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Systematic Palaeontology (Palaeobotany)

Chemotaxonomical investigations of fossil and extant beeches. II. Leaf lipids of Pliocene *Fagus* from the Upper Valdarno Basin, central Italy

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Abstract

The chemical composition of fossil plants can provide useful data for taxonomy and palaeoecological reconstructions. The Upper Valdarno Basin deposit (central Italy) shows a great diversity of well-preserved fossils. The first results of the analysis of Pliocene *Fagus* leaf lipids are reported here. Series of long chain aliphatic lipids (*n*-alkanes, *n*-alcohols, *n*-aldehydes, *n*-ketones) dominated the extract. Their distributions differ for the fossil and the extant European beech, suggesting that they may belong to different species. The occurrence of compounds sensitive to degradation such as *n*-aldehydes and monoglycerids shows the excellent chemical preservation of the fossils. Two long-chain *n*-alkyl-1,15-diols were identified. They may come from Eustigmatophyceae microalgae, and their occurrence is in agreement with a freshwater deposit ion environment. Various polycyclic terpenoids were detected (e.g., native plant sterols, triterpenoids of the oleanane type). The occurrence of compounds that do not correspond to *Fagus* lipids is discussed. ***To cite this article: F. Zanetti et al., C. R. Palevol 6 (2007).***

Résumé

Étude chimiotaxonomique de hêtres fossiles et actuels. II. Lipides foliaires de *Fagus* du Pliocène (bassin du Valdarno supérieur, Italie centrale). La composition chimique des plantes fossiles peut fournir des informations utiles pour la taxinomie et les reconstructions paléoécologiques. Le gisement du Valdarno supérieur, en Italie centrale, montre une grande diversité de fossiles remarquablement préservés. Les premiers résultats de l'analyse des lipides foliaires de *Fagus* du Pliocène sont présentés. Des séries de lipides aliphatiques (*n*-alcanes, *n*-alcools, *n*-aldéhydes, *n*-cétones) ont été identifiées. Leurs distributions diffèrent entre le fossile et le hêtre commun actuel, suggérant leur appartenance à deux espèces distinctes. La présence de composés sensibles à la dégradation, comme les *n*-aldéhydes et des monoglycérides, montre l'excellente préservation chimique des fossiles. Deux *n*-alkyl-1,

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15-diols à longue chaîne ont été identifiés. Ils proviennent probablement de microalgues Eustigmatophyceae et corroborent un environnement de dépôt en eau douce. Divers terpénoïdes polycycliques ont été détectés (par exemple, stérols végétaux natifs, triterpénoïdes de type oléanane). La présence de composés ne correspondant pas aux lipides de *Fagus* est discutée. ***Pour citer cet article : F. Zanetti et al., C. R. Palevol 6 (2007).***

Keywords: Chemotaxonomy; Diols; *Fagus*; Lipids; Monoglycerids; Middle Pliocene; Upper Valdarno; Italy

Mots clés : Chimiotaxinomie ; Diols ; *Fagus* ; Lipides ; Monoglycérides ; Pliocène moyen ; Valdarno supérieur ; Italie

1. Introduction

Chemical investigations of fossil plants can provide interesting information useful in several fields. Comparing fossils with their extant counterparts, and with other fossils, can help to clarify their taxonomic affinities (e.g., [9,18,23,25–27]). Moreover, fossil plants chemicals allow a better understanding of the origin and of the fate of organic matter in sediments, and may also provide palaeoenvironmental proxies (e.g., [15–17,28]).

Lipids are the most commonly used molecules in palaeobiology. It is therefore crucial to assess the nature and extent of the changes in lipid composition associated with diagenesis and fossilization. Furthermore, additional data on the fossil plants chemical composition are still needed.

The genus *Fagus* L. (Fagaceae) is well represented in extant temperate ecosystems and in the fossil record since the Early Cenozoic (e.g., [5]). On the one hand, fossil and extant beeches display a high morphological variability, and on the other hand many diagnostic characters are not preserved during fossilization ([5] and references therein). This raises important issues for the systematics of this group and chemical data may provide some help in solving such problems.

Hence, this work aimed at determining the lipid composition of Pliocene *Fagus* leaves collected in the deposits of the first lacustrine cycle of the Upper Valdarno Basin (central Italy). The latter is one of the best-known European continental successions (Middle Pliocene–Middle Pleistocene) for the occurrence of numerous vertebrate and vegetal micro- and macroremains (e.g., [1–3]). Lipids were extracted by organic solvents (i.e. dichloromethane and methanol) and characterized by gas chromatography coupled with mass spectrometry. The results of this preliminary study are reported here. The discussion focuses on taxonomy and early taphonomy. Detailed study of the embedding sediment and biogeochemical considerations (e.g., diagenetic implications of the terpenoids profiles) will be discussed elsewhere (Zanetti et al., in preparation).

2. Experimental

2.1. Sampling site

The upper Valdarno, located ca 30 km southeast of Florence (central Italy), is one of the best-known intermontane basins of the northern Apennine. It has been filled by fluvio-lacustrine deposits starting from the Middle Pliocene, as documented by several geological, sedimentological, and stratigraphical studies (e.g., [1] and references therein).

