Anal. Chem. **2002,** *74,* 965−975

Identification of Archaeological Adhesives Using Direct Inlet Electron Ionization Mass Spectrometry

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Adhesives made from natural substances such as resins, tars, and waxes are found during excavations on archaeological sites dating back to prehistoric periods. Until now, their analysis was mainly performed by gas chromatography, possibly coupled to mass spectrometry, after extraction, purification, and derivatization of the samples. To minimize sampling and sample preparation of ancient organic remains, which are often preserved in tiny amounts, we have directly analyzed archaeological samples from Bronze and Iron Age periods by direct inlet electron ionization mass spectrometry. A series of contemporary natural and synthetic substances, including pine and pistacia resins, birch bark tar, beeswax, and plant oils, possibly used for adhesive fabrication during ancient times, was also investigated with the same technique as reference materials. Despite the complexity of their chemical composition, pine resin and birch bark tar were clearly identified in archaeological samples. Furthermore, mass spectrometry has been shown to be efficient for the identification of glues made of a mixture of beeswax, presenting a series of mass spectral peaks assigned to long-chain esters, and birch bark tar, whose mass spectrum presents characteristic peaks of lupane compounds. The intentional mixing of birch bark tar and beeswax during prehistory is reported here for the first time.

Adhesive materials have been produced as early as prehistoric times, 45 000 years ago.[1,2] Despite their high sensitivity to natural biodegradation processes linked to their organic composition, some of them are still preserved on archaeological objects[3−10] or in works of art.[11] Since the Neolithic period (ca. 5500−2000 years before Christ in western continental Europe), they have been used for hafting arrowheads or other bone and flint tools,[1,3,8] for repairing ceramic vessels,[5] or, later, for embalming mummies in Egypt.[12] Their study has focused on the chemical identification of natural substances involved in their fabrication in order to understand the way they were produced and to enhance the conservation, restoration, and storage procedures in museums. Analyses of adhesives, dating back from the Middle Palaeolithic to the Roman period, have already been performed using gas chromatography/mass spectrometry.[1−10] However, such analyses are necessarily preceded by extraction, purification, and derivatization of the samples. Because a wide range of natural products may have been used to produce adhesives, sample preparation and analytical conditions, especially for gas chromatography, have to be adapted to the specificity of each material and several analyses are usually necessary to determine the molecular composition of ancient adhesives. In the case of very tiny and precious archaeological samples, for which a single analysis is permitted, a method minimizing sampling and allowing the identification of several organic materials mixed in a same adhesive is still needed.

The present work, based on direct inlet mass spectrometry of archaeological organic residues from Bronze and Iron Age periods, reveals the use of a wide range of natural substances and methods of fabrication for the production of adhesives in ancient times. To better interpret the results obtained on archaeological samples, a series of contemporary natural products possibly used in the making of ancient glues was also investigated. Among the contemporary natural substances, pine and pistacia resins, beeswax, and plant oils were analyzed by direct inlet electron ionization mass spectrometry. Birch bark tar, a synthetic adhesive, which is known to have been produced since the Middle

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10.1021/ac0155862 CCC: $22.00
Published on Web 02/02/2002

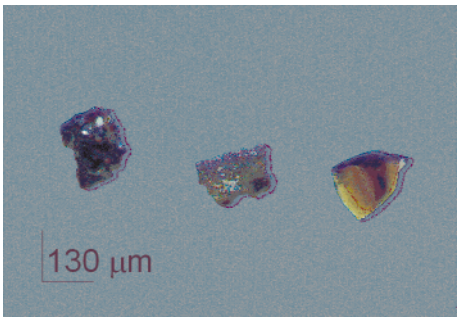

Figure 1. Microsamples of organic archaeological residues analyzed by direct inlet mass spectrometry. From left to right: black residue from a chape of a sword (first Iron Age, Moselle, France), brownish residue adhering to a ceramic vessel from the site of Grand Aunay (second Iron Age, Sarthe, France), and translucent orange-yellowish residue removed from a ceramic vessel on the Bronze Age site of La Fangade (Herault, France). © Odile Guillon, *Centre de Recherche et de Restauration des Musées de France*.

Palaeolithic in Europe,[2] has been experimentally produced in the laboratory. The birch bark tar obtained was used as a contemporary reference and submitted to mass spectrometric analysis. The mass spectra obtained were found to be characteristic of each material and allowed us to clearly identify the nature of ancient adhesives using a microsample without any chemical preparation.

EXPERIMENTAL SECTION

Pine (*Pinus sylvestris*) and pistacia (*Pistacia lentiscus*) resins and oils (olive, linen seed, and poppy seed oils) were sampled in the collection of natural substances available in the laboratory. Beeswax was provided by an apiarist working in Gironde (Lugos, France).

Birch bark tar was produced in the laboratory by heating 1 g of outer white birch bark, previously cut in small pieces, in a glass tube with a Bunsen flame. The temperature was estimated to be ~600 °C using an Fe−Ni thermocouple gauge. After a few minutes, a tarry black and odoriferous material was formed at the bottom of the tube which was thereafter analyzed by mass spectrometry.

One molecular constituent of beeswax was synthesized in the laboratory in order to obtain its mass spectrum. The procedure was adapted from previous publications[13,14] as follows: a mixture of palmitic acid (10 μL of a 100 μmol/mL solution in CH_2Cl_2, 1 μmol), tetracosan-1-ol (100 μL of a 10 μmol/mL solution in CH_2Cl_2, 1 μmol), (dimethylamino)pyridine (DMAP, 0.05 μmol), and *N*,*N*-dicyclohexylcarbodiimide (DCC, 10 μL of a 100 μmol/mL solution in CH_2Cl_2, 1 μmol) is evaporated under a stream of nitrogen until the evaporation of half of the solvent and then stirred continuously at room temperature for 2 h. The organic phase is washed with a saturated solution of citric acid (3 × 1 mL) and with a saturated solution of $NaHCO_3$ (3 × 1 mL), dried on $MgSO_4$, and filtered before analysis by mass spectrometry. All the solvents were of HPLC grade and the products were purchased from Sigma-Aldrich.

The mass spectra were recorded on a Finnigan GCQ mass spectrometer by electron ionization at 70 eV. The samples were placed in a sample vial fixed on the probe. The probe was programmed in temperature as follows: 50 °C for 30 s, from 50 to 350 °C in 2 min, and 120 s at 350 °C. The mass range was scanned from 50 to 950 for all samples and then on a more restricted range in order to get better information on the mass pattern of samples characterized by the absence of high molecular mass. The source temperature was fixed at 200 °C.

The natural substance model compounds were analyzed after dissolution in dichloromethane. The concentration of the solutions obtained was 1 mg/mL, and a volume of 1 μL of each solution was introduced in the sample vial.

