**Beginning of viniculture in France**

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Chemical analyses of ancient organic compounds absorbed into the pottery fabrics of imported Etruscan amphoras (ca. 500–475 B.C.) and into a limestone pressing platform (ca. 425–400 B.C.) at the ancient coastal port site of Lattara in southern France provide the earliest biomolecular archaeological evidence for grape wine and viniculture from this country, which is crucial to the later history of wine in Europe and the rest of the world. The data support the hypothesis that export of wine by ship from Etruria in central Italy to southern Mediterranean France fueled an ever-growing market and interest in wine there, which, in turn, as evidenced by the winepress, led to transplantation of the Eurasian grapevine and the beginning of a Celtic industry in France. Herbal and pine resin additives to the Etruscan wine point to the medicinal role of wine in antiquity, as well as a means of preserving it during marine transport.

**Ancient medicine | biomolecular archaeology | viticulture | Western Mediterranean**

Much is already known about the initial domestication of the Eurasian grapevine (*Vitis vinifera sp. vinifera*) and the emergence of a "wine culture" in the mountainous Near East, as early as the Neolithic period (1, 2). Less is known about how viniculture moved from east to west across the Mediterranean Sea, eventually reaching Italy and France. Merchant seafarers, including Canaanites and later Phoenicians and Greeks, were the principal conveyors, who progressively established colonies along the coasts and on one island after another.

By at least 800 B.C., the Etruscans of central Italy along the Tyrrenhian Sea had come in contact with the Phoenicians, as shown by their “Orientalizing” industries of metals, pottery, glass, ivories, and a species of *Vitis vinifera*. This amphora (Fig. L1) was the prototype for the Etruscan amphora (Fig. 1B), and, where a similarity of form exists, most likely a similar function was intended: primarily to hold grape wine (3), which was supplied by a nascent local industry.

Such wine amphoras eventually filled the holds of Etruscan ships, some of which sank along the Italian and French coasts on their way to southern Mediterranean France, beginning ca. 625–600 B.C. (4–7). On land, the Celts, the native inhabitants of large parts of Western Europe in the first millennium B.C., were lured into the wine culture and eventually saw the advantages of local production to promote their own trading interests. The Gallic wine culture spread inland after the Roman conquest up the Rhone and Rhine rivers to the rest of Europe where, centuries later, primarily monasteries, such as the Cistercian abbey of Vougeot in Burgundy, refined viniculture to such a degree that it became a model for the rest of the world.

**Archaeological Samples Chosen for Analysis**

The coastal site of Lattara, near the modern town of Lattes south of Montpellier, is key to understanding the transference of the wine culture to Mediterranean France (8). Merchant quarters for the storage, preparation, and transport of imported and exported goods were newly constructed inside a walled settlement ca. 525 B.C. (Fig. 2). Multiroom buildings along the southwestern wall gave direct access to a lagoon (now partly silted up) connecting to the sea, where boats could have been moored and protected.

Etruscan amphoras, believed to contain wine on archaeological grounds, had already been arriving along the coast of France since the end of the seventh century B.C. Their importation, however, dramatically decreased at many sites after ca. 525 B.C. when the Greek colony of Massalia, founded in 600 B.C. by Phocaean Greeks coming from western Anatolia, began to produce its own wine amphoras. These people began producing a distinctively shaped Massaliote amphora (Fig. 3C) in the third quarter of the sixth century B.C., thought to have been used to export locally produced wine so as to compete with the Etruscan market. Lattara was the exception to the rule; Etruscan amphoras and other artifacts from Italy, attesting to close commercial contacts, continued to be imported during the heyday of activity in the merchant quarters from about 525–475 B.C.

The critical issue addressed by this study is whether these Etruscan and Massaliote amphoras did indeed contain wine. A biomolecular archaeological argument, as the phrase implies, entails a rigorous assessment of the chemical, archaeological, and, in this instance, archaeobotanical evidence separately and in combination. Absolute certainty is unattainable in a biomolecular archaeological investigation because it is an inherently probabilistic historical field of inquiry. The probability of a solution to an archaeologically relevant problem increases with ever-accumulating data, with the refinement of chemical, archaeological, and archaeobotanical methods, and as more natural products are analyzed and become available for bioinformatics searches.

On this basis, amphora samples were selected for chemical analysis based on whether it (a) was an Etruscan or Massaliote type; (b) was excavated from an undisturbed, sealed context; (c) was part of a whole vessel, with base sherds available for analysis; (d) had remnants of a possible residue on its interior; and (e) was unwashed. Only 13 Etruscan amphoras, lined up in two rows in the southeastern part of the storeroom of a merchants’ building in zone 27 (Figs. S1 and S2), met all these criteria. They were clearly in situ and sealed off from later intrusions by a ca. 475 B.C. destruction layer. Another 22 amphoras in this room were more haphazardly arranged and might have been secondarily disturbed.

The 13 Etruscan amphoras belonged to a very specific pottery type (9), amphore étrusque 4 (A-ETR 4), which was likely...


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manufactured at the Etruscan city of Cisra (modern Cerveteri) ca. 525–475 B.C. (10). The archaeological consensus is that this type was primarily used to transport wine from Etruria to southern France and elsewhere. Three of the 13 amphoras (Dataset S1; nos. 4, 5, and 7) were chosen as representative samples for analysis. These were base sherds because precipitates of liquids settle out and, upon evaporation, concentrate organic compounds there. Two of the sherds (nos. 4 and 5) had small, darkened areas on their interiors, possibly residues of the original contents. Another amphora base (no. 10) of the same Etruscan type from a secure context—the construction level of the building—completed our Etruscan analytical corpus.

