Organic Mass Spectrometry in Art and Archaeology

Edited by

MARIA PERLA COLOMBINI AND FRANCESCA MODUGNO
University of Pisa, Pisa, Italy

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List of Contributors

Alessia Andreotti, Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy

Jean Bleton, Groupe de Chimie Analytique de Paris-Sud 11 (LETIAM) EA 4041, Institut Universitaire de Technologie d'Orsay, Plateau de Moulon, 91400 Orsay, France

Ilaria Bonaduce, Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy

Oscar Chiantore, Department of IPM Chemistry and Centre of Excellence NIS, University of Torino, Via Giuria 7, 10125 Torino, Italy

Maria Perla Colombini, Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy

Patrick Dietemann, Doerner Institut, Bayerische Staatsgemäldesammlungen, Barer Str. 29, D-80799 München, Germany

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

Mariaelena Fedi, INFN Sezione di Firenze, Via Bruno Rossi 1, 50019 Sesto Fiorentino, Italy

Gwénaëlle Gautier, The Art Institute of Chicago, 111 South Michigan Avenue, Chicago, IL 60603-6404, USA

Gianluca Giorgi, Department of Chemistry, University of Siena, Via Aldo Moro, 53100 Siena, Italy

Christoph Herm, Studiengang Restaurierung, Hochschule für Bildende Künste Dresden, Güntzstr. 34, D-01307 Dresden, Germany

Radovan Hynek, Institute of Chemical Technology, Department of Biochemistry and Microbiology, Technická 3, 166 28 Prague 6, Czech Republic

Maciej Jarosz, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

Milan Kodicek, Institute of Chemical Technology, Department of Biochemistry and Microbiology, Technická 3, 166 28 Prague 6, Czech Republic

Stepanka Kuckova, Charles University, Department of Chemistry and Chemical Education, M.D. Rettigové 4, 116 39 Prague 1, Czech Republic and Institute of Chemical Technology, Department of Biochemistry and Microbiology, Technická 3, 166 28 Prague 6, Czech Republic

Katarzyna Lech, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

Vincent Mazel, Laboratoire Matériaux et Santé, UFR de Pharmacie, Université Paris-sud XI, 92296 Chatenay Malabry Cedex, France

Francesca Modugno, Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy

Katarzyna Połeć-Pawlak, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

Martine Regert, CEPAM (Centre d'Etudes Préhistoire, Antiquité, Moyen Âge), UMR 6130, Université Nice Sophia Antipolis – CNRS, Bât. 1; 250, rue Albert Einstein, F-06560 Valbonne, France

Erika Ribechini, Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy

Pascale Richardin, C2RMF–CNRS UMR 171, Centre de Recherche et de Restauration des Musées de France, Palais du Louvre, Porte des Lions, 14, Quai François Mitterrand, 75001 Paris, France

Dominique Scalarone, Department of IPM Chemistry and Centre of Excellence NIS, University of Torino, Via Giuria 7, 10125 Torino, Italy

Alain Tchapla, Groupe de Chimie Analytique de Paris-Sud 11 (LETIAM) EA 4041, Institut Universitaire de Technologie d'Orsay, Plateau de Moulon, 91400 Orsay, France

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Preface

Early applications of chemical and physical analytical methods in studies on art materials and archaeological objects demonstrated that scientific investigation is an essential tool for acquiring information on the materials that make up an artwork and for assessing their decay, in order to plan restoration approaches. Chemical diagnosis, together with the investigation carried out by historians, archaeologists and art experts, is a valuable contribution to help identify the materials in paintings and ancient artefacts, as well as their state of conservation.

The development of scientific procedures that are able to use very minute samples (a few micrograms), together with the increased availability of advanced analytical instrumentation, have led to great interest in the chemical study of materials used in cultural heritage. This has given rise to a sharp increase in research studies at the interface between art, archaeology, chemistry and the material sciences. As a result, successful multidisciplinary collaborations have flourished among researchers in museums, conservation institutions, universities and scientific laboratories.

Thus, a new science, called Conservation Science, was born. This term came into use in the 1980s and is now widely adopted: the field includes both pure and applied research. Fundamental research is required specifically where knowledge gaps exist, for instance in the behaviour under natural ageing of new synthetic materials used both for restoration and for art purposes.

Organic substances can be identified both as the main constituents of an artwork or a cultural heritage object, and as secondary components, mixed with inorganic compounds. Organic materials can be found in the finish or decoration of the surfaces, or as residues of commodities, such as in ceramic or glass vessels. Moreover, the majority of restoration products applied as consolidants, adhesives, restoration paints and varnishes are of an organic nature.

Investigation into this wide range of substances is crucial for conservation since organic constituents are more prone to degradation due to the effects of light, temperature, moisture, biological agents and environmental oxidizing conditions. Their degradation pathways are thus often key in determining the overall decay of an object.

Over the past decade, particular attention has been focused on the characterization of organic materials occurring, for example, as the residues of food, medicines and balms in archaeology, as adhesives, and as binders in paints. The mixture of many materials in ancient recipes and technologies, and the chemical changes induced by ageing make it even more difficult to study these samples.

In this respect, research relies heavily on structural information at a molecular level, and thus the application of mass spectrometry plays a prominent role. This is mainly due to its

ability to obtain detailed compositional information of complex mixtures. In addition, the possibility of coupling mass spectrometry with chromatographic techniques, such as gas chromatography and high-performance liquid chromatography, make mass spectrometry the most powerful tool to investigate the complex and aged mixtures of organic molecules that are currently encountered as constituents of artistic, historic and archaeological objects.

