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.

Acknowledgment. We thank Drs. G. Cohen, H. Lambert, and H. Lowrie for their continuous encouragement. We also thank Drs. R. Mikulec and A. Soms for their commenting upon the manuscript.

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

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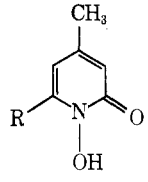
In view of the fact that several derivatives of the general type I in the series of *N*-hydroxypyridones synthesized by Lohaus[1,2] had been found to possess antimycotic activity, we decided to find out what relationship existed between substitution of the basic structure and chemotherapeutic activity. 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.



I

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-

Table I. Substituents and Parameters for Regression Analysis

	Substituent R	Mol wt	π[a]	E_s[b]	σ^*[c]	d.p.[d]	D,[e] mm	Log $1/rD$
1	H	125	0.00	1.24	0.49	0	14.4 (8.7)[f]	0.939 (1.157)[f]
2	CH_3	139	0.50	0.00	0.00	0	12.3 (8.4)	1.053 (1.219)
3	C_2H_5	214	1.00	−0.07	−0.10	1	10.5 (7.4)	1.309 (1.462)
4	i-C_3H_7	228	1.37	−0.47	−0.19	1	11.0 (7.0)	1.317 (1.513)
5	n-C_7H_{15}	223	3.50	−0.40	−0.13	0	8.5 (7.2)	1.419 (1.491)
6	n-$C_{11}H_{23}$	279	5.50	−0.40	−0.09	0	5.4 (4.7)	1.714 (1.775)
7	C_6H_{11}	268	2.39	−0.79	−0.15	1	8.0 (5.5)	1.526 (1.688)
8	$CH_2C_6H_{11}$	221	2.89	−0.98	−0.06	0	8.2 (6.4)	1.431 (1.539)
9	$CH_2CH_2C_6H_{11}$	235	3.39			0	7.8 (6.6)	1.479 (1.551)
10	p-$CH_2C_6H_4Cl$	310.5	3.40			1	7.5 (6.4)	1.618 (1.686)
11	2-Furyl	191	1.60			0	10.1 (7.7)	1.277 (1.395)

[a] See ref 4. [b] See ref 5. [c] See ref 6. [d] Dummy parameter. [e] Diameter of alopecias in millimeters. [f] Data obtained with the 3 × 1% application are shown in parentheses.

Table II. Substituents and Parameters for Regression Analysis

	Substituent R	Mol wt	π	E_s	d.p.	D, mm	Log $1/rD$ Obsd	Log $1/rD$ Calcd[a]
1	H	125	0.00	1.24	0	14.4	0.939	1.010
1a	H	125	0.00	1.24	0	11.6	1.032	1.010
2	CH_3	139	0.50	0.00	0	12.3	1.053	1.077
3	C_2H_5	214	1.00	−0.07	1	10.5	1.309	1.297
3a	C_2H_5	214	1.00	−0.07	1	11.4	1.274	1.297
4	i-C_3H_7	228	1.37	−0.47	1	11.0	1.317	1.346
4a	i-C_3H_7	228	1.37	−0.47	1	9.2	1.394	1.346
5	n-C_7H_{15}	223	3.50	−0.40	0	8.5	9.419	1.474
6	n-$C_{11}H_{23}$	279	5.50	−0.40	0	5.4	1.714	1.739
7	C_6H_{11}	268	2.39	−0.79	1	8.0	1.526	1.481
7a	C_6H_{11}	268	2.39	−0.79	1	9.0	1.474	1.481
8	$CH_2C_6H_{11}$	221	2.89	−0.98	0	8.2	1.431	1.393
8a	$CH_2C_6H_{11}$	221	2.89	−0.98	0	8.1	1.436	1.393
9	$CH_2CH_2C_6H_{11}$	235	3.39		0	7.8	1.479	1.460
10	p-$CH_2C_6H_4Cl$	310.5	3.40		1	7.5	1.618	1.615
10a	p-$CH_2C_6H_4Cl$	310.5	3.40		1	7.6	1.611	1.615
11	2-Furyl	191	1.60		0	10.1	1.277	1.222
12	p-C_6H_4Cl	296.5	2.84		1	9.1	1.513	1.540
13	$CH_2C_6H_5$	276	2.69	−0.69	1	7.8	1.549	1.521
14	p-$C_6H_4CH_3$	276	2.36		1	10.0	1.441	1.477
15	p-$SCH_2C_6H_4Cl$	342.5	3.41		1	9.1	1.576	1.616
16	p-$OCH_2C_6H_4Cl$	326.5	2.75		1	9.1	1.551	1.528

