Analysis of Ten Absorbed Residues from Hiwassee Island Pottery

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Absorbed residues were analyzed from ten sherds from Hiwassee Island, an important archaeological site in central Tennessee. Four of the sherds were undecorated, and six were from decorated vessels. One of the submitted sherds was too contaminated by plasticizers to be interpreted. All residues in the remaining nine sherds showed evidence for microbial degradation of lipids and plasticizer contamination, but information could be gleaned from all of them. The decorated sherds contained ricinoleic acid, often used as a biomarker for castor oil. It is postulated that this substance may have been used postdepositionally in some way on the decorated sherds. The potential use of castor oil complicated interpretation further; however, some interpretation was still possible. Coniferous resin and meat compounds were present in four of the six decorated sherds, while coniferous resin probably without meat was present in one of the decorated sherds and one of the undecorated sherds. Biomarkers for oil were found in two of the decorated sherds suggesting either modern contamination by diesel oil, or some form of prehistoric trade in bitumen or tar.

Introduction

Absorbed residues were analyzed from ten Hiwassee Island sherds, an important archaeological site in Central Tennessee. The site was primarily excavated as a WPA project during the 1930s, and the sherds had been stored since that time. Of the sherds submitted, one was a Mississippian frog effigy, three were plain shell-tempered sherds, one was shell-tempered net-impressed, two were Hiwassee Island Complicated Stamped, two were Hiwassee Island Red-Filmed, and one was Hiwassee Island Red on Buff. This allowed a comparison between the more decorated pottery types at the site with the less decorated types. The primary purpose of the project was to determine if absorbed residue analysis can successfully be performed on sherds in legacy museum collections. The secondary purpose was to determine if a difference could be ascertained in pot contents between the more and less highly decorated wares. A tertiary purpose was to look for potential evidence for bean, *Phaseolus vulgaris,* presence in the sherds.

Absorbed residue analysis involves the extraction of compounds extracted through cooking or other pot use and absorbed within the ceramic matrix of a potsherd. They are generally the result of the slow absorption of chemical components of resources processed in a

pottery vessel over its use-lifetime. In order to be preserved within the matrix of the pottery, components must be hydrophilic enough to dissolve in cooking liquid, but hydrophobic enough that they do not wash out of the pot during archaeological deposition. Lipids chemically fit this description most closely, and lipids therefore make up the large majority of chemical components in absorbed pottery residues.

In all varieties of residue analysis, the compounds extracted from the residues are analyzed chemically, with gas chromatography/mass spectrometry (GC/MS) being one of the preferred methods, and the one used in this study. This method allows for the separation of complex mixtures of compounds, and the identification of a wide range of compounds. This is performed by means of the chemical fingerprint, or mass spectrum, of each separate type of molecule that is separated by the gas chromatograph. Once the compounds have been identified, the analyst tries to identify their source or sources, keeping in mind that the lipids probably underwent some degree of hydrolysis, oxidation, or microbial breakdown over the period of archaeological deposition.

In general, all lipid residues can be interpreted using two major techniques; biomarker and relative compound abundances. Biomarkers are compounds unique to a certain resource or class of resources, and the biomarker approach allows the identification of specific resources or classes of resources. Cholesterol, a sterol found in meat and animal lipids, is a biomarker for meat, for example. The relative abundance approach utilizes the relative amounts of various common compounds to give general interpretations about the composition of the residue. For example, the presence of large abundances of unsaturated fatty acids often indicates that a residue originated primarily in either plant or marine resources. Both the biomarker and the relative abundance approach were used while interpreting residues in this project.

Methods

Absorbed residues were extracted using the methodology published by Evershed, et al. (1990). Sherds were cleaned with a solvent-washed model drill to remove surface impurities. This surface cleaning was more vigorous than usual because all the sherds had been extensively labelled, with nail polish, whiteout, ink, and occasionally pencil. The cleaned sherds were then crushed in a solvent-washed mortar and pestle, an internal standard of 20µL *n*-tetratriacontane was added, and the sherd was extracted with approximately 10 mL of 2:1 v/v chloroform/methanol per 2 g of powdered sherd.

Each sample vial was then ultrasonicated for 20 min x 2, with a 10 min cooling period. The samples were centrifuged at 2000 rpm for 20 min, the supernatant was pipetted into solvent-washed vials, and samples were then filtered through solvent-washed 220-440 mesh amorphous silica gel to remove the remaining fine particles from the residue-impregnated solvent.

The clean solvent/residue mixture was evaporated under N_2 gas and mild heat to dryness. An aliquot of this residue was derivatized with approximately 200 µl N,O-bis(trimethylsilyl)fluoroacetamide (BSTFA) +1% trimethylchlorosilane (TMCS) and analyzed in a Fisons 8065 gas chromatograph interfaced to a Trio 1000 mass spectrometer, using a DB-1HT 15 m x .32 mm column with .1µl film thickness and with a column head pressure of 7.5 psi. The temperature was held at 50˚ for 2 min, then ramped at 10˚/min until 350˚, followed by a 10 min hold at that temperature. Total runtime was 42 min. Prior to analysis each day, the GC/MS was tuned with DFTPP to EPA standards to ensure consistent and precise mass spectrometry. This portion of the analysis is called the total lipid extract (TLE) since it contains all the components in the residue without saponification.

Residue samples were also separated into neutral and fatty acid (FA) fractions for better quantification and analysis of the various compounds in the residue. Approximately 60% of the total residue extracted from sherds was transferred to solvent-washed culture tubes, then saponified with 2 mL NaOH/methanol and heated at 75˚ for 1 h. The saponified residues were then extracted with 3 x 2 mL hexane, which was blown down. This extraction became the neutral fraction, and contained compounds such as alkanes, long-chain alcohols, sterols, and terpenoids. This fraction was stored under N_2 gas and refrigeration until analyzed using the same instrument and temperature program as the TLE.

The remainder of the residue, containing primarily free fatty acids, was acidified to pH 3-4 with 2 M HCl, and extracted with 3 x 2mL hexane into cleaned and solvent-rinsed culture tubes. This solution was evaporated, stored under N_2 and refrigerated until analyzed. Approximately half of the fatty acid fraction was derivatized to trimethylsilyl esters with BSTFA and analyzed using the same instrument and column as the TLE, but with a temperature program ramping from 50-150˚ C at 15˚C min^{-1}, followed by 150-250˚ C at 3˚ C min^{-1}, and a 10 min hold at 250˚ C.

A quick quantification was done for the absolute amount of non-plasticizer residue present in each sample by adding the amount of all identified lipids from the TLE fraction that were not plasticizer biomarkers, and calculating the amount based on the amount of the internal standard known to be present in the sample. This quantification was an interesting, but not conclusive measure of viable residue present in a potsherd. Richard Evershed has suggested that 5 μg/g is the lower limit for correct interpretation of an archaeological sample (2008). In this study, all interpretable samples included more lipid than 5 μg/g—the amounts ranged from 9-167 μg/g.

