

VERY HIGH MOLECULAR WEIGHT ORGANIC MARKER (>C₄₀) EMISSIONS FROM BIOMASS BURNING IN AMAZONIA

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ABSTRACT - High temperature gas chromatography (HTGC) and HTGC coupled to mass spectrometry (HTGC-MS) analyses of smoke samples taken by particle filtration from combustion of different species of plants, provided for the first time the characterization of various classes of high molecular weight (HMW) compounds in emissions from controlled biomass burning of plants from Amazonia, Brazil. Among these components are a series of wax esters (long chain alcohols esterified with long chain fatty acids) with up to 58 carbon numbers, *n*-alkanes (C₁₅-C₄₀, C_{max} = 31; odd predominance), triglycerides, long chain *n*-alkan-2-ones (up to 37 carbons, C_{max} = 33, odd predominance), *n*-alkanols (up to 40 carbons, C_{max} = 32, even predominance), series of triterpenyl fatty acid esters (e.g., α - and β -amiryl stearate), and various unknown HMW compounds. Long chain fatty acids >C₃₂ are not present in the smoke samples analyzed. The organic compounds with highest concentrations extracted from smoke consist of a series of lower molecular weight volatile polar components, which are the result of thermal breakdown of cellulose and lignin (i.e., natural product derivatives). In contrast, the HMW compounds in smoke samples from burning of Amazon biomass indicate the input of directly-volatilized natural products in the original plants during their combustion. These HMW natural products may be suitable tracers for specific sources of vegetation combustion because they are emitted as particles in the smoke.

RESUMO - Análises de cromatografia gasosa de alta temperatura (CGAT) e CGAT acoplada à espectrometria de massas (CGAT-EM) de amostras de fumaças, coletadas por filtração de partículas da queima de diferentes espécies de plantas, possibilitou a caracterização de várias classes de compostos de alto peso molecular, que foram detectados pela primeira vez em emissões da queima controlada de biomassa de plantas da Amazônia, Brasil. Entre esses componentes estão uma série de ésteres graxos (álcoois de cadeia longa esterificados com ácidos de cadeia longa) com até 58 números de carbono, *n*-alcanos (C₁₅-C₄₀, C_{max} = 31, predominância ímpar), triglicerídeos, *n*-alcan-2-onas de cadeia longa (com até 37 carbonos, C_{max} = 33; preferência ímpar), *n*-alcanóis (com até 40 carbonos, C_{max} = 32, predominância par), séries de triterpenil ésteres de ácidos graxos (p. ex., α - e β -amiril-estearato) e vários compostos de alto peso molecular com estruturas desconhecidas. Ácidos graxos de cadeia longa >C₃₂ não estão presentes nas amostras de fumaça analisadas. Os compostos, extraídos em maior concentração das amostras de fumaças, consistem de uma série de componentes polares voláteis de baixo peso molecular derivados da degradação térmica da celulose e lignina (i.e., derivados de produtos naturais). No entanto, os componentes de alto peso molecular, identificados na fumaça da queima de biomassa Amazônica, indicam um influxo de produtos naturais que foram diretamente volatilizados durante a combustão das plantas. Os produtos naturais de alto peso molecular identificados por CGAT-EM, apesar de minoritários, são mais adequados para traçar fontes específicas da combustão de vegetação, porque eles são emitidos como partículas na fumaça das plantas.

KEY WORDS: HTGC-mass spectrometry, lipids, wax esters, triglycerides, smoke.

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INTRODUCTION

Biomarkers may be used to trace point source emissions and distinguish between anthropogenic and biogenic organic matter in atmospheric chemistry (e.g., Simoneit, 1989; Simoneit and Mazurek, 1982; Rogge et al., 1993; Schauer et al., 1996). Good examples are the markers retene and dehydroabietic acid, derived from the thermal degradation of diterpenoids, which can be used for tracing the burning of conifers in temperate regions (Ramdahl, 1983; Standley and Simoneit, 1994). Although various molecular markers have been proposed for correlations, additional indicators should be characterized in order to enlarge the availability of tracers for specific source correlations. In this regard, high molecular weight (HMW) compounds may be preferable as chemical fingerprints because they have a longer residence time in the atmosphere and occur solely in the particle phase. The analysis of organic tracers by high temperature high resolution gas chromatography (HTGC) and HTGC-mass spectrometry (MS) may enable the characterization of HMW organic compounds not previously reported in aerosols due to their non-elution on conventional GC columns with lower temperature limits.

