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**Pentacyclic triterpene methyl ethers in recent lacustrine sediments
(Lagoa do Caçó, Brazil).**

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Abstract

The lipid extracts of sediments collected from the Quaternary filling of a tropical lacustrine series (Lagoa do Caçó, Brazil) were investigated by gas chromatography-mass spectrometry (GC-MS). Various pentacyclic triterpene 3-methyl ethers (PTMEs) were present in the neutral fraction. Comparison of retention times and mass spectra with available standards allowed us to certify the presence of olean-12-en-3 β -ol ME (β -amyryn ME), olean-18-en-3 β -ol ME (miliacin), taraxer-14-en-3 β -ol ME (crusgallin), fern-9(11)-en-3 β -ol ME (arundoin) and arbor-9(11)-en-3 β -ol ME (cylindrin). The following other compounds could also be tentatively identified from their GC-MS characteristics: urs-12-en-3 β -ol ME, bauer-7-en-3 β -ol ME and fern-8-en-3 β -ol ME. Other compounds such as possible 3 α isomers of the PTMEs as well as di- or tri-unsaturated counterparts might be PTME diagenetic derivatives. According to previous chemotaxonomic studies, all these compounds most probably originate from Gramineae that used to colonize the savannas of Northern Brazil at the time of deposition.

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1. Introduction and setting

Since the isolation of isoarborinol from the Messel Oil Shale (Albrecht and Ourisson, 1969), the literature has been continuously enriched with information on new pentacyclic triterpenes which have been used in petroleum exploration or paleoenvironmental studies (Cranwell, 1984). These compounds comprise higher plants triterpenes (e.g. oleanane, lupane, ursane derivatives) which occur widely in the plant kingdom in the free form or bound to glycosyl or phenolic moieties through a functional group (Pant and Rastogi, 1979; Das and Mahato, 1983; Mahato et al., 1988; 1992; Mahato and Sen, 1997). The complex molecular skeleton and the different functionality of the triterpenes make this family of compounds one of the most diversified in Nature. Their chemotaxonomic potential, their ability to degrade via specific diagenetic routes and their widespread occurrence in the sedimentary record can provide key information on floral changes and early diagenesis (e.g. Killips et al., 1995). As noted by van Aarssen et al. (2000), few studies have screened for these compounds or their diagenetic derivatives in order to reconstruct past environmental changes and climatic fluctuations. Other relevant work was limited to assessing terrestrial input versus phytoplankton organic matter production (Peters and Moldowan, 1993).

Recent tropical lacustrine sedimentary records are usually poorly described with regard to their biomarker content. Nevertheless, they provide unique botanical and geological records which encourage the identification of new compounds. In addition, studies on biomarkers in these areas could reveal useful information for calibrating geochemical studies on older sediments deposited in similar settings.

Lagoa do Caçó is a small oligotrophic lake located in northeast Brazil, close to the Equator (Fig. 1). The sedimentary filling has been studied using various approaches to better document palaeoclimatic development since the Last Glacial Maximum (LGM) in the Tropics (Ledru et al., 2001; 2002; Sifeddine et al., 2003; Jacob, 2003; Jacob et al., 2004a and b). Here we report on the GC-MS characteristics of several series of pentacyclic triterpene methyl ethers (PTMEs) that are present in the lipid extracts of Quaternary sediments from this tropical setting and their significance as plant source indicators or diagenetic indicators.

2. Materials and methods

2.1. Sediment samples

The context and sample handling have been described (Jacob et al., 2004a). Briefly, a core (MA98-3; 6 m long) was divided into two main intervals. The lower one (ca. 3 m long), which dates back to the end of the LGM, consists of fine-grained sands and silts. The upper

half (ca. 3 m long), consisting of organic matter-rich silts, is divided into greenish-brown organic silts (Late Glacial) and black organic silts (Holocene). Two samples, which best illustrate the distribution of PTMEs, were selected following sedimentology and Rock-Eval 6 screening. Sample 170 (352 cm depth) belongs to a section dated back to the LGM, whereas sample 073 (150 cm depth) is from sediments deposited during the Holocene.

2.2. Extraction and separation of free lipids

The method for lipid extraction and separation was based on that of Logan and Eglinton (1994), with some modifications. One gram of dried sediment was ultrasonically extracted with acetone-pentane 1:1. The mixture was then separated into a neutral and an acidic fraction by solid phase extraction using AminoPropyl Bond Elute® cartridges. Neutral compounds were eluted with DCM:CH₃OH (1:1) and acidic compounds with ether after acidification of the medium with ether:formic acid 9:1. The neutral fraction was submitted to further fractionation on activated Florisil® to give aliphatic hydrocarbons (eluted with heptane), aromatic hydrocarbons and ethers (DCM) and polar compounds (DCM:CH₃OH 1:1).

2.3. GC-MS

GC-MS analysis was performed on a ThermoFinnigan TRACE-PolarisGCQ gas chromatograph-mass spectrometer. The gas chromatograph was fitted with an Rtx®-5Sil MS capillary column (30 m x 0.32 mm i.d., 0.25 µm film thickness) with 5 m of guard column. The GC operating conditions were as follows: temperature hold at 40°C for 1 min, then increase from 40 to 120°C at 30°C.min⁻¹, 120 to 300°C at 3°C.min⁻¹ with final isothermal hold at 300°C over 30 min. The sample was injected splitless, with the injector temperature set at 280°C. Helium was the carrier gas. The mass spectrometer was operated in the electron ionisation (EI) mode at 70 eV ionization energy and scanned from 50 to 650 Dalton. Where possible, the structure of individual compounds was ascertained with authentic standards isolated from extant plants that were analysed with the same conditions. The remaining compounds were tentatively identified by comparison of mass spectra with literature data, relative retention times and interpretation of mass spectrometric fragmentation patterns.

3. Results

Total extract yields ranged between 0.5 and 4.2 mg.g⁻¹ sediment. The neutral fraction afforded between 0.4 and 3.5 mg.g⁻¹ sediment. The dominant compounds in this fraction are PTMEs and hopanoids. In total, at least sixteen compounds were distinguished by GC-MS. Two

examples of the distribution of these compounds in two extracts from the sedimentary fill of Lagoa do Caçó are shown in Fig. 2 together with a reconstructed total ion current (TIC) trace of reference compounds.

