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Separation of keto–enol tautomers of chlortetracycline and 4-epichlortetracycline by liquid chromatography on poly(styrene–divinylbenzene)copolymer*

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Abstract: Keto and enol tautomers of chlortetracycline and 4-epichlortetracycline were successfully separated by low temperature high-performance liquid chromatography on poly(styrene–divinylbenzene)copolymer. The keto–enol tautomerism occurs between C-11a and C-12. The elucidation of the chemical structure of the tautomers was realized with the help of on-line and off-line UV-spectrophotometry and of ^{13}C and ^{1}H NMR spectrometry. The kinetics of the equilibrium reaction were investigated.

Keywords: *Keto–enol tautomers; chlortetracycline; 4-epichlortetracycline; liquid chromatography; poly(styrene–divinylbenzene)copolymer; UV spectrophotometry; ^{13}C and ^{1}H NMR.*

Introduction

Chlortetracycline (CTC) has been in use for more than 40 years and is still one of the more important tetracycline antibiotics. CTC like other tetracyclines, undergoes epimerization at position C-4 forming 4-epichlortetracycline (ECTC) [1]. During the development of a liquid chromatography (LC) method for the analysis of CTC on poly(styrene–divinylbenzene)copolymer (PSDVB) as the stationary phase, it was observed that the peaks corresponding to CTC or ECTC were much broader than those corresponding to other tetracyclines [2]. This phenomenon was further investigated and it was observed that by using the same LC method at 10°C instead of 60°C, each of the peaks corresponding to CTC or ECTC was split in two peaks. Fresh solutions of CTC or ECTC showed only one peak, but upon standing an additional peak was formed. When either of the two peaks was collected, the other was formed again upon standing. The phenomenon was not observed on classical silica based reversed-phase stationary phases. It was believed that an equilibrium was formed between tautomers. An indication for the existence of such an equilibrium can be found in the early literature. McCormick *et al.* showed the UV spectra of ECTC and CTC, recorded after standing of the solutions for 30 min, whereas the UV spectra of the other tetracyclines were recorded immediately [1]. Here the phenomenon was examined by UV spectrophotometry and ^{1}H and ^{13}C NMR spectrometry. It is concluded that keto–enol tautomerism occurs between C-11a and C-12 (see Fig. 1). The kinetics of the equilibrium reaction are also discussed.

It is believed that the separation of the tautomers on PSDVB is due to π–π interactions between ring D of CTC or ECTC and the aromatic rings of the stationary phase. Such interactions have been reported previously [3, 4]. The separation of tautomers by LC has also been mentioned in literature previously [5, 6].

Experimental

Samples and reagents

Purified chlortetracycline hydrochloride (CTC·HCl), 4-epichlortetracycline hydrochloride (ECTC·HCl), anhydrochlortetracycline hydrochloride (ACTC·HCl), 4-epianhydrochlortetracycline hydrochloride (EACTC·

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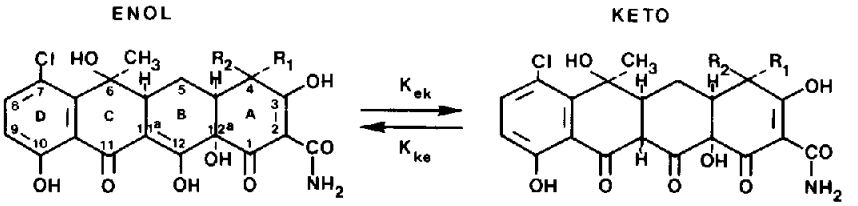
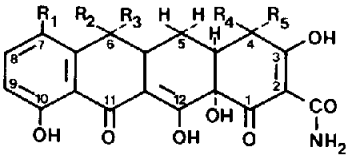
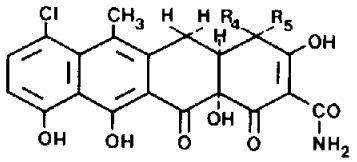


	R_1	R_2
Chlortetracycline (CTC)	$N(CH_3)_2$	H
4-Epichlortetracycline (ECTC)	H	$N(CH_3)_2$

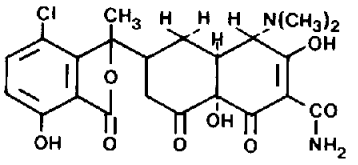
Figure 1
Structures of the tautomers of chlortetracycline and 4-epichlortetracycline.



