the rate constant for the change as well as the half-time for the change were found. The latter is listed in Table I for the compounds studied in this way.

Acute Toxicities. The single ip dose that will kill half the animals tested was used as a measure of the toxicities of these compounds. The protocol and the results are shown in Table II.

Inhibition of Ascitic Sarcoma 180 in Mice. Swiss mice obtained from Mid-Continent Research Animals, Inc., Shawnee Mission, Kan., were used in these experiments. The ascitic sarcoma 180 cells were obtained from Frederic A. French, Chemotherapy Laboratory, Mount Zion Hospital, San Francisco, Calif. The LD_{50} provides an estimate for the first dose used in the determination of ascitic sarcoma 180 activity; subsequent determinations are made with smaller or larger doses of the compounds to avoid toxicity and yet retain activity. The values given for the daily dose in Table II are those that gave the largest values of T/C.

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Structure–Activity Relationships in Antitumor Aniline Mustards[1]

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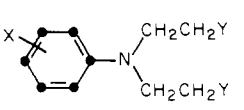
Quantitative structure–activity relationships (QSAR) have been formulated for the hydrolysis of aniline mustards and their antitumor activity against Walker 256 tumor and L1210 and P388 leukemia. In general, the antitumor activity parallels hydrolysis under the conditions defined by Ross; toxicity (LD_{50}) parallels antitumor efficacy. Chlorambucil is an exception. A most important finding is that ideal lipophilicity for effectiveness against Walker tumor appears to be much higher than for the leukemias which suggests that solid tumors may, in general, require more lipophilic drugs than leukemias.

It has become clear in recent years that even that most difficult area of chemotherapy, cancer chemotherapy, can be studied using the techniques of QSAR.[2] In this report we wish to analyze data from the literature as well as those collected over the years by the National Cancer Institute on aniline mustards of type I. It is our hope in making such retrospective studies that some generalizations can be uncovered which will be of help in the design of better antitumor drugs.



I

These compounds, referred to as alkylating agents, fall into the must studied class of antitumor compounds and are one of the few classes of cancer drugs about which we have a general understanding of the mechanism of action.[3] A large amount of evidence has accumulated to suggest that the antitumor as well as the carcinogenic activity of the alkylating agents is brought about by their interaction with DNA and RNA.[4]

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Table I. X-$C_6H_4N(CH_2CH_2Y)_2$ vs. Walker 256 Rat Tumor

No.	X	Y	Log $1/C_{ED_{90}}$ Obsd[a]	Log $1/C_{ED_{90}}$ Calcd[b]	Log $1/C_{LD_{50}}$ Obsd[a]	Log $1/C_{LD_{50}}$ Calcd[c]	σ^-	π	I
1	4-SO_2NH_2	Cl	2.82	2.79	2.94	2.70	0.94	−1.82	0
2	4-$CONH_2$	Cl	2.92	3.41	2.92	3.30	0.63	−1.49	0
3	4-CHO	Cl	2.93	2.92	2.78	2.69	1.13	−0.65	0
4	4-COOH	I	3.26[d]	3.19	3.30	3.00	0.77	−0.32	0
5	4-COOH	Cl	3.29[d]	3.19	3.03	3.00	0.77	−0.32	0
6	H	Cl	3.39	3.84	3.43	3.77	0.00	0.00	0
7	4-$SCOCH_3$	Cl	3.42	3.20	3.16	3.07	0.46	0.10	0
8	4-SO_2NH_2	Br	3.68	3.54	3.13	3.30	0.94	−1.82	1
9	H	I	4.08	3.84	3.85	3.77	0.00	0.00	0
10	4-$SCOCH_3$	Br	4.09	3.92	3.79	3.67	0.46	0.10	1
11	4-COOH	Br	4.18[d]	3.94	3.55	3.60	0.77	−0.32	1
12	H	Br	4.30	4.58	4.40	4.36	0.00	0.00	1
13	4-$NHCOCH_3$	I	4.30	4.31	4.50	4.22	0.00	−0.97	0
14	4-$NHCOCH_3$	Cl	4.46	4.31	3.99	4.22	0.00	−0.97	0
15	4-OH	Cl	4.49	4.46	4.13	4.38	−0.16	−0.67	0
16	4-NH_2	Cl	4.69	4.44	4.82	4.40	−0.15	−1.23	0
17	4-$NHCOCH_3$	Br	5.04	5.05	4.82	4.81	0.00	−0.97	1
18	4-$(CH_2)_3COOH$	Cl	5.12[d]	3.60	4.03	3.56	0.00	0.21	0

[a] From ref 6. [b] Calculated using eq 3. [c] Calculated using eq 6. [d] These points not used to derive eq 1–6; note, however, that they are, in general, well fit.

Table II. Activity ED_{90} of RO-C_6H_4-$N(CH_2CH_2Y)_2$ against Walker 256 Rat Tumor and Toxicity LD_{50} in Rats

No.	R	Y	Log $1/C_{ED_{90}}$ Obsd[a]	Log $1/C_{ED_{90}}$ Calcd[b]	$\lvert\Delta$ log $1/C\rvert$	Log $1/C_{LD_{50}}$ Obsd[a]	Log $1/C_{LD_{50}}$ Calcd[c]	$\lvert\Delta$ log $1/C\rvert$	π	σ^- [d]	E_s	I[e]
1	H	Cl	4.44	4.54	0.10	4.07	4.00	0.07	−0.67	−0.37	0.00	0
2	H	Br	5.00	4.90	0.10	5.00	5.09	0.09	−0.67	−0.37	0.00	1
3	OCC_6H_5	Br	5.45	5.22	0.23	5.45	5.28	0.17	1.46	0.13	0.00	1
4	OCC_6H_5-2-CH_3	Br	4.96	4.64	0.32	4.96	4.84	0.12	2.02	0.03	−1.24	1
5	OCC_6H_3-2,6-CH_3	Br	3.81	3.98	0.17	3.58[f]	4.34	0.76	2.58	−0.07	−2.48	1
6	OCC_6H_4-2-Et	Br	4.96[f]	4.51	0.45	4.64	4.72	0.08	2.48	−0.02	−1.31	1
7	OCC_6H_4-2-*i*-Pr	Br	4.39	4.13	0.26	4.66	4.46	0.20	2.99	−0.03	−1.71	1
8	OCC_6H_4-3-CH_3	Br	5.18	5.17	0.01	5.16	5.21	0.05	2.02	0.06	0.00	1
9	OCC_6H_4-4-CH_3	Br	4.94	5.21	0.27	5.32	5.21	0.11	2.02	−0.04	0.00	1
10	OCC_6H_4-3-Cl	Br	4.96	4.97	0.01	5.11	5.18	0.07	2.17	0.50	0.00	1
11	OCC_6H_4-4-Cl	Br	4.66[f]	5.03	0.37	5.18	5.18	0.00	2.17	0.36	0.00	1
12	OCC_6H_4-4-OCH_3	Br	5.12	5.32	0.20	5.21	5.28	0.07	1.44	−0.14	0.00	1
13	OCC_6H_4-3-CF_3	Br	4.99	4.91	0.08	5.11	5.15	0.04	2.34	0.56	0.00	1
14	OCC_6H_4-3-NO_2	Br	4.67	4.96	0.29	5.07	5.30	0.23	1.18	0.84	0.00	1
15	OCC_6H_4-4-NO_2	Br	4.86	4.93	0.07	5.37	5.30	0.07	1.18	0.91	0.00	1
16	OCC_6H_5	Cl	4.86	4.85	0.01	4.06	4.18	0.12	1.46	0.13	0.00	0
17	OCC_6H_4-2-CH_3	Cl	4.55	4.28	0.27	3.71	3.74	0.03	2.02	0.03	−1.24	0
18	OCC_6H_4-4-CH_3	Cl	4.78	4.85	0.07	4.20	4.12	0.08	2.02	−0.04	0.00	0
19	OCC_6H_3-2,6-CH_3	Cl	3.31	3.62	0.31	3.10	3.24	0.14	2.58	−0.07	−2.48	0
20	OCC_6H_4-3-Br	Cl	4.22	4.57	0.35	3.84	4.06	0.22	2.32	0.52	0.00	0
21	OCC_6H_4-4-Br	Cl	4.84	4.63	0.21	4.19	4.06	0.13	2.32	0.36	0.00	0
22	OCC_6H_4-3-Cl	Cl	4.55	4.61	0.06	3.95	4.09	0.14	2.17	0.50	0.00	0
23	OCC_6H_4-3-F	Cl	4.88	4.72	0.16	4.38	4.17	0.21	1.60	0.47	0.00	0
24	OCC_6H_4-4-F	Cl	4.90	4.82	0.08	4.21	4.17	0.04	1.60	0.19	0.00	0
25	OCC_6H_4-4-OCH_3	Cl	4.89	4.96	0.07	4.11	4.19	0.08	1.44	−0.14	0.00	0
26	OCC_6H_4-3-NO_2	Cl	4.81	4.59	0.22	4.40	4.20	0.20	1.18	0.84	0.00	0
27	OCC_6H_4-3-OCH_3	Br				5.25	5.28	0.03	1.44	0.25	0.00	1

[a] From ref 7. [b] Calculated using eq 7. [c] Calculated using eq 9. [d] σ constants for the substituents on the benzoyl moiety plus σ for $C_6H_5COO^-$ (0.13) have been used except in the case of compounds 1 and 2 where σ is for OH; although this is not strictly correct, dropping compounds 1 and 2 did not change the quality of the correlation or the size of the parameters which would seem to justify our approximation. [e] I = 1 when Y = Br and 0 when Y = Cl. [f] These points not used in deriving correlation equations.

