Synthesis and Characterization of New Octadecanoid Standards

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KEY FINDING

Twelve new oxylipin standards derived from ALA and GLA have been synthesized, purified, and characterized by LC-MS/MS and NMR

INTRODUCTION

Oxylipins, produced by the oxidation of polyunsaturated fatty acids (PUFAs), are lipid mediators involved in a variety of biological mechanisms of health and disease. They can be produced by enzymatic or nonenzymatic pathways, both as free acids or esterified as part of lipids such as glycerophospholipids. The best studied are eicosanoids, 20-carbon compounds derived from arachidonic or eicosapentaenoic acids, and which include mediators of inflammation such as prostaglandins, leukotrienes, thromboxanes, eicosatrienoic acids (EETs), hydroxyeicosatetraenoic acids (HETEs), and E-series resolvins. Their involvement in a broad spectrum of diseases is well documented and continues to be actively investigated. Docosanoids, 22-carbon oxylipins derived from docosahexaenoic acid, have also been studied extensively as mediating the resolution of inflammation. More recently, attention has been directed to octadecanoids, 18-carbon oxylipins derived from linoleic or linolenic acids. These compounds share enzymatic pathways with their longer counterparts, including lipoxygenases and cytochrome P450 enzymes, and they are increasingly recognized as having important effects in biological responses. However, the availability of authentic standards for these mediators is rather limited, which makes it difficult to identify and quantify them in experimental models and patient samples. This study shows the synthesis and characterization of twelve new standards for epoxy-octadecadienoic acids (EpODEs) and dihydroxy-octadecadienoic acids (DiHODEs) derived from α-linolenic acid (ALA) and γ-linolenic acid (GLA), which expand the availability of biochemical tools to further investigate the biological roles of octadecanoids.