In addition, because of the large amount of plasticizers in some of the residues, the total percentage of plasticizer in each overall residue was calculated. This was done by quantifying known biomarkers for plasticizers, calculating the μg plasticizer/g sherd, and then dividing this by the total μg lipid/ g of sherd. Results of this quantification are given in Table 1. In addition, the amount of microbial digestion products was also roughly quantified, due to the abundance of microbial biomarkers, and the unusual alkane series. This was done by quantifying some of the known biomarkers for microbial digestion—Cyclohexane alkanes and branched alkanes—and then calculating a percentage divided by the total non-plasticizer lipid in the residue. It should be noted that this calculation undercounted the total presence of microbial lipids in the residue, because other possible microbial lipids were not included—most importantly iso- and anteiso-fatty acids and alkanols. We used the percentage of microbial lipids, given in Table 1, as a rough guideline for microbial contribution.

Solvent blanks were run in parallel with the archaeological samples and used to control for laboratory contamination. Blanks were generally clean for this project. Samples were run in a semi-blind fashion, in that each sample was assigned a lab number for analysis, and the true provenience of the samples was never used until interpretation began to take place. Lab numbers and the original sample numbers, sherd descriptions, sherd provenance, and a basic interpretation of all residues in the project with comments, are given in Table 1.

How to Interpret a Lipid Residue

When interpreting a lipid residue, several different classes of compounds are examined. The fatty acid relative abundances, particularly in terms of chain length and saturation, are examined to determine the general overall composition of the residue, as described above.

Saturation is the number of double bonds present in a carbon chain. Fatty acids are generally written in the form $C_{\text{carbon chain length: \# of double bonds}}$. Fatty acids most commonly occur linked to a glycerol backbone in the form of triacylglycerols, which are the most abundant constituents of fats and oils in nature. Free fatty acids, although present in normal lipids, occur in only small amounts and tend to dissolve in water more easily than the glycerol forms (Evershed 1993; Evershed, et al. 1992) and many others.

In most cases, fatty acids with more unsaturated fatty acids, particularly $C_{16:1}$ and $C_{18:1}$, and more $C_{16:0}$ than $C_{18:0}$, tend to originate in either vegetables or fish. Fatty acids with less unsaturated fatty acids and more $C_{18:0}$ than $C_{16:0}$ tend to be comprised primarily of meat lipids. Odd chain fatty acids often originate in bacterial or fungal lipids. Also, fatty acids with shorter chain lengths tend to wash out of absorbed residues earlier, while more unsaturated fatty acids are more prone to hydrolysis, oxidation, and microbial degradation (Evershed 2008; Reber and Evershed 2004). Due these and other issues described at length in other publications, this preliminary interpretation of fatty acid composition must be paired with the interpretation of other compound types. In most cases, a residue containing highly unsaturated fatty acids can only be interpreted as 'primarily plant/fish' in origin, due to the difficulty of distinguishing between unsaturated fatty acids originating in plants and fish. For this reason, fatty acid ratios cannot be used to definitively identify any one resource type, including beans.

Due to the tendency of unsaturated fatty acids to undergo hydrolysis, oxidation, and microbial breakdown, it is unusual for an archaeological residue to be very strongly unsaturated. This can be defined such that an unsaturated fatty acid makes up more than 50% of the total fatty acid fraction of a residue. If a residue is that strongly unsaturated, it suggests either that the residue was comprised almost completely of plant or marine resources, or that the residue was

contaminated. Modern oils and lotions are often very highly unsaturated. If a residue with a strongly unsaturated fatty acid fraction is present in a residue also containing biomarkers of modern contamination, such as DEET, vitamin E, or sunscreen compounds, then it must be classified as so contaminated that interpretation is either very difficult or impossible. If those biomarkers of modern contamination are not present, however, it is possible that the archaeological residue was comprised almost entirely of plant, fish, or shellfish resources. One of the suggested indicators for a primarily fish/shellfish residue is a high degree of unsaturation, a $C_{18:0}/C_{16:0}$ ratio lower than .48, and the presence of cholesterol (Isaksson and Hallgren 2012). Other, less common indicators for fish and shellfish are the presence of isoprenoid fatty acids, (Baeten, et al. 2013; Corr, et al. 2008), and the presence of ω-(o-alkylphenyl) alkanoic acids, pyrolytically formed from isoprenoid fatty acids (Hansel, et al. 2004).

Sterols are one of the compound types most likely to produce general category biomarkers. Cholesterol, as mentioned above, is a biomarker for the presence of meat resources, while there is a series of plant biomarkers, including sitosterol, campesterol, and stigmasterol that indicate the presence of plant resources. The presence of cholesterol or plant sterols can help support a fatty acid composition interpretation, as well as definitively determining whether plant and meat resources were present in the lipid residue. Unfortunately, sterols are not as common as fatty acids, and are not always present. When they are present, however, they provide valuable and clear information concerning vessel contents. Since cholesterol is found in human skin lipids, however, the possibility of cholesterol contamination by handling should also be considered during interpretation.

Terpenoids are another compound type particularly useful in interpreting residues. They are plant biomarkers; pentacyclic triterpenoids are commonly found in non-pine plant resins and

surface waxes (Glastrup 1989; Harborne and Tomas-Barberan 1991; Langenheim 2003). Diterpenoids, particularly those with the pimarane and abietane carbon skeletons, are often biomarkers for pine resin. Labdane diterpenoids occur both in pine resins and in resin from other plants, and thus can be used as a category biomarker for plant resin, but not for any particular class of plants.

Alkanols are long-chain alcohols—carbon chain lengths of 12-34 are often found in lipid residues. Alkanols often originate in wax esters, linked to alkanes. As such, alkanols give valuable information concerning the presence of waxes in the lipid residue. Waxes occur in all resource types, but even-chain alkanols are particularly prevalent in higher plant waxes (Kolattukudy 1976). In this report, alkanols will be notated by the form $OL_{\text{chain length}}$. By carefully examining references on plant waxes, sometimes a plant resource or a range of resources may be identified partially through alkanol composition. For example, very long-chain alkanols, such as OL_{32} are rare in most plants but relatively common in panicoid grasses (Bianchi, et al. 1984; Reber, et al. 2004). Panicoid grasses are a large subfamily of about 2000 grasses, including maize and many other grasses from around the world. The presence of this compound indicates that a panicoid grass or grasses may be present in the residue. Additionally, most (but not all) plant waxes consist of a small number of alkanols esterified with a range of alkanes, or of a range of alkanols with a gradual increase in abundance of chain length to the most abundant alkanol, followed by a gradual decrease in chain length abundances (Kolattukudy 1976). Residues containing a wide range of alkanols, particularly those of very different chain length and not fitting either of these patterns, probably indicate that more than one plant resource is present.