This work reports the characterization of HMW tracers from smoke aerosols by HTGC and HTGC-MS. Smoke samples were taken by particle filtration from combustion of different species of plants so that the organic compounds adsorbed to particles may be used for source correlation to distinguish specific inputs of natural and altered products from source specific biomass combustion. Various extract fractions of differing polarities have been analyzed. Many HMW compounds were characterized for the first time in smoke aerosols, which illustrates HTGC and HTGC-MS as powerful techniques in the detection of novel biomarkers.

EXPERIMENTAL METHODS

Controlled fires were set by burning (both flaming and smoldering) dry leaf and stem litter of individual plant species and sampling the smoke particles by high volume filtration on quartz filters. Details for sample preparation, extraction, separation, and derivatization are given elsewhere (Abas et al., 1995; Elias et al., 1999; Simoneit and Mazurek, 1982). HTGC-MS analyses were carried out with a Hewlett-Packard (HP) model 5973 MSD coupled to a HP 6890 gas chromatograph equipped with an on-column injector (Carlo Erba). HTGC was performed on a 20 m x 0.3 mm i.d. custom-made glass capillary column coated with 0.1 mm of OV-

1701-OH (88% methyl, 7% cyanopropyl, 5% phenylpolysiloxane; Ohio Valley Specialty Chemical Co.) (Elias et al., 1998). The column temperature was maintained at 40°C for 2 min, then programmed to 390°C at 10°C/min and held isothermal for 20 min. The on-column injector and the transfer line temperatures were set at 40°C and 390°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode with an ion source energy of 70 eV. The detailed analytical technique is reported elsewhere (Elias et al., 1997; 1998; 1999).

RESULTS AND DISCUSSION

HTGC-MS analyses enabled the analytical extension of the homologous compound series, as well as the identification of HMW tracers not previously detected in smoke aerosols. Nine plant smoke extracts and 81 fractions of differing polarities have been analyzed. However, only representative examples containing HMW compounds are described in this paper. HMW components are present in smoke from all the plants analyzed, but differ in abundance, chemical functionality and structure. This is due to the varying combustion conditions and the different natural product compositions of the fuels. Technically, HMW compounds are defined and characterized as those components eluting above 300°C in the HTGC or HTGC-MS analyses. These components are determined by analysis of the derivatized total extracts or separated fractions of the smoke samples, which denotes their importance in the lipid fractions. Thus, by increasing the temperature of the GC analysis we were able to detect, for example, series of triterpenyl fatty acid esters (e.g., α - and β -amyryl stearate) which have been characterized for the first time in smoke aerosols (Elias et al., 1997), series of wax esters (long chain alcohols esterified with long chain fatty acids) with up to 58 carbon numbers, n -alkanes (C_{15} - C_{40} , $C_{max} = 31$, odd predominance), triglycerides, n -alkan-2-ones (up to 37 carbons, $C_{max} = 33$, odd predominance) and n -alkanols (up to 40 carbons, $C_{max} = 32$, even predominance).

The total extracts of smoke from burning of plant matter generally present series of compounds eluting above 350°C as shown in the HTGC traces (e.g., Fig. 1). However, the major compounds <300°C consist of levoglucosan (1,6-anhydro- β -D-glucose) and other monosaccharide derivatives (e.g., mannosan and galactosan) from the thermal breakdown of cellulose (cf., Fig. 1) (Simoneit et al., 1999). The second most abundant class of compounds comprises various methoxyphenols which are produced by thermal degradation of lignin (Hawthorne et al., 1992; Simoneit et al., 1993). Typically, these two classes of components