In recent years, specialized journals and congresses have presented an increasing number of papers and case studies. However, with a few exceptions, due to its specialized and fragmentary nature such information is difficult to access.

This book offers an overview of the use of the techniques based on mass spectrometry for the analysis of organic substances in art and archaeological materials. The fundamental principles are illustrated along with procedures and mass spectrometric techniques. Case studies and examples show how these techniques can be used to reveal the history of the objects and how they were produced. A key issue is the search for fingerprints of the organic materials, which might reveal the techniques and the technologies used in the past. Another is the study of the decay processes of the constituent materials of heritage objects, which is crucial in terms of their conservation.

The examples given in the chapters start from sampling and sample pretreatments, and include data interpretation. Conclusions are then drawn which bring together chemical and archaeological data.

Since many difficult problems arise from the complex composition and alteration pathways of the materials considered, the book begins with a survey of organic materials used both in art and archaeology (Chapter 1) and a discussion of their behaviour under natural and artificial accelerated ageing and burial conditions. To help in understanding the various mass spectrometric methods and instruments, the main concepts in mass spectrometric instrumentation are discussed in Chapter 2. The wide variety of features offered by mass spectrometry makes the range of organic materials that can be studied very broad: it includes small volatile molecules such as monoterpenes in essential oils as well as macromolecules and proteins. Mass spectrometric based techniques are used for the accurate quantitative analysis of specific, well known species, and also for the identification of unknown, unexpected compounds such as degradation products on the basis of the mass spectra. The adoption of proteomic techniques in archaeometry has opened up new horizons in the study of biological residues.

Chapters 3–6 deal with direct mass spectrometric analysis highlighting the suitability of the various techniques in identifying organic materials using only a few micrograms of samples. Due to the intrinsic variability of artefacts produced in different places with more or less specific raw materials and technologies, complex spectra are acquired. Examples of chemometric methods such as principal components analysis (PCA) are thus discussed to extract spectral information for identifying materials.

Chapters 7–13 discuss several analytical approaches based on gas chromatography/mass spectrometry, pyrolysis-gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry. Biomarkers for material identification are assessed and degradation mechanisms are examined, giving an in-depth insight of natural and synthetic resins, proteinaceous binders, plant gums, vegetable oils and waxes.

Finally, Chapters 14–16 deal with innovative applications in this field such as mass spectrometric compound-specific isotopic analysis, secondary ion mass spectrometry

(SIMS) to map specific compounds in cross-sections, and atomic isotopic analysis for dating using accelerator mass spectrometry (AMS).

This multi-authored book is aimed at:

– researchers who exploit analytical chemistry based on mass spectrometry to improve the knowledge and conservation of cultural heritage;
– students in instrumental analyses, chemical science and conservation science;
– archaeologists, art historians and museum conservators who cooperate with scientists in the study of materials.

We hope that this book will provide a useful source of information for people involved in conservation sciences and as a springboard for those who are just starting out in this fascinating field.

Maria Perla Colombini
Francesca Modugno
University of Pisa, Italy

Part I
Introduction

1

Organic Materials in Art and Archaeology

Maria Perla Colombini and Francesca Modugno

1.1 Introduction

Men have always used the natural materials around us to produce functional objects and works of art. Paintings and other objects that are part of our cultural heritage, including textiles, books, sculptures, archaeological objects, furniture and the organic residues found in association with them (e.g. cosmetics, medicines, perfumes, food), contain a wide variety of organic materials from natural to synthetic.

Since ancient times, natural organic materials have been employed as paint binders, adhesives, waterproofing materials and so on, as reported in classical literature by Plinius the Elder and Vitruvius. Archaeological excavations often bring to light a wide variety of objects and materials that have been collected, processed and used by humans over time. Due to their long period underground, some of these materials and objects, especially those of an organic nature, have been partially or totally altered.

Being able to identify natural substances and their degradation products is a challenge. If we manage to do so, we then can shed light on the nature and the origin of the material employed, the artistic techniques used and the state of conservation. Organic materials are more subject to degradation than inorganic ones, so if we can understand their composition then we can ensure that ancient artefacts will remain part of our cultural heritage. This chapter outlines the main organic materials encountered in artistic and archaeological objects, along with their composition, basic behaviour and degradation pathways related to ageing. Table 1.1 summarizes the organic materials and how they were once used.

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Table 1.1 *Category, organic materials and uses*

