Evaluation of the Potential of Cutin Hydroxyacids as Paleoecological Markers
Sedimentary Diagenesis And Occurrence - Part 2

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Abstract

The analyses of a 6 m sediment core (corresponding to approx. 12 000 yr. of sedimentation) taken from Voua de la Motte (France), showed that m-hydroxyhexadecandioic acid (m=7 or 8) is present in all sections of the core and its isomeric composition appears to correlate with the higher plant distributions obtained from palynology. The vertical variation of the isomeric composition reflects the past modifications in the higher plant input, which, in turn, are related to the paleoclimate changes. On the other hand, m,16-dihydroxyhexadecanoic acid which is generally much more abundant in cutins of higher plants, could not be detected in several sections. The carbon number distributions of sedimentary ω-hydroxyacids, also present in all sections, do not correlate with palynologic data, presumably because of other input sources than higher plant cutins.

Keywords: *cutin acids, higher plants, mid-chain hydroxy- and dicarboxy-fatty acids, ω-hydroxy acids, lake sediments, paleoecology, Voua de la Motte, Léman Basin*

Résumé

Evaluation du potentiel des hydroxyacides cuticulaires pour l'estimation des conditions paléoécologiques. 2.- Présence et diagenèse des hydroxyacides dans les sédiments. – *Les analyses d'une carotte de sédiment de 6 m (correspondant à approximativement 12 000 ans de la sédimentation) provenant du lac de Voua de la Motte (France), ont révélé la présence d'acide m-hydroxyhexadécandioique (m=7 ou 8) dans toutes les sections de la carotte ainsi qu'une corrélation entre cette composition isomérique et les distributions observées pour les arbres (obtenues par palynologie).*

La variation verticale de composition isomérique reflète les modifications passées subies par les arbres, qui, à leur tour, sont liées aux changements de paleo-climatiques. Cependant, l'acide m,16-dihydroxyhexadécanoique qui est généralement très abondant dans les cutines des arbres, n'a pu être détecté dans plusieurs sections. De même, la distribution des ω-hydroxyacides sédimentaires, présents dans toutes les sections, ne semble pas se corréler avec les données palynologiques, ce qui semble indiquer une contribution au sédiment lacustre, autre que celle de la cutine des arbres.

Mots clefs: *acides cuticulaires, dicarboxyacides, dihydroxyacides ω-hydroxyacides, sédiments, paléoécologie, Voua de la Motte, bassin du Léman*

Introduction

Variations in the ancient climate of the Earth have been elucidated using diverse approaches to which new methodologies continue to be added (Hecht, 1985). The first paleoclimatic techniques commonly used were based on microscopic observations (paleontology, palynology, dendrochronology…) which yield qualitative information to distinguish warm and cold episodes but do not furnish a precise temperature record. Later, more sensitive methods involving molecular variations (elemental, isotopic and molecular characterisation of sediments) lead to more reliable paleothermometers (e.g. Bradley,R.S. and references therein). One of the most powerful and widely used tools in the assessment of paleotemperatures, is

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the U^k_{37} index derived from the distribution of C_{37} di- tri- and tetraunsaturated methylketones (Killops and Killops 1993). These compounds are found almost exclusively in the marine unicellular alga *Emiliana huxleyi* (Brassell et al. 1986; Marlowe et al. 1990) for which it has been shown that the U^k_{37} index is linearly related to the growth temperature. As these ketones are sufficiently stable to survive sedimentary diagenesis, the U^k_{37} index of Quaternary sediments allows the deduction of the sea surface temperatures at the time of deposition with a precision of $\pm 0.5°C$ (Prahl and Wakeham 1987). Unfortunately, no equivalent method exists for freshwater sediments.