Numerous leaf remains were collected in the upper silty strata of the first lacustrine cycle of the upper Valdarno throughout the Santa Barbara succession (Middle Pliocene). In the latter, detailed palynological and macrobotanical studies were previously carried out in order to reconstruct the flora, vegetation and climate change [2,3]. They documented, at the base of the succession, a rich flora indicative of warm humid forest vegetation (*Taxodium*/*Glyptostrobus* type, *Nyssa*, *Engelhardia*, Arecaceae, *Itea*, *Symplocos*, *Cephalanthus*, Clethraceae, Cyrillaceae, *Myrica*, *Carya*, *Quercus*, *Carpinus*, *Ulmus*, *Zelkova*), typical of subtropical to warm–temperate climate during the development of a swamp in a lacustrine system. A progressive expansion of a forest of cooler aspect (from about 2.7 Ma), characterized mainly by *Picea* and *Fagus*, followed, coincident with a rapid and intense subsidence, causing the deepening of the lake. During this gradual trend toward cooler conditions, herbs remained a minor component. Steppe taxa significantly increase only later, coincident with the maximal expansion of the Arctic glaciation at ca 2.6 Ma. However, this event is not recorded in the Santa Barbara succession, but in the overlain deposits.

Stratigraphically the selected leaf remains are from the transitional cooler, but yet humid, interval before the first significant glacial event at 2.6 Ma. The excellent

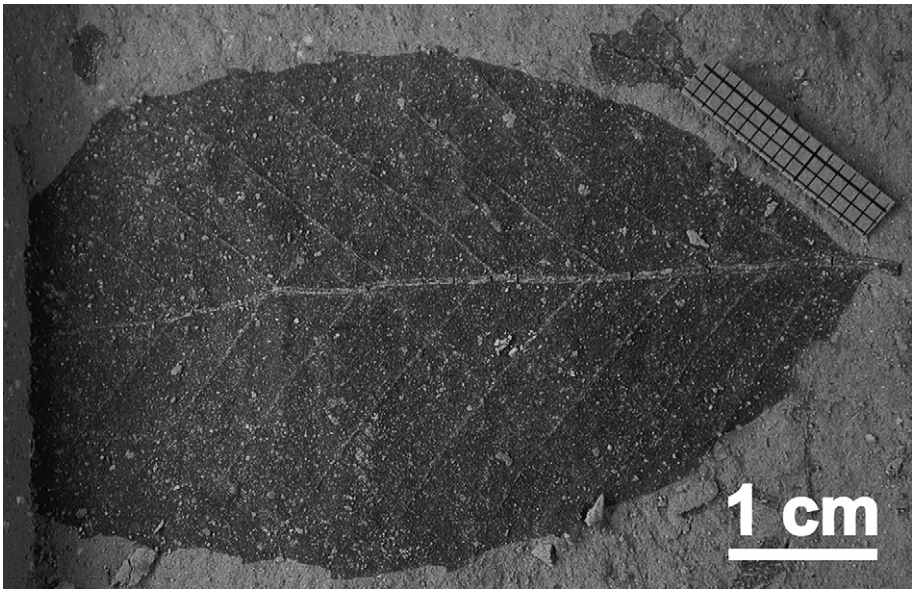

Fig. 1. Fossil beech leaf (∼2.7 Ma) studied as a complete single leaf.
Fig. 1. Feuille fossile de hêtre (∼2.7 Ma) étudiée en tant que feuille complète isolée.

morphological preservation of leaf remains suggests that burial probably occurred near the vegetation zone.

2.2. Samples

Among the rich and diversified fossil leaf remains, beech leaves very similar to those of extant European beech (*Fagus sylvatica* L., Fagaceae) were selected (Fig. 1). They were tentatively assigned to the morphotaxon *Fagus gussonii* A. Massal. (P. Roiron, pers. commun.). Considering the few morphological characters available for this work, this attribution could be consistent with a recent work by Denk [5] on *Fagus* from the Cenozoic of Europe. However, in this work the author considered that *F. gussonii* is restricted to the Late Miocene, and that it hybridized with, or was "replaced" by *Fagus haidingeri* Kováts 1856 sensu Knobloch 1969 during the Pliocene in Italy. Moreover, Denk considered that fossil leaves from the Middle Pliocene of Meleto (in the upper Valdarno Basin) "clearly belong to *F. haidingeri*" [5]. The author referred to a previous work [8] that could not be examined in the present study. Nevertheless, due to close geographic and stratigraphic occurrence, it could be considered that the fossil leaves studied here and those of that previous work [8] probably correspond to the same morphotaxon, i.e. *F. haidingeri*, following the conception of Denk [5].

At the site, blocks of sediment were split open to reveal the plant compressions. Resulting blocks were taken from the field and stored in the dark at ∼5 °C until analysis. Fossil leaves were separated from the embedding sediment in the laboratory by scrapping them with a solvent-washed scalpel [20]. A small amount of sediment was thus incorporated in the fossil samples. In order to study a possible variability among leaves and the possibility to combine different fossils, two types of fossil samples were analysed: a single complete leaf (Fig. 1) and five incomplete leaves grouped together. A sediment sample was analysed separately to investigate possible contributions of the sedimentary lipids to the fossils. The samples were crushed in a mortar and sieved (500 μm).

