_{k2}^2 + \ldots + y_M'\sum_k n_{k2}m_{kM} = \sum_k n_{k2}f_k$$

$$\vdots \qquad \vdots \qquad \vdots$$

$$\mu'\sum_k m_{kM} + x_2'\sum_k m_{kM}n_{k2} + \ldots + y_M'\sum_k m_{kM}^2 = \sum_k m_{kM}f_k \tag{6}$$

which are solved for μ', x_2', . . . , y_M'. It is clear that trouble will arise if a pair of substituents X_i and Y_j each appear only once and are in the same molecule, since the one experimental value of f cannot give two parameters x_i' and y_j'. More complicated examples can occur which are not so easy to recognize on sight.[5] The general test for this kind of difficulty is obtained from the determinant of the coefficients of the unknowns in eq 6 (see Appendix). If this determinant equals 0, the compounds are not sufficient for determining all the parameters; either new compounds must be tested, or some of the compounds and parameters dropped. Equations 1–6 generalize straightforwardly for any number of substituents.

Now suppose the two sites in I are equivalent as they would be in, for example, a disubstituted benzene. Each substituent X_i has only a single constant, not two, for the two positions. The activity of the kth molecule is

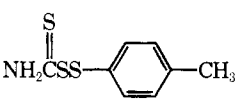
$$f_k = \mu + \sum_{i=1}^{N} n_{ki}x_i \tag{7}$$

with the single constraint

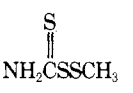
$$\sum_{i=1}^{N} n_{ki} = 2 \tag{8}$$

since each molecule must contain exactly two substituents. This is generalized to P equivalent substituent sites simply by replacing the 2 in eq 8 by P.

Compounds Studied. p-Tolyl trithiopercarbamate (II) is typical of the 77 disulfides tested. These can be treated as a case of substitution at two symmetric sites on the –SS– residue. Thus X = $H_2NC(S)$–, Y = p-$CH_3C_6H_4$–, and II = X–SS–Y. An alternative which was also examined is to dissect X and Y further so that X_1 = NH_2–, X_2 = –C(S)–, X_3 = 1,4-C_6H_4–, X_4 = $-CH_3$, and II = X_1–X_2–SS–X_3–X_4. This is a symmetric 4-substituent case slightly modified by the fact that substituents in positions 1 and 4 cannot be interchanged with those in 2 and 3. A more serious modification appears at first sight to be the fact that the equation of constraint (eq 8) no longer holds since the permissible compound (III) is obtained from II by dropping X_3. However, this case can be reduced easily to the standard form by treating a missing X_2 or X_3 as a kind of special substituent and computing its substituent constant along with the others.



II



III

The disulfides studied are listed in Table I. Details of synthesis and biological testing are in ref 6–12 where bio-

Table I. Inhibitory Effects of Organic Disulfides on the Growth of *Histoplasma capsulatum* (Strain H-7)