encompass approximately 85% of the total extractable organic compounds in the smoke samples analyzed. Compounds of relatively HMW (e.g., triterpenoids and fatty acids) and eluting earlier than 310°C (temperature limit of conventional GC columns) are also present in the total extracts but at lower abundances than the lower molecular weight compounds discussed above. Considering the scope of this report, compounds eluting earlier than 300°C are not discussed further. Various compounds elute late in the GC traces (>350°C) of the total extracts. The detection of HMW compounds in some samples (with no prior fractionation) demonstrates their magnitude in smoke from biomass burning (e.g., Fig. 1). Because many of these compounds have not been reported previously, it was necessary to separate the total extracts into different polarity fractions in order to increase the confidence in the identifications as well as minimize background in the mass spectra.

Long chain wax esters (LCWE) are present in smoke from burning of some plant species. For example, smoke aerosol from burning of Cupuaçu showed LCWE from 38 up to 58 total carbon numbers with a strong even carbon number predominance (Fig. 2). This is typical as reported for plant wax (Kolattukudy, 1970). This LCWE series is comprised of mainly palmitic acid esterified with the fatty alcohols ranging from C₂₂ to C₃₄ and minor amounts of stearic and arachidic acids esterified with the C₃₂ and C₃₄ alcohols (Fig. 2, wax esters C₃₈–C₅₈). Although wax esters have been described extensively in the literature, LCWE are reported as such only for a few cases of higher plant waxes (Kolattukudy, 1970; Boon and de Leeuw, 1979) despite their likely widespread occurrence. The reason for this may be that they were simply not detected when analyzed on conventional GC columns used to analyze lipid mixtures. LCWE are described here for the first time to occur in smoke aerosols. These natural products are found in abundances comparable to the *n*-alkanes (e.g., Fig. 2) in some smoke samples indicating their magnitude in biomass burning emissions. This compound signature is further evidence for direct volatilization of HMW compounds into smoke. In this regard other classes of compounds, as discussed below, may follow the same mechanism of introduction into smoke aerosols (Mazurek and Simoneit, 1997).

Intact triglycerides elute late (>350°C) in the HTGC and HTGC-MS analyses of the polar fractions of some smoke samples (e.g., Fig. 3). The major compounds are dipalmitoylolein (C₅₃H₁₀₀O₆, MW 832), 1-palmitoyl-2-oleoyl-3-stearin (C₅₅H₁₀₄O₆, MW 860), and triolein (C₅₇H₁₀₄O₆, MW 884). The mass spectra of these compounds (<800 dalton)

match with data for authentic standards. No fragments above 800 dalton (e.g., the M⁺ ion) could be obtained due to the technical constraint of the HP 5973 MSD instrument which limits the MS data acquisition to a maximum of 800 daltons. The GC retention indices of the triglycerides found in the smoke samples are the same as those obtained for triglyceride standards. Many plants have triglycerides in their lipids. It is interesting to note that these common energy storage lipids are rarely present in epicuticular waxes (Kolattukudy, 1970). They are commonly found in seeds, but are probably also internal constituents of the plants. Triglycerides have a very low vapor pressure and their presence in smoke is evidence of their direct volatilization as HMW natural products during burning of plants. As in the case of the LCWE, this is the first report on the presence of these compounds in smoke aerosols.

Ester fractions separated from extracts of smoke particulate matter from burning of different species of plants contained compounds, in relatively high abundance, eluting late in the total ion current traces (TIC) of HTGC-MS analyses (Fig. 4). The most intense peaks of these HMW components have mass spectra resembling the characteristic fragmentation pattern of pentacyclic triterpenoids with a double bond in their structure. For instance, the most intense peaks at long retention times are a homologous series with fragment ions at *m/z* 218 (base peak), 189, 203 and 409 typical of pentacyclic triterpenoids with the Δ^{12} -ursene (α -amyrin) and Δ^{12} -oleanene (β -amyrin) structures (cf. examples in Fig. 5) (Elias et al., 1997). However, the retention indices (up to ~5700) and molecular ions (up to 720 da) indicate the structures to be triterpenyl fatty acid esters (TFAE). Essentially, the fragmentation patterns of the TFAE consist of molecular ion (M⁺), M-CH₃, M-fatty acid, and then fragments characteristic of the esterified triterpenol (Fig. 5). The TFAE have acyl carbon chain lengths extending from 5 up to 20 carbon numbers (Elias et al., 1997). The dominant esters are with α - and β -amyrin and a minor amount with taraxasterol. The complete characterization as well as interpretation of the mass spectra is given elsewhere (Elias et al., 1997). These compounds are reported for the first time in smoke aerosol and are novel natural products believed to be constituents of the plants.

