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Correlation of Biological Activity and High-Pressure Liquid Chromatographic Retention Index for a Series of Propranolol, Barbiturate, and Anthranilic Acid Analogues

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The antiarrhythmic activity of propranolol analogues, the inotropic activity of propranolol analogues, the anti-inflammatory activity of anthranilic acids, the hypnotic activity of barbiturates, and the inhibition of cell division by barbiturates were correlated with either octanol–water partition coefficients or with high-pressure liquid chromatographic retention indices. The retention index, which was a scale based on the relative retention of the drug and a series of C_3–C_{23} 2-ketoalkanes, was found to give higher correlations with biological activity than was found between octanol–water partition coefficients and biological activity. Only in the case of the anthranilic acids was the retention index found to give the lower correlation.

The use of octanol–water partition coefficients (log *P*) in quantitative structure–activity studies, as pioneered by Hansch, has become a standard method in drug design studies. Numerous workers have realized that there is a close parallel between the retention of drugs on reverse-phase high-pressure liquid chromatographic (LC) columns and the octanol–water partition coefficients and they have tried to link this correlation to biological activity in two distinct ways. The more common approach to the problem has been to use high-pressure LC as a tool to obtain estimates of octanol–water partition coefficients,[1-4] which can then be used in structure–activity studies. The other approach to the problem has been to relate the retention time of drugs on reverse-phase high-pressure LC columns directly to biological activity.[5,6]

In most of the studies where high-pressure LC has been used as a technique to estimate log *P*, various types of "octanol-like" reverse-phase high-pressure LC columns have been used with mobile phases that were largely water.[1-3] These methods have several advantages over the classical shake-flask method of obtaining log *P* values; in that only extremely small quantities of the drug are needed, the drug doses do not need to be extremely pure, and the method is much faster. However, because these methods have been restricted to mobile-phase systems with a high water content, very lipophilic drugs may not be detected due to extremely long retention times. Even if different mobile-phase systems were used, each column mobile-phase system must be calibrated with a number of compounds with known log *P* values.

As indicated earlier, the other basic approach to the problem is to relate high-pressure LC retention times directly to biological activity. It is of considerable importance to note that "octanol-like" reverse-phase high-pressure LC systems have been shown to give inferior correlations with biological activity than commercially available C-18 reverse-phase systems.[6] One of the objectives of the present study was to test the hypothesis that C-18 reverse-phase high-pressure LC systems not only give better correlations with biological activity than the "octanol-like" systems but that it might also be a better model for biological interactions than the direct octanol–water partitioning model.

One of the advantages that the classical log *P* measurement system has over all of the previous high-pressure LC systems is that the classical system provides a *single*, continuous scale for the measurement of the lipophilicity of all drugs. In contrast, most of the previous high-pressure LC measurements of drug lipophilicity used scales that are unique to each column–solvent system that was used. Recently, a retention index scale suitable for use with reverse-phase high-pressure LC systems has been introduced.[7] The retention scale is based on the relative retention times of a series of 2-ketoalkanes (C_3–C_{23}). A given column–solvent combination is calibrated by chromatographing the 2-keto standards and relating the logarithm of the observed capacity factor with the defined retention indices (2-butanone = 400, etc.) in a linear manner.

It has been shown that the retention index of a given drug is fairly constant even when large changes in the composition of the mobile phase were made.[7] It was also found that acetonitrile could be substituted for methanol in the mobile phase, and nearly identical retention indices were obtained for the drugs. Because of these properties, the retention index scale could provide a single, uniform scale for the measurement of the lipophilicity of drugs.

