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Thermally produced ω-(*o*-alkylphenyl)alkanoic acids provide evidence for the processing of marine products in archaeological pottery vessels

Fabricio A. Hansel,[a,b] Mark S. Copley,[b] Luiz A. S. Madureira[a] and Richard P. Evershed[b,*]

[a]*Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina 88040 900, Brazil*
[b]*Organic Geochemistry Unit, Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK*

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Abstract—ω-(*o*-Alkylphenyl)alkanoic acids with 16, 18 and 20 carbon atoms were identified in archaeological pottery vessels from coastal sites in Southern Brazil. Such compounds are presumed to form during heating of triunsaturated fatty acids ($C_{16:3}$, $C_{18:3}$ and $C_{20:3}$), which are commonly found in the tissues of marine animals. The detection of these unusual cyclic compounds together with the isoprenoid fatty acids, 4,8,12-trimethyltetradecanoic acid and phytanic acid and substantial quantities of archaeological bones of fish and mollusca, provides evidence for the processing of marine animal products in archaeological pottery.

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Organic residue analysis is now a recognized approach for determining the uses of archaeological vessels. During the processing of foodstuffs (e.g., cooking), in porous unglazed pottery vessels, organic components are released and absorbed into the vessel wall. These may survive over considerable archaeological time periods (e.g., several thousand years). Lipids can be extracted and characterized using a range of analytical techniques.[1a–c] Thus, it has been possible to detect chemical indicators of terrestrial-based animals, for example, ruminant adipose and dairy fats, porcine fats, plant oils and waxes and other commodities, such as bee-products.[2a–d] One group of commodities that has up to now eluded definitive detection by this approach is marine products.[3]

One factor that must be considered in this work is the potential for structural transformations of the original components as a result of the heating/processing of foodstuffs during the lifetime of use of pottery vessels. For example, a series of mid-chain secondary ketones formed through the condensation of fatty acids provided evidence for the heating of vessels to >300 °C.[4a,b] Other thermally-induced reactions may result in oxidations, polymerizations, hydrolyzes, isomerizations and cyclizations, as seen in frying involving modern oils.[5a,b]

Organic residue analyses have been performed of 31 potsherds[6] recovered from coastal precontact archaeological sites situated on Santa Catarina Island (21° 66″ South, 48° 55″ East). These sites were inhabited by the Itararé people and date to ca. 10th century AD. Importantly, there is evidence for the consumption of large quantities of marine commodities, as indicated by the high abundances of fish bone and mollusca shells recovered during excavations.[7]

Extracts of 14 of the vessels yielded unusual lipid distributions, typified by the total ion current (TIC) shown in Figure 1A. The extract is dominated by saturated fatty acids ($C_{16:0}$, with lower abundances of $C_{14:0}$ and $C_{18:0}$), which is typical of degraded oils.[2c] Significantly, isoprenoid fatty acids [4,8,12-trimethyltridecanoic acid (4,8,12-TMTD) and 3,7,11,15-tetramethylhexadecanoic (phytanic acid)] were detected, albeit in lower abundance than the *n*-alkanoic acid components. These isoprenoid compounds are common constituents of marine animals.[8] In additon, eluting after $C_{18:0}$, are a number of ω-(*o*-alkylphenyl)alkanoic acids, recognized by their

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* Corresponding author. Tel.: +44-117-9287671; fax: +44-117-92512-95; e-mail: r.p.evershed@bristol.ac.uk

