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R_M VALUES OF XANTHONE DERIVATIVES IN STRUCTURE–ACTIVITY STUDIES

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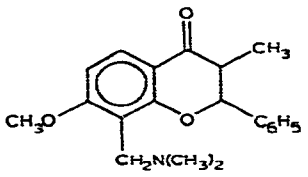
SUMMARY

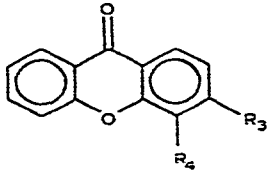
The R_M values of a series of xanthone derivatives obtained in a chromatographic system were correlated with the calculated log *P* values. The relationship between lipophilic character and acute toxicity in mice was also studied. The equations describing the structure–activity relationship indicate the importance of lipophilic character in determining the acute toxicity of xanthone derivatives in mice.

INTRODUCTION

In continuation of research on central nervous system (CNS) stimulating drugs of the benzopyrone series, the most significant of which was dimefline, *i.e.*, 3-methyl-7-methoxy-8-dimethylaminomethylflavone I[1], a series of xanthone derivatives II was synthesized and the pattern of their CNS excitation described[2–5].



I



II

The acute toxicity in mice was considered as a good index of their stimulating power[2–5].

The purpose of the present work was to study the relationship between the chromatographic R_M values of xanthone derivatives and their calculated log *P* values. The relationship between lipophilic character and acute toxicity in mice was also studied.

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EXPERIMENTAL

Determination of R_M values

The basic reversed-phase thin-layer chromatographic (TLC) technique for the determination of R_m values has been described previously[6,7]. The polar mobile phase was glycine buffer (0.1 *M*) of pH 13.0 (ref. 8) alone or in various mixtures with methanol.

Because of their basic character, at pH 13.0 the compounds should be mainly in the undissociated form. On the other hand, preliminary experiments at pH 7.4 or 9.0 provoked migrations characterized by tailing.

The non-polar stationary phase was a silica gel GF_{254} layer impregnated with a 5% (v/v) solution of silicone oil [silicone DC 200 (350 cSt); Applied Science Labs., State College, PA, U.S.A.] in diethyl ether. The concentration of methanol in the mobile phase ranged from 40 to 85%. The xanthone derivatives were dissolved in methanol or ethanol (1 mg/ml) and 1–5 μl volumes were spotted on the plates in random locations. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. The compounds were also visible under an ultraviolet lamp.

Calculation of log P values

The log *P* value of xanthone was calculated as follows

log *P* xanthone = log *P* thioxanthone − (log *P* thiophene − log *P* furan) = 3.99 − (1.81 − 1.34) = 3.52

log *P* xanthone = log *P* thioxanthone − (log *P* benzothiophene − log P benzofuran) = 3.99 − (3.09 − 2.67) = 3.57

$\bar{X}$ = 3.54

where the log *P* values of thioxanthone, thiophene, furan, benzothiophene and benzofuran were taken from Hansch and Leo[9].

The log *P* values of xanthone derivatives were calculated by adding to 3.54 the π values for each substituent, as taken or calculated from Hansch and Leo[9].

Biological data

The log $1/C$ values of Table I, where C is the LD_{50} (m$M \times 10^{-1}$/kg), were determined by an intraperitoneal route in mice[2–5].

RESULTS

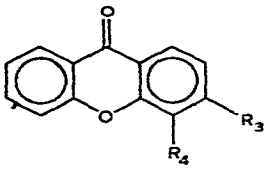
R_M and log P values

In the system 5% silicone oil–glycine buffer none of the compounds migrated when the mobile phase was only buffer. Therefore the addition of methanol was necessary to obtain suitable R_F values for each compound. The range of linear relationship between R_M values and methanol concentration in the mobile phase was used to calculate a theoretical R_M value at 0% methanol (Table I).

The ΔR_M values of Table II were calculated by difference from the R_M values of the compounds indicated in the third column of Table II. The π values were taken or

TABLE I

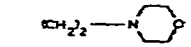
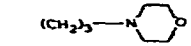
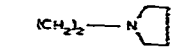
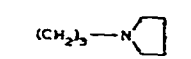
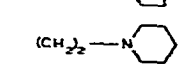
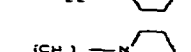
R_M LOG *P* AND LOG 1/*C* VALUES



