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Mass Spectra of Triterpenyl Alkanoates, Novel Natural Products

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A series of high molecular mass pentacyclic terpenoid wax esters in smoke from biomass combustion were characterized by high-temperature, high-resolution gas chromatography–mass spectrometry. Based on their mass spectra, they are interpreted as being a series of triterpenyl fatty acid esters (e.g. α- and β- amyryl palmitate). The carbon chain length of the fatty acids ranges from 5 to 20. This interpretation is supported by retention indeces, molecular ions and typical fragments of pentacyclic triterpenoids. Essentially, the mass spectra are simple and composed of the molecular ion, M − CH_3, M − fatty acid and fragments characteristic of the esterified triterpenoid moieties. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Triterpenoids are common constituents of plants.[1–3] In smoke samples from biomass burning, these compounds are used as organic tracers for input of directly volatilized natural products from plants during their combustion.[4] There is a need to demonstrate and characterize additional specific organic tracers for this input process to the global atmosphere. Various molecular markers have been proposed,[5–9] but additional indicator compounds should be characterized to increase the sensitivity for the detection and the range of atmospheric residence times. In this regard, the analysis of organic tracers by high-temperature, high-resolution gas chromatography–mass spectrometry (HTHRGC/MS) may permit the characterization of higher molecular mass (HMM) organic compounds not previously reported.

HTHRGC is an established technique for separation of complex mixtures containing HMM compounds. It has been used to characterize HMM components in different matrices, such as petroleum[10,11] and natural products.[12] The heavy compounds in these samples elute at much higher temperatures than the upper limit of conventional GC columns.

In this work, a series of triterpenols esterified to fatty acids were characterized for the first time during routine HTHRGC and HTHRGC/MS analyses of smoke samples from biomass burning. Although steroidal fatty acid esters (e.g. cholesteryl palmitate) are common lipids, we are not aware of any report on triterpenoidyl esters as reported here. Because of the importance of this finding, the structural elucidation of these compounds using electron impact (EI) ionization MS was examined.

EXPERIMENTAL

Controlled fires were set by burning different species of dry leaf and stem litter and the smoke particles were sampled by high-volume filtration on quartz filters. Detailed sample preparation extraction, separation and derivatization procedures are given elsewhere.[4]

HTHRGC/MS analyses of ester fractions from smoke sample extracts were carried out on a Hewlett-Packard (HP) Model 5973 mass-selective detector coupled to an HP Model 6890 gas chromatograph equipped with an on-column injector (Carlo Erba). HTHRGC was performed on a 20 m × 0.3 mm i.d. custom-made glass capillary column coated with 0.1 μm of OV-1701-OH (88% methyl-, 7% cyanopropyl-, 5% phenylpolysiloxane)

