$MR(8) = MR(7) + 2MR(CH_2) = 59.46 + 2 \times 4.65 = 68.76$ ...

MV Parameters. We followed exactly the method described by Exner.[33] For example:

$MV(7) = MV(C_6H_5) + MV(CH_2) + MV(CO) + MV(N) + 2MV(CH_3) = 74.65 + 2 \times 16.58 + 7.30 + 10.21 - 5.09 - 2 \times 31.48 = 183.19$

$MV(8) = MV(7) + 2MV(CH_2) = 183.19 + 2 \times 16.58 = 216.35$ ...

$^1\chi^v$, Connectivity Index. The calculations were performed as described in ref 34 and 35.

Regressions Analysis. The multiple linear regressions were performed on a CII 10070 computer. The bilinear regressions were carried out on an IBM 360 by means of a program adapted after Kubinyi[39] by the Department of Statistics of Metabio-Joullié Laboratories. n is the number of data, r the regression coefficient, s the standard deviation, F the F test after;[47] each regression coefficient has its 95% confidence interval in parentheses.

Pharmacology. Chloroform-Induced Fibrillation in Mice after Vargaftig and Coignet.[48] Fibrillation was induced in mice by mortal inhalation of chloroform with groups of 10 animals. An aqueous solution of the drug was orally administrated 30 min before the beginning of the test. The heart was exteriorized and fibrillation controlled before death; prolongation of survival time was checked.

Corneal Anesthetic Activity in Rabbit Adapted from the Method of Régnier.[49] An aqueous solution of the drugs (0.25 mL) was applied on the cornea for 1 min and carefully wiped. The active minimal dose was estimated as the concentration which nullified the corneal reflex after 5 min, under the influence of 100 regular mechanical stimulations. The measurements were repeated until 10 concordant results were obtained and the experimental error was not more than 10%.

Intraveneous Acute Toxicity in Mice. Aqueous solutions of the drug were administrated intraveneously in a volume of 0.2 mL/20 g of the corporal weight. Mice were divided in groups of 10 randomized animals. The percentage of dead mice was obtained 5 days after the beginning of the experiment. LD_{50} was calculated by the usual statistical method.[50]

Acknowledgment. We are grateful to Dr. D. Moccatti and Dr. M. Bonnet for assistance in calculation on bilinear regressions. We are also indebted to Professor R. F. Rekker for communication of his new set of f values.

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R_m Values and Structure-Activity Relationship of Benzodiazepines

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Quantitative structure–activity relationships (QSAR) have been formulated for the activities of a series of benzodiazepines in rats. The lipophilic character of molecules was expressed by means of the chromatographic R_m values which were very well correlated with experimental or calculated log P values. The ideal lipophilic character for activity of benzodiazepines in the exploratory behavior test is not far from that of compounds acting in the central nervous system as unspecific depressant agents. The results of both the conflict and exploratory behavior studies might support the hypothesis of different sites of action for the antianxiety and sedative effects of benzodiazepines.

QSAR studies pointed out the importance of the physicochemical parameters of active CNS agents in determining activities such as protein binding of benzodiazepines,[1] MAO inhibition of many classes of compounds,[2,3] inhibition of oxidative metabolism,[4] mice hypnosis by barbiturates,[5] etc. Although Barfknecht et al.[6] found a correlation between lipophilic character and hallucinogenic activity in a series of psychotomimetic phenylisopropylamines, there is a persisting lack of QSAR reports correlating structure and more specific CNS activity in vivo.

The purpose of the present work was an attempt to correlate structure and behavioral activity in a series of benzodiazepines. As an expression of the lipophilic character of molecules, the chromatographic R_m values were used and compared with the experimental or calculated log P values from an 1-octanol–water system. The benzodiazepines reported in Table I were obtained from commercial sources.

Methods

Determination of R_m Values. The R_m values were measured by means of a reversed-phase thin-layer chromatography technique, which allowed the partitioning of benzodiazepines between a polar mobile phase and a nonpolar stationary phase. The mobile phase consisted of H_2O in various mixtures (v/v) with Me_2CO. The stationary phase was obtained by impregnating with a 5% (v/v) silicone oil or 1-octanol solution in ether and a layer of silica gel G F_{254} (Type 60) from Merck Co., Darmstadt. Silicone DC 200 (350 cSt) from Applied Science Laboratories and 1-octanol ("Baker analyzed" reagent) were used. The method of impregnating the silica gel G plates and other details of the chromatography technique were already described.[7,8] In particular, the mobile phase was saturated with silicone or 1-octanol. The

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compounds were detected in UV light. The benzodiazepines were dissolved in H_2O, Me_2CO, MeOH, or $CHCl_3$ (1 mg/mL), and 1 μL of solution was spotted on the plates in randomized allocations. When using silica gel G F_{254} layers impregnated with 1-octanol, the mobile phase was represented by H_2O or sodium acetate–Veronal buffer ($^1/_7$ M) at pH 7.4 in various mixtures (v/v) with Me_2CO. In the case of the aqueous buffer, in order to have a better control of the pH, the slurry of silica gel G F_{254} was obtained with 0.09 N NaOH. In this way it was possible to also study the influence of the pH of the chromatography system on the partitioning between the mobile and stationary phases.

Finally, the R_m values of benzodiazepines were measured on unimpregnated silica gel G F_{254} and polyamide layers by using a mobile phase represented by H_2O in various mixtures (v/v) with Me_2CO.

Biological Activity Data. The CNS activities of several benzodiazepines were investigated by means of two psychopharmacological techniques investigating the exploratory, and the conflict, behavior in rats. All the compounds were suspended in a 5% acacia gum solution, containing 1.25% of Me_2SO, and injected intraperitoneally. The data regarding some benzodiazepines had been already published by Babbini et al.[9-12]

The exploratory activity was recorded by means of 6 actometers already described,[9] which registered the horizontal displacements of the animals inside a plastic cage. Rats were placed into the activity cage 30 min after the drug treatment, and motility was recorded for 10 min. Each compound was tested at four to seven doses, using five to six animals per dose level; another group of rats treated with the vehicle alone served as control. For each drug an ED_{50} was calculated by the Spearman–Kärber method[13] as the dose which would decrease to a half the locomotor activity in 50% of rats.