Archaeological organic remains were found adhering to either ceramic vessels or bronze objects. They were black, brown, or orange amorphous residues. To avoid any surface contamination due to contact with archaeological sediment or to handling of the

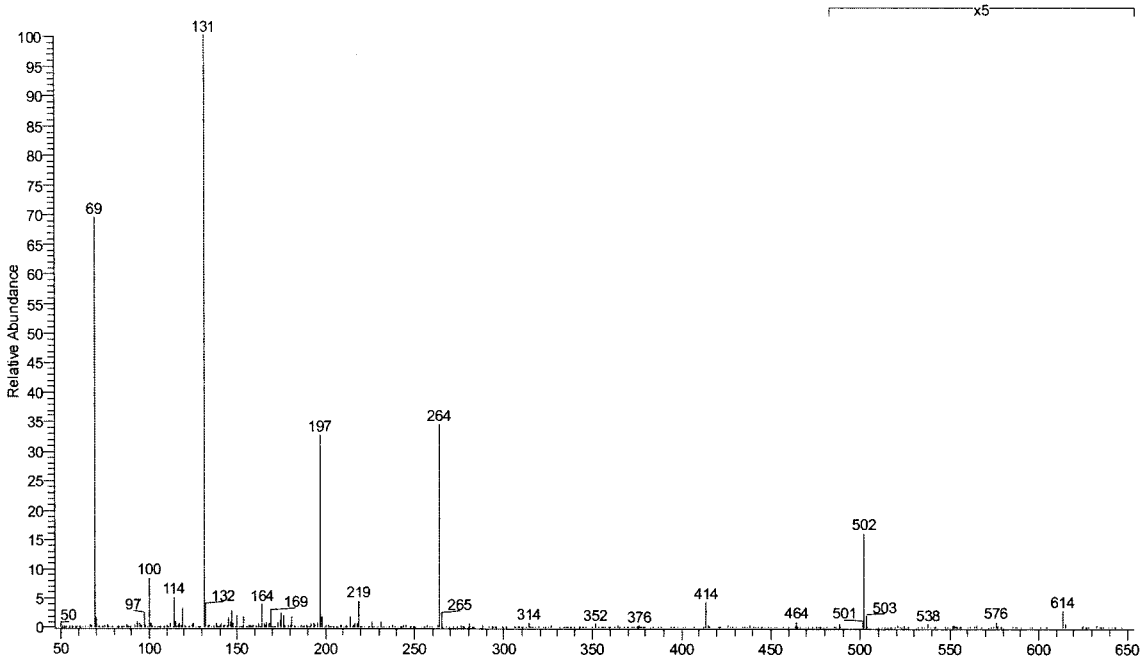

Figure 2. Mass spectrum of the calibration gas perfluorotributylamine (PFTBA; FC43) provided by the Finnigan GCQ mass spectrometer.

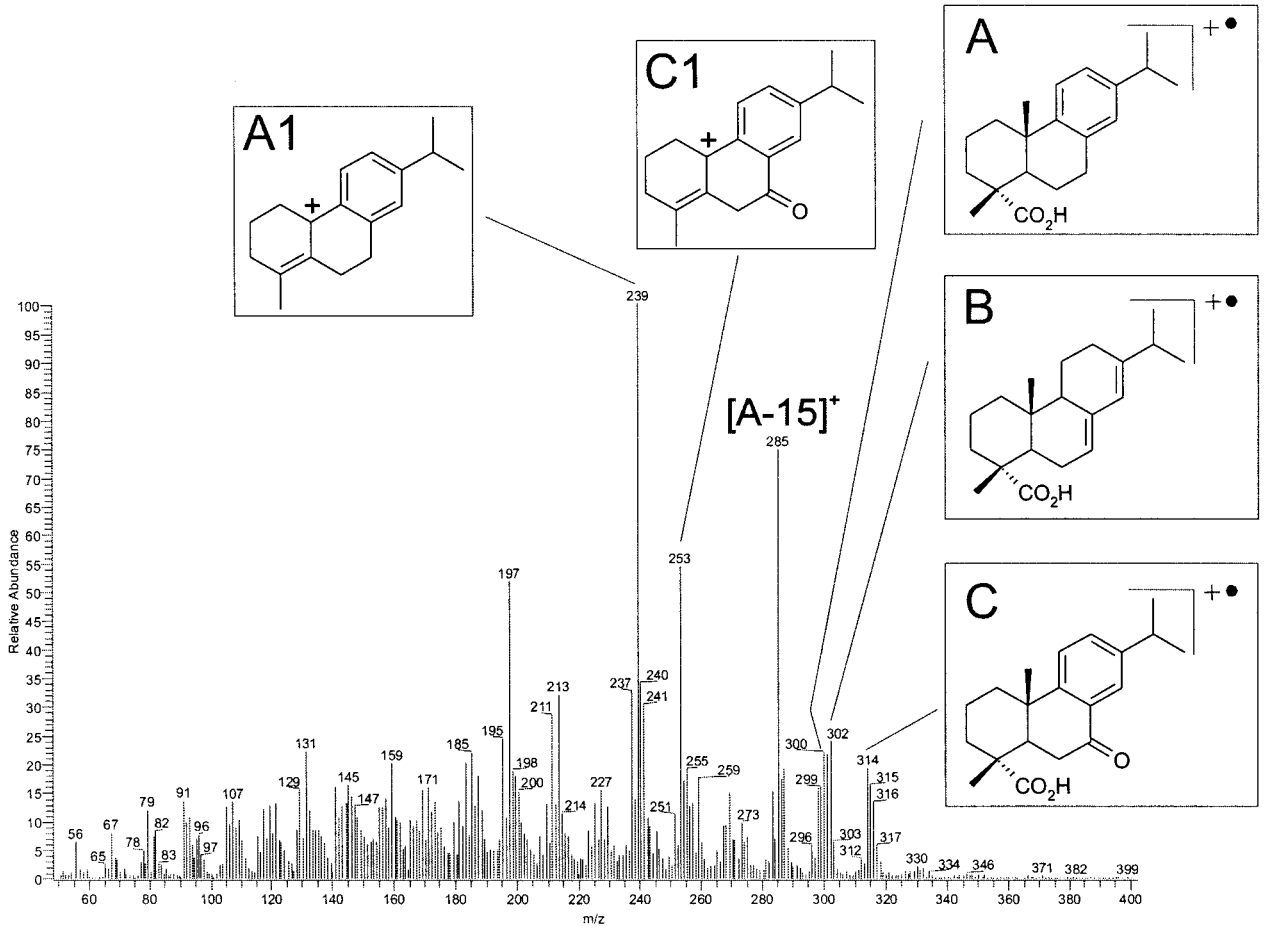

Figure 3. Mass spectrum of a pine resin (*Pinus sylvestris*) obtained by direct inlet electron ionization mass spectrometry using an *m*/*z* scan range of 50−400. The diterpenoid compounds are as follows: (A) dehydroabietic acid, (B) abietic acid, and (C) 7-oxodehydroabietic acid. A1 and C1 correspond to characteristic fragments of, respectively, dehydroabietic acid and 7-oxodehydroabietic acid.

preceding sample, the residues were first cautiously cleaned by scraping with a sterile scalpel blade under a microscope. A microsample measuring from 100 to 200 μm long was then removed with the head of the scalpel blade and placed into the sample vial for mass spectrometric analysis (Figure 1). Because of the very tiny size of the samples, this operation was performed under an optical microscope. These solid microsamples were directly analyzed by mass spectrometry immediately after sampling with no further preparation.

RESULTS AND DISCUSSION

Mass Spectra of Contemporary Reference Samples. All the contemporary samples analyzed by direct inlet mass spectrometry provided very complex mass spectra because of the large number of molecular components present in natural substances. However, despite this complexity, these materials were characterized by different mass spectral patterns.

Before presenting the mass spectra obtained on natural substances, it appears interesting to provide the mass spectrum of the calibration gas, perfluorotributylamine (PFTBA; FC43) (Figure 2). Indeed, the Finnigan GCQ mass spectrometer, equipped with an ion trap analyzer, is noted for its discrimination against ions with *m*/*z* values below 100 and the spectrum shown in Figure 2 would help in providing a proper perspective for the other spectra presented here. In particular, one must note that the base peak is at *m*/*z* 131 whereas it is observed at *m*/*z* 69 with a magnetic sector instrument. The peak at *m*/*z* 197, also present on the reference mass spectrum produced by ThermoFinnigan, must be noted since it is absent from the mass spectrum obtained with a quadrupole or a magnetic analyzer. To the best of our knowledge, there is no report about this ion in the literature. MS/MS experiments on the GCQ showed that ion *m*/*z* 197 fragments to ions *m*/*z* 169 ($C_3F_7^+$), 147, and 119 ($C_2F_5^+$). From the CO losses (*m*/*z* 197 to 169 and 147 to 119) and the usual peaks in FC43 (*m*/*z* 181 and 131), we can guess that ion *m*/*z* 197 results from the addition of one oxygen atom to ion *m*/*z* 181 ($C_4F_7^+$), leading to attribution of the elemental composition $C_4F_7O^+$ to ion *m*/*z* 197. Ion *m*/*z* 181 is produced by dissociation of the ion *m*/*z* 219 ($C_4F_9^+$), which is particularly fragile[15] and very small in the GCQ tuning spectrum during the transfer between the electron impact source and the ion trap.

