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Chemistry and Physics of Lipids 134 (2005) 161–171
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Free radical oxidation of coriolic acid (13-(*S*)-hydroxy-9*Z*,11*E*-octadecadienoic Acid)

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Received 25 June 2004; received in revised form 12 January 2005; accepted 12 January 2005

Abstract

The reaction of (13*S*,9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoic acid (**1a**), one of the major peroxidation products of linoleic acid and an important physiological mediator, with the Fenton reagent (Fe^{2+}/EDTA/H_2O_2) was investigated. In phosphate buffer, pH 7.4, the reaction proceeded with >80% substrate consumption after 4 h to give a defined pattern of products, the major of which were isolated as methyl esters and were subjected to complete spectral characterization. The less polar product was identified as (9*Z*,11*E*)-13-oxo-9,11-octadecadienoate (**2**) methyl ester (40% yield). Based on 2D NMR analysis the other two major products were formulated as (11*E*)-9,10-epoxy-13-hydroxy-11-octadecenoate (**3**) methyl ester (15% yield) and (10*E*)-9-hydroxy-13-oxo-10-octadecenoate (**4**) methyl ester (10% yield). Mechanistic experiments, including deuterium labeling, were consistent with a free radical oxidation pathway involving as the primary event H-atom abstraction at C-13, as inferred from loss of the original *S* configuration in the reaction products. Overall, these results provide the first insight into the products formed by oxidation of **1a** with the Fenton reagent, and hint at novel formation pathways of the hydroxyepoxide **3** and hydroxyketone **4** of potential (patho)physiological relevance in settings of oxidative stress.

Keywords: Linoleic acid; Fenton reagent; Deuterium labeling; (1/2)D NMR; Lipid peroxidation

Abbreviations: 13-(*S*)-HODE, (13*S*,9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoic acid; 13-(*S*)-HPODE, (13*S*,9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid; LDL, low-density lipoprotein; PPAR-γ, peroxysome proliferator-activated receptor; BSTFA, bis(trimethylsilyl)-trifluoroacetamide; EDTA, ethylendiaminotetraacetic acid; ABAP, 2,2′-azobis(2-amidinopropane); HRP, horseradish peroxidase; COSY, correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond correlation; TOCSY, total correlation spectroscopy; PICI/MS, positive chemical ionization/mass spectrometry; tetramethylsilane, TMS

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doi:10.1016/j.chemphyslip.2005.01.005

1. Introduction

(13*S*,9*Z*,11*E*)-13-Hydroxy-9,11-octadecadienoic acid (13-(*S*)-HODE, **1a**), commonly referred to as coriolic acid, is a major product of lipid metabolism which arises by 15-lipoxygenase-catalyzed oxidation of linoleic acid followed by reduction of the resulting hydroperoxide ((13*S*,9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid, 13-(*S*)-HPODE) (Kühn, 1996). Additional routes to **1a** involve cycloxygenase-catalyzed oxidation of linoleic acid (Hamberg and Samuelsson, 1980) as well as non-enzymatic peroxidation (Nemann and Khenkin, 1997). In these latter cases, however, the *R* enantiomer is also formed to comparable extents, along with the 9-hydroxy isomers, due to the lack of stereo- and regioselectivity of these reactions.

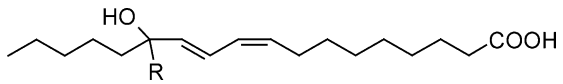
Both *S*- and racemic 13-HODE are produced by a variety of cell types, including polymorphonuclear leukocytes (Soberman et al., 1985), eosinophils (Engels et al., 1996), breast carcinoma cells (Reddy et al., 1997), and human dermal fibroblasts (Godessart et al., 1996). Racemic 13-HODE has been identified as a component of oxidized low-density lipoprotein (LDL) (Jira and Spiteller, 1996; Jira et al., 1997) and is the chief hydroxylated fatty acid in human psoriatic skin scales, where it occurs in levels of 115 ng/mg (Baer et al., 1990).

In mammalian cells **1a** acts as a physiological mediator, being involved in signal transduction and gene expression. It potentiates the mitogenic signal produced by epidermal growth factor in human breast carcinoma cells (Reddy et al., 1997), displays chemotactic properties toward polymorphonuclear leukocytes (Henricks et al., 1991), modifies inflammatory cell activity and is a ligand to peroxysome proliferator-activated receptor (PPAR-γ) (Nagy et al., 1998; Marx et al., 1999).

In the skin, **1a** has been ascribed antiinflammatory and antiproliferative properties, whereas in the vascular system it acts as a chemorepellant, reducing the adhesion of platelets (Buchanan et al., 1985; Haas et al., 1988). It also causes relaxation of coronary arteries (De Meyer et al., 1992) and may have both pro- and anti-atherogenic effects, depending on the rate of generation by the 15-lipoxygenase expressed in the macrophages recruited at sites of atherosclerotic lesions (Simon et al., 1989).

Although the (patho)physiological routes leading to **1a** production in settings of oxidative stress now appear to be well established, much less is known about the effects of reactive oxygen species on the ultimate fate and biological activity of this important lipid mediator. Knowledge of the oxidative chemistry of **1a** is currently limited to formation of (9*Z*,11*E*)-13-oxo-9,11-octadecadienoic acid (**2**) by the action of a NAD(+)-dependent dehydrogenase (13-HODE dehydrogenase) (Bronstein and Bull, 1997), and no other oxidation product has been isolated in pure form and chemically characterized. Notably enough, **1a** and its congeners have been reported to be stable to various oxidizing systems, including air, air/Fe^{2+}/ascorbate, air/Fe^{2+}, air/Fe^{2+}/H_2O_2, air/Fe^{3+}, and have therefore gained the reputation of excellent markers of lipid peroxidation (Spiteller and Spiteller, 1997).

In the present paper, we have investigated the reaction of **1a** with Fe^{2+}/EDTA/H_2O_2 (the Fenton reagent), which is widely used to model non-enzymatic oxidative processes, as well as with other oxidizing systems of physiological relevance. Aim of the work was to assess whether **1a** displays patterns of reactivity other than conversion to the keto compound **2**, and to provide a detailed structural characterization of the oxidation products



1a: R = H
1b: R = ^{2}H

2. Materials and methods

2.1. Materials

Linoleic acid (99%), hydrogen peroxide (water solution, 33%), D-mannitol, 2-iodobenzoic acid, oxone ($2KHSO_5$–$KHSO_4$–K_2SO_4), sodium borohydride, sodium borodeuteride and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were from Aldrich Chemie; $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ was from Carlo Erba; ethylenediaminetetraacetic acid (EDTA) was from Fluka; 2,2′-azobis(2-amidinepropane) (ABAP) chlorhydrate was from Polysciences. Horseradish peroxidase (HRP) (H_2O_2 oxidoreductase; E.C. 1.11.1.7) type II, catalase (H_2O_2:H_2O_2 oxidoreductase; EC