Zeitschrift: Archives des sciences [2004-ff.]
Herausgeber: Société de Physique et d'histoire Naturelle de Genève
Band: 60 (2007)
Heft: 1

Artikel: Evaluation of the potential of cutin hydroxyacids as paleoecological markers : chemotaxonomy of higher plants. Part 1
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DOI: https://doi.org/10.5169/seals-738409

Evaluation of the Potential of Cutin Hydroxyacids as Paleoecological Markers

Chemotaxonomy Of Higher Plants Part 1

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Ms received 28.10.2006, accepted 3.3.2007

Abstract

Hydroxylated fatty acids present in needles and leaves of the most abundant higher plants growing in the Lake Léman basin (SW-Switzerland/France) have been analyzed. Among the constituent acids, the positional isomeric compositions of m,16-dihydroxyhexadecanoic acid (m = 8, 9 or 10) and m-hydroxyhexadecan-1,16-dioic acid (m = 7 or 8) as well as the carbon number distribution of ω-hydroxyacids were found to have distinct characteristics for gymnosperms and angiosperms.

Gymnosperm samples are characterized by a high predominance (≥93%) of 9,16-dihydroxyhexadecanoic acid positional isomer, except for Juniperus communis which contains almost exclusively 10,16-dihydroxy- isomer (99%). The samples of angiosperms are characterized by a co-occurrence of all three 8,16-, 9,16- and 10,16-dihydroxy- positional isomers in significant proportions.

The positional isomeric composition of m-hydroxyhexadecan-1,16-dioic acid shows a correlation with that of m,16-dihydroxyhexadecanoic acid, suggesting a biosynthetic relationship between these two components. Thus, gymnosperms contain mainly 8-hydroxyhexadecan-1,16-dioic acid, presumably originated from ω-oxidation of 9,16-dihydroxyhexadecanoic acid. However, Juniperus communis contains mainly (93.4%) 7-hydroxyhexadecandioic acid isomer. Angiosperms have mixtures of 7-hydroxy- and 8-hydroxyhexadecandioic acid positional isomers in various proportions.

Another characteristic feature of the cutin acid composition of higher plants is that only gymnosperms contain significant amounts of $<C_{16}$ ω-hydroxyacids.

Keywords: *cutin acids, higher plants, mid-chain hydroxy- and dicarboxy-fatty acids, ω-hydroxy acids, angiosperms, gymnosperms.*

Résumé

Evaluation du potentiel des hydroxyacides cuticulaires pour l'estimation des conditions paléoécologiques. 1.- Chemotaxonomie des plantes supérieures. – *Les acides gras hydroxylés présents dans les aiguilles et les feuilles des arbres les plus abondants du bassin du bassin Lémanique ont été analysés. Les compositions en isomères de position des acides m,16-dihydroxyhexadécanoique (m = 8, 9 ou 10) et m-hydroxyhexadécane-1,16-dioique (m = 7 ou 8) ainsi que les distributions en ω-hydroxyacides se sont révélés très différentes pour les gymnospermes et les angiospermes. Les échantillons de gymnospermes sont caractérisés par la prédominance de l'acide 9,16-dihydroxyhexadécanoique (≥93%), excepté dans le cas Juniperus communis qui contient presque exclusivement l'isomère 10,16-dihydroxy- (99%). Par contre, les échantillons d'angiospermes sont caractérisés par une co-occurrence des trois isomères (8,16-, 9,16- et 10,16-) dans des proportions significatives.*

