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Chemical Composition of Leaf Lipids of Angiosperms: Origin of Land Plant- Derived Hydrocarbons in Sediments and Fossil Fuel

Oluwadayo O. Sonibare
Petroleum and Environmental Geochemistry Research Group
Chemistry Department, University of Ibadan, Nigeria
E-mail: sonibaredayo@yahoo.com
Tel: +2348023278234

Olatunbosun S. Sojinu
Redeemer's University, Lagos-Ibadan Express way, Nigeria
E-mail: sojinuok2000@yahoo.com
Tel: +2348038104649

Abstract

The chemical compositions of leaf lipids of two angiosperms plant species (*Ficus. elasticoides* and *Phyllanthus amaraus*) have been analyzed by gas chromatography-mass spectrometry. The distributions and abundance of compounds that can serve as precursors for higher plant biomarker hydrocarbons commonly found in sediments and fossil fuels were assessed. Aliphatic lipids such as *n*-alkanes, *n*-alkanols and *n*-alkanoic acid were detected in the lipid extracts. Triterpenoids of the oleanane (28-norolean-17-en-3-one, β -amyrin), ursane (α -amyrin) and lupane (lupeol, betulin) series, as well as steroids (α-sitosterol and stigmasterol) were also detected in the extracts. These compounds can serve as precursors for several triterpanes and steranes found in sedimentary records.

Keywords: Angiosperms, Hydrocarbons, Aliphatic lipids, Triterpenoids, Fossil fuels

1. Introduction

Several aliphatic and aromatic hydrocarbons occurring in sediments and crude oils have been related to biological precursors present in higher plants, most especially the angiosperms (Whitehead, 1974; Eglinton and Hamilton, 1967; Riva et al., 1988; Bianchi, 1995; Nytoft et al., 2002; Jansen et al., 2006). These compounds, know as biomarkers, are useful in assessing the biological origin and thermal maturity of organic matter in geological samples and in paleoenvironmental reconstruction (e.g. Mello et al., 1988; Riva et al., 1988; Ekweozor and Udo, 1988; Riva et al., 1988; Ekweozor and Telnaes, 1990; Seifert and Moldowan, 1978; 1981). Land plants have been major sources of long chain *n*-alkanes, *n*-alkanols and *n*-alkanoic acids in the range of C_{22} to C_{32} (Douglas and Eglinton, 1966; Eglinton and Hamilton, 1967; Baker, 1982; Barthlott et al., 1998; Chikaraishi and Naraoka, 2007). Distributions of *n*-alkanes with odd over even carbon number predominance in the C_{25} to C_{35} carbon number range are usually attributed to epicuticular wax from higher plants (Eglinton and Hamilton, 1963; Rieley et al., 1991; Collister et al., 1994). The relative amount of odd/even carbon number *n*-alkane or the Carbon preference index (CPI) is to some extent used to obtain information on the

relative maturity of oil and rock extract (Bray and Evas, 1961; Eglinton and Hamilton, 1967; Barthlott et al., 1998).

Diterpenoids are excellent biomarkers derived from terrestrial plants but are found mostly in gymnosperm and in only a few angiosperms among contemporary plants. Diterpenoids are present in high concentrations in several conifer species (Otto and Simoneit, 2001; Otto et al., 2003; Otto and Wilde, 2001). The triterpenoids of the oleanane, ursane and lupane classes derive essentially from various oxygenated triterpenoids in angiosperms (Simoneit, 1986; Woolhouse et al., 1992; Rullkötter et al., 1994). Angiosperms contain triterpenoids of the β-amyrin which upon diagenesis will ultimately produced the C_{30} triterpane known as oleanane. Oleanane has been used as a reliable marker for higher plants input into oil source rock and as well as maturity indicator (Hill and Whitehead, 1966; Ekweozor and Udo, 1987; Ekweozor and Telnaes, 1990). Lupane, a C_{30} pentacyclic triterpane is considered as common constituents of coal biomarkers and more often have been detected in low rank coals as their corresponding unsaturated analogs (Wang and Simoneit, 1990). The probable biogenic source for the lupane skeleton has been proposed to be angiosperms.

