62. 8-Substituted Xanthines as Phosphodiesterase Inhibitors: Conformation-Dependent Lipophilicity and Structure-Activity Relationships[1])

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The 8-substituted xanthines **1**–**21** (including compound S 9795), caffeine (**22**), and the three isomeric dimethylxanthines **23**–**25** (see *Table 1*), were examined for their lipophilic behaviour using a reversed-phase HPLC technique. A number of flexible compounds showed a smaller-than-expected lipophilicity which based on conformational and tautomeric calculations were ascribed to the predominance of folded forms. A QSAR analysis of the phosphodiesterase-inhibitory potency of several compounds showed favourable factors to be a low lipophilicity and the absence of a substituent on the N^7 position.

Introduction. – Xanthine (=3,7-dihydro-1*H*-purine-2,6-dione) derivatives are a class of bioactive compounds the pharmacological profile of which is markedly influenced by the substitution pattern of the xanthine moiety. *N*-Alkyled xanthines such as theophylline, caffeine, and 3-isobutyl-1-methylxanthine are well known as inhibitors of cyclic nucleotide phosphodiesterase activity [1]. It has been shown that 7-alkyl, 7-arylalkyl or 8-alkyl derivatives of 3-isobutyl-1-methylxanthine are compounds with much increased inhibitory potency relative to theophylline. In addition, such compounds are relatively selective for calmodulin-stimulated phosphodiesterase activity in smooth muscles. Promising antiasthmatic activity has been obtained with novel 1,3,7,8-substituted xanthines which demonstrated a potent and even long-lasting (> 48 h) antibronchoconstrictive effect in guinea pigs [2].

Structure-activity relationships are not readily apparent in this series of 1,3,7,8-substituted xanthines and are the object of the present study. Using a classical reversed-phase(RP) HPLC technique, the lipophilicity of twenty-one 1,3,7,8-substituted xanthines was measured and compared with calculated partition coefficient values. Relationships were sought between structural properties and inhibition of rat brain phosphodiesterase activity [2].

Materials and Methods. – *Compounds.* The 8-substituted xanthines **1**–**21** used in this study (*Table 1;* compound **1** is also known as S 9795) were kindly supplied by the *Institut de Recherches Servier* (Suresnes, France). Their synthesis has been described [2]. The compounds were of pharmaceutical purity and used without further purification. Anal. grade MeOH, 3-morpholinopropanesulfonic acid (MPS), and methylated xanthines of pharma-

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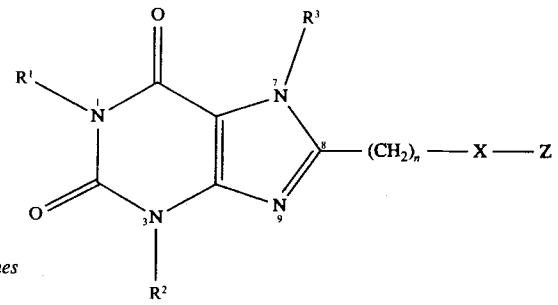

Table 1. *Investigated 1,3,7,8-Substituted Xanthines*

	R^1	R^2	R^3	*n*	X	Z
1	Me	i-Bu	H	2	Piperazine-1,4-diyl	Ph_2CH
2	Me	i-Bu	H	2[a])	Piperazine-1,4-diyl	Ph_2CH
3	H	i-Bu	H	2	Piperazine-1,4-diyl	Ph_2CH
4	Me	i-Bu	Me	2	Piperazine-1,4-diyl	Ph_2CH
5	Me	Me	H	2	Piperazine-1,4-diyl	Ph_2CH
6	Me	i-Bu	H	2	Piperazine-1,4-diyl	H
7	Me	i-Bu	H	2	Piperazine-1,4-diyl	$(4\text{-F}-C_6H_4)_2CH$
8	Me	i-Bu	Me	2	Piperazine-1,4-diyl	$(4\text{-F}-C_6H_4)_2CH$
9	Me	i-Bu	H	1	Piperazine-1,4-diyl	Ph_2CH
10	Me	Me	H	1	Piperazine-1,4-diyl	Ph_2CH
11	Me	Me	H	3	Piperazine-1,4-diyl	Ph_2CH
12	Me	Me	Me	3	Piperazine-1,4-diyl	Ph_2CH
13	Me	i-Bu	Me	3	Piperazine-1,4-diyl	$(4\text{-F}-C_6H_4)_2CH$
14	Me	Ph	Me	3	Piperazine-1,4-diyl	Ph_2CH
15	Me	Pr	Me	3	Piperazine-1,4-diyl	Ph_2CH
16	Et	i-Bu	H	3	Piperazine-1,4-diyl	Ph_2CH
17	Et	i-Bu	Me	3	Piperazine-1,4-diyl	Ph_2CH
18	Me	i-Bu	H	3	Piperazine-1,4-diyl	Ph_2CH
19	Me	i-Bu	Me	3	Piperazine-1,4-diyl	Ph_2CH
20	Me	i-Bu	H	2	Piperidine-1,4-diyl	Ph_2CH
21	Me	i-Bu	H	2	$-N(Et)CH_2CH_2N(Et)-$	$(4\text{-F}-C_6H_4)_2CH$