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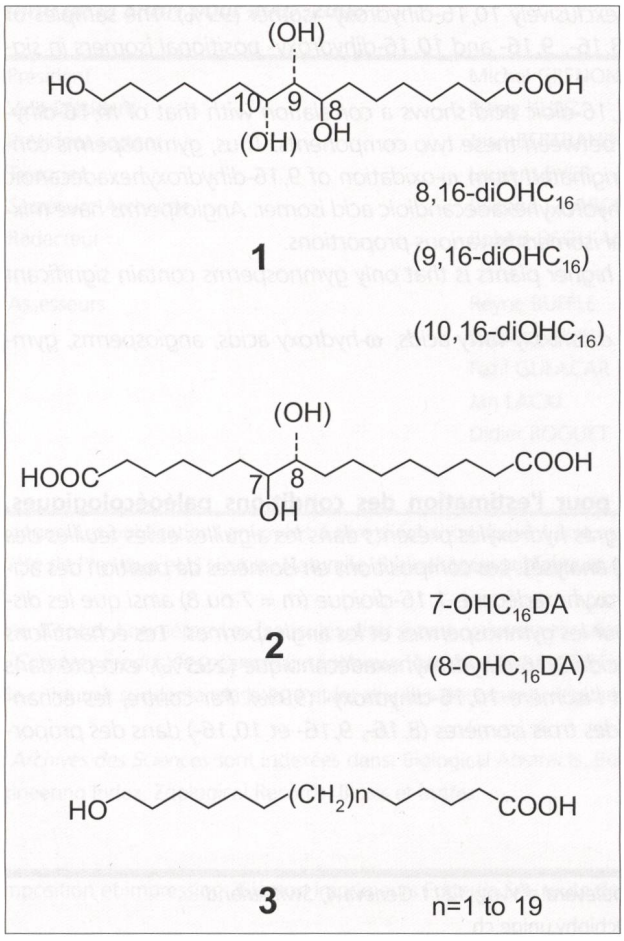
La composition en isomères de l'acide de m-hydroxyhexadécan-1,16-dioique montre, une corrélation avec celle de l'acide m,16-dihydroxyhexadécanoique correspondant, suggérant une relation biosynthétique entre ces deux composants. Ainsi, les gymnospermes contiennent principalement l'acide 8-hydroxyhexadécan-1,16-dioique, vraisemblablement issu de l'ω-oxydation de l'acide 9,16-dihydroxyhexadécanoique. Juniperus communis (Cupressacea) contient, toutefois, principalement l'isomère 7-hydroxyhexadecandioique) et les angiospermes contiennent des mélanges d'acides 7- et 8- hydroxyhexadécandioique. Une autre caractéristique distinctive pour les angiospermes et les gymnospermes est que seulement ces derniers contiennent des quantités significatives de ω-hydroxyacides inférieurs à C_{16}.

Mots clefs: *Acides cuticulaires, gymnospermes, angiospermes, ω-hydroxyacides, dicarboxyacides, dihydroxyacides.*

Introduction

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 (Wünsche et al. 1988; Blum et al. 1995). In previous work we have also shown that some of the cutin acids from vascular plants have 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**) seemed to be species specific on the basis of the results obtained from four angiosperms and two gymnosperms among the most common plants in the Léman Basin. 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. In this paper we report complementary data on several other vascular plants which were abundant during the last millennia in the Léman Basin.



Material and methods

Leaves and needles from trees growing naturally in the Léman Basin were collected and lyophilized to constant weight. For each plant, 0,5 to 1.0 g of dried leaves or needles were finely ground and saponified in a 5 % KOH/MeOH solution at reflux temperature for 12 h. The solution was acidified to pH 3, filtered and the filtrat and the residue were then extracted with methylene chloride (3 x 15 min., ultrasonication for the residue). The organic phases were combined, concentrated and the acidic fraction was isolated by chromatography on a silica gel column (20 x 1 cm, 70-230 mesh) impregnated with a 6,5% p/v KOH / isopropanol solution (McCarthy and Duthie 1962). After methylation with BF_3 - MeOH, the acidic fraction was further separated into two fractions by flash chromatography on a 15 cm long, 0,6 cm i.d. column filled with silica gel (230-400 mesh). The first fraction, containing the unsubstituted fatty acids, was eluted with a 3:1 mixture of hexane and methylene chloride. The second fraction, eluted with a 9:1 solution of methylene chloride and ethyl acetate, contains the hydroxylated acids and the diacids and will be referred to as cutin acids (CA). The hydroxyacids fraction was treated (1h at 100°C) with bis (trimethylsilyl) trifluoroacetamid (BSTFA) to form the trimethylsilyl ethers (TMS), and was analyzed by gas chromatography-mass spectrometry (GC/MS) on a Hewlett Packard 5890 series II gas chromatograph coupled to a VG-TRIO2 quadrupole mass spectrometer. The chromatographic analyses were performed on a Altech SE-54 capillary column (30 m x 0.32 mm, 0.25µm) using helium as the carrier gas (2.5 ml/min). The column temperature was programmed from 60 to 180°C at 10°C/min and from 180°C to 280°C at 3°C/min. The mass spectrometer was operated at a nominal ionizing potential of 70 eV with an ion source temperature of 220°C.

