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Supplementary issue paper

Beyond the basics: A systematic approach for comprehensive analysis of organic materials in Asian lacquers

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Identification of organic materials in Asian lacquers presents many challenges due to their complex formulations and the limited solubility of the main component, which is catechol-rich sap from three species of *Anacardiaceae* trees that crosslinks to form a hard film. Pyrolysis-gas chromatography–mass spectrometry with thermally assisted hydrolysis and methylation using tetramethylammonium hydroxide (THM-Py-GC–MS) has been shown to produce a wide range of marker compounds useful for identifying the catechol components and lacquer additives such as drying oils, natural resins, proteins, starch, and colorants. However, interpreting the test results is quite challenging because of the sheer number of compounds produced by pyrolysis and the wide range of materials that have been used in traditional Asian lacquer formulations. An expert system developed by scientists at the Getty Conservation Institute and conservators at the J. Paul Getty Museum for a workshop entitled 'Recent Advances In Characterizing Asian Lacquers' (RAdICAL) utilizes software tools to overcome challenges in data analysis and marker compound interpretation, making it possible for even relative newcomers to Py-GC–MS to identify materials in lacquered objects systematically. Automated Mass spectral Deconvolution and Identification System (AMDIS), a freeware program developed by the National Institute of Standards and Technology (NIST), systematizes GC–MS data analysis by rapidly deconvoluting chromatograms, identifying individual peaks, and then searching the results against a user library of marker compounds, producing a simple report that lists the names, retention indices, and peak areas for all the compounds identified in the sample. The authors have produced a custom RAdICAL compound library, compiled from in-house studies of reference samples made from mixtures of raw or processed lacquer mixed with additives, and supplemented by published work from other researchers. The lists include numerous oxidation products of the alkyl- and alkenyl-substituted catechols, and alkyl- and alkenyl-substituted benzenes in the tree saps identified in studies of aged lacquer replicas, many of which have diagnostic purposes. A specialized Excel workbook developed for RAdICAL can import the AMDIS report, organize the marker compound results by class of artists' materials, and perform automatic calculations to display sorted information for each material in specialized diagnostic graphs. Expert knowledge relating raw materials to their associated marker compounds, obtained from in-house research, publications and personal communications has been embedded into the individual Excel worksheets. This aids users of the workbook in verifying the presence or absence of materials in their lacquer samples, based on the marker compound distributions. All of the final results are presented in a pre-formatted comprehensive analytical report. Extensive lists of analytical data for marker compounds from the major classes of organic additives used in Asian lacquer formulations provide researchers the information needed to identify these markers in unknown lacquer samples. As other researchers contribute marker compound information for further lacquer materials and the knowledge for interpreting them, the capabilities of the RAdICAL expert system will continue to expand.

Keywords: *Anacardiaceae*, AMDIS, Arlenic acid, Asian lacquer, Gestalt graph, Mazzeic acid, Pyrolysis-gas chromatography–mass spectrometry, RAdICAL, Thermally assisted hydrolysis and methylation, *Thitsi*, *Urushi*

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Introduction

Currently, pyrolysis-gas chromatography–mass spectrometry with thermally assisted hydrolysis and methylation (THM-Py-GC–MS) is the best analytical technique for characterizing the full range of organic materials present in Asian lacquers. It provides the most detailed compositional information about the catechol-rich saps from the three main species of trees in the *Anacardiaceae* family used in Asian lacquers (abbreviated to Anacards), as well as a wide range of lacquer additives. Although the analysis itself is easy to conduct, Py-GC–MS data evaluation can be quite challenging, especially for newcomers to the technique. The key difficulty in data evaluation is recognizing the connection between artists' materials present in the lacquer sample and the sets of associated marker compounds formed by pyrolysis of these materials in the presence of tetramethylammonium hydroxide (TMAH).

An expert system developed by scientists at the Getty Conservation Institute (GCI) and conservators at the J. Paul Getty Museum (JPGM) to characterize organic materials in Asian lacquers overcomes many challenges of Py-GC–MS data interpretation. The two components in the expert system are:

1. Processing of the GC–MS data with Automated Mass spectral Deconvolution and Identification System (AMDIS) to identify marker compounds systematically and calculate peak areas;
2. evaluation using Excel to interpret and present peak area results comprehensively.

This article describes the expert system, provides complete tables of marker compounds for identification of artists' materials used in Asian lacquer formulations, explains in some detail how software tools can be used to facilitate data analysis, and presents important graphs and tables used for data interpretation. It is intended to be a companion to the other articles in this supplement to *Studies in Conservation*. Marker compounds and information for identifying European lacquer materials are also in the system, but are beyond the scope of this article.

The expert system is one component of a GCI workshop entitled 'Recent Advances In Characterizing Asian Lacquers' (RAdICAL), in which sampling of lacquer layers by micro-excavation of flake samples and the use of histochemical stains for mapping organic materials in cross sections are the other two topics. RAdICAL provides a unique opportunity for scientists and conservators to work in close collaboration using analytical techniques to aid the understanding of lacquered objects in their institutions (Getty Conservation Institute, 2016). Venues for RAdICAL have included the GCI (2012), the Institute for Preservation of Cultural Heritage at Yale (2013) and the Centre for Research and Restoration of the Museums of France (2014).[1]

THM-Py-GC–MS analytical procedure

At the GCI, THM-Py-GC–MS analyses were performed on a Frontier PY-2020D microfurnace pyrolyzer interfaced to an Agilent 7890A GC/5975C inert MSD. Samples placed into 50 µl stainless steel Eco-cups were treated with 3 µl of 25% TMAH in methanol and pyrolyzed at 550°C.[2] A J&W DB-5MS-UI capillary column (30 m × 0.25 mm × 0.25 µm) attached to a Frontier Vent-Free adaptor was used (40 m effective column length), with the helium flow set to 1 ml per minute. The split injector was set to 320°C with a split ratio of 20:1. The oven of the GC was held at 40°C for two minutes then ramped to 320°C at 6°C per minute, followed by a nine-minute isothermal hold.

Use of this expert system will ensure that highly comparable results will be obtainable by analysts working with Py-GC–MS instrumentation from different manufacturers, thus maintaining reproducibility between laboratories.

Reference samples

Careful study of reference materials is, of course, critical to sophisticated interpretation of chromatograms. Only through such study can we characterize the full range of marker compounds produced by the wide variety of raw materials used in lacquer production. Over the course of the last decade, and in collaboration with generous collaborators, we have built a reference collection of various lacquer types including urushiol-, laccol- and thitsiol-based lacquers from China, Japan, Korea, Taiwan, Thailand, Cambodia, and Burma. In Py-GC–MS, marker compounds for materials may vary with the sample matrix, so we have prepared standards made with a variety of additives mixed into Anacard lacquers, including a range of vegetable oils, natural resins, essential oils, protein-based materials, starchy materials, and natural dyes. Most of the standards were coated onto glass microscope slides in batches of four to five, and cured at approximately 80% relative humidity for several days. In some instances, standards were prepared on other substrates, such as wood or traditional ground materials. One

[1]The appendices provide information, encourage readers to attend a RAdICAL workshop where they will receive training and copies of the Excel workbook and AMDIS library. Eventually, there are plans to write a book on RAdICAL that will contain all of this information.

[2]A limited study was carried out on the effects of pyrolysis temperature on the peak area results for THM-Py-GC–MS of Japanese *roiro* lacquer in order to ascertain the optimum pyrolysis temperature of Anacards in the presence of TMAH. The yields for selected marker compounds were measured for identical powder samples that were pyrolyzed at 400, 450, 500, and 550°C. Overall, the yield of Anacard markers relative to the oil content was reduced nearly by half, largely due to an almost complete loss of hydrocarbons, whereas no significant improvement was observed in the yield of acid catechols (an important set of marker compounds) compared to saturated catechols. Thus, there seems to be no advantage in using a pyrolysis temperature below 550°C for the identification of Anacards in lacquer samples.

example of each standard was then exposed to light in an east-facing window at the Getty Museum for up to two years; the remaining examples are kept in dark storage and are available for future study.

Expert system for data processing

AMDIS is a freeware program developed by the National Institute of Standards and Technology (NIST) that automates the process of identifying compounds in GC–MS sample data files by means of mass spectral library searching. Detailed explanations of how AMDIS processes data are available elsewhere (Stein, 1999), but a brief description will help explain its most powerful feature, deconvolution. When AMDIS is run on a data file for a sample, it scans the entire chromatogram and groups together (or extracts) into a single mass spectrum all those ions that rise and fall at the same retention time and which exhibit the same peak shape. This is obviously the case for ions originating from an eluted compound (which AMDIS terms a component). Conversely, ions exhibiting constant intensity over the same time period are excluded from the extracted mass spectrum for the component. Sources for such ions include siloxane compounds present in column bleed (m/z 207, 281, and 355) and air in the helium carrier gas (m/z 18, 28, 32, and 40). Consideration of peak shape in the deconvolution process leads to vast improvements in the quality and purity of the extracted mass spectra for partially co-eluting components when compared with manual background subtraction results. After compiling the entire set of component mass spectra in the GC–MS results, automated searching of each extracted mass spectrum against a user-generated library identifies compounds (termed targets) in the chromatogram and tabulates the search results. Users can scroll through the target compound list and review the accuracy of the library match results. The AMDIS report lists the library match factor and peak area for each target compound identified in the sample, plus a number of other evaluated results, sorted by retention time or retention index (RI) (if applicable). RI is a useful sorting parameter for most purposes because the target compound list relates directly to the peak order in the chromatogram. Typically, it takes less than a minute for AMDIS to process a data file, which is an enormous practical benefit of using the program for data analysis.

AMDIS version 2.70 bundled with the NIST MS library version 2.0 g was used in this study. Although AMDIS is also available for free download from the NIST website (NIST, 2016), one distinct advantage of the bundled version is the capability of performing NIST searches within AMDIS. For most lacquer samples, default program parameters for resolution, sensitivity and component width are adequate. RI may be used as a compound identification parameter in AMDIS analysis, which greatly improves the match accuracy for homologous series of target compounds, such as hydrocarbons, because the members tend to have similar mass spectra. Accordingly, RI-type analysis is recommended for lacquers. This feature requires that users first analyze a mixed hydrocarbon standard using the same GC–MS method in order to calibrate RI for the method. The AMDIS library developed in the Getty research, compiled from test results from numerous lacquer standards mixed with additives, contains mass spectra for more than 700 target compounds. Regarding library matching, users must specify a minimum match factor above which AMDIS will report spectral matches to library compounds. A value of 80% works well for RI-type analyses of lacquer, but for simple (non-RI) analyses of other sample types the value should be set higher.

As mentioned in the introduction, compound identification is only the first step in identifying materials with Py-GC–MS. A list of compounds present in the test result for a sample is not necessarily helpful to analysts who lack an in-depth understanding of the materials that form them, especially in complex materials such as lacquer. Typically, an expert level of understanding in lacquer analysis comes only after years of effort and practical Py-GC–MS experience, so that newcomers to the technique face a formidable knowledge barrier before attaining competence. Relevant publications by experts are useful for narrowing the knowledge gap, but comprehensive information is difficult to obtain because the lacquer literature is highly diverse, spread over many different publications and written in many languages. Marker compounds identified in publications may be difficult to locate in users' chromatograms because retention indices are seldom published.

An innovative approach to Py-GC–MS data interpretation developed in our research utilizes expert knowledge integrated into a Microsoft Excel 2013 workbook to process target compound results from AMDIS reports for the purpose of identifying artists' materials in lacquers. The workbook accepts the AMDIS report, sorts the target compound information, reorganizes it by class of material, and presents interpreted results with graphs and tables. Expert knowledge culled from scientific publications and from interviews with researchers experienced in lacquer materials provide marker compound lists for various lacquer materials and tools for interpreting the results.

One difficulty encountered in developing the workbook was identifying a field in the AMDIS report that could serve as a sorting parameter to make the target compound results fit into pre-formatted Excel worksheet templates. Each AMDIS library entry has

several fields into which users enter relevant data for the target compound: Chemical Abstracts Service number (CAS #), compound name, molecular formula, RI, notes, and an ion for calculating signal-to-noise ratio (termed 'Ref. Ion'). Although a CAS number is itself an unambiguous descriptor for a chemical compound, it does not easily lend itself to sorting. Because signal-to-noise data were deemed unessential for our study, we decided to use this field (Ref. Ion) to assign a library entry number for each target compound in the RAdICAL library. The library entry number is simply a convenient way to allow our Excel workbook to search the list of target compounds reported by AMDIS quickly and match them with marker compounds in the RAdICAL library.

The RAdICAL Excel workbook contains multiple worksheets that handle specific tasks in the semi-automated data evaluation process. The AMDIS sample report is pasted into one worksheet, and the CSV file of the corresponding GC–MS chromatogram is pasted into a second. The target compound results in the AMDIS report are automatically processed by the lookup formula, extracted and reorganized in a separate 'Material Verification' worksheet in which all of the identified compounds are grouped according to their likely material of origin. In this worksheet, users decide whether or not that material is present in the sample based on the peak area, RI and library match factor data for each of the compounds identified in a given material.

To make this task easier, expert knowledge is provided the 'Interpretation Tools' worksheet, giving users the information they need to verify if a representative number of marker compounds were detected. For example, 21 marker compounds found to be associated with pine resin are listed together in a single column in the 'Material Verification' worksheet. Expert information indicated that of these 21 compounds, methyl dehydroabietate (DHA), methyl 7-methoxy-tetradehydroabietate and methyl 7,15-dimethoxytetradehydroabietate must be present in a sample for users to conclude that aged pine resin is indeed present. If so, then users would choose to 'verify' pine resin as an identified artists' material present in the sample, and the peak area results for the compound group will then be added together into the tables and charts that follow. If, on the other hand, these compounds are not present, the user chooses not to verify and the peak area results for any markers in the pine resin category will not be tallied.