Category	Organic materials	Uses
Proteins	Egg, milk and casein, animal glue, silk, wool, vegetable proteins (e.g. garlic, beans), human and animal tissues (e.g. mummies)	Paint binders, adhesives, textiles, commodities, parchment
Glycerolipids	Animal fats, vegetable oils (e.g. palm oil, olive oil) including drying oils (e.g. linseed, walnut, poppy seed)	Paint binders, varnishes, illuminants, commodities, ingredients of cosmetic and pharmaceutical preparations
Waxes	Beeswax, spermaceti, Chinese wax, lanolin (animal waxes); carnauba, candelilla, Japan wax, esparto (vegetable waxes); paraffin, ozokerite (fossil waxes)	Paint binders, coatings, sealants, writing tablets, ingredients of cosmetic and pharmaceutical preparations, sculptures
Natural resins	Pine resins, sandarac, copals, mastic, dammar, amber, frankincense, benzoe, styrax, myrrh, (plant resins); shellac (animal resin); tar and pitch (from thermal treatment of plant resins or wood)	Varnishes, coatings, waterproofing materials, paint binders, ingredients of cosmetic and pharmaceutical preparations
Polysaccharide materials	Starch, cellulose, plant gums (arabic gum, tragacanth, karaya, ghatti, guar, locust bean, fruit tree gum)	Paper, paint binders, adhesives
Bituminous materials	Bitumen, asphalt	Moulding materials, adhesive, pigment
Organic dyes	Cochineal, madder, kermes, saffron, purple, indigo, synthethic dyes	Colourants for dyeing textiles, paint materials
Synthetic polymers	Polyacrylates, cellulose nitrate, phenolic resins, polyethylene, poly(vinyl acetate), polystyrene	Paint binders, varnishes, coatings, consolidants, sculptures

1.2 Proteins

Proteins are macromolecules made up of one or more unbranched chains of amino acids which are joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues. Several amino acids are commonly found in animal and vegetable proteins: glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), methionine (Met), proline (Pro), hydroxyproline, (Hyp), threonine (Thr), asparagine (Asn), glutamine (Gln), tyrosine (Tyr), cysteine (Cys), lysine (Lys), arginine (Arg), aspartic acid (Asp), phenylalanine (Phe), tryptophan (Trp), serine (Ser), glutamic acid (Glu), and hystidine (His) [1].

The number and the type of amino acids and their sequence determine the surface charge of the protein, its molecular configuration and its unique chemical and physical properties. The function of a protein is dependent on its three-dimensional structure. A number of agents can disrupt this structure thus denaturing it, for example changes in pH, temperature, salt concentration, and the presence of reducing substances.

Both vegetable and animal proteins are encountered in art as textiles, leather and parchment, paint binders, and adhesives, and in archaeological objects as organic residues of commodities, or of human or animal tissues. Aged proteins are denaturized: as a result of the loss of water and of ageing, the tertiary and quaternary structures change, through the rearrangement of internal bonds between functional groups. Thus, their solubility and reactivity differ from the native ones, whereas in favourable cases the amino acid composition can remain mostly unchanged. Microbic degradation of protein is quite fast in the burial environment, whereas proteins are often present in quite a good state of conservation in the paint layers of paintings.

The determination of the amino acid profile of proteins after hydrolysis of peptide bonds can be used in specific cases for the differentiation and the identification of proteins in paint samples [2–7] . In paintings, animal proteins such as egg, casein and glue, were frequently used as binders for pigments in the tempera technique. Egg was mainly used as whole egg and egg yolk. A whole dry hen egg contains about 45% protein, 41% lipid and 2% cholesterol [2,8]. Milk is an aqueous emulsion of proteins and lipids: dry cow milk contains about 26% protein, 26% lipid, and sugars [2,8]. Casein is obtained by the acidic, enzymatic or thermal treatment of milk, and its main constituents are α-casein, β-casein, δ-casein, and γ-casein. Animal glue was obtained by boiling the skin, bones or cartilaginous parts of mammals and fish. It is made up of collagen, a protein characterised by the presence of a high content of glycine, proline and hydroxyproline [2]. One of the most important vegetable proteins is garlic (*Allium sativum*), a member of the Liliaceae family. Garlic contains 0.1–0.4% volatile oil, carbohydrates (making up 75% of the dry matter), and proteins (15–17% of the dry matter) [9] and was used as an adhesive in gildings [10]. Also plant gums such as arabic gum, which is mainly composed of polysaccharides, contain a minor proteinaceous fraction [11].

Table 1.2 gives the average amino acid composition of some animal and vegetable proteins found in art and archaeology.

During ageing, proteins react with other materials in the historical/archaeological object and, for instance, condensation and cross-linking reactions between proteins and glycerolipids may occur. Amino malonic aldehyde has been identified as a possible product of oxidative degradation of serine, phenylalanine and cysteine [13]. The further oxidation of this compound can lead to the formation of amino malonic acid. This compound has been detected in paint samples, and its presence increases in the course of ageing. Another important factor of protein degradation is pH changes which, in the presence of moisture, can cause the hydrolysis of peptide bonds. As a consequence, the molecular weight may change, and the serine and threonine may dehydrate. Alkaline treatments (commonly used in restoration) can partially hydrolyse proteins and produce cystine and dehydroalanine from cysteine [14]. The formation of oxalate salts on paint surfaces has also been observed, suggesting some kind of photo-oxidation [15]. The reduced solubility of proteins in ancient samples is related to denaturation and cross-linking processes during ageing: cations may act as catalysers for the protein oxidation, thus enhancing this phenomenon. Anaerobic degradation of proteins by micro-organisms may lead to the formation of molecules such as piperidone, benzoic acid and *p*-hydroxyphenylacetate [16].

