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Original paper

Correlation between lipophilicity and reactivity under biomimetic conditions of β-hydroxybarbiturates

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Summary — The effects of lipophilicity are studied on a chemical equilibrium between β-hydroxybarbiturates and corresponding allophanoyl γ-lactones under biomimetic conditions, in order to show that this physicochemical parameter is not only active *in vivo* on drug disposition, but also on the kinetics of chemical reactions.

Résumé — Corrélation entre la lipophilie et la réactivité de β-hydroxybarbituriques dans des conditions biomimétiques. *Les effets de la lipophilie sont étudiés sur un équilibre chimique entre des barbituriques β-hydroxylés et les lactones γ-allophaniques correspondantes, dans des conditions biomimétiques, dans le but de montrer que ce paramètre physicochimique n'est pas actif,* in vivo, *uniquement au niveau de la distribution dans l'organisme des molécules mais aussi sur la cinétique des réactions chimiques.*

lipophilicity / correlation / kinetics / hydroxybarbiturates / allophanoyl γ-lactones / biomimetic conditions

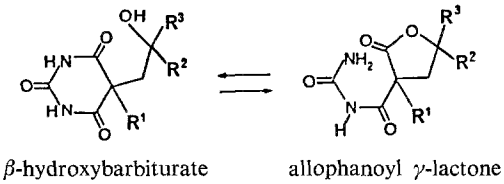
Introduction

Lipophilicity has been extensively used in quantitative structure—activity relationship (QSAR) studies, even if spatial or topological molecular descriptors seem to be as effective in biological correlations [1,2]. The influence of this physicochemical parameter on drug metabolism and pharmacokinetics has also been pointed out, especially in the case of barbituric acid derivatives [3—10]. Most often, lipophilic character is related to the ability of compounds to pass through biological membranes [10,11]. For example, in the three compartment model system, according to Hyde and Lord [11]: "the members of a drug series are assumed to have the same intrinsic activity and mechanism of action, and variations in activity derive from the variations in Drug Receptor binding and Drug distribution equilibrium", but the partition equilibrium between *n*-octanol and water is only considered as a model for partition *in vivo* between organic and aqueous phases. Dependence of observed drug activity on log *P* is undoubtly proved, but one must not forget that drug—receptor interaction can be interpreted in terms of chemical reactivity and that the influence of lipophilicity can be effective also at this particular level and not on only drug disposition.

In order to test this hypothesis, the effects of lipophilicity were studied on a chemical equilibrium, considered as a model of drug—receptor interaction.

It was necessary to choose a reaction occurring under biomimetic conditions. The metabolism of barbiturates could offer such a model.

One of the main metabolic pathways for these xenobiotics consists of oxidation of one of the side chains by liver cytochrome P-450. If the hydroxylation occurs in the β-position, a chemical equilibrium takes place *in vivo* or *in vitro* between the hydroxybarbiturate **A**, and the corresponding allophanoyl γ-lactone **B**, [12—18], (Scheme 1).



β-hydroxybarbiturate (**A**) ⇌ allophanoyl γ-lactone (**B**)

Scheme 1.

The present paper deals with the study of the relation between the lipophilicity of β-hydroxybarbiturates and the rate of this chemical equilibrium.

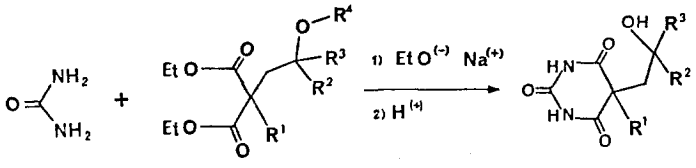
A set of 10 hydroxycompounds presenting various R^1, R^2 and R^3 substituents were synthesized, in order to make a series of regularly increasing lipophilicities.

The time necessary to reach equilibrium under biomimetic conditions was then related to the variation in the lipophilic character.