Steranes found in sediments and fossil fuels are derived from sterols, which are widely distributed in plants and microorganism. Sterols present in resins and essential oils of higher plants have been reported to be the major source of C_{28} and C_{29} steroids and steranes/sterenes found in sediments and fossil fuels (Baker, 1982; Harwood and Russell, 1984; Bianchi, 1995; Huang et al., 1995). The aim of this study was to assess the distributions and abundance of land plant biomarker precursors in two angiosperm species (*Ficus. elasticoides* and *Phyllanthus amaraus*).

2. Experimental

2.1. Sample Preparation

Fresh leaves of *Ficus. elasticoides* and *Phyllanthus amaraus* were collected in Ibadan, Nigeria. The leaves were freeze-dried and crushed to a fine powder. Prior to lipid extraction, the powdered leaves were stored at -20ºC to prevent degradation by bacteria and fungi.

2.2. Extraction and Derivatisation

The freeze–dried samples were extracted ultrasonically three times for 10mins with dichloromethane/methanol (1:1; v/v). The combined solvent extracts were filtered and concentrated by use of a rotary evaporator and then blow-down under nitrogen gas. Aliquots of the lipid extracts were converted to trimethylsilyl derivatives by reaction with N, O-bis-(trimethylsilyly) trifluoroacetamide (BSTFA) and pyridine for 3 hrs at 70ºC.

2.3. Gas Chromatography-Mass Spectrometry

Gas chromatography-mass spectrometry (GC-MS) analyses of the derivatized lipid extracts were performed on an Agilent Model 6890 GC with split/splitless injector interfaced to an Agilent 5973 Mass Selective Detector. (electron input energy 70Ev, filament current 220Ma, source temperature 160ºC, multiplier voltage 1500V, interface temperature 300ºC). Data were acquired and process by a HP Vectra 486 Chemstation computer in full scan mode (50-650) or selected ion mode.

GC-MS analyses in full scan mode (*m/z* 50-650) were performed on a Varian CP-3800 gas chromatograph, interfaced to a Varian 1200 mass spectrometer (EI mode, 70 eV). Separation was achieved on a VF-1MS fused silica capillary column (50 m x 0.25 mm i.d., 0.25 μm thickness), with helium as the carrier gas, and an oven programme of 50°C (hold for 2 min) to 300°C (hold for 20.5 min) at 4°C min^{-1}. Compound identification was based on comparison of mass spectra with literature and library data and interpretation of fragmentation pattern. Components in the mass chromatograms of the lipid extracts were quantified by integration of their peak areas.

3. Results

3.1. Chemical Composition of the Total Extracts

The total ion chromatograms (TIC) of the lipid extracts of the samples are shown in Fig. 1. The identified compounds with their relative abundance are given in Table 1.

The extracts contained aliphatic lipids (*n*-alkanes, *n*-alkanols, *n*-alkanoic acid), triterpenoids, steroids, carbohydrates and some unidentified compounds.

