Supporting Information

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SI Text

Extraction of the Gebel Adda sample for LC/MS/MS analysis was carried out by pulverizing ≈50 mg of the residue in 5 mL of 0.1% formic acid in water/methanol 80:20 (vol/vol), stirring overnight, and ultrasonicating for 1 h. The suspension was concentrated by evaporating off the methanol, followed by filtration through a 0.45-μm Nylon Acrodisc filter (Waters Corp.).

An additional special pretreatment was discovered to be critical in detecting tartaric acid in the Abydos sample by LC/MS/MS. The same extraction steps were followed as for the Gebel Adda sample, except that after pulverization of the ≈200-mg sample, 3 mL of a 1-mM solution of ammonium hydroxide was added to the very small Abydos sample. Because of enhanced dissolution of tartaric acid in basic solution, it could then be detected as the negative ion and its fragments. Tartaric acid could not be detected in this sample using positive-mode ion monitoring.

A 35-ng/mL limit of detection was estimated from the signal-to-noise ratio of the MRM chromatogram of tartaric acid standard, similar to the findings of other researchers (1). Guasch-Jané et al. (2) reported a 0.05-ng/mL sensitivity.

It was calculated from the tartaric acid peak areas of the standard and archaeological samples that the acid was present at less than 1 ppm for the Abydos sample and at less than 32 ppm for the Gebel Adda sample. Because the latter was a younger sample, the acid might have been better preserved.

Headspace SPME experiments were performed with a DVB/CAR/PDMS 50/30-μm fiber on a MPS2 Multipurpose Autosampler (Gerstel, Inc.). Fifty micrograms of the Abydos and Gebel Adda samples were pulverized and suspended in water, to which 0.5 g of NaCl was added. The fiber was exposed to the headspace of the saline suspension at 80 °C for 40 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.

TD experiments were carried out on a TDS2 Therm-Desorption System and Autosampler (Gerstel, Inc.). A total of 5–10 mg of the pulverized archeological samples was heated from 50–250 °C at 50 °C/min. The volatiles were released into a Cooled Injection System (CIS 3, Gerstel, Inc.) at −70 °C and heated at the rate of 12 °C/min before GC/MS analysis. Blank controls were run before and after each sample.

For both SPME and TD, GC/MS analysis was carried out on a 6890 GC with a 5973 mass selective detector (Agilent Technologies), equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 μm). The mass spectrometer was operated in the scan mode from 40–400 atomic mass units. The oven was heated for 1 h from 60 to 240 °C at 3 °C/min, and a constant pressure flow rate of 1.2 mL/min was maintained on the column. The compounds were identified using the NIST05 mass spectral library and, where available, the Agilent's Flavor2 database in the retention time lock (RTL) feature of the instrument.

For the liquid-injection GC/MS analysis of the Abydos sample, it was extracted with a 1:1 mixture of chloroform and methanol, sonicated for 15 min, heated for 1 h at 60 °C, centrifuged, concentrated down, and derivatized with Me-Prep II (Grace). The sample was injected splitless onto an HP-5MS column (5% w/w phenyl methyl siloxane). Compound identification was made by retention time and mass spectrum using NIST05. Our attempt to identify tartaric acid/tartrate by GC-MS was hindered by the polarity of tartrate.

It should be noted that some compounds, which might be anticipated in a fermented fruit beverage made from grapes, have not yet been definitively detected in our ancient wine samples, *viz*., resveratrol, other stilbenes, and flavonoids. Unfortunately, these compounds are not readily detectable by GC/MS because of their polarity and/or low volatility. They can also be anticipated to be present in very small amounts in ancient samples. Ongoing research, as outlined in the main text, should eventually lead to their detection.

1. Stern B, Heron C, Tellefsen T, Serpico M (2008) New investigations into the Uluburun resin cargo. *J Archaeol Sci* 35:2188–2203.
2. Guasch-Jane MR, Ibern-Gomez M, Andres-Lacueva C, Jauregui O, Lamuela-Raventos RM (2004) Liquid chromatography with mass spectrometry in tandem mode applied for the identification of wine markers in residues from ancient Egyptian vessels. *Anal Chem* 76:1672–1677.