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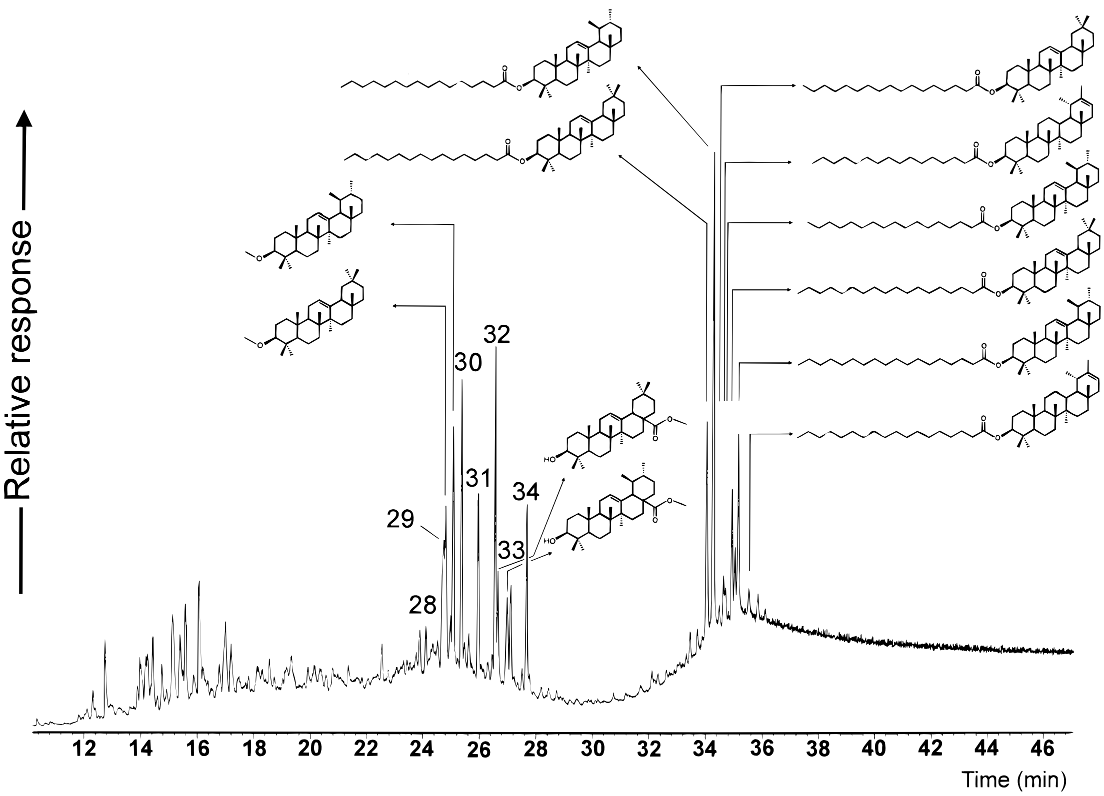

Figure 1. Representative HTHRGC/MS total ion current trace of the ester fraction from smoke particle extract of Castanha-do-Pará. Numbers refer to carbon chain length of free fatty acids (analyzed as the methyl esters).

(Ohio Valley Specialty Chemical). The column temperature was maintained at 40 °C for 2 min, then programmed to 390 °C at 10 °C min^{-1} and held isothermal at 390 °C for 20 min. The on-column injector and the transfer line temperatures were set at 40 and 390 °C, respectively. The mass spectrometer was operated in the EI mode with a 70 eV ion source energy.

RESULTS AND DISCUSSION

Ester fractions separated from extracts of smoke particulate matter from burning of different species of plants showed the presence of compounds, in relatively high abundance, eluting late in the total ion current (TIC) traces of GC/MS analyses (Fig. 1, >10 min). Conventional GC/MS detection usually ends at ~ 32 min for such samples. The high abundance of these compounds is an indication of their importance in these samples. The HMM components comprise up to 0.5% of the total lipids extracted from smoke aerosols. However, they are believed to be present in the plants at much higher abundances because additional pyrolytic degradation products (e.g. of cellulose and lignin) formed during biomass combustion are the major components in the total extracts. The most intense peaks in the TIC trace of these HMM components have mass spectra which resemble the characteristic fragmentation of pentacyclic triterpenoids with a double bond in their structure. For instance, among the most intense peaks of the TIC is a homologous series with fragment ions at *m/z* 218 (base peak, Fig. 2), 189, 203 and 409, which are typical of pentacyclic triterpenoids with Δ^{12}-ursene and Δ^{12}-oleanene structures. However, the retention indices (up to ~ 5700) and molecular ions (up to 720) are too high for these compounds to be considered pentacyclic triterpenoids.

Figure 3 shows examples of mass spectra of the triterpenols esterified to palmitic acid (triterpenyl palmitates) as representative of the triterpenyl fatty acid ester (TFAE) series. The interpretation of the mass spectra of the HMM compounds (e.g., Fig. 3) clearly indicates a TFAE structure. This is based on the molecular ions, fragmentation patterns and retention indices of the various TFAEs from the different species of plants. The mass spectra of these esterified triterpenols are fairly simple, considering their relatively complex structures.