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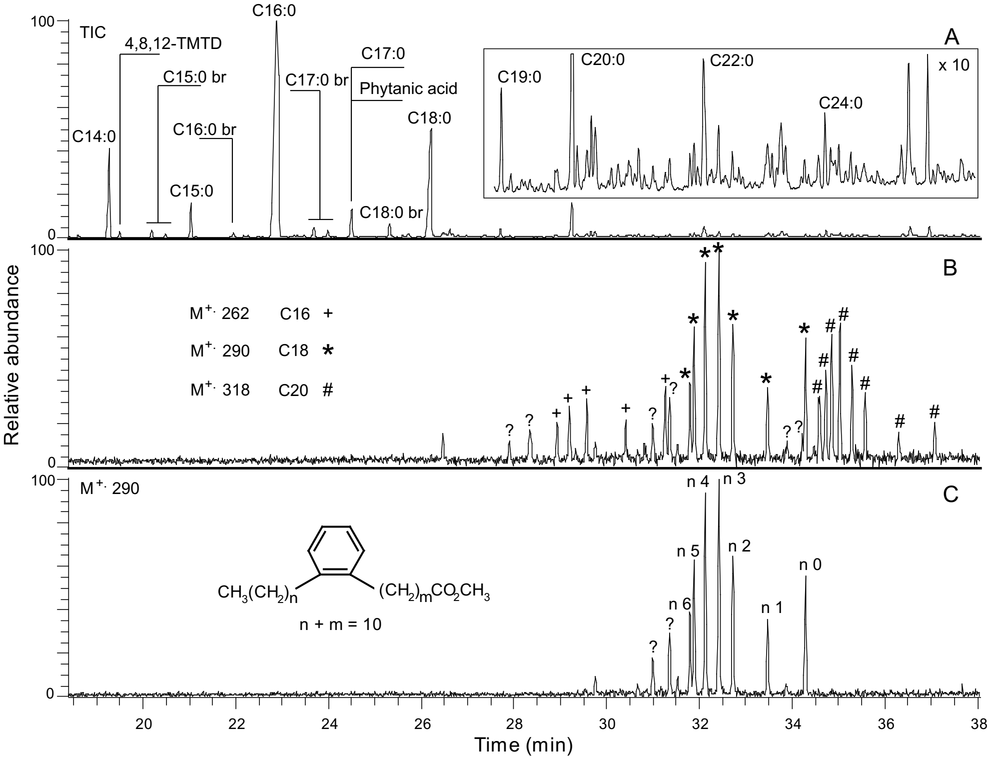


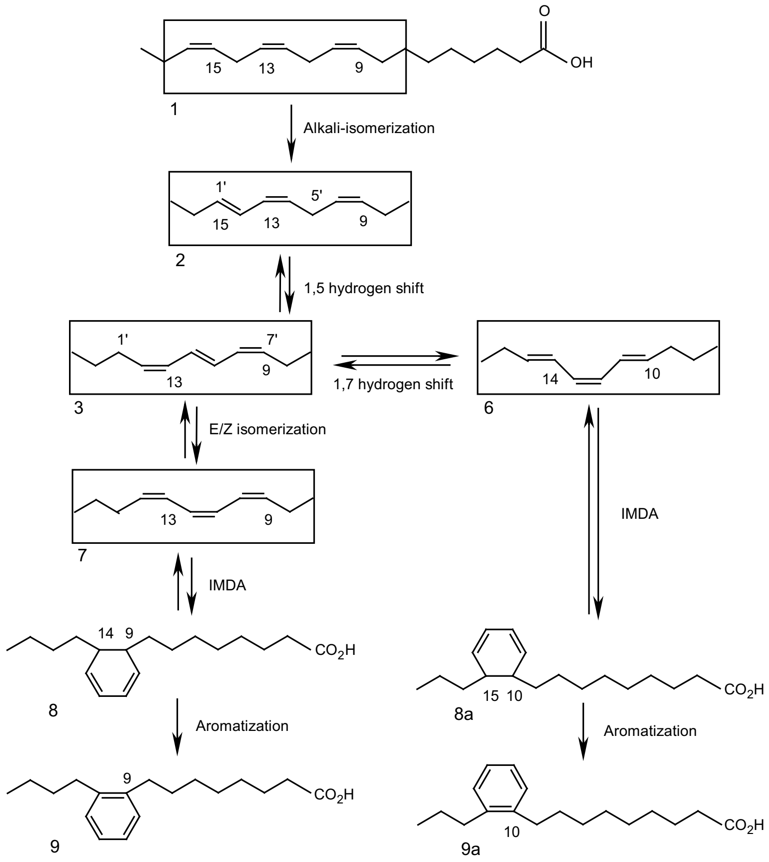
Figure 1. Typical partial total ion current (TIC, A), summed mass chromatogram of $M^{+\cdot}$ 262, 290 and 318 (B) and mass chromatogram of $M^{+\cdot}$ 290 (C) of total acid fraction (as methyl ester derivatives) of archaeological potsherd interpreted to contain lipid evidence of processing of marine animal commodities.

characteristic mass spectra.[9] Figure 1B shows a series of isomers of these cyclic compounds containing 16, 18 and 20 carbon atoms exhibiting molecular weights ($M^{+\cdot}$) of 262, 290 and 318, respectively. The mass spectra of such compounds (as methyl ester derivatives) have been reported previously.[10a,b] Figure 1C illustrates the wide range of positional isomers for the C_{18} ω-(*o*-alkylphenyl)alkanoic acid. Similarly wide distributions were detected for the C_{16} and C_{20} homologues (Fig. 1B). In the case of the C_{18} ω-(*o*-alkylphenyl)alkanoic acids the alkyl side-chain length ranged from $n = 6$ to $n = 0$ (Fig. 1C), with isomers containing alkyl side chains $>n = 6$ not detectable, possibly due to steric effects restricting their formation.