[a]) $CH_2CH(OH)$.

ceutical purity (caffeine (= 1,3,7-trimethylxanthine; **22**), theophylline (= 1,3-dimethylxanthine; **23**), theobromine (= 3,7-dimethylxanthine; **24**), and paraxanthine (= 1,7-dimethylxanthine; **25**)) were obtained from commercial sources.

Determination of log k_w^o *Values by RP-HPLC.* A *Siemens S101* chromatograph equipped with an *Orlita DMP-AE* 10.4 pump was used. The detector was a *Uvikon 740 LC* (*Kontron*, Zurich, Switzerland) operating at 254 nm; the column (25 cm × 4 mm i.d.) was prepacked with *Lichrosorb RP-18*, particle size 10 μm (*Knauer*, Bad Homburg, FRG). A *Hewlett-Packard-3390A* integrator was used for peak registration and calculation of retention times. The mobile phases were 10–80% MeOH/H_2O *(v/v)* mixtures buffered with MPS (0.02M, pH 7.4). Decylamine (0.2%, *v/v*) was added as a masking agent to eliminate silanophilic interactions [3]. All eluent solutions were degassed by ultrasonication and filtered under vacuum using a *Millipore-Q* system. Retention times (t_R) were measured at r.t., the flow rate was 1.5 ml/min, and the column dead time (t_0) was determined using MeOH as the non-retained compound. The isocratic capacity factor (k_i) is defined as

$$k_i = (t_R - t_o)/t_o \quad (1)$$

Such capacity factors (log k_i) determined at various MeOH/H_2O ratios were extrapolated to 100% H_2O (log k_w values) and corrected for solute ionization (linear extrapolation to 100% neutral species, log k_w^o values). Further experimental details can be found in previous works [3] [4].

log P *Calculations.* Octanol/H_2O partition coefficients were estimated as log P_{calc} values starting from the experimental log *P* value of theophylline (**23**; log $P = -0.02$ [6]) or caffeine (**22**; log $P = -0.07$ [6] [7]) depending on the *N*-alkylation pattern of the compound, and adding *Rekker's* hydrophobic fragmental constants and proximity correction factors [5]. The value for the piperazine ring was taken as -1.52 [8].

Determination of Ionization Constants. Solns. (final concentration 7.5×10^{-4} M) were prepared in distilled H_2O which had been boiled to remove O_2 and CO_2 and saturated with N_2. The ionic strength was fixed at 0.1M using KCl. An excess of HCl was added, and the solns. were back-titrated with 0.01N NaOH using a *Metrohm 670* titroprocessor. Titration curves were determined in triplicate for each compound and the pK_a calculated using a non-logarithmic linearization of the titration curve proposed by *Benet* and *Goyan* [9] and modified by *Leeson* and *Brown* [10] to overcome the problem of dilution during titration. The temp. was 21 ± 1°.

For 8 compounds which proved poorly soluble, pK_a values were determined from solubility data at 21 ± 1°. For each compound, 5–7 buffer solns. (1/15M phosphate) were prepared in the pH range 3.5–7.5. These solns. were saturated by addition of an excess of the compound and vigorously shaken for 3 h. The pH was then measured, the suspension filtered, and the solute concentration measured by RP HPLC under the following conditions: 25 cm × 4 mm column packed with *Lichrosorb RP-18*, particle size 10 μm; eluent: buffer (MPS; 0.02M, pH 7.4)/MeCN in various proportions (4:6 *(v/v)* for **2**, **3**, and **10**; 3:7 for **11**, **12**, and **19**; 1:3 for **1** and **9**); flow rates 1 ml/min (**2**, **3**, and **10**), 2 ml/min (**11**, **12**, and **19**), or 1.5 ml/min (**1** and **9**); detection at 254 nm. The pK_a values were then calculated according to *Zimmerman* [11].

Biochemical Assays. Phosphodiesterase-inhibition data were taken from *Regnier et al.* [2]. Briefly, phosphodiesterase activity was determined in brain preparations from adult *Sprague-Dawley* rats according to the spectrophotometric method of *Butcher* and *Sutherland* [12].

