CHROMATOGRAPHIC PARAMETERS, pK_a, AND SURFACE ACTIVITY OF A SERIES OF HYDROCHLORIDES OF PERHYDROAZEPINYLETHYL ESTERS OF ALKOXYPHENYLCARBAMIC ACIDS AND THEIR RELATION TO ANAESTHETIC ACTIVITY*

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Hydrochlorides of perhydroazepinylethyl esters of alkoxyphenylcarbamic acids were separated by adsorption and partition TLC. The surface tensions, γ, of aqueous solutions of the compounds were measured and the values of pK_a were determined. The relation between structure and physico-chemical properties has been confirmed by the dependences of R_M and γ on the number of carbon atoms in the alkoxyl chain. Employing the method of regression analysis, the physico-chemical parameters of lipophilicity and the surface activity were correlated, and their effect on biological activity (relative local anaesthetic efficacy) has been assessed.

The series of hydrochlorides of perhydroazepinylethyl esters of alkoxyphenylcarbamic acids was prepared[1] as part of a systematic study of local anaesthetics, of the group of basic esters of alkoxy-substituted phenylcarbamic acid. In pharmacological tests all the compounds were efficacious in both the surface and the infiltration application. The maximum efficacy was observed with alkoxy substituents having 5 to 7 carbon atoms, further elongation of the substituent led to diminishing and finally disappearance of the local anaesthetic activity. The perhydroazepine analogues were more efficaiious than the corresponding piperidino derivatives[2]. The generally higher efficacy of the 3-substituted derivatives compared to the 4-substituted ones is evidently due to different mesomeric effects and to different conformation of the molecules[1]. For an anaesthetic to penetrate from the spot of application to the site of action the following physico-chemical properties are important: solubility in lipids and in water (the partition coefficient), the dissociation constant and the surface tension of the substance[3]. Synthesis of new, biologically active compounds has in recent years been accompanied by studies aimed at correlation of structural alternations with changes in physico-chemical properties and in biological activity[4]. Making use of these findings, we have applied regression analysis to a new series of compounds, in an attempt to describe the relation of biological activity to physico-chemical properties and structure.

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EXPERIMENTAL

The hydrochlorides of perhydroazepinylethyl esters of alkoxy phenylcarbamic acids are listed in Table I.

The equipment for chromatography was a Kavalier product, Votice, Czechoslovakia; the foils used were Silufol^R UV 254 with silica gel Silpearl^R, and foils with cellulose Lucefol^R Quick, 200 mm by 200 mm. The solutions of the compounds (1% in methanol) were applied to the foils with a capillary, volume 2 μl. For detection in UV light (universal UV lamp Camag, Switzerland) it was necessary to apply 2 to 4 μl, for detection with the Dragendorff agent (on foils without and indicator) it was sufficient to apply 1 to 2 μl. The chromatography ran in glass boxes 190 × × 180 × 80 mm, lined with filtration paper. on a path of 12 cm. Prior to a run the chamber was saturated for 30 min with the system S_1 or S_4. The time of chromatography was 20 to 30 min with Silufol and 20 to 50 min with Lucefol. The cellulose layers for partition chromatography were impregnated with 40% formamide in 96% ethanol, containing an admixture (1%) of tris-(hydroxymethyl)aminomethane. The impregnation was carried out after application of a sample by immersing a part of the foil into the impregnation solution and the excess was blotted with a filtration paper. The region of the start was impregnated by spraying. After the impregnation the layer was dried 1 h in the air. Mobil phases: cyclohexane–diethylamine 7 : 1 (S1), cyclohexane–diethylamine 9 : 2 (S2), toluene–diethylamine 7 : 1 (S3), pentane (S4) and pentane–diethylamine 9 : 0·05 (S5).

From R_F (average value from six chromatograms) we calculated R_M and ΔR_M using the equations $R_M = \log [(1/R_F) - 1]$ and $\Delta R_M = R_{MX}$, where R_{MX} and R_{MH} denote R_M values of the substituted and the non-substituted substances.