3.2. Aliphatic Lipids

The C_{28} and C_{31} homologues were the only *n*-alkanes detected in the two samples. *n*-Alkanes are one of the most abundant lipid molecules biosynthesized by terrestrial plants and are usually characterized by odd over even carbon number predominance in the C_{25} to C_{35} carbon number range (Rieley et al., 1991; Collister et al., 1994). The abundance of C_{31} relative to C_{28} *n*-alkanes in the extracts further confirm the use of odd over even carbon predominance of *n*-alkanes distribution as indicator of terrestrial organic matter contributions to geological materials (Eglinton and Hamilton, 1967; Pancost and Boot, 2004; Jansen et al., 2006; Chikaraishi and Naraoka, 2007).. The *F. elasticoides* extracts contains *n*-C_{16} and *n*-C_{28} alkanol while *P. amarus* has only the *n*-C_{16} alkanol. The *n*-alkanols in the extracts are also associated with pentinol, arabitol and inositol, even at very high concentration. The *n*-alkanoic acids in the extracts range from C_2 to C_{24} with C_{16} and C_{18} as the major components in *F. elasticoides* and *P.amarus* extracts, respectively. This observation agreed with previous reports of *n*-alkanoic distributions in epicuticular leaf waxes of higher land plants where *n*-alkanoic acids maximizing at C_{16} or C_{18} are ubiquitous compounds originating from higher plant lipids (Gupta et al., 2007; Chikaraishi and Naraoka, 2007). Succinic, gluconic and oleic acids were also detected along side the *n*-alkanoic acids in the lipid extracts.

3.3. Triterpenoids distribution

The major triterpenoids identified in the extract of *Ficus elasticoides* were 28-norolean-17-en-3-one, lup-20(29)-ne-3β-ol (lupeol), β –amyrin, α-amyrin and lup-20(29)-en-3β-28-diol (betulin). Lup-20(29)-ne-3β-ol (lupeol), β –amyrin and α-amyrin were the only triterpenoids detected in the extract of *P. amarus*. These triterpenoids can serve as precursors for many biomarkers found in geological samples. Triterpanes of the oleanane, ursane and lupane series reported in crude oils and sediments are believed to be derived from oxygenated triterpenoids in angiosperms (Simoneit, 1986; Woolhouse et al., 1992;

Rullkötter et al., 1994; Otto and Simoneit, 2001; Otto and Wilde, 2001). β -amyrin has been reported to be the major precursor of oleanane and a number of polycyclic hydrocarbons. 1,2,5 and 1,2,7-trimethylnaphthalene have been suggested to be degraded products of β-amyrin (Strachan et al., 1985). The degradation of the β-amyrin via 8,14-seco-triterpenoids is supposed to additionally yield 1,2,5,6-tetramethylnapthalene (Puttman and Villa, 1987). Lupanoids (e.g. lupan-3β, 20,28-triol, lup-20(29)-ne-3β-ol, lup-20(29)-en-3β-28-diol) are believed to be major precursors of lupane found in geological samples. It has been shown that acid catalysed isomerisation of lup-20(29)-ene can also produce oleananes (Rullkotter et al., 1994). Lupane occurs mostly in coals and lignites but has not been detected in petroleum (Wang and Simoneit, 1990; Peters and Moldowan, 1993; Stefanova et al., 1995).

