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Significance of the High Abundance of Pentacyclic Triterpenyl and Hopenyl Acetates in *Sphagnum* Peat Bogs from Northern Spain

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Abstract: Global warming is expected to increase the rate of CH_4 emission from acidic peatlands leading to an increased interest on its mechanisms of formation. The main routes are through the reduction of CO_2 by molecular hydrogen and through the cleavage of acetate. A predominance of the former, a reaction which also competes with homoacetogenesis to form acetate, may enrich the media in acetate, which could potentially be incorporated in the peat molecular markers. Acetates of triterpenoid biomarkers have been identified in peats and lake sediments and related to the input of higher plants. Nevertheless, the acetyl derivatives are found in very low amounts in fresh plants and in much lower amount than other derivatives with alcohol or ketone functional groups. The dichloromethane/methanol extracts of Asturian peat bog profiles (North Spain) were analyzed using gas chromatography/mass spectrometry (GC/MS) and compound-specific-isotope-analysis (CSIA). They show abundance of acetates of compounds with oleanane, ursane, and lupane skeletons derived from higher plants and with hopane skeleton, which can be considered a characteristic of these peats. Two families of 3-oxyhopenyl acetates with -17(21)- and -22(29)- configurations were detected in the upper part of the peat profiles, having a $\delta^{13}C$ isotopic composition enriched by 4‰ compared with that of higher plant triterpenoids, and similar to that of microorganism-derived regular hopanoids. Both the acetate and ketone derivatives with the oxygenated functionality at C-3 were generally present in a given extract and tended to accumulate at certain depth in the profiles and in specific levels. The widespread occurrence of acetyl-derivatives, their higher concentration in the deeper layers of the peat, the fact that the acetates correspond to different compound families of diverse source and the very low amount of acetates identified in Ericaceae-contributing to the peat compared to the alcohols suggest that they were formed in the peat under particularly favorable environmental conditions. We postulate that these conditions could have been the existence of a medium enriched in acetic acid produced by the dominance of hydrogenotrophic methanogenesis and/or homoacetogenesis over acetoclastic methanogenesis. This phenomenon that has been preferentially described in *Sphagnum* bogs at high latitudes, and in the deeper layers of peat, appears to be also present in the temperate peats of the Asturian coast.

Keywords: temperate peat; acetyl-triterpenoids; hydrogenotrofic methanogenesis; *Sphagnum*-peat; 3-oxyhopanoids; acetyl-hopanoids

1. Introduction

Acidic wetlands are the most extensive type of northern wetlands in Europe and North America and contribute up to 50% of the global production of the atmospheric greenhouse gas, CH_4 [1,2]. Global warming is expected to increase the rate of emission of CH_4 from these peats and for this reason research into the mechanisms of CH_4 production has received widespread attention in recent years [3–6]. Methane can either be generated by hydrogenotrophic methanogenesis through the reduction of CO_2 by molecular hydrogen ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) or by acetoclastic methanogenesis through acetate cleavage ($CH_3COOH \rightarrow CH_4 + CO_2$). Generally, hydrogenotrophic methanogenesis and acetoclastic methanogenesis are expected to contribute to CH_4 production in most methanogenic habitats by as much as one-third and two-thirds, respectively [7–9]. However, there are conflicting findings with respect to the relative contributions of these mechanisms to CH_4 formation in anoxic peat. Hydrogenotrophic methanogenesis (H_2/CO_2) has been shown to be depth-dependent [10] accounting for 50%–100% of the production of methane in the deeper anoxic layers [11,12]. In addition, *Sphagnum*-containing wetlands such as bogs that typically develop at high latitudes are known to favor methanogenesis from H_2/CO_2 [13,14]. A recent study on the dominant methanogenic route as a function of vegetation type in Alaskan wetlands found that *Sphagnum*-rich sites tend to be enriched in acetate, whereas vascular plant-rich sites favor acetoclastic methanogenesis [15]. CO_2-reduction was shown to dominate not only in acidic *Sphagnum*-rich peats, but also in a moderately acidic bog in Germany, where CH_4 formation was favored by the addition of H_2/CO_2 and inhibited when there was an excess of acetic acid [16]. The absence of acetoclastic methanogenesis may result in acetate becoming a terminal product that will accumulate over time [17]. Acetate accumulation has been observed in peatlands, particularly in bogs [14,18–20]. Alternatively, acetate can be generated from CO_2 and H_2 by homoacetogenesis (the reduction of CO_2 with H_2 to acetate via the acetyl-CoA pathway; $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$). This mechanism has recently shown to compete with hydrogenotrophic methanogenesis in bogs, swamps and fens, although it was initially considered less thermodynamically favorable [5].

Sphagnum peats in the North of Spain represent the southernmost limit for the common existence of this type of peats in Europe, which develop in temperate and humid climates. Reports of acetylated terpenols in some peats in the region [21–24] suggest that the accumulation of acetates is a widespread phenomenon. Generally, acetyl triterpenoids are not reported in sediments or peats. One of the reasons may be that a widely spread procedure for extract pretreatment prior to gas chromatography/mass spectroscopy analysis involves acetylation [25], which would mask the naturally occurring acetyl-derivatives. Among the few records of triterpenyl acetates in sediments, it can be mentioned those recorded in a peatland topsoil with an acetate group at C-3 and with oleanane, ursane, friedelane, and isobauerane skeleton [26], and their MeO counterparts that have been recorded in Gramineae [27] and in turf and related lake sediments [28,29]. Bauerenyl and hopenyl with an acetate group at C-3 have also been recorded in Asteraceae plants [30,31] and hopenyl and lupenyl acetates have been detected in large amounts in propolis extracted from beehives [32,33]. The present study focuses on the variation in concentrations of triterpenyl acetates in various peat profiles from the North of Spain with the aim of establishing the conditions under which natural acetylation might occur in Asturian *Sphagnum* peat environments.

2. Experimental

2.1. Description of the Sampling Sites

The mires considered in this study are located in Asturias (North Spain), in the coastal high plains, which are thought to be erosion surfaces representing ancient coastlines [34]. The studied sites are Roñanzas (UTM 30 T; 365083; 4803428), La Borbolla (UTM 30 T 365314 4803546), Pendueles (UTM 30 T 368243 4805879), and Buelna (UTM 30 T 369898 4805333), which are located in an area of around 25 km^2 close to the boundary between Asturias and Cantabria. The peat bogs developed over low

permeability quartzitic substrates at heights ranging from 134 to 256 m above sea level (masl) in a strictly continental setting (Figure 1). They have limited areal extent (2–9 ha) and depths ranging from 56 to 254 cm. The climate in this region is of the Atlantic type with humid temperate winters and occasional droughts in the summer. The vegetation of the mires is dominated by *Sphagnum* with some subordinate herbaceous plants and heather [35]. The studied mires correspond to the Erico mackaianae-Sphagnetum papillosi association [36] and are of the Cantabro-Atlantic type, according to the classification proposed by Fernandez Prieto et al. [36] for northern Spain peats. They are characterized by the presence of bryophytes: *Sphagnum* (*S. papillosum* and *S. tenellum*), *Odontoschisma sphagni*, and *Aulacomnium palustre*, and plants like *Erica mackaiana*, *Erica ciliaris*, *Drosera rotundifolia*, *Molinia caerulea*, *Potentilla erecta*, and *Arnica montana* subsp. atlantica [37].

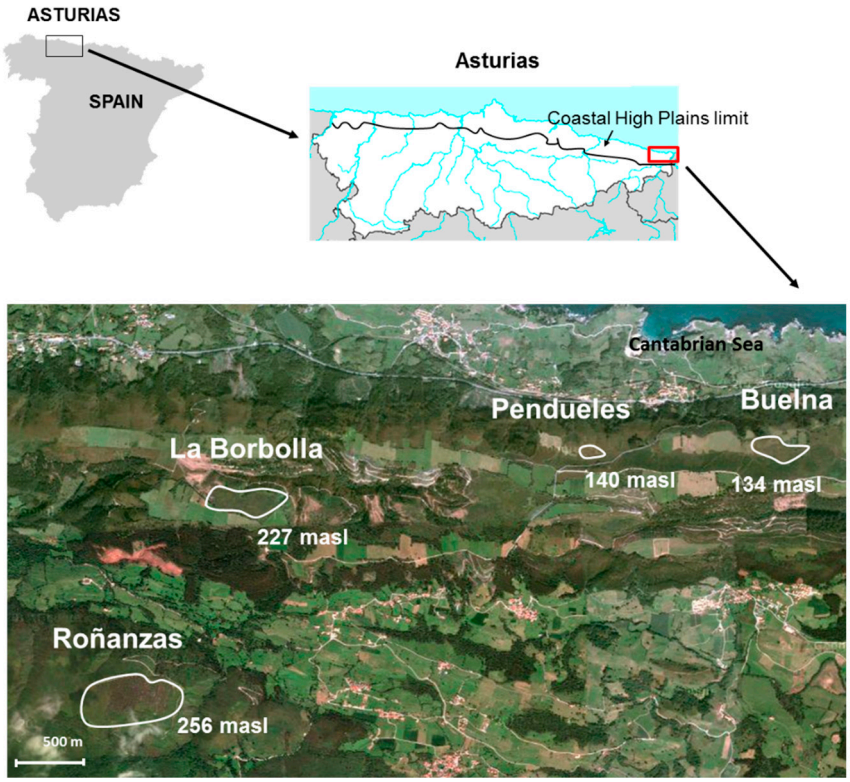

Figure 1. Location of the Roñanzas, La Borbolla, Buelna, and Pendueles mires.