In total, five out of the sixteen compounds were identified by coelution with available standards, the identification of other compounds remaining tentative. The main characteristics of all the studied compounds are summarized in Table 1. Thirteen out of the sixteen have a molecular mass of 440 amu (Table 1). Compared to these compounds, two others with a molecular mass of 438 and another with 436, most probably have one and two additional double bonds, respectively. For most of the compounds having a molecular mass of 440 amu, the presence of fragments at m/z 408 $[(M-32)^+]$ and m/z 393 $[(M-15)-32]^+$ points to the loss of a methoxy group as a neutral methanol molecule during fragmentation (Bryce et al., 1967b). These characteristics, plus the fragmentation pattern typical for triterpene ethers as discussed below, led us to propose that the compounds with a molecular mass of 440 are unsaturated methoxy triterpenes having the formula $C_{31}H_{52}O$. At first sight the compounds can be separated into two main groups, namely those whose mass spectra are dominated by fragments at m/z 218, 203/204 and 189 (compounds 2-7) and those giving a base peak at m/z 273 and a major fragment at m/z 241 (compounds 8, 10, 13, 14 and 15). We discuss first the possible identity of the compounds belonging to these two different groups, then, we consider those having different general characteristics (compounds 1, 9, 11, 12 and 16).

3.1. Compounds 2 to 7

The intense fragments at m/z 218, 203/204 and 189 in the mass spectra of compounds 2 to 7 (Fig. 3) can arise from the D/E moiety of normal and D-friedo- triterpenes after C-ring breaking and possible retro-Diels Alder rearrangement (Budzikiewicz et al., 1962; Djerassi et al., 1962).

In addition to intense ions at m/z 218, 204 and 189, the mass spectra of compounds 2 and 4 (Fig. 4) also show intense fragments at m/z 316, 301, 284 and 269 typical for taraxer-14-en-3 β -ol methyl ether (i.e. sawamilletin or crusgallin; Bryce et al., 1967a). As a matter of fact, comparison of retention time and mass spectrum with an authentic standard allowed us to identify compound 4 as taraxer-14-en-3 β -ol ME (Fig. 4). Compound 2 has a comparable mass spectrum to compound 4 except for the fragment at m/z 190, which could arise from hydrogen rearrangement on the m/z 189 moiety or from a possible coeluting compound. Because only the mass spectrum of taraxer-14-en-3-ol ME is presently known to display fragments at m/z 316, 301, 284 and 269, it can thus be supposed that compound 2 could correspond to the

taraxer-14-en-3-ol methyl ether isomer evidenced by Bryce et al. (1967a). The simplest change that taraxer-14-en-3 β -ol ME (compound 4) could undergo, without significantly affecting the fragmentation pattern, is an epimerisation of the methyl ether group, at the C-3 position. We can therefore hypothesize that compound 2 is taraxer-14-en-3 α -ol ME.

Co-injection with an authentic standard (Fig. 3) allowed us to identify compound 6 as olean-12-en-3 β -ol ME (β -amyrin ME or isosawamilletin; Fig. 5). In a similar way as for compounds 2 and 4 discussed above, the difference in retention time and spectral resemblance between compounds 3 and 6 (Fig. 5) led us to hypothesize that compound 3 could be the 3 α - epimer of compound 6, i.e. olean-12-en-3 α -ol ME.

In the absence of an available standard but according to mass spectral resemblance (Bryce et al., 1967b), compound 5 is tentatively identified as urs-12-en-3 β -ol ME (α -amyrin ME; Fig. 5). Identical retention time (Fig. 3) and mass spectrum with an authentic standard allowed us to identify compound 7 as olean-18-en-3 β -ol ME (miliacin or germanicol ME; Fig. 6).

3.2. Compounds 8, 10, 13, 14 and 15

Compounds 8, 10, 13, 14 and 15 (Fig. 7) display very similar mass spectral features, with significant fragments at m/z 393, 287, 273, 255 and 241 in comparable proportions in the five spectra (Fig. 8). The dominant fragment at m/z 273 $[M-167]^+$ is typical of D:C- or E:C-friedo triterpenes of the fernane, arborane, bauereane, or multiflorane type (Nishimoto et al., 1968; Shiojima et al., 1992). By loss of CH_3OH , this latter fragment produces the other important fragment at m/z 241. A small fragment at m/z 365 could be attributed to the loss of an isopropyl group from the m/z 408 $[(M-32)^+]$ fragment. Therefore, compounds 8, 10, 13, 14 and 15 can be identified as D:C- or E:C-friedo triterpene methyl ethers with an isopropyl group on ring E and a double bond in the $\Delta^{9(11)}$ or Δ^8 positions (Nishimoto et al., 1968; Bryce et al., 1967a). By comparison of retention times (Fig. 7) and mass spectra with authentic standards, compounds 14 and 15 can be identified as fern-9(11)-en-3 β -ol ME (arundoin) and as arbor-9(11)-en-3 β -ol ME (cylindrin), respectively (Fig. 8).

Finally, there are few spectroscopic features to distinguish between the three remaining compounds (8, 10 and 13). In the same way as for taraxer-14-en-3 β -ol ME (compound 4) and its possible 3 α epimer (compound 2) discussed above, comparable differences in retention time between compounds 8-14 and 10-15 (1.51 min and 1.78 min, respectively) and mass spectral resemblances lead us to propose that compounds 8 and 10 could be the 3 α - epimers of fern-9(11)-en-3-ol ME and arbor-9(11)-en-3-ol ME, respectively. The remaining

compound 13 could be the fern-8-en-3 β -ol ME, previously described by Nishimoto et al. (1968).

3.3. Compounds 11 and 1

Compound 11 coelutes with compound 10 under the GC temperature program used routinely (Fig. 7). The mass spectra of 10 and 11 were obtained on samples containing greater proportions of one of these two compounds than of the other, and by background subtraction. The mass spectrum of 11 is dominated by a strong doublet at m/z 261 and m/z 229 (Fig. 9). Small fragments at m/z 273 and m/z 241 may originate from the coeluting compound 10 (Fig. 7). No fragment indicative of the loss of an isopropyl group from the ion at m/z 408 was detected. From this evidence and according to Nishimoto et al. (1968) and Bryce et al. (1967b), compound 11 is tentatively identified as bauer-7-en-3 β -ol ME.

Compound 1 (Fig. 3) shows a mass spectrum with a base peak at m/z 163 and intense ions at m/z 191 and m/z 205 (Fig. 10). Minor doublets at m/z 229 and 261, m/z 241 and 273, m/z 255 and 287 and m/z 323 and 355 suggest a D:C- or E:C-friedo structure with a methoxy group on ring A. Ions at m/z 365 and m/z 163 indicate the loss of an isopropyl group from fragments at m/z 408 and 206, respectively. The relatively short retention time of compound 1 could be indicative of a tetracyclic triterpene methyl ether. However, significant differences in retention times or fragmentation patterns, exclude the possibility that compound 1 could be cycloartenol ME (Fig. 3), or parkeol ME (Russell et al., 1976), respectively. Because lanostene-type molecules have a similar fragmentation behaviour as that of arborenes/ferrenes (ascribed to the presence of methyl groups located at the C/D-ring junction), compound 1 is tentatively assigned as a lanostadienol ME (Fig. 10; Uyeo et al., 1968).

