

New Antiarrhythmic Agents. 6. Quantitative Structure-Activity Relationships of Aminoxyliidides

Paul A. Tenthorey,* Alan J. Block,[†] Robert A. Ronfeld,
Astra Research Laboratories, Framingham, Massachusetts 01701

Paul D. McMaster,
College of the Holy Cross, Worcester, Massachusetts 01610

and Eugene W. Byrnes

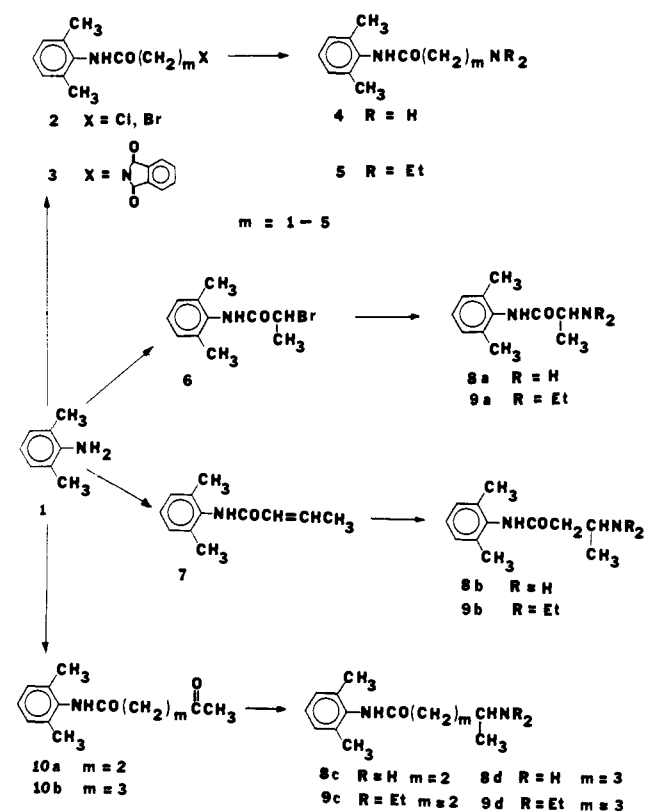
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The synthesis and pharmacological evaluation of primary and tertiary aminoxyliidides with the amino group in the 2-7 position of the acyl chain are described. 2,6-Xylidine was acylated with haloacyl halides and converted to the target compounds by direct amination or by the Gabriel procedure. Alternatively, 2,6-xylidine was coupled with keto acids, and the ketoxyliidides were converted to the amines by reductive amination. The target compounds were evaluated in mice both for antiarrhythmic efficacy against chloroform-induced tachycardia and for central nervous system toxicity. Experimentally determined values of partition coefficients and pK_a values were used for quantitative structure-activity analyses. While the antiarrhythmic activity could be described as a function of $\log P$ alone, the CNS toxicity was best described as a function of both $\log P$ and pK_a . The results suggest that antiarrhythmic potency can be increased by increasing lipophilicity, while the therapeutic index can be improved by increasing the pK_a .

In a search for new orally active antiarrhythmic agents with pharmacodynamic properties similar to those of lidocaine (5a), we found that most primary amine analogues of lidocaine have antiarrhythmic activity in animals.^{1,2} One of those amines, tocainide (8a), was selected for clinical trials³ on the basis of its antiarrhythmic efficacy,^{1,4} long half-life,⁵ and high oral bioavailability.⁶ We also found that many primary β -amine⁷ analogues of lidocaine (5a) have antiarrhythmic properties like the corresponding α -amines but generally appear to be less toxic to the central nervous system (CNS). With the objective of obtaining agents with higher selectivity for antiarrhythmic over CNS effects, we synthesized a series of primary and tertiary aminoxyliidides (Table I) with increasing length of the intermediate chain between amide and amine functions. Increasing the chain length was expected to increase the basicity due to decreasing electron withdrawal on the amine by the amide. Such increases in basicity, we hoped, would lead to reduced CNS toxicity without adversely affecting the antiarrhythmic activity, as we had observed when α - and β -amines were compared. In order to gain insight into the effect of basicity and lipophilicity on biological properties, the target structures were chosen so that the results would be amenable to a quantitative structure-activity relationship (QSAR) analysis, i.e., partition coefficients and pK_a values independent. QSAR analysis of both antiarrhythmic and CNS activities was expected to point out areas of high selectivity and provide leads to further synthesis. We report here the synthesis, biological evaluation, and QSAR analysis of a series of primary and tertiary amine analogues of lidocaine and tocainide.

Chemical Methods. Synthetic routes to the target amines are shown in Scheme I. 2,6-Xylidine (1) was acylated with ω -haloacyl halide in a mixture of acetic acid and sodium acetate⁸ to give the corresponding ω -haloacyl-2,6-xylidides (2, $m = 1-5$). Treatment of 2 with potassium phthalimide⁹ in dimethylformamide and subsequent hydrazinolysis⁹ produced the primary amines 4. The tertiary amines 5 were prepared by treating 2 with diethylamine in acetonitrile, anhydrous ethanol, or benzene.

Scheme I



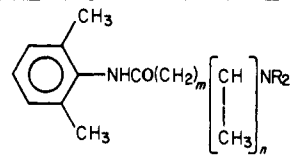
Amines with branched intermediate chains (8 and 9) were prepared in two ways. Direct amination of 2-bromo-

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* Address correspondence to: Bristol-Meyers Co., New York, NY 10022.

[†] EG & G Mason Research Institute, Worcester, MA 01608.

Table I. Structures, Physiochemical Properties, and Biological Effects of Aminoxylylides



no.	m	n	R	pK _a	log P	ED ₅₀ , ^a mmol/kg		therapeutic index ^b	p[BH ⁺ ₅₀] ^d
						protection against tachycardia	ataxia		
4a	1	0	H	7.42	0.45	0.84 (0.58-1.21)	0.62 (0.40-0.84)	0.7	0.37
4b	2	0	H	8.75	0.62	0.72 (0.59-1.04)	2.30 (1.05-4.44)	3.2	0.16
4c	3	0	H	9.35	0.71	0.80 (0.43-1.50)	3.25 (2.81-3.80)	4.1	0.10
4d	4	0	H	9.81	1.02	0.31 (0.23-1.12)	1.81 (1.46-2.24)	5.8	0.51
4e	5	0	H	10.05	1.29	0.27 (0.06-1.16) ^c	2.07 (1.71-2.51)	7.7	0.57
5a	1	0	Et	7.63	2.36	0.21 (0.19-0.23)	0.18 (0.17-0.20)	0.9	0.88
5b	2	0	Et	8.83	2.21	0.13 (0.08-0.18)	0.21 (0.15-0.26)	1.6	0.90
5c	3	0	Et	9.52	2.06	0.14 (0.06-0.26)	0.76 (0.50-1.17)	5.4	0.86
5d	4	0	Et	9.96	2.42	0.12 (0.08-0.17)	0.66 (0.49-1.28)	5.5	0.92
5e	5	0	Et	10.24	2.95	0.13 (0.04-0.24)	0.67 (0.50-0.95)	5.2	0.89
5f	6	0	Et	10.24	3.48	0.04 (0.01-0.68)	0.83 (0.46-1.51) ^c	21	1.40
8a	0	1	H	7.54	0.76	0.55 (0.43-0.66)	0.76 (0.63-0.89)	1.4	0.50
8b	1	1	H	8.71	0.84	0.25 (0.08-0.81)	1.64 (1.02-2.63) ^c	6.6	0.62
8c	2	1	H	9.36	1.08	0.36 (0.20-0.69)	2.66 (2.31-2.90)	7.4	0.45
8d	3	1	H	9.79	1.41	0.19 (0.00-0.41)	1.17 (0.88-1.39)	6.2	0.72
9a	0	1	Et	7.93	2.73	0.17 (0.10-0.26)	0.13 (0.07-0.20)	0.8	0.88
9b	1	1	Et	9.03	2.63	0.05 (0.01-0.14)	0.09 (0.05-0.13)	1.8	1.31
9c	2	1	Et	9.87	2.66	0.21 (0.09-0.38)	1.15 (0.88-2.68)	5.5	0.68
9d	3	1	Et	10.10	2.64	0.10 (0.06-0.17)	0.79 (0.51-0.97)	7.9	1.00
11	0	1	Me	7.33	1.53	0.25 (0.15-0.39)	0.20 (0.14-0.25)	0.8	0.94

