135. The Reversible Proxibarbal-Valofan Isomerisation

Part II

Kinetic Studies in a Biphasic Octanol/Water System

by **H. Harald Wittekind**[a]), **Bernard Testa**[a])*, and **Jurek Estreicher**[b])

[a]) Ecole de Pharmacie, Université de Lausanne, Place du Château, CH–1005 Lausanne

[b]) Division d'Informatique, Hôpital Cantonal Universitaire, Rue Micheli-du-Crest, CH–1211 Genève 4

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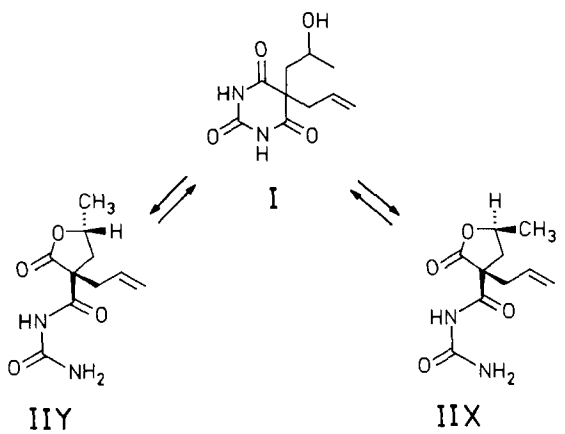
The tautomerisation between proxibarbal (**I**) and the two diastereoisomers of valofan (**IIX** and **IIY**) was investigated at pH 7.4 in a biphasic octanol/H_2O system. The rate constants of isomerisation and the equilibrium constants of partitioning (partition coefficients) were calculated by compartmental analysis. The rate constants of isomerisation were comparable with those determined in monophasic aqueous solutions, whereas at pH 7.4 and 37° the duration necessary for a global equilibrium to be reached was 4–6 times longer in the biphasic system. These reduced rates and the higher lipophilicity of **IIX** and **IIY** as compared to **I** may be of pharmacokinetic and pharmacodynamic significance. They may also have relevance for a number of drugs known or suspected to form lactonic or lactamic metabolites.

Introduction. – The two antimigraine drugs proxibarbal (**I**; *5-(2-hydroxypropyl)-5-(2-propenyl)-2,4,6(1H,3H,5H)-pyrimidinetrione*) and valofan (**II**; N-*(aminocarbonyl)-tetrahydro-5-methyl-2-oxo-3-(2-propenyl)-3-furancarboxamide*) are tautomers that readily interconvert in aqueous solution. Proxibarbal contains one chiral centre and has been studied mainly as the racemate. Valofan on the other hand contains two chiral

Scheme 1. *The Interconversion of Proxibarbal* (**I**) *and Valofan Diastereoisomers* **IIX** *and* **IIY**. Only relative configurations are indicated.



centres and exists as the diastereoisomers **IIX** and **IIY** [1]. Diastereoisomer **IIX** is the more lipophilic of the two, since it is more retained in reversed-phase HPLC and elutes faster from silica columns. The configuration of **IIX** has been established as *r*-2-allophanoyl-2-allyl-*t*-4-methylbutyrolactone (*i.e.* having the Me and allophanoyl groups in a *trans*-configuration), while in the less lipophilic **IIY** diastereoisomer the two groups are *cis*-configurated *(Scheme 1)* [2].

In aqueous solutions under physiological conditions, the interconversion reaches a proxibarbal/valofan equilibrium of 84:16 with half-lives in the range 20–40 min [3]. The reaction also occurs in biological systems and is of particular importance for the human metabolism of these drugs [4–7]. The mechanism of action of the two drugs is unknown, but there is some evidence to indicate that valofan (or a metabolite thereof) may be the active form in that it crosses the blood-brain barrier and exerts metabolic effects on central serotonin and dopamine [8–10].

The proxibarbal-valofan isomerisation is, thus, of importance from both a pharmacokinetic and a pharmacodynamic viewpoint, but the fast rates of interconversion observed in aqueous solutions are difficult to reconcile with some *in vivo* findings. For example, only proxibarbal and practically no valofan is found in the urine of humans dosed with either drug [7]. Also, the LD_{50} values of proxibarbal and valofan in mice have been reported to differ (2.5 g/kg and 1.0 g/kg, respectively) [11] [12]. A differential lipid/H_2O partitioning of the two drugs would not be without pharmacodynamic and pharmacokinetic consequences, but the partition coefficient of labile compounds cannot be measured by straightforward shake-flask methods [13]. The present study was, thus, undertaken to examine the proxibarbal-valofan isomerisation reaction in biomimetic biphasic octanol/H_2O systems.