To gain a fuller perspective on the possible importation and production of wine at Lattara, two base sherds (nos. 8 and 9) from complete Massaliote amphoras from later (ca. 475–450 B.C.), nearby contexts were also analyzed. No. 9 had a resin-like deposit covering its interior. Archaeologists are in agreement that Massaliote amphoras were almost certainly used for wine.

Additionally, a limestone installation (11) (Fig. 3), dated to ca. 425–400 B.C. and found in situ in a courtyard built over the destroyed merchants’ quarters, was analyzed. It has been interpreted as a pressing platform for processing olives or grapes (5–7). Contemporaneous Greek vase paintings (e.g., see Fig. S6) show how such platforms supported baskets of grapes for stomping and collecting the juice. Excavated samples are common throughout the ancient Mediterranean world (1, 7) up until today. Our goal was to determine whether the platform had been used in local production of wine or olive oil.

Archaeobotanical Evidence

The overwhelming predominance of domesticated grape (V. vinifera sp. vinifera) remains at Lattara, beginning as early as ca. 500 B.C., lends further support to the archaeological interpretation that specific imported amphora types contained wine and that the domesticated grapevine was eventually transplanted to southern France and its grapes pressed to make local wine at the site.

The same merchants’ room with the Etruscan amphoras, as well as nearby buildings of the same period, yielded numerous grape seeds, pedicels, and even fruit (skin). For the site as a whole, 15–25% of the cultivated plants and 80% of the fruits were of grape. To date, the only attested fruits other than grape are fig (Ficus carica), blackthorn or sloe (Prunus spinosa), blackberry (Rubus fruticosus), and olive (Olea europaea var. europaea). The latter occur in very small amounts and with rare exceptions are post-fifth century B.C. Other plants that contain tartaric acid (a principal biomarker for grape—see below and SI Text), such as pomegranate or exotic fruits from distant countries, are totally absent from the site.

A cluster of several thousand carbonized grape seeds, which were found inside a clay container in an earlier phase (ca. 435 B.C.) of the same area in which the pressing platform was excavated, provides compelling evidence that the latter was used for grapes (12). Masses of grape remains often point to grape pressing and stomping for winemaking (13). By contrast, no olive pits were found near the platform. In general, they are extremely uncommon until Roman times and nearly always occur whole; i.e., they had not been pressed.

Chemical Results

After sample extraction, ancient organic compounds were identified by a combination of chemical techniques: Fourier-transform infrared spectrometry (FT-IR), gas chromatography-mass spectrometry (GC-MS), ultraHPLC tandem mass spectrometry (LC/MS/MS), HPLC with a linear ion trap-Orbitrap mass spectrometry (Orbitrap LC/MS), and headspace solid phase micro-extraction (SPME) coupled to GC-MS (SI Text).

FT-IR showed that nos. 4, 5, 7, 9, and 10 had the characteristic absorptions for a tree resin, according to the results of previous studies (14, 15). Only the spectra for no. 8 and the platform sample were ill-defined. Samples comprised of complex mixtures can be equivocal for FT-IR, and the spectra must be deconvoluted and examined closely for the presence/absence of key absorptions; if a known absorption for a compound is not observed, then that compound is likely not present.

GC-MS (Datasets S1 and S2; Fig. S3) revealed that a tree resin was attested for all of the amphoras, irrespective of whether a possible resin-like residue or resin-like soil inclusions on their interiors were observed. Only the platform lacked resin compounds. The detected compounds, which belong to the abietic acid family (namely, abietic acid and its oxidation products when aged or heat-treated) and the pimaric/sandaracopimaric acid family (pimaric acid, isopimaric acid, and sandaracopimaric acid), are best explained as originating from pine (Pinaceae) resin. The pimaric acid family is lacking for nos. 7, 8, and 10, which might be interpreted as true absence, very low concentration, or differential preservation. Tartaric acid, a principal biomarker for grape wine (see below and SI Text), was weakly detected by this method only in no. 8.

LC/MS/MS demonstrated that tartaric acid/tartrate was unquestionably present in nos. 4 and 8 (Fig. S4), likely present in
Discussion and Conclusions
Fermented beverages, especially wine, have long played a crucial role in the transfer of culture from one people to another around the world (2, 4, 6). The wine trade was one of the principal incentives for the Canaanites and Phoenicians, followed by the Greeks, Etruscans, and Romans, to expand their influence in the Mediterranean Sea. Where wine went, so other cultural elements eventually followed. Technologies of all kinds and new social and religious customs took hold in regions where another fermented beverage made from different natural products had long held sway.

It is not surprising then that the Celts or Gauls along the shore of Mediterranean France between ca. 625 and 400 B.C. should have become equally entranced by the cultural and economic possibilities for wine and begun to substitute it for their native beverages, which were likely beers, meads, and mixed fermented beverages (2). This hypothesis, however, has never been tested by biomolecular archaeological methods. Based on our findings, it is now highly probable that (a) the Etruscan amphoras arriving in ports of Mediterranean France, specifically Lattara, contained wine; (b) this wine was pine-resinated; (c) additional botanicals, probably including rosemary, basil and/or thyme, had been added to the wine; and (d) the importation of the Etruscan wine eventually led in a relatively short period to the transplantation of the domesticated Eurasian grapevine and to local wine production in southern France, probably in its initial stages under Etruscan tutelage. These findings bear importantly on the subsequent course of the wine culture in Europe and ultimately the New World.