In the course of our ongoing studies of biological markers in lacustrine sediments we have observed that the cutin acids (CA) from higher plants survive early diagenesis and may be encountered in sediments deposited several thousand years (ky) ago (Blum et al. 1995; Wünsche et al. 1988). In a previous work we have also shown that some of the cutin acids from vascular plants have a taxonomic value (Hu et al. 1988). Particularly, the positional isomeric compositions of m,16-dihydroxyhexadecanoic acid (**1**, m = 8, 9 or 10) and m-hydroxyhexadecane-1,16-dioic acid (**2**, m = 7 or 8) and the carbon number distribution of ω-hydroxyacids (**3**) seems to be species

specific on the basis of results obtained with the most common angiosperms and gymnosperms growing in the Léman Basin (Blum et al.; this issue). These results suggest that the variation of the positional isomeric composition of **1** (m,16-diOH-C_{16}) and **2** (m-OHC$_{16}$DA), and of the chain length distribution of **3** in a sediment profile could reflect the variations of the relative contribution of gymnosperms *vs.* angiosperms. Since the plant cover of a basin evolve with the climate, we pointed out the potential of the above acids as molecular indicators for paleoenvironmental studies. We report here the results of the analysis of several sections of a 6 m lacustrine sediment core, corresponding to approx. 12 000 years of sedimentation, taken from Voua de la Motte, a small pond near Thonon (France), in order to verify this hypothesis.

Material and methods

A 6 m long sediment core was taken from the deepest point (9 m) of Voua de la Motte (46° 20' 27" N, 6° 29' 52" E) where the water-sediment interface remains permanently anoxic, by means of a Mackereth pneumatic corer (Mackereth 1958). The sediment was immediately frozen and stored at -20°C. Twenty samples regularly spaced along the whole core were studied by palynology. For fatty acids analyses, sections about 5 cm long were cut off, hydrolysed with 6N HCl at reflux for 12h followed by a saponification with aqueous NaOH at reflux for 4h. Extractions, separations and analyses of hydroxyacid fractions were performed as described for plant samples (Blum et al., Part 1, this issue).

1

8,16-diOHC$_{16}$

(9,16-diOHC$_{16}$)

(10,16-diOHC$_{16}$)

2

7-OHC$_{16}$DA

(8-OHC$_{16}$DA)

3   n=1 to 19

Results and discussion

Table 1 reports the results of the analyses of a 6 m sediment core taken from Voua de la Motte (Wünsche et al. 1988) for the isomeric compositions of **1** and **2** and the distribution of ω-hydroxyacids. The first important observation is about the relative abundances of **1** and **2**. In all plants we analysed, **1** was much more abundant than **2** (Blum et al., Part 1, this issue). However, although **2** is present all along the sediment core to a depth of 5.75 m, **1** was below the detection limit in several sections along the core. This inversion of the relative abundances from plants to sediments suggests either a better preservation of the diacids in the sedimentary column or, more probably, a diagenetic transformation of the dihydroxyacids to the diacids by ω-oxidation. Based on the biosynthetic relationship between **1** and **2** as pointed out previously (Blum et al., Part 1, this issue), the chemotaxonomic potential of the sedimentary m-OHC$_{16}$DA would not be lost.
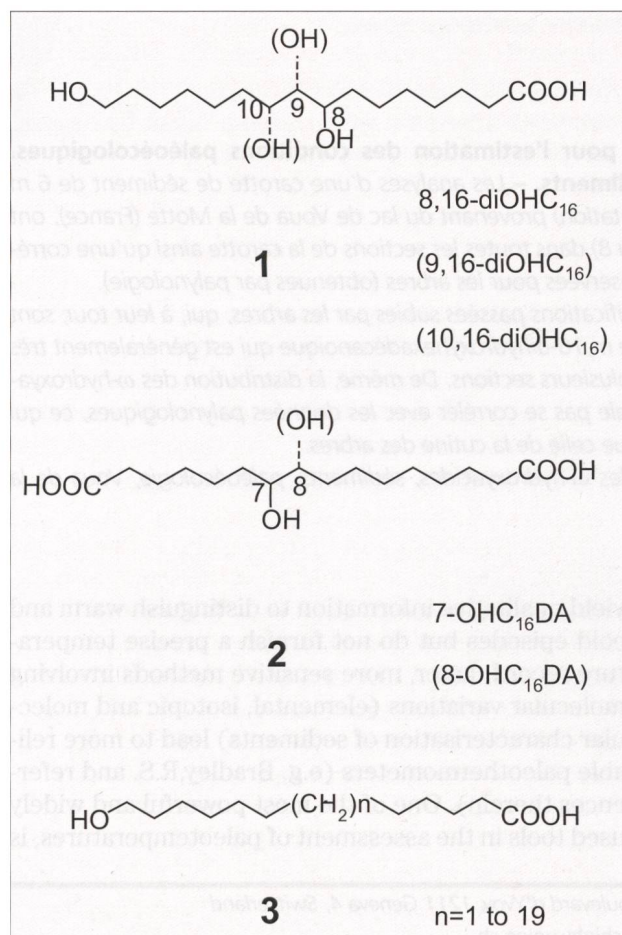
Table 1. *Structural isomeric compositions of 1 and 2 and normalized distribution of C12-C16 ω-Hydroxyacids in the 6 m sediment core from Voua de la Motte.*

