134. The Reversible Proxibarbal-Valofan Isomerisation

Part I

Kinetic and Thermodynamic Studies in Aqueous Solutions

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The tautomerisation between proxibarbal (I) and the two diastereoisomers of valofan, IIX and IIY, was investigated in aqueous solutions, and various rate and equilibrium constants were calculated by compartmental analysis. The proportion of I at equilibrium was found to increase with pH, and indeed, the two equilibrium constants are linear functions of pH. In contrast, the equilibrium-concentration ratio of IIY/IIX was close to 63:37 and remained constant in the pH range investigated. The rate constants were also determined as a function of temperature, allowing calculation of the thermodynamic parameters. Under physiological conditions, the difference in free energy favouring I vs. IIX and IIY is 6.7 and 5.4 kJ·mol^{-1}, respectively.

Introduction. – 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 two drugs used in the treatment of migraine and cephalea. While chemically distinct, the two compounds are in fact tautomers that readily interconvert by ring opening followed by ring closure, a behaviour also displayed by a number of analogues [1–4]. Proxibarbal contains one chiral centre and has been studied mainly in racemic form. Valofan on the other hand contains two chiral centres and exists as two pairs of enantiomers, namely the diastereoisomers designated as IIX and IIY [5]. We have demonstrated that the more lipophilic diastereoisomer IIX is *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 diastereoisomer IIY the two groups are *cis*-configurated *(Scheme)* [6].

The interconversion of valofan (II) and proxibarbal (I) occurs in aqueous solution [5] as well as in biological systems and is of particular significance for the human metabolism of these drugs [7–10]. The mechanisms of action of the two drugs are 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 [11–13].

The proxibarbal-valofan isomerisation is, thus, of importance from both a pharmacokinetic and a pharmacodynamic viewpoint. Nevertheless, the kinetics of this non-enzymatic reaction remains poorly understood, the reason presumably lying in the analytical difficulties involved. Indeed, the rate of the reaction as well as the irreversible hydrolysis of valofan *(Scheme 1)* under unfavourable conditions call for a fast, direct and accurate

Scheme 1. *The Interconversion of Proxibarbal* (**I**) *and Valofan Diastereoisomers* **IIX** *and* **IIY**. Only relative configurations are indicated [6]. The irreversible hydrolysis of valofan is also included.
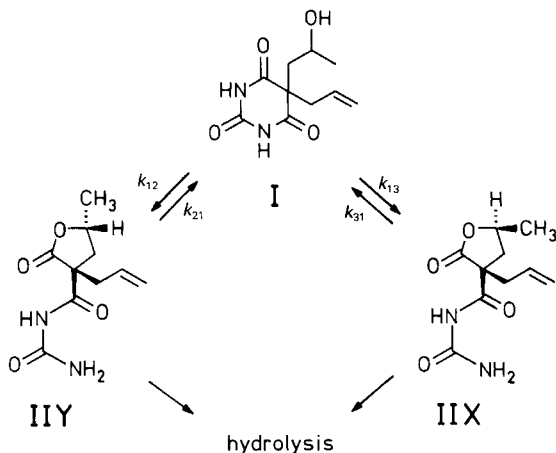
method avoiding sample extraction and the danger of artefactual results. Such a method [5] is used here to investigate the kinetics and thermodynamics of the interconversion between proxibarbal and valofan diastereoisomers in aqueous solutions as a function of pH and temperature. In the subsequent paper, the reaction in biomimetic biphasic octanol/H_2O media will be reported.

Material 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 reactions were carried out in 100-ml stirred solns. containing the starting compound (proxibarbal or valofan) at a 0.8-mM concentration and the internal standard at a 0.05-mg/ml concentration. It was verified that the internal standard had no detectable influence on the reaction. The solns. were buffered with 0.05M triethanolamine and adjusted with HCl to the desired pH (±0.05), measured at the temp. of study. The reactions vessels were placed in a thermostated water bath (±0.1°) (Type *1420; Braun*, Melsungen, FRG) and the reaction was initiated by the addition and immediate dissolution of the solute.

