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Formation of dihydroxy acids from *Z*-monounsaturated alkenoic acids and their use as biomarkers for the processing of marine commodities in archaeological pottery vessels

Fabricio A. Hansel [†], Richard P. Evershed [*]

Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

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ABSTRACT

Distributions of *erythro* and *threo* dihydroxy acids, extracted from 'bound' forms of organic residues preserved in archaeological pottery cooking vessels are shown to be the direct products from *Z*-monounsaturated alkenoic acids present in the original commodities, and are useful biomarkers for detecting marine animal product processing/consumption by ancient people.

Biomarkers at archaeological sites are those compounds whose carbon skeletons suggest a link with natural products associated with human activity in the past.[1] Potsherds (fragments of ceramic containers) in particular, recovered from favourable burial environments, provide a unique medium for the preservation of a wide range of biomarkers, via strong surface adsorption, trapping within molecular-sized pores or covalent bonding within polymeric networks. Importantly, it has been shown that the chemical compositions of solvent extracts of potsherds provide lipid distributions that can directly reflect components of the original contents of pottery vessels. Based on these lipid biomarkers, a wide range of terrestrial animal and plant products have been recognised as being processed in archaeological pottery vessels.[2] Recently, isoprenoid acids [i.e., 4,8,12-trimethyltridecanoic acid (4,8,12-TMTD); phytanic acid and pristanic acid], and $>C_{20}$ ω-(*o*-alkylphenyl)alkanoic acids have been demonstrated to be important biomarkers for the processing of marine commodities in archaeological pottery vessels.[3] Since the exploitation of marine organisms by ancient people is of current interest, the recognition of new classes of marine biomarker is of particular significance.

Further work has shown that hydroxylated alkanoic acids produced during the oxidative degradation of unsaturated fatty acids have the potential to survive and can be used to distinguish sources of organic residues preserved in archaeological potsherds.[4] For example, Copley et al.[4a] have shown that vicinal dihydroxy acids indicate the original position of the double bond in the precursor fatty acids, and more specifically, that these degradation products can be used to distinguish contributions from oils/fats from different terrestrial animal and plant products processed in vessels in antiquity. These hydroxy acid oxidation products are most commonly detected in potsherds in 'bound' or polymerised forms, in many cases involving ester linkages,[5] only being released from solvent extracted potsherds by strong alkaline treatment, and normally found to comprise series of different classes of oxygenated products, including mono- and dihydroxy alkanoic acids and α,ω-dicarboxylic acids.[4,5]

Marine oil/fats are rich in monounsaturated ($C_{16:1}$, $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$) and polyunsaturated ($C_{20:5}$ and $C_{22:6}$) fatty acids.[6] However, these compounds are poorly preserved in potsherds, with polyunsaturated fatty acids rarely, if ever, being observed due to their inherent susceptibility to oxidation during vessel use and burial.[2] However, vicinal dihydroxy acid oxidation products hold promise as potential recorders of the original monounsaturated fatty acid composition of the fat/oil components of marine commodities processed in archaeological pottery vessels. In order to test this hypothesis, the molecular compositions of solvent and alkaline extracts of potsherds recovered from coastal sites situated in Santa Catarina State, Brazil and interpreted as being likely to contain degraded marine oils/fats, were investigated.[3a,7]

The solvent extracts were dominated by palmitic acid, with lower abundances of myristic and stearic acids, which is typical

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[*] Corresponding author. Tel.: +44 117 92876721; fax: +44 117 925 1295.
E-mail addresses: hansel@cnpf.embrapa.br (F.A. Hansel), r.p.evershed@bristol.ac.uk (R.P. Evershed).

[†] Present address: Embrapa Florestas, Colombo, Paraná, Brazil, 83411 000. Tel.: +55 41 3675 5624; fax: +55 41 3675 5601.

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of degraded oil/fats.[2] The alkaline and solvent extracts of individual potsherds contained similar distributions of the aforementioned *n*-alkanoic acids, strongly indicating the same source. Significantly, at least one of the following isoprenoid fatty acids: 4,8,12-TMTD, phytanic acid and/or pristanic acid were detected in all the alkaline extracts, together with complex mixtures of ω-(*o*-alkylphenyl)alkanoic acid isomers, albeit in lower abundance than the *n*-alkanoic acid components; the latter strongly suggesting a marine contribution to the oil/fat residues preserved in the potsherds.[3] Significantly, the alkaline extracts comprised complex mixtures of oxygenated fatty acid derivatives, including α,ω-dicarboxylic and hydroxy acids, recognised by their mass spectra obtained by GC/MS of their methyl ester-trimethylsilyl (TMS) ether derivatives.[8]