^a Measured in mice; 95% Fieller limits in parentheses. ^b ED₅₀ (ataxia)/ED₅₀ (protection). ^c Approximate 95% Fieller limits. ^d -Log [fraction ionized × ED₅₀ (protection)].

propionoxylylide (6) or crotono-2,6-xylylide (7) led to the lower homologues 8a,b and 9a,b. The oxoacylylides 10 were synthesized by coupling levulinic acid or 5-oxohexanoic acid with 1 by the dicyclohexylcarbodiimide method.¹⁰ Reductive amination¹¹ of 10 with sodium cyanoborohydride led to the higher homologues 8c,d and 9c,d. Two additional target compounds (5f and 11) were synthesized in order to test the predictive validity of the QSAR equations. Their structures are listed in Table I; the synthetic methods correspond to those utilized in the preparation of 5, viz., amination of the corresponding haloacylylides with the appropriate secondary amines.

Several of the target compounds have been described previously (see Chemical Results). These compounds were synthesized by the published routes, except for 4e¹² for

which 6-phthalimidohexanoyl chloride was treated with 1 followed by hydrazinolysis of the Gabriel intermediate.

Pharmacological Methods. The antiarrhythmic activity and acute CNS toxicity of the target compounds were determined as described under Experimental Section. The antiarrhythmic activity was determined in mice according to modification of the method by Lawson.¹³ The modifications arose in part from recent evidence from this laboratory¹⁴ indicating that fibrillation due to chloroform inhalation might not occur in this species. Thus, prevention of fibrillation could no longer be considered a valid criterion of efficacy. Instead, efficacy was based upon reduced incidence of ventricular tachycardia. The revised procedure had been validated with currently available standard antiarrhythmic agents, which evoked dose-dependent efficacy and has been used previously in these laboratories.¹⁵

Results

Chemistry. The structures and the physiochemical parameters of the target compounds are reported in Table I, together with their pharmacological properties. Several of the primary amines have been reported by us previously (4b,⁷ 8a,¹ and 8b⁷), but an alternate and more efficient synthesis of 8a is reported here. Several of the target compounds have been prepared previously by others (4a,¹⁶ 4e,¹¹ 5b,¹⁷ 5c,¹⁸ 9a,¹⁷ 9b¹⁹).

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Table II. Correlation Matrix

	protection ^a	ataxia ^b	ther index ^c	log P	(log P) ²	pK _a
protection	1.00	0.61	0.14	0.85	0.80	0.40
ataxia	0.61	1.00	-0.69	0.64	0.65	-0.41
ther index	0.14	-0.69	1.00	0.03	0.08	0.88
log P	0.85	0.64	-0.03	1.00	0.99	0.31
(log P) ²	0.80	0.65	-0.08	0.99	1.00	0.26
pK _a	0.40	-0.41	0.88	0.31	0.26	1.00

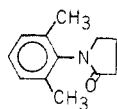
^a Protection against chloroform-induced tachyarrhythmia, as $-\log \text{ED}_{50}$ (protection). ^b $-\log \text{ED}_{50}$ (ataxia). ^c Log [ED₅₀ (ataxia)/ED₅₀ (protection)].

Table III. QSAR Analysis of Antiarrhythmic and CNS Toxic Effects^a in Mice

effect	eq	$Y = a_0 + a_1(\log P) + a_2(\log P)^2 + a_3(\text{pK}_a)$					<i>r</i> ^b	<i>s</i> ^c
		<i>a</i> ₀	<i>a</i> ₁	<i>a</i> ₂	<i>a</i> ₃			
protection, ^d $Y = -\log \text{ED}_{50}$	1	0.08 (±0.10) ^e	0.33 (±0.05)				0.85	0.03
	2	-0.66 (±0.75)			0.14 (±0.08)		0.40	0.10
	3	-0.35 (±0.20)	0.99 (±0.30)	-0.19 (±0.09)			0.89 ^f	0.03
	4	-0.38 (±0.43)	0.31 (±0.05)		0.05 (±0.05)		0.87 ^g	0.03
	5	-0.54 (±0.40)	0.93 (±0.32)	-0.18 (±0.09)	0.03 (±0.05)		0.90 ^h	0.03 ^h
ataxia, $Y = -\log \text{ED}_{50}$	6	-0.46 (±0.19)	0.34 (±0.10)				0.64	0.13
	7	1.97 (±1.02)			-0.20 (±0.11)		0.41	0.19
	8	-0.27 (±0.47)	0.04 (±0.69)	0.09 (±0.20)			0.65	0.14
	9	2.37 (±0.48)	0.45 (±0.06)		-0.33 (±0.05)		0.91 ⁱ	0.04
	10	2.27 (±0.50)	0.83 (±0.40)	-0.11 (±0.11)	-0.35 (±0.06)		0.92 ^k	0.04
ther index, ^l $Y = \log$ (ther index)	11	0.54 (±0.20)	-0.01 (±0.10)				0.03	0.14
	12	-2.62 (±0.43)			0.35 (±0.05)		0.88	0.03
	13	-0.07 (±0.47)	0.95 (±0.67)	0.28 (±0.20)			0.35	0.13
	14	-2.75 (±0.33)	-0.14 (±0.04)		0.38 (±0.04)		0.94 ^m	0.02
	15	-2.81 (±0.34)	0.10 (±0.27)	-0.07 (±0.08)	0.37 (±0.04)		0.94	0.02

^a Based on results with compounds 4a-e, 5a-e, 8a-d, and 9a-d (*n* = 18). ^b Correlation coefficient. ^c Variance of the error. ^d Antiarrhythmic effect, viz., protection against chloroform-induced tachyarrhythmia. ^e Standard error estimated coefficient. ^f Significant improvement over eq 1, *p* = 0.05. ^g Not significantly different from eq 1. ^h Not significantly different from eq 3 and 4. ⁱ Significant improvement over eq 6, *p* = 0.001. ^k Not significantly different from eq 9. ^l Therapeutic index = ED₅₀ (ataxia)/ED₅₀ (protection). ^m Significant improvement over eq 12, *p* = 0.005.

The scope of the synthetic procedures used by Löfgren^{8,17} for the preparation of α- and β-aminoacyl-xylidides has been extended in our laboratories to the synthesis of analogues with longer intermediate chains. Tertiary as well as primary amines (the latter via the corresponding phthalimides) were prepared in good yields, except for 3c and 5c (yields of 44 and 31%, respectively). These two syntheses produced appreciable amounts of 1-(2,6-xylyl)-2-pyrrolidinone (12), which was formed by cyclization of the starting material 2c either thermally or in basic media. The pyrrolidinone 12 was isolated by distillation and characterized by NMR, IR, and UV spectroscopy.