The soln. to be analysed was driven continuously by a peristaltic pump across a pneumatic six-channel valve (*Rheodyne* model *70-10*) equipped with a 10-μl loop. A complete passage through the circuit took less than 20 s with negligble cooling of the soln. Every 10 min, an injection was made into a *Siemens-S-101* high-performance liquid chromatograph and analysed using a *Lichrosorb RP-8* column and UV detection as described in [5]. Under these conditions, the order of elution was: internal standard, **I**, **IIY**, and **IIX** (full separation); each run was complete within less than 8 min.

Kinetic and Thermodynamic Calculations. The experimental data were analysed with compartment models, the values of the transfer rates constants k_{ij} being estimated by means of the optimisation package LINDE (linear differential equations) [14] run on the *CDC Cyber-840* computer of the Geneva University Hospital.

The various thermodynamic parameters were calculated from the rate and equilibrium constants by means of well known equations. For a first-order reversible reaction characterised by the rate constants k_{ij} and k_{ji}, the time necessary for the reaction to proceed halfway to the equilibrium is given by:

$$t_{\frac{1}{2}} = 0.693/(k_{ij} + k_{ji})\tag{1}$$

Results and Discussion. – *Influence of pH on the Rate of Reaction at 37°.* The reaction of isomerisation proceeded at rates which were strongly pH-dependent. The equilibrium was reached in *ca.* 60 min and 240 min at pH 8 and 6.75, respectively. This is illustrated in *Figs. 1* and *2*, which show the reversible proxibarbal/valofan transformation at pH 7.4
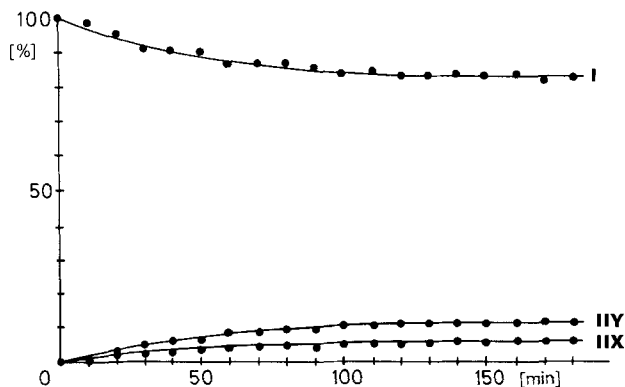
Fig. 1. *Reversible isomerisation of proxibarbal* (**I**) *to valofan diastereoisomers* **IIX** *and* **IIY** *in aqueous solution at pH 7.4 and 37°*
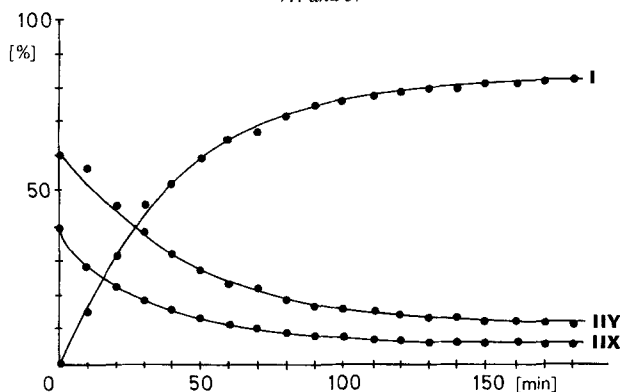

Fig. 2. *Reversible isomerisation of valofan diastereoisomers* **IIX** *and* **IIY** *to proxibarbal* (**I**) *in aqueous solution at pH 7.4 and 37°*

and 37° starting with proxibarbal *(Fig.1)* or with valofan *(Fig.2)*. From the experimental data, rate constants were calculated using a three-compartment model *(Scheme 1)*; *i.e.* the compartments **I**, **IIX**, and **IIY**. At the higher pH values, a five-compartment model was also used, *i.e.* taking the irreversible hydrolysis of **IIX** and **IIY** into account. Only at pH 8.0 and above, the rate constants were sufficiently different to justify the lengthier calculations.