2.2. Analytical Methods

Samples were collected with a manual corer (5 cm diameter, 50 cm length). Once a description of the profile had been carried out, they were sliced into 1 cm-thick portions within a period of 24 h after their arrival at the laboratory and then freeze-dried before analysis to prevent alteration of organic matter. The same freeze-drying procedure was applied to representative peat-forming plants collected at the sampling sites. The studied plants comprise examples of the most common heather (*Calluna vulgaris*, *Erica mackaiana*), sedge (*Carex* sp., *Eriophorum angustifolium*), grass (*Molinia caerulea*), rush (*Juncus articulatus*), fern (*Osmunda regalis*), and moss (*Sphagnum cuspidatum*, *S. capillifolium*, *S. papillosum*, *S. denticulatum*, *S. tenellum*).

The organic carbon content (Corg) was measured in a LECO CHN-2000 instrument to determine the quantities of individual compounds in the extracts normalized to the bulk organic carbon in the samples. Ashing at a moderate temperature (600 °C) was applied to estimate the amount of mineral matter throughout the profile. Accelerator mass spectrometry ^{14}C dating was carried out by Beta-analytic in Miami and CNA in Seville to determine the age of up to 28 samples taken from the various profiles. The numerical data including calibrated and uncalibrated figures are published

elsewhere [23,38] and will not be tabulated here, although the mid value of the 2σ interval is included in Figure 2.

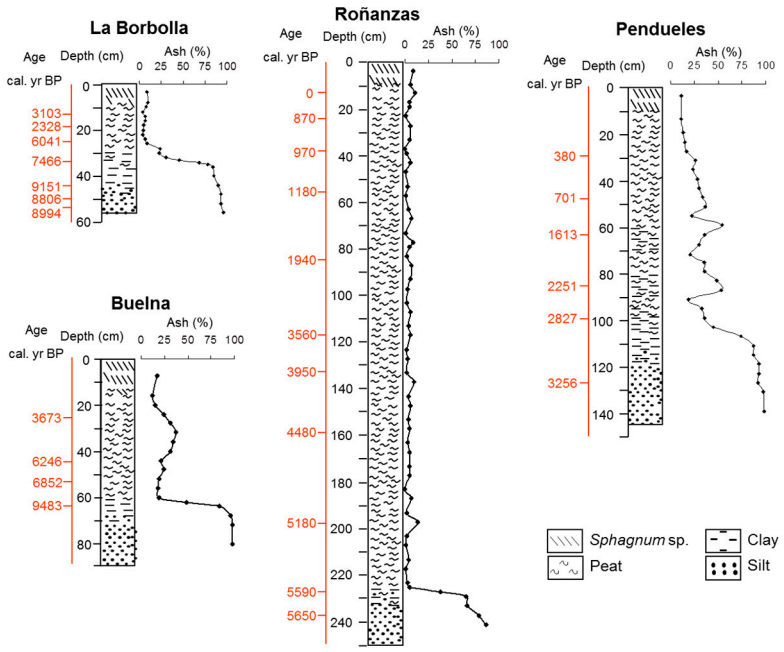

Figure 2. Lithology of the peat profiles with indications of depth and age and the ash content.

For the organic geochemical analysis, fresh plants and peat samples were ultrasonically extracted for 1 h in a mixture (3/1) of dichloromethane (DCM)/methanol (MeOH) at a sample/solvent ratio of 1:20. The solutions were filtered and the solvent removed by means of a rotary evaporator followed by drying under a N_2 flow. The total and underivatized extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890A gas chromatograph attached to an Agilent 5975C mass spectrometer. The GC was equipped with a HP-5 GC column (30 m × 320 µm × 0.25 µm) using He as carrier gas and with an oven temperature program raising from 35 to 300 °C (held for 15 min) at 3 °C min^{-1}. The internal standard was d_{48}-*n*-tricosane. Some triterpenoids were identified by comparing them with authentic standards (α-amyrenone, α-amyrin, β-amyrenone, β-amyrin, and lup-20(29)-en-3β-ol). The standards of triterpenyl acetates were prepared in the laboratory by mixing 520 µL of the corresponding alcohol for α-amyrin and β-amyrin and 770 µg for lup-20(29)-en-3β-ol with 200 µL of pyridine and 200 µL acetic anhydride in an injection vial. After 90 min the triterpenols were completely transformed into triterpenyl acetates. Then, 1 µL of the mixture was diluted with 400 µL of dichloromethane for injection into the GC-MS spectrometer. Other compounds were identified on the basis of their mass spectra and their retention times by comparing them with mass spectra published in the literature and library spectra. Integration was performed using the suitable *m*/*z* mass chromatograms and the results were converted to the total ion current (TIC) integration data using a rate calculated from the standard of each compound.

Selected peat extracts, with a particularly high abundance of some triterpenoids, were submitted to $\delta^{13}C$ compound specific isotope analysis (CSIA) at the Stable Isotope Laboratory of the Department of Applied Geosciences and Geophysics at the University of Leoben (Austria). These samples were fractionated prior to CSIA using column chromatography. Extracts (5 mg) were separated in a column packed with silica gel 60 into four fractions according to their polarity: Aliphatic (eluted with 4 mL n-hexane), aromatic (4 mL *n*-hexane/DCM 9:1), ester (4 mL DCM), and polar (4 mL MeOH). A GC-C-IRMS instrument was used for this CSIA analyses. The mass spectrometer was a Thermo Fisher Delta V mass spectrometer connected to an Ultra series gas chromatograph, fitted with a similar column to that of GC-MS. The GC oven temperature program, injection conditions and carrier gas flow

were also the same as those used for the GC-MS analyses. The standard was an external mixture of *n*-C_{15}, *n*-C_{20}, and *n*-C_{25} and the values are reported relative to Pee Dee Belemnite (PDB).

3. Results

3.1. Description of the Profiles

All the profiles have distinct intervals very rich in organic matter, indicating periods of accumulation under an ombrothrophic regime (Figure 2). Roñanzas and La Borbolla can be classified as raised bogs, whereas Pendueles and Buelna fit in the definition of a blanket bog [23,38]. Generally, the profiles start at the bottom with an interval of light-grey limolite with ash percentages typically over 80%. In the case of the Roñanzas and La Borbolla profiles, the sharp decrease in mineral matter led to the instauration of ombrothrophic conditions of peat accumulation with mineral matter contents of below 10% (Figure 2). The amount of mineral matter in the two raised bogs varies within narrow limits for most of the section. The Buelna profile also shows a decrease in mineral matter content at a depth of 60 cm, although in this case the mineral matter content of the peaty part of the profile is generally higher (ca. 20%), reaching 40% at specific intervals. In Pendueles, with a peat profile that developed on a slope, the mineral matter content of the profile is generally higher and only in the topmost 50 cm is the mineral matter content less than 25%. The intervals, rich in organic matter, are dominated by reddish bryophytic peat.

The age of the samples (Figure 2) indicates that Buelna and La Borbolla have condensed profiles in which 87 and 56 cm of sediment, respectively, accumulated over a period of around 10,000 cal. yr BP (Before Present), whereas the Roñanzas profile extends for 254 cm over 5700 cal. yr BP and Pendueles profile extends for 145 cm over 3500 cal. yr BP.

3.2. Triterpenoids in the Major Peat-Forming Plants in the Region

The extracts of the major plants contributing to the peats in the studied sites were analyzed to identify the triterpenoid derivatives that could be the source of these compounds in the peat extracts. Higher plant triterpenoids derive from the enzymatic cyclization of 2,3-oxidosqualene present in plants, giving rise to different structures with an oxygenated functionality in position C-3 [39]. Those with lupane, ursane, and oleanane skeletons are generally considered as markers for angiosperms [40–45]. More specifically, both β-amyrin and α-amyrin have been found in the roots of *Erica tetralix* and in the leaves of *Calluna vulgaris* and in both cases the former was less abundant than the latter [46]. Higher plants have shown to synthesize not only alcohols but also friedelan-3-one and lupen-3-one and both may be transformed into lupan-3-one by biological and biochemical reduction [47], although lupenone may also derive from the corresponding lupenol. Other less widespread triterpenoids, such as D:C-Friedours-7-en-3-ol acetate, has recently been reported in significant amounts in the catchment area of Lake Aydat in the French Massif Central and it has been claimed to originate from Asteraceae [31]. Peat-forming plants as a source of molecular markers have been studied in various locations [31,48] and also in Roñanzas, but with the focus on *n*-alkanes and *n*-alkan-2-ones [49].

Table 1 shows the abundance of triterpenoids and steroids in the fresh plants collected for this study. The assemblages are dominated by alcohol derivatives, whereas ketone and acetates are found, if present, in significant lower amounts. Stigmasterol was found to be the dominant compound in *Sphagnum* sp., which predominated over other steroid compounds with lower specificity. Triterpenoids with ursane, oleanane, and lupane skeletons were detected in most of the species with the largest concentrations being found in Ericaceae, where ursanoids predominated over oleanoids. *C. vulgaris* contained as major compounds α-amyrin and β-amyrin, the corresponding ketones were absent and the acetates were present in amounts 6 to 12 times lower than those of the alcohols (Table 1). Friedelin was the ketone derivative identified in this fresh plant.

Table 1. Concentrations of triterpenoid and steroid derivatives (μg/g) in the Roñanzas peat-forming plants.