	R_1	R_2	R_3	R_4	R_5
Tetracycline (TC)	H	OH	CH_3	H	$N(CH_3)_2$
4-Epitetracycline (ETC)	H	OH	CH_3	$N(CH_3)_2$	H
Demethylchlortetracycline (DMCTC)	Cl	OH	H	H	$N(CH_3)_2$
4-Epidemethylchlortetracycline (EDMCTC)	Cl	OH	H	$N(CH_3)_2$	H



	R_4	R_5
Anhydrochlortetracycline (ACTC)	H	$N(CH_3)_2$
4-Epianhydrochlortetracycline (EACTC)	$N(CH_3)_2$	H



Isochloortetracycline (ISOCTC)

Figure 2
Chemical structures of other tetracycline and related substances examined.

HCl), isochlortetracycline hydrochloride (ISOCTC·HCl), tetracycline hydrochloride (TC·HCl) and 4-epitetracycline hydrochloride (ETC·HCl) were provided from Janssen Chimica (Beerse, Belgium). Demeclocycline hydrochloride (DMCTC·HCl) and 4-epi-demeclocycline hydrochloride (EDMCTC·HCl) were purified in the laboratory. The structures of these substances are shown in Fig. 2.

Organic solvents were from Janssen Chimica. Double distilled water was used throughout. Other reagents were of pro analysi quality (Merck, Darmstadt, FRG). Buffers for the calibration of pH measurements were

prepared following instructions of the *European Pharmacopoeia* [7].

Sample preparation and apparatus

The HPLC apparatus consisted of a L-6200 intelligent pump (Merck-Hitachi, Tokyo, Japan), an injector model CV-6-UHPa-N60 (Valco, Houston, TX, USA) equipped with a 20-μl loop, a Merck–Hitachi L-4000 UV detector set at 260 nm and an integrator model 3390 A (Hewlett–Packard, Avondale, PA, USA). A Waters model 990 photodiode-array detector (Milford, MA, USA) was used to measure the on-line UV spectra. The 100 × 4.6 mm i.d. column was packed in the laboratory with PLRP-S 100 Å 8 μm (Polymer Labs, Church Stretton, Shropshire, UK). The material was moistened with acetone R and then water was added to prepare a slurry which was packed upwards into the column at a pressure of 48 MPa with water as the pressurizing solvent.

The mobile phase was 2-methyl-2-propanol-1 M perchloric acid–water (16:5:79%, m/v/v). The mobile phase was degassed by ultrasonication. The flow rate was 1.0 ml min^{-1}. The column was maintained at 10°C by means of a water jacket connected with a cooling system (Julabo, Seelbach, FRG).

A sample aliquot (5.0 mg) was dissolved in 20.0 ml of the specified solvent and the time clock was started at the moment the solvent was added. For qualitative experiments 0.01 N HCl was used as the solvent. For kinetic work sodium citrate–HCl buffers were used in the pH range 2–4. The ionic strength of the buffers was adjusted with potassium chloride. In some experiments the solvent was water which was adjusted to the pH indicated with 0.01 N HCl or 0.01 N NaOH. The solvents and the solutions were maintained at the temperature indicated in the text.

Off-line UV spectra were recorded on a PU 8740 UV/vis scanning spectrophotometer (Philips–Pye Unicam, Cambridge, UK) using a 10 mm cell. A sample aliquot (5.0 mg) was dissolved in 500.0 ml of 0.01 N hydrochloric acid. The UV absorbance–time profile was followed. The time clock was started at the moment the solvent was added. NMR spectra (^1H and ^{13}C) were taken on a FT 90 MHz FX90Q instrument (Jeol, Tokyo, Japan) operating at 89.60 MHz (^1H) or 22.53 MHz (^{13}C). ECTC·HCl (70 mg) was dissolved in 1.0 ml of H_2O or D_2O. The spectrum profile was obtained at 20°C. CTC·HCl was not investigated because of its poor solubility in water.