Alkanes are unsaturated carbon chains, usually originally found linked to alkanols in waxes, or to sterols. Alkanes will be described in this paper in the form $AL_{\text{carbon chain length}}$. Like alkanols, they occur in all resource classes. Higher plant alkanes usually have odd carbon chains; highly branched alkanes often indicate microbial or fungal breakdown of the original wax ester, and were used as biomarkers of microbial action during the quantification phase of this project. Furthermore, the alkane AL_{29} can be used as a biomarker for higher plant epicuticular wax (Evershed 2008: 898). They can also be used to determine whether more than one resource source is present in a lipid similarly to the way alkanols are used.

It is important to remember that all residue interpretation must be done with some knowledge of the local biome of the site being investigated, or at least with the knowledge that more knowledge of the local biome is needed. For example, coniferous resins can be easily identified in a residue through the presence of abietane and pimarane diterpenoids, which are well-established biomarkers for this type of resin. Determining the source of such a resin, however, requires knowledge of what coniferous trees would be found near the site and likely to be utilized by the ancient inhabitants of the site. From a residue standpoint, a coniferous resin from Connecticut and one from Tennessee look identical, but the interpretation of the source and use of the resin would be different in the two places, based on environmental and cultural considerations. This is why collaboration between residue analysts, site archaeologists, and paleoethnobotanists is so crucial to a successful residue analysis.

Results and Discussion

Since the sherds were already labeled with ink, nail polish, white-out, and occasionally pencil and had been stored in plastic, contamination was an issue for many of the residues. Only

one sherd, a plain shell-tempered sherd from 40MI5 F8-7 (lab # RL 363) was so contaminated by plasticizers that it could not be interpreted. As shown in Table 1, plasticizer biomarkers made up 95% of the total lipids in the residue, suggesting that the residue was so contaminated that it could not be interpreted. Other residues in the project contained between 0.7% and 10.2% plasticizer biomarkers. Obviously, these percentages should be kept in mind during residue interpretation. Possibly due to the early excavation date, however, bugspray and sunscreen contamination was not present in any of the residue in this project.

All the residues in the project contained an unusual quirk in the alkane series; natural alkane series, such as those present in beeswax and plant waxes, are primarily comprised of odd-chain alkanes (Evershed, et al. 1997; Kolattukudy 1976:216). The residues in this project contained almost equal amounts of even- and odd- chain alkanes. Three of the decorated sherds also contained a longer-than-usual sequence, extending up to A_{L39} in the most extreme cases, as discussed below. This type of even-odd alkane series may occur naturally in fungal lipids, hydrocarbons such as oil and gasoline, and synthetic waxes. It is not possible to determine the origin of the alkanes in these residues, although oil may be responsible for the longer-chain sequences in two of the decorated sherds. The ubiquity of these compounds in all residues in the study, however, suggests that the compounds derive from some sort of depositional or postdepositional contamination.

Table 1. Sherd provenance, lipid quantification, % plasticizers, % microbial lipid contribution, laboratory description, and residue description and interpretation for all samples in the project.

Sample #	Provenance	µg Lipid/g sherd	% Plast-icizers	% Micr. Lip.	Laboratory description	Residue description	Residue Interpretation
RL 362	37M631 309 side slope of mound levels A,B,C	87.8	3.0	2.6	Shell-tempered burnished black rimsherd with embossed chevron on neck. Carinated? Labelled with white ink on interior	TLE contained a lg lump containing alkanes, C16:0 and C18:0; fatty acid series of C6:0-C22:0, some plasticizers, labelling compounds, DAGs, TAGs, wax, ricinoleic acid, DHA, Juvabione, and 4-anisaldehyde. Neutral contained cholesterol, 3 abietane diterpenoids, one PAH, and alkanol series OL18-OL32. Fatty acid fraction contained 5% ricinoleic acid, fatty acids similar to plant.	Residue contained mixture of meat and plant resources, including tree resin from Pinaceae, and some fragrance contamination. Small amount of soot present. Ricinoleic acid present, suggesting castor oil contamination? This makes interpretation difficult.
RL 363	40MI5 F8-7	9.0	95.0	11.0	Shell-tempered, indeterminate surface, 3 sherds glued together, labelled in black ink on interior, buff paste	Plasticizers made up 95% of this residue.	Too contaminated by glue and plasticizers to interpret accurately.

Sample #	Provenance	µg Lipid/g sherd	% Plast-icizers	% Micr. Lip.	Laboratory description	Residue description	Residue Interpretation
RL 364	37M631 346 L-E jar	59.5	0.7	8.4	Shell-tempered plain everted collar rimsherd, labelled on int. with nailpolish and white ink.	TLE has lg lump containing long alkane series, C6:0-C24:0 fatty acid series, one PAH. No biomarkers present in Neutral, microbial presence, fatty acids unusually short, look like primarily plant or fish.	Residue definitely present, but lack of biomarkers make interpretation difficult. May be degraded by oxidation and microbial breakdown, might have contained plant or fish?
RL 365	355/37M631 Fea. 40-L E1	20.2	2.2	12.1	Net-impressed (net S-twist), shell-tempered sherd, labelled on back wit white ink and nail polish. Sherd hard to break, but crumbly when broken Hard to sample cleanly.	TLE contained lg. lump containing alkanes, C16:0 and C18:0, long alkane series, 1 PAH present in TLE and N. 4-anisealdehyde in TLE, No biomarkers in N, Fatty acids similar to plant, C9:0-C20:0.	Residue present, but lack of biomarkers makes interpretation difficult, except for presence of a small amount of soot and fragrance contamination. Degraded by oxidation and microbial breakdown, might have contained plant?

Sample #	Provenance	µg Lipid/g sherd	% Plast-icizers	% Micr. Lip.	Laboratory description	Residue description	Residue Interpretation
RL 366	355/37M631 Fea. 40 L. E	11.2	9.0	12.3	Plain, shell-tempered bodysherd from vessel collar--cordmarked at base. Labelled on interior with white ink and nail polish	Less residue in this than other residues in the project. TLE contained a lg. lump containing alkanes, $C_{16:0}$ and $C_{18:0}$, long alkane series. PAH and other biomarker for burning in TLE, 4-anisaldehyde in TLE, 2 abietane diterpenoids and 1 labdane diterpenoid in N, ketone in N, Fatty acids primarily plant or fish; C_8-$C_{22:1}$, waxes present in TLE.	Probable resin from coniferous tree, soot present, some fragrance contamination, possible residue from waxy plant? Possible other plant or fish, but degraded by microbial action.
RL 367	369/37Mg31 Phase E1	67.4	1.3	22.9	Shell-tempered chevron stamping, labelled on interior with white ink and nail polish	TLE has lg lump with very long alkane sequence, DAG present, $C_{8:0}$-$C_{26:0}$ fatty acid series in TLE. Cholesterol in N, 2 abietane diterpenoids, 1 unidentified diterpenoid, 1 PAH. Fatty acid fraction included 6% ricinoleic acid and other hydroxy acids, otherwise $C_{12:0}$-$C_{24:0}$ Fatty acid sequence plantlike.	Residue was highly degraded by microbes, but may have contained a mixture of meat and and plant resources, including resin from a member of Pinaceae, small amount of soot present. Ricinoleic acid suggests castor oil contamination? This makes interpretation difficult.