The conflict behavior was studied by means of six homemade Skinner boxes supplied with a lever, a dispenser for 70 mg of food pellets, and a panel for light stimuli presentation. Programming and recording of the experiments were automated with electromechanical and solid-state control equipment, digital and printing counters, and pen recorders. Rats previously trained to a conflict behavior according to Geller and Seifter[14] were used. Periods of 3 min of punished schedule were alternated with periods of 12 min of nonpunished schedule. In particular during the punished schedule period, a light stimulus was on in the conditioning chamber and the animals avoided pressing the lever, since each lever pressing was rewarded with food but also punished with an electric shock to the animal's feet. During the nonpunished period, pressing the lever was occasionally followed only by a delivery of food. Each test session included 2 days: on the first day the animals were treated with the vehicle 30 min before being placed in the Skinner box and then their behavior was recorded for 60 min. On the second day, the same procedure was repeated, replacing the vehicle by a drug. Test sessions were spaced at least 1 week apart. Four to seven doses of a given drug were tested using five to six animals per dose. The response of each animal was expressed as the difference between the log of the total number of lever pressing obtained under control or drug conditions. For the punished schedule, an ED_{50} was computed as the dose which caused a fourfold increase of lever pressing in 50% of the rats. For the nonpunished schedule, the ED_{50} represented the dose which would decrease to a half the lever pressing in 50% of the animals.

In Table IV, the ED_{50} values (mg/kg) were reported as log (1/C) data, where C is the ED_{50} expressed in mmol/kg. The equations correlating structure and activity were calculated by means of a multiple regression analysis.[15]

Results

R_m Values on Silica Gel G F_{254} Layers Impregnated with Silicone Oil. The experimental R_m values were plotted against the acetone concentration in the mobile phase. For each compound, there was a range of linear relationships between R_m values and composition of the mobile phase. The equations describing the linear relationship are reported in Table I, where their intercepts represent the theoretical R_m values at 0% acetone in the mobile phase. Extrapolated R_m values had been already obtained for several series of drugs, such as penicillins and cephalosporins,[16] phenols,[17] and steroids.[18] The *b*'s (i.e., slopes) of the equations in Table I show a substantial parallelism among different straight lines. Exceptions are represented by compounds **9** and **25–27** with *b*'s between −0.015 and −0.021 and compounds **33** and **38** with *b*'s of −0.075 and −0.070, respectively. All other *b*'s range from −0.037 to −0.055. The compounds with the lowest *b*'s were characterized by the presence of a $(CH_2)_2N(C_2H_5)_2$ group. Because of the substantial parallelism among straight lines, the extrapolated R_m values at 0% were considered as suitable for structure–activity studies and for considering the influence of substituent groups in determining the lipophilic character of the whole molecule. In Table II are reported the ΔR_m values for 20 substituent groups as obtained from the experimental R_m values in the silicone system. The introduction of a CH_3 group or a Cl atom increases the R_m value. This is lowered by the introduction of $(CH_2)_2N(C_2H_5)_2$, $(CH_2)_2OH$, or OH group. A similar influence is shown by the $OCO(CH_2)_2COOH$ and $(CH_2)_3$-OH groups. An exception is represented by **21**, where the introduction of a $(CH_2)_2N(C_2H_5)_2$ group as in **26** provokes an increase of the R_m value. The introduction of a F atom does not provoke a significant change in the lipophilic character of the molecule. An increase of the R_m value is provoked by other groups listed in Table II. Clearly, the replacement of a Cl atom with a NO_2 group decreases the hydrophilic character of the molecule; nitrazepam is more hydrophilic than *N*-demethyldiazepam. The R_m values of clonazepam and demoxepam (**40** and **41**), as reported in Table I for the silicone system, were calculated by means of the ΔR_m values of Table II. In particular, a R_m value of 1.65 for clonazepam was obtained by adding a ΔR_m value of 0.18 for Cl to the measured R_m value of nitrazepam; a R_m value of 1.10 for demoxepam was calculated by substracting a ΔR_m value of −0.10 for $(CH_2)_2OH$ from the measured R_m value of **37**.

R_m Values on Unimpregnated Silica Gel G F_{254} Layers. The R_m values measured on unimpregnated layers were plotted against the acetone concentration in the mobile phase. Although for most of the compounds it was possible to obtain an experimental R_m value at 0% acetone, i.e., with a mobile phase represented by only water, in Table I are reported both the experimental and the extrapolated R_m values at 0% on unimpregnated layers, the latter being represented by the intercepts of the equations describing the linear relationship between R_m values and acetone concentration. Since in most cases the experimental R_m values are very close to the R_m values calculated from the TLC equation, the present experiment

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could support the validity of the extrapolation technique.

Equation 1 shows that there is a poor relationship between the extrapolated R_m values at 0% acetone on silicone-impregnated layers and those on unimpregnated ones. In fact, eq 1 explains only 37% of the variability in the R_m(unimpregn) values.

$$R_m(\text{unimpregn}) = -0.165(\pm 0.180) + 0.467(\pm 0.099)R_m(\text{sil}) \quad (1)$$

$$n = 39;\ r = 0.612;\ s = 0.285;\ F = 22.16;\ p < 0.005$$

The lipophilic character of substituents does not seem to influence the migration of compounds in the same way on impregnated and unimpregnated layers. It can be noted that all the compounds with a $(CH_2)_2N(C_2H_5)_2$ group at R_1 are among those with higher R_m values on unimpregnated silica gel G. This could be due to a stronger interaction with the unimpregnated silica gel G provoked by the presence of such a group at R_1.

All this clearly shows the qualitative influence of the impregnation technique which is really able to change the physicochemical properties of the stationary phase. On the other hand, the different degree of interaction with the stationary phase is shown by the b's of the equations reported in Table I. In fact, comparison with the b's of the equations calculated with the R_m values measured on silicone-impregnated layers shows that on unimpregnated layers, by increasing the acetone concentration in the mobile phase, the R_m values decrease faster than on impregnated layers.

