

# Study of *Burseraceae* resins used in binding media and varnishes from artworks by gas chromatography–mass spectrometry and pyrolysis–gas chromatography–mass spectrometry

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## Abstract

In the present work, a study attempting to characterize the Manila elemi and Mexican copal resins from the *Burseraceae* family, which are used as components of varnishes and binding media of artworks, has been carried out. A new GC–MS method involving the use of methyl chloroformate as derivatization reagent has been proposed. A second method which uses pyrolysis–GC–MS and hexamethyldisilazane as derivatization reagent has also been applied. Characterization of the main components of the mono-, sesqui- and triterpenoid fractions occurring in the raw materials has been achieved. Both  $\alpha$ - and  $\beta$ -amyrin have been established as the major triterpenoid compounds occurring in these resins together with hop-22(29)-en-3 $\beta$ -ol, found only in the Mexican copal. Artificially aged samples of Manila elemi and Mexican copal have also been analysed in order to study the stability of the triterpenoid components of the resins and their possible use as marker molecules. The results obtained indicate that these molecules, in particular,  $\alpha$ - and  $\beta$ -amyrin undergo oxidation processes during both artificial and natural ageing. Nevertheless, hop-22(29)-en-3 $\beta$ -ol could be selected as marker compound for Mexican copal. The proposed methods of analysis have been applied to real paint samples extracted from paintings in which Mexican copal was present as the main component of an “oleoresin” binding medium to assess their ability for identifying this product when used in artworks. Satisfactory identification of this resin is obtained by means of GC–MS whereas Py–GC–MS provides, in general, weaker signals for the components of the resin. Additionally, the influence of the pigments present in real samples on the resin ageing process has been considered.

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**Keywords:** Gas chromatography; Mass spectrometry; Pyrolysis; Methyl chloroformate; Hexamethyldisilazane; *Burseraceae* resin; Manila elemi; *Canarium luzonicum*; Mexican copal; *Bursera cuneata*; Artwork

## 1. Introduction

Triterpenic resins have been traditionally used as the main components of pictorial varnishes and binding media and, accordingly, the analysis of these natural products has claimed considerable attention in the field of cultural heritage in recent years. Among them, elemi, generic term applied to a

large number of resins from the *Burseraceae* family, has been used as component of varnishes, where other terpenoid products are included, to modify their consistency. These resins have been included in a number of recipes for preparing varnishes from the 19th century [1]. Over the 20th century elemi resin was added to waxes in relining processes (Dutch Process) for increasing the tackiness [2–4]. Moreover, elemi resin is frequently included in recipes of varnishes for toys, furniture, wagons and carriages, metals and swords, violin and musical instruments, gildings, etc. [5].

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The *Burseraceae* family includes over 600 species of plants yielding resins that are used, in some extent, for artistic purposes. The genus *Canarium* grows in Australia, South-East Asia and Africa. Several resinous species from this genus have, at various times, been placed in the European market under the name elemi. In particular, the product known as Manila elemi from the Philippine Islands, yielded from the species *Canarium luzonicum* or *Canarium commune* [4,6], has been mostly used in Fine Arts. These materials were imported to Europe, at latest, by the early 17th century although references to them are found in documents from 15th century [7].

Various species of the genera *Amyris*, *Bursera* and *Icica*, are largely distributed from central and south America. The products from *Bursera cuneata* (Schl) Engl., *Elaphirium Jacquinianum* (*Bursera tomentosa*), *Amyris elemifera* and *E. elemifer* occur in Mexico and California in opaque (sometimes translucent) fragments or semi-cylindrical pieces in pale-yellow or nearly white colour and have an agreeable odour. These products are known in Mexico under the name “copal” related with “copalli” that was the Aztec name for resinous materials, in general [7]. Nowadays, Mexican artists use these resins, which are easily available in the market, as binding medium for paintings together with linseed oil, wax and other triterpenic resins such as Manila elemi, dammar or mastic. Use of Mexican copal, as binding medium, by outstanding Mexican painters such as Gerardo Murillo (1875–1964) and Diego Rivera (1886–1957) has been reported. This last artist used Mexican copal in the wall painting “La Creación” placed in the San Ildefonso College (Mexico DF) among other works. In this case D. Rivera combined elemi resin, Mexican copal and wax (wax-oil of spike-elemi solved in oil of spike-Mexican copal solved in petroleum ether, 1:1:1:1) [8].

Brazilian elemi is a greenish-yellow, fragrant, translucent resin exuding from trunks of several species of *Icica* (e.g. *I. icicariba*) and *Protium* [9]. A number of resins employed for artistic purposes are obtained from the genus *Protium*. Thus, the Caraná balsam used as frankincense and as varnish ingredient is obtained from *Protium carana* (Humb.) L. Similarly, a solid light yellow resin obtained from *P. icicaribo* [10],

*P. guianense* and *P. leica elemifera*, is used as plasticizer of varnishes [11].

African elemi or oriental elemi, exuded from *Boswellia Frereana* [12], occurs in tears, fragments or large stalactitic pieces whose fracture is shell-like, exhibiting a transparent amber yellow interior.

The *Burseraceae* family also yields several resins, which contain water-soluble gums in some extent. Myrrhs from the genus *Commiphora* (*C. abyasimica*, *C. Schimperii*) and Olibanum (frankincense) from the genus *Boswellia* (*B. carteri*, *B. papyrifera*) are resinous products that have been chiefly used in medicine and cosmetics [3,11].

Despite the variety of uses given to *Burseraceae* resins, only a number of species have been systematically investigated. Thus, studies have been reported on essential oils (i.e. *C. luzonicum* [13]) or on the triterpenoid fraction (i.e. *C. Zeylanicum* [14]).  $\alpha$ - and  $\beta$ -amyrin ( $C_{30}H_{50}O$ ) (Fig. 1), formerly isolated from *Amyris* resins, have been identified as the main components of the triterpenoid fraction of elemi resins. These compounds together with different elemi acids ( $C_{30}H_{48}O_3$ ) from the euphane group have been reported as compounds occurring only in Manila elemi [7,15].