Compd no.[a]	Structure[b,c] X–SS–X′ or X_1–X_2–SS–X_3–X_4				Solvent[d]	Exptl MIC,[e,f] μg/ml	Computer input MIC	Calcd act., μg/ml			
								X–SS–X′ model		X_1–X_2–SS–X_3–X_4 model	
	X_1	X_2	X_3	X_4				1/MIC	Log (1/MIC)	1/MIC	Log (1/MIC)
1[r]	$-SC(S)N(CH_2)_5$	$-(CH_2)_2-$	$-(CH_2)_2-$	-NHAc	M, T	–[g]	30	40	41	33	40
2[s]	$-NH_2$	-C(S)-	–	$-C(CH_3)_3$	M, T	Inactive	50	–19	39	–9	42
3[r]	$-SC(S)N(CH_2)_5$	$-(CH_2)_2-$	$-(CH_2)_2-$	$-SC(S)N(CH_2)_5$	M, T	–[h]	48	40	41	45	42
4[s]	$-NH_2$	-C(S)-	*p*-Ph-	$-CH_3$	M, T	Good	8.75	5	11	4	10
5[s]	$-NHCH_3$	-C(S)-	$-(CH_2)_2-$	-NHAc	M, T	Good	8.75	–55	15	–19	17
6[s]	$-NHCH_3$	-C(S)-	–	$-C(CH_3)_3$	M, T	Fair to good	10	16	12	49	13
7[s]	$-NHCH_3$	-C(S)-	*p*-Ph-	$-CH_3$	M, T	Good, p 5[t]	7.5	3	4	3	3
8[s]	$-N(CH_3)_2$	-C(S)-	-C(S)-	$-N(CH_3)_2$	T[i]	Good	8.75	3	3	3	3
9[s]	$-N(CH_3)_2$	-C(S)-	$-(CH_2)_2-$	-NHAc	M, T	Good	8.75	5	11	5	11
10[u]	$-SO_2Na$	$-(CH_2)_4-$	-C(S)-	$-N(CH_3)_2$	W	2.5	2.5	4	7	4	7
11[s]	$-N(CH_3)_2$	-C(S)-	*p*-Ph-	$-CH_3$	M, T	Good,[j] 1.0	1.0	2	3	2	2
12[r]	$-N(CH_2)_5$	-C(S)-	–	$-C(CH_3)_3$	M, T	p 15	17.5	18	18	18	18
13[t]	$-SO_2Na$	$-(CH_2)_4-$	-C(S)-	$-N_2C_4H_8Ac$	W	–	50	16	31	15	31
14[r]	$-N_2C_4H_8-Ac$	-C(S)-	-C(S)-	$-N_2C_4H_8Ac$	M, T	–[k]	48	–1099	61	–481	61
15[u]	$-SO_2Na$	$-(CH_2)_4-$	-C(S)-	$-NC_4H_8O$	W	2.5	2.5	3	3	3	3
16[v]	$-CH_3$	-C(O)-	–	$-CH_3$	M, W	10	10	14	15	12	14
17[v]	$-CH_3$	-C(O)-	–	$-C_2H_5$	M, T	10	10	14	13	12	13
18[v]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	-Cl	M, T	8, p 5[t], 7.5[t]	7.5	8	8	8	8
19[w]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	$-NH_2 \cdot HCl$	W	15	15	40	21	42	22
20[t]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	-NHAc	M, W	–	50	31	24	28	25
21[w]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	$-CH_3$	M, T	16, –,[t] p 15[t]	16	16	16	20	20
22[w]	$-CH_3$	-C(O)-	–	$-CH_2CH=CH_2$	M, W	5–10, p 10,[t] 15[t]	12.5	13	13	13	13
23[r]	$-CH_3$	-C(O)-	$-CH_2-$	$-C(O)CH_3$	M, T	10[l]	10	10	10	10	10
24[u]	$-CH_3$	-C(O)-	$-(CH_2)_4-$	$-SO_2Na$	W	20[m]	30	12	15	12	15
25[v]	$-C(CH_3)_3$	–	-C(O)-	$-CH_3$	M, T	30[n]	22.5	9	19	9	18
26[v]	-Ph	–	-C(O)-	$-CH_3$	M, T	8, p 5[t]	6.25	10	10	12	12
27[v]	-3,4-Cl_2Ph	–	-C(O)-	$-CH_3$	M, T	15	15	15	15	15	15
28[r]	$-C_6Cl_5$	–	-C(O)-	$-CH_3$	M, T	20[h,k]	18	18	18	18	18
29[v]	*p*-Tolyl	–	-C(O)-	$-CH_3$	M, T	8, p 7.5[t]	8.75	3	6	5	6
30[w]	-COOH	*o*-Ph-	-C(O)-	$-CH_3$	M, T	–[k]	48	–63	45	–73	47
31[w]	$-CH_3$	-C(O)-	$-CH_2-$	-COOH	M, W	20	20	20	20	22	26
32[w]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	-COOH	M, W	–	50	50	50	31	39
33[w]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	$-COOCH_3$	M, T	15	15	15	15	15	15
34[w]	$-CH_3$	-C(O)-	$-(CH_2)_3-$	-COOH	M, W	–	50	50	50	50	50
35[w]	-COOH	$-(CH_2)_4-$	-C(O)-	$-CH_3$	M, W	–	50	50	50	78	49
36[w]	$-CH_3$	-C(O)-	$-(CH_2)_2-$	-NH-*n*-decyl·HCl	M, T	10, p 10[t]	12.5	13	13	8	11
37[v]	-*n*-Dodecyl	–	-C(O)-	$-CH_3$	M, T	–	50	29	28	36	29
38[r]	$-CH_3$	-C(O)-	-C(O)-	$-CH_3$	M, T	10 (p 5)	7.5	25	14	30	16
39[v]	$-CH_3$	-	-C(O)-	$-C_2H_5$	M, T	20	20	17	18	12	13
40[v]	-Ph	–	-C(O)-	$-C_2H_5$	M, T	10	10	11	11	12	11