Materials and Methods. – *Experimental and Analytical Conditions.* Proxibarbal (**I**) and valofan (**II**; a 64:36 mixture of **IIY** and **IIX**) were kindly donated by *Hommel AG* (Adliswil, Switzerland). The internal standard 1,4-bis(hydroxymethyl)benzene is commercially available.

The pK_a value of proxibarbal was determined by potentiometry and UV spectrophotometry at 20° and a ionic strength of 0.2. The method and equipment used for the potentiometric pK_a determination have been described previously [14]. The spectrophotometric pK_a determination was performed using a *Beckman 25* spectrophotometer. Proxibarbal was dissolved in 14 buffered solns. of pH ranging from 6.0 to 8.9, and the absorbance measured within 2 min at 238 nm. Non-linear calculation based on the *Henderson-Hasselbach* equation yielded the pK_a value.

The distribution and isomerisation studies were performed in mixtures of equal volumes of octanol and H_2O buffered at pH 7.4 with 0.05M triethanolamine/HCl [3]. The two solvents were mutually saturated at the temp. of study, with the internal standard having partitioned between the two solvents (mean concentration 0.05 mg/ml). The starting compound (**I** or **II**) was dissolved in the org. phase at a concentration of 0.5 mg/ml, and 5-ml samples of this soln. were distributed in *Sovirel* tubes immersed in the water bath. Additional tubes were used as controls to ascertain the absence of isomerisation in monophasic octanol solns.

At time zero, 5 ml of the aq. phase (at the temp. of study) were added to each tube, and shaking (frequency *ca.* 200 min^{-1}) started immediately. During each experiment, 8–10 tubes were collected at various time intervals and immediately centrifuged at 1000 rpm for 1 min. The phases were then carefully separated, and *both* were analysed for compounds **I**, **IIX**, and **IIY** as described in [3]. Following the analysis of each octanolic sample, the non-eluted octanol had to be removed by washing the HPLC column with MeOH (1.5 ml/min for 4 min) and reconditioning it with the usual eluent for 10 min.

Kinetic Calculations. The concentrations of **I**, **IIX**, and **IIY** were determined in both phases, and, hence, a six-compartment model was used in the kinetic calculations *(Fig. 1)*. Since the proxibarbal/valofan isomerisation cannot be detected in octanol (see later), the model could be simplified by considering only ten rate constants, *i.e.* four rate constants of isomerisation and six rate constants of partitioning. The ten rate constants were calculated by compartmental analysis of experimental data using the LINDE package as previously described [3]. In a number of cases, the rate constants were repeatedly calculated from the same set of data, starting the optimisation from

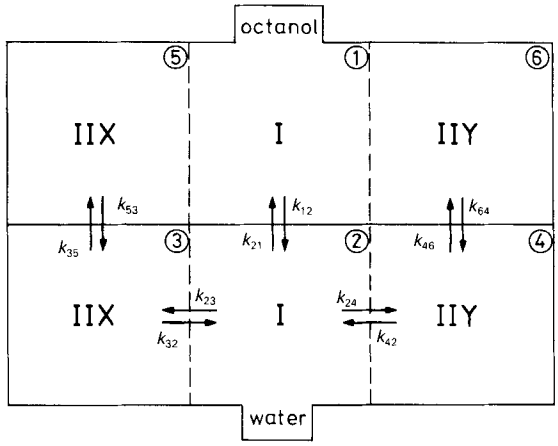


Fig. 1. *Compartmental model describing the proxibarbal-valofan isomerisation and octanol/H_2O partitioning.* The numbers *1–6* refer to the six theoretical compartments in the model.

various initial values. These repetitions yielded consistent equilibrium constants in all cases as well as consistent rate constants of isomerisation, but not consistent rate constants of partitioning. This indicates that there are strong correlations between some of these parameters (geometrically, the 'surface' to be minimised presents 'ridges' instead of 'peaks'), and these partitioning parameters cannot be considered as well-defined by the data collected. As a consequence, only equilibrium constants, but not rate constants of partitioning, are reported.

