



Photochemical alteration of 3-oxygenated triterpenoids: Implications for the origin of 3,4-*seco*-triterpenoids in sediments

Bernd R.T. Simoneit^{a,b}, Yunping Xu^{b,1}, Renato R. Neto^{b,2}, Joshua B. Cloutier^b, Rudolf Jaffé^{b,*}

^a Department of Chemistry, Oregon State University, Corvallis, OR 97331, USA

^b Southeast Environmental Research Center, Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA

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ABSTRACT

The reactivity of higher plant derived 3-oxy-triterpenoids to sunlight was investigated using a series of pure reference standards both under simulated and real solar exposure. The majority of the exposed compounds showed reactivity to light, particularly to simulated sunlight and among others generated *seco*-derivatives. While photochemical processes have been suggested for the formation of such compounds, their abundances in some sediments have often been assumed to be the result of diagenetic reworking of parent triterpenoids. Analyses of mangrove leaf waxes, an important known source of taraxerol in coastal ecosystems, showed the presence of the 3,4-*seco*-derivative dihydrolacunolic acid, which could represent an important biotic source for des-A-triterpenoid precursors to such sediments, and is unrelated to aquatic organic matter diagenesis.

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1. Introduction

Many 3,4-*seco*-triterpenoidal acids have been characterized as natural or secondary products from epicuticular waxes of higher plants. For example, canaric acid (I), the structures of all numbered compounds are shown in Appendix A) from *Canarium muelleri* or *Canarium zeylanicum* (Carman and Cowley, 1964, 1965; Ekong and Okogun, 1969; Bandaranayake, 1980), nyctanthic acid (II) from *Nyctanthus arbortristis* (Arigoni et al., 1960; Witham, 1960), roburic acid (III) from gall wax of *Quercus robur* (Mangoni and Belardini, 1963; Mangoni and Belardini 1964), methyl dihydronyctanthate (IV, R = CH₃) from *Hoya* species (Baas, 1982, 1983, 1991; Baas et al., 1992), or dammarenic acid (V) from *Shorea* species (Arigoni et al., 1960; Bisset et al., 1971) were identified initially. The current list of 3,4-*seco*-triterpenoids comprises acids, aldehydes, alcohols and hydrocarbons, with some compounds glycosylated, and also less commonly some 2,3-*seco*-triterpenes (Baas, 1985). However, the predominant naturally occurring *seco*-triterpenoids are 3,4-*seco*-3-acid methyl esters or their free acids with a double bond

at C-4(23). The botanical interpretation of the utility of these compounds is attributed to their bitter taste and metabolic effects as a defense against predation and pathogenic microorganisms (Baas, 1985). These natural products have generally not been reported in sediments, but their analogues with the C-4(23) double bond hydrogenated are common (Trendel et al., 1989; Huang et al., 1996; Yanes et al., 2006).

The *seco*-triterpenes reported from sediments are all 3,4-*seco*-3-acids or methyl esters and derivatives. Some examples are dihydrocanaric acid (VI, 3,4-*seco*-lup-29-en-3-oic acid), tetrahydrocanaric acid (VII, 3,4-*seco*-lupan-3-oic acid), dihydronyctanthic acid (IV, R = H, 3,4-*seco*-olean-12-en-3-oic acid), dihydroroburic acid (VIII, 3,4-*seco*-urs-12-en-3-oic acid), dihydroputranjivic acid (IX, 3,4-*seco*-friedelan-3-oic acid), with minor amounts of the corresponding A-nor compounds (Corbet et al., 1980; Hoffmann et al., 1984; Trendel et al., 1989; Huang et al., 1996; Yanes et al., 2006). The 3,4-*seco*-3-acids form by photolysis in solution under UV as the acids and methyl esters (Corbet et al., 1980, and references therein). In addition, numerous des-A-triterpene hydrocarbons have been characterized and range from the fully hydrogenated tetracyclanes to the triaromatic derivatives (e.g., des-A-lupane, X vs. 1-methyl-7,8-(isopropylcyclopenta)chrysene, XI) (Trendel et al., 1989). It has been shown that minor amounts of these des-A-triterpenes also form by photolysis under UV from the triterpen-3-ones by losses of dimethylketene and ethylene, although at low efficiency (Corbet et al., 1980).

* Corresponding author. Tel.: +1 305 348 24 56; fax: +1 305 348 40 96.

E-mail address: jaffer@fiu.edu (R. Jaffé).

¹ Present address: Department of Physical and Environmental Science, University of Toronto at Scarborough, 1265 Military Trail, Toronto, Ontario, Canada M1C 1A4.

² Present address: Departamento de Ecologia e Recursos Naturais, Universidade Federal de Espirito Santo, Av. Fernando Ferrari 514, Vitória, ES 29075-910, Brazil.

Des-A-triterpenoids have also been suggested to be formed through diagenetic transformations of their parent triterpenoids (Corbet et al., 1980; Jaffé and Hausmann, 1995; Jaffé et al., 1996, 2006; Yanes et al., 2006). Here it is assumed that oxidation of the oxygenated functional group in position 3 to the corresponding *seco*-acid is favored under reducing environmental conditions (Trendel, 1985). As a result, tetracyclic, des-A degradation products of the pentacyclic 3-oxy-triterpenoids are often observed in anoxic sediments (Jaffé and Hausmann, 1995) or in seasonally flooded organic-rich soils (Jaffé et al., 1996). Thus, a combination of photochemical reactions and diagenetic transformations of higher plant triterpenoids may generate *seco*-acids and their derivatives in natural environments.

Here we reexamine the photochemistry of triterpen-3-ols and triterpen-3-ones under direct and simulated sunlight to compare the products with those observed in certain tropical surface sediments. We will also assess the presence or formation of photoproducts directly on plant leaves as part of their epicuticular waxes to compare with those found in tropical sediments receiving such a detrital input.

2. Experimental methods

2.1. Sediment samples

Lake Leopoldo is located on the summit of the Cuao-Sipapo Massif (latitude 800 m a.s.l.) in SW Amazonas, Venezuela (Yanes et al., 2006). It is highly oligotrophic and the sediments derive from a small drainage basin of weathering sandstones and higher plant detritus. A sediment sub-sample collected at 20 m depth was used for biomarker analysis.

The Shark River and Slough and the Taylor Slough are the natural sheet flow drainage systems of Everglades National Park to the Gulf of Mexico and Florida Bay, respectively. They are highly oligotrophic ecosystems exposed to alternating wet and dry seasons, with unique flora and fauna (Jaffé et al., 2001). The study region where the sediment samples were collected is dominated by mangrove forests (Jaffé et al., 2006). Samples used in this study were collected at ca. 16 m and 1 m depths using an Eckman Dredge (Wildco, Michigan) for the Shark and Taylor rivers respectively. All sediment samples were placed in clean glass jars covered with Teflon lids and kept frozen at -20°C until analyzed.