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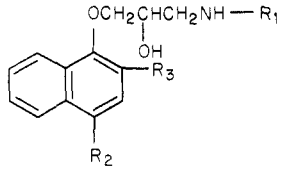
Table I. Physical and Biological Properties of Propranolol Analogues



compd	R_1	R_2	R_3	retention index	log P^a	log P^b (calcd)	$-\log ED_{40}{}^c$ (MDF)	$-\log ED_{40}{}^d$ (contract.)
P-1	$(CH_2)_3COOH$	H	H	546	0.21 ± 0.01[e]	2.03	3.41 ± 0.28[e]	3.02 ± 0.24[e]
P-2	$CH(CH_3)_2$	SO_2NH_2	H	561	−0.51 ± 0.02	1.23	3.58 ± 0.10	3.35 ± 0.02
P-3	$CH(CH_3)_2$	NH_2	H	590	−0.27 ± 0.02	1.79	5.02 ± 0.12	4.92 ± 0.20
P-4	$CH(CH_3)_2$	OH	H	667	0.39 ± 0.01	2.48	4.60 ± 0.03	4.39 ± 0.20
P-5	$(CH_2)_3C{=}ONH_2$	H	H	720	0.31 ± 0.09	1.58	4.89 ± 0.10	4.79 ± 0.06
P-6	$(CH_2)_3OCH_3$	H	H	901	1.28 ± 0.01	2.82	5.45 ± 0.15	5.40 ± 0.26
P-7	$CH(CH_3)_2$	H	H	912	1.08 ± 0.01	3.09	5.43 ± 0.07	5.21 ± 0.10
P-8	$(CH_2)_3CH_3$	H	H	950	2.08 ± 0.02	3.79	5.64 ± 0.08	5.38 ± 0.08
P-9	$CH(CH_3)_2$	Cl	H	1047	2.00 ± 0.01	3.82	5.20 ± 0.06	5.26 ± 0.12
P-10	$(CH_3)_2NH_3^+$	H	H	1104	−0.19 ± 0.01	2.10	4.50 ± 0.10	4.47 ± 0.04
P-11	$CH(CH_3)_2$	Br	Br	1215	2.71 ± 0.01	5.12	5.10 ± 0.15	5.53 ± 0.04
P-12	$(CH_2)_3N^+(CH_3)_3H$	H	H	1316	−1.89 ± 0.09	−2.41	3.08 ± 0.16	3.06 ± 0.06

[a] Experimentally measured octanol/pH 7.0 buffer distribution coefficient. [b] Calculated octanol/water partition coefficient; amine group assumed to be in the nonionic form. [c] Negative log of the molar drug concentration necessary to reduce the maximum driving frequency 40% (ref 9). [d] Negative log of the molar drug concentration necessary to reduce the tissue contractility 40% (ref 9). [e] Standard deviation.

Experimental Section

Chromatographic Conditions. A 3.9 mm i.d. × 30 cm C_{18} reverse-phase column (μ-Bondapak C_{18}, Waters Associates Inc.) with a 10-μm particle size was used for the study. The mobile-phase flow rate was 2.0 mL/min and was prepared using 6.6 g of K_2HPO_4, 8.4 g of KH_2PO_4, 1.6 L of CH_3OH, and 2.4 L of H_2O. The pH of the mobile phase was 7.0 before the addition of CH_3OH.

A Waters Associates Inc. M-6000 pump, U6K injector, and Model 440 dual-wavelength ultraviolet detector (254 and 280 nm) were used. Though the dual-wavelength detector was not essential, measurements of the 254/280 nm absorbance ratio greatly facilitated the identification of the drug in the mixture with the 2-ketoalkane standards.[7]

Materials. The 2-ketoalkane standards (C_3–C_{23}) were obtained from Analabs and the barbiturates from the Theta Corp. The anthranilic acid derivatives were synthesized[8] by R. F. Borne, Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi. The propranolol analogues were synthesized in these laboratories.[9] The methanol used in the mobile phase was freshly distilled, while all other chemicals were of reagent grade and were used as obtained.