A series of earlier works focused on the determination of the chemical composition of triterpenic resins from the *Burseraceae* [13–22] and *Anacardiaceae* [23–30] families and the *Dipterocarpaceae* sub-family [31–42] have been reported. There, the analytical techniques used for characterizing the triterpenoid compounds present were X-ray diffraction, infrared (IR) spectroscopy, UV–visible spectrophotometry (UV–vis), nuclear magnetic resonance, mass spectrometry and thin-layer chromatography. In particular, MS has been proposed as a powerful tool for the structural study of polycyclic triterpenoids, being especially useful for the elucidation of the skeleton and for the location of double bonds in the triterpenoid molecules [43–45]. Ageing of triterpenoids has attracted attention of scientists in the field of conservation of artistic and cultural heritage. Studies dealing with photochemical and thermal degradation of triterpenoid resin films have been performed by means of UV–vis, IR and fluorescence spectrophotometry [46,47]. More recently, Fourier transform infrared and micro-Raman spectroscopies

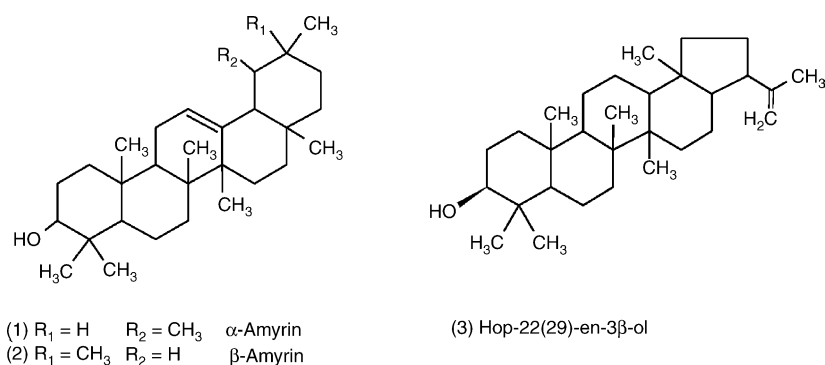


Fig. 1. Chemical structure of the main components of the triterpenoid fraction of *Burseraceae* resins: (1)  $\alpha$ -amyrin, (2)  $\beta$ -amyrin and (3) hop-22(29)-en-3 $\beta$ -ol.

have been proposed as alternative spectroscopic techniques for the analysis of varnishes composed by triterpenoid compounds [48,49]. Chromatographic techniques have also been extensively used in the characterization of ageing processes of triterpenic resins. GC–MS has been applied to the study of triterpenoid compounds [50–58]. The analysis is based on the formation of methyl esters from the carboxylic groups present in the triterpenoid components of resins using basic methanolysis [57,59], trimethylsilyldiazomethane [54], diazomethane [7,51,53,56,58] or  $\text{CH}_3\text{I}$  [55] as derivatisation reagents. Silylating reagents such as hexamethyldisilazane (HMDS) [50] or trimethylsilylchloride [50] have been also proposed.

Pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) is a typical hyphenated analytical technique that couples a pyrolyzer, a gas chromatograph and a mass spectrometer. Analytical pyrolysis was first applied in 1860 to help elucidate that the structure of natural rubber is polyisoprene [60]. Pyrolysis, which is defined as the chemical transformation of a sample when heated at a temperature significantly higher than ambient, is used in the Py-GC–MS technique as a procedure to load the sample into the GC–MS system [61]. Many molecules, in particular, those with polymeric structure, undergo decomposition and generate smaller, volatile molecules when pyrolysis is carried out, and thus they can be analysed using GC–MS. Pyrolysis has also been applied to the analysis of triterpenoid compounds present in artworks [62–65]. Methylation of pyrolysates with quaternary ammonium hydroxides has been proposed for the analysis of triterpenoid resins, especially, tetramethylammonium hydroxide [66,67] and trimethyl( $\alpha,\alpha,\alpha$ -trifluoro-*m*-tolyl)ammonium hydroxide [68]. Silylation has also proved useful to the analysis of terpenoid compounds. Among other silylating reagents, HMDS has been proposed for the analysis of di- and triterpenoid resins used in works of art [68].

Direct temperature-resolved mass spectrometry (DT-MS) has been recently applied to the analysis of triterpenoid resins [54,69–71]. During DT-MS measurements samples are gradually heated inside the ion source and thus, low molecular mass compounds are volatilised at low temperature whereas polymeric materials undergo pyrolytic degradation at high temperature under vacuum and their thermal dissociation products are quickly evaporated. Moreover, the vacuum conditions under which the pyrolytic process takes place reduce the probability of molecular collisions and hence the occurrence of secondary reactions. The volatile compounds formed are ionised and, finally, a summation spectrum corresponding to the multi-component mixture of volatilisation and thermal degradation products is obtained.

Other techniques that have also been successfully applied to the analysis of triterpenoid resins are liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry [72], graphite assisted laser desorption/ionisation [73,74], mass spectrometry, electron paramagnetic resonance [75] and direct inlet electron ionization mass spectrometry [76].

The general aim of the present work was to develop analytical methods able to characterize resins from the *Burseraceae* family in paint samples using GC–MS and Py-GC–MS. Particular targets of the research were the assay of methyl chloroformate (MCF) as derivatisation reagent for GC–MS as well as the study of pyrolysis in combination with “on line” trimethylsilylation with HMDS. Another goal was a comparison study of the results obtained from both GC–MS and Py-GC–MS techniques. Lastly, the developed analytical methodology was applied to various samples to know their terpenoid composition. These samples were Mexican copal from *Bursera cuneata* and Manila elemi from *C. luzonicum*. Accelerated light-aged samples of these resins, thermally treated samples of Mexican copal and paint samples in which Mexican copal had been applied as binding medium have been used to perform the proposed study.

## 2. Experimental

### 2.1. Solvents and reagents

The following reagents and solvents were used: hexamethyldisilazane and methyl chloroformate (purity 99%), absolute pyridine (Fluka, Buchs, Switzerland), chloroform (98% purity) for GC (Acros, Cambridge, USA), absolute methanol for analysis, ethanol, sodium hydrogencarbonate for analysis and turpentine (Panreac, Barcelona, Spain).

### 2.2. *Burseraceae* resin samples

#### 2.2.1. Raw materials

Two different *Burseraceae* resins have been studied in this paper as summarized in Table 1. Two samples of Manila elemi from the specie *C. luzonicum*, obtained from two different suppliers of artists' materials, were analysed. Agar-Agar (Vigo, Spain) provided a Manila elemi supplied by Kremer Pigmente (Aichstten/Allgäu, Germany) (sample A) and Droguería Riesgo (Madrid, Spain) provided a second sample of Manila elemi that has been stored in the laboratory (average storage conditions: 22 °C, 65% RH, darkness) since 1992 (sample B). Two samples of Mexican copal from *Bursera cuneata* from the supplier of artists' materials (Casa Sierra, Mexico DF, Mexico) (sample C) and from Sonora market (Sonora, Mexico) (sample D) were also analysed. Sample D was stored for 5 years in the studio of the Mexican artist Mrs Carmen López.