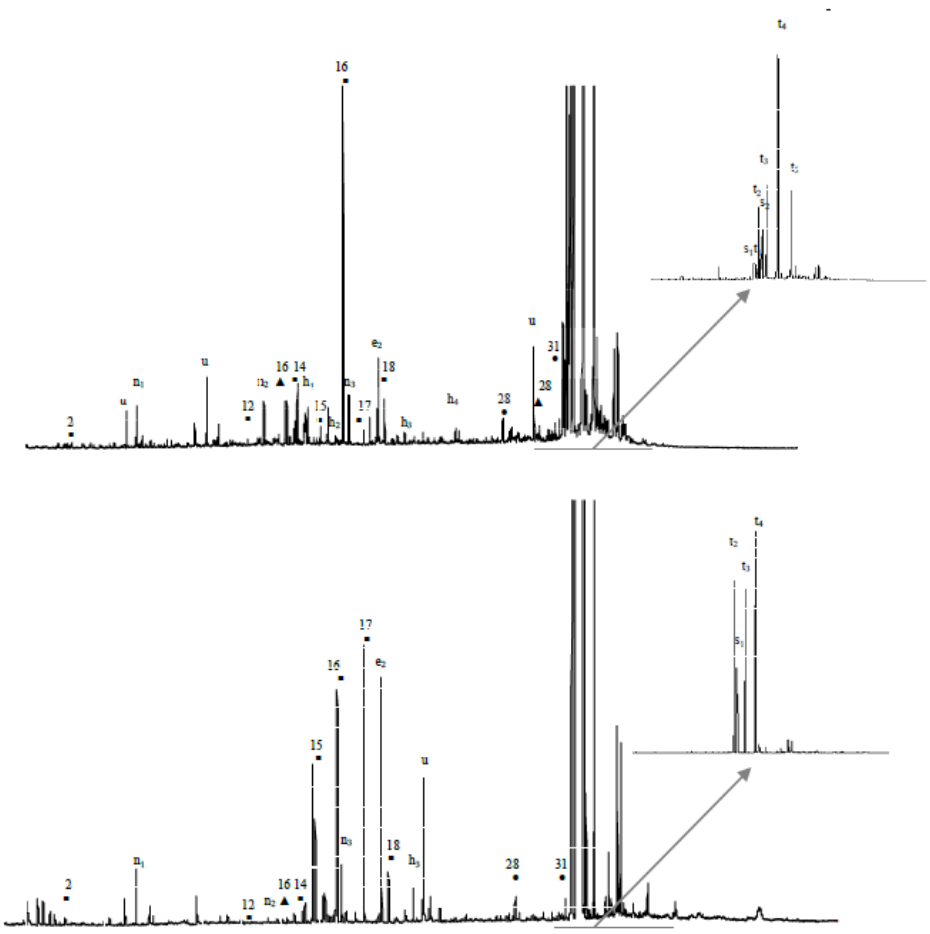

Figure 1: Total ion current (TIC) chromatograms of the leaf extracts of (a) *Ficus elasticoides* and (b) *Phyllanthus amarus*

3.4. Steroid Distribution

Sitosterol and stigmasterol were the steroids identified in the extract of *F. elasticoides* while *P. amarus* has only the stigmasterol. α-sitosterol and stigmasterol have been reported to be widely distributed among the plant kingdom and the most common steroids in the epicuticular waxes of higher plants (Baker, 1982; Harwood and Russell, 1984; Bianchi, 1995). C_{28} and C_{29} steroids and their saturated counterpart (sterane) in oils and sediments are derived mainly from degradation of α-sitosterol and stigmasterol (Harwood and Russell, 1984; Bianchi, 1995; Huang et. al., 1979; Huang et al., 1995; Brassel et al., 1983). Steranes and diasteranes are formed from steroid diagenesis processes that involve defunctionalization (loss of a hydroxyl group), loss of hydrogen and oxygenation.

Table 1: Compounds identified in the lipid extracts of *Ficus. elasticoides* and *Phyllanthus amaraus*