Proteins are synthesized from L-amino acids. When the living organism has died, they start to spontaneously convert to the D-form through a process called racemization. The extent of racemization is measured by the ratio of D/L isomers and increases as a function of time and temperature, and can be used for geochronology or palaeothermometry. The longer racemization continues, the closer to 1 the ratio between the D- and L-forms

Table 1.2 *Amino acid composition of proteinaceous materials (w/w %) [2,12]*

Amino acid	Egg white	Egg yolk	Casein	Animal glue (collagen)	Wool (keratin)	Silk (fibroin)	Garlic
Glycine	3.6	3.5	1.7	26.6	6.0	42.8	4.9
Alanine	6.3	5.6	2.7	10.3	3.9	33.5	6.2
Valine	8.3	6.4	7.2	2.5	5.5	3.3	5.8
Leucine	10.3	9.2	9.0	3.7	7.9	0.9	5.8
Isoleucine	6.2	5.1	6.0	1.9	3.8	1.1	3.0
Proline	4.5	4.5	13.2	14.4	6.7	0.5	3.1
Phenylalanine	5.2	3.9	5.1	2.3	3.7	1.3	4.9
Tyrosine	1.4	2.8	5.5	1.0	5.2	11.9	2.1
Serine	5.8	9.1	4.0	4.3	8.4	16.3	10.9
Threonine	3.7	5.6	2.7	2.3	6.6	1.4	—
Cystine	1.9	1.9	0.0	0.0	12.8	0.0	—
Methionine	1.2	2.3	2.3	0.9	0.6	0.0	0.8
Arginine	6.8	5.5	4.0	8.2	9.9	1.0	—
Histidine	2.4	2.4	3.6	0.7	3.0	0.4	—
Lysine	8.0	5.7	6.7	4.0	0.9	0.6	6.1
Aspartic acid	10.5	11.5	6.1	6.9	6.9	2.2	16.7
Glutamic acid	13.9	15.0	20.2	11.2	14.5	1.9	29.3
Hydroxyproline	0.0	0.0	0.0	12.8	0.0	0.0	0.3

becomes. Although it is not an absolute dating method, the extent of amino acid racemization has been used to date organic materials such as well-preserved fossils, teeth, bones, egg and mollusc shells, plants, calcium-rich soil sediments, as well as rock paintings and to evaluate the state of degradation of proteinaceous matter. The racemization of some amino acids can also be used to estimate the age of animals at the time of their death [17–20].

1.3 Glycerolipids

Oils and fats are mixtures of triglycerides, also known as triacylglycerols. They are basically esters of glycerol with fatty acids, and contain smaller amounts of other compounds, which include sterols and vitamins.

The physical and chemical properties of individual oils and fats are determined by the nature and proportions of fatty acids that enter into the triglycerides composition. Animal and dairy fat like plant oils are dominated by triacylglycerols, with steroids present as minor components, cholesterol and its esters being the most significant. The triacylglycerols of animal fats differ from plant oils since they contain more of the saturated fatty acids and consequently are solid at room temperature.

The fatty acid percentage composition of some fresh lipids which may be encountered in an archaeological context or in a painting is reported in Table 1.3.

Vegetable oils and dairy and animal fats were used extensively in ancient times in cookery, for lighting, and as ingredients of cosmetics, balms and medications [21–29]. Olive, almond, balanos, castor, coconut, linseed, moringa, palm, poppy, radish, safflower, and sesame oils were well known oleiferous species in the Mediterranean [21,30]. Data on

Table 1.3 *Fatty acid percentage composition of some fresh vegetable oils and of animal lipids*

Oil	Palmitic acid (esadecanoic acid)	Stearic acid (octadecanoic acid)	Oleic acid (9-octadecenoic acid)	Linoleic acid (9,12-octadecadienoic acid)	Linolenic acid (9,12,15-octadecatrienoic acid)	Elaeostearic acid (9,11,13-octadecatrienoic acid)	Ricinoleic acid (12-hydroxy-(*Z*)-9-octadecenoic acid)	Gondoic acid (11-eicosenoic acid)	Erucic acid (13-docosenoic acid)
Linseed	6–8	3–6	14–24	14–19	48–60	—	—		
Walnut	3–7	0.5–3	9–30	57–76	2–16	—	—		
Poppyseed	8–12	2–3	12–17	55–65	3–8	—	—		
Olive	8–18	2–5	56–82	4–19	0.5–1	—	—		
Sunflower	5–6	4–6	17–51	38–74	—	—	—		
Castor	1–2	1–2	3–6	4–7	—	—	83–89		
Tung	3–5	2–4	8–11	12–15	0–3	75–85	—		
Palm	43–46	4–10	35–40	7–10	—	—	—	—	—
Rapeseed	2–6	1–3	20–30	17–22	6–10	—	—	13–16	20–40
Hen's egg	25–27	9–12	38–44	13–15	0–1	—	—	—	—
Lard	20–27	13–19	37–45	7–10	0–1	—	—	—	—

the use of oils are derived from papyri and from texts written by Theophrastus, Dioscorides and Pliny, which help to clarify the identification of plants cultivated for their oily seeds [30]. Lipids radically alter their original chemical composition as a consequence of degradation reactions [29,31,32]. The hydrolysis of triacylglycerols is a common process that leads to the formation of free fatty acids. Monoacylglycerols and diacylglycerols, which are produced by the partial hydrolysis of triacylglycerols, can survive in archaeological samples [32].

