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EFFECT OF PHLORETIN ON CHLORIDE PERMEABILITY: A STRUCTURE-ACTIVITY STUDY

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Summary

On the basis of data obtained with thin lipid membranes, phloretin's inhibition of chloride, urea and glucose transport in biological membranes has been suggested to be due to the effects of interfacial dipole fields on the translocator of these molecules (Andersen, O.S., Finkelstein, A., Katz, I. and Cass, A. (1976) J. Gen. Physiol. 67, 749—771).

From the systematic analysis made in the present paper it effectively appears that the ability of phloretin analogs to inhibit chloride permeability in red-cell membrane depends on the capacity they have to alter the interfacial dipole potential: the magnitude of the potential change depending on the dipole moment of the molecule and its membrane concentration, it follows that the inhibitory capacity of a phloretin analog is a function of the dipole moment and the lipid solubility of the compound.

Phloretin, the aglycone of phloridzin, is known to inhibit several energy-independent transport processes in red-cell membrane: hexoses [1,2], chloride [3], organic anions [4], glycerol and urea [5]. It also affects different transports across other biological membranes: sugar in fat cells [6] and rabbit heart muscle [7], urea in toad bladder [8]. Despite extensive studies to establish a correlation between the molecular structure of phloretin derivatives and their capacity to inhibit sugar transport in red cells, the mechanism of action of phloretin is not yet understood [1].

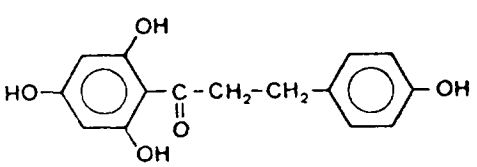
Recently, Andersen et al. [9] investigated the action of phloretin on artificial lipid membranes. They demonstrated that this compound increases lipophilic cation conductance and decreases lipophilic anion conductance. To explain these effects the authors assume that a positive potential difference of several hundred mV exists between the hydrocarbon interior and

the adjacent aqueous phases. This potential would arise from oriented dipoles near the lipid-water interface. They suggested that phloretin and other dipole organic molecules adsorb at the membrane surface and introduce a dipole potential of opposite polarity to the preexisting one; the reduction of internal potential so obtained allows, in the membrane, an increased concentration of cations and a decreased concentration of anions. The authors extended this interpretation to the biological transport processes, suggesting that inhibition of chloride, urea and hexose transport could also be explained by the effects of interfacial dipole fields on the translocator of these molecules.

It is tempting to speculate that such a simple physicochemical mechanism is responsible for all the effects of phloretin on a large diversity of biological transport systems and on bimolecular lipid membranes. The study we made of the inhibitory effect of several phloretin analogs on the chloride transport in red-cell membrane was performed to test this possibility.

Chloride permeability was measured by determining the rate of tracer efflux from radioactively labelled ox red cells under steady state conditions of anion concentration. For all the compounds the molar concentration causing a 50% inhibition for chloride transport (I_{50}) was determined. The inhibitory activity of each of them can be expressed in $1/I_{50}$.

In the hope of identifying the physicochemical parameter(s) responsible for the various effects of phloretin on red-cell transport processes, we decided to analyse, in quantitative terms, the relation between the inhibitory effects of phloretin-related compounds and their molecular properties. To quantify the molecular properties of compounds we have to use homologous series of congeners.



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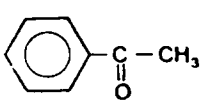

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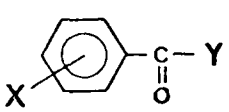

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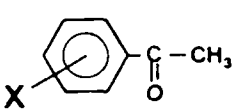

IV

Phloretin (formula I) has a rather complex structure and can be considered as a derivative of acetophenone (formula II) containing two kinds of substituents: one, X, on the ring and another, Y, on the carbon chain (formula III). So, let us consider acetophenone, which also decreases chloride per-

meability in red cell, as the parent compound of the series to study. When substituent X is introduced into acetophenone (II) to yield derivative IV, a compound showing a new inhibitory activity, one could anticipate at least three changes in II to which the difference in the biological response might be attributed: there could be a change in the dipole moment, μ, in the electron distribution within the molecule (this change can be quantified by the well-known Hammett constant σ)* and in the hydrophobicity of the molecule (this modification can be quantified by the Hansch's constant π)**.