Our biomolecular archaeological methodology for arriving at these conclusions is very straightforward: (a) carefully articulate the archaeological problem to be solved; (b) select the best-provenienced, best-dated, and best-preserved archaeological samples for chemical analysis; (c) propose a hypothesis that best explains the interrelated archaeological, archaeobotanical, and chemical data; and (d) subject this hypothesis to ever-more-exacting testing by the same disciplines.

The presence/absence of tartaric acid/tartrate, as a key biomarker of the Eurasian grape, is obviously important to the hypothesis we propose. Based on a thorough bioinformatics search, other compounds, such as malvidin, are less definitive for grape (SI Text). One can also legitimately ask whether our detection of this compound necessarily derives from the Eurasian grape and, if it does, whether it is present as grape juice, syrup, or vinegar rather than wine. Archaeological and enological considerations come into play in answering these questions, not just chemical analysis (also see SI Text).

A crucial archaeological fact is that the narrow-mouthed, complete amphoras of this study are ideal for preserving tartaric acid/tartrate. Tartaric acid will be absorbed into the pottery, depending on its porosity, and form ionic bonds with the clay, thus helping to preserve the compound. Tartaric acid also readily precipitates out of wine as potassium bitartrate as part of the wine lees. These precipitates collect either as a residue on the bases of the amphoras, which were targeted, or are absorbed into the pottery fabric. In the calcareous geological environment of southern coastal France, tartaric acid also would have been readily converted to insoluble calcium tartrate, further assuring a residue accumulation and/or absorption into the pottery.

Moreover, because the amphoras were likely stoppered (below), any cross-contamination between amphoras would also have been minimized. If tartaric acid escaped from the amphoras into the groundwater, it would have been quickly bound up with calcium and other metallic ions in the calcareous soil, precipitate out, and not have been transported far. It would have been consumed by microorganisms in the soil, especially in relatively anaerobic conditions underground, at a more rapid rate than it was produced by microbes (17). This conclusion was borne out by Orbitrap LC/MS analyses of soil and limestone control samples from the same area and approximate time period as the amphora and pressing platform samples (Dataset S4). The latter had tartaric acid levels that significantly exceeded those of the control samples (SI Text).

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The SPME results for nos. 4 and 5 (Datasets S1 and S3) are also consistent with grape being the source of the tartaric acid. Using standard bioinformatics tools to search the chemical literature (14), constituents of modern grape wine (18) were identified in one or both of the ancient samples tested, including alcohols, esters, aldehydes, and terpenoids. Any ancient ethanol would have been metabolized by microorganisms. Although benzaldehyde, 2-ethyl-1-hexanol, and nonanal might derive from wine, they could also be contaminants. Other compounds might derive either from ancient and/or modern “background contaminants” due to groundwater percolation or sample handling (e.g., plasticizers and antioxidants from plastic, including compounds in the phthalate family). Possibly, some of the low-boiling compounds up to hexenal were also contaminants, but, more likely, they were preserved within the ionic clay structure.

Botanical additives to the wine in nos. 4 and 5 were also identified. Three natural products account for the greatest number of compounds that are not naturally ubiquitous and are therefore most likely: rosemary, basil, and thyme. These herbs are native to central Italy where the wine was likely made. Rosemary (*Rosmarinus officinalis*) (labeled 3 in Dataset S3), which is widespread throughout the Mediterranean region, accounts for the most number of volatile compounds in Dataset S3, namely, the monoterpenes D-limonene, fenchol (only in no. 4), camphor, borneol and menthol (only in no. 5), the sesquiterpene copaene (only in no. 4), and cuminaldehyde, a benzaldehyde derivative. A previous study of Egyptian wine (14) showed chemically that rosemary had been added to the wine in a Byzantine amphora from Egypt. Basil (*Ocimum basilicum*), a native western Mediterranean plant, can account for the same compounds except copaene and cuminaldehyde; additionally, it contains the sesquiterpene calamanene (only in no. 4) in the naphthalene family, which is rare in the plant world. Although estragole makes up more than half the content of fresh basil, its allylic and benzylic structure makes it highly unstable to bio- and photodegradation, and it would not be expected to survive for thousands of years. Thyme (*Thymus vulgaris*), which grows widely around the Mediterranean, is another possibility, but it lacks calamanene and copaene.

All of the Lattara amphoras contained compounds (labeled 4) from pine resin. Natural untreated pine resin also contains the monoterpenes fenchol, camphor, and borneol (19). This resin is still used today to make Greek *retsina*, the only modern carryover of ancient tradition.

Resinated wines with many of the same compounds as those attested for the Lattara amphoras are reported for a bronze cauldron (*situla*), part of the drinking equipment in a wealthy Etruscan tomb, dated to ca. 450–400 B.C., at the Adriatic Sea port of Spina at the mouth of the Po River in Italy (20). DNA analyses (20) of amphoras, which were recovered from shipwrecks found in the Aegean Sea and off the coasts of western Anatolia and Corfu (fifth–third centuries B.C.), further substantiate the presence of similar botanicals to those in the Lattara amphoras—namely, rosemary, thyme, and pine resin. An SPME study of a Greco-Roman amphora from Campania in Italy, dated ca. 200 B.C.–A.D. 200, from a shipwreck in the Adriatic Sea off the coast of Croatia, yielded a suite of compounds (21) that is consistent with a pine-resinated herbal wine like those in the Lattara Etruscan amphoras. The compounds include alcohols, esters, ketones, and aldehydes characteristic of wine, the monoterpenes fenchone, camphor, and borneol, the sesquiterpene calamanene, and members of the abietic and pimaric/isopimaric acid families, together with possible naphthalene and phenanthrene-related derivatives originating from heat processing and/or oxidative aging of pine resin.