Unsaturated and especially polyunsaturated fatty acids in the triacylglycerol molecule are commonly subject to oxidation [33–37] via radical reactions with the inclusion of oxygen in the acyl chain, carbon-carbon bond cleavage, and the formation of lower molecular weight species. This phenomenon causes polymerization and cross-linking processes during the curing of drying oils (linseed oil, poppy seed oil, walnut oil, tung oil), highly polyunsaturated oils widely used as paint binders, varnishes and coatings. It leads to the formation of a polymeric network, generating a solid paint film.

The amount of free fatty acids increases with ageing and reflects the extent of hydrolysis of the triacylglycerols. The uptake of oxygen by double bonds leads to the formation of new oxygen containing functional groups and to the oxidative cleavage of fatty acid hydrocarbon chains. The products of the oxidation processes of lipids are generally α,ω-dicarboxylic fatty acids, hydroxycarboxylic acids and dihydroxycarboxylic acids [32,38,39]. Due to their relatively high solubility in water, which facilitates leaching once they have been buried, they are rarely detected in ancient artefacts [32].

Particular conservation conditions such as very arid environments, the absence of percolating water, and controlled storage conditions (e.g. paintings in museums) mean that relatively high amounts of hydroxyacids can be recovered along with dicarboxylic acids and dihydroxycarboxylic acids [29,39,40–42]. Aged drying oil paint films generally contain substantial amounts of dicarboxylic acids such as pimelic (1,7-heptanedioic, 7di), suberic (1,8-octanedioic, 8di), azelaic (1,9-nonanedioic, 9di) and sebacic (1,10-decanedioic, 10di) acid, with azelaic acid being the most abundant.

The natural degradation processes of lipids can be accelerated or modified if the material is exposed to oxidizing conditions or to high temperatures, which occurs when cooking pottery, with oils used as illuminants, with drying oil prepolymerized by heating before use as paint binders, and with paint layers that are exposed to light and oxygen. Thus, the nature of degradation products depends on the composition of the original material, on the treatment of the material before or during its use, the presence of interacting species in the material, and on the environmental conditions.

It is thus quite difficult to distinguish between different degraded oils and fats on the basis of their fatty acid composition. The similarities in the composition of many vegetable oils used in ancient times and the way they might have been mixed together, means that degraded oils exhibit complex molecular patterns that usually prevent us from identifying the original botanical source.

Nevertheless, there are some vegetable oils that have a very specific composition. For example, castor oil consists of large amounts (83–89%) of 12-hydroxy-(*Z*)-9-octadecenoic acid (ricinoleic acid) which is not found in other natural lipids [21]. Ricinoleic acid produces a very characteristic oxidation product, 9,12-dihydroxyoctadecanoic acid [43], and both of these compounds can be considered as specific biomarkers for castor oil and have been used to assess its presence in ceramic lamps [43] and mummification balms [23].

Other oils show a very distinctive saturated fatty acid profile which in theory could be used for identification purposes in archaeological samples; for example moringa oil, which contains about 8% of long-chain saturated fatty acids (eicosanoic acid and docosanoic acid) which usually survive ageing [21,44], and coconut oil, which mainly consists of saturated triglycerides in which dodecanoic acid (lauric) and tetradecanoic acid (myristic) are the principal fatty acids [21]. All oils obtained from the seeds of Cruciferae such as rapeseed oil, turnip oil and radish oil, which were used in North Africa and in large areas of Europe and Asia [21,45], are characterised by a fatty acid profile showing some distinctive features: 5–15% (*Z,Z*)-9,12-octadecadienoic acid (linoleic acid), 10–30% (*Z*)-9-octadecenoic acid (oleic acid), 5–20% (*Z*)-11-eicosenoic acid (gondoic acid), 20–60% (*Z*)-13-docosenoic acid (erucic acid), 0.1–3% (*Z*)-15-tetracosenoic acid (nervonic acid), and about 4% long chain saturated fatty acids (eicosanoic acid, docosanoic acid, tetracosanoic acid) [21,46]. Mid to long chain α,ω-dicarboxylic fatty acids together with 11,12-dihydroxyeicosanoic acid and 13,14-dihydroxydocosanoic acid in ceramic vessels and oil lamps from Egypt have been reported [29,43,47]. These works demonstrated that the formation of these α,ω-dicarboxylic and dihydroxycarboxylic acids is related to the oxidation of erucic and gondoic acids, susceptible to degradation due to the presence of the double bond, so that a vegetable oil obtained from Brassicaceae seeds was identified.

A particular case is that of lipid materials as paint binders. Over the centuries, painting techniques have restricted the range of materials used to egg and drying oils [2]. The short list of possible candidates means that lipid materials in paint samples can generally be identified on the basis of their fatty acid profile. After ageing, drying oils are characterised by a higher amount of dicarboxylic acids than in egg lipids [40,41,48,49]. Moreover, steroids can survive in their original form or as degraded steroids such as cholesta-3,5-dien-7-one and 7-ketocholesterol [50–53]. Different drying oils can be distinguished on the basis of their palmitic over stearic acid (C16:0/C18:0) ratio [2,40]. This parameter is not significant in the case of mixtures of binders such as in the presence of *tempera grassa* (whole/yolk egg and drying oil) or in the presence of beeswax.