As the first step let us consider the modifications due to the introduction of OH groups as substituents on the ring of acetophenone. The effects of such substitutions on the inhibitory activity of acetophenones can be correlated with the dipole moment μ, and with the substituent constants σ and π. From the data in Table I (compounds 1—6) we have derived Eqns. 1—3 by the method of least squares (π and σ are logarithmic terms).

$$\log \frac{1}{I_{50}} = 2.81 - 1.40\,\pi \qquad n = 6 \qquad r = 0.837 \qquad S = 0.574 \qquad (1)$$

$$\log \frac{1}{I_{50}} = 3.04 - 1.79\,\sigma \qquad n = 6 \qquad r = 0.833 \qquad S = 0.579 \qquad (2)$$

$$\log \frac{1}{I_{50}} = -0.01 + 6.79 \log \mu \qquad n = 6 \qquad r = 0.971 \qquad S = 0.249 \qquad (3)$$

In the above equations, n is the number of points used in the regression, r is the correlation coefficient and S the standard deviation.

Comparison of Eqns. 1—3 indicates that it is the dipole moment effect of substituent which is of primary importance: indicated by r^2, Eqn. 3 'explains' 94% of the variance in the data, while the hydrophobic effect of substituent (Eqn. 1) and the electronic effect of substituent (Eqn. 2) account only for 70 and 69%, respectively.

This result could indicate, as postulated by Andersen et al. [9] for phloretin, that the biological effect of acetophenones on membrane permeability is essentially controled by the dipole moment of the molecule. However, it must be emphasized that the variation of dipole moment in the above experiments, as in the work of Andersen et al., is exclusively obtained by adding and moving OH groups around the ring. So the question arises: is the modification of the biological activity really due to a variation of the dipole moment or to a variation in the spatial distribution of groups able to realize hydrogen bonds? The answer can be obtained by modifying the dipole moment with other chemical groups.

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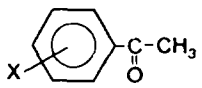
*σ is defined by the equation $\log k/k_0 = \rho\sigma$ where k is a constant describing a reaction of substituted aromatic compound, k_0 is the corresponding for the parent compound, ρ is a constant characteristic of the reaction.

**π constant can be defined as $\pi = \log P_X - \log P_H$, where P_H is the octanol-water partition coefficient of the acetophenone, P_X the octanol-water partition coefficient of the acetophenone derivative. Thus, π is the logarithm of the partition coefficient of the substituent X. Octanol-water partition coefficient is an index of lipophilicity.

TABLE I

PHYSICOCHEMICAL PROPERTIES AND INHIBITORY ACTIVITIES ON CHLORIDE TRANSPORT OF ACETOPHENONE DERIVATIVES



No.	X	π^a	σ^b	$\log \mu^c$	$\log \frac{1}{I_{50}}^d$
1	H	0.00	0.00	0.45	2.72
2	2-OH	−0.49	−0.36	0.48	3.42
3	3-OH	−0.49	0.00	0.47	3.45
4	2,4-OH	−1.10	−0.72	0.57	3.76
5	2,6-OH	−0.98	−0.72	0.74	5.15
6	2,4,6-OH	−1.58	−1.08	0.74	4.90
7	2-CH_3	0.68	−0.17	0.42	3.19
8	4-CH_3	0.52	−0.17	0.51	3.19
9	2,4-CH_3	1.20	−0.34	0.47	3.47
10	3,4-CH_3	1.03	−0.24	0.53	3.80
11	2,4,6-CH_3	1.88	−0.31	0.45	3.55
12	2-OCH_3	−0.33	−0.27	0.60	3.43
13	3-OCH_3	0.12	0.12	0.46	3.18
14	4-OCH_3	−0.04	−0.27	0.54	3.04

[a]The Hansch constant taken from refs. 11—13.
[b]The Hammett constant, from refs. 11—13.
[c]The dipole moment, from ref. 14.
[d]Experimental values. I_{50} represents the molar concentration of compound necessary to reduce the chloride transport by 50%.