Chemistry

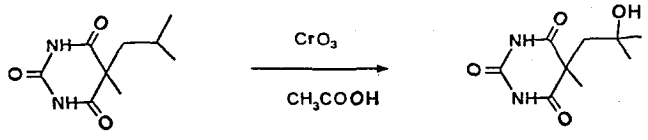
Synthesis of β-hydroxybarbiturates **A1—A2**

When the compounds were not substituted on the side chain bearing the alcohol function ($R^2 = R^3 = H$; **A1**, **A3**, **A9**) the hydroxyethyl group was introduced as a vinyl (**A3—A9**) or methyl (**A1**) ether in corresponding monoalkyl-2-ethyl malonate and the 2,2-disubstituted ethyl malonate obtained was then condensed with urea in alkaline medium. Acid hydrolysis then destroyed the ether function and led to the hydroxybarbiturate (Scheme 2).



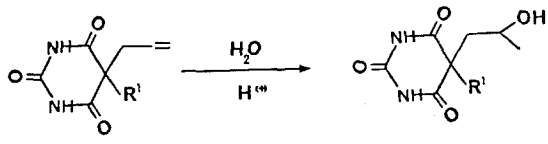
Scheme 2.

Compound **A4** ($R^2 = R^3 = CH_3$) was synthesized by chromic oxidation of corresponding barbituric acid (Scheme 3).



Scheme 3.

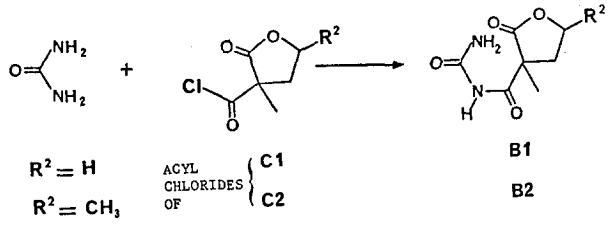
When the R^2 group is methyl and the R^3 hydrogen (**A2**, **A5**, **A6**, **A7**, **A8** and **A10**), the hydration of corresponding allyl-barbiturates was performed in acidic medium (Scheme 4).



Scheme 4.

Synthesis of allophanoyl γ-lactones

Compounds **B1** and **B2** were prepared from corresponding γ-lactone carboxylic acids **C1** and **C2** which were converted into acyl chlorides and then condensed with urea (Scheme 5).



Scheme 5.

Allophanoyl γ-lactones **B3—B10** were synthesized from corresponding β-hydroxybarbiturates. The chemical equilibrium (Scheme 1) was shielded by extraction with an organic solvent.

Study of chemical equilibrium

Under biomimetic conditions (37°C, pH 7.40) eight β-hydroxybarbiturates (**A3—A10**) were converted into a mixture of 67—74% **A** and 33—26% corresponding allophanoyl γ-lactone **B**. Times necessary to reach the equilibrium are listed in Table I.

Table I. Log P_0/w variations and modifications of the equilibrium between β-hydroxybarbiturates **A** and corresponding allophanoyl γ-lactones **B**.

No.	R^1	R^2	R^3	Δ log P*	Δ log P**	Equilibrium	
						A%	time h
1	CH_3	H	H	0	0	100	—
2	CH_3	CH_3	H	0.3	0.2	100	—
3	$CH_2—CH_3$	H	H	0.5	0.46	74	22
4	CH_3	CH_3	CH_3	0.6	0.58	73	9
5	$CH_2—CH_3$	CH_3	H	0.8	0.66	70	1.5
6	$CH_2—CH=CH_2$	CH_3	H	1	0.87	74	1
7	$CH_2—CH_2—CH_3$	CH_3	H	1.3	1.1	67	12
8	$CH_2—CH(CH_3)_2$	CH_3	H	1.6	1.3	73	28
9	$CH_2—CH_2—CH(CH_3)_2$	H	H	1.8	1.6	75	40
10	$CH(CH_3)—CH_2—CH_2—CH_3$	CH_3	H	2.1	1.8	72	60

Satisfactory analytical data (± 0.4% for C, H, N) were reported for all new compounds listed in the table.
*Calculated according to [19, 20].
**Calculated according to [21].

On the other hand, neither **A1** nor **A2** could be converted into corresponding **B1** or **B2**.

In contrast, compounds **B1** and **B2** were immediately converted into corresponding **A1** and **A2**, equally well after dissolution in water at room temperature and under biomimetic conditions.