The mass spectrum of pine resin (Figure 3) presents a base peak at *m*/*z* 239 and peaks at *m*/*z* 253 and 285 with high intensity

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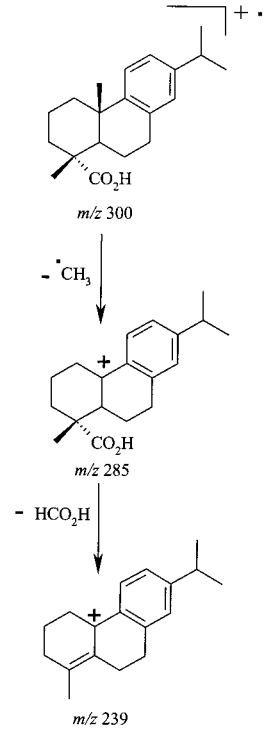
of, respectively, 54 and 74% of the base peak. The ions detected in the region m/z 300−320 of the mass spectrum are consistent with the molecular composition of pine resins, which mainly contain tricyclic diterpenoid acids with abietane, pimarane, and isopimarane skeletons, abietic acid being predominant in pine resin.[16] All these acids are characterized by a molecular weight of 302. The peak at m/z 300 corresponds to the molecular weight of dehydroabietic acid, a degradation marker of abietic acid, but it could also be formed under the electron ionization of abietic acid in the source of the mass spectrometer. However, the ratio of peaks at m/z 302 and 300 in the mass spectrum of pine resin is of 85/100 whereas it is 7/100 in that of abietic acid under the same experimental conditions. This observation leads us to conclude that the peak at m/z 300 mainly results from the presence of dehydroabietic acid in the resin. This compound naturally occurs in conifer resins but is also known to be formed from abietic acid by the oxidative process named dehydrogenation.[16−20] It may also be formed by heating pine resin, but this hypothesis has to be excluded here because the reference sample is a pine resin that has not been heated before. This attribution is reinforced by the presence of the fragments at m/z 285 and 239, respectively, formed by the loss of a methyl radical from the molecular ion of dehydroabietic acid and by the successive loss of the methyl group in position 20 and the neutral fragment [HCO_2H] as shown in Scheme 1.[21,22] The 7-oxodehydroabietic acid, an oxidation product of abietic acid,[16,21,23] can be considered as responsible for the formation of the molecular ion peak at m/z 314. The fragmentation of this radical ion, with the pathway similar to that described in Scheme 1, is responsible for the formation of the fragment ion at m/z 253.[22] The mass spectrum obtained by direct inlet electron ionization of a pine resin clearly exhibits characteristic peaks of diterpenoid components and gives evidence for the degradation processes that occurred through time by aromatization and oxidation of the main constituent.

Both pistacia resin and birch bark tar present several similarities in the mass range 50−300, but their patterns fairly differ for m/z higher than 400 (Figures 4 and 5). These two materials contain triterpenoid components, characterized by a common structure on rings A, B, and C, which belong to three different skeletons: lupane, lanostane, and oleanane (Figure 6).[25,26] The ion at m/z 454 on the mass spectrum of the pistacia resin corresponds to the molecular ion of the main triterpenoid acids present in this resin, namely, moronic, mastica, and isomastica-

Scheme 1. Fragmentation Mechanism Responsible for the Formation of the *m/z* 239 Fragment in Dehydroabietic Acid from Diterpenoid Pine Resins[21]



dienoic acids (Figure 6).[16,29−31] This resin also produces a significant fragment at m/z 439 formed by the loss of a methyl radical from the molecular ion of the main biomarkers. In the case of the birch bark tar mass spectrum, the region in the range m/z 420−450 presents characteristic ions that can be attributed to the molecular ions of three triterpenoid components from the lupane family, betulin (m/z 442), lupeol (m/z 426), and lupenone (m/z 424), which are known as biomarkers of birch bark tar (Figure 6).[3,5,7,8,27,28] The other fragments above m/z 390 are formed by the loss of a methyl group from lupeol (m/z 411) and lupenone (m/z 409) or by the loss of $^{\bullet}CH_2OH$ and H_2O (m/z 393) from betulin in birch bark tar.

The main peaks observed in the mass range 50−300 on spectra of both birch bark tar and pistacia resin at m/z 189 and 203 are related to their triterpenoid composition.[24,25] The base peak of the mass spectrum of birch bark tar at m/z 189 arises by fragmentation of the ring system[24] followed by the loss of a neutral fragment H_2O. In the case of pistacia resin, the presence of oleanoic acid, a triterpenoid biomarker with a C−C double bond at position 12−13, is responsible for a retro Diels−Alder fragmentation of ring C (radical ion at m/z 248), followed by the loss of $^{\bullet}CO_2H$ from ring E. This fragmentation pathway gives rise to the base peak at m/z 203.[24]

Although birch bark tar, pistacia, and pine resins contain biomarkers characterized by quite similar terpenoid structures,

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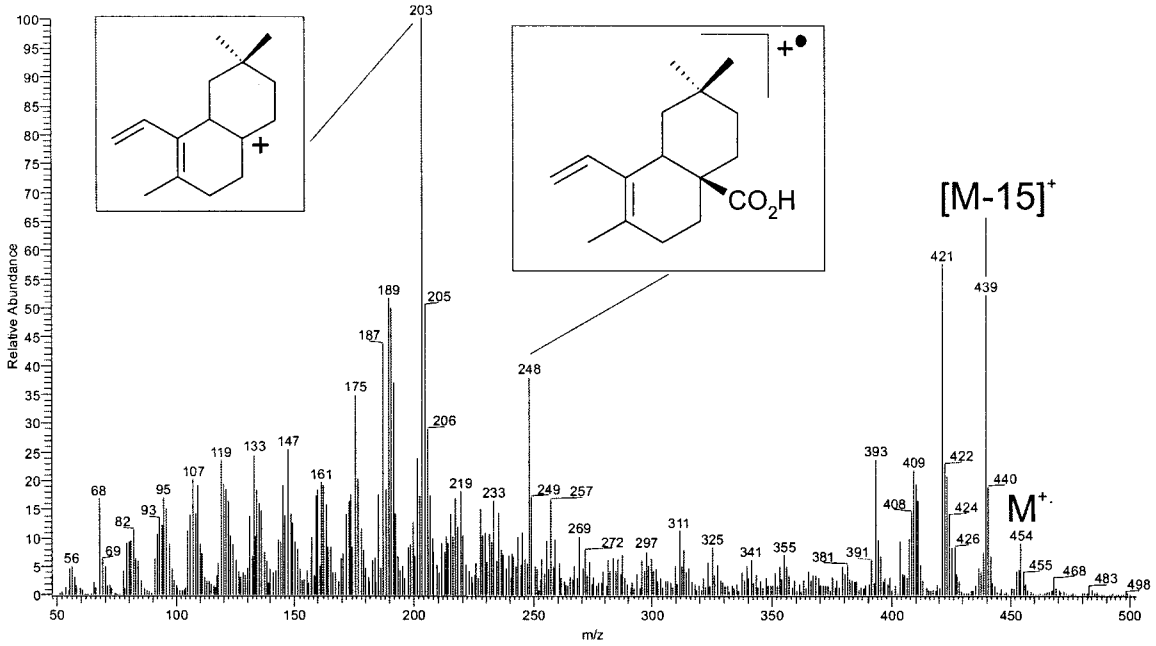


Figure 4. Mass spectrum of a contemporary pistacia resin obtained by direct inlet electron ionization mass spectrometry using an *m*/*z* scan range of 50−500. M corresponds to the molecular weight of the main triterpenoids.