Table 1. *Rate Constants* (k values $\times 10^3$ [min^{-1}]) *and Half-lives* ($t_{1/2}$ [min]) *for the Reversible Transformation of Proxibarbal* (**I**) *into Valofan Diastereoisomers* **IIX** *and* **IIY** *as a Function of pH at 37°*

pH	k_{21}	k_{12}	$t_{1/2}$	k_{31}	k_{13}	$t_{1/2}$
6.75	5.75	1.04	102.1	6.15	0.67	101.6
6.95	9.33	1.53	63.8	10.6	0.98	59.8
7.05	14.2	2.03	42.7	15.8	1.28	40.6
7.15	19.7	2.68	31.0	23.0	1.82	27.9
7.30	26.5	3.32	23.2	28.8	2.10	22.4
7.40	29.9	3.59	20.7	34.1	2.42	19.0
8.00[a])	96.1	8.44	6.6	112.0	5.74	5.9

[a])   Five-compartment model.

Table 2. *Rate Constants (k values* $\times 10^3$ *[min^{-1}]) and Half-lives (t$_{1/2}$ [min]) for the Reversible Transformation of Valofan Diastereoisomers* **IIX** *and* **IIY** *into Proxibarbal* (**I**) *as a Function of pH at 37.0°*

pH	k_{21}	k_{12}	$t_{1/2}$	k_{31}	k_{13}	$t_{1/2}$
6.00	0.78	0.20	707.1	1.16	0.17	521.1
7.00	7.50	0.95	82.0	9.22	0.65	70.2
7.40	15.1	1.78	41.0	18.8	1.38	34.3
8.00[a])	87.5	7.75	7.3	86.4	4.39	7.6

[a]) Five-compartment model.

Table 1 reports the rate constants and corresponding $t_{1/2}$ values for the isomerisation of proxibarbal to valofan diastereoisomers, *i.e.* the set of experiments where proxibarbal was added to the solution at time zero. *Table 2* shows the values for the set of experiments where valofan was added to the solution at time zero. The pH values investigated in both sets of experiments were not identical, but in both cases a pH range from 6.0 to 8.0 was covered. Each log k_{ij} is a linear function of pH, with r^2 values ranging from 0.972 to 0.997, and for each k_{ij} the difference between the equations calculated from *Tables 1* and *2* is not significant in statistical terms (not shown).

From the rate constants in *Tables 1* and *2*, equilibrium constants can be calculated at each pH value *(Table 3)*. The equilibrium constants are linear functions of pH, as shown by *Eqns. 2–5* (95% confidence limits in parentheses):

$$K_Y = 4.73(\pm 0.43)\,\text{pH} - 26.5(\pm 3.1) \tag{2}$$
$$n = 7;\, r^2 = 0.994$$

$$K_X = 7.85(\pm 1.95)\,\text{pH} - 43.8(\pm 13.9) \tag{3}$$
$$n = 7;\, r^2 = 0.956$$

$$K_Y = 3.91(\pm 1.25)\,\text{pH} - 20.0(\pm 8.9) \tag{4}$$
$$n = 4;\, r^2 = 0.989$$

$$K_X = 6.39(\pm 4.69)\,\text{pH} - 32.0(\pm 32.0) \tag{5}$$
$$n = 4;\, r^2 = 0.944$$

Table 3. *Equilibrium Constants for the Reversible Proxibarbal-Valofan Transformation as a Function of pH at 37.0°* $(K_Y = k_{21}/k_{12};\, K_X = k_{31}/k_{13})$

pH	K_Y[a])	K_X[a])	K_Y[b])	K_X[b])	K_X/K_Y
6.00			3.85	6.63	1.72
6.75	5.53	9.21			1.66
6.95	6.10	10.8			1.77
7.00			7.89	14.2	1.80
7.05	7.00	12.3			1.75
7.15	7.35	12.6			1.71
7.30	7.98	13.7			1.72
7.40	8.33	14.1			1.69
7.40			8.48	13.6	1.61
8.00	11.4	19.5			1.71
8.00			11.3	19.7	1.74

[a]) Calculated from the rate constants in *Table 1*. [b]) Calculated from the rate constants in *Table 2*.

One can see that, at the 95% level of confidence, there are no significant differences between the regression coefficients in *Eqns. 2* and *4*, or in *Eqns. 3* and *5*. Thus, *Eqns. 2* and *4* do not differ, and neither do *Eqns. 3* and *5*. As for the ratio K_X/K_Y, *Table 3* clearly shows that it remains constant (1.72 ± 0.05) in the pH range investigated.