Results and Discussion

Structure analysis

Figure 3 shows chromatograms of freshly prepared and equilibrated solutions of CTC and ECTC in 0.01 N HCl obtained with a mobile phase at 10°C. This low temperature allows the separation of the keto–enol tautomers, but as a consequence the back pressure is rather high (about 20 MPa). It is observed that in the freshly prepared solutions (chromatograms A), the first peak is very small. Its presence is due to the fact that the solution was injected only 2 min after the first contact with the solvent. The chromatograms B show the situation at equilibrium. More of the first peak is formed for ECTC (50%) than for CTC (20%). When the concentration of CTC or ECTC was increased in acid aqueous solution the ratio of the newly formed peak in the mixture decreased. For CTC the equilibrium was reached much faster. When either of the two peaks of CTC or ECTC was collected and reinjected, the same equilibrium was reached again. This clearly shows that the phenomenon is not caused by decomposition. That the appearance of the additional peak is not due to chromatographic splitting is shown by the UV spectra in Fig. 3, which were recorded at the apex of the peak. Tautomers of CTC are well separated from tautomers of ECTC. Under the experimental conditions, epimerization was not observed. To compensate for differences in concentration the spectra were normalized at 260 nm. Analogous spectra were obtained throughout the peak, which indicates that each peak corresponds to one single substance.

Figure 4 shows off-line spectra of CTC or ECTC obtained 2 min after the first contact with the solvent and at equilibrium (120 min). Analogous LC and UV experiments were carried out with the tetracyclines and their derivatives shown in Fig. 2. None showed the same behaviour as that observed for CTC or ECTC. Since the major differences in the UV-spectra in Figs 3 and 4 are situated at longer wavelength (340–380 nm) it can be concluded that the rings B-C-D-chromophore is involved in the observed phenomenon. Apparently the A ring chromophore, contributing only to the absorbance at shorter wavelength (250–300 nm), is not affected [8]. This is further

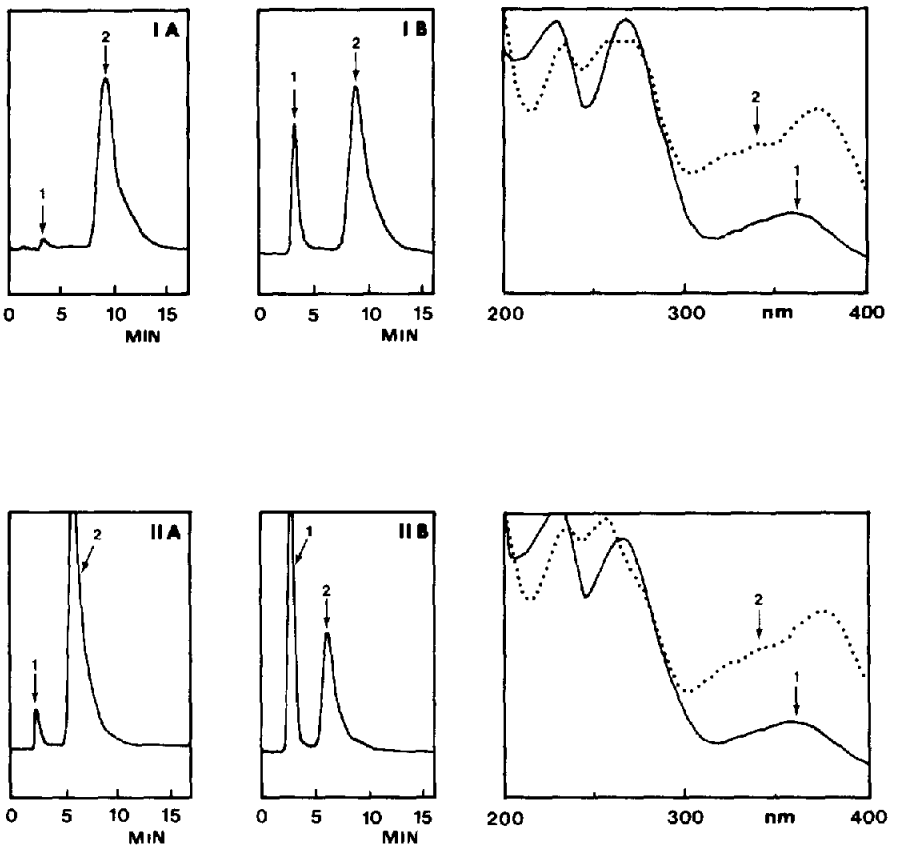


Figure 3
LC chromatograms of chlortetracycline and 4-epichlortetracycline and the corresponding on-line UV spectra. Solutions examined: IA, CTC 2 min after dissolution; IB, CTC after 60 min (equilibrium); IIA, ECTC 2 min after dissolution; IIB, ECTC after 120 min (equilibrium); solvent, 0.01 N HCl; temperature, 20°C. HPLC conditions: column, PLRP-S 100 Å (100 × 4.6 mm i.d.); mobile phase, 2-methyl-2-propanol-1 M perchloric acid–water (16:5:79%, m/v/v); temperature, 10°C, detection at 260 nm; flow rate, 1.0 ml min^{-1}. The UV spectra are taken at the apex of the peaks and are normalized at 260 nm.