After the user verifies the presence or absence of each of the classes of materials in the lacquer sample that are represented by the AMDIS library, the total peak area is calculated for each of the identified classes, and a pie chart presents the peak area percentage of each class of material that was detected in the sample. Peak areas are related to concentration by detector response factors, which can be obtained only through the use of calibration standards for every compound identified. Of course, this is an impossible task when dealing with complex, multi-component pyrograms from natural products such as Asian lacquer. Thus, the peak area pie charts are currently the best way of presenting overall composition of the sample. They are most useful for showing relative amounts of material classes in comparisons of test results for multiple samples taken from a single object. For example, an upper lacquer layer on an object might contain more Anacard sap and less oil than a lower lacquer layer from the sample object, or an upper lacquer layer might contain more *urushi* and less *thitsi* than a lower lacquer layer. There is greater risk involved in comparing sample results between objects because of gross differences in the formulations.

The RAdICAL workbook developed at the Getty also contains worksheets that automatically produce pre-formatted report templates. This means that headers, tables, and graphs of results appear in the report as soon as the AMDIS report and CSV file are pasted in and the materials present in the sample are verified. The report templates include blank fields where the analyst's comments and interpretations may be added. Many additional formulas used in the Excel workbook are beyond the scope of this article, especially in the area of graphing and automation.

In the subsequent sections of this article, examples of the processed data are presented for each class of artists' material represented by marker compounds in the RAdICAL workbook. Graphs are important interpretive tools for identifying oils and Anacards, whereas for other materials, data tables are currently the most relevant format. Tables of selected marker compounds for the main material classes are presented in the Appendices. The term 'unverified' is used in the appendices to denote marker compounds that are reliably present in test results for a particular material but for which the molecular structure is unknown. The status of unverified markers may change in the future, depending on the outcomes of further research on the materials, and chemical structures may eventually be assigned to them. Each section contains specific expert knowledge needed to interpret the marker compound results for a sample and verify the presence of the materials.

Drying oils

The vast majority of oils and lipids are composed of glycerol esters of fatty acids. Appendix 1 lists four classes of marker compounds for oils and lipids: methyl esters of monocarboxylic and dicarboxylic

fatty acids, methyl ether derivatives of glycerol and methyl alkylphenyl alkanoates (APAs), which are formed from highly unsaturated linolenic and eleostearic acids by bodying linseed, perilla or tung oils at elevated temperatures (Evershed, 2007). When interpreting sample results in the RAdICAL workbook for markers associated with oils and other lipids, the presence of dicarboxylic and monocarboxylic fatty acids together is indicative of drying oils; for samples with significant oil content, glycerol may also be detected.

Generally, fatty acid peak area ratios developed originally for identifying oils in Western easel paintings (Mills, 1966) have been found to be applicable to Asian lacquer materials. For instance, because drying oils produce high levels of azelaic acid during the aging process, the A/P ratio (dimethyl azelate to methyl palmitate) broadly differentiates drying oils from other materials that contain fatty acids (semi- and non-drying oils or fats from egg, animal glue, blood, and raw Anacard lacquers). We have discovered that the ratio of APAs to monocarboxylic fatty acids reveals additional information about the heat-bodying conditions because the APA content increases with temperature. Another important ratio for lacquers is the total of fatty acids to glycerol. Raw Anacard lacquers contain measurable amounts of native fatty acids but little or no detectable glycerol, so fatty acid to glycerol ratios are reduced in samples containing raw lacquer mixed with triglyceride oils.

Table 1 Compositions of materials in Asian lacquers that contain fatty acids

Product	*P/S*	Characteristic ratios and compounds
Tung oil	1–1.2	High A/P
Heat-bodied tung oil	1–1.2	High A/P; presence of APAs
Linseed	1.2–2.5	High A/P
Tallow tree oil[a]	~3	High A/P
Perilla oil	2–4	High A/P
Sesame oil	1.5–2	Low A/P
Rapa oil	2–3	Low A/P; presence of C_{22-1}, C_{22}, C_{20-1}, C_{20}, C_{24-1}, C_{24} monocarboxylic fatty acids; C_{11} dicarboxylic fatty
Blood	~2	Low A/P; presence of blood markers and cholesterol
Urushi	~2	Low A/P; presence of C_{15} Anacard markers
Animal glue	~4	Low A/P; presence of glue markers
Laccol	~7	Low A/P; presence of C_{17} Anacard markers
Thitsi	~7	Low A/P; presence of C_{15} Anacard markers and alkylphenyl catechols

[a]Ratio calculated from data by Jamieson and McKinney (1938) and Chen *et al.* (1987).

Mills discovered the dimethyl suberate to dimethyl azelate ratio (di-C_8/di-C_9) is elevated for bodied drying oils and low for cold-pressed drying oils in easel paintings (Mills, 1966), but comparable data for Anacard lacquers has yet to be compiled.

Table 1 lists information useful for interpreting test results for materials in Asian lacquer formulations that contain fatty acids. Four oils that produce high A/P ratios in lacquers are classified as drying oils. The ratio of P/S (methyl palmitate to methyl stearate) is used for oil identification. The unusually low P/S ratio of one makes tung oil from *Vernicia fordii* easy to differentiate from all other oils in lacquers. Partial overlap in P/S ranges makes it impossible to differentiate unambiguously between linseed oil, perilla oil, and tallow tree oil in samples. Chinese tallow tree oil is a drying oil also referred to as stillingia oil (from *Sapium Sebiferum*). Its excellent drying properties are attributed to high concentrations of 2,4-decadienoic acid and 8-hydroxy-5,6-octadienoic acid, which are unusual fatty acids that are not present in other oils (Chen *et al.*, 1987). Because of difficulties in obtaining pure Chinese tallow tree oil in our research, we were unable to confirm the fatty acid compositions given in the references. Given its unusual fatty acid composition, it may be possible eventually to identify specific pyrolysate markers in lacquers made with this oil. Two non-drying oils are also associated with lacquer making. Rapa oil, also known as rapeseed oil, is used for cleaning lacquer brushes (Webb, 2000). Because it contains dodecenoic acid, dried films containing rapa oil have unusually high levels of undecanedioic acid relative to the other dicarboxylic fatty acids (van Keulen, 2014a). Sesame oil, which has a relatively low P/S ratio, is discussed in documents from the Northern Song Dynasty (Heginbotham *et al.*, 2016). Obviously, more work needs to be done on testing botanically verified specimens of drying oils from Asia in order to define the statistical ranges of P/S more accurately and to look for additional marker compounds that might help differentiate oils.

It should be noted that ester-type waxes such as beeswax and carnauba wax, used in furniture polishes, contain significant amounts of fatty acids that may skew fatty acid ratios, making drying oil identification difficult at best. Additional markers include long-chain fatty alcohols (and their methyl ethers) and hydrocarbons (some of which will appear in graphs presented later in this article).

AMDIS peak area results for fatty acid methyl esters can be arranged by carbon number and plotted in a bar graph format, as shown in Figs. 1 and 2 for samples of red and black lacquer from a late-eighteenth-century Chinese carved lacquer screen in the collections of the Weltmuseum Wien in Vienna (Pitthard *et al.*, 2016).

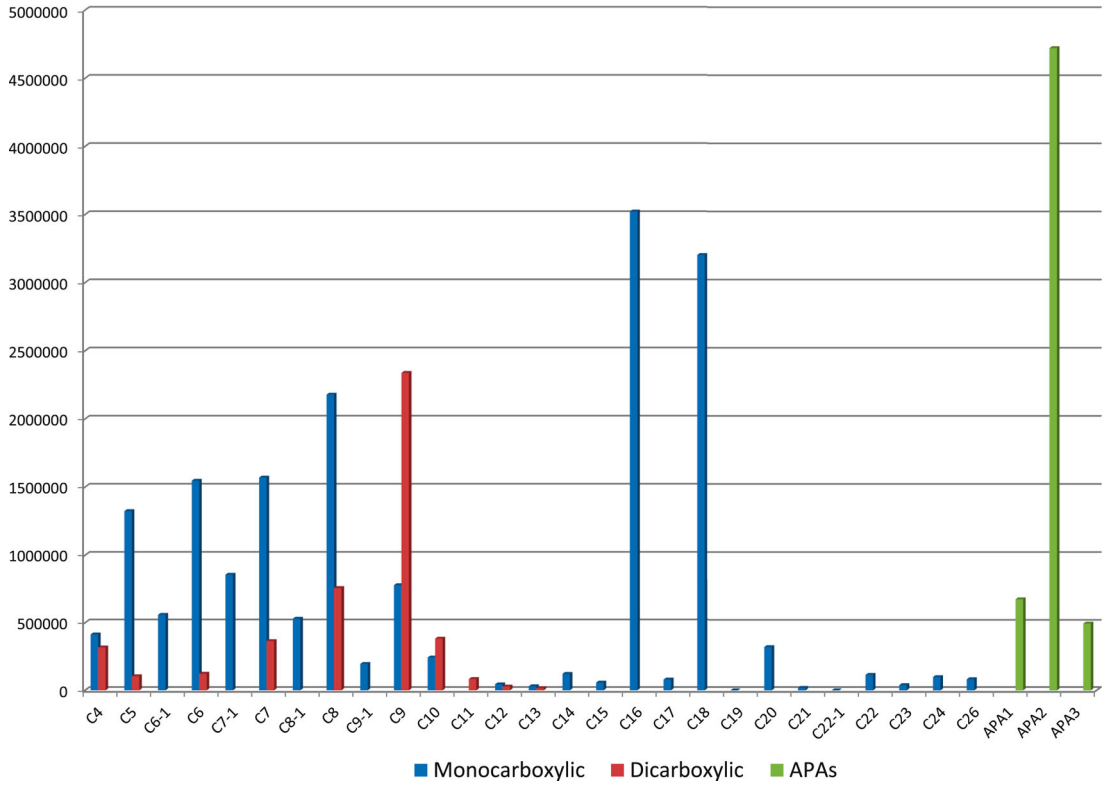

Figure 1 Fatty acid bar graph for red lacquer from an eighteenth-century Chinese lacquer screen, Weltmuseum Wien, Vienna.

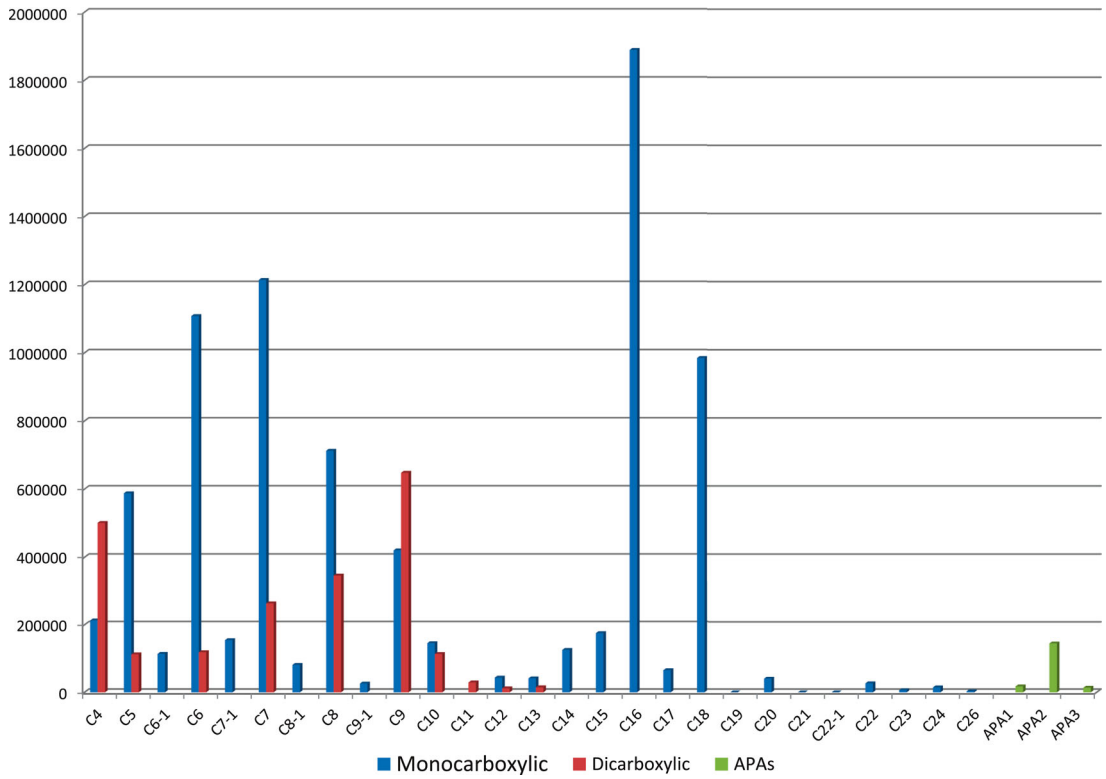

Figure 2 Fatty acid bar graph for black lacquer from an eighteenth-century Chinese lacquer screen, Weltmuseum Wien, Vienna.

The bar graph format shows the full range of fatty acid marker compounds and obvious differences in their prevalence between the samples; Table 2 lists the relevant ratios for these samples.