Structural Calculations. Molecular modeling was performed using the SYBYL software *(Tripos Associates)* running on a *VAX-8550* computer. The standard *Tripos* force field [13] [14] was used. The search option on SYBYL permitted the simultaneous conformational minimization of 2, 3, or 4 single bonds depending on the chain length between the xanthine and the piperazine rings. Determination of the minimal energy of the tautomers of **1** was made using full geometry optimization with the semi-empirical AM1 quantum mechanical method [15] (program AMPAC, QCPE No. 506) also running on a *VAX-8550* computer.

Molecular and H_2O-accessible surface areas were calculated with the program MOLSV (QCPE No. 509) running on an *Apollo DN 3000* workstation. *Van der Waals* radii were taken from [16], and the H_2O molecule was assumed to be a sphere of radius 1.5 Å. The H_2O-accessible surface area was calculated by the program as being the ensemble of all the points occupied by the centre of the H_2O sphere when it rolls on the molecular surface.

Results and Discussion. – *p*K_a *Values.* The investigated xanthines **1**–**21** behave as relatively weak bases and display pK_a (in fact, pK_{a1}) values in the range 5.2–7.9, in agreement with literature data for 1,2-dialkylated piperazines (see *Table 2*). Removing the benzhydryl moiety to yield compound **6** markedly increases the pK_a. Overall, compounds with $n = 3$ appear to have slightly higher pK_a values than their lower homologues, compound **18** being an unexplained exception. However, seeking further relationships between pK_a variations and structural features would be meaningful only if the actual site(s) of protonation (*i.e.* micro pK_a values) were known.

Comparison Between log k_w^o *and log* P *Values.* Four xanthines were included in this study in addition to the 8-substituted derivatives **1**–**21** *(Table 1)*, namely caffeine (**22**) and the three isomeric dimethylxanthines **23**–**25**. From the experimentally determined pK_a values and apparent lipophilicity at pH 7.4, the lipophilicity of the neutral species were calculated as log k_w^o *(Table 2)*. Due to the weak basic or acidic character of the compounds, the correction for ionization is very small or negligible in most cases.

Octanol/H_2O partition coefficients were calculated according to *Rekker's* fragmental method [5] as explained in the *Exper. Part* (see *Table 2*). There are abundant reports in the literature exemplifying excellent correlations between retention on RP-HPLC (log k_w^o values) and measured or calculated octanol/H_2O partition coefficients (log *P*) (*e.g.* [4]

Table 2. *Physicochemical and Biological Properties of Compounds* **1**–**25**

	$\log k_w$[a])	$\log k_w^o$[b])	$\log P_{calc}$[c])	pK_a[e])	pIC_{50}[k])
1	4.01	4.02	4.89	6.0[f])	5.221
2	5.21	5.21	3.82	5.6[f])	not det.
3	4.58	4.58	4.41	5.2[f])	5.000
4	5.58	5.62	4.84	6.4[g])	4.220
5	4.17	4.18	3.34	5.6[g])	not det.
6	1.32	1.91	1.15	7.9[g])	not det.
7	4.16	4.16	5.31	5.2[g])	5.013
8	4.96	4.97	5.26	6.1[g])	3.795
9	5.23	5.23	4.38	5.4[f])	not det.
10	3.88	3.88	2.82	5.3[f])	not det.
11	3.67	3.73	3.86	6.6[g])	not det.
12	3.92	3.94	3.81	6.1[f])	4.086
13	4.80	4.81	5.78	5.6[g])	3.744
14	4.23	4.27	4.95	6.8[g])	4.427
15	5.35	5.39	4.84	6.4[g])	4.279
16	4.90	5.02	5.93	6.9[g])	4.397
17	4.72	4.74	5.88	6.2[g])	< 4
18	4.29	4.29	5.41	5.4[g])	5.387
19	4.89	4.93	5.36	6.3[f])	4.309
20	5.05	5.10	6.74	6.5[g])	4.301
21	5.79	5.88	6.71	6.7[g])	4.036
22 (Caffeine)	0.94	0.94	–0.07[d])	0.6[h])	not det.
23 (Theophylline)	1.10	1.11	–0.02[d])	8.6[i])	not det.
24 (Theobromine)	0.38	0.41	–0.78[d])	10.0[i])	not det.
25 (Paraxanthine)	0.95	0.97	–0.22[d])	8.5[i])	not det.

[a]) Apparent lipophilicity at pH 7.4, measured by RP HPLC.
[b]) Lipophilicity, measured by RP HPLC and corrected for ionization.
[c]) log *P* (octanol/H_2O), estimated according to *Rekker* [5].
[d]) Experimental log *P* (octanol/H_2O) values, taken from [6] [7].
[e]) pK_a of the base.
[f]) Determined using solubility data.
[g]) Determined by titration.
[h]) Weak base pK_a, taken from [17].
[i]) Weak acid pK_a, taken from [17].
[k]) Brain phosphodiesterase inhibition data, taken from [2]; not det. = not determined.

