commenting upon the manuscript. He is very grateful to Mrs. B. Frimark for the preparation of the manuscript.

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Quantum Statistical Calculation for the Correlation of Biological Activity and Chemical Structure. 3. Diisopyramide Phosphate and Related Compounds as Antiarrhythmic Agents

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Diisopyramide phosphate has been recognized as a new antiarrhythmic agent.[1] Our screening program for antiarrhythmic activity has generated a large amount of biological data on compounds having structural variations. We consider the data appropriate for analysis of the structure–activity relationship employing the quantum statistical concepts.[2,3] In doing this, we gain insight into the antiarrhythmic action, in the hope of predicting the drug potency before a potential antiarrhythmic agent is synthesized.

The compounds shown in Table I have the structural variations near the carbonyl group and show a wide variance in the ir absorption peak of the carbonyl group. The wave numbers ranging from as low as 1622 cm^{-1} (compound **10**) up to as high as 1735 cm^{-1} (compound **12**) represent the absorption of the fundamental vibrational transitions ($l = 0 \rightarrow l = 1$, and l is the vibrational quantum number). This difference in wave numbers measures a contribution to the variation in the drug potency.

The partition coefficients of the compounds shown in Table I were experimentally determined in an octanol–pH 7.4 phosphate buffer system[4] and found to be substantially influenced by the chemical structures. However, the partition coefficients alone did not correlate significantly with the drug potency. At this point, the lipophilicity and C=O absorption frequency are only useful correlative variables, and this study does not imply that these variables are the only variables of importance in drug–transfer and drug–receptor interaction.

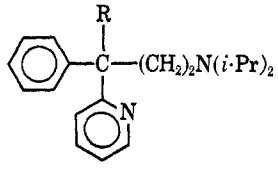
Theoretical Method. The ventricular arrhythmia induced in the unanesthetized dog by ligation of the anterior descending coronary artery was reduced by intravenous treatment with the compounds in Table I. If antiarrhythmic potency (AP) is defined as mean maximal reduction in extrasystoles per mean effective dose, eq 2 of part 1[2] must be slightly modified as

$$\mathrm{AP} = \frac{\text{mean maximal reduction in extrasystoles}}{\text{mean effective dose}} = \frac{\mathrm{d(response)}/\mathrm{d}t}{1000C} = Ap^{a1}(\text{Brownian}) \times p^{a2}(\text{binding}) \quad (1)$$

where C is the molar concentration and A is the proportionality constant, which has the same unit as d(response)/Cdt. Substituting eq 5 of part 1[2] and eq 18 of part 2[3] in eq 1, taking logarithms on both sides, and collecting constants in one term, we obtain

$$\ln \mathrm{AP} = k_1 + k_2\pi - k_3\pi^2 + k_4 \ln [\lambda\xi^{-1}(\partial\xi/\partial\lambda)_T] \quad (2)$$

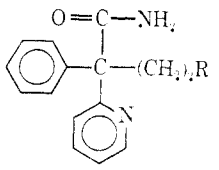
Table I. Data for Observed and Calculated Antiarrhythmic Potency



Compd	R	π	Ir, cm^{-1}, $\nu_{C=O}$	Ln AP[a] Obsd	Ln AP[a] Calcd[b]	Ln AP[a] Calcd[c]
1	-CONMeNMe$_2$	0.30	1640	2.254	2.276	2.276
2	-CONHEt	0.21	1656	1.891	1.936	1.965
3	-CONHNHMe	−0.35	1645	1.711	1.601	1.649
4	-CO$_2$Et	1.11	1733	1.677	1.518	1.603
5	-CONHNHAc	−0.57	1624	1.715	1.738	1.769
6	-COMe	0.75	1712	1.403	1.527	1.604
7	-NHAc	0.05	1679	1.313	1.419	1.492
8	-CONHN=CMe$_2$	0.21	1680	1.374	1.549	1.613
9	-CONH$_2$(H$_3$PO$_4$) (Norpace)	0.00	1682	2.195		1.405
10	-CONHNH$_2$	−0.73	1622	1.407	1.624	1.663
11	-CONHNMe$_2$ (oxalate MeOH)	−0.59	1656	1.514	1.204	1.284
12	-CO$_2$Me	0.61[d]	1735	0.910	1.028	1.148
13	-CONHPh ($H_2SO_4 \cdot 0.5H_2O$)	1.65	1683	2.935	2.818	2.795
14	-CONHMe	−0.31[e]	1661	1.404	1.379	1.449
15	-CONHCH$_2$OH	−0.82	1657	1.085	0.977	1.073