Lipophilicity evaluation

When studying a series of compounds, it is more important to evaluate the variations in log P_0/w values than these values themselves. These variations, which are listed in Table I, were calculated according to the classical method of Hansch *et al.* and Leo *et al.* [19, 20], as well as to a more recent system of atomic contributions [21]. Compound **A1** which has the lower log P value was chosen as the zero point of the scale.

Thus, values of Δ log P_0/w increase from **A1** to **A10**. The same evolution is observed for both calculation methods, even if some slight differences appear.

Qualitatively, one can notice that there is no relationship between the percentage of each form, **A** or **B**, at equilibrium and Δ log P, except for **A1** and **A2**, which are not converted into the corresponding **B1** or **B2**

The time necessary to reach equilibrium seems to depend upon log P: it is infinite when lipophilicity is low, and then decreases when log P increases, to reach a minimum for equilibrium **A6** $\rightleftarrows$ **B6**, and then increases again with log P values.

Kinetics and lipophilicity

Before any quantitative approach, it can be noted that the compounds can be divided into two groups: those (**1** and **2**) for which **A** cannot be transformed into **B**, while **B** is immediately converted into **A**, and those which lead to an equilibrium of about 70% **A** and 30% **B**.

For this second group, the value of Δ log P can be related to the time, t_e, necessary to reach the equilibrium, by a polynomial regression (equation 1):

$$t_e = \sum_{n=0}^{6} a_n(\Delta \log P)^n \qquad \text{eqn. 1}$$

Table II regroups the values of a^n and the experimental points are presented in Figs. 1 and 2.

There is thus a particular value of Δ log P for which t_e is minimal. The aspect of the curve suggests the competition between two antagonist properties. Nevertheless, this approach is not sufficient to take account of all the compounds of the series, and, on the other hand, it consists of the comparison of Δ log P, which is an energy, and of a time which is not directly proportional to an energy.

Another approach is thus necessary. Considering that the equilibrium between **A** and **B** is composed of two opposite first order reactions and that equilibrium rates are similar for compounds **3**—**10**, t_e is proportional to $1/k_1$, if k_1 and k_{-1}, are the reaction rates of the equilibrium:

$$\mathbf{A} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathbf{B}$$

The kinetics are then expressed by equation 2:

$$\frac{dx}{dt} = -k_{-1}x + k_1(a - x) = (k_1 + k_{-1})(m - x) \qquad \text{eqn. 2}$$

with $m = k_1a/(k_1 + k_{-1})$ and the initial concentration of **B** = 0; x: concentration of **B** at time t; a: initial concentration of **A**; m: concentration of **B** at $t = \infty$.

If one considers $x(t_e) = m/p$ with p close to 1 ($p > 1$), t_e becomes in equation 3:

$$t_e = \frac{1}{k_1} \cdot \frac{K}{1 + K} \cdot \mathrm{Ln}\frac{p}{p - 1} \qquad \text{eqn. 3}$$

where $K = k_1/k_{-1}$.

Table II. Values of a_n in eqn. 1.

	a_0	a_1	a_2	a_3	a_4	a_5	a_6	r^2
Values calculated according to Hansch and coworkers [19, 20]	397.83	—1829.31	3527.91	—3668.40	2155.87	—666.10	83.90	0.999
T	2.04	—1.59	1.30	—1.13	1.04	—0.97	0.93	—
Values calculated according to Moreau and coworkers [21]	301.10	—1528.47	3591.67	—4978.27	4054.78	—1729.96	294.88	0.999
T	0.65	—0.49	0.43	—0.44	0.49	—0.56	0.64	—