R_m Values on Silica Gel G F_{254} Layers Impregnated with 1-Octanol. At the lower acetone concentrations not all the compounds migrated in a suitable way. Practically, only at 35–40% acetone concentrations was it possible to obtain R_m values for all the compounds. On the other hand, at 45–50% acetone concentrations most of the compounds tended to migrate with the solvent front. On silica gel G F_{254} layers impregnated with silicone oil at 25–30% all the compounds did migrate and most of them still provided reliable R_m values at 65% acetone concentration. The series of experiments carried out by using a mobile phase represented by sodium acetate–Veronal buffer at pH 7.4 yielded R_m values not differing from those obtained with a mobile phase represented by water. At least in the range taken into consideration, a change in the pH of the mobile phase did not affect the migration of the compounds. In Table I the intercepts of the equations represent the extrapolated R_m values. The slopes of the straight lines are much more different in the 1-octanol system than in the silicone one. In particular, the high b's of compounds **7**, **8**, **12**, **32** and **34–36** caused the very high extrapolated R_m values of these compounds. As in the case of the silicone system (eq 1), eq 2, which shows a low correlation coefficient between the R_m values on unimpregnated plates and those on plates impregnated with 1-octanol, points out the influence of the stationary phase. However, the correlation coefficient provided by eq 3, which was calculated with the extrapolated R_m values from both systems, is not very good.

$$R_m(\text{unimpregn}) = 0.345(\pm 0.062) + 0.177(\pm 0.027)R_m(\text{oct}) \quad (2)$$

$$n = 39;\ r = 0.733;\ s = 0.246;\ F = 42.69;\ p < 0.005$$

$$R_m(\text{oct}) = -3.023(\pm 0.479) + 2.720(\pm 0.263)R_m(\text{sil}) \quad (3)$$

$$n = 39;\ r = 0.861;\ s = 0.757;\ F = 106.53;\ p < 0.005$$

The reason for such a different behavior of benzodiazepines in the octanol system could be due to the fact that the impregnation carried out with 1-octanol does not provide a system where only partitioning of the compounds between the stationary phase and the mobile phase takes place, without any adsorption at the support. In fact, Hulshoff et al.[19] did not consider 1-octanol as a suitable stationary phase in TLC of benzodiazepines.

R_m Values on Polyamide Layers. The R_m values on unimpregnated polyamide layers were plotted against the acetone concentration in the mobile phase. Most of the compounds did migrate with only water and, therefore, the extrapolation could not affect the results in a substantial way. In fact, eq 5 calculated with the experimental R_m values of 35 compounds at 0% acetone in the mobile phase is very similar to eq 4 which was calculated with the extrapolated ones. However, both eq 4 and 5 provided very low correlation coefficients.

$$R_m(\text{polyamide}) = -0.089(\pm 0.550) + 0.405(\pm 0.310)R_m(\text{sil}) \quad (4)$$

$$n = 38;\ r = 0.213;\ s = 0.799;\ F = 1.71;\ p < 0.25$$

$$R_m(\text{polyamide 0\%}) = -0.091(\pm 0.524) + 0.399(\pm 0.294)R_m(\text{sil}) \quad (5)$$

$$n = 35;\ r = 0.230;\ s = 0.662;\ F = 1.85;\ p < 0.25$$

The reason for the low correlation coefficients of eq 4 and 5 is in the different mechanism by which polyamide separates compounds. Polyamide acts by formation of a hydrogen bond between the amide linkage in the macromolecule polyamide and the compound; the steric effects of substituents seem to be very important in determining the chromatographic behavior.[20] On the contrary, on silica gel G layers impregnated with silicone oil the main factor in determining the migration of compounds would be their lipophilic character. The different mechanism of separation should be a major factor, particularly in the case of complex molecules such as benzodiazepines. On polyamide, the strongest interaction with the stationary phase, i.e., shortest migration, was observed with compounds **2**, **7**, and **8**. However, a shorter migration could be due to a decrease in the interaction between water and compound rather than to an increase in the interaction between the solute and polyamide. From a chromatographic point of view, polyamide layers can be useful in separating benzodiazepine compounds. As an example, it can be noted that compounds **25** and **28** with very close extrapolated R_m values in the silicone oil system show more different R_m values on polyamide layers.

Relationship between R_m and Log P Values. The log P values of 11 benzodiazepines as determined by Müller et al.[1] in an octanol–water system are reported in Table III in parentheses (**3**, **8–10**, **15**, **18**, **35**, **36** and **39–41**). A very good correlation between the experimental log P values for 9 of the above 11 compounds and the corresponding experimental R_m values in the silicone system from Table I are shown by eq 6.

$$\log P = -0.439(\pm 0.202) + 1.674(\pm 0.104)R_m(\text{sil}) \quad (6)$$

$$n = 9;\ r = 0.987;\ s = 0.118;\ F = 258.59;\ p < 0.005$$

Clonazepam (**40**) and demoxepam (**41**) were not used in calculating eq 6 because their experimental R_m values were

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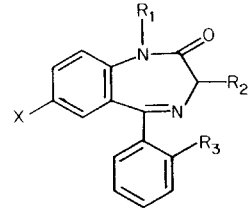
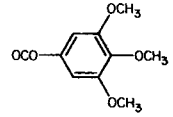
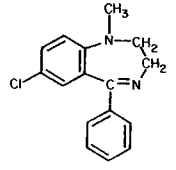
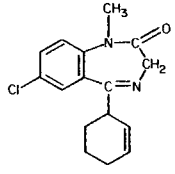
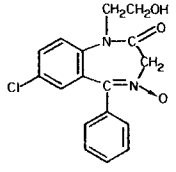
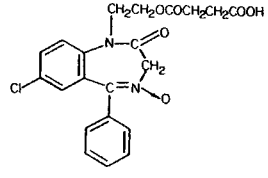
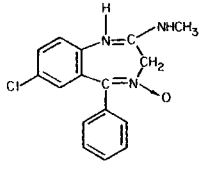
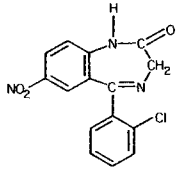
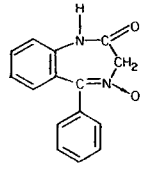
Table I. Structure and R_m Values of Benzodiazepines in Different TLC Systems