Compound	*C. Vulgaris*	*E. Mackaiana*	*M. Caerulea*	*S. Cuspidatum*	*S. Denticulatum*	*S. Papillosum*	*S. Tenellum*
Ergost-5-en-3β-ol (Campesterol)			0.09	69.70	0.39	0.53	0.51
Stigmast-5, 22-en-3β-ol (Stigmasterol)				177.56	6.41	3.49	1.49
γ-Sitosterol (Stigmast-5-en-3β-ol)			1.12	18.49	0.79	0.30	0.22
Ergost-4-en-3-one			0.08		0.28		
5α-Stigmastanone			0.10				
24-Etil-cholestan-3, 5-dien-7-one			0.06	0.70		0.07	
Stigmast-4-en-3-one	5.18		1.24		0.42	0.22	
β-Amyrin	55.65	110.41		4.07	0.14	2.80	0.45
β-Amyrenone (olean-12-en-3-one)							0.13
β-Amyrin Acetate (olean-12-en-3b-ol Acetate)	4.64	1.49				0.22	
α-Amyrin	135.30	233.82		9.00		6.29	1.35
α-Amyrenone (urs-12-en-3-one)				1.82		0.63	0.22
α-Amyrin Acetate (Urs-12-en-3b-ol Acetate)	9.12	8.60				0.23	
Friedelin (D:A-Friedooleanan-3-one)	0.68						
Lup-20 (29)-en-3-one				0.16	0.01	0.08	0.02
Lup-20(29)-en-3β-ol Acetate		6.42					
hop-22 (29) en-3-ol		0.07					
hop-22 (29) en-3-one							0.04

In the case of *Erica mackaiana*, an endemic species from the north of Spain, the major triterpenoids were α-amyrin, lupeol, and β-amyrin, while their acetates were present in amounts that were between 50 and 100 times lower. Low concentrations of hop-22(29)-en-3β-ol were present in both species. These results agree with those from Bargerveen peat profile and the Ericaceae plants at the site [48]. No higher plants triterpenoids were detected in *Molinia caerulea*. In general, the alcohol derivatives predominated over ketone and acetates in the fresh plants, whereas in the peats of the region alcohols were restricted to the upper layers [23]. Low amounts of hopanoids functionalized in C-3 have been observed in these plants, apparently having a low specificity.

3.3. Higher Plant Triterpenoids in the Peat Extracts

The relative abundance of the various higher plant triterpenoid families and the type of derivative (ketone, acetate) will be presented in this section in order to establish their distribution in the different peat profiles. Compounds belonging to similar families to those identified in the fresh plants were identified in the peat extracts as shown in the higher retention time sections of the total ion current (TIC) chromatograms of selected extracts from Roñanzas (as an example, TIC traces of samples R39 and R163 are shown in Figure 3). The higher plant triterpenoids found in Roñanzas profile are generally functionalized at the C-3 position with ketone or acetyl groups (Table 2). Among these compounds, lupane derivatives are generally the most abundant ones and are represented by lupan-3-one (ranging from 0.5 to 78 μg/g Corg) and lup-20(29)-en-3β-ol acetate (ranging from 0.5 to 127.5 μg/g Corg).

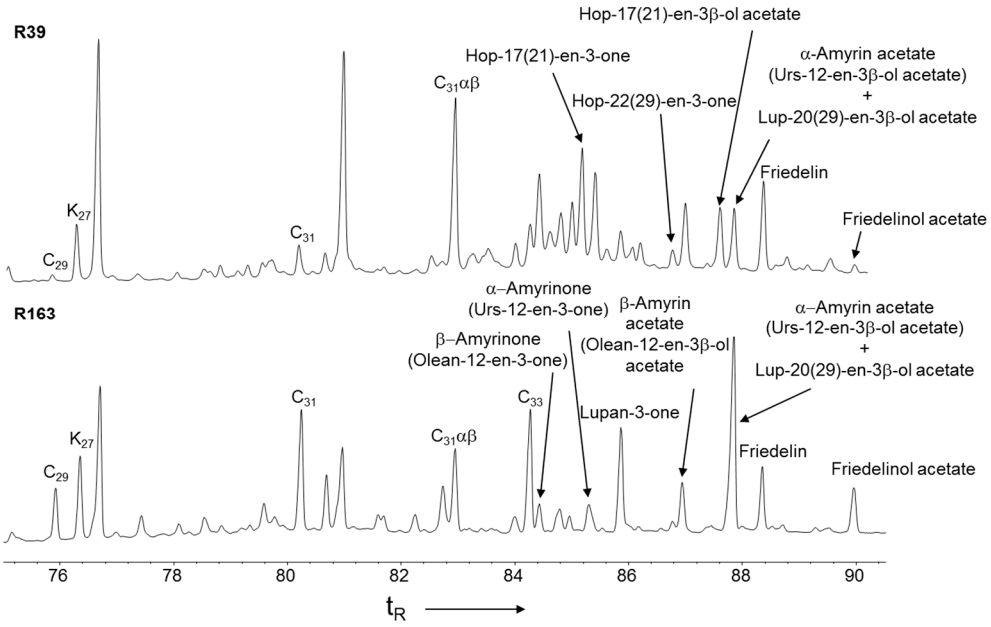

Figure 3. Total Ion Current (TIC) chromatographic traces of two selected samples from the upper and lower part of the Roñanzas profile at 39 and 163 cm (R39 and R163) in the high retention time (t_R) region where triterpenoid acetates appear. In the chromatogram are also identified some of the *n*-alkanes (C_n) and the C_{31} αβ R-Homohopane (C_{31} αβ R).

Ursanoids are represented by α-amyrenone and D:C-friedours-7-en-3-one and their acetyl derivatives in the peat extracts. The abundances of α-amyrenone (0.3 to 27 μg/g Corg) and its acetate (0.1 to 23.5 μg/g Corg), being of similar order of magnitude (Table 2).

Oleanoids and friedelanoids are represented in the Roñanzas profile by β-amyrenone (0.1 to 27 μg/g Corg), friedelin (0.4 to 84 μg/g Corg), and their acetyl derivatives. The β-amyrin acetate concentrations (0.3 to 43 μg/g Corg) are nearly twice as large as those of β-amyrenone (Table 2), whereas the opposite occurs for friedelanyl acetate throughout most of the profile (0.2 to 42 μg/g Corg). Contrarily to the fresh plants, the oleanane-derivatives predominate over ursane-derivatives in Roñanzas peat (Tables 1 and 2), a characteristic known to be shared by other bogs and fens in the region [21–23,50].

Table 2. Concentrations of triterpenoid derivatives (μg/g Corg) in the Roñanzas profile.

Depth	C_{31} αβ R-Homopane	22, 29, 30-Trisnorhopan-21-one	Hop-17(21)-en-3-one	Hop-17(21)-en-3β-ol Acetate	Hop-22(29)en-3-one	Hop-22(29)en-3β-ol Acetate	β-Amyrenone (olean-12-en-3-one)	β-Amyrin Acetate (olean-12-en-3β-ol Acetate)	Friedelin (D:A-Friedooleanan-3-one)	Friedelinol Acetate (D:A-Friedooleanan-3β-ol Acetate	α-Amyrenone (urs-12-en-3-one)	α-Amyrin Acetate (Urs-12-en-3β-ol Acetate)	Lupan-3-one	Lup-20(29)-en-3β-ol Acetate)	D:C-Friedours-7-en-3-one (Bauerenone)	D:C-Friedours-7-en-3β-ol Acetate (Bauer-7-en-3β-ol Acetate)
3.5	0.6		16.7	10.0	3.3	1.0	0.1		1.3		0.5					
9.5		0.4	34.6	17.2	11.9	2.3			38.8		0.8	0.5				0.6
13	2. 7	1.7	1.6		3.6	0.8			17.5	0.8						2.8
19	70.6	18.5	64.0	25.5	15.3	1.9			17.1	1.6	2.7					
23	39.1	71.8	27.5	13.5	3.5	0.8								7.3		10.5
27	5.5	9.0	28.1	12.0	2.0	0.5										
33	420.1	62.6	67.2	50.9	8.4	2.7			14.3	5.1		3.0		40.0		
39	168.7	3.7	102.4	66.8	13.8	4.6		5.2	83.9	8.3	8.4	8.5	11.8	63.7		
43	269.4	3.3	15.8	16.9	6.3	2.9		2.9	26.5	15.4	11.2	6.3	18.9	78.0		
47	84.2	0.5	2.0	2.0		0.2		2.1	1.5	2.3	1.2	1.2	2.2	12.9		
53	376.9	2.5	27.1	13.3		0.8		0.5	5.3	4.0	3.6	2.9	5.6	24.4		
57	127.5	0.5	1.2	1.5				2.5	4.6	2.4	1.1	1.7	2.9	15.5		
63	207.1	0.7	6.3	1.2	1.3		2.3	5.9	2.7	7.2	3.2	1.9	7.7	33.7		
67	258.0	0.9	55.9	14.3	12.6	1.5	3.2				6.3		5.8			3.9
73	392.3	3.5	28.8	16.4	10.4	1.6	10.5		12.3		13.5	2.2	14.8		7.7	85.5
79	33.7	1.6	47.8	41.7	9.4	4.8	3.9		11.3		3.9		5.6		6.1	112.9
83	12.8	2.5	69.0	19.4	9.7	1.4	1.2		3.5						0.6	16.3
87	37.7	3.5	76.5	31.9	13.6	4.5	3.3		72.8		3.8				1.2	15.5
93	11.2	9.8	39.7	23.8	5.8	1.7	2.2		48.7	1.8	1.8				0.2	3.3
97	32.9	1.8	5.4	2.7	1.2	0.2	3.5		67.3		0.7					
103	171.3	0.9					1.1	4.9	9.3	5.4	1.1	3.4	1.6	30.3		
107	111.3	1.5					1.3		17.4	2.8	1.3	0.5		6.3		
113	52.7		2.9	2.3	1.6	0.6	3.7	15.1	22.1	9.5	2.7	4.9	6.0	48.7		
117	52.7						2.1	4.7	17.0	3.7		1.5	3.9	20.4		
123	55.8	0.4					2.8	4.1	15.3	3.9	1.1	1.1	6.3	18.2		
127	54.6	0.4					8.0	11.4	40.3	8.9	2.7	6.3	7.7	45.8		
133	18.1	0.3					2.6	9.0	11.5	6.4	1.2	2.4	6.4	44.3		