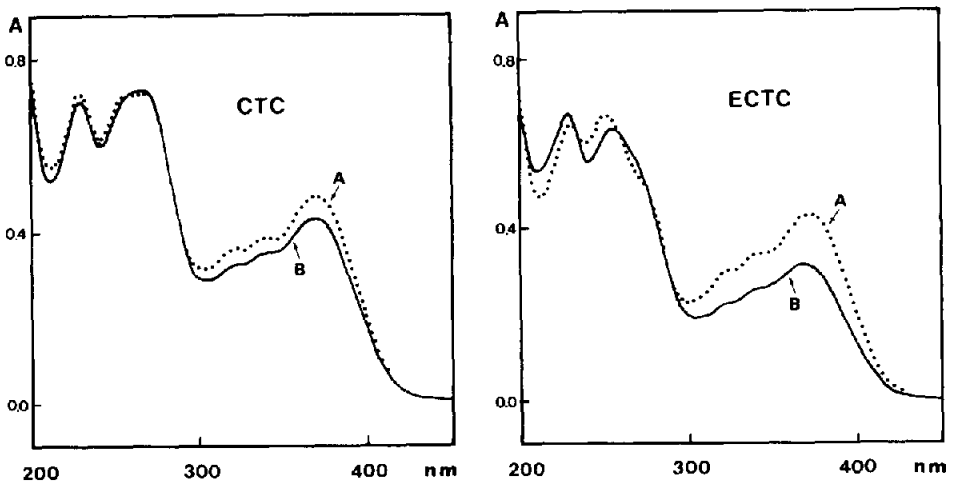


Figure 4
UV spectra of chlortetracycline and 4-epichlortetracycline: A, 2 min after dissolution; B, after 120 min (equilibrium). 0.001% (m/v) in 0.01 N HCl at 20°C.

indicated by the fact that the phenomenon was not observed with the anhydro- or iso-derivatives of CTC or ECTC, though they all have an intact A ring. It is therefore suggested that in solution the compound may be considered to be a tautomeric mixture, as shown in Fig. 1.

Conclusive structural evidence was obtained from ^{13}C NMR spectrometry, which clearly indicates, as will be discussed below, the presence of a 11,12-β-diketo tautomer in the mixture. Another possible tautomeric structure having the 11-enol, 12-keto unit [9] instead of the normal 11-keto, 12-enol form which is usually retained for tetracyclines [10] could not be detected here. The proton–noise decoupled spectrum of a sample of ECTC·HCl in D_2O after equilibration for 120 min showed two different sets of resonances in a ratio of about 2:3. The chemical shifts are summarized in Table 1, and the assignments were based largely on a comparison with published values for tetracycline derivatives [11]. The shifts for the major isomer compare very well with those of CTC taking into account the specific changes for C-4, C-4a and C-5 induced by epimerization at C-4 and exemplified by the pair tetracycline–4-epitetracycline. This major tautomer, which is represented by formula ENOL in Fig. 1 is thus the normal form of ECTC and seems to be also the form of the crystalline substance as shown by the rapidly recorded ^1H NMR spectrum of a freshly prepared sample.

