Discriminant-Analytical Investigation on the Structural Dependence of Hyperglycemic and Hypoglycemic Activity in a Series of Substituted *o*-Toluenesulfonylthioureas and *o*-Toluenesulfonylureas

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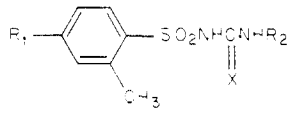
The influence of a series of substituted *o*-toluenesulfonylthioureas and *o*-toluenesulfonylureas on the level of blood sugar was investigated in rats. According to the observed response the compounds were divided into three classes corresponding to hypoglycemic, hyperglycemic, and no activity. The distribution of the compounds over these classes can be described by discriminant functions using substituent constants, R_M values, and indicator variables. Most important for the separation of classes are hydrophobic and/or steric properties as well as the presence or absence of the thioamide group. The results indicate that two different mechanisms of action with opposite effect overlap in the case of the series studied.

It is well known that certain sulfonamides are able to decrease the level of blood sugar.[1-6] In some cases, however, a hyperglycemic activity has also been observed.[7,8] We became interested in the question in which way the direction of the influence on blood sugar concentration depends on the properties of substituents. In order to analyze such substituent effects it seemed suitable to start with sulfonamides of moderate hypoglycemic activity. Therefore, a series of 22 substituted *o*-toluenesulfonylthioureas and *o*-toluenesulfonylureas of general structure



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I was investigated (see Table I); these compounds show a much weaker hypoglycemic activity than the corresponding *p*-methyl derivatives.[9,10]

Three types of effects on blood sugar concentration were observed: (1) increase, (2) no effect, and (3) decrease. Thus, the compounds can be divided into three classes according to their activity, and such a situation can be treated by discriminant analysis, which should yield information regarding relationships between chemical structure and the type of biological effect.[11-14]

Methods and Theory. Blood sugar levels were measured prior and 90 min after intraperitoneal administration of the substances to narcotized rats (six animals in each experiment) in doses of 70 and 100 mg/kg. Determination of blood glucose was performed by means of the Bio-La-Test-Glucose set[15] which is based on the reaction of glucose with *o*-toluidine in acid medium. A Student's *t* test leads to the following classification of the compounds: class 1, significant increase of blood glucose level; class 2, no significant change of blood glucose level; class 3, significant decrease of blood glucose level.

For the two doses used the classification of compounds was practically the same so that it is sufficient to consider one dose only. The classification used in discriminant analysis refers to the dose of 70 mg/kg. Levels of blood sugar before and after application and statistical criterions for classification are presented in Table I.

Discriminant analysis was performed with the help of an R-20 computer using the program of Läuter.[16] This program consists of two parts. In the first part, the parameter space is reduced by multivariate variance analysis in a stepwise (step-up) procedure, in which the most dispensable variables are eliminated using an approximate *F* test based on Hotelling's T^2 as test statistics. The test quantity $\tilde{F}$ in this multivariate *F* test is defined as

$$\tilde{F} = \frac{n - m - p + 1}{(m - 1)p(n - m)} \sum_{j=1}^{m} n_j (x_{j\cdot} - x_{\cdot\cdot}) S^{-1} (x_{j\cdot} - x_{\cdot\cdot}) \quad (1)$$

n = number of compounds in the training series, m = number of classes, p = number of variables, n_j = number of compounds in class j, $x_{j\cdot}$ = vector of class means of the variables in class j, $x_{\cdot\cdot}$ = vector of total means of the variables, and S = covariance matrix averaged over all classes.

The null hypothesis is that all class mean vectors are equal; in that case no separation of classes in the parameter space considered is possible. It is rejected if the test quantity $\tilde{F}$ is greater than the corresponding *F* quantile (g_1,g_2 = degrees of freedom, α = critical probability of error)

$$\tilde{F} > F_{g_1,g_2,\alpha} \quad (2)$$

with

$$g_1 = \frac{(m - 1)p(n - m - p)}{n - (m - 1)p - 2} \text{ if } n - (m - 1)p - 2 > 0$$

$$g_1 = \infty \text{ if } n - (m - 1)p - 2 \leq 0$$

$$g_2 = n - m - p + 1$$

In the variance analysis the variables are added (and removed) step by step, and after each step α is calculated from eq 1 and 2. The variables are selected in such a way that α is minimized, and the resulting set of variables is optimal with respect to the separation of classes in the training series.