Fig. S1. Tide-line of a yellowish residue, similar to that in jar no. 115 (Fig. 2), on the interior of jar no. 50 from chamber 7 of the tomb of Scorpion I (U-j) at Abydos (Egypt), *ca*. 3150 B.C. [Hartung U, ed (2001) *Umm el-Qaab II: Importkeramik aus dem Friedhof U in Abydos (Umm el-Qaab) und die Beziehungen ägyptens zu Vorderasien im 4. Jahrtausend v. Chr*. (P. von Zabern, Mainz, Germany): cat. no.156, pls. 58: and 94:156]. Height of sherd: 33.5 cm. Drawing courtesy of German Archaeological Institute in Cairo.

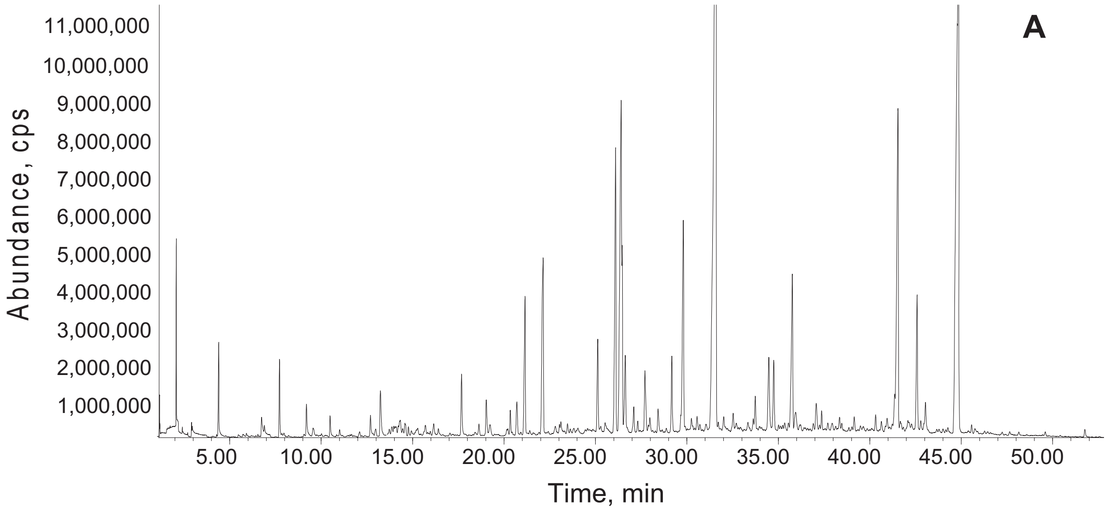


Fig. S2. SPME total ion chromatogram (*A*) of the ancient Abydos sample, with the *m/z* 95 extracted ion chromatogram expanded in the 12.00–13.60-min range (*B*) and showing the experimental electron impact (70 eV) mass spectra of camphor (*C*), borneol (*D*), and L-menthol (*E*).