#### 2.2.2. Aged model varnishes

In addition to the analysis of the pieces of resin described above, three samples, to which two different artificial treatments were applied, were also analysed. A series of specimens were obtained with model spirit varnishes prepared by dissolving Manila elemi A and Mexican copal C in the appropriate solvents. The Manila elemi varnish (A.1) was prepared by dissolving 10 g of the resin in 100 ml ethanol

Table 1  
List and description of analysed samples

Group of samples	Reference	Resin type	Supplier	Storage time	Storage conditions	Artificial treatment conditions
Pure (untreated) resins	A	Manila elemi	Agar-Agar (Vigo, Spain) (original supplier: Kremer Pigmente Aichstten/Allgäu, Germany)	—	—	—
	B	Manila elemi	Droguería Riesgo (Madrid, Spain)	In the laboratory since 1992	22 °C, 65% RH, darkness	—
	C	Mexican copal	Casa Sierra (Mexico DF, Mexico)	—	—	—
	D	Mexican copal	Sonora Market (Sonora, Mexico)	In the artist's studio since 1999	—	—
Treated resins	A.1	Manila elemi A	—	—	—	Irradiation of thin film samples with a 36 W fluorescent lamp (maximum emission intensity between 350 and 400 nm) at a distance of 12 cm for 4 weeks
	C.1	Mexican copal C	—	—	—	As in A.1
	C.2	Mexican copal C	—	—	—	100 °C (heating block) for 10 min

whereas the Mexican copal varnish (C.1) was prepared by dissolving 67 g of resin in 100 ml turpentine. The freshly prepared varnishes were spread as a thin layer (average thickness, 50–80  $\mu\text{m}$ ) on glass slides. Thickness measurements of the varnish films were performed using a Cryo-Scanning Electron Microscope (Jeol JSM 5410). The prepared specimens were artificially light-aged in order to make an approach to the effect of ageing on the chemical composition of the resin. UV light ageing consisted of irradiating the samples with a 36 w OSRAM L36/73 fluorescent lamp, which emits UV light with maximum intensity between 350 and 400 nm, at a distance of 12 cm for 4 weeks. The environmental conditions within the box were recorded as about 3 °C above the room temperature and approximately 5% below ambient relative humidity (RH). Room conditions during this treatment were: 22 °C and 65% RH.

In order to reproduce the heating treatment applied by Mexican artists to Mexican copal resins during the preparation procedure of a binding medium, a piece of Mexican copal C was heated in a heating block at 100 °C for 10 min (sample C.2).

### 2.2.3. Paint samples

Six samples provided by Mrs López, from a number of 1-year-old paintings, where the “oleoresin” technique had been used, were analysed attempting to establish the influence of pigments and other components of the medium on the identification of the triterpenoid compounds from the resin. Mexican copal from *Bursera cuneata* was used as component of the binding medium. For preparing the varnish, the pieces of resin are dissolved in turpentine and then, the pertinent amount of beeswax is added to the solution, which is heated in water bath for 10 min to melt the wax. The obtained solution is added to a paint previously prepared where the pigment was dispersed in linseed oil (samples E.1–E.6). Amounts of the components used for preparing the paint, which were supplied by Casa Sierra, were not indicated by the artist [77].

## 2.3. Instrumentation

### 2.3.1. GC–MS

Experiments were carried out with a GC Agilent 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with an on-column injection system and coupled to an Agilent 5973N mass spectrometer (Agilent Technologies). A capillary column HP-5MS (5% phenyl–95% methylpolysiloxane, 30 m  $\times$  0.25 mm I.D., 0.25  $\mu\text{m}$  film thickness, Agilent Technologies) was used in order to provide adequate separation of components. Agilent ChemStation software G1701CA MSD was used for GC–MS control, peak integration and mass spectra evaluation.

The chromatographic conditions were as follows: the oven initial temperature was 170 °C with a gradient of 5 °C/min up to 185 °C, then a ramp of 15 °C/min to 250 °C and finally with a gradient of 5 °C/min to 295 °C which was held for



20 min. The injector temperature was 250 °C. The carrier gas was He with inlet pressure of 72.5 kPa and 1:20 split ratio. The electronic pressure control was set to constant flow mode with vacuum compensation.

Ions were generated by electron ionisation (70 eV) in the ionisation chamber of the mass spectrometer. The mass spectrometer was scanned from  $m/z$  20 to 800, with a cycle time of one second. Tuning of the mass spectrometer was checked using perfluoro-tributylamine. EI mass spectra were acquired by total ion monitoring mode and peak area data from total ion current (TIC) chromatograms were used for quantitative analysis. The temperatures of the interface and the source were 280 and 150 °C, respectively. Wiley Library of Mass Spectra was used for identifying compounds.

### 2.3.2. Pyrolysis-GC-MS

Experiments were carried out with an integrated system composed of a CDS Pyroprobe 1000 heated filament pyrolyser (Analytical Inc., New York, USA), and the GC-MS system described above equipped with a pyrolysis injection system. The capillary column and working conditions described in Section 2.3.1 were also used.

Different pyrolysis trials were performed at 300, 400, 500, 600 and 700 °C for 10 s. Pyrolysis was carried out using pre-calibrated Pt coil type pyrolysers (CDS pyroprobe). The pyrolyser interface and the inlet were set at 250 °C. The samples were injected in the split mode (split ratio 1:80). The chromatographic conditions were as follows: the oven initial temperature of 100 °C, was increased at 5 °C/min to 295 °C where it was held for 8 min. Helium gas flow was set at 1.6 ml min<sup>-1</sup>. The inlet pressure of the carrier gas was 72.5 kPa. The electronic pressure control was set to constant flow mode with vacuum compensation.

## 2.4. Preparation of samples

### 2.4.1. Off-line derivatization

The derivatization procedure was based on a previous methodology for the analysis of diterpenoid resins developed in our laboratory [78]. Samples were dissolved in 50 µl methanol-pyridine (4:1, v/v). Then, 10 µl MCF was added. The reaction mixture was vortexed for about 10 s and the derivatives were extracted in 50 µl chloroform containing 1% MCF. After that, 50 µl of a saturated solution of NaHCO<sub>3</sub> was added and the mixture was shaken carefully. A 1 µl aliquot of the organic phase was injected into the GC-MS system.

### 2.4.2. On-line derivatization-pyrolysis

Samples were directly pyrolysed in order to obtain the chromatograms (pyrograms) of the resins. One milligram of sample was placed in a quartz tube for pyrolysis with 20 µl HMDS and positioned in the platinum coil probe, which was then inserted into the interface. Then, samples were pyrolysed at 600 °C for 10 s.