The surface tension was determined stalagmometrically at 22°C, the Traube stalagmometer and aqueous solutions of the substances ($c = 0{\cdot}002$ mol/l) being used. The reference liquid was water, its surface tension, γ, at this temperature is 0·07244 N m^{-1}. From the measured values we calculated decreases of the surface tension as against water, $\Delta\gamma$.

The dissociation constants were determined potentiometrically; pK_a was determined as pH of the solution of a compounds titrated to 50% with an alkali hydroxide. Because of poor solubility in water of the bases liberated in the course of the titration the medium used was a mixture water–methanol 2 : 3 (v/v) (in calculating pK_a correction was made for the volume of methanol). The course of a titration was followed on a pH meter (Precision digital pH-meter OP-208, Radelkis, Hungary).

The values of relation efficacy in the surface and the infiltration anaesthesis were taken from the literature[1].

RESULTS AND DISCUSSION

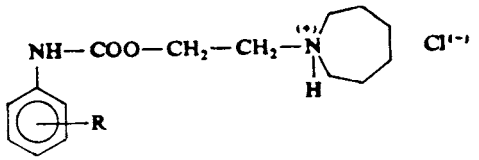
To study the relation between local anaesthetic activity and physico-chemical properties we used R_M (calculated from R_F), surface activity of the aqueous solutions and the degrees of dissociation. Lipophilicity was evaluated from the correlation of the chromatographic parameter R_M and the partition coefficient P (ref.[5]). Validity of the equation $R_M = a \log P + b$ had been verified on several series of similar structures[6–8], demonstrating that R_M can be used as a parameter of lipophilicity.

In chromatography on both Silufol, where the principle of separation was mainly adsorption, and on Lucefol, where it was supposed to be partition, we succeeded in selecting such eluting systems for homologic series of 2-, 3- and 4-substituted

derivatives that the value of R_F steadily increased with elongation of the carbon chain of the alkoxyl (Table I). The calculated R_M values from both the adsorption and the partition chromatography were correlated by regress equations with the

TABLE I

pK_a, R_F and R_M of hydrochlorides of perhydroazepinylethyl esters of alkoxyphenylcarbamic acids



Compound	R	pK_a	Adsorption chromatography		Partition chromatography		
			R_F	R_M	R_F	R_M	ΔR_M
1	H	8·19	0·267[a]	0·439	0·050[d]	1·279	
2	2-OC_3H_7	8·12	0·478	0·038	0·085	1·032	−0·247
3	2-OC_4H_9	8·07	0·500	0·000	0·183	0·650	−0·629
4	2-OC_5H_{11}	7·93	0·520	−0·035	0·342	0·284	−0·995
5	2-OC_6H_{13}	8·06	0·535	−0·061	0·498	0·003	−1·276
6	2-OC_7H_1a	7·94	0·550	−0·097	0·655	−0·278	−1·557
7	2-OC_8H_{17}	7·99	0·570	−0·122	0·755	−0·489	−1·768
1	H		0·440[b]	0·105	0·057[e]	1·219	
8	3-OC_3H_7	8·08	0·443	0·099	0·183	0·650	−0·569
9	3-OC_4H_9	8·10	0·458	0·073	0·278	0·414	−0·805
10	3-OC_5H_{11}	7·99	0·475	0·043	0·372	0·227	−0·992
11	3-OC_6H_{13}	7·96	0·485	0·026	0·512	−0·021	−1·240
12	3-OC_7H_{15}	7·90	0·510	−0·017	0·775	−0·537	−1·756
13	3-OC_8H_{17}	7·93	0·522	−0·038	0·920	−1·061	−2·280
1	H		0·527[c]	−0·047	0·048[f]	1·297	
14	3-OC_3H_7	8·05	0·538	−0·066	0·092	0·994	−0·303
15	4-OC_4H_9	7·94	0·548	−0·094	0·172	0·683	−0·614
16	3-OC_5H_{11}	7·95	0·561	−0·106	0·248	0·482	−0·815
17	4-OC_6H_{13}	8·07	0·575	−0·131	0·412	0·154	−1·143
18	4-OC_7H_{15}	8·10	0·585	−0·149	0·662	−0·292	−1·489
19	4-OC_8H_{17}	8·16	0·598	−0·172	0·863	−0·799	−2·096