2.3. Leaf lipids' extraction

Total lipids were obtained after seven successive extractions as follows and by combining the extracts. A mixture of dichloromethane/methanol (2:1, v/v) was added to the sample. The mixture was sonicated during 20 min and then centrifuged during 10 min at 4000 rpm. Lipids were recovered in the supernatant. The amount of total lipids was determined by weighing after solvent evaporation by rotary evaporation under reduced pressure.

2.4. Extract fractionation

In order to facilitate the molecular analyses, the total lipid extract was fractionated by column chro-

matography. The solid phase consisted in alumina (10 g per g of dry extract; Sigma-Aldrich 507C ~150 mesh) deactivated to Brockmann grade IV by adding 10% (w/w) of distilled water. The elutant consisted in various organic solvents of increasing polarity, yielding three fractions as follows: fraction 1 (*n*-heptane), fraction 2 (toluene), fraction 3 (dichloromethane/methanol 9:1, v/v). Each fraction was dried by rotary evaporation under reduced pressure and then dissolved again in a small volume of its appropriate solvent.

2.5. Molecular analyses

The so-obtained fractions were analysed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC–MS). The first fraction (in *n*-heptane) was directly analysed, whereas the second (in toluene) and the third (in dichloromethane) were derivatized prior to analysis. Aliquots of fractions 2 and 3 were dried under a gentle flow of dinitrogen and trimethylsilylated by heating (1 h, 80 °C) with BSTFA (i.e. *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane, Fluka/Sigma-Aldrich, Switzerland). Then they were dried again under dinitrogen and dissolved in the appropriate solvent.

GC analyses were carried out using a Varian 3900 chromatograph fitted with a VF5-MS fused silica capillary column coated with 5% phenylated polymethylsiloxane (50 m × 0.32 mm i.d., 0.25-μm film thickness). Helium was used as carrier gas at a constant flow of 2 ml min^{-1}. Both injector (splitless) and detector (FID) were heated at 350 °C. The temperature program consisted in heating at 10 °C min^{-1} from 80 °C to 100 °C, then at 4 °C min^{-1} to 325 °C, and finally in an isothermal period at 325 °C for 30 min.

GC–MS analyses were performed using an Agilent 6890N chromatograph coupled with an Agilent 5973N quadrupolar mass spectrometer, scanning the range *m*/*z* 40–800 (scan time 0.5 s). Chromatographic conditions were the same as above (except the He flow: 1 ml min^{-1}). The temperature of the transfer line to the spectrometer was 250 °C. The ionization cell of the electron impact source was heated at 220 °C (electron energy 70 eV). The quadrupole temperature was 120 °C. The compounds were identified by interpretation of their mass spectrometry fragmentation patterns and by comparison of their retention time and mass spectra with those of reference compounds or from the literature (e.g., [4,15,21,25]) and databases (NIST Mass Spectral Search Program Version 2.0 a, 2002).

3. Results and discussion

The two fossil samples (i.e. one single complete leaf and five incomplete leaves grouped together) yielded very similar chemical compositions. Thus, it can be considered that there is no significant variability between the leaves. Therefore, the samples investigated can be considered as representative of the fossil beech. Moreover, this shows that different fossil fragments belonging to the same species can be combined if needed in further studies. Consequently, the following discussion refers to the fossil as both the single leaf and the combined leaves samples.

The leaf lipids extracted from the fossil beech appeared as a complex mixture of a number of diversified constituents upon GC–MS characterization. The identified compounds are listed in Table 1 and their molecular structures are shown in Appendix A.

3.1. Acyclic lipids

The extracts appeared to be dominated by various aliphatic lipids (Fig. 2).

3.1.1. Diols

Two *n*-alkyl-diols were strikingly abundant (Fig. 2) in both the fossil and sediment extracts: triacontan-1,15-diol (C_{30}) and dotriacontan-1,15-diol (C_{32}). Previous studies showed that these compounds can be synthesized by Eustigmatophyceae phytoplanctonic microalgae, but another microbial source cannot be excluded [32–35]. The C_{30} homologue predominated over the C_{32}. Following the empiric rule proposed by Versteegh et al. [32], their relative abundances may indicate a freshwater or restricted marine deposit environment. This is consistent with previous palaeoenvironmental studies that showed that the sediment deposited in a lacustrine setting (e.g., [3] and references therein).

3.1.2. Aliphatic alcohols

Long-chain primary *n*-alcohols were identified. They ranged from C_{20} to C_{32}. In this series, even carbon-numbered homologues predominate over the odd ones, in the fossil as well as in the embedding sediment. Long-chain even primary *n*-alcohols are characteristic of Embryophytes epicuticular waxes (e.g., [6,28]). Thus, it can be assumed that these compounds mainly originate from land plants. The *n*-alcohol profile of the fossil leaves shows a maximum at C_{30} with a significantly abundant C_{28} (Fig. 3c), whereas no homologue dominates that of the sediment (Fig. 3d). Short chain *n*-alcohols were also detected in very low amounts. They ranged from C_{14} to