The relative prominence of retene in the Lattara amphoras might imply that a heated tree tar or pitch was applied to their interiors or to a now-disintegrated stopper (22). Only one amphora body sherd (no. 9), however, appeared to have a tar or...
resin lining on its entire interior surface. An accumulation of resin at the bottom of the base with none continuing up the side wall (no. 7), isolated small darkened areas (nos. 5 and 9), and resin-like particles dispersed in soil on the inside of nos. 4 and 8 are better interpreted as resulting from the precipitation of a resin or tar added as a preservative or flavorant to the wine, with subsequent degradation to the oxidized diterpene acid forms. Wine transported by ship also kept better when it was resinated.

Perhaps the most important finding of this study, with obvious implications for the beginning of winemaking in France and Europe as a whole, is that the pressing platform at Lattara was already being used to stomp grapes and to produce local wine ca. 425–400 B.C. To date, nothing comparable has been reported from the region, especially at Massalia, which is believed to have begun exporting native wine in its distinctive amphorae as much as a half century earlier. The pressing platform is remarkably like the grape-stomping platform that is shown on a black-figured vase (Fig. S6) by the Amasis Painter of sixth century B.C. Athens, recovered from the Etruscan site of Vulci. This ceramic masterpiece is the earliest depiction in the Greek world that shows a sequence of vinicultural activities (picking, treading, and fermentation) and uniquely illustrates the intimate association of wine with the arts.

The question remains whether similar archaeological, chemical, and botanical evidence for local wine production as that from Lattara will be forthcoming from Massalia or another site in the region. It is reported that large quantities of presumably domesticated grape seeds have been recovered from sixth century B.C. levels at Massalia, and by the end of the century, the production of Massaliote amphorae, probably for transporting local wine, had sky-rocketed (9, 23). Could it be that the Phocaenaeans brought a tradition of winemaking with them from Anatolia when they founded Massalia or adopted it early on from the Etruscans? Large numbers of grape remains, including seeds, pedicels, and grape skins, are also reported from fifth century B.C. Coudounèu (24), a site within the economic sphere of Massalia, 75 km to the northwest. At the same time at Roquepertuse (25), even closer to Massalia, pips of the domesticated Eurasian grape have been reported.

The real issue, however, is not whether Lattara, Massalia, or another French site proves to have the earliest evidence for local wine production according to the evidence that has been presented here, we can now state that local winemaking was in place in Mediterranean France by at least the fifth century B.C., and that the groundwork for this crucial development was preceded by a trade in wine amphorae coming from Etruria where local winemaking was already well-established.

Similarly to the transfer of winemaking by the Canaanites to the Egyptian Nile Delta millennia earlier (1, 2), the native Celts at Lattara would have needed the expertise and knowledge of the Etruscans to plant their own vineyards and begin making wine. They might have had general knowledge of the Eurasian grape, which grew wild along the northern Mediterranean shore and which they might have used to make a native fermented beverage. However, such exploitation and the morphological transition between wild and domestic grapes is not attested until at least the third century B.C., particularly at Port Ariane, about a half kilometer distant from Lattara (26). Moreover, much more agricultural knowledge and technological proficiency would have been needed to transplant the domesticated grapevine, successfully tend it, vinish the grapes into wine using specialized equipment, and preserve the wine in sealed vessels with tree resins.

Plantings of the domesticated Eurasian grapevine in Mediterranean France were probably transported on Etruscan ships. A fourth century B.C. Punic shipwreck off the coast of Mallorca at El Sec (27, 28) illustrates how it might have been accomplished: grapevines on this ship were embedded in soil in the cool hull of the ship, which would have enabled them to travel well and be replanted. This ship also carried numerous amphorae from throughout the Mediterranean and Black Sea, specialized drinking vessels, and cauldrons and buckets of types well-documented elsewhere in Europe for making and serving a mixed fermented beverage.

The Etruscan shipwreck of Grand Ribaud F (27, 29), found off the coast of the Hyères Islands, east of Marseilles, and dated to ca. 515–475 B.C., is especially pertinent to the transfer of winemaking to Mediterranean France. Its hold was filled with grapevines, which the excavator argues were for cushioning the shipment (dunnage) of some 700–800 amphorae rather than for transplantation. Significantly, all of the Etruscan amphorae on board this ship, which had been carefully stopped with cork (among the earliest evidence for this technology, which is also attested by two examples from Lattara, datable ca. 475 B.C.) and stacked at least five layers deep in the hull, are of the same period type (A-ETR-4) and consignment with the Etruscan amphorae analyzed and reported on here. The ship’s final destination was quite possibly Lattara.

Finally, it should be stressed that ancient wine, such as that imported into Lattara and later made there, served as more than a social lubricant or aromatic beverage, as is customary today. In addition to its eventual role as a powerful religious symbol, grape wine and other alcoholic beverages were the medicines of antiquity, as evidenced by the pharmacopoeias of Egypt, China, Greece, and Rome (30) (ST Text). Alcoholic beverages were an excellent means to dissolve and administer botanical concoctions externally and internally.