The compounds were identified by comparing the GC equivalent chain length values (ECL) and mass spectrometric data with those of authentic samples or data from the literature (Eglinton et al. 1968; Holloway and Deas 1971; Hunneman and Eglinton 1972). Quantitative analyses were done by comparing the GC peak areas of components relative to that of 2-hydroxytetradecanoic acid (added as an internal standard before methylation). The isomeric compositions of the dihydroxyhexadecanoic and hydroxyhexadecan-1,16-dioic acids were determined by GC/MS by measuring the abundances of the characteristic ions arising from the cleavage α to the secondary TMS ether groups (Holloway and Deas 1971).

C_{12}-C_{26} range, and saturated ω-hydroxyacids in the C_{16}-C_{26} range. ω-Hydroxy-$C_{18:1}$ and ω-hydroxy-$C_{18:2}$ acids may also be quite abundant in some plants (Kolattukudy 1980). ω-Hydroxyacids are generally in minor or trace amounts; they are probably originating from parasitic microorganisms since they have not been previously reported to occur in isolated cutins. **1** and **2** are generally a mixture of GC coeluting positional isomers. At the beginning of this study, we analyzed leaves and needles from several plants after sampling them in different periods of the growth cycle (Blum and Dong, unpublished results). Although the qualitative data of CA remained almost the same, the proportions of the different constituents varied considerably from spring to autumn. Analyses of stems and barks from some of the plants lead to the same observation. However, a remarkable feature remained constant for a given plant whatever the sampling period or the origin of the sample, i.e. the relative amounts of the positional isomers of **1** and **2** as shown in Table 1. All angiosperm species are characterized by the presence of significant amounts of at least two positional isomers, while the gymnosperms contain almost exclusively one isomer. Pinaceae species (*Larix decidua M., Abies alba M., Picea abies* and *Pinus sylvestris*) contain mainly

Table 1. Positional isomer composition (%) of dihydroxyhexadecanoic (m,16-diOHC16) and hydroxyhexadecan-1,16-dioic (m-OHC16DA) acids in the spring tissues of the most abundant higher plant species of the lake Léman Basin.

Plants	ΣCA	1: m,16-diOHC_{16}				2: m-OHC_{16}DA		
	mg/g dry weight	% of CA	m=8	m=9	m=10	% of CA	m=7	m=8
Angiosperms								
Ulmus campestris L.	0.37	10.8	15.0	47.5	37.5	7.1	31.5	68.5
Tilia platyphyllos	5.25	12.2	4.6	45.6	49.8	12.0[b]	19.6	80.4
Castanea sativa M.	1.10	22.7	9.2	20.8	70.0	14.1[b]	94.8	5.2
Fagus silvatica L.	4.50	14.5	9.8	26.0	64.2	7.5[b]	40.7	59.3
Juglans regia	7.61	25.6	6.8	22.4	72.8	2.5[b]	49.0	51.0
Alnus viridis	12.6	5.2	4.1	66.9	29.0	0.9	45.4	54.6
Humulus lupulus	23.7	21.0	28.7	54.5	16.8	3.6	19.9	80.1
Betula verrucosa[a]	15.9	89.0	4.4	72.9	22.7	2.5	15.3	84.7
Quercus petraea[a]	2.28	66.3	-	8.6	91.4	5.2	73.8	26.2
Corylus avellana[a]	5.81	41.2	-	27.6	72.4	13.9	40.6	59.4
Populus nigra[a]	3.88	77.6	-	12.9	87.1	2.9	37.5	62.5
Gymnosperms								
Larix decidua M.	16.58	15.9	2.4	94.1	3.5	9.6[b]	6.1	93.9
Abies alba M.	3.76	34.1	1.8	94.6	3.6	5.3[b]	2.7	97.3
Picea abies	19.5	9.0	1.9	93.2	4.9	3.8[b]	3.4	96.6
Pinus sylvestris[a]	12.8	31.1	-	98.2	1.8	3.4	1.0	99.0
Juniperus communis[a]	11.4	54.7	-	1.0	99.0	4.3	93.4	6.6

[a] *Taken from (Hu et al., 1988a).*

[b] *These values represent upper limits because of coeluting peaks.*

CA: Cutin acids; see Material and Methods.