3.4. Compounds 9, 12 and 16

The spectra of compounds 9 and 12 have comparable features: a molecular ion at m/z 438, fragments at m/z 423 $[M-15]^+$, and m/z 391 $[(M-15)-32]^+$ (Fig. 11). Fragments at m/z 425 and 408 in the mass spectra of compound 9 arise from coeluting compound 8. The fragment at m/z 363 can be explained by loss of an isopropyl group from ion m/z 406, well expressed in the spectra of 12. The spectra of both compounds exhibit doublets at m/z 285 and 253 and 271 and 239, indicative of an additional double bond on A, B or C-ring as compared with the spectra of compounds 8, 10, 13, 14 and 15 (Fig. 8). Accordingly, 9 and 12 are thought to be di-unsaturated D:C- or E:C-friedo PTMEs with a fernane or arborane skeleton. Given the

earlier elution of fernane-type compared to arborane-type compounds, we propose a fernane-7,9(11)-dien-ol ME structure for 9 and an arborane-7,9(11)-dien-ol ME structure for 12.

Compound 16 (Fig. 7) has a molecular ion at m/z 436, which is consistent with a structure of PTME with three double bonds (Fig. 11). Its mass spectrum points to a D:C- or E:C-friedo structure. Comparison with the spectra of compounds 9 and 12 (Fig. 11) suggests that the additional double bond is located on ring A or B. From this evidence, an arboratriene or fernatriene ME structure is proposed for compound 16.

4. Discussion

To our knowledge, the only previous record of methoxy triterpenes in geological samples was made by Ries-Kautt (1986), who identified in soils one compound displaying a similar mass spectrum to fern-9(11)-en-3 β -ol ME and another unidentified one that could be related to taraxer-14-en-3 β -ol ME.

4.1. Biological sources of PTMEs

The following discussion on PTME biological sources is mainly based on available information on the occurrences of these compounds in living organisms and on a comparison with those detected from Lagoa do Caçó (Table 2). Pentacyclic triterpenes bearing an oxygenated group at position 3 are widely distributed in the plant kingdom (Pant and Rastogi, 1979; Das and Mahato, 1983; Mahato et al., 1988; 1992; Mahato and Sen, 1997). Nevertheless, methyl ethers are rather uncommon. Most of the PTME plant sources are monocots and belong to Gramineae (Poaceae). PTMEs from Gramineae have been largely studied during the 1960s (review in Martin-Smith et al., 1967; Ohmoto et al., 1970). Ohmoto et al. (1970) reported on the occurrence of nine PTME structures from thirty one species of Gramineae belonging to fourteen tribes. Little chemotaxonomic evidence is available from these studies due to the few plants studied. More recently, arbor-9(11)-en-3 β -ol ME, lupanol ME and lupeol ME have been identified in palm trees (*Butia capitata* and *Orbignya sp.*; Garcia et al., 1995; *Elaeis guineensis*; Goh et al., 1988). Taraxer-14-en-3 β -ol ME, arbor-9(11)-en-3 α -ol ME and arbor-9(11)-en-3 β -ol ME have also been isolated from various dicots (*Diospyros ferrea*, *Bosistoa sp.* and *Mimusops littoralis*; Deshmane and Dev, 1971). Finally, Pinaceae (Rowe and Bower, 1965) and Burseraceae (Uyeo et al., 1968) have been cited as sources of PTMEs but, in the case of Pinaceae, the compounds were of the serratane-type. Injection of authentic lupeol ME (Bryce et al., 1967a) proves its absence in the analysed

samples (Fig. 2). In addition, none of the following compounds have been detected in our samples: hop-22(29)-en-3 β -ol ME (Rowan and Russell, 1992; Paupit et al., 1984), friedelenol ME (Oros and Simoneit, 2001) and serratenol ME (Rowe and Bower, 1965).

It is clear from the data in Table 2 that none of the species cited in the literature affords a range of PTMEs as wide as that encountered in our samples. Fern-9(11)-en-3 β -ol ME, arbor-9(11)-en-3 β -ol ME, taraxer-14-en-3 β -ol ME, olean-12-en-3 β -ol ME and olean-18-en-3 β -ol ME only co-occur in Poaceae species. The taxon displaying the distribution of PTMEs which is the more comparable to that found in our samples is Andropogonae. As a matter of fact, plants belonging to this genus produce taraxer-14-en-3 β -ol ME, olean-18-en-3 β -ol ME, fern-9(11)-en-3 α -ol ME, bauer-7-en-3 β -ol ME, fern-9(11)-en-3 β -ol ME and arbor-9(11)-en-3 β -ol ME but not olean-12-en-3 β -ol ME. Therefore, although Andropogonae could appear as a likely source for the sedimentary PTMEs, at least one additional source must also be envisaged. While screening chemotaxonomic relationships in the Cortaderia genus, Martin-Smith et al. (1967) noted that the South American strains (*C. selloana* and *C. atacamensis*) did not contain any PTMEs, in contrast to New Zealand species. It must thus be considered that the biological precursor(s) of the PTMEs identified in our samples could also originate from at least one of the twenty Cortaderia species that have not been analysed yet.

4.2. Diagenesis of PTMEs

PTMEs are minor components in living organisms and are defined as secondary metabolites (H. Connor, pers. comm.). These compounds are found in leaf epicuticular waxes, where they play a crucial role in defending the organism against external agents (e.g. microbes, fungi, U.V). In the upper section of Lagoa do Caçó sediments (i.e. since the Late Glacial), the OM is dominantly terrestrial in origin and is subjected to strong reworking in aerobic waters (Jacob, 2003; Jacob et al., 2004a). Therefore, the relatively high abundance of PTMEs in the core section, is probably due to their relative resistance to early degradation processes and hence a better preservation than many other biomarkers.

Despite this relative resistance, PTMEs could undergo alteration to their structure that might account for the enhanced diversity observed in the sediments of Lagoa do Caçó, as compared to the reported occurrences in the plant kingdom.

Pentacyclic triterpenes are known to undergo structural rearrangements (Courtney et al., 1958; Coates, 1967; Chatterjee et al., 1976; Ageta et al., 1987; Rullkötter et al., 1994). For example, ten Haven et al. (1992) have shown that taraxerane-type structures are converted into

thermodynamically more stable oleanane-type compounds. Similarly, Ageta et al. (1987) have demonstrated that fern-9(11)-ene is converted into fern-8-ene when treated with BF_3 . The presence of olean-12-en-3 β -ol ME, together with olean-18-en-3 β -ol ME in the sediments of Lagoa do Caçó could hence result from the diagenetic rearrangement of taraxer-14-en-3 β -ol ME. Similarly, the supposed fern-8-en-3 β -ol ME could be produced from fern-9(11)-en-3 β -ol ME.