Sample #	Provenance	µg Lipid/g sherd	% Plast-icizers	% Micr. Lip.	Laboratory description	Residue description	Residue Interpretation
RL 368	355/37Mg31 Level E.I	167.3	0.7	3.3	Shell-tempered, red-filmed body sherd, labelled on interior with white ink and nail polish 355 written on interior of sherd in pencil	Lots of residue present, DAGs present in TLE, 4-anisaldehyde. Neutral contained stigmasterol, saccharostenone, 5-α stigmastanol, 2 abietane diterpenoids, 1 PAH, good alkanol series $OL_{20\text{-}32}$. Fatty acids looked primarily meat-based, included small amount of ricinoleic acid, $C_{9:0}$-$C_{22:0}$.	Plant and meat resources present, small amount of soot, possible castor oil contamination?
RL 369	349/37Mg31 Fea. 39 L. E-I	23.3	6.5	4.4	Red-slipped, shell-tempered bodysherd labelled on interior with white ink and nail polish, 2 pieces glued	TLE had lg lump with long alkane series, juvabione, 4-anisaldehyde, ketone. Neutral contained cholesterol, 2 hopanoids, 1 abietane diterpenoid, 1 ketone. Fatty acids looked primarily plant-based, $C_{8:0}$-$C_{18:0,}$ some ricinoleic acid present.	Mixture of meat and plant resources including possible resin from Pinaceae, oil present, fragrance contamination, castor oil contamination?

Sample #	Provenance	µg Lipid/g sherd	% Plast-icizers	% Micr. Lip.	Laboratory description	Residue description	Residue Interpretation
RL 370	346/37Mg31 Level EI	18.2	10.2	2.9	red-filmed, shell-tempered bodysherd, labelled on interior with white ink, nail polish, and pencil under nail polish	TLE contained lump with long alkane series, 4-anisaldehyde. Neutral contained 2 abietane diterpanoids and 1 unidentified diterpenoid, 2 ketones, 1 PAH, 2 hopanoids. Fatty acid contained indeterminate fatty acids $C_{8:0}$-$C_{18:0}$, and 3% ricinoleic acid	Plant resins present, including coniferous and non-coniferous, oil present, soot contamination, fragrance contamination, castor oil contamination?
RL 371	355/37Mg31 Fea. 40 L. E	57.3	6.8	14.1	Complicated stamped, shell-tempered collar sherd, labelled on interior with white ink and nail polish	TLE contained a long, extensive alkane series and 4-anisaldehyde. Neutral contained almost completely alkanes and branched alkanes. Fatty acids looked indeterminate $C_{9:0}$-$C_{20:0}$, ricinoleic acid present, some biomarkers for plastic burning?	Indeterminate residue containing some fragrance and castor oil contamination, residue degraded by microbial action.

Plain and Net-Impressed Sherds

For the remaining two plain and one net-impressed sherds in the project, residue interpretation was difficult. None of them contained any sterol biomarkers, which would have given a definite indication for the presence of animal or plant-based lipids. All these sherds showed a strong contribution of microbial lipids, suggesting that microbes had digested some of the original lipid contents. Microbial lipid contribution (again, more of a guideline than a specific number) was from 8.4-12.3%, which manifested most clearly in a large number of unusually short-chain compounds and branched alkanes.

These residues also contained relatively few alkanols, suggesting either that these compounds had been degraded by microbes or that the residues contained relatively few epicuticular leaf waxes or other natural waxes. The fatty acid abundances in these residues looked similar to plant-based residues. However, due to the microbial presence and lack of biomarkers, this interpretation is quite tentative.

RL 366, a plain shell-tempered sherd from 355/37M631 Fea. 40 L. E, contained highly oxidized abietane biomarkers for coniferous tree resin as well as polyaromatic hydrocarbon (PAH) biomarkers for soot and a short ketone. Such ketones may derive from plant waxes, from contaminants, or from pyrolysis of fatty acids. In this case, a derivation from plant wax, tree resin, or contaminants appears most likely, as the ketone is not made up of common fatty acid chain lengths. The soot may have derived from the firing of the pot, from its use over a fire, or from its decoration. In general, the three interpretable residues from undecorated Hiwassee Island wares were somewhat disappointing in terms of interpretability. All could be uncertainly interpreted as containing plant-resources, but were highly degraded by microbes and contained relatively few biomarkers.

Table 2. Percentage of Total Lipid Extraction (TLE) fraction for each compound in each residue in the project. Compounds are organized by variety, and then by chain length; fatty acids, alkanols, and alkanes are labelled as described in the 'How to Interpret a Lipid Residue in the Absence of Serious Contamination' section of the paper. Unknowns are labeled by elution time, and then by important fragments and tentative interpretation of compound class. DAG stands for diacylglycerol, TAG stands for triacylglycerol, and MAG stands for monoacylglycerol.

Compound	**RL 362**	**RL 363**	**RL 364**	**RL 365**	**RL 366**	**RL 367**	**RL 368**	**RL 369**	**RL 370**	**RL 371**
$C_{6:0}$	1	-	-	-	-	-	1	-	-	1
$C_{7:0}$	-	-	-	-	-	-	1	-	-	-
$C_{8:0}$	1	-	1	-	-	-	2	-	-	1
$C_{9:0}$	1	-	1	-	1	1	4	-	1	4
$C_{10:0}$	-	-	1	-	-	-	1	-	-	1
$C_{12:0}$	1	-	2	1	1	1	1	3	3	1
$C_{13:0}$	-	-	1	-	-	1	-	-	1	-
$C_{14:0}$	1	-	2	1	1	1	1	4	3	1
$C_{15:0}$	1	-	-	-	-	1	-	2	-	1
$C_{16:1}$	-	1	-	-	-	-	-	-	-	-
$C_{16:0}$	17	1	2	3	3	8	23	-	9	3
$C_{17:0}$	2	-	-	-	-	1	1	2	2	-
$C_{18:1}$	-	-	-	-	-	4	-	-	-	-
$C_{18:0}$	18	-	1	3	3	8	27	13	10	3
$C_{20:0}$	1	-	-	-	-	1	3	2	-	-
$C_{22:0}$	1	-	-	-	-	1	1	1	-	-
$C_{23:0}$	-	-	-	-	-	1	-	-	-	-
$C_{24:0}$	-	-	-	-	-	1	-	-	-	-
C_{14}br	-	-	-	-	-	1	-	-	-	-
C_{15}br	-	-	-	-	-	-	-	-	5	-
C_{17}br	-	-	-	-	-	-	-	-	1	-
C_{22}br	-	-	-	-	-	1	-	-	-	-
C_{24}br	-	-	-	-	-	1	-	-	-	-
di-$C_{9:0}$	-	-	-	-	-	1	-	-	-	-
12-hydroxy $C_{18:1}$	1	-	-	-	-	1	-	-	1	-
Hydroxy di-$C_{18:1}$	-	-	-	-	-	2	1	-	-	-
MAG 14	-	-	-	-	-	1	-	-	-	-
TAG tri-12-hydroxy C18:1?	Tr	-	-	-	-	Tr	Tr	-	-	-