41[v]	$-CH_3$	–	$-C(O)-$	$-C(CH_3)_3$	M, T	30,[m] p 20[t]	22.5	**16**	20	9	18
42[v]	$-C(CH_3)_3$	$-C(O)-$	$-CH_2-$	$-CH_3$	M, T	30,[m] p 20[t]	22.5	16	18	12	17
43[v]	–Ph	–	$-C(O)-$	$-C(CH_3)_3$	M, T	15 (p 5, 10)[t]	7.5	**11**	13	9	15
44[v]	–*n*-Dodecyl	–	$-C(O)-$	$-C(CH_3)_3$	M, T	50	45	38	37	20	37
45[t]	$-C_{10}H_{15}$	$-C(O)-$	$-CH_2-$	$-CH_3$	M, T	20	20	16	22	17	21
46[v]	$-C_{10}H_{15}$	$-C(O)-$	$-C(O)-$	$-C_{10}H_{15}$	M, T	–[o]	40	52	38	47	39
47[v]	$-CH_3$	–	$-C(O)-$	$-(CH_2)_{16}CH_3$	M, T	–	50	41	41	24	36
48[v]	–Ph	–	$-C(O)-$	$-(CH_2)_{16}CH_3$	M, T	–[o]	40	18	26	23	30
49[v]	–*n*-Dodecyl	–	$-C(O)-$	$-(CH_2)_{16}CH_3$	M, T	–[o]	40	–96	75	–67	74
50[s]	–Ph	–	$-C(O)-$	–Ph	D, T	Fair	15	15	15	12	10
51[v]	–2,4,6-Me_3Ph	$-C(O)-$	–	–Ph	M, T	20, p 15[t]	17.5	18	18	18	18
52[v]	–3,4,5-$(MeO)_3$Ph	$-C(O)-$	$-CH_2-$	$-CH_3$	M, T	30,[m] p 25[t]	27.5	28	28	28	28
53[t]	$-NH_2 \cdot HCl$	$-(CH_2)_2-$	$-(CH_2)_4-$	$-SO_2Na$	W	20	20	14	22	14	21
54[x]	$-NH_2 \cdot HCl$	$-(CH_2)_2-$	$-(CH_2)_4-$	$-SO_2CH_2Ph$	W	–	50	50	50	50	50
55[s]	$-NH_2 \cdot HCl$	$-(CH_2)_2-$	$-CH_2-$	$-CH(NH_2)COOH$	W	Inactive	50	24	33	26	33
56[s]	$-NH_2 \cdot HCl$	$-(CH_2)_2-$	–	–2,6-$(MeO)_2$Ph	W	Fair, 20[t]	15	15	15	15	15
57[x]	–NHAc	$-(CH_2)_2-$	$-(CH_2)_4-$	$-SO_2Na$	W	p 20	22.5	13	25	12	24
58[x]	–NHAc	$-(CH_2)_2-$	$-(CH_2)_4-$	$-SO_3Na$	W	–	50	50	50	50	50
59[r]	–COOH	*o*-Ph–	$-CH_2-$	$-CH_3$	M, T	–	50	61	43	61	40
60[r]	–COOH	*o*-Ph–	$-CH_2-$	$-CH(NH_2)COOH$	M, T	–[k]	48	–2500	72	588	73
61[r]	–COOH	*o*-Ph–	–	$-C(CH_3)_3$	M, T	–[k]	48	17	61	16	55
62[r]	–COOH	*o*-Ph–	–	–Ph	D, W	–[k]	48	22	31	26	35
63[r]	–COOH	*o*-Ph–	–	$-C_6H_{10}Cl$	M, T	–	50	50	50	50	50
64[x]	–NHAc	$-(CH_2)_2-$	$-(CH_2)_2-$	–NH-*n*-decyl[p]	M, T	10	10	10	10	10	10
65[s]	–NH-*n*-decyl·HCl	$-(CH_2)_2-$	$-CH_2-$	–Ph	M, T	Good	8.75	9	9	7	7
66[s]	–NH-*n*-decyl·HCl	$-(CH_2)_2-$	$-CH_2-$	–*p*-Tolyl	M, T	Very good	3	3	3	4	4
67[s]	–NH-*n*-decyl·HCl	$-(CH_2)_2-$	$-CH_2-$	*p*-MeOPh	M, T	Very good	3	3	3	3	3
68[s]	–NH-*n*-decyl·HCl	$-(CH_2)_2-$	$-CH_2-$	*p*-CNPh	M, T	Good	8.75	9	9	9	9
69[s]	–NH-*n*-decyl·HCl	$-(CH_2)_2-$	$-CH_2-$	*m*-NO_2Ph	M, T	Good	8.75	9	9	9	9
70[x]	–NHAc	$-(CH_2)_2-$	$-(CH_2)_2-$	–NHAc	W	–	50	**41**	40	26	38
71[s]	$-CH_3$	*p*-Ph–	–S–	–*p*-Tolyl	D, T	Fair, p 10[t]	12.5	13	13	13	13
72[s]	$-CH(NHAc)COOCH_3$	$-CH_2-$	$-CH_2-$	$-CH(NHAc)COOCH_3$	W	Inactive	50	50	50	50	50
73[x]	$-CH_3$	$-(CH_2)_4-$	$-(CH_2)_4-$	$-CH_3$	M, T	–	40	40	40	35	40
74[x]	–Ph	–	–	–Ph	M, T	7.5	7.5	6	7	7	9
75[x]	*n*-Dodecyl	–	–	–*n*-Dodecyl	M, T	–	50	35	54	45	53
76[r]	$-CH(NH_2)COOH$	$-C(CH_3)_2-$	$-C(CH_3)_2-$	$-CH(NH_2)COOH$	M, T	–	50	50	50	50	50
77[s]	"*o*-Benzoylthiamine"	–	–	"*o*-Benzoylthiamine"	W	–	50	50	50	50	50
						Standard deviation of MIC[q]		574	15	151	14
						Standard deviation of 1/MIC[q]		0.14	0.15	0.12	0.13

[a]Superscripts indicate references. [b]NC_5H_{10} = piperidyl; $N_2C_4H_8$ = piperazinyl; NC_4H_8O = morpholino; $C_6H_{10}Cl$ = *trans*-2-chlorocyclohexyl; $C_{10}H_{15}$ = 1-adamantyl; – = no group. [c]Groups X_1 and X_4 must not be used in place of groups X_2 and X_3 (see text). [d]Compounds dissolved in methanol (M) or dioxane (D) were diluted either with water (W) or an aqueous Tween 80 solution (T) so that the following concentrations were not exceeded: M, 0.25%; D, 0.1%; T, 0.008%. [e]In transforming activities reported earlier, which were not given numerically, to a numerical input for the least-squares program, the following estimates were made: inactive or – = 50 μg/ml; fair = 15; fair to good = 10; good = 8.75; very good = 3. A numeral preceded by the letter "p" indicates that inhibition was partial at the concentration tested; MIC then was set equal to the average of this and the next higher concentration tested. Concentrations tested were 1, 2.5, 5, 10, 15, 20, and 25 μg/ml. If the compounds did not dissolve completely, 2 μg/ml arbitrarily was subtracted from the MIC. [f]MIC = minimum inhibitory concentration. [g]MIC for compound 1 was p20 against strain H-25. MIC for strain H-7 is usually about 5–10 μg/ml greater than for strain H-25. [h]Samples of compounds **3** and **28** were sterilized in an autoclave; some destruction of the compound may have resulted. [i]Compound suspended in aqueous Tween 80 solution. [j]A later test of compound **11** indicated MIC = 1.0.[12] [k]Compound did not dissolve completely. [l]Efforts to purify compound **23** were unsuccessful, and assay was done with crude material.[10] [m]MIC = 10 against strain H-25. [n]Compounds that showed activity on the fourth day, but not thereafter, were arbitrarily assigned an MIC of 30; those that were inactive on the fourth day, an MIC of 50. [o]Sparing solubility prevented tests above 10 μg/ml at which concentration the compound was completely inactive.[8] [p]See footnote *c*, Table II. [q]See text for a discussion of these standard deviations. [r]See ref 10. [s]See ref 6. [t]See ref 12. [u]See ref 9. [v]See ref 8. [w]See ref 11. [x]See ref 7.