12

The halo acids necessary for the synthesis of 8c,d and 9c,d were not available, so an alternate synthetic sequence was developed. This sequence, viz., coupling of 2,6-xylidine with either levulinic acid or 5-oxohexanoic acid and subsequent reductive amination, was convenient but gave poor yields of the intermediate ketones 10a and 10b due to the

competing formation of *N*-acylureas. Attempts to improve these yields using different solvents, concentrations, or *N*-hydroxybenzotriazole²⁰ were unsuccessful. The reductive aminations of 10a and 10b to the primary amines 8c and 8d were successful, although both required long reaction times. The tertiary amines 9c and 9d could also be prepared by reductive amination in methanol, but reaction times increased to 20 and 17 days, respectively. These times decreased to 24 h in refluxing acetonitrile, giving about the same yield of crude base. The major side reaction in the preparation of 9d was the reduction of the ketone (10b) to 5-hydroxyhexano-2',6'-xylidide (13).

The partition coefficients (*P*) required for the QSAR analyses were determined experimentally rather than by calculation.²¹ A plot of log *P* vs. the molecular weight (Figure 1) strongly suggested that the total number of methylene groups was the major factor influencing the partition coefficient. A least-square regression was run on compounds with *m* + *n* > 2 and is also shown. Compounds with *m* + *n* ≤ 2 (4a,b, 5a,b, 8a,b, 9a,b, and 11) were found to be more lipophilic than expected on the basis of their molecular weights. Lumley-Jones²² analyzed IR data of lidocaine base (5a) and suggested that it exists in a five-membered, intramolecularly hydrogen-bonded conformation in carbon tetrachloride. Since the hydrophilic groups are thus turned in toward the center of the molecule, increased lipophilicity might be expected. Waraskiewicz et al.^{18d} studied 5a-c by IR and NMR spectroscopy; they confirmed Lumley-Jones' conclusion for 5a and proposed

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Table IV. Calculated Activities Using the Preferred Equation

no.	protection ^a		ataxia ^a		therapeutic index	
	measured	calcd ^b	measured	calcd ^c	measured	calcd ^d
4a	0.84	0.87	0.62	0.78	0.7	1.1
4b	0.72	0.64	2.30	1.81	3.2	3.4
4c	0.80	0.55	3.25	2.61	4.1	5.7
4d	0.31	0.35	1.81	2.69	5.8	7.7
4e	0.27	0.25	2.07	2.45	7.7	8.8
5a	0.21	0.13	0.18	0.13	0.9	0.7
5b	0.13	0.13	0.21	0.37	1.6	2.2
5c	0.14	0.14	0.76	0.74	5.4	4.3
5d	0.12	0.13	0.66	0.72	5.5	5.6
5e	0.13	0.13	0.67	0.51	5.2	6.1
8a	0.55	0.51	0.76	0.62	1.4	1.1
8b	0.25	0.45	1.64	1.40	6.6	3.1
8c	0.36	0.32	2.66	1.79	7.4	5.1
8d	0.19	0.22	1.17	1.78	6.2	6.7
9a	0.17	0.13	0.13	0.11	0.8	0.8
9b	0.05	0.12	0.09	0.28	1.8	2.3
9c	0.21	0.13	1.15	0.52	5.5	4.8
9d	0.10	0.12	0.79	0.63	7.9	5.9

^a Expressed as ED₅₀, in mmol/kg. ^b Using eq 3. ^c Using eq 9. ^d Using eq 14.

Table V. Predicted and Measured Antiarrhythmic and Toxic Effects for Compounds 5f and 11

no.	protection ^a			ataxia ^a			therapeutic index		
	measured	predicted		measured	predicted		measured	predicted	
		eq 3	eq 1		eq 9	eq 10		eq 14	eq 17
5f	0.04	0.19	0.06	0.83	0.30	0.57	20.8	5.1	11.4
11	0.25	0.20	0.27	0.20	0.24	0.19	0.8	0.7	0.6

^a Expressed as ED₅₀, in mmol/kg.

an associated *cis*-amide conformation for 5b and a non-associated *trans*-amide conformation for 5c. Our results in Figure 1 suggest that α - and β -amines may be conformationally different from the longer chain analogues 4c–e, 5c–f, 8c,d, and 9c,d. Studies are under way to evaluate this hypothesis.

Pharmacology. The results of QSAR analyses of antiarrhythmic and CNS effects of compounds 4a–e, 5a–e, 8a–d, and 9a–d are listed in Tables II and III. In Table II are listed the correlation coefficients among all of the physicochemical parameters and the pharmacological variables. The physicochemical parameters log *P* and p*K*_a had a sufficiently low correlation to be considered independent variables. Among the pharmacological properties, protection (as –log ED₅₀) correlated well with log *P* but not so well with p*K*_a; ataxia (as –log ED₅₀) correlated directly with log *P* but inversely with p*K*_a; the therapeutic index [as log [ED₅₀ (ataxia)/ED₅₀ (protection)]] correlated very well with p*K*_a but not with log *P*.

In Table III are listed the mathematical coefficients for the equations relating the pharmacological properties to physicochemical parameters, along with the correlation coefficient and the variance of the error for each equation. Protection was described satisfactorily by an equation linear in log *P* (eq 1), but the use of an equation quadratic in log *P* (eq 3) resulted in a significant increase in the correlation coefficient. Attempts to improve the correlation by addition of a term for p*K*_a (eq 2, 4, and 5) were not fruitful. By way of contrast, ataxia could not be described by an equation linear in either log *P* or p*K*_a alone (eq 6 and 7, respectively) nor by an equation quadratic in log *P* (eq 8); rather, a good correlation was found for an equation linear in both log *P* and p*K*_a (eq 9). Equation 10, linear in p*K*_a and quadratic in log *P*, did not give a significantly better correlation than eq 9. Although the therapeutic index was described well by an equation linear in p*K*_a (eq 12), the addition of a term for log *P* gave a

significantly better correlation (eq 14). Thus, eq 3, 9, and 14 were the simplest equations that gave the best correlations of the pharmacological properties with the physicochemical parameters. Table IV tabulates the measured and calculated values for protection, ataxia, and therapeutic index using eq 3, 9, and 14, respectively.

As a test of the predictive value of these equations, two additional substances, 5f and 11, were investigated. 5f is the next homologue in its series; 11 is the dimethyl analogue related to 8a and 9a. The test results and the values predicted by the equations are listed in Table V. The antiarrhythmic potency of 11 was predicted reasonably well by eq 3, whereas that of 5f was not. The log *P* value for 11 fell within the limits of the QSAR analyses but that of 5f was higher than any used in the analyses. Apparently, the negative term for (log *P*)² in eq 3 caused a significant underestimation of the potency of 5f. By way of contrast, eq 1, linear in log *P*, predicted both values quite well. The predictions of CNS toxicity based on eq 9 were reasonably good, but eq 10, which had one more term, gave better predictions of the toxicity. When all 20 compounds were included in the regression analysis, eq 16, with coefficients

$$-\log \text{ED}_{50} (\text{ataxia}) = 2.22 (\pm 0.44) + 0.94 (\pm 0.25) \log P - 0.15 (\pm 0.07) (\log P)^2 - 0.35 (\pm 0.05) \text{p}K_a \quad (16)$$

$$r = 0.92; s = 0.04$$

very close to those of eq 10, was obtained. Equation 14 predicted the therapeutic index of 11 (low p*K*_a) quite well but underpredicted the therapeutic index of 5f (high p*K*_a and lipophilicity). This was probably a consequence of inaccuracies in predicting potency and toxicity. Statistical analysis of all 20 compounds led to the satisfactory eq 17.