Depth (cm)	m,16-diOHC$_{16}$ (%)			m-OH C$_{16}$DA (%)		ω-OH C$_{12-16}$ (%)			
	m=8	m=9	m=10	m=7	m=8	C$_{12}$	C$_{14}$	C$_{16}$	Σ<C$_{16}$/C$_{16}$
0	24.3	25.2	50.5	18.6	81.4	0	0.6	99.4	0.006
10	29.5	23.6	46.9	15.3	84.7		na		-
20	36.2	23.0	40.8	16.3	83.7	0	0.1	99.9	0.001
50	21.3	47.3	31.4	18.6	81.4	1.5	4.0	94.5	0.058
70	47.4	29.4	23.1	16.0	84.0	0.5	0.2	99.3	0.007
80	nd	nd	nd	12.2	87.8	0	0.5	99.5	0.005
100	24.5	27.0	47.5	17.1	82.9	0.7	2.3	97.0	0.031
120	30.9	25.0	44.1	15.5	84.5	0	0	100	0
140	34.6	26.2	39.2	14.9	85.1	0.6	1.7	97.7	0.023
170	nd	nd	nd	11.9	88.1	0	0.3	99.7	0.003
190	37.1	30.7	32.2	9.7	90.3	0	0	100.0	0
210	31.0	45.7	23.3	7.0	93.0	1.3	4.4	94.3	0.060
230	nd	nd	nd	11.8	88.2	0	0.2	99.8	0.002
250	27.5	21.4	51.1	24.4	75.6	0	0	100.0	0
260	32.0	40.0	23.0	19.9	80.1	1.4	2.2	96.4	0.037
290*	nd	nd	nd	0	100.0	0	0	100.0	0
300	nd	nd	nd	19.6	80.4	0.2	0.4	99.4	0.006
320	19.6	39.0	41.4	26.6	73.4	1.5	1.7	96.8	0.033
340	9.8	49.1	41.1	23.2	76.8	2.6	4.9	92.5	0.081
360	9.5	41.1	49.4	26.3	73.7	1.6	4.6	93.8	0.066
380	0	57.0	43.0	31.9	68.1	0.1	0.8	99.1	0.099
400	9.3	42.9	47.8	22.5	77.5	0.4	14.3	85.3	0.172
410	8.4	66.6	25.0	30.5	69.5	0	17.0	83.0	0.204
420	15.9	30.8	53.3	34.7	65.3	0.8	3.0	96.2	0.040
440	nd	nd	nd	26.1	73.9	0	0.8	99.2	0.008
450	18.8	32.2	49.0	22.0	78.0	0.3	1.1	98.6	0.014
475	15.3	34.8	49.9	22.9	77.1	4.0	5.0	91.0	0.099
500	10.3	45.3	44.4	22.6	77.4	20.3	11.7	68.0	0.471
520	nd	nd	nd	17.6	82.4	17.3	10.3	72.4	0.381
540	7.0	44.5	48.5	21.9	78.1	16.7	11.5	71.8	0.393
575	9.0	33.0	58.0	24.3	75.7	40.0	12.5	47.5	1.105
590*	nd	nd	nd	nd	nd	0	0	100.0	-

* results not reliable because of the low concentrations.
nd: not detected.
na: not analyzed.