Results and Discussion. – *Ionisation Constant of Proxibarbal.* The potentiometric and spectrophotometric methods yielded at 20° for proxibarbal pK_a values of 7.89 ± 0.03 and 7.92 ± 0.12, respectively. The two results are, thus, perfectly consistent, indicating a mean pK_a value of 7.90. This compares well with the pK_a values of various hydroxylated barbiturates at 38°, which range from 7.50 to 7.75 [15].

Equilibrium and Rate Constants in Octanol/H_2O. The isomerisation and partitioning of proxibarbal and valofan diastereoisomers were investigated in octanol/H_2O (pH 7.4) systems at 20° and 37° with either proxibarbal or valofan as the starting compound dissolved in the octanolic phase. No isomerisation was detectable in this solvent, as long as it was not in contact with the H_2O phase, in agreement with the finding that the rate of isomerisation in pure solvents declines sharply with decreasing dielectric constant of the medium [16]. This allows the isomerisation and partitioning processes to be described by the compartmental model shown in *Fig. 1.*

Typical concentration profiles obtained at 37° are presented in *Figs. 2* and *3.* From these data, rate and equilibrium constants were calculated, but, as explained above, only the equilibrium contants are reported for the processes of partitioning. The results are collected in *Table 1,* showing that similar values are obtained whether starting with proxibarbal or valofan. The only noteworthy difference is displayed by k_{24} and hence K_Y values at 20°, but the reason for this discrepancy is not known (see also later).

It is interesting to compare the $t_{1/2}$ values of isomerisation obtained in the biphasic system with those found in aqueous solutions under identical conditions of pH and temperature (Tables 5 and 6 in [3]). In each case, the corresponding values are essentially similar, allowing for experimental scattering of results. Indeed, the $t_{1/2}$ values at 37° in the monophasic and biphasic systems are in the range 27–40 min^{-1} and 37–55 min^{-1}, respectively. At 20°, the corresponding values are in the range 147–179 min^{-1} and 149–183 min^{-1},

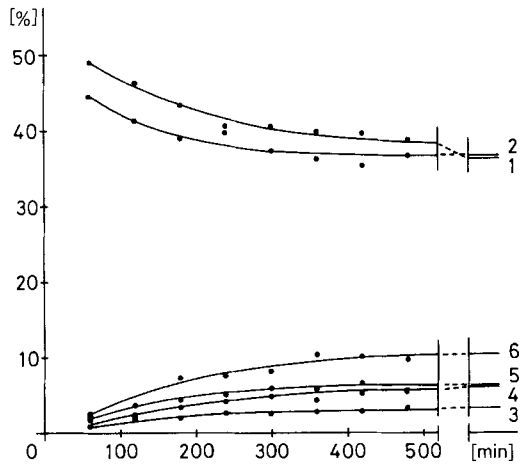


Fig. 2. *Concentration profiles of proxibarbal* (**I**) *and valofan diastereoisomers* **IIX** *and* **IIY** *in an octanol/H_2O* (pH 7.4) *system at 37°, the starting compound being proxibarbal.* The numbers *1–6* refer to the six theoretical compartments in the model (see *Fig. 1*). The relative concentrations at equilibrium are calculated values (see *Table 2*).

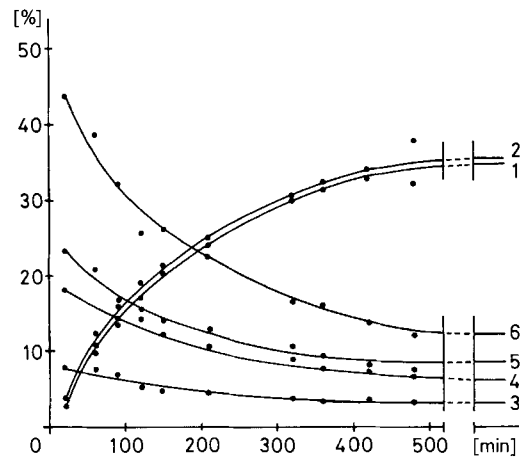


Fig. 3. *Concentration profiles of proxibarbal* (**I**) *and valofan diastereoisomers* **IIX** *and* **IIY** *in an octanol/H_2O* (pH 7.4) *system at 37°, the starting compound being valofan.* The numbers *1–6* refer to the six theoretical compartments in the model (see *Fig. 1*). The relative concentrations at equilibrium are calculated values (see *Table 2*).

respectively. There is no theoretical reason to expect a meaningful difference between monophasic and biphasic systems in the rate constants of isomerisation, and the non-significant differences noted here merely show the limits of the analytical precision.