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
Wax 17.63	-	-	-	-	1	-	-	-	-	-
8-heneicosanone	1	-	-	-	1	-	-	-	-	-
9-docosanone	-	-	-	-	-	-	-	2	-	-
Glycerol	-	-	-	-	-	1	2	-	-	6
4-Anisaldehyde	1	-	-	2	3	-	1	1	1	5
Juvabione	1	-	-	-	1	-	-	2	-	-
Dehydroabietate	-	-	-	-	-	-	-	-	2	-
Dehydroabietic acid	1	-	-	-	1	-	-	-	1	-
Oxo-dehydroabietate	-	-	-	-	2	-	-	2	1	-
Phenanthrene or anthracene	-	-	1	1	1	1	-	-	-	-
Trisnorhopane	-	-	-	-	-	-	-	-	1	-
Norhopane	-	-	-	-	-	-	-	1	1	-
OL_{14}	-	-	-	-	-	-	1	-	-	-
OL_{16}	-	-	-	-	-	-	-	-	1	-
OL_{18}	-	-	-	1	-	-	-	1	1	-
OL_{32}	-	-	-	-	-	-	1	-	-	-
AL_{16}	-	-	1	1	1	-	-	-	-	-
AL_{17}	1	-	1	2	1	1	1	-	-	1
AL_{18}	1	-	2	4	2	1	1	-	1	1
AL_{19}	-	-	2	7	-	-	2	-	-	-
AL_{21}	6	-	14	15	18	5	3	4	2	4
AL_{22}	13	-	20	15	0	6	3	9	7	6
AL_{23}	7	-	15	8	17	2	1	7	6	6
AL_{24}	3	-	8	3	6	1	1	6	6	5
AL_{25}	1	-	4	1	2	2	-	5	4	3

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
AL_{26}	1	-	2	-	1	1	-	4	2	2
AL_{27}	-	-	1	-	-	1	-	2	1	1
AL_{28}	-	-	1	-	-	1	-	2	-	1
AL_{29}	-	-	-	-	-	2	-	1	1	1
AL_{30}	-	-	-	-	-	1	-	1	-	1
AL_{31}	-	-	-	-	-	1	-	1	1	1
AL_{32}	-	-	-	-	-	1	-	1	1	1
AL_{33}	-	-	-	-	-	1	-	1	1	1
AL_{35}	-	-	-	-	-	1	-	1	1	1
AL_{36}	-	-	-	-	-	1	-	1	1	1
AL_{37}	-	-	-	-	-	1	-	1	-	1
AL_{38}	-	-	-	-	-	-	-	-	-	1
AL_{40}	-	-	-	-	-	1	-	-	-	-
AL_{17}br	-	-	1	2	2	-	-	-	-	-
AL_{18}br	1	-	2	3	3	1	1	-	-	-
AL_{19}br	-	-	-	1	-	-	-	-	-	-
AL_{20}br	4	-	5	11	7	1	2	2	2	2
AL_{21}br	2	-	-	2	2	-	-	-	-	-
AL_{22}br	-	-	1	-	1	-	-	-	-	-
AL_{23}br	-	-	1	1	1	1	-	-	-	-
AL_{24}br	-	-	1	-	1	-	-	-	-	-
AL_{25}br	-	-	1	-	-	-	-	-	-	-
AL_{26}br	-	-	1	-	-	-	-	-	1	-
AL_{27}br	-	-	-	-	-	-	-	1	1	-
AL_{32}br	-	-	-	-	-	1	-	-	-	-
AL_{33}br	-	-	-	-	-	1	-	-	-	-
AL_{34}br	-	-	-	-	-	3	-	-	-	1
AL_{35}br	-	-	-	-	-	3	-	-	-	1
AL_{36}br	-	-	-	-	-	2	-	-	-	1
AL_{37}br	-	-	-	-	-	4	-	-	-	1
AL_{38}br	-	-	-	-	-	-	-	1	-	1
AL_{39}br	-	-	-	-	-	-	-	1	-	1
AL_{40}br	-	-	-	-	-	2	-	-	-	1
AL_{42}br	-	-	-	-	-	1	-	-	-	1
? 7.47 73, 145, 161, 177	-	-	-	-	-	-	-	-	-	2
? 8.88 132, 145, 149, 119, 159, 75, 250	-	-	-	-	-	-	2	1	4	4

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
Plasticizer 11.86	-	-	-	-	-	-	-	1	-	1
Plasticizer 12.69	-	-	-	-	-	-	-	1	10	-
Plasticizer 12.83	-	-	-	-	-	-	-	5	-	3
Plasticizer 13.08	3	-	-	-	6	-	-	-	-	-
Plasticizer 16.92	-	-	-	-	-	-	-	-	-	-
Plasticizer 17.90	-	94	-	-	-	-	-	1	-	-
FAA $C_{22:1}$	-	-	-	1	2	-	1	-	-	1
? 13.37 Cyclo AL	-	-	-	-	-	1	-	-	-	-
? 19.23(amide)	-	-	-	-	-	1	-	-	-	-
? 195 13.70	-	-	-	-	-	1	-	1	1	-
? 14.62 cyclo AL	-	-	1	1	1	-	-	-	-	-
? 15.27 Cyclo AL	-	-	-	-	-	-	-	-	1	-
? 15.54 Cyclo AL	-	-	1	-	-	-	-	-	-	-
? 15.80 Cyclo AL	-	-	-	1	1	-	-	-	-	-
? 20.05 v. branchy	1	-	-	-	1	-	-	-	1	-

Decorated Wares

The remaining six sherds in the study were from a variety of forms of decorated wares, including a frog effigy, two Hiwassee Island Complicated Stamped sherds, two Hiwassee Island Red Filmed sherds, and one Hiwassee Island Red on Buff sherd. All residues from decorated sherds in the study had one unusual characteristic in common—they contained measurable amounts of ricinoleic acid. This unusual fatty acid is formally termed 12-hydroxy-9-cis-

octadecenoic acid. It is found primarily in castor bean oil, although it may also occur in some fungal lipids (Kolattukudy 1976:370), most notably ergot (Kren, et al. 1985), as well as other plant lipids. None of the known sources for ricinoleic acid is native to North America, however, suggesting that the fatty acid derived from either modern castor oil use, or fungus. The lack of ergosterol, and the fact that the ricinoleic acid was present only in decorated sherds in the project, suggests that castor oil may have been used as a museum or archaeological treatment, either to bring out a shine on the artifacts, or as a fungicide.