Essentially, the fragmentation pattern consists of molecular ion ($\mathrm{M}^{+\cdot}$), $\mathrm{M}-\mathrm{CH}_3$, M − fatty acid and fragments characteristic of the esterified triterpenol. The basic fragmentation pattern of the proposed structures is illustrated in Fig. 4. The dominant fragmentation is cleavage of the ester bond directly or by H-transfer via a McLafferty-type rearrangement to yield the triterpenyl ions (*m/z* 408 and 409, respectively). This is followed by fragmentation of the triterpadiene moiety. The general fragmentation patterns of triterpenoid compounds have been reported previously[13] and are not discussed here.

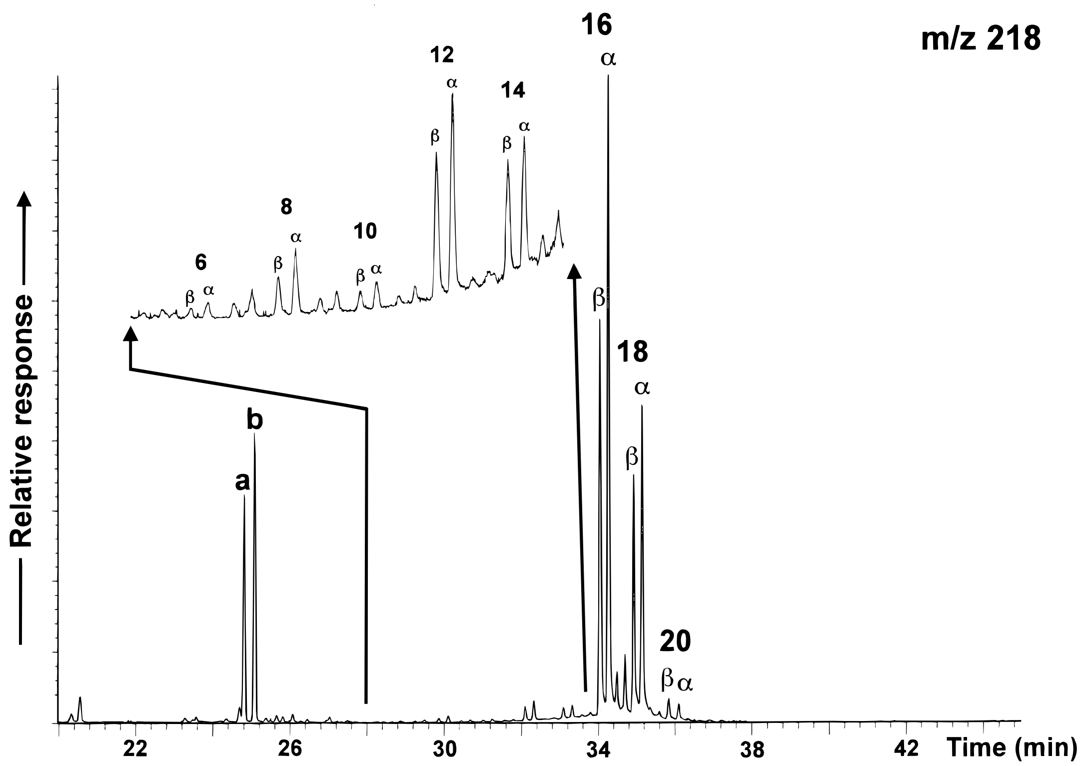

Figure 2. Representative HTHRGC mass fragmentogram (*m*/*z* 218) showing the homologous series of amyryl alkanoates. Numbers refer to carbon chain length of the esterified fatty acids; α and β are the esterified triterpenols α-amyrin and β-amyrin, respectively; a and b are β-amyrin and α-amyrin methyl ether, respectively.