[a] AP = 5 × MW × AR/1000, where MW is the molecular weight and AR is the activity ratio, which is described in a paper by Yen, Lowrie, and Dean (*J. Med. Chem.*, submitted for publication). [b] The values are computed from eq 9. [c] The values are computed from eq 10. [d] This value was estimated by subtracting 0.5 from compound **4**. [e] This value was estimated by subtracting 0.5 from compound **2**.

Table II. Data for Observed Antiarrhythmic Potency



Compd	R	AP
1	$-N(i\text{-}Pr)_2$	8.5
2		5.1
3	$-NH(i\text{-}Pr)$[a]	2.1

[a]For more information about this compound, see A. Karim, R. Ranney, and S. Kraychy, *J. Pharm. Sci.*, **61**, 888 (1972).

The right-hand side of eq 2 is identical with eq 20 of part 2.[3] Although the left-hand side of the two equations represents different biological data, they both indicate relative drug potencies. The difference between the present treatment and early treatments of parts 1 and 2 is that in this case the rate of the biological response is not treated as a constant, because laboratory controls of the constant reduction in extrasystoles are time consuming and difficult.

This study is based on the assumption that the frontier electron density of N in the common group $-N(i\text{-}Pr)_2$ of Table I is unaffected by the substituent in the amide group. Further study (molecular orbital methods) on charge density around N of the tertiary amine will be reported elsewhere, because the change in the electronic structure around that site has produced different potency (see Table II).

Equations 5 and 9 of part 1[2] can be immediately applied here for the case of one-site binding

$$p(\text{binding}) = B\lambda\xi^{-1}(\partial\xi/\partial\lambda)_T = Bq\lambda/(1 + q\lambda) \quad (3)$$

where q is the total site partition functions of >C=O and the receptor site, and λ is the absolute activity which is expressed as $\lambda = \exp(\mu/kT)$ and μ is the chemical potential which is set to zero in this case; hence, λ is equal to 1. Moreover, q is a small number; therefore, $q\lambda \ll 1$ and eq 3 is then approximated as follows

$$p(\text{binding}) = B\lambda\xi^{-1}(\partial\xi/\partial\lambda)_T \simeq B\lambda q \quad (4)$$

Substituting eq 4 in the last term of eq 2, we obtain

$$\ln \text{AP} = k_1' + k_2\pi - k_3\pi^2 + k_4 \ln q \quad (5)$$

Now, employing eq 19 of part 1, we get

$$\ln \text{AP} = k_1' + k_2\pi - k_3\pi^2 + k_4 \ln (aq_{\nu 1}^{r1}q_{\nu 2}^{r2}) \quad (6)$$

where $q_{\nu 1}$ and $q_{\nu 2}$ are respectively the site partition functions of the drug molecule and the receptor. If $k_2 \gg k_3$, the π^2 term can be omitted, and eq 6 can be written as

$$\ln \text{AP} = k_1'' + k_2\pi + k_3' \ln [q_{\nu 1}] \quad (7)$$

where $q_{\nu 2}$, the partition function of the receptor site, is constant and is incorporated into the constant term k_1''.