[a] With eq 10.

tions. As the measure of the influences exerted by substituents on the lipohydrophilic behavior of the derivatives we used the parameter π.[4] The parameter E_s[5] served to determine steric influences and the parameter σ^* [6] to determine electronic influences of the substituents.

For the first regression analysis, only those eight derivatives were used for which all three parameters were available. We obtained eq 1-3. Here n denotes the number of

$$\log 1/rD = 0.125\ (\pm 0.024)\ \pi + 1.071 \quad (1)$$
$$n = 8;\ s = 0.113;\ r = 0.907\ (\rho \neq 0 \text{ with } p < 0.01)$$

$$\log 1/rD = -0.280\ (\pm 0.096)\ E_s + 1.273 \quad (2)$$
$$n = 8;\ s = 0.173;\ r = 0.746\ (\rho \neq 0 \text{ with } p < 0.05)$$

$$\log 1/rD = -0.806\ (\pm 0.332)\ \sigma^* + 1.315 \quad (3)$$
$$n = 8;\ s = 0.191;\ r = 0.704\ (\rho \text{ not significantly} \neq 0)$$

derivatives used, s the standard deviation, and r the correlation coefficient. In addition, the 95% confidence interval of the regression coefficient is given and the probability of error p for the hypothesis that the correlation coefficient ρ of the statistical universe differs significantly from zero.

The best correlation is obtained with the lipohydrophilic substituent parameter π. Equation 1 explains 82% of the variance ($r^2 = 0.823$). Equation 2 with the steric parameter E_s is still just significant but explains only 48% of the variance. As shown by eq 3, electronic influences of substituents apparently are of no importance for the activity.

A considerable improvement of the correlation as against eq 1 was obtained when we examined the derivatives separately, depending on whether they were present as free acids (eq 4) or as ethanolamine salts (eq 5). (For this purpose all 11 derivatives were used.) Such a separation had no influence on the correlation with E_s and σ^*, respectively.

$$\log 1/rD = 0.136\ (\pm 0.011)\ \pi + 0.993 \quad (4)$$
$$n = 7;\ s = 0.051;\ r = 0.984\ (\rho \neq 0 \text{ with } p < 0.001)$$

$$\log 1/rD = 0.140\ (\pm 0.019)\ \pi + 1.157 \quad (5)$$
$$n = 4;\ s = 0.036;\ r = 0.982\ (\rho \neq 0 \text{ with } p < 0.05)$$

Comparison of eq 4 and 5 shows that they represent functions of two parallel straight lines. By using a "dummy" parameter, which was set equal to 0 for the free acids and equal to 1 for the ethanolamine salts, both equations can be combined into one.

Equation 6 "explains" 97% of the variance and represents a significant improvement at the 0.999 level compared

$$\log 1/rD = 0.137\ (\pm 0.009)\ \pi + 0.173\ (\pm 0.028)\ \text{d.p.} + 0.991 \quad (6)$$
$$n = 11;\ s = 0.045;\ r = 0.985\ (\rho \neq 0 \text{ with } p < 0.001)$$

with the corresponding equation without dummy parameter ($F_{1,8} = 37.5$; $F_{1,8,\alpha=0.999} = 25.42$).

We then repeated the guinea pig test with six of the former derivatives and five that had not previously been tested. On the one hand we wanted to investigate the reproducibility of the experimental data and, on the other, whether eq 6 was also suitable for predicting the activity of new derivatives.