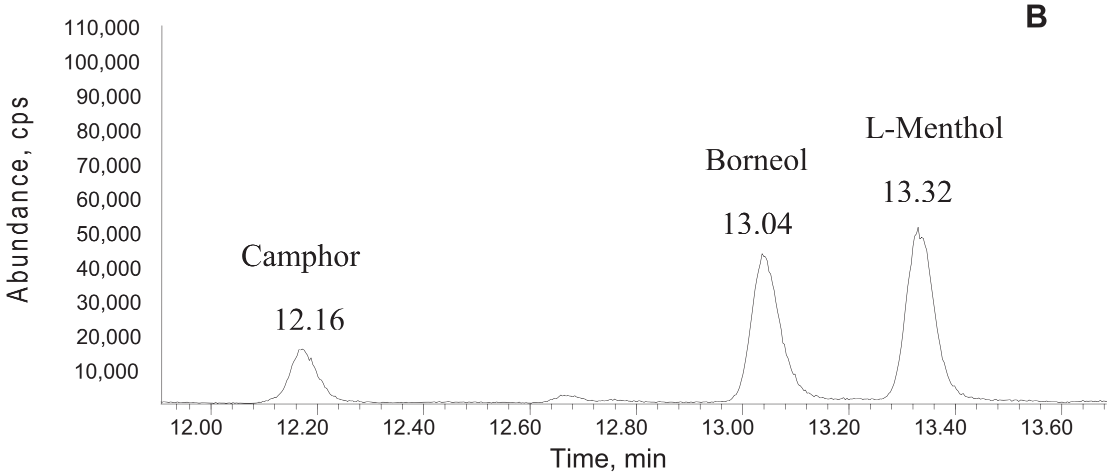
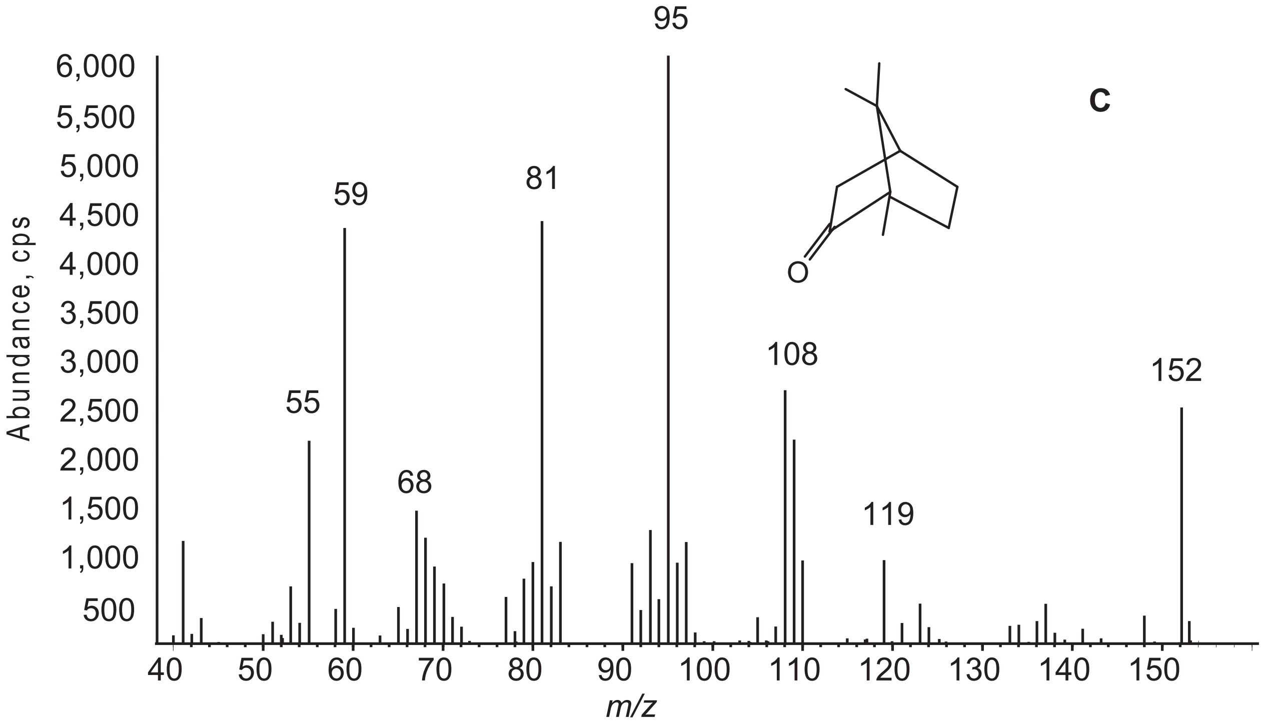


Fig. S2. Continued.