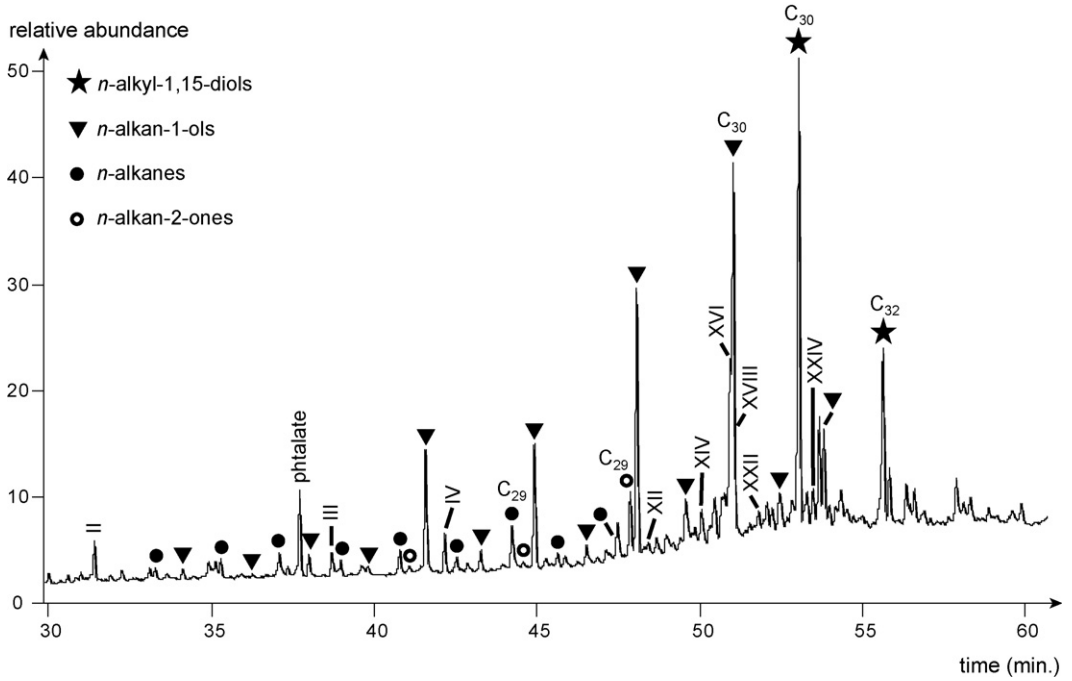

Fig. 2. Chromatogram of fraction 3 from the single fossil leaf extract. Identified compounds are listed in Table 1 and their structures are shown in Appendix A (phtalate = pollutant related to analytical procedure).
Fig. 2. Chromatogramme de la fraction 3 de l'extrait de la feuille fossile isolée. La liste des composés identifiés figure dans le Tableau 1 et leurs structures dans l'annexe A (phtalate = contaminant lié au protocole analytique).

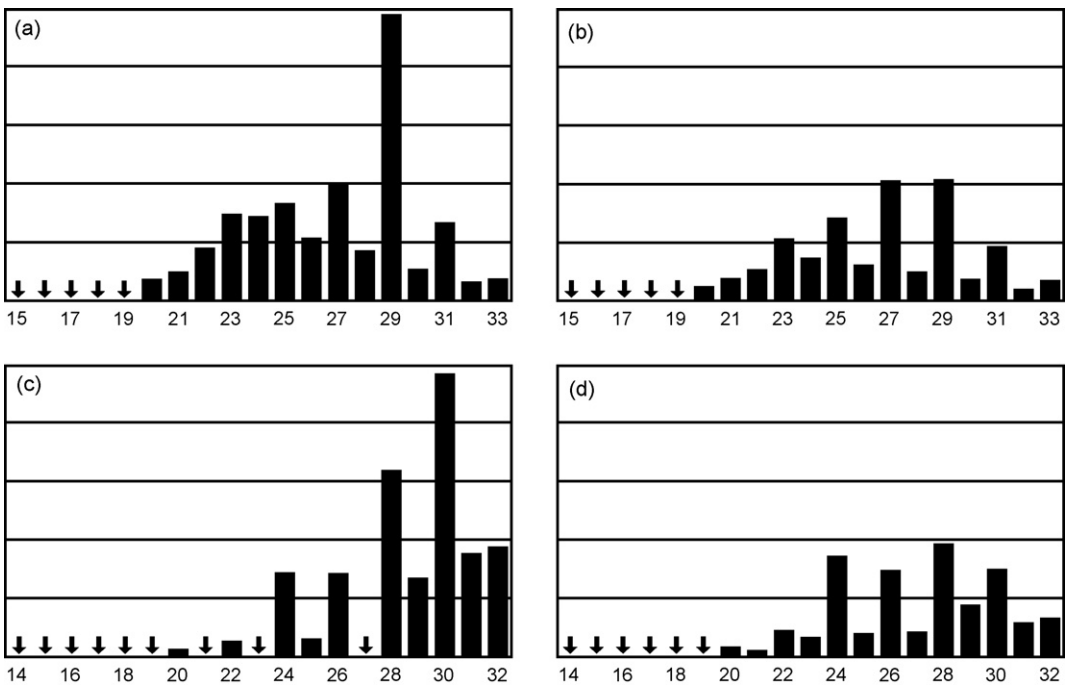

Fig. 3. Fossil leaves (**a**, **c**) and sediment (**b**, **d**) *n*-alkanes (**a**, **b**) and *n*-alkan-1-ols (**c**, **d**) distributions (abscissa: number of carbon atoms of the alkyl-chain; ordinate: relative abundance) Arrows indicate compounds detected by mass spectrometry but below the quantification level.
Fig. 3. Distributions des *n*-alkanes (**a**, **b**) et des *n*-alkan-1-ols (**c**, **d**) des feuilles fossiles (**a**, **c**) et du sédiment (**b**, **d**) (abscisse: nombre d'atomes de carbone de la chaîne alkyle; ordonnée: abondance relative) Les flèches indiquent des composés détectés en spectrométrie de masse, mais en dessous du seuil de quantification.

