Kinetics and Mechanism of the Base-Catalyzed Rearrangement and Hydrolysis of Ezetimibe

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Received 21 October 2013; revised 1 April 2014; accepted 4 June 2014

Published online 1 July 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24070

ABSTRACT: The pH-rate profile of the pseudo-first-order rate constants for the rearrangement and hydrolysis of Ezetimibe giving (2*R*,3*R*,6*S*)-*N*,6-bis(4-fluorophenyl)-2-(4-hydroxyphenyl)-3,4,5,6-tetrahydro-2*H*-pyran-3-carboxamide (**2**) as the main product at pH of less than 12.5 and the mixture of **2** and 5-(4-fluorophenyl)-5-hydroxy-2-[(4-fluorophenylamino)-(4-hydroxyphenyl)methyl]-pentanoic acid (**3**) at pH of more than 12.5 in aqueous tertiary amine buffers and in sodium hydroxide solutions at ionic strength $I = 0.1$ mol L^{-1} (KCl) and at 39°C is reported. No buffer catalysis was observed and only specific base catalysis is involved. The pH-rate profile is more complex than the pH-rate profiles for the hydrolysis of simple β-lactams and it contains several breaks. Up to pH 9, the log k_{obs} linearly increases with pH, but between pH 9 and 11 a distinct break downwards occurs and the values of log k_{obs} slightly decrease with increasing pH of the medium. At pH of approximately 13, another break upwards occurs that corresponds to the formation of compound **3** that is slowly converted to (2*R*,3*R*,6*S*)-6-(4-fluorophenyl)-2-(4-hydroxyphenyl)-3,4,5,6-tetrahydro-2*H*-pyran-3-carboxylic acid (**4**). The kinetics of base-catalyzed hydrolysis of structurally similar azetidinone is also discussed. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2240–2247, 2014

Keywords: ezetimibe; rearrangement; azetidinone; NMR; kinetics; Liquid chromatography; acid-base catalysis; pH rate profile; chemical stability; degradation products; UV-VIS spectroscopy

INTRODUCTION

Ezetimibe (Zetia®, Ezetrol®), (3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one (**1**), is a selective cholesterol absorption inhibitor, which significantly lowers levels of the biliary and dietary cholesterol in the small intestine.[1] The inherent stability of Ezetimibe has been determined under a variety of conditions,[2–4] but there is no information about the structure of degradation products in those reports. Cited articles[2–4] mainly report on the relative or absolute retention times of degradation products under various HPLC conditions. The formation of (2*R*,3*R*,6*S*)-*N*,6-bis(4-fluorophenyl)-2-(4-hydroxyphenyl)-3,4,5,6-tetrahydro-2*H*-pyran-3-carboxamide (**2**) was briefly mentioned by Singh et al.,[2] but no proper characterization of this compound was described. Moreover, the correct structure of the main alkaline degradation product has been the subject of controversy. At the beginning of 2011, Gajjar and Shah[5] reported 5-(4-fluorophenyl)-2-[(4-fluorophenylamino)-(4-hydroxyphenyl)methyl]-pent-4-enoic acid (Scheme 1) to be the major alkaline degradation product. As early as 1 week after the publication of this article, Barhate and Mohanraj[6] pointed out in their *Letter to the Editor*, that the interpretation of spectral data in the original paper[5] was wrong. The correct structure of the alkaline degradant—compound (**2**)—was published a few months later by Filip et al.[7] and Sánta et al.[8] (Scheme 1). The formation of the same compound (**2**) under strong acid conditions was reported earlier and its absolute structure was confirmed by X-ray analysis.[9] However, the determination of the structure of other degradation products formed in alkaline medium and the dependency for their formation at different pH were not described. To get a better insight into the reaction mechanism of the unusual Ezetimibe transformation to (**2**) and other not yet published degradation products (**3**) and (**4**) (Scheme 1), we performed a detailed kinetic study whose results we wish to report in this paper.

MATERIALS AND METHODS

Materials and Reagents

Tertiary amines (triethylamine, *N*-methylmorpholine, *N*-methyldiethanolamine, and *N*,*N*-dimethylaminoethanol) and hydrochloric acid used for the preparation of buffer solutions and KCl for ionic strength adjustment were of extra pure grade and were obtained from commercial suppliers (Sigma–Aldrich Company LLC, Prague, The Czech Republic, and Acros Organics, part of Thermo Fisher Scientific, Geel, Belgium). All the buffer solutions were freshly prepared just before kinetic measurements. The water used for the kinetic and product studies was distilled twice. Pure (>99.9%) Ezetimibe was obtained from Zentiva-Prague, Czech Republic (part of the Sanofi-Aventis group). All other solvents for preparative/analytical chromatography were purchased from Merck or Sigma–Aldrich and were of HPLC grade.