Results

The first QSAR formulated on aniline mustards were those by Lien and Tong[2b] using data from a study by Bardos and Chmielewicz[5] on compounds of type I where Y = Cl. Data for ED_{90} with rat Walker 256 carcinoma and also toxicity (LD_{50}) data were analyzed. Although the variation in substituents was not well suited for QSAR, it was clear that electron-releasing groups increased both potency and toxicity in an almost parallel manner. It is noteworthy that for substituents (COOH, $NHCOCH_2NH_2$) which are ionized under physiological conditions, Lien and Tong found good correlations using π and σ for the unionized substituents. We have also noted this phenomenon in our data.

An earlier study by Bardos et al.,[6] which was not considered by Lien and Tong, actually contains a rather good distribution of substituents from the π and σ point of view. We have formulated eq 1–6 from these data (Table I) on ED_{90} for Walker 256 cancer and toxicity (LD_{50}) (Table I) for congeners of I. The indicator variable I takes the value of 0 in both equations (3 and 6) when Y = Cl or I and the value of 1 when Y = Br. The positive coefficient with I shows that Br is the best group. One must bear in mind that π_0 applies strictly to the cases where Y = Cl. Although

ED_{90} Walker tumor

$$\log 1/C = -1.25\ (\pm 0.61)\ \sigma^- + 4.28\ (\pm 0.32) \quad (1)$$
$$n = 14;\ r = 0.791;\ s = 0.453;\ F_{1,X} = 20.0$$

$$\log 1/C = -1.30\ (\pm 0.49)\ \sigma^- + 0.61\ (\pm 0.47)\ I + 4.12\ (\pm 0.28) \quad (2)$$
$$n = 14;\ r = 0.887;\ s = 0.357;\ F_{1,X} = 8.36$$

$$\log 1/C = -1.19\ (\pm 0.51)\ \sigma^- + 0.75\ (\pm 0.41)\ I - 1.00\ (\pm 0.87)\ \pi - 0.53\ (\pm 0.55)\ \pi^2 + 3.84\ (\pm 0.33) \quad (3)$$
$$n = 14;\ r = 0.940;\ s = 0.291;\ \pi_0 = -0.95$$

$$\log 1/C = -1.28\ (\pm 0.55)\ \sigma^- + 0.63\ (\pm 0.50)\ I - 0.31\ (\pm 0.99)\ \pi - 0.19\ (\pm 0.60)\ \pi^2 + 4.17\ (\pm 0.38) \quad (3a)$$
$$n = 18;\ r = 0.856;\ s = 0.428;\ \pi_0 = -0.81$$

LD_{50}

$$\log 1/C = -1.33\ (\pm 0.55)\ \sigma^- + 4.17\ (\pm 0.29) \quad (4)$$
$$n = 14;\ r = 0.838;\ s = 0.405;\ F_{1,X} = 28.3$$

$$\log 1/C = -1.36\ (\pm 0.48)\ \sigma^- + 0.47\ (\pm 0.46)\ I + 4.04\ (\pm 0.28) \quad (5)$$
$$n = 14;\ r = 0.893;\ s = 0.349;\ F_{1,X} = 5.20$$

$$\log 1/C = -1.31\ (\pm 0.51)\ \sigma^- - 0.90\ (\pm 0.86)\ \pi + 0.59\ (\pm 0.41)\ I - 0.45\ (\pm 0.55)\ \pi^2 + 3.78\ (\pm 0.33) \quad (6)$$
$$n = 14;\ r = 0.941;\ s = 0.289;\ \pi_0 = -1.00$$

$$\log 1/C = -1.31\ (\pm 0.35)\ \sigma^- - 0.69\ (\pm 0.63)\ \pi + 0.51\ (\pm 0.31)\ I - 0.35\ (\pm 0.39)\ \pi^2 + 3.87\ (\pm 0.24) \quad (6a)$$
$$n = 18;\ r = 0.932;\ s = 0.272;\ \pi_0 = -0.99$$

technically it also applies to iodine since iodine has a different π from Cl, another factor must be associated with π to fortuitously allow the use of the same indicator variable for iodine and chlorine. Log P for the parent compound X = H, Y = Cl is 2.90; this suggests that maximum efficacy and toxicity for "normal" aniline mustards occur with rather lipophilic compounds (log $P \sim 2$).

The above equations show the stepwise development of eq 3 and 6 for efficacy and toxicity. Equations 3a and 6a include all data, while four data points were omitted in eq 3 and 6. In the case of eq 6 and 6a, there is little difference because the ionized carboxylate congeners are well fit if we use π and σ^- for the un-ionized form of COOH. There is considerable difference in eq 3a because chlorambucil (18) is very poorly fit. The calculated values in Table I have been obtained using eq 3 and 6. Note that the ionizable congeners (**4**, **5**, and 11) are all reasonably well fit. This treatment of the data brings out the unique specificity of chlorambucil and explains why this congener has found extensive use in the clinic. Including chlorambucil in eq 3a does not make significant changes in the coefficients of σ^-, I, or in π_0; however, the π terms are greatly changed. If 17 data points are employed with 18 omitted, an equation almost identical with eq 3 is obtained.

Adding π terms one at a time to eq 2 or 5 does not make a significant reduction in the variance in log $1/C$; adding both terms together does. For eq 3, $F_{2,9} = 3.79$ and for eq 6, $F_{2,9} = 3.51$ ($F_{2,9;\alpha=0.1} = 3.01$); hence, the two π terms are significant. Equation 3a is not a significant improvement over eq 2a (not shown). The one aberrant point (18) destroys the significant parabolic relationship found for "normal" aniline mustards (eq 3). We believe that eq 3 will give reasonable predictions for the activity of new "normal" aniline mustards; it will not predict, nor will any other correlation equation, highly specific activity not present in the majority of the congeners on which the correlation equation is based. At this stage of development of structure–activity study when so little is known about the mechanism of drug action at the molecular level, it can be quite misleading to force poorly predicted congeners into a correlation equation. In fact, one of the greatest assets of a correlation equation is that it brings to light unusual congeners which can then be studied for new leads.

Slightly better results were obtained with σ compared to σ in eq 3 and 6. The reason for dropping congeners with the COOH function in Table I in deriving eq 3 and 6 was that we have used π for the un-ionized form of these molecules as did Lien and Tong, despite the fact that they are largely ionized at physiological pH. The fact that these congeners are well fit would tend to justify the use of un-ionized π values; however, we believe that this is probably fortuitous. It seems likely that COO^- has some inherent activity-increasing characteristic which offsets its π values of −4.3 in such a way that the π of −0.32 "works".

In an interesting attempt[7] to make more stable, more lipophilic esters which would be converted to highly active mustards on hydrolysis, a set of benzoates [4-(X-$C_6H_4COO)C_6H_4N(CH_2CH_2Y)_2$] were tested against Walker 256 tumors (ED_{90}); the toxicity of the compounds against rats was also measured (Table II, LD_{50}). Equation 7 has

$$\log 1/C = 0.34\ (\pm 0.27)\ \pi - 0.13\ (\pm 0.12)\ \pi^2 + 0.44\ (\pm 0.17)\ E_s\text{-}2 - 0.37\ (\pm 0.33)\ \sigma + 0.36\ (\pm 0.19)\ I + 4.69\ (\pm 0.24) \quad (7)$$
$$n = 24;\ r = 0.899;\ s = 0.224;\ \pi_0 = 1.27\ (0.70\text{–}3.5)$$

$$\log 1/C = 0.35\ (\pm 0.29)\ \pi - 0.14\ (\pm 0.13)\ \pi^2 + 0.40\ (\pm 0.18)\ E_s\text{-}2 - 0.40\ (\pm 0.36)\ \sigma + 0.36\ (\pm 0.21)\ I + 4.69\ (\pm 0.26) \quad (7a)$$
$$n = 26;\ r = 0.862;\ s = 0.245;\ \pi_0 = 1.25\ (0.61\text{–}4.1)$$

been derived from data in Table II.