The residues from all of these sherds except the Frog effigy also contained a the unusually long even-odd alkane series described above and shown in Table 3. Because the even-odd alkane series was present in all residues in the study, as described above, these compounds were tentatively postulated to derive from some form of contamination. That said, very long alkane series of this type, which were found in five of the decorated sherds, can derive from oil, and there were hopane biomarkers for oil in two of these sherds, as discussed below. Those two sherds did contain the longest alkane series in the project, so in those two cases, it is likely that at least some of the longer alkanes derived from oil in some form or another.

Frog Effigy

The residue extracted from the Frog Effigy sherd, RL 362, contained cholesterol, three abietane diterpenoid biomarkers for coniferous resin, a PAH compound that indicates soot, and one of the most complete alkanol series in the project, suggesting a wax of some type, probably deriving from a plant. It also contained 4-anisaldehyde which probably indicates some contamination from modern fragrance. The fatty acids for this residue were typical of plants; however, it can be assumed that much of this plant contribution derived from castor oil. Discounting the fatty acid contribution, therefore, the vessel had contained meat and possible

coniferous resin, although this resin might have been used to seal or decorate the pot. Alternatively, it could have been used as a flavoring, medicinal, or ritual agent. The residue analysis cannot distinguish the purpose of the resin.

Table 3. Percentage of Neutral fraction for each compound in each residue in the project. Compounds are organized by variety, and then by chain length. Unknowns are labeled by elution time and significant peak as in Table 2.

Compound	**RL 362**	**RL 363**	**RL 364**	**RL 365**	**RL 366**	**RL 367**	**RL 368**	**RL 369**	**RL 370**	**RL 371**
Cholesterol	1	-	-	-	-	1	-	1	-	-
Campesterol	-	-	-	-	-	-	1	-	-	-
Sitosterol	-	1	-	-	-	-	-	-	-	-
Saccharostenone	-	-	-	-	-	-	1	-	-	-
5α-Stigmastanol	-	-	-	-	-	-	2	-	-	-
Dehydroabietic acid	1	-	-	-	-	1	4	-	5	-
Methyl deabietate	2	-	-	-	1	2	-	-	-	-
Oxo-deabietate	1	-	-	-	1	-	1	-	-	-
Oxo-deabietic acid	-	-	-	-	-	-	-	-	1	-
Diterp. 15.43	-	-	-	-	-	1	-	-	-	-
Diterp. 16.52	-	-	-	-	-	-	-	-	2	-
Labdane? 13.29	-	-	-	-	1	-	-	-	-	-
9-Heneicontanone	-	-	-	-	1	-	-	-	1	-
9-Docontanone	-	-	-	-	-	-	-	-	3	-
9-Tricontanone	-	-	-	-	-	-	-	3	-	-
Anthracene	1	-	-	1	-	1	1	-	-	-
Pyrene	-	-	-	-	-	-	-	-	4	-
Trisnorhopane	-	-	-	-	-	-	-	1	1	-
Norhopane	-	-	-	-	-	-	-	1	1	-
OL_{16}	-	-	-	-	-	-	-	-	1	-
OL_{18}	1	5	-	-	-	-	-	-	1	-
OL_{20}	1	1	-	-	-	-	3	-	-	-
OL_{21}	-	-	-	-	-	-	1	-	-	-
OL_{22}	1	1	-	-	-	-	2	-	-	-
OL_{23}	-	-	-	-	-	-	1	-	-	-
OL_{24}	1	-	-	-	-	-	2	1	-	-
OL_{26}	1	-	-	-	-	-	2	-	-	-
OL_{28}	1	-	-	-	-	-	2	-	-	-
OL_{30}	1	-	-	-	-	-	2	-	-	-
OL_{32}	1	-	-	-	-	-	3	-	-	-
OL_{16} br	1	-	-	-	-	-	-	-	-	-
OL_{20}br	1	-	-	-	-	-	1	-	-	-
OL_{22}br	-	-	-	-	-	-	1	-	-	-
OL_{24}br	-	-	-	-	-	-	1	-	-	-

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
AL_{16}	-	2	-	1	-	-	-	-	-	-
AL_{17}	-	4	-	1	1	1	3	1	1	1
AL_{18}	1	8	1	2	1	1	5	-	1	1
$AL_{19:1}$	-	-	-	-	-	-	-	-	2	-
AL_{19}	5	10	2	7	3	-	-	-	3	2
AL_{20}	8	6	2	10	6	3	6	3	5	3
$AL_{21:1}$	-	-	-	-	-	-	-	1	-	-
AL_{21}	13	-	4	18	17	3	8	4	3	5
$AL_{22:1}$	-	-	-	1	-	-	-	-	-	-
AL_{22}	14	13	10	18	24	3	8	6	6	8
AL_{23}	10	4	-	12	17	3	7	8	12	11
AL_{24}	6	3	27	4	8	3	4	11	6	8
AL_{25}	3	2	17	2	2	3	2	10	6	4
AL_{26}	2	2	11	1	1	2	1	4	4	3
AL_{27}	1	1	4	-	-	2	1	3	1	2
AL_{28}	1	-	2	-	-	2	3	2	1	1
AL_{29}	-	-	1	-	-	2	1	2	1	1
AL_{30}	1	-	1	-	-	2	3	2	1	1
AL_{31}	-	-	1	-	-	3	-	1	1	1
AL_{32}	-	-	-	-	-	3	-	2	1	2
AL_{33}	-	-	-	-	-	3	-	2	1	2
AL_{35}	-	-	-	-	-	3	1	2	1	2
AL_{36}	-	-	-	-	-	2	1	2	1	2
AL_{37}	-	-	-	-	-	1	-	1	1	1
AL_{38}	-	-	-	-	-	-	-	1	1	1
AL_{39}	-	-	-	-	-	-	-	1	-	1
AL_{17}br	1	14	-	2	1	1	-	-	1	-
AL_{18}br	3	13	2	4	2	1	-	1	2	1
$AL_{19:1}$br	-	-	-	-	-	-	-	-	-	1
AL_{19}br	-	5	-	-	1	-	6	3	-	1
AL_{20}br	-	-	1	-	-	-	-	-	-	-
$AL_{21:1}$br	-	-	-	-	-	-	-	-	1	-
AL_{21}br	1	-	-	1	-	1	-	-	2	2
AL_{22}br	4	-	-	1	2	3	-	-	2	3
AL_{23}br	3	-	-	-	1	4	-	-	1	-
AL_{24}br	1	-	-	1	1	-	-	-	3	1
$AL_{25:1}$br	-	-	-	-	-	-	-	-	-	1
AL_{25}br	1	-	-	-	-	-	-	-	-	-
AL_{26}br	-	-	3	-	-	-	-	-	-	-
AL_{27}br	-	-	8	-	-	1	-	-	1	1
AL_{28}br	-	-	-	-	-	1	-	-	-	-
AL_{29}br	-	-	1	-	-	1	-	-	-	-
AL_{30}br	-	-	-	-	-	1	-	1	-	-