Measurement of Retention Indices. The capacity factor (k') of the drugs and standards were determined from the observed retention time (t_R) using eq 1, where t_0 was the void volume as

$$k' = \frac{t_R - t_0}{t_0} \quad (1)$$

detected by the solvent front. The retention index of a given 2-ketoalkane standard was by definition equal to 100 times the number of carbons in the compound. Thus, 2-butanone was assigned a value of 400. The retention index (I) of a given drug or other test compound was calculated from the observed capacity factor for the drug (k_D'), the capacity factor for a 2-ketoalkane standard eluting just before the test compound (k_N'), and the capacity factor of next higher homologue (k_{N+1}') using eq 2.

$$I = 100\left[\frac{\log k_D' - \log k_N'}{\log k_{N+1} - \log k_N'}\right] + 100N \quad (2)$$

Measurement of Octanol/Water Partition Coefficients. The details of the measurement of the octanol/water partition coefficients of the propranolol series are described elsewhere.[9] Basically, the method consisted of the measurement of the distribution of the drug between an octanol phase and a pH 7.0 phosphate buffer as measured by ultraviolet spectroscopy. The partition coefficients of the anthranilic acid series[8] were determined in a similar manner; however, a pH of 8.0 was used.

The majority of the partition coefficients of the barbiturates have been previously published[10] and the values were determined for the nonionized form of the drug. The remainder of the partition coefficients were calculated using Hansch additivity constants.[17]

Measurement of Biological Activity. The antiarrhythmic activity of the propranolol derivatives was measured using the maximum driving frequency (MDF) test developed by Vaughan Williams and Szekeres[11] and the details of the biological evaluation of these specific propranolol derivatives have been previously reported.[9] In this test, an isolated rabbit atrium was electrically stimulated with a gradually increasing frequency until the physical contraction of the atrium could no longer follow the stimulus. The antiarrhythmic activity of the drug was then expressed as the molar concentration of the drug necessary to reduce the MDF by 40%.

The negative inotropic activity of the propranolol derivatives were made in these laboratories, and the details of these studies have been previously reported.[9] In this test, the isolated rabbit atrium was stimulated at a constant frequency and the activity was expressed as the molar concentration of the drug necessary to reduce the strength of the contraction 40%.

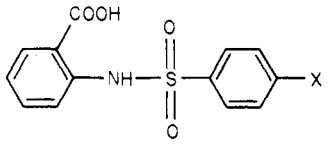
The antiinflammatory activity of the anthranilic acid derivatives has been previously reported.[8] The activity was expressed in terms of dose (mol/kg) of the drug necessary to inhibit the carrageenin-induced edema in the hindpaw of a Sprague–Dawley rat by 50%.

The biological activities of the barbiturates were not measured in these laboratories. The sources of the experimental data are given in Table III.

Results and Discussion

The propranolol derivatives were found to have a very wide range of lipophilicity, as measured by either log P or by high-pressure LC retention indices (Table I). It should also be noted that the series contained monocations, dications, and amphoteric compounds, and the structural variations were at a variety of sites on the parent compound. All of these factors would have been expected to decrease the statistical correlation of a simple drug activity–lipophilicity model. However, it was observed that antiarrhythmic activity of the drugs could be satisfactorily correlated with high-pressure LC retention indices when a parabolic model was used (Table IV, eq 4). The parabolic model for propranolol analogues has been previously reported,[18] and the addition of the second term to the re-

Table II. Physical and Biological Properties of Anthranilic Acid Derivatives



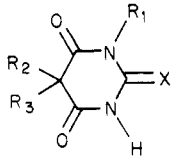
compd	X	retention index (obsd)	log P^a	π^b	$-\log I_{50}{}^c$
A-1	H	530	−0.46	0	3.24
A-2	F	565	0.03	0.15	3.49
A-3	NO_2	586	−0.03	0.24	3.44
A-4	CH_3	606	0.05	0.52	*d*
A-5	Cl	656	0.54	0.70	3.51
A-6	Br	678	0.83	1.02	3.90

[a] Log octanol/pH 8.0 buffer partition coefficient. Data from ref 8. [b] Standard aromatic substituent constants (ref 17). [c] Negative log of mol/kg of the drug necessary to reduce rat paw edema 50%. [d] Found to be an agonist.