[a] Elution system S_1 (standard deviation of R_F 0·018), [b] S_2 (0·011), [c] S_3 (0·020), [d] S_4 (0·032), [e] S_5 (0·060), [f] S_E (0·043).

number of carbon atoms in the alkoxyl side chain, linear inverse proportions being found. For the adsorption chromatography and for $n = 6$, the absolute values of the correlation coefficients were $r = 0{\cdot}995$ to $0{\cdot}999$ and $s = 0{\cdot}002$ to $0{\cdot}005$. For the partition chromatography and $n = 6$ they were $r = 0{\cdot}975-0{\cdot}995$, $s = 0{\cdot}059-0{\cdot}142$.

As for the surface activity it was found that with the increasing number of carbon atoms in the alkoxyl from C_3 to C_8 the surface tension (γ) of the aqueous solutions decreased (Table II). The dependence was linear and the absolute values of the correlation coefficients were $r = 0{\cdot}957-0{\cdot}973$ and $s = 0{\cdot}003-0{\cdot}004$. By introduction of a quadratic term., and by calculation of a non-linear regression equation, the correlation coefficient improved to $r = 0{\cdot}993-0{\cdot}998$, so that the relation of the surface tension to the number of carbon atoms in the alkoxyl had better be considered to be a parabolic one (Fig. 1).

TABLE II

Surface tension (γ), its decrease related to water ($\Delta\gamma$) and log of relative anaesthetic efficacy of hydrochlorides of perhydroazepinylethyl esters of alkoxyphenylcarbamic acids

Compound	γ, N m^{-1}	$\Delta\gamma$, N m^{-1}	log U^a	log U^b
1	0·0788	−0·0064	irritation	1·05690
2	0·0766	−0·0041	0·55145	1·26717
3	0·0724	0·0000	1·01703	1·18469
4	0·0705	0·0019	1·76567	1·87967
5	0·0670	0·0054	1·79588	1·41497
6	0·0570	0·0154	2·06070	2·27875
7	0·0487	0·0237	1·04532	0·91908
8	0·0705	0·0019	1·43136	1·96848
9	0·0670	0·0054	1·75587	1·6206
10	0·0638	0·0086	1·81291	1·77815
11	0·0570	0·0154	1·78247	1·43775
12	0·0462	0·0262	[c]	[c]
13	0·0367	0·0357	0·07555	0·82608
14	0·0705	0·0019	0·69897	1·34242
15	0·0670	0·0054	0·68124	0·65321
16	0·0654	0·0071	0·91908	1·22789
17	0·0583	0·0142	1·31806	1·12385
18	0·0479	0·0246	[c]	[c]
19	0·0362	0·0362	[c]	[c]

[a] Surface anaesthesia, [b] infiltration anaesthesia, [c] for poor solubility the compound was not tested.

The values of pK_a (Table I) and the number of carbon atoms in the alkoxyl do not seem to be in any correlation. The differences between pK_a values of the individual hydrochlorides of perhydroazepinylethyl esters of alkoxyphenylcarbamic acids often neared the range of error of the determination. For this reason we also refrained from any attempt to correlate them with chromatographic parameters or surface tension.

In evaluating the relation of the surface tension (γ) to lipophilicity we obtained, for a linear function $\gamma = f(R_M)$ and a non-linear function $\gamma = F(R_M, R_M^2)$ in homologic series, the following equations:
for 2-derivatives:

$$\gamma = 1{\cdot}710 \,.\, 10^{-2} R_M + 6{\cdot}194 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}933\,; \quad s = 0{\cdot}379 \,.\, 10^{-2}\,; \quad F = 26{\cdot}7 \qquad (1)$$

$$\gamma = 2{\cdot}439 \,.\, 10^{-2} R_M - 1{\cdot}362 \,.\, 10^{-2} R_M^2 + 6{\cdot}476 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}988\,; \quad s = 0{\cdot}207 \,.\, 10^{-2}\,; \quad F = 62{\cdot}7 \qquad (2)$$

for 3-derivatives:

$$\gamma = 2{\cdot}045 \,.\, 10^{-2} R_M + 5{\cdot}798 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}988\,; \quad s = 0{\cdot}083 \,.\, 10^{-2}; \quad F = 996{\cdot}0 \qquad (3)$$

$$\gamma = 2{\cdot}050 \,.\, 10^{-2} R_M + 0{\cdot}019 \,.\, 10^{-2} R_M^2 + 5{\cdot}795 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}998\,; \quad s = 0{\cdot}106 \,.\, 10^{-2}\,; \quad F = 476{\cdot}8 \qquad (4)$$

for 4-derivatives:

$$\gamma = 1{\cdot}972 \,.\, 10^{-2} R_M + 5{\cdot}353 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}990\,; \quad s = 0{\cdot}190 \,.\, 10^{-2}\,; \quad F = 188{\cdot}3 \qquad (5)$$

$$\gamma = 2{\cdot}060 \,.\, 10^{-2} R_M - 0{\cdot}496 \,.\, 10^{-2} R_M^2 + 5{\cdot}537 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}998\,; \quad s = 0{\cdot}101 \,.\, 10^{-2}\,; \quad F = 422{\cdot}3 \qquad (6)$$

It is seen that with the 3-alkoxy derivatives the relations are linear, whereas with the 2- and 4-derivatives they are rather parabolical (Fig. 2). If ΔR_M, expressing the increase in lipophilicity caused by the alkoxyl, is substituted for R_M in the equations, then,

for the 2-derivatives chromatographed in S_4, it applies that:

$$\gamma = 1{\cdot}710 \,.\, 10^{-2}\, \Delta R_M + 8{\cdot}381 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}933\,; \quad s = 0{\cdot}379 \,.\, 10^{-2}\,; \quad F = 26{\cdot}7 \tag{7}$$

$$\gamma = -1{\cdot}044 \,.\, 10^{-2}\, \Delta R_M - 1{\cdot}362 \,.\, 10^{-2}\, \Delta R_M^2 + 7{\cdot}68 \,.\, 10^{-2}$$

$$n = 6\,; \quad r = 0{\cdot}988\,; \quad s = 0{\cdot}207 \,.\, 10^{-2}\,; \quad F = 62{\cdot}7 \tag{8}$$

For the 3- and 4-derivatives:

$$\gamma = 1{\cdot}971 \,.\, 10^{-2}\, \Delta R_M + 8{\cdot}053 \,.\, 10^{-2}$$

$$n = 12\,; \quad r = 0{\cdot}986\,; \quad s = 0{\cdot}207 \,.\, 10^{-2}\,; \quad F = 355{\cdot}0 \tag{9}$$

$$\gamma = 0{\cdot}202 \,.\, 10^{-2}\, \Delta R_M - 0{\cdot}660 \,.\, 10^{-2}\, \Delta R_M^2 + 7{\cdot}114 \,.\, 10^{-2}$$

$$n = 12\,; \quad r = 0{\cdot}982\,; \quad s = 0{\cdot}258 \,.\, 10^{-2}\,; \quad F = 124{\cdot}7 \tag{10}$$

The results were analogous if the values of $\Delta\gamma$ (Table II) instead of γ were used.

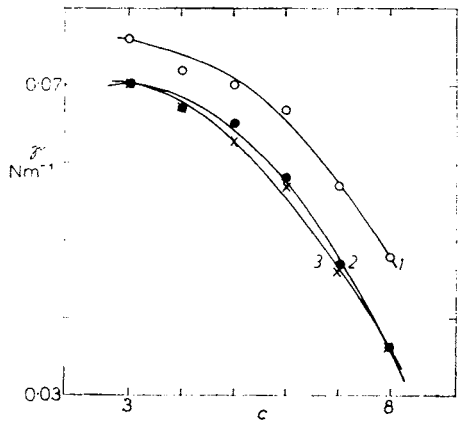


FIG. 1

Surface tension (γ) in relation to the number of carbon atoms in the alkoxyl chain. *1* 2-derivatives, *2* 4-derivatives, *3* 3-derivatives