Extended homologous series of aliphatic HMW components, as for example *n*-alkanes, *n*-alkanones, *n*-alkanols, etc., are not major components of the smoke samples analyzed. Thus, conventional GC and GC-MS analyses of aerosol lipid matter is adequate to screen these aliphatic compound series.

CONCLUSION

HTGC and especially HTGC-MS analyses have enabled the identification of various HMW compounds reported for the first time in smoke aerosols. This demonstrates the potential utility of these techniques in the detection of HMW compounds of geochemical and environmental interest. HMW components are present at varying concentrations in most smoke samples from burning of the few Amazonian vegetation species described here. These HMW compounds are in general natural products and are emitted as such exclusively in the particle phase. In contrast to the lower molecular weight components which are typically degradation products from thermal alteration of cellulose and lignin, comprise the major organic components of the smoke extracts. Both low and high molecular weight compounds are specific tracers for emissions from biomass burning and the HMW compounds provide additional taxonomic source confirmation.

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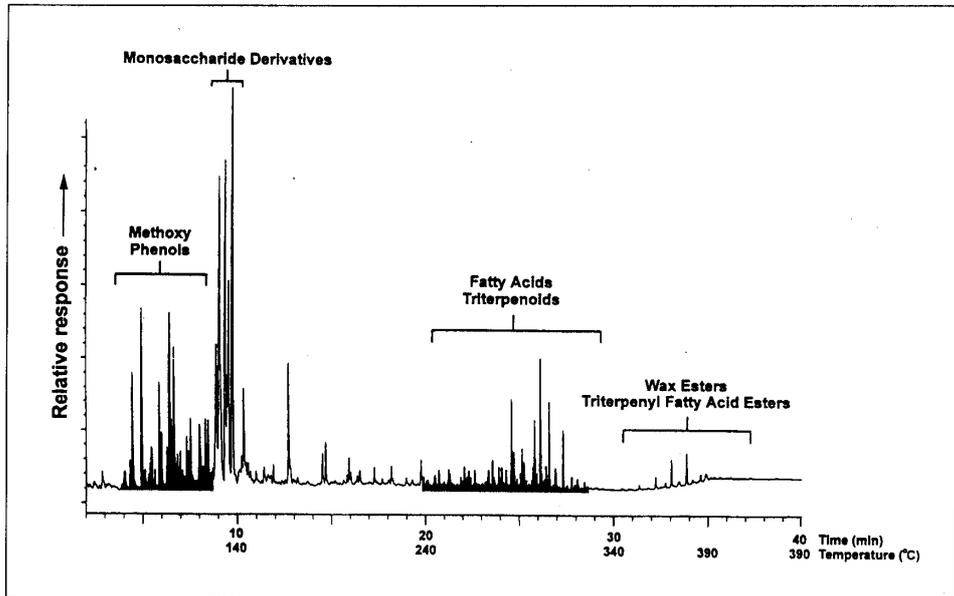


Fig 1: Typical gas chromatogram (HTGC) of the total extract (methylated and silylated) from fine particles of smoke from burning of *Vismia*.