Anacards

Saps from lacquer-producing trees in the *Anacardiaceae* family are complex, water-in-oil emulsions consisting of substituted catechols, substituted

Table 2 Characteristic ratios for lacquer samples from Figs. 1 and 2

Ratios for identification	Red	Black
A/P	0.66	0.34
P/S	1.10	1.92
di-C8/di-C9	0.32	0.53
APAs/monocarboxylic	0.32	0.02
Total fatty acids/glycerol	91	61

phenols, carbohydrates, glycoproteins, and laccase enzyme (Kumanotani, 1995). Using Py-GC–MS, Miyakoshi and colleagues elucidated all the major pyrolytic pathways that lead to the formation of homologous series of marker compounds for Anacard lacquers: catechols, phenyl catechols, phenols, phenyl phenols, alkyl benzenes, and hydrocarbons. For each homologous series there is a maximum side chain length and a predominant member. For example, chromatograms of *urushi* (from *Toxicodendron vernicifluum*) show series of catechols, phenols, and hydrocarbons in which each has a maximum side chain length of 15 carbons and the most abundant member has seven carbons. For laccol (from *Toxicodendron succedanea*), the same series are present, but the maximum chain length is 17 carbon atoms and the most abundant member has nine carbons. The characteristic features of *thitsi* (from *Gluta usitata* or *Gluta laccifera*) are significant amounts of alkyl benzenes, with smaller amounts of phenyl catechols and phenyl phenols (Niimura *et al.*, 1999; Niimura & Miyakoshi, 2000; Frade *et al.*, 2009; Honda *et al.*, 2010, 2015). These persistent Anacard markers have been detected in ancient Jōmon period lacquerware (Yuasa *et al.*, 2015), Han dynasty burial objects (Khanjian *et al.*, 2011) and ancient Chinese objects from Sichuan (Tamburini *et al.*, 2015). In THM-Py-GC–MS, the homologous series of anisoles and dimethoxybenzenes, which form from phenols and catechols respectively, proved to be equally as characteristic of the three Anacard species as were their underivatized precursors (Heginbotham & Schilling 2011; Petisca *et al.*, 2011; Le Hô *et al.*, 2012).

One significant advantage afforded by TMAH in pyrolysis is that it permits the detection of a number of homologous series formed by aging and oxidation of all three species of Anacard lacquers. For example, Chiavari and Mazzeo (1999) reported an unidentified compound that they detected routinely in Chinese lacquered artifacts; its mass spectrum appears in Fig. 3. This compound, identified in our research as methyl-8-(2,3-dimethoxyphenyl)octanoate, is the $n = 8$ member of a series we have termed acid catechols. They are products of oxidation reactions along the unsaturated side chains of substituted catechols, which are analogous in structure to dicarboxylic fatty acids that form by oxidation of unsaturated fatty acids in drying oils. Figure 3 also provides the general structure of methylated acid catechols. Mazzeic acid ($n = 8$) is the dominant member of the

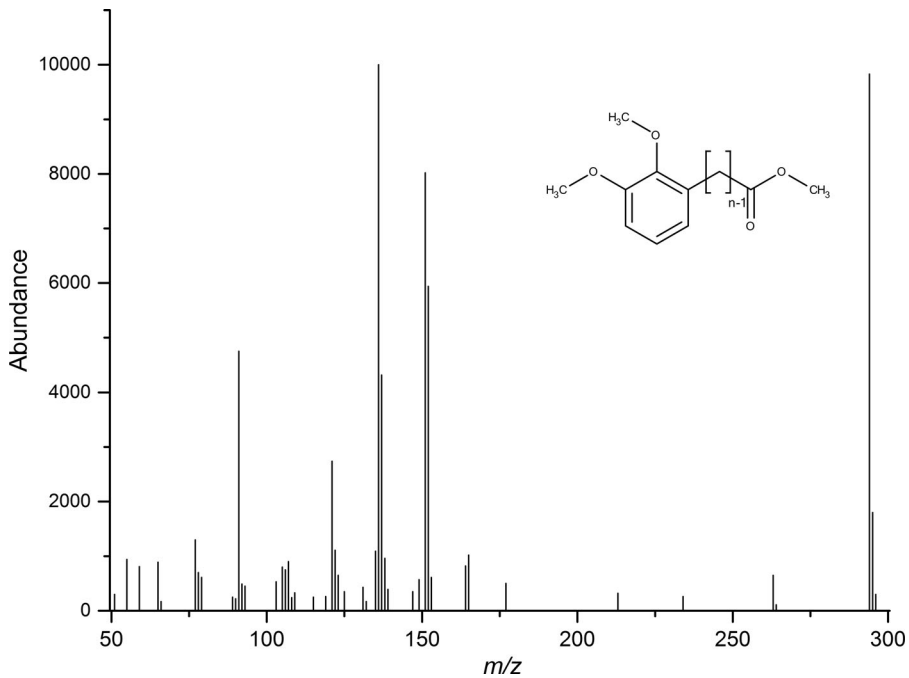

Figure 3 Structure for methyl-*n*-(2,3-dimethoxyphenyl)alkanoate and the mass spectrum for the compound with $n = 8$.

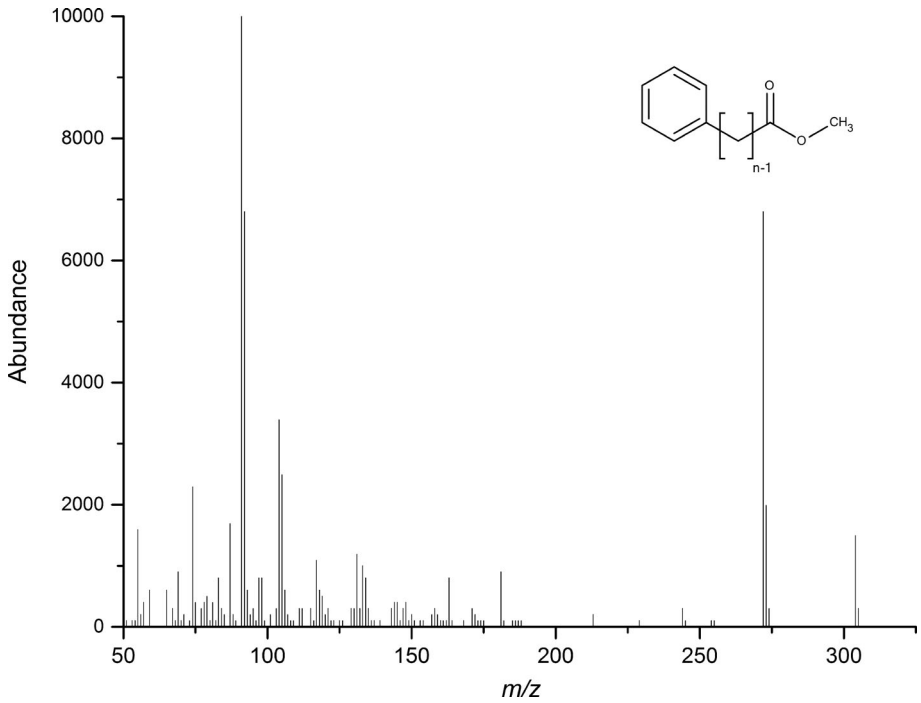

Figure 4 Structure for methyl *n*-phenyl alkanoate and the mass spectrum for the compound with $n = 13$.

acid catechol series formed in aged *urushi* and aged *thitsi*, whereas arlenic acid ($n = 10$) is most abundant in aged laccol lacquers.[3]

A related series of compounds forms in aged *thitsi* lacquers from oxidation of the unsaturated side chains in alkenyl- benzenes (Fig. 4). In addition, two homologous series of ketones form in aged *thitsi* by oxidation of the side chains at the alpha-position to the benzene rings (Figs. 5 and 6).

Appendix 2 lists the important markers for Anacard lacquers: dimethoxybenzenes, acid catechols, anisoles, alkyl- and alkenyl-benzenes and their oxidation products, and cashew nut shell liquid (CNSL). Hydrocarbons were omitted from the list because their mass spectra are present in the NIST spectral database, and considering that they define the RI scale (RI = 100 × carbon number; e.g., RI for decane is 1000, dodecane is 1200, etc.), their RI data are already known.

In the literature, extracted ion chromatograms are the most common method of presenting multiple series of Py-GC–MS marker compound classes present in Anacards, yet peaks for unrelated compounds interrupt the regular patterns exhibited by the homologous series and cause confusion. A new option developed in the Getty research utilizes the stacked area graph format in Excel to present peak area data for homologous series of marker compounds. The number of carbon atoms and double bonds is designated on the *x*-axis (i.e., C_{17} is heptadecane, and C_{17-1} is heptadecene), and peak area is plotted on the *y*-axis.[4] Each compound series is represented by one color in the stacked area graph. We use the term 'gestalt graph' to describe this format because the sum of the many compounds represented creates a recognizable visual form that can be used to distinguish the major Anacard types quickly and intuitively.[5]

Figure 7 shows the gestalt graphs for reference samples of aged *urushi* and aged laccol. In urushi, the homologous series for catechols and hydrocarbons each have a maximum side chain length of C_{15} with C_7 as the most abundant member of both series, and C_8 (mazzeic acid) is the most abundant acid catechol. In contrast, in aged laccol the chain lengths are C_{17}, C_9, and C_{10} (arlenic acid) respectively. Both Anacard lacquers give a series of alkyl benzenes from C_3 to C_7,

[3]The authors named the acid catechols after important people in the history of Asian lacquer analysis: Kisaburo Miyama from Imperial University, Tokyo; Ju Kumanotani from the University of Tokyo; Rocco Mazzeo from the University of Bologna; Tetsuo Miyakoshi from Meiji University; Giuseppe Chiavari from the University of Bologna; and Tim Whalen from the GCI. *Thitsi* oxidation products were named after colleagues who have supported our research: Dr Chu Watanabe; Henk van Keulen; Chris Petersen; and Ulrike Körber. Methyl 13-oxo-13-phenyltridecanoate (tamburinic acid), a *thitsi* oxidation product, is named after Diego Tamburini, who attended the RAdICAL workshop in Paris and made contributions to our study of tannins. Methyl 2-methoxy-6-(8-methoxy-8-oxooctyl)benzoate, the characteristic marker for cashew nut shell liquid, is named after Bettina Ebert, a conservator who studied Vietnamese lacquer paintings in the Witness Collection.

[4]Any problems of incomplete derivatization of catechols by the TMAH reagent in sample preparation may be overcome in data processing by totaling the peak areas for each type of derivative and the underivatized compound into a single value for each member of a homologous series.

[5]The term 'gestalt' means something that is made of many parts and yet is somehow more than or different from the combination of its parts. To us, this term seemed apt because users can clearly and easily see the important information for homologous series of lacquer marker compounds when they are represented in Excel stacked area graph format, whereas other graph formats lack such clarity.

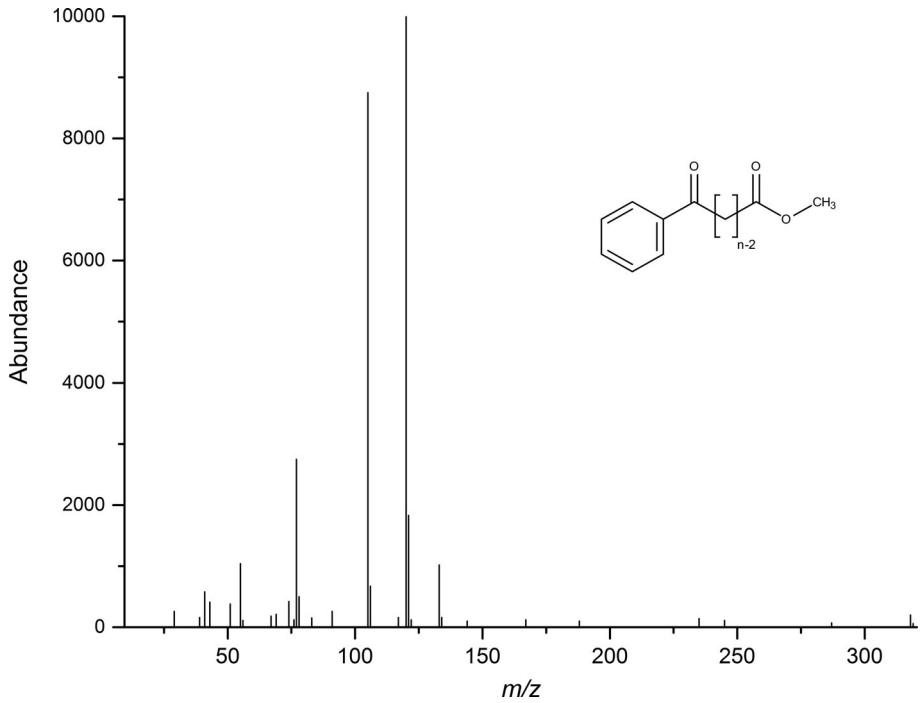

Figure 5 Structure for methyl *n*-oxo-n-phenyl alkanoate and the mass spectrum for the compound with $n = 13$.