The reproducibility (see Table II) was found to be good and also the predictive value, as can be seen in Table III from the comparison between calculated and observed log $1/rD$ values.

Of these new substituents, R, only π-benzyl had been referred to in the literature.[7] The remaining π values were obtained as follows. For π(*p*-chlorophenyl) the log *P* value of chlorobenzene was taken from the Hansch tables.[8] π(*p*-tolyl) = π(phenyl) + π(*p*-CH_3) = 1.85 + 0.51 = 2.36. π(*p*-chlorobenzylthio) = π(*p*-chlorophenyl) + π(*p*-SCH_3) − [$\pi(CH_3)$ − $\pi(CH_2)$] = 2.84 + 0.62 − (0.51 − 0.46) = 3.41. π(*p*-chlorobenzyloxy) = π(*p*-chlorophenyl) + π(*p*-OCH_3) − [$\pi(CH_3)$ − $\pi(CH_2)$] = 2.84 − 0.04 − 0.05 = 2.75.

In eq 7 we have combined all the dermatophytosis data of both experiments (Table II) and again subjected them to a Hansch analysis. By separating into free acids and ethanolamine salts, eq 8 and 9 were obtained; and by introduction of the dummy parameter, in analogy to eq 6, it was possible to combine both groups, preserving the good correlation coefficient and the small standard deviation (eq 10).

$$\log 1/rD = 0.134\ (\pm 0.014)\ \pi + 1.097 \quad (7)$$
$$n = 22;\ s = 0.087;\ r = 0.903\ (\rho \neq 0 \text{ with } p < 0.001)$$

$$\log 1/rD = 0.134\ (\pm 0.009)\ \pi + 1.007 \quad (8)$$
$$n = 9;\ s = 0.048;\ r = 0.984\ (\rho \neq 0 \text{ with } p < 0.001)$$

$$\log 1/rD = 0.128\ (\pm 0.010)\ \pi + 1.174 \quad (9)$$
$$n = 13;\ s = 0.031;\ r = 0.967\ (\rho \neq 0 \text{ with } p < 0.001)$$

$$\log 1/rD = 0.133\ (\pm 0.006)\ \pi + 0.154\ (\pm 0.017)\ \text{d.p.} + 1.010 \quad (10)$$
$$n = 22;\ s = 0.038;\ r = 0.983\ (\rho \neq 0 \text{ with } p < 0.001)$$
$$t_1 = 21.27\ (p < 0.001);\ t_2 = 9.31\ (p < 0.001)$$

The additional t test shows that both regression coefficients differ significantly from 0, the probability of error being very small. Equation 10 explains 97% of the variance and compared with eq 7 represents a highly significant improvement at the 0.999 level ($F_{1,19} = 88.65$; $F_{1,19,\alpha=0.999} = 15.08$). With the greater number of derivatives now in hand we investigated whether the importance of the steric parameter E_s, which had been indicated in eq 2, is really significant.

Neither in the group of free acids nor in that of the ethanolamine salts or by combined treatment with the dummy parameter (d.p.) was it possible to achieve an improvement of the correlation by the additional use of E_s. Therefore, it can be concluded that steric influences of the substituent R are of no consequence for the activity in the guinea pig dermatophytosis assay.

The group of 11 derivatives that was investigated first (Table I) was subjected in addition to a test in which a solution containing 1% of the active principle was applied three times (instead of 5 × 0.1%). In this case, too, the same regularity was found to exist. The combined treatment of acids and ethanolamine salts resulted in eq 11.