Eqn. 4 is derived from the data obtained using acetophenone derivatives with methyl and methoxy groups as ring substituents (Table I).

$$\log \frac{1}{I_{50}} = 2.52 + 1.56 \log \mu \qquad n = 9 \qquad r = 0.281 \qquad S = 0.324 \tag{4}$$

From this relation it appears that the ability of acetophenones to inhibit chloride transport is not related to their dipole moment. However, it can be objected that this result is not sufficient to consider that alterations of the interfacial dipole potential are not involved in the inhibiting effect of acetophenones: the modification in the dipole potential between membrane interior and aqueous phase induced by a compound in fact results both from its dipole moment and its concentration in the membrane, which in turn depends on its lipid solubility. Thus, to consider the possibility of such an additive effect of liposolubility and dipole moment an equation relating $\log 1/I_{50}$ to both π and $\log \mu$ has been obtained by least squares analysis of the same data used in Eqn. 4:

$$\log \frac{1}{I_{50}} = 1.13 + 0.41\,\pi + 3.91 \log \mu \qquad n = 9 \quad r = 0.869 \quad S = 0.181 \tag{5}$$

By comparison with Eqn. 4, this relation shows that the correlation is greatly improved when the lipophilicity of the compounds is considered in addition to their dipole moment (lipophilicity by itself cannot explain this correlation, as shown by Eqn. 6)

$$\log \frac{1}{I_{50}} = 3.14 + 0.27\,\pi \qquad n = 9 \qquad r = 0.600 \tag{6}$$

Combining $\log \mu$, π and σ results in no better correlation than that of Eqn. 5, suggesting that the electronic effect of substituents in this series of compounds is not significant (yet it must be noticed that the values of σ, for all these compounds, are quite similar).

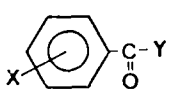
Thus, Eqn. 5 strongly suggests that the inhibitory activity of acetophenone derivatives on chloride transport is, at least in part, due to an alteration of the interfacial dipole potential.

Consequently, the highly significant correlation of inhibitory activity with dipole moment we observed when the ring substituent is an OH group (see Eqn. 3) very likely reflects a real dependence of the biological activity on the dipole potential and not a spatial position of OH group in the ring, suggesting a structural requirement.

We achieved a similar conclusion on the role of dipole potential in the inhibiting effect of acetophenone derivatives by using the following argument: if we consider a series of congeners having identical dipole moments but different lipid solubility, it is expected that the inhibitory activity will be strictly correlated with lipid solubility supposing that dipole potential only is involved in inhibition. To test this argument we made an analysis of derivatives in which the substituent on carbon chain, Y, was varied so as to increase lipophilicity, but the ring substituent, X, was constant so

TABLE II

PHYSICOCHEMICAL PROPERTIES AND INHIBITORY ACTIVITIES ON CHLORIDE TRANSPORT OF ACETOPHENONE DERIVATIVES AND ANALOGS



No.	X	Y	$R_{m\,H_2O}$[a]	σ[b]	$\log \frac{1}{I_{50}}$[c]
1	2,4,6-OH	H	0.38	0.49	4.13
2	2,4,6-OH	CH_3	0.66	0.00	4.90
3	2,4,6-OH	C_6H_5	0.89	0.60	5.00
4	2,4,6-OH (phloretin)	$(CH_2)_2C_6H_4OH$	1.28	0.08	5.70
5	2,6-OH	CH_3	1.05	0.00	5.15