Although the presence of 3 α - isomers in the sediments of Lagoa do Caçó remains speculative, it constitutes the simplest way to explain the diversity of PTMEs present in the sediment, especially with regard to their known occurrences in the plant kingdom. Few 3 α -PTMEs have been isolated from living sources (Table 2). Arborinol ME (arbor-9(11)-en-3 α -ol methyl ether) was isolated from thirteen plant species and fern-9(11)-en-3 α -ol methyl ether from two plant species, 3 α -friedelin methyl ether has been found in leaves of *Humboldtia laurifolia* (Samaraweera et al., 1983) and was suspected in aerosols from burnings of *Tsuga mertensiana* (Oros and Simoneit, 2001). If demonstrated, the presence of several 3 α - PTMEs in Lagoa do Caçó sediments would suggest a process of epimerisation during diagenesis. Such a possibility is supported by the observations of Bryce et al. (1967a) on the possible isomers of arundoin and sawamilletin. Acid catalysed epimerisation at position 3 of pentacyclic triterpenes is well known for alcohols (e.g. arborinol/isoarborinol: Pakrashi and Samanta, 1967; friedel-8-en-3 β -ol/ friedel-8-en-3 α -ol: Chatterjee et al., 1976). Although there is no report of β to α isomerisation for PTMEs, this transformation could occur at the C-3 position, especially in an acidic medium (G. Eglinton, pers. comm.). It can be hypothesized that this process is promoted by microbial activity or mineral catalysis as in the case for alcohols (ten Haven et al., 1992). As a matter of fact, the decrease in the taraxer-14-en-3 β -ol / taraxer-14-en-3 α -ol ratio along the whole core suggests a precursor/byproduct relationship and the conversion from 3 β to 3 α configuration during early diagenesis (Jacob, 2003).

Bryce et al. (1967a) reported three synthetic di-unsaturated PTMEs, namely multiflor-7,9(11)-dien-3 β -ol, bauera-7,9(11)-dien-3 β -ol ME and ferna-7,9(11)-dien-3 β -ol ME. However, no natural di- or tri-unsaturated PTME having been reported yet, compounds 9, 12 and 16 could then merely correspond to early diagenetic products deriving from mono-unsaturated PTMEs rather than be biological constituents.

4.5. Implications for paleoenvironmental and phytochemical studies

Paleoenvironmental reconstructions in tropical continental series are often focussed mostly on the alternation of savannas and forests that document climate change from dry to humid and conversely. Contrary to pollens that record vegetation from a rather large surrounding area, biomarkers can certify autochthonous production at the site of deposition or, at least, within the catchment area. Furthermore, the homogeneity in morphology of South American pollens from Gramineae (Salgado-Labouriau, 1997) prevents distinction between ecologically significant species, genus or families. Carbon isotopic ($\delta^{13}\text{C}$) and lignin analyses, which have been used to distinguish between savannas and forests (Guillet et al., 2000), were performed on bulk OM and thus did not offer the possibility of tracking ecologically significant species or genus as PTMEs might probably do. Some other key information could be gained from these biomarkers. As noticed above, they seem to be resistant to degradation and may therefore be preserved even under highly degrading conditions, as opposed to Gramineae pollens that are easily destroyed (Ledru, pers. comm.).

Another field of investigation concerns the ecological role of PTMEs. Ohmoto et al. (1970) pointed out that the PTME content of Cortaderiae changes with season, probably as a consequence of a given physiological function of these compounds in plants. This assumption is also evidenced in Zoysieae, where arundoin dominates in inland species while sawamilletin dominates in plants growing on sandy seashores. Although the physiological role of PTMEs is not fully understood, their relative abundance in epicuticular waxes of a given species suggests that they could represent a response to environmental stress. As noticed by Martin-Smith et al. (1967), the co-occurrence of different PTME skeletons implies the existence of several types of oxido-squalene cyclases and methyl transferases in parent plants. The involvement of these enzymes, and thus, PTMEs synthesis, could be controlled by environmental stress. Finally, it is worthwhile noting that, while biochemical-phytochemical investigations are the main sources of information on plant-biomarker relationships in organic geochemistry, the detection of PTMEs in Lagoa do Caçó sediments might be a prelude to the discovery of yet unknown South American PTME plant producers.

4.6. Fernane and arborane type compounds in the sedimentary record

The origin of fernane and arborane derivatives in sedimentary series is still a matter of debate. Arborinone and isoarborinol have been identified together with des-A-arborenes in Lake Valencia sediments (Jaffé and Hausmann, 1995). Ourisson and Rohmer (1982) assumed a microbial origin for these compounds. This assumption was later supported by stable carbon isotopic data that were consistent with an algal origin (Hauke et al., 1992). Components of

these molecular groups that have been recorded up to the Upper Carboniferous (Vliex et al., 1994) have been assigned a higher plant origin. This was consistent with the initial hypothesis of Albrecht and Ourisson (1969). Our findings provide further support to this interpretation and suggest that Gramineae might be an important source of fernane and arborane-type compounds in the sedimentary record.

Conclusion

The Quaternary filling of Lagoa do Caçó afforded a wide range of pentacyclic triterpene methyl ethers. These compounds dominate the neutral solvent-extractable fraction. The identity of five of them has unambiguously been assigned with available reference compounds. Based on an inventory of plants from which PTMEs have already been isolated, Gramineae that grew in the watershed at the time of sediment deposition appear as the most probable sources of these compounds. The presence of PTMEs in Lagoa do Caçó sediments witnesses to their peculiar resistance to degradation. Their structural diversity could result from: (a) a diversified set of plant producers; (b) the ability of an unknown species to produce several compounds; (c) early diagenetic transformation; (d) a combination of these three hypotheses. From a more general point of view, because: (i) of the existence of only few reports of PTMEs in plants (mostly in Gramineae but none from South America); (ii) only two or three Gramineae species dominate savanna ecosystems in the tropics (D. Schwark, pers. comm.), it can be hypothesized that the PTMEs discussed in this study might have been produced by a single species of Gramineae. As a consequence, PTMEs might appear as a unique example of specific higher plant-derived biomarkers.

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Table:

Table 1: Mass spectral data for PTMEs.

Table 2: Sources inventory of PTMEs.

Figure captions :

Fig. 1: Lagoa do Caçó position and location of the coring site.

Fig. 2: a- Total ion current (TIC) chromatogram in the 55-65min time range of the DCM fraction isolated from two sediment samples from Lagoa do Caçó.

b- Reconstructed TIC chromatogram of available reference compounds.

Fig. 3: a- Specific ion mass chromatograms of DCM fraction isolated from sediments of Lagoa do Caçó.

b- Reconstructed chromatogram from selected fragments of available reference compounds.

Fig. 4: Mass spectra of compounds 2 and 4 and proposed structures.

Fig. 5: Mass spectra of compounds 3, 5 and 6 and proposed structures.

Fig. 6: Mass spectrum of compound 7 and proposed structure.

Fig. 7: Specific ion chromatograms illustrating the distribution of D:C- and E:C-friedo PTMEs in Lagoa do Caçó sediments (a: sample 073 and b: sample 170) and (c) reconstructed mass chromatogram of available authentic products.

Fig. 8: Mass spectra of compounds 8, 14, 10, 15 and 13 and proposed structures.