Table III. Comparison between Calculated and Observed Log $1/rD$ Values

	Substituent R	Mol wt	π	d.p.	*D*, mm	Log $1/rD$ Obsd	Log $1/rD$ Calcd[a]
12	*p*-C_6H_4Cl	296.5	2.84	1	9.1	1.513	1.553
13	$CH_2C_6H_5$	276	2.69	1	7.8	1.549	1.533
14	*p*-$C_6H_4CH_3$	276	2.36	1	10.0	1.441	1.487
15	*p*-$SCH_2C_6H_4Cl$	342.5	3.41	1	9.1	1.576	1.631
16	*p*-$OCH_2C_6H_4Cl$	326.5	2.75	1	9.1	1.551	1.541

[a] With eq 6.

$$\log 1/rD = 0.099\ (\pm 0.021)\ \pi + 1.268 \quad (11)$$
$$n = 11;\ s = 0.107;\ r = 0.843\ (\rho \neq 0 \text{ with } p < 0.01)$$

Separation into free acids and ethanolamine salts gave eq 12 and 13, and these could be combined, by using the dummy parameter, to form eq 14.

$$\log 1/rD = 0.108\ (\pm 0.009)\ \pi + 1.178 \quad (12)$$
$$n = 7;\ s = 0.043;\ r = 0.983\ (\rho \neq 0 \text{ with } p < 0.001)$$

$$\log 1/rD = 0.100\ (\pm 0.030)\ \pi + 1.384 \quad (13)$$
$$n = 4;\ s = 0.056;\ r = 0.921\ (\rho \neq 0 \text{ with } p < 0.1)$$

$$\log 1/rD = 0.107\ (\pm 0.009)\ \pi + 0.188\ (\pm 0.028)\ \text{d.p.} + 1.181 \quad (14)$$
$$n = 11;\ s = 0.044;\ r = 0.978\ (\rho \neq 0 \text{ with } p < 0.001)$$
$$t_1 = 12.233\ (p < 0.001);\ t_2 = 6.713\ (p < 0.001)$$
$$F_{1,8} = 44.0;\ F_{1,8,\alpha=0.999} = 25.42$$

Thus again, by using the dummy parameter (eq 14) the improvement as against the simple correlation with π (eq 11) is significant at the 0.999 level. The additional use of the steric parameter does not bring about a further improvement of the correlation; this finding underlines once more the statement that in this test steric substituent influences are of no importance.

Summary and Discussion

Evaluation of the Hansch analysis shows that *one* parameter (π) is sufficient to define the activity of this series. The activity increases in the same graduation as the lipophilicity of the compounds is raised by the substituent R. It is surprising that this dependence is linear over such an extremely wide range. On comparing the π values of the two extreme substituents, π(H) = 0 and $\pi(C_{11}H_{23})$ = 5.50, it is seen that the $C_{11}H_{23}$ radical increases the lipophilicity by more than the 100,000-fold of substituent H. With *in vivo* experiments the activity normally passes through a maximum with increasing lipophilicity.

Compared with other observed correlations the coefficient of π in our equations (0.08–0.14) seems to be unusually low. However, such a comparison with other examples of a correlation with π would be misleading in our opinion.

Normally log $1/C$ serves as a measure for the biological response with *C* indicating a characteristic *dose*, whereas we have used log $1/rD$ instead with *rD* indicating a characteristic *effect*. The possible scope of the biological effect of course is small, compared with the scope of the dose, and consequently a small coefficient of π must be expected here.

When comparing the coefficients of the dummy parameters in the different equations, the remarkable constancy

is apparent. Whether treatment with 5 × 0.1% solution or 3 × 1% solution is concerned, a coefficient of about 0.17 is obtained. This means that in each of the experiments evaluated, the activity of the ethanolamine salts is about 50% higher than that of the corresponding free acids (the antilogarithm of 0.17 is 1.47).

If the general term "activity" has been used so far, this—strictly speaking—is not quite correct. Defined precisely, one ought to state that the rate-determining step in the mechanism of action of this experiment is controlled exclusively by the parameter π.

However, in this relatively simple animal model only the penetration into the *stratum corneum* can be thought of as the rate-determining step.

We have no satisfactory mechanistic explanation at the moment as to why the activity of a compound can be increased by about 50% if it is used not in its free form but as the ethanolamine salt. A possible explanation would be that there is an influence by the ethanolamine on environmental factors in the area of infection or on the motility of the compounds in the aqueous epidermal layer.