Table II. Substituent Constants for 2-Group Model (X–SS–X′)

Group no.	Structure[a]	No. of times used in data	Substituent constants from calcn using 1/MIC[b]	Substituent constants from calcn using Log (1/MIC)	Group no.	Structure[a]	No. of times used in data	Substituent constants from calcn using 1/MIC[b]	Substituent constants from calcn using Log (1/MIC)
1	$-C(S)NC_4H_8O$	1	0.2925	0.8363	30	$-(CH_2)_4CH_3$	2	−0.0310	−0.1627
2	$-p$-$C_6H_4CH_3$	5	0.2884	0.4670	31	$-(CH_2)_2SC(S)NC_5H_{10}$	3	−0.0310	−0.1692
3	$-p$-$CH_2C_6H_4CH_3$	1	0.2296	0.6842	32	$-(CH_2)_2NHAc$	9	−0.0313	−0.1634
4	$-p$-$CH_2C_6H_4OCH_3$	1	0.2296	0.6842	33	$-C(O)C(CH_3)_3$	4	−0.0315	0.0597
5	$-C(S)N(CH_3)_2$	5	0.1472	0.4020	34	$-(CO)C_2H_5$	2	−0.0334	−0.0021
6	$-(CH_2)_2Cl$	1	0.0700	0.3373	35	$-CH_2CH(NHCOCH_3)$-$COOCH_3$	2	−0.0335	−0.2111
7	$-C(CH_3)_3$	5	0.0509	−0.0653					
8[c]	$-(CH_2)_2NH$-n-decyl	1	0.0443	0.4402	36	$-C(CH_3)_2CH(NH_2)COOH$	2	−0.0335	−0.2111
9	$-C_6H_5$	9	0.0370	0.2266	37[d]	"o-Benzoylthiamine"	2	−0.0335	−0.2111
10	$-CH_2C(O)CH_3$	1	0.0367	0.2124	38	$-C_{10}H_{15}$	3	−0.0340	−0.1522
11	$-(CH_2)_4SO_2Na$	6	0.0204	0.0425	39	$-(CH_2)_4SO_3Na$	1	−0.0357	−0.2588
12	$-2,6$-$(CH_3O)_2C_6H_3$	1	0.0173	0.2049	40	$-(CH_2)_2NH_2 \cdot HCl$	5	−0.0377	−0.1042
13	$-CH_2CH{=}CH_2$	1	0.0167	−0.0653	41	$-(CH_2)_2COOH$	1	−0.0433	−0.4866
14	$-(CH_2)_2NH$-n-decyl·HCl	6	0.0167	0.1155	42	$-(CH_2)_3COOH$	1	−0.0433	−0.4866
15	$-C_6H_{10}Cl$	1	0.0123	0.0202	43	$-(CH_2)_4COOH$	1	−0.0433	−0.4866
16	$-CH_2C_6H_5$	1	0.0106	0.2193	44	$-C(S)N_2C_4H_8C(O)CH_3$	3	−0.0440	−0.2547
17	$-p$-$CH_2C_6H_4CN$	1	0.0106	0.2193	45	$-C(O)C_6H_5$	1	−0.0573	0.1259
18	$-m$-$CH_2C_6H_4NO_2$	1	0.0106	0.2193	46	$-C(O)$-$3,4,5$-$(CH_3O)_3$-C_6H_2	1	−0.0594	−0.2488
19	$-C_2H_5$	5	0.0087	0.0862					
20	$-CH_3$	4	0.0058	0.0300	47	$-C(O)$-$2,4,6$-$(CH_3O)_3$-C_6H_2	1	−0.0668	−0.1929
21	$-(CH_2)_2COOCH_3$	1	0.0033	0.0363					
22	$-3,4$-$Cl_2C_6H_3$	1	0.0033	0.0363	48	$-C(O)(CH_2)_{16}CH_3$	3	−0.0682	−0.3667
23	$-(CH_2)_2CH_3$	1	−0.0008	0.0083	49	$-C(S)NHCH_3$	3	−0.0738	0.2583
24	$-C_6Cl_5$	1	−0.0078	−0.0429	50	$-m$-C_6H_4COOH	6	−0.0793	−0.4424
25	$-CH_2CH(NH_2)COOH$	2	−0.0081	−0.1400	51	$-NC_5H_{10}$	1	−0.0808	0.0990
26	$-CH_2COOH$	1	−0.0133	−0.0886	52	$-C(S)NH_2$	2	−0.1895	−0.2446
27	$-C(O)CH_3$	24	−0.0237	0.0644	53	$-p$-$SC_6H_4CH_3$	1	−0.2954	−0.2871
28	$-n$-Dodecyl	5	−0.0292	−0.2295		μ (molecular residue = SS)	77	0.0870	−1.2767
29	$-(CH_2)_4SO_2CH_2C_6H_5$	1	−0.0294	−0.3180					