Fig. 9: Mass spectrum of compound 11 and proposed structure.

Fig. 10: Mass spectrum of compound 1 and proposed structure.

Fig. 11: Mass spectra of compound 9, 12 and 16 and proposed structures. Additional double bond (dashed line) in compound 16 can be located either in Δ^2 or Δ^{15} position.

Table 1

Ret. Time	Peak n°	Structure	M ⁺	Most characteristic fragments*
56,3	1	<i>Lanostadien-3-ol ME</i>	440	425, 408, 393, 365, 355, 323, 287, 273, 261, 255, 241, 229, 215, 205, 191, 177, 163 (100)
57,59	2	<i>Taraxer-14-en-3α-ol ME</i>	440	425, 393, 355, 316, 301, 284, 269, 218 (100), 204, 190, 189, 175, 159
57,86	3	<i>Olean-12-en-3α-ol ME</i>	440	393, 325, 257, 218 (100), 203, 189, 175, 161
58,4	4	Taraxer-14-en-3β-ol	440	425, 408, 393, 355, 316, 301, 284, 269, 218, 204 (100), 189, 175, 159
58,68	5	<i>Urs-12-en-3β-ol ME</i>	440	425, 408, 393, 355, 255, 243, 229, 218 (100), 203, 189, 175, 161
58,84	6	Olean-12-en-3β-ol ME (b)	440	425, 408, 393, 323, 257, 243, 229, 218, 204, 189 (100), 177, 161
59,01	7	Olean-18-en-3β-ol ME (c)	440	425, 408, 393, 371, 339, 257, 243, 229, 218, 204, 189 (100), 177, 161
59,79	8	<i>Fern-9(11)-en-3α-ol ME</i>	440	425, 408, 393, 365, 323, 287, 273 (100), 261, 255, 241, 229, 215, 201, 189, 175, 159
59,87	9	<i>Arbor- or fern-7,9(11)-dien-3-ol ME</i>	438	423, 406, 391 (100), 363, 355, 323, 285, 267 (100), 253, 239, 227, 213, 199, 185, 171, 159
60,27	10	<i>Arbor-9(11)-en-3α-ol ME</i>	440	425, 408, 393, 365, 273 (100), 261, 255, 241, 229, 215, 201, 189, 175, 163
60,27	11	<i>Bauer-7-en-3β-ol ME</i>	440	425, 408, 393, 273, 261 (100), 255, 241, 229, 215, 201, 189, 175, 163
60,6	12	<i>Arbor or fern-7,9(11)-dien-3-ol ME</i>	438	423, 406, 391, 363, 321, 295 (?), 285 (100), 271, 253, 239, 225, 213, 197, 183, 171, 159
60,8	13	<i>Fern-8-en-3β-ol ME</i>	440	425, 408, 393, 365, 287, 273 (100), 261, 255, 241, 229, 213, 199, 187, 175, 159
61,3	14	Fern-9(11)-en-3β-ol ME (d)	440	425, 408, 393, 365, 355, 323, 287, 273 (100), 261, 255, 241, 229, 215, 201, 189, 175, 159
62,05	15	Arbor-9(11)-en-3β-ol ME (e)	440	425, 408, 393, 365, 355, 323, 287, 273 (100), 261, 255, 241, 229, 215, 201, 187, 173, 161
62,7	16	<i>Arbor- or Ferna-trien-3-ol ME</i>	436	421, 404, 389, 321, 283, 269, 257, 251 (100), 237, 225, 209, 195, 183, 169, 155

Legend: - Ret. time = retention time

- ME = methyl ether

- M⁺=molecular mass

- (a-e): compound trivial names : (a) sawamilletin or crusgallin; (b) isosawamilletin or b-amyrin ME; (c) miliacin or germanicol ME; (d) arundoin; (e) cylindrin.

- Compounds in bold are those identified with authentic standardss. The identification of the other compounds is only speculative, based on their mass spectral characteristics and retention times.

- * The abundance of the base peak is reported as (100).

Table 2

		Monocots		Compound structure	
Family	Genus	Species			
Arecaceae		<i>Butia capitata</i>		Taraxer-14-en-3 β -ol ME	
		<i>Elaeis guineensis</i>			
		<i>Orbignya sp.</i>			
	Danthonieae		<i>Cortaderia totoe*</i>	X X	Urs-12-en-3 β -ol ME
			<i>Cortaderia fulvida</i>	X X X	Olean-12-en-3 β -ol ME
			<i>Cortaderia splendens</i>	X X X	Olean-18-en-3 β -ol ME
			<i>Cortaderia richardii</i>	X	Fern-9(11)-en-3 α -ol ME
			<i>Chionocloa sp.</i>		Arbor-9(11)-en-3 α -ol ME
	Bambuseae		<i>Arundinaria chino</i>		Bauer-7-en-3 β -ol ME
	Oryzeae		<i>Oryza sativa</i>	X	Fern-8-en-3 β -ol ME
Centothaceae		<i>Lophaterum gracile</i>	X X X X X X X	Fern-9(11)-en-3 β -ol ME	
Cynodonteae		<i>Cynodon dactylon</i>	X X X X	Arbor-9(11)-en-3 β -ol ME	
Eragrostae		<i>Eragrostis curvula</i>	X	Lup-20(29)-en-3 β -ol ME	
		<i>Eragrostis ferrigunea</i>	X X X	Hop-22(29)-en-3 β -ol ME	
Zoysiaeae		<i>Zoysia japonica</i>	X	Friedelane-type Mes	
		<i>Zoysia macrostachia</i>		Serratane-type Mes	
		<i>Zoysia matrella</i>		Cycloartenol ME	
		<i>Zoysia tenuifolia</i>		Parkeol ME	
Andropogoninae		<i>Arthraxon hispidus</i>			
		<i>Imperata cylindrica</i>			
		<i>Microstegium vimineum</i>	X		
		<i>Miscanthus floridulus</i>			
		<i>Miscanthus sacchariflorus</i>	X X X X		
		<i>Saccharum spontaneum</i>	X X X X		
		<i>Saccharum officinarum</i>	X X X X		
		<i>Saccharum robustum</i>	X X X X		
		<i>Saccharum edule</i>	X X X X		
		<i>Hemarthria sibirica</i>	X X X X		
		<i>Phacelurus latifolius</i>	X X X X		
	Arundinelleae		<i>Arundinella hirta</i>		
	Panicaceae		<i>Echionocloa crusgalli</i>	X	
		<i>Oplismenus undulatifolius</i>			
		<i>Panicum miliaceum</i>	X X X X		
		<i>Panicum dichotomiflorum</i>	X X X X		
		<i>Paspalum dilatatum</i>	X X X X X		
		<i>Syntherisma sanguinalis</i>	X		
Aveneae		<i>Agrostis alba</i>			
		<i>Alopercurus aequalis</i>			
		<i>Phalaris arundinacea</i>	X X X X X		
		<i>Calamagrostis epigeios</i>	X X X X X		
Meliceae		<i>Glyceria acutifolia</i>			
Poeae		<i>Festuca arundinacea</i>	X X X X X X X X		
		<i>Poa pratensis</i>	X X X X X X X X		
		<i>Poa sphondylodes</i>	X X X X X X X X		
Dicots	Rubiaceae	<i>Hedyotis acutangula</i>			
		<i>Rubia oncotricha</i>	X X		
	Ebenaceae	<i>Diospyros ferrea</i>			
	Fabaceae	<i>Humboldtia laurifolia</i>			
	Rutaceae	<i>Bosistoa sp.</i>			
	<i>Glycosmis arborea</i>	X			
Sapotaceae	<i>Mimusops littoralis</i>	X			
Bursseraceae					
Gymnosperms		<i>Picea jezoensis</i>			
		<i>Tsuga mertensiana**</i>			