## 3. Results and discussion

### 3.1. Analysis of raw materials

#### 3.1.1. GC-MS

The TIC chromatogram obtained by GC-MS from Manila elemi A is shown in Fig. 2a and the identified compounds are summarized in Table 2. The sesquiterpenoid fraction ranges from  $t_R$  3.50 to 6.00 min whereas the triterpenoid fraction is located at  $t_R$  > 20 min. The main sesquiterpenoids found in samples A and B are elemol (21), which was the most prominent peak in the sesquiterpenoid fraction,  $\gamma$ -eudesmol (22) and  $\beta$ -eudesmol (23) (see Fig. 3). It should be noted that in previous studies of various samples of Manila elemi resins [13,68] the same sesquiterpenoids have been isolated together with some others. In addition to the previously mentioned sesquiterpenoid compounds, the phenyl propanoid elemicin (20) was identified. The intense peak (24), which exhibited characteristic fragment ions  $m/z$  59, 149 and 189, has been tentatively attributed to a compound with structure related to  $\beta$ -eudesmol.

Samples C and D exhibited mostly peaks corresponding to monoterpenoids and a few sesquiterpenoids. The chromatogram obtained by GC-MS from sample C of Mexican copal is shown in Fig. 2b and the identified compounds are summarized in Table 2. The monoterpenoid and sesquiterpenoid fraction ranges from  $t_R$  2.50 to 20.00 min whereas the triterpenoid fraction is located at  $t_R$  > 20 min. The main monoterpenoids occurring in sample C are  $\alpha$ -pinene (3), *o*-cymene (2), limonene (8), sabinol (13) and 4-terpineol (14). The monoterpenoids verbenene (1), camphene (4),  $\beta$ -pinene (5),  $\alpha$ -phellandrene (6),  $\alpha$ -terpinene (7),  $\gamma$ -terpinene (9), verbenone (11),  $\alpha$ -terpinolene (10), carvacrol (12) and carvacrol methyl ether (15) and the sesquiterpenoids fenchyl acetate (16), *cis*-calamenene (17) and isolodene (18) (see Fig. 3) were also identified in this sample. The number of monoterpenoids was lower in sample D than in sample C (Table 2). Interestingly, the peak corresponding to the sesquiterpenoid *trans*-caryophyllene (19), which does not appear in sample C, was found in sample D. In addition to the mentioned compounds, sample D exhibited a prominent peak of hexanedioic acid, bis(2-ethylhexyl) ester (25).

The study carried out has been focused on the triterpenoid fraction due to its major analytical interest for characterizing triterpenoid resins. Likewise, the triterpenoids are present in larger amounts than the mono- and sesquiterpenoids owing to their lower volatility and, as consequence, they are more indicative of the presence of *Burseraceae* resin in paintings [79].

The GC-MS analysis carried out on samples A and B of Manila elemi reveals that the triterpenoid fraction consists of pentacyclic molecules with oleanane and ursane skeletons. The principal triterpenoid compounds are  $\alpha$ -amyrin (urs-12-en-3-ol) (35) and  $\beta$ -amyrin (olean-12-en-3-ol) (33). Sample B exhibits, in addition to these, a small peak at 15.10 min which is tentatively assigned to A-neooleana-3(5),12-diene

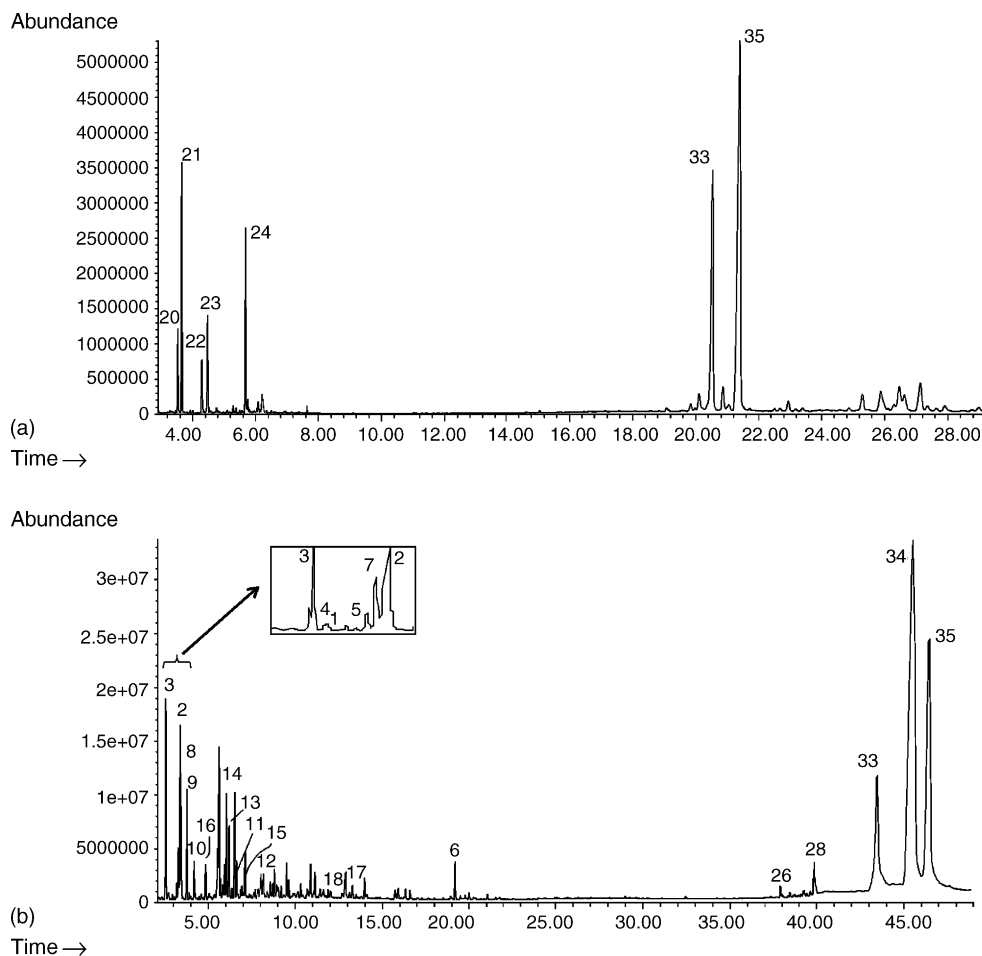


Fig. 2. TIC chromatogram of: (a) sample A of Manila elemi and (b) sample C of Mexican copal. Initial temperature 100 °C during 2 min. Ramp 5 °C/min until 295 °C held for 8 min.