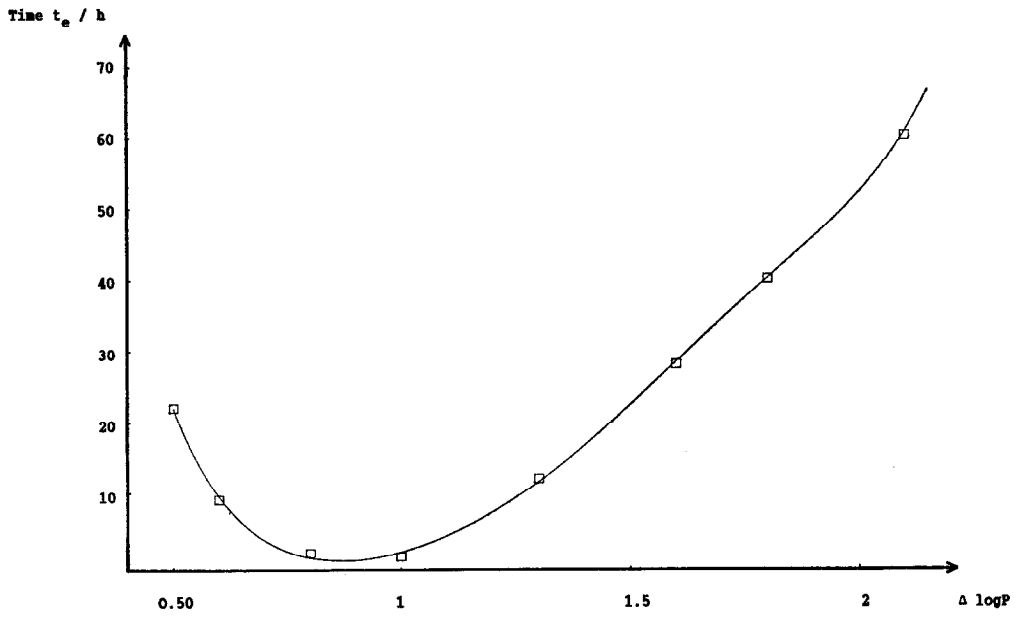


Fig. 1. Regression curve from experimental points computed according to Hansch and coworkers [19, 20].

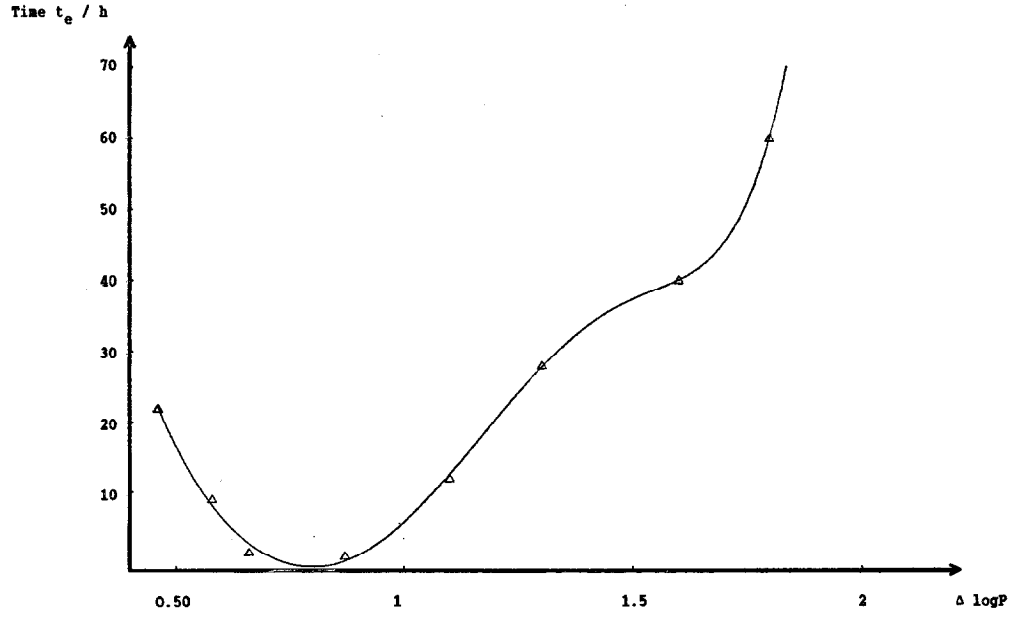


Fig. 2. Regression curve from experimental points computed according to Moreau and coworkers [21].