[18–20]). However, these relationships have been established for relatively small and rigid compounds, whereas the present study deals with comparatively large and predictably flexible compounds *(Table 1)*. In such cases, the correlation may be of lesser quality (*e.g.* [21]).

For the 25 compounds in *Table 2*, the relationship between calculated log *P* values and experimental log k_w^o values is expressed by *Eqn. 2* (95% confidence limits in parentheses):

$$\log P_{calc} = 1.30(\pm 0.22) \log k_w^o - 1.22(\pm 0.95) \quad (2)$$

$$n = 25; r = 0.929; s = 0.840; F = 146$$

Superficially, such a correlation appears reasonable, but a closer look at the data *(Fig. 1)* reveals their dissymmetric distribution which artificially 'boosts' the correlation coeffi-

cient. For the twenty lipophilic derivatives (*i.e.* for **1**–**5** and **7**–**21**), the correlation is a very poor one ($r = 0.53$), but a close inspection of the data in *Table 2* shows that a number of compounds have log k_w° values that are either similar to or somewhat larger than log P_{calc} values, whereas other compounds display an experimental lipophilicity which is markedly smaller than the calculated one. Compounds in the former group have at least one of the following structural features (see *Table 1*): *i)* $n = 1$ (**9**, **10**); *ii)* $n = 2$ and an OH group in the side-chain (**2**), a H–N(1) (**3**), a substituent at N(3) smaller than isobutyl (**5**), or a Me–N(7) (**4**, **8**); *iii)* $n = 3$ and a substituent at N(3) smaller than isobutyl (**11**, **12**, **15**). In contrast, compounds in the latter group have the following characteristics: *i)* $n = 2$ and the simultaneous presence of Me–N(1), i-Bu–N(3), H–N(7) (**1**, **7**); *ii)* $n = 3$ and a substituent at N(3) being isobutyl or larger (**13**, **14**, **16**–**21**).

For the ten compounds of the former group, the correlation between log P_{calc} and log k_w° has a coefficient $r = 0.718$. Adding the five more hydrophilic compounds **6**, **22**–**25** yields *Eqn. 3:*

$$\log P_{calc} = 1.11(\pm 0.15) \log k_w^\circ - 1.11(\pm 0.59) \quad (3)$$

$$n = 15; r = 0.976; s = 0.487; F = 256$$

In contrast, the ten compounds in the latter group above yield *Eqn. 4:*

$$\log P_{calc} = 1.00(\pm 0.47) \log k_w^\circ + 0.98(\pm 2.24) \quad (4)$$

$$n = 10; r = 0.866; s = 0.343, F = 24.0$$

Graphical representation of *Eqns. 2–4 (Fig. 1)* suggests that *Eqn. 3* corresponds to compounds displaying a 'normal' (*i.e.* predictable) lipophilicity, whereas *Eqn. 4* corresponds to ten compounds whose lipophilicity is smaller than predicted by *ca.* 1.5 log *P* unit (mean distance between *lines 2* and *3* in *Fig. 1*).

The origin of such a marked reduction in lipophilicity is intriguing. *Gaspari* and *Bonati* [7] have shown that in *N*-methylated xanthines, the three Me groups make different contributions to the lipophilicity of the molecule (*i.e.* marked, minute, and nil

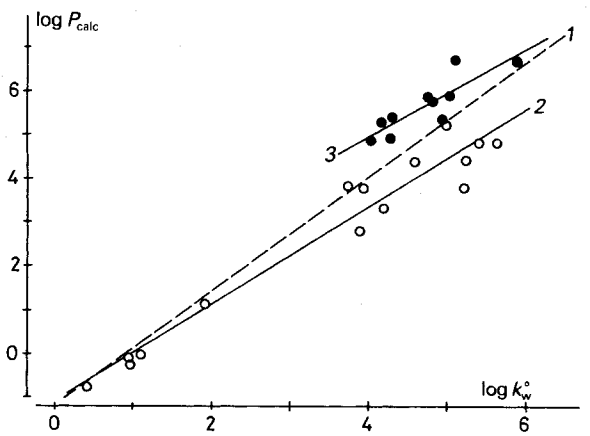

Fig. 1. *Plot of experimental* (log k_w°) vs. *calculated* (log *P*) *lipophilicity values. Line 1* corresponds to *Eqn. 2* (all 25 compounds), *Line 2* to *Eqn. 3* (○; $n = 15$), and *Line 3* to *Eqn. 4* (●; $n = 10$).