Table 1
Compounds identified in the fossil beech leaves. Molecular structures are shown in Appendix A
Tableau 1 Composés identifiés dans les feuilles fossiles de hêtre. Les structures figurent dans l'annexe A

No.[a]	Name	Series distribution[b]	Formula	MW[c]
Lipids with *n*-alkyl chain				
	n-alkanes	C_{21}–C_{33} (C_{29})	C_nH_{2n+2}	(408)
	polymethylalkanes	n.d.	C_nH_{2n+2}	
	n-alkan-1-ols	C_{20}–C_{32} (C_{30})	$C_nH_{2n+2}O$	(438)
	n-alkan-2-ols	C_{25}, C_{27}, C_{29} (C_{29})	$C_nH_{2n+2}O$	(424)
	n-nonacosan-10-ol		$C_{29}H_{60}O$	424
I	*n*-alkyl-1,15-diols	C_{30}, C_{32} (C_{30})	$C_nH_{2n+2}O_2$	(454)
	n-alkanals	n.d.	$C_nH_{2n}O$	
	n-alkan-2-ones	C_{25}, C_{27}, C_{29} (C_{29})	$C_nH_{2n}O$	(422)
	6,10,14-trimethylpentadecan-2-one (C_{18} isoprenoid ketone)		$C_{18}H_{36}O$	268
	hexadecanoic acid (palmitic acid)		$C_{16}H_{32}O_2$	256
II	octadecanoic acid (stearic acid)		$C_{18}H_{36}O_2$	270
III	1-hexadecanoylpropan-1,2,3-triol (1-hexadecanoylglycerol, 1-monopalmitin)	$C_{19}H_{38}O_4$	328	
IV	1-octadecanoylpropan-1,2,3-triol (1-octadecanoylglycerol, 1-monostearin)	$C_{21}H_{42}O_4$	358	
Diterpenoids				
V	diaromatic totarane		$C_{19}H_{24}$	252
VI	phyllocladane (or *ent*-kaurane isomer)		$C_{20}H_{34}$	274
VII	ferruginol (phenolic abietane)		$C_{20}H_{30}O$	286
Hopanoids				
VIII	22,29,30-trisnorhopan-21-one		$C_{27}H_{44}O$	384
IX	hop-17(21)-ene		$C_{30}H_{50}$	410
X	homomoretane		$C_{31}H_{54}$	426
Steroids				
XI	cholestan-3-one (cholestanone)		$C_{27}H_{46}O$	386
XII	cholestan-3-ol (cholestanol)		$C_{27}H_{48}O$	388
XIII	24-ethylcholest-3,5-diene (sitost-3,5-diene, stigmast-3,5-diene)		$C_{29}H_{48}$	396
	24-ethyl-x-methylcholestan-3-one (methylcholestanone)		$C_{28}H_{60}O$	400
XIV	24-ethylcholest-5,22-dien-3-ol (stigmasterol)		$C_{29}H_{48}O$	412
XV	24-ethylcholest-4-en-3-one (sitost-4en-3-one, stigmast-4-en-3-one)		$C_{29}H_{48}O$	412
XVI	24-ethylcholest-5-en-3-ol (sitosterol)		$C_{29}H_{50}O$	414
XVII	24-ethylcholestan-3-one (sitostanone, stigmastanone)		$C_{29}H_{50}O$	414
XVIII	24-ethylcholestan-3-ol (sitostanol, stigmastanol)		$C_{29}H_{52}O$	416
Other triterpenoids				
XIX	*des*-A-olean-9,13(18)-diene		$C_{24}H_{38}$	326
XX	olean-12-en-3-one β-(amyrenone)		$C_{30}H_{48}O$	424
XXI	arbor-9(11)-en-3-one (arborenone)		$C_{30}H_{48}O$	424
XXII	olean-12-en-3-ol β-(amyrin)		$C_{30}H_{50}O$	426
XXIII	friedelan-12-en-3-one (friedelin)		$C_{30}H_{50}O$	426
XXIV	isoarbor-9(11)-en-3-ol (isoarborinol)		$C_{30}H_{50}O$	426

n.d. = not determined.
n.d. = non déterminé.
[a] number in Appendix A and label in Fig. 3
[a] numéro dans l'annexe A et marque dans la Fig. 3.
[b] carbon number range (maximum).
[b] intervalle de nombre d'atomes de carbone (maximum).
[c] molecular weight, weight in parentheses corresponds to the series maximum (g mol^{-1})
[c] masse moléculaire, la masse entre parenthèses correspond au maximum de la série (g mol^{-1})

C_{19}. They correspond to rather ubiquitous compounds and can originate from various organisms such as plants and microorganisms (e.g., [28]).