gression equation in the present study was justified by an improvement of the correlation coefficient and the standard error of the estimate (eq 3 vs. 4; eq 5 vs. 6). There was also a slight improvement following the addition of the second lipophilicity term to the other propranolol series as well (eq 8, 10, 12, and 14). Though the inclusion of squared term in the latter four equations was not strongly justified, they were reported primarily to facilitate the comparison of retention index and log *P* as measures of lipophilicity using the same regression model. It was also found that the antiarrhythmic activity showed a higher correlation with the retention index than with the experimentally measured octanol–water partition coefficients measured at pH 7.0 (Table IV, eq 8) or with calculated lipophilicities (Table IV, eq 12). In the examination of the data for specific compounds in the series, it was observed that the retention index correctly predicted the relative activity of the two amphoteric compounds P-1 and P-2. In the case of compounds P-4 and P-5, it was also noted that the retention index measurements showed the correct relative biological activity, while both experimentally measured or calculated log *P* values would have predicted the wrong relative activity. In the case of the compound P-3, both the retention index and log *P* models would have predicted lower activity than was actually observed.

Despite the small differences noted above, there was a fairly close correlation between retention index and log *P*; thus, both measures of lipophilicity showed the same general parabolic relationship with biological activity. The largest discrepancies between the two lipophilicity measurements were observed for compounds P-10 and P-12. The retention index of both compounds was considerably higher than would have been expected from either measured or calculated log *P* values. It has been previously reported that protonated amines generally show longer retention times than would be anticipated, and this has been attributed to the interaction of the cationic amine and anionic sites on the column resulting from free silanol

Table III. Physical and Biological Properties of Barbiturates



barbiturate	R_1	R_2	R_3	X	retention index	log P^a	$-\log C^b$ (hypnosis)	$-\log ED_{50}{}^c$ (Arbacia egg division)
barbituric acid	H	H	H	O	53	−1.35		
barbital	H	CH_2CH_3	CH_2CH_3	O	423	0.65	3.09[d]	1.49
allobarbital	H	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	O	511	1.05	3.54[e]	1.79
phenobarbital	H	CH_2CH_3		O	523	1.42	3.32[e]	2.02
metharbital	CH_3	CH_2CH_3	CH_2CH_3	O	532	1.21	3.12[f]	
aprobarbital	H	$CH_2CH{=}CH_2$	$CH(CH_3)_2$	O	552	1.15	3.60[e]	2.01
butabarbital	H	CH_2CH_3	$-CH(CH_3)CH_2CH_3$	O	600	1.45	3.63[d]	
cyclobarbital	H	CH_2CH_3		O	604	1.86		2.24
butethal	H	CH_2CH_3	$CH_2CH_2CH_2CH_3$	O	610	1.65	3.72[d]	2.40
butalbital	H	CH_2CH_3	$CH_2CH(CH_3)_2$	O	616	1.45	3.63[d]	
hexobarbital	CH_3	CH_3		O	648	1.92	4.37[f]	
amobarbital	H	CH_2CH_3	$CH_2CH_2CH(CH_3)_2$	O	681	1.95	3.75[d]	2.82
pentobarbital	H	CH_2CH_3	$-CH(CH_3)CH_2CH_2CH_3$	O	686	1.95	4.05[g]	2.92
secobarbital	H	$CH_2CH{=}CH_2$	$-CH(CH_3)CH_2CH_2CH_3$	O	728	2.15	4.20[g]	3.62
thiopental	H	CH_2CH_3	$-CH(CH_3)CH_2CH_2CH_3$	S	732	3.00	3.98[h]	
thiamylal	H	$CH_2CH{=}CH_2$	$-CH(CH_3)CH_2CH_2CH_3$	S	760	3.20	4.15[h]	
methohexital	CH_3	$CH_2CH{=}CH_2$	$-CH(CH_3)C{\equiv}CH$	O	776	2.19	4.74[h]	

[a] Log of the octanol/water partition coefficient of the nonionic form. The majority of the values are taken from ref 10; however, a few were calculated using the standard substituent constants. [b] Negative log of minimum effective dose (mol/kg) in rabbits. [c] Negative log of molar drug concentration necessary to reduce cell division 50%. Data from ref 11. [d] Data from ref 12. [e] Data from ref 13. [f] Data from ref 14. [g] Data from ref 15. [h] Data from ref 16.

