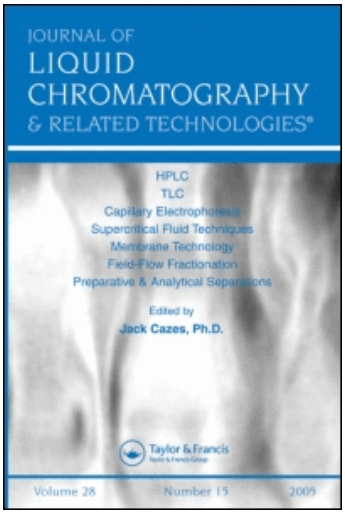

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I. Forfar[a]; J. J. Bosc[a]; C. Jarry[a]
[a] Laboratoire de Chimie Physique et Minérale, Université Victor Segalen Bordeaux 2, Bordeaux Cedex, France

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KINETICS OF 5-(1-PHENYL-4-PIPERAZINYL)METHYL-2-AMINO-2-OXAZOLINE HYDROLYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

I. Forfar, J. J. Bosc, C. Jarry

Laboratoire de Chimie Physique et Minérale
Université Victor Segalen Bordeaux 2
146 rue Léo Saignat
33076 Bordeaux Cedex, France

ABSTRACT

The effect of pH on the stability of 5-(1-phenyl-4-piperazinyl)methyl-2-amino-2-oxazoline (COR 3224), a novel antidepressant, was determined by HPLC at different pH (1, 3, 6, 7.4) at 100°C. One degradation product was isolated at pH 1 and identified as the 5-(1-phenyl-4-piperazinyl)methyl-2-oxazolidinone. A hydrolysis kinetic study was carried out over a range of pH (1-7.4). The degradation followed a pseudo-first-order kinetics with respect to COR 3224 and was accelerated by an increase in pH.

INTRODUCTION

COR 3224 (5-[1-phenyl-4-piperazinyl]methyl-2-amino-2-oxazoline) is the lead compound of a series of 2-amino-2-oxazolines synthesized in our group, which has been shown in clinical studies to present antidepressant properties.[1,2]

Different analytical methods have been proposed for its quantitation in biological samples. They include gas chromatography after derivatization[3] and high performance liquid chromatography.[4] All procedures revealed a partial stability of COR 3224 in solution, in relation to the possible opening of the heterocyclic ring. It was already reported that 2-amino-2-oxazolines underwent degradation under hydrolytic conditions leading to a mixture of compounds keeping or not a cyclic moiety.[5] This sensitivity towards hydrolysis has been used for pharmaceutical applications. The oxazolidines of the two ephedrine isomers, hydrolyzed quantitatively in the pH range 1-11, were reported as possible pro-drug models for the β-aminoalcohol moiety.[6]

The pharmaceutical importance of COR 3224 prompted us to undertake the study of the chemical stability of this compound in aqueous medium. Recently, we have developed a RP-HPLC analytical study with intent to determine the capacity factors (log k'_w) of 5-(1-aryl-4-piperazinyl)methyl-2-amino-2-oxazolines.[7] These lipophilicity indexes were discussed in regard to the ionization constants measured by a potentiometric method. The purpose of the present work was to determine the effect of pH on the stability of COR 3224 and to report the degradation kinetics of COR 3224 in acidic medium studied by HPLC. These analytical studies were deemed necessary for the preclinical development of COR 3224.

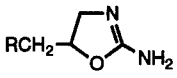
MATERIALS AND METHODS

Apparatus and Chromatographic Conditions

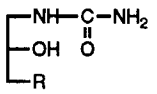
A Waters HPLC apparatus equipped with a Model 501 pump, a Model 455 ultraviolet detector operating at 254 nm and an U_6K manuel injector was used for analytical purpose. The compounds were chromatographied on a Nova Pak C-18 column (3,9 mm x 150 mm, 4 µm particle size) (Waters). The mobile phase was methanol/0.06 mol L^{-1} phosphate buffer 25/75 (v/v) adjusted at pH 5.4 with orthophosphoric acid. It was filtered through a 0.45 µm membrane filter. The flow rate was 1.0 mL · $min.^{-1}$ The detector output was recorded on a Model 746 data Module integrator.