2.2. Solar irradiation

Known concentrations of standard triterpenol and triterpenone solutions (see Table 1, only results for triterpenones are listed) were spiked onto glass fiber filters and the solvent (dichloromethane) allowed to evaporate. Filters were exposed to direct sunlight for 14 days (at Oregon State University; α -amyronone only) and to simulated sunlight in the laboratory for a period of 7 days (cumulative light exposure). The simulated solar irradiation was carried out with a Suntest XLS⁺ solar simulator (Atlas Material Testing Technology LLC, Chicago, IL) set at 765 Wm^{-2} (approximately solar noon sun at mid latitude) and 20°C .

2.3. Mangrove leaf waxes

Mangroves identified as red mangrove (*Rhizophora mangle*), the dominant species in the estuaries of the Florida Everglades, were sampled to obtain leaves representative of live (green) and senescent (yellow) status. Both sets were taken as growth in full exposure to solar radiation and primarily shaded. The samples were taken to the laboratory for extractions of both bulk leaves (dried and powdered) and surficial (epicuticular) wax.

Table 1

Products from solar photolysis of standard triterpenoids

Precursor/products	Composition	Structure (cf. Appendix A)	Concentration (relative%)
<i>Friedelin</i>	$\text{C}_{30}\text{H}_{50}\text{O}$	XIII	76
4-Epi-friedelin	$\text{C}_{30}\text{H}_{50}\text{O}$		3.8
Methyl dihydroputranjivate	$\text{C}_{31}\text{H}_{54}\text{O}_2$	IX, R = CH_3	11
3-Nor-4 β -friedelane	$\text{C}_{29}\text{H}_{50}$	XV	8.8
Conversion (%)			24
<i>Lupenone</i>	$\text{C}_{30}\text{H}_{48}\text{O}$	XII	4.5
Methyl dihydrocanarate	$\text{C}_{31}\text{H}_{52}\text{O}_2$	VI, R = CH_3	90
Methyl canarate	$\text{C}_{31}\text{H}_{50}\text{O}_2$	I, R = CH_3	5.5
Conversion (%)			96
α -Amyronone ^a	$\text{C}_{30}\text{H}_{48}\text{O}$	XIV	21 (66)
Methyl dihydroborurate	$\text{C}_{31}\text{H}_{52}\text{O}_2$	VIII, R = CH_3	79 (1)
Ursa-9(11),12-dien-3-one	$\text{C}_{30}\text{H}_{46}\text{O}$		-(7)
Ursa-9(11),12-dien-3-on-11-ol	$\text{C}_{30}\text{H}_{46}\text{O}_2$		-(11)
Urs-12-ene-3,11-dione	$\text{C}_{30}\text{H}_{46}\text{O}_2$		-(4)
Urs-12-en-3-on-11-ol	$\text{C}_{30}\text{H}_{48}\text{O}_2$		-(6)
Ursa-9(11),12-dien-3 β -ol	$\text{C}_{30}\text{H}_{48}\text{O}$		-(5)
Conversion/oxidation (%)			79 (1/33)

^a Data are for exposure of 7 days in simulator and in parentheses to direct sunlight (Oregon) for 14 days.

2.4. Sample extraction

Sediments (typically 5 g dry) were extracted three times with dichloromethane/methanol (3:1, vv, Optima Fisher, USA) under ultrasonication, aliquots combined, filtered and concentrated to about 1 mL. Filters were extracted under ultrasonication three times with dichloromethane, and extracts combined, filtered and concentrated. The leaves from the red mangroves were air dried and one set was dipped (about 3 s each) into the same dichloromethane/methanol mixture as above to dissolve the surface epicuticular wax. Another dried leaf set was powdered and extracted by ultrasonication with the same solvent mixture. This method does not dissolve interior lipids as is evident by the absence of saccharides in the extracts. All extracts were filtered and concentrated. Aliquots of the solvent extracts were derivatized with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) prior to instrumental analysis. Typically 20 μL aliquots of the respective extracts or fractions were treated with 20 μL BSTFA containing 1% trimethylchlorosilane and 5 μL pyridine for 3 h at 70°C . This mixture was evaporated and the products dissolved in hexane prior to instrumental analysis. Some of these extracts were also fractionated as described below (see also Jaffé et al., 2001).

2.5. Extract fractionation

Sub-samples of sediments were extracted for 24 h in a Soxhlet apparatus with dichloromethane (Optima Fisher, USA), in contact with activated copper to remove elemental S. Total extracts were saponified with 1 N KOH to isolate the acid and neutral lipid fractions. The neutral fraction (organic phase) was fractionated by adsorption chromatography over silica gel into aliphatic hydrocarbons, aromatic hydrocarbons, ketones, alcohols and sterols according to a method described by Wakeham et al. (1984). The acid fraction (aqueous phase) was acidified with HCl to a pH of 1–2 and extracted with dichloromethane to obtain the organic acids, which were transformed into their methyl esters by the addition of freshly distilled diazomethane. The alcohol and sterol fractions were also derivatized with BSTFA prior to GC–MS analysis (Jaffé et al., 2001). Blanks were run throughout the procedure and no significant contamination was observed. Lipid recoveries were better than 85% and detection limits were at $0.02\text{ }\mu\text{g g}^{-1}$.

2.6. Instrumental analyses

Sample extracts and fractions were analyzed directly and as the silylated mixtures by gas chromatography–mass spectrometry (GC–MS). The instrument was a Hewlett–Packard (Model HP6890) gas chromatograph interfaced with a Model HP5973 MSD and equipped with a Grob injector operated in the pulsed splitless mode at 1.7 bar until 1.0 min. The injector temperature was set to 300 °C. A DB5-MS capillary column (30 m × 0.25 mm i.d. and film thickness of 0.25 μm, Agilent) was used with the column temperature program consisting of injection at 60 °C, held for 2 min, temperature increase of 6 °C min⁻¹ to 300 °C, followed by an isothermal period for 15 min. The MSD was operated in the electron impact mode with an ionization energy of 70 eV. The scan range was set from 50 to 650 Da and 1.27 scan s⁻¹.

Data were acquired and processed with the HP-Chemstation software. Individual compounds were identified by comparison of mass spectra with literature and library data, comparison of mass spectra and GC retention times with those authentic standards and/or interpretation of mass spectrometric fragmentation patterns. Relative response factors were determined and identifiable compounds quantified as relative concentrations using the MS-data system.

3. Results and discussion

The data from the sunlight exposure experiments will be discussed first, and then coupled with the results for the mangrove wax analyses. The examples of sediment extracts are presented last to ascertain the efficacy of photochemical biomarker alteration.