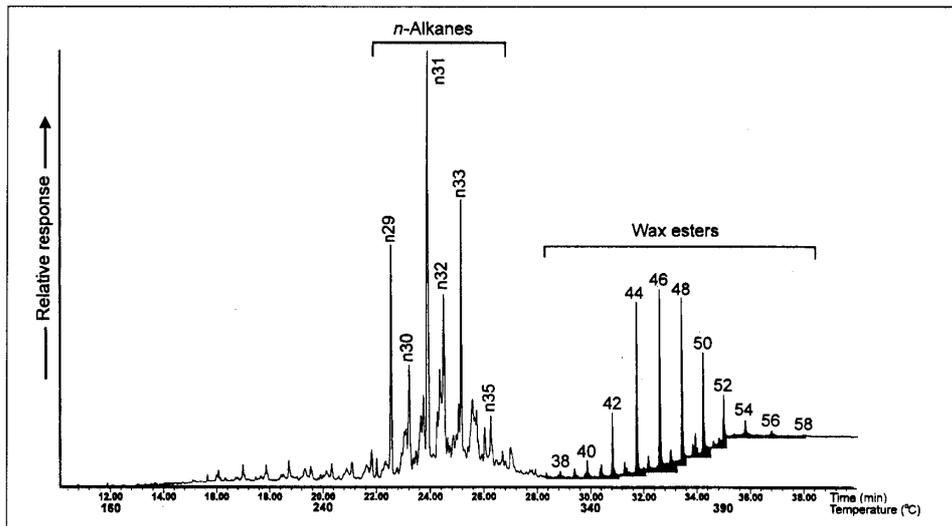


Fig. 2: Representative total ion current trace (HTGC-MS) of the fraction containing wax esters from smoke extract from burning of *Cupuaçu*. Numbers refer to the total carbon chain length of the wax esters, n_i - normal alkane carbon chain length.

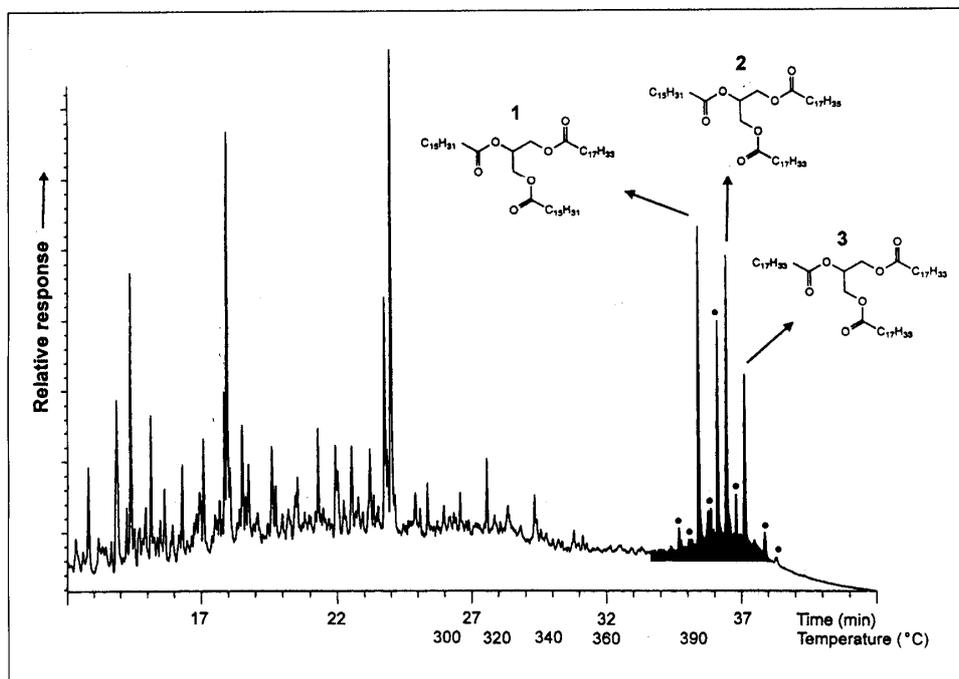


Fig. 3: Representative gas chromatogram (HTGC) of the fraction containing triglycerides in smoke extract from burning of Andiroba. (1 = dipalmitoyl-olein, 2 = 1-palmitoyl-2-oleoyl-3-stearin, 3 = triolein, · = unknown triglycerides).