$$\log (\text{therap index}) = -2.65 (\pm 0.40) - 0.50 (\pm 0.22) \log P + 0.11 (\pm 0.06) (\log P)^2 + 0.40 (\pm 0.04) \text{p}K_a \quad (17)$$

$$r = 0.93; s = 0.03$$

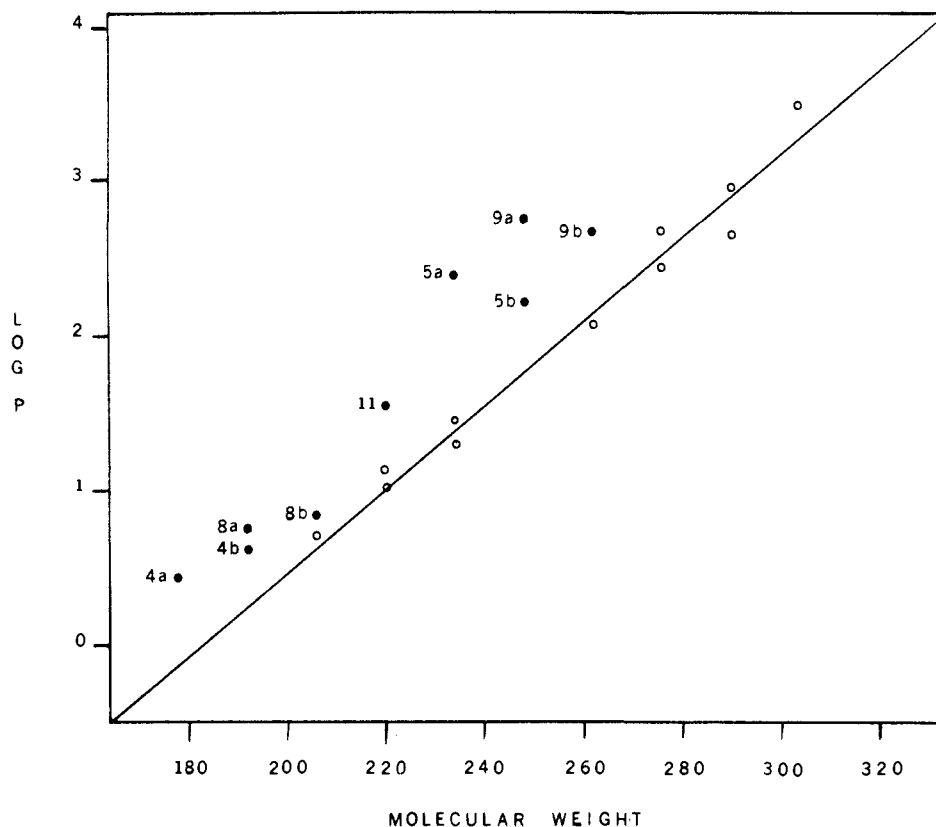


Figure 1. Log P vs. molecular weight: (●) $m + n \leq 2$; (○) $m + n > 2$. Regression only with $m + n > 2$, giving equation $\log P = 0.027(\text{MW}) - 4.86$, $r = 0.99$.

Discussion

Several recent articles have dealt with QSAR analyses of antiarrhythmic effects. Hellenbrecht et al.²³ studied the effects of a series of β -blocking agents on the conduction velocity in frog hearts (antiarrhythmic effect) and on the action-potential amplitude in frog sciatic nerve (local anesthetic effect). Both effects were found to be highly correlated with partition coefficients. Subsequently, Hellenbrecht et al. reported²⁴ on other β blockers in the same models and found that equations linear in $\log P$ or, depending on the incubation period, quadratic in $\log P$ described the antiarrhythmic properties quantitatively. Close examination of their experimental method for determining partition coefficients revealed that their P (which we will call P') corresponded to the distribution coefficient (D) as defined by Scherrer and Howard,²⁵

$$D = \frac{[\text{B}]_{\text{org}}}{([\text{HB}^+]_{\text{aq}} + [\text{B}]_{\text{aq}})}$$

rather than to the usual definition²⁶

$$P = \frac{[\text{B}]_{\text{org}}}{[\text{B}]_{\text{aq}}}$$

where $[\text{B}]$ is the concentration of the nonionized form and $[\text{HB}^+]$ is that of the protonated form. Our own investigations have shown a poor correlation ($r = 0.41$) between antiarrhythmic effect and $\log D$. Nevertheless, Hellenbrecht's findings are similar to ours. Since the $\text{p}K_a$ values differ very little within their set of compounds, the $\log P$ and $\log P'$ values are directly proportional. As a result, their finding that the antiarrhythmic effect correlates highly with $\log P'$ means that the antiarrhythmic effect

would also correlate with $\log P$.

Rauls and Baker²⁷ have investigated the effects of a series of β -blocking agents on the maximum driving frequency and the contractility of isolated rabbit atria. Using the same definition of distribution coefficient as Hellenbrecht et al., they reported QSAR equations linear in $\log P'$ or quadratic in $\log P'$. For one set of compounds, in which the amine substituent was kept the same and, thus, $\text{p}K_a$ values would be expected to be the same, antiarrhythmic activity correlated well ($r = 0.869$) with $\log P'$. Here again, since $\log P'$ and $\log P$ are linearly related, the correlation should also be high with $\log P$. In a second set of compounds, the amine substituent was varied, giving rise to variations in the $\text{p}K_a$ values. In this case, the correlation coefficients were considerably lower; $r = 0.626$ for a linear equation and $r = 0.787$ for a quadratic equation. Perhaps this is an indication that $\log P$ would be a more appropriate variable than $\log P'$.

Ehrhardt et al.²⁸ have presented antiarrhythmic and local anesthetic data obtained with 2,6-dichloro analogues of lidocaine with variation of the substituents on the amine nitrogen and on the para position. Antiarrhythmic effect was expressed as a 25% reduction of the difference between the maximum driving rate and the spontaneous rate of guinea pig atria. The effect was well described by an equation linear in $\text{p}K_a$ ($r = 0.973$). These results contrast markedly with our own, in which we found a low correlation (eq 2; $r = 0.40$) with $\text{p}K_a$. This difference may be a consequence of differences in the species (mouse vs. guinea pig), the type of the arrhythmia (catechol induced vs. electrically induced), or the testing procedure (in vivo vs. in vitro).

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In addition, the pK_a range of their test compounds (4.8–8.0) was different from ours (7.4–10.2). Assuming a constant overall concentration of drug and a physiological pH of 7.4, one can calculate that the concentration of the ionized form varies by 2.5 log units and the concentration of the nonionized form by 0.7 log unit for their compounds. Any pK_a change thus leads to a dramatic change of the concentration of the ionized species. Ehrhardt et al. found the antiarrhythmic activity to be highly correlated with the pK_a and concluded that the ionized form was the active one. For our series of compounds, the concentration of the ionized form varied by only 0.3 log unit, whereas the concentration of the nonionized form varied by 2.5 log units under the conditions described above. Our investigation did not show a correlation of antiarrhythmic effect with pK_a , and we therefore conclude that the nonionized form is not of importance in overall activity, a complementary finding to Ehrhardt's. However, CNS toxicity was correlated to pK_a , possibly indicating the importance of the concentration of the nonionized form in passing the blood-brain barrier.

Ehrhardt et al.²⁸ derived a term $\log [BH^+]$, which was the negative logarithm of the concentration of the ionized form at the effective concentration. They found that this term remained effectively constant over the range of compounds tested. This constancy was not observed for the conjugate base. From this they concluded that the conjugate acid was the active form. We calculated a similar term ($p[BH^+_{50}]$, Table I) using our ED_{50} values and the fraction ionized at pH 7.4. We found that the $p[BH^+_{50}]$ values varied only over 1.2 log units, whereas $\log [B_{50}]$ varied over 3.5 log units. This may indicate that the role of the pK_a is only to increase the amount of BH^+ at the active site and that pK_a becomes self-limiting as the fraction ionized approaches 1. For example, in the series 5a–e, the pK_a changed dramatically (7.63–10.24). However, $p[BH^+_{50}]$ remained constant throughout the series and activity became constant when the pK_a was greater than 8.8 or when the fraction ionized was approximately 1.0. Note that $\log P$ was also relatively constant throughout this series. When the pK_a was constant (e.g., 4b, 5b, and 8b or 5e and 5f), both $p[BH^+_{50}]$ and activity were solely dependent on lipophilicity. This can be further demonstrated by regression of compounds a and b in each series ($N = 8$), which results in an equation of activity dependent on both $\log P$ and pK_a . These eight compounds were chosen since the fraction ionized varied from 0.5 to 0.98. However, the pK_a dependence became insignificant with regression of all 18 compounds, since the fraction ionized was 1.0 for 80% of the compounds. Thus, for our series of compounds, the critical factor controlling relative activity was lipophilicity.