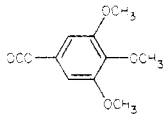


		substituents				silica gel G F_{254} impregnated with silicone oil: eq from TLC		silica gel G F_{254} unimpregnated			silica gel G F_{254} impregnated with 1-octanol		polyamide	
								exptl	eq from TLC					
no.	compound	X	R_1	R_2	R_3	$a = R_m$	b	R_m[a]	a	b	a	b	a	b
1	*N*-demethyldiazepam	Cl	H	H	H	1.772	−0.047	0.599	0.553	−0.095	1.897	−0.036	1.186	−0.035
2		Cl	H	H	Cl	1.939	−0.051	0.605	0.564	−0.092	2.525	−0.058	1.382	−0.038
3	diazepam	Cl	CH_3	H	H	1.952	−0.050	0.792	0.762	0.104	1.949	−0.038	0.924	−0.034
4		Cl	CH_3	H	Cl	2.150	−0.054	0.740	0.704	−0.089	1.960	0.036	1.165	−0.038
5		Cl	H	H	F	1.732	0.047	0.602	0.556	−0.098	1.758	−0.036	1.248	−0.041
6		Cl	CH_3	H	F	1.905	−0.051	0.635	0.601	−0.091	1.665	−0.032	0.943	−0.035
7		Cl	CH_2CF_3	H	H	2.540	−0.055	0.676	0.603	−0.082	3.955	−0.085	1.353	−0.040
8	prazepam	Cl	CH_2-c-C_3H_5	H	H	2.410	−0.054	0.899	0.848	−0.089	3.873	−0.084	1.361	−0.040
9	flurazepam	Cl	$(CH_2)_2N(C_2H_5)_2$	H	F	1.679	−0.021		1.320	−0.035	1.545	−0.020	−0.907	−0.012
10	nitrazepam	NO_2	H	H	H	1.470	−0.045	0.242	0.216	−0.073	1.035	−0.015	0.923	−0.031
11		NO_2	$CONHCH_3$	H	H	1.457	−0.045	0.503	0.468	−0.099	1.086	−0.020	0.947	−0.032
12		Cl	$(CH_2)_2Cl$	OC_2H_5	H	2.322	−0.053	0.861	0.798	−0.096	2.880	−0.059	1.044	−0.032
13		Cl	$(CH_2)_2OCOCH_3$	OC_2H_5	H	1.875	−0.047	0.829	0.720	−0.110	1.135	−0.015	0.317	−0.023
14		Cl	$(CH_2)_2OH$	OC_2H_5	H	1.788	−0.052	0.662	0.596	−0.100	1.072	−0.021	0.357	−0.020
15	oxazepam	Cl	H	OH	H	1.510	−0.046	0.443	0.397	−0.100	1.239	−0.026	1.126	−0.033
16	temazepam	Cl	CH_3	OH	H	1.580	−0.045	0.542	0.542	−0.100	1.162	−0.022	0.697	−0.028
17		Cl	$(CH_2)_2OH$	OH	H	1.345	−0.044	0.503	0.458	−0.100	0.682	−0.018	0.456	−0.023
18	lorazepam	Cl	H	OH	Cl	1.645	−0.048	0.419	0.378	−0.098	1.366	−0.029	1.224	−0.035
19	methyllorazepam	Cl	CH_3	OH	Cl	1.818	−0.050	0.624	0.583	−0.090	1.437	−0.030	1.041	−0.038
20		Cl	$(CH_2)_2OH$	OH	Cl	1.653	−0.051	0.470	0.456	−0.096	1.026	−0.025	0.864	−0.031
21		Cl	H	OH	F	1.481	−0.046	0.459	0.412	−0.100	1.185	−0.029	1.021	−0.034
22		Cl	CH_3	OH	F	1.621	−0.048	0.633	0.598	−0.104	1.186	−0.026	0.742	−0.032
23		Cl	$(CH_2)_2OH$	OH	F	1.341	−0.045	0.468	0.439	−0.098	0.582	−0.017	0.483	−0.026
24		NO_2	$(CH_2)_2OH$	OH	H	0.868	−0.037	0.237	0.130	−0.088	0.067	−0.008	0.177	−0.016
25		Cl	$(CH_2)_2N(C_2H_5)_2$	OH	H	1.427	−0.015		1.050	−0.027	1.263	−0.012	−0.961	−0.010
26		Cl	$(CH_2)_2N(C_2H_5)_2$	OH	F	1.520	−0.020		1.067	−0.029	1.333	−0.021	−1.070	−0.010
27		Cl	$(CH_2)_2N(C_2H_5)_2$	OH	Cl	1.459	−0.017		1.112	−0.032	1.381	−0.017	−0.784	−0.015
28		Cl	$(CH_2)_3OH$	OH	H	1.420	−0.049	0.572	0.464	−0.108	0.663	−0.018	0.503	−0.026
29		Cl	$(CH_2)_3OCOCH_3$	$OCOCH_3$	H	2.104	−0.052	0.714	0.626	−0.105	1.538	−0.023	0.582	−0.028

30		Cl	$(CH_2)_2OCOCH_3$	$OCOCH_3$	H	1.935	−0.050	0.633	0.536	−0.104	1.235	−0.018	0.426	−0.025
31		Cl	$(CH_2)_2OH$	$OCOCH_3$	H	1.795	−0.053	0.491	0.420	−0.098	1.046	−0.020	0.527	−0.026
32		Cl	H		H	2.430	−0.048		1.120	−0.080	5.828	−0.132	1.780	−0.023
33		Cl	H	$OCOCH_2CH_2$-COOH	H	1.332	−0.075	0.107	0.039	−0.113	−0.308	−0.033	−1.830	−0.030
34		Cl	H	$OCOCH(CH_2CH_3CH_3)_2$	H	3.012	−0.049		2.035	−0.074	6.968	−0.160		
35	medazepam					2.648	−0.055		1.020	−0.057	3.883	−0.082	−0.090	−0.014
36	tetrazepam					2.002	−0.046	0.949	0.846	−0.115	2.884	−0.059	0.597	−0.020
37						1.000	−0.041	0.597	0.496	−0.105	1.658	−0.025	1.215	−0.034
38						0.775	−0.070	0.523	0.407	−0.145	−0.507	−0.025	1.800	−0.032
39	chlordazepoxide					1.813	−0.050	0.676	0.634	−0.092	1.333	−0.023	0.370	−0.019
40	clonazepam					(1.65)[a]								
41	demoxepam					(1.10)[a]								

[a] Since the experimental R_m values for clonazepam and demoxepam were not determined in the silicone system, they were calculated as described in the text.