Much more remains to be discovered about the progress of viticulture, winemaking, and the cultural impact of grape wine in France and Europe beginning with the Celts of Mediterranean France. Future biomolecular archaeologists will increasingly be called upon not only to identify biomarker compounds by ever more sensitive techniques, but also to correlate and assess their findings in light of ever more precise archaeological and archaeobotanical data.

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Supporting Information

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SI Text

Sample Preparation and Extraction
The amphora sherds were first examined macroscopically and under low magnification. Soil adhering to the amphora sherds was then physically removed, followed by light washing with distilled water. Resin-like particles were noted in the interior soil of nos. 4 and 8. The interiors of nos. 4 and 5 had small, darkened areas in places, possibly remnants of ancient residues. Only no. 9 had a black resin-like deposit covering its entire interior surface. No. 7 had a yellowish clump of resin-like material filling the toe of its base, which did not extend up the sides of the interior. Even in the absence of visible residues, the aluminosilicate structure of pottery is ideal for absorbing and retaining ancient organic compounds, especially those with polarity.

The interior surfaces of the sherds were ground down to a depth of 1–3 mm with a Dremel rotary grinder with a tungsten-carbide Burr. To remove and discard this interior surface, as some researchers do (1), would have been largely to destroy the samples. It should also be noted that the amphora interiors were less exposed to any ground-water contamination. Samples of ground-down pottery, soil containing resin-like particles (nos. 4 and 8), the resin-like material in no. 7, and the pressing platform sample were pulverized with an agate mortar and pestle.

For the ground-down pottery, our standard chloroform/methanol procedure (2, 3) by either Soxhlet extraction or boiling in borosilicate glassware for 30 min, combining and evaporating to dryness, was used. The latter procedure was sometimes preferable because of the build-up of fine clay particles in the Soxhlet apparatus.

The platform, which had only been cleaned by physical means and water since its excavation, was sampled by chiseling away an ∼5 × 5-cm interior area of the limestone, which had a reddish coloration on its surface, to a depth of 2–3 mm, and pulverizing.

The samples weighed about 3–5 g and yielded from <5–400 mg of extract. The highly sensitive Fourier-transform infrared spectrometry (FT-IR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) analyses required very small amounts of these samples (0.1–0.2 mg). Three extractions of 14 g of the platform sample yielded a total extract of 9 mg for the FT-IR and GC-MS analyses.

FT-IR Databases and Searches
FT-IR spectra were searched for “matches” against large databases of relevant natural products and processed organic materials, synthetic compounds, modern wine samples, and “ancient wine reference samples.” The latter were residues from ancient vessels that likely originally contained wine, based on strong archaeological criteria or exterior inscriptions that recorded their contents. All of the samples, except no. 8, provided matches to ancient and modern wine samples, especially those that were resinated, to a high level of probability (90 or above on a scale of 100, according to Thermo Scientific’s proprietary OMNIC algorithm).

The primary IR data are not presented here because of limitations of space. Moreover, for the purpose of this paper, the pertinent compounds are much more exactly characterized by gas chromatography-mass spectrometry (GC-MS), ultraHPLC tandem mass spectrometry (LC/MS/MS), HPLC with a linear ion trap-Orbitrap mass spectrometry (Orbitrap LC/MS), and headspace solid phase microextraction (SPME) coupled to GC-MS. Suffice it to say that the higher-polarity tartaric acid, which was extracted by methanol, has a distinctive doublet in the 1,740–1,720 cm⁻¹ carbonyl region, with a less intense shoulder at the lower wave number (frequency). Its hydroxyl absorption occurs in the 1,450–1,430 cm⁻¹ region. By contrast, the carbonyl of lower-polarity resinous acids, which were extracted by chloroform, has a single intense absorption at 1,720–1,700 cm⁻¹, and its hydroxyl absorption is in the 1,470–1,455 cm⁻¹ region. Some researchers claim that resin absorption overlaps with tartaric acid in the 1,740–1,720 cm⁻¹ region; their own spectra (figure 4 in ref. 4), however, belie this assertion in showing a significantly lower carbonyl peak (1,710–1,700 cm⁻¹).

GC-MS Extractions and Analyses
For the liquid-injection GC-MS analyses, already extracted samples were taken up in a 1:1 mixture of chloroform and methanol, heated for 1 h at 60 °C, centrifuged, the solubles concentrated down, and derivatized by either methylation with Alltech II Me-Prep or by silylation with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide). The silylated samples were treated with a small amount of formic acid to acidify any tartrate present to tartaric acid. One-microliter samples were injected splitless onto a 30 m × 250 μm × 0.25 μm film thickness HP-5MS column (5% phenyl methyl siloxane) of an Agilent HP 6890 GC, run at a 1.5 mL/min flow rate. An HP 5973 mass selective detector was used with the injector port at 325 °C. The oven temperature was held at 50 °C for 2 min, then programmed to increase at 10 °C/min to 325 °C where it was held for 10.5 min for a total run time of 40 min. The transfer line to the mass spectrometer was at 300 °C. The key silylated tartaric acid ion at m/z 219 was detected by selected ion monitoring, which enhances sensitivity. Compound identification was made by retention time and mass spectrum using National Institute of Standards and Technology (NIST) 05.

Some of the GC-MS analyses were overloaded (e.g., peak B in Fig. S3, representing the dominant compound, dehydroabetic acid, in the residue). Despite overloading, the compound eluted at the correct retention time and with the correct masses. If the sample had been diluted to prevent overloading, the terpenoid components present in lower concentrations would not have been detected.