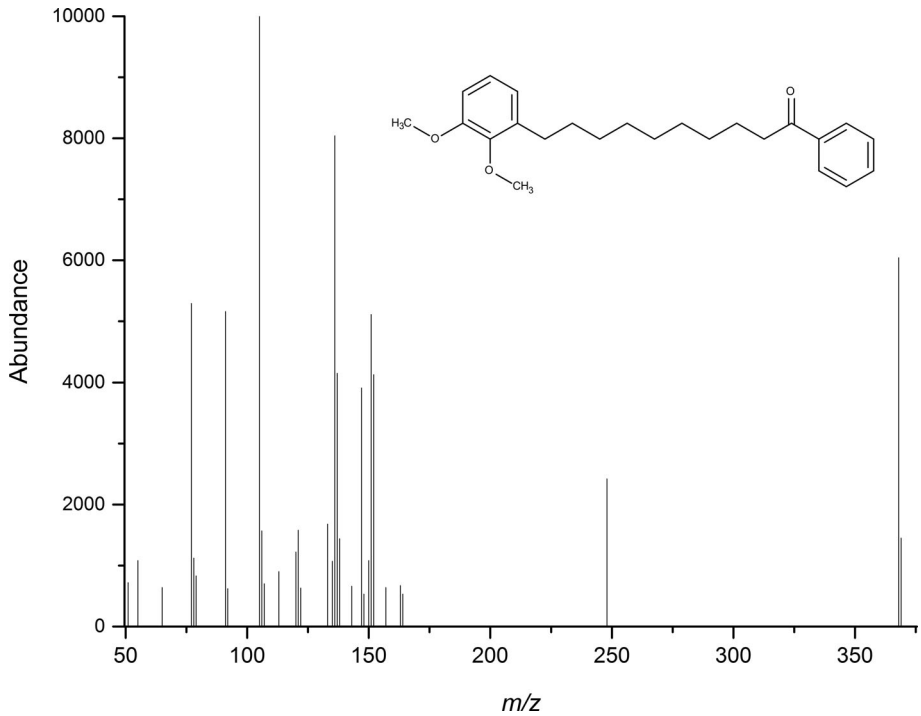

Figure 6 Structure and mass spectrum for 10-(2,3-dimethoxyphenyl)-1-phenyldecan-1-one.

which are thought to form by dehydration of alkyl phenols (Le Hô *et al.*, 2012).

Figure 8 shows the gestalt graphs for *thitsi* lacquer and CNSL lacquer. Phenyl catechols and phenyl phenols are unique markers in *thitsi* lacquer and alkyl benzenes dominate the gestalt graph. CNSL from *Anacardium occidentale* is a modern, less expensive substitute for traditional Asian lacquer (Niimura & Miyakoshi, 2003). The gestalt graph for CNSL lacquer is quite unique with its abundance of phenols compared to the other markers. CNSL phenols are *meta*-substituted, so they elute consistently after the *ortho*-substituted phenols present in the other Anacard lacquers. Additionally, this lacquer has three marker compounds not present in other Anacards and which do not appear in the gestalt graphs. CNSL has been

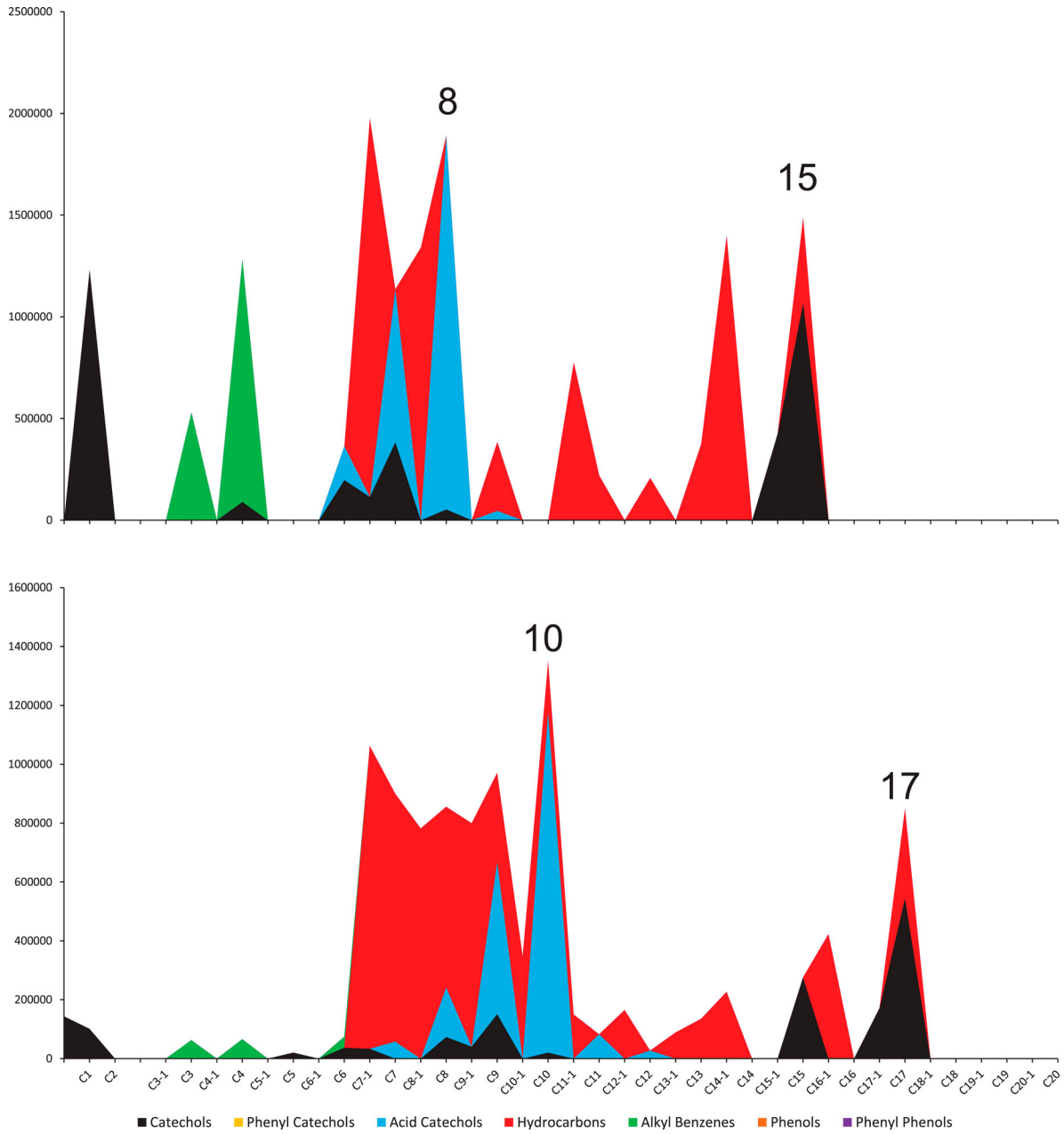

Figure 7 **Gestalt graphs for: (top) aged *urushi*; and (bottom) aged laccol.**

discussed in relation to its usage in Vietnamese lacquer paintings in some detail (Ebert & Schilling, 2016).

Gestalt graphs also make it possible to recognize mixtures of Anacards in lacquer samples. Figures 9 and 10 show results for a French commode (*c*.1750) decorated with Japanese lacquer panels that were made *c*.1675. It is clear that the relative proportions of phenyl catechols to acid catechols differ between the two samples. The foundation layer is enriched in phenyl catechols that originate from *thitsi*, whereas the finish layer has a greater proportion of acid catechols, far more than the amount that forms in aged thitsi. Since mazzeic acid is the dominant species, the acid catechols must come from aged *urushi*; if instead arlenic acid predominated, laccol would have been identified as the source (Heginbotham & Schilling, 2011).

In reviewing marker compound results for samples, the presence of Anacard lacquer is confirmed when catechols, hydrocarbons and alkyl benzenes are all present. Gestalt graphs are consulted to identify the specific type of Anacard lacquer and acid catechols are considered the most distinctive markers.

Anacard oxidation products

Although Asian lacquer is an exceptionally durable and stable material, as is evident from its preservation on ancient burial objects, its sensitivity to light is well-documented (Keneghan, 2011). Exposure to light causes profound changes to its surface appearance and sensitivity to solvents (McSharry *et al*., 2011). To better understand this phenomenon, THM-Py-GC–MS was performed on residues extracted by

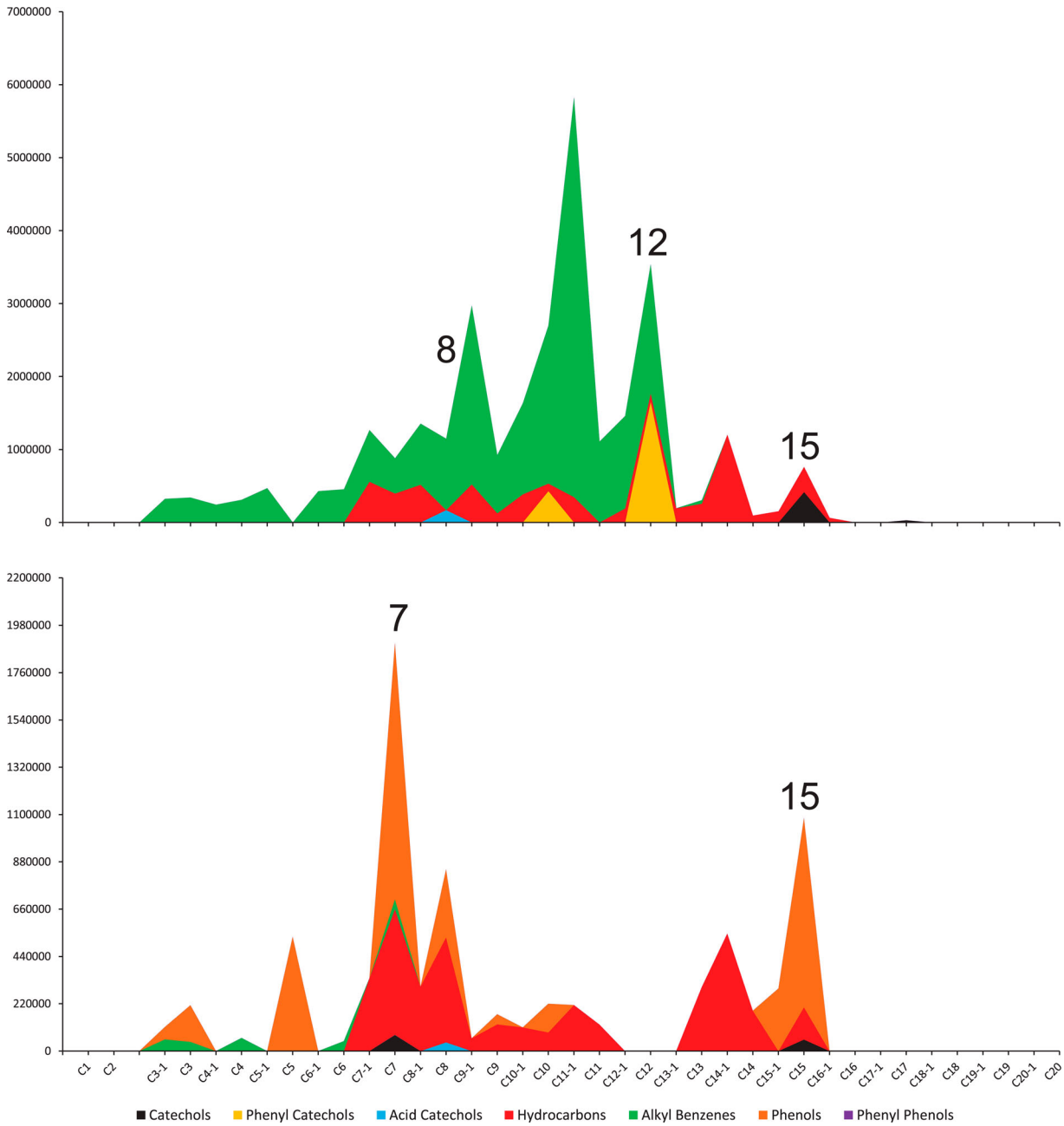

Figure 8 Gestalt graphs for: (top) aged *thitsi*; and (bottom) aged cashew nut shell liquid.

droplets of water applied to the surfaces of Chinese and Japanese export lacquer replica panels that had been exposed to light in an Atlas weatherometer chamber (Webb *et al.*, 2016). Surprisingly, the water extracts were composed almost entirely of isomers of mono-, di-, tri-, and tetra-carboxylated benzene (see Appendix 2), and the lacquer composition affected the final product distribution. These compounds may be largely responsible for the reduction in surface pH as lacquer ages (Webb, 2000). Although the exact mechanism for the formation of these compounds in lacquer is unknown, research on the photochemistry of secondary organic aerosols has shown highly oxidized species form from catechols and substituted phenolics, and that the product distributions are affected by the presence of environmental nitrogen oxides and ozone (Schilling, 2015). Given the relative stability of the catechol ring structures and the absence of phenolic groups in the products, it may be that the side chains are responsible for the formation of carboxylated benzenes in the water extracts of the lacquers (Webb, 2011). Research is needed to investigate the reaction mechanisms for the formation of these compounds and how the residual aged lacquer matrix responds upon their removal during exposure to solvents, which is especially relevant to assessments of conservation treatments involving water or other solvents.