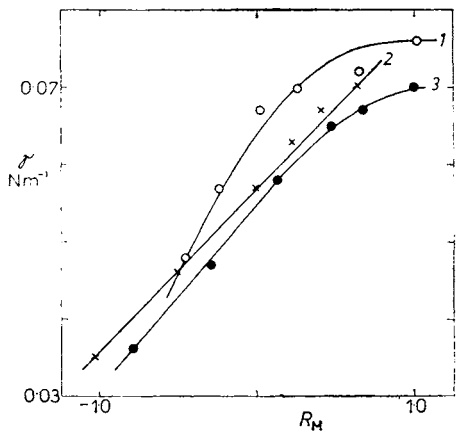


FIG. 2

Surface tension (γ) in relation to R_M from partition chromatography. For symbols see Fig. 1

The results of local anaesthetic, surface and infiltration activity are given in Table II. Regression equations (*11*)–(*16*) described the surface anaesthetic activity in relation to physico-chemical properties:

a) as a function of ΔR_M from partition chromatography for 2-substituted derivatives:

$$\log U = -0{\cdot}607\,\Delta R_M + 0{\cdot}718$$
$$n = 6\,;\quad r = 0{\cdot}594\,;\quad s = 0{\cdot}471\,;\quad F = 2{\cdot}18 \qquad (11)$$

$$\log U = -3{\cdot}679\,\Delta R_M - 1{\cdot}519\,\Delta R_M^2 - 0{\cdot}412$$
$$n = 6\,;\quad r = 0{\cdot}884\,;\quad s = 0{\cdot}353;\quad F = 5{\cdot}36 \qquad (12)$$

for 3- and 4-derivatives:

$$\log U = 0{\cdot}347\,\Delta R_M + 1{\cdot}502$$
$$n = 9\,;\quad r = 0{\cdot}326\,;\quad s = 0{\cdot}573\,;\quad F = 0{\cdot}83 \qquad (13)$$

$$\log U = -2{\cdot}929\,\Delta R_M - 1{\cdot}236\,\Delta R_M^2 - 0{\cdot}159$$
$$n = 9\,;\quad r = 0{\cdot}811\,;\quad s = 0{\cdot}390\,;\quad F = 6{\cdot}70 \qquad (14)$$

b) as a function of the decrease in surface tension, $\Delta\gamma$, for all the compounds:

$$\log U = -11{\cdot}142\,\Delta\gamma + 1{\cdot}350$$
$$n = 15\,;\quad r = 0{\cdot}196\,;\quad s = 0{\cdot}575\,;\quad F = 0{\cdot}52 \qquad (15)$$

$$\log U = 87{\cdot}657\,\Delta\gamma - 3\,250{\cdot}6\,\Delta\gamma^2 + 1{\cdot}038$$
$$n = 15\,;\quad r = 0{\cdot}734\,;\quad s = 0{\cdot}430\,;\quad F = 7{\cdot}02 \qquad (16)$$

It can be seen from equations (*11*), (*13*) and (*15*) that the relations between the surface local anaesthetic efficacy and ΔR_M or $\Delta\gamma$ are not linear, but are described by the non-linear equations (*12*), (*14*) and (*16*).

In the case of the infiltration local anaesthetic efficacy the relations to the physico-chemical properties cannot be regarded as linear even at higher lipophilicity (r = = 0·181, 0·364) or surface activity (r = 0·298), and the correlations are not much better even after introduction of a quadratic term (r = 0·469, 0·445, 0·417).

From equations (*12*) and (*14*) we calculated the optimum value of lipophilicity, ΔR_M, from equation (*16*) the optimum decrease of the surface tension of an aqueous solution related to water, $\Delta\gamma$. For the 2-derivatives $(\Delta R_M)_{opt} = -1{\cdot}211$, for the 3-

and 4-derivatives $(\Delta R_M)_{opt} = -1{\cdot}185$, and for all the compounds $(\Delta\gamma)_{opt} = 0{\cdot}0135$. On the basis of this analysis it can be stated that lipophilicity and surface tensions are important factors for local anaesthetic activity. According to these parameters, the most efficacious compounds of this group are those containing six carbon atoms in the alkoxyl chain.

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