for Me–N(1), Me–N(3), and Me–N(7), resp.). Electronic and intermolecular H-bonding effects were postulated [7]. While such effects may be operative in the compounds studied here, their contribution is partial at best. The case of **2** is particularly spectacular: introducing an OH group into compound **1** to yield **2** increases the lipophilicity by 1.2 units when a 1.1-units *decrease* is expected *(Table 2)*. Similarly, methylating compound **1** at N(7) to obtain **4** should have a negligible effect on lipophilicity, while a marked increase (1.6 units) is seen. Note that the same structural change elicits smaller lipophilicity increases in the pairs **7/8** and **18/19** and quite modest changes in the pairs **11/12** and **16/17**. The loss of the benzhydryl moiety in compound **1** to yield **6** should be accompanied by a very large lipophilicity decrease (3.7 units), while a relatively small one is seen (2.1 units). Another example is the replacement of i-Bu–N(3) by a Me–N(3); the expected decrease in lipophilicity is seen with the pairs **9/10**, **19/12**, and **18/11**, while the pair **1/5** shows an unexpected increase in lipophilicity. And finally, side-chain shortening in the pair **1/9** is not accompanied by the expected decrease in lipophilicity (0.5 units), but by a marked increase (1.2 units), whereas the pair **5/10** behaves as expected.

Interpretation of the above deviations is not straightforward. Certainly, intramolecular electronic effects of the type discussed by *Gaspari* and *Bonati* [7] do not afford a suitable explanation. Conformational effects, thus, appear as the only alternative mechanism able to account, at least in part, for the unexpectedly low lipophilicity of several compounds in *Table 2* [19]. To examine the possibility of such effects, the conformational behaviour and H_2O-accessible surface area of compound **1** were investigated as described below.

Conformational Analysis. Molecular models show that compounds **9** and **10**, which have one C-atom between the xanthine and piperazine rings, have comparatively limited flexibility and preferably exist in extended conformations. In contrast, the homologues with two or three C-atoms between the xanthine and piperazine rings are much more flexible and can exist as folded (compact) and extended conformers. Indeed, preliminary calculations revealed the existence of 20 or 30 conformations in the range 0–2 kcal/mol, respectively.

In addition, N^7-unsubstituted xanthines can undergo prototropic tautomerism to N(9)H tautomers. The full geometry of both tautomers of compound **1** (S 9795) was optimized by the semi-empirical MO method AM1, and their conformational behaviour was then calculated by a force-field method. In the extended conformers **1A** and **1C**, interactions between the side-chain and the xanthine part of the molecule are minimized, and the relative energies of the N(7)H and N(9)H tautomer (*Fig. 2*, **1A** and **1C**, resp.; *Table 3*) indicate the former to be intrinsically more stable than the latter. In the folded conformers **1B** and **1D** (compact forms), nonbonded intramolecular interactions can occur between the xanthine moiety and the 8-substituent. The folded N(7)H tautomer **1B**

Table 3. *Properties of the Conformers and Tautomers of Compound* **1** *Shown in* Fig. 2

Isomers	Heat of formation [kcal/mol]	Molecular surface area [Å^2]	H_2O-accessible surface area [Å^2]
1A	56.5	643	936
1B	59.9	641	901
1C	60.2	631	946
1D	56.1	620	873

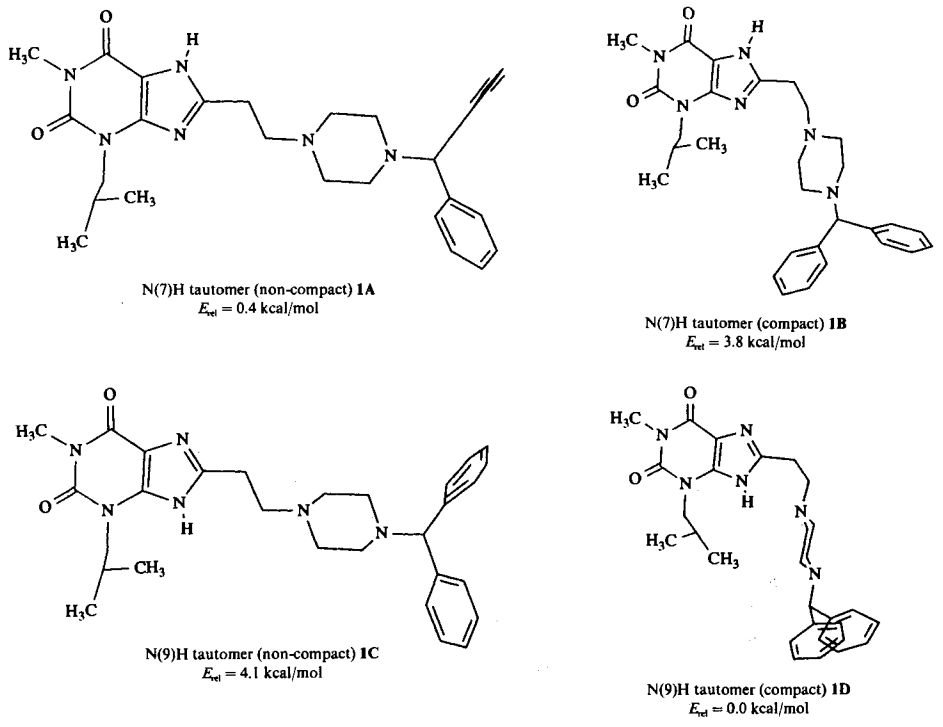

Fig. 2. *Relative energies of the N(7)H* (**1A** and **1B**) *and N(9)H* (**1C** and **1D**) *tautomers in their extended* (**1A** and **1C**) *and folded* (**1B** and **1D**) *conformations.* Form **1D** represents the global energy minimum of compound **1**, forms **1A**, **1B**, and **1C** represent local minima.