With regard to animal and dairy fats, the ratio of C16:0/C18:0 fatty acids has been used to identify animal fats and distinguish them from plant oils [21,43,54,55]. A content of C16:0 lower than that of C18:0 generally indicates an animal fat. The presence of odd numbered carbon straight chain fatty acids (C15, C17 and C19) and of significant amounts of branched chain fatty acids are considered characteristic of ruminant fats (sheep, cattle, goats, etc.) [56,57]. Ruminant fats also display a complex mixture of positional isomers of octadecenoic acid, resulting from the biohydrogenation of unsaturated dietary fatty acids in the rumen, characterised by double bonds located in various positions. This means a ruminant fat can be distinguished from non-ruminant fats (such as pig), which contain only the C18:1 Δ9 isomer [56,57].

Lipids from marine products have been studied less frequently. The detection of ω-(*o*-alkylphenyl)alkanoic acids with 16, 18 and 20 carbon atoms together with isoprenoid fatty acids (4,8,12-trimethyltetradecanoic acid and phytanic acid) and substantial quantities of bones from fish and molluscs has provided evidence for the processing of marine animal products in vessels [58–60]. C16, C18, and C20 ω-(*o*-alkylphenyl)alkanoic acids are presumed to be formed during the heating of tri-unsaturated fatty acids (C16:3, C18:3 and C20:3), fatty acyl components of marine lipids, involving alkali isomerization, pericyclic (intermolecular Diels-Alder reaction) and aromatization reactions.

1.4 Natural Waxes

Natural waxes are highly heterogeneous lipid materials containing esters of long chain carboxylic acids, which are solid at room temperature and highly hydrophobic. Waxes of animal origin (beeswax, Chinese wax, lanolin, spermaceti wax), vegetable origin (carnauba, candelilla, esparto wax, Japan wax) and fossil waxes (paraffin wax, montan wax, ceresine) [2] have been used for many purposes such as sealants, surface coatings and polishes, casting and modelling materials, ingredients of balms and cosmetics, and lighting candles. Some chemical and physical features of natural waxes are reported in Table 1.4, and more details can be found in Chapter 4. In addition to natural waxes, a wide variety of synthetic waxes are used in restoration such as silicon waxes and polyethylene glycol.

1.4.1 Animal Waxes

Beeswax, obtained from the hives of bees, is the most commonly used natural wax for manufacturing works of art. Since prehistory, beeswax has been used as a waterproofing and sealing agent. The Egyptians used it in balms for mummies, in shipbuilding, to polish the surface of paintings, for lighting, and to make statues and writing tablets [2,61–66]. It was used by the Greeks and Romans to waterproof stone surfaces, as a protective agent and as a varnish [67]. Until the Middle Ages beeswax was used as a binder in a painting technique referred to as the encaustic technique [65,67]. Between the seventeenth and twentieth centuries the ceroplastic technique was developed for the realization of anatomical sculptures and botanical models [68].

Table 1.4 *Chemical and physical features of natural waxes*

Wax	Melting range (°C)	Saponification number[a]	Iodine number[b]
Animal			
Beeswax	66–71	17–21	8–11
Chinese wax	80–83	11–15	1–2
Spermaceti	42–50	1–3	3–4
Lanolin	35–42		18–36
Ambergris	60–80	—	—
Vegetable			
Carnauba	82–86	4–8	12–15
Candelilla	67–79	16	14–37
Japan wax	50–60	206–237	4–13
Mineral			
Ceresin	54–77		7–9
Montan	76–92	23–27	10–16
Paraffin	46–68	—	—

[a]The saponification number is an indication of the number of acidic functionalities, and is the amount of KOH or NaOH (in mg) required to neutralize the acids in 1 g of lipid material.

[b]The iodine number is an indication of the degree of unsaturation of triglycerides, and is the amount of iodine (in mg) required to react with 1 g of lipid material.

The qualitative average composition of beeswax is quite constant and is made up of hydrocarbons (14%), monoesters (35%), diesters (14%), triesters (3%), hydroxymonoesters (4%), hydroxypolyesters (8%), monoacid esters (1%), acid polyesters (2%), free acids (12%) and free alcohols (1%) [2,69–73].

The aliphatic chains of beeswax compounds are mainly saturated and consequently extremely resistant to ageing. However, depending on the treatments undergone by the wax and the conservation conditions, some modifications to the original composition may occur. Since beeswax is solid at room temperature, thermal treatments were very commonly used to obtain a softened material to act as a binding medium or to be mixed with other materials. As a result, partial sublimation of the constituents can occur, leading to a change in the relative amounts of alkanes and esters [61,71,74–77]. Moreover, ambient humidity can cause a partial hydrolysis of beeswax esters, leading to the formation of free palmitic acid and long chain alcohols, which may also partially sublime, leading to changes in the acid and alcohol profiles.

Chinese wax is a white to yellowish-white, gelatinous, crystalline, water-insoluble substance obtained from the secretion of the scaled insect *Coccus ceriferus*, common in China and India. Chinese wax is used chiefly in the manufacture of polishes, sizes, and candles and is traditionally employed in Chinese medicine. It is basically made up of ceryl cerotate (esacosanoyl esacosanoate) and esacosanol [78,79].

Spermaceti wax (ambergris) is obtained from the precious oil in the head cavity of the sperm whale *Physeter macrocephalus*, and was used chiefly in balms, ointments, cosmetic creams, fine wax candles, pomades, textile finishing, and as a fuel for oil burning lamps. Nowadays, due to the current ban on whaling, authentic spermaceti wax is unavailable and the synthetic cetyl esters wax is used as a replacement for the naturally occurring material. The spermaceti wax composition includes cetyl palmitate (esadecanoyl esadecanoate), cetyl myristate, cetyl laurate, octyl stearate, octyl palmitate and cetyl alcohol [80,81]. Ambergris occurs as a biliary secretion of the intestines of the sperm whale and contains 46% cholestanol-type sterols [82].