Table 2. *Cont.*

Depth	C_{31} αβ R-Homopane	22, 29, 30-Trisnorhopan-21-one	Hop-17(21)-en-3-one	Hop-17(21)-en-3β-ol Acetate	Hop-22(29)en-3-one	Hop-22(29)en-3β-ol Acetate	β-Amyrenone (olean-12-en-3-one)	β-Amyrin Acetate (olean-12-en-3β-ol Acetate)	Friedelin (D:A-Friedooleanan-3-one)	Friedelinol Acetate (D:A-Friedooleanan-3β-ol Acetate	α-Amyrenone (urs-12-en-3-one)	α-Amyrin Acetate (Urs-12-en-3β-ol Acetate)	Lupan-3-one	Lup-20(29)-en-3β-ol Acetate)	D:C-Friedours-7-en-3-one (Bauerenone)	D:C-Friedours-7-en-3β-ol Acetate (Bauer-7-en-3β-ol Acetate)
137	21.0						1.5		8.3							
143	61.4	0.8					13.9	3.1	60.0	5.7	2.6	1.2	5.9	12.3		
147	67.1	0.6					10.2	21.7	19.2	39.9	8.2	10.9	24.1	85.8		
153	32.1	0.3					9.0	18.9	14.6	21.5	5.5	7.6	28.7	88.1		
157	36.8	0.6					15.7	28.7	23.0	23.1	12.1	11.3	53.7	127.4		
163	23.0	0.3					9.0	14.7	15.5	13.4	8.3	7.7	25.2	68.6		
167	88.3	0.6					26.7	31.3	35.7	24.3	24.8	9.4	77.9	97.1		
173	80.2	0.8					3.2	2.6	4.5	3.5	2.3	0.8	7.6	7.1		
177	41.9	0.9					15.6	22.8	17.1	16.4	14.6	11.1	38.1	69.1		
183	33.9	0.2					8.4	4.6	38.2	3.3	2.9	1.7	9.4	18.5		
187	30.8	0.1					2.0	1.0	3.3	0.7	0.8	0.2	2.8	3.9		
193	41.7	0.2					7.3	11.5	6.7	15.3	8.8	6.4	22.2	36.6		
197	70.4	0.4					7.1	5.3	10.6	5.8	4.4	1.5	23.6	28.5		
203	114.1						26.0	43.3	32.7	42.2	26.9	23.5	67.6	117.2		4.1
207	21.9	0.3					2.0	2.4	2.6	2.3	1.5	0.7	4.7	6.6		1.4
213	10.9	0.3					0.6	0.3	1.1	0.6	0.4	0.1	1.5	1.0		
217	7.0	0.3							0.6				0.5	0.6		0.4
223	34.8	0.3					0.6						1.2		0.3	12.4
227	20.2	0.3					0.8		1.0	0.8	0.9	0.4	1.6	3.2	1.4	15.2
233	12.3	0.2					0.3					0.2	0.4	0.5	0.8	9.4
237	3.1	0.3					0.2	1.3	0.4	0.2	0.3	2.0	0.5	1.4	3.3	12.2

In the Roñanzas profile D:C-Friedours-7-en-3-one (bauerenone) and D:C-Friedours-7-en-3β-ol acetate (bauerenyl acetate) are both detected in two distinct intervals (at a depth of 70–90 cm for the highest concentrations, and below 200 cm). In addition, the acetyl derivative is also present in the upper 20 cm. Whereas Bauerenone reaches a maximum concentration of 8 μg/g Corg, the bauerenyl acetate goes up to 113 μg/g Corg (Table 2). The restricted distribution of this compound family in the Roñanzas profile compared to other higher plant triterpenoids could indicate a more specific source. Both *Asteraceae tubuliflorae* and *Asteraceae liguliflorae* pollen have been reported in this profile at distinct intervals [51], which could be the source of bauerenyl derivatives according to Lavrieux et al. [31].

The variations in the concentration of the higher plants triterpenoids (lupanoids, ursanoids, or oleanoids) in the Roñanzas profile show remarkable similarities regardless of the structure of the compound and the type of derivative (Table 2). It can be seen that most of them accumulate preferentially in the lower half of the profile (below 120 cm) and show maxima at 120–130, 140–170, 175–185, 195–205 cm (Figure 4). These intervals, with the exception of the first one, correspond to dry periods identified for this bog on the basis of the *n*-alkane ratios [23,24,52,53]. The 120–130 cm interval, where there is a moderate increase in higher plant triterpenoids, corresponds to a transition zone between vegetation with an abundance of woody plants and vegetation dominated by *Sphagnum*, and it coincides with high values of humidity proxies [23,52]. In the upper part of the profile, higher plant triterpenoids are generally found in lower concentrations suggesting a lower input of woody plants to the peat. Only friedelin is present in high concentrations at specific intervals (depths of 35–45 cm and 85–100 cm) within this section and also D:C-fridours-7-en-3β-ol acetate is concentrated at around 80 cm.

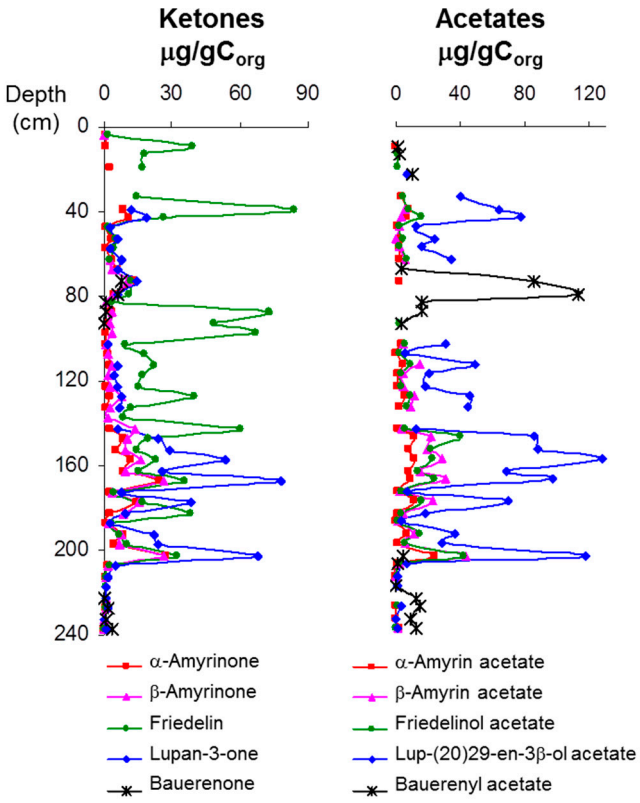

Figure 4. Variation of the concentration of higher plant triterpenoid ketones and acetates in the Roñanzas profile (μg/g Corg).

Similar higher plant triterpenoids to those quantified in Roñanzas are also quantified in the Buelna, La Borbolla, and Pendueles profiles. Both the oleanane and ursane derivatives generally tend to concentrate at specific intervals in the different profiles (Figure 5), whereas the bauerane, lupane, and friedelane derivatives exhibit different concentration patterns. The concentration of friedelin in

La Borbolla (0.3 to 114 μg/g Corg) is significantly higher than that of the other ketones (0.1 to 6 μg/g Corg). The differences are smaller in Buelna (0.5–11.2 vs. <2.6 μg/g Corg), where also lupan-3-one show higher concentrations than both α- and β-amyrenone (Table 3). In the Pendueles profile, both lupan-3-one and friedelin have uneven concentration patterns and predominate over other ketones, the former being the most abundant one. Lupenol acetate could not be quantified in this profile due to its co-elution with hop-17(21)-en-3β-ol acetate. Bauerenyl acetate is present throughout the Buelna, La Borbolla, and Pendueles profiles in significant concentrations (Table 3), whereas in Roñanzas it is restricted to specific intervals. Common features to all the profiles are that friedelin and lupan-3-one are generally more abundant than α-amyrenone and β-amyrenone, and that oleanoid concentrations are slightly higher than those of ursanoids (Table 3, Figure 5). Generally, higher plant triterpenoid concentrations are lower in the Buelna and Pendueles profiles and higher in the Roñanzas profile (Figures 4 and 5, Tables 2 and 3).