3.1. Triterpenoid exposure to solar radiation

Numerous reports describe the products formed by ultraviolet irradiation of triterpenoids and the highest yields are derived from the C-3 ketones (e.g., Tsuyuki et al., 1968; Kohen et al., 1969; Shirasaki et al., 1975). However, previous irradiation experiments were carried out with standard compounds in organic solvent solutions exposed to ultraviolet light (e.g., Turro, 1965; Kan, 1966). We carried out experiments where solutions of standard triterpenols and triterpenones were evaporated on silica surfaces (i.e., glass fiber filter matrix) and then irradiated for various times with simulated and direct sunlight. The purpose of this procedure is to closer replicate the exposure of triterpenoids on mineral/organic particles to sunlight during sediment transport in river systems.

The results from the irradiation experiments are given in Table 1 and typical examples summarized in Fig. 1. The triterpenols, as expected, did not react within the the time period of exposure to simulated sunlight. All triterpen-3-ones generated photoproducts but with differing yields, as for example, lupenone (XII) has a maximal 96% conversion to *seco*-products, friedelin (XIII) 24%, and α-amyronone (XIV) 79% in the solar simulator, but only 1% in direct sunlight (Oregon) with 33% other oxidation products (Table 1). The dominant product from lupenone is methyl dihydrocanarate (VI, methyl ester) with a minor amount of methyl canarate (I, methyl ester) (Fig. 1a). No des-A-lupane hydrocarbons were detectable. Friedelin converts mainly to dihydroputranjivic acid (IX), its methyl ester, and 3-nor-4β-friedelane (XV) (Fig. 1b). The products from friedelin are the same as reported earlier (Kohen et al., 1969). It should be noted that the chemistry of β-amyronone is the same as α-amyronone. β-Amyronone is not available commercially, so we used the α-isomer for the simulation experiment. Also, there is no commercial source for taraxerone, thus it could not be tested in the solar simulator to confirm the inferred dihydrolacunosic acid.

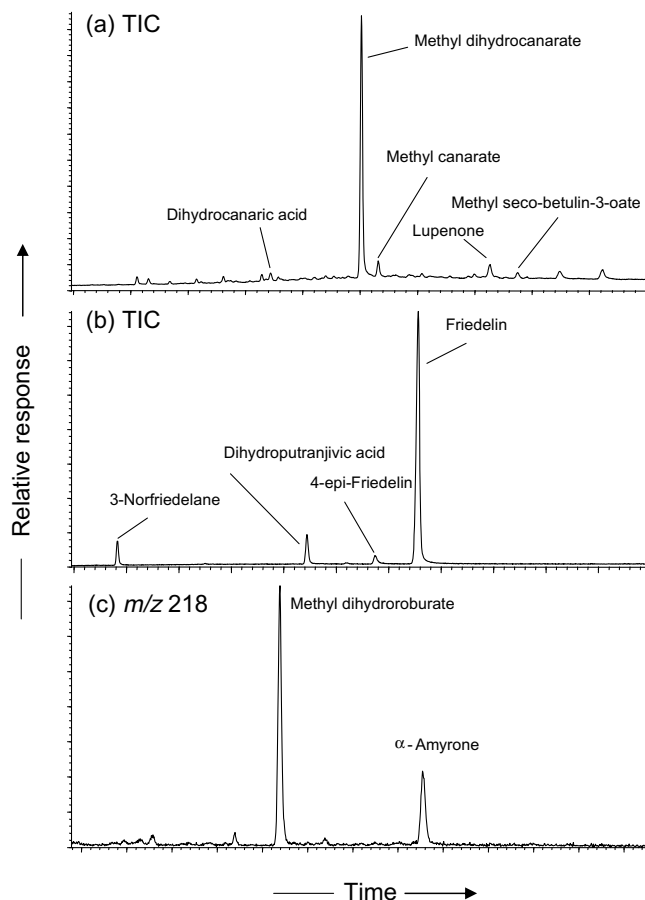


Fig. 1. Salient features of the GC–MS data for the solar exposure experiments with standards: (a) total ion current (TIC) trace for lupenone products, (b) TIC trace for friedelin products (as TMS), and (c) TIC trace for α-amyronone products.

3.2. Mangrove leaf wax

The extracts from mangrove leaves and of surface epicuticular wax taken in both sun-exposed and shaded, under dense foliage, locations, and either senescent (yellow) or live (green), all contain *seco*-derivatives of triterpenones. The results of the GC–MS analyses of both extract sets are summarized in Table 2. Dihydrolacunosic acid, both as the methyl ester and free compound (XVI, Setzer et al., 2000), was the major *seco*-derivative derived from taraxerone. This is the first report of dihydrolacunosic acid from mangroves and it was previously found in sediments from a mangrove-fringed river (Harney River, Everglades, Jaffé et al., 2006). Lacunosic acid (XVII) and traces of canaric acid (I) were also present in the sun-exposed leaf wax extract. Germanicol and β-amyrin are present in the waxes, but germanicone and β-amyronone were not detected. However, their derivatives 3,4-*seco*-germanic-3-oic acid (XVIII) and dihyroncyanthanic acid (IV, R = H) are minor coeluting components as the methyl esters. No des-A-triterpenoid hydrocarbons were detectable in any leaf wax mixture. The amounts of *seco*-products are greater in the wax from the senescent, sun-exposed than the generally shaded leaves, whereas the opposite is the case for the green leaves (see Table 2). This supports in part the enhancement of the photochemical reaction by sunlight in the environmental alteration of triterpenones to the *seco*-derivatives. Photochemical alteration is strengthened further by the cumulative increase in the *seco*-derivative concentration with time, where the highest values are observed for the wax from sun-exposed senescent (i.e., old) leaves (91 μg g⁻¹, Table 2).