[a] See footnote *b*, Table I. [b] In decreasing order of effectiveness. [c] The one compound on which these values are based is 64 in Table I. We suspect that the compound actually tested may have been the HCl salt, not the free base, so that 64 perhaps should have been included in computing the (quite similar) substituent constants of 14. There is no way to check what may have been an old clerical error, but the first recognition at this point illustrates another advantage of least-squares systematization. [d] See ref 6.

logical activities usually are given as minimum inhibitory concentration (MIC) against the growth of *H. capsulatum*. Precise values of MIC were not always given for the less active compounds and, for example, "inactive" was arbitrarily translated for the least-squares program as MIC = 50 μg/ml. This should not lead to serious error since it is only the compounds with low MIC values that are of interest.

It is not obvious what function of MIC should be fitted to eq 1. The simplest choice, $f_k = \mathrm{MIC}_k$, has the disadvantage of giving too much weight to the inactive compounds. That is, the difference between MIC = 50 and 48 would be considered more important than the difference between MIC = 0.1 and 2.0, but these lower values are more accurately known and of far greater interest. Using the reciprocal, $f_k = (1/\mathrm{MIC})_k$, overcomes these objections and, effectively, puts f_k in terms of biological activity rather than concentration. We have also used $f_k = \log (1/\mathrm{MIC})_k$. This is a plausible choice if one supposes the biological activity to be proportional to the rate of some unspecified biochemical reaction involving the drug and that the various substituents contribute additively to the free energy of activation of this reaction. Activities predicted by fitting (1/MIC) and log (1/MIC) with both the 2- and 4-substituted models are compared with measured activities in Table I. Although the least-squares fitting was done with 1/MIC or log (1/MIC), predicted activities are in all cases recalculated to and listed as MIC since this is the familiar measure. Predictions are reasonably accurate for the more active compounds, although the unattractive inactive compounds are often wildly in error. For example, fitting 1/MIC gave a meaningless predicted MIC = −1099 for compound 14. This is as expected since use of reciprocal MIC deemphasizes the inactive, and therefore uninteresting, compounds. More precise estimates of the fit are listed in the last two lines of Table I and were computed from

$$\text{std deviation} = \left[\frac{\sum_{k=1}^{K}[f_k(\text{exptl}) - f_k(\text{calcd})]^2}{K - F}\right]^{1/2} \quad (9)$$

where f_k(exptl) is the measured activity, f_k(calcd) is the calculated activity, F is the number of parameters fitted in the least-squares process, and K is the number of experimental points. Standard deviations using f = MIC are high owing to poor fit of inactive compounds. Those from f = 1/MIC are more satisfactory.

Substituent constants for the two- and four-group treatments are in Tables II and III, in order of decreasing activity in the 1/MIC column. Although all computed activities in Table I are transformed to MIC, the constants in Tables

Table III. Substituent Constants for 4-Group Model (X_1–X_2–SS–X_3–X_4)

Group no.	Structure[a]	No. of times used in data	Substituent constants from calcn using 1/MIC[b]	Substituent constants from calcn using Log (1/MIC)
	Groups for Central Positions X_2 and X_3[c]			
1	–C(S)–	15	1.0353	–30.2477
2	–1,4-C_6H_4–	4	0.6751	–7.0109
3	– (*i.e.*, bond)	32	0.2936	–7.5106
4	–CH_2–	15	0.2720	–7.4869
5	–$(CH_2)_2$–	28	0.2592	–7.6669
6	–$C(CH_3)_2$–	2	0.2500	–7.5514
7	–$(CH_2)_3$–	1	0.2470	–7.7706
8	–C(O)–	39	0.2420	–7.5549
9	–$(CH_2)_4$–	11	0.2397	–7.7601
10	–1,2-C_6H_4–	6	0.2132	–7.7460
11	–S–	1	–0.2506	–8.3459
	Groups for Outer Positions X_1 and X_4[c]			
1	–*p*-$C_6H_4OCH_3$	1	–0.0859	8.0784
2	–*p*-$C_6H_4CH_3$	3	–0.1627	7.9420
3	–Cl	1	–0.1859	8.0285
4	–NH-*n*-decyl·HCl	6	–0.1989	7.8751
5	–SO_2Na	6	–0.2165	7.8075
6[d]	–NH-*n*-decyl	1	–0.2216	8.0962
7	–C(O)CH_3	1	–0.2321	7.7236
8	–$C(CH_3)_3$	9	–0.2459	7.4864
9	–$COOCH_3$	1	–0.2526	7.7275
10	–C_6H_5	11	–0.2686	7.6819
11	–CH_3	39	–0.2689	7.5947
12	–$SO_2CH_2C_6H_5$	1	–0.2704	7.4334
13	–C_2H_5	3	–0.2704	7.6180
14	–$CH_2CH{=}CH_2$	1	–0.2737	7.6504
15	–$C_{10}H_{15}$	3	–0.2748	7.3984
16	–2,6-$(H_3CO)_2C_6H_3$	1	–0.2776	7.7069
17	–SO_3Na	1	–0.2821	7.4906
18	–$CH(NH_2)COOH$	4	–0.2835	7.3403
19	–NHAc	9	–0.2838	7.5142
20	–$C_6H_{10}Cl$	1	–0.2868	7.5262
21	–3,4-$Cl_2C_6H_3$	1	–0.2870	7.5714
22	–COOH	10	–0.2870	7.3083
23	–$SC(S)NC_5H_{10}$	3	–0.2915	7.4956
24	–NH_2·HCl	5	–0.2956	7.5713
25	–3,4,5-$(H_3CO)_3C_6H_2$	1	–0.2957	7.2845
26	–2,4,6-$(CH_3)_3C_6H_2$	1	–0.2969	7.4175
27	–C_6Cl_5	1	–0.2981	7.4922
28	–*p*-C_6H_4CN	1	–0.3050	7.6135
29	–*m*-$C_6H_4NO_2$	1	–0.3050	7.6135
30	–CH(NHAc)-$COOCH_3$	2	–0.3055	7.2760
31	–$(CH_2)_{16}CH_3$	3	–0.3114	7.1870
32	–*n*-Dodecyl	5	–0.3261	7.2861
33	"*o*-Benzoyl-thiaminyl"	2	–0.3271	7.2996
34	–NC_4H_8O	1	–0.7456	31.0789
35[e]	–$N(CH_3)_2$	5	–0.9063	30.6304
36	–$N_2C_4H_8C(O)CH_3$	3	–1.0799	29.9920
37	–NC_5H_{10}	1	–1.1129	30.3057
38[e]	–$NHCH_3$	3	–1.1496	30.4494
39[e]	–NH_2	2	–1.2822	29.9238
	μ (molecular residue = –SS–)	77	0.0870	–1.2767