Lanolin is a wax secreted by the sebaceous glands of sheep; it is obtained from wool and it has been used as a lubricant and as an ingredient in pharmaceutical preparations. It contains esters of long chain alkanoic acids, both linear and branched, and of hydroxyacids, cholesterol and lanosterol [2,83].

1.4.2 Plant Waxes

These waxes are biosynthesized from plants and mainly contain esters made from long chain alcohols (C_{22}–C_{34}) and fatty acids with even carbon numbers.

Carnauba wax is obtained from the leaves of several species of palm trees in South America, such as *Copernicia cerife*ra which grows in Brazil. It is made up of esters of long chain alcohols and acids with high carbon number, high molecular weight polyesters of hydroxyacids, and derivatives of *p*-hydroxy- and *p*-methoxycinnamic acid [84].

Ouricuri wax is an exudate on the underside of the leaves of the *Syagrus coronata* palm in northeastern Brazil and has similar properties and composition to Carnauba wax. Ouricouri wax is used in the manufacture of carbon paper, mould release agents and inks.

Candelilla wax is extracted from *Euphorbia cerifera* and *Euphorbia antisyphilitica*, which grow mainly in Mexico. The wax is collected from the root surface where the wax

acts as a protective coating. It is a dark yellow, hard and fragile solid, and it has been used to harden other waxes such as beeswax. Candelilla wax is used as a polishing material and in the manufacture of candles and sealing paper. Its main constituents are hydrocarbons (about 50%, between C29 and C33), esters (28–29%), alcohols, free fatty acids (7–9%) and triterpenoid esters (12–14%). Entriacontane and miricylic alchol [$CH_3(CH_2)_{28}CH_2OH$] are the most abundant and characteristic compounds.

Many other plant waxes have also been exploited for various uses, including esparto wax, from esparto grass *Stipa tenacissima*, and Japan wax, from plants of the *Rhus* species.

1.4.3 Fossil Waxes

Ceresine is the white end-product of the purification of the fossil wax ozokerite, which is found in Miocene lignite deposits at considerable depths, by the separation of foreign and resinous matter and decolorisation by active agents. It is harder than paraffin wax, and has linear and cyclic hydrocarbons with high molecular weight [2]. It is used for waterproofing and oil absorption.

Paraffin waxes are also considered of mineral origin and are obtained from petroleum. The petroleum is distilled and the white colour of the wax is obtained by acid washing and purification. It has a typical melting point between about 47 °C and 64 °C. Its uses include candle making, casting and as a solidifier/stabilizer. The wax is composed of C20–C36 n-alkanes (40–90%), isoalkanes and cycloalkanes.

Montan wax is obtained by solvent extraction of certain types of lignite or brown coal. It has a dark colour when not treated, but it is lighter when refined. Its chemical composition includes esters of C22–C32 acids (53%), free acids (17%), free alcohols (1–2%), ketones (3–6%) and terpenoids (20–23%) [85].

Microcrystalline wax is found worldwide as a constituent of crude oil. It is removed by solvent extraction and distillation. The colour varies, depending on grade, from white to brown–black. It has many uses, including waterproofing paper and textiles, and as a sealant. This wax consists of a mixture of long chain (C41–C57) unsaturated hydrocarbons with an average molecular weight of 500–800.

1.5 Natural Resins

Plant resins are lipid-soluble mixtures of volatile and nonvolatile terpenoid and/or phenolic secondary compounds that are usually secreted in specialized structures located either internally or on the surface of the plant. Although terpenoid resins constitute the majority of the resins produced and used, some other important resins are phenolic. Phenolic resin components, which occur on the surfaces of plant organs, have been used particularly in medicines [86].

Natural terpenoid resins and resinous materials played a prominent role in ancient times – their intrinsic properties meant that they were used as adhesives, hydro-repellents, and coating and sealing agents [87–92]. They produced incense when burnt [92] and due to their antitoxic and antioxidant properties, they were also added to wine. In ancient Egypt, vegetable resins along with other natural organic compounds such as waxes, gums, oils and bitumen, were used to prepare mummification balms [23,88,93–97]. Resins and wood

from birch, pine and firs were used to produce tar and pitch in various regions of Europe and the Mediterranean.

Natural resins are substances with a high viscosity, semisolids or solid and insoluble in water. They are formed in the so-called 'resiniferous canals' of several trees. Many varieties of plants spontaneously exude resins as a product of their metabolism, to protect themselves against excessive loss of water and attack from micro-organisms.

From a chemical point of view, vegetable resins are a complex mixture of mono-, sesqui-, di- and triterpenes, which have, respectively, 10, 15, 20 and 30 carbon atoms per molecule. The mono- and sesquiterpenes are both present in most resins. The di- and triterpenes are rarely found together in the same resin, which means that terpenic resins can be divided into two main classes. Table 1.5 lists the botanical origin and the kind of terpenoid compounds of some natural resins.