Discriminant analysis (second part of the program) is concerned with calculating so-called nonelementary discriminant functions according to eq 3. In this equation,

$$w_{ih} = \sum_{k=1}^{p} a_{ik} x_{kh} \quad (3)$$

w_{ih} is the value of the ith discriminant variable (i = 1, ..., J - 1, if there are J classes) for a substance h, a_{ik} is the weight coefficient of the kth variable in the ith discriminant function, and x_{kh} is the value of the variable k for the substance h. Discriminant functions are linear combinations of those variables appearing in the optimal set. Mathematically, these functions have the highest separating power among all possible discriminant functions.

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The reclassification of each substance of the training series as well as the classification of any nontested substance is performed as shown in eq 4. In this equation,

$$F_{h/j} = \frac{n - 2(J-1)}{(J-1)(n-J)} \frac{n_j}{n_j + 1} \sum_{i=1}^{J-1} (w_{ih} - \bar{w}_{ij})^2 \quad (4)$$

w_{ih} is the value of the ith discriminant variable for the hth substance, $\bar{w}_{ij}$ is the mean value of the ith discriminant variable for the jth class of the training series, n is the number of substances in the training series, n_j is the number of substances belonging to class j, J is the number of classes, and p is the number of variables of the optimal set. A substance h is classified into class j, if

$$\tilde{F}_{h/j} \leq F_{J-1,n-2(J-1);\alpha} \quad (5)$$

where $F_{J-1,n-2(J-1);\alpha}$ is a value resulting from F statistics with $J - 1$ and $n - 2(J - 1)$ as degrees of freedom and α as the known probability of error (mostly 0.05).

Quite frequently there are ranges in which different classes overlap each other. For compounds falling into these ranges no clear classification is possible. It is common practice to classify such compounds into the class with the smallest F value. After all, it should be pointed out that slight distinctions of classification are possible depending on the procedure used for the discriminant analysis.

The substances of the training series and all data used for the calculations are summarized in Table I. Substituent constants were used to describe hydrophobic (π), electronic (σ_p), and steric (molar refraction, MR) effects. Properties of the whole molecule were characterized by R_M values calculated from the results of thin-layer chromatography. Two indicator variables were added. The first (I_1) was assigned a value of "0" for the N-amide and a value of "1" for the N-thioamide group, and the second (I_2) expresses the electronic influence of phenyl substituents ("-1" for electron-releasing substituents, "1" for electron-attracting substituents, and "0" for substituents with negligible electronic influence). For substituents at the nitrogen no electronic parameters were used, since all substituents occurring in this position have nearly the same electronic properties.

Results

(1) Simultaneous Separation of All Classes. Table II summarizes the results of the step-up procedure in multivariate variance analysis. MR_2 is sufficient to separate class 1 from both other classes but not for the separation of classes 2 and 3. In order to separate class 3 from the two other classes addition of the variables I_1 and R_M is necessary; in that case, however, the separation of classes 1 and 2 is lost. The optimal set for the simultaneous separation of all classes includes the variables MR_2, I_1, R_M, and π_1. Again this set does not significantly separate classes 1 and 2 at the 0.05 level. The whole picture indicates that hypo- and hyperglycemic activity is connected with different molecular properties. Hyperglycemic activity depends exclusively on the properties of R_2. It is difficult, however, to draw definite conclusions on the nature of these properties since the variables are interrelated (see Table III). This is especially true for MR_2 and π_2 so that no decision is possible whether hydrophobic or steric effects are of primary importance. The most important variable for hypoglycemic activity is R_M since R_M produces the highest increase of T^2 and the most pronounced decrease in α for the separation of classes 2 and 3. For the substituents in the training series, R_M can be considered a measure of hydrophilicity as shown in eq 6. The contribution of the indicator variable I_1 to the

$$R_M = -0.110(\pi_1 + \pi_2) + 0.502 \quad (6)$$
$$n = 22;\ r = -0.859;\ s = 0.006$$

separation of hypoglycemic from inactive compounds is relatively small but statistically significant so that the nature of the group X in structure I seems to be important.