In order to explore the compositions of these complex mixtures of oxygenated alkanoic acids more fully the alkaline extracts were fractionated.[7] The hydroxy acid fraction was dominated by dihydroxy acids with carbon numbers ranging from C_{16} to C_{22}, with lower abundances of mid-chain monohydroxy acids (C_{16} and C_{18}), α- and β-hydroxy acids (C_{14} to C_{18}) and α,ω-dicarboxylic acids (C_7 to C_{14}). All the dihydroxy acids identified were vicinal substituted, and diastereoisomeric mixtures were always present (Fig. 1). As had been anticipated, the isomer distributions of the most prominent dihydroxy acids were very similar to those of the monounsaturated fatty acids present in the oil/fats of modern marine animals.[6]

The distributions of dihydroxy acids observed can be rationalised by considering the hydroxylation reactions of alkenes that lead to vicinal diols. These can be produced synthetically by treatment with either dilute alkaline permanganate or peracids. In the case of the former, a *Z*-alkene leads to an *erythro* diol via *syn* addition. In contrast, treatment of a *Z*-alkene with peracid proceeds via an epoxy intermediate and *anti* addition, to yield a *threo* diol.[9] Since hydroxylation of *Z*-alkenes by peracids or dilute alkaline permanganate is stereospecific reactions which occur under contrasting chemical conditions, similar pathways involving both reactions are unlikely to lead to the formation of *erythro* and *threo* diols in pottery vessels.

The major monounsaturated fatty acids in oil/fats possess the *Z*-configuration, and it has been found that heating either pure methyl oleate or commercial oils gave mixtures of *E*-9,10- and *Z*-9,10-epoxy compounds.[10] Since, long-chain *Z*- and *E*-epoxides do not isomerise and undergo stereospecific ring-opening by hydration, they must be the direct precursors of *threo* and *erythro* dihydroxy acids, respectively.[8] Through heating experiments, it has been shown that methyl stearate epoxides can be produced either: (i) from hydroperoxides of methyl oleate itself, or (ii) through secondary reactions between oleate and the hydroperoxides. In the first case, a series of isomers: 8,9-, 9,10- and 10,11-substituted, resulting from double-bond migration, would be expected. In the second case, the 9,10-isomers would be the sole products.[11] The distributions of dihydroxy acids detected in the extracts of archaeological pottery indicate that they are formed predominantly via secondary reactions with hydroperoxides.

A potential source of hydroperoxides is via auto-oxidation of the co-existing polyunsaturated fatty acid present in marine oils processed in the vessels.[6] These hydroperoxides would be very unstable and react rapidly with the monounsaturated fatty acid. Thus, the formation of diastereoisomeric mixtures of dihydroxy acids from *Z*-monounsaturated fatty acids in pottery vessels can be explained by the three pathways depicted in Scheme 1: (i) a stereospecific *Z*-epoxidation through oxidation by a hydroperoxide species giving the *Z*-epoxide (route A, **2**); (ii) a non-stereospecific two-step reaction leading preferentially to *E*-epoxides (more stable product); addition of a radical ($^{\cdot}O_2R$) to one end of the double bond gives a free radical in which rotation about the position of the original double bond can occur freely (route B, **3**) and (iii) *Z/E* isomerisation of the alkenoic acid catalysed by metal ions, common in clays, followed by epoxidation either by a hydroperoxide species or by peroxide radicals (route C, **4** and **3**).[10a,12] After the formation of *Z*- and *E*-epoxy acids, *anti* addition by hydration yields *threo* and *erythro* dihydroxy acids **5** and **6**, respectively. The reaction pathways explaining the formation of *threo* and *erythro* 9,10-dihydroxystearic acids from oleic acid (**1**) are summarised in Scheme 1. Given the operation of these pathways the presence of C_{16}, C_{18}, C_{20} and C_{22} dihydroxy acids can be explained by formation from their monounsaturated fatty acid precursors with the same carbon number, and more importantly, the position of the hydroxy groups mirrors the double-bond position in the original monounsaturated alkenoic acids. Hence, it can be concluded that the distribution of dihydroxy acids in archaeological potsherds is highly dependent on commodities processed in the original pottery vessels, and their formation is a consequence of the commodities, mode of vessel use and burial conditions.