The relative abundances of the characteristic fragments in the mass spectra of the HMM triterpenyl esters which are detectable are given in Table 1. The TFAEs have acyl carbon chain lengths extending from 5 to 20 (Fig. 2), but only data for the most intense peaks without other co-eluting compounds are given in Table 1. The retention indices of the TFAEs detected are summarized in Table 2. The dominant esters are those of α- and β-amyrin with a minor amount of those of taraxasterol.

CONCLUSION

A series of HMM triterpenoid wax esters in smoke from biomass burning were characterized by HTHRGC/MS. The simple mass spectra consist of the molecular ion, $M - CH_3$, M − fatty acid and typical triterpenoid fragments. The carbon chain lengths of the fatty acids range from C_5 to C_{20} and these esters are novel natural products.

Table 1. Main fragments (*m*/*z*) and their relative abundances (%) (in parentheses) in the mass spectra of triterpenyl fatty acid esters

Compound	$M^{+\cdot}$	Base peak (100)	$M - CH_3$	M − fatty acid	Other significant fragments
Taraxasterol esters:					
Taraxasteryl hexadecanoate	664 (1.7)	189	649 (0.4)	408 (13)	393 (7), 175 (17)
Taraxasteryl octadecanoate	692 (1.2)	189	649 (0.2)	408 (14)	393 (11), 175 (15)
Amyrin esters:					
β-Amyryl dodecanoate	608 (0.5)	218	593 (0.7)	409 (4)	203 (37), 189 (17)
α-Amyryl dodecanoate	608 (1.0)	218	593 (0.4)	409 (2)	203 (18), 189 (22)
β-Amyryl tetradecanoate	636 (0.8)	218	621 (0.1)	409 (2)	203 (36), 189 (17)
α-Amyryl tetradecanoate	636 (0.7)	218	621 (0.6)	409 (7)	203 (17), 189 (21)
β-Amyryl hexadecanoate	664 (0.7)	218	649 (0.3)	409 (3)	203 (34), 189 (16)
α-Amyryl hexadecanoate	664 (1.3)	218	649 (0.5)	409 (5)	203 (18), 189 (23)
α-Amyryl heptadecanoate	678 (1.1)	218	663 (0.3)	409 (4)	203 (15), 189 (22)
β-Amyryl octadecanoate	692 (0.6)	218	677 (0.3)	409 (3)	203 (32), 189 (17)
α-Amyryl octadecanoate	692 (0.7)	218	677 (0.3)	409 (4)	203 (14), 189 (17)
α-Amyryl nonadecanoate	706 (0.8)	218	691 (0.8)	409 (9)	203 (20), 189 (43)
β-Amyryl eicosanoate	720 (0.7)	218	705 (0.3)	409 (6)	203 (32), 189 (16)
α-Amyryl eicosanoate	720 (0.9)	218	705 (0.4)	409 (3)	203 (12), 189 (17)