Only the first of the two nonelementary discriminant functions calculated with the optimal set of variables is statistically significant at the 0.05 level:

$$w_1 = 0.258MR_2 + 2.297I_1 + 14.65R_M + 1.170\pi_1 \quad (7)$$

The results of reclassification are summarized in Table I. Three out of the 22 compounds of the training series are misclassified (error of reclassifications, 13%). As was to be expected these compounds belong to the classes 2 and 3 which are not sharply separated by the optimal set for the simultaneous separation of all classes.

The class means of the nonelementary discriminant variable w_1 are

	$\bar{w}_1$
class 1	8.549
class 2	9.499
class 3	11.93

Considering eq 4 and 5 it follows that high values of MR_2, R_M, and π_1 as well as the presence of the thioamide group instead of the amide group are favorable for hypoglycemic activity. For hyperglycemic activity the reverse is true. The simultaneous occurrence of MR_2 and π_1 together with the hydrophilicity parameter R_M may indicate the existence of an optimum in the hydrophobicity of the whole molecule with respect to hypoglycemic activity. It may also be, however, that this optimum simply reflects the unfavorable influence of large alkoxy groups in position R_1.[21]

Since different molecular properties are connected with hypo- and hyperglycemic activity, all these conclusions must be considered with caution. Obviously, different optimal sets of variables are required to separate blood sugar increasing and blood sugar decreasing from inactive compounds. Thus, the results of the step-up procedure for the simultaneous separation of all classes need not be truly representative for neither the separation of hyperglycemic nor of hypoglycemic from inactive compounds. In order to get a deeper insight the separation of classes 1 and 2 on the one hand and of classes 2 and 3 on the other hand must be treated separately. The results of this treatment will be presented in the following sections.

(2) Separation of Hyperglycemic from Inactive Compounds. In agreement with the results presented in Table II the variable MR_2 is sufficient to separate these two classes ($T^2 = 0.90$; $\alpha = 0.02$). Addition of the indicator variable I_1 increases the critical probability of error ($\alpha = 0.073$) so that the nature of X is not important for hyperglycemic activity. Discriminant analysis yields the following nonelementary discriminant function

$$w = 0.143MR_2 \quad (8)$$

with the class means $\bar{w} = 1.474$ for class 1 and $\bar{w} = 2.740$ for class 2. With this discriminant function, 14 out of the 17 compounds considered are correctly reclassified. Three compounds (see Table IV) are misclassified. This misclassification, however, is equivocal since these compounds fall into the region of overlap between the two classes so that the classification had to be based on the smallest $\tilde{F}$ values and not on the correct F test according to eq 5.

Table I. Substances of the Training Series, Blood Sugar Levels before and after Application, Values of Variables Used, Classification According to Experimental Results, and Reclassification Resulting from Discriminant Analysis of All Classes