(26). Formation of this compound from  $\beta$ -amyrin as a result of thermal alteration has been suggested in literature [80]. Presence of this compound in sample B (12-year-old), suggests that a chemical oxidation process could have started in the raw material at a low rate even though the storage temperature has not exceeded 22 °C. The compound appearing at 20 min is tentatively assigned to ursa-9(11),12-dien-3-ol (30). Similarly, compounds appearing at  $t_R$  from 24.00 to 28.00 min in chromatograms of both samples A and B have been assigned to the oleane/ursane group. Finally, it should be noted that elemi acids from the euphane group were not found in any of the analysed samples.

Samples C and D from Mexican copal exhibited a triterpenoid fraction simpler than that from the Manila elemi. The most prominent peaks correspond to  $\beta$ -amyrin (33), hop-22(29)-en-3 $\beta$ -ol (34) and  $\alpha$ -amyrin (35). Small amounts of urs-20-en-3-ol (28) and A-neoleana-3(5),12-diene (26) were also detected in sample C.

### 3.1.2. Py-GC-MS

Analysis of *Burseraceae* resins using the Py-GC-MS technique combined with use of a trimethylsilylating reagent was carried out in order to compare its results with those from

GC-MS. Use of Py-GC-MS technique allows the rapid analysis of solid samples, which are directly placed in the quartz tube and no treatment apart from addition of the derivatisation reagent is required.

First of all, a series of analysis was performed on raw materials to determine the temperature for performing the pyrolysis experiments on raw materials, aged samples and real samples. Thus, pyrograms were obtained at temperatures of 300, 400, 500, 600 and 700 °C. Fig. 4 shows the pyrograms obtained at 300, 400 and 600 °C on samples of Manila elemi A. In general, pyrograms in the studied range of temperatures exhibited a similar profile. The pyrogram obtained at 300 °C exhibited some weak peaks in the 14–20 min  $t_R$  range, which were not identified. They could tentatively be associated to decomposition products of the polymeric fraction of the resin (Fig. 4a). Their signals increased slightly when temperature was increased to 400 °C (Fig. 4b), 500 and 600 °C (Fig. 4c). Finally, pyrograms obtained at 700 °C were very similar to the ones obtained at 600 °C.

Trials on Mexican copal C in this same range of temperatures 300–700 °C provided pyrograms that show no significant changes, as can be seen in Fig. 5a and b, which display the pyrograms obtained at 300 and 600 °C, respectively.

Table 2

Main compounds identified by GC–MS in samples A, A.1 and B of Manila elemi and samples C, C.1, C.2 and D of Mexican copal

Peak	Assignment <sup>a</sup>	$M_w$	Manila elemi, <i>Canarium luzonicum</i>			Mexican copal, <i>Bursera cuneata</i>				Mass spectra data (70 eV), Characteristic ions: $m/z$ (%)
			A	A.1	B	C	C.1	C.2	D	
Monoterpenoid fraction										
1	Verbenene	134				✓				134 (3), 119 (18), 92 (44), 91 (100)
2	<i>o</i> -Cymene	134				✓	✓	✓	✓	134 (28), 119 (100), 91 (24)
3	$\alpha$ -Pinene	136				✓			✓	136 (9), 121 (15), 93 (100), 77 (30)
4	Camphene	136				✓			✓	136 (13), 121 (60), 107 (25), 93 (100), 79 (42)
5	$\beta$ -Pinene	136				✓			✓	136 (7), 121 (10), 93 (100), 69 (51), 41 (66)
6	$\alpha$ -Phellandrene	136				✓			✓	136 (22), 119 (13), 93 (100), 91 (75), 77 (43)
7	$\alpha$ -Terpinene	136				✓			✓	136 (50), 121 (100), 105 (20), 93 (84), 91 (49)
8	Limonene	136				✓	✓	✓	✓	136 (24), 121 (27), 107 (25), 93 (80), 79 (40), 68 (100)
9	$\gamma$ -Terpinene	136				✓		✓	✓	136 (37), 121 (31), 93 (100), 77 (35)
10	$\alpha$ -Terpinolene	136				✓	✓	✓	✓	136 (88), 121 (100), 93 (99)
11	Verbenone	150				✓				150 (37), 135 (70), 107 (100), 91 (74)
12	Carvacrol	150				✓				150 (34), 135 (100), 91 (18)
13	Sabinol	152				✓				134 (31), 92 (100), 91 (95), 81 (51)
14	4-Terpineol	154				✓			✓	154 (15), 111 (56), 93 (55), 71 (100)
15	Carvacrol methyl ether	164				✓				164 (35), 149 (100), 91 (23)
Sesquiterpenoid fraction										
16	Fenchyl acetate	196				✓				154 (9), 136 (36), 121 (45), 108 (32), 95 (100)
17	<i>cis</i> -Calamenene	202				✓				202 (10), 159 (100)
18	Isolatedene	204				✓				204 (54), 161 (100), 119 (41), 105 (44), 81 (53)
19	<i>trans</i> -Caryophyllene	204							✓	41 (100), 69 (77), 93 (91), 133 (78), 161 (29), 204 (6)
20	Elemicin <sup>b</sup>	208	✓		✓					208 (100), 193 (56)
21	Elemol	222	✓	✓	✓					204 (7), 189 (22), 161 (56), 93 (77), 59 (100)
22	$\gamma$ -Eudesmol	222	✓	✓	✓					222 (7), 204 (64), 189 (100), 161 (80)
23	$\beta$ -Eudesmol	222	✓	✓	✓					222 (4), 204 (25), 189 (29), 161 (32), 149 (73), 59 (100)
24	Product related to $\beta$ -eudesmol	?	✓	✓	✓					189 (9), 164 (35), 149 (100), 59 (73)
25	Hexanedioic acid, bis(2-ethylhexyl) ester	370							✓	259 (4), 241 (6), 147 (20), 129 (100), 112 (27), 57 (36)
Triterpenoid fraction										
26	A-neooleana-3(5), 12-diene	408			✓	✓				408 (8), 393 (12), 365 (100), 175 (48)
27	Ursa-9(11), 12-dien-3-one	422		✓			✓			422 (100), 281 (24), 269 (49), 255 (62), 207 (63)
28	Urs-20-en-3-ol	424				✓				408 (20), 393 (13), 218 (34), 207 (61), 189 (100)
29	Olean-9(11), 12-dien-3-ol	424		✓			✓			424 (100), 281 (23), 255 (62), 207 (47)
30	Ursa-9(11), 12-dien-3-ol	424		✓	✓		✓			424 (100), 295 (14), 255 (56), 207 (15)
31	$\beta$ -Amyrone	424		✓			✓			424 (5), 218 (100), 203 (52), 189 (16)
32	$\alpha$ -Amyrone	424		✓			✓			424 (8), 218 (100), 203 (27), 189 (23)
33	$\beta$ -Amyrin	426	✓	✓	✓	✓	✓	✓	✓	426 (3), 218 (100), 203 (48), 189 (15)
34	Hop-22(29)-en-3 $\beta$ -ol	426				✓	✓	✓	✓	426 (25), 218 (45), 207 (59), 189 (100), 135 (58)
35	$\alpha$ -myrin	426	✓	✓	✓	✓	✓	✓	✓	426 (7), 218 (100), 203 (23), 189 (24)

?, un-determined  $M_w$ .<sup>a</sup> Wiley Spectra Library was used for identifying compounds.<sup>b</sup> Phenyl propanoid compound.