[a] See footnote *b*, Table I. [b] In decreasing order of effectiveness. [c] Groups in positions 1 and 4 should not be interchanged with those in positions 2 and 3. [d] See footnote *c*, Table II. [e] The low values of these constants may be misleading. These substituents appear in combination with the –C(S)– group in compounds of relatively high activity; however, the least-squares program fitted this high activity by combining a large positive constant for –C(S)– with large negative constants for these groups. This is a good illustration of a dangerous property of least-squares fits that must be kept in mind.

II and III are those appropriate to the actual function fitted. The use of substituent constants from these two tables to calculate the MIC values of Table I and, of course MIC for any other combinations desired, can be illustrated as follows for compound 4 of Table I using the two-group model

$$1/\text{MIC} = 0.0870\ (\mu \text{ from last line, Table I}) - 0.1895\ (\text{group 52}) + 0.2884\ (\text{group 2}) = 0.1859$$

which gives MIC = 5.38 as shown rounded to 5 in Table I. Similarly, with the four-group model

$$1/\text{MIC} = 0.0870\ (\mu) + 1.0353\ (\text{central group 1}) + 0.6751\ (\text{central group 2}) - 0.2689\ (\text{outer group 11}) - 1.2822\ (\text{outer group 39}) = 0.2463$$

or MIC = 4.06.

The number of times each substituent appeared in the set of input data is also given in Tables II and III as a rough index of reliability. The more times a substituent appears, the more reliable its computed constant. Constants for substituents that appear only once are to be used with particular caution. These constants reproduce the corresponding experimental activities exactly, but their reliability cannot be estimated. Their inclusion in the least-squares fit does not affect the values of the other parameters.

Results

Table I does not suggest any very compelling reason for preferring either the two-group model or the four-group model over the other, nor for choosing between the fit of 1/MIC and log (1/MIC). Because of the large standard deviations of MIC obtained in the 1/MIC fits, compared to those from the log (1/MIC) fits, it might appear that the latter are to be preferred. But, as stated above, the large standard deviations are due to a poor fit of the inactive compounds. We have a slight preference for the 1/MIC fits because, in both the two- and four-group models, they give somewhat better accuracy for the more active compounds. The four-group model has some advantage over the two-group model since it gives a slightly better fit with slightly fewer parameters, and it also allows more imaginative combinations of the functional groups.

The two-group treatment suggests that morpholino is the most active of all substituents fitting either 1/MIC or log (1/MIC), but very different results are obtained from the four-group fits. In any case, not much importance should be attached to these morpholino constants since the group appears only once among the compounds tested. The *p*-tolyl group appears close to the top of both lists and was used five times in the two-group fits and three times in the four-group fits (where, for flexibility, it was sometimes en-

tered as a combination of phenyl and methyl). This substituent therefore is probably more worthy of further testing than morpholino. Other groups that appear to be good include phenyl (strong agreement between the various fits, high on activity lists, used many times), 2-chloroethyl (high on all lists, but used only once), and diethylamino (high on three of the four lists, used many times).

One can estimate the most attractive candidates by combining substituents that have the largest positive substituent constants in Table II or Table III. Intuition, of course, must play a role, since some combinations would be expected to lead to compounds that would be poor drugs because of sparing solubility or for other reasons. Alternatively, it is also possible to use these substituent constants in a computer program that calculates the activity of possible new disulfides and lists them in order of decreasing activity based on the mathematical model. Synthesis and testing of some of the most promising disulfides so predicted is now underway.

