Kinetics of isomerization of tetracyclic spiro oxindole alkaloids

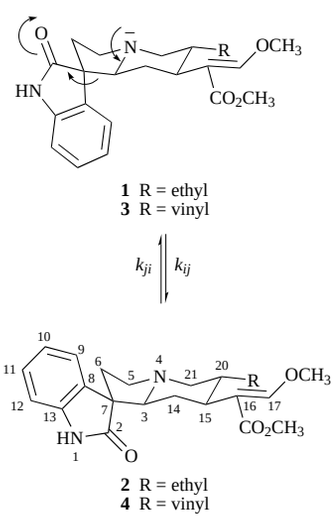
Gerhard Laus

Immodal Pharmaka GmbH, Bundesstrasse 44, A-6111 Volders, Austria

The effects of temperature, pH and solvent polarity on the rate of isomerization and the equilibrium composition of the tetracyclic spiro oxindole alkaloids rhynchophylline, isorhynchophylline, corynoxeine and isocorynoxeine have been investigated.

Introduction

In a recent work the kinetics of the isomerization of six pentacyclic spiro oxindole alkaloids was investigated.[1] Now data for the related tetracyclic alkaloids rhynchophylline **1**, isorhynchophylline **2**, corynoxeine **3** and isocorynoxeine **4** are available. The isomerization at the spiro centre of the alkaloids **1** and **2** was recognized as early as 1959 and a retro-Mannich ring opening, rotation and Mannich ring closure was proposed as the mechanism (Scheme 1).[2] This behaviour complicates the evaluation of pharmacological properties of single isomers which are expected to exhibit different activities. From the present results the course of isomerization can be predicted and used to produce a desired isomer, while on the other hand the isomerization can be inhibited by the proper selection of a solvent.



Scheme 1

Results and discussion

Effect of temperature

The isomerization at the spiro centre C-7 proceeds in a pseudo-first-order process with respect to each isomer and can be described by the familiar rate law in eqn. (1).† The open zwitterionic species is treated as a steady-state intermediate and cancelled. The rate coefficients at three temperatures (25, 37 and 50 °C) were obtained by fitting the calculated curves to the experimental data by a least-squares method. The results and activation parameters are summarized in Table 1.

$$\frac{d[\mathbf{1}]}{dt} = -k_{12}[\mathbf{1}] + k_{21}[\mathbf{2}] \quad (1)$$

† The numbering system is based on that customarily used for the hetero-yohimbinoid alkaloids.

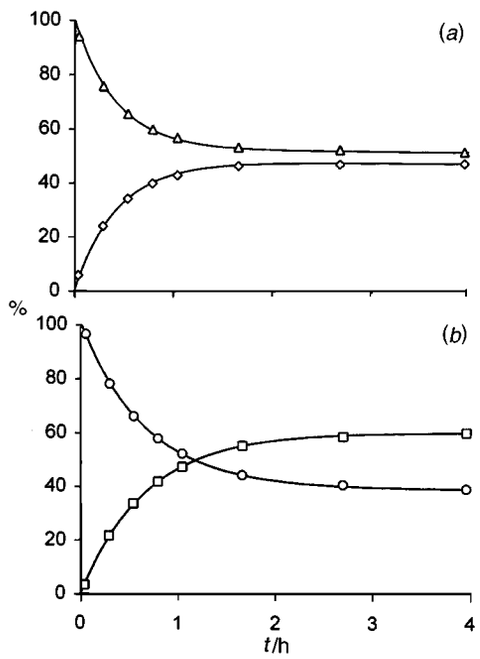


Fig. 1 Isomerization of tetracyclic spiro oxindole alkaloids in aqueous buffer pH 7 at 50 °C, as monitored by HPLC. Percent of **1** (△), **2** (◇), **3** (○) and **4** (□) as a function of time, starting with either (*a*) **1** or (*b*) **3**. The curves are calculated using the coefficients given in Table 1.