Legend

X Compound described in taxa

* *Cortaderia Totoe* is the same species as *Arundo conspicua*

** Reported in burning aerosols

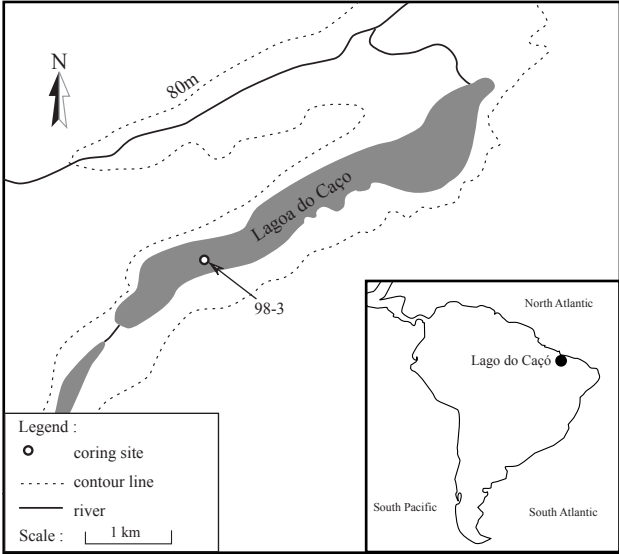


Figure 1

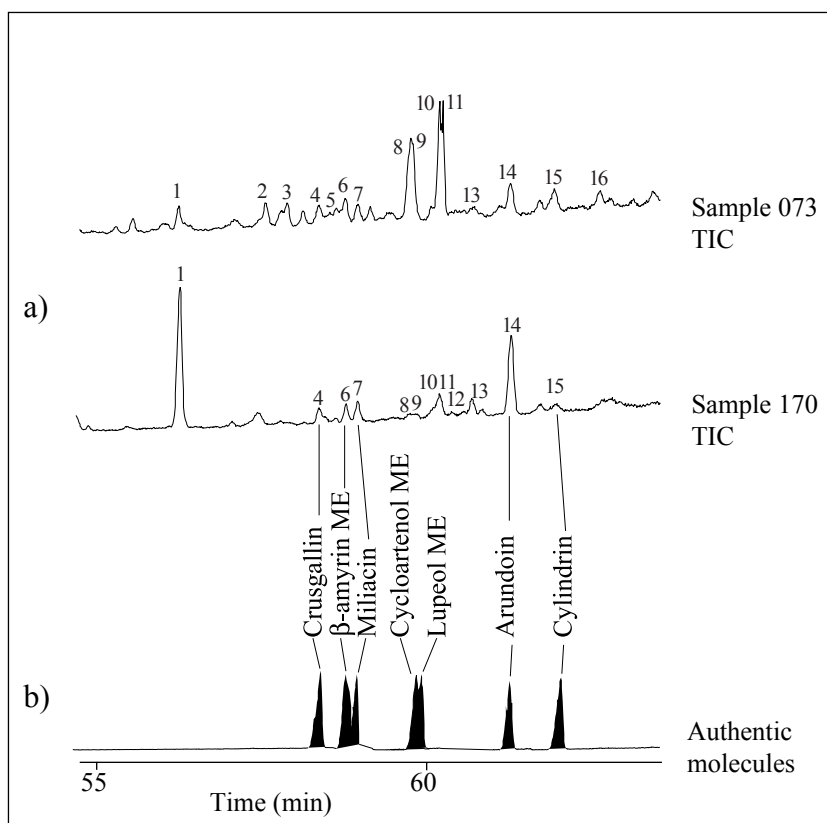


Figure 2

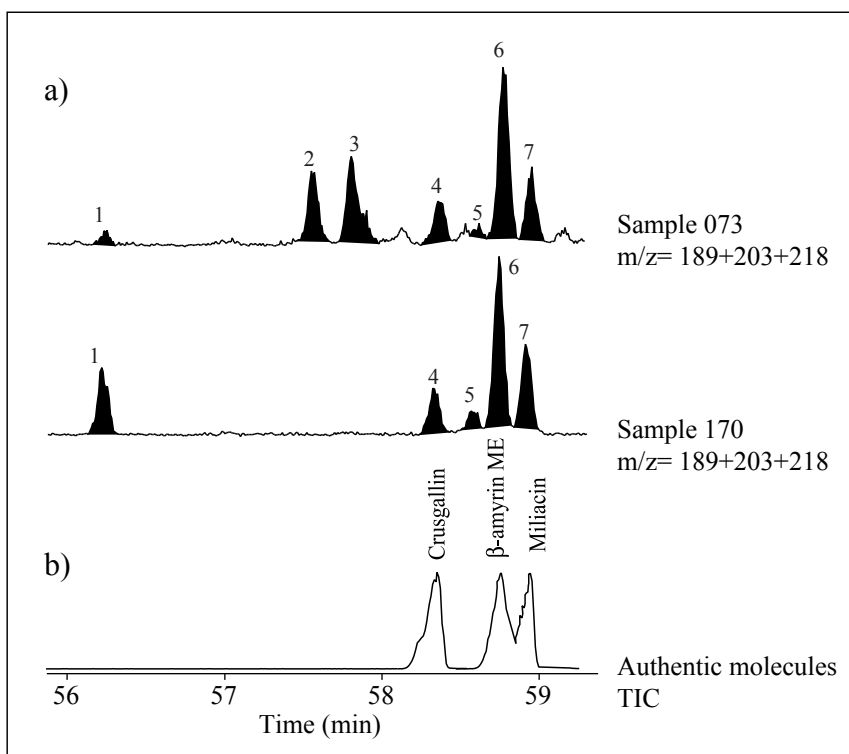


Figure 3

Figure 4

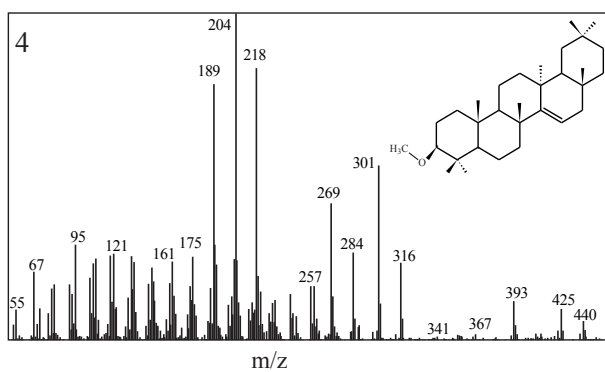
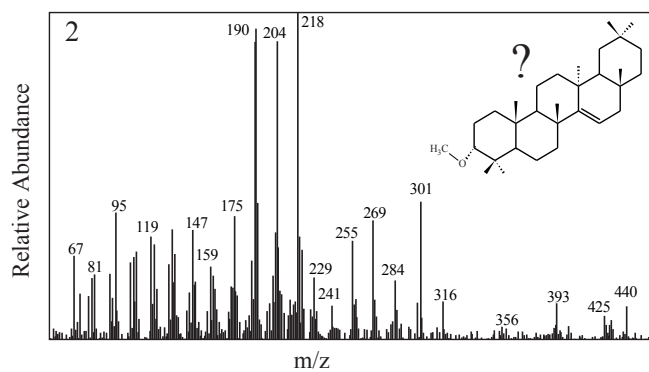


Figure 5

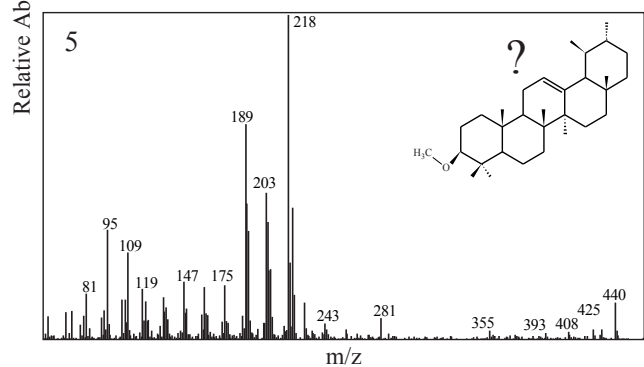
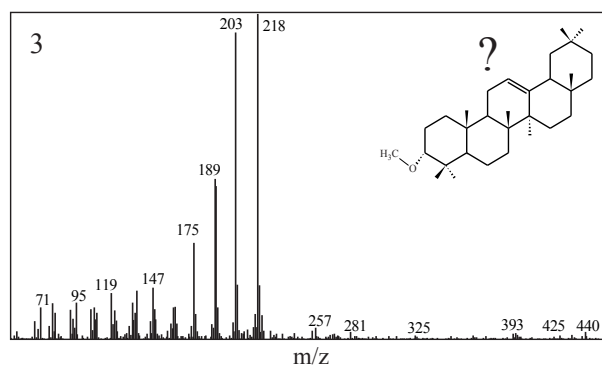
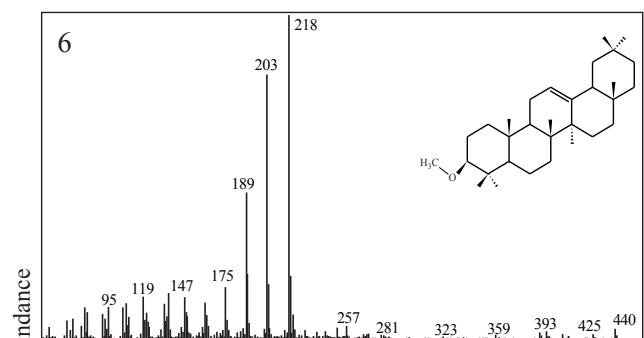