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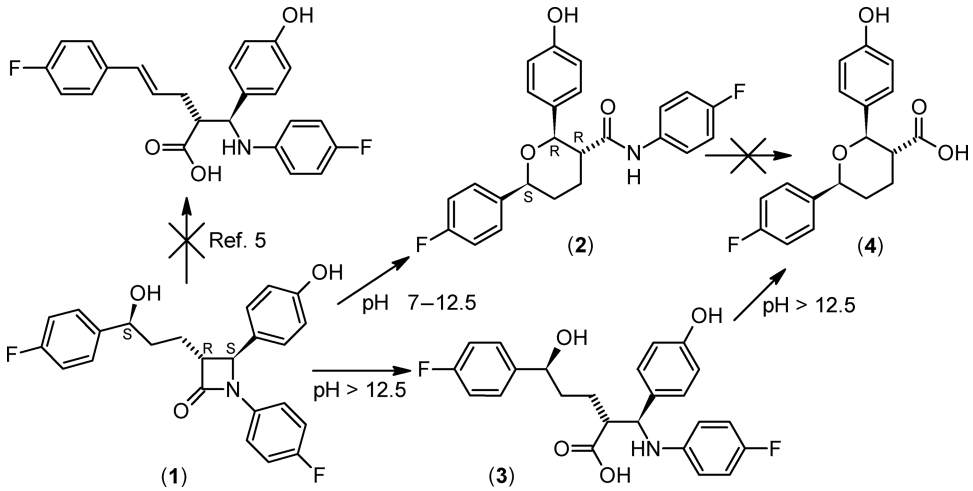
Dedicated to Professor Vladimír Macháček on the occasion of his 70th birthday.

This article contains supplementary material available from the authors upon request or via the Internet at http://wileylibrary.com.

Journal of Pharmaceutical Sciences, Vol. 103, 2240–2247 (2014)



Scheme 1. Ezetimibe alkaline degradation pathways.

Nuclear Magnetic Resonance Spectroscopy

The ^{1}H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Bruker Avance 3–400 MHz (Billerica, Massachusetts, USA) instrument in hexadeuteriodimethyl sulfoxide (DMSO-d_6) solution. Chemical shifts δ are referenced to the solvent residual peaks δ(DMSO-d_6) = 2.50 (^{1}H) and 39.6 (^{13}C) ppm. Coupling constants J are quoted in Hz. Proton–proton connectivities were found by gs-COSY. Protonated carbon atoms were assigned by inspection of gs-HSQC spectra. ^{13}C NMR spectra were also measured in a standard way and by means of the APT (attached proton test) pulse sequence to distinguish CH, CH_3 and CH_2, and C_{quart}. All NMR experiments were performed with the aid of the manufacturer's software.

HPLC Measurements

The samples were taken directly from the reaction mixture containing 5 mg of Ezetimibe and 6 mL of 0.1 or 0.01 M NaOH, respectively, and analyzed on the LaChrom Elite HPLC System (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA) equipped with a diode array UV–Vis detector MultiChrom 5 (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA) using chromatographic column Purospher RP8e, 250 × 4.0 mm^2, 5 μm (Merck KGaA, Darmstadt, Germany) and a mobile phase composed of phosphate buffer (0.02 M KH_2PO_4, pH 2.7 ± 0.05) and acetonitrile (both Merck KGaA). The mobile phase flow rate was kept at 1 mL/min using gradient elution [gradient ranging from 70% (v/v) buffer +30% (v/v) acetonitrile to 30% (v/v) buffer +70% (v/v) acetonitrile during 25 min). Representative chromatograms can be found in the Supplementary Information.

Liquid Chromatography–Mass Spectrometry Measurements

High-resolution liquid chromatography–mass spectrometry (LC–MS) analysis of the sample taken from reaction mixture after 4 h in 0.1 M NaOH was performed on an linear trap quadrupole (LTQ) Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to a HPLC system (CTC, Basel, Switzerland). LC analysis of the prepared samples was performed on a Kinetex C18 column, 150 × 4.6 mm^2, 2.6 μm (Phenomenex, Inc., Torrance, California, USA) using 0.6 mL min^{-1} flow rate. The mobile phase consisted of 10 mM ammonium formate pH 6.3 and acetonitrile (gradient ranging from 30% to 100% acetonitrile in 18 min). For ionization of eluted analytes, an APCI ion source operated in positive mode was employed (vaporizer temperature 400°C, capillary temperature 300°C, discharge current 4 μA, and tube lens voltage 40 V).

Chiral HPLC

The optical purity of Ezetimibe and the alkaline degradation product (**2**) were determined with a HPLC system consisting of a high-pressure pump (LCP 4000; ECOM-Prague, The Czech Republic), an autosampler and a UV detector (LCD 2082; ECOM-Prague), and employing a Chiralcel® OD-H (Chiral Technologies Europe, Illkirch, Cedex, France) column (250 × 4.6 mm^2). The mobile phase consisted of hexane–propan-2-ol mixture (85:15, v/v). The flow rate was kept at 0.8 mL min^{-1} and UV detection was at 230 nm. The retention time of Ezetimibe was 20.72 min, and the retention time of (2*R*,3*R*,6*S*)-*N*,6-bis(4-fluorophenyl)-2-(4-hydroxyphenyl)-3,4,5,6-tetrahydro-2*H*-pyran-3-carboxamide (**2**) was 16.82 min. Both samples provided single peaks that proved their chemical as well as optical purity.

Optical Rotatory Power

The optical rotatory power of (2*R*,3*R*,6*S*)-*N*,6-bis(4-fluorophenyl)-2-(4-hydroxyphenyl)-3,4,5,6-tetrahydro-2*H*-pyran-3-carboxamide (**2**) $[\alpha]_D^{20} = -114.4^\circ$ was measured in methanol on a Perkin Elmer 341 (Perkin Elmer, Inc., Alameda, California, USA) instrument at the concentration 1.2 g/100 mL.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

Mass spectra were recorded on a MALDI LTQ Orbitrap XL (Thermo Fisher Scientific) equipped with nitrogen UV laser (337 nm, 60 Hz, 8–20 μJ) in positive ion mode. For the CID experiment using the LTQ helium was used as the collision gas and 2,5-dihydroxybenzoic acid or *trans*-2-[3-(4-*tert*-butylphenyl)-2-methylprop-2-en-1-yliden]malononitrile as the MALDI matrix.