Mono- and sesquiterpenoids are of limited use for the identification and classification of aged resins. Due to their volatility, they are rarely found in ancient samples except when they have been conserved in very particular conditions [88,98]. On the other hand, the di- and triterpenoids enable us to identify resins thereby identifying their botanical origin [2,99]. Figures 1.1 and 1.2 show the main diterpenoid and triterpenoid structures.

Table 1.5 *Botanical origin and chemical composition of terpenic resins*

Class	Family	Genus (type of resin)	Composition
Coniferales	Pinaceae	*Pinus* (pine resin, colophony)	Abietadienic acids, pimaradienic acids
		Abies (Strasbourg turpentine)	Abietadienic acids, pimaradienic acids, *cis*-abienol
		Larix (Venice turpentine)	Abietadienic acids, pimaradienic acids, epimanool, larixol, larixyl acetate
	Cupressaceae	*Juniper, Cupressus, Tetraclinis articulata* (sandarac)	Pimaradienic acids (sandaracopimaric acid), communic acid, totarol
Guttiferales	Dipterocarpaceae	*Hopea* (dammar)	Dammaranes (hydroxydammarenone, dammaradienol), ursanes (ursonic acid, ursonaldehyde)
Terebinthales	Anacardiaceae	*Pistacia* (mastic)	Euphanes (masticadienonic acid, isomasticadienonic acid), oleanananes (oleanonic acid, moronic acid), dammaranes
	Burseraceae	*Commiphora* (myrrh)	α- and β-amyrin, euphanes, oleananes
		Boswellia (olibanum or frankincense)	
		Canarium (elemi)	

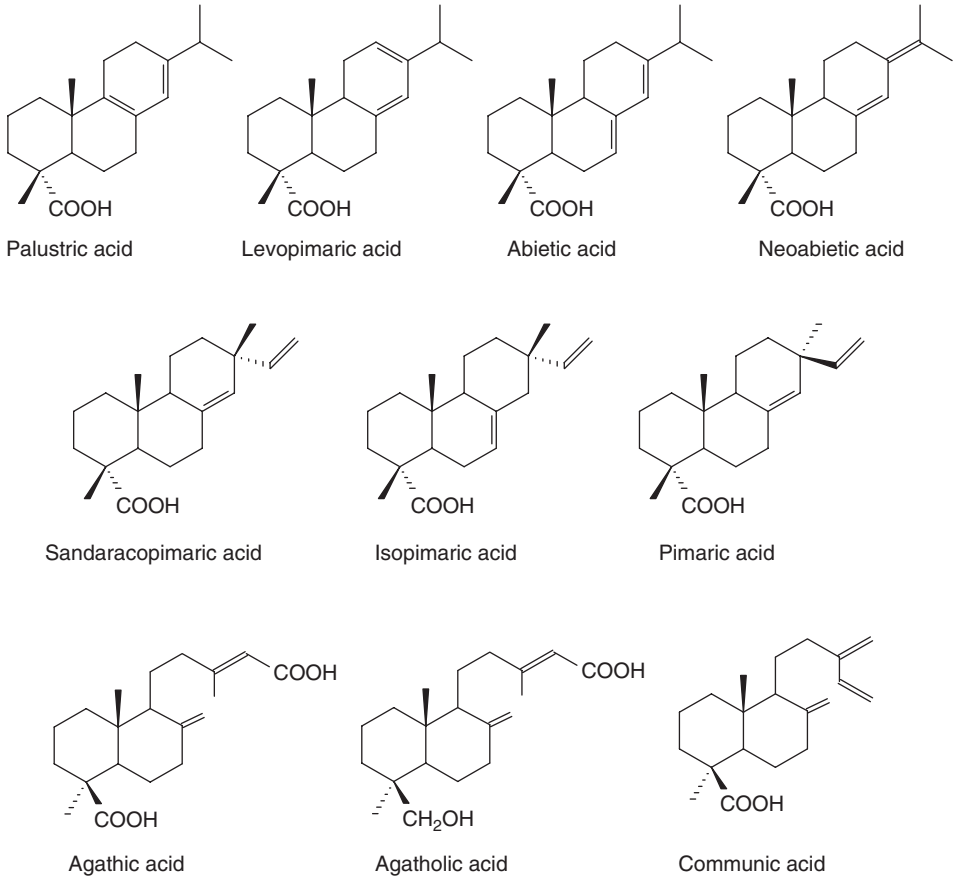

Figure 1.1 *Characteristic diterpenoid compounds of Pinaceae and Cupressaceae resins*

Diterpenoid and triterpenoids in natural resins generally lead to one, two or three oxygen atoms in the form of acidic, carboxylic or alcoholic functionality and a variable degree of unsaturation.

1.5.1 Diterpenoid Resins

The plants that exude diterpenoid resins belong to the order of conifers. Pine resins (from the *Pinus* genus), Strasburg turpentine (from the *Abies* genus), Venice turpentine (from *Larix decidua*) were extracted from Pinaceae. Sandarac, juniper and cypress resins were extracted from trees of the Cupressaceae family: *Tetraclinis articulata*, *Juniperus* spp. and *Cupressus sempervirens*, respectively. Moreover, labdanum resin from the Cistaceae family (*Cistus* spp.) also belongs to the diterpenoid resins.

Pine resin, namely rosin or colophony, is one of the most widespread diterpenoid resins and has been used for waterproofing, for treating wood and paper, as varnish, as incense and as an ingredient in scented ointments. The main compounds present in fresh Pinaceae resins