Figure 6

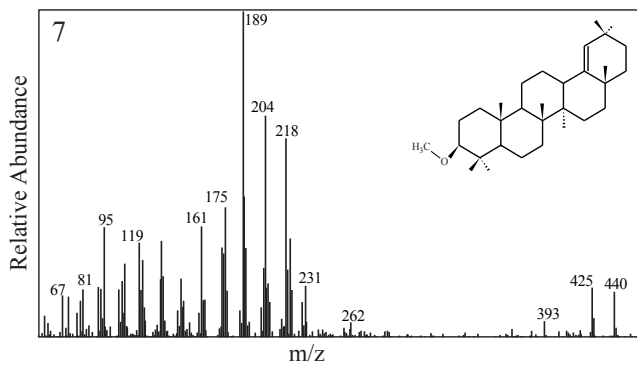


Figure 7

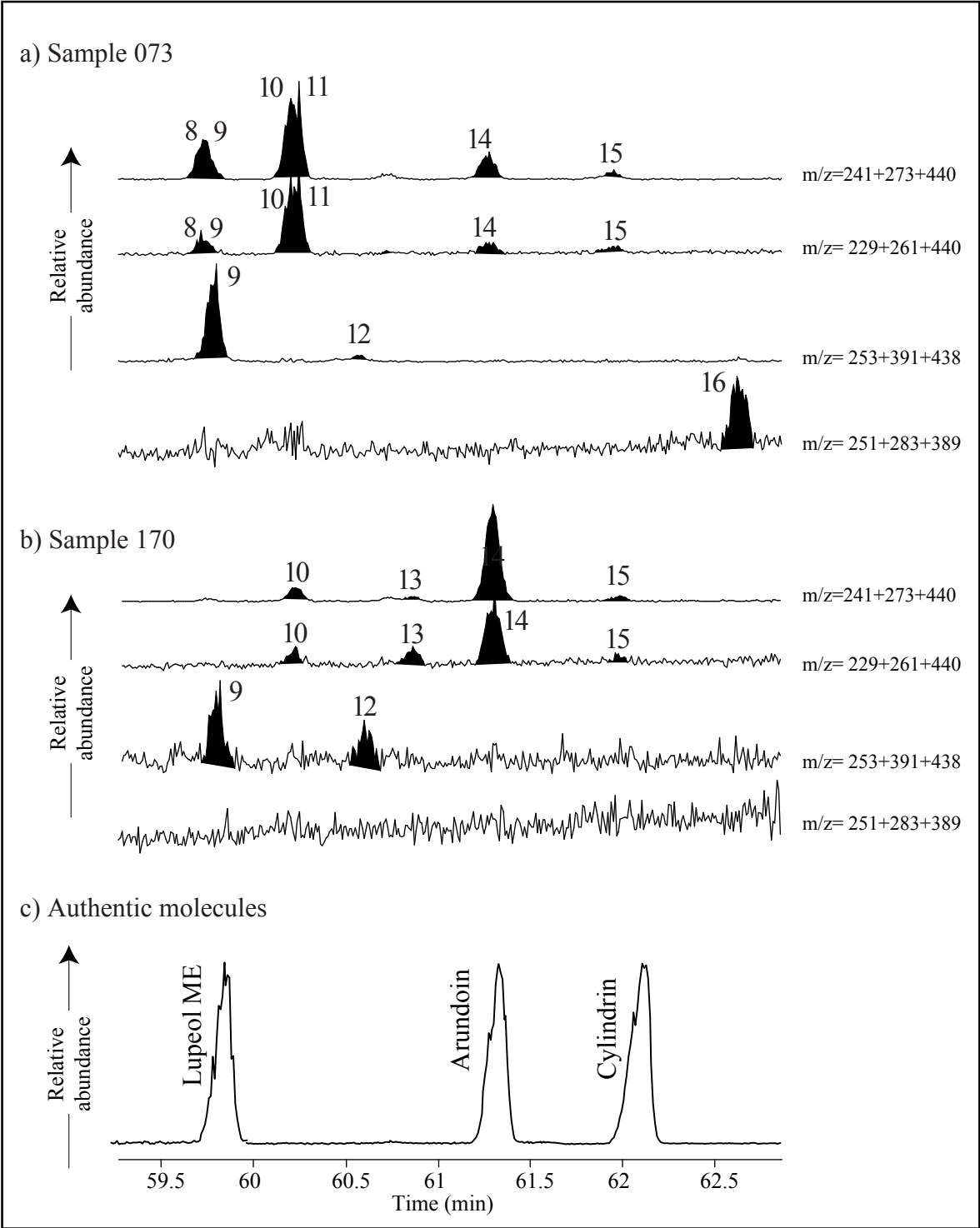


Figure 8

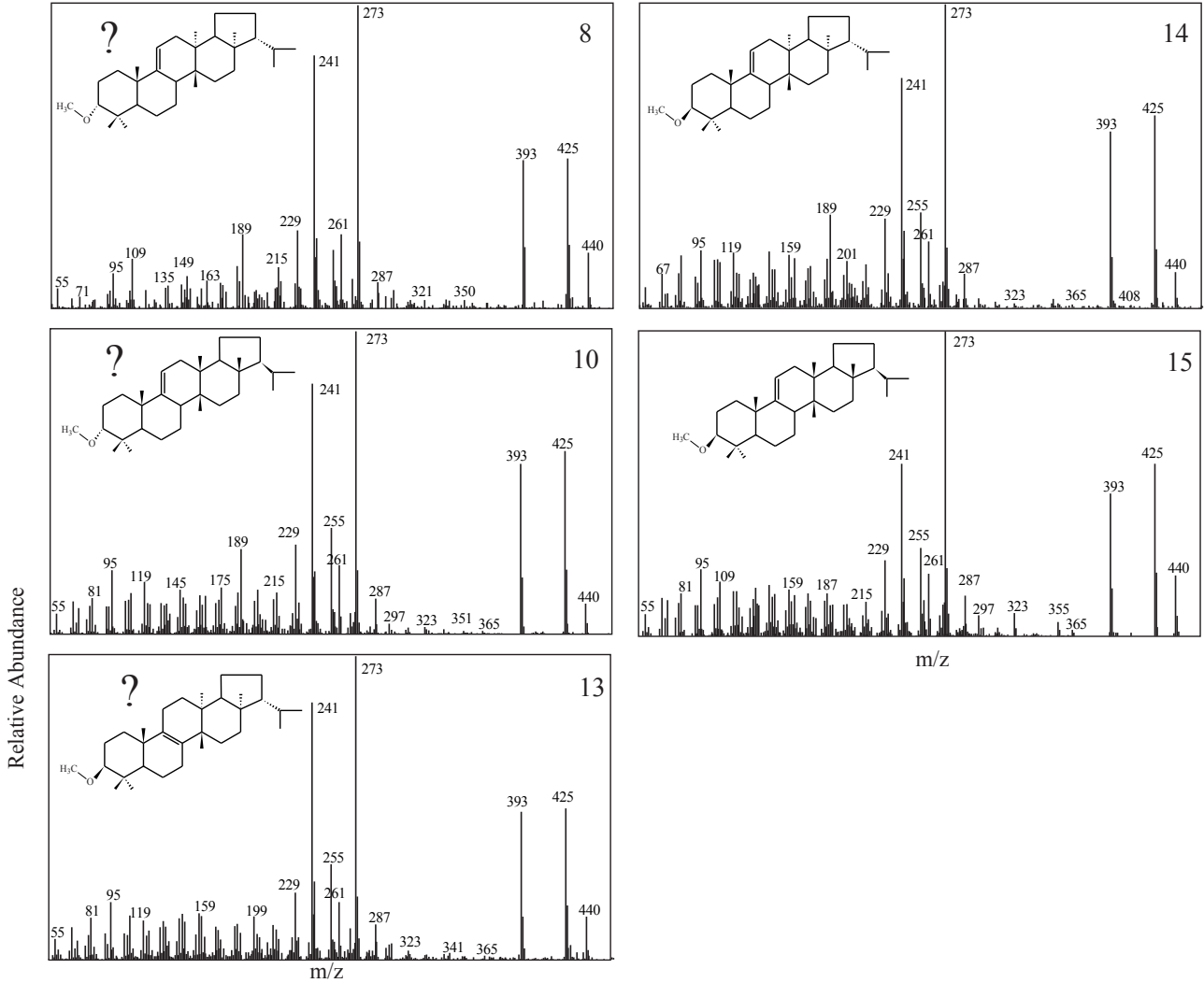