Table II. ΔR_m[x] and π Values in Benzodiazepines

group	position	ΔR_m	π values obsd	π values calcd (eq 7)	$\Delta\pi$
N ⟶ O		−0.76[a]	−1.58[b]	−1.56	−0.02
$OCO(CH_2)_2COOH$	R_2	−0.44[c]	−0.73[d]	−0.84	0.11
OH	R_2	−0.30[e]	−0.67[f]	−0.53	−0.14
$(CH_2)_2OCO(CH_2)_2COOH$	R_1	−0.12[g]	−0.37[h]	−0.13	−0.24
$(CH_2)_2OH$	R_1	−0.10[i]	−0.12[j]	−0.08	−0.04
$(CH_2)_3OH$	R_1	−0.09[k]	0.40[l]	−0.06	0.46
$(CH_2)_2N(C_2H_5)_2$	R_1	−0.07[m]	0.20[n]	−0.01	0.21
$(CH_2)_2OCOCH_3$	R_1	−0.02[o]	0.35[p]	0.10	0.25
$CONHCH_3$	R_1	−0.01[q]	−0.17[r]	0.10	−0.27
F	R_3	0.00[s]	0.01[t]	0.14	−0.13
$OCOCH_3$	R_2	0.03[u]	−0.01[v]	0.21	−0.22
OC_2H_5	R_2	0.12[w]	0.38[y]	0.41	−0.03
CH_3	R_1	0.16[z]	0.52[aa]	0.50	0.02
Cl	R_3	0.18[bb]	0.59[cc]	0.54	0.05
$(CH_2)_3OCOCH_3$	R_1	0.33[dd]	0.87[ee]	0.88	−0.01
$(CH_2)_2Cl$	R_1	0.43[ff]	1.43[gg]	1.10	0.33
CH_2-c-C_3H_5	R_1	0.64[hh]	1.58[ii]	1.57	0.01
	R_2	0.66[jj]	1.40[kk]	1.62	−0.22
CH_2CF_3	R_1	0.77[ll]	1.59[mm]	1.87	−0.28
$OCOCH(CH_2CH_2CH_3)_2$	R_2	1.24[nn]	3.11[oo]	2.92	0.19
NO_2	X		−0.04[pp]		

[a] Calculated using the measured R_m for 37, subtracting the R_m for 1 and the ΔR_m for $(CH_2)_2OH$. [b] See ref 21. [c] Calculated from the measured R_m values of 33 and 1. [d] Calculated using a π value of −0.01 for $OCOCH_3$ and adding a π value of −0.72 for CH_2COOH (ref 21). [e] Calculated using the measured R_m for 3 – 16, 2 – 18, 1 – 15, 4 – 19, 22 – 26, and 21 – 25. [f] See ref 23. [g] Calculated using the measured R_m for 38, subtracting the R_m for 37 and the ΔR_m for $(CH_2)_2OH$. [h] Calculated adding a π value of 0.35 for $(CH_2)_2OCOCH_3$ to a π value of −0.72 for CH_2COOH (ref 22 and 24). [i] Calculated using measured R_m for 15 – 17, 20 – 18 and 21 – 23. [j] See ref 23. [k] Calculated using the measured R_m for 28 and 15. [l] Calculated by adding a π value of 0.52 to the π value of $(CH_2)_2OH$. [m] Calculated using the measured R_m for 9 – 5, 25 – 15, 21 – 26, and 27 – 18. [n] Calculated using the measured log P for 9 minus measured log P for 3 and π values for F and CH_3 (ref 22 and 1). [o] Calculated using the measured R_m value for 13, subtracting the measured R_m for 1 and the ΔR_m for OC_2H_5. [p] Calculated using the π value for CH_2OCOCH_3 and adding the π value for CH_3 (ref 22 and 24). [q] Calculated using the R_m for 11 and 10. [r] Calculated using a π value of −1.92 for $CONHNH_2$, subtracting the π value for NH_2 (−1.23), and adding the π value for CH_3 (ref 22 and 25). [s] Calculated from the measured R_m values for 5 – 1, 6 – 3, 21 – 15, 22 – 16, 23 – 17, and 26 – 25. [t] See ref 22. [u] Calculated for the measured R_m value for 31 by subtracting the measured R_m for 1 and the ΔR_m for $(CH_2)_2OH$. [v] Calculated from the π value for $OCOC_2H_5$ by subtracting the π value for CH_3 (ref 26). [w] Calculated from the measured R_m for 14 by subtracting the measured R_m for 1 and the ΔR_m for $(CH_2)_2OH$. [x] Calculations were based on the R_m values from the silicone system. [y] See ref 24. [z] Calculated from the measured R_m values for 16 – 15, 19 – 18, 22 – 21, 3 – 1, 4 – 2, and 6 – 5. [aa] See ref 22. [bb] Calculated from the measured R_m for 18 – 15, 19 – 16, 27 – 25, 20 – 17, 2 – 1, and 4 – 3. [cc] See ref 22. [dd] Calculated from the measured R_m for 29 by subtracting the measured R_m for 1 and the ΔR_m for $OCOCH_3$. [ee] Calculated from the π for $(CH_2)_2OCOCH_3$ by adding the π value for CH_3 (ref 22 and 24). [ff] Calculated from the measured R_m for 12 by subtracting the R_m for 1 and the ΔR_m for OC_2H_5. [gg] Calculated from the π value for $(CH_3)_2$ plus the π value for Cl (ref 22). [hh] From measured R_m for 8 minus 1. [ii] From the measured log P for 8 by subtracting the measured log P for 3 and the π value for CH_3 (ref 25). [jj] Calculated from the measured R_m for 32 – 1. [kk] Calculated from the π value for $OCOC_6H_5$ plus the π value for $(OCH_3)_3$ (ref 24). [ll] Calculated from the measured R_m for 7 – 1. [mm] Calculated from the π value for CH_3 plus the π value for CF_3 (ref 22). [nn] Calculated from the measured R_m for 34 – 1. [oo] Calculated from the π value for $OCOCH_3$ plus the π value for $(CH_3)_6$ (ref 22 and 24). [pp] See ref 22.

not available (see Table I). As a further step in our analysis, the π values for the 21 substituent groups of Table II were calculated from the 11 experimental log P values of Table III or taken from the literature. The experimental R_m values of Table I did not allow the calculation of the ΔR_m value for the NO_2 group. Therefore, eq 7 was calculated with 20 data points.

$$\pi = 0.142(\pm 0.050) + 2.239(\pm 0.109)\Delta R_m(\text{sil}) \quad (7)$$

$$n = 20;\ r = 0.979;\ s = 0.215;\ F = 420.89;\ p < 0.005$$

Finally, by taking advantage of the additive property of the lipophilic character, the log P values for the other 30 compounds were calculated (Table III).