Appendix

We thank one of the Reviewers for the suggestion that the connection between linear dependence of a set of linear equations and vanishing of the coefficient determinant be demonstrated. The result is not new, but, as the Reviewer points out, it is surprisingly difficult to extract from most textbooks.

Consider a set of n linear equations in n unknowns

$$\begin{aligned} a_{11}x_1 + a_{12}x_2 + \ldots + a_{1n}x_n &= c_1 \\ a_{21}x_1 + a_{22}x_2 + \ldots + a_{2n}x_n &= c_2 \\ \vdots \qquad\quad \vdots \qquad\qquad\quad \vdots \qquad &\vdots \\ a_{n1}x_1 + a_{n2}x_2 + \ldots + a_{nn}x_n &= c_n \end{aligned} \tag{A1}$$

Suppose the left side of one of these equations (say the second) is a linear combination of others (say it equals the third plus twice the fourth); there are then two possibilities. If the right sides are not in the same relation as the left sides (say $c_2 \neq c_3 + 2c_4$), the set of equations is inconsistent and cannot be solved. This will never occur in least-squares equations. If the left and right sides do show the same linear dependence, then the equations are consistent; but there is no single unique solution. This case can occur and has occurred in least-squares fitting of drug activity. To diagnose it, one examines the determinant

$$\det |a_{ij}| = \begin{vmatrix} a_{11} & a_{12} & \ldots & a_{1n} \\ a_{21} & a_{22} & \ldots & a_{2n} \\ \vdots & \vdots & & \vdots \\ a_{n1} & a_{n2} & \ldots & a_{nn} \end{vmatrix} \tag{A2}$$

A determinant is defined as the sum of permutations

$$\det |a_{ij}| = \sum_P (-1)^p P a_{11}a_{22}\ldots a_{nn} \tag{A3}$$

where P is an operator that permutes the column indices; p is its parity and equals the number of pair exchanges necessary to rearrange a permutation from natural (*i.e.*, 1, 2, 3, . . . , n) row order to natural column order. Equivalently, the determinant can be expanded by cofactors along any row

$$\det |a_{ij}| = \sum_{\alpha=1}^{n} a_{r\alpha}A_{r\alpha} \tag{A4}$$

The required test for linear dependence is derived as follows.

(a) *A determinant with an entire row of zeros equals zero.* Proof: expand by that row using eq A4 to give

$$\det |a_{ij}| = \sum_{\alpha=1}^{n} 0 \times A_{r\alpha} = 0$$

(b) *Interchanging any two rows in a determinant changes its sign.* Proof: suppose $\det |a'_{ij}|$ is obtained from $\det |a_{ij}|$ by interchanging rows r and s. Equation A3 shows that, except for sign, both determinants contain the same terms. Natural row order for $\det |a'_{ij}|$ is obtained from that of $\det |a_{ij}|$ by the single exchange of elements from rows r and s. Therefore p for each term in $\det |a'_{ij}|$ is one greater than that for the same term in $\det |a_{ij}|$. Therefore, $\det |a'_{ij}| = -\det |a_{ij}|$.

(c) *A determinant with two identical rows vanishes.* Proof: suppose rows r and s are identical in $\det |a_{ij}|$. Interchanging them has no effect. Therefore by (b) $\det |a_{ij}| = -\det |a_{ij}|$. Hence, $\det |a_{ij}| = 0$.

(d) *Adding any multiple of any row to any other row does not change the value of the determinant.* Proof: consider the determinant formed by adding λ times the rth row to the sth row of $\det |a_{ij}|$

$$\det |a'_{ij}| = \begin{vmatrix} a_{11} & a_{12} & \ldots & a_{1n} \\ \vdots & \vdots & & \vdots \\ a_{r1} & a_{r2} & \ldots & a_{rn} \\ \vdots & \vdots & & \vdots \\ (a_{s1} + \lambda a_{r1}) & (a_{s2} + \lambda a_{r2}) & \ldots & (a_{sn} + \lambda a_{rn}) \\ \vdots & \vdots & & \vdots \\ a_{n1} & a_{n2} & \ldots & a_{nn} \end{vmatrix}$$

Expand by the sth row

$$\det |a'_{ij}| = \sum_{\alpha=1}^{n} (a_{s\alpha} + \lambda a_{r\alpha})A_{s\alpha} = \sum_{\alpha=1}^{n} a_{s\alpha}A_{s\alpha} + \lambda \sum_{\alpha=1}^{n} a_{r\alpha}A_{s\alpha}$$

$$= \det |a_{ij}| + \text{a determinant with two identical rows}$$

$$= \det |a_{ij}| + 0$$

(e) Now suppose one has a determinant where the rth row = λ_1 (row 1) + λ_2 (row 2) + . . . λ_{r-1} (row $r-1$) + λ_{r+1} (row $r+1$) + . . . + λ_n(row n). Subtract λ_1 times the first row + λ_2 times the second row + . . . from the rth row to give a determinant with a row of zeros. By (a) this determinant equals 0; by (d) it equals the original determinant.

This completes the proof that linear equations which are linearly dependent have a zero coefficient determinant.

In applying this test, it is often necessary to carry more figures in the computation than one might expect. For example, computer evaluation of

$$D = \begin{vmatrix} 1 & 0 & -2 & 4 & 8 \\ 6 & 9 & 1 & -1 & 2 \\ 3 & 7 & 5 & 1 & 3 \\ 23 & 8 & 22 & 12 & -19 \\ 7 & 5 & 8 & 6 & 1 \end{vmatrix}$$

in which (row 4) = −2 (row 1) + (row 2) − 3 (row 3) + 4 (row 5) gives $D = 0.000000$ if the computation is done in double precision. But due to round-off error one obtains $D = 0.017$ if single precision only (*i.e.*, six figures) is used.