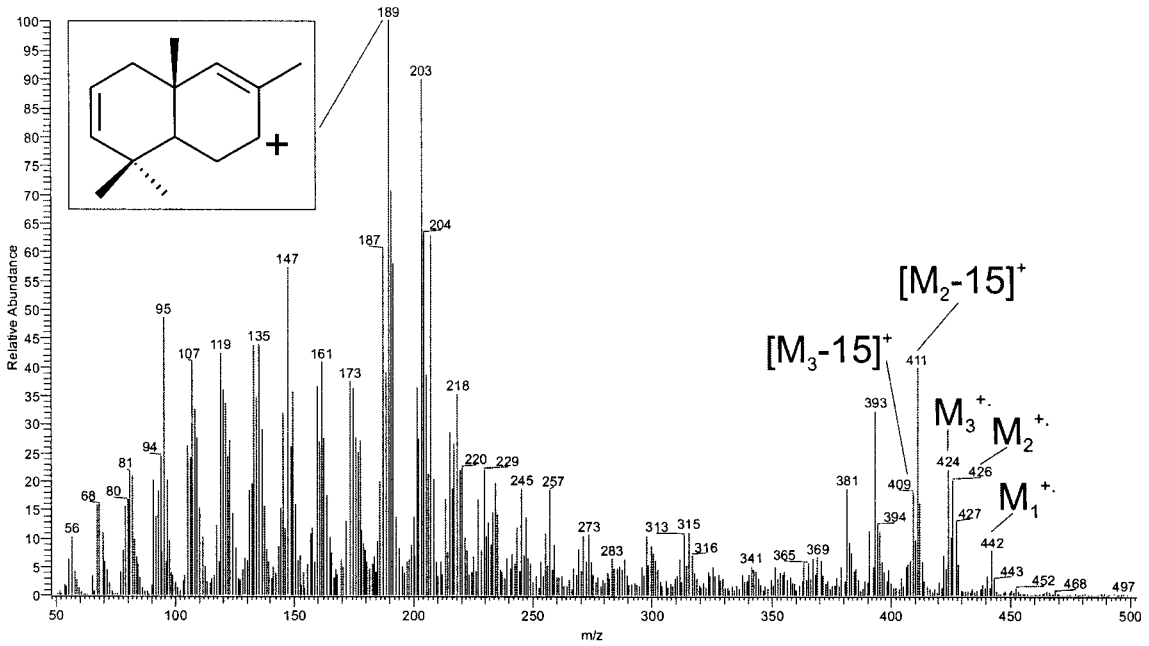
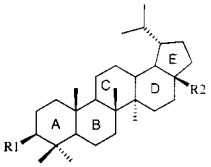


Figure 5. Mass spectrum of synthetic birch bark tar obtained by direct inlet electron ionization using an *m*/*z* scan range of 50−500. M1, M2, and M3 respectively correspond to biomarkers of molecular weight 442 (betulin), 426 (lupeol), and 424 (lupenone).

these results clearly show that mass spectrometry is a powerful technique to characterize di- and triterpenoid materials but also to distinguish different botanical sources containing biomarkers presenting very similar triterpenoid skeletons with different molecular weights. Moreover, the results obtained on pine resin provide information on the presence of degradation markers formed by chemical transformation of resin through time. It may thus be assumed that direct inlet electron ionization mass spectrometry of ancient samples will not only permit one to identify tars and resins involved in adhesive fabrication but also to assess their degree of degradation or transformation under anthropogenic actions.

If plant tars and resins may have been used as major commodities in ancient glues for their adhesive properties, other materials that played the role of plasticizers or binders may have been added to improve the quality of the glue. To identify such materials in archaeological adhesives, beeswax and different plant oils were also analyzed by direct inlet electron ionization mass spectrometry.

The beeswax mass spectrum is dominated by two main peaks at *m*/*z* 256 (base peak) and 257 (Figure 7). In the low-*m*/*z* range of the spectrum, it presents a series of peaks regularly spaced by 14 mass units, indicative of the presence of alkane structure. The region of high *m*/*z* values, above 500, shows a specific pattern of

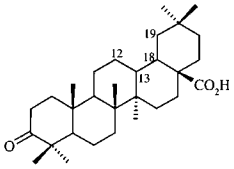


Lupane skeleton

betulin R1: OH, R2: CH_2OH

lupeol R1: OH, R2: CH_3

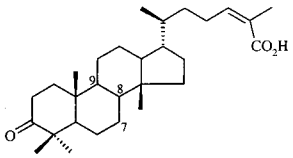
lupenone R1: =O, R2: CH_3



Oleanane skeleton

moronic acid: double bond C-C on position 18-19

oleanoic acid: double bond C-C on position 12-13



Lanostane skeleton

masticadienoic acid: double bond C-C on position 7-8

isomarticadienoic acid: double bond C-C on position 8-9

Figure 6. Skeleton of the main triterpenoid biomarkers of birch bark tar (lupane skeleton) and pistacia resin (oleanane and lanostane skeletons).[26]

peaks regularly spaced every 28 m/z units, from m/z 592 to 732. This mass spectrum is perfectly compatible with the molecular composition of beeswax, which mainly contains a series of odd-numbered linear hydrocarbons ($C_{21}-C_{33}$) and long-chain palmitate esters in the carbon range $C_{40}-C_{52}$.[32−34] All the esters present the same acid moiety, namely, palmitic acid, and differ by the length of the alcohol moiety, which varies from 24 to 34 carbon atoms. With the purpose to better interpret the origin of the main fragments at m/z 256 and 257, one of the esters present in beeswax, the tetracosyl palmitate, was synthesized in our laboratory as described in the Experimental Section. This ester was analyzed with both an ion trap analyzer (Finnigan GCQ device) and a magnetic sector instrument (JEOL MS700) in order to compare the fragmentation mechanisms in these two systems (Figures 8 and 9). As in the case of the mass spectrum of beeswax, the peaks at m/z 256 and 257 are observed on the two spectra. These fragments are respectively formed by migration of one or two hydrogen atoms corresponding to $[C_{15}H_{31}CO_2H]^{\bullet+}$ and $[C_{15}H_{31}CO_2H + H^+]$ fragments,[35,36] and their presence on both spectra indicates that they probably do not result from any ion/molecule reaction in the ion trap analyzer. However, in the case of the analysis on a magnetic sector instrument, the base peak is observed at m/z 57 instead of m/z 256 with the ion trap analyzer. It may be assumed that the difference in the mass spectrum obtained between an ion trap and a magnetic sector is due to a discrimination against the m/z values below 100 in the ion trap. One must note that the ratio 256/257 seems to vary according to the type of analyzer used and the analytical conditions, which does not allow us to use this ratio for beeswax identification. Indeed, the ion at m/z 257 can become predominant versus the ion at m/z 256, especially in the case of beeswax, which contains an important number of molecular compounds.[36,37] Other peaks at m/z 239, 336, 381, and 592 respectively correspond to the fragments $[C_{15}H_{31}CO]^+$, $[C_{24}H_{48}]^+$, $[CO_2C_{24}H_{49}]^+$, and $M^{\bullet+}$.[37] All these fragments are also present in the mass spectrum of beeswax in which we also observe peaks at m/z 409, 437, 465, and 493 respectively corresponding to the ion fragments $[CO_2C_{24}H_{49}]^+$, $[CO_2C_{26}H_{53}]^+$, $[CO_2C_{28}H_{57}]^+$, $[CO_2C_{30}H_{61}]^+$, and $[CO_2C_{32}H_{65}]^+$. The series of peaks in the mass range 592−732 correspond to the molecular ions of the different long-chain esters present in beeswax (Table 1).