In our investigations, relatively simple equations using $\log P$ or pK_a adequately described both antiarrhythmic activity and CNS toxicity. The use of only two independent variables minimizes the probability of chance correlations²⁹ within a small group of compounds. In consideration of the relatively narrow range of potencies (differing by a factor of 17 for antiarrhythmic effect and by a factor of 36 for CNS toxicity), correlation coefficients of 0.89 to 0.94 were deemed satisfactory. Moreover, the good correlations gave an indication of the reasonable precision of the biological results.

Conclusions

As stated in the introduction, the objective of this study was to find leads to new agents with higher selectivity for

antiarrhythmic effect over CNS toxicity. We expected that QSAR analysis would provide these leads by producing descriptions of the influence of lipophilicity and basicity on both effects. Clearly, guidelines useful in the design of more selective aminoxylic antiarrhythmics have been obtained: potent antiarrhythmic compounds can be produced by increasing lipophilicity, and compounds of low CNS toxicity can be produced by increasing pK_a . In order to understand the significance of these results, the following limitations have to be considered: (1) the results apply to the "chloroform–mouse" model only, (2) other signs of toxicity (e.g., conduction disturbances in the heart) have not been assessed in this study but would add further constraints, and (3) pharmacokinetic data have not been gathered for these compounds but constitute an essential consideration in the design of orally effective drugs. Further investigations with other animal models, studies of the antiarrhythmic efficacy, the toxicity, and the pharmacokinetic properties, would be required to establish the usefulness of these compounds. Nevertheless, we consider these leads to be important for the design of new antiarrhythmic drugs, since the chloroform–mouse model is widely accepted, and its use as a primary screen has already led to agents of interest, e.g., tocainide and flecainide.³⁰

Experimental Section

Melting points (uncorrected) were determined on a Thomas–Hoover Mel-Temp apparatus. The microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and Galbraith Laboratories, Inc., Knoxville, TN. Where analyses are indicated by symbols of elements, the analytical results were within $\pm 0.4\%$ of the theoretical values. Partition coefficients and pK_a values were determined by Dr. Douglas R. Flanagan, University of Iowa, College of Pharmacy, Iowa City, IA. All new compounds were characterized by IR spectra (Perkin-Elmer Model 257 spectrophotometer). Target compounds were additionally characterized by elemental analyses, NMR spectra (Hitachi Perkin-Elmer Model R-20 or Perkin-Elmer Model R24B), and in many cases by mass spectra (Finnigan 10 15 D quadrupole GC–MS). All spectra were in accord with the assigned structures. Progress of reactions and purity of products were determined by gas chromatography (Varian 1200, OV-101 1.5%; Varian 200, OV-17 3%; or JXR 3%) or by high-pressure liquid chromatography (Waters μ Bondapak C_{18} column, LDC Spectromonitor III UV detector, 205 nm; solvent, mixtures of MeCN and 0.05 M $NaClO_4$, pH 4).

4-Chlorobutyro-2',6'-xylylidide (2c). A solution of 156.4 g (1.29 mol) of 1 in 1100 mL of HOAc was cooled to 0 °C. 4-Chlorobutyryl chloride (200.0 g, 1.42 mol) and then a solution of 428.0 g (3.15 mol) of NaOAc·3H₂O in 1780 mL of H₂O were added, and the mixture was shaken for 30 min. The precipitate was filtered off, washed with water (6 × 1 L), and dried to give 213 g (73%) of 2c, mp 100.5–101.5 °C, homogeneous according to HPLC analysis.

4-Phthalimidobutyro-2',6'-xylylidide (3c). A mixture of 10.0 g (0.044 mol) of 2c, 9.0 g (0.049 mol) of potassium phthalimide, and 50 mL of DMF was heated under reflux for 2 h. After the mixture cooled 20 mL of HOAc and 50 mL of H₂O were added, and the precipitate was filtered and washed with 3 M NaOH and H₂O. The precipitate was dried to give 6.5 g (44%) of 3c, mp 211–213 °C, pure according to HPLC analysis. The filtrate contained a considerable amount of 1-(2,6-xylyl)-2-pyrrolidinone (12) and some phthalimide.

4-Aminobutyro-2',6'-xylylidide (4c). 3c (14.6 g, 0.043 mol) was heated in 100 mL of 95% EtOH, 5 mL of 85% N₂H₄·H₂O was added, and the mixture was heated under reflux for 75 min. Concentrated HCl (10 mL) was added and the heating was continued for 1 h. After the mixture cooled, the precipitate was filtered off, washed with 25 mL of EtOH, and dried to give 7.0 g (99%) of phthalhydrazide. The filtrate was concentrated to

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dryness and the residue was dissolved in 50 mL of H₂O. Aqueous NaOH (15%, 25 mL) was added, the solution was saturated with NaCl, and **4c** was extracted with CH₂Cl₂. After the organic phase was dried, ethereal HCl solution was added, and the precipitate was filtered and dried to yield 5.7 g of **4c**·HCl (mp 242–243 °C). Recrystallization from anhydrous EtOH/Et₂O gave 5.6 g of pure **4c**·HCl (53%), mp 243–243.5 °C. Anal. (C₁₂H₁₈N₂O·HCl) C, H, N, Cl.

5-Chloropentano-2',6'-xylylidide (2d). A solution of 100.0 g (0.65 mol) of 5-chloropentanoyl chloride in 365 mL of anhydrous Et₂O was cooled to 0 °C. A solution of 156.6 g (1.29 mol) of **1** in 437 mL of Et₂O was added dropwise with stirring. After the solution was stirred for 1 h, the precipitate was filtered off and washed with Et₂O and 6 N HCl. The precipitate was dissolved in CH₂Cl₂ and dried. Removal of solvent gave 130.6 g (84%) of **2d**, mp 86–86.5 °C.

5-Phthalimidopentano-2',6'-xylylidide (3d). A mixture of 56.9 g (0.24 mol) of **2d**, 44.9 g (0.24 mol) of potassium phthalimide, and 500 mL of DMF was heated under reflux for 7 h. After the mixture cooled, 285 mL of 4.5 M HOAc was added, and the mixture was stirred for 30 min. The precipitate was filtered off, washed with H₂O, and dried, yielding 66.0 g (79%) of **3d**, mp 174–174.5 °C.

5-Aminopentano-2',6'-xylylidide (4d). **3d** (66.0 g, 0.20 mol) was dissolved in 500 mL of 95% EtOH, 16.2 mL of N₂H₄·H₂O was added, and the solution was heated under reflux for 2 h. Concentrated HCl (25 mL) was added, and the mixture was heated under reflux for 1 h. After the mixture cooled, the precipitated phthalhydrazide was filtered off and washed with EtOH. The solvent was evaporated, the residue was treated with hot EtOH, and the insoluble N₂H₄·2HCl was filtered off. Evaporation of solvent gave 40.7 g (80%) of slightly impure **4d**·HCl. Recrystallization from EtOH/Et₂O gave an analytical sample, mp 198–199 °C. Anal. (C₁₃H₂₀N₂O·HCl) C, H, N, Cl.

