Determination of Lipophilic Character of a Series of Dermorphin-Related Oligopeptioes by Means of Reversed-Phase HPLC

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DETERMINATION OF LIPOPHILIC CHARACTER OF A SERIES OF DERMORPHIN-RELATED OLIGOPEPTIDES
BY MEANS OF REVERSED-PHASE HPLC

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In recent times there has been a growing interest in the determination of chromatographic
parameters of lipophilicity with regard to their use in the study of quantitative structu-
re-activity relationship (1, 2). Very good correlations had been shown between the chroma-
tographic parameters and the log P or π values as a measure of the partition coefficient
between octanol and water (2). The reversed phase TLC R_m values in two different chromato-
graphic systems and the reversed-phase HPLC log k' values of a series of dermorphin-rela-
ted oligopeptides have been previously determined (3, 4). The purpose of the present
work was to study the relationship between log k' values on one hand and R_m or $\Sigma\pi$ values
on the other one in view of QSAR studies. In fact the discovery of enkephalin and endor-
phins with high affinities for opioid receptors added new dimensions to the study of
structure-activity relationship of opioid agonists (5, 6, 7, 8).

MATERIALS and METHODS

The test compounds are reported in Table 1 (9, 10). The HOPL reversed-phase chromatography
was performed on a Waters 6000 A chromatograph using a μ Bondapak C_{18} column (300 x 3.9 mm
I.D.) (Waters), packed with Silica Gel (particle size 10 μm) with a C_{18} chemically bonded
non-polar stationary phase. A UV detector (Waters Model 480) at 214 μm and Hamilton 802
chromatographic syringes (25 μl) were also used. the dermorphin derivatives were sepa-
rated using methanol-water mixtures as the mobile phase at a flow-rate of 1 ml/min. The me-
thanol concentration ranged from to 80%. Samples were dissolved in methanol (1 mg/ml) and
applied to the column in 5 μl volumes. The solutions were first filtered to reduce con-
tamination. The experiments were performed at room temperature (20-22 °C). The retention
times were expressed as log capacity factor (k') were $k' = \dfrac{Tx - To}{To}$

The R_m values had been previously determined by means of a reversed-phase TLC technique
(4). A stationary non-polar phase was obtained by impregnating a Silica Gel g layer with
silicone oil DC 200 (350 cS) from Applied Science Laboratories. The polar mobile phase

saturated with silicone oil was an aqueous buffer (sodium acetate-veronal buffer 1/7 M
at pH 7.0) alone or mixed with various quantities of methanol or acetone.
The $\Sigma\pi$ values have been calculated from the data of Hansch et al. (11) and Fauchére
et al. (12).

RESULTS and DISCUSSION

The linear relationship between the TLC R_m values and the composition of the mobile phase
has been previously described as a condition which allows to calculate on extrapolated
R_m value at 0% of organic solvent in the mobile phase (13, 14, 15). The extrapolated R_m
values should be an expression of the partitioning of compounds between silicone oil
and water, i.e. in a standard system where all the compounds could be compared. The li-
near relationship has been described in liquid-liquid partition chromatography (16)
and in reversed-phase HPLC (17). In particular it was pointed out that the extrapolated
hydrophobic parameters are practically independent of the organic solvent in the mobile
phase (17, 4). The plots of Fig. 1 show a linear relationship between the log k' values
and the methanol concentration in the mobile phase. The equations describing such linear
relationship yielded the log k' values at 0% reported in Table 1 are also reported the
R_m values from the two TLC systems previously described (4), as well as the $\Sigma\pi$ values.
The R_m $_{MeOH}$ and R_m $_{Me_2CO}$ values are the intercepts of the equations describing the li-
near relationship between the R_m values and the concentration of methanol and acetone
respectively in the mobile phase (4). A very good correlation is shown by both eq. 1
and 2 between the R_m values from the two TLC HPLC systems and the log k' values from
the HPLC system.

	n	r	s

$$\log k' = 0.553 + 1.117\ R_m\ _{MeOH} \qquad 23 \quad 0.922 \quad 0.464 \quad (1)$$
(F=119.18; P<0.005)

$$\log k' = 0.456 + 1.238\ R_m\ _{Me_2CO} \qquad 23 \quad 0.921 \quad 0.466 \quad (2)$$
(F=118.00; P<0.005)