The variation of the relative proportion of the 7-OHC$_{16}$DA isomer with sediment depth is shown in the Figure 1. The different stages of the Holocene and the climatic data from palynology are also reported on the same figure. The highest proportions of 7-OHC$_{16}$DA are found in sections deposited during the warmest Atlantic and Subboreal periods (~8000-2500 yr. BP) which correspond to a maximum development of angiosperms forests in the Léman Basin (Olive 1972). In sections deposited during the colder Subatlantic and Boreal periods, with a higher spreading of pinaceous forests towards lower altitudes, the relative abundance of 7-OHC$_{16}$DA remains low. Despite the dispersion of the points, the trend of the variation of 7-OHC$_{16}$DA correlates quite well with the palynological information on the past climate of the basin. In sections corresponding to the Boreal period, however, we expected to find smaller values for the relative abundance of 7-OHC$_{16}$DA. It is probable that the contribution from *Juniperus communis* was not negligible during this period, since this species had its maximum development at the very beginning of the sediment deposition, more than 12 000 years ago. Considering that angiosperms spread during hot periods and gymnosperms during cold ones, the data for the sediment core from Voua de la Motte show that it would be possible to obtain climatic information through the analysis of the hydroxyacid isomeric compositions. However, for a general application this requires that several criteria be met:
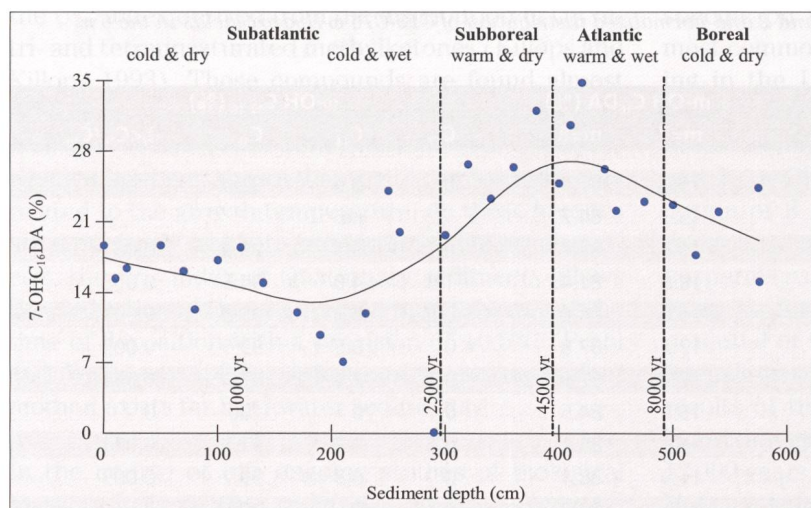
Figure 1. Variation of the relative proportion of 7-OHC16DA with sediment depth. The trend line was obtained using SYSTAT version 10 for Windows by line fitting via DWLS (diagonally weighted least squares) algorithm.

i)   other input sources than vascular plants do not contribute significantly to sedimentary 1 and 2,

ii)   gymnosperms other than Pinaceae do not contribute to sedimentary 1 and 2,

iii) the relative contributions of divers species of angiosperms do not vary much during sediment deposition,

iv)   and sedimentary diagenesis of 1 and 2 do not modify the isomeric composition.