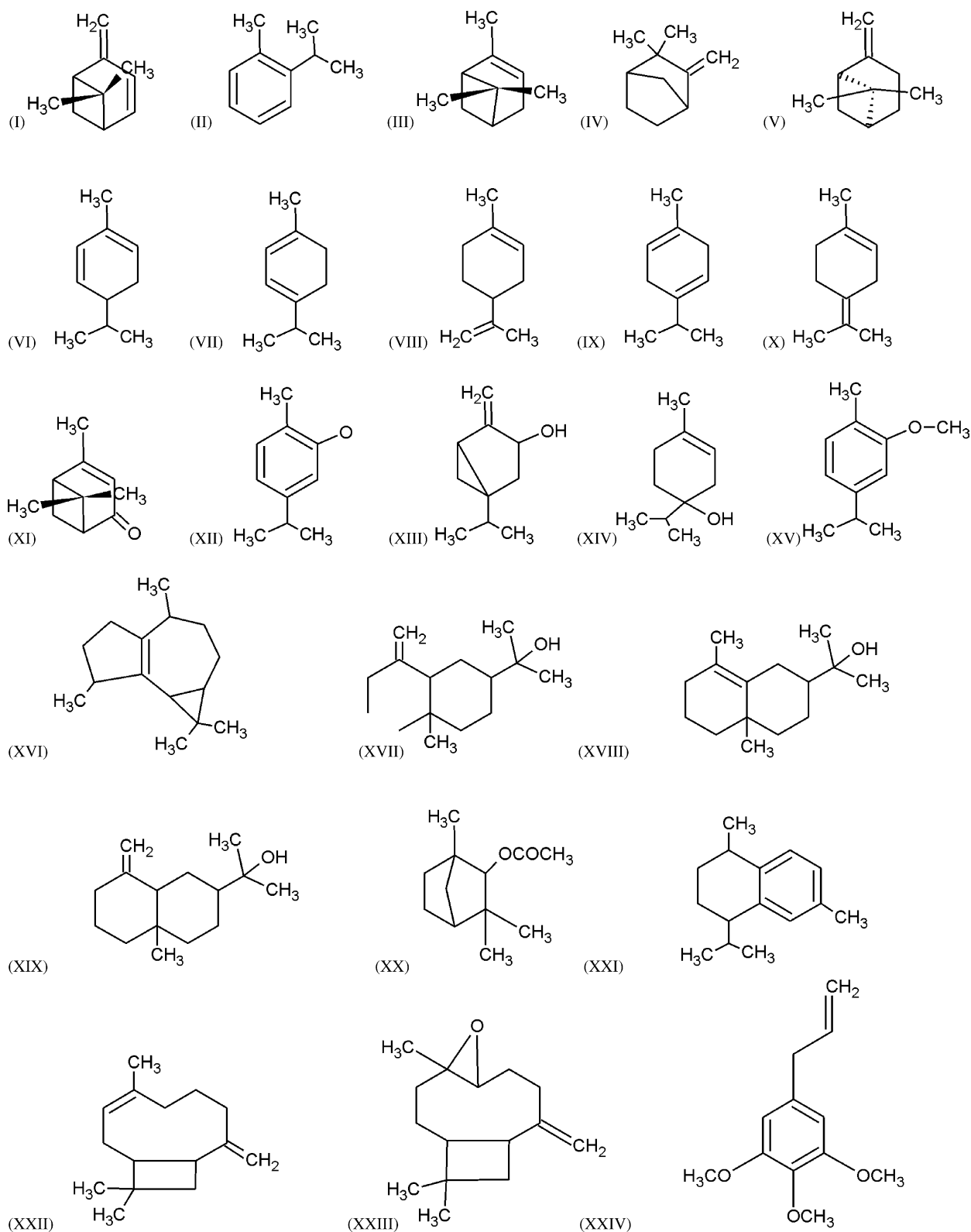


Fig. 3. Chemical structure of the main components of the mono and sesquiterpenoid fractions of studied resins: monoterpenoids: (I) verbenene; (II) *o*-cymene; (III)  $\alpha$ -pinene; (IV) camphene; (V)  $\beta$ -pinene; (VI)  $\alpha$ -phellandrene; (VII)  $\alpha$ -terpinene; (VIII) limonene; (IX)  $\gamma$ -terpinene; (X)  $\alpha$ -terpinolene; (XI) vervenone; (XII) carvacrol; (XIII) sabinol; (XIV) 4-terpineol; (XV) carvacrol methyl ether. Sesquiterpenoids: (XVI) isoleudene; (XVII) elemol; (XVIII)  $\gamma$ -eudesmol; (XIX)  $\beta$ -eudesmol; (XX) fenchyl acetate; (XXI) *cis*-calamenene; (XXII) *trans*-caryophyllene; (XXIII) caryophyllene oxide. A compound appearing in the monoterpenoid and sesquiterpenoid intervals: (XXIV) elemicin.