corresponding to a local energy minimum is characterized by an energy of 3.8 kcal/mol above the global minimum which indicates unfavourable interactions between the side-chain and the rest of the molecule.

The N(9)H tautomer **1D** in the folded conformation represents the global energy minimum, as stabilized by attractive nonbonded interactions which overcome the unfavourable tautomeric form. *Fig. 2* reveals these interactions to be an intramolecular H-bond and weak attractive forces (presumably *Van der Waals* forces) between the benzhydryl and isobutyl moieties. Space-filling representations (not shown) indicate a close fit and suggest that hydrophobic forces must also exist in aqueous solution between the benzhydryl and isobutyl moieties, but our calculations cannot take this contribution into account.

Conformational calculations with analogues displaying 3 C-atoms between the xanthine and piperazine rings yielded comparable results (not shown). In addition, space-filling representations indicate that in folded forms (and independently of the tautomeric form) an N^3-alkyl group of sufficient bulk can fit into a pocket created by the benzhydryl moiety, suggesting a marked stabilization by *van der Waals* and predominantly hydrophobic forces. Such a compact conformation is shown in *Fig. 3* for compound **19**.

Lipophilicity and Conformation of the Investigated Xanthines: a Structural Interpretation. Our calculations and rationalizations suggest that folded (compact) conforma-

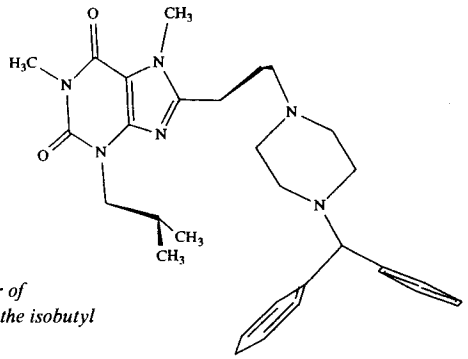

Fig. 3. *Low-energy compact conformer of compound* **19**, *showing the fit between the isobutyl and benzhydryl groups*

tions are stabilized by the following intramolecular interactions: *i)* When $n = 2$ (see *Table 1*), by a H-bond between N(7)H and the proximal N in the piperazine ring and by hydrophobic and *Van der Waals* forces between a sufficiently large N^3-alkyl group and the benzhydryl moiety. *ii)* When $n = 3$, by hydrophobic and *van der Waals* forces between a sufficiently large N^3-alkyl group and the benzhydryl moiety.

These intramolecular interactions are precisely those which can occur in compounds **1**, **7**, **13**, **14**, and **16–21** (*i.e.* those compounds whose lipophilicity is described by *Eqn.* 4), but not in compounds **2–5**, **8–12**, and **15** (which belong to *Eqn. 3*). We are thus led to postulate that the 'deviant' (smaller-than-expected) lipophilicity of the former ten compounds is due to the existence of folded (compact) conformers of relatively low energy, while the ten compounds in the second group lack the structural features necessary to stabilize folded forms.

A smaller-than-predicted lipophilicity of folded forms can be explained (at least in part) by their decreased H_2O-accessible surface area. As shown in *Table 3*, the molecular surface area of the folded forms **1B** and **1D** is 0.3 and 1.7% smaller than that of the extended forms **1A** and **1C**, respectively, while the H_2O-accessible surface areas are 5.5 and 7.7% smaller. The decrease in H_2O-accessible surface area mainly affects the apolar parts of the molecule, and its relevant effect in the aqueous phase is to render the compound less hydrophobic. In the organic phase, lipophilic fragments in folded forms are partly masked from the solvent and thus unable to fully express their lipophilicity. A few similar cases are documented in the literature (reviewed in [19]). For example, we have shown that in some flexible molecules, the free energy of transfer of a CH_2 group from H_2O to dibutyl ether is normally 2 kJ/mol, but is dimished to *ca.* 1 kJ/mol when the side chain is maintained in a folded conformation by an internal H-bond [22].

Chemical changes which destabilize folded conformers result in a better solvent exposure of lipophilic groups and hence to a 'normal' (predictable) lipophilicity. In this way, the unexpected changes in lipophilicity discussed above (*e.g.* between compounds **1** and **2**, **1** and **4**, **1** and **6**, **1** and **9**) can be understood and ascribed to an 'unfolding' upon chemical modification.