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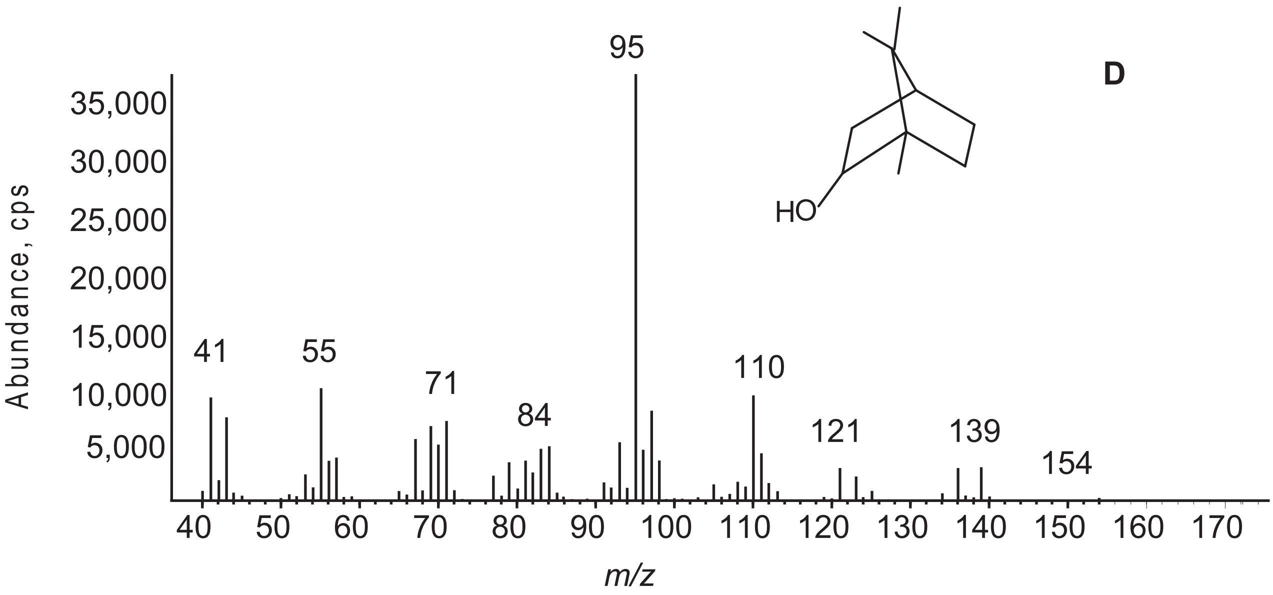

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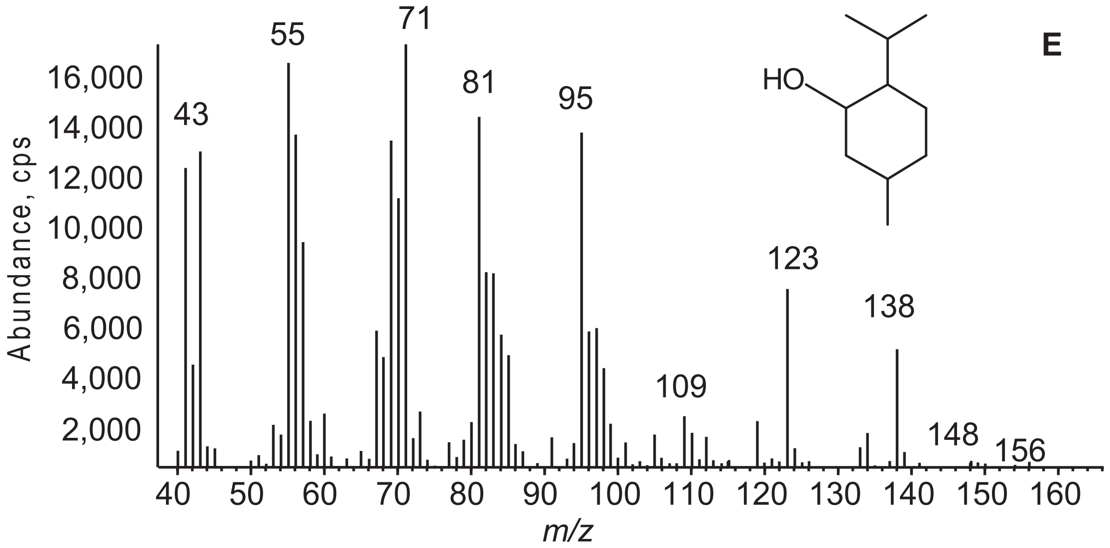