Table IV. Correlation of Biological Activity with the Retention Index and with the Partition Coefficients

$$-\log C = \alpha X + \delta X^2 + \beta$$

drug series	biol act.	physical property (X)	α	δ	β	r	F	N	eq
propranolol	MDF	ret index	0.0034 ± 0.0010		−4.36 ± 0.90[b]	0.104	0.11	12	3
			0.0236 ± 0.0054	$-1.29 \times 10^{-5} \pm 0.25 \times 10^{-5}$[a]	−5.29 ± 0.47	0.866	13.5	12	4
propranolol	contract.	ret index	$8.8 \times 10^{-4} \pm 10.9 \times 10^{-4}$		3.79 ± 0.95	0.248	0.65	12	5
			0.0234 ± 0.0056	$-1.25 \times 10^{-5} \pm 0.31 \times 10^{-5}$	−5.58 ± 0.59	0.818	9.1	12	6
propranolol	MDF	log P_{expl}	0.51 ± 0.13		4.35 ± 0.57	0.772	14.7	12	7
			0.60 ± 0.17	−0.067 ± 0.079	4.44 ± 0.58	0.791	7.51	12	8
propranolol	contract.	log P_{expl}	0.63 ± 0.16		4.13 ± 0.62	0.772	14.8	12	9
			0.81 ± 0.24	−0.101 ± 0.099	4.23 ± 0.62	0.799	7.93	12	10
propranolol	MDF	log P_{calcd}	0.349 ± 0.097		3.86 ± 0.59	0.753	13.1	12	11
			0.38 ± 0.13	−0.0163 ± 0.035	3.90 ± 0.62	0.760	6.14	12	12
propranolol	contract.	log P_{calcd}	0.38 ± 0.10		3.69 ± 0.64	0.759	13.6	12	13
			0.35 ± 0.14	0.015 ± 0.038	3.65 ± 0.66	0.764	6.31	12	14
anthranilic acids	I_{50}	ret index	0.0033 ± 0.0012		1.54 ± 0.14	0.853	7.98	5	15
anthranilic acids	I_{50}	log P_{expl}	0.43 ± 0.11		3.44 ± 0.11	0.913	15.0	5	16
anthranilic acids	I_{50}	π_{calcd}	0.51 ± 0.14		3.30 ± 0.12	0.898	15.0	5	17
barbiturates	hypnosis	ret index	0.00389 ± 0.00060		1.36 ± 0.23	0.875	42.6	15	18
barbiturates	hypnosis	log P	0.47 ± 0.13		2.97 ± 0.34	0.707	13.0	15	19
barbiturates	A, egg div	ret index	0.00641 ± 0.00071		−1.42 ± 0.20	0.959	80.9	9	20
barbiturates	A, egg div	log P	1.18 ± 0.21		0.56 ± 0.30	0.903	30.9	9	21

[a] Standard error of regression coefficient. [b] Standard error of estimate.

sites.[1,2] This effect can be reduced by either adding a competing lipophilic cation to the mobile phase of the high-pressure LC column or by vigorous silylation of the column. Though these two techniques may give better correlations between high-pressure LC retention and log P values, it does not necessarily follow that this would lead to better correlations between high-pressure LC retention and biological activity. In fact, for several series of compounds, better correlations have been found for standard C-18 reverse-phase columns than for "octanol-like" columns.[5,6] In the case of the propranolol series, both the antiarrhytmic activity and the inotropic activity were found to have better correlations with the high-pressure LC retention index than with the experimentally measured or calculated log P values.