In contrast to comparable rate constants of isomerisation, we note a significant difference in the durations necessary for the monophasic and biphasic systems to reach a global equilibrium. In monophasic aqueous solutions (pH 7.4, 37°), equilibrium was reached in 120–150 min (Fig. 1 and 2 in [3]), whereas in biphasic systems (*Figs. 2* and *3*) durations of 500 min and more were seen. Under our conditions of study, it took 4–6 times longer for an equilibrium to be reached in a biphasic octanol/H_2O system than in a monophasic aqueous solution. Because proxibarbal and valofan essentially escape isom-

Table 1. *Equilibrium Constants (K) and Chemical Rate Constants* (k values $\times 10^3$ [min^{-1}], $t_{1/2}$ [min]) *for the Reversible Proxibarbal-Valofan Isomerisation in a Biphasic Octanol/H_2O System at pH 7.4* (see *Fig. 1*)

	20°		37°	
	A[a])	B[b])	A[a])	B[b])
Partitioning				
k_{21}/k_{12}	0.88	0.91	1.00	0.98
k_{35}/k_{53}	2.43	1.88	1.95	2.60
k_{46}/k_{64}	1.92	1.43	1.71	2.01
Isomerisation				
k_{32}	3.61	4.03	14.0	17.4
k_{23}	0.18	0.38	1.16	1.28
K_X (k_{32}/k_{23})	20.1	10.6	12.1	13.6
$t_{1/2}$	182.9	157.1	45.7	37.1
k_{42}	4.10	3.76	15.2	11.1
k_{24}	0.35	0.88	1.85	1.39
K_Y (k_{42}/k_{24})	11.7	4.27	8.22	7.99
$t_{1/2}$	155.7	149.4	40.6	55.5

[a]) Proxibarbal (**I**) as the starting compound. [b]) Valofan (**II**) as the starting compound.

erisation in the organic phase, the rate of the chemical reaction is considerably decreased in biphasic systems.

Lipophilicity of Proxibarbal and Valofan Diastereoisomers. The equilibrium concentrations of proxibarbal and valofan diastereoisomers, as calculated by compartmental analysis, are shown in *Table 2*. Some differences are apparent depending on either proxibarbal or valofan being the starting compound. These differences may be genuine, which would imply that the compartmental model used here is oversimplified. However, these differences may just as well be ascribed to the limits of the experimental precision, and in the absence of decisive factors only mean values are considered here.

At both temperatures, the proxibarbal/valofan ratio is constant for each solvent, namely 66:34 (±6.9) in octanol and 79:21 (±6.5) in H_2O. The latter value is not sig-

Table 2. *Relative Concentrations* [%] *of Proxibarbal and Valofan Diastereoisomers at Equilibrium in a Biphasic Octanol/H_2O System at pH 7.4* (calculated values)

	20°		37°	
	A[a])	B[b])	A[a])	B[b])
Octanol				
Proxibarbal (**I**)	37.7	30.6	36.3	34.7
Valofan-Y (**IIY**)	7.8	15.1	10.5	12.2
Valofan-X (**IIX**)	5.7	6.8	6.5	8.5
Ratio **I**/**II**	74:26	58:42	68:32	63:37
Ratio **IIY**/**IIX**	57:43	65:35	61:39	59:41
Water				
Proxibarbal (**I**)	42.5	33.5	37.0	35.5
Valofan-Y (**IIY**)	4.1	10.3	6.3	6.1
Valofan-X (**IIX**)	2.2	3.6	3.4	3.1
Ratio **I**/**II**	87:13	71:29	79:21	79:21
Ratio **IIY**/**IIX**	65:35	74:26	65:35	66:34

[a]) Proxibarbal (**I**) as the starting material. [b]) Valofan (**II**) as the starting material.