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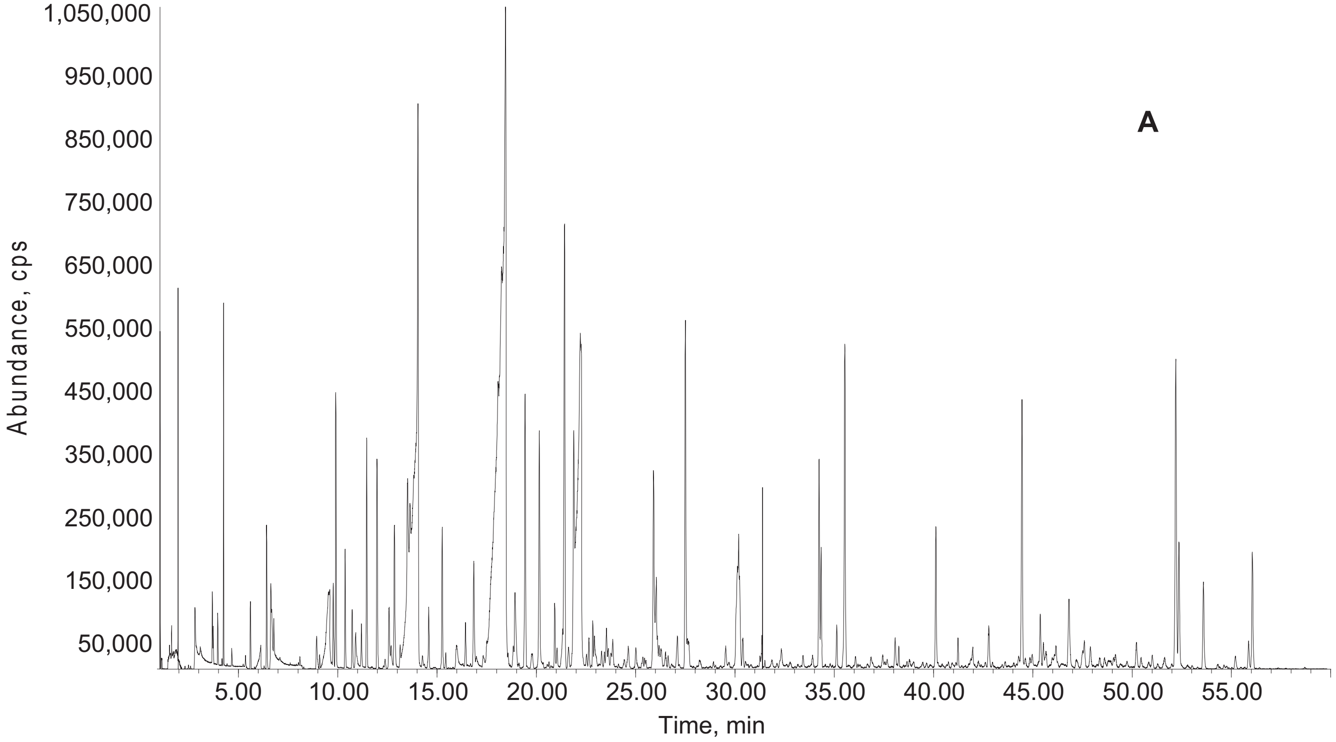
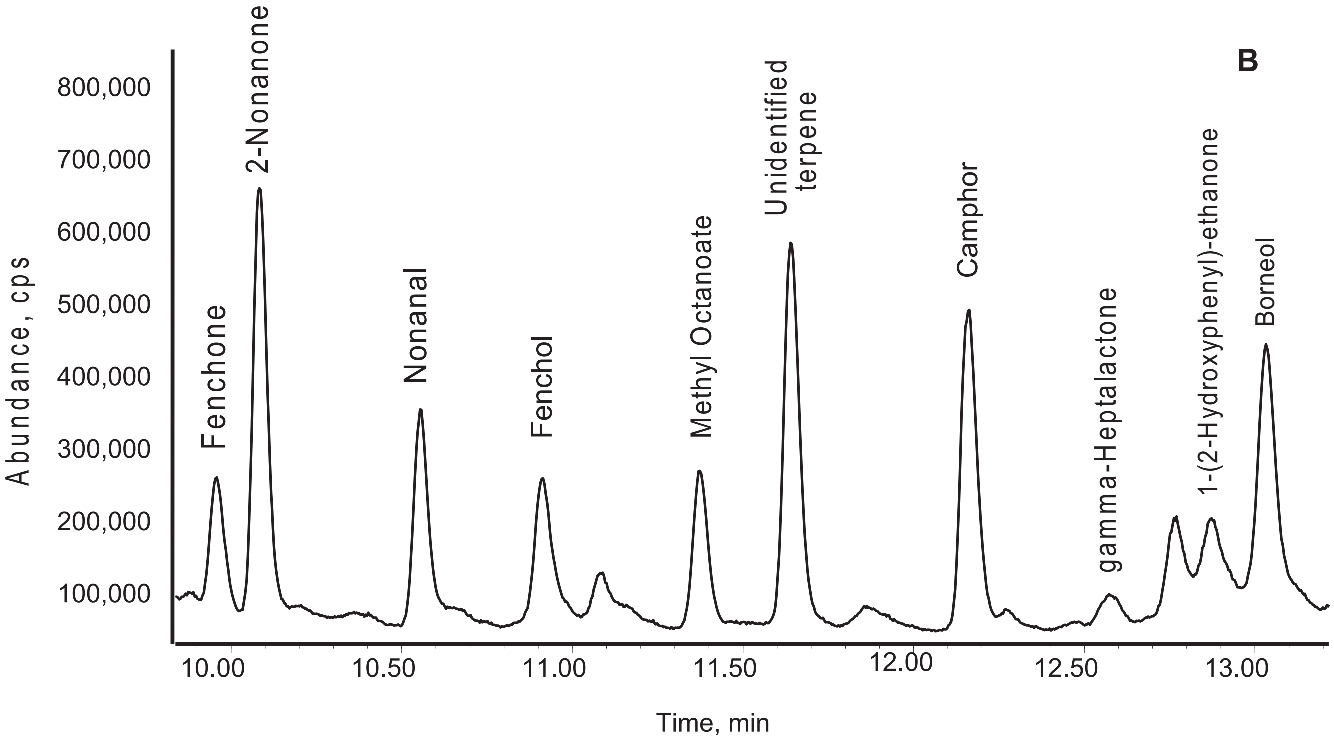