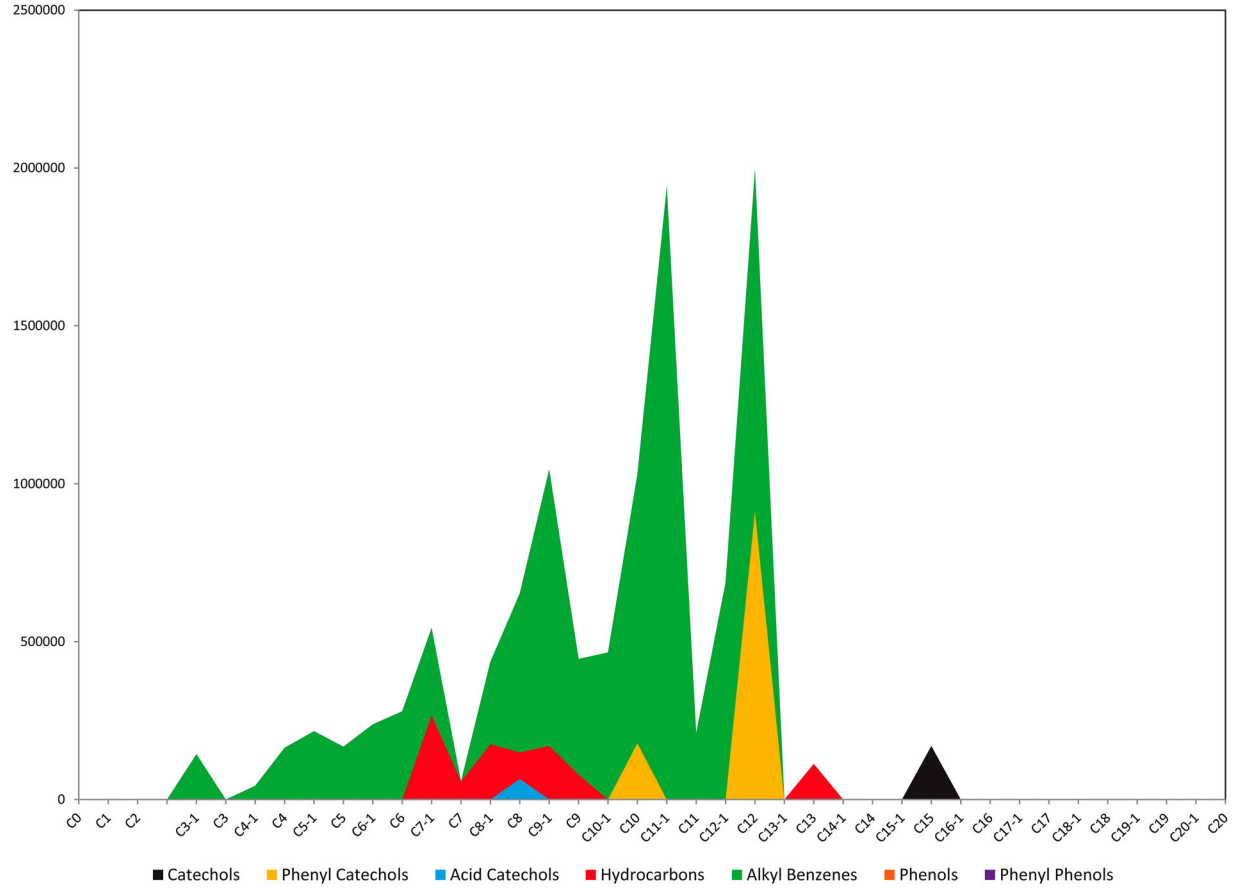

Figure 9 Gestalt graph for the ground layer from the Joseph Commode (55.DA.2) in the collection of the JPGM.

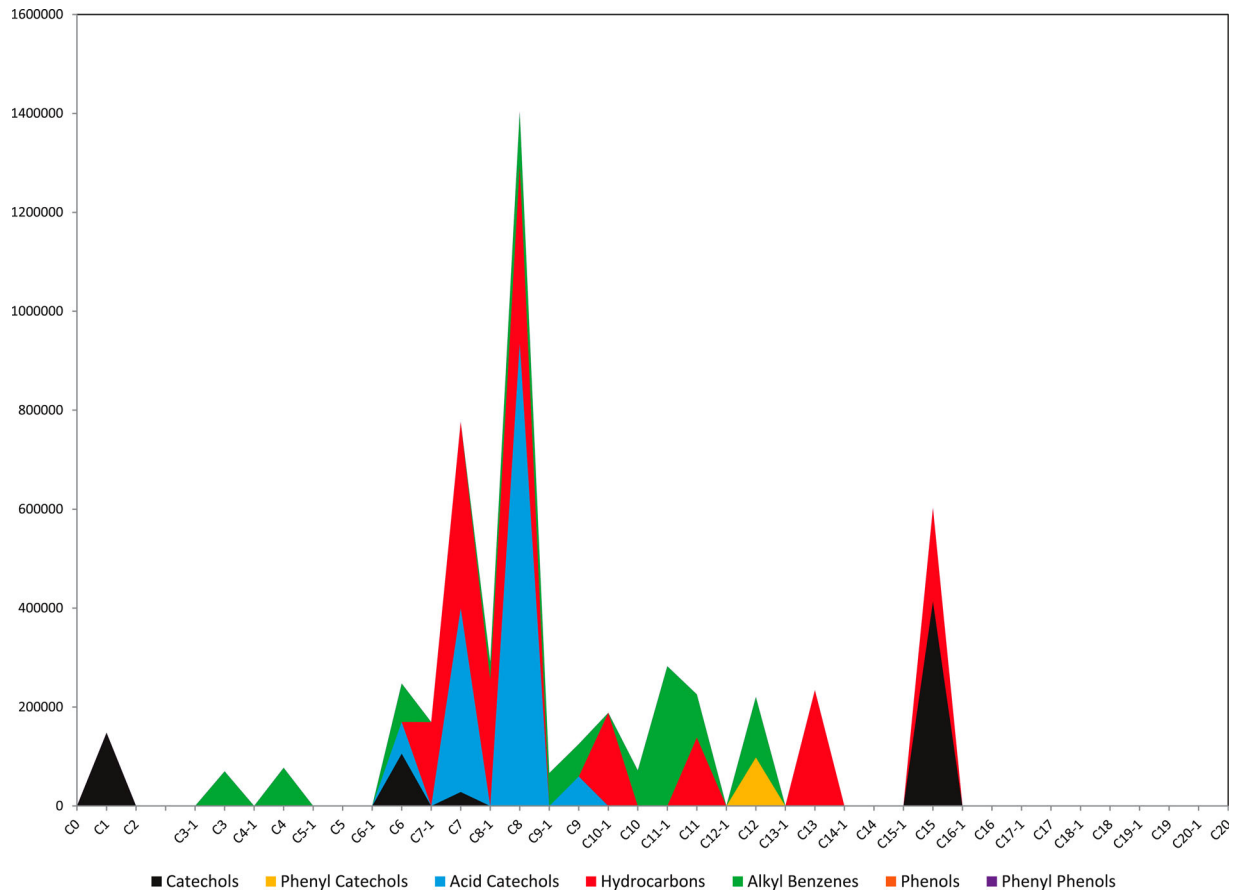

Figure 10 Gestalt graph for finish layer on the Joseph Commode (55.DA.2) in the collection of the JPGM.

Proteins

Several proteinaceous materials have been used in Anacard lacquer formulations. Chinese lacquer recipes dating back to the Northern Song dynasty mention the use of whole egg (Chang & Schilling, 2016). Lighter colored grounds on Asian lacquered objects from many countries may contain animal glue (Webb, 2000), whereas traditionally blood has been used in dark ground layers from Chinese and Ryukyuan objects (Körber *et al.*, 2016). At a lacquer craft workshop held at Buffalo State College (Buffalo State College, 2013), Japanese lacquer masters demonstrated the use of tofu as a lightweight filler material that increases the volume of underlying lacquer layers. Markers from all of these materials, sorted by material type in Appendix 3, form by pyrolysis of polypeptides in the presence of TMAH; it should be noted that some are formed by more than one type of protein.

Protein identification in the RAdICAL workbook involves reviewing tables of marker compounds and considering their total peak area in the sample. Other marker compounds that may help confirm the identification are listed in Table 1. Additionally, small amounts of dimethyl sulfide and dimethyl disulfide are formed by the sulfur-containing amino acids in animal glue. Confidence in the identification results is greater when multiple marker compounds for a particular material are present and their total peak area exceeds a few tenths of a percent. Experience has demonstrated that identification of blood and glue in a sample is relatively straightforward because multiple markers are formed by these materials. To date, we have yet to identify tofu or egg in samples from lacquered objects, which may indicate that a broader range of markers is needed. Finally, proteins would not be confirmed in samples if only one or two marker compounds are present at low concentration, because these may originate from naturally occurring enzymes and glycoproteins in the Anacard saps.

Carbohydrates

Carbohydrate marker compounds are listed in Appendix 4. The Chinese lacquer literature mentions rice starch as a common additive in grounds. The history and uses of carbohydrate materials in Japanese lacquer grounds has been explored elsewhere (Heginbotham & Schilling, 2011). Furfural, a generic marker for carbohydrates that appears consistently in pyrograms for starch, is accompanied by a few unverified markers. One such compound was identified in many samples of Chinese and Japanese ground layers supplied to the authors by Nanke Schellmann, who used histochemical stains to study lacquer grounds on objects in the Victoria & Albert Museum (Schellmann, 2012). Ground layers that stained positively for starch with Lugol's reagent showed an abundant compound in the THM-Py-GC–MS results at 1395 RI units that had a base peak of m/z 101. The molecular structure of this compound is presently unknown, but the closest NIST library match is 2,3,4-trimethyllevoglucosan. This compound was named Schellmannose to reflect its discovery. A marker named 'glucoside' is another generic marker for carbohydrates.

Another category of carbohydrate markers originates from gums and glycoproteins naturally present in Anacard saps. The carbohydrate content of laccol sap is nearly three times higher than that of *urushi* sap (Kumanotani, 1995). Undetectable without the use of TMAH, these markers have not been reported previously in Py-GC–MS studies of lacquered objects. Two main clusters of carbohydrate peaks appear in THM-Py-GC–MS chromatograms for laccol lacquers. One set that elutes at around 1500 RI units with a base peak of m/z 129 is likely to relate to pyrolysates that originate from monosaccharides and uronic acids, whereas the second set eluting near 2500 RI units with significant ions at m/z 101 and m/z 88 are presumably disaccharide pyrolysates. These compounds are abundant in laccol-containing lacquered objects, often comprising the majority of the total peak area. Conversely, *urushi* lacquers seldom show measurable amounts of any of these markers. Accordingly, these markers have been designated in Appendix 4 as laccol carbohydrates.

Interpretation and verification of carbohydrate marker compound results in the RAdICAL workbook follow the recommendations described in the section on proteins concerning the presence of multiple markers at levels above a few tenths of a percent.

Resins

Natural resins are an important class of additives in Asian lacquer formulations that affect leveling, gloss, drying rate, aging behavior, and solvent sensitivity. Resins identified in studies of Asian lacquered objects carried out at the Getty include camphor, cedar oil, exudates from *Dipterocarpus* genus (such as wood oil and dammar), benzoin resin, resins from the *Pinaceae* family and shellac (Heginbotham *et al.*, 2008; Heginbotham & Schilling, 2011; Heginbotham *et al.*, 2016).

Published research on natural resin composition has been an important source of marker compound information in the AMDIS database and the RAdICAL workbook (van Keulen 2014b). Additionally, a number of experts have been especially generous in sharing AMDIS library spectra and interpretation schemes for resins with the authors: Ken Sutherland (shellac); and Chris Petersen (triterpenes). Marker compounds for a number of natural resins identified

in Asian lacquers are listed in Appendix 5. In the RAdICAL workbook, markers for each resin are tabulated and their total peak areas measured. Marker compounds for resins used exclusively in European lacquers, which are included in the Excel workbook distributed at the RAdICAL workshop, do not appear in Appendix 5 due to the focus of this article.

Pinaceae resin was studied extensively by van den Berg and other researchers in the MOLART project (Pastorova *et al*., 1997); mass spectra for numerous oxidized markers appear in the NIST database. *Pinaceae* resin is confirmed in a sample if, at minimum, the following markers are present: methyl DHA; tetradehydroabietic acid, 7-methoxy-, methyl ester; and 7,15-dimethoxytetradehydroabietic acid, methyl ester. In many Asian lacquer samples, DHA may be present at low concentrations without the other key markers, which suggests the compounds originate from non-resinous materials. In contrast, large proportions of pine resin are customarily added to modern commercial sources of artists' lacquers in Vietnam (Ebert & Schilling, 2016).

Characterization of shellac has been the focus of research at the Philadelphia Museum of Art by Sutherland, who published mass spectra for numerous marker compounds from different types and sources of shellac (Sutherland & del Rio, 2014). Shellac is confirmed in a sample by the presence of aleuritic acid derivatives, plus aliphatic and cyclic hydroxyacids. Aging or oxidation is indicated by elevated levels of shellolic and laccishellolic acids, as compared to jalaric and laccijalaric acids. Bleached shellac is indicated by a chlorinated lac resin terpene marker with elevated levels of shellolic and laccishellolic acids.

The identification of most of the marker compounds for benzoin resin originated from studies of Egyptian embalming materials (Colombini *et al*., 2000). These employed a specialized GC–MS procedure involving alkaline hydrolysis and trimethylsilylation to differentiate the two main types of benzoin resin: styrax and benzoe. One limitation of the RAdICAL protocol is that TMAH converts monomethoxy- benzoin marker compounds to dimethoxy- derivatives, which are less characteristic than the original markers for differentiating the two types of benzoin resin. Notwithstanding, the TMAH derivatives in Appendix 5 were identified in a reference standard of Chinese *urushi* mixed with benzoin resin. Gum benzoin is confirmed in lacquer samples if a major amount of 2-propenoic acid, 3-phenyl-, methyl ester is present with three or more other benzoin marker compounds.

Miscellaneous materials

Appendix 6 lists marker compounds for a variety of miscellaneous materials that are tabulated in the RAdICAL workbook.

