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Kinetics of thermal decomposition of talampicillin hydrochloride in the solid state

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In continuation of studies of the stability of the ampicillin esters bacampicillin [1–2] and talampicillin [3] the stability of talampicillin hydrochloride (**1**) in the solid state was investigated. The drug contains four chemical groups: β-lactam, ester, lactone and amide, sensitive to the actions of various factors.

In the kinetic studies two methods were used for the determination of changes of content and concentration of **1**: the well known iodometric method [4] based on the reactivity of the β-lactam bond, and the previously developed selective spectrophotometric method using UV ($\lambda_{max} = 254.5$ nm) which allows the stability of the lactone bond to be studied. In acid medium, **1** is present in the lactone form as a cation absorbing at $\lambda_{max} = 231.6$ nm, while in alkaline medium the lactone bond is hydrolysed with formation of the hydroxy acid anion absorbing at $\lambda_{max} = 254.5$ nm [3].

In the studies it was established that the process takes place according to the first order equation: $\log c = b + a \times t$ (where c is the concentration of **1**, and t is time). In the case of lactone bond hydrolysis the results were interpreted according to the equation: $\log (A_t - A_\infty)_{254.5} = b + a \times t$ [3]. The plots of the semilogarithmic relationship of **1** concentration changes expressed as absorbance ($A_{254.5}$) vs. time were in that case not rectilinear, since the $A_{254.5}$ value does not tend to zero but reaches a constant asymptotic value A_∞ (after time $t \rightarrow \infty$ the value of $A_{254.5} \rightarrow \infty$). The calculated values of the difference: $\Delta A_{254.5} = (A_t - A_\infty)_{254.5}$ were marked on the plots of the relationship: $\log \Delta A_{254.5} = f(t)$ and linear plots were obtained. The values of the rate constants of the **1** decomposition reaction (k in s^{-1}) were calculated from the slope (a) of the plots of the above relationships. They served to establish the influence of temperature changes on **1** stability on the basis of the Arrhenius equation: $\log k = b + a \times 1/T$, where $b = \log A$, A is frequency coefficient, $a = E_A/(2.303 \times R)$; $R = 8.3144\ J \times K^{-1}$. The coefficients of the equation of the linear plot (a, b) were used to calculate the following thermodynamic parameters characterizing the reaction of **1** decomposition in the solid state: activation energy (E_A), enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$). They were used to calculate the value of free enthalpy according to the equation: $\Delta G^{\neq} = \Delta H^{\neq} - T \times \Delta S^{\neq}$ (Table 1).

Values were also calculated for the rate constant k at 20 °C which allowed calculation of the $t_{0.1}$ value, that is the so called expiry date of the preparation of **1** investigated under the experimental conditions. This showed that the analysed substance **1** could be stored at room temperature in dry air for about 10 years and would retain its therapeutic usefulness for that period.

Table 2: Regression parameters for log k as a function of relative humidity

Conditions RH (%)	Rate constant k (s^{-1})	
	iodometric method	spectrophotometric UV method
56.2	2.568×10^{-5}	1.121×10^{-5}
76.4	7.488×10^{-5} [3]	3.475×10^{-5} [3]
90.0	1.186×10^{-4}	4.844×10^{-5}
	$\log k = b + a \times RH$	
n	3	3
a	1.992×10^{-2}	1.922×10^{-2}
b ($\log k_o$)	−5.692	−6.001
r	0.994	0.981
k_o (s^{-1})*	2.03×10^{-6}	9.98×10^{-7}
$t_{0,1}$*	14 h	29 h

* RH = 0

Table 1: Thermodynamic Parameters

Conditions Temperature (°C)	Rate constant k (s^{-1})		
	Iodometric method	75% RH Iodometriic method	75% RH spectrophotometric UV method
60	–	1.505×10^{-5}	6.507×10^{-6}
70	–	3.635×10^{-5}	2.088×10^{-5}
80	5.510×10^{-8}	7.488×10^{-5} [3]	3.475×10^{-5} [3]
90	1.203×10^{-7}	–	–
100	2.112×10^{-7}	–	–
	$\log k = b + a \times 1/T$		
n	3	3	3
r	−0.997	−0.999	−0.979
b (log A)	3.650	7.491	7.744
a	−3846	−4098	−4292
E_A (kJ × mol^{-1})	73.65	78.46	82.17
$\Delta H^{\neq}$ (kJ × mol^{-1})*	71.22	76.03	79.74
$\Delta S^{\neq}$ (J×K^{-1} × mol^{-1})*	−175	−101	−97
$\Delta G^{\neq}$ (kJ × mol^{-1})*	122	106	108
k (s^{-1})*	3.3×10^{-10}	3.2×10^{-7}	1.3×10^{-7}
$t_{0,1}$	10 years	3.8 d	11 d

* 20 °C

A harmful effect of humidity was observed on the stability of the studied penicillin (**1**) in the solid state. It was found, e.g. that at 80 °C and 75% relative humidity (RH) the hydrolysis of the β-lactam bond in **1** occurred at a rate nearly 1000 times that found in the absence of humidity. The $t_{0.1}$ value was then from 4 (iodometric method) to 11 days (spectrophotomeric UV method) (Table 1).
The rate constant k was also analysed as a function of RH using the equation: $\log k = b + a \times RH$. Rectilinear plots were obtained by both of the method used, and their slopes (a) had similar values (Table 2). This shows that the β-lactam as well as lactone groups are sensitive to a similar degree to the action of changes in humidity.
The process of hydrolysis of the lactone groups at 20 °C in the presence of humidity occurs at a rate three times (Table 1) and at 80 °C about two times (Table 2) slower than hydrolysis of the β-lactam bond.