The development of eq 7 (Table III) is instructive. E_s of the ortho substituents is the most important variable (this steric effect was noted by the authors[7]). The indicator variable I is assigned the value of 1 for Y = Br and 0 for Y = Cl. Adding the variables σ, π, and π^2 one at a time or two at a time to the two-variable equation ($E_s + I$) does not result in a significant reduction in the variance; however, adding all three at once not only produces a significant reduction in the variance, but also allows one to estimate π_0. This value is higher than one would expect from eq 3 but again underscores the importance of lipophilic drugs for action against Walker tumor. A better selection of substituents with lower π values would have enabled us to make a better definition of π_0. Assuming no hydrolysis, the size and sign of the σ term seem reasonable. Electronic effects of X would be poorly transmitted to the mustard moiety.

The development of eq 7 does illustrate the advantage of looking at all possible regression equations. Using stepwise regression analysis, this equation might well have been missed.

Two data points in Table II were not included in the formulation of eq 7. There is no obvious reason why they are aberrant. If these two points are included (eq 7a), the

Table III. Development of Equation 7

Intercept	E_s-2	I	σ	π	π^2	r	s	$F_{1,X}$	Eq
4.88	0.42					0.752	0.304	28.6	A
4.72	0.44	0.34				0.843	0.254	10.5	B
4.77	0.47	0.34	−0.18			0.854	0.252	1.39	C
4.70	0.38	0.35		0.20	−0.09	0.864	0.250	1.37[a]	D
4.69	0.44	0.36	−0.37	0.34	−0.13	0.899	0.224	3.04[b]	7

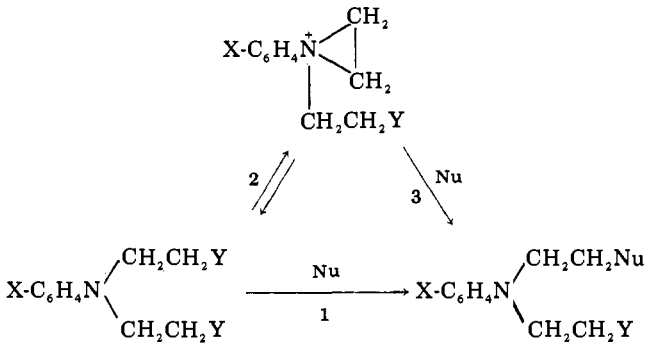
[a] This is $F_{2,19}$ obtained by comparison with B. [b] This is $F_{3,18}$ obtained by comparison with B; the F test shows that neither eq C nor D is a significant improvement over B. However, eq 7 is: $F_{3,18;\alpha=0.1} = 2.42$, $F_{3,18;\alpha=0.05} = 3.16$.

Table IV. Enzymatic Hydrolysis of $X\text{-}C_6H_4COOC_6H_4N(CH_2CH_3)_2$

No.	X	Log k Obsd[a]	Log k Calcd[b]	$\lvert\Delta \log k\rvert$	σ
1	4-NO_2	0.67	0.71	0.04	0.78
2	3-NO_2	0.55	0.63	0.08	0.71
3	4-CF_3	0.45	0.45	0.00	0.54
4	3-CF_3	0.34	0.32	0.02	0.43
5	3-Cl	0.32	0.26	0.06	0.37
6	4-Cl	0.20	0.10	0.10	0.23
7	4-CH_3	−0.41	−0.34	0.07	−0.17
8	3-CH_3	0.32[c]	−0.23	0.55	−0.07
9	4-OCH_3	−0.49	−0.45	0.04	−0.27
10	3-OCH_3	0.15	−0.02	0.17	0.12
11	H	−0.26	−0.15	0.11	0.00

[a] From ref 7. [b] Calculated using eq 8. [c] This point not used in deriving eq 8.

Scheme I



resulting coefficients are all almost identical with eq 7. The correlation is somewhat poorer (r = 0.862, s = 0.247).

It should be emphasized that points are not dropped just to obtain a better correlation coefficient. One wants to highlight unusual compounds. Understanding is the name of the game, not high correlation coefficients.

The authors of this work hoped that hydrolysis of the benzoates near the site of action would produce a very active 4-hydroxyaniline mustard. To obtain a better understanding of the characteristics of enzymic hydrolysis, they subjected a similar set of esters [$X\text{-}C_6H_4COOC_6H_4N(CH_2CH_3)_2$] to liver esterases. Equation

$$\log k_{rel} = 1.11\ (\pm 0.20)\ \sigma - 0.15\ (\pm 0.09) \quad (8)$$
$$n = 10;\ r = 0.976;\ s = 0.093$$

$$\log k_{rel} = 0.97\ (\pm 0.38)\ \sigma - 0.07\ (\pm 0.16) \quad (8a)$$
$$n = 11;\ r = 0.887;\ s = 0.189$$

8 has been derived from their data (Table IV). Adding a term in π to eq 8 did not improve the correlation. The use of σ^- in place of σ gave a poorer correlation (r = 0.919, s = 0.170).

In this correlation, k is the relative amount of *N,N*-diethylphenol produced in 5 min at 32 °C. The positive coefficient with σ is expected; however, it is interesting that the hydrophobicity of X is not involved.

The results of eq 7 and 8 suggest that it is not the hydrolyzed form of the ester that is the antitumor agent. If this were so, one would expect a large positive ρ for σ (comparable to that for ester hydrolysis) in eq 7 while, in fact, ρ has a negative sign and a small value in this QSAR. One data point (8) has been omitted in deriving eq 8. Including this point, eq 8a is obtained.

Equation 9, similar to eq 7, has been derived from the

$$\log 1/C = 0.15\ (\pm 0.14)\ \pi - 0.08\ (\pm 0.07)\ \pi^2 + 0.30\ (\pm 0.11)\ E_s + 1.10\ (\pm 0.11)\ I + 4.13\ (\pm 0.15) \quad (9)$$
$$n = 26;\ r = 0.979;\ s = 0.140;\ \pi_0 = 0.96\ (0.2\text{–}2)$$

$$\log 1/C = 0.14\ (\pm 0.19)\ \pi - 0.07\ (\pm 0.09)\ \pi^2 + 0.42\ (\pm 0.13)\ E_s + 1.06\ (\pm 0.15)\ I + 4.13\ (\pm 0.20) \quad (9a)$$
$$n = 27;\ r = 0.962;\ s = 0.190;\ \pi_0 = 1.05$$

toxicity data in Table II. Except for the lack of the σ term (which is of low significance), eq 9 is quite similar to eq 7. The π_0 are also close. The significant difference between eq 7 and 9 is in the intercepts. The ED_{90} dose is, on the average, about 3.5 times less than the LD_{50} dose which is not a favorable therapeutic ratio. The only significant difference between eq 9 and 9a is that confidence limits cannot be placed on π_0 for eq 9a.

From Table V, NCI data on L1210 leukemia for congeners of I where Y = Cl have been used to derive eq 10–12.

$$\log 1/C = -0.32\ (\pm 0.17)\ \pi + 4.20\ (\pm 0.32) \quad (10)$$
$$n = 19;\ r = 0.679;\ s = 0.571;\ F_{1,X} = 14.5$$

$$\log 1/C = -0.32\ (\pm 0.16)\ \pi - 0.95\ (\pm 0.88)\ \sigma^- + 4.28\ (\pm 0.31) \quad (11)$$
$$n = 19;\ r = 0.771;\ s = 0.510;\ F_{1,X} = 5.3$$

$$\log 1/C = -0.31\ (\pm 0.10)\ \pi - 0.96\ (\pm 0.54)\ \sigma^- + 0.86\ (\pm 0.37)\ I_0 + 4.07\ (\pm 0.21) \quad (12)$$
$$n = 19;\ r = 0.926;\ s = 0.313;\ F_{1,X} = 27.6$$

$$\log 1/C = -0.28\ (\pm 0.14)\ \pi - 0.55\ (\pm 0.67)\ \sigma^- + 0.74\ (\pm 0.49)\ I_0 + 4.02\ (\pm 0.29) \quad (12a)$$
$$n = 21;\ r = 0.815;\ s = 0.455$$

C in these equations is the dose (mol/kg) required to produce T/C of 125 (25% increase in life span). This low T/C was used so that the maximum number of compounds could be included without large extrapolations of dose–response curves. In eq 12, I_0 takes the value of 1 for ortho substituents; other factors being equal, ortho substitution produces more active congeners. Following our observation with eq 1–6 and that of Lien and Tong, we have used π and σ constants for the un-ionized form of COOH. These give better correlations in eq 10–12 than constants for COO^-.