No.	*Compound*	R_3	R_4	R_M	*log P*	*Log 1/C*
1	Xanthone	H	H	1.84	3.54	–
2	3-Methoxy	OCH_3	H	2.06	3.52	–
3	3-Chloro	Cl	H	2.50	4.25	–
4	4-Morpholinomethyl	H	CH_2—N⟨O⟩	1.65	3.21	–
5	3-Methoxy-4-morpholinomethyl	OCH_3	CH_2—N⟨O⟩	1.85	3.19	1.764
6	3-Chloro-4-morpholinomethyl	Cl	CH_2—N⟨O⟩	1.83	3.92	1.019
7	3-Amino-4-morpholinomethyl	NH_2	CH_2—N⟨O⟩	1.83	1.98	0.921
8	3-Nitro-4-morpholinomethyl	NO_2	CH_2—N⟨O⟩	1.63	2.93	0.628
9	3-Isopropoxy-4-morpholinomethyl	$OCH(CH_3)_2$	CH_2—N⟨O⟩	1.85	3.57	1.623
10	4-Pyrrolidinomethyl	H	CH_2—N⟨ ⟩	2.82	4.56	–
11	3-Methoxy-4-pyrrolidinomethyl	OCH_3	CH_2—N⟨ ⟩	2.87	4.54	1.907
12	3-Chloro-4-pyrrolidinomethyl	Cl	CH_2—N⟨ ⟩	2.86	5.27	1.646
13	3-Amino-4-pyrrolidinomethyl	NH_2	CH_2—N⟨ ⟩	2.69	3.33	2.031
14	3-Nitro-4-pyrrolidinomethyl	NO_2	CH_2—N⟨ ⟩	2.71	4.28	1.951
15	3-Isopropoxy-4-pyrrolidinomethyl	$OCH(CH_3)_2$	CH_2—N⟨ ⟩	2.76	4.92	1.947
16	4-Piperidinomethyl	H	CH_2—N⟨ ⟩	3.10	4.94	–
17	3-Methoxy-4-piperidinomethyl	OCH_3	CH_2—N⟨ ⟩	2.74	4.92	1.708
18	3-Chloro-4-piperidinomethyl	Cl	CH_2—N⟨ ⟩	3.12	5.65	1.084
19	3-Amino-4-piperidinomethyl	NH_2	CH_2—N⟨ ⟩	2.77	3.71	0.990
20	3-Nitro-4-piperidinomethyl	NO_2	CH_2—N⟨ ⟩	3.22	4.66	0.696
21	3-Isopropoxy-4-piperidinomethyl	$OCH(CH_3)_2$	CH_2—N⟨ ⟩	2.76	5.30	1.815
22	4-Dimethylaminomethyl	H	$CH_2N(CH_3)_2$	2.47	3.80	–
23	3-Methoxy-4-dimethylaminomethyl	OCH_3	$CH_2N(CH_3)_2$	2.53	3.78	2.444
24	3-Chloro-4-dimethylaminomethyl	Cl	$CH_2N(CH_3)_2$	2.58*	4.51	2.481
25	3-Amino-4-dimethylaminomethyl	NH_2	$CH_2N(CH_3)_2$	2.16**	2.57	2.200

(Continued on p. 4)

TABLE I *(continued)*

No.	*Compound*	R_3	R_4	R_M	*log P*	*Log 1/C*
26	3-Nitro-4-dimethylaminomethyl	NO_2	$CH_2N(CH_3)_2$	2.22	3.46	2.638
27	3-Isopropoxy-4-dimethylaminomethyl	$OCH(CH_3)_2$	$CH_2N(CH_3)_2$	2.59	4.10	2.538
28	4-Diethylaminomethyl	H	$CH_2N(C_2H_5)_2$	3.02	4.80	–
29	3-Methoxy-4-diethylaminomethyl	OCH_3	$CH_2N(C_2H_5)_2$	2.82	4.78	1.851
30	3-Chloro-4-diethylaminomethyl	Cl	$CH_2N(C_2H_5)_2$	2.69	5.53	2.252
31	3-Amino-4-diethylaminomethyl	NH_2	$CH_2N(C_2H_5)_2$	2.05	3.59	1.818
32	3-Nitro-4-diethylaminomethyl	NO_2	$CH_2N(C_2H_5)_2$	2.96	4.54	1.446
33	3-Isopropoxy-4-diethylaminomethyl	$OCH(CH_3)_2$	$CH_2N(C_2H_5)_2$	2.60	5.18	1.955
34	3-Methoxy-4-β-morpholinoethyl	OCH_3		2.43	3.75	–
35	3-Methoxy-4-γ-morpholinopropyl	OCH_3		2.74	4.31	–
36	3-Methoxy-4-β-pyrrolidinoethyl	OCH_3		3.24	5.10	–
37	3-Methoxy-4-γ-pyrrolidinopropyl	OCH_3		3.85	5.66	–
38	3-Methoxy-4-β-piperidinoethyl	OCH_3		3.48	5.48	–
39	3-Methoxy-4-γ-piperidinopropyl	OCH_3		3.65	6.04	–
40	3-Methoxy-4-β-dimethylaminoethyl	OCH_3	$(CH_2)_2$-$N(CH_3)_2$	2.93	4.34	–
41	3-Methoxy-4-γ-dimethylaminopropyl	OCH_3	$(CH_2)_3$-$N(CH_3)_2$	3.19	4.90	–
42	3-Methoxy-4-β-diethylaminoethyl	OCH_3	$(CH_2)_2$-$N(C_2H_5)_2$	3.07	5.34	–
43	3-Methoxy-4-γ-diethylaminopropyl	OCH_3	$(CH_2)_3$-$N(C_2H_5)_2$	3.37	5.90	–