6-Bromohexano-2',6'-xylylidide (2e). A solution of 109.0 g (0.51 mol) of 6-bromohexanoyl chloride in 288 mL of anhydrous Et₂O was cooled to 0 °C. A solution of 123.9 g (1.02 mol) of **1** in 346 mL of Et₂O was added dropwise with stirring and cooling. Thereafter, the mixture was allowed to warm to room temperature. The precipitate was filtered off, washed with Et₂O, slurried with 0.3 N HCl, filtered off, and dried, giving 140.4 g (92%) of **2e**, mp 158.5–159.5 °C.

6-Phthalimidohexano-2',6'-xylylidide (3e). A mixture of 10.0 g (0.034 mol) of **2e**, 6.9 g (0.037 mol) of potassium phthalimide and 50 mL of DMF was heated under reflux for 2 h. After the mixture cooled, 42 mL of 4.3 M HOAc was added and the mixture was heated under reflux for 30 min. After the mixture cooled, the precipitate was filtered off, washed with water, and dried, giving 11.4 g (93%) of **3e**, mp 153.5–154.5 °C.

6-Aminohexano-2',6'-xylylidide (4e). N₂H₄·H₂O (2.7 mL) was added to a solution of 11.4 g (0.031 mol) of **3e** in 100 mL of hot EtOH, and the mixture was stirred and heated under reflux for 2 h. After the mixture cooled, 5 mL of concentrated HCl was added, and the mixture was heated under reflux for 30 min. It was cooled to room temperature, the precipitated phthalhydrazide was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in H₂O, the solution was treated with NaOH, and the desired material was extracted with CHCl₃. The CHCl₃ was evaporated, and the hydrochloride was prepared by addition of aqueous HCl. After evaporation of the solvent, the residue was recrystallized from 95% EtOH/EtCOMe to give 6.1 g (73%) of **4e**·HCl·0.5H₂O, mp 149.5–150.5 °C. Anal. (C₁₄H₂₂N₂O·HCl·0.5H₂O) C, H, N, Cl.

4-(Diethylamino)butyro-2',6'-xylylidide (5c). A mixture of 10.0 g (0.044 mol) of **2c** and 8.5 g (0.16 mol) of Et₂NH in 12 mL of PhMe was heated under reflux for 2 h. After the mixture cooled, precipitated Et₂NH·HCl (4.8 g after drying, 99%) was filtered off, and the solvent was evaporated. The dark oily residue was taken up in 1 M HCl and extracted with Et₂O. The aqueous phase was brought to pH 5.5 and again extracted with Et₂O. After the Et₂O phases were dried and evaporated, 1-(2,6-xylyl)-2-pyrrolidinone (**12**) was obtained (0.4 g in the first extraction and 4.7 g in the second, representing a yield of 60%; the second ether phase contained a trace of **1**). The remaining aqueous phase was made alkaline with NaOH (pH 11) and extracted with Et₂O. The Et₂O was dried and evaporated to give 3.6 g (31%) of oily **5c**,

homogeneous on GLC (3% OV 17). An analytical sample was obtained by adding HCl gas to an Et₂O solution of **5c**, filtering, treating the hydrochloride with charcoal, and recrystallizing from anhydrous EtOH: mp 137.8–138.5 °C. Anal. (C₁₆H₂₆N₂O·HCl) C, H, N, Cl.

5-(Diethylamino)pentano-2',6'-xylylidide (5d). A mixture of 152.0 g (0.64 mol) of **2d**, 272.8 g (3.74 mol) of Et₂NH, 190.0 g (1.27 mol) of NaI, and 1915 mL of anhydrous EtOH was heated under reflux for 48 h. After the mixture cooled, the precipitated NaCl was filtered off and washed with anhydrous EtOH, and the combined filtrates were evaporated. The residue was treated with 1.5 L of 1 M HCl, 300 mL of CHCl₃, and 400 mL of Et₂O, and the insoluble material was filtered off and washed with 2 M HCl. The aqueous phase of the filtrate was brought to pH 11 with 7 M NaOH and extracted with three portions of Et₂O. After the organic phase was dried and evaporated, 150.0 g (86%) of yellowish, crude **5d** was obtained, which contained a small amount of **1** according to GLC analysis. To remove **1**, 143.0 g of crude **5d** was dissolved in Et₂O and extracted with four portions of H₂O at pH 7.6–8.2. The aqueous phases were brought to pH 11 and the free base was extracted with ClCH=CH₂ to give 136.4 g (82%) of oily **5d**, homogenous according to GLC analysis. Analytical samples were obtained by (1) recrystallization of the tartrate salt from EtOH/Et₂O, conversion to the free base, and recrystallization from petroleum ether [mp 76.5–77 °C. Anal. (C₁₇H₂₈N₂O) C, H, N] or (2) recrystallization of the hydrochloride salt from *i*-PrOH/*i*-Pr₂O [mp 105–106 °C. Anal. (C₁₇H₂₈N₂O·HCl) C, H, N, Cl].

6-(Diethylamino)hexano-2',6'-xylylidide (5e). A mixture of 30.0 g (0.10 mol) of **2e**, 44.3 g (0.61 mol) of Et₂NH, 30.3 g (0.20 mol) of NaI, and 305 mL of anhydrous EtOH was refluxed for 4 h and allowed to cool to room temperature. The precipitate was filtered off and washed with EtOH, and the combined filtrates were evaporated. Since the residue was not completely soluble in a mixture of 1 M HCl and Et₂O, 7 M NaOH was added to bring the pH to 11 and the free base was extracted with Et₂O to give 29.6 g (>100%) of a brown oil. It was taken up in Et₂O and extracted with 1 M HCl. The aqueous phase was brought to pH 11 with NaOH and extracted with Et₂O to give 23.6 g of crude **5e** (81%). Recrystallization of the oxalate salt and reconversion to the free base produced pure **5e**, mp 49–50 °C. Anal. (C₁₈H₃₀N₂O) C, H, N.

7-Bromoheptano-2',6'-xylylidide (2f). A solution of 21.8 g (0.96 mol) of 7-bromoheptanoyl chloride in 54 mL anhydrous Et₂O was cooled to 0 °C. A solution of 23.3 g (0.19 mol) **1** in 65 mL of Et₂O was added dropwise with stirring and cooling over 1 h. After standing at room temperature over the weekend, the mixture was filtered and the precipitate was dried, giving 40.7 g (90%) of colorless **2f** together with some 1·HCl. The crystals were suspended in 18 mL of 6 M HCl, stirred for 2 h, filtered, and washed with water. After drying, 26.0 g (87%) of **2f** was obtained, mp 111.5–112.5 °C, pure according to HPLC analysis.

7-(Diethylamino)heptano-2',6'-xylylidide (5f). A mixture of 8.5 g (0.027 mol) of **2f**, 12.0 g (0.16 mol) of Et₂NH, and 83 mL of anhydrous EtOH was heated under reflux for 7.5 h. EtOH and excess Et₂NH were evaporated, and the residue was treated with 1 M HCl and filtered. The filtrate was made alkaline with 7 M NaOH and extracted with Et₂O (3 × 50 mL). After drying and evaporating, 7.0 g of amber oil was obtained. Attempts to recrystallize the hydrochloride, hexamate, and tartrate salts failed. Recrystallization of the oxalate from anhydrous EtOH/Et₂O gave 4.7 g (44%) of **5f**·H₂C₂O₄, mp 109.5–110.5 °C. For biological testing, the material was converted to the base **5f**. Anal. (C₁₉H₃₂N₂O) C, H, N. Alternatively, purification of a similar batch by preparative HPLC (silica gel, C₆H₁₄/EtOAc, 5:3, with 1% Et₂NH) yielded 59% of **5f**, as base, pure according to HPLC analysis.