Fig. S3. SPME total ion chromatogram (*A*) of the ancient Gebel Adda sample, expanded in the 9.80–13.20-min range (*B*) and showing the experimental electron impact (70 eV) mass spectra of fenchone (*C*) and fenchol (*D*).



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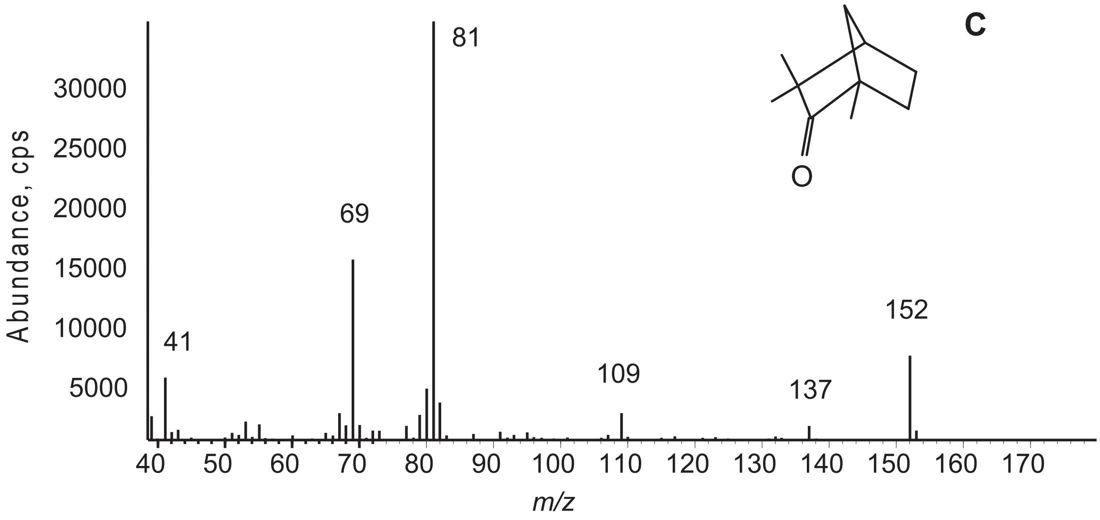