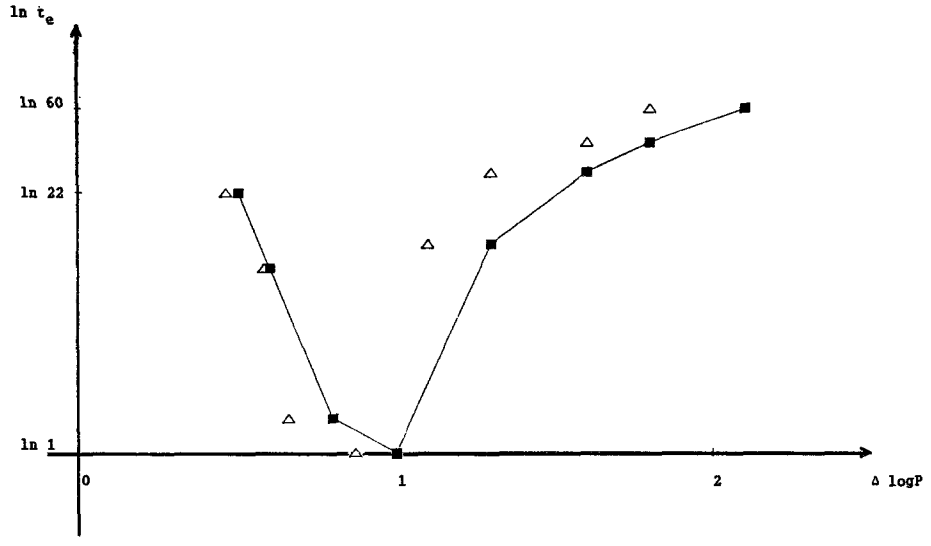
Inside this second group of compounds, one can consider that K and p are nearly constants. Then Ln t_e is proportional to E^* activation energy of reaction **A** $\rightarrow$ **B**, according to Arrhenius equation (equation 4):

$$k_1 = k' \exp(-E^*/RT) \qquad \text{eqn. 4}$$

The variation of Ln t_e *versus* Δ log P (Fig. 3) looks like the evolution of the potential energy U of NaCl as a function of internuclear distance (Fig. 4) which is expressed by equation 5:

$$U = qq'/r + b' \cdot \exp(-a'r) \qquad \text{eqn. 5}$$

Fig. 3. Ln t_e *versus* Δ log P.



■ computed according to Hansch and coworkers [19, 20].
△ computed according to Moreau and coworkers [21].

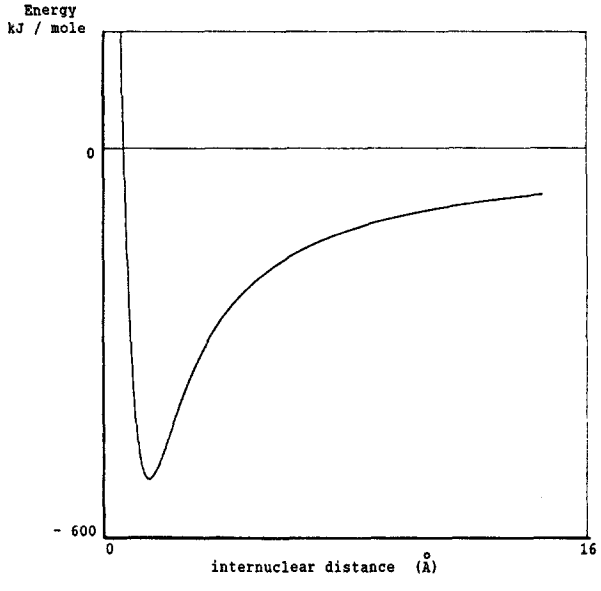


Fig. 4. Potential energy U of NaCl *versus* internuclear distance.

where q and q': electric charges of ions; r: distance between the two nuclei; a' and b': constants.

The first term of equation 5 represents coulombic attraction while the second term describes the repulsion of electrons (Born and Mayer [22]).

Considering that Ln $t_e = E^*/RT$ + constant, equation 6 describes the results of our experiments for the two groups of compounds:

$$E^* = -l/\Delta \log P + \beta \cdot \exp(-\alpha \cdot \Delta \log P) + \gamma \qquad \text{eqn. 6}$$

l, α, β, γ: constants.