[a] An index of liposolubility equivalent to the logarithm of the partition coefficient (see ref. 10). Experimental data.
[b] The Hammett constant (from refs. 11–13).
[c] See Table I.

as to keep the dipole moment practically constant. We have studied two series of congeners: the 2-OH derivatives (Table III) with a dipole moment of about 3.2; the 2,4,6-OH derivatives (Table II), in which phloretin belongs, with a dipole moment of about 5.5. (for example, according to Andersen et al. the dipole moment for compounds Nos. 2, 4 and 5 in Table II is 5.5, 5.6 and 5.5, respectively). In these series the substituent constant π for the most complex compounds cannot be found in literature. Therefore, it was necessary to determine experimentally the hydrophobic properties of all the compounds of the series. This has been done by the measurement of the R_m values by reversed phase thin-layer chromatography. Thus, in Tables II and III the index of hydrophobicity is $R_{m\ H_2O}$ which is a measure of the partition data equivalent to the logarithm of the partition coefficient [10].

From the data on 2,4,6-OH derivatives we have derived Eqns. 7 and 8 by the method of least squares.

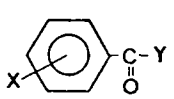
$$\log \frac{1}{I_{50}} = 3.64 + 1.57\, R_{m\ H_2O} \quad n = 5 \quad r = 0.966 \quad S = 0.168 \tag{7}$$

$$\log \frac{1}{I_{50}} = 5.22 - 1.03\, \sigma \quad n = 5 \quad r = 0.525 \quad S = 0.555 \tag{8}$$

Comparisons of Eqns. 7 and 8 indicates that electronic effects of the substituents are not significant, whereas the relative ability of these compounds to alter chloride permeability is highly correlated with their liposolubility: Eqn. 7 accounts for 93% of the variability in the data (combining $R_{m\ H_2O}$ and σ results in no better correlation). The highly dependent correlation between inhibitory activity and liposolubility is illustrated in Fig. 1, curve a.

TABLE III

PHYSICOCHEMICAL PROPERTIES AND INHIBITORY ACTIVITIES ON CHLORIDE TRANSPORT OF ACETOPHENONE DERIVATIVES AND ANALOGS



No.	X	Y	$R_{m\ H_2O}$ [a]	$\log \frac{1}{I_{50}}$ [b]
1	2-OH	H	0.42	3.08
2	2-OH	NH_2	0.73	3.20
3	2-OH	CH_3	1.10	3.42
4	2-OH	NH-C_6H_5	2.13	4.60
5	2-OH-3,5-Br	NH-C_6H_4-3'-CF_3	3.45	5.80
6	2-OH-3,5-Br	NH-C_6H_4-4'-Br	3.05	5.87

[a] See Table II.
[b] See Table I.

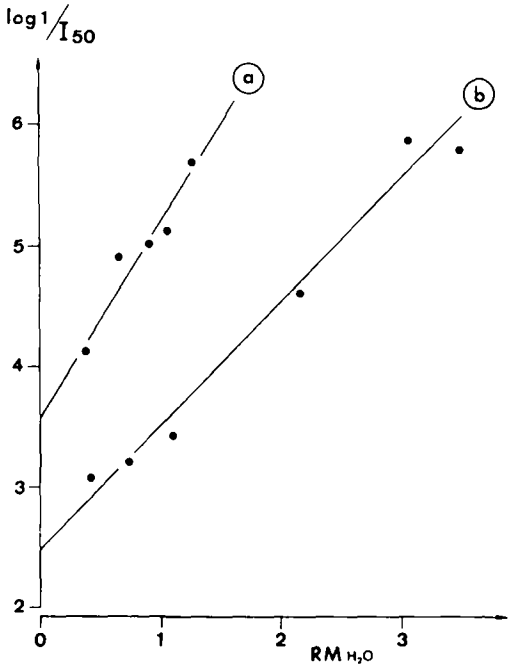


Fig.1. Relation between inhibition of chloride permeability and lipophilic character (quantified by $R_{m\ H_2O}$) of 2,4,6-OH acetophenone derivatives (curve a) and 2-OH acetophenone derivatives (curve b). Least squares regression line is superimposed on the data.