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Substituted Thiadiazolines as Inhibitors of Central Nervous System Carbonic Anhydrase

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A series (**24–30**) of substituted thiadiazolines was synthesized and tested for *in vitro* carbonic anhydrase inhibition and for protective ability against pentylenetetrazole-induced convulsions. ED_{50} (pentylenetetrazole protection), TD_{50}, and LD_{50} values are reported for each compound. With the exception of **30**, all compounds approximated the model compound methazolamide as *in vitro* carbonic anhydrase inhibitors. Several of the compounds produced extended protection against pentylenetetrazole-induced convulsions. Ring methoxy substitution in the ortho position appeared to produce maximum activity.

Mann and Kellin[1] demonstrated the inhibitory effects of unsubstituted sulfonamides on carbonic anhydrase in 1940. Subsequent work led to the development of clinically important diuretics, a few of which revealed potential clinical usefulness as anticonvulsants. In a series of thiadiazole derivatives methazolamide (**1**) (2-acetylimino-3-methyl-Δ^4-1,3,4-thiadiazoline-5-sulfonamide, Neptazane, Lederle Laboratories) showed the highest concentration in the brain.[2] This compound served as a model for the design of the compounds in this paper. Previous work[3] had revealed increased carbonic anhydrase inhibition in those compounds bearing an aromatic ring in the 2-substituents. Aromatic ring methoxy substitutions were modeled after known hallucinogenic compounds. Halogen substitutions were prepared to provide opposite electronic effects for SAR comparisons and the unsubstituted compound was prepared as a standard.

Experimental Section

Methazolamide was obtained through the courtesy of Lederle Laboratories. Melting points were observed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were taken on a Beckman Microspec Model 1485 in Nujol mull. Nmr spectra were determined on a Hitachi Perkin-Elmer spectrometer Model R-24. Elemental analyses were performed by Baron Consulting Company, Orange, Conn. Synthesized compounds are summarized in Tables I and II.

2-Amino-5-benzylmercapto-1,3,4-thiadiazole (2).[4] KOH pellets (24 g, 0.43 mol) were added to a slurry of 2-amino-1,3,4-thiadiazole-5-thiol (40 g, 0.3 mol) in 100 ml of water. After cooling, 50 ml EtOH was added and benzyl chloride (48 ml, 0.38 mol) was added dropwise. The mixture became viscous and a "curdled" white product separated. After stirring for an additional 30 min at 10°, the mixture was diluted with 200 ml of water. Filtration yielded 64 g (95%) of white crystals (EtOH) melting at 157–158°. *Anal.* ($C_9H_9N_3S_2$) C, H, N.

Substituted 2-Benzamido-5-benzylthio-1,3,4-thiadiazoles (3–9)[5] **(General Procedure).** To 25 ml of pyridine was added the appropriate acid (0.02 mol) and **2** (4.5 g, 0.02 mol). With constant stirring $SiCl_4$ (2 g, 0.023 mol) was added dropwise resulting in temperature elevation to *ca.* 85°. Stirring was continued at room temperature for 10 hr and the mixture was poured into ice water. Silica was removed by filtration and the filtrate was concentrated. The residual solid was recrystallized from EtOH–H_2O. Yields ranged from 46 to 55%.

The same amides were synthesized by reacting **2** with acid anhydrides in aqueous acid (5–16% yield), with acid chlorides in aqueous hydroxide (14–32% yield), and with acid chlorides in pyridine (16–22% yield).

Substituted 2-Benzoylimino-3-methyl-5-benzylthio-Δ^4-1,3,4-thiadiazolines (10–16) (General Procedure). The appropriate thiadiazole **3–9** (0.01 mol) was dissolved in 60 ml of water containing KOH (0.7 g, 0.0125 mol) and the solution was diluted with 35 ml of EtOH. Dimethyl sulfate (1.3 g, 0.01 mol) was added, the mixture was refluxed for 20 min, and it was then cooled to 10°. Cold NaOH solution (100 ml, 1.5 *M*) was added until a slurry formed. Water (150 ml) was added and the mixture refrigerated for 1 hr. The precipitate was collected by filtration and recrystallized from MeOH–H_2O. Yields ranged between 46 and 62%. Similar methylations with CH_3I and K_2CO_3 in acetone resulted in yields between 29 and 43%.

Substituted 2-Benzoylimino-3-methyl-Δ^4-1,3,4-thiadiazoline-5-sulfonyl Chloride (17–23) (General Procedure). The appropriate thiadiazoline **10–16** (0.05 mol) was added to 40 ml of HOAc in a three-necked flask fitted with a thermometer, an inlet tube, and an outlet tube leading to a water trap. The reaction was maintained at 5° as chlorine was introduced by the inlet tube terminating about 1 in. above the reaction mixture. Chlorine was added in excess over a period of 15 min until the compound went into solution and the solution attained a yellow color. The solution was poured in water and the precipitate filtered and blotted dry. Yields ranged from 47 to 58%. The compounds were not purified for subsequent reactions.

Substituted 2-Benzoylimino-3-methyl-Δ^4-1,3,4-thiadiazoline-5-sulfonamide (24–30) (General Procedure). The appro-