QSAR of Rat Brain Phosphodiesterase Inhibition. Phosphodiesterase-inhibition potency of 15 xanthines out of the 21 compounds in *Table 1* has been reported [2] and can be found as pIC_{50} values in *Table 2*. The value for **17** is not usable in a QSAR analysis and

will be discarded. A total of 14 observations was thus available, *i.e.* a set of seven compounds with H–N(7), and a set of seven compounds with Me–N(7).

Plotting pIC_{50} values against log k_w° values *(Fig. 4)* suggested a distinct structure-activity relationship for the H–N(7) and Me–N(7) sets. For the former set, a good linear relationship exists between experimental lipophilicity and activity:

$$pIC_{50} = -0.731(\pm 0.329) \log k_w^\circ + 8.21(\pm 1.57) \quad (5)$$

$$n = 7;\ r = 0.931;\ s = 0.206;\ F = 32.5$$

For these compounds, and within the explored property space, activity increases with decreasing lipophilicity. In contrast, the compounds in the Me–N(7) set display activities that are completely independent from their lipophilicity, and a line with zero slope and total lack of statistical significance can be calculated. Thus, the most active compounds in *Table 2* are characterized both by a relatively low lipophilicity (log $k_w^\circ < 5$) and by the presence of an H–N(7) group. The H–N(7) group ceases to be a favourable feature when lipophilicity reaches values of log $k_w^\circ > 5$. For these compounds of higher lipophilicity, no difference in activity exists between the H–N(7) and Me–N(7) analogues, but it is impossible from *Fig. 4* to know whether the poor activity of the more lipophilic Me–N(7) analogues is due to their lipophilicity and/or the presence of Me–N(7) group.

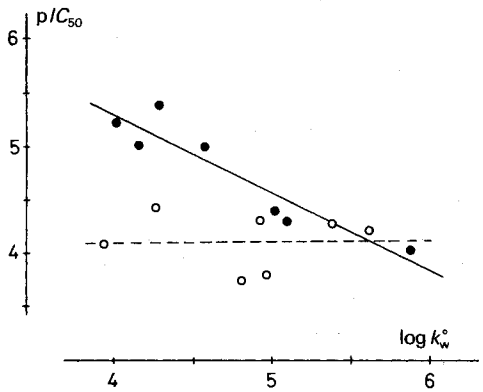

Fig. 4. *Plot of the experimental lipophilicity* (log k_w°) vs. $p\,IC_{50}$ (inhibition of phosphodiesterase) *values.* The full line (—●—) corresponds to *Eqn. 5* and describes the compounds with H–N(7). The broken line (– – ○ – –) is purely indicative and corresponds to the compounds with Me–N(7).

Note that among the four most active compounds, three (namely **1**, **7**, and **18**) have a smaller-than-expected experimental lipophilicity, while one (compound **3**) has identical log k_w° and log P_{calc} values. This indicates that lipophilicity as such, and not conformation, influences activity.

In the above analysis, log k_w° rather than log k_w values were used simply to allow direct comparison with an analysis based on log P_{calc} values (see below). In the present series of compounds, the log k_w° and log k_w values are so similar that they lead to identical correlations (not shown) and allow no conclusion regarding the relative biological role of the protonated and unprotonated species.

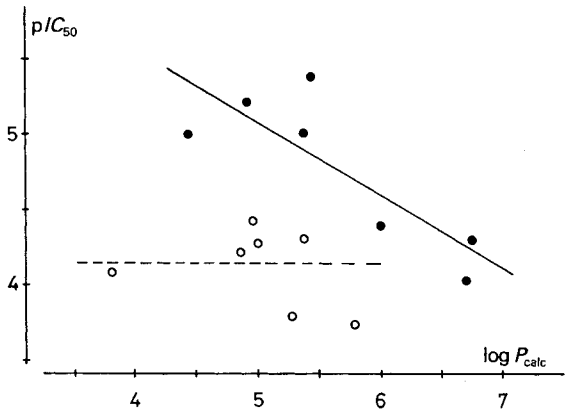

Fig. 5. *Same plot as* Fig. 4, *but for calculated lipophilicity values* (log *P*). The full line is characterized by $r = 0.83$.

A plot of pIC_{50} *vs.* log P_{calc} values *(Fig. 5)* proved to be quite revealing. Indeed, a relationship comparable to *Eqn. 5* but of smaller significance ($r = 0.83$) exists for the H–N(7) set. In addition, the Me–N(7) set again shows a lack of dependence from lipophilicity. However, the two sets are well separated in *Fig. 5*, and the Me–N(7) analogues now appear poorly active despite a favourable lipophilicity. This leads to the unambiguous conclusion that a Me–N(7) is the sole cause of the poor activity of the N^7-methyl analogues. Thus, calculated and experimental lipophilicity values lead to diverging conclusions regarding the biological significance of the N^7-methyl group. This revealing example illustrates the danger of reaching misleading conclusions when basing QSAR studies on calculated log *P* values only.