An alternative approach using ionized π values plus an indicator variable to compensate for our lack of under-

standing of the relative partitioning of a set of mixed ionized and un-ionized congeners did not give a better result. We believe that it is simply fortuitous that π for neutral COOH works in these systems. Adding a term in π^2 to eq 12 does not reduce the variance so that we cannot even make an estimate of the ideal log P. However, the negative coefficient with π in eq 12, the π values of Table V, and the fact that log P for $C_6H_5N(CH_2CH_2Cl)_2$ is 2.90 make it clear that log P_0 for this set of congeners must be less than 0. We have previously noted[8] low log P_0 for alkylating agents acting against L1210 leukemia. The electronic effect of substituents plays a less important role in eq 10–12 than in eq 1–6 which in part is due to less variation in σ in Table V. For this set of congeners, the mean and standard deviation is 0.07 ± 0.28, while it is 0.30 ± 0.45 for Table I. The parameter σ^- gives slightly better results than σ in eq 10–12.

Including two points not included in deriving eq 12 yields eq 12a. One of these points (4-COOH) is extremely poorly fit, so much so that although the coefficients in eq 12a are similar to eq 12, the confidence limits on σ destroy the credibility of this term. This compound is mispredicted by almost 5 standard deviations and we believe this is most likely due to poor biological testing since this function is not poorly predicted in other examples.

Data in Tables VI and VII are for similar sets of congeners acting against P388 leukemia in mice. Equations 13–15 are for the endpoint of T/C = 125, while eq 16–18

T/C 125, P388 leukemia

$$\log 1/C = -0.39\ (\pm 0.14)\ \pi + 4.67\ (\pm 0.22) \quad (13)$$
$$n = 19;\ r = 0.822;\ s = 0.423;\ F_{1,X} = 35.3$$

$$\log 1/C = -0.40\ (\pm 0.08)\ \pi - 1.00\ (\pm 0.35)\ \sigma + 4.59\ (\pm 0.13) \quad (14)$$
$$n = 19;\ r = 0.949;\ s = 0.240;\ F_{1,X} = 36.8$$

$$\log 1/C = -0.39\ (\pm 0.08)\ \pi - 1.01\ (\pm 0.35)\ \sigma + 0.19\ (\pm 0.32)\ I_0 + 4.56\ (\pm 0.13) \quad (15)$$
$$n = 19;\ r = 0.954;\ s = 0.236;\ F_{1,X} = 1.5$$

$$\log 1/C = -0.24\ (\pm 0.17)\ \pi - 1.13\ (\pm 0.83)\ \sigma + 0.36\ (\pm 0.76)\ I_0 + 4.57\ (\pm 0.30) \quad (15a)$$
$$n = 22;\ r = 0.708;\ s = 0.578$$

T/C 180, P388 leukemia

$$\log 1/C = -0.34\ (\pm 0.13)\ \pi + 0.428\ (\pm 0.23) \quad (16)$$
$$n = 16;\ r = 0.820;\ s = 0.406;\ F_{1,X} = 28.8$$

$$\log 1/C = -0.35\ (\pm 0.11)\ \pi - 1.38\ (\pm 1.0)\ \sigma + 4.17\ (\pm 0.20) \quad (17)$$
$$n = 16;\ r = 0.897;\ s = 0.325;\ F_{1,X} = 8.8$$

$$\log 1/C = -0.34\ (\pm 0.11)\ \pi - 1.39\ (\pm 0.97)\ \sigma + 0.30\ (\pm 0.44)\ I_0 + 4.13\ (\pm 0.21) \quad (18)$$
$$n = 16;\ r = 0.914;\ s = 0.311;\ F_{1,X} = 3.07$$

$$\log 1/C = -0.22\ (\pm 0.14)\ \pi - 1.45\ (\pm 1.5)\ \sigma + 0.53\ (\pm 0.67)\ I_0 + 4.04\ (\pm 0.32) \quad (18a)$$
$$n = 18;\ r = 0.731;\ s = 0.507$$

are for T/C of 180. In these two sets, σ gives slightly better correlations than σ^- which at least in part is due to the fact that there are very few groups with σ^- values significantly different from σ. As in eq 10–12, I_0 is an indicator variable for ortho substitution. Although this parameter does give a slight improvement in eq 15 compared to eq 14, it is not statistically significant. This term is significant in eq 18 although it is of marginal value. One of the reasons for low significance of I_0 in these two equations is that few (three) congeners in Tables VI and VII have ortho substituents.

Three points in Table VI (**2**, **4**, **20**) are extremely poorly fit, being off by 3.7, 8.3, and 5 standard deviations, respectively. If these three points are included, one obtains the poor correlation of eq 15a. Although the correlation is poor, values predicted by eq 15a would not be greatly different from eq 15. One of these compounds (**20**) is an aliphatic aldehyde which would be rapidly converted to the uniquely active chlorambucil. The other two compounds (**2** and **4**) are not very different structurally from other congeners of Table VI and, hence, there is no obvious explanation for their poor behavior.

Equations 13–18 are very similar to eq 10–12; however, P388 leukemia is more sensitive to the aniline mustards which allows us to use a higher T/C (180). Five of the congeners were not active enough to analyze at the T/C 180 level without large extrapolations of dose–response curves. As with L1210 leukemia, the results show that hydrophilic drugs are most potent. Again, π_0 cannot be established. The difference in the intercepts of eq 15 and 18 shows that, on the average, 2.5 higher dose is needed to produce the higher T/C.

Two data points in Table VII have not been used to derive eq 16–18. Including these points yields eq 18a. Equation 18 mispredicts the activity of compounds **2** and **12** by 5.9 and 2.4 standard deviations, respectively. Compound **2** is also poorly fit by eq 15 which suggests that the β-NH_2 function somehow destroys activity.

One might be inclined, after seeing eq 15a and 18a, to throw out eq 13–18 as being of no value. We do not take this view. Biological testing of antitumor compounds is notoriously poor. We believe eq 15 and 18 are the best guides available for further work on aniline mustards. We are now making a new series of aniline mustards which will all be tested at the same time in the same laboratory.

Equations 19–21 have been derived from the data in

$$\log 1/C = 2.09\ (\pm 0.63)\ I_2 + 4.06\ (\pm 0.32) \quad (19)$$
$$n = 16;\ r = 0.884;\ s = 0.511;\ F_{1,X} = 50.1$$

$$\log 1/C = 2.83\ (\pm 0.91)\ I_2 - 0.60\ (\pm 0.58)\ \sigma^- + 4.37\ (\pm 0.40) \quad (20)$$
$$n = 16;\ r = 0.918;\ s = 0.450;\ F_{1,X} = 5.04$$

$$\log 1/C = 3.03\ (\pm 0.68)\ I_2 - 0.94\ (\pm 0.48)\ \sigma^- - 0.86\ (\pm 0.53)\ I_1 + 4.78\ (\pm 0.31) \quad (21)$$
$$n = 16;\ r = 0.960;\ s = 0.329;\ F_{1,X} = 12.3$$

$$\log 1/C = 3.11\ (\pm 0.87)\ I_2 - 1.11\ (\pm 0.59)\ \sigma^- - 1.04\ (\pm 0.66)\ I_1 + 4.96\ (\pm 0.45) \quad (21a)$$
$$n = 17;\ r = 0.932;\ s = 0.426$$

Table VIII for congeners of I where Y = OSO_2R (R = CH_3 or $-C_6H_4CH_3$) acting against L1210 leukemia (T/C = 125). In this set of congeners, a number contain the 4-nitroso group and I_2 is the indicator variable for this highly activating group. The variable I_1 is assigned a value of 1 where Y = $-OSO_2C_6H_4CH_3$. This leaving group produces less active congeners. A surprising result of the study leading to eq 19–21 is that π terms play no role in the QSAR. This may be due to the much greater activity of the leaving group Y ($CH_3SO_3^-$). The rapid formation of the extremely hydrophilic aziridinium intermediate may preclude disadvantageous hydrophobic interactions. The most interesting aspect of this set of drugs is the presence