no.	R_1	R_2	X	yield, %	mp, °C	blood sugar levels[b] (mg/100 mL) before appl	blood sugar levels[b] (mg/100 mL) after[c] appl	α[d]	I_1	R_M[e]	I_2	π_1[f]	σ_{p_1}[g]	MR_1[h]	π_2[i]	MR_2[h]	classification I[k]	classification II[m]
1	CH_3O	CH_3	O	87.6	188-189	91 ± 1.0	142 ± 12.2	<0.001	0	0.477	1	0.02	0.27	7.87	0.5	5.65	1	1
2	CH_3O	C_2H_5	O	86.9	176-177	113 ± 12.0	135 ± 15.6	<0.001	0	0.432	1	0.02	0.27	7.87	1.0	10.3	1	1
3	CH_3O	C_3H_7	O	83.9	182-183	115 ± 13.1	135 ± 9.6	<0.001	0	0.327	1	0.02	0.27	7.87	1.5	14.96	1	1
4	CH_3O	C_3H_7	S	73.3	105-106	134 ± 5.4	153 ± 8.9	<0.001	1	0.213	1	0.02	0.27	7.87	1.5	14.96	1	1
5	CH_3O	H	O	68.8	190-191	84 ± 6.2	97 ± 9.3	<0.001	0	0.501	1	0.02	0.27	7.87	0.0	1.03	1	1
6	C_2H_5O	H	O	67.9	184-185	88 ± 11.4	103 ± 18.7	<0.01	0	0.454	1	0.38	0.24	12.47	0.0	1.03	1	3
7	C_3H_7O	C_4H_9	S	67.3	68-69	133 ± 9.3	160 ± 10.6	<0.001	1	0.176	1	1.05	0.25	17.06	2.0	19.59	1	1
8	C_3H_9O	CH_3	O	85.3	156-157	78 ± 22.3	96 ± 11.6	<0.01	0	0.327	1	1.6	0.32	21.66	0.5	5.65	1	2
9	C_4H_9O	C_4H_9	O	81.0	161-162	85 ± 13.0	113 ± 1.0	<0.001	0	0.035	1	1.6	0.32	21.66	2.0	19.59	1	1
10	CH_3O	C_4H_9	O	84.4	164-165[a]	114 ± 2.5	114 ± 4.9	1	0	0.288	1	0.02	0.27	7.87	2.0	19.59	2	2
11	CH_3O	CH_2Ph	O	71.4	177-178	106 ± 5.5	104 ± 14.0	<0.50	0	0.213	1	0.02	0.27	7.87	2.01[j]	30.01	2	2
12	C_2H_5O	C_4H_9	O	82.4	153-154	119 ± 4.6	114 ± 9.1	<0.05	0	0.269	1	0.38	0.24	12.47	2.0	19.59	2	2
13	C_3H_7O	C_4H_9	O	81.6	156-157	138 ± 11.7	130 ± 8.1	<0.05	0	0.250	1	1.05	0.25	17.06	2.0	19.59	2	2
14	C_4H_9O	CH_3	S	78.5	94-95	82 ± 9.0	77 ± 4.7	<0.05	1	0.269	1	1.6	0.32	21.66	0.5	5.65	2	1
15	$C_5H_{11}O$	C_4H_9	O	84.1	167-168	73 ± 3.1	79 ± 9.7	<0.02	0	0.017	1	2.1	0.34	26.26	2.0	19.59	2	2
16	$C_5H_{11}O$	C_4H_9	S	55.9	83-84	132 ± 6.1	129 ± 10.4	<0.25	1	0.017	1	2.1	0.34	26.26	2.0	19.59	2	2
17	CH_3CONH	C_4H_9	O	75.1	188-189	113 ± 5.3	112 ± 4.1	<0.50	0	0.389	1	0.97	0.00	14.93	2.0	19.59	2	2
18	CH_3O	CH_2Ph	S	45.6	138-139	117 ± 6.0	97 ± 4.0	<0.001	1	0.176	1	0.02	0.27	7.87	2.01[j]	30.01	3	3
19	C_2H_5O	C_4H_9	S	77.9	78-79	110 ± 2.9	99 ± 2.9	<0.001	1	0.213	1	0.38	0.24	12.47	2.0	19.59	3	3
20	CH_3CONH	C_4H_9	S	74.1	142-144	131 ± 15.4	116 ± 8.0	<0.002	1	0.347	1	0.97	0.00	14.93	2.0	19.59	3	3
21	CH_3	C_4H_9	O	70.4	156-157[l]	123 ± 2.6	102 ± 3.1	<0.001	0	0.432	0	0.56	0.17	5.65	2.0	19.59	3	3
22	CH_3	C_4H_9	S	65.3	110-112	131 ± 4.9	119 ± 6.4	<0.001	1	0.327	0	0.56	0.17	5.65	2.0	19.59	3	3

[a] Lit.[25] gives mp 163-165 °C by different synthesis. [b] Mean blood sugar concentrations of six rats ± 2 s. [c] Levels were measured 90 min after intraperitoneal administration of 70 mg/kg. [d] Critical probability of error for Student's t test regarding significant differences between blood sugar levels before and after application. [e] R_M values calculated from measured R_f values (see Experimental Section). [f] From ref 20. [g] From ref 19. [h] From ref 18. [i] From ref 17. [j] From ref 20. [k] I = experimental. Classification according to results of Student's t test; if $\alpha < 0.01$, the substances were classified into class "hyperglycemic" or "hypoglycemic" with respect to an increase or decrease of the mean blood sugar levels. [l] Lit.[25] gives mp 156-157 °C by different synthesis. [m] II = discriminant function (eq 7).