Chemicals

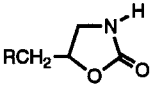
All chemical and solvents were of analytical or HPLC grade. Methanol was purchased from SDS (Peypin, France). Water was deionized and doubly glass-distilled. Acetic acid, sodium acetate, potassium chloride, sodium



5-(1-phenyl-4-piperazinyl)methyl-
2-amino-2-oxazoline (COR 3224)



1-(phenyl-4-piperazinyl)-
3-ureidopropan-2-ol (I)



5-(1-phenyl-4-piperazinyl)
methyl-2-oxazolidinone (II)

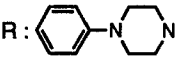


Figure 1. Formulae of compounds.

hydroxide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate trihydrate (Merck), hydrogen chloride (Aldrich), sodium citrate (Fluka) were used to prepare the buffers solutions. The chemical formulae of studied compounds are depicted in Figure 1. COR 3224 was prepared according to an already reported method.[2] The ionization constants (pK_a = 8.88, 4.92) were measured by a classical method.[7] The degradation products **I** and **II** were identified in reference to compounds prepared by alternative methods. 1-(Phenyl-4-piperazinyl)-3-ureidopropan-2-ol (**I**) was obtained by hydrolysis of COR 3224 in basic aqueous medium at pH 13 according to reference.[8] The 5-(1-phenyl-4-piperazinyl)methyl-2-oxazolidone (**II**) was prepared from COR 3224 by heating in acidic aqueous medium at pH 1 for 15 hours. After cooling, the mixture was basified to pH 9 with NaOH 1N and **II** was extracted with chloroform. The organic layer was dried, then evaporated to dryness and recrystallized from ethanol. The structure of this original compound was supported by IR, ^1H and ^{13}C NMR spectral data.[9]

Hydrolysis Kinetic Studies

All kinetic experiments were carried out in aqueous boiling buffers (potassium chloride/hydrochloric acid, sodium acetate/acetic acid, sodium citrate/sodium hydroxide, phosphate buffers). Solutions of COR 3224 (0.2 mol $\cdot$ L^{-1}) at different pH values (1, 3, 6, 7.4) and different hydrolysis times were chromatographied using the liquid chromatograph described above.

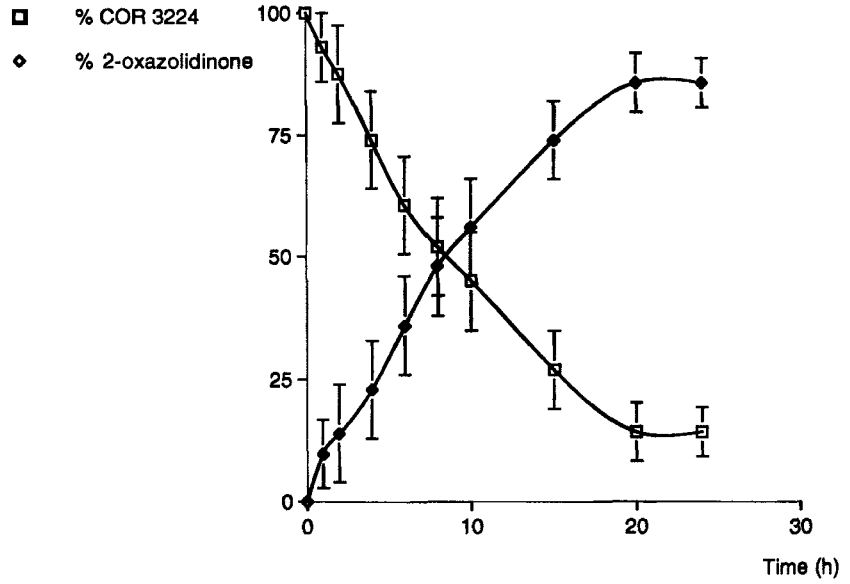


Figure 2. Plot showing concomitant degradation of COR 3224 and formation of compound **II** at pH 1.