Table 2
Triterpenoids in extracts of mangrove leaves and epicuticular wax ($\mu\text{g g}^{-1}$ dry weight)

Compound	Composition	Green leaves ^a		Senescent leaves ^a	
		Sun-exposed	Shaded	Sun-exposed	Shaded
Taraxerol	C ₃₀ H ₅₀ O	1481(28)	1767(50)	2632(70)	2375(52)
Iso-ursenol	C ₃₀ H ₅₀ O	1	2	7	3
β -Amyrin	C ₃₀ H ₅₀ O	273(3)	976(15)	1664(18)	1741(12)
Lupeol	C ₃₀ H ₅₀ O	521(4)	976(11)	2359(28)	2268(15)
Germanicol	C ₃₀ H ₅₀ O	84(0.7)	354(6)	544(9)	544(5)
Taraxerone	C ₃₀ H ₄₈ O	8(1)	19	20(2)	24(1)
Taraxera-2,14-diene	C ₃₀ H ₄₈	6	11(1.2)	5(1.8)	12(2.4)
Taraxeryl acetate	C ₃₂ H ₅₂ O ₂	3	9	14(3.3)	16(3.4)
Methyl dihydrolacunosate (XVI, R = CH ₃)	C ₃₁ H ₅₂ O ₂	7(12)	40(30.8)	54(90.4)	27(58)
Methyl 3,4- <i>seco</i> -germanican-3-oate (XVIII, R = CH ₃)	C ₃₁ H ₅₂ O ₂	0.4(0.3)	1(3)	2(6.4)	1(3)
Methyl dihydronyctanthate (IV, R = CH ₃)	C ₃₁ H ₅₂ O ₂	1(0.05)	2(5)	9(18)	4(9)
Methyl lacunosate (XVII, R = CH ₃)	C ₃₁ H ₅₀ O ₂			20	1
Methyl nyctanthate (II, Me ester)	C ₃₁ H ₅₀ O ₂			2	
Methyl canarate (I, R = CH ₃)	C ₃₁ H ₅₀ O ₂		1	2	1
Methyl 3,4- <i>seco</i> -betulin-3-oate	C ₃₁ H ₅₂ O ₃		3	4	2
Total precursors		2367	4094	7226	6955
Total <i>seco</i> -compounds		8	47	91	35
Relative proportions (%)		0.3	1.2	1.3	0.5

^a Values are for total leaf extracts and those in parentheses for surface wax wash.

Baas (1985) proposed that 3,4-*seco*-triterpenoic acids with a saturated C-4(23) bond occurring in leaves were of a biosynthetic and not photochemical origin, because photochemical alteration of 3-oxy-triterpenoids in oxic conditions would form 3,4-*seco*-triterpenoic acids with an isopropenyl or ethenyl group at C-5. Here, the photochemical alteration of 3-oxy-triterpenoids adsorbed on a glass fiber matrix under atmospheric conditions yielded 3,4-*seco*-triterpenoic acids with a saturated C-4(23) bond and only a minor amount of C-4(23) unsaturated *seco*-acids normally expected when irradiation is performed under exposure to oxygen. This suggests that diffusion of oxygen is limited in the triterpenones crystallized on the glass fiber surfaces, although some oxidation products were formed. A similar phenomenon might occur to triterpenoids within epicuticular wax exposed to sunlight. The wax lipids might thus protect triterpenoids from oxygen and enhance the formation of C-4(23) saturated 3,4-*seco*-triterpenoids. Therefore, the dihydrolacunosic acid in the mangrove wax is of a predominantly photochemical origin.

The relative proportions of the natural product precursors (triterpenols via triterpenones) to the photoproducts (*seco*-derivatives) vary from 0.34% to 1.26% for wax from live (green-shaded) to senescent (yellow, sun-exposed) leaves (Table 2). This indicates that the reaction *in situ* on the source vegetation is quite efficient and that such photoproducts may be a direct source of triterpenoid degradation products in sediments and soils.

3.3. *seco*-Triterpenoids in sediments

The *seco*-derivatives from α - and β -amyrone, lupenone, lupanone, friedelin and/or arborinone have been reported in sediments (e.g., Corbet et al., 1980; Jaffé and Hausmann, 1995; Jaffé et al., 1996; Yanes et al., 2006). Although, it has been shown that 3,4-*seco*-triterpenoids are direct biosynthetic and bacterial alteration products (Baas, 1985; Trendel, 1985), we will argue in favor of photochemical formation in this study. Baas (1985) proposed that the biosynthetic pathway produces 3,4-*seco*-triterpenoids with a saturated C-4(23) bond and photochemical alteration generates the same compounds with an unsaturated C-4(23) bond. Bacterial incubation of lupanone in an anoxic sediment formed tetrahydrocanaric acid (VII, 3,4-*seco*-lupan-3-oic acid, Trendel (1985)). Thus, bacterial alteration might occur in sedimentary environments to supplement photochemical formation of *seco*-compounds. Here, we compare the *seco*-derivatives from friedelin in sediments of

Lake Leopoldo, Venezuela, and from taraxerol (mangrove source) in the Everglades of Florida.

The physiography and general geochemistry of Lake Leopoldo and the Everglades have been described in detail earlier (Jaffé et al., 2001; Neto et al., 2006; Yanes et al., 2006). The salient features of the GC-MS data for the total extract and hydrocarbon, acid and alcohol fractions for Lake Leopoldo are shown in Fig. 2. The compound identifications and relative concentrations are given in Table 3, along with those of typical indicator compounds from plant wax and other lipids for reference. The predominant compound in the mixture is friedelin (Yanes et al., 2006), with significant amounts of *n*-alkanols, *n*-alkanoic acids and *n*-alkanes from plant waxes, friedelanol, β -amyrin, *epi*-friedelin, aromatic triterpenoid hydrocarbons and *seco*-derivatives from friedelin (Fig. 2a). An unknown compound tentatively identified as 3,4-*seco*-friedelanol (XIX) is the major derivative inferred from friedelin, with methyl dihydroputanjivite (IX, R = CH₃) and other known friedelin photoproducts as lesser components. The hydrocarbon fraction (Fig. 2b) contains friedel-2-ene, 3-nor-2,4-*seco*-friedelane (XX) and 4 α - and 4 β -(methyl)-3-norfriedelanes (XV). The presence of 3-nor-2,4-*seco*-friedelane and 4 α - and 4 β -(methyl)-3-norfriedelanes in the sediments and their production in the photodegradation of friedelin are strong supporting evidence that sedimentary dihydroputanjivic acid is also formed by photochemical processes. There is no evidence that those hydrocarbons form by biosynthetic or enzymatic reactions. The relative proportion of precursors to the *seco*-compounds is 28% for this locale (Table 3).