In conclusion, dihydroxy acids present in extracts of archaeological pottery are the direct degradation products of *Z*-monounsaturated alkenoic acids formed via secondary reactions with hydroperoxides. Moreover, this provides unambiguous evidence that the dihydroxy acids preserved in polymerised or 'bound' fractions of organic residues preserved in archaeological cooking vessels, can be used as biomarkers for the processing of marine products. While C_{16} to C_{22} monounsaturated alkenoic acids are not solely restricted to marine organisms they do not occur in terrestrial animals and even their occurrence in plants is rather restricted.[6] Significantly, the co-occurrence of C_{16} to C_{22} dihydroxy acids, isoprenoid acids (abundant in marine oil/fats) and $>C_{20}$ ω-(*o*-alkylphenyl)alkanoic acids, would constitute unequivocal evidence of marine commodity processing in archaeological pottery vessels.

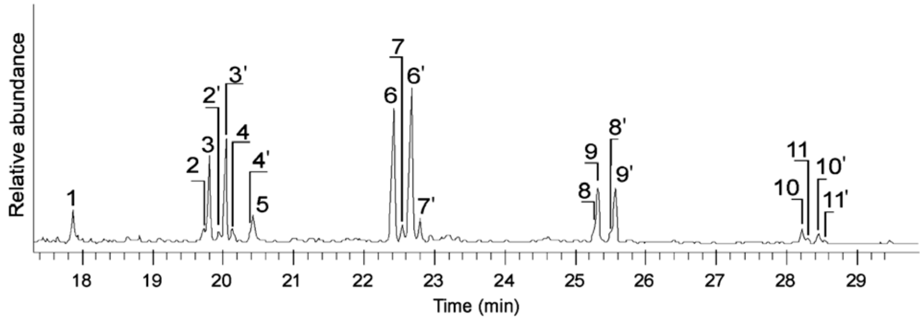
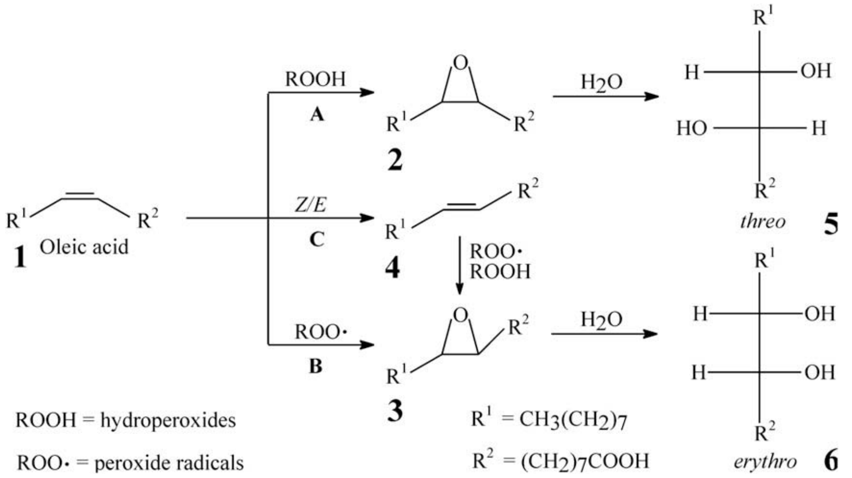


Figure 1. Partial total ion current (TIC) showing the hydroxy acid distribution (as methyl ester-TMS derivatives). Compound **1**: 10-hydroxypalmitic acid, **5**: 10-hydroxystearic acid; and *threo* (×) and *erythro* (×′) diastereoisomers **2**, **3** and **4**: 7,8-, 9,10-, 11,12-dihydroxypalmitic acids; **6** and **7**: 9,10-, 11,12-dihydroxystearic acids; **8** and **9**: 9,10-, 11,12-dihydroxyarachidic acids; **10** and **11**: 11,12-, 13,14-dihydroxybehenic acids.



Scheme 1. Reaction pathways leading to the formation of vicinal dihydroxy acids from *Z*-monounsaturated alkenoic acids in organic residues preserved in archaeological potsherds.

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7. Alkaline treatment: NaOH (5 mL, 0.5 mol L^{-1} in MeOH:H_2O (9:1 v/v), 70 °C, 1 h). Alkaline extracts of potsherds were methylated using BF_3/MeOH (14% w/v) (100 μL, 70 °C, 1 h) and an aliquot of methylated extract was separated into four fractions using silica gel column chromatography. The hydroxy acid fraction was eluted with dichloromethane:methanol (1:1 v/v). Total alkaline extracts and hydroxy acid fractions were converted into their TMS ethers and were analysed by GC–MS.
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