At specific intervals of time, aliquot samples (100 μL) were taken, diluted in 1 mL of methanol, 10 mL of water, and 10 mL of eluent. Then 20 μL of this solution were injected in the chromatograph. Kinetics of COR 3224 hydrolysis was followed by measuring the concentrations of the reactant and of compounds **I** and **II** at known intervals of time, and plotting the concentrations vs time. The peak area ratios of all compounds were normalized. The data points in Figure 2 are the means of triplicate measurements and the errors represent standard deviations.

RESULTS AND DISCUSSION

Influence of the pH on the Nature of Degradation Products

Chromatograms depicting typical degradation profiles of COR 3224 at the different pH solutions are presented in Figure 3. Homogeneous acidic COR 3224 solutions showed initially one peak corresponding to the reagent (peak 1 at retention time 8.2 min). After a one-hour heating at pH 1 and 3, another

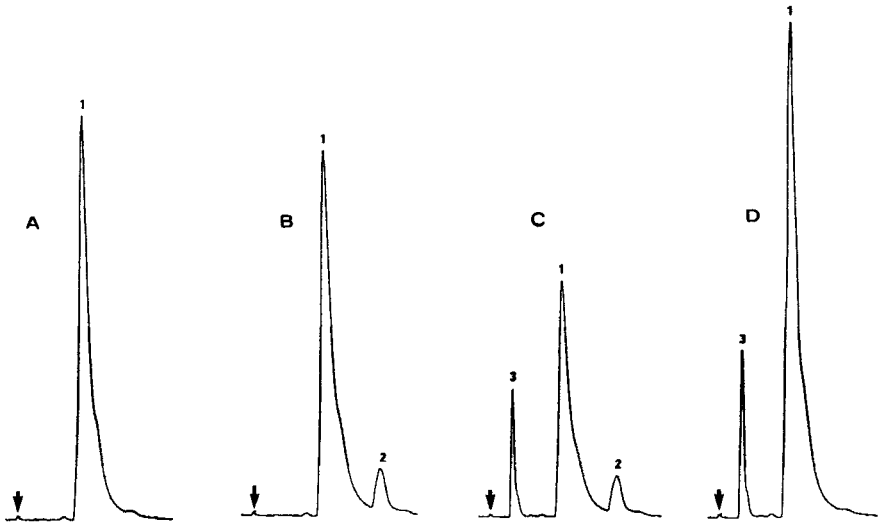


Figure 3. HPLC analysis. (A) Chromatogram of a solution of COR 3224 (1). (B), (C) and (D) Typical chromatographic degradation profiles of 1 at different pH (B : pH 1 or 3; C : pH 6 ; D : pH 7.4).

peak (peak 2 at retention time 12.6 min) was detected in addition to the peak for COR 3224 (Figure 3B). This peak corresponds to the 5-(1-phenyl-4-piperazinyl)methyl-2-oxazolidinone (**II**) which represents the major degradation product in strong acidic medium. At pH = 6 (Figure 3C), as the hydrolysis advanced two peaks appear simultaneously at retention times 3.2 and 12.6 min, corresponding respectively to the 1-(phenyl-4-piperazinyl)-3-ureidopropan-2-ol (**I**) (peak 3) and to **II**. These two degradation products were obtained in similar proportions after twenty-hours of heating.

Another hydrolysis experiment was achieved by heating COR 3224 in aqueous medium at pH 7.4. Under these conditions COR 3224 was at first quite insoluble at 25°C, but heated under reflux, the mixture became homogeneous. In addition to the peak due to COR 3224, only one peak corresponding to **I** was detected (Figure 3D).