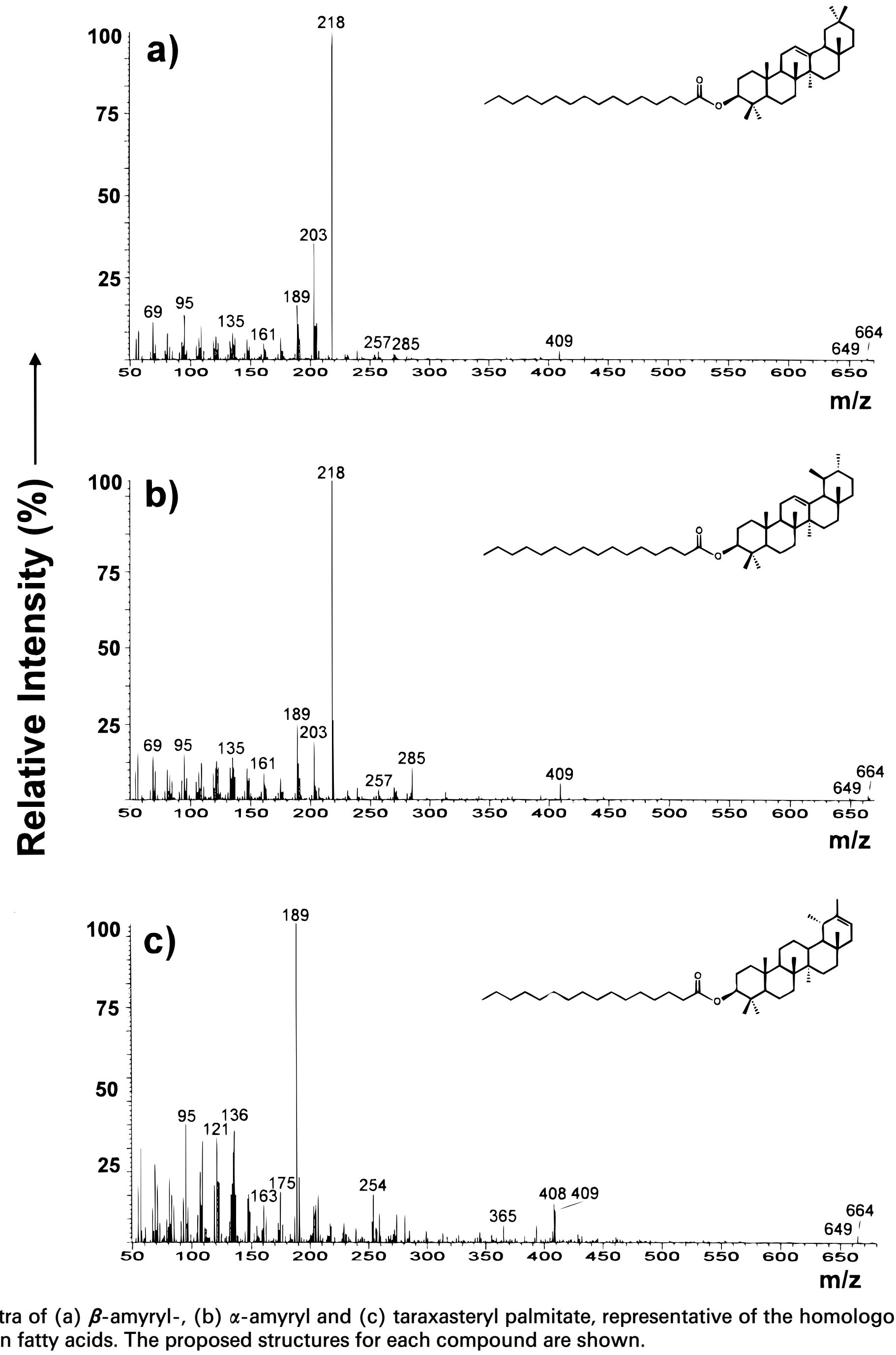

Figure 3. Mass spectra of (a) β-amyryl-, (b) α-amyryl and (c) taraxasteryl palmitate, representative of the homologous series of triterpenols esterified to long-chain fatty acids. The proposed structures for each compound are shown.