For example, Fig. 5 shows the curve obtained for: $l = 0.01$; $\alpha = 4100$; $\beta = 100$; and $\gamma = -8.23$; *i.e.*, $Y = -l/x + \beta \cdot \exp(-\alpha \cdot x) + \gamma$. The thin peak, near the asymptote constitutes the frontier between the two classes of compounds. The rapid decrease of E^* coincides with the variation of K (0.7 → 1).

Discussion—Conclusion

It is important to point out that experimental data cannot be explained by the influence of inductive effects of the substituents. Compounds **A1**, **A2** and **A4** have the same R^1 substituent (CH_3), yet they behave quite differently. The same observation can be made for **A3** and **A5**.

On the other hand, the alcohol function is primary for **A1**, **A3** and **A9**, secondary for **A2**, **A5**, **A6**, **A7**, **A8** and **A10** and tertiary for **A4**. These chemical differences and the electronic effects that they induce are not related to equilibration times.

However, the steric hindrance of the substituents is closely related to the increase of the number of carbon atoms and thus to lipophilicity, and the contribution of conformational energies to the kinetics is certainly important.

Nevertheless, as lipophilicity results from the addition of many factors, it is a good property to take account of all these factors.

This study shows that log P is related to the kinetics of a chemical reaction. It is thus impossible to neglect the influence of this chemicophysical parameter at the level of drug—receptor interaction.

On the other hand, the distribution of compounds into two groups can be considered as a model of the existence of active and non-active drugs in a homogeneous family of chemical compounds.

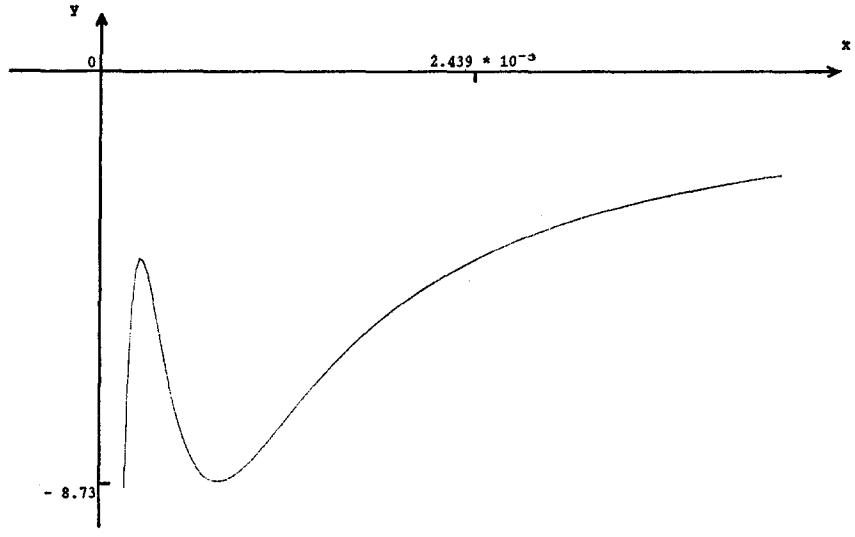


Fig. 5. $y = -l/x + \beta \exp(-\alpha x) + \gamma$.

Experimental protocols

The compounds were characterized by ^{1}H NMR DMSO-d$_6$ (Varian T 60), elemental analyses (Perkin—Elmer 240) (within $\pm 0.4\%$ of the theoretical values) and uncorrected melting point determinations (Kofler apparatus).

Synthesis of β-hydroxybarbiturates A1—A10

Compounds **A1, A3** and **A9** were synthesized using the method described for **A9** in [16] (for **A1** a methyl ether was used instead of a vinyl ether).

Compound **A4** was synthesized according to the same chromic oxidation method as that used for γ-hydroxyamobarbital in [16].

Compounds **A2, A5, A6, A7, A8** and **A10** were prepared by hydration of the corresponding allyl barbiturates using the method described for **A6** in [12] and for **A8** in [15].