LC/MS/MS Extractions and Analyses
Because previous analyses of the extracted powders had been negative, separate extractions of soil containing resin-like particles (nos. 4 and 8), the resin-like material in no. 7, and the platform sample were carried out at the Alcohol and Tobacco Tax and Trade Bureau (TTB). Approximately 50–75 mg of the soil and resin-like material and 620 mg of the platform were mixed in 5 mL of 1% to 2.8% ammonium hydroxide in water/methanol (80:20, vol/vol), stirred overnight, and ultrasonicated for 1 h. Two milliliters of methylene chloride were added to samples that appeared to be more resinous. Ammonium hydroxide enhances dissolution of tartaric acid in basic solution so that the latter can be detected as the negative ion and its fragments. All aqueous extracts/suspensions were concentrated by evaporating off the methanol and/or reducing the water content, followed by filtration through a 0.45-μm Nylon Acrodisc filter. It should also be noted that short retention times are typical for ultrahigh performance LC methods and present no problem in separating tartaric acid from other compounds that elute at later retention times. More importantly, our identification techniques relied on multiple factors, including retention times and accurate mass measurements that enable the unambiguous identification of tartaric acid.

Orbitrap LC/MS Extractions and Analyses
Samples of Lattara nos. 4 and 7 were also analyzed by Orbitrap LC/MS using the same extract solutions as for LC/MS/MS. The
LC/MS/MS extract of the platform sample was also purified by solid phase extraction before analysis. After conditioning with 2 mL of methanol and 2 mL of ultrapure water, ~600 μL of extract was loaded onto a Waters Oasis Max 3-cc cartridge and rinsed with 2 mL of 5% ammonia in water followed by 2 mL of methanol. Tartaric acid (and other organic acids) were then eluted using 2 mL of 5% formic acid in methanol. The eluate was dried in a CentriVap (Labconco), resuspended in 100 μL of 2.8% NH₄OH in water, and transferred to an HPLC vial.

A Thermo Scientific Accela High Speed LC coupled to a Thermo Scientific LTQ Orbitrap XL hybrid mass spectrometer was used for the analyses. HPLC separation was achieved with a Phenomenex Luna 5 μm phenyl-hexyl column (1.00 mm × 250 mm) maintained at 40 °C and a flow rate of 0.10 μL/min. Mobile phase (A) was composed of 10 mM ammonium formate, pH 8.4, and mobile phase (B) was acetonitrile. Mobile phase (B) was ramped from 0% to 85% over 5 min, held constant at 85% until 11 min, then ramped back down to reequilibrate the column. A 10-μL sample injection was used.

The experimental parameters were optimized as follows: spray voltage 2.2 kV, tube lens 85 V, ion transfer capillary voltage of −26 V, ion transfer capillary temperature 275 °C, sheath gas 30 (arbitrary unit, a.u.), and auxiliary gas 5 (a.u.). Both the sheath gas and auxiliary gas were nitrogen. Full scan spectra were acquired over a mass range of m/z 50–250. To maintain a sufficient number of data points across chromatographic peaks, a mass resolution setting of 15,000 (at full-width-half-maximum for m/z 400) was used, which resulted in a mass resolution of ~27,000 for tartaric acid. Automated gain control (AGC) was set to 5 × 10^5 ions with a maximum injection time of 1 s. For MS/MS measurements, the AGC was set to 1 × 10^4 ions with a maximum injection time of 100 ms, and the mass window for precursor ion selection was set to 1.0. Parent mass selection, collision induced dissociation (CID), and fragment mass detection all occurred in the ion trap. For tartaric acid, the collision energy was set to 28%; the compound was monitored for the molecular fragment at m/z 87.

External calibration for negative ion mode in the range of m/z 150–2,000 was performed using a mixture of SDS, sodium taurocholate, and Ultrasym 1621 in an acetonitrile-methanol solution containing 1% acetic acid. A formic acid dimer (m/z 112.98563, [M₂ + Na – 2H]⁻) in the background was used as an internal lock mass, which resulted in a typical mass accuracy of less than 1.0 ppm.

Tartaric acid, malic acid, succinic acid, and citric acid in the sample extracts were identified by (i) correlating sample compounds with known standards at the experimentally determined chromatographic retention times, and (ii) comparing accurate mass measurements with theoretical exact masses for the organic acids. Elemental compositions were calculated from the deprotonated molecule with introduced limits of carbon (0–30), hydrogen (0–60), nitrogen (0–10), and oxygen (0–15), with a mass tolerance of 2 ppm. Peak areas were obtained by either manual integration or by the ICIS peak algorithm in the Xcalibur software package.

Orbitrap LC/MS has been applied to the study of highly complex samples, including meteorites (5), petroleum (6), humic substances (7), and here to the analysis of archaeological samples, for which it proved to be well-suited.

**Soil and Stone Control Samples**

Orbitrap LC/MS was also used to assess the background levels of tartaric acid produced by microbial activity. Two soil samples (dated ca. 425–400 B.C. and 400–350 B.C.) from the same courtyard where the platform was located (zone 27, sector 9), close to the merchants’ room, were sampled and sent in March 2013. Similarly, a limestone fragment, mineralogically comparable to the limestone of the pressing platform, was obtained from the nearby city wall (dated ca. 475–400 B.C.). After removing vegetation and foreign materials, the soil and limestone control samples were pulverized with a ceramic mortar and pestle. Heterogeneity effects were minimized by grinding and mixing 650- to 750-mg portions of each sample. A second sample of the ancient platform (no. 2) was also run to assure uniform procedure.