Table II. Step-Up Procedure of Multivariate Variance Analysis; Calculation of an Optimal Set of Variables for All Classes

set of variables	comparison of classes	Hotelling's T^2	critical probability of error
MR_2	all[a]	1.947	*0.009*[c]
	1-2[b]	1.212	*0.012*
	1-3	1.52	*0.006*
	2-3	0.072	0.508
MR_2, I_1	all	2.823	*0.013*
	1-2	1.212	*0.047*
	1-3	2.243	*0.007*
	2-3	0.774	0.127
MR_2, I_1, R_M	all	5.175	*0.004*
	1-2	1.216	0.114
	1-3	4.126	*0.002*
	2-3	2.724	*0.01*
MR_2, I_1, R_M, π_1 (optimal set)	all	6.861	*0.004*
	1-2	1.505	0.142
	1-3	5.814	*0.001*
	2-3	3.385	*0.012*

[a] Simultaneous comparison of all classes together. [b] Single comparison of each class with each other. [c] Critical probability of error belonging to $\tilde{F}$ values calculated from Hotelling's T^2; italicized values are significant at the 0.95 level.

Table III. Correlation Coefficients of Closely Correlated Variables ($|r| > 0.4$)

	I_1	σ_{P_1}	π_1	MR_1	π_2	MR_2
R_M	0.47	0.53	-0.63	-0.61	-0.59	-0.58
I_2		0.92	-0.49			
MR_1		-0.71	0.72			
π_2						0.88

Strictly speaking, these compounds cannot be classified, and these misclassifications can thus not be considered real errors.

In order to check the stability and discriminating power of the discriminant function the leave-one-out technique was applied. In this technique each of the compounds is once left out from the analysis and is then classified with the discriminant function obtained from the other compounds. In this way a real classification is simulated. The results are summarized in Table IV. It is evident that the discriminant function and the statistical data are very stable. Only three of the left-out compounds are not correctly classified, but again this is not a true misclassification since these three compounds fall into the region of overlap between the two classes.

The results clearly indicate the importance of the substituents at the nitrogen for hyperglycemic activity. In blood sugar increasing compounds these substituents should be small and/or hydrophilic. This conclusion might also have been drawn by simply looking at Table I, but discriminant analysis now substantiates this picture and yields the additional information that other properties of the compounds considered are not important for hyperglycemic activity.

(3) Separation of Hypoglycemic from Inactive Compounds. The optimal set for the separation of classes 2 and 3 includes the variables MR_2, I_1, and R_M ($T^2 = 4.06$; $\alpha = 0.015$), in agreement with the results presented in Table II. The variable π_1, however, which occurred in the optimal set for the simultaneous separation of all classes and which also slightly increased T^2 for the separation of classes 2 and 3 in the step-up procedure for the all-class problem is no longer significant. Since R_M and π_1 are correlated, replacing R_M by π_1 was tried in the optimal set. With this modified set of variables no significant separation was possible. The nonelementary discriminant function obtained from the optimal set reads

$$w = 0.15MR_2 + 2.82I_1 + 8.53R_M \quad (9)$$

with the class means $\bar{w} = 5.399$ for class 2 and $\bar{w} = 8.092$ for class 3. With this discriminant function 92% of the compounds considered are correctly reclassified. One compound falls in the region of overlap between the two classes and is "misclassified" if the criterium of the smallest $\tilde{F}$ value is applied. The results of the leave-one-out analysis are summarized in Table V. Optimal set, discriminant function, and statistical data are quite stable. Three of the left-out compounds are not correctly classified; again

Table IV. Leave-One-Out Technique for Hyperglycemic and Inactive Compounds

no. of compd left out[a]	α[b]	coeff a_{ik} of discrim function MR_2	F[c]	classification I[d]	II[e]	III[f]
1	0.036	0.14	0.493	1	1	1
2	0.028	0.14	0.000	1	1	1
3	0.019	0.14	0.480	1	1	1
4	0.019	0.14	0.480	1	1	1
5	0.039	0.15	2.162	1	1	1
6	0.039	0.15	2.162	1	1	1
7	0.010	0.14	2.134	1	2	2[g]
8	0.036	0.14	0.493	1	1	1
9	0.010	0.15	2.134	1	2	2[g]
10	0.030	0.14	0.003	2	2	2
11	0.044	0.15	3.209	2	2	2
12	0.030	0.14	0.003	2	2	2
13	0.030	0.14	0.003	2	2	2
14	0.004	0.16	5.645	2	1	1
15	0.030	0.14	0.003	2	2	2
16	0.030	0.14	0.003	2	2	2
17	0.030	0.14	0.003	2	2	2