In the anthranilic acid series, the lipophilicity of the compounds was found to cover only about a fourth of the range (Table II) as the propranolol series. The composition of the anthranilic acid series had been originally designed to detect electronic effects, while, in fact, lipophilicity was found to play the major role.[8] In this series of compounds, it was found that the antiinflammatory activity had a slightly higher correlation with log P than with the retention index (Table IV, eq 15–17). The biological activities of all of the compounds in the series were very closely grouped, but the retention index correlated with the order of potency, with the exception of the order of A-2 and A-3.

In the barbiturate series (Table III), the lipophilicities of the drugs were found to cover a range larger than that for the anthranilic acid series but smaller than that of the propranolol series. The ability of the barbiturates to inhibit the division of *Arbacia* eggs was found to have a higher correlation with the retention index than with log P (Table IV; eq 20 and 21). Specific examples of discrepancies in the log P correlation were that the activity of cyclobarbital was considerably lower than would be expected and that the activity of secobarbital was higher than could be accounted for by the log P measurement of lipophilicity.

The data for the hypnotic activity of the barbiturates (Table III) had been taken from a variety of literature sources and it was not unexpected to find that the correlation coefficients for the regression of this activity with either measure of lipophilicity were poorer (Table IV, eq 18 and 19). As with the previous biological activity of the barbiturates, the hypnotic activity was found to have a better correlation with the retention index than with log P. Specific examples of discrepancies in the log P sequence were noted for hexobarbital and methohexital, both of which were found to be more active than would have been expected.

Of the five different series of biological activities in the study (antiarrhythmic activity of propranolol analogues, inotropic activity of propranolol analogues, antiinflammatory activity of anthranilic acids, hypnotic activity of barbiturates, and the inhibition of cell division by bar-

Table V. Comparison of Observed and Calculated Biological Activities

compd	$-\log C_{obsd}$	$-\log C_{calcd}$ (octanol model)	$-\log C_{calcd}$ (HPLC model)
A. propranolol series, antiarrhythmic			
P-1	3.41 ± 0.24	4.56[c]	3.75[b]
P-2	3.58 ± 0.10	4.12	3.89
P-3	5.02 ± 0.12	4.27	4.14
P-4	4.60 ± 0.03	4.66	4.72
P-5	4.89 ± 0.10	4.62	4.84
P-6	5.45 ± 0.15	5.10	5.50
P-7	5.43 ± 0.07	5.01	5.50
P-8	5.64 ± 0.08	5.55	5.49
P-9	5.20 ± 0.06	5.37	5.30
P-10	4.50 ± 0.10	3.29	5.04
P-11	5.10 ± 0.15	5.57	4.34
P-12	3.08 ± 0.16	3.06	3.43
B. propranolol series, inotropic act.			
P-1	3.02 ± 0.24	4.39[c]	3.47[d]
P-2	3.35 ± 0.02	3.77	3.61
P-3	4.92 ± 0.20	4.00	3.87
P-4	4.39 ± 0.20	4.53	4.46
P-5	4.79 ± 0.06	4.47	4.79
P-6	5.40 ± 0.26	5.10	5.36
P-7	5.21 ± 0.10	4.99	5.37
P-8	5.38 ± 0.08	5.48	5.37
P-9	5.26 ± 0.12	5.45	5.22
P-10	4.47 ± 0.04	4.07	5.02
P-11	5.53 ± 0.04	5.68	4.40
P-12	3.06 ± 0.06	2.34	3.57
C. anthranilic acid series			
A-1	3.24	3.24[e]	3.29[f]
A-2	3.49	3.45	3.40
A-3	3.44	3.43	3.47
A-4	[g]	3.46	3.54
A-5	3.51	3.67	3.70
A-6	3.90	3.80	3.78
D. barbiturates, hypnotic act.			
barbital	3.09	3.28[h]	3.01[i]
allobarbital	3.54	3.46	3.35
phenobarbital	3.32	3.64	3.39
metharbital	3.12	3.54	3.43
aprobarbital	3.60	3.51	3.51
butabarbital	3.63	3.65	3.69
butethal	3.72	3.75	3.73
butalbital	3.63	3.65	3.76
hexobarbital	4.37	3.87	3.88
amobarbital	3.75	3.89	4.01
pentobarbital	4.05	3.89	4.03
secobarbital	4.20	3.98	4.19
thiopental	3.98	4.38	4.21
thiamylal	4.15	4.47	4.32
methohexital	4.74	4.00	4.38
E. barbiturates, inhibn of egg division			
barbital	1.49	1.33[j]	1.29[k]
allobarbital	1.79	1.80	1.86
phenobarbital	2.02	2.23	1.93
aprobarbital	2.01	1.92	2.12
cyclobarbital	2.24	2.75	2.45
butethal	2.40	2.51	2.49
amobarbital	2.82	2.86	2.95
pentobarbital	2.92	2.86	2.98
secobarbital	3.62	3.10	3.25