Some long-chain secondary *n*-alcohols were detected in small quantities: pentacosan-2-ol (C_{25}), heptacosan-2-ol (C_{27}), nonacosan-2-ol (C_{29}), nonacosan-10-ol (C_{29}). The latter is mainly produced by 'Gymnosperms', but it was also found in some Angiosperms (e.g., [13]), in particular in extant European beech [24]. The *n*-alkan-2-ols are considered to be degradation products

of *n*-alkanes (e.g., [30], and references therein) and their distribution is consistent with this origin, as discussed below. At least a part of all these secondary *n*-alcohols may thus correspond to the fossil leaves lipids.

3.1.3. Aliphatic alkanes

Long-chain *n*-alkanes were identified. They ranged from C_{21} to C_{33}. In this series, odd carbon numbered homologues predominate over the even ones, in the fossil as well as in the embedding sediment. Long-chain odd *n*-alkanes are characteristic of Embryophytes epicuticular waxes (e.g., [6,28]). Thus, it can be assumed that these compounds mainly originate from land plants. The *n*-alkane profile of the fossil shows a strong maximum at C_{29} (Fig. 3a), whereas that of the sediment displays two slightly dominating homologues, C_{27} and C_{29} (Fig. 3b). Short-chain *n*-alkanes were also detected in very low amounts. They ranged from C_{15} to C_{20}. They correspond to rather ubiquitous compounds and can originate from various organisms such as plants and microorganisms (e.g., [28]).

Numerous branched alkanes occurred in small quantities. Some of them constituted a series of homologues with mass spectrometry fragmentation patterns similar to polymethylated alkanes studied by Greenwood [10]. The origin of such compounds remains controversial: microbial lipids or artificial pollutants such as degradation products from synthetic polypropylene-like polymers [10]. All steps of sampling, storage and analyses were carefully carried out (i.e. use of solvent washed, aluminium and glass material). Therefore, if these compounds correspond to contaminants, then they probably reflect environmental pollution at the site.

3.1.4. Fatty acids and monoglycerids

Two fatty acids were identified in both the fossil and the embedding sediment: palmitic acid (C_{16}) and stearic acid (C_{18}). Their monoacylglycerols (1-monopalmitin and 1-monostearin) also occurred in significant amounts (Fig. 2). All these fatty lipids are ubiquitous: they are produced by various organisms, including plants and microorganisms. However, since they are significant constituents of extant European beech leaves [24], at least a part of them may come from the fossil beech leaves themselves.

3.1.5. Other aliphatic lipids

Various aliphatic lipids were detected in low amounts.

A series of long chain *n*-aldehydes was detected. Previous studies reported that a large part of the aldehydes occurring in a sample may be lost when extracts are analysed after silylation (e.g., [22]); therefore, the *n*-aldehydes could be more abundant in the studied fossil leaves. These fatty lipids occur in Embryophytes epicuticular waxes (e.g., [6]) and some of them were detected in extant European beech leaves (e.g., [11,24]). At least a part of them may thus come from the fossil beech leaves themselves.

Some *n*-alkan-2-ones were detected, i.e. pentacosan-2-one (C_{25}), heptacosan-2-one (C_{27}), nonacosan-2-one (C_{29}). Their occurrence is consistent with that of *n*-alkanes and secondary *n*-alcohols, because they and the latter are usually considered as degradation products of the former (e.g., [30] and references therein).

Another ketone was identified: 6,10,14-trimethylpentadecan-2-one (C_{18} isoprenoid ketone). It is a common degradation product of phytyl compounds, such as chlorophyll, tocopherol, or various esters (e.g., [31] and references therein).

3.2. Polycyclic lipids

The extracts contained various polycyclic lipids, but in lower amounts than acyclic lipids (Fig. 2).

3.2.1. Diterpenoids

Some diterpenoids were detected in low amounts, in both the fossil and the embedding sediment. Three of them were identified: a phyllocladane (or an isomer: *ent*-kaurane), a diaromatic totarane, and the abietane-type ferruginol. They are characteristic of some conifers belonging to the Cupressaceae *s.l.* (including Taxodiaceae) (e.g., [16]). This is consistent with the fossil micro- and macroflora of the site. Indeed, they can reflect the contribution of those conifers to the sedimentary organic matter that was incorporated in the fossil samples (§ 2.2. and 3.3.). Nevertheless, the abundance of compounds characteristic of conifers is not representative of the abundance of these plants in the site flora [27].

3.2.2. Triterpenoids

Various tetracyclic and pentacyclic triterpenoids were detected.

Three hopanoids were identified: hop-17(21)-ene, homomoretane, and 22,29,30-trisnorhopan-21-one. These compounds are characteristic of bacteria (e.g., [28]).

Typical plant sterols (i.e. sitosterol and stigmasterol) were identified, as well as their degradation products (i.e. sitostanol, sitostanone, sitost-4-en-3-one, sitost-3,5-diene). These steroids probably come from the flora of the site, but they could also originate from algae (e.g., [27] and references therein). At least a part of them may come from the fossil leaves. Other steroids occurred:

cholestanol, cholestanone, and a methylcholestanone. They are generally considered to come from various non-plant Eukaryotes (including algae), in this case maybe from various planktonic organisms.