The three different oils analyzed by mass spectrometry are characterized by very similar mass spectra (Figure 10). Common features are in particular observed in the regions m/z 300−350, 560−620, and 800−890, respectively, due to monoacylglycerols, diacylglycerols, and triacylglycerols. Contrary to terpenoid tars and resins, which can be easily distinguished by mass spectrometry, the mass spectra obtained on oils do not seem to be characteristic of the plants they were extracted from. However, these spectra should be helpful in the detection of plant oils in archaeological samples even if more detailed investigations would be necessary to precise the exact origin of the oil.

Mass Spectra and Identification of Archaeological Adhesives. Archaeological remains investigated in this study were sampled at the end of the 1990s either directly after detection on the excavation site or during the restoration procedure. Three samples (Figure 1) were analyzed by direct inlet electron ionization mass spectrometry in order to determine their composition and to identify their natural origin.

The first of them, dated from the Final Bronze Age (12th−11th century B.C.), was found on the site of La Fangade, located on the pond of Thau (Hérault, France), near the Mediterranean sea. A brownish-orange deposit, characterized by fractures presenting a yellowish brightness, was observed on a ceramic sherd measuring 5 × 4 cm (archaeological reference: FG97, S2−NE, 12−c. III). The distribution of the organic residue on the sherd was quite heterogeneous and its thickness varied from less than 1 mm to a few millimeters. A microsample was submitted to mass spectrometric analysis in order to understand the role of the pottery containing this amorphous substance.

The second arose from a black and amorphous residue observed, still adhering to a bronze object (the chape of a sword, which is a metal piece placed at the base of the sheath of a sword in order to decorate the sheath and protect the sword) during its

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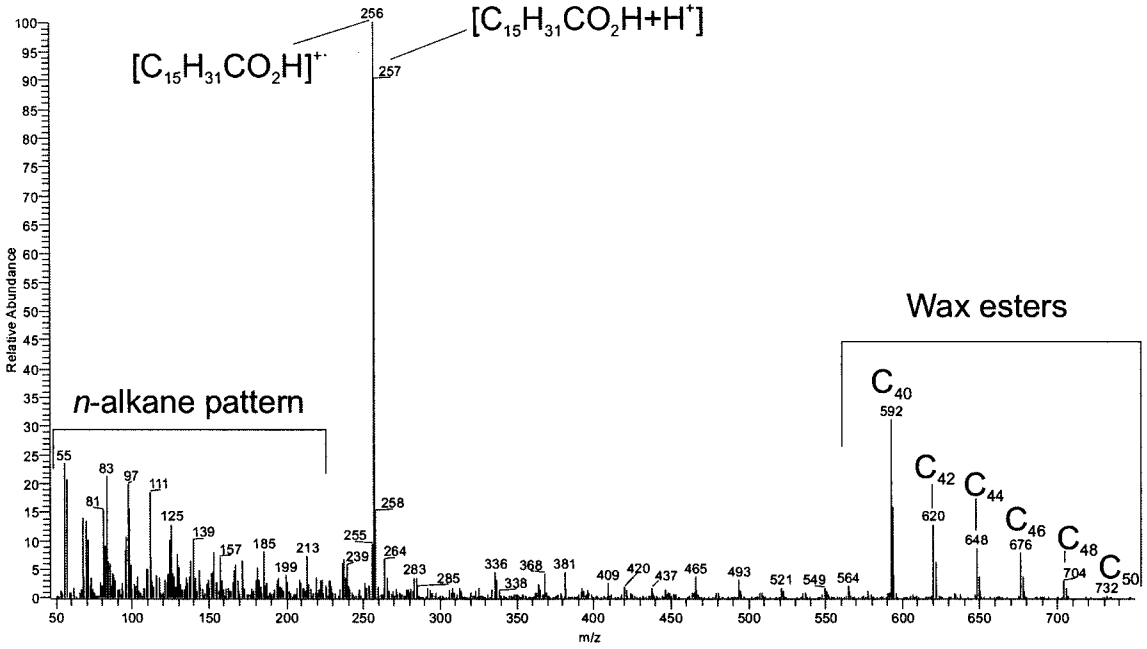

Figure 7. Mass spectrum of a contemporary beeswax obtained by direct inlet electron ionization mass spectrometry using an *m*/*z* scan range of 50−800. C_x corresponds to palmitate wax esters with *x* carbon atoms.

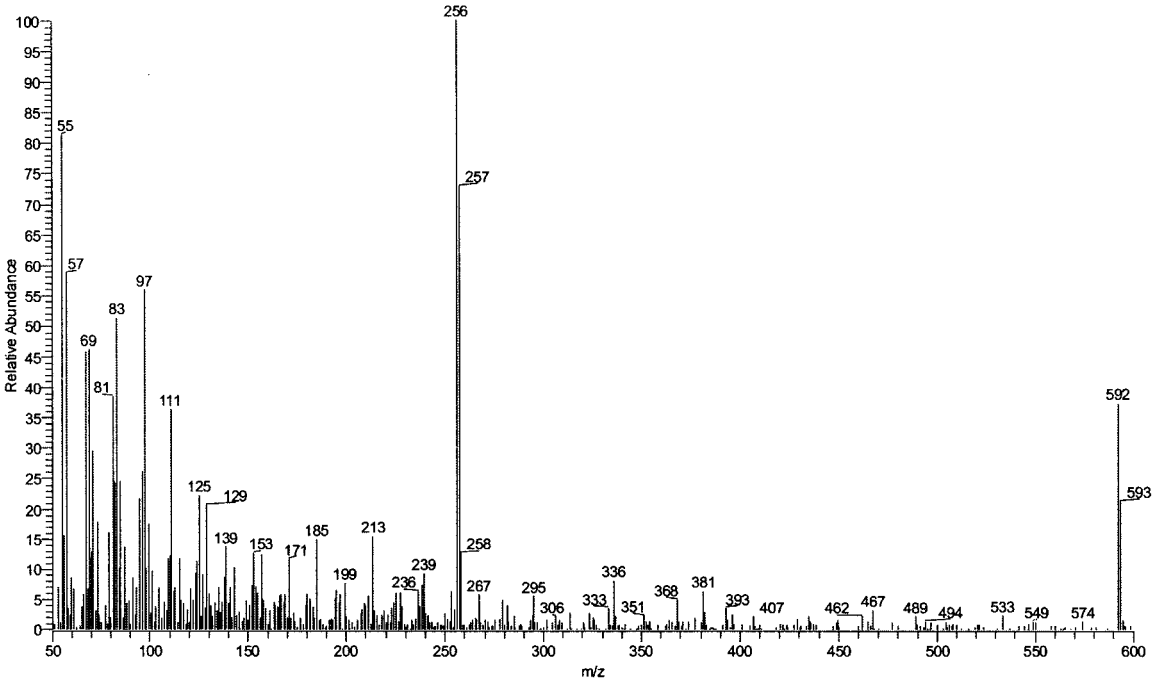

Figure 8. Mass spectrum of a tetracosanyl palmitate synthesized in the laboratory, obtained by direct inlet electron ionization mass spectrometry using an *m*/*z* scan range of 50−800. This spectrum was performed on the GCQ Finnigan device equipped with an ion trap analyzer.

restoration in 1998 (Figure 11). This matter was partly sampled with a scalpel blade before the restoration occurred and stored in a sealed glass tube in order to avoid any contamination by contemporary organic substances. This bronze object was discovered in a tomb from the first Iron Age (Hallstatt period, ca. 800−700 B.C.) on the site of Argancy (Moselle, France) during a rescue excavation.

The third archaeological sample consisted of a thin brownish deposit of less than 1-mm thickness adhering on the inner surface of a ceramic vessel discovered on the site of Grand Aunay, located at Yvré-l'Evêque, near Le Mans (Sarthe, France), from the second Iron Age dated from the second century B.C.