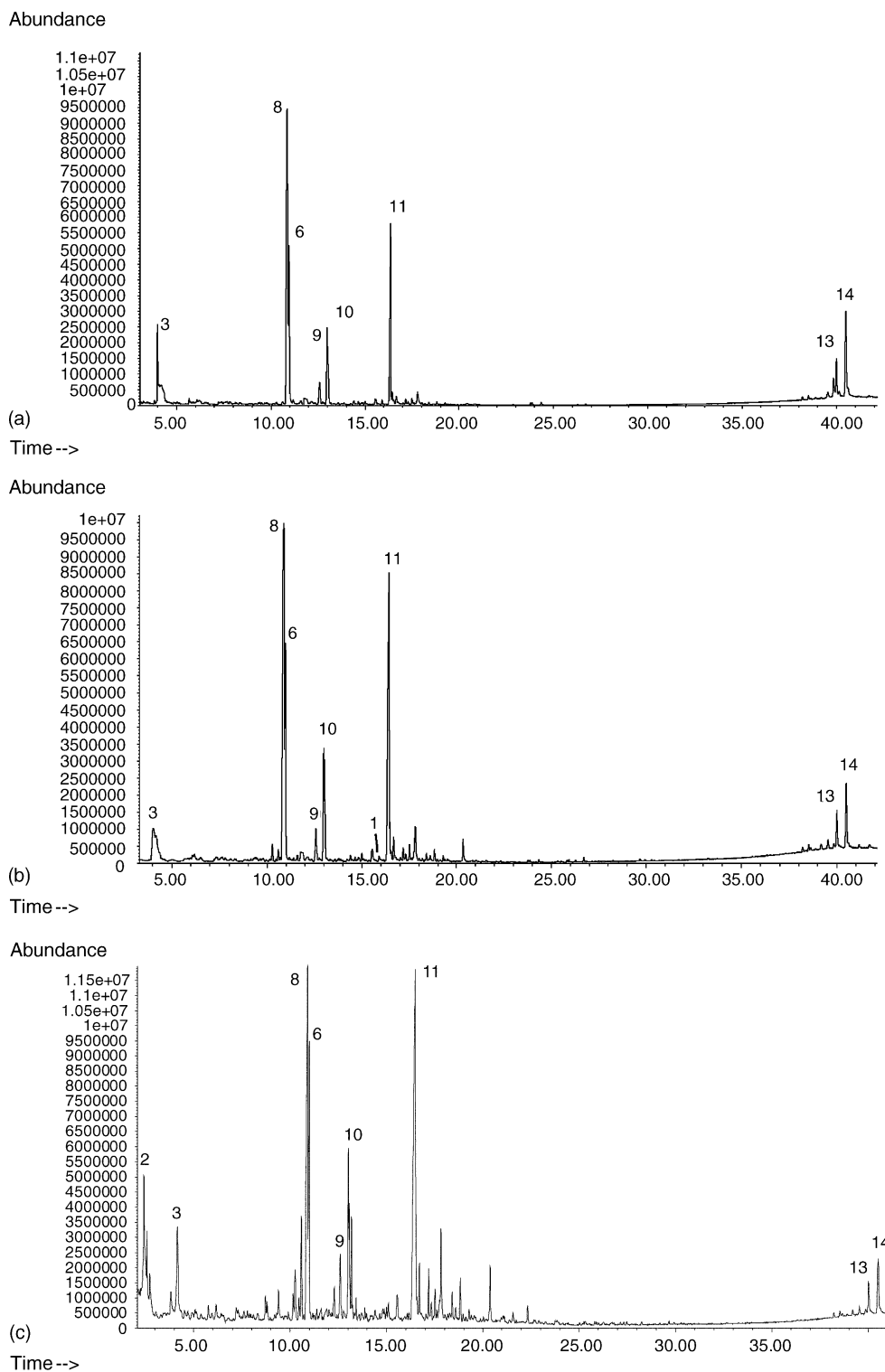


Fig. 4. Pyrograms corresponding to the Manila elemi A obtained at different pyrolyzer temperatures: (a) 300 °C, (b) 400 °C and (c) 600 °C.

As a result of this prior series of experiments, 600 °C was fixed as temperature for performing the Py-GC-MS analyses of the studied resins. In addition to the good resolution and sensitivity obtained at this temperature for the different terpenoid fractions considered in the Manila elemi samples, this

temperature is also suitable for analysing other compounds present in artworks such as diterpenoid resins, drying oils, waxes or natural dyes, when HMDS is used as derivatisation reagent [81,82]. Use of the same experimental conditions is valuable when an unknown sample is analysed, in particular,

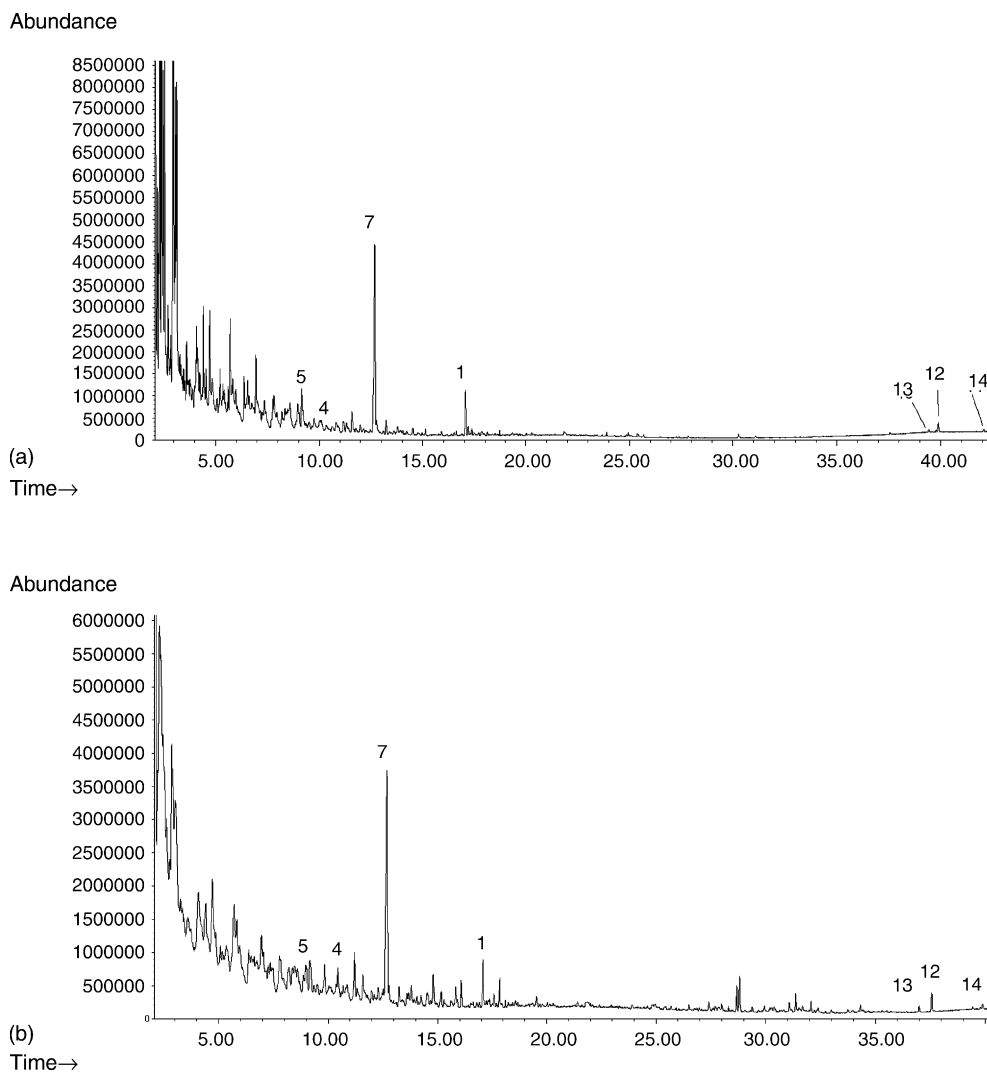


Fig. 5. Pyrogram of TMS derivatives corresponding to the Mexican copal C obtained at different pyrolyzer temperatures: (a) 300 °C and (b) 600 °C.

when a mixture of different natural products is suspected to be in the medium or varnish present in the painting.