Various other triterpenoids were detected. They notably belong to the oleanane-type (i.e. β-amyrin, β-amyrenone, *des*-A-oleana-9,13(18)-diene), the friedelane-type (i.e. friedelin) and the arborane-type (i.e. arborenone and isoarborinol). The oleanane-type and friedelane-type triterpenoids are characteristic of Angiosperms (e.g., [27]) and some of them occur in the extant European beech (e.g., [24,29]). At least a part of them may thus come from the fossil leaves themselves. The arborane-type triterpenoids can be produced by both plants and microorganisms, so their precise origin cannot be determined (e.g., [15,27]). Nevertheless, no study reports their occurrence in the beech.

3.3. Beech versus sediment signal

All of the molecules from the fossil leaves were also detected in the sediment sample. As previous studies suggested that there are no significant lipid exchanges between fossil leaves and their embedding sediment (e.g., [20]), it can be hypothesized that the beech molecules identified in the embedding sediment come at least partly from microscopic remains of beech leaves. Indeed, studies showed that *Fagus* was an important component of the site flora [3]. Thus, it is not surprising that the beeches contributed to the sedimentary organic matter of the layer.

The occurrence of exogenous compounds in the fossil extract can be explained by several facts:

- the analytical procedure incorporated some sedimentary organic matter to the fossil samples, although the sediment probably contains much less organic matter than the fossil leaves themselves (e.g., [18]) – *all lipids, notably the diols and some terpenoids*;
- Logan et al. [20] emphasized that organic sedimentation may occur on the leaves, i.e. that organic matter from the water column could deposit and adsorb on the leaves during their sinking and burial – *all lipids, notably the diols and some terpenoids*;
- the leaves could be colonized by microalgae during their transport and sinking in the water column (some kind of aquatic epiphytism) as well as before their death (unpublished observations) – *notably the diols*;
- the leaves could be colonized by bacteria before, during and after their death, transport and burial – *notably the hopanoids*;
- sedimentary lipids could diffuse into the fossil leaves during diagenesis and compaction, even if studies tend to demonstrate that there are no significant molecular exchanges between the fossil leaves and their embedding sediment [20].

The comparison of alkyl series distributions between the fossil leaves and the sediment provides further insight into the origin of the lipids in these two pools. The *n*-alkane and *n*-alcohol profiles of the fossils and sediment exhibit the same chain-length ranges and could thus comprise respective contributions from each other. However, contrary to the profiles of the sediment, those of the fossil leaves are clearly dominated by a single homologue (C_{29} for *n*-alkanes and C_{30} for *n*-alcohols, Fig. 3). Therefore, the sedimentary contribution to the fossil leaves organic matter appears to be minor. This allows chemotaxonomic interpretations of the fossil leaves profiles.

3.4. Chemotaxonomic implications of the aliphatic lipids profiles

Several studies showed that the wax profiles of leaves could vary during the development of the latter (e.g., [12,14]). Differences in lipid distributions between specimens could thus be explained by development stage differences. However, these variations were observed during the early development of the leaves, whereas it is generally considered that fossil leaves from deciduous trees most probably correspond to be mature or senescent leaves. Indeed, due to massive abscission during autumn, this leaf stage exhibits a higher probability to be fossilized than stages that are more immature do (e.g., [7]). Therefore, fossil leaves from deciduous trees may have retained a chemotaxonomic signal, provided that they are compared with the distribution of extant species obtained from mature leaves.

The *n*-alkane profiles of mature leaves of the extant European beech (*Fagus sylvatica* L.) and some other extant beeches that were investigated for lipid distributions (i.e. *F. crenata* Blume, *F. grandifolia* Ehrh., *F. japonica* Maxim., *F. orientalis* Lipsky) display various features and differ from each other (e.g., [11,19,24]): they maximize either at C_{27} or C_{29} and exhibit either a strong maximum or a maximum with significant amounts of other homologues. All these *n*-alkane distribution patterns of extant beeches are different from that of the fossil beech, which clearly maximizes at C_{29} (Fig. 3a). Furthermore, the *n*-alcohol profile of the fossil leaves shows a maximum at C_{30} with a significantly abundant C_{28} (Fig. 3c), whereas that of the extant European beech (*Fagus sylvatica* L.) shows a strong

maximum at C_{28} (e.g., [11,24]). All these differences in *n*-alkanes and *n*-alcohols distribution patterns can reflect taxonomic differences. In other words, the studied fossil beech may belong to a different species than those cited and especially *Fagus sylvatica*.

3.5. Preservation state

Various compounds rather sensitive to degradation occurred in the fossil leaves: *n*-aldehydes, monoglycerids, native sterols (sitosterol and stigmasterol). To the best of our knowledge, this is the first report of *n*-aldehydes and monoglycerids in fossil leaves. This shows that the excellent morphological preservation of these fossil beech leaves is associated with a rather good chemical preservation. Thus, it strengthens the importance of this exceptional fossiliferous layer in the Upper Valdarno Basin. This good chemical preservation also indicates that the leaves were exposed to mild physicochemical conditions during their fossilization and not to strong degrading agents. Such biogeochemical and taphonomical consideration will be discussed elsewhere, with the detailed study of the sediment (Zanetti et al., in preparation).