In both equations the slopes are rather close to 1 and therefore the TLC and HPLC systems
are similar in measuring the lipophilic character of the present series of compounds. It
can be pointed out that the log k' values range from 0.88 to 5.24 with a difference of
4.36 which is much larger than that shown by the R_m values in both TLC systems.
In fact while the R_m values in the methanol system range from 0.94 to 4.06 with a diffe-
rence of 3.12, in the acetone system they range from 0.92 to 4.19 with a difference of
3.27. This should be an advantage of the HPLC data over the R_m values in QSAR studies.
In Table 1 are also reported the $\Sigma\pi$ values as calculated from the data of Hansch (11)
and Fauchére (12). In a previous paper a highly significant relationship has been shown
between the R_m and the $\Sigma\pi$ values (4). As regards the present data eq. 3 shows a rather
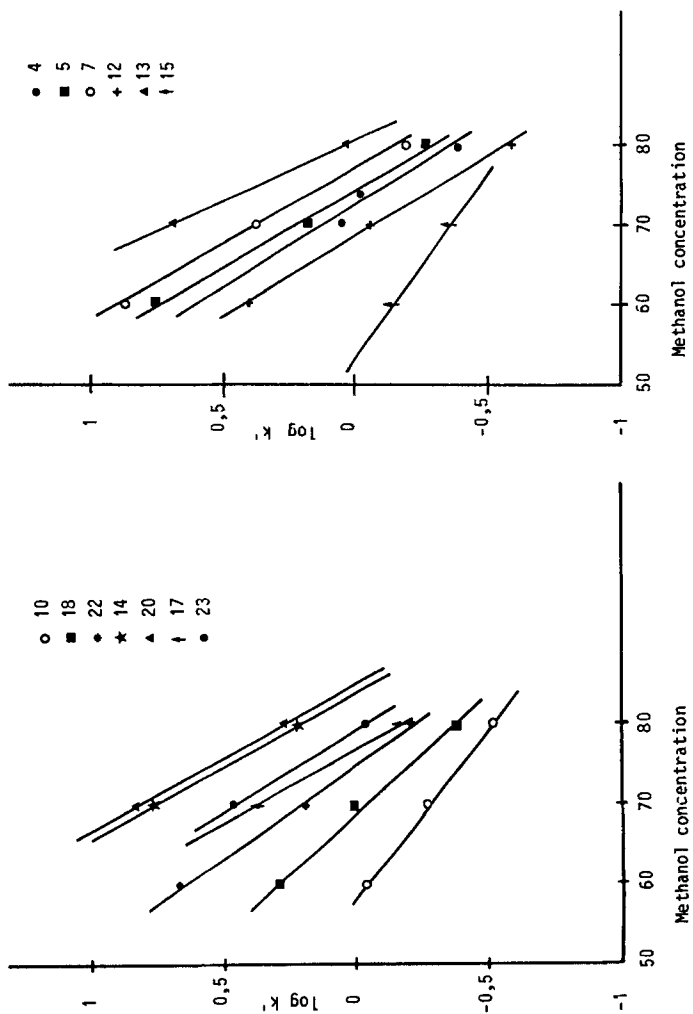lower correlation coefficient between log k' and $\Sigma\pi$ values.

Fig. 1. Relationship between log k' values and methanol concentration in the mobile phase.

Table 1 - log k' R_m and $\Sigma\pi$ values of dermorphin-related oligopeptides

$$H - \underset{\underset{\underset{OH}{|}}{\overset{|}{\underset{NH_2}{|}}}{CH} - \underset{NH_2}{\overset{|}{CH}} - \underset{O}{\overset{\parallel}{C}} - NH - \underset{CH_3}{\overset{|}{CH}} - \underset{O}{\overset{\parallel}{C}} - NH - \underset{CH_2}{\overset{|}{CH}} - \underset{O}{\overset{\parallel}{C}} - NH - R_1 - \underset{O}{\overset{\parallel}{C}} - R_2$$