It can be seen that the equilibrium composition of the tetracyclic isomers in water is more sensitive towards a change of temperature than it was in the case of the related pentacyclic alkaloids mitraphylline and isomitraphylline, where the molar heat change of reaction was found to be only 3.5 kJ mol^{-1}.[1] From a plot of ln K against $1/T$ it was deduced that at pH 7 the equilibrium constant K is unity for the rhynchophyllines at 54 °C and for the corynoxeines at 23 °C, respectively. The course of the reactions in water at 50 °C is depicted in Fig. 1. In methanol the isomerization is considerably slower and the effect of temperature on the equilibrium is reversed which is reflected by the negative molar heat change of reaction. The decrease of the rate in methanol can be largely attributed to the negative entropy of activation due to charge separation in a solvent with a small relative permittivity. It is remarkable how different the activation parameters of the saturated and unsaturated pairs of isomers are in water while nearly identical in methanol.

Effect of pH

The effect of pH on the rate of isomerization was studied in the range pH 4–9 at 50 °C. Protonation at N-4 inhibits the formation of the intermediate immonium ion and therefore slows the reaction. The simplified rate law for the initial rate is given in eqn. (2) where k_{12} applies to unprotonated molecules only.

$$\frac{d[\mathbf{1}]}{dt} = -k_{12}[\mathbf{1}]_{free} = -k'_{12}[\mathbf{1}]_{total} \quad (2)$$

Table 1 Common logarithms of the rate coefficients k_{ij} and Arrhenius activation parameters for the isomerization of **1**–**4** in aqueous buffer pH 7 and in MeOH at 25, 37 and 50 °C

		log (k/s^{-1})					
Solvent	k_{ij}	T = 298 K	310 K	323 K	E_a/kJ mol^{-1}	log (A/s^{-1})	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}
Water	k_{12}	−5.21	−4.41	−3.53	124	16.5	63
	k_{21}	−4.96	−4.28	−3.49	108	14.0	15
	k_{34}	−5.05	−4.27	−3.60	107	13.7	9
	k_{43}	−5.08	−4.36	−3.79	95	11.6	−32
Methanol	k_{12}	−6.02	−5.33	−4.68	99	11.3	−37
	k_{21}	−6.56	−5.84	−5.16	103	11.5	−33
	k_{34}	−6.49	−5.79	−5.11	101	11.3	−37
	k_{43}	−7.06	−6.32	−5.63	106	11.5	−33

		Equilibrium data			
		mol%[a]			
Solvent	Isomer	T = 298 K	310 K	323 K	ΔH_r/kJ mol^{-1}
Water	**1**	64.1	57.3	52.1	16 (for **1**→**2**)
	2	35.9	42.7	47.9	
	3	48.8	45.0	39.3	12 (for **3**→**4**)
	4	51.2	55.0	60.7	
Methanol	**1**	22.3	23.6	24.7	−4.1 (for **1**→**2**)
	2	77.7	76.4	75.3	
	3	21.1	22.9	23.4	−4.3 (for **3**→**4**)
	4	78.9	77.1	76.6	

[a] Mean values of at least three experiments, rel. s.d. = 0.8%.

From the initial rates at 50 °C the effective rate coefficients k' were determined which apply to the total concentration of (protonated and free) alkaloid. The nature of the pH–log k' profile, *e.g.* for **1**, can be predicted by eqn. (3). For sufficiently acidic

$$\log k'_{12} = \log k_{12} + \log \frac{[\mathbf{1}]_{free}}{[\mathbf{1}]_{total}} \quad (3)$$

solutions eqn. (3) simplifies to eqn. (4), giving a linear dependence of log k' on pH, where K_a is the acidity constant of the protonated species.

$$\log k' = \log k - pK_a + pH \quad (4)$$

Above pH 8 the reaction proceeds without hindrance, and k' becomes equal to k. The observed pH–log k' profiles of the isomerizations of **1**–**4** are described by eqns. (5)–(8).

$$\text{for } \mathbf{1} \quad \log(k'/s^{-1}) = -3.00 - 0.98 \log(1 + 10^{(7.5-pH)}) \quad (5)$$

$$\text{for } \mathbf{2} \quad \log(k'/s^{-1}) = -3.35 - 0.81 \log(1 + 10^{(6.8-pH)}) \quad (6)$$

$$\text{for } \mathbf{3} \quad \log(k'/s^{-1}) = -2.90 - 1.00 \log(1 + 10^{(7.7-pH)}) \quad (7)$$

$$\text{for } \mathbf{4} \quad \log(k'/s^{-1}) = -3.78 - 0.78 \log(1 + 10^{(6.7-pH)}) \quad (8)$$