Acknowledgment. We are indebted to Professor von Wasielewski and D. M. Vanderbeke for stimulating discussions of these results.

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Nitrofurans with High Renal Excretion

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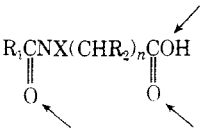
During the last 20 years a considerable number of nitrofurans with high antibacterial activity have been synthesized, but only a few of these are found to be excreted in the urine in an antibacterially active form.[1,2] Nitrofurantoin is one example of a nitrofuran compound with a high urinary excretion. This may partly be ascribed to its tubular excretion.[3,4] Other urinary tract antibacterial agents of sulfonamide[5] and penicillin[6] type are also excreted tubularly. To obtain new urinary tract antibacterial nitrofurans, therefore, it would be advantageous to synthesize nitrofuran compounds which are likely to be excreted in the tubules.

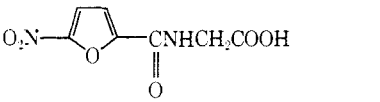
Despopoulos has systematized various compounds having a renal tubular excretion similar to that of 4-aminohippurate.[7] All these compounds are acids. The renal tubular excretion transport of these organic acids is described as a substrate-specific process involving an interaction between a transported anion and a receptor molecule in the renal cell. Despopoulos gave the general structural requirements (structure 1) for compounds with tubular excretion. The two oxygens of the carboxyl group and the oxygen of the carbonyl group were assumed to be essential for the three-point contact with the receptor.



1. X = H, alkyl; n = 1–5

On the basis of Despopoulos's general formula 1, we embarked on the synthesis of the nitrofurans 2–4 containing those groups considered essential for tubular excretion (Chart I and Table I). Since the acids 3 and 4 were synthesized *via* their esters, the latter compounds were included for comparison.

Chart I



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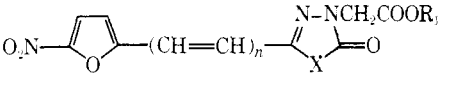

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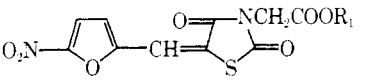

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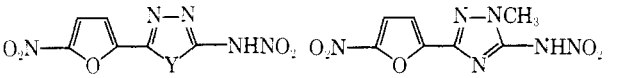

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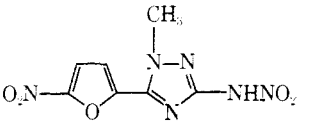

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X = O, S, NCH_3; Y = NH, NCH_3, S; R_1 = H, ethyl

In addition, a series of nitrofurans with a nitroamino group (5–7) was synthesized since we had found earlier that 4-methyl-3-nitroamino-5-(5-nitro-2-furyl)-1,2,4-triazole (5b) is excreted tubularly (Chart I and Table I).[8] The antibacterial activity *in vitro* and the urinary excretion of the new nitrofurans 2–7 were examined.

Synthesis and Chemical Data. The nitrofurans 3 and 4 were synthesized from the corresponding nitrofuran compounds in which the heterocyclic ring had a NH adjacent to the C=O group. The sodium salt was prepared and then allowed to react with ethyl chloroacetate or ethyl bromoacetate. The ester group was then hydrolyzed in concentrated H_2SO_4-formic acid. The nitrofurans 5–7 with a nitroamino group were obtained by nitration of the corresponding amino compounds.[8]

The pK_a values of the acidic nitrofurans were determined by potentiometric titration and are reported in Table I. All the nitrofurans 2–7 were stronger acids than nitrofurantoin and exist at physiological pH (7.2) mainly as anions.

Biological Data. I. Antibacterial Activity *in Vitro.* The minimal inhibitory concentrations (MIC values) of the nitrofurans 2–7 against *Staphylococcus aureus*, *β-haemolytic streptococcus*, and *Escherichia coli* were determined by the twofold serial dilution technique.[9] Furthermore, some of the nitrofurans were tested using the same