A small number of pigments associated with lacquer are known to give rise to distinctive marker compounds. For example, the pyrolysis of arsenic pigments such as orpiment produces As_4O_6 and As_4 (Chiavari & Mazzeo, 2003; Kamiya *et al*., 2015); moreover, in the presence of TMAH, the sulfide anion in these pigments forms dimethyl sulfide. Pyrolysis of mercuric sulfide produces elemental mercury vapor, which is evident as a broad baseline rise early in the chromatogram (Chiavari & Mazzeo, 2003; Kamiya *et al*., 2015). Unfortunately, because elemental mercury elutes as a rise in baseline and not as a well-resolved peak, AMDIS is incapable of identifying it in lacquer analysis results. Its presence can be verified in an extracted ion chromatogram for m/z 202, then noted in the comments section of the RAdICAL workbook. Additionally, the sulfide anion in this pigment also produces dimethyl sulfide and dimethyl disulfide in the presence of TMAH. Three markers for indigo, identified by Poulin (2007) in GC–MS analysis of dyed textiles, were found for green Ryukyuan lacquer that was analyzed using THM-Py-GC–MS (Körber *et al*., 2016). Finally, bone white and bone black produce trimethyl phosphate, a compound also formed by phospholipids in blood and egg.

Contamination from fingerprints may be detected in lacquer samples by the presence of squalene, cholesterol, and fatty acids. Caffeine may be identified in certain domestic lacquered objects associated with tea, as was the case for a sample from a Taiwanese lacquered tea tray. Presently, there are no known lacquer recipes that mention tea, although the chemical structures of tea catechins are loosely comparable with lacquer components that contain tannins. Finally, Crown 18 is a specific crown ether occasionally detected in Anacard lacquer samples, although a precise mechanism for its formation is currently unknown.

All of the marker compounds tabulated thus far in this article have been verified through extensive research on reference standards and verified in multiple samples from lacquer objects; moreover, the chemical structures for the vast majority have been ascertained and expert knowledge for interpreting the results is available. Accordingly, identification of the corresponding materials in lacquer samples can be carried out with a high degree of certainty using the RAdICAL workbook.

Notwithstanding, there remain a few lacquer materials for which additional research is still needed to validate their corresponding sets of marker compounds. Conversely, there are some markers for which there is insufficient information linking them to a single material. In the RAdICAL workbook, materials and markers in these categories are designated as 'Provisionally-identified materials'. One important group of substances in this category is tannins, which are complex polyphenolic substances

that are produced by plants and are often attached to carbohydrate rings (Nakatsubo *et al.*, 2002). Tannins are ubiquitous substances in the plant kingdom and are present in various tissues, including leaves, buds, seeds, roots, stems, and wood. Lacquer-related materials that are known to contain tannins include laccol lacquer (tapped from a *Toxicodendron succedanea* tree growing in the Los Angeles County Arboretum), fermented persimmon juice (which has been used as a binder in ground layers of Japanese lacquer) and fruit obtained from Bhutanese lacquer trees (*da-se*) (Kitagawa, 2012). Studies of Chinese and Ryukyuan objects made with laccol revealed the presence of tannin markers at significant levels relative to the other lacquer components. In the RAdICAL workbook, tannin is confirmed in lacquer samples if four or five marker compounds listed in Appendix 6 are present, although with our current set of marker compounds and state of knowledge, the specific plant source for the tannin cannot be identified. Further research on this topic may help differentiate the tannin-containing materials used in lacquers.

Ox gall, mentioned in recipes for black Chinese lacquer (Zhu, 2002), is another provisionally identified material that has been detected in a number of Chinese lacquered objects, including the Chinese lacquered screen mentioned earlier in the section on oils. It was possible to find high probability NIST spectral matches to some of the GC–MS peaks associated with ox gall (Casas-Catalán *et al.*, 2004), whereas lower-quality matches for other peaks correlated to NIST library compounds with similar molecular structures. The gall markers are late-eluting and, with few other compounds within this RI region, they are easy to detect visually in chromatograms. Phosphate and sterol markers often accompany the ox gall markers.

One method for coloring lacquer black is through the addition of a carbon black, in the form of lamp black, soot or bone black. These materials are extremely complex and their formulations depend on the source material, heating conditions and method of collection (Watson & Valberg, 2001; Achten *et al.*, 2015). A small number of polycyclic aromatic compounds have been observed in black lacquers that are absent in transparent lacquers, and these have been assigned to soot in the RAdICAL workbook. Often, these markers are accompanied by small amounts of methyl DHA and other *Pinaceae* resin markers. If instead trimethyl phosphate is present, this is indicative of bone black.

Gamboge is another material for which the set of marker compounds in Appendix 6 requires confirmation. The gamboge markers in Appendix 6 originate from late-eluting peaks in the chromatogram for a yellow-tinted Japanese lacquered object for which compounds with similar, but not identical, mass spectra were identified in reference samples of Asian gamboge mixed into Anacard lacquer (Heginbotham & Schilling, 2011). Further research into the identification of this material is needed.

Conclusions

The combination of THM-Py-GC–MS with the RAdICAL expert system for data evaluation represents a powerful tool for characterizing organic materials in Asian lacquers. Compared to Py-GC–MS, pyrolysis in the presence of TMAH yields a much broader range of characteristic marker compounds. No other analytical technique is capable of identifying Anacard saps and the full range of known additives in Asian lacquers, even in objects buried for more than 2000 years. Improvements in pyrolyzer technology have reduced the required sample size, making it possible to analyze powder samples taken from individual lacquer layers as small as 20 μm in thickness.

The RAdICAL expert system overcomes many difficulties encountered in evaluating Py-GC–MS data. AMDIS is, without question, the best way of processing Py-GC–MS data files to identify compounds in lacquer samples. The program is fast, precise, accurate and systematic, and the results are repeatable. Based on our experience of teaching AMDIS in RAdICAL workshops, even newcomers to Py-GC–MS are capable of using the program to identify hundreds of marker compounds routinely and accurately in unknown lacquer samples. The only limit to the applicability of AMDIS to material characterization is the breadth of the target compound library. AMDIS components not matched to a RAdICAL library spectrum are clearly marked in the program window, allowing users the option of searching the full NIST database to identify the component, or adding the mass spectrum for the unknown component to a library. Substitution of a library entry number into the 'Ref. Ion' field for each target compound in the AMDIS library has opened the possibility of systematic processing of the AMDIS report by Excel. The downside of this substitution is the loss of automated signal-to-noise calculations for the target compounds. It would be beneficial if NIST would add a library entry number field to the next revision of AMDIS. The customized RAdICAL Excel workbook accepts the AMDIS report that lists marker compounds identified in a sample and reorganizes them by class of artists' materials, making it possible to perform calculations and display sorted information for each material in graphs and tables. Expert knowledge relating materials to their associated marker compounds, obtained from publications and personal communications and which has been embedded into the Excel worksheets, permits researchers to make informed decisions about the presence or absence of materials in their lacquer samples.

Identification of additives in Asian lacquers continues to be an important research topic. As demonstrated by the work of Chang and Schilling (2016), much relevant information on lacquer formulations and additives has been published in languages other than English. Therefore, a systematic review of lacquer literature from Japan, Korea, Vietnam, Burma, and Thailand, and translation into English would make this vast body of knowledge accessible to researchers outside these countries. Such work would expand the known range of lacquer materials and formulations that should be studied, and provide complementary information to existing technical studies of objects from these countries. From the analytical perspective, it should be recognized that pyrolysis pathways leading to marker compound formation are affected by the sample matrix, meaning that marker compounds formed by a material may differ from those produced by the same material mixed with raw lacquer. Thus, the identification of lacquer materials will improve through study of lacquer replicas formulated using a broad range of additives mixed into lacquer.

The standardized data formats of AMDIS and Excel facilitate the sharing of results with other researchers using the RAdICAL protocol, thereby encouraging cross-comparison of interpreted results, collaboration and expansion of the lacquer community. AMDIS was designed to open data files from most GC–MS instrument manufacturers, and has the option of opening the generic format net cdf files from any instrument so that, theoretically, anyone analyzing Asian lacquer with Py-GC–MS may benefit from the RAdICAL protocol. We encourage researchers of Asian lacquer to share their marker compounds and schemes for interpreting the results with us, so their expert knowledge can be integrated into the expert system and improve its current capabilities, and shared with RAdICAL alumni and others in the lacquer community. As marker compounds for new materials are added to the library, and supplemental markers are added to improve the identification of materials already in the database, the lacquer community will be presented with opportunities for the periodic re-evaluation of existing test data in order to search for the suite of new materials and markers in their lacquered objects. One hope is that this process will lead to a shared database of raw Py-GC–MS data files and RAdICAL reports for objects that are accessible to all lacquer researchers. Finally, RAdICAL alumni familiar with Excel have developed other presentation formats better suited to represent their test results, which highlights the flexibility of the system.

This article provides supplementary information for readers to better understand the analytical results presented in other articles in this issue that were obtained from the RAdICAL protocol. It is hoped that readers will appreciate the breadth and depth of information that can be obtained by the protocol and the value of presenting test results for lacquered objects in standardized formats that facilitate comparisons.

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gardenia extract; and Samnang Huot, National Museum of Cambodia, for wood oil and Cambodian lacquer.

Suppliers

Raw laccol from Phú Thọ province in northern Vietnam, *son điều* lacquer from the south of Vietnam, Cambodian lacquer: Asiarta Foundation.

Seed lac: H. Behlen (www.shellac.net/behlen-wood-finishing.html).
Gum benzoin, gamboge: Kremer Pigmente (http://kremer-pigmente.de/en).

Japanese *urushi*, Chinese *urushi*, Taiwanese laccol, tung oil: Long Nan Museum of Natural Lacquerware, No. 211-1 Beiping St., Puli Township, Nantou, Taiwan.

Sesame oil: Sigma-Aldrich (www.sigmaaldrich.com/united-states.html).

Tung oil, perilla oil: Tokyu Hands Store, 1-9-1 Marunouchi, Chiyoda, Tokyo 100-6701, Japan.

Chinese raw *urushi*, Japanese *roiro*, fermented persimmon juice: Watanabe-Shoten, 6-5-8 Ueno, Taito-ku, Tokyo 110-0005 Japan.

Purple Shiso seeds (*Perilla frutescens nankinensis*): Whatcom Seed Company, 4419 Hyacinth St, Eugene, OR 97404, USA.

Ox gall: Winsor & Newton (www.winsornewton.com/na/).

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Appendix 1. THM-Py-GC–MS marker compounds for oils and lipids

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Monocarboxylic fatty acid methyl esters								
Butanoic acid, methyl ester	720	$C_5H_{10}O_2$	102	43	74	71	59	87
Pentanoic acid, methyl ester	830	$C_6H_{12}O_2$	116	74	57	85	87	101
5-Hexenoic acid, methyl ester	938	$C_7H_{12}O_2$	128	74	43	41	68	128
Hexanoic acid, methyl ester	952	$C_7H_{14}O_2$	130	74	87	43	99	59
6-Heptenoic acid, methyl ester	1046	$C_8H_{14}O_2$	142	74	41	55	68	110
Heptanoic acid, methyl ester	1054	$C_8H_{16}O_2$	144	74	87	43	113	144
7-Octenoic acid, methyl ester	1135	$C_9H_{16}O_2$	156	74	55	43	40	125
Octanoic acid, methyl ester	1142	$C_9H_{18}O_2$	158	74	87	127	115	158
8-Nonenoic acid, methyl ester	1218.1	$C_{10}H_{18}O_2$	170	74	55	96	138	121
Nonanoic acid, methyl ester	1226.5	$C_{10}H_{20}O_2$	172	74	87	129	141	172
Decanoic acid, methyl ester	1325.1	$C_{11}H_{22}O_2$	186	74	87	143	155	186
Dodecanoic acid, methyl ester	1525	$C_{13}H_{26}O_2$	214	74	87	143	183	214
Tridecanoic acid, methyl ester	1623.2	$C_{14}H_{28}O_2$	228	74	87	143	185	228
Tetradecanoic acid, methyl ester	1726	$C_{15}H_{30}O_2$	242	74	87	143	199	242
Pentadecanoic acid, methyl ester	1827	$C_{16}H_{32}O_2$	256	74	87	143	213	256
Hexadecanoic acid, methyl ester	1928	$C_{17}H_{34}O_2$	270	74	87	143	270	227
Heptadecanoic acid, methyl ester	2025	$C_{18}H_{36}O_2$	284	74	87	143	284	241
9-Octadecenoic acid (Z)-, methyl ester	2103	$C_{19}H_{36}O_2$	296	55	83	97	264	296
Octadecanoic acid, methyl ester	2128	$C_{19}H_{38}O_2$	298	74	87	143	298	255
8,11-Octadecadienoic acid, methyl ester	2192	$C_{19}H_{34}O_2$	294	67	81	95	109	294
Nonadecanoic acid, methyl ester	2206	$C_{20}H_{40}O_2$	312	74	87	143	312	269
Eicosanoic acid, methyl ester	2329	$C_{21}H_{42}O_2$	326	74	87	143	326	283
Heneicosanoic acid, methyl ester	2417	$C_{22}H_{44}O_2$	340	74	87	143	340	297
13-Docosenoic acid, methyl ester	2495.3	$C_{23}H_{44}O_2$	352	43	42	55	320	352
Docosanoic acid, methyl ester	2530	$C_{23}H_{46}O_2$	354	74	87	143	354	311
Tricosanoic acid, methyl ester	2627	$C_{24}H_{48}O_2$	368	74	87	143	368	325
Tetracosanoic acid, methyl ester	2730	$C_{25}H_{50}O_2$	382	74	87	143	382	339
Hexacosanoic acid, methyl ester	2932	$C_{27}H_{54}O_2$	410	74	87	143	410	367
Octacosanoic acid, methyl ester	3125	$C_{29}H_{58}O_2$	438	74	87	143	438	395
Triacontanoic acid, methyl ester	3337	$C_{31}H_{62}O_2$	466	74	87	143	466	423
Dotriacontanoic acid methyl ester	3538	$C_{33}H_{66}O_2$	494	74	87	143	494	451
Tetratriacontanoic acid methyl ester	3719.6	$C_{35}H_{70}O_2$	522	74	87	143	522	479
Dicarboxylic fatty acid dimethyl esters								
Butanedioic acid, dimethyl ester	1061	$C_6H_{10}O_4$	146	115	55	59	114	87
Pentanedioic acid, dimethyl ester	1152	$C_7H_{12}O4$	160	59	100	129	55	128
Hexanedioic acid, dimethyl ester	1249	$C_8H_{14}O_4$	174	59	114	101	111	143
Heptanedioic acid, dimethyl ester	1350	$C_9H_{16}O_4$	188	115	74	55	125	157
Octanedioic acid, dimethyl ester	1451	$C_{10}H_{18}O_4$	202	129	138	74	97	171
Nonanedioic acid, dimethyl ester	1551	$C_{11}H_{20}O_4$	216	152	55	111	143	185
Decanedioic acid, dimethyl ester	1652	$C_{12}H_{22}O_4$	230	55	125	98	199	157
Undecanedioic acid, dimethyl ester	1753	$C_{13}H_{24}O_4$	244	98	139	171	213	152
Dodecanedioic acid, dimethyl ester	1853	$C_{14}H_{26}O_4$	258	55	74	98	153	227
Tridecanedioic acid, dimethyl ester	1953	$C_{15}H_{28}O_4$	272	98	74	112	241	199
Alkylphenyl alkanoates (markers for heat-bodied oils)								
Heptanoic acid, 7-(o-pentylphenyl)-, methyl ester	2116.4	$C_{19}H_{30}O_2$	290	105	41	290	91	106
Methyl alkylphenyl alkanoate 2	2130	$C_{19}H_{30}O_2$	290	105	202	290	187	91
Nonanoic acid, 9-(o-propylphenyl)-, methyl ester	2135.7	$C_{19}H_{30}O_2$	290	105	91	133	290	258
Methyl alkylphenyl alkanoate 1	2150	$C_{19}H_{30}O_2$	290	105	91	290	197	119
Methyl alkylphenyl alkanoate 3	2180.9	?	?	105	91	276	183	133
Glycerol markers								
2-Propanol, 1,3-dimethoxy-	890	$C_5H_{12}O_3$	120	45	75	43	71	102
Propane, 1,2,3-trimethoxy-	916.4	$C_6H_{14}O_3$	134	59	89	45	58	102
2,3-Dimethoxypropan-1-ol	930	$C_4H_{10}O_3$	106	45	58	75	89	59