* The R_M value was calculated by adding to the experimental R_M value of compound 22 the R_M value for the Cl group (see Table II).

** The R_M value was calculated by adding to the experimental R_M value of compound 22 the R_M value for the NH_2 group (see Table II).

calculated from Hansch and Leo[9] as described in Table II.

The log P values reported in Table I were calculated by adding the π values of Table II to the calculated log P value of xanthone, as described in the Experimental section.

Eqn. 1 shows a very good correlation between π and ΔR_M values:

$$\pi = -0.069 + 1.456\, R_M \qquad (F = 124.6;\ P < 0.005) \qquad (1)$$

n	r	s
22	0.928	0.363

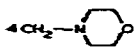
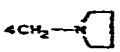
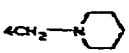
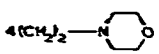
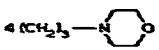
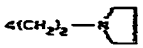
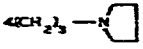
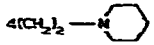
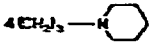
The Cl and NH_2 substituents show the greatest deviations from linearity. However, eqn. 2 calculated without the π and ΔR_M values for the Cl and NH_2 groups is quite similar to eqn. 1:

$$\pi = -0.021 + 1.399\, \Delta R_M \qquad (F = 128.5;\ P < 0.005) \qquad (2)$$

n	r	s
20	0.936	0.310

TABLE II

ΔR_M AND π VALUES

Substituent	ΔR_M	*Calculation of* ΔR_M ††	*Obs.*	*Calc.*	*Obs. – calc.*
$3OCH_3$	−0.005	2-1, 5-4, 11-10, 17-16, 23-22, 29-28	−0.02*	−0.08	0.06
3Cl	0.11	3-1, 6-4, 12-10, 19-16, 30-28	0.71*	0.09	0.62
$3NH_2$	−0.31	7-4, 13-10, 19-16, 31-28	−1.23*	−0.52	−0.71
$3NO_2$	0.05	8-4, 14-10, 20-16, 32-28, 26-22	−0.28*	0.00	−0.28
$3OCH(CH_3)_2$	−0.10	9-4, 15-10, 21-16, 33-28, 27-22	0.36**	−0.21	0.57
	−0.36	4-1, 5-2, 6-3	−0.33***	−0.59	0.26
	0.72	10-1, 11-2, 12-3	1.02§	0.98	0.04
	0.85	16-1, 17-2, 18-3	1.40§§	1.17	0.23
	0.37	34-2	0.23***	0.47	−0.24
	0.68	35-2	0.79***	0.92	−0.13
	1.18	36-2	1.58§	1.65	−0.07
	1.79	37-2	2.14§	2.54	−0.40
	1.42	38-2	1.96§§	2.00	−0.04
	1.59	39-2	2.52§§	2.25	0.27
$4CH_2$	0.47	34-5, 36-11, 38-17, 40-23, 42-29	0.56*	0.61	−0.05
$4(CH_2)_2$	0.80	35-5, 37-11, 39-17, 41-23, 43-29	1.12*	1.10	0.02
$4CH_2N(CH_3)_2$	0.55	22-1, 23-2	0.26§§§	0.73	−0.47
$4(CH_2)_2N(CH_3)_2$	0.87	40-2	0.82§§§	1.20	−0.38
$4(CH_2)_3N(CH_3)_2$	1.13	41-2	1.38§§§	1.58	−0.20
$4CH_2N(C_2H_5)_2$	0.97	38-1, 29-2	1.26†	1.34	−0.08
$4(CH_2)_2N(C_2H_5)_2$	1.00	42-2	1.82†	1.39	0.43
$4(CH_2)_3N(C_2H_5)_2$	1.31	43-2	2.38†	1.84	0.54

* Taken from Table VI-1 of ref. 9.

** Taken from p. 106 of ref. 9.

*** Calculated by adding a π value of 0.56 for each CH_2 group in the case of ethyl or propyl derivatives to the log *P* of morpholine, 4-methyl (see p. 192 of ref. 9).