The analysis of the archaeological materials was performed on a solid microsample directly introduced in the mass spectrometer. All the samples provided quite intense mass spectra with a very particular pattern. The mass spectrum of the sample from the Final Bronze Age recovered on a ceramic sherd was characterized by a base peak at *m*/*z* 239 and a main fragment at *m*/*z* 285 (Figure 12). Both fragments have been shown to be the main peaks of the mass spectrum of a pine resin. In particular, the base

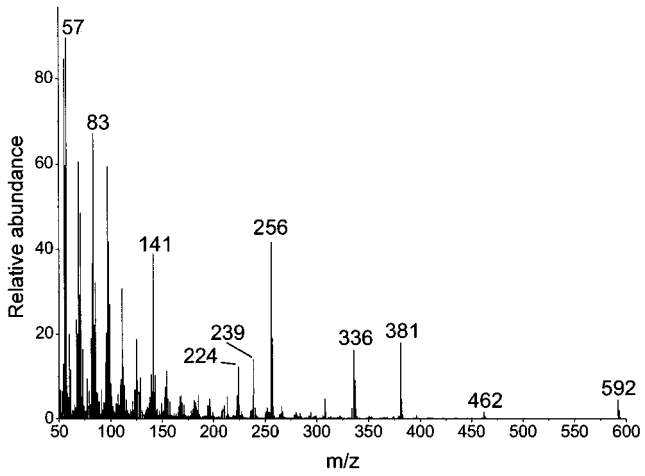

Figure 9. Mass spectrum of a tetracosanyl palmitate synthesized in the laboratory, obtained by direct inlet electron ionization mass spectrometry on a JEOL B/E mass spectrometer (JEOL MS700) using an *m*/*z* scan range of 50−800.

Table 1. General Formula and Molecular Weight of the Main Long-Chain Esters Present in Beeswax

long-chain ester name	molecular formula	MW
tetracosanyl palmitate	$C_{15}H_{31}CO_2C_{24}H_{49}$	592
hexacosanyl palmitate	$C_{15}H_{31}CO_2C_{26}H_{53}$	620
octacosanyl palmitate	$C_{15}H_{31}CO_2C_{28}H_{57}$	648
triacosanyl palmitate	$C_{15}H_{31}CO_2C_{30}H_{61}$	676
dotriacosanyl palmitate	$C_{15}H_{31}CO_2C_{32}H_{65}$	704
tetratriacosanyl palmitate	$C_{15}H_{31}CO_2C_{34}H_{69}$	732

peak at *m*/*z* 239 is known to be characteristic of the fragmentation of dehydroabietic acid (Scheme 1), a degradation marker formed by oxidation of abietic acid, the main biomarker in pine resins. Contrary to what was observed on the mass spectrum of the contemporary resin, it is difficult to precisely identify the degradation stage of this archaeological sample, based only on study of its mass spectrum. Indeed, the peaks observed in the region of the molecular weight of the diterpenoids present in pine resin are not intense enough (*m*/*z* 300 and 302) to be interpreted without doubt as the molecular ions of diterpenoid compounds. However, the presence of a significant peak at *m*/*z* 285, presumably formed by the loss of a methyl group, reinforces the hypothesis of the presence of dehydroabietic acid in the sample. This molecular compound may have been formed either by purposeful heating or by some other act during burial. The similarities of this mass spectrum to that of a pine resin (Figure 3) indicate that a conifer resin was contained in the ceramic vessel. The low intensity of fragments at *m*/*z* 253 and of molecular peak at *m*/*z* 314, provided by the 7-oxodehydroabietic acid, as discussed previously, gives rise to the assumption that the pine resin has not been submitted to any significant oxidation process. This may be explained by the anaerobic burial environment of the pond of Thau. The mass spectrum obtained does not allow detection of any additive to the pine resin and indicates that the ceramic vessel studied contained a pure resin that had been stored in this pottery before use.

The mass spectrum of the sample associated with bronze material from the first Iron Age was dominated by a fragment at *m*/*z* 189, characteristic of triterpenoid compounds (Figure 13). The general pattern of the mass spectrum appears to be very similar to that of birch bark tar, especially in the range *m*/*z* 350−450 region of the molecular ions of lupeol (*m*/*z* 424), lupenone (*m*/*z* 426), and betulin (*m*/*z* 442), three tripernoids of the lupane family, biomarkers present in outer birch bark. Birch bark tar was produced and used for a long time by prehistoric people since the most ancient samples chemically identified date back to ca. 45 000 years before Christ.[2] However, our findings result in the first report that birch bark was used for assembling bronze tools during the Iron Age in Europe.

The brownish material sampled on a ceramic vessel from the site of Grand Aunay revealed a complex mass spectrum that exhibits the characteristic pattern of both birch bark tar and beeswax (Figure 14). Indeed, the peaks at *m*/*z* 256, 257, 592, 620, 676, and 704 present a remarkable similarity to that of beeswax whereas the peak at *m*/*z* 189 and the pattern in the mass range 350−450 may be attributed to a triterpenoid tar and probably a birch bark tar. The mass spectrum of this sample may thus be considered as characteristic of a mixture of birch bark tar and beeswax.

Birch bark tar and beeswax are known to have been used at least since the Neolithic period.[8,9,36−39] Birch bark tar was mainly used as an adhesive for hafting lithic tools or repairing ceramics, but it has usually not been mixed with other materials. To our knowledge, this is the first report of a mixture of birch bark tar with beeswax during ancient times. It may be supposed that beeswax was added to birch bark tar in order to increase its plasticity. It must be noted that it is still difficult to precisely determine whether beeswax was intentionally added and mixed with birch bark tar or if the vessel was first used to contain beeswax and then birch bark tar or vice versa. However, previous results showing that ancient ceramic vessels were systematically used for the same purpose[38] led us to conclude that the association of beeswax and birch bark tar in a pottery probably results from an intentional mixture. The only other material shown to be mixed with birch bark tar during antiquity was made of animal fats, as reported recently.[10] Our results provide a new explanation for the use of beeswax, which was extensively used during ancient times but heretofore has never been identified in any adhesives from prehistoric periods.

CONCLUSION

Analysis by direct inlet mass spectrometry using electron ionization can be employed to detect the presence of natural substances in ancient adhesives and to obtain molecular information on these substances in order to identify them. This method has been successfully applied for the first time to the study of ancient adhesives by comparison with spectra obtained on contemporary reference samples. Among the analytical techniques that can be directly applied on a microsolid archaeological sample, infrared spectroscopy and pyrolysis-gas chromatography/mass spectrometry have already been performed. However, the results obtained by infrared spectroscopy are much less precise than those obtained by mass spectrometry because many different natural substances may present quite similar infrared spectra

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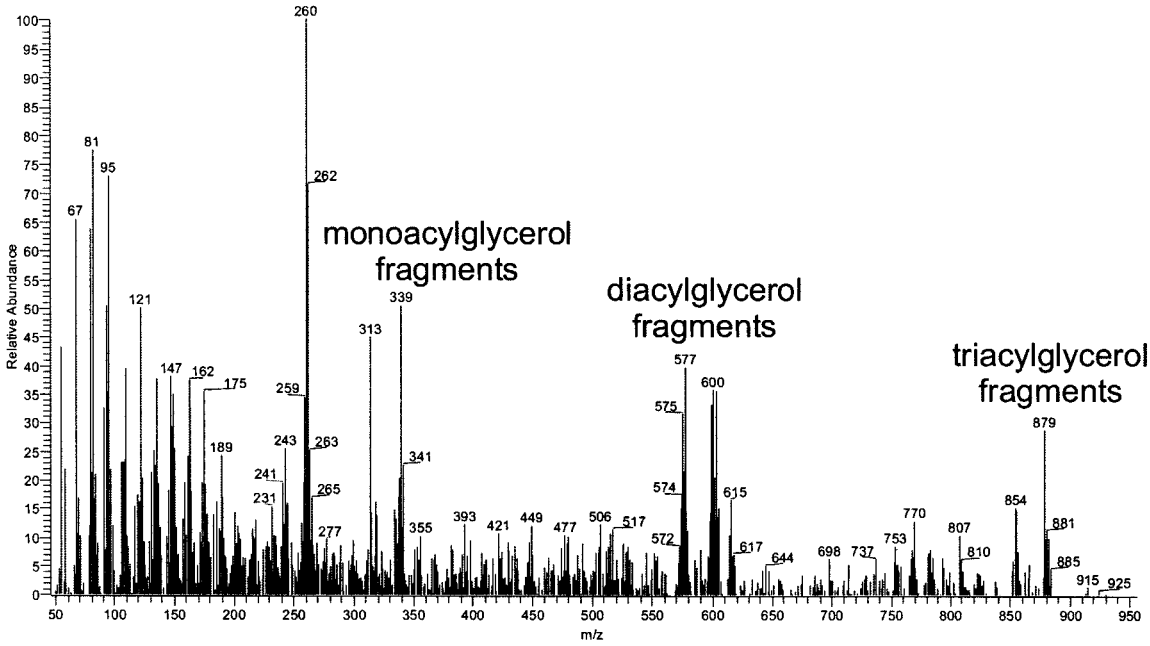