Relative concentrations at equilibrium were also calculated from the experimental data *(Table 4)*. The proportion of proxibarbal increases with pH, being 70% at pH 6.0, 84% at pH 7.4, and 88% at pH 8.0. At pH 9.0, no equilibrium exists due to marked hydrolysis of valofan, but the proxibarbal/valofan ratio is close to 97:3. In contrast to the variable proxibarbal/valofan ratio, the proportion of the two valofan diastereoisomers remains the same in the pH range 6.0–8.0, the percentage of **IIY** and **IIX** being 63.2 ± 1.0 and 36.8 ± 1.0, respectively.

Table 4. *Relative Equilibrium (or Quasi-equilibrium) Concentrations [%] of Proxibarbal* (**I**) *and Valofan Diastereoisomers* **IIX** *and* **IIY** *as a Function of pH at 37°*

pH	**I**[a])	**I**[b])	**IIX**	**IIY**	**IIY/IIX**
6.00		70.5	10.9	18.6	64/36
6.75	78.1		8.3	13.6	62/38
6.95	79.6		7.4	13.0	64/36
7.00		83.5	5.9	10.6	64/36
7.05	81.7		6.6	11.7	64/36
7.15	82.3		6.5	11.2	63/37
7.30	83.5		6.1	10.4	63/37
7.40	83.9		6.0	10.1	63/37
7.40		84.3	6.0	9.7	61/39
8.00	87.4		4.7	7.9	63/37
8.00		87.9	4.1	7.1	64/36
9.00		97.5	0.8	1.7	66/34

[a]) Proxibarbal = 100% at time zero.　[b]) Valofan = 100% at time zero.

Influence of Temperature on the Rate of Reaction at pH 7.4. In a second set of experiments, the isomerisation was studied as a function of temperature at pH 7.4 to assess its thermodynamic parameters. The individual rate constants and the $t_{1/2}$ values

Table 5. *Rate Constants (k values* $\times 10^3$ *[min^{-1}]) and Half-lives ($t_{1/2}$ [min]) for the Reversible Transformation of Proxibarbal* (**I**) *into Valofan Diastereoisomers* **IIX** *and* **IIY** *as a Function of Temperature at pH 7.40*

Temp [°C]	k_{21}	k_{12}	$t_{1/2}$	k_{31}	k_{13}	$t_{1/2}$
20	3.49	0.40	178.1	4.45	0.26	147.1
30	9.50	1.29	64.2	10.8	0.85	59.5
37	21.1	2.81	29.0	24.0	1.84	26.8
45	27.9	4.49	21.4	34.0	3.29	18.6

Table 6. *Rate Constants (k values* $\times 10^3$ *[min^{-1}]) and Half-lives ($t_{1/2}$ [min]) for the Reversible Transformation of Valofan Diastereoisomers* **IIX** *and* **IIY** *into Proxibarbal* (**I**) *as a Function of Temperature at pH 7.40*

Temp [°C]	k_{21}	k_{12}	$t_{1/2}$	k_{31}	k_{13}	$t_{1/2}$
20	3.49	0.38	179.1	4.03	0.27	161.2
30	10.4	1.21	59.7	13.0	0.95	49.7
37	15.4	1.77	40.4	18.8	1.38	34.3
45	31.2	5.05	19.1	43.6	3.73	14.6

Table 7. *Thermodynamic Parameters for the Reversible Proxibarbal-Valofan Transformation at pH 7.40 and 37°*

		k_{21}	k_{12}	k_{31}	k_{13}
E_a [kJ·mol^{-1}]	[a]	67.0	76.4	65.4	79.1
	[b]	66.5	77.2	71.6	78.5
$\Delta H^{\neq}$ (kJ·mol^{-1})	[a]	64.4	73.8	62.9	76.5
	[b]	63.9	74.6	69.1	76.0
$\Delta S^{\neq}$ [J·mol^{-1}·K^{-1}]	[a]	−103.8	−90.3	−107.4	−85.1
	[b]	−108.0	−91.6	−89.6	−89.0
$\Delta G^{\neq}$ [kJ·mol^{-1}]	[a]	96.6	101.8	96.2	102.9
	[b]	97.4	103.0	96.9	103.6