In accordance with the LC/MS/MS extraction method, precisely weighed samples were then stirred overnight in a 2.8% ammonium hydroxide in water/methanol (80:20, vol/vol) solution. Each solution was filtered using a Monocrop 1 mL syringe equipped with a Pall Life Sciences Acrodisc 25-mm syringe filter with 0.2-μm Supor membrane. Before the sample solution was filtered, we prewet the syringe filter by filtering ~1 mL of 2.8% NH₄OH: MeOH solution through it. Sample solutions usually required two syringe filters due to build up of solid material on the syringe filter. All sample solutions appeared clear and colorless after filtration. Following the protocol described above, and which we used previously, they were then purified by solid phase extraction with ~100% recovery of tartaric acid based on standards, and analyzed.

It should be noted in Dataset S4 that the ancient pressing platform samples, when averaged, have a tartaric acid amount that is more than four times that of the city wall control sample. The ancient Lattara amphorae exceed the amount of tartaric acid in the soil samples, when averaged, by more than two orders of magnitude (Lattara no. 4) and by about three times (Lattara no. 7). These are significant differences, especially when other considerations are taken into account. Because the control samples were gathered during the rainy season, when microbial activity is more intense, their tartaric acid contents can be expected to be higher than usual. It is also likely that the amount of tartaric acid in the platform has declined following its excavation in 1998 and especially after it was moved to the excavation storehouse (1999–2008) and then to the museum (2008–present). Particularly in the climate-controlled environment of the museum, any tartaric acid produced by microbial activity would be minimized.

**SPME Extractions and Analyses**

Using fresh powdered samples, the headspace SPME analyses were carried out on an Agilent HP 6890 GC with a 5973 mass selective detector, equipped with an HP-5MS column (30 m × 250 μm × 0.25 μm) and Gerstel MPS2 Multipurpose Autosampler with a divinylbenzene/carboxen/polydimethylsiloxane 50/30/70 fiber. Fifty milligrams of sample were sampled in 1 mL of deionized water, to which 0.5 g of NaCl was added. The fiber was exposed to the headspace of the saline suspension at 70 °C for 10 min, followed by 3 min desorption and splitless injection into the GC-MS at 250 °C. To identify possible carryover compounds or contaminants, blank control samples, consisting of only the aqueous saline solutions, were run between the analyzed samples. The mass spectrometer was operated in the scan mode from 40 to 400 atomic mass units. The oven was heated for 29 min from 50 °C to 250 °C at 7 °C/min, and a constant pressure flow rate of 1.2 mL/min was maintained on the column. The compounds were identified by matching scores of 80 or above to those in the NIST 05 and 08 mass spectral libraries (comprising more than 160,000 compounds).

This method is of great utility in biomolecular archaeological studies. It requires only milligram quantities of valuable archaeological samples, and analyses can be performed rapidly, at lower detection limits, in an aqueous saline solution without prior extraction in an organic solvent.

**Tartaric Acid as the Principal Grape Biomarker in the Near East and Mediterranean**

Barnard et al. (8) recently claimed that malvidin is a better biomarker than tartaric acid/tartrate for identifying the Eurasian grape and its products in the Near East and Mediterranean regions, including Italy. However, a recent, very thorough bioinformatics search confirms the long-established and general

McGovern et al. www.pnas.org/cgi/content/short/1216126110
reliability of Singleton’s data (9), namely, that the concentration of tartaric acid in grape (4,000 mg/L) is twenty times that of malvidin (200 mg/L), as a conservative estimate. Natural sources for malvidin, as might be expected for a pigment, are also much more broadly distributed than plants with tartaric acid. They include pomegranate (Punica granatum), carrot (Daucus carota), apple (Malus domestica), whortleberry/ bilberry (Vaccinium myrtillus), red clover (Trifolium pratense), and dill (Anethum graveolens).

Ref. 8 also incorrectly states that Middle Eastern hawthorn fruit has high amounts of tartaric acid. Although the tartaric acid concentrations in two Chinese hawthorn species (Crataegus pinnatifida and C. cuneata) do exceed those of grape (10), the chemistries of different species of the same genus in different regions of the world can vary enormously. Unless trade relations can be established by archaeological evidence between diverse regions at the time under consideration, other plants with high tartaric acid—e.g., tamarin from the Indian subcontinent, hawthorn fruit and star fruit from east Asia, or yellow plum from malic and citric, as unambiguously identified especially together with other organic acids (including succinic, fumaric, malic, and citric), as determined by Orbitrap LC/MS here; also see ref. 12)—are irrelevant. For the period of this paper, ca. 525–400 B.C. in southern France and Etruria, no archaeobotanical evidence exists for these nonnative plants.

Pomegranate is the only close contender to grape in having relatively large amounts of both tartaric acid and malvidin. Aarabi et al. (11) state that pomegranate has about 600 mg/L of tartaric acid and 650 mg/L of malvidin (200 mg/L), as a conservative estimate. Natural sources in southern France and Etruria, no archaeobotanical evidence exists for these nonnative plants.

Thus, if tartaric acid/tartrate is present in an ancient sample, especially together with other organic acids (including succinic, malic, and citric), as unambiguously identified by Orbitrap LC/MS here; also see ref. 12) and alcohols, esters, aldehydes, and terpenoid compounds characteristic of modern grape (as identified by SPME here), then the probability increases for a grape product.