[a] See Table I. [b] Critical probability of error belonging to $\tilde{F}$ values calculated from Hotelling's T^2. [c] F statistics according to eq 4 for compounds left out regarding their experimental class; critical $F_{1,15;0.05} = 4.54$. [d] Experimental class of the substances. [e] Reclassification using discriminant function (eq 8). [f] Classification by the leave-one-out technique. [g] Closer to the range of class 2.

Table V. Leave-One-Out Technique for Hypoglycemic and Inactive Compounds

no. of compd left out[a]	α[b]	coeff a_{ik} of discrim functions MR_2	I_1	R_M	$\tilde{F}$[c]	classification I[d]	II[e]	III[f]
10	0.028	0.15	2.65	8.34	0.002	2	2	2
11	0.013	0.21	2.66	8.61	3.042	2	2	3[g]
12	0.030	0.15	2.66	8.26	0.015	2	2	2
13	0.030	0.15	2.68	8.23	0.091	2	2	2
14	0.014	0.06	3.15	8.60	5.163	2	2	3
15	0.005	0.21	4.02	14.76	16.512	2	2	2[h]
16	0.024	0.14	2.90	6.70	0.598	2	2	2
17	0.016	0.15	2.63	9.75	1.549	2	2	2
18	0.043	0.15	2.78	8.46	0.736	3	3	3
19	0.026	0.15	2.61	8.75	0.397	3	3	3
20	0.043	0.15	2.75	8.32	0.520	3	3	3
21	0.005	0.18	3.67	6.86	7.500	3	2	2
22	0.042	0.15	2.71	8.23	0.280	3	3	3

[a] See Table I. [b] Critical probability of error belonging to $\tilde{F}$ values calculated from Hotelling's T^2. [c] F statistics according to eq 4 for compounds left out regarding their experimental class; critical $F_{1,11;0.05} = 4.84$. [d] Experimental class of the substances. [e] Reclassification using discriminant function (eq 9). [f] Classification by the leave-one-out technique. [g] Closer to the range of class 3. [h] Closer to the range of class 2.

these compounds fall into the region of overlap between the two classes.

According to the class means, high values of MR_2 and of R_M as well as the presence of the thioamide group instead of the amide group are favorable for hypoglycemic activity. Thus, substituents at R_2 should be large and/or hydrophobic, and the hydrophobicity of the whole molecule should not be too high. As already pointed out the simultaneous occurrence of MR_2 and of the hydrophilicity parameter R_M might indicate the existence of an optimum in overall hydrophobicity, but it may also simply reflect the unfavorable effect of large alkoxy groups on hypoglycemic activity.[21]

Discussion

The main purpose of this work was to gain some understanding of the reason why opposite effects on the blood sugar level are present in a homologous series with only minor structural differences. Therefore, we were primarily interested in describing the distribution of the compounds over the three classes of hyperglycemic, inactive, and hypoglycemic activity *within* the training series and not so much in the use of the resulting discriminant functions for the design of new compounds. In fact, the discriminant functions obtained cannot be recommended for predictions because of the small sample size and the uniformity of the substituents used. Within the series, however, the descriptive power of these functions is satisfactory, and they do yield information which could not have been obtained in any other way. First of all, the outstanding role of substituents at position R_2 is revealed. For hyperglycemic activity these substituents should be small and/or hypophilic, whereas large and/or hydrophobic substituents are favorable for hypoglycemic activity. This conclusion is in agreement with results of Ruschig and co-workers,[2,3] indicating that optimal hypoglycemic activity of alkyl-substituted sulfonylureas requires substituents with three to six carbon atoms in this position. The situation with substituents in the phenyl ring is somewhat more complicated. As was shown by Seydel et al.[22] hypoglycemic activity in a series of benzenesulfonamidopyrimidines increases with increasing hydrophobicity of these substituents. At the first look the results summarized in Table II and eq 7 seem to indicate a similar trend, but the contribution of π_1 to the separation of classes is very small, and it vanishes completely if the two-class problem, hypoglycemic/inactive compounds, is considered. This may be due to the fact that the most hydrophobic substituents at R_1 are alkoxy groups. Large alkoxy groups are known to diminish hypoglycemic activity,[21] and this unfavorable effect probably outweighs a possible positive hydrophobic influence.