Appendix 2. THM-Py-GC–MS marker compounds for *Anacardiaceae* tree saps

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Substituted dimethoxybenzenes (from catechols)								
1,2-Dimethoxy-3-ethylbenzene	1259	$C_{10}H_{14}O_2$	166	166	136	118	120	162
1,2-Dimethoxy-3-propylbenzene	1338	$C_{11}H_{16}O_2$	180	180	136	151	91	
1,2-Dimethoxy-3-butenylbenzene	1401.6	$C_{12}H_{16}O_2$	192	136	151	91	131	192
1,2-Dimethoxy-3-butylbenzene	1430	$C_{12}H_{18}O_2$	194	136	194	91	151	152
1,2-Dimethoxy-3-pentylbenzene	1519	$C_{13}H_{20}O_2$	208	208	136	151	152	
1,2-Dimethoxy-3-hexenylbenzene	1615	$C_{14}H_{20}O_2$	220	136	151	152	220	91
1,2-Dimethoxy-3-hexylbenzene	1622	$C_{14}H_{22}O_2$	222	136	222	152	91	77
1,2-Dimethoxy-3-heptenylbenzene	1715	$C_{15}H_{22}O_2$	234	136	91	151	152	234
1,2-Dimethoxy-3-heptylbenzene	1723	$C_{15}H_{24}O_2$	236	136	236	152	151	91
1,2-Dimethoxy-3-octenylbenzene	1815	$C_{16}H_{24}O_2$	248	136	248	151	152	91
1,2-Dimethoxy-3-octylbenzene	1823	$C_{16}H_{26}O_2$	250	250	136	151	152	91
1,2-Dimethoxy-3-nonenylbenzene	1921	$C_{17}H_{26}O_2$	262	136	151	262	137	
1,2-Dimethoxy-3-nonylbenzene	1924	$C_{17}H_{28}O_2$	264	264	136	152	137	
1,2-Dimethoxy-3-decylbenzene	2140	$C_{18}H_{30}O_2$	278	278	136	151		
1,2-Dimethoxy-3-pentadec-8-enylbenzene	2533	$C_{23}H_{38}O_2$	346	346	136	151	152	91
1,2-Dimethoxy-3-pentadecylbenzene	2552	$C_{23}H_{40}O_2$	348	348	151	136	152	91
1,2-Dimethoxy-3-heptadec-10-enylbenzene	2707	$C_{25}H_{42}O_2$	374	374	151	136	152	91
1,2-Dimethoxy-3-heptadecylbenzene	2755	$C_{25}H_{44}O_2$	376	376	151	136	152	91
1,2-Dimethoxy-10-phenyldecylbenzene	2791	$C_{24}H_{34}O_2$	354	354	91	136	151	152
1,2-Dimethoxy-12-phenyldodecylbenzene	3007	$C_{26}H_{38}O_2$	382	382	91	136	151	152
Acid catechols (catechol oxidation products)								
Methyl 6-(2,3-dimethoxyphenyl)hexanoate (miyamic acid)	1954	$C_{15}H_{22}O_4$	266	136	151	266	91	121
Methyl 7-(2,3-dimethoxyphenyl)heptanoate (kumanotanic acid)	2056	$C_{16}H_{24}O_4$	280	136	280	151	91	121
Methyl 8-(2,3-dimethoxyphenyl)octanoate (mazzeic acid)	2158	$C_{17}H_{26}O_4$	294	136	294	151	91	121
Methyl 9-(2,3-dimethoxyphenyl)nonanoate (miyakoshic acid)	2261	$C_{18}H_{28}O_4$	308	308	136	151	91	121
Methyl 10-(2,3-dimethoxyphenyl)decanoate (arlenic acid)	2364	$C_{19}H_{30}O_4$	322	322	136	151	152	91
Methyl 11-(2,3-dimethoxyphenyl)undecanoate (whalenic acid)	2468	$C_{20}H_{32}O_4$	336	336	136	151	91	121
Methyl 12-(2,3-dimethoxyphenyl)dodecanoate (chiavaric acid)	2567	$C_{21}H_{34}O_4$	350	350	136	151	152	91
Anisoles (from phenols)								
2-Hexylanisole	1495.9	$C_{13}H_{20}O$	192	122	192			
2-Heptylanisole	1595	$C_{14}H_{22}O$	206	122	121	151	206	
2-Octylanisole	1698.7	$C_{15}H_{24}O$	220	122	186	93	220	65
2-Pentadecenylanisole	2440	$C_{22}H_{36}O$	316	122	121	135	316	41
2-Pentadecylanisole	2424.7	$C_{22}H_{38}O$	318	122	121	318	107	
2-Heptadecenylanisole	2614.3	$C_{24}H_{40}O$	344	215	344	345		
12-Phenyldodecylanisole	2901	$C_{25}H_{36}O$	352	122	91	121	352	65
Alkyl- and alkenyl-benzenes								
Benzene, 1-propenyl-	983	C_9H_{10}	118	117	118	91	115	103
Benzene, propyl-	992	C_9H_{12}	120	91	120	92	65	105
Benzene, 3-butenyl-	1079	$C_{10}H_{12}$	132	91	132	65	104	51
Benzene, butyl-	1091	$C_{10}H_{14}$	134	91	92	134	65	105
Benzene, 4-pentenyl-	1163	$C_{11}H_{14}$	146	104	91	92	117	146
Benzene, pentyl-	1177	$C_{11}H_{16}$	148	91	92	148	65	105
Benzene, 4-hexenyl-	1270	$C_{12}H_{16}$	160	104	91	92	117	160
Benzene, hexyl-	1277	$C_{12}H_{18}$	162	91	92	162	105	133
Benzene, 5-heptenyl-	1369	$C_{13}H_{18}$	174	104	91	117	92	174
Benzene, heptyl-	1377	$C_{13}H_{20}$	176	92	91	176	105	133
Octenyl benzene	1474	$C_{14}H_{20}$	188	91	104	117	188	131
Benzene, octyl-	1482	$C_{14}H_{22}$	190	91	92	190	105	133
Nonenyl benzene	1577	$C_{15}H_{22}$	202	91	104	117	202	131
Nonyl benzene	1584	$C_{15}H_{24}$	204	92	91	204	105	133
Decenyl benzene	1684	$C_{16}H_{24}$	216	91	104	92	117	216

Continued

Appendix 2. *Continued*

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Benzene, decyl-	1690	$C_{16}H_{26}$	218	92	91	218	105	133
Undecenyl benzene	1786	$C_{17}H_{26}$	230	91	104	117	230	131
Benzene, undecyl-	1793	$C_{17}H_{28}$	232	92	91	232	105	133
Dodecenyl benzene	1894	$C_{18}H_{28}$	244	91	104	92	117	244
Benzene, dodecyl-	1900	$C_{18}H_{30}$	246	92	91	246	105	133
Tridecyl benzene	2003	$C_{19}H_{32}$	260	92	91	260	57	119
Oxidation products of alkenyl-benzenes								
Methyl 9-phenyl-nonanoate (poulinic acid)	1925	$C_{16}H_{24}O_2$	248	91	92	104	216	248
Methyl 10-phenyl-decanoate (körberic acid)	2029	$C_{17}H_{26}O_2$	262	91	92	104	230	262
Methyl 11-phenyl-undecanoate (watanabic acid)	2136	$C_{18}H_{28}O_2$	276	91	92	104	244	276
Methyl 12-phenyl-dodecanoate (szelewskic acid)	2241	$C_{19}H_{30}O_2$	290	91	92	104	258	290
Methyl 13-phenyl-tridecanoate (keulenic acid)	2347	$C_{20}H_{32}O_2$	304	91	272	92	104	304
Methyl 14-phenyl-tetradecanoate (petersenic acid)	2452	$C_{21}H_{34}O_2$	318	91	92	104	286	318
Ketone oxidation products of thitsi								
Methyl 9-oxo-9-phenylnonanoate	2141	$C_{16}H_{22}O_3$	262	120	105	77	133	262
Methyl 10-oxo-10-phenyldecanoate	2250	$C_{17}H_{24}O_3$	276	120	105	77	133	276
Methyl 11-oxo-11-phenylundecanoate	2355	$C_{18}H_{26}O_3$	290	120	105	77	133	290
Methyl 12-oxo-12-phenyldodecanoate	2462	$C_{19}H_{28}O_3$	304	120	105	77	133	304
Methyl 13-oxo-13-phenyltridecanoate (tamburinic acid)	2569	$C_{20}H_{30}O_3$	318	120	105	77	133	318
10-(2,3-Dimethoxyphenyl)-1-phenyldecan-1-one	3000	$C_{24}H_{32}O_3$	368	105	136	368	151	248
12-(2,3-Dimethoxyphenyl)-1-phenyldodecan-1-one	3235	$C_{26}H_{36}O_3$	396	105	136	151	396	276
Cashew nut shell liquid markers								
Methyl 8-(3-methoxyphenyl)octanoate	2049	$C_{16}H_{24}O_3$	264	122	121	264	135	91
Methyl 2-methoxy-6-(8-methoxy-8-oxooctyl)benzoate (ebertic acid)	2329.1	$C_{18}H_{26}O_5$	322	161	291	290	272	175
Isomer of cashew nut shell liquid oxidation product	2397.3	$C_{18}H_{26}O_5$	322	161	290	291	162	272
Anacard oxidation products								
Benzoic acid, methyl ester	1126	$C_8H_8O_2$	136	105	77	136	51	106
1,4-Benzenedicarboxylic acid, dimethyl ester	1514	$C_{10}H_{10}O_4$	194	163	135	194	103	76
1,3-Benzenedicarboxylic acid, dimethyl ester	1525	$C_{10}H_{10}O_4$	194	163	135	194	76	103
1,2,3-Benzenetricarboxylic acid, trimethyl ester	1806.1	$C_{12}H_{12}O_6$	252	221	222	104	149	252
1,2,4-Benzenetricarboxylic acid, trimethyl ester	1845	$C_{12}H_{12}O_6$	252	221	119	162	222	252
1,3,5-Benzenetricarboxylic acid, trimethyl ester	1914	$C_{12}H_{12}O_6$	252	221	75	193	252	147
1,2,3,4-Benzenetetracarboxylic acid, tetramethyl ester	2096.2	$C_{14}H_{14}O_8$	310	279	280	162	104	310
1,2,4,5-Benzenetetracarboxylic acid, tetramethyl ester	2127.7	$C_{14}H_{14}O_8$	310	279	280	162	75	310
1,2,3,5-Benzenetetracarboxylic acid, tetramethyl ester	2163.3	$C_{14}H_{14}O_8$	310	279	280	233	162	310