Table V. X-$C_6H_4N(CH_2CH_2Cl)_2$ vs. L1210 Leukemia; T/C = 125

No.	X	Log 1/C Obsd[a]	Log 1/C Calcd[b]	|Δ log 1/C|	π or Σπ	σ⁻	I[c]	Regimen[d]	NSC no.
1	4-SO_2NH_2	3.55	3.73	0.18	−1.82	0.94	0	Z	77647
2	3-OCH_2CO_2Et	3.60	3.95	0.35	0.03	0.12	0	Z	41449
3	4-OC_4H_9	3.76	3.75	0.01	1.55	−0.16	0	Z	43814
4	3-OCH_2COOH	3.87	4.23	0.36	0.87	0.12	0	Z	41447
5	3-COOH	3.91	3.84	0.07	−0.32	0.35	0	Z	240365
6	H	4.00	4.07	0.07	0.00	0.00	0	9	18429
7	3-CH=CHCOOH	4.05	3.94	0.11	0.00	0.14	0	Z	45631
8	4-OCH_3	4.28	4.23	0.05	−0.02	−0.16	0	9	31577
9	3-CH_2CH_2COOH	4.30	4.19	0.11	−0.29	−0.03	0	Z	41457
10	2-CH=CHCOOH	4.64	4.34	0.30	0.00	0.62	1	Z	44440
11	2-OCH_2CH_2COOEt	4.67	4.97	0.30	0.37	−0.16	1	Z	42345
12	4-CH_2COOH	4.79	4.36	0.43	−0.72	−0.07	0	15	71964
13	4-$CH_2CH_2NH_2$	4.83	4.46	0.37	−0.72	−0.17	0	9	240533
14	2-OCH_2CH_2COOH	4.89	5.14	0.25	−0.18	−0.16	1	Z	42343
15	3-$CH(NH_2)CH_2COOH$	4.89	5.30	0.41	−3.96	0.00	0	Z	44424
16	4-OCH_2COOEt	4.91[e]	4.22	0.69	0.03	−0.16	0	Z	44426
17	4-$CH_2CH(NH_2)COOH$	4.94	5.24	0.30	−3.56	−0.07	0	9	35051
18	4-COOH	4.94[e]	3.43	1.51	−0.32	0.77	0	9	240367
19	3-$CH_2CH(NH_2)COOH$	5.71	5.18	0.53	−3.56	0	0	9	27381
20	4-$CH_2CH(NH_2)COOH$,2-Cl	5.83	5.66	0.17	−2.85	0.16	1	Z	43348
21	2-$CH_2CH(NH_2)COOH$	6.18	6.10	0.08	−3.56	−0.07	1	Z	57199

[a] NCI data. [b] Calculated using eq 12. [c] $I = 1$ for ortho substitution. [d] Number in this column represents the number of injections given on consecutive days; Z indicates that in these instances, injections were given daily until death. [e] These points not used in deriving equations.

of the 4-NO group and its ability to greatly increase the potency of the aniline mustards. The reason for this great activating effect may lie in its metabolic fate (i.e., possible in vivo reduction near the site of action to 4-NHOH and then to 4-NH_2). Activation may also be associated with the long recognized[9] ambivalent electronic effects of the nitroso group. For –N=O, σ_p is only 0.12, while σ_p^- is 1.60. This is one of the highest known σ^- values; unfortunately, its σ^+ has not been determined but it would not be surprising if it turned out to be negative.

The difference between eq 21 and 21a, containing all data points, is not really significant. Equation 21 highlights the poor fit of compound **13**.

One of the difficulties frequently encountered in drug research programs extending over many years is that of obtaining results under a uniform set of conditions. In the present case we have been limited by the many different regimens employed. In Tables V–VIII, a number of different regimens have been used in administering drugs. A number such as 9 or 15 means that an injection was given daily for 9 or 15 consecutive days. The Z regimen means the animals received a daily dose until death. For our purposes, there are only a few days difference between 9 and death so that it seems safe to treat these examples in a single equation.

Discussion

Ideas on the mechanism of alkylation by aniline mustards have varied over the years. One of the most careful studies was that of Bardos et al. The most recent evidence[10,11] favors an aziridinium intermediate (see Scheme I). For very active nucleophiles, route 1 is a possible mechanism; for less active nucleophiles, route 2–3 is more likely. The formation of the aziridine would, of course, be highly dependent on the electron density on nitrogen and, hence, associated with a large ρ for the σ parameter in the Hammett equation. Early in the study of the aniline mustards Ross appreciated the importance of substituent effects on the nucleophilic character of nitrogen and undertook an extensive study of substituent effects on their hydrolysis.[12] The hydrolyses were carried out under a set of standard conditions in which the percent hydrolysis in 30 min at 66 °C in 50% aqueous acetone was measured. Strangely, Ross never attempted to fit his data to the Hammett equation.

We have derived eq 22 from the data in Table IX. In

$$\log \%\ \text{hyd} = -1.42\ (\pm 0.18)\ \sigma + 0.45\ (\pm 0.15)\ I_0 + 0.70\ (\pm 0.11)\ I + 1.21\ (\pm 0.06) \quad (22)$$

$$n = 42;\ r = 0.952;\ s = 0.157$$

$$\log \%\ \text{hyd} = -1.49\ (\pm 0.22)\ \sigma + 0.46\ (\pm 0.18)\ I_0 + 0.74\ (\pm 0.13)\ I + 1.19\ (\pm 0.07) \quad (22a)$$

$$n = 43;\ r = 0.939;\ s = 0.190$$

this equation, I_0 is assigned the value of 1 for compounds having an ortho substituent. The positive slope of this term shows that ortho substitution increases the rate of hydrolysis, no doubt by twisting the nitrogen out of conjugation with the π electrons of the ring, thus making nitrogen a better nucleophile. The indicator variable I is assigned the value of 1 for derivatives of I where Y = Br. These congeners are more rapidly hydrolyzed than the corresponding Cl derivatives. The coefficient for σ in eq 22 agrees well with those in eq 3 and 6, showing that biological activity parallels activity as measured by hydrolysis. The coefficient for I_0 in eq 22 agrees reasonably well with the corresponding coefficient in eq 12, considering the confidence limits on these terms. However, the corresponding coefficient in eq 15 and 18, although positive, is much smaller and less significant. The better leaving group in these congeners is less affected by ortho substitution. The coefficient with the σ term is similar to those in the antitumor equations, although it is a bit larger. Antitumor activity is a little less dependent than hydrolysis is on the electronic effect of substituents. The use of σ^- in eq 22 in place of σ gives a poorer correlation ($r = 0.902$).

Ross et al.[12b] determined the rate constants for hydrolysis for a smaller set of aniline mustards. We have derived eq 23 from their data in Table X. The variation

$$\log k = -1.84\ (\pm 0.40)\ \sigma - 4.02\ (\pm 0.08) \quad (23)$$

$$n = 11;\ r = 0.961;\ s = 0.116$$

$$\log k = -1.76\ (\pm 0.63)\ \sigma - 3.97\ (\pm 0.13) \quad (23a)$$

$$n = 12;\ r = 0.893;\ s = 0.184$$

Table VI. X-$C_6H_4N(CH_2CH_2Cl)_2$ vs. P388 Leukemia; T/C = 125

No.	X	Log 1/C Obsd[a]	Log 1/C Calcd[b]	|Δ log 1/C|	σ	π or Σπ	Regimen[c]	NSC no.
1	4-CH=C(CN)$_2$	3.62	3.86	0.25	0.70	0.05	9	48841
2	3-CH_2CH_2COOH	3.86[d]	4.73	0.87	−0.03	−0.29	9	41457
3	4-OC_6H_5	3.95	3.79	0.16	−0.03	2.08	9	58426
4	3-CH(NH_2)CH_2COOH	4.18[d]	6.15	1.97	0.01	−3.96	9	44424
5	3-OCH_2COOEt	4.31	4.45	0.14	0.12	0.03	9	41449
6	4-OC_4H_9	4.38	4.24	0.14	−0.27	1.55	9	43814
7	3-CH=CHCOOH	4.38	4.45	0.07	0.14	0.00	9	45631
8	3-OCH_2CH_2COOH	4.41	4.54	0.13	0.12	−0.18	9	41448
9	H	4.49	4.59	0.10	0	0	9	18429
10	3-OCH_2COOH	4.81	4.81	0.00	0.12	−0.87	9	41447
11	2-CH=CHCOOH	4.48	4.42	0.06	0.17	0	9	44440
12	4-$NHCOCH_2Cl$	4.89	4.81	0.08	−0.03	−0.50	9	260490
13	2-OCH_2CH_2COOH	4.89	4.93	0.04	−0.27	−0.18	9	42343
14	4-$N(CH_3)_2$	4.89	5.34	0.45	−0.83	0.18	9	260516
15	4-CH_2CH_2COOH	5.06	4.77	0.29	−0.07	−0.29	9	71965
16	4-CH_2COOH	5.19	4.94	0.25	−0.07	−0.72	9	71964
17	4-N=CHC_6H_5	5.33	5.25	0.08	−0.55	−0.29	9	240391
18	4-$CH_2CH(NH_2)COOH$	5.61	6.07	0.46	−0.07	−3.56	10	35051
19	4-NH_2,3-CH_3	5.68	5.60	0.08	−0.73	−0.73	9	260510
20	4-$(CH_2)_3CHO$	5.96[d]	4.48	1.48	−0.17	−0.65	18	138101
21	3-$CH_2CH(NH_2)COOH$	6.09	6.03	0.06	−0.03	−3.56	10	27381
22	2-$CH_2CH(NH_2)COOH$	6.49	6.07	0.42	−0.07	−3.56	10	57199

[a] NCI data. [b] Calculated using eq 14. [c] See Table V. [d] These points not used in the formulation of equations.