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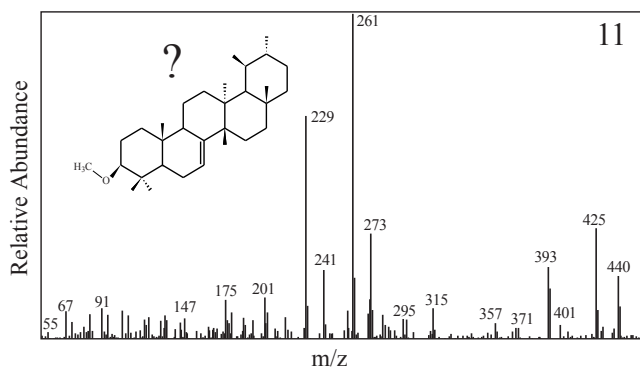


Figure 10

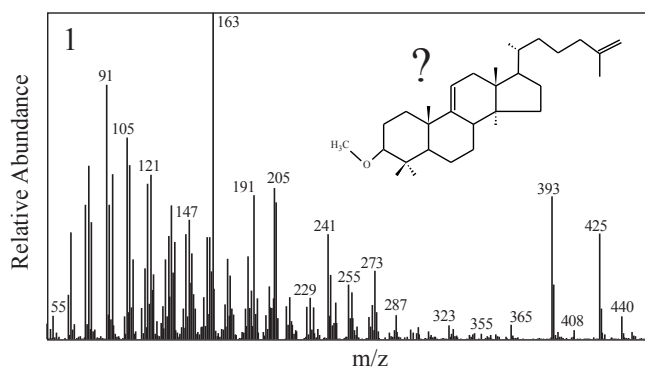


Figure 11