If these criteria are met, then a decrease in the percentage of 10,16-diOHC$_{16}$ in **1** or of 7-OHC$_{16}$DA in **2** in the sediment column would signify an increasing input from Pinaceae and, consequently a colder climatic period. It is well known that cutin acids are produced in significant amounts only by vascular plants with the exception of some tropical monocotyledons (Goni and Hedges 1990a). Criterion i) is therefore generally satisfied. For the basin of the lake Voua de la Motte, which we chose for our studies, we can consider that ii) is also satisfied since the palynological analysis of the sediment core showed that *Juniperus* counted for only 0 to 10% of total gymnosperm pollen count during the whole 12 ky of sedimentation. On the other hand, pollen analysis showed also that major angiosperm species in the basin were *Quercus, Corylus, Betula, Tilia* and *Fagus,* with variable proportions throughout the sedimentation period. However, we think this does not necessarily invalidate the criterion iii). The pollen may travel long distances and the palynology gives the information on the vegetation cover of a geographical area. Debris of leaves, needles, barks or stems, transporting cutins acids to the sediment, on the other hand, come certainly from a much more restricted area around the lake. Finally, regarding the criterion iv), because of the close similarity of the chemical structures, it is unlikely that a sedimentary diagenesis affects differently the positional isomers of **1** or **2**. However, it should be noted in this context, that, although a com-

parison of the isomeric compositions of **1** in fresh and sedimentary needles of cedar showed no significant difference, a slight difference was observed for a mixture of needles of fir/hemlock (Goni and Hedges 1990b). This difference was ascribed to analytical reasons and to a possible contamination of sedimentary needles by tissues from other species with different isomeric composition of **1**.

The sedimentary distributions of C$_{12-16}$ ω-hydroxyacids (Table 1) appear to have no relation with the relative contributions of gymnosperms and angiosperms. The ratio Σ<C$_{16}$/C$_{16}$, which should increase with increasing contribution from gymnosperms, remains close to 0 throughout the core except for sections 400-410 cm and sections below 500 cm. If higher plants were the only significant sources for them we would expect the lowest ratios around 400 cm and highest ratios around 200 cm and below 500 cm. One reasonable explanation would be the contribution of other additional sources for ω-hydroxyacid-C$_{16}$. Plankton is known to be devoid of ω-hydroxyacids. We have analyzed the plankton of Léman lake and Motte lake at various seasons for several years and never detected hydroxyacids (Mermoud 1982). On the other hand, Goni and Hedges (Goni and Hedges 1990a) reported that lower vascular plants, such as ferns and clubmosses have ω-OH-C$_{16}$ as their main acid component, but these organisms also contain ω-OH C$_{14}$, so, even an important contribution from them cannot lower Σ<C$_{16}$/C$_{16}$ ratio toward 0. To our knowledge, no other macroorganism has been reported to contain significant amounts of ω-hydroxyacids. We believe therefore that a diagenetic formation of ω-hydroxyacids, for example by a microbially mediated or chemical ω-oxidation of monocarboxylic acids, may be the reason for the observed distributions of these acids. Another argument in support of this hypothesis is the sedimentary distribution of the whole series of the ω-

hydroxyacids which extends up to C_{30} (not shown in Table 1). Although, in almost all analyzed sections of the core, ω-OH C_{16} acid was the most prominent member, the distribution was bimodal as for the unsubstituted monocarboxylic acids (Wünsche et al. 1988) with the second maximum frequently at C_{22} to C_{24}.

❚ Conclusions

Positional isomeric compositions of mid-chain hydroxyacids **1** and **2** in higher plant cutins are species specific and furnish a chemotaxonomic tool to distinguish angiospermes from gymnosperms. However, once incorporated into the sediment, dihydroxyacid **1** is not stable enough to be used as a molecular marker for palaeoecological studies. On the other hand, dicarboxyacid **2** is stable enough to survive diagenesis for several thousand years, and its isomeric composition remains the same as at the time of deposition. If only species from Pinaceae are the significant gymnosperm contributors to the sediment input, then the variation of the isomeric composition of **2** may reflect the variation of the input of gymnosperms relative to angiosperms and, as a result, the variation of the paleoclimate. Contrary to the U^k_{37} method, the information obtained by this method is only qualitative; no absolute values of the temperature can be deduced, but it has the advantage of being applicable to any depositional environment (except perhaps open sea sediments).

The cutin distributions of ω-hydroxyacids in the range C_{12}-C_{16} also yield a very reliable parameter to distinguish gymnosperms and angiosperms. However, this parameter can not be applied to the sedimentary distributions because of the contribution of sources other than higher plant cutins.

❚ Acknowledgments

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