Table VII. X-$C_6H_4N(CH_2CH_2Cl)_2$ vs. P388 Leukemia; T/C = 180

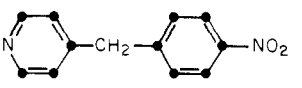
No.	X	Log 1/C Obsd[a]	Log 1/C Calcd[b]	|Δ log 1/C|	σ	π or Σπ	I[c]	Regimen[d]	NSC no.
1	4-OC_6H_5	3.35	3.46	0.11	−0.03	2.08	0	9	58426
2	3-CH(NH_2)CH_2COOH	3.65[e]	5.45	1.80	0.01	−3.96	0	9	44424
3	3-OCH_2COOEt	3.71	3.95	0.24	0.12	0.03	0	9	41449
4	3-CH_2CH_2COOH	3.81	4.26	0.45	−0.03	−0.29	0	9	41457
5	H	4.02	4.12	0.10	0.00	0.00	0	9	18429
6	4-OC_4H_9	4.02	3.97	0.05	−0.27	1.55	0	9	43814
7	3-OCH_2CH_2COOH	4.04	4.02	0.02	0.12	−0.18	0	9	41448
8	3-OCH_2COOH	4.29	4.25	0.04	0.12	−0.87	0	9	41447
9	4-CH_2CH_2COOH	4.36	4.32	0.04	−0.07	−0.29	0	9	71965
10	2-CH=CHCOOH	4.39	4.19	0.20	0.17	0.00	1	9	44440
11	2-OCH_2CH_2COOH	4.41	4.86	0.45	−0.27	−0.18	1	9	42343
12	4-$CH_2C(CH_3)(NH_2)COOH$	4.55[e]	5.29	0.74	−0.07	−3.00	0	9	260629
13	4-$(CH_2)_3CHO$	4.72	4.14	0.58	−0.17	−0.21	0	18	138101
14	4-CH_2COOH	4.87	4.47	0.40	−0.07	−0.72	0	9	71964
15	4-N=CHC_6H_5	5.00	4.98	0.02	−0.55	−0.29	0	9	240391
16	4-$CH_2CH(NH_2)COOH$	5.18	5.43	0.25	−0.07	−3.56	0	10	35051
17	3-$CH_2CH(NH_2)COOH$	5.39	5.37	0.02	−0.03	−3.56	0	10	27381
18	2-$CH_2CH(NH_2)COOH$	5.98	5.73	0.25	−0.07	−3.56	1	10	57199

[a] NCI data. [b] Calculated using eq 18. [c] I = 1 for ortho substituents. [d] See Table V. [e] These points not used in formulation of equations.

in σ is poor for this set of congeners. Although the slope of the σ term in eq 23 is of somewhat greater magnitude than eq 22, we cannot place much reliance on it because of the rather large confidence limits. In general, Ross' results, and especially eq 22, parallel rather well antitumor activity in this series.

One point in Table X (10) is badly fit, as can be seen from a comparison of eq 23 and 23a; however, the parameters in these equations are so similar that either one could be used for predictive purposes.

Bardos et al.[6] used another model system to gain insight into the antitumor activity of aniline mustards. They determined the rate constants for the nucleophilic attack of



on derivatives of I where Y = Cl, Br, I. Omitting ionizable substituents and groups for which σ^- values are lacking, we have used their data (Table XI) to formulate eq 24.

$$\log k = -1.92\ (\pm 0.34)\ \sigma^- + 1.12\ (\pm 0.31)\ I - 1.77\ (\pm 0.21) \quad (24)$$
$$n = 14;\ r = 0.972;\ s = 0.251$$

Using σ in place of σ^- yields a much poorer correlation (r = 0.905; s = 0.455). While eq 24 has a rather large value for ρ, it is not as high as one often finds for the reactions of aniline.[13] In eq 24, I is given the value of 1 for Y = Br or I; these leaving groups are about ten times as active as Cl. The addition of the term in I to the simple σ^- equations is quite significant ($F_{1,11}$ = 62.3).

In using indicator variables to study the effect of the leaving groups Y on activity, we have assigned Cl the value of 0 in each instance. In the case of eq 1–6, iodine was also assigned a value of 0 since, by observation, it was apparent that this gave a good fit. Br, with an indicator variable of 1, is the most effective form of Y for antitumor activity. This is also true for antitumor activity of eq 7 and toxicity,

Table VIII. X-$C_6H_4N(CH_2CH_2OSO_2R)_2$ vs. L1210 Leukemia; T/C = 125

No.	X	Log 1/C Obsd[a]	Log 1/C Calcd[b]	|Δ log 1/C|	σ^-	*I*-1[c]	*I*-2	Regimen[d]	NSC no.
1	3-Cl,4-NO_2	3.02	3.24	0.22	1.61	0	0	15	104965
2	3-F	3.31	3.57	0.26	0.34	1	0	15	105786
3	4-NO_2	3.74	3.58	0.16	1.24	0	0	10	101673
4	H	3.80	3.89	0.09	0.00	1	0	15	75326
5	3-NO_2	3.93	4.08	0.15	0.71	0	0	10	100774
6	H	3.97	4.75	0.78	0	0	0	15	71035
7	4-CO_2Et	4.07	4.15	0.08	0.64	0	0	15	59643
8	3-CF_3	4.42	4.34	0.08	0.43	0	0	15	102468
9	3-CO_2Et	4.63	4.40	0.23	0.37	0	0	10	101674
10	3-Cl	4.85	4.40	0.45	0.37	0	0	10	101671
11	4-NH_2,3-CO_2Et	4.85	4.54	0.31	0.22	0	0	15	103962
12	4-F	4.20	3.85	0.35	0.05	1	0	9	102322
13	3-$NHCOCH_3$	5.67[e]	4.55	1.12	0.21	0	0	9	104967
14	3-Cl,4-NO	6.03	5.93	0.10	1.97	0	1	15	102467
15	3-$NHCOCH_3$,4-NO	6.05	6.08	0.03	1.81	0	1	15	104964
16	4-NO	6.13	6.27	0.14	1.60	0	1	8	79423
17	3-CH_3,4-NO	6.40	6.34	0.06	1.53	0	1	10	101672

[a] NCI data. [b] Calculated using eq 21. [c] *I*-1 = 1 when R = $-C_6H_4CH_3$; 0 when R = CH_3. [d] See Table V. [e] These points not used in formulation of equations.

Table IX. Hydrolysis of X-$C_6H_4N(CH_2CH_2Y)_2$ in 50% Acetone at 66 °C

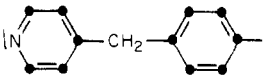
No.	X	Y	Log % hydrolysis Obsd[a]	Log % hydrolysis Calcd[b]	|Δ log % hydrolysis|	σ[c]	*I*-1	*I*-2	Ref
1	H	Cl	1.30	1.22	0.08	0.00	0	0	12a
2	2-CH_3	Cl	1.92	1.80	0.12	−0.10	1	0	12a
3	3-CH_3	Cl	1.32	1.32	0.00	−0.07	0	0	12a
4	4-CH_3	Cl	1.58	1.46	0.12	−0.17	0	0	12a
5	2-OCH_3	Cl	1.95	2.19	0.24	−0.37	1	0	12a
6	4-OCH_3	Cl	1.76	1.60	0.16	−0.27	0	0	12a
7	4-Cl	Cl	0.95	0.89	0.06	0.23	0	0	12a
8	2,3-CH=CHCH=CH	Cl	1.70	1.61	0.09	0.04	1	0	12a
9	3,4-CH=CHCH=CH	Cl	1.18	1.16	0.02	0.04	0	0	12a
10	4-CH=CHC_6H_5	Cl	1.26	1.32	0.06	−0.07	0	0	12a
11	H	Br	1.90	1.92	0.02	0.00	0	1	12a
12	3,4-CH=CHCH=CH	Br	1.90	1.86	0.04	0.04	0	1	12a
13	4-OCH_3	Br	1.99	2.30	0.31	−0.27	0	1	12a
14	4-Br	Cl	0.78	0.89	0.11	0.23	0	0	12b
15	4-Bu	Cl	1.56	1.44	0.12	−0.16	0	0	12b
16	4-NHAc	Cl	1.62	1.22	0.40	0.00	0	0	12b
17	4-OEt	Cl	1.72	1.56	0.16	−0.24	0	0	12b
18	4-OH	Cl	1.75	1.74	0.01	−0.37	0	0	12b
19	4-*t*-Bu	Cl	1.46	1.50	0.04	−0.20	0	0	12c
20	4-CO_2Et	Cl	0.18	0.58	0.40	0.45	0	0	12c
21	4-Cl	Br	1.82	1.59	0.23	0.23	0	1	12c
22	2-Ph	Cl	1.49	1.36	0.13	0.21	1	0	12c
23	2-Ph	Br	1.97	2.07	0.10	0.21	1	1	12c
24	4-Ph	Cl	1.08	1.23	0.15	−0.01	0	0	12c
25	4-Ph	Br	1.85	1.93	0.08	−0.01	0	1	12c
26	4-SH	Cl	1.18	1.01	0.17	0.15	0	0	12d
27	4-$SCOCH_3$	Cl	0.48	0.59	0.11	0.44	0	0	12d
28	4-SCH_3	Cl	1.11	1.22	0.11	0.00	0	0	12d
29	4-SCN	Cl	0.48	0.48	0.00	0.52	0	0	12d
30	4-$SCONH_2$	Cl	0.90	0.81	0.09	0.29	0	0	12d
31	4-SH	Br	1.85	1.71	0.14	0.15	0	1	12d
32	4-SCN	Br	1.28	1.18	0.10	0.52	0	1	12d
33	4-$SCONH_2$	Br	1.60	1.51	0.09	0.29	0	1	12d
34	4-SO_2NH_2	Cl	0.30	0.41	0.11	0.57	0	0	6
35	4-$CONH_2$	Cl	0.00	0.71[d]	0.71	0.36	0	0	12e
36	4-NH_2	Cl	2.00	2.15	0.15	−0.66	0	0	12e
37	4-$NHCOCH_3$	Br	1.93	1.92	0.01	0.00	0	1	6
38	4-$SCOCH_3$	Br	1.41	1.30	0.11	0.44	0	1	6
39	4-SO_2NH_2	Br	0.90	1.11	0.21	0.57	0	1	6
40	3-CO_2Et	Cl	0.65	0.69	0.04	0.37	0	0	12b
41	4-CH_2COOEt	Cl	1.19	1.32	0.13	−0.07	0	0	12b
42	4-CH_2CH_2COOEt	Cl	1.32	1.32	0.00	−0.07	0	0	12b
43	4-$(CH_2)_3COOEt$	Cl	1.36	1.46	0.10	−0.17	0	0	12b