From a plot of log k' against pH (Fig. 2) it can be seen that the curves of two isomers intersect at a definite pH value. Thus, at 50 °C the equilibrium constant becomes unity at pH 7.20 for the rhynchophyllines and at pH 6.55 for the corynoxeines, respectively, explaining the considerable difference between the course of isomerization of the rhynchophylline and the corynoxeine isomers at pH 7. At both higher and lower pH values the course of reaction is similar for the two groups of isomers. This can also be seen from the distribution of the alkaloids in equilibrated aqueous solutions at different pH values (Table 2). Obviously, the base strengths of rhynchophylline and corynoxeine are sufficiently different to account for these results.

Table 2 Distribution of alkaloids **1**–**4** in equilibrated aqueous solutions as a function of pH at 50 °C

	mol%					
Isomer	pH = 4	5	6	7	8	9
1	77	77	73	52	34	26
2	23	23	27	48	66	74
3	75	75	69	39	32	28
4	25	25	31	61	68	72

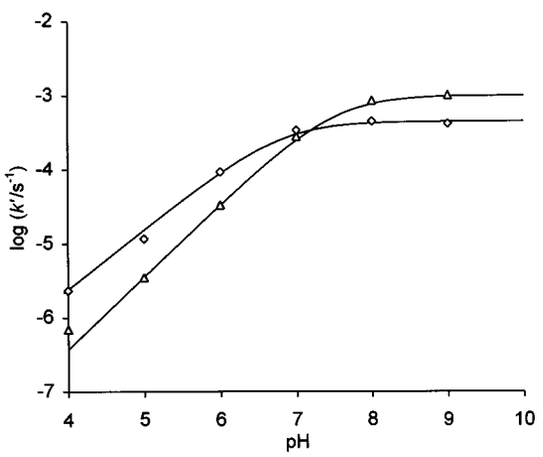


Fig. 2 pH–rate coefficient profiles of the isomerization of the spiro oxindole alkaloids rhynchophylline **1** (△) and isorhynchophylline **2** (◇) measured in aqueous buffer at 50 °C. The curves are calculated from eqns. (5) and (6).

Influence of solvent polarity

From the initial rates at 50 °C the dependence of the rate coefficients on the solvent polarity of six organic solvents was studied. Highly satisfactory correlations between the Dimroth–Reichardt solvent polarity parameter[3] E_T^N and the logarithm of the rate coefficients k were obtained. By linear regression including the values in water the following eqns. (9)–(12) were derived:

$$\text{for } \mathbf{1} \quad \log(k/s^{-1}) = 5.2\, E_T^N - 8.7 \quad r^2 = 0.976 \quad (9)$$

$$\text{for } \mathbf{2} \quad \log(k/s^{-1}) = 7.2\, E_T^N - 10.8 \quad r^2 = 0.991 \quad (10)$$

Table 3 Logarithmic correlation of the rate coefficients of the isomerization of the alkaloid isomers **1**–**4** at 50 °C with solvent polarity using the Dimroth–Reichardt polarity scale

Solvent	Polarity E_T^N (323 K)	log (k/s^{-1}) **1**	**2**	**3**	**4**
Water	1.00	−3.53	−3.49	−3.60	−3.79
Methanol	0.76	−4.68	−5.16	−5.11	−5.63
Ethanol	0.64	−5.58	−6.20	−6.06	−7.16
Propan-2-ol	0.54	−6.08	−7.28	−6.41	−7.73
Dimethyl sulfoxide	0.42	−6.28	−7.71	−7.20	—[a]
Pyridine	0.31	−7.45	−8.33	—[a]	—[a]
Dioxane	0.17	−7.64	—[a]	—[a]	—[a]

[a] Too small to be determined.