Table 1
^{13}C chemical shifts*† and peak assignments for 4-epichlortetracycline hydrochloride tautomers in D_2O solution

Position	Enol tautomer	Keto tautomer
1	191.4 (S)	187.6 (S)
2	96.6 (S)	97.7 (S)
3	188.4 (S)	188.6 (S)
4	67.8 (D)	68.4 (D)
4a	42.2 (D)	42.7 (D)
5	20.9 (T)	22.3 (T)
5a	40.2 (D)	48.4 (D)
6	72.1 (S)	72.8 (S)
6a	142.7 (S)	142.4 (S)
7	123.7 (S)	123.7 (S)
8	141.5 (D)	142.7 (D)
9	120.2 (D)	119.7 (D)
10	161.5 (S)	161.7 (S)
10a	117.4 (S)	115.9 (S)
11	193.4 (S)	196.7 (S)
11a	106.1 (S)	61.7 (D)‖
12	173.2 (S)‡	200.2 (S)
12a	74.8 (S)	81.5 (S)
CO-amide	173.5 (S)‡	172.2 (S)
4-NMe_2	§	§
6-Me	24.9 (O)	26.4 (Q)

*Expressed in ppm downfield from tetramethylsilane (TMS); peak positions were measured relative to the centre of the multiplet of internal DMSO-d_6, set at 39.6 ppm versus TMS.

†The multiplicity of the signals in the OFR-spectrum is indicated in parentheses: S, singlet for quaternary carbon; D, doublet for methine; T, triplet for methylene; and Q, quadruplet for methyl carbon.

‡Assignments may be interchanged.

§Very broad signal at about 40 ppm.

‖Not visible in D_2O solution, but only in H_2O solution.

Spectral analysis of the minor component in the mixture revealed that most of the chemical shifts are only slightly different from those of the major isomer except for an additional ketonic absorption in the range 180–200 ppm, the disappearance of a signal in the enolic region (160–180 ppm) and the absence of another minor signal in the lower sp^2-region (95–115 ppm). When the spectrum was taken in H_2O instead of D_2O, the missing signal appeared at 61.7 ppm and was further split into a doublet when the spectrum was recorded in the "off resonance" mode (OFR). This all clearly points to a β-diketo structure as represented in formula KETO in Fig. 1. Indeed, the shift and multiplicity of the signal at 61.7 ppm is characteristic for a methine carbon flanked by two carbonyl groups and the signal is thus assigned to C-11a, showing a resonance at about 106.1 ppm in the normal tautomer. Also, as could be expected, the signal should disappear from the spectrum by deuteration of the carbon and this is what happened when the spectrum was taken in D_2O. Further, C-12 is ketonic and is accordingly assigned to the lowest field resonance at 200.2 ppm. The 196.7 ppm signal is then assigned to C-11 which in comparison with the normal tautomer, is less conjugated and thus shifts slightly downfield. The 3.8 ppm upfield shift for C-1 which is now assigned to the 187.6 ppm signal may be caused by the lack of H-bonding with the 12-OH group. Other shift differences, especially for adjacent carbons C-12a or C-5a, may be explained by direct electronic effects due to different environments in the two tautomers. Also conformational changes in the A ring orientation similar to those reported previously for 4-epimeric tetracyclines [12] could have a substantial influence upon the chemical shifts of the carbons in question. However, since the 90 MHz ^1H NMR spectrum did not show clearly resolved peak patterns, except for H-4 and for the aromatic hydrogen H-8 and H-9, no relevant coupling information could be extracted from the spectrum and consequently no

conclusions could be drawn concerning the exact conformation of the rings nor for the configuration of the newly formed asymmetric centre C-11a.

It can be concluded that in the solid state CTC·HCl like ECTC·HCl exists as the enol, in acid solution it exists as the keto–enol tautomers. It was not possible to isolate the keto tautomer in the solid state. When an aqueous solution of CTC was slowly evaporated the pure enol was obtained and when such a solution was quickly evaporated *in vacuo*, a mixture of the tautomers was obtained.

Kinetic studies

In order to obtain quantitative results for the keto–enol tautomerism of CTC it was necessary to validate further the LC method. The tautomerism of ECTC was not examined quantitatively because ECTC is only a microbiologically less active impurity of CTC. The detector was set at 260 nm since this is the isobestic point for the CTC tautomers. Calibration curves for CTC enol (CTC e) and CTC keto (CTC k) were prepared with solutions containing known amounts of CTC·HCl in 0.01 N HCl up to 0.5 mg ml^{-1}. After equilibration the solutions were analysed and the mass corresponding to the enol or keto tautomers was calculated from the total mass injected, the area of each of the two peaks and the sum of the area of the two peaks. In all, four solutions were analysed three times. The following relationships were found, where y = peak area, x = amount (in micrograms) of hydrochloride salt injected, r = coefficient of correlation, $S_{y,x}$ = standard error of estimate, CR = range of injected mass examined. For CTC e, $y = -164 + 6772x$, $r = 0.9997$, $S_{y,x} = 50$, CR = up to 7.5 μg, for CTC k, $y = -66 + 6792x$, $r = 0.9997$, $S_{y,x} = 56$, CR = up to 2.5 μg. The limits of quantitation were 0.1 μg for CTC e and 0.05 μg for CTC k.