Figure 10. Mass spectum of a contemporary poppy seed oil obtained by direct inlet electron ionization mass spectrometry using an *m*/*z* range of 50−950.

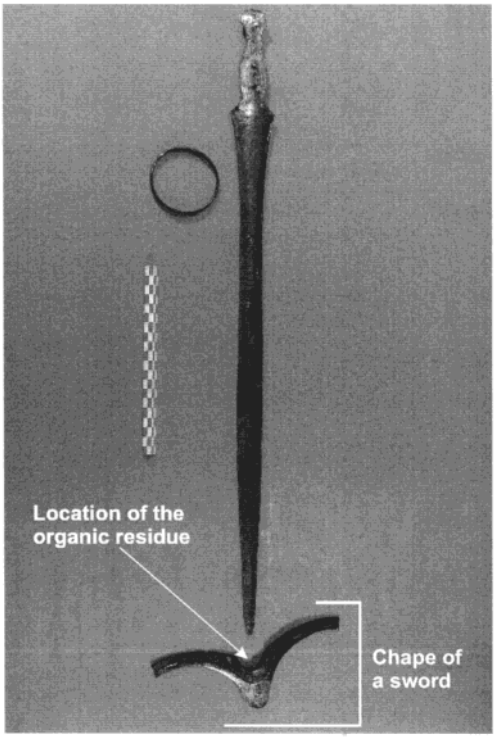

Figure 11. Chape of a sword dating from the first Iron Age in which an organic residue was identified as birch bark tar. © François Bargain, *laboratoire d'archèologie des mêtaux*.

(Figure 15). In most cases, C—H and C═O vibrations correspond to the most intense bands on the IR spectrum whereas the region between 1500 and 500 cm^{-1} is usually complex and difficult to interpret. As shown in Figure 15, distinguishing triterpenoid tars or resins by this method is not often possible whereas mass spectrometry has been shown to be successful for this task (Figures 4 and 5) because of the information obtained on the molecular weights of the compounds contained in such materials.

In the case of mixtures of various materials, infrared spectroscopy does not allow us to detect any material in low amounts (less than 5−10% of the total amount) whereas mass spectrometry is also efficient in this case if the different materials involved in the mixture are characterized by peaks corresponding to different mass-to-charge ratios. Pyrolysis-GC/MS may also be used to identify ancient organic materials,[40] but the analysis time is significantly longer than by direct inlet mass spectrometry and the interpretation more complicated because of the different rearrangements involved by pyrolysis, which is a very damaging method for structural analysis in organic chemistry.

Compared to Py-GC/MS and IRTF techniques, the analyses by direct inlet electron ionization mass spectrometry are particularly rapid, with data acquisition occurring in less than 5 min, and only require a minuscule sample, which is very important for the analysis of precious ancient materials. Moreover, this technique is very adapted to the identification of archaeological adhesives, even when they result from a mixture of different natural resources.

However, it must be noted that such a method has to be completed by other analyses when more information is necessary on the degradation stage of the sample. In that case, mass spectrometric analysis may be followed by GC/MS analyses, and when enough matter is available, the mass spectrometric analysis may be helpful in order to choose adequate solvent and derivatization reagent for preparing sample before GC analysis and to adapt the chromatographic conditions to the sample specificity.

The results obtained on archaeological samples showed that diterpenoid materials such as pine resin or triterpenoid birch bark tar could be directly identified by mass spectrometry, avoiding any sample preparation, which is always time and sample consuming. The use of additives such as beeswax to the main adhesive

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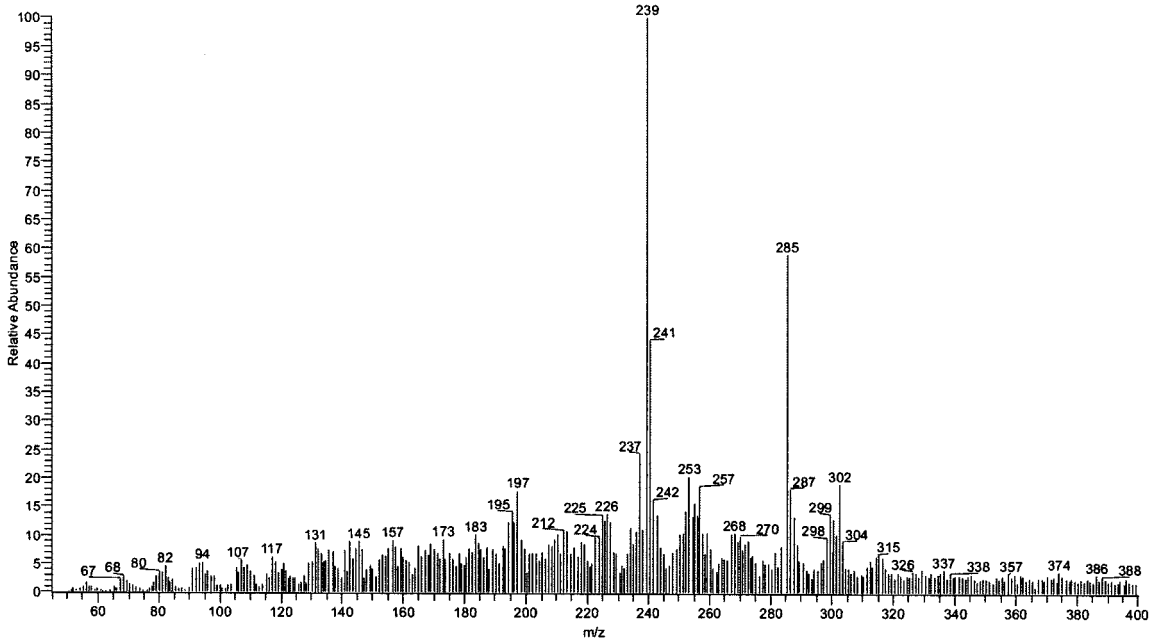

Figure 12. Mass spectrum of an archaeological material sampled on a ceramic sherd of the Bronze Age site of La Fangade showing a characteristic pattern of pine resin.