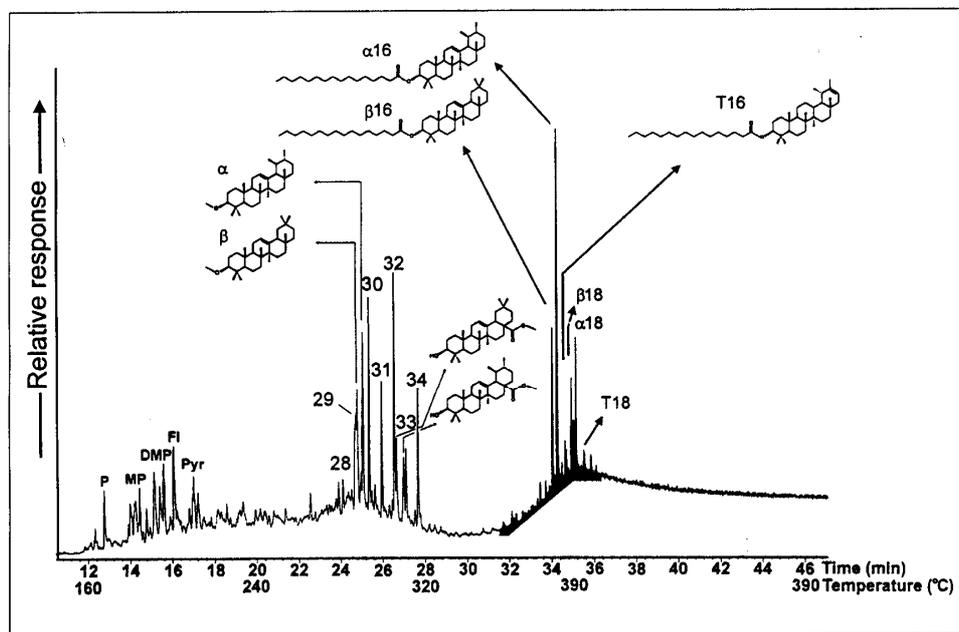


Fig. 4: Representative total ion current trace (HTGC-MS) of the ester fraction from smoke extract from burning of Castanha-do-Pará. Numbers refer to the carbon chain length of free fatty acids (analyzed as the methyl esters). P = phenanthrene; MP = methylphenanthrenes; DMP = dimethylphenanthrenes; FI = fluoranthene and Pyr = pyrene. α , β and T are the esterified triterpenols α -amyrin, β -amyrin and taraxasterol, respectively.

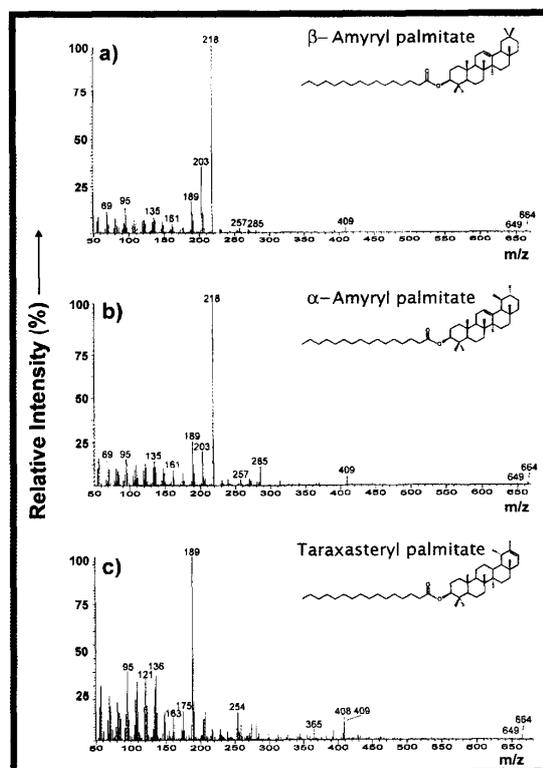


Fig. 5: Mass spectra of (a) β -amyryl-, (b) α -amyryl- and (c) taraxasteryl palmitate, representative of the homologous series of triterpenyl esters (structures are indicated on the figure).