Dihydroputanjivic acid (IX) is the sole *seco*-product in the fatty acid fraction (Fig. 2c). The fatty acids from autochthonous microbiota and higher plant wax are dominant with minor amounts of $\beta\beta$ -bishomohopanoic acid and oleanolic acid confirming those sources. A significant component in the alcohol fraction is tentatively identified as 3,4-*seco*-friedelanol (XIX) (Fig. 2d). This is based on interpretation of the MS data and GC retention time. The mass spectrum of 3,4-*seco*-friedelanol-TMS (Fig. 3a) exhibits a M^+ at m/z 502 (C₃₃H₆₂OSi); $M-CH_3$ at m/z 487; $[M-(CH_3)_3SiOH]^+$ to m/z 412; $[M-(CH_3)_3SiOH-CH_3]^+$ to m/z 397; $[M-(CH_3)_3SiOH-CH_2CH_2]^+$ to m/z 383, which in turn rearranges with loss of C₁₁H₂₂ (B ring) to a hydrocarbon fragment at m/z 229 (C₁₇H₂₅, C-E ring); and $[M-C1-C3-OTMS]$ to m/z 373 (B-E ring fragment). Thus, we tentatively interpret this compound as *seco*-friedelanol and it could be derived from dihydroputanjivic acid by reductive diagenesis. 3,4-*seco*-friedelanol is not observed in the photolysis experiment of

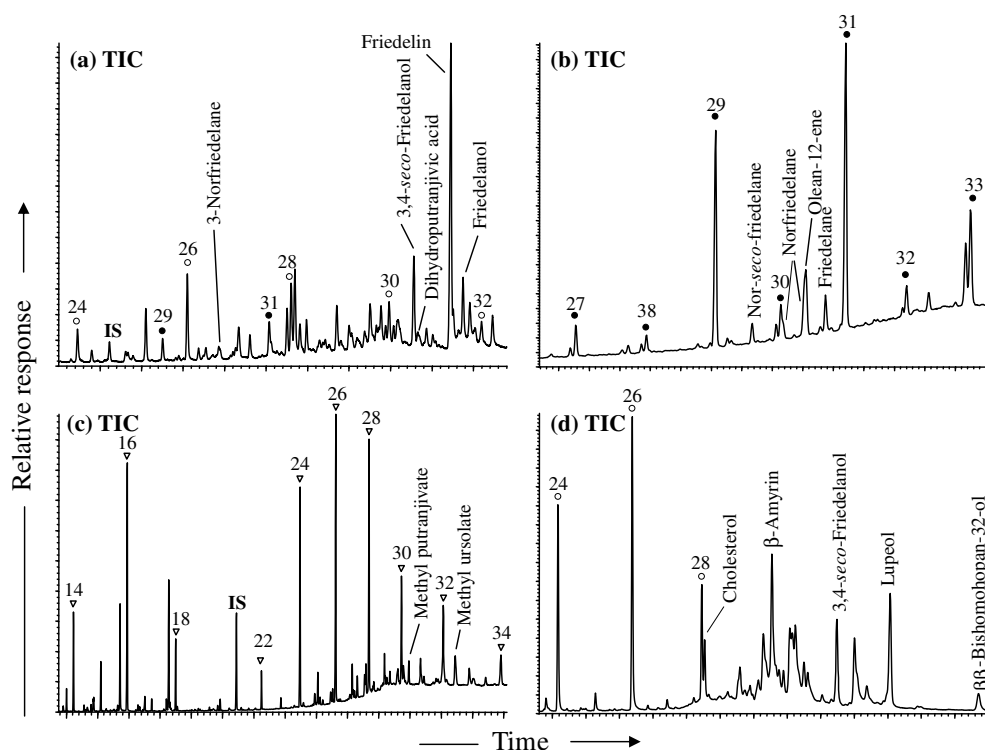


Fig. 2. Salient features of the GC-MS data for a sediment extract from Lake Leopoldo, Venezuela: (a) TIC trace for total extract (as TMS), (b) TIC trace of the hydrocarbons, (c) TIC trace of the carboxylic acids (as methyl esters), and (d) TIC trace of the alcohol fraction (as TMS). Numbers refer to the carbon chain length.

friedelin nor has it been reported in the early experiments (Kohen et al., 1969).

The Taylor Slough and Harney River in Everglades National Park have been described earlier as typical shallow water drainage systems of a semi-tropical environment (e.g., Jaffé et al., 2001; Neto et al., 2006). The fatty acid fraction of flocculent surface organic matter in Taylor Slough (Fig. 4a) has a significant amount of dihydrolacunonic acid (XVI, Fig. 3b as methyl ester) and minor dihydr-

onyctanthic acid (IV, R = H, Fig. 3c as methyl ester) compared to the autochthonous microbial fatty acids <C₂₀. This sample site is located at the mouth of the Taylor River to Florida Bay, and is influenced by marine derived organic matter in addition to organic matter from the adjacent fringe mangrove swamps (Jaffé et al., 2001; Xu et al., 2006). As such, and particularly during the dry season, floc at this location is expected to contain planktonic detritus, which consequently features the relative high abundances of shorter chain fatty acids (C₁₂–C₁₈) depicted in Fig. 4a. The presence of significant amounts of bishomo-hopanoic and 2-methylbishomohopanoic acids in this sample suggests an important bacterial input, possibly derived from cyanobacterial mats, which are abundant in the Bay (Xu et al., 2006). Furthermore, elevated levels of higher plant derived fatty acids (C₂₄–C₃₀), likely from a mangrove origin, are also present in the sample together with the taraxerol-derived *seco*-acid (XVI). The relative proportion of precursors to *seco*-products is 0.79% for this case (Table 3).

The total extract of sediments from the Harney River is dominated by taraxerol and *n*-alkanols (Fig. 4b). The *seco*-products from the triterpenones are trace constituents in the total mixture (Table 3) but are readily quantitated in the separated fractions. The relative proportion of precursors to the *seco*-products is 2.2% for this sample (Table 3). As mentioned above, taraxerol is a biomarker for mangroves in South Florida coastal environments (Jaffé et al., 2006; Neto et al., 2006; Xu et al., 2006) and was observed to be present at elevated concentrations in this sample. The presence of dihydrolacunonic acid (XVI) and minor amounts of dihyronyctanthic acid (IV, R = H) could result from a combination of mangrove leaf-derived photoproducts and diagenetic reworking of mangrove detritus. However, these *seco*-products were not reported in a selective microbial degradation study on mangrove derived triterpenols (Koch et al., 2005). The dihyronyctanthic acid (Table 3) is inferred to be derived from β -amyrin, because dihydroroburic acid (VIII, R = H) was observed as a

Table 3
Triterpenoids of some tropical sediments (normalized values)

Compound	Lake Leopoldo	Taylor Slough	Harney River
4 α -Friedelin (epi)	5		
4 β -Friedelin (XIII)	100		
Friedelanol	19		
Taraxerol		100	100
Taraxer-3-one		6	2
β -Amyrin	7	3	13
Lupeol	6		4
Myricadiol			7
Dihydroputranjivic acid (IX, R = H)	4		
Tentative 3,4- <i>seco</i> -friedelanol (XVI)			
(XII)	28		
Friedel-2-ene	1.5		
3-Nor-2,4- <i>seco</i> -friedelane (XX)	0.8		
3-Nor-4 α -friedelane	0.2		
3-Nor-4 β -friedelane (XV)	0.6		
Dihydrolacunonic acid (XVI, R = H)		0.84	2.1
Dihyronyctanthic acid (IV, R = H)		0.09	0.13
Relative proportions (%)	28	0.8	2.2
<i>Other reference lipids</i>			
<i>n</i> -Nonacosane	8	1.3	0.8
Methyl palmitate	20	15	19
<i>n</i> -Hexacosanol	31	10	6