Experimental

1. Material

The experiments were conducted using bacampicillin hydrochloride (**1**) substance. The mean content of **1** in the preparation was $99.53 \pm 0.35\%$ $C_{24}H_{33}N_3O_6S \cdot HCl$ (iodometric method: $n = 7$, $s = 0.382$, $s^2 = 0.146$, $s_r = 0.38\%$), molecular mass 518.0, Yananouchi Pharmaceutical Co., Tokyo, batch N 24 A.

2. Methodology

Samples of **1** were kept at elevated temperature (60 °C−100 °C) in anhydrous conditions and also at raised humidity (RH 56% to 90% [5]), their colour changed through yellow to brown.
For spectrophotometric testing samples of 1.0 ml of 0.125% water − methanol [5 : 2(V/V)] solution of **1** were taken. The contents of a flask was made up to volume with 0.1 mol/l solution of sodium hydroxide (reference standard).
The method for kinetic studies and analytical procedures using iodometric and spectrophotometric methods have been described in detail in an earlier study [3].

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Received August 4, 1999
Accepted October 15, 1999

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HPLC identification and determination of flavone aglycones in *Helichrysum plicatum* DC. (Asteraceae)

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Helichrysum plicatum DC. has been used in Macedonian folk medicine for a long time, for treatment of gastric and hepatic disorders, usually in combination with other plants with similar effects [1]. Another species that occurs in this climate is identified as *H. zivojinii* Cernjavski and Soska, representing an endemic, while *H. arenarium* has not been recognized in the flora of Macedonia [2].
The chemical composition of *H. plicatum* has been investigated in Turkey, where Mericli et al. isolated and identified chelipiron and 5-methoxy-7-hydroxy-phthalid from *H. plicatum* ssp. *plicatum* and chelipiron from ssp. *polyfyllum* [3]. Smirnov et al. showed that alcoholic extracts of *H. plicatum* possess bactericidal activity against *Corynebacterium michiganese, Xanthomonas malvacearum, Staphylococcus aureus* and *Bacillus subtilis* [4]. Among the extensive literature about the species of *Helichrysum*, flavonoids seem to be the most frequently investigated constituents. Thus, in three various subspecies of *H. arenarium* (ssp. *aucheri*, ssp. *erzincanicum* and ssp. *rubicundum*) apigenin, luteolin, naringenin, kaempferol and 3,5-dihydroxy-6,7,8-trimethoxy-flavone were identified as well as seven different glycosides [5]. Almost the same flavone aglycones were identified in few other *Helichrysum* species, in *H. pallasii* [6], *H. noeanum* [7], *H. stoechas* ssp. *barrelieri* [8], *H. graveolens* [9], *H. nitens* [10], *H. decumbens* [11], *H. armenium* ssp. *armenium* and ssp. *araxinum* [12], etc. Apigenin, naringenin, 3,5-dihydroxy-6,7,8-trimethoxy-flavone are mainly found in the flowers of *Helichrysum* sp. very often kaempferol and rarely luteolin and quercetin. The yellow colour of the flower is due to the halcone isosalipurposid [13]. Steams and leaves of *Helichrysum*, on the other hand, contain mainly quercetin and kaempferol glycosides, then naringenin and luteolin glycosides, and rarely astragalin [7, 9, 12], hispidulin, skutelarein and glycosides of gosipetin [14].
Up to now the chemical composition of *Helichrysum* species from Macedonia has not been investigated. In the present study flavone aglycones were identified and determined in different parts of *H. plicatum*. The plant material was collected during summer 1996, on the Golak Mountain in Eastern Macedonia. Extractions were performed using dried material previously separated into flowers, steams and leaves. The flavone aglycones in these extracts were analysed by HPLC. Identification was made according to the retention times and UV spectra of the components compared to those of authentic samples of available flavonoids (luteolin-7-glycoside, eriodictiol, qvercetin, luteolin, naringenin, apigenin, kaempferol and chrysoeriol). Chromatograms obtained using HPLC are presented in the Fig., where a stands for a mixture of authentic samples, b and c for ethylacetate extracts (glycosides previously hydrolised) from flowers and steams + leaves, respectively. Calibration curves for the flavones apigenin, naringenin, luteolin, quercetin and kaempferol were made for quantitative measurements. The results of the identification and determination of flavone aglycones in *H. plicatum* from Macedonia are presented in the Table.