For new compounds **(A1, A2, A3, A4, A5, A7, A10),** last step yields were respectively: 62, 69, 69, 30, 68, 66 and 72% and melting points: 188, 215, 174, 228, 180, 195 and 191°C. Anal. (C, H, N): **A1**: $C_7H_{10}N_2O_4$; **A2**: $C_8H_{12}N_2O_4$; **A3**: $C_8H_{12}N_2O_4$; **A4**: $C_9H_{14}N_2O_4$; **A5**: $C_9H_{14}N_2O_4$; **A7**: $C_{10}H_{16}N_2O_4$; **A10**: $C_{12}H_{20}N_2O_4$.

Synthesis of allophanoyl γ-lactones

Preparation of compounds B1 and B2. In an erlenmeyer flask, 0.00485 mol of γ-lactonecarboxylic acids **C1** (0.70 g) or **C2** (0.76 g) were dissolved in 2 ml of thionyl chloride (0.027 mol). The mixture was stirred for 48 h at room temperature. Then the excess thionyl chloride was distilled off and 0.00485 mol of urea (0.291 g) were added. The mixture is stirred for 48 h and compounds **B1** (yield = 100%; mp°C = 135) or **B2** (yield = 100%; mp°C = 126) were obtained as solids. Anal. (C, H, N): **B1**: $C_7H_{10}N_2O_4$; **B2**: $C_8H_{12}N_2O_4$.

Preparation of compounds B6, B8 and B9. Compounds **B6, B8** and **B9** were prepared as reported previously [12, 15, 16].

Preparation of compounds B4 and B5. Compounds **A4** and **A5** were dissolved in water (1 g, 500 ml) (*i.e.*, 0.0094 mol·l^{-1}). The temperature was allowed to rise to 0°C. After 1 h, extraction with chloroform was performed. For **B4,** the aqueous phase was stirred for 2 h more and, every hour, a new extraction was performed. Compounds **B4** (mp°C = 164) and **B5** (mp°C = 109) were obtained after evaporation of the organic solvent (yields = 80%). Anal. (C, H, N): **B4**: $C_9H_{14}N_2O_4$; **B5**: $C_9H_{14}N_2O_4$.

Preparation of compounds B3 and B7. Compounds **A3** and **A7** (1 g, 500 ml) (*i.e.*, respectively, 0.01 mol·l^{-1} and 0.0087 mol·l^{-1}) were dissolved in a solution (500 ml) of sodium hydroxide in water (pH 10). Chloroform (100 ml) was then added. Reaction medium was heated and stirred (80°C). Every 30 min, the chloroform was removed and 100 ml were added. After 5 h, **(B3)** and 3 h, **(B7)** organic solutions were pooled and evaporated under reduced pressure. Compounds **B3** (yield = 75%; mp°C = 148) and **B7** (yield = 72%; mp°C = 118) were then isolated. Anal. (C, H, N): **B3**: $C_8H_{12}N_2O_4$; **B7**: $C_{10}H_{16}N_2O_4$.

Preparation of compound B10. Compound **A10** (1 g, 500 ml) (*i.e.*, 0.0078 mol·l^{-1}) was dissolved in a solution of sodium hydroxide in water (pH 14) and the procedure was then the same as that used for **B3** and **B7**. The reaction yield **B10** (65%) (mp°C = 163). Anal. (C, H, N): **B10**: $C_{12}H_{20}N_2O_4$.

^{1}H NMR data critical to identification of compounds A and B

When $R^2 = R^3 = H$ **(A1, A3** and **A9),** hydroxybarbiturates presented a triplet (δ = 3.4 ppm) corresponding to the two protons situated on the carbon atom bearing the hydroxyl group. Corresponding protons in allophanoyl γ-lactones also presented a triplet but the signals were deshielded (**B1**: δ ppm = 4.3) (**B3**: δ ppm = 4.4) and (**B9**: δ ppm = 4.2).

When $R^2 = CH_3$ and $R^3 = H$ **(A2, A5, A6, A7, A8** and **A10),** hydroxybarbiturates gave a doublet (δ ppm = 1—1.1) corresponding to the three protons of R^2. The spectrum of allophanoyl γ-lactones **(B2, B5, B7, B8, B10)** was characterized by a doublet (δ = 1.3—1.5) attributed to corresponding protons of R^2.

When $R^2 = R^3 = CH_3$, the singlet corresponding to R^2 and R^3 (δ = 1.1) in the hydroxybarbiturate form, **A4,** became two singlets in the spectrum of allophanoyl γ-lactone, **B4,** (δ = 1.3 and 1.4).