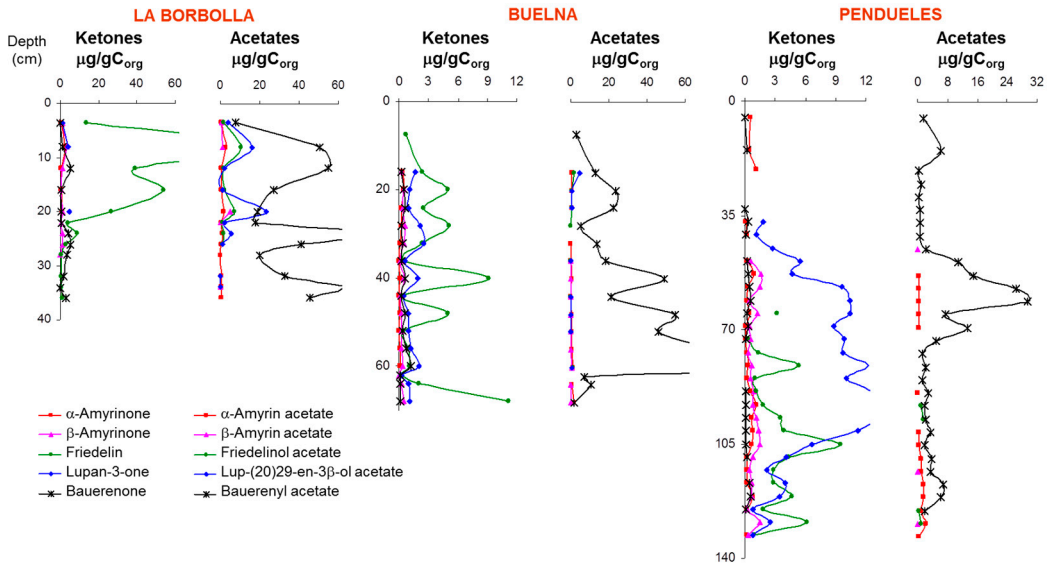

Figure 5. Variation of the concentration of higher plant triterpenoid ketones and acetates in the Buelna, La Borbolla, and Pendueles profiles (μg/g Corg).

Table 3. Concentrations of triterpenoid derivatives (μg/g Corg) in the Buelna, La Borbolla, and Pendueles profiles.

Depth	C_{31} αβ R-Homopane	22, 29, 30-Trisnorhopan-21-one	Hop-17(21)-en-3-one	Hop-17(21)-en-3β-ol Acetate	Hop-22(29)en-3-one	Hop-22(29)en-3β-ol Acetate	β-Amyrenone (olean-12-en-3-one)	β-Amyrin Acetate (olean-12-en-3β-ol Acetate)	Friedelin (D:A-Friedooleanan-3-one)	Friedelinol Acetate (D:A-Friedooleanan-3β-ol Acetate	α-Amyrenone (urs-12-en-3-one)	α-Amyrin Acetate (Urs-12-en-3β-ol Acetate)	Lupan-3-one	Lup-20(29)-en-3β-ol Acetate)	D:C-Friedours-7-en-3-one (Bauerenone)	D:C-Friedours-7-en-3β-ol Acetate (Bauer-7-en-3β-ol Acetate)
BUELNA																
7.5	9.6	0.7	4.6	5.5	2.2	0.8			0.7							3.1
16	13.4	1.6	20.0	12.2	3.4	1.2	0.3		2.3	1.8	0.4	0.4	1.6	4.5	0.2	12.9
20	12.4	1.7	20.6	12.6	2.8	0.9	0.5		5.0	0.7	0.5	0.4	1.1	0.6	0.5	23.6
24	3.3	1.3	4.9	2.4	1.1	0.3	0.3		2.5	0.4	0.2	0.3	1.0	0.3	0.5	22.4
28	0.6	0.9	0.4		0.1	0.0	0.6		5.1	0.3	0.2		2.2		0.2	5.2
32	0.3	0.6					0.5		2.3		0.1	0.2	2.6		0.4	13.3
36	0.1	0.2					0.1	0.1	0.6	0.1	0.0	0.2	0.6	0.1	0.3	18.4
40	0.3	0.6					0.5	0.1	9.2		0.1	0.4	1.9		0.7	48.8
44	0.1	0.2					0.1	0.1	0.4	0.1	0.0	0.2	0.5	0.1	0.2	20.9
48	0.6	0.7					0.3	0.1	5.0		0.1	0.5	1.0	0.3	0.5	54.6
52	0.2	0.7					0.2	0.2	0.5		0.1	0.4	0.9	0.3	0.4	46.0
56	6.3	0.7					0.3	0.1	1.0		0.1	0.6	1.2		0.7	75.8
60	3.0	2.0					0.4	0.6	1.0	1.2	0.1	1.1	2.0	0.8	1.2	105.4
62	0.5	0.3					0.1		0.3				0.3		0.1	7.1
64	1.4	1.0					0.2	0.0	2.0			0.4	0.9		0.2	10.3
68	0.6	0.7					0.4	0.0	11.2			1.7	1.1		0.1	1.6
LABORBOLLA																
3.5	16.8	2.5	26.0	19.1	2.7	1.4	0.9	0.3	13.5	1.8	0.9	0.4	0.9	3.8	0.2	7.9
8	31.5	9.7	45.5	31.3	12.8	4.0	3.8	1.0	114.2	10.7	2.3	2.5	4.2	16.0	1.4	50.6
12	10.2	8.3	108.2	38.8	23.5	8.8	1.2		39.0	1.5	0.5	0.5		2.0	5.6	54.9
16	9.5	7.1	50.9	22.0	10.7	2.1	0.5		54.0	2.2	0.9	0.5		1.4	0.7	27.5
20	19.0	3.0	8.0	4.8	2.5	0.9	1.1	5.0	26.6	7.4	1.3	1.9	4.5	23.3	0.7	18.7
22	4.2	5.8	0.5	0.3			0.7	0.1	4.2	0.9		0.4		2.3	0.7	17.6
24	2.3	17.1					1.1		8.8	1.8		1.0		5.5	4.4	81.3
26	4.0	5.6					0.9		3.1	1.1		0.5		0.9	5.5	41.2
28	4.1	3.1					0.1		0.9			0.2			3.4	20.2
32	1.5	1.9							0.3			0.4		0.2	1.8	32.7
34	1.0	1.1						0.1	0.5			0.7		0.3	0.2	62.9
36	2.1	2.2							1.0			0.8			3.1	45.6

Table 3. *Cont.*

Depth	C_{31} αβ R-Homopane	22, 29, 30-Trisnorhopan-21-one	Hop-17(21)-en-3-one	Hop-17(21)-en-3β-ol Acetate	Hop-22(29)en-3-one	Hop-22(29)en-3β-ol Acetate	β-Amyrenone (olean-12-en-3-one)	β-Amyrin Acetate (olean-12-en-3β-ol Acetate)	Friedelin (D:A-Friedooleanan-3-one)	Friedelinol Acetate (D:A-Friedooleanan-3β-ol Acetate	α-Amyrenone (urs-12-en-3-one)	α-Amyrin Acetate (Urs-12-en-3β-ol Acetate)	Lupan-3-one	Lup-20(29)-en-3β-ol Acetate)	D:C-Friedours-7-en-3-one (Bauerenone)	D:C-Friedours-7-en-3β-ol Acetate (Bauer-7-en-3β-ol Acetate)
PENDUELES																
5	0.3	0.2									0.6				0.0	1.5
15		0.4			1.2						0.5				0.2	6.3
21	2.8	1.0			1.4	0.3					1.2					0.4
25	4.1	2.1														1.0
29	3.1	5.4	3.1	1.7	5.2	1.4										0.2
33	4.3	2.9	13.4	4.5	8.7	1.6									0.0	0.5
37		6.2	3.3	2.7	1.8						0.1		1.8		0.3	0.5
41	0.1	7.4	5.3	1.8	1.1						0.3		1.1		0.1	0.7
45	0.5	6.1		2.1	2.7			0.1					2.7			2.3
49	14.9	1.7	7.1	5.0	31.6	8.3	0.6				0.3		5.5		0.2	11.0
53	21.3	3.9	16.4		49.3	9.1	1.6				0.9	0.3	4.7		0.3	15.0
57	13.2	3.1	21.6	11.9	23.5	6.4	1.5				0.3	0.4	9.6		0.5	26.4
61	5.8	4.5	8.1	6.2	11.2	4.2	0.5				0.3	0.5	10.4		0.6	29.5
65	2.3	2.9			11.5	3.9	1.2		3.2		0.5	0.2	10.4		0.2	7.3
69	1.1	2.6	7.2	5.5	5.7	2.3	0.5				0.1	0.2	8.8		0.3	13.4
73	1.8	2.3	10.7	4.6	6.8	2.2	0.6		0.3		0.2		9.8		0.1	4.8
77	3.8	2.2	4.7	3.9	10.4	2.5	0.3		1.3		0.2		9.7			1.2
81	11.2	1.8	4.1	2.4	2.5	0.7	0.7		5.4		0.3		12.2			2.3
85	3.6	0.9	1.6	1.3		0.3	0.6		1.0		0.2		10.1			1.3
89	4.4	0.6	0.3				0.9		1.1		0.6	0.1	12.6		0.1	2.8
93	4.3	0.4	12.3	16.1	1.9	0.9	0.9		1.9	0.8	1.2		13.9		0.1	2.0
97	4.1	0.7	7.9	2.6	1.7	0.3	1.2		3.5	1.6	0.7		13.2		0.1	2.1
101	3.7	0.8	4.0	0.6	3.9	0.6	1.4		3.9		0.8	0.3	11.2		0.2	3.3
105	1.3	0.7	2.1		7.0	0.6	1.5		9.5		0.7	0.3	6.6		0.1	1.8
109	0.4	0.3	0.2		0.6	0.2	0.8		4.3		0.3	0.9	4.1		0.3	3.9
113		0.2			0.3	0.1	0.4	0.0	2.9		0.2	0.9	2.2			3.5
117		0.3					0.7		2.9		0.2	1.6	4.0		0.5	6.8
121		0.3	0.5		0.5	0.3	0.7		4.6		0.8	1.7	3.4		0.5	6.3
125		0.1				0.1	0.3		1.8	0.2	0.2	0.9	0.8		0.2	2.0
129		0.4				0.3	1.5	0.1	6.1	0.9		2.3	2.5			
133	0.3	0.3				0.2	0.4		0.9		0.3	0.3	0.9			