[a] From ref 12. [b] Calculated using eq 22. [c] The σ values are from ref 17 except for 2-CH_3 [from H. W. Thompson and G. Steel, *Trans. Faraday Soc.*, **52**, 145 (1956)], 2-C_6H_5 [from E. M. Tribble and J. Traynham, *J. Am. Chem. Soc.*, 91, 379 (1969)], and $SCONH_2$ whose value we have estimated as follows: $\sigma_{SO_2CH_3} - \sigma_{SO_2NH_2} = \Delta\sigma = 0.72 - 0.57 = 0.15$. $\sigma_{SCOCH_3} = 0.44$; therefore, $\sigma_{SCONH_2} = 0.44 - 0.15 = 0.29$. [d] This point omitted in the derivation of eq 22.

Table X. Hydrolysis of X-$C_6H_4N(CH_2CH_2Cl)_2$ in 50% Acetone at 66 °C

No.	X	Log k Obsd[a]	Log k Calcd[b]	$\lvert\Delta \log k\rvert$	σ
1	3-CO_2Et	−4.59	−4.69	0.10	0.37
2	4-Br	−4.46	−4.44	0.02	0.23
3	4-CH_2CO_2Et	−4.04	−3.89	0.15	−0.07
4	4-CH_2COOH	−3.99	−3.89	0.10	−0.07
5	4-CH=CHC_6H_5	−3.95	−3.89	0.06	−0.07
6	4-$CH_2CH_2CO_2$Et	−3.88	−3.89	0.01	−0.07
7	4-$(CH_2)_3CO_2$Et	−3.84	−3.70	0.14	−0.17
8	4-$(CH_2)_3$COOH	−3.65	−3.70	0.05	−0.17
9	4-Bu	−3.61	−3.72	0.11	−0.16
10	4-NHAc	−3.52[c]	−4.01	0.49	0.00
11	4-OEt	−3.38	−3.57	0.19	−0.24
12	4-OH	−3.32	−3.33	0.01	−0.37

[a] From ref 12b. [b] Calculated using eq 23. [c] This point not used in deriving eq 23.

Table XI. Reaction of  with X-$C_6H_4N(CH_2CH_2Y)_2$ at 80 °C

No.	X	Y	Log k_{rel} Obsd[a]	Log k_{rel} Calcd[b]	$\lvert\Delta \log k_{rel}\rvert$	σ^-
1	H	Cl	−1.89	−1.77	0.12	0.00
2	4-CHO	Cl	−3.48	−3.75	0.27	1.03
3	4-$CONH_2$	Cl	−3.30	−2.98	0.32	0.63
4	4-SO_2NH_2	Cl	−4.00	−3.57	0.43	0.94
5	4-OH	Cl	−1.31	−1.46	0.15	−0.16
6	4-NH_2	Cl	−1.37	−1.48	0.11	−0.15
7	4-$NHCOCH_2F$	Cl	−1.78	−1.77	0.01	0.00
8	4-$NHCOCH_3$	Cl	−1.53	−1.77	0.24	0.00
9	4-$SCOCH_3$	Cl	−2.56	−2.65	0.09	0.46
10	H	Br	−0.91	−0.65	0.26	0.00
11	4-$SCOCH_3$	Br	−1.41	−1.53	0.12	0.46
12	4-SO_2NH_2	Br	−2.12	−2.45	0.33	0.94
13	H	I	−0.80	−0.65	0.15	0.00
14	4-SO_2NH_2	I	−2.50	−2.45	0.05	0.94

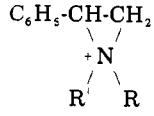
[a] From ref 7. [b] Calculated using eq 24.

eq 9. The in vitro result with eq 24 is different from the biological data of eq 1–6 and 9 since here both Br and I are assigned an indicator variable value of 1 for I. This is another discrepancy between in vivo and in vitro data, the cause of which cannot be given at present.

The observed ρ of eq 22–24 is, of course, the resultant value of the ρ's for steps 2 and 3 in Scheme I. If step 3 has a positive value of ρ, this could account for the somewhat lower ρ with aniline mustards than that found for simple reactions of anilines.

Bardos et al. commented that, "Contrary to expectation, the correlation between 'alkylating activity' and molar antitumor activity data appears to be somewhat less than satisfactory." Of course, some of this dissatisfaction is now accounted for in terms of π. However, all of the ρ values found in this study are below those of eq 22–24 but are not as low as one would expect for route 1. The small values of ρ seem best accounted for by assuming that step 3 is more important in the in vivo alkylation inhibiting cell growth. It is possible that adsorption of the aziridinium ions to a macromolecule would somewhat disperse the plus charge and thus make the ion more resistant to nucleophilic attack than if it were in solution.

Some light can be shed on this problem from the studies by Chapman and Triggle[14] on the solvolysis in 1:1 acetone–water of



Their results have yielded[15] the following QSAR. While this example is not strictly analogous to the present aziridinium compound and a relatively poor selection of substituents was used, it does suggest that, after correcting for steric effects via E_s^c, ring opening depends on electron withdrawal by R and R′. It is also of interest that E_s^c in eq 25 has a negative coefficient, showing that large groups

$$\log k = -1.10\,E_s^c + 4.47\,\sigma^* - 1.85 \qquad (25)$$
$$n = 6;\ r = 0.998;\ s = 0.031$$

sterically assist ring opening. The activating effect of ortho substitution which we have uncovered via indicator variables may be similar to that involved in the solvolysis of the aziridines. Ortho substituents also help localize the lone pair electrons on nitrogen by twisting the amino moiety partially out of conjugation. Since eq 25 is based on only three data points per variable, a great deal of weight cannot be placed upon it.

Conclusions

Our analysis shows that, in broad outline, the mechanism of action of this extensively studied class of compounds is rather well accounted for in terms of electron density on nitrogen and lipophilicity as modeled by octanol–water partition coefficients. We have yet to develop a satisfactory explanation for the unusual activity of chlorambucil.

Our most interesting finding is that log P_0 for activity against a solid tumor (Walker 256) appears to be quite different from log P_0 for leukemia. Although this point needs further study with better sets of congeners, it is not unexpected since the penetration of the Walker tumor by drugs may well involve passage through more lipophilic

barriers than in the case of leukemia. We feel confident that this finding about the difference in ideal lipophilicity for drugs acting against leukemia and solid tumors will be found in other types of antitumor drugs. Our own study of drug activity against leukemia convinces us that, for some unknown reason, the most effective compounds usually have low log P values—much lower than one would expect, for example, for penetration of the CNS. Excessive dependence on leukemia as a means for screening antitumor drugs tends to develop drugs which will not be ideally lipophilic for the penetration of other types of tumors. Cain and Atwell have also noted[16] that more lipophilic drugs are required to effectively attack tumors localized remotely from the site of drug injection.

Method

The values of σ and π are largely from ref 17; σ^- values are from ref 18. The following calculations were made for π.