C_{18} ω-(*o*-alkylphenyl)alkanoic acids (**9** and **a**; Scheme 1) have been shown to form via alkali isomerization of methyl linolenoate[11] (**1** and **2** drawn as the free acids; 260–270 °C overnight), followed by a 1,5 hydrogen shift yielding a fully conjugated triene system **3**, with an intramolecular Diels–Alder (IMDA) reaction yielding, via *E*/*Z* isomerization products **7**, the cyclic products **8**. It appears that following a 1,5 hydrogen shift, a 1,7 hydrogen shift occurs to produce the additional triene isomers **6** and thus the additional cyclic products **8a** (Scheme 1). These rearrangements require protracted heating and result in the formation of cyclohexadienoic structures (**8** and **a**), and then successive aromatizations to yield the different aromatic isomers (**9** and **a**).[12a,b] The overall reaction pathway leading to formation of the C_{18} ω-(*o*-alkylphenyl)alkanoic acids, whereby the double bond in the Δ^{15} position of the original fatty acid becomes conjugated with the Δ^{12} double bond, is summarized in Scheme 1. Given this pathway of formation, the higher the carbon number of the precursor fatty acid, the greater the number of positional isomers that will be produced; this phenomenon is evident in Figure 1B.

In experiments involving deep fat frying (heating vegetable oils without prior alkali isomerization) the major cyclic products are nonaromatic five- and six-membered ring monocyclic acids.[13] Such compounds arise through allylic radicals followed by ring formation but do not involve the isomerizations required to produce a phenyl ring. The presence of a wide array of ω-(*o*-alkylphenyl)alkanoic acids in potsherds suggests that some special conditions exist within pottery that promotes their formation. It is known that clays can act as an acid or base catalyzing agent,[14] and thus has the potential for promoting the isomerization of double bonds.

Since vegetable oils are often high in triunsaturated fatty acids, an obvious conclusion would be that these were the source of the ω-(*o*-alkylphenyl)alkanoic acids. However, if this was the case only the C_{18} isomers, and in some cases C_{16} isomers, would have been observed. Thus, given the array of C_{20} isomers detected, together with the archaeological evidence,[7] $C_{16:3}$, $C_{18:3}$, $C_{20:3}$ fatty acyl components of marine products[15a,b] are the most likely source. Moreover, the identification of two isoprenoid acids (4,8,12-TMTD and phytanic acid; Fig. 1A) reinforces the utilization of marine commodities in the vessels studied herein. These findings provide com-



Scheme 1.

pelling chemical evidence for the processing of marine foods (e.g., fish) in pottery at archaeological sites, and thus it is now possible to use these biomarkers to explore the extent of marine resource exploitation in antiquity.

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6. Two gram fragments were surface cleaned and ground to a fine powder and extracted with chloroform/methanol (2:1 v/v, 2×10 mL) using ultrasonication (15 min). Base treatment: NaOH (2 mL, 0.5 mol L^{-1} in methanol/water (9:1 v/v), 70 °C, 1 h). The acid fraction was recovered with hexane after extraction of the neutral fraction, followed by acidification (pH = 3). Methyl ester derivatives were prepared using 100 μL of BF_3/MeOH (14% w/v) complex (70 °C, 1 h).
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9. Instrumentation: Trace GC with PTV injector coupled to a Finningan Trace MS. Electron ionization (70 eV). GC column used: VF3-23ms, high cyanopropyl modified methyl polysiloxane capillary column (60 m × 0.32 mm, 0.15 μm); oven temperature programme 40–100 °C at 10 °C min^{-1} then to 240 °C at 4 °C min^{-1}, isotherm for 20 min; helium used as gas carrier. Principal fragment ions: m/z 105, ($C_8H_9^+$), m/z 91 ($C_7H_7^+$), $M^{+\cdot}$ 262 (C_{16}), 290 (C_{18}), 318 (C_{20}), m/z [M-(CH_3O or CH_3OH)]$^{+\cdot}$, loss of alkyl side chain, loss of alkyl side chain plus CH_4O, loss of alkyl side chain plus CH_4O and H_2O, loss of substituent containing ester moiety.
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