RESULTS

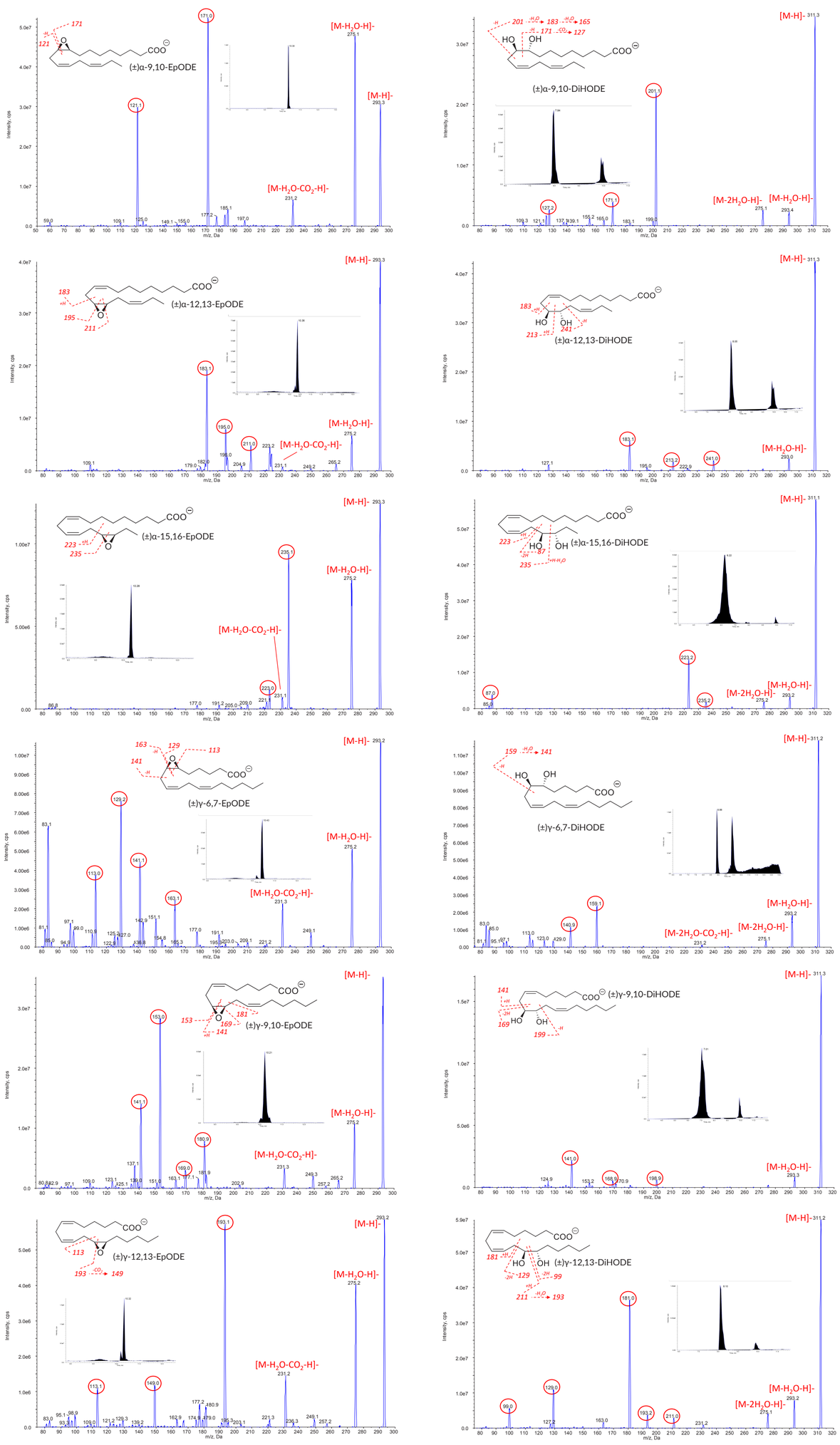


FIGURE 1 – **LC-MS/MS Analysis of Octadecanoid Standards.**
Each octadecanoid standard was analyzed individually by reversed-phase HPLC coupled with tandem mass spectrometry. The corresponding MS/MS spectra are shown, along with annotation of several fragments that confirm the identities of the different isomers. Please note that only relative stereochemistry is shown, and that MS data were obtained in a low-resolution triple-quadrupole mass spectrometer. Insets show the corresponding chromatograms. Some of the standards required further purification.