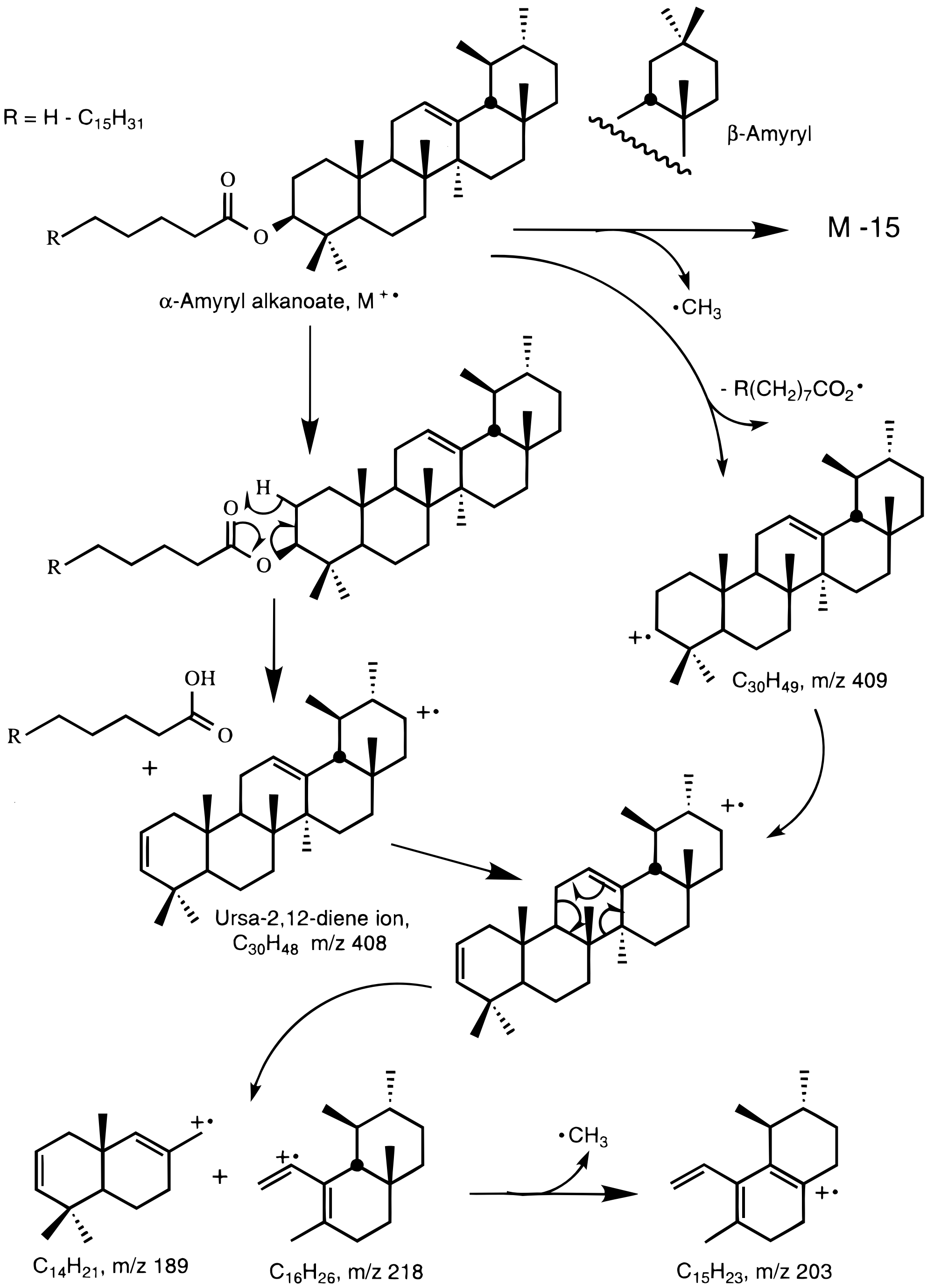

Figure 4. Scheme summarizing the mass spectrometric fragmentation pattern of triterpenyl fatty acid esters.

Table 2. Retention indices of the triterpenyl fatty acid esters

Triterpenyl fatty acid ester	Retention index[a]		
	β-Amyryl	α-Amyryl	Taraxasteryl
Pentanoate	3892	3944	—
Hexanoate	4027	4077	—
Heptanoate	4153	4207	—
Octanoate	4287	4339	—
Nonanoate	4415	4465	—
Decanoate	4539	4589	—
Undecanoate	4657	4704	—
Dodecanoate	4772	4820	—
Tridecanoate	4883	4930	—
Tetradecanoate	4989	5037	—
Pentadecanoate	5094	5141	—
Hexadecanoate	5196	5246	5324
Heptadecanoate	5291	5339	—
Octadecanoate	5387	5435	5517
Nonadecanoate	5480	5533	—
Eicosanoate	5585	5641	—

[a] GC stationary phase OV-1701-OH.

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