Although the log P values of Table III range from 0.19 to 5.25 and the R_m values in the silicone system range from 0.90 to 2.65, eq 8 shows a very good correlation.

$$\log P = -1.220(\pm 0.144) + 2.045(\pm 0.080)R_m(\text{sil}) \quad (8)$$

$$n = 41;\ r = 0.971;\ s = 0.236;\ F = 651.76;\ p < 0.005$$

Despite their very good correlation coefficients, eq 7 and 8 need some comment. In fact, a scatter diagram of the data of Table II shows that the *N*-oxide and 2-propylpentanoic acid moieties are at two extremes. However, dropping the π values for these two substituents transforms eq 7 into eq 9, which is very similar to eq 7.

$$\pi = 0.148(\pm 0.055) + 2.115(\pm 0.160)\Delta R_m \quad (9)$$

$$n = 18;\ r = 0.957;\ s = 0.219;\ F = 174.02;\ p < 0.005$$

In calculating the π values of Table II, we did not take into account any possible effect of steric twisting or shielding or intramolecular hydrogen bonding.[23]

In other words, π values used sometimes in the same way as fragmentation values are not always the same as partial values.[27,28] On the other hand, the π values of Table II

(23) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(24) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, 16, 1207 (1973).

Table III. Log P Values of Benzodiazepines

no.	log P obsd	log P calcd (eq 8)	Δ log P
1	2.14	2.40	−0.26
2	2.73	2.74	−0.01
3	(2.66)	2.77	−0.11
4	3.25	3.18	0.07
5	2.15	2.32	−0.17
6	2.67	2.68	−0.01
7	3.73	3.97	−0.24
8	(3.72)	3.71	0.01
9	(2.35)	2.21	0.14
10	(2.12)	1.79	0.33
11	1.95	1.76	0.19
12	3.95	3.53	0.42
13	2.87	2.61	0.26
14	2.40	2.44	−0.04
15	(2.17)	1.87	0.30
16	1.99	2.01	−0.02
17	1.35	1.53	−0.18
18	(2.38)	2.14	0.24
19	2.58	2.50	0.08
20	1.94	2.16	−0.22
21	1.48	1.81	−0.33
22	2.00	2.09	−0.09
23	1.36	1.52	−0.16
24	0.72	0.55	0.17
25	1.67	1.70	−0.03
26	1.68	1.89	−0.21
27	2.26	1.76	0.56
28	1.87	1.68	0.19
29	3.00	3.08	−0.08
30	2.48	2.74	0.26
31	2.01	2.45	−0.44
32	3.54	3.75	−0.21
33	1.41	1.50	−0.09
34	5.25	4.94	0.31
35	(4.05)	4.19	−0.14
36	(2.76)	2.87	−0.11
37	0.44	0.82	−0.38
38	0.19	0.36	−0.17
39	(2.50)	2.49	0.01
40	(2.41)	2.15	0.26
41	(1.46)	1.03	0.43

were used in order to calculate the log P values for the remaining 30 compounds of Table III. The very good correlation coefficient provided by eq 8 between the experimental R_m values of Table I (with the only exceptions being 40 and 41, which were calculated) and the log P values of Table III could further support the present choice of π values.

Finally, a comparison of eq 8 with equations 10 and 11,

$$\log P = 1.354(\pm 0.139) + 0.569(\pm 0.061)R_m(\text{oct}) \quad (10)$$

$$n = 39;\ r = 0.838;\ s = 0.551;\ F = 87.33;\ p < 0.005$$

$$\log P = 2.222(\pm 0.205) + 0.078(\pm 0.205)R_m(\text{pol}) \quad (11)$$

$$n = 39;\ r = 0.838;\ s = 0.551;\ F = 0.146;\ p \text{ not significant}$$

calculated with the experimental R_m values in the octanol and polyamide system, respectively, clearly shows that the log P values of Table III are best correlated with the R_m values in the silicone system.

Structure–Activity Relationship. The equations describing the quantitative structure–activity relationships had been formulated by means of the R_m values from the above three different chromatographic systems. However, since the R_m values from the silicone system showed to be the best correlated with biological activity, the equations obtained with the R_m values from the octanol and polyamide systems were not reported.

Exploratory Behavior Test. The biological data obtained in the exploratory behavior test were used in order to calculate equations 12 and 13 describing their relationship with lipophilic character, as represented by R_m values from the silicone system.

$$\log (1/C) = 1.853(\pm 0.600) - 0.142(\pm 0.324)R_m \quad (12)$$

$$n = 28;\ r = 0.086;\ s = 0.892;\ F = 0.19;\ p \text{ not significant}$$

$$\log (1/C) = -2.994(\pm 1.249) + 5.546(\pm 1.381)R_m - 1.532(\pm 0.366)R_m^2 \quad (13)$$

$$n = 28;\ r = 0.645;\ s = 0.697;\ F = 8.93;\ p < 0.005$$

Equation 13 explains only 42% of the variability in the log $(1/C)$ data. In fact, in the range of intermediate R_m values there is a striking variability in activity. However, since the least active compounds are found among those characterized by the lowest or respectively highest R_m values, eq 13 suggests that an ideal lipophilic character is necessary but not sufficient to provide higher activity.

As a further step in the analysis, the substituents in position R_3 were considered. The electron-withdrawing effects of halogen substituents in the R_3 position could influence the benzodiazepines activity. In fact, it is well known that halogenation of the R_3 position increases activity.[29] In particular, halogenation in R_3 could influence activity through an electron-withdrawing effect. On the other hand, meta and para substituents on the same ring have an undesirable effect on activity.[30] However, in the present series of compounds only two substituents were available for the R_3 position and, therefore, instead of a correlation by means of linear free energy-related parameters, we turned our attention to the use of an indicator variable, I_3, for position R_3 in order to calculate eq 14.

$$\log (1/C) = -1.276(\pm 0.923) + 2.912(\pm 1.076)R_m - 0.800(\pm 0.288)R_m^2 + 1.132(\pm 0.213)I_3 \quad (14)$$

$$n = 28;\ r = 0.855;\ s = 0.482;\ F = 21.81;\ p < 0.005$$

A comparison of eq 14 and 13 illustrates the superiority of eq 14. In fact, an analysis of variance[15] shows that the introduction of the I_3 term into eq 13 provokes a significant improvement in eq 14 ($F_{1,24} = 28.15$; $F_{1,24;\alpha=0.005} = 9.55$). In particular, eq 14 shows a significant dependence on R_m ($t_{R_m} = 2.70$; $p < 0.025$), R_m^2 ($t_{R_m^2} = -2.77$; $p < 0.025$), and I_3 ($t_{I_3} = 5.31$; $p < 0.001$). On the other hand, the regression on R_m and R_m^2 after I_3 in eq 14 is significant ($F_{2,24} = 3.86$; $F_{2,24;\alpha=0.05} = 3.40$). It should be pointed out that collinearity cannot be shown between independent variables in eq 14.