For an equilibrium reaction the following equations can be written [13]:

$$\text{CTC e} \underset{k_{ke}}{\overset{k_{ek}}{\rightleftarrows}} \text{CTC k}$$

k_{ek} = rate constant for transformation of CTC e to CTC k

k_{ke} = rate constant for transformation of CTC k to CTC e

$$\log \frac{\text{CTC e (O)} - \text{CTC e (eq)}}{\text{CTC e } (t) - \text{CTC e (eq)}} = \frac{k_{ek} + k_{ke}}{2.303} t$$

CTC e (O) = content (%) of CTC e at time O = 100%

CTC e (eq) = content (%) of CTC e at equilibrium

CTC e (t) = content (%) of CTC e at time t

$$K_{eq} = \frac{k_{ek}}{k_{ke}} = \frac{\text{CTC } k \text{ (eq)}}{\text{CTC e (eq)}}$$

From these equations values for K_{eq}, k_{ek} and k_{ke} were obtained for pH 2.0 at different ionic strengths. The results are reported in Table 2. It can be concluded that the influence of the ionic strength is negligible in the range examined.

The influence of the buffer concentration was investigated in the pH range 2–4. The results are reported in Table 3. The buffer concentration had only a slight influence on the equilibrium constants, but the pH value affected the equilibrium constants. At higher pH the equilibrium was shifted towards the keto tautomer. A catalytic effect of the buffer on the rate constants was observed at the three pH values examined. Linear relationships existed between the observed rate constants and the total citrate concentration. The intercept provided buffer-independent apparent first-order rate constants (k_O). Table 4 shows the relation-

Table 2
Observed rate constants (min^{-1}) and equilibrium constants for the tautomerism of chlortetracycline at pH 2.0 as a function of the ionic strength

Ionic strength (μ)	$k_{ek} \times 10^2$	$k_{ke} \times 10^2$	$K_{eq} \times 10^2$
0.025	2.74 ± 0.06	11.61 ± 0.24	23.60 ± 0.71
0.1	2.73 ± 0.13	11.50 ± 0.54	23.74 ± 1.59
0.5	2.82 ± 0.09	11.70 ± 0.37	24.10 ± 1.08

Solvent: 0.765×10^{-2} M citrate buffer pH 2.0, with the ionic strength indicated and at 20°C.

Table 3
Observed rate constants (min^{-1}) and equilibrium constants for the tautomerism of chlortetracycline at different pH values as a function of the concentration of the citrate buffer, with an ionic strength of 0.1 and at 20°C

	pH 2.0				pH 3.0				pH 4.0		
Buffer concentration ($M \times 10^2$)	$k_{ek} \times 10^2$	$k_{ke} \times 10^2$	$K_{eq} \times 10^2$	Buffer concentration ($M \times 10^2$)	$k_{ek} \times 10^2$	$k_{ke} \times 10^2$	$K_{eq} \times 10^2$	Buffer concentration ($M \times 10^2$)	$k_{ek} \times 10^2$	$k_{ke} \times 10^2$	$K_{eq} \times 10^2$
0.459	2.78 ± 0.04	11.36 ± 0.16	24.47 ± 0.49	0.605	3.11 ± 0.07	11.08 ± 0.23	28.07 ± 0.86	0.840	3.08 ± 0.08	8.17 ± 0.20	37.70 ± 1.35
0.765	2.73 ± 0.13	11.50 ± 0.54	23.74 ± 1.59	1.010	3.53 ± 0.10	13.28 ± 0.36	26.58 ± 1.04	1.400	4.98 ± 0.03	13.45 ± 0.09	37.03 ± 0.61
1.53	2.80 ± 0.02	12.24 ± 0.10	22.88 ± 0.25	2.020	4.49 ± 0.14	16.99 ± 0.53	26.43 ± 1.17	2.800	7.96 ± 0.24	21.64 ± 0.64	36.78 ± 1.55
2.30	3.01 ± 0.03	13.44 ± 0.14	22.40 ± 0.32	3.020	5.32 ± 0.19	20.53 ± 0.73	25.91 ± 1.31				
3.06	3.17 ± 0.09	14.33 ± 0.40	22.12 ± 0.08								
Intercept ($k_O \times 10^2$)	2.63 ± 0.06	10.65 ± 0.15			2.59 ± 0.06	9.09 ± 0.37			1.27 ± 0.52	3.20 ± 1.48	
Slope	0.16 ± 0.03	1.18 ± 0.08			0.91 ± 0.03	3.84 ± 0.19			2.42 ± 0.28	6.68 ± 0.79	
r	0.9472	0.9933			0.9991	0.9976			0.9933	0.9930	