Results and discussion

The major components of the hydroxyacid fractions from plant cutins include, besides the previously mentioned m,16-dihydroxyhexadecanoic (**1**: m,16-diOHC_{16}) and m-hydroxyhexadecanedioic (**2**: m-OHC_{16}DA) acids, 9,10-epoxy-18-hydroxyoctadecanoic acid (analyzed as a GC coeluting mixture of 9-methoxy-10,18-dihydroxy- and 10-methoxy, 9, 18-dihydroxyoctadecanoic acids formed during the saponification), 1,16-hexadecanedioic acid, C_{15} analogs of **1** and **2**, saturated ω-hydroxyacids in the

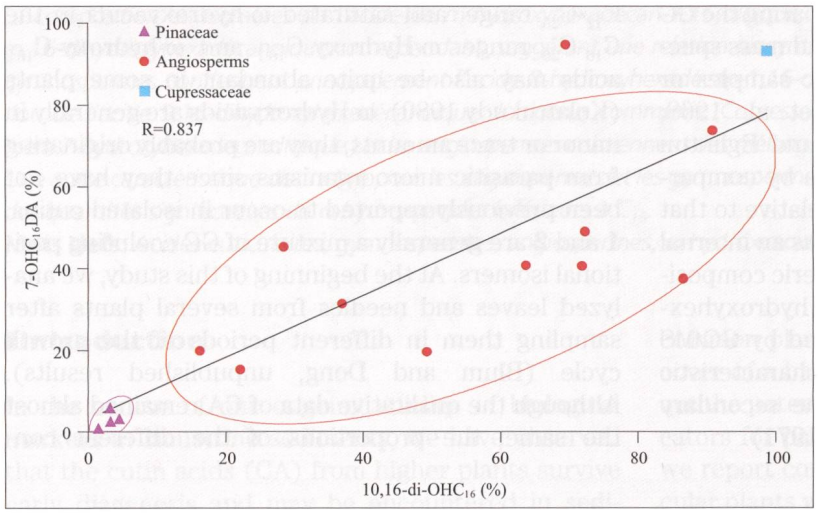
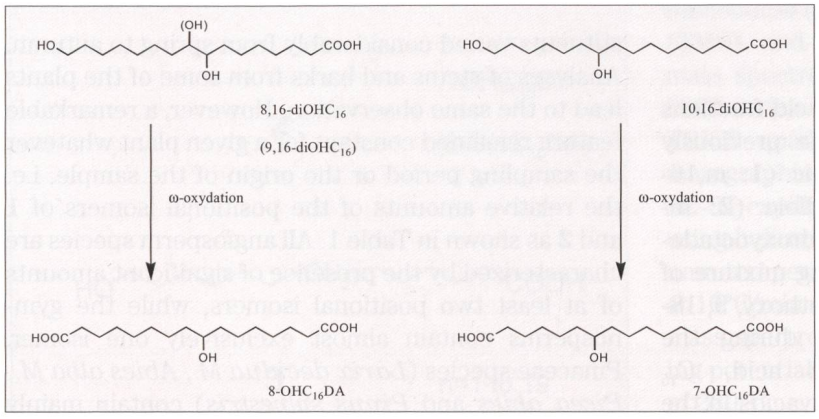


Figure 1. Correlation between the contents in 10,16-diOHC16 and 7-OHC16DA isomers. Confidence ellipses for angiosperms and pinaceae were obtained using Systat statistical software for default p value (0.6827)

9,16-diOHC$_{16}$ and 8-OHC$_{16}$DA, and the Cupressacea *Juniperus communis*, a particular case in the gymnosperm species, has mainly 10,16-diOHC$_{16}$ and 7-OHC$_{16}$DA. These results are comparable with those obtained by Goni and Hedges (Goni and Hedges 1990a; Goni and Hedges 1990b) on a sample set including 16 species of angiosperms and 15 gymnosperms from NW American plants. It should be noted however that, among the gymnosperms they analyzed, three species from Taxodiaceae (*Sequoia sempervirens, Sequoiadendron giganteum* and *Taxodium distichum*) and one species from Cupressaceae (*Thuja plicata*) were also characterized by a predominance of 10,16-diOHC$_{16}$ and 7-OHC$_{16}$DA like *Juniperus communis*. Although, at the present, one can find some specimen in the Léman basin belonging to these four gymnosperm species, they are not indigenous; they were imported during the last centuries.