3.4. Hopanoid Derivatives in the Peat Extracts

Hopanoids are pentacyclic triterpenoids produced by a wide range of bacteria [54] and by a handful of lichens and plants where they modulate the fluidity and permeability of membranes [55]. The relative abundance of the various hopanoids families, the type of derivative (ketone, acetate), and the relation to higher plant triterpenoids will be presented and discussed in this section. Compounds belonging to similar families to those identified in the fresh plants were identified in the peat extracts as shown in Figure 6. The regular hopane structure, commonly associate to bacterial source, is formed by the cyclization of the branched terpenoid hydrocarbon squalene. These compounds have a biological configuration 17β, 21β(H)-hopanoid, which is transformed into the more stable 17α, 21β(H)-hopanoid [56] with increasing thermal maturity as occurs in old sediments. The biological configurations are expected to dominate in recent peat sediments, although dominance of the homopane C_{31} αβ R is frequently reported. Explanations for the source of thermally stable hopanoid configurations in peat comprise microbially mediated synthesis, a phenomenon which frequently occurs in decaying organic matter [57], direct input of bacterial biomass—after the finding of the stable αβ configuration in typical soil bacteria such as *Frankia* spp. [58]—and acid-catalyzed degradation [59,60]. The latter was recently confirmed by the statistically significant correlation of C_{31} hopane isomerisation ratio with pH [61]. Also, simple C_{30} biohopanoids such as diploptene (hop-22(29)-ene) are commonly reported in peats, where it is thought to be directly inherited from living or recently decayed microorganisms [60]. In addition to bacterial sources, diploptene has also been reported to occur in some ferns [62] and in mosses [63], including some common peat-forming species such as *Polytrichum commune* and *Hypnum revolutum* [64].

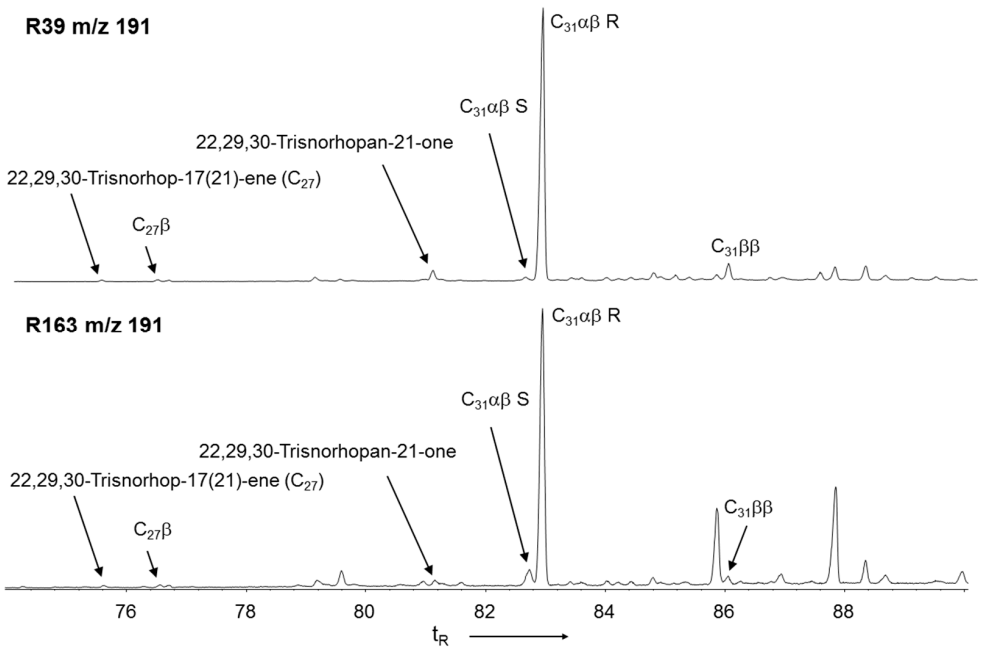

Figure 6. *m/z* 191 traces of two selected samples from the upper and lower part of the Roñanzas profile at 39 and 163 cm (R39 and R163) in the high retention time where hopanoids appear.

The presence of an oxygenated functionality at C-3 in hopanoids is not a common phenomenon [65], and when it occurs, it is usually associated with a plant origin [66,67], where oxidosqualene, instead of squalene, is the biosynthetic precursor [68]. Among the reports of a plant origin for 3-oxyhopanoids is that of 22-Hydroxihopan-3-one, which has been detected in dammar resin and it is transformed through dehydration in either hop-21(22)-en-3-one or hop-22(29)-en-3-one, possibly yielding hop-17(21)-en-3-one through isomerization in an acidic environment [66]. Hopen-3-ones, including hop-17(21)- and/or -22(29)- have been found in leaves of *Euphorbia lathyris* [69] in *Maprounea*

guianensis from Brazil [70], in Gentianaceae family plants in China [71], in the *Quercus* species in Hong Kong [72,73], and in peatland topsoil in a French bog study [26]. The methyl ether derivative of the hopanoid -22(29)- has been isolated from the epicuticular waxes of a graminea -*Chionochloa*- [74] while its acetyl derivative has been isolated from a Vietnamese Asteraceae plant [30].

The *m*/*z* 191 mass chromatograms for samples R39 and R163 are shown in Figure 6, where regular hopanes from C_{27} to C_{31} (except C_{28}) can be appreciated corresponding to the α and β configurations at positions C-17 and C-21, respectively. The most prominent peak of the regular hopanes is the homopane C_{31} αβ R (Figure 6).

All the studied peat profiles contain diploptene (not quantifiable due to co-elution with taraxerone), hop-22(29)-en-3-one and its corresponding acetate. It also contains hop-17(21)-ene, hop-17(21)-en-3-one and its acetate. The 3-oxyhopanoids are particularly abundant in the upper part of the profiles (Figures 7 and 8), with exception of Pendueles profile where these compounds extend to deeper layers. In both configurations, ketone predominates over the acetate derivative (Tables 2 and 3). Of the regular hopanoids, the 17α(H), 21β(H)-homohopane R (C_{31} αβ R hopane) is present throughout the Roñanzas profile (ranging from 0.6 to 420 μg/g Corg), maximizing in the 30–80 cm interval (Figure 7 and Table 2). The concentrations of C_{31} αβ R hopane in La Borbolla and Buelna profiles (ranging from 1.0 to 31.5 μg/g Corg and 0.1 to 13.4 μg/g Corg, respectively) reach their maximum in the uppermost 20 cm, whereas in Pendueles profile this hopanoid (ranging from 0.1 to 21.3 μg/g Corg) maximize at the interval 50-60 cm and around the centimeter 80 (Figure 8 and Table 3). Hopanones with oxygenated functionality in position C-21 and C-22 can also be appreciated. The distribution of 22, 29, 30-trisnorhopan-21-one (ranging from 0.1 to 72 μg/g Corg) is different to that of the other hopanoids and is only found in significant amounts in the 15–40 cm interval in Roñanzas profile (Figure 7 and Table 2). The distribution of this trisnorhopanone is also different to the other hopanoids for La Borbolla, Buelna and Pendueles profiles (Figure 8 and Table 3) and its concentration is lower than in Roñanzas profile (ranging from 1.1 to 17.1 μg/g Corg, from 0.2 to 2 μg/g Corg and from 0.1 to 7.4 μg/g Corg, respectively).

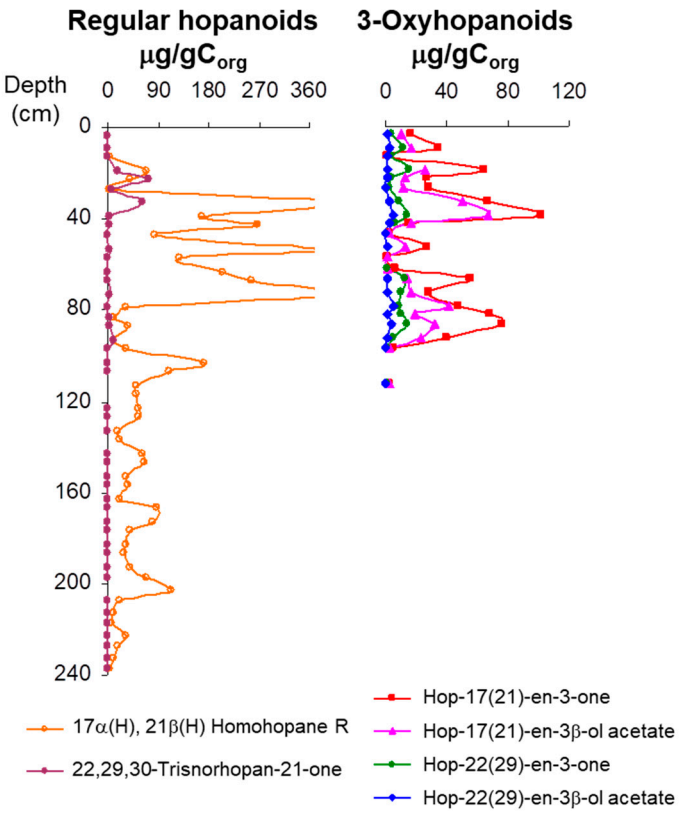

Figure 7. Variation of the concentration of regular and 3-oxyhopanoid derivatives in the Roñanzas profile (μg/g Corg).