[a] Calculated using eq 8. [b] Calculated using eq 4. [c] Calculated using eq 10. [d] Calculated using eq 6. [e] Calculated using eq 16. [f] Calculated using eq 15. [g] Found to be an agonist. [h] Calculated using eq 19. [i] Calculated using eq 18. [j] Calculated using eq 21. [k] Calculated using eq 20.

biturates), only the antiinflammatory activity of the anthranilic acids were found to show a higher correlation with log P than with the retention index. One possible explanation for this observation may be that the precision of the retention index measurements is greater than the precision of the log P measurements. The standard deviations of the propranolol log P values (Table I) were very small and were of the same order as the errors for the retention index measurements (typically ±10 units). The propranolol analogues in Table I are listed in order of increasing retention index, and it can be seen that, although there is a general correlation between these two measures of lipophilicity, the ordering noted for the log P values is statistically different in the majority of the cases. Thus, it would appear that the superiority of the retention index measurements in the biological activity correlations is not simply the result of a higher precision in the measurements. With the limited data at hand, it is not possible to generalize this observation to other series of compounds at this time, but there are reasons to believe that the retention index model may be generally superior to the log P model.

In most bilayer biomembrane systems, there are ordered arrays of hydrocarbon side chains facing an aqueous interface on one side and facing a polar core structure in the center. In the C-18 reverse-phase high-pressure LC columns, the alkyl side chains are probably ordered in similar arrays facing an aqueous phase on one side and facing a polar silica surface on the other. There are also free silanol sites on the high-pressure LC column that could participate in hydrogen bonding as might be found in the core structure of bilayer biomembranes or in the core structure of enzyme sites. The octanol–water model also measures the lipophilicity of the drug, and the alcohol group also takes into account the hydrogen-bonding interactions, but there is no ordering of the alkyl chains. In the octanol–water model the drug is transferred from one bulk phase to the other, while in the high-pressure LC system the transfer is from a bulk aqueous phase to what is essentially a monolayer system.

If the observations found in the present study are found to be general, the efforts that have been directed at making the high-pressure LC more "octanol-like", or using other techniques to give high-pressure LC measurements that more closely follow log P measurements, may have been misguided. That is, it is quite possible that as one develops high-pressure LC systems that show better correlations with the classical log P model, one may actually decrease the correlation between the high-pressure LC model and biological activity. The findings of the present study are consistent with those of Henry et al.,[6] who found that biological activity showed better correlation with retention times obtained with Corasil C-18 columns than with octanol-treated Corasil columns.

One of the other advantages of the retention index system over the classical shake-flask measurements is that only very small quantitities of the drug are required. If melting point capillary tubes are used for containers, a total of 100 μg of the drug can be dissolved in a few microliters of solvent from which many replicate determinations can be made.

When ultraviolet spectroscopy is used to make the classical log P measurements, small impurities with strong chromophones can produce large errors in the measurements. In contrast, the samples used for the retention index measurements do *not* need to be of ultrahigh purity. One needs only to be able to correctly identify the drug peak in the chromatogram.