Table 3. *log P_{app} Values* (log of octanol/H_2O partition coefficients at pH 7.4) *of Proxibarbal* (**I**) *and Valofan Diastereoisomers* **IIY** *and* **IIX** *Calculated from the Results in* Table 2

	20°		37°	
	A[a])	B[b])	A[a])	B[b])
Proxibarbal (**I**)	–0.05	–0.04	–0.01	–0.01
Valofan-Y (**IIY**)	0.28	0.17	0.22	0.30
Valofan-X (**IIX**)	0.41	0.28	0.28	0.44

[a]) Proxibarbal (**I**) as the starting material. [b]) Valofan (**II**) as the starting material.

nificantly different from the 84:16 ratio found in H_2O (monophasic system). In contrast, the 66:34 ratio indicates a valofan enrichment in octanol as a consequence of its lipophilicity (see below). As a result of this preferential distribution, the entire system is enriched in valofan, the overall proxibarbal/valofan ratio being 72:28 (±6.7).

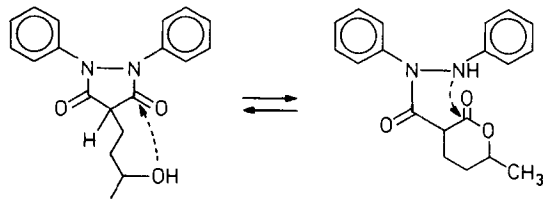
Apparent octanol/H_2O partition coefficients at pH 7.4 can be calculated from the data in *Table 2*, yielding the log P_{app} values shown in *Table 3*. The mean values for proxibarbal, valofan-Y, and valofan-X are, thus, –0.05, 0.22, and 0.34 at 20°, and –0.01, 0.26, and 0.36 at 37°. Valofan diastereoisomers do not ionise at neutral pH and hence the apparent partition coefficient is equal to the 'true' partition coefficient (expressed as log P). For proxibarbal at 20°, correction for ionisation yields a log P value of 0.07. The lipophilicity sequence for proxibarbal, valofan-Y and valofan-X is the same as that deduced from reversed-phase and normal-phase liquid chromatography elution [1].

Conclusion. – A comparison of the previous [3] and present study reveals some marked differences in the proxibarbal/valofan isomerisation in H_2O and octanol/H_2O systems. Firstly, the overall rate of reaction is considerably slower in biphasic systems due to partitioning into a solvent where the reaction is very slow. And secondly, the proxibarbal/valofan ratio is shifted towards valofan due to the higher lipophilicity of the latter.

From a physicochemical viewpoint, biphasic solvent systems resemble biological systems much more than monophasic solvent systems do. As a consequence, studying the proxibarbal/valofan isomerisation in aqueous solutions leads to reaction rates and product ratios of lesser biological relevance. In contrast, the present study suggests that valofan will be enriched relatively to proxibarbal in lipophilic tissues and organs such as the brain, in agreement with the suggestion that the former may be the active tautomer (see *Introduction*). Furthermore, the considerably reduced rate of isomerisation in biphasic systems is compatible with fragmentary evidence suggesting some differences in the *in vivo* activity of proxibarbal and valofan (see *Introduction*).

Beyond proxibarbal and valofan, the previous and present studies also have relevance for a number of drugs shown or suspected to form lactonic or lactamic metabolites. Thus, α-phenyl-γ-butyrolactone has been found in human urine following the ingestion of intoxicating amounts of phenobarbital, primidone and glutethimide [17]. This metabolite is generated by β-hydroxylation of the ethyl side-chain, followed by lactone formation and loss of the amidic side-chain. Conflicting results have been reported regarding its toxicity [18] [19]. An analogous metabolite is also formed in humans chronically treated with aminoglutethimide [20]. Interestingly, a similar metabolic pathway has recently been characterized for phenylbutazone [21]; following administration to the rat, the drug

Scheme 2. *Reversible Lactonisation of the γ-Hydroxylated Metabolite of Phenylbutazone* [21]



yields among others a γ-hydroxylated metabolite which slowly isomerizes to a δ-lactone *(Scheme 2)*.

In a large number of barbiturates, the 5-alkyl side-chain has been shown to undergo metabolic hydroxylation in the 2′- and/or 3′-position. Examples include allobarbital, amobarbital, barbital, brallobarbital, butalbital, nealbarbital, pentobarbital, secobarbital, and secbutobarbital [22–26]. Such hydroxylated metabolites are candidate genitors of lactones and could help to explain the large fraction of dose (usually 20–30%) which remains unaccounted for in the *in vivo* metabolic studies of most barbiturates.

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