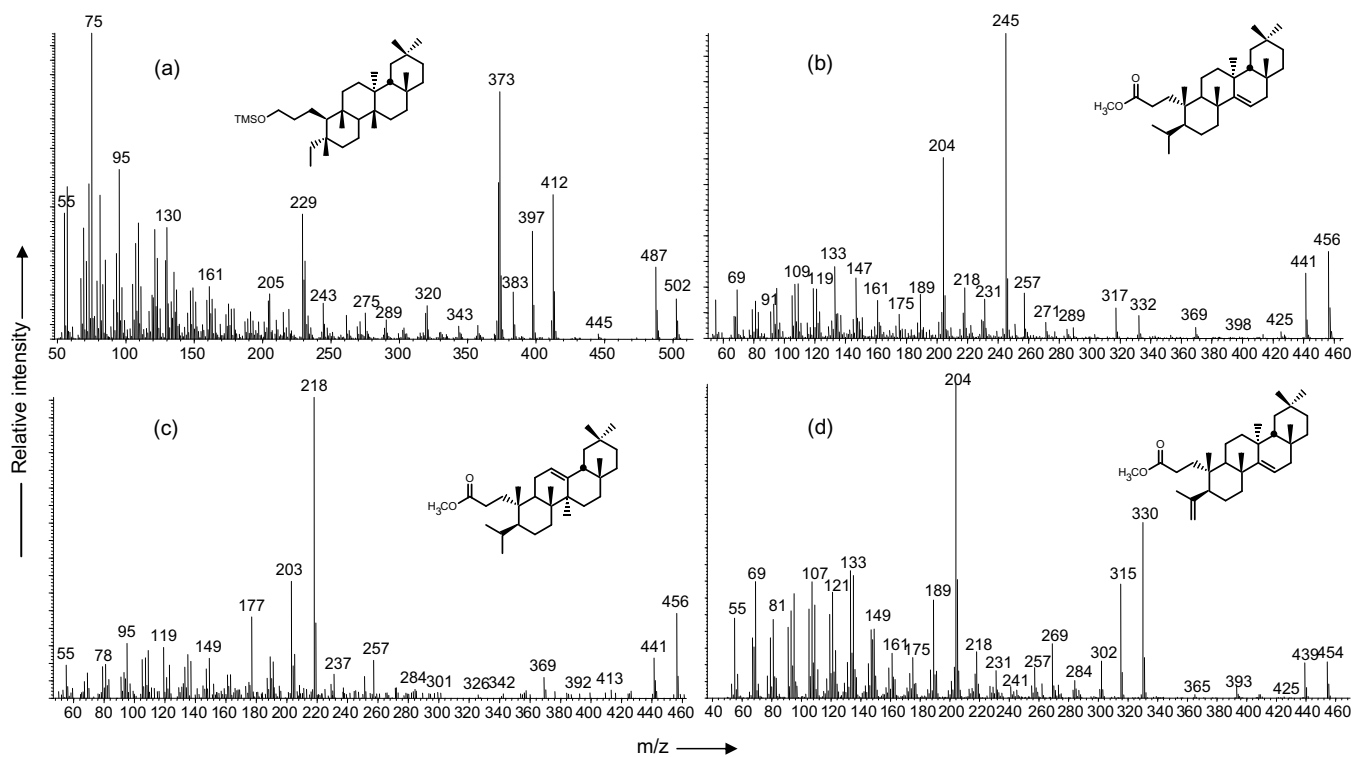


Fig. 3. Mass spectra of *seco*-derivatives: (a) tentative 3,4-*seco*-friedelanol (XIX, as TMS), (b) methyl dihydrolacunosate (XVI, R = CH₃), (c) methyl dihydroncyntanthate (IV, R = CH₃), and (d) methyl lacunosate (XVII, R = CH₃).