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
AL_{32}br	-	-	-	-	-	-	-	1	1	1
$AL_{33:1}$br	-	-	-	-	-	-	-	1	-	-
AL_{33}br	-	-	-	-	-	2	-	-	-	1
AL_{34}br	-	-	-	-	-	2	-	-	1	1
AL_{35}br	-	-	-	-	-	4	-	2	-	2
AL_{36}br	-	-	-	-	-	4	-	1	1	2
$AL_{37:1}$br	-	-	-	-	-	1	-	-	-	-
AL_{37}br	-	-	-	1	-	3	-	1	1	2
AL_{38}br	-	-	-	-	-	3	-	2	1	-
AL_{39}br	-	-	-	-	-	3	-	1	1	2
AL_{40}br	-	-	-	-	-	2	-	1	-	1
AL_{41}br	-	-	-	-	-	2	-	1	-	1
AL_{42}br	-	-	-	-	-	1	-	1	-	1
AL_{43}br	-	-	-	-	-	1	-	-	-	1
AL_{44}br	-	-	-	-	-	-	-	-	-	1
Cyclo AL 11.39	-	-	-	-	-	1	-	-	-	-
Cyclo AL 12.39	-	-	-	1	-	-	-	-	-	-
Cyclo AL 13.65	-	-	-	-	-	1	-	-	-	-
Cyclo AL 14.18	-	-	-	-	-	-	-	-	-	1
Cyclo AL 14.53	-	-	-	1	-	-	-	-	-	-
Cyclo AL 21.60	-	-	-	-	-	1	-	-	-	-
? 8.93 132, 145, 119, 165, 56	-	-	-	-	-	-	2	1	2	-
? 13.87 123, 69, 253	-	-	-	-	-	1	-	-	-	-
? 17.73 191, 109, 369	-	-	-	-	-	-	1	-	-	-
? Very branched 19.92	2	2	-	1	-	-	2	-	-	1

Hiwassee Island Complicated Stamped

The two residues extracted from Hiwassee Island Complicated Stamped sherds were not particularly easy to interpret. RL 371, from 355/37Mg31 Fea. 40 L. E, was an indeterminate residue, containing indeterminate fatty acids, some castor oil contamination, and no biomarkers. There were some unusual biomarkers that may have derived from burning coal, oil, or plastic, but presumably these derived from some form of unusual modern contamination. The residue therefore had no definite interpretation.

RL 367, from 369/37Mg31 Phase E1, did contain cholesterol, suggesting that meat was processed or served in the vessel, as well as two abietane biomarkers for conifer resin. There was a good deal of microbial lipid presence, however, and ricinoleic acid made up 6% of the total fatty acids, which was the most of any residue in the study (see Table 4). Meat and coniferous resin were processed or served in this complicated stamped vessel, similar to the frog effigy described above.

Table 4. Percentage of fatty acid fraction for each compound in each residue in the project. Compounds are organized by variety, and then by chain length. Unknowns are labeled by elution time and significant peaks similarly to Tables 2 and 3.

Compound	**RL 362**	**RL 363**	**RL 364**	**RL 365**	**RL 366**	**RL 367**	**RL 368**	**RL 369**	**RL 370**	**RL 371**
$C_{7:0}$	-	-	1	-	-	-	-	-	-	-
$C_{8:0}$	-	-	4	-	1	-	-	1	2	-
$C_{9:0}$	1	-	8	2	1	-	1	6	8	1
$C_{10:0}$	-	-	4	1	1	-	-	2	2	1
$C_{11:0}$	-	-	3	1	1	-	-	1	1	-
$C_{12:0}$	1	1	9	4	4	1	1	8	6	5
$C_{13:0}$	-	-	4	1	1	1	-	1	1	1
$C_{14:1}$	-	-	-	1	-	-	-	-	-	-
$C_{14:0}$	3	2	7	6	-	4	1	9	6	7
$C_{15:0}$	1	1	6	2	3	2	-	2	2	2
$C_{16:2}$	-	3	-	-	-	-	-	-	-	-
$C_{16:1}$	2	37	2	3	6	2	-	2	1	2
$C_{16:0}$	39	33	24	31	26	28	41	28	22	27
$C_{17:0}$	1	1	1	1	1	1	1	1	1	1
$C_{18:2}$	-	1	-	-	-	-	-	-	1	-
$C_{18:1}$	3	8	1	6	7	6	1	3	4	4
$C_{18:0}$	33	9	12	26	21	21	43	16	18	20
$C_{20:0}$	2	-	1	1	-	3	3	-	-	-
$C_{22:1}$	-	-	-	-	1	-	-	-	-	-
$C_{22:0}$	1	-	1	-	-	2	1	-	-	-
$C_{24:0}$	-	-	1	-	-	1	-	-	-	-
C16 hydroxy	-	-	-	-	-	2	-	1	1	-
b-C16 hydroxy	-	-	-	-	-	1	-	-	-	-
Ricinoleic acid	5	-	-	-	-	6	1	2	3	1
11-oxo-$C_{18:0}$	1	-	-	-	-	-	-	-	-	-
C_{12}br	-	-	-	-	-	-	-	1	-	-

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
C_{13}br	-	-	-	-	-	-	-	1	1	-
C_{14}br	-	-	2	1	-	-	-	1	1	1
C_{15}br	1	1	6	2	2	1	-	3	1	2
C_{16}br	-	-	1	-	-	1	-	-	-	-
$C_{17:1}$br										
C_{17}br	1	2	1	-	-	1	-	3	2	1
C_{18}br	-	-	-	1	-	-	-	-	-	-
$C_{18:1}$br	-	-	-	-	-	1	-	-	-	-
C_{21}br	-	-	-	-	-	1	-	-	-	-
C_{22}br	-	-	-	-	-	2	-	-	-	-
C_{23}br	-	-	-	-	-	1	-	-	-	-
C_{25}br	-	-	-	-	-	1	-	-	-	-
FAA $C_{22:1}$	-	-	-	-	12	1	-	-	-	-
FAA $C_{22:0}$	-	-	-	-	-	1	-	-	-	-
Phenylhexanoic acid	-	-	-	-	-	-	-	-	2	-
Didehydroabietic acid	-	-	-	-	1	-	-	-	-	-
Dehydroabietic acid	-	-	-	-	1	1	-	-	1	-
Oxo-dehydroabietic acid	-	-	-	-	4	-	-	1	1	1
Juvabione	-	-	-	-	1	-	-	1	-	-
CAS# 86-00-0	-	-	-	-	-	-	-	-	-	3
Malonic acid										
CAS# 1620-98-0 (pl.)	-	-	-	-	-	-	-	-	-	1
2,6-ditert butyl 4 nitrophenol [PVC and coal burning]	-	-	-	-	-	-	-	-	-	5
4-anisaldehyde	-	-	-	2	-	-	-	-	-	1
? 6.95 132, 119, 145, 117, 159	-	-	-	-	-	-	-	2	3	4
? 9.01 93, 73, 323, 321, 325	-	-	-	-	3	-	-	-	-	-
? 9.86 212, 73, 297	-	-	-	-	-	-	-	-	3	-