		K_Y	K_X		
$\Delta G°$ [kJ·mol^{-1}]	[a]	5.2	6.6		
	[b]	5.6	6.7		

[a]   Proxibarbal = 100% at time zero.
[b]   Valofan = 100% at time zero.

were determined with proxibarbal and valofan as the starting compound (*Tables 5* and *6*, respectively). The corresponding rate constants in *Tables 5* and *6* are closely related, and a marked increase in reaction rates is seen. The log k_{ij} values are linearly correlated with $1/T$ ($r^2 = 0.97$ or better), allowing calculation of the activation energies E_a from the *Arrhenius* equation (*Table 7*).

The changes in the enthalpies, entropies, and free energies of activation, and the standard free-energy changes, are reported in *Table 7*. It is apparent that practically identical values are obtained from the experiments with either proxibarbal or valofan as the starting compound, the only exceptions being some entropy values. In particular, the changes in the free energy of activation ($\Delta G^{\neq}$) differ by 0.7 to 1.2 kJ·mol^{-1}, and the standard free-energy changes ($\Delta G°$) differ by 0.1 and 0.4 kJ·mol^{-1}. It is, thus, permissible to calculate average values for $\Delta G^{\neq}$ and $\Delta G°$, and to draw the energy profile of the reaction as shown in *Fig. 3*.
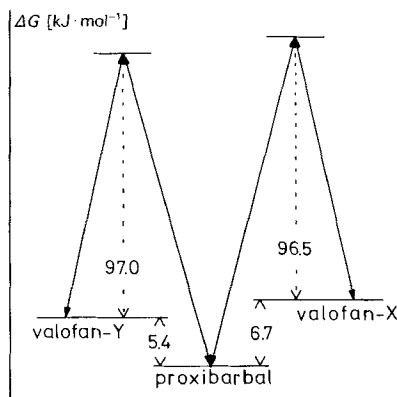

Fig. 3. *Free-energy profile of the reversible proxibarbal-valofan isomerisation in aqueous solution at pH 7.4 and 37°.* The energy differences shown are mean values taken from *Table 7*.

At pH 7.4 and 37°, proxibarbal (**I**) is thus more stable than valofan-Y (**IIY**) and valofan-X (**IIX**) by 5.4 and 6.7 kJ·mol⁻¹, respectively. The difference in standard free energy between **IIY** and **IIX** is −1.3 kJ·mol⁻¹, which corresponds to a calculated **IIY/IIX** ratio of 62:38 (observed value: 63.2:36.8).

Mechanism of the Proxibarbal-Valofan Isomerisation. In slightly acidic, neutral, or alkaline solutions, the ring opening of barbiturates is dominated by a HO⁻-catalysed hydrolysis [15–18]. A comparable situation is encountered for lactones in neutral and alkaline solutions [19]. If the same mechanism is postulated here, the proxibarbal-valofan isomerisation must follow a two-step reaction, in other words a reaction path with two transition states: *a)* a HO⁻-catalysed opening of the barbiturate or lactone ring (proxibarbal or valofan, respectively), leading to a common acyclic intermediate. *b)* An intramolecular nucleophilic attack on the carboxylate group by either the amido or the OH group, leading to the formation of a barbiturate or a lactone ring (*i.e.* to proxibarbal or valofan, respectively).

Such a mechanism does not appear likely under the conditions of the present study, since the latter shows the proxibarbal-valofan isomerisation to proceed at rates several orders of magnitude faster than those of HO⁻-catalysed barbiturate-ring hydrolysis [16–18]. Furthermore, the step *b* above involves a highly unlikely nucleophilic attack on an electron-rich carboxylate group.

These arguments point, in our opinion, to a simpler reaction path involving an intramolecular nucleophilic attack on a C=O group, namely: *a)* Attack of a lactam C=O by the OH group (proxibarbal-to-valofan reaction), or *b)* attack of the lactone C=O by the amido group (valofan-to-proxibarbal reaction). Both these intramolecular reaction lead to the same bicyclic transition state already postulated by *Bobranski* [1] *(Scheme 2)*. The latter intermediate can rearrange either to valofan *(Reaction 1, Scheme 2)* or to proxibarbal *(Reaction 2, Scheme 2)*. As the pH increases, N−C cleavage *(Reaction 1)* becomes less likely due to the progressive deprotonation of the NH group, and the proxibarbal/valofan equilibrium must be shifted towards proxibarbal as observed.