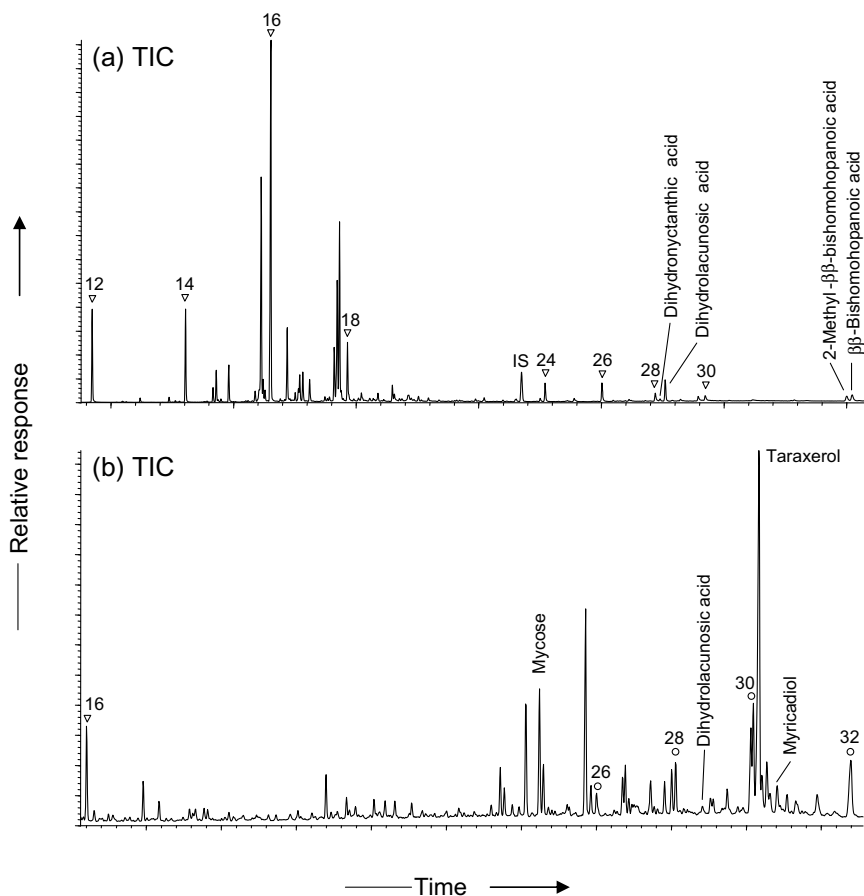
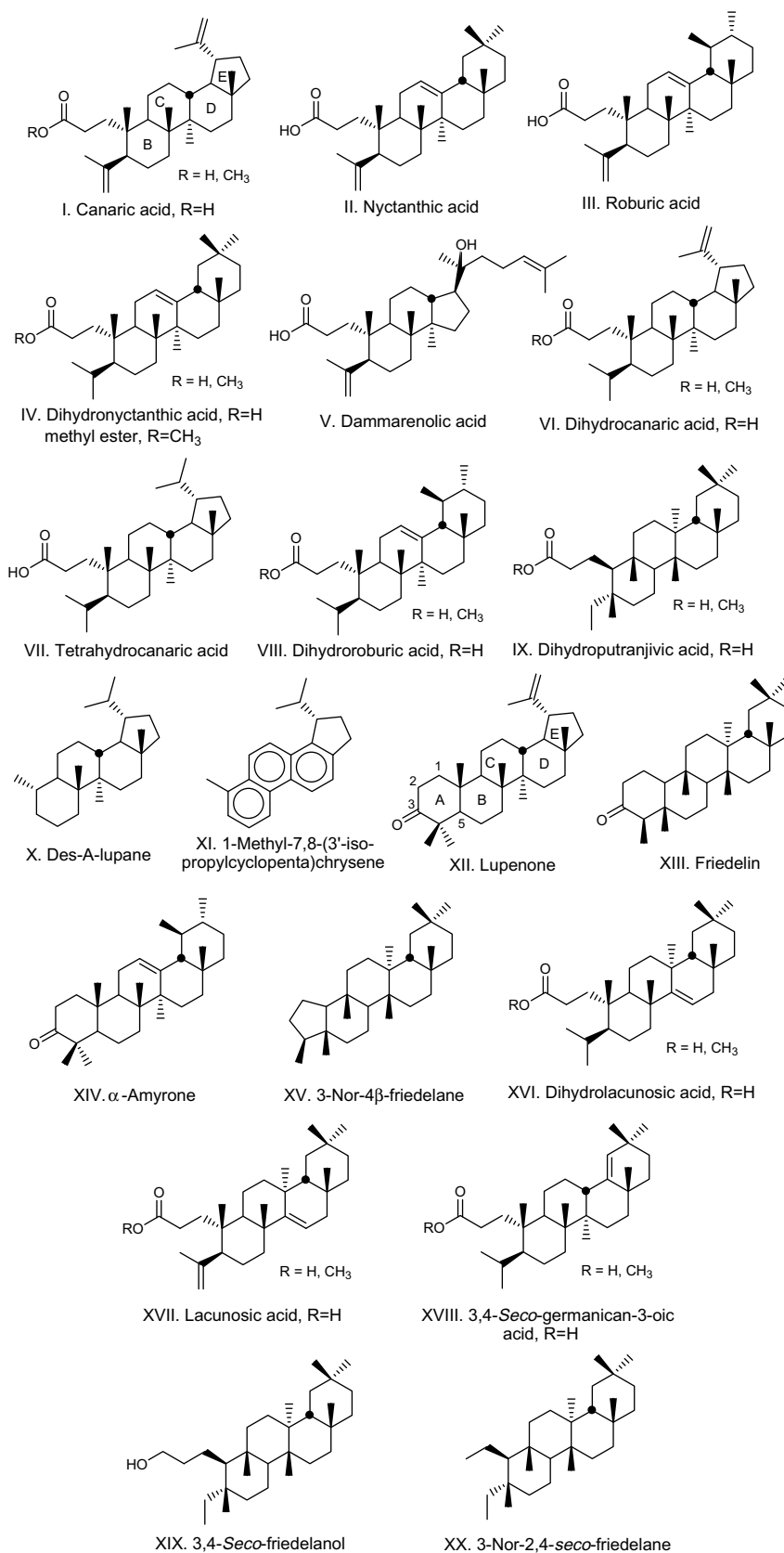


Fig. 4. Salient features of the GC-MS data for sample extracts from the Florida Everglades: (a) Taylor Slough, floc, acid fraction methylated, and (b) Harney River, sediment, total extract as TMS. Numbers and symbols as in Fig. 2.

photoproduct of α -amyrone (XIV) (Table 1), and β -amyrin is present in mangrove leaf waxes (Table 2). The possibility that dihydronyctanthic acid is formed by acid catalyzed rearrangement of dihydrolacunolic acid in the environment was checked. That rear-

angement should also produce the more stable 3,4-*seco*-olean-13(18)-en-3-oic acid which was not detectable. Therefore, dihydronyctanthic acid is likely an analogous alteration product formed by photochemistry or in the anoxic sediments of the mangrove



swamps through oxidative degradation (Trendel, 1985; Jaffé et al., 1996).

The relative proportions of the natural product precursors to the photoproducts (i.e., *seco*-compounds) on the mangrove source plants vary from 0.3% to 1.3% (ave. 0.8%) in mangrove leaves (Table 2) versus 0.8–28% (ave. 10%) in the sediments (Table 3). Although these data are limited, one interpretation suggests that additional photochemical or microbial alterations occur in the sediment during transport, deposition and diagenesis.

4. Conclusions

The photo-reactivity of several common higher plant, 3-oxy-triterpenoids were tested and all, except bauerenol, were found to be reactive to photochemical processing and generated *seco*-derivatives that have been reported in sediments and soils as diagenetic byproducts from the biodegradation of plant derived organic matter. In this study, the analyses of mangrove leaves, known to contain high concentrations of taraxerol, showed the presence of *seco*-derivatives of taraxerol (e.g., dihydrolacunolic acid, XVI), and minor amounts of such compounds from other 3-oxy-triterpenoids (β -amyrin, lupeol and germanicol). The *seco*-compounds were present in higher relative abundances in sun-exposed, senescent leaves. Exposure of 3-oxy-triterpenoid standards to simulated sunlight conditions suggests that *seco*-derivatives can potentially be generated at very favorable conversion ratios. While no quantitative assessments were made in this study, inputs of such photoproducts to sediments and soils could potentially supply des-A triterpenoid precursors commonly assumed to be derived during diagenesis of higher plant organic matter under reducing conditions (e.g., Corbet et al., 1980; Jaffé et al., 1996). While it remains to be determined how much these photoproducts can contribute to the formation of des-A triterpenoids found in sediments and soils, this study suggests that photochemical exposure of 3-oxy-triterpenoids could be a potential source in the environment.

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Appendix A

Chemical structures cited in the text.