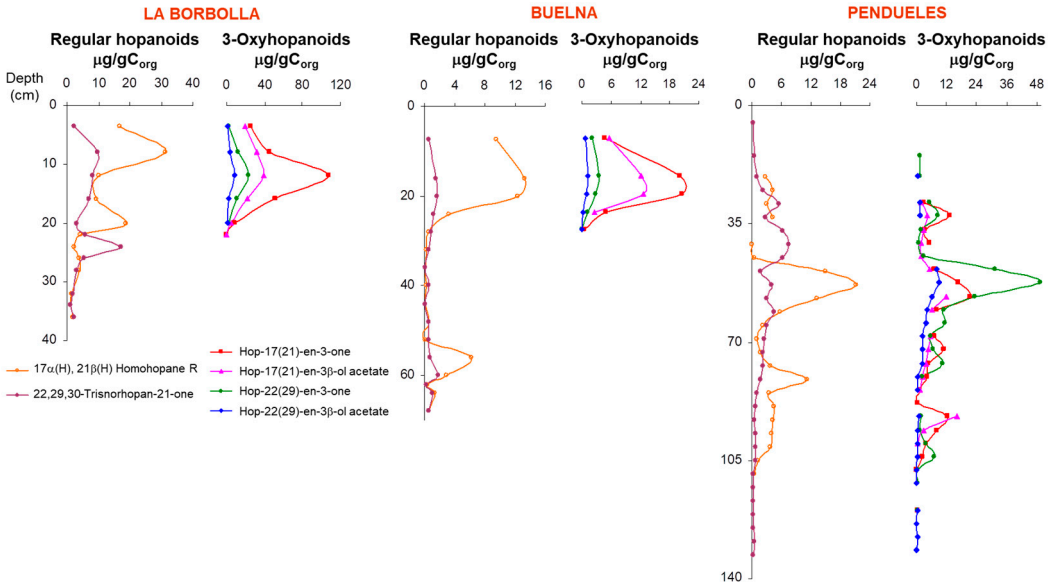

Figure 8. Variation of the concentration of regular and 3-oxyhopanoid derivatives in the Buelna, La Borbolla, and Pendueles profiles (µg/g Corg).

3.5. $\delta^{13}C$ Values of Specific Hydrocarbons

The stable carbon isotopic composition ($\delta^{13}C$) of triterpenoids can provide insights into the origin of these compounds. In modern peat-forming environments, the $\delta^{13}C$ values of extended regular hopanes are enriched by around 6‰ compared to long chain *n*-alkanes derived from higher plants [75,76]. As such, they have been attributed to heterotroph organisms, which were consuming ^{13}C-enriched carbohydrates [77]. This difference is greater than the one between cyclic triterpenoid compounds and linear *n*-alkane from the same source, which has been established on 2.5‰ in average [78]. Also records of lower $\delta^{13}C$ values in C_{27} to C_{30} hopanes/hopenes have been found in peat and attributed to mixed bacterial sources consuming both ^{13}C-enriched carbohydrates and ^{13}C-depleted, methane-derived CO_2 [79,80]. Therefore, the $\delta^{13}C$ signature will support either a bacterial or higher plant source for the studied triterpenoids.

Table 4 provides the average $\delta^{13}C$ values of some of the families of compounds in the different profiles. The $\delta^{13}C$ values of *n*-alkanes and *n*-alkan-2-ones are also shown as a reference, in addition to the triterpenoids discussed in this work. The values of the odd *n*-alkanes thought to derive from higher plants (*n*-C_{27}-*n*-C_{31}) or *Sphagnum* (*n*-C_{23} and *n*-C_{25}) are similar and the average values range from −32.6‰ to −33.4‰. These values are also similar to those reported for *n*-alkane in other peat extracts [76,81–83]. The $\delta^{13}C$ values of the lupanoid, ursanoid, oleanoid, and friedelinoid compounds range between −29.5‰ and −31.9‰ (Table 4) with average values of −30.6‰ and with lupanoids presenting the most enriched values.

Table 4. Average $\delta^{13}C$ values (‰) of some compound families in the different peat profiles with the corresponding standard deviations (σ).

Title	Roñanzas	Pendueles	Buelna	La Borbolla
n-Alkanes	−32.9	−33.0	−33.4	−32.6
σ *n*-Alkanes	0.5	0.6	1.0	0.5
n-Alkan-2-ones	−29.5	−30.3	−31.5	−31.2
σ *n*-Alkan-2-ones	0.4	0.6	0.6	0.3
Hopanoids	−25.9	−27.2	−27.9	−27.5
σ Hopanoids	0.5	1.1	1.0	0.4
Higher Plants Triterpenoids	−30.7	−31.3	-32.2	−32.3
σ Higher Plants Triterpenoids	2.4	1.2	1.0	0.8

In the present study, the scarce data available for acetates and their corresponding ketones indicate similar $\delta\ ^{13}C$ values for both types of compound (Table 5). The D:C Friedours 7 en 3 ol acetate and its isomer show $\delta\ ^{13}C$ depleted values (−32.4‰) compared to those of other higher plant triterpenoids and they are similar in all the five samples analyzed.

As expected, enriched $\delta\ ^{13}C$ values were obtained for hopanoids (−25.9‰ to −28.4‰) compared with higher plant triterpenoids (Table 4). They are also more enriched than those of *n*-alkan-2-ones (−29.5‰ to −31.2‰), for which a bacterial origin has been suggested by López-Días et al. [82] on the basis of the difference between the $\delta\ ^{13}C$ values of *n*-alkane and *n*-alkan-2-ones. In addition, the values are similar for C_{30} hopanoids with an oxygenated functionality in position C-3 and regular extended hopanoids (Table 5), and also similar to those previously reported for microorganism-derived compounds in soils [84,85]. The enriched $\delta\ ^{13}C$ values of the hopane derivatives compared to those of the higher plant triterpenoids support their microbial source, even if they have an oxygenated functionality in position C-3. Other potential sources for hopanoids, such as methanotrophs, which have been described as *Sphagnum* symbionts, are not supported by the enriched $\delta\ ^{13}C$ signature [79,86].

Table 5. δ ^{13}C values (‰) of some higher plant triterpenoids and hopanoid derivatives in the peat samples (R = Roñanzas; PE = Pendueles; B = La Borbolla; and BU = Buelna).

Compound	R33	R93	R123	R127	R163	R203	R233	PE25	PE53	PE77	PE105	PE121	B216	B228	BU120	BU132	BU160
Olean-12-en-3β-ol acetate					−30.6												
Urs-12-en-3β-ol acetate												−30.8					
Lup-20(29)-en-3β-ol acetate					−30.4												
Lupan-3-one			−29.8		−30.0	−30.3				−29.8	−29.6						−30.6
Friedelinol					−30.9												
Friedelin			−32.1	−31.8	−31.5						−30.9				−31.9		
Friedelinol acetate				−29.5	−30.1												
Bauer-7-en-3-one														−31.4			
Bauer-7-en-3β-ol acetate							−32.3					−32.2	−33.1			−32.6	−33.3
Bauerenol acetate isomer							−32.9					−31.5				−32.5	
β C_{27} Hopane									−28.1	−27.9				−28.0	−27.6	−26.3	
αβ C_{31} Hopane	−25.9	−25.4	−24.9	−26.6	−26.2	−25.7	−26.0	−25.8	−27.9	−28.4	−26.3		−27.2	−27.4	−28.3		
ββ C_{31} Hopane	−26.3	−26.0	−25.3			−26.3											
22,29,30-Trisnorhopan-21-one	−27.8													−27.1			
Hop-22(29)-ene								−26.1									
Hop-22(29)-en-3-one									−26.4	−26.2	−27.7				−27.4		
Hop-17(21)-en-3β-ol acetate		−26.5											−27.3				

4. Discussion: Relative Abundance of Acetate vs. Ketone Triterpenoids

Acetyl derivatives of molecular markers are rarely reported in sediments. In this section, the records of these occurrences will be reviewed highlighting the similarities or differences with the peatlands studied in this work. Of the few recorded cases of the presence of triterpenyl acetates in sediments, the study of different soils of the Vosges Massif in France [87] deserves special mention for the similarity of climate conditions in the area. In that case, the presence of naturally occurring 3β-friedelanyl acetate has been reported to be apparently restricted to grasslands. Decayed sycamore leaves [88] and senescent *Acer* leaves [89] have been considered the source of β-amyrin acetate in Rothamsted Station. A greater variety of triterpenyl acetates has been reported in the catchment area of the Lake Ayat in the French Massif Central. From these compounds, C3-acetates with bauerane, taraxastane, swertane, and pichierane skeletons have been unequivocally associated to the *Asteraceae* family, while other triterpenyl acetates have been considered to be less specific [31]. A recent study of peats in central France has also reported a variety of triterpenyl acetates [26]. Olean-12-en-3β-yl acetate, D:C-friedo-olean-7-en-3β-yl Acetate, Urs-12-en-3β-yl acetate, and D:A-friedo-olean-3β-yl acetate and their corresponding ketones are also prominent compounds in this work. The simultaneous detection of some of the acetates in *Calluna vulgaris* and in its peat and their absence in other peat-forming plants led the authors of the French study to consider some triterpenyl acetates as markers for *C. vulgaris*. The amounts reported for the French soil were significantly lower than the ones observed in the Roñanzas profile. Pentacyclic triterpenol derivatives (*α*- and *β*-amyrinyl and lupenyl acetates) have also been detected in in the sediments of the Cross-River system in Nigeria. In this case they were mainly considered as markers for higher plants that have been preserved due to favorable conditions [90]. Nevertheless, the authors also pointed out that acetyl derivatives may be more frequent than has been reported for recent sediments, being masked by the derivatization procedure used for the analysis of acids. Triterpenones can be synthesized by plants and then form saturated ketones via biological or biochemical oxidation [47], but generally they are considered to derive from oxidation of their alcohol counterparts [91].