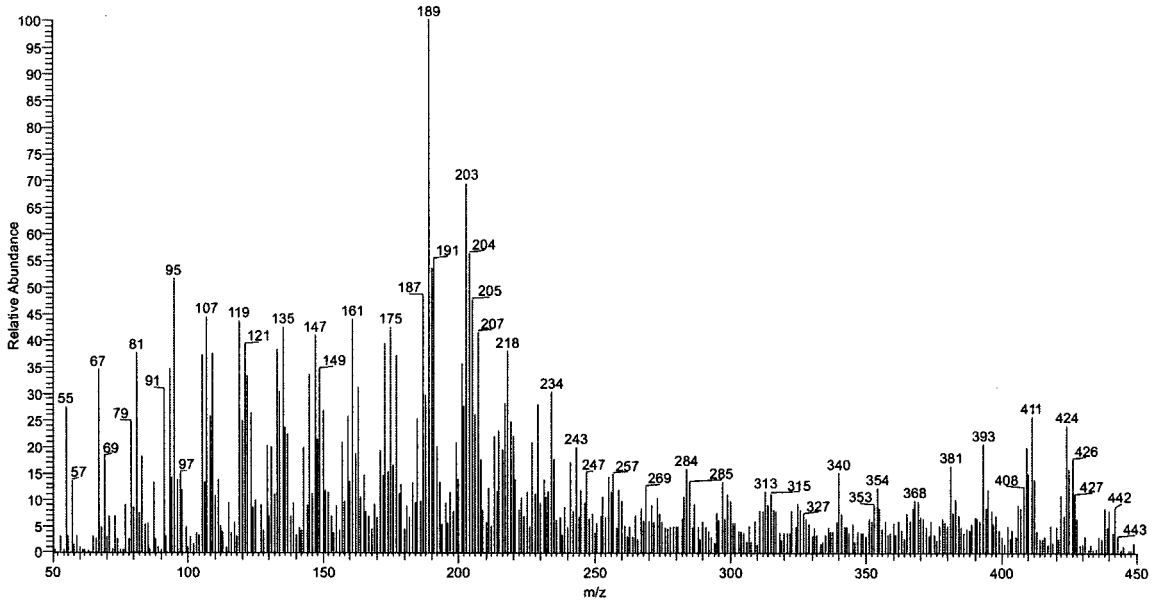

Figure 13. Mass spectrum of an archaeological black material sampled in a bronze chape of a sword of the Iron Age period, showing a characteristic pattern of birch bark tar.

material may also be easily detected. Direct inlet mass spectrometry has considerable potential for extending our knowledge on the different natural and synthetic resources used by prehistoric people for making adhesives and it should now be used more commonly before the conventional analysis performed on such ancient materials by gas chromatography.

From an historical point of view, our results shed new light on the evolution of the adhesive process during ancient times and these data tend to show that the variety of natural resources for the production of adhesives during the Bronze and Iron Ages is greater than before. While mainly birch bark tar or other derived-bark tars were produced during the Neolithic period, the finding of a conifer resin in a ceramic vessel in the south of France shows that local other substances could also have been collected. We can thus imagine that, as early as this period, cultural and technical differences were born between northern and southern Europe. Indeed, whereas pine resin is collected in the south of France, birch bark tar is still produced during the Iron Age period in the northern half of France, as shown by the analysis of two adhesives presented here. However, whereas adhesives were produced by the simple heating of birch bark tar during the Neolithic era, the people from the Iron Age were looking for adhesives with specific properties. They incorporated beeswax into birch bark tar, probably to increase the plasticity of the final product. To summarize, these results tend to show for the first time a diversification of the raw materials used in adhesive making and

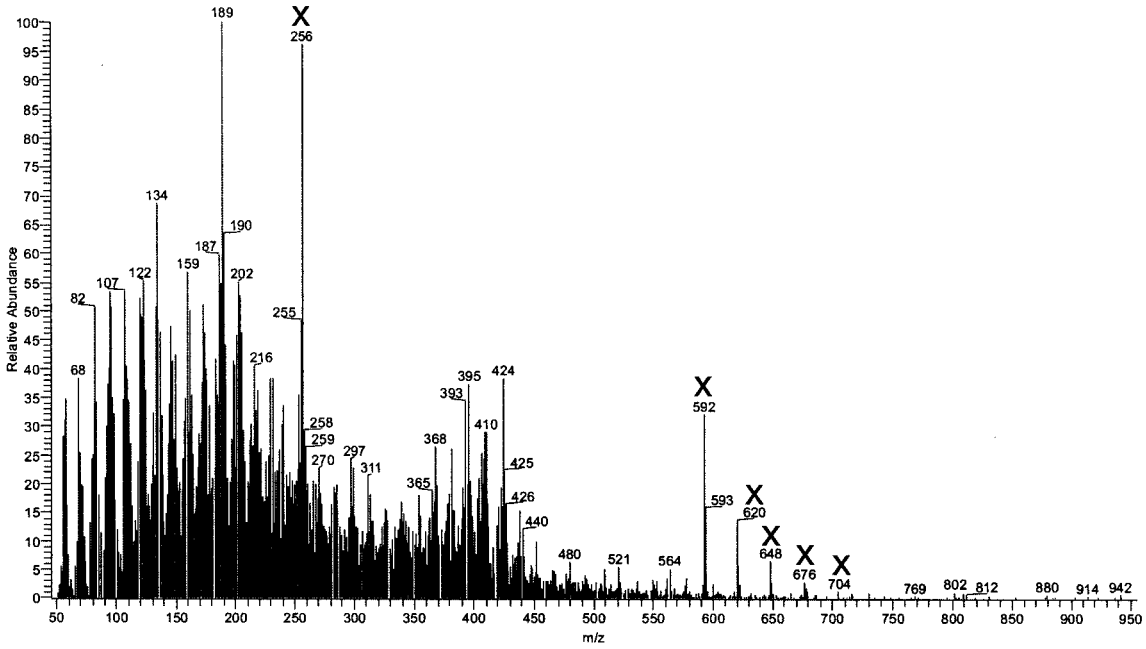

Figure 14. Mass spectrum obtained by direct inlet electron ionization mass spectrometry on a solid microsample of a brownish residue from the Iron Age site of Grand Aunay characteristic of a mixture of birch bark tar (*m*/*z* 189, 424, and 426) and beeswax. The peaks due to the presence of beeswax are indicated with a cross.

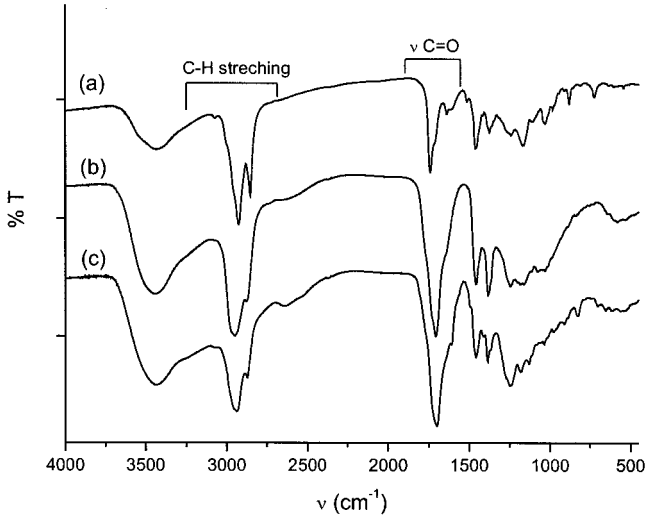

Figure 15. Infrared spectra of three different contemporary natural materials: (a) birch bark tar, (b) pistacia resin, and (c) pine resin.

innovation in the association of natural substances which were used separately before. One must note that this increased complexity in adhesive making seems to appear during the period when metallurgy was developed, i.e., when people had acquired a better control of temperature conditions and alloy fabrication.

ACKNOWLEDGMENT

The authors thank Thierry Leroy and Stéphane Vacher (AFAN) for providing samples, respectively, from the sites of La Fangade and Le Grand Aunay. We also express our gratitude to Marielle Bouchara and Dr. François Mirambet, from the "Laboratoire d'archéologie des métaux", for detecting the organic residue in the chape of the sword and for all the information they provided us on this object. This material was discovered during a rescue excavation, decided by the "Service régional de Lorraine" and carried out in 1998 by the archaeologist Francesca Schembri (AFAN). We gratefully acknowledge the support of the GDR CNRS 2114 "Physico-Chimie des Matériaux du Patrimoine Culturel" (CHIMART).

Received for review August 5, 2001. Accepted November 26, 2001.

AC0155862