When drugs with either very high or very low lipophilicities are examined, great care must be taken when the classical method is used. Frequently, either the

aqueous or organic phase may become saturated with the drug and can produce large errors in the log P measurements. Also, very lipophilic drugs may form micelles or microemulsions which would also produce errors with very high or very low lipophilicities that can be measured very easily, and because the quantitities of the drug used are extremely small, micelles or microemulsions present no problems.

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Antidotes to Organophosphate Poisoning. 2.[1] Thiadiazole-5-carboxaldoximes

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Three new nonquaternary oximes have been evaluated with respect to their antidotal activities against organophosphate poisoning. The oximes 1,2,3-thiadiazole-5-carboxaldoxime (1, pK_a = 7.6), 2-methyl-1,3,4-thiadiazole-5-carboxaldoxime (**2**, pK_a = 8.4), and 3-methyl-1,2,4-thiadiazole-5-carboxaldoxime (**3**, pK_a = 7.0) were prepared in yields of 56, 24, and 58%, respectively, by nitrosation of the corresponding methyl derivatives with isoamyl nitrite in the presence of potassium ethoxide. The new compounds are substantially more lipophilic and much less toxic than the well-known antidote P2S. They reactivate phosphylated AChE slowly. In the five animal species investigated, they disappear from the blood stream after iv administration at approximately the same rates as Obidoxime. It was shown that the disappearance of 1 from rabbit blood is not due to rapid renal excretion of the unchanged oxime, as is the case for Obidoxime and P2S. Since an unidentified derivative of 1 has been found in the urine, rapid metabolic conversion of 1 is most likely responsible. Therapeutic administration (ip) of **2–3** at 250 mg/kg, in combination with atropine, saves mice from four to five times the LD_{50} of sarin. Much lower doses (33–75 mg/kg) of 1 save rats and mice from five times the LD_{50} of sarin, whereas P2S cannot save mice from the same challenge dose. In contrast herewith, 1 does not save rats from five times the LD_{50} of paraoxon, whereas P2S is effective in this case. For oral prophylaxis against sarin, 1 is inferior to P2S. This inferiority may be partly ascribed to the combined effects of rapid absorption of 1 from the gastrointestinal tract and rapid elimination.

The oximes 2-[(hydroxyimino)methyl]-1-methylpyridinium methanesulfonate (P2S) and 1,1′-[oxybis(methylene)]bis[4-[(hydroxyimino)methyl]pyridinium dichloride (Obidoxime) are valuable antidotes in organophosphate poisoning.[2,3] Their therapeutic usefulness depends primarily on their abilities to reactivate the phosphylated[4] enzyme acetylcholinesterase (AChE), thereby restoring the normal transmission of nerve impulses.

In a previous paper,[1] we reported the synthesis and therapeutic activity of quaternary [(hydroxyimino)methyl]-2-methylisothiazolium salts, which are isosteric with the aforementioned pyridinium compounds. Unexpectedly, we also found that the nonquaternary isothiazole-5-carboxaldoxime reactivates phosphylated AChE, in spite of its rather high pK_a value (8.6). Moreover, in combination with atropine this oxime saved rats given several times the LD_{50} of isopropyl methylphosphonofluoridate (sarin). These results encouraged us to investigate related thiadiazole-5-carboxaldoximes. The introduction of an extra nitrogen atom into the ring of isothiazole-5-carboxaldoxime was expected to lower the pK_a toward the range 7.5–8.0, which is considered to be optimal for reactivation of inhibited AChE at physiological pH.[5,6] The presence of two nitrogen atoms and one sulfur atom in a five-membered (unsaturated) ring can lead to four different thiadiazole molecules, three of which were synthetically accessible. One oxime derivative of each of the three isomeric ring systems was synthesized for pilot studies of their various (physico) chemical and biological properties.

Results and Discussion

Synthesis and pK_a Values. The new oximes 1,2,3-thiadiazole-5-carboxaldoxime (**1**) and 2-methyl-1,3,4-thiadiazole-5-carboxaldoxime (**2**) were prepared in yields of 56 and 24%, respectively, by the same method as used by Goerdeler and Hammen[7] for 3-methyl-1,2,4-thiadia-

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