Conclusion. – This study suggests that in large and flexible molecules, the conformational behaviour is a feature able to markedly influence lipophilicity. Reliable lipophilicity data are indispensable if meaningful conclusions are to be reached, and the reversed-phase HPLC technique again confirms its value. Comparison with calculated log *P* values was the approach followed here to detect compounds displaying a smaller-than-predicted lipophilicity which could then be explained in terms of the predominance of folded (compact) conformations able to decrease hydrophobicity in H_2O and lipophilicity in organic phases. The investigated compounds are thus believed to illustrate the import of self-coiling of organic molecules on their hydrophobic-lipophilic interactions, as cogently discussed by *Jiang* [23].

The compounds investigated are inhibitors of phosphodiesterase, and published data on their inhibitory potency were applied to a QSAR investigation. Structural properties contributing to a increased potency were shown to be a comparatively low lipophilicity and, only for the less lipophilic compounds, the presence of an unsubstituted N^7 position. The use of calculated log *P* values led to a misleading structure-activity rationalization.

With other studies of this type (*e.g.* [24]), this work should contribute to a better understanding of the molecular properties of synthetic xanthine derivatives and of the structure-activity relationships of phosphodiesterase inhibitors, a class of agents of considerable therapeutic potential.

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REFERENCES

[1] J.N. Wells, J.E. Garst, G.L. Kramer, *J. Med. Chem.* **1981,** *24,* 954.
[2] L.G. Regnier, C.G. Guillonneau, J.L. Duhault, F.P. Tisserand, G. Saint-Romas, S.M. Holstorp, *Eur. J. Med. Chem.* **1987,** *22,* 243.
[3] N. El Tayar, H. van de Waterbeemd, B. Testa, *J. Chromatogr.* **1985,** *320,* 293.
[4] D. Reymond, G.N. Chung, J.M. Mayer, B. Testa, *J. Chromatogr.* **1987,** *391,* 97.
[5] R.F. Rekker, H.M. de Kort, *Eur. J. Med. Chem.* **1979,** *14,* 479.
[6] A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* **1971,** *71,* 525.
[7] F. Gaspari, M. Bonati, *J. Pharm. Pharmacol.* **1987,** *39,* 252.
[8] J.M. Mayer, H. van de Waterbeemd, B. Testa, *Eur. J. Med. Chem.* **1982,** *17,* 17.
[9] L.Z. Benet, J.E. Goyan, *J. Pharm. Sci.* **1967,** *56,* 665.
[10] L.J. Leeson, M. Brown, *J. Pharm. Sci.* **1966,** *55,* 431.
[11] I. Zimmerman, *Int. J. Pharm.* **1983,** *13,* 57.
[12] R.H. Butcher, E.W. Sutherland, *J. Biol. Chem.* **1968,** *237,* 1244.
[13] I. Motoc, R.A. Dammkoelher, D. Mayer, J. Labanowski, *Quant. Struct.-Act. Relat.* **1986,** *5,* 99.
[14] J. Labanowski, I. Motoc, C.B. Naylor, D. Mayer, R.A. Dammkoehler, *Quant. Struct.-Act. Relat.* **1986,** *5,* 138.
[15] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, *J. Am. Chem. Soc.* **1985,** *107,* 3902.
[16] B. Testa, P. Seiler, *Arzneim.-Forsch. (Drug Res.)* **1981,** *31,* 1053.
[17] D.W. Newton, R.B. Kluza, *Drug Intell. Clin. Pharm.* **1978,** *12,* 546.
[18] N. El Tayar, H. van de Waterbeemd, B. Testa, *Quant. Struct.-Act. Relat.* **1985,** *4,* 69.
[19] H. van de Waterbeemd, B. Testa, in 'Advances in Drug Research', Ed. B. Testa, Academic Press, London, 1987, Vol. 16, pp. 85–225.
[20] Th. Braumann, *J. Chromatogr.* **1987,** *373,* 191.
[21] N. El Tayar, H. van de Waterbeemd, B. Testa, *J. Chromatogr.* **1985,** *320,* 305.
[22] Ch. Repond, J.M. Mayer, H. van de Waterbeemd, B. Testa, W. Linert, *Int. J. Pharm.* **1987,** *38,* 47.
[23] X.-K. Jiang, *Acc. Chem. Res.* **1988,** *21,* 362.
[24] A. Davis, B.H. Warrington, J.G. Vinter, *J. Comput.-Aided Molec. Design* **1987,** *1,* 97.