Determination of the equilibrium A ⇆ B

In an erlenmeyer flask, 1 g of β-hydroxybarbiturate **(A1—A10)** or allophanoyl γ-lactone **(B1—B10)** was dissolved in 1 l of an acid buffer, pH 7.4, 0.1 M citric acid:0.1 M disodium phosphate (9.15:90.85). The temperature was adjusted to 37°C±0.5 and the solution was stirred. 50 ml samples were collected using various time sequences, appropriate for each compound. 5 drops of 1 M hydrochloric acid were added to each sample, in order to stop the equilibrium. The sample was extracted 3 times with ethyl acetate (25 ml). Organic solutions were dried on sodium sulfate and ethyl acetate was evaporated under reduced pressure. The residue was then studied by ^{1}H NMR DMSO-d$_6$. Integrations of signals characteristic of corresponding hydroxybarbiturate A and of allophanoyl γ-lactone **B** were then recorded. The ratio of their values allows the determination of percentage of each form (Table I).

References

1 Basak S. C., Harriss D. K. & Magnuson V. R. (1984) *J. Pharm. Sci.* 73, 429—437

2 Broto P., Moreau G. & Vandyke C. (1984) *Eur. J. Med. Chem.* 19, 61—65

3 Gaudette L. E. & Brodie B. B. (1959) *Biochem. Pharmacol.* 2, 89—96
4 Lin Y.-J., Awazu S., Hanano M. & Nogami H. (1973) *Chem. Pharm. Bull.* 21, 2749—2756
5 Kaneniwa N., Hiura M. & Nakagawa S. (1979) *Chem. Pharm. Bull.* 27, 1501—1510
6 Kakemi K., Arita T., Hori R. & Konishi R. (1967) *Chem. Pharm. Bull.* 15, 1705—1712
7 Lambrey B., Lafont O. & Jacquot C. (1984) *Lyon Pharm.* 35, 11—16
8 Lambrey B., Lafont O., d'Athis P. & Jacquot C. (1984) *J. Pharmacol. (Paris)* 15, 223—230
9 Mayer J. M. (1985) *in: Molecular Approaches and Pharmacological Implications* (Siest G., ed.), Pergamon Press, Oxford, pp. 187—194
10 Janssen H. M. (1987) *Eur. J. Med. Chem.* 22, 131—134
11 Hyde R. & Lord E. (1979) *Eur. J. Med. Chem.* 14, 199—202
12 Bobranski B., Prelicz D., Syper L. & Wojtowski R. (1960) *Bull. Acad. Pol. Sci. Ser. Sci. Chim.* 8, 475—479
13 Lafont O., Lambrey B., Jacquot C., Rapin J. R., d'Athis P. & de Lauture D. (1979) *Eur. J. Med. Chem.* 14, 143—150
14 Lafont O., Lambrey B., d'Athis P., de Lauture D. & Jacquot C. (1982) *Eur. J. Med. Chem.* 17, 181—186
15 Lafont O., Cavé C., Lambrey B., Briffaux J. P., Jacquot C. & Miocque M. (1983) *Eur. J. Med. Chem.* 18, 163—168
16 Lafont O., Cavé C., Lambrey B., Ménager S. & Miocque M. (1985) *Eur. J. Med. Chem.* 20, 339—344
17 Lafont O., Cavé C., Lambrey B., Ménager S. & Miocque M. (1985) *Biochem. Pharmacol.* 34, 423—424
18 Cavé C., Lafont O., Ménager S., Lambrey B. & Miocque M. (1986) *Eur. J. Med. Chem.* 21, 487—492
19 Hansch C., Steward A. R., Anderson S. M. & Bentley D. (1968) *J. Med. Chem.* 11, 1—11
20 Leo A., Hansch C. & Elkins D. (1971) *Chem. Rev.* 71, 525—616
21 Broto P., Moreau G. & Vandycke C. (1984) *Eur. J. Med. Chem.* 19, 71—78
22 Born M. & Mayer E. (1932) *Z. Phys.* 75, 1—18