§ Calculated by adding a π value of 0.56 for each CH_2 group to the log *P* of pyrrolidine (see p. 186 of ref. 9).

§§ Calculated by adding a π value of 0.56 for each CH_2 group to the log *P* of piperidine (see p. 192 of ref. 9).

§§§ The π value of $N(CH_3)_2$ was taken from p. 97 of ref. 9 and then added with a π value of 0.56 for each CH_2 group.

† Calculated from the corresponding $N(CH_3)_2$ derivatives by adding an aliphatic π value of 1.00 for the two CH_3.

†† The pairs of numbers refer to the compounds listed in Table I.

The log P and R_M values of Table I were used in order to calculate eqn. 3

$$\log P = 0.530 + 1.445\ R_M \quad (F = 96.0;\ P < 0.005) \qquad n = 43,\ r = 0.837,\ s = 0.517 \tag{3}$$

which shows a fairly low correlation coefficient, at least when compared with eqn. 1. This is mainly due to the fact that the compounds with the Cl or NH_2 substituents are among those which deviate most from linearity. In fact eqn. 4

$$\log P = 0.870 + 1.327\ R_M \quad (F = 139.3;\ P < 0.005) \qquad n = 32,\ r = 0.907,\ s = 0.354 \tag{4}$$

calculated without the log P and R_M values for the compounds bearing a Cl or NH_2 group explains 82% of the variability in the log P data instead of the 71% of eqn. 3.

One must conclude that in this series of xanthone derivatives the correlation between the experimental R_M values and the calculated log P values is not as high as shown for other series of compounds[10–12]. However, in the case of 5-nitroimidazoles the correlation was even lower and an interaction with the silica gel G layer was suggested[13].

Relationship between R_M values and biological activity

In Table I are reported the log $1/C$ values determined for 25 compounds. The structure–activity relationship is described by eqns. 5 and 6:

$$\log 1/C = 1.408 + 0.130\ R_M \quad (F = 0.239;\ \text{not significant}) \qquad n = 25,\ r = 0.101,\ s = 0.581 \tag{5}$$

$$\log 1/C = -13.526 + 13.096\ R_M - 2.712\ R_M^2 \quad (F = 28.1;\ P < 0.005) \qquad n = 25,\ r = 0.848,\ s = 0.316 \tag{6}$$

The biological activity is parabolically related with the R_M values. In fact the introduction of the R_M^2 term into eqn. 6 significantly improved the correlation coefficient. However, the correlation coefficient of eqn. 6 is not very high. Therefore compound 19, showing the greatest deviation from eqn. 6, was not used in calculating eqn. 7

$$\log 1/C = -14.102 + 13.567\ R_M - 2.800\ R_M^2 \quad (F = 51.3;\ P < 0.005) \qquad n = 24,\ r = 0.911,\ s = 0.243 \tag{7}$$

which shows quite a higher correlation coefficient.

The ideal lipophilic character for the acute toxicity in mice is represented by $R_{M_0} = 2.42$. This is not very far from the $R_{M_0} = 1.82$ previously reported as the ideal lipophilic character for benzodiazepines[10], *i.e.*, for relatively non-specific CNS-active compounds, for which Hansch and Clayton[14] reported log $P_0 \approx 2.00$. However, while in the case of benzodiazepines our R_{M_0} value corresponded to a log P_0 value of 2.50, in the present case substituting the R_{M_0} value into eqn. 4 yields a log P_0 value of

4.08. This is a fairly uncommon log P_0 value for drugs acting in whole animals. The explanation could be that the present xanthone derivatives are basic compounds which should be almost completely ionized at physiological pH[14]. As a consequence the true log P_0 value could be quite lower than the above calculated value of 4.08 and closer to the log $P = 2.52$ previously calculated for benzodiazepines. At the same time the above $R_{M_0} = 2.42$ should be lower and therefore closer to the $R_{M_0} = 1.82$ found for benzodiazepines.

However, the difference between the R_{M_0} values of xanthone derivatives and benzodiazepines (R_{M_0} xanthones $- R_{M_0}$ benzodiazepines $= 2.42 - 1.82 = 0.60$) is smaller than that found between the corresponding log P_0 values (log P_0 xanthones $-$ log P_0 benzodiazepines $= 4.08 - 2.52 = 1.56$). The reason could be the narrower range of the R_M values when compared with that of the log P values. In fact while the R_M values for xanthone derivatives range between 1.63 and 3.85, the log P values range from 1.98 to 6.04.

In conclusion, the lipophilic character of molecules seems to play an important role in determining the acute toxicity of xanthone derivatives in mice. In particular the ideal lipophilic character for xanthone derivatives is fairly close to that of other CNS-active drugs. Finally the usefulness of chromatographic R_M values in structure–activity studies is confirmed.

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