According to what was found by means of GC–MS, elemol (8),  $\gamma$ -eudesmol (9) and  $\beta$ -eudesmol (10), the phenyl propanoid elemicin (6) and a compound related to  $\beta$ -eudesmol (11) were the main sesquiterpenoids found in samples A and B, as can be seen in Fig. 4c and Table 3. Interestingly, the monoterpenoids limonene (2) and 4-terpineol (3) were identified in Manila elemi A whereas this last compound was the only one identified in Manila elemi B.

The pyrogram of Mexican copal C at 600 °C (Fig. 5b) showed only the monoterpenoid  $\alpha$ -phellandrene (1) and the sesquiterpenoids *cis*-calamenene (4), *trans*-caryophyllene (5) and caryophyllene oxide (7) whereas only  $\alpha$ -phellandrene (1) and caryophyllene oxide (7) appear in Mexican copal D. No compound corresponding to the polymeric fraction of the Mexican copal or their decomposition products has been identified by using Py-GC–MS.

The triterpenoid fractions of the Manila elemi A and B exhibited peaks corresponding to  $\beta$ -amyrin (13), and  $\alpha$ -amyrin (14). Additionally, pyrograms of Mexican copal C and D showed peak corresponding to hop-22(29)-en-3 $\beta$ -ol (12) in good agreement to the results from GC–MS. The ability of the HMDS as derivatisation reagent is put in evidence with these three triterpenoids which were found as trimethylsilyl derivatives.

### 3.2. Analysis of artificially aged samples

GC–MS and Py-GC–MS analysis of artificially aged samples from resins of both origins have been compared to identify possible decomposition or oxidation products formed as consequence of degradation processes that can take place during resin ageing. Accelerated light aged Manila elemi A.1 and Mexican copal C.1 have been studied. Additionally, thermally treated Mexican copal C.2 has been also analysed by the two proposed chromatographic methods.

Table 3

Main compounds identified by Py-GC–MS in samples A, A.1 and B of Manila elemi and samples C, C.1, C.2 and D of Mexican copal

Peak	Assignment <sup>a</sup>	$M_w$	Manila elemi, <i>Canarium luzonicum</i>			Mexican copal, <i>Bursera cuneata</i>				Mass spectra data (70 eV), Characteristic ions: $m/z$ (%)
			A	A.1	B	C	C.1	C.2	D	
Monoterpenoid fraction										
1	$\alpha$ -Phellandrene	136				✓		✓	✓	136 (22), 119 (13), 93 (100), 91 (75), 77 (43)
2	Limonene	136	✓							136 (24), 121 (27), 107 (25), 93 (80), 79 (40), 68 (100)
3	4-Terpineol	154	✓		✓					154 (15), 111 (56), 93 (55), 71 (100)
Sesquiterpenoid fraction										
4	<i>cis</i> -Calamenene	202				✓		✓		202 (10), 159 (100)
5	<i>trans</i> -Caryophyllene	204				✓		✓		41 (100), 69 (77), 93 (91), 133 (78), 161 (29), 204 (6)
6	Elemicin <sup>b</sup>	208	✓		✓					208 (100), 193 (56)
7	Caryophyllene oxide	220				✓		✓	✓	190 (14), 175 (42), 121 (49), 107 (55), 93 (88), 79 (100), 93 (88)
8	Elemol	222	✓	✓	✓					204 (7), 189 (22), 161 (56), 93 (77), 59 (100)
9	$\gamma$ -Eudesmol	222	✓	✓	✓					222 (7), 204 (64), 189 (100), 161 (80)
10	$\beta$ -Eudesmol	222	✓	✓	✓					222 (4), 204 (25), 189 (29), 161 (32), 149 (73), 59 (100)
11	Product related to $\beta$ -Eudesmol	?	✓	✓	✓					189 (9), 164 (35), 149 (100), 59 (73)
Triterpenoid fraction										
12	Hop-22(29)-en-3 $\beta$ -ol TMS ether	498				✓	✓	✓	✓	498 (10), 393 (12), 207 (89), 189 (100), 73 (69)
13	$\beta$ -Amyrin TMS ether	498	✓	✓	✓	✓	✓	✓	✓	218 (100), 207 (44), 203 (42), 189 (20), 73 (24)
14	$\alpha$ -Amyrin TMS ether	498	✓	✓	✓	✓	✓	✓	✓	218 (100), 207 (25), 203 (19), 189 (15), 73 (26)

?, un-determined  $M_w$ .<sup>a</sup> Wiley Spectra Library was used for identifying compounds.<sup>b</sup> Phenyl propanoid compound.

### 3.2.1. GC–MS

Fig. 6a shows the chromatogram corresponding to the aged Manila elemi A.1. The sesquiterpenoid fraction of the chromatogram from sample A.1 is identical to that from the un-aged resin apart from the decrease in the intensity of peaks and the absence of the peak corresponding to elemicin. The triterpenoid fraction was characterised by the presence of new compounds assigned to urs-9(11),12-dien-3-one (27), olean-9(11),12-dien-3-ol (29), urs-9(11),12-dien-3-ol (30),  $\beta$ -amyron (31) and  $\alpha$ -amyron (32) (Table 2). According to Oros et al. [80], oxidation processes occurring on  $\beta$ -amyrin and  $\alpha$ -amyrin during the artificial ageing could be on the basis of the appearance of these two last compounds in the aged sample.

The results obtained on the light aged and thermally treated samples of Mexican copal C.1 and C.2 are listed in Table 2. In both cases, the number of monoterpenoids appearing in their chromatograms was notably lower than in chromatograms of the raw materials. Thus, *o*-cymene (2), limonene (8) and  $\alpha$ -terpinolene (10) were found in both samples and  $\gamma$ -terpinene (9) was additionally appearing in thermally treated sample C.2. Absence of sesquiterpenes

in both artificially treated samples was another interesting aspect to note in the results concerning the aged samples. Finally, the triterpenoid fraction of sample C.2 showed the same peaks than the raw resin whereas urs-9(11),12