The amide group is not essential for blood sugar decreasing sulfonamide-like substances. Rather than the amide group the presence of an sp^2-hybridized carbon connected with nitrogen is important, the thioamide group being somewhat more favorable for hypoglycemic activity than the amide group as follows from the positive sign of the indicator variable I_1 in the discriminant functions (eq 7 and 9). Comparison of compounds 11, 12, and 17 with compounds 18, 19, and **20** clearly shows the activity promoting effect of X = S as compared to X = O.

More detailed interpretations cannot be made because of the limited number of substances within the training series. Another restriction follows from the small range of electronic properties covered by the substituents. Furthermore, an exact separation of hydrophobic and steric influences is nearly impossible since MR and π are highly correlated.

Since different variables are responsible for the separation of hyperglycemic and of hypoglycemic compounds, respectively, from the two other classes, it may be concluded that two different mechanisms of action are responsible for hypoglycemic and hyperglycemic activity. This conclusion is supported by experimental data from the literature. Gutsche and co-workers[23] assumed that hypoglycemic activity of sulfonylureas is produced by binding to receptors occurring in β cells, whereas the hyperglycemic effect is attributed to an activation of the suprarenal gland leading to an increased production of adrenaline.[9,24] These two opposite effects obviously overlap, and it depends on their relative strength whether a particular compound will be hypoglycemic, hyperglycemic, or inactive. Since the influence of substituent properties on the two mechanisms is different, the fact that structurally similar sulfonamides influence the level of blood sugar in different ways can easily be explained.

Experimental Section

Preparation of *o*-Toluenesulfonylthioureas and *o*-Toluenesulfonylureas. The thioureas have been synthesized from the substituted benzenesulfonamides and the corresponding isothiocyanates.[25] Subsequent oxidation by H_2O_2 in alkaline medium[26] produced the analogous benzenesulfonylureas.

4-Methoxy-*o*-toluenesulfonyl-*N'*-propylthiourea (Compound 4, Table I). 4-Methoxy-*o*-toluenesulfonamide (0.04 mol) was dissolved in 70 mL of $(CH_3)_2CO$. Aqueous NaOH (10 mL, 16%) and 0.044 mol of propyl isothiocyanate were added to this solution, and the mixture was refluxed for 6 h. Then the solvent was removed and the residue was dissolved in H_2O (100 mL). The solution was acidified with HCl (pH 2–2.5), filtered, and crystallized (EtOH, 70%). The other *o*-toluenesulfonylthioureas were prepared by the same procedure.

4-Methoxy-*o*-toluenesulfonyl-*N'*-propylurea (Compound 3, Table I). 4-Methoxy-*o*-toluenesulfonyl-*N'*-propylthiourea (0.02 mol) was dissolved in 0.5 N NaOH (100 mL). H_2O_2 (30%) was added dropwise so that the temperature was maintained at 45–55 °C. During the last 30 min the temperature was kept at 45 °C. The reaction mixture was then acidified to pH 2.5–3. The white residue was collected by filtration, washed (H_2O), and crystallized (EtOH, 70%).

Other *o*-toluenesulfonylureas with the exception of the following two compounds were prepared as described above.

4-Methoxy-*o*-toluenesulfonylurea (Compound 5, Table I). The sodium salt of 4-methoxy-*o*-toluenesulfonamide (0.1 mol) and urea (0.1 mol) dissolved in chlorobenzene were heated during 20 h at 110–115 °C. The solvent was removed in vacuo and the residue was diluted with H_2O (70 mL). The solution was decolorized, filtered, acidified with HCl (pH 2.5–3), and crystallized (CH_3OH). Compound 6 in Table I has been obtained in an analogous manner.

All compounds were analyzed for C, H, N, and S; analytical results were within ±0.4% of the theoretical values. Yields and melting points are presented in Table I. Melting points are uncorrected, capillary melting points.