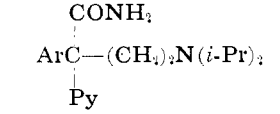
Substituting the following equation

$$q_{\nu 1} = \exp(-hc\nu_{C=O}/2kT)$$

in eq 7, and changing the notation of the constants, we have

$$\ln \text{AP} = C_1 + C_2\pi - C_3\nu_{C=O} \quad (8)$$

Table III. Data for Observed Antiarrhythmic Activity



Ar	Py	π	Ir, cm^{-1}, $\nu_{C=O}$	Acon,[a] obsd
C_6H_5	3-Py	0.443	1689	4.8
C_6H_5	4-Py	0.172	1701	1.9
$4\text{-}FC_6H_5$	2-Py	0.210	1689	2.2
$4\text{-}ClC_6H_5$	2-Py	0.730	1683	6.0

[a]The test animal was an anesthetized dog, in which atrial flutter was induced by application of aconitine nitrate to the atrium. The figures show the relative potency.

Equation 8 is the final equation which is specifically derived for the correlation of the ventricular antiarrhythmic activity of the compounds listed in Table I. The constants C_1, C_2, and C_3 of eq 8 are obtained by a multiple regression.

Results and Discussion

The AP, π values, and ir spectroscopic data were experimentally observed in our laboratories. These data are shown in Table I and analyzed statistically by multiple regression. The resulting equations are

excluding diisopyramide phosphate

	n	r	s	
$\ln \text{AP} = 1.576 + 0.352\ \pi$	14	0.51	0.42	
$\ln \text{AP} = 28.440 + 0.915\ \pi - 16.121 \times 10^{-3}\nu_{C=O}$	14	0.96	0.14	(9)

including diisopyramide phosphate

$\ln \text{AP} = 1.618 + 0.343\ \pi$	15	0.48	0.43	
$\ln \text{AP} = 26.044 + 0.851\ \pi - 14.649 \times 10^{-3}\nu_{C=O}$	15	0.86	0.25	(10)

where n is the number of compounds used for the analysis, r is the correlation coefficients, and s is the standard deviation. It is seen that high values of π and low values of $\nu_{C=O}$ are the essential elements for a high AP according to either eq 9 or 10.

Looking only at the π values in Table I, it is easy to make a wrong judgment about the AP of compound **4**. In fact, this very favorable π value is cancelled by the very unfavorable $\nu_{C=O}$ value, and its antiarrhythmic activity is a moderate one. On the other hand, looking only at $\nu_{C=O}$ values, it is easy to be misled about compound **10**, because it has the lowest $\nu_{C=O}$ (1622 cm^{-1}) among the compounds shown, but this favorable fact is cancelled by its unfavorable π value (−0.73), and again its antiarrhythmic activity turns out to be moderate. There are many other similar examples in the tables (*e.g.*, compound **5**). Compounds **1**, **3**, **5**, **10**, and **11** have the substituent R = CON(X)N(Y)Z and have low $\nu_{C=O}$, which is a favorable factor. However, except for compound **1**, their π values are negative, and their overall antiarrhythmic effects are moderate. We also observed that those compounds with R = COX (compounds **4**, **6**, and **12**) have high π values, but this favorable fact was cancelled by the unfavorable $\nu_{C=O}$ values (higher than 1700 cm^{-1}). From the data of this study, a potential antiarrhythmic agent could be discovered, if one could find a particular substituent R with an increased π value and a decreased $\nu_{C=O}$ value.

If one looks at Table III, in which the substituent variations are far away from the carbonyl group and the variations in $\nu_{C=O}$ are minor, compared to the dramatic ones in

Table I, one would find that the observed atrial antiarrhythmic activity follows the trend of the π values.

The inclusion of diisopyramide phosphate in the regression analysis reduces the correlation coefficient (eq 10). Since only this compound has two amide hydrogens, the antiarrhythmic activity may depend heavily on this singular structural feature. Further study of the high-resolution spectra of the N-H vibration is desirable.

In conclusion, the development of this quantitative structure-activity relationship based on quantum statistical mechanics probably provides a tractable approach for complicated problems.