Appendix 3. THM-Py-GC–MS marker compounds for proteins

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Blood								
Blood – unverified 4	1093	?	?	113	56	28	72	131
Benzyl nitrile	1145	C_8H_7N	117	117	90	116	89	63
Blood – unverified 5	1336	?	?	128	42	127	113	170
Blood – unverified 6	1435	?	?	128	127	42	57	102
Blood – unverified 8	1774	?	?	150	165	68	122	94
Blood – unverified 7	1815	?	?	91	127	42	218	65
Blood – unverified 9	2269	?	?	139	70	167	42	258
Glue								
Glue – unverified 1	1347	?	?	98	42	41	70	114
Glue – unverified 2	1474	?	?	42	113	156	56	141
Glue – unverified 3	1871	?	?	198	112	168	44	139

Continued

Appendix 3. *Continued*

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Blood & glue								
Blood – unverified 1	1563	?	?	98	42	41	70	156
Blood – unverified 10	1220	?	?	55	140	54	56	141
Protein 3 – blood & glue	1702	?	?	83	168	42	70	112
Tofu & glue								
Protein 4 – tofu & glue	1423	?	?	101	88	45	158	172
Tofu & blood								
Protein 1 – tofu & blood	1725	?	?	98	42	41	72	170
Egg								
Indole	1307.2	C_8H_7N	117	117	90	89	63	58
Egg – unverified 1	2196	?	?	59	72	43	137	202
Tofu & egg								
Protein 2 – tofu & egg	1350	?	?	98	42	131	70	160
Protein – various								
L-Proline, 1-methyl-, methyl ester	1072	$C_7H_{13}NO_2$	143	84	31	42	59	143
Protein – unverified 7	1163	?	?	135	136	134	77	90
Protein – unverified 8	1225	?	?	42	127	142	56	57
Benzenepropanenitrile	1246.9	C_9H_9N	131	91	131	65	92	77
Protein – unverified 1	1495	?	?	114	43	56	127	155
1-Piperidinepropanenitrile	1588	$C_8H_{14}N_2$	138	98	41	204	137	70
Protein – unverified 3	1679.8	?	?	128	44	99	42	129
Protein – unverified 4	1686.7	?	?	91	155	198	65	213
Glue marker – Mazzeo	1740	?	?	186	93	65	130	65
Blood – unverified 11	1274	?	?	131	130	89	77	132
1H-Indole, 1,3-dimethyl-	1371.5	$C_{10}H_{11}N$	145	144	145	102	103	115
1H-Pyrrole, 1-methyl-	736	C_5H_7N	81	81	80	53	42	39

Appendix 4. THM-Py-GC–MS marker compounds for carbohydrates

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Furfural	833	$C_5H_4O_2$	96	96	95	39	29	38
Starch – unverified 2	1322	?	?	87	101	45	115	124
Schellmannose	1395	?	?	101	45	127	71	88
Starch – unverified 1	1395	?	?	101	45	75	127	88
Starch – unverified 3	1423	?	?	101	88	141	73	156
Carbohydrate 1 – starch and tofu	1439	?	?	101	74	45	180	205
Tofu – unverified 1	1457	?	?	101	45	72	87	218
Starch – unverified 4	1530	?	?	45	89	101	75	161
Glucoside – tofu and carbohydrates	2234	?	?	187	101	111	45	219
Starch – unverified 5	2544	?	?	88	45	263	71	175
Laccol carbohydrate – unverified 14	1483.5	?	?	129	75	101	161	191
Laccol carbohydrate – unverified 1	1491	?	?	129	101	45	59	147
Laccol carbohydrate – unverified 13	1497	?	?	129	75	101	161	191
Laccol carbohydrate – unverified 3	1501	?	?	129	75	101	161	191
Laccol carbohydrate – unverified 2	1515	?	?	129	45	101	146	177
Laccol carbohydrate – unverified 4	1526	?	?	129	75	101	161	191
Laccol carbohydrate – unverified 5	1532	?	?	129	75	101	145	161
Laccol carbohydrate – unverified 6	1554	?	?	129	75	101	145	161
Laccol carbohydrate – unverified 9	2457.3	?	?	88	101	129	219	75
Laccol carbohydrate – unverified 10	2467	?	?	88	101	129	219	75
Laccol carbohydrate – unverified 7	2484	?	?	129	101	201	75	219

Continued

Appendix 4. *Continued*

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Laccol carbohydrate – unverified 15	2500.8	?	?	129	101	201	161	219
Laccol carbohydrate – unverified 8	2523	?	?	101	129	201	88	219
Laccol carbohydrate – unverified 11	2544	?	?	101	88	59	75	246
Laccol carbohydrate – unverified 12	2570	?	?	101	88	59	75	201

Appendix 5. THM-Py-GC–MS marker compounds for natural resins

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Camphor								
Camphene	990	$C_{10}H_{16}$	136	93	79	121	107	136
Camphor	1158	$C_{10}H_{16}O$	152	95	81	108	69	152
Cedar oil								
Alpha-cedrene	1418.8	$C_{15}H_{24}$	204	119	93	105	161	204
Beta-cedrene	1427.3	$C_{15}H_{24}$	204	161	204	93	120	147
Cedrol	1612.3	$C_{15}H_{26}O$	222	95	150	151	207	222
Dipterocarpus								
Dipterocarp – unverified 1	3278.5	?	?	143	191	125	385	367
Dipterocarp – unverified 6	3337	?	?	143	85	109	175	385
Dipterocarp – unverified 2	3352.8	?	?	143	99	125	399	424
Dipterocarp – unverified 4	3399.3	?	?	143	59	85	107	161
Dipterocarp – unverified 3	3417.9	?	?	143	125	413	112	144
Dipterocarp – unverified 7	3440	?	?	143	85	125	161	429
Dipterocarp – unverified 8	3492	?	?	143	191	107	71	383
Dipterocarp – unverified 5	3572	?	?	143	125	85	205	399
Dipterocarp – unverified 9	3572	?	?	143	125	205	399	367
Dipterocarp – unverified 10	3604	?	?	143	125	191	81	383
Benzoin resin								
Benzoic acid, methyl ester	1126	$C_8H_8O_2$	136	105	77	136	51	106
Benzaldehyde, 4-methoxy-	1218	$C_8H_8O_2$	136	135	136	77	92	107
Benzoic acid, 4-methoxy-, methyl ester	1354	$C_9H_{10}O_3$	166	135	166	77	92	107
2-Propenoic acid, 3-phenyl-, methyl ester	1402	$C_{10}H_{10}O_2$	162	131	103	162	77	51
Benzaldehyde, 3,4-dimethoxy-	1490	$C_9H_{10}O_3$	166	166	165	95	77	151
Methyl p-methoxycinnamate, cis	1683	$C_{11}H_{12}O_3$	192	161	192	133	89	118
Pinaceae								
Methyl neoabietate	2207	$C_{21}H_{32}O_2$	316	135	316	148	181	257
Methyl pimarate	2233	$C_{21}H_{32}O_2$	316	121	180	257	316	301
Methyl isopimarate	2346	$C_{21}H_{32}O_2$	316	241	257	316	187	287
Methyl palustrate	2354.5	$C_{21}H_{32}O_2$	316	43	149	241	301	316
Tetra decahydro abietic acid methyl ester	2369	$C_{21}H_{36}O_2$	320	163	123	109	261	320
Methyl-6-dehydrodehydroabietate	2377	$C_{21}H_{28}O_2$	312	237	312	197	141	165
Methyl DHA	2383	$C_{21}H_{30}O_2$	314	239	299	314	197	173
Methyl abietate	2384	$C_{21}H_{32}O_2$	316	43	316	121	256	301
Mercusic acid dimethyl ester	2456.4	$C_{22}H_{36}O_4$	364	121	304	305	181	364
3-Hydroxy dehydroabietic acid methyl ester	2461	$C_{21}H_{30}O_3$	?	237	195	312	41	238
Tetradehydroabietic acid, 7-methoxy-, methyl ester	2481	$C_{22}H_{30}O_3$	342	342	267	282	227	327
Methyl 12-methoxyabieta-8,11,13-trien-20-oate	2500	$C_{22}H_{32}O_3$	344	269	344	270	227	329
15-Methoxydehydroabietic acid, methyl ester	2545	$C_{22}H_{32}O_3$	344	329	330	269	313	344
15-Hydroxydehydroabietic acid, methyl ester	2573	$C_{21}H_{30}O_3$	330	315	255	330	316	237
7,15-Dimethoxytetradehydroabietic acid, methyl ester	2611	$C_{23}H_{32}O_4$	372	372	297	340	313	357
7-Oxodehydroabietic acid, methyl ester	2624	$C_{21}H_{28}O_3$	328	253	328	187	313	269
15-Hydroxy-7-oxodehydroabietic acid, methyl ester	2811	$C_{21}H_{28}O_4$	344	329	269	330	344	187

Continued

Appendix 5. *Continued*

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Shellac								
Butolic acid, 6-methoxy, methyl ester	1863	$C_{16}H_{32}O_3$	272	127	159	69	158	83
Butolic acid lactone	1883.9	?	?	85	55	84	113	67
Laccijalaric acid, trimethyl isomer	2067	?	?	75	151	306	183	274
Shellac marker 1	2187	?	?	247	187	159	276	306
Shellac marker 2	2197	?	?	247	244	159	187	276
Jalaric acid tetramethyl	2206	?	?	75	45	291	304	336
Shellolic acid dimethyl ester, dimethyl ether	2246	?	352	320	201	228	261	289
Aleuritic acid methyl ester, trimethoxy	2375	?	?	95	201	71	159	137
Aleuritic acid, trimethyl isomer	2448	?	?	95	71	145	187	201

Appendix 6. THM-Py-GC–MS marker compounds for miscellaneous materials

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Gall								
Gall – unverified 1	2984.3	?	?	370	147	105	249	213
Gall – unverified 2	3121.6	?	?	94	386	371	148	430
Deoxycholic acid, methyl ester, dimethyl ether	3163.5	$C_{27}H_{46}O_4$		255	287	370	355	402
Gall – unverified 5	3238.8	?	?	253	285	400	368	385
Gall – unverified 3	3256	?	?	370	355	255	213	402
Methyl 7.alpha.-hydroxy-3.alpha.-methoxy-5.beta.-cholanoate	3291	$C_{26}H_{44}O_4$	420	370	255	355	287	402
Cholan-24-oic acid, 3,7-dihydroxy-, methyl ester, (3.alpha.,5.beta.,7.alpha.)-	3344	$C_{25}H_{42}O_4$	406	370	355	388	255	406
Methyl cholate	3394.1	$C_{25}H_{42}O_5$	422	55	271	253	386	368
Methyl hyodesoxycholate	3444.6	$C_{25}H_{42}O_4$	406	55	213	255	370	388
Sterols								
Cholest-5-ene, 3-methoxy-, (3.beta.)-	3072	$C_{28}H_{48}O$	400	368	400	353	275	385
Cholesterol	3170	$C_{27}H_{46}O$	386	43	386	275	301	368
Cholesta-3,5-dien-7-one	3258	$C_{27}H_{42}O$	382	382	174	161	269	367
Tannins								
Acetic acid, methoxy-, methyl ester	754	$C_4H_8O_3$	104	45	74	29	59	104
1,2,3-Trimethoxybenzene	1318	$C_9H_{12}O_3$	168	168	153	110	125	95
1,2,4-Trimethoxybenzene	1377	$C_9H_{12}O_3$	168	153	168	125	110	69
1,2,3,4-Tetramethoxybenzene	1452	$C_{10}H_{14}O_4$	198	198	183	140	155	168
Benzoic acid, 2,3-dimethoxy-, methyl ester	1487	$C_{10}H_{12}O_4$	196	163	164	196	107	122
Benzoic acid, 3,4,5-trimethoxy-, methyl ester	1726	$C_{11}H_{14}O_5$	226	226	211	195	155	183
Arsenic pigments								
Dimethyl-methylthio-arsine	842.1	C_3H_9AsS	152	137	152	109	121	89
Arsenic – As_4	1378.9	As_4	300	300	225	150	75	
Arsenic – As_4O_6	1442.2	As_4O_6	395.7	396	91	289	182	75
Indigo								
Methyl-2-aminobenzoate	1345.5	$C_8H_9NO_2$	151	119	151	92	120	65
Benzoic acid, 2-(methylamino)-, methyl ester	1407.4	$C_9H_{11}NO_2$	165	165	105	104	132	77
2-bis-(N-methylindole-3-methoxy)	2648.8	$C_{20}H_{20}N_2O_2$	320	320	305	275	290	146
Soot								
Cadinene	1417.6	$C_{15}H_{24}$	204	161	189	204	105	133
Cadalene	1683	$C_{15}H_{18}$	198	183	198	168	153	165
Pyrene	2070	$C_{16}H_{10}$	202	202	203	200	101	88
Fluoranthene	2071	$C_{16}H_{10}$	202	202	203	200	201	101
Indeno[1,2,3-cd]pyrene	3262	$C_{22}H_{12}$	276	276	138	277	274	124

Continued

Appendix 6. *Continued*

Target compound name	RI	Formula	MW	Ion 1	Ion 2	Ion 3	Ion 4	Ion 5
Sulfur markers from minerals & proteins								
Dimethyl sulfide	553	C_2H_6S	62	62	47	45	61	46
Disulfide, dimethyl	749	$C_2H_6S_2$	94	94	45	79	46	61
Dimethyl sulfate	868	$C_2H_6O_4S$	126	95	96	45	66	125
Others								
Trimethyl phosphate	968	$C_3H_9O_4P$	140	110	79	109	95	140
Caffeine (tea)	1830	$C_8H_{10}N_4O_2$	194	194	109	55	67	82
Squalene (fingerprints)	2805.5	$C_{30}H_{50}$	410	69	95	121	137	149
Crown 18 (Anacards)	3028	$C_{20}H_{24}O_6$	360	121	136	80	360	109