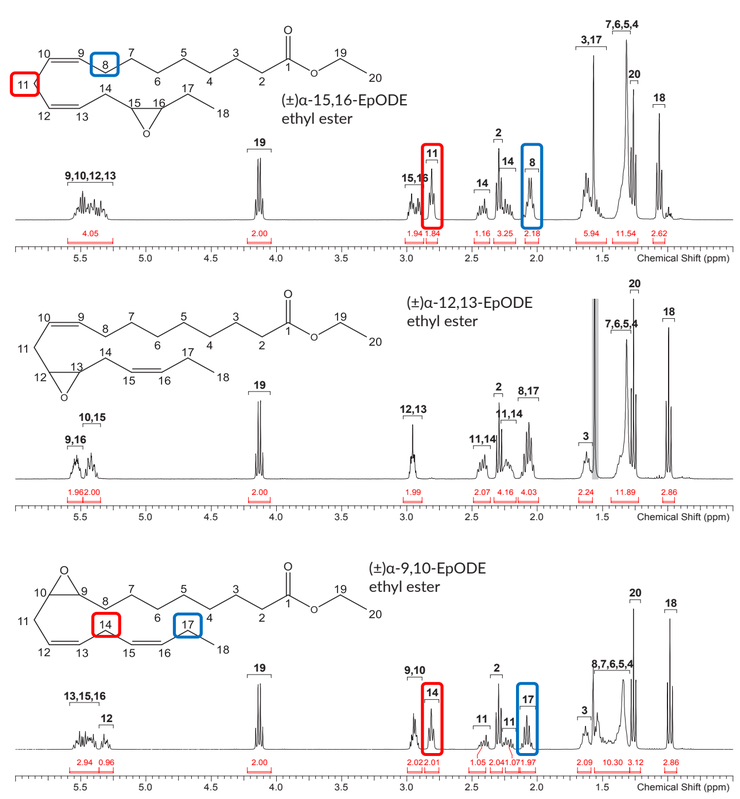


FIGURE 2 – **Annotated ^{1}H NMR Spectra (400 MHz, $CDCl_3$, δ 7.27 ppm) of the Three Epoxides Derived from the Ethyl Ester of α-Linolenic Acid.**
The signals at 2.8 ppm (in red boxes), notably absent from the 12,13-EpODE, correspond to the skipped diene methylene groups. The coupling patterns of the allylic methylene group signals at 2.05 ppm (in blue boxes) distinguish 9,10-EpODE from 15,16-EpODE.

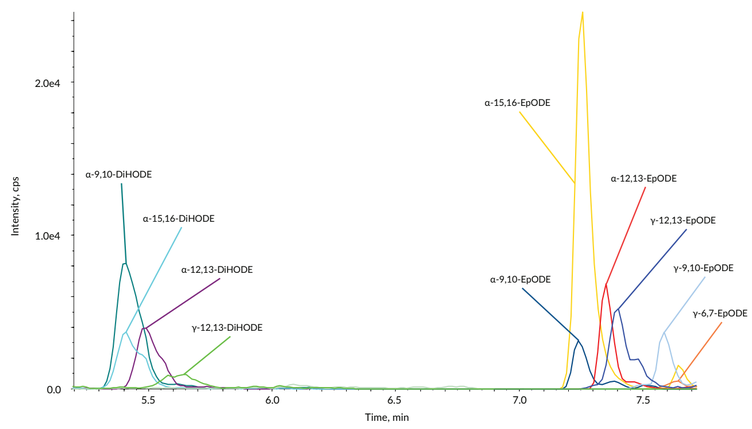
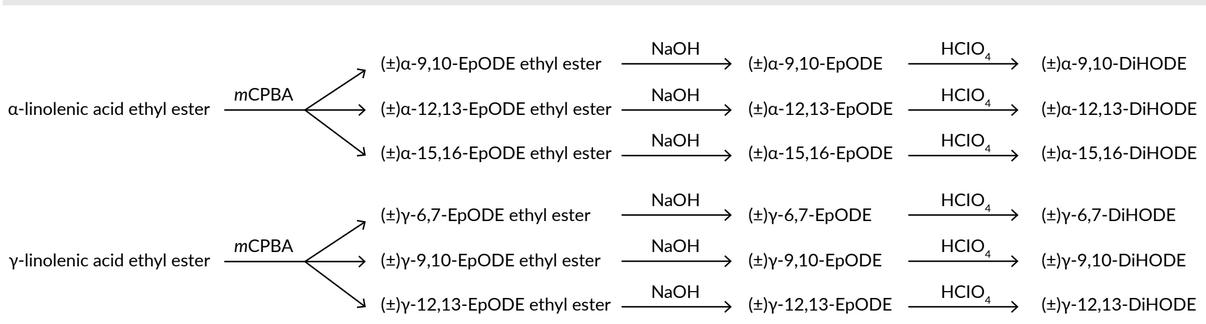


FIGURE 3 – **Detection of Octadecanoids in a Biological Sample.**
A solid-phase extract of rat plasma was analyzed by reversed-phase HPLC and multiple-reaction monitoring (MRM) based on the MS/MS spectra from the authentic standards shown in **Figure 1**.

SYNTHESIS OF STANDARDS DERIVED FROM ALA AND GLA



CONCLUSIONS

- Twelve new octadecanoid standards derived from ALA and GLA were synthesized and purified.
- Standards were characterized unequivocally by LC-MS/MS and NMR.
- Interestingly, NMR data helped establish the positions of the epoxide groups, which were confirmed by the LC-MS/MS data.
- Using a multiple-reaction monitoring (MRM) LC-MS/MS method developed using the parameters established with the new standards, several 18-carbon octadecanoids were detected in rat plasma.
- These standards will help investigate the roles of octadecanoids in patient samples and experimental models.