4. Conclusion

The analysis of the lipids of Middle Pliocene beech leaves from the upper Valdarno Basin allowed the identification of various compounds, which helped to clarify their taxonomic position and their deposit environment.

Series of long chain aliphatic lipids (*n*-alkanes, *n*-alcohols, *n*-aldehydes, *n*-ketones) occurred. They are characteristic of Embryophytes' epicuticular waxes. Their distributions differ between the fossil beech and several extant European beeches, suggesting that they may belong to different species. The occurrence of compounds rather sensitive to degradation such as *n*-aldehydes and monoglycerids shows the excellent chemical preservation of the fossils. Two long-chain *n*-alkyl-1,15-diols (C_{30} and C_{32}), probably originating from Eustigmatophyceae microalgae, were identified, and their relative abundances are consistent with a freshwater deposit ion environment. Native plant sterols and their degradation products were identified. Triterpenoids of the oleanane type and friedelane type also occurred. They are characteristic of the Angiosperms and some of them may come from the fossil leaves. Further work is needed to identify several other triterpenoids and to establish profiles that could be useful to clarify the taxonomic affinities and the diagenetic history of these plant fossils.

The occurrence in the fossil leaves lipids of compounds characteristic of bacteria (i.e. hopanoids) and microalgae (i.e. *n*-alkyl-1,15-diols) revealed a potential contribution from epiphytic microflora and/or sedimentary lipids to the leaf lipids pool. Organic sedimentation could also add various compounds from the site flora (e.g., diterpenoids and some triterpenoids) to this lipids pool.

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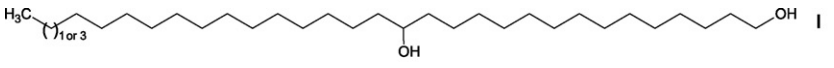
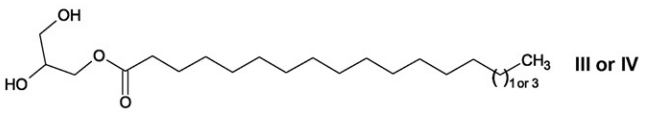
Appendix A. Compounds identified in the fossil beech leaves.

Names of the structures are shown in Table 1.

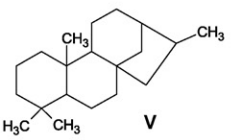
Annexe A. Composés identifiés dans les feuilles fossiles de hêtre.

Les noms des structures figurent dans le Tableau 1.





III or IV



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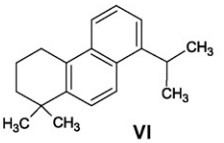

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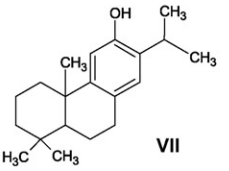
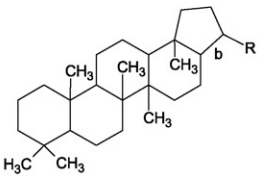
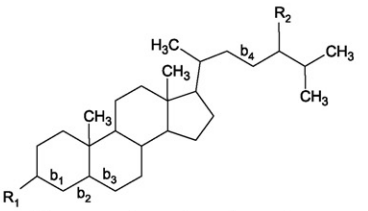


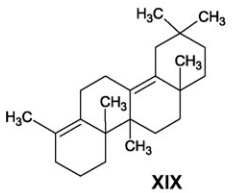
VII



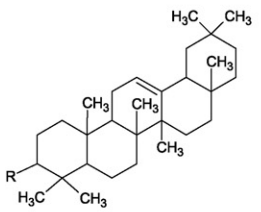
R	b	Nb
=O	-	**VIII**
-CH(CH$_3$)$_2$	=	**IX**
-CH(CH$_3$)(CH$_2$CH$_3$)	-	**X**



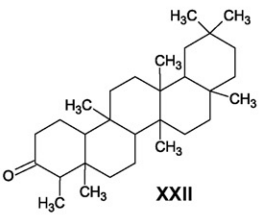
R$_1$	R$_2$	b$_1$	b$_2$	b$_3$	b$_4$	Nb
=O	-H	-	-	-	-	**XI**
-OH	-H	-	-	-	-	**XII**
-H	-CH$_2$CH$_3$	=	-	=	-	**XIII**
-OH	-CH$_2$CH$_3$	-	-	=	=	**XIV**
=O	-CH$_2$CH$_3$	-	=	-	-	**XV**
-OH	-CH$_2$CH$_3$	-	-	=	-	**XVI**
=O	-CH$_2$CH$_3$	-	-	-	-	**XVII**
-OH	-CH$_2$CH$_3$	-	-	-	-	**XVIII**



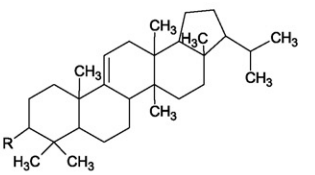
XIX



R	b	Nb
=O	-	**XX**
-OH	=	**XXII**



XXII



R	b	Nb
=O	-	**XXI**
-OH	=	**XXIV**

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