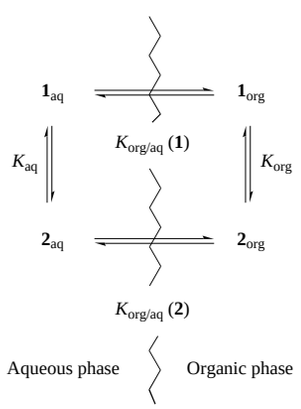
$$\text{for } \mathbf{3} \quad \log(k/s^{-1}) = 6.2\, E_T^N - 9.8 \quad r^2 = 0.994 \qquad (11)$$

$$\text{for } \mathbf{4} \quad \log(k/s^{-1}) = 8.8\, E_T^N - 12.6 \quad r^2 = 0.987 \qquad (12)$$

From these results (Table 3) reaction rates in other solvents or solvent mixtures can be predicted. For example, log k for the isomerization of **1** at 50 °C in a mixture of dioxane–methanol (1 : 1) with $E_T^N = 0.68$ was estimated from eqn. (9) as −5.2 and was found to be −5.0 experimentally. The polarity of the solvent also affects the composition of the equilibrium mixture, as already seen for methanol in Table 1. The equilibrium in more apolar solvents can be calculated from the rate coefficients in Table 3. Thus, the isorhynchophylline content of an equilibrated solution increases with diminishing polarity, *e.g.* 81% in ethanol, 96% in dimethyl sulfoxide and 99% in dioxane. In conclusion, the polar solvents reduce $\Delta G^{\ddagger}$ of the zwitterionic intermediate, while on the other hand apolar solvents stabilize the more lipophilic isomer in the equilibrium.

Water-immiscible solvents

The equilibrium constant in water-immiscible solvents cannot be determined directly owing to the small rate of reaction, but it can be calculated from distribution ratios between the organic and the aqueous phases (Scheme 2) using eqn. (13), wherein



Scheme 2

$$K_{org} = \frac{K_{org/aq}(\mathbf{2})}{K_{org/aq}(\mathbf{1})} K_{aq} \qquad (13)$$

K_{org} and K_{aq} are the equilibrium constants **[2]**/**[1]** in the organic and aqueous phases, respectively, and $K_{org/aq}$ is the distribution ratio for each isomer between the organic and the aqueous phase. Distribution experiments showed that those isomers which cannot be stabilized by an intramolecular hydrogen bond between the protonated N-4 and the carbonyl oxygen of the oxindole moiety, *i.e.* isorhynchophylline **2** and isocoryn-

Table 4 Distribution ratios $K_{org/aq}$ of the alkaloids **1**–**4** between organic solvents and aqueous buffer as a function of pH at 50 °C

Organic solvent	Isomer	log $K_{org/aq}$ pH = 5	6	7	8
n-Hexane	**1**	−1.6	−1.3	−0.81	−0.36
	2	−1.0	−0.005	0.34	0.46
	3	−1.9	−1.5	−0.76	−0.46
	4	−0.82	−0.18	0.20	0.27
$CHCl_3$	**1**	1.8	2.2	2.7	3.1
	2	2.2	2.5	2.9	3.6

oxeine **4**, are more lipophilic than the corresponding 7-epimers (Table 4).

Experimental

Materials

The alkaloids **1**–**4** were obtained by extraction of *Uncaria rhynchophylla* (Miq.) Havil. (Rubiaceae) leaves,[4] followed by conventional acid–base work-up and separation by column chromatography (silica gel, AcOEt–MeOH = 95 : 5). The structures of the oxindole alkaloids were corroborated by IR, UV, MS and NMR spectroscopy. Solvents were used as supplied (Merck, ≤0.02% water). Phosphate buffer concentration was 0.065 mol dm^{-3}.

Kinetic procedures

The isomerization was followed by monitoring the composition of the thermostatted (±0.1 K) reaction mixture by HPLC analysis, using a RP-18 (5 μm) column (125 × 4 mm id, Merck) and a mixture of acetonitrile and 0.01 M aqueous phosphate buffer pH 7.0 (40 : 60, 52 °C) as the eluent with a flow of 1.3 cm^3 min^{-1}. Detection was carried out at 247 nm. Retention times were as follows: isocorynoxeine 5.9 min, corynoxeine 6.2 min, isorhynchophylline 7.2 min and rhynchophylline 8.1 min. Typically, solutions of 0.1 mg alkaloid in 10 cm^3 solvent were prepared (2.6×10^{-5} mol dm^{-3}). Solutions in miscible organic solvents were diluted with buffer, immiscible solvents were evaporated and the residue reconstituted with eluent, and acidic solutions were neutralized immediately prior to analysis. Relative standard deviation of the primary HPLC measurements was 0.8%, standard deviation of log (k/s^{-1}) was 0.03.

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