2-Phthalimidopropiono-2',6'-xylylidide (15). A mixture of 19.8 g (0.077 mol) of **6**, 15.8 g (0.085 mol) of potassium phthalimide, and 70 mL of DMF was heated under reflux for 2 h. After the mixture cooled, 97 mL of 4.2 M HOAc was added, and the mixture was warmed and stirred for 1 h. The solid was filtered, washed with water, and dried, giving 22.2 g (89%) of **15**, mp 202–203.5 °C.

2-Aminopropiono-2',6'-xylylidide (8a). N₂H₄·H₂O (1.0 mL, 85%) was added to a hot mixture of 2.0 g (0.006 mol) of **15** and

50 mL of 95% EtOH. Concentrated HCl (3.5 mL) was added, and the mixture was heated under reflux for 30 min. After the mixture cooled, the precipitated phthalhydrazide was filtered, and the filtrate was evaporated. The residue was treated with 50 mL of H₂O, filtered, and washed with CH₂Cl₂. The aqueous phase was brought to pH 11 with 7 M NaOH, the base was salted out with K₂CO₃, and the amine was extracted with CH₂Cl₂. Treatment of the organic extracts with HCl gas gave 1.1 g (78%) of 8a·HCl, mp 247–248 °C (lit.¹ 246–247 °C).

4-Oxopentano-2',6'-xylylide (10a). Dicyclohexylcarbodiimide (61.8 g, 0.30 mol) was added in portions to a solution of 31.2 g (0.27 mol) of levulinic acid and 36.3 g (0.30 mol) of 1 in 100 mL of CH₂Cl₂ while cooling and stirring. After 3.5 h, precipitated dicyclohexylurea (43.5 g, 72%, mp 229–230 °C) was filtered off, and the filtrate was washed with 1 M HCl, 0.05 M NaOH, and H₂O. After drying and evaporation of CH₂Cl₂, 76.2 (>100%) of red oil was obtained. Addition of 30 mL of EtOAc and then 140 mL of petroleum ether led to the separation of an oil, which crystallized upon scratching. The crystals (21.9 g, mp 92–92.5 °C) were filtered off and recrystallized from EtOAc/petroleum ether to give 17.6 g (30%) of 10a, mp 94–95 °C.

4-Aminopentano-2',6'-xylylide (8c). A solution of 7.9 g (0.036 mol) of 10a, 28.5 g (0.37 mol) of NH₄OAc, and 1.7 g (0.027 mol) of NaBH₃CN in 125 mL was stirred for 24 h at room temperature. An additional 1.7 g of NaBH₃CN was added, and the stirring was continued for 3 days. The mixture was filtered, and the solvent was evaporated. The residue was taken up in Et₂O and H₂O (100 mL each) and the pH was adjusted to 1 with HCl. After filtration, the aqueous phase was separated, and the water was evaporated. The residue was treated with anhydrous EtOH, and the insoluble material was filtered off. The solvent was evaporated, MeCN was added to induce crystallization, and the resulting crystals were filtered off, yielding 5.6 g (61%) of crude 8c·HCl, mp 176–179 °C, containing traces of impurities according to HPLC analysis. An analytical sample was prepared by recrystallization from EtOH/Et₂O, mp 182.5–184 °C. Anal. (C₁₃H₂₀N₂O·HCl) C, H, N, Cl.

5-Oxohexano-2',6'-xylylide (10b). A solution of 24.7 g (0.19 mol) of 5-oxohexanoic acid and 25.4 g (0.21 mol) of 1 in 100 mL of CH₂Cl₂ was cooled to 5 °C. With cooling and stirring of the solution, 43.2 g (0.21 mol) of molten dicyclohexylcarbodiimide was added. The mixture was stirred for 2 h, and allowed to stand at 25 °C overnight. The precipitate was filtered off (24.5 g of dicyclohexylurea, 58%, mp 230–232 °C), and the filtrate was washed with 1 M HCl, 0.5 M NaOH, and water. Drying and evaporating the CH₂Cl₂ gave 58.7 g (>100%) of a yellow oil, which was treated with EtOAc/petroleum ether to give 17.5 g (40%) of 10b, mp 103–105 °C. An analytical sample was prepared by recrystallization from EtOAc, mp 104–106 °C. Anal. (C₁₄H₁₉NO₂) C, H, N.

5-Aminohexano-2',6'-xylylide (8d). A solution of 10.0 g (0.043 mol) of 10b, 35.0 g (0.46 mol) of NH₄OAc, and 2.0 g (0.032 mol) of NaBH₃CN in 500 mL of MeOH was stirred for 2 days at room temperature. The solution was brought to pH 2 by addition of concentrated HCl, a colorless precipitate was filtered off, and the solvent was evaporated. The residue was dissolved in water, the solution was washed with Et₂O and CH₂Cl₂, and the aqueous phase was filtered through Celite. After the addition of 7 M NaOH (to pH 11), the base was extracted with CH₂Cl₂ to yield 8.9 g (88%) of a colorless oil. It was dissolved in 40 mL of 1 M HCl and filtered, and the water was evaporated. The residue was recrystallized from anhydrous EtOH/Et₂O, yielding 6.0 g (52%) of 8d·HCl, mp 175–177 °C. An analytical sample was prepared by recrystallization from EtOH/Et₂O and MeCN, mp 178–179 °C. Anal. (C₁₄H₂₂N₂O·HCl) C, H, N, Cl.

4-(Diethylamino)pentano-2',6'-xylylide (9c). A solution of 14.0 g (0.064 mol) of 10a, 18.7 g (0.26 mol) of Et₂NH, 14.2 g (0.13 mol) of Et₂NH·HCl, and 2.9 g (0.046 mol) of NaBH₃CN in 250 mL of MeCN was heated under reflux for 25 h. The solvent was evaporated, 2 M HCl was added, and the aqueous phase was extracted with Et₂O and CH₂Cl₂. The aqueous phase was made alkaline (7 M NaOH), to pH 11, and the product was extracted with Et₂O, yielding 9.2 g (53%) of crude 9c. The preparative HPLC of the base and recrystallization of the cyclamate salt gave an analytical sample of 9c·C₆H₁₃NSO₃, mp 130–131 °C. Anal. (C₁₇H₂₈N₂O·C₆H₁₃NSO₃) C, H, N, S.

5-(Diethylamino)hexano-2',6'-xylylide (9d). 10b (12.8 g, 0.055 mol), Et₂NH (16.5 g, 0.23 mol), Et₂NH·HCl (12.5 g, 0.12 mol), and NaBH₃CN (2.5 g, 0.04 mol) were suspended in 250 mL of MeCN. The mixture was heated under reflux for 24 h. HPLC analysis (μ Bondapak C₁₈ column) indicated no further change on continued heating. The solvent was evaporated, the residue was dissolved in 2 M HCl, and the aqueous phase was extracted with 2 × 50 mL of Et₂O and 2 × 50 mL of CH₂Cl₂. The combined organic phases were washed with 2 × 10 mL of 2 M HCl and dried (Na₂SO₄), and the solvent was evaporated to give 5-hydroxyhexano-2',6'-xylylide (13; 2.9 g, 23%) of greater than 95% purity by HPLC. The combined aqueous phases were made alkaline with 7 N NaOH, extracted with 3 × 100 mL of Et₂O, and dried (K₂CO₃), and the solvent was evaporated, yielding 9d (11.0 g, 69%) as a yellow oil which solidified on standing (purity >95% by HPLC). Attempts to recrystallize the hydrochloride salt failed. Preparative HPLC (silica gel) of the base, recrystallization of the cyclamate salt from EtOH/Et₂O, conversion to the free base, and recrystallization from MeCN gave pure 9d, mp 73–74 °C. Anal. (C₁₈H₃₀N₂O) C, H, N.