The difference in these results illustrates an important principle in stability studies, i.e., the stability of a chemical depends on the number of potential reactants with it.[10] A possible transformation of the 3-ureidopropan-2-ol (**I**) in the corresponding 2-oxazolidinone (**II**) favoured in acidic conditions could proceed in parallel with the initial degradation of COR 3224.

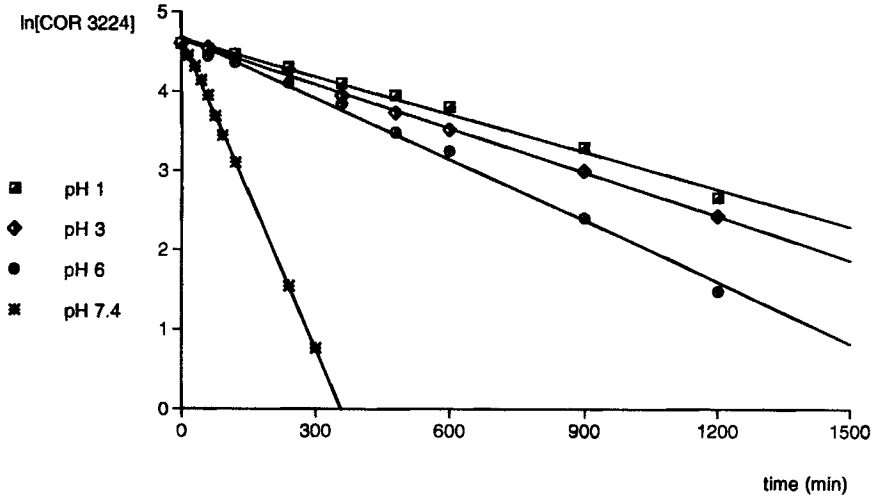


Figure 4. First-order decomposition plots for COR 3224 at different pH values.

Table 1

Rate Constants and Half-Lives at the Different pH

	$k \times 10^{-3}$ (min^{-1})	$t_{1/2}$ min
pH 1	1.62	428
pH 3	1.82	380
pH 6	2.6	267
pH 7.4	12.8	54

Rate of COR 3224 Hydrolysis

Typical semilogarithmic plots of the percentage of residual COR 3224 versus time for the degradation kinetics of COR 3224 are shown in Figure 4 at the different pH. In pH 1, 3, and 6 buffers, the evolution of the reaction is rather the same during the first 240 minutes. The reaction was then performed at pH 7.4. In all cases the results indicate that the COR 3224 hydrolysis performed in aqueous solution followed a pseudo-first-order kinetics.

The rate constants were computed by using least-squares linear regression analysis. These lines exhibited excellent linearity ($r>0.98$). The half-lives were calculated and reported in Table 1. The most rapid evolution is observed at pH 7.4.

These preliminary results, determined at different pH values, seem to indicate the rate of breakdown of COR 3224 to be pH dependent.

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9. Physical and spectral data for compound **II** : F(°C) 153 (Ethanol) ; Formula : $C_{14}H_{19}N_3O_2$; IR (KBr) ν cm^{-1} : 1730 (C=O), 3200 (NH) ; NMR ^1H ($CDCl_3$) δ ppm : 7.05 (m, 5H, Har), 5.10 (s, 1H, NH), 4.82 (m, 1H, 5-H), 3.63 (m, 2H, 4-H), 3.2 (m, 4H, $CH_{2piperazine}$), 2.72 (m, 6H, $CH_{2piperazine}$ and NCH_2) ; NMR ^{13}C ($CDCl_3$) δ ppm : 157.7 (C=O), 148.8, 129.1, 120.6, 116.2 (C_{phenyl}), 70.3 (C-5), 58.1 (CH_2), 51.1 and 45.4 ($C_{piperazine}$), 43.2 (C-4).

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