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Quantitative Structure-Activity Analysis in a Series of Antimycotically Active *N*-Hydroxypyridones

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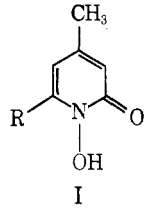
In view of the fact that several derivatives of the general type I in the series of *N*-hydroxypyridones synthesized by Lohaus[1,2] had been found to possess antimycotic activity, we decided to find out what relationship existed between substitution of the basic structure and chemotherapeutic activity. It was quite clear that an interrelation had to exist, for the graduated activity of the individual derivatives was certainly greater than the experimental error that arose in determining the activity.



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This series of congeners lends itself to an exact structure-activity analysis, since from the chemical viewpoint the variation of the basic structure took place uniformly only in one place and, on the other hand, because a good animal model was available providing quantitative data with satisfying reproducibility. With the aid of Hansch analysis[3] we correlated the experimental data for the biological response with physicochemical parameters for various substituent influences.

It was the aim of our experiments first to ascertain the factors which are significant for the biological effect as well as their relative importance and, second, to be able to make quantitative predictions as to the activity of new compounds of this series in order to optimize the subsequent synthesis work.

In Vivo **Experiments. (a) General.** We used as biological reference values the diameters of the alopecias (bald spots) which were formed in guinea pigs as the final stage of experimentally induced dermatophytoses (skin disease caused by fungi).[2] The fungal foci, in their early stage, *i.e.*, as from the third day after infection, were treated locally with the compounds under test.

The greater the inhibitory activity of the preparation applied (dissolved in 2-propanol) on the mycotic foci, the smaller the alopecias will be. In order that inhibition is obtained at all, it is necessary for the compound to penetrate the *stratum corneum* of the animal's skin sufficiently rapidly so that it can be active in the skin tissue, before the spread of the fungal mycelium is stopped by the developing immunity.

The experiments have the advantage over other *in vivo* experiments that the drugs on their way to the site of action are not subject to the elimination processes which must otherwise be taken into account, *e.g.*, metabolization and excretion.

(b) **Procedure.** Male albino guinea pigs (Pirbright white) with no history of mycosis, weighing 350–400 g, were cropped to 1 mm on both sides of the back 1 day before infection. Four animals were used as a treatment unit for each preparation. Infection was carried out on both sides of the animals' backs in three skin areas 25 mm distant from one another, using a suspension of about 10^7 microconidia (asexual spores) of the fungus *Microsporum canis*, strain 559, per milliliter of physiological saline. The microconidia were obtained from 4-week-old surface cultures on malt extract–dextrose–peptone agar.

We applied 0.05 ml of the spore suspension to each inoculation point. By revolving a pipette tip (external diameter 3 mm) with slight pressure in the individual 0.05-ml droplets, the site of inoculation was slightly roughened.

After 3 days a slight reddening and scurf formation was observed on all inoculation sites, this being a sure sign of the development of the infection. In a first (Table I) and a second (Table II) series of tests 1 ml of a 0.1% solution of the preparation in 2-propanol was applied once daily to the inoculation sites on the right side, from the third through the seventh day after infection. In a third series (Table I, values are in parentheses) of tests (eq 11–14) 1 ml of a 1% solution of the preparation in 2-propanol was applied once daily from the third through the fifth day after infection. In each test control animals treated with 2-propanol only were used.

The infection sites on the left side were not treated; they served to check whether the infection had in fact taken a regular course in each animal. During the second and third week after infection, encrusted mycotic foci about 20 mm in diameter developed. Toward the end of the third week, on the skin area where the hair roots had been damaged by the penetration of the fungal hypha, the hair and crusts had fallen off and a sharply defined alopecia 15–20 mm in diameter was present on each mycotic site. Three and a half weeks after infection the diameters of the alopecias were measured accurately to 0.5 mm. For noncircular alopecias a mean value was calculated from several diameters.

As each animal had three measurements taken on the right side, $n = 12$ data were obtained for each treated group from which the arithmetical mean was then derived. In order to relate the effect to a molar basis, we divided this diameter in each case by the molecular weight of the respective derivative. These values were designated "reduced" diameters, rD, and $\log 1/rD$ was taken to be the expression of the biological response.

(c) Evaluation by Hansch Analysis. First, we checked various substituent parameters as to their possible significance for the biological activity, using simple linear equa-