Compound	RL 362	RL 363	RL 364	RL 365	RL 366	RL 367	RL 368	RL 369	RL 370	RL 371
? 12.39 134, 107, 73, 234	-	-	-	3	-	-	-	-	-	-
? 12.58 117, 247, 232, 327, 346	-	-	-	1	-	-	-	-	-	-
? 12.70 262, 275, 303, 73, 360	-	-	-	3	1	-	-	-	-	-
Dihydroxy FA 14.28	-	-	-	-	-	2	-	-	-	-
? 16.27 Hydroxy acid	1	-	-	-	-	-	-	-	-	-
? 25.11 Amide	-	-	-	-	-	1	-	3	3	5
? 31.13 233	-	-	-	-	-	-	-	-	-	1

Hiwassee Island Red Filmed

Of the two residues extracted from Hiwassee Island Red Filmed sherds, one contained the most sterol biomarkers of any residue in the study. The sherd found at 355/37Mg31 Level E.I (RL 368), contained stigmasterol, a plant sterol; saccharostenone, an unusual sterone found in plants; and 5-α stigmastanol, a common breakdown product of stigmasterol. The residue also contained abietane biomarkers for coniferous resins, and an unusually complete alkanol series for this group of residues, including OL_{20-32}. This suggests a plant-derived wax of some type was present in the residue. Also unusually for this study, the fatty acids appeared to derive primarily from meat, despite the absence of cholesterol in the residue. This residue was therefore interpreted as containing a mixture of plant and meat components, including coniferous resin. Like the other decorated sherds, there was some castor oil contamination.

The residue extracted from the sherd found at 346/37Mg31 Level EI (RL 370) contained no sterol biomarkers. It did contain two hopane terpenoids, however, trisnorhopane and norhopane. These compounds are generally used as biomarkers for the presence of oil—either in

the form of an oil spill, natural oil in the dirt of a site, or modern diesel or oil contamination from poor vehicle maintenance (Reddy, et al. 2012; Seidel, et al. 2016). Alternatively, oil could have been contained within the vessel. At the present time, it is impossible to determine if the oil derived from modern contamination or from the vessel contents—if possible, a soil sample from the site might assist with this determination. This residue also contained abietane biomarkers for coniferous resin, polyaromatic hydrocarbon biomarkers for soot, and castor oil contamination. The fatty acids were indeterminate. This residue was therefore interpreted as containing coniferous plant resin, oil (either as a contaminant or content) and soot, as well as fragrance and castor oil contamination.

Hiwassee Island Red on Buff

The only Hiwassee Island Red on Buff sherd extracted in the study was found at 349/37Mg31 Fea. 39 L. E-I, (RL 369), and contained hopane biomarkers for oil, as well as abietane biomarkers for coniferous resin. Cholesterol was present in the neutral fraction, and although the fatty acids did appear to be primarily plant-based, the residue also showed evidence for castor oil contamination. The residue was therefore interpreted as containing meat, coniferous resin, and perhaps oil, although the caveats about oil contamination mentioned above are still in force.

Presence of Beans?

At the present time, we could not determine whether beans were present in any of the residues. Fatty acid ratios are not an effective means of detecting a single resource in a mixed residue, especially given the issues of microbial degradation, oxidation, and hydrolysis discussed above. Some preliminary analyses of modern *Phaseoleus* did suggest some target compounds

(Hunt 2016). However, we did not detect any of them in the residues from the site. For future research, we would suggest looking both for the nitrogenous compounds present in modern *Phaseolus*, as well as likely breakdown or polymerized products of these compounds. It would then be possible to identify Phaseolus through either a straight biomarker approach, or via compound-specific nitrogen isotope analysis of common nitrogen-containing compounds from the residue. Concentrated research should be able to help with this problem, but preliminary studies on this research should probably utilize a less complicated series of residues than the Hiwassee Island legacy collection. When and if the technique is established, it could then be used on more complicated types of residues, such as those that have undergone long-term museum storage.

Discussion and Conclusions

The residues in these sherds were clearly complicated by their history of storage and conservation. Assuming that the ricinoleic acid derived from some historical use of castor oil, it's clear that past archaeological or conservation techniques, as well as storage, are a potential stumbling block to the interpretation of archaeological residues. The high microbial lipid contribution in many of these residues, as well as the unusual alkane series present in all of them, likewise probably derive from long storage in some form or another.

Given these complications, what meaningful archaeological information can we derive from the residue extracted from this collection of sherds? Perhaps the most important result is the presence of coniferous resin. Coniferous resin was present in five of the six decorated sherds, and in one of the three interpretable undecorated sherds. Four of the six decorated sherds contained a mixture of meat (indicated by cholesterol) and coniferous resin. The sample size in

this project was too small to determine if this is a meaningful pattern, but it is a potentially interesting finding. Future studies would allow a more accurate statistical analysis of whether coniferous resin is found more commonly in decorated wares than undecorated wares, and whether it is found most often in association with meat or animal-based contents in decorated pots.

Secondly, the source of the oil present in RL 369 and 370 could ideally be tracked either to vessel contents or to some form of contamination. Oil biomarkers could clearly derive from modern diesel fuel or gasoline; however, the biomarkers were not present on all sherds at the site, which might be expected in the case of widespread contamination of this type. Further, prehistoric peoples are known to have occasionally made use of bitumen and tar, both of which are found at locations such as the Louisiana coast and western Pennsylvania, and might have been traded into the region. If a soil sample was preserved from some of these features, this would help with this determination. Failing that, if all the residues from pottery found in a particular feature contained hopanoids, one could safely assume that the feature fill was contaminated. If only a few of the sherds from a feature contained these biomarkers, one might be able to argue for an archaeological source for the compounds.

In terms of the overall goals of the project, the results were mixed. It was possible to derive some useful information from absorbed residues extracted from legacy pottery that had been stored long-term and heavily labelled. The quality of this information was compromised by microbial action on the lipids, and the apparent use of castor oil as a curatorial or fungicidal tool, but there was still some interpretable information derived from the residues. The sample size in the project was too small to determine content difference between decorated and undecorated sherds, particularly since one of the undecorated sherds was so heavily contaminated by

plasticizers that it couldn't be interpreted. The attempt to locate the presence of *Phaseolus vulgaris* in the residues did not succeed, and will probably require a concentrated research project.

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