Table 4
Relationship between the fraction of citric acid in the buffers and the slope of the observed rate constants for the tautomerism of chlortetracycline in citrate buffers at different pH, with an ionic strength of 0.1 and at 20°C

pH	Fraction of citric acid	Slope of k_{ek}	Slope of k_{ke}
2.0	0.93	0.16 ± 0.03	1.18 ± 0.08
3.0	0.59	0.91 ± 0.03	3.84 ± 0.19
4.0	0.12	2.42 ± 0.28	6.68 ± 0.79
		Slope −2.82 ± 0.28	−6.74 ± 0.50
		Intercept 2.70 ± 0.18	7.59 ± 0.32
		r −0.9951	−0.9973

ship between the slopes of k_{ek} or k_{ke} at different pH values, as obtained in Table 3, and the fraction of citrate present in the acid form (citric acid) in the buffers at these different pH values. The correlation for these relationships is negative indicating that the reactions are susceptible to general base catalysis rather than to general acid catalysis. This can be explained by the fact that keto–enol tautomerism involves proton transfer from C-12 to C-11a, which is facilitated by bases like the citrate ion. The intercept of these regression curves provides a second-order catalytic rate constant (mol^{-1} min^{-1}) for citrate ions, i.e. 2.70 mol^{-1} min^{-1} for the enol–keto tautomerism and 7.59 mol^{-1} min^{-1} for the keto–enol tautomerism.

The pH-rate profile is shown in Fig. 5. Since the ionic strength did not affect the rate constants as described above, these experiments were carried out using non-buffered aqueous solutions, adjusted to the pH indicated with 0.01 N hydrochloric acid or 0.01 N sodium hydroxide. The k_O values obtained as the intercepts in Table 3 are also added to this figure. Good agreement between both series of values is observed. In both directions the reaction rate is higher at lower pH. At higher pH the reaction rate drops and the equilibrium shifts towards the keto tautomer. When some

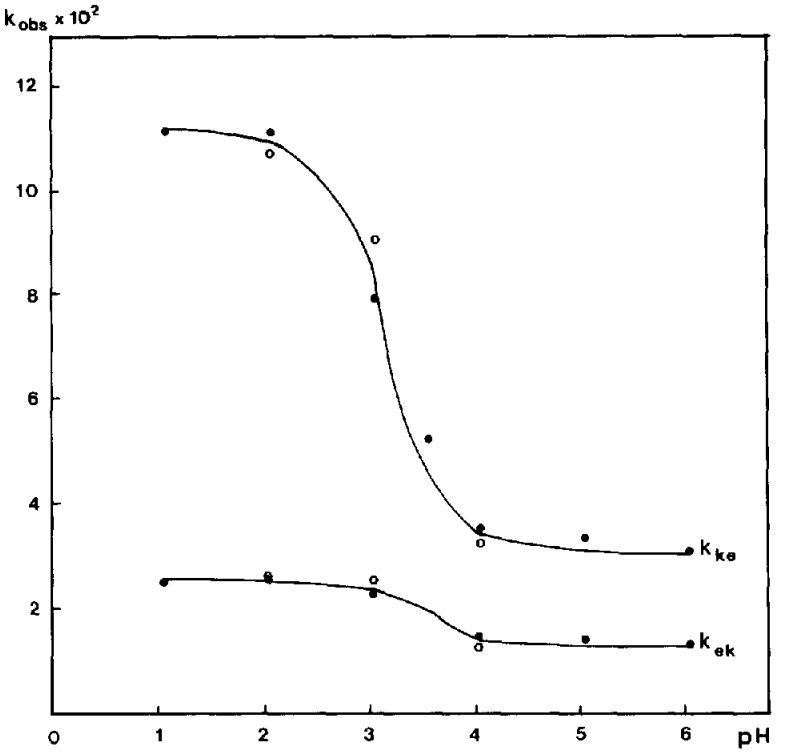


Figure 5
pH-rate profile for the tautomerism of chlortetracycline: ●, obtained in non-buffered solutions; ○, obtained in buffered solutions.