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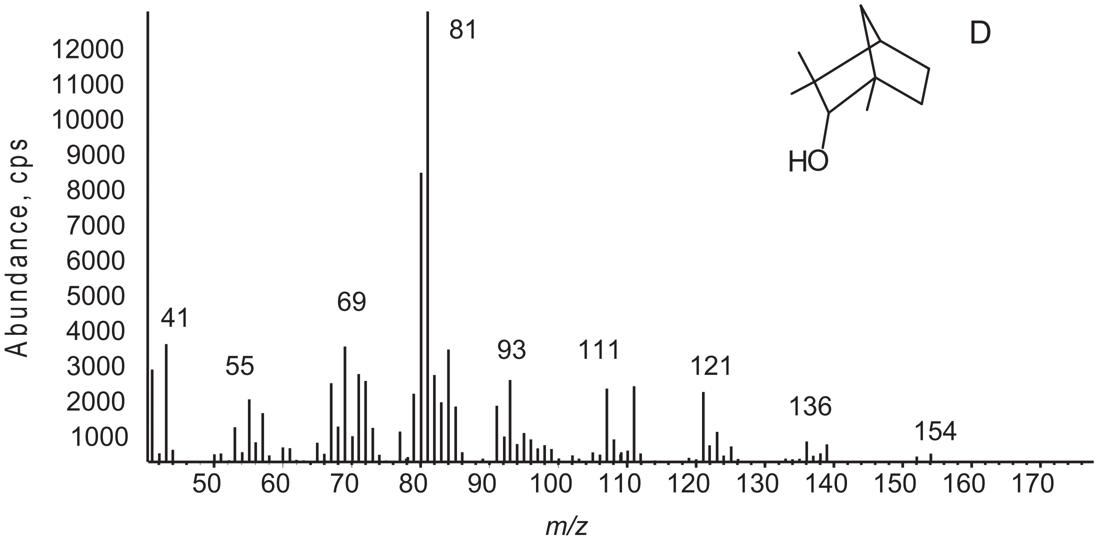

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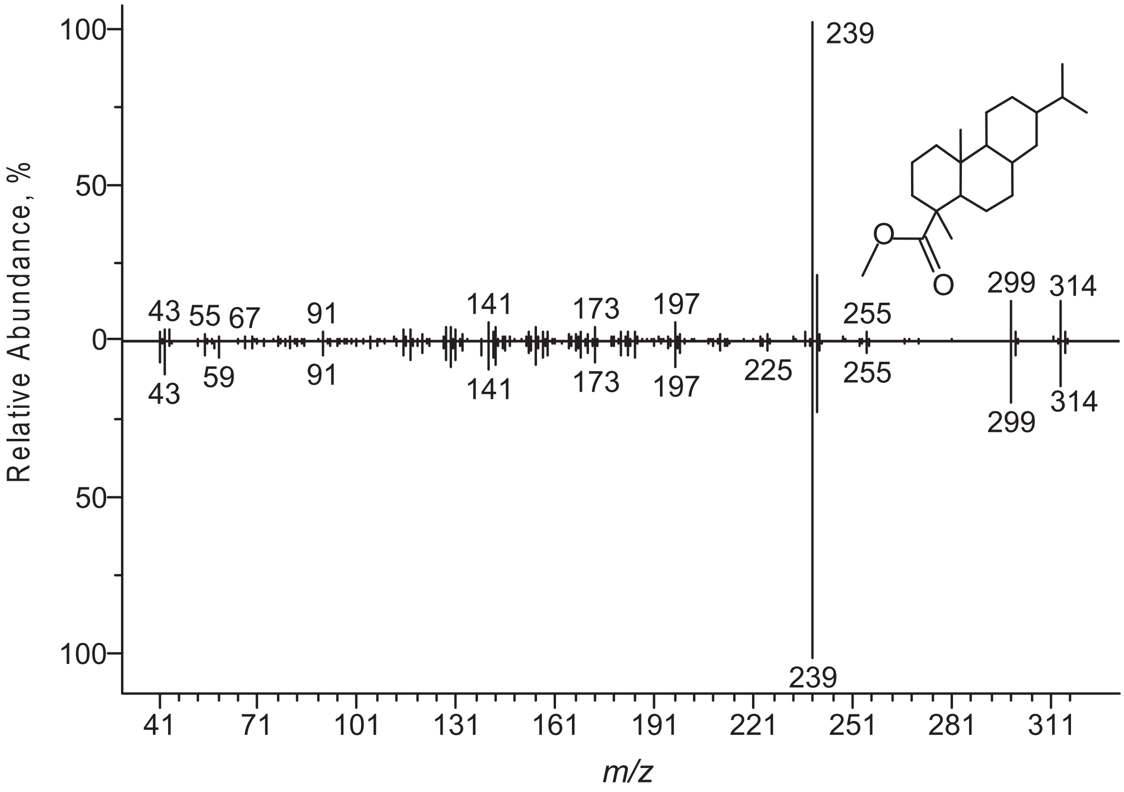

Fig. S4. Comparison of the experimental (*Upper*) and library (*Lower*) spectra of methyl dehydroabietate at 56.24 min.

Other Supporting Information Files

Table S1