Tyr   D - Ala   Phe   Gly or β - Ala

Cpd. No.	R_1	R_2	Log k'	R_m Methanol	R_m Acetone	$\Sigma\pi$
1	CH_2	$N\!\!\begin{smallmatrix}H\\H\end{smallmatrix}$	2.35	1.36	1.28	-1.23
2	CH_2	$N\!\!\begin{smallmatrix}H\\CH_2CH_3\end{smallmatrix}$	2.86	1.41	1.44	0.08
3	CH_2	$N\!\!\begin{smallmatrix}CH_2CH_3\\CH_2CH_3\end{smallmatrix}$	2.69	1.95	1.94	1.18
4	CH_2	$N\!\!\begin{smallmatrix}H\\CH_2\text{-}⬡\end{smallmatrix}$	3.56	2.24	2.17	0.78
5	CH_2	$N\!\!\begin{smallmatrix}H\\CH_2CH_3\text{-}⬡\end{smallmatrix}$	3.87	3.09	2.56	1.34
6	CH_2	$N\!\!\begin{smallmatrix}H\\CH\text{-}⬡\\CH_3\end{smallmatrix}$	3.48	3.07	2.75	1.34
7	CH_2	$N\!\!\begin{smallmatrix}CH_3\\CH_2\text{-}⬡\end{smallmatrix}$	4.03	2.93	2.69	1.34
8	CH_2	$N\!\!\begin{smallmatrix}CH_3\\CH_2CH_2\text{-}⬡\end{smallmatrix}$	3.57	2.99	2.73	1.90
9	CH_2	$N\!\!\begin{smallmatrix}H\\◻\end{smallmatrix}$	3.20	2.30	2.23	0.91
10	CH_2	$N\!\!\begin{smallmatrix}H\\CH_2CH_2OH\end{smallmatrix}$	1.40	1.42	1.34	-0.59
11	CH_2	$N\!\!\begin{smallmatrix}H\\CH_2CH_2OCH_3\end{smallmatrix}$	1.77	1.56	1.41	0.06
12	CH_2	$N\!\!\begin{smallmatrix}H\\CH_2CH_2\text{-}⬡\text{-OH}\end{smallmatrix}$	3.43	1.93	2.17	0.67

(continued)

Table 1 (continued)

Cpd. No.	R_1	R_2	log k'	R_m Methanol	R_m Acetone	$\Sigma\pi$
13	CH_2	N-H, adamantyl	5.24	3.73	3.62	2.14
14	CH_2	N-H, CH_2-adamantyl	4.61	4.01	4.19	2.70
15	CH_2	OH	1.18	0.94	0.92	-0.67
16	CH_2	O-CH_2CH_3	2.26	1.45	1.50	0.38
17	CH_2	O-CH_2-⟨O⟩	4.30	3.13	2.59	1.66
18	$(CH_2)_2$	N-H,H	2.35	1.36	1.31	-0.95
19	$(CH_2)_2$	N-H, CH-⟨O⟩, CH_3	3.99	3.14	2.62	1.62
20	$(CH_2)_2$	N-H, adamantyl	4.82	4.06	3.54	2.42
21	$(CH_2)_2$	OH	0.88	1.03	0.96	-0.39
22	$(CH_2)_2$	O-CH_2CH_3	3.30	1.91	1.87	0.66
23	$(CH_2)_2$	O-CH_2-⟨O⟩	4.04	3.09	2.74	1.94

$$\log k' = 2.407 + 0.924\ \Sigma\pi \qquad \begin{array}{ccc} n & r & s \\ 23 & 0.871 & 0.390 \end{array} \quad (3)$$

(F=65.86; P<0.005)

However the slope is close to 1 which should indicate that the HPLC system is very similar to the π system in measuring the lipophilic character of the present series of compounds. The range of the log k' values is still wider than that of the $\Sigma\pi$ values which range from -1.23 to 2.70 with a difference of 3.93.

On the other hand it has been pointed out that the range of the $\Sigma\pi$ values is wider than that of the R_m values in both TLC systems (4). In particular it was pointed out that this was mainly due to the $\Sigma\pi$ values for compounds 1 and 18 which are characterized by an amino group. In the π system the amino group seemed to be more hydrophilic than in the TLC systems. A similar result seems to arise from the present HPLC data. In fact cpds 1 and 18 are among those showing the largest deviations from the linear regression described by eq. 3, in the π system being more hydrophilic than in the HPLC one. When eq. 4 was calculated without cpds 1 and 18 a higher correlation coefficient was obtained.

$$\log k' = 2.095 + 1.140\ \Sigma\pi \qquad \begin{array}{ccc} n & r & s \\ 21 & 0.920 & 0.433 \end{array} \quad (4)$$

(F=104.21; P<0.005)

The slope still close to 1 confirms the similarity of the two systems in measuring the lipophilic character.

In conclusion the log k' values are very well correlated on one hand with the TLC R_m values and on the other one with the calculated $\Sigma\pi$ values. This should show their reliability as an expression of the lipophilic character of drugs in QSAR studies. An advantage of the HPLC procedure at least for the present series of dermorphin-related oligopeptides might be represented by the wider range of the log k' values which should allow better correlation studies with biological data.

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