Scheme 2. *Postulated Mechanism of the Proxibarbal/Valofan Isomerisation under Near-Physiological Conditions*
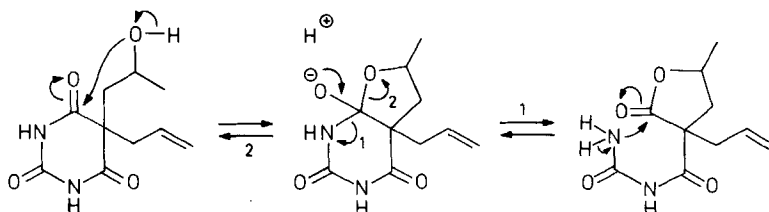
Conclusion. – The present study brings evidence for the pH and temperature dependence of the reversible proxibarbal-valofan isomerisation and for a reaction mechanism involving intramolecular nucleophilic attack. At pH 7.4 and 37° (*i.e.* under physiological conditions), an equilibrium was reached in *ca.* 120 min, yielding a proxibarbal/valofan ratio of 84:16. Such results may be of interest from a pharmacodynamic and pharmacokinetic viewpoint, but their biological relevance is limited by the fact that all experiments were conducted in monophasic aqueous systems. In a biological environment, lipophilic-

ity-dependent transport phenomena can markedly influence a drug's behaviour. In the subsequent study, this situation is demonstrated by a model using a biphasic octanol/H_2O system.

REFERENCES

[1] B. Bobranski, *Farmaco, Ed. Sci.* **1966**, *21*, 310.
[2] B. Bobranski, *Farmaco, Ed. Sci.* **1966**, *21*, 394.
[3] B. Bobranski, *Farmaco, Ed. Sci.* **1972**, *27*, 429.
[4] B. Bobranski, E. Wagner, *Roczniki Chemii* **1969**, *43*, 341.
[5] H. H. Wittekind, B. Testa, *J. Chromatogr.* **1979**, *179*, 370.
[6] J. Lauterwein, A. A. Pinkerton, H. H. Wittekind, B. Testa, *Helv. Chim. Acta* **1984**, *67*, 1662.
[7] B. Bobranski, L. Syper, *Arch. Immunol. Ther. Exp.* **1961**, *9*, 579.
[8] E. Frochaux-Zeidler, B. Testa, *Pharm. Acta Helv.* **1980**, *55*, 79.
[9] B. Lambrey, O. Lafont, C. Jacquot, *Eur. J. Med. Chem.* **1980**, *15*, 463.
[10] H. H. Wittekind, B. Testa, L. P. Balant, *Eur. J. Drug Metab. Pharmacokin.* **1984**, *9*, 117.
[11] A. Clow, P. Jenner, C. D. Marsden, J. Pratt, C. Reavill, C. Thanki, A. Theodorou, *Br. J. Pharmacol.* **1979**, *66*, 478P.
[12] B. Lambrey, P. L. Compagnon, C. Jacquot, *Eur. J. Drugs* **1981**, *6*, 161.
[13] U. Traversa, P. Puppini, C. Florio, R. Vertua, *Farmaco, Ed. Sci.* **1987**, *42*, 755.
[14] J. Estreicher, C. Revillard, J. P. Scherrer, *Comput. Biol. Med.* **1979**, *9*, 49.
[15] S. O. Eriksson, *Scient. Pharm.* **1966**, *2*, 255.
[16] E. R. Garrett, J. T. Bojarski, G. J. Yakatan, *J. Pharm. Sci.* **1971**, *60*, 1145.
[17] T. Hermann, *Pharmazie* **1974**, *29*, 453.
[18] T. Hermann, *Pharmazie* **1976**, *31*, 368.
[19] G. M. Blackburn, H. L. H. Dodds, *J. Chem. Soc., Perkin Trans. 2* **1974**, 377.