A close examination of the isomeric compositions of **1** and **2** in Table 1 suggests a correlation between the two components. Samples which have a low relative abundance of 7-hydroxy isomer of **2** also have a low relative abundance of 10-hydroxy isomer of **1** and vice versa. Indeed, the relative amounts of 10,16-diOHC$_{16}$ and 7-OHC$_{16}$DA isomers show a clear positive correlation (r = 0.837, p-value<10^{-3}; Fig. 1). Fig. 1 also shows graphically the difference between angiosperms and gymnosperms. While the angiosperms are spread around the center of the graph, the gymnosperms are all grouped on the left end, with the exception of *Juniperus communis* which is located at the opposite right end. The correlation between 10,16-diOHC$_{16}$ and 7-OHC$_{16}$DA isomers suggests a biosynthetic relationship between **1** and **2**. This may be, for example, the production of **2** by an ω-oxidation of **1** as shown in Scheme 1. An ω-oxidation of the 10,16-diOHC$_{16}$ acid would yield the 7-OHC$_{16}$DA, while ω-oxidation of both 8,16-diOHC$_{16}$ and 9,16-diOHC$_{16}$ acids would give the same isomer, *i.e.*, 8-OHC$_{16}$DA.

Another characteristic feature of the CA contents of the higher plants is that only gymnosperms contain significant amounts of <C$_{16}$ ω-hydroxyacids (Table 2). Moreover, the total amount of saturated ω-hydroxyacids (as % of CA) in gymnosperms is much higher than in angiosperms. Finally, unlike gymnosperms, most of angiosperms contain appreciable



Scheme 1. Biosynthetic relation between m,16-diOHC16 and m-OHC16DA.

Table 2. Distribution of saturated ω-hydroxyacids acids in the spring tissues of the most abundant higher plant species of the lake Léman Basin.

Plants		ω-OH acids								
	% of CA	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	$\Sigma<C_{16}/C_{16}$
Angiosperms										
Ulmus campestris L.	30.6	-	0.1	92.0	2.6	0.7	3.9	0.7	-	~ 0
Tilia platyphyllos	9.6	-	-	49.8	3.9	1.1	13.7	31.4	0.1	0
Castanea sativa M.	25.4	-	-	92.1	-	2.3	5.1	0.5	-	0
Fagus silvatica L.	31.8	-	0.7	43.6	5.3	15.7	31.8	2.9	-	0
Juglans regia	9.5	-	-	97.1	1.7	0.5	0.7	-	-	0
Alnus viridis	5.5	-	-	83.8	8.1	1.4	6.7	-	-	0
Humulus lupulus	17.8	-	-	97.3	2.7	-	-	-	-	0
Betula verrucosa[a]	4.8	-	-	100.0	-	-	-	-	-	0
Quercus petraea[a]	3.5	-	-	100.0	-	-	-	-	-	0
Corylus avellana[a]	10.4	-	-	100.0	-	-	-	-	-	0
Populus nigra[a]	6.2	-	-	100.0	-	-	-	-	-	0
Gymnosperms										
Larix decidua M.	20.0	-	3.0	78.2	9.1	5.1	4.3	0.3	-	0.04
Abies alba M.	57.7	-	32.3	60.5	1.7	4.8	0.7	-	-	0.53
Picea abies	72.2	-	25.4	71.2	0.7	1.6	1.1	-	-	0.36
Pinus sylvestris[a]	52.8	35.6	22.2	42.2	-	-	-	-	-	1.37
Juniperus communis[a]	45.8	39.9	17.3	42.7	-	-	-	-	-	1.34

[a] *Taken from (Hu et al., 1988a).*

proportions of higher homologues. As a result, the sum of ω-hydroxyacids C_{12} and C_{14} relative to ω-hydroxy C_{16} is a very sensitive parameter to distinguish between angiosperms and gymnosperms ($\Sigma<C_{16}/C_{16}$ in Table 2).

Conclusion

Positional isomeric compositions of mid-chain hydroxyacids **1** and **2** in higher plant cutins are species specific and may be used as a chemotaxonomic tool to distinguish angiosperms and gymnosperms. The cutin distributions of ω-hydroxyacids (**3**) in the range C_{12}-C_{16} also yield a very reliable parameter to distinguish the gymnosperms and angiosperms.

Acknowledgments

The authors thank the «Fonds national suisse pour la recherche scientifique» for partial financial support (Grant N°20-52429.97 and 20-057201.99)

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