Methodological Approach to Identifying an Ancient Grape Product as Wine

Assuming that tartaric acid/tartrate has been identified in an ancient vessel, then several other archaeological and enological factors must be assessed, to determine whether the intended product was wine and not another grape product. A syrup, produced by heating grape juice and concentrating it down, was unfermented to all intents and purposes, and if the viscosity was high, it would have left a uniform coating of residue on the inside of the vessel, which was absent. Minimally, then, the amphoras and pressing platform had contained or had come in contact with grape juice. However, any grape juice would not have remained nonalcoholic for long had contained or had come in contact with grape juice. However, any grape juice would not have remained nonalcoholic for long, with the result that any grape product would have become alcoholic. A vineyard product would have potentially wide-ranging medicinal benefits (13).

Adding a tree resin to wine, to protect against wine disease as well as for medicinal purposes and covering up off-tastes and off-odors, was a popular and widespread practice throughout the ancient world (14). Later literary references in Pliny the Elder, Strabo, Cato, and others make it abundantly clear that Etruscan wine was often mixed with both fresh pine resin and processed pitch to make vinum picatum (Latin, “pitched wine”) (15), which left resinous sploths on sidewalks and accumulations on the bases of bronze wine cauldrons at sites throughout Etruscan and Ligurian Italy and Celtic Gaul as early as the fifth century B.C. (16). A metal such as bronze did not need to be sealed with tar, as became more customary for pottery amphoras and other containers in later periods. Resinated wines were still being made in the Middle Ages, according to the extensive agricultural and medical compilations based on classical writings, collectively known as the Geoponica (e.g., ref. 17).

Other researchers have begun to report botanical and chemical evidence for herbal concoctions in alcoholic beverages. Far in advance of the Etruscan evidence, native rosemary and mint, together with thyme, were added to a fermented emmer wheat and barley beverage at Geno, near Barcelona in Spain, around 3000 B.C. (18). Mugwort (Artemisia vulgaris) in the wormwood family), also detected in some of the early Spanish brews, was hypothesized to have been an additive, together with carrot, in a dark, sour barley beer (19) at the settlement of Hochdorf, located next to the tumulus burial for the Celtic prince who was honored in death by a cauldron filled with meal. Wild rosemary continued to be an ingredient in gruit, the principal bittering agent in early medieval European beer, along with bog myrtle, yarrow, and other herbs (20).


Fig. S1. Two analyzed Lattara samples, according to their representative archaeological types: no. 4 (Upper), an Etruscan amphora, and 8 (Lower), a Massaliote amphora (photograph and drawings by B.P.L.).

Fig. S2. Remains of the foundations of the Etruscan merchants’ quarters in zone 27 of Lattara, dated ca. 525–474 B.C. Amphora nos. 4, 5, and 7 came from the concentration of amphoras in room 15 (Inset). Photographs courtesy of Michel Py, copyright l’Unité de Fouilles et de Recherches Archéologiques de Lattes.
Fig. S3. GC-MS chromatogram for Lattara no. 4, an Etruscan amphora. A, abietic acid; B, dehydroabietic acid; C, tetradehydroabietic acid; D, hexadehydroabietic acid; E, 7-oxo-dehydroabietic acid; F, 15-hydroxy-dehydroabietic acid; G, retene; H, pimaric acid; I, isopimaric acid; J, sandaracopimaric acid.

Fig. S4. Multiple reaction monitoring LC/MS/MS traces of l-tartaric acid corresponding to m/z 149→87 molecular ion fragmentation for an Etruscan amphora, Lattara no. 4 (A) and a Massaliote amphora, Lattara no. 8 (B), compared with standard solutions of l-tartaric acid and calcium tartrate (C and D, respectively). The 4-s earlier retention time for sample no. 4 is due to a slightly different extraction procedure.
Fig. S5. SPME total ion chromatogram (A) of Lattara sample no. 4, with the chromatogram expanded in the 9.2–12.2 min range (B) and showing the experimental electron ionization (70 eV) mass spectra of nonanal (C), fenchol (D), and cuminaldehyde (E). The Upper traces of C–E are the experimental mass spectra; the Lower traces are NIST 08 database matches. Representative mass spectra of camphor and borneol are published in ref. 2.

Fig. S6. Black-figured vase by the Amasis Painter of sixth century B.C. Athens, recovered from the Etruscan site of Vulci, shows a busy winemaking scene in the vineyard. A hairy satyr merrily stomps away inside an open basket, filled with grapes, from which yellowish juice runs out through the spout of a flat basin, shaped like the Lattara wine pressing platform, into a large jar or pithos buried up to its shoulders in the floor. Note the grapevine, supported on poles and trained vertically and horizontally—this trellis method is useful in opening the grapes up to greater airflow and more sunlight for ripening and easy care and harvesting. The yellowish juice points to a white wine and grape, rare in the pre-Roman ancient world. This ceramic masterpiece is the earliest depiction in the Greek world that shows a sequence of vinicultural activities (picking, treading, fermentation) and highlights the close connection of winemaking to music, dance, religion, and celebration. Photograph courtesy of the Martin von Wagner Museum, University of Würzburg. Photograph by P. Neckermann (redrawn and adapted by B.P.L.).
Dataset S1. Description and primary chemical compounds/families of analyzed amphora and pressing platform samples from Lattara

Dataset S2. Pine tree resin compounds identified by GC-MS for amphora and platform samples from Lattara

Dataset S3. Chemical compounds identified by SPME for Etruscan amphora nos. 4 and 5 from Lattara

Dataset S4. Orbitrap LC/MS data for soil and limestone control samples, ancient amphoras, and pressing platform from Lattara