(1) $\pi(-OCH_2CO_2Et) = \pi(-OCH_2COOH) + \Delta\pi$ (ethyl ester to acid)

(ester → acid) = log P (CH_3COOEt) − log P (CH_3COOH) = 0.73 + 0.17 = 0.90

$\pi(-OCH_2CO_2Et) = -0.87 + 0.90 = 0.03$

(2) $\pi[-NH-C(=O)CH_2F] = \log P(Ph-NHCOCH_2Cl) + \Delta\pi$ ($NH_2COCH_2F \rightarrow NH_2COCH_2Cl$) − log P (C_6H_6) = 1.63 + (−0.52) − 2.13 = −1.02

$NH_2COCH_2F \rightarrow NH_2COCH_2Cl$

$-1.05 \rightarrow -0.53$

$\therefore \Delta\pi = -0.52$

(3) $\pi(-CH_2CH_2NH_2) = \log P(Ph-CH_2CH_2NH_2)$ − log P (C_6H_6) = 1.41 − 2.13 = −0.72

(4) $\pi[-CH(NH_2)CH_2COOH] = \pi[-CH_2CH(NH_2)COOH] + \Delta F$ ($P_1 \rightarrow P_2$) = −3.56 − 0.4 = 3.96

(5) $\pi[-CH=C(CN)_2] = \log P[Ph-CH=C(CN)_2]$ − log P (C_6H_6) = 2.18 − 2.13 = 0.05

(6) $\pi(-NH-COCH_2Cl) = \log P(Ph-NHCOCH_2Cl)$ − log P (C_6H_6) = 1.63 − 2.13 = −0.50

(7) $\pi[-(CH_2)_3COOH] = \pi[(CH_2)_2COOH] + \pi(-CH_2)$ = −0.29 + 0.50 = 0.21

(8) $\pi(-CH_2CHO) = \log P(Ph-CH_2CHO)$ − log P (C_6H_6) = 1.78 − 2.13 = −0.35

$\pi[-(CH_2)_3CHO] = -0.35 + 1.00 = 0.65$

(9) log P ($Ph-OCH_2CH_2COOH$) = log P ($Ph-OCH_2CH_3$) − f_H + f_{CO_2H} + fb + f_{P_2} + f_H bond = 2.51 − 0.23 − 1.11 − 0.12 + 0.4 + 0.5 = 1.95

$\pi(-OCH_2CH_2COOH) = \log P(Ph-OCH_2CH_2COOH)$ − log P (C_6H_6) = 1.95 − 2.13 = −0.18

(10) log P ($Ph-OCH_2CH_2CO_2Et$) = log P ($Ph-OCH_2CH_3$) − f_H + f_{CO_2} + f_{CH_2} + f_{CH_3} + $2fb$ + f_{P_2} = 2.51 − 0.23 − 1.49 + 0.66 + 0.89 − 0.24 + 0.4 = 2.50

$\pi(-OCH_2CH_2CO_2Et) = 2.50 - 2.13 = 0.37$

We have calculated log P using fragment constants[19] in a number of the above examples. The fragment symbol f represents log P for an atom or fragment. F_p represents the *factor* for the proximity of the two H-bonding groups. The subscript refers to the number of carbon atoms separating them. In some cases we have had to estimate σ values. There are really no good models for some of the constants; however, we believe that the differences for the types of groups involved will not be serious. We have taken σ for $CH_2CH(NH_2)COOH$ to be the same as σ for CH_2CH_2COOH. σ for OCH_2CH_2COOH is taken to be the same as for OCH_3 and σ for $(CH_2)_3CHO$ is taken as σ for CH_3. We believe in any case that errors in estimated σ constants will be less than errors in the biological data.

To establish log $1/C$ values producing the same T/C (T = survival of test animal compared to control animal C), the "best" straight line was drawn through the response plotted against dose. T/C was selected so that it was not necessary to make large extrapolations. Antitumor in vitro data are inherently variable and tend to exhibit considerable scatter.

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(1) This investigation was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Contract No. NO1-CM-67062.

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Structure and Tumor-Promoting Activity of Analogues of Anthralin (1,8-Dihydroxy-9-anthrone)

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Seventeen analogues of the tumor-promoting agent anthralin were tested for the same biological property by repeated skin application on mouse skin using female ICR/Ha Swiss mice, after a single application of a subcarcinogenic dose of 7,12-dimethylbenz[*a*]anthracene. Seven of the compounds tested are new compounds. They are 1,8-diacetoxy-9-anthrone, 1,8-dimyristoyloxy-9-anthrone, 1,8-dihydroxy-10-acetyl-9-anthrone, 1,8-dihydroxy-10-myristoyl-9-anthrone, 1,8,10-trihydroxy-9-anthrone, 1,8-dihydroxy-9,10-dihydroanthracene, and myristoyljuglone. All compounds were used in pure form for the bioassays. Of the 17 test compounds four showed notable tumor-promoting activity. They are 1,8-dihydroxy-10-acetyl-9-anthrone, 1,8-dihydroxy-10-myristoyl-9-anthrone, 1-hydroxy-9-anthrone, and juglone. In order to determine whether there is any relationship between tumor-promoting activity and metal chelation in this series, the chelating abilities of anthralin and of its inactive analogue 1,8-dihydroxyanthraquinone were examined using the bivalent metal ions Cu(II), Zn(II), Mn(II), Mg(II), and Ca(II). No relationship between chelation and tumor-promoting ability was found.

Anthralin (1,8-dihydroxy-9-anthrone) has been used for many years in the treatment of psoriasis and related skin diseases.[1] This compound attracted our attention because of its reported tumor-promoting activity on mouse skin.[2] In subsequent work in this laboratory, its tumor-promoting activity in two-stage carcinogenesis on mouse skin was confirmed.[3] It was also shown to be cocarcinogenic; i.e., it enhanced remarkably the carcinogenic activity of a low dose of benzo[*a*]pyrene on mouse skin when the two agents were applied simultaneously and repeatedly on mouse skin.[4] The compound is less active as a tumor promotor and cocarcinogen than the phorbol esters of croton oil, e.g., phorbol myristate acetate.[4] Because it is a comparatively simple molecule, anthralin is useful for studies on mode of action and structure–activity relationships of tumor promoters and cocarcinogens.

This report describes the tumor-promoting activity of a series of 17 analogues of anthralin. A number of these are new compounds, synthesized specifically for this study.

It has been suggested that metal chelation plays a role in chemical carcinogenesis.[5-7] As part of the present study it was, therefore, of interest to examine the chelating abilities of some representative compounds in this group. The present report includes the chelation characteristics of the tumor promoter anthralin and its biologically inactive analogue 1,8-dihydroxyanthraquinone[3] with Cu(II), Zn(II), Mn(II), Mg(II), and Ca(II) ions.

In the course of the synthesis of the acetate and myristate esters of anthralin for bioassay, it was found that hydrogen bonding of the protons of the C-1 and C-8 hydroxyl groups to the C-9 carbonyl oxygen had unusual effects on the acylation reactions of anthralin. These findings are also given in this report.

Results and Discussion

Chemistry. In the course of the synthesis of the acetate and myristate esters of anthralin, it was found that the molecule reacted to the acylating agents in an unusual manner. In previous experiments from this laboratory[8] it was found that the reaction of pyrogallol (1,2,3-trihydroxybenzene) with acyl chlorides under basic conditions (pyridine as solvent) gave a mixture of mono-, di-, and triesters; when the reaction was carried out in benzene solution, pyrogallol was acylated at C-4. Similar results with phenol esterification were obtained by others.[9] When anthrone was allowed to react with acyl chlorides in pyridine O-acylation occurred, suggesting that the enolate form of anthrone predominated. In the absence of base and in hydrocarbon solvents the keto form of anthrone is more stable than the enol form.[10]

When anthralin was allowed to react with acyl chlorides under alkaline conditions, the expected esters were not formed; instead the 10-acyl derivatives, 10-acetylanthralin (**4**) and 10-myristoylanthralin (**5**), were isolated. Under acid conditions, anthralin reacted with acetic anhydride and myristoyl chloride to give the 1,8-diesters, i.e., the diacetate **2** and the dimyristate **3**, rather than the acyl derivative.

In basic solution (pyridine), anthralin exists primarily in the enolate form with the charge being distributed between the three oxygens on C-1, C-8, and C-9 as well as on C-10 through resonance. The resonance interactions involving the three oxygens and two protons associated with them inhibit acylation at these positions, thus acylation takes place at the C-10 position via the carbanion. In acid solution anthralin exists in the keto form (1,8-dihydroxy-9-anthrone) with protonation of the C-9 carbonyl oxygen possibly disrupting intramolecular hydrogen bonding and facilitating ester formation.

Tumor-Promoting Activity. The results of the two-stage carcinogenesis experiments with 17 analogues of anthralin are summarized in Table I. Anthralin was used as a positive control. In these experiments anthralin did not result in carcinomas, as were observed in our earlier experiments.[3] The earlier experiments[3] were continued for 490 days, whereas most of the experiments shown in

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