UV and IR Spectroscopy. Each compound was identified by UV and IR spectra. IR spectra showed absorption bands at 3360–3340, 3100–3120 (NH), 1140, and 1320 cm^{-1} (SO_2). Urea derivatives have a characteristic absorption band at 1660 cm^{-1} (C=O). UV spectra showed a maximum at 252 nm (log ϵ 4.3) for the thiourea and at 244 nm (log ϵ 4.1) for the urea derivatives.

Thin-Layer Chromatography. Thin-layer chromatography of the compounds was conducted on "Silufol UV 254" plates (aluminum oxide, silica gel, starch) from Chemapol, CSSR. The mobile phase consisted of 1-butanol and 7 N ammonia (7:3). The substances were detected in UV light.

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Studies on Biologically Active Halogenated Compounds. 1. Synthesis and Central Nervous System Depressant Activity of 2-(Fluoromethyl)-3-aryl-4(3*H*)-quinazolinone Derivatives

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Some 2-(fluoromethyl) analogues of 2-methyl-3-aryl-4(3*H*)-quinazolinones have been synthesized and screened for CNS activities. It was shown that the 2-(fluoromethyl) analogues possess in general more potent CNS depressant activities and less toxicities than their parent compounds. Of particular interest were the 2-(fluoromethyl) analogues (**22**, **24**, and **31**) of methaqualone and 6-aminomethaqualone. Compound **24** was more potent in CNS depressant activity and less toxic than methaqualone. Compound **31** exhibited potent central muscle relaxing activity and markedly reduced toxicity as compared with 6-aminomethaqualone.

Numerous synthetic efforts on halogen-containing compounds have been made to exploit the pharmacophoric effect of halogenation. The most fruitful success has been achieved by the introduction of a fluorine atom into an already active compound, resulting in the following beneficial changes in molecular properties: (1) higher fat solubility giving different absorption and transport rate; (2) altered electronic effects; (3) improved stability; (4) equivalent steric size.

In recent years we have investigated the synthesis[1] and biological activity of 2,3-dihydro-4(1*H*)-quinazolinone derivatives and their related compounds in order to develop potent analgesics. During these studies it was found incidentally that some 1-(*tert*-aminoacetyl)-2-methyl-3-phenyl-2,3-dihydro-4(1*H*)-quinazolinones possessed strong choleretic activity.[1c] The highest activity was exhibited with 1-(morpholinoacetyl)-2-methyl-3-phenyl-2,3-dihydro-4(1*H*)-quinazolinone. From a study of the structure–activity relationship, it has been found that the introduction of a substituent into the fused benzene ring generally reduced the activity. Elongation of the alkyl chain at C-2 also resulted in a significant decrease in activity and an increase in toxicity. However, the 2-(fluoromethyl) analogue showed equipotent activity, suggesting an important role of steric size at C-2 for the activity. This is in accord with an earlier observation[2a] on the steric requirement at C-2 of 2-methyl-3-(*o*-tolyl)-4-(3*H*)-quinazolinone (methaqualone) for hypnotic activity. In view of these results it became of interest to synthesize 2-(fluoromethyl) analogues of 2-methyl-3-aryl-4(3*H*)-quinazolinone derivatives in an attempt to prepare improved neurotropic drugs. We describe here our findings in the synthesis of some 2-(fluoromethyl)-3-aryl-4(3*H*)-quinazolinones and their pharmacological activities.

The structural modifications[2] of methaqualone reported to date involve mainly the additional substitution with various functional groups on the 3-tolyl moiety and/or on the fused benzene ring and the replacement of the 2-methyl group with longer alkyl groups. After our preliminary communication[3] on the hypnotic activity of 2-(fluoromethyl)-3-(*o*-tolyl)-4(3*H*)-quinazolinone (**22**) appeared, a number of new derivatives bearing halogen, amino, alkoxy, and sulfide groups on the 2-methyl group were reported by Taylor[4] and his co-workers.

Chemistry. The 2-(fluoromethyl)-3-aryl-4(3*H*)-quinazolinones IV were prepared as shown in Scheme I via three steps from the corresponding anthranilic acids. A fusion reaction of anilines with isatoic anhydride is well-known as a general method[5] for the preparation of anthranilanilides. However, this method requires handling of phosgene in the preparation of isatoic anhydride. In order to avoid the hazards with phosgene, we attempted the alternate reaction of anthranilic acids I with thionyl chloride in boiling benzene, followed by treatment with

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