References

- Arigoni, D., Barton, D.H.R., Bernasconi, R., Djerassi, C., Mills, J.S., Wolff, R.E., 1960. The constitutions of dammarenolic and nyctanthic acids. *J. Chem. Soc. (London)*, 1900–1905.
- Baas, W.J., 1982. Investigations on leaf waxes III. Pentacyclic triterpenes, *seco*-triterpenes and non-volatile aliphatics of four *Hoya* species and *Ficus benjamina* in relation to leaf age. *Acta Bot. Neerlandica* 31, 449–476.
- Baas, W.J., 1983. Dihydronyctanthic acid methyl ester and other 3,4-*seco*-pentacyclic triterpenoids from *Hoya lacunosa*. *Phytochemistry* 22, 2809–2812.
- Baas, W.J., 1985. Naturally occurring *seco*-ring-A-triterpenoids and their possible biological significance. *Phytochemistry* 24, 1875–1889.
- Baas, W.J., 1991. 3,4-*seco*-Triterpenoid acids and other constituents of the leaf wax of *Hoya naumanii*. *Phytochemistry* 30, 1625–1628.
- Baas, W.J., van Berkel, I.E.M., Versluis, C., Heerma, W., Kreyenbroek, M.N., 1992. Ring-A fissioned 3,4-*seco*-3-nor-triterpene-2-aldehydes and related pentacyclic triterpenoids from the leaf wax of *Hoya australis*. *Phytochemistry* 31, 2073–2078.
- Bandaranayake, W.M., 1980. Terpenoids of *Canarium zeylanicum*. *Phytochemistry* 19, 255–257.
- Bisset, N.G., Chavanel, V., Lantz, J.-P., Wolff, R.E., 1971. Chemotaxonomic study of the Dipterocarpaceae family. 4. Sesqui- and diterpenic constituents of the resins of the genus *Shorea*. *Phytochemistry* 10, 2451–2463.
- Carman, R.M., Cowley, D.E., 1964. Canaric acid – a 3,4-*seco*triterpene acid from *Canarium muelleri*. *Tetrahedron Lett.* 5, 627–629.
- Carman, R.M., Cowley, D., 1965. The structure and partial synthesis of canaric acid. *Aust. J. Chem.* 18, 213–217.
- Corbet, B., Albrecht, P., Ourisson, G., 1980. Photochemical or photomimetic fossil triterpenoids in sediments and petroleum. *J. Am. Chem. Soc.* 102, 1171–1173.
- Ekong, D.E.U., Okogun, J.I., 1969. Terpenoids of *Dacryoides Edulis*. *Phytochemistry* 8, 669–673.
- Hoffmann, C.F., Mackenzie, A.S., Lewis, C.A., Maxwell, J.R., Oudin, J.L., Durand, B., Vandembrouck, M., 1984. A biological marker study of coals, shales and oils from the Mahakam Delta, Kalimantan, Indonesia. *Chem. Geol.* 42, 1–23.
- Huang, Y.-S., Lockheart, M.J., Logan, G.A., Eglinton, G., 1996. Isotope and molecular evidence for the diverse origins of carboxylic acids in leaf fossils and sediments from the Miocene Lake Clarkia deposit, Idaho, USA. *Org. Geochem.* 24, 289–299.
- Jaffé, R., Hausmann, K.B., 1995. Origin and early diagenesis of arborinone/isoarborinol in sediments of a highly productive freshwater lake. *Org. Geochem.* 22, 231–235.
- Jaffé, R., Elisme, T., Cabrera, A.C., 1996. Organic geochemistry of seasonally flooded rain forest soils: molecular composition and early diagenesis of lipid components. *Org. Geochem.* 25, 9–17.
- Jaffé, R., Mead, R., Hernandez, M.E., Peralba, M.C., DiGuida, O.A., 2001. Origin and transport of sedimentary organic matter in two subtropical estuaries: a comparative, biomarker-based study. *Org. Geochem.* 32, 507–526.
- Jaffé, R., Rushdi, A.I., Medeiros, P.M., Simoneit, B.R.T., 2006. Natural product biomarkers as indicators of sources and transport of sedimentary organic matter in a subtropical river. *Chemosphere* 64, 1870–1884.
- Kan, R.O., 1966. *Organic Photochemistry*. McGraw-Hill Book Co., New York, NY. p. 71.
- Koch, B.P., Harder, J., Lara, R.J., Kattner, G., 2005. The effect of selective microbial degradation on the composition of mangrove derived pentacyclic triterpenols in surface sediments. *Org. Geochem.* 36, 273–285.
- Kohen, F., Samson, A.S., Stevenson, R., 1969. Friedelin and related compounds. X. Products from ultraviolet irradiation of friedelin. *J. Org. Chem.* 34, 1355–1358.
- Mangoni, L., Belardini, M., 1963. Roburic acid, a new triterpene 3,4-*seco*-acid. *Tetrahedron Lett.* 4, 921–924.
- Mangoni, L., Belardini, M., 1964. Sui componenti di alcune galle di quercia – Nota I. Acido nictantico ed acido roburico. *Gazz. Chim. Ital.* 94, 382–392.
- Neto, R.R., Mead, R.N., Louda, J.W., Jaffé, R., 2006. Organic biogeochemistry of detrital flocculent material (floc) in a subtropical, coastal wetland. *Biogeochemistry* 77, 283–304.
- Setzer, W.N., Shen, X.-M., Bates, R.B., Burns, J.R., McClure, K.J., Zhang, P., Moriarity, D.M., Lawton, R.O., 2000. A phytochemical investigation of *Alchornea latifolia*. *Fitoterapia* 71, 195–198.
- Shirasaki, H., Tsuyuki, T., Takahashi, T., 1975. Photochemical reaction of friedelin with acetone. *Tetrahedron Lett.* 16, 2271–2274.
- Trendel, J.M., 1985. Degradation de triterpenes dans les sediments – aspects photochimiques et microbiologiques. Ph.D. Thesis, Université Louis Pasteur, Strasbourg.
- Trendel, J.M., Lohmann, F., Kintzinger, J.P., Albrecht, P., Chiarone, A., Riche, C., Cesario, M., Guilhem, J., Pascard, C., 1989. Identification of des-A-triterpenoid hydrocarbons occurring in surface sediments. *Tetrahedron* 45, 4457–4470.
- Tsuyuki, T., Yamada, S., Takahashi, T., 1968. The photochemical cleavage of friedelin. *B. Chem. Soc. Jpn.* 41, 511–512.
- Turro, N.J., 1965. *Molecular Photochemistry*. W.A. Benjamin, Inc., New York, NY. p. 224.
- Wakeham, S.G., Farrington, J.W., Gagosian, R.B., 1984. Variability in lipid flux and composition of particulate matter in the Peru upwelling region. *Org. Geochem.* 6, 203–215.
- Witham, G.H., 1960. The structure of nyctanthic acid. *J. Chem. Soc. (London)*, 2016–2020.
- Xu, Y., Mead, R.N., Jaffé, R., 2006. A molecular marker-based assessment of sedimentary organic matter sources and distribution in Florida Bay. *Hydrobiologia* 569, 179–192.
- Yanes, C., Alvarez, H., Jaffé, R., 2006. Geochemistry of a tropical lake (Lake Leopoldo) on pseudo-karst topography within the Roraima Group, Guayana Shield, Venezuela. *Appl. Geochem.* 21, 870–886.