The extraction of Ericaceae fresh peat-forming plants in this study indicated that *C. vulgaris* contained as major compounds the alcohol derivatives α-amyrin and β-amyrin, that the ketones were absent and that their acetates were present in amounts 6 to 12 times lower than those of the alcohols (Table 1). A similar situation is observed in the case of *Erica mackaiana*, where the major triterpenoids were the alcohols (α-amyrin, lupeol, and β-amyrin), while their acetates were present in amounts that were between 50 and 100 times lower. Low concentrations of hop-22(29)-en-3β-ol were present in both species. The absence of alcohol derivatives in the peat (found only in the uppermost samples of some profiles) and the high amounts of the ketone and acetates led us to postulate that they were probably formed from alcohols present in the sediments. A common source for both derivatives would be also supported by their similar $\delta\ ^{13}C$ values. The formation of one or another could then depend on the availability of acetate and/or the redox conditions.

The concentration of triterpenoid acetates in the studied profiles is significant (Tables 2 and 3) and in many cases, it is within the range of *n*-alkane and *n*-alkan-2-one concentrations in the same extracts [82]. A simple plot of the acetate/ketone ratio for each family of compounds allows the predominance of one or the other derivative for each compound throughout the profiles to be appreciated (Figure 9). In most of the cases, the acetate/ketone ratios for the 3-oxyhopanoids are below one, indicating the predominance of ketone. In addition, in all the profiles the ratios for the -17(21)- pair are greater than for the -22(29)- one, suggesting preference for the formation of the hop-17(21)-en-3β-ol acetate. This is not necessarily related to the relative abundance of the configurations -17(21)- and -22(29)- in the profiles because it applies to profiles where the -17(21)- configuration is dominant (Roñanzas, Buelna and La Borbolla) and to the Pendueles profile where the -22(29)- configuration is dominant. The 3-oxyhopanoids tend to concentrate in the upper part of the profiles and therefore, their acetates were also concentrated in the same area.

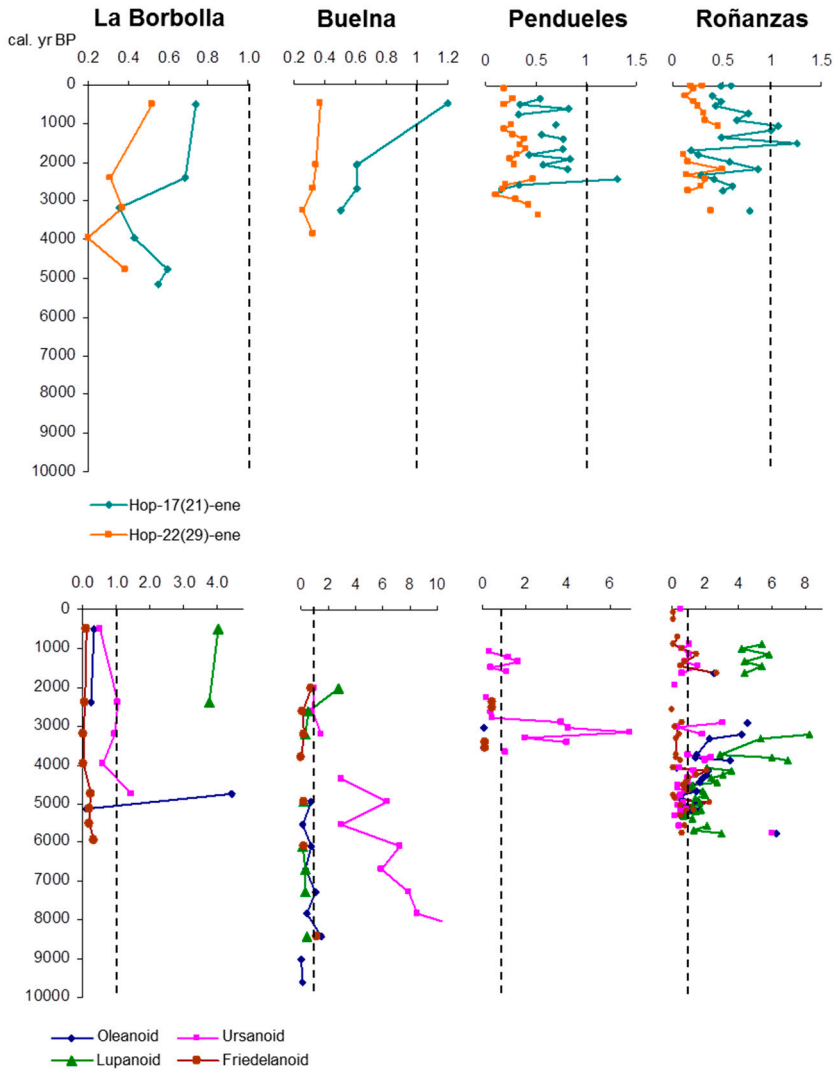

Figure 9. Variation of the acetate/ketone ratio with age for each family of compounds in the studied peat profiles.

In the case of higher plant triterpenoids, a different behavior is observed between friedelanoid derivatives on the one hand and oleananoid, ursanoid, and lupanoid derivatives on the other. The acetate/ketone ratio is <1 for the friedelanoids in most of the profiles, although the ratio increases towards the bottom. The acetate/ketone ratios are well over one for ursanoids in the lower part of the Buelna, Pendueles, and Roñanzas profiles. This ratio is also high for oleanoids in Roñanzas, whereas it is close to 1 in Buelna. In the case of lupanoids, the acetate/ketone ratio may have a different significance because it was calculated with the saturated ketone instead of the unsaturated one, which was not quantifiable due to co-elution in most of the cases. The high acetate/ketone ratios for lupanoids in the Roñanzas profile, and also at few points in La Borbolla indicate, in any case, the formation of a significant amount of lupenyl acetate derivatives. The fact that acetyl derivatives are found in large amounts in the total extracts and that they are not restricted to a single family of compounds but involve triterpenoids from different sources suggests that the formation of these compounds might be environmentally related.

The environmental reason could be the presence of acetate in peats, which in turn may reflect the dominance of hydrogenotrophic methanogenesis in which CO_2 is reduced by molecular hydrogen over acetoclastic methanogenesis, where acetate cleavage occurs. The complex process involved in the production of CH_4 requires the breakdown of recalcitrant organic carbon into simpler substrates to be used by methanogens. As the accumulation of humus in litter and soils is favored in colder

regions such as the boreal forests and peatlands and also the anaerobic degradation by fermenting bacteria [92], the end products of these processes (acetate, CO_2 and H_2) tend to accumulate at high latitudes, in cold climates, in acidic conditions, in *Sphagnum*-dominated peats, and preferentially in the deeper peat layers [11,13,15]. This is nevertheless not exclusive of these settings because it has also been reported in some subtropical sediments of Florida Everglades soils [93].

Triterpenyl acetates can be appreciated throughout all the profiles (Tables 2 and 3 and Figure 9), although they are generally more abundant in the lower part of the profiles (Table 2). A similar result to that of Cadillo-Quiroz et al. [94], who found that although hydrogenotrophic methanogenesis can predominate in a bulk peat bog, in the upper part of the profile, with high amounts of roots, acetoclasctic methanogenesis has significantly higher contribution, lowering the availability of acetate and the possibility of acetate derivatives formation.

5. Conclusions

A high abundance of acetylated derivatives of pentacyclic triterpenols/hopenols originating from different precursors (higher plant and bacterial origin) was detected in *Sphagnum*-rich bogs of the north of Spain. The similar distribution of the ketone and acetate derivatives of most of the triterpenoid families in the profiles and their similar $\delta\ ^{13}C$ values of acetate/ketone pair suggests that both derivatives have a common source. Although a direct input from plants cannot be completely ruled out for the origin of acetyl-derivatives of triterpenoids in these peats, their similar distribution for various compounds and the fact it affects compounds from different sources points to a widespread process for acetyl-derivatives formation. The predominance of acetyl- or keto-triterpenoids might be related to the excess amount of acetic acid in the medium, and the relative stability of the functionality. An excess of acetic acid in the environment indicates the predominance of either hydrogenotrophic methanogenesis or homoacetogenesis over acetoclastic methanogenesis, extending the predominance of this process to acidic *Sphagnum*-rich peats in temperate climatic regions. A specific plant input is only required for bauerenol acetate, for which the Asteraceae family has been suggested in the literature.

The existence of an oxygenated functionality at C-3 apparently favors the formation of acetyl derivatives, because all the triterpenyls identified have the acetyl group in this position. Among the higher plant triterpenoids, the formation of acetates appears to be favored in the case of ursanoids and lupanoids. Within the 3-oxyhopanoid group, the hopenyls with a -17(21)- configuration, typical of more acidic environments, were generally more abundant than those with a -22(29)- configuration.

The 3-oxyhopanoids, which were very abundant in the upper part of the profiles, have $\delta\ ^{13}C$ values similar to those of regular hopanoids ($C_{31}\alpha\beta R$) of unequivocally prokaryotic origin. Both are enriched by around 4‰ compared to the $\delta\ ^{13}C$ values of the lupane, ursane, and oleanane derivatives. This fact leaves open the question of a prokaryotic origin also for the 3-oxyhopanoids present in these profiles.

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