An alternative development of eq 14 is given in Table V. The indicator variable I_3 is the most important one. Adding the variables R_m and R_m^2 one at a time does not result in a significant improvement; however, adding the

(25) C. Hansch, S. D. Rockwell, P. Y. C. Jow, A. Leo, and E. E. Steller, *J. Med. Chem.*, **20**, 304 (1977).
(26) P. N. Craig, *J. Med. Chem.*, **14**, 680 (1971).
(27) A. Leo, P. Y. C. Jow, C. Silipo, and C. Hansch, *J. Med. Chem.*, **18**, 865 (1975).
(28) R. F. Rekker, "The Hydrophobic Fragmental Constant", Elsevier, Amsterdam, 1977.
(29) L. H. Sternbach, L. O. Randall, R. Banziger, and H. Lehr, in "Drugs Affecting the Central Nervous System", Medical Research Series, Vol. 2, A. Burger Ed., Marcel Dekker, New York, 1968.
(30) L. H. Sternbach, in "The Benzodiazepines", S. Garattini, E. Mussini, and L. O. Randall, Eds., Raven Press, New York, 1973.

Table IV. Activity ED_{50} of Benzodiazepines in Rats[a]

compound	no.	exploratory behavior: log (1/C) obsd	exploratory behavior: log (1/C) calcd (eq 14)	punished schedule: log (1/C) obsd	punished schedule: log (1/C) calcd (eq 18)	nonpunished schedule: log (1/C) obsd	nonpunished schedule: log (1/C) calcd (eq 20)	I_3	I_X
N-demethyldiazepam	1	1.247	1.372	1.830	1.629	0.832	1.184	0	0
	2	2.790	2.495	2.678	2.697	2.509	2.380	1	0
diazepam	3	1.793	1.360	1.876	1.915	1.659	1.383	0	0
	4	2.409	2.421	2.824	3.034	2.409	2.614	1	0
	5	2.046	2.500	2.284	2.368	1.825	2.151	1	0
	6	2.598	2.500	2.721	2.643	2.417	2.343	1	0
	7	1.097	0.960					0	0
prazepam	8	0.709	1.094					0	0
flurazepam	9	1.943	2.490	1.745	2.283	1.291	2.093	1	0
nitrazepam	10	2.326	1.278					0	1
	12	0.193	1.174					0	0
oxazepam	15	1.191	1.297	1.192	1.211	0.961	0.894	0	0
temazepam	16	1.629	1.325	1.883	1.323	1.228	0.971	0	0
	17	0.797	1.193	0.361	0.948	0.420	0.711	0	0
lorazepam	18	3.145	2.478	2.444	2.229	2.897	2.055	1	0
methyllorazepam	19	2.594	2.502	2.658	2.504	2.394	2.246	1	0
	20	2.005	2.486	1.959	2.242	1.711	2.064	1	0
	21	2.501	2.417	2.356	1.968	2.045	1.873	1	0
	22	2.812	2.473	2.585	2.191	2.371	2.028	1	0
	23	2.239	2.321	1.648	1.744	1.697	1.718	1	0
	24	^ 340	0.652					0	1
	32	358	1.080					0	0
	33	381	1.187					0	0
	34	503	0.239					0	0
medazepam	35	443	0.827					0	0
	37	346	0.836					0	0
	38	0.762	0.501					0	0
chlordiazepoxide	39	1.628	1.371	1.578	1.694	1.277	1.235	0	0

[a] The R_m values from the silicone system for the QSAR equations are reported in Table I.

two variables at once not only produces a significant reduction in the variance but also allows one to estimate R_{m_0}. The above development of eq 14 does illustrate the advantage of looking at all possible regression equations. A forward stepwise addition could not have given this information.

An indicator I_X was given the value of 1 or 0 for congeners containing, respectively, a NO_2 or a Cl group in position X. However, the introduction of the indicator variable I_X did not improve the correlation coefficient of eq 15.

$$\log (1/C) = 1.613(\pm 0.951) + 3.156(\pm 1.081)R_m - 0.844(\pm 0.287)R_m^2 + 1.184(\pm 0.214)I_3 + 0.484(\pm 0.386)I_X \quad (15)$$

$$n = 28;\ r = 0.865;\ s = 0.477;\ F = 17.14;\ p < 0.005$$

The positive sign associated with the I_X coefficient could indicate a positive influence of nitro substitution in position X. This should be in agreement with Sternbach et al.[29] who pointed out that contribution to the overall activity of X substituents should be mainly due to their electron-withdrawing properties. The lack of a significant improvement in the present analysis is mainly due to the fact that only two compounds were NO_2 substituted while all other ones were Cl substituted. Moreover, one of the NO_2-substituted compounds, i.e., **24**, was characterized by the second lowest R_m value. This could mask the positive influence of the NO_2 group. Because of the difficulty in taking into account the NO_2 substituents in position X, eq 16 was calculated with only 26 compounds, i.e., with exclusion of **10** and **24**.

$$\log (1/C) = -0.800(\pm 0.952) + 2.255(\pm 1.078)R_m - 0.620(\pm 0.284)R_m^2 + 1.244(\pm 0.200)I_3 \quad (16)$$

$$n = 26;\ r = 0.878;\ s = 0.441;\ F = 24.76;\ p < 0.005$$

Equation 16 shows a better correlation coefficient and a significant dependence on R_m ($t_{R_m} = 2.09$; $p < 0.05$), R_m^2 ($t_{R_m^2} = -2.18$; $p < 0.005$), and I_3 ($t_{I_3} = 6.22$; $p < 0.001$). In the present instance about 23% in the variance in the data is not accounted for. However, when considering the experimental error involved in this type of testing, the correlation coefficient of eq 16 is not bad.