				Occurrence and Relative Abundance[a]	
Peak	**Compound name**	**MW**	**Composition**	**F. elasticoides**	**P. amarus**
	n-Alkanes				
•	Octacosane	394	$C_{28}H_{58}$	12.6	8.2
•	Heneicosane	436	$C_{31}H_{64}$	10.7	7.5
	n-alkanols				
n_1	Glycerol	92	C_3H_8O	10.9	11.2
▲	Hexadecanol	296	$C_{16}H_{34}O$	12.4	22.2
▲	Octacosanol	410	$C_{28}H_{58}O$	8.5	0
n_2	Arabitol	152	$C_5H_{12}O_5$	10.4	2.0
n_3	Inositol	180	$C_6H_{12}O_6$	12.4	10.6
	n-alkanoic acids				
▪	Acetic acid	60	$C_2H_4O_2$	0.9	1.3
▪	Dodecannoic acid	200	$C_{12}H_{24}O_2$	2.0	0.7
▪	Tetradecannoic acid	228	$C_{14}H_{28}O_2$	7.0	9.6
▪	Pentadecannoic acid	242	$C_{15}H_{30}O_2$	3.0	4.2
▪	Hexadecannoic acid	256	$C_{16}H_{32}O_2$	45.2	28.2
▪	Heptadecannoic acid	270	$C_{17}H_{34}O_2$	2.5	9.2
▪	Octadecannoic acid	424	$C_{18}H_{36}O_2$	5.6	5.2
e_1	Gluconic acid	196	$C_6H_{12}O_7$	0	5.7
e_2	Oleic acid	282	$C_{18}H_{34}O_2$	18.0	0
	Terpenoids				
t_1	28-norolean-17-en-3-one	410	$C_{29}H_{46}O$	27.6	0
t_2	lup-20(29)-ene-3β-ol	424	$C_{30}H_{48}O$	40.4	65.2
t_3	β -Amyrin	426	$C_{30}H_{50}O$	47.4	6.05
t_4	α -Amyrin	426	$C_{30}H_{50}O$	100	100
t_5	lup-20(29)-en-3β-28-diol	442	$C_{30}H_{48}O_2$	41.1	0
	Steroids				
s_1	Stigmasterol	412	$C_{29}H_{48}O_2$	28.7	40.3
s_2	α -Sitosterol	414	$C_{29}H_{50}O$	30.8	0
	Carbonhydrate				
h_1	Galactose	180	$C_6H_{12}O_6$	8.6	25.2
h_2	L-Mannopyranose	180	$C_6H_{12}O_6$	1.3	0
h_3	Maltose	342	$C_{12}H_{22}O_{11}$	5.4	0
h_4	D-Turanose	342	$C_{12}H_{22}O_{11}$	10.8	1.8

[a] Relative abundance normalized to major peak = 100

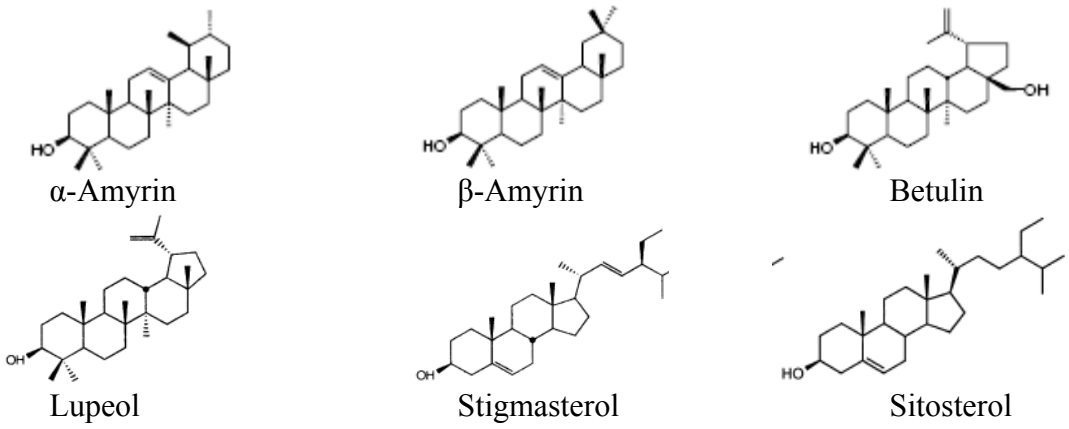

Figure. 2: Structure of polar triterpenoids and steroids identified in the leaf lipids of *Ficus elasticoides* and *Phyllanthus amarus*

3.4. Other Compounds

Some free carbohydrates were detected in the extracts. Galactose, L-manopyranose, maltose and D-Turanose were detected in *F. elasticoides* while galactose and D- Turanos were present in *P. amarus* (Table 1).

4. Conclusions

Gas chromatography-Mass spectrometry analyses of total lipid extracts of *Ficus. elasticoides* and *Phyllanthus amaraus* leaves were performed to determined their chemical composition. The extracts contained aliphatic lipids (n-alkanes, n-alkanols, n-alkanoic acid), triterpenoids and steroids that can serve as precursors for most of the higher plant derived biomarkers and polycyclic hydrocarbons found in fossil fuels and sediments.

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