Table 5
Observed rate constants (min^{-1}), equilibrium constants and Arrhenius parameters for the tautomerism of chlortetracycline as function of temperature, in water at pH 3.5

Temperature (°C)		$k_{ek} \times 10^2$	$k_{ke} \times 10^2$	$K_{eq} \times 10^2$
0		0.27 ± 0.02	0.95 ± 0.07	28.42 ± 2.97
10		0.60 ± 0.00	1.91 ± 0.02	31.41 ± 0.33
20		1.85 ± 0.13	5.28 ± 0.37	35.04 ± 3.48
37		6.42 ± 0.24	16.51 ± 0.61	38.89 ± 2.04
	r	−0.9993	−0.9998	
	Slope ($-E_{obs}/R$)	−7356 ± 378	−6630 ± 348	
	E_{obs} (kJ mol^{-1})	61.1 ± 3.1	55.1 ± 2.9	

experiments were carried out in the presence of methanol it was observed that k_{ek} decreased and k_{ke} increased. At pH 3.5 K_{eq} shifted from 35 to 12.5 upon addition of 50%, v/v of methanol.

The influence of temperature was investigated in the range 0–37°C using an aqueous solution of CTC adjusted to pH 3.5. Table 5 shows the obtained rate constants and equilibrium constants. The natural logarithms of the rate constants were plotted versus $1/T$. From the slopes of the straight lines the apparent activation energies were calculated.

Conclusion

Tautomers of chlortetracycline or 4-epi-chlortetracycline were separated by LC at 10°C on poly(styrene–divinylbenzene)copolymer. By NMR spectroscopy it was confirmed that keto–enol tautomerism occurs between C-11a and C-12. The equilibrium reaction is subject to general base catalysis. In aqueous solutions the rate constants are directly proportional and the equilibrium constant is indirectly proportional to the proton concentration.

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References

[1] J.R.D. McCormick, S.M. Fox, L.L. Smith, B.A. Bitler, J. Reichenthal, V.E. Origoni, W.H. Muller, R. Winterbottom and A.P. Doerschuk, *J. Am. Chem. Soc.* **79**, 2849–2858 (1957).
[2] Weng Naidong, E. Roets and J. Hoogmartens, *Chromatographia*. In press.
[3] F.F. Cantwell and S. Puon, *Anal. Chem.* **51**, 623–632 (1979).
[4] J.M. Joseph, in *Chromatography and Separation Chemistry*, *ACS Symposium Series* 297. American Chemical Society, Washington, DC (1986).
[5] M. Moriyasu, A. Kato and Y. Hashimoto, *J. Chromatogr.* **400**, 143–148 (1987).
[6] M. Moriyasu, A. Kato and Y. Hashimoto, *J. Chromatogr.* **411**, 466–471 (1987).
[7] *European Pharmacopoeia*, 2nd edn. V.6.3.1, Maisonneuve, Sainte Ruffine, France (1980).
[8] C.R. Stephens, L.H. Conover, R. Pasternack, F.A. Hochstein, W.T. Moreland, P.P. Regna, F.J. Pilgrim, K.J. Brunings and R.B. Woodward, *J. Am. Chem. Soc.* **76**, 3568–3575 (1954).
[9] S. Hirokawa, Y. Okaya, F.M. Lovell and R. Pepinsky, *Acta Cryst.* **12**, 811–812 (1959).
[10] J. Donohue, J.D. Dunitz, K.N. Trueblood and M.S. Webster, *J. Am. Chem. Soc.* **85**, 851–856 (1963).
[11] A.F. Casy and A. Yasin, *J. Pharm. Biomed. Anal.* **2**, 19–36 (1984).
[12] A.F. Casy and A. Yasin, *Magn. Res. Chem.* **23**, 767–770 (1985).
[13] A.N. Martin, J. Swarbrick and A. Cammarata, in *Physical Pharmacy*, pp. 365–366. Lea & Febiger, Philadelphia (1969).

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