5-Hydroxyhexano-2',6'-xylylide (13). 10b (0.57 g, 0.024 mol) and NaBH₄ (0.15 g, 0.004 mol) were dissolved with stirring in a two-phase system (2:1 Et₂O/H₂O) and stirred continuously for 2 h. The ether phase was separated, and the aqueous phase was brought to pH 2 and extracted with 3 × 10 mL of Et₂O. The combined ether phases were dried (Na₂SO₄) and the solvent was evaporated, giving 13 (0.52 g, 91%) as a white oil which was pure according to HPLC. The oil was dissolved in MeOH and filtered, and the solvent was evaporated to give 13 (0.44 g, 77%), mp 79–82 °C. Recrystallization from EtOAc/C₅H₁₂ gave 0.28 g (49%), mp 82–85 °C with decomposition. Anal. (C₁₄H₂₁NO₂) C, H, N.

Antiarrhythmic Activity and Acute CNS Toxicity. Groups of 10 female mice (Charles River, CD 1) weighing 18–24 g were injected subcutaneously with drug solution delivered in a volume of 0.1 mL/10 g of body weight. After a 20-min period, the mice were placed one at a time in an atmosphere of chloroform. Each mouse was removed when respiratory arrest occurred (about 35–45 s later) and was pinned to a cork board with dissecting needles which also served as electrodes. After a thoracotomy was performed, three different electrocardiograms were recorded until 90 s from the time the mouse had been placed in the chloroform. Leads I and II were obtained via the needle electrodes, and an epicardial electrocardiogram was obtained from a wick electrode placed on the exposed surface of the right ventricle. All electrocardiograms were displayed on a Grass Model 7 pen recorder as well as Tektronix 5100 series oscilloscope. Ventricular rates (beats per minute) were measured directly from the clearest electrocardiogram by counting ventricular depolarizations for several seconds and extrapolating to beats per minute (bpm). A mouse was assumed to have a tachycardia if the 30 to 40 s recording contained at least 5 s in which the ventricular rate exceeded 520 bpm or 2 s in which the rate exceeded 600 bpm. Conversely, a mouse was "protected" from the arrhythmogenic effects of chloroform if these criteria were not fulfilled. Antiarrhythmic efficacy was based upon the percentage of mice "protected" at each dose. Doses were varied by at least 0.15 log unit in an up/down fashion; the intent was to achieve a dose-response curve in which the doses elicited a low, medium, and high incidence of protection. Thereafter, the ED₅₀ for protection was calculated according to the logit Chi square method of Berkson.³¹

Acute CNS toxicity was assessed during the 20-min period prior to exposure to chloroform. Any mouse which displayed either staggered gait, splayed limbs, or hypertonia was assumed to have acute CNS toxicity in the form of ataxia. In general, doses were increased until at least eight animals displayed these symptoms. The ED₅₀ for ataxia was calculated also according to the method of Berkson.³¹

Determination of pK_a and Partition Coefficients. For the pK_a determination a 50-mL aliquot of a 1 nM solution of the compound was titrated with 0.1 N NaOH at 37 °C. The solution was stirred vigorously during the titration in order to ensure rapid equilibration and reproducibility. The stirring was stopped for

(31) Berkson, J. *J. Am. Stat. Assoc.* 1953, 48, 565.

each pH reading. In general, 15 to 20 pH readings were taken for each titration. The pK_a was calculated by the nonlogarithmic method of Benet and Goyan.³²

The partition coefficients (1-octanol/water) of the test compounds were determined by a similar titration method. A 10- to 40-mL aqueous aliquot of the test compound (1 mM) and 4 to 25 mL of 1-octanol was stirred vigorously and titrated with 0.1 N NaOH. From these data an apparent pK_a (pK_a') was calculated. The partition coefficient (P) of the free base was calculated from the pK_a and pK_a' according to the following formula:³³

$$P = \frac{V_w}{V_0} (10^{pK_a' - pK_a} - 1)$$

where V_w = volume of aqueous solution and V_0 = octanol volume.

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QSAR Analysis. The coefficients for the independent parameters in the QSAR equations were arrived at by multiple regression using a standard matrix inversion technique.^{34,35} A sequential F test was used to test for significant improvement when adding another term to the regression equation.³⁶

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Methylangelicins: New Potential Agents for the Photochemotherapy of Psoriasis. Structure-Activity Study on the Dark and Photochemical Interactions with DNA

Francesco Dall'Acqua,* Daniela Vedaldi, Adriano Guiotto, Paolo Rodighiero, Francesco Carlassare, Francarosa Baccichetti, and Franco Bordin

Institute of Pharmaceutical Chemistry of the Padova University, Centro di Studio sulla Chimica del Farmaco e dei Prodotti Biologicamente Attivi del CNR, 35100 Padova, Italy. Received August 20, 1980

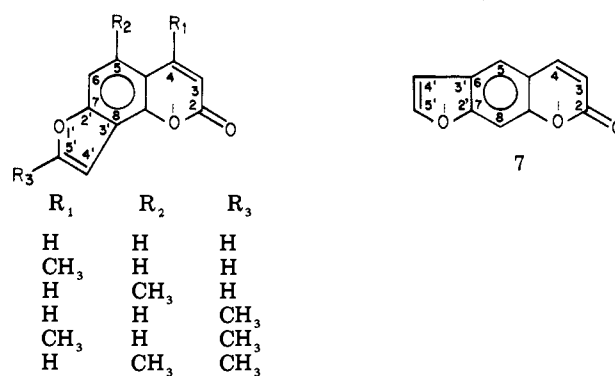
The interactions both in the ground and in the excited state between various methylangelicins, previously prepared with the aim to increase the low photobiological activity of the parent angelicin 1, and DNA have been studied. In general, the new methylangelicins show an increased capacity to photobind monofunctionally to DNA and a parallel increment of photobiological activity in comparison with the parent 1. This increase appears to be connected with various factors, such as the augmented affinity toward DNA for the dark complex formation and the electronic effect connected with the introduction into 1 of one or two methyl groups. The new compounds, on the basis of their photobiological activity and their lack of skin phototoxicity, appear as possible agents for the photochemistry of skin diseases characterized by cell hyperproliferation.

Photochemotherapy of psoriasis and other skin diseases characterized by hyperproliferation of the cutaneous cells is realized by oral or topical administration of psoralens to the patient and successive irradiation (UV-A) of the ill areas of the skin.¹⁻³ Psoralens, linear furocoumarins, are able to induce mono- and bifunctional photodamage to the DNA of the cutaneous cells in a selective way, thus inhibiting DNA functions and, as a consequence, the cell proliferation.¹

The photodamages consist of the products of photocycloaddition between one molecule of psoralen and either one (monoadducts) or two pyrimidine bases of DNA (biadducts);^{4,5} in the latter case, the pyrimidines belong to the two different complementary strands of DNA and their photocycloaddition with psoralens induces the formation of interstrand cross-linkages in the macromolecule.^{5,6}

This therapeutical treatment, however, is accompanied by some undesired side effects, such as skin phototoxicity and risk of skin cancer.^{7,8} In this connection, skin phototoxicity is strictly connected with the bifunctional lesions in DNA;⁹ on the other hand, bifunctional lesions, in connection with the error-prone repair systems involved for their remotion, seem to be the main cause of the risk of

Chart I. Molecular Structures of Angelicin Derivatives 1-6 and Psoralen (7; Reported for Comparison)



skin cancer, while monoadducts, repaired through error-free systems, appear much less involved in this process.^{10,11}

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* Address correspondence to Istituto di Chimica Farmaceutica Università, 35100 Padova via F. Marzolo, 5, Italy.