The ideal lipophilic character for the exploratory activity as calculated from eq 14 or 16 is represented by $R_{m_0} = 1.82$. The substitution into eq 8 provides a $\log P_0 = 2.50$. This is not very far from the $\log P_0 \approx 2$ indicated by Hansch[31] as the ideal lipophilic character for relatively unspecific CNS-active compounds. On the other hand, this could be in agreement with the fact that a depressive effect upon the exploratory behavior might be considered as a measure of an unspecific depressant action. However, in the present case an optimum lipophilic character is necessary but not sufficient to provide higher activity. A somewhat similar conclusion could be drawn from the work of Barfknecht et al.,[6] who showed a significant parabolic relationship between log *P* and activity in a series of psychotomimetic amines. In other words, for more specific compounds, such as benzodiazepines, after they have reached their site of action, electronic and/or steric contributions such as those suggested for the R_3 and X positions would be then necessary for the interaction with specific receptors. In fact, it is known that the highly selective and saturable action of benzodiazepines on GABAergic synaptic inhibition is proposed as the basis of many central effects of this class of drugs.[32] The involvement of serotonin and/or noradrenaline in the behavioral action of benzodiazepines has been also repeatedly postulated.[33]

(31) C. Hansch, *Acc. Chem. Res.*, **2**, 232 (1969).
(32) W. E. Haefely, *Agents Actions*, **7**, 353 (1977).
(33) E. Costa and P. Greengard, Eds., *Adv. Biochem. Psychopharmacol.*, **14** (1975).

Table V. Development of Equation 14

intercept	R_m	R_m^2	I_3	r	s	F	eq
1.853	-0.142			0.086	0.892	0.19	A
1.904		-0.088		0.201	0.877	1.10	B
-2.994	5.546	-1.532		0.645	0.697	8.93	C
1.044			1.418	0.803	0.533	48.28	D
1.105	-0.034		1.415	0.803	0.543	0.03[a]	E
1.155		-0.030	1.398	0.806	0.540	0.33[b]	F
-1.275	2.912	-0.800	1.132	0.855	0.482	3.86[c]	14

[a] This is $F_{1,25}$ obtained by comparison with eq D. [b] This is $F_{1,25}$ obtained by comparison with eq D; the F test showed that neither eq E nor eq F is a significant improvement over eq D. [c] This is $F_{2,24}$ obtained by comparison with eq D. The F test showed that eq 14 is a significant improvement over eq D.

Conflict Behavior Tests. The punished schedule test had been carried out with only 17 compounds (Table IV). The following equations show the development of eq 18.

$$\log (1/C) = -1.106(\pm 1.011) + 1.846(\pm 0.589)R_m \quad (17)$$

$$n = 17;\ r = 0.629;\ s = 0.515;\ F = 9.81;\ p < 0.01$$

$$\log (1/C) = -1.195(\pm 0.648) + 1.593(\pm 0.382)R_m + 0.803(\pm 0.169)I_3 \quad (18)$$

$$n = 17;\ r = 0.876;\ s = 0.330;\ F = 23.17;\ p < 0.005$$

The introduction of the I_3 term into eq 17 improved the correlation coefficient in a significant way. For eq 18, $F_{1,14} = 22.49$ ($F_{1,14;\alpha=0.005} = 11.06$). On the other hand the regression on R_m after I_3 in eq 18 is significant ($F_{1,14} = 17.44$).

Similarly, a regression analysis carried out for the nonpunished schedule yielded eq 19 and 20.

$$\log (1/C) = -0.658(\pm 1.248) + 1.421(\pm 0.728)R_m \quad (19)$$

$$n = 17;\ r = 0.450;\ s = 0.636;\ F = 3.81;\ p < 0.10$$

$$\log (1/C) = -0.777(\pm 0.773) + 1.107(\pm 0.454)R_m + 1.012(\pm 0.202)I_3 \quad (20)$$

$$n = 17;\ r = 0.845;\ s = 0.394;\ F = 17.54;\ p < 0.005$$

The introduction of the I_3 term improved in a significant way the correlation coefficient in eq 20 ($F_{1,14} = 25.06$). The regression on R_m after I_3 in eq 20 is also significant ($F_{1,14} = 5.92$; $F_{1,14;\alpha=0.05} = 4.60$).

In order to compare the QSAR equation for the exploratory behavior test with those for both conflict behavior tests, eq 21 was calculated with the data from the

$$\log (1/C) = 0.361(\pm 0.713) + 0.613(\pm 0.419)R_m + 1.043(\pm 0.186)I_3 \quad (21)$$

$$n = 17;\ r = 0.850;\ s = 0.363;\ F = 18.30;\ p < 0.005$$

exploratory behavior test for the 17 compounds used in calculating eq 17 and 18 and 19 and 20.

The different slopes of the R_m term in eq 18 and 20 might point out a different dependence on lipophilic character for activity in the punished- and in the nonpunished-schedule test. This could suggest that two different mechanisms are involved in the above-mentioned activities; this is in agreement with the hypothesis of Stein et al.,[34] who proposed different neurological structures and biochemical mechanisms for the antianxiety and sedative action of benzodiazepines. However, because of their large confidence limits, eq 18 and 20 do not allow any conclusion.

On the other hand, because of the lower slope of its R_m term, eq 21 seems to be closer to eq 20 than to eq 18. This should be in agreement with the fact that the depressive effect upon the exploratory behavior, i.e., an unspecific depressant effect, should be more related to the sedative effect obtained in the unpunished schedule.

In conclusion, the slope of the R_m term in the QSAR equations might indicate a relationship between lipophilic character and different CNS effects of benzodiazepines. At least one might suggest that the antianxiety effect measured in the punished-schedule test is more dependent on the lipophilic character than the unspecific depressant effect measured in the exploratory behavior test. Moreover, the present data seem to point out the usefulness of R_m values as an expression of the lipophilic character of complex molecules such as benzodiazepines.

(34) L. Stein, C. D. Wise, and J. D. Belluzzi, in "Mechanism of Action of Benzodiazepines", E. Costa and P. Greengard, Eds., Raven Press, New York, 1975.

Antiamebic Amidines and Sulfonamides of 5- and 6-Amino-2,3-bis(4-alkyl-1-piperazinyl)quinoxalines

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A series of amidines and sulfonamides of 5- and 6-amino-2,3-bis(4-alkyl-1-piperazinyl)quinoxalines was synthesized and tested against cecal and hepatic forms of *Entamoeba histolytica* infections in rats and hamsters, respectively. Four compounds (**5**, **6**, **8**, and **9**) were found to have acceptable activity against infections in both species but were too toxic to be considered for use in man.

The current primary drug[2] used in the treatment of human amebiasis, metronidazole, a nitroimidazole, has demonstrated carcinogenicity in test animals.[3,4] In our research efforts to find antiamebic agents which do not

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