From the data on 2-OH derivatives we have derived Eqn. 9 which also clearly shows that the more lipophilic the substituent in the carbon chain the more active the derivative (Eqn. 9 accounts for 98% of the variability in the data):

$$\text{Log}\ \frac{1}{I_{50}} = 2.50 + 1.01\ R_{m\ H_2O} \quad n = 6 \quad r = 0.989 \quad S = 0.213 \tag{9}$$

This relation is illustrated in Fig. 1, curve b.

Thus, as expected, it appears that at a given constant dipole moment of the different inhibitors there is a highly significant correlation between inhibitory activity and lipid solubility (i.e., membrane concentration) which means, in fact, a correlation between inhibitory activity and interfacial dipole potential.

In summary, the ability of acetophenone derivatives, and among them phloretin, to inhibit chloride permeability quite clearly seems to depend on the capacity they have to alter the interfacial dipole potential. As the magnitude of the potential change induced by a compound is a function of its dipole moment and its membrane concentration, it means that the inhibitory capacity of acetophenone derivatives depends on two physicochemical parameters: the dipole moment and the lipid solubility

of the compound. Every substitution which increases one of these parameters will increase the inhibitory activity.

It is premature to extend this conclusion to the inhibitory action of phloretin analogs on sugar transport. However, the fact that the inhibitory activity is stimulated [2] by the presence of an hydrophobic tail and OH groups in *ortho* position (which greatly increases the dipole moment) seems to indicate that the mechanism of action could be similar.

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References

1 Le Fevre, P.G. (1961) Pharmacol. Rev. 13, 39—70
2 Kotyk, A., Kolinska, J., Veres, K. and Szammer, J. (1965) Biochem. Z. 342, 129—138
3 Wieth, J.O., Dalmark, M., Gunn, R.B. and Tosteson, D.C. (1972) in Erythrocytes, Thrombocytes, Leucocytes (Gerlach, E., Moser, K., Deutsch, E. and Wilmans, W., eds.), pp. 71—76, Georg Thieme Verlag, Stuttgart
4 Deuticke, B. (1975) in Biophysics of Membrane Transport, 2nd Winter School on Biophysics of Membrane Transport, Poland (Gomulkewicz, J. and Tomicki, B., eds.), pp. 177—202, Agricultural University Wroclaw, Poland
5 Macey, R.I. and Farmer, R.E.L. (1970) Biochim. Biophys. Acta 211, 104—106
6 Czech, M.P., Lynn, D.G. and Lynn, W.S. (1973) J. Biol. Chem. 248, 3636—3641
7 Bihler, J., Cavert, H.M. and Fisher, R.B. (1965) J. Physiol. (Lond.) 180, 168—177
8 Levine, S., Franki, N. and Hays, R.M. (1973) J. Clin. Invest. 52, 1435—1442
9 Andersen, O.S., Finkelstein, A., Katz, I. and Cass, A. (1976) J. Gen. Physiol. 67, 749—771
10 Motais, R. and Cousin, J.L. (1976) Am. J. Physiol. 231, 1485—1489
11 Hansch, C. (1973) in Structure-Activity Relationship (Cavallito, C.J., ed.), Vol. 1, pp. 75—165, Pergamon Press, New York
12 Kutter, E. and Hansch, C. (1969) Arch. Biochem. Biophys. 135, 126—135
13 Hansch, C. and Deutsch, E.W. (1966) Biochim. Biophys. Acta 126, 117—128
14 McClellan, A.L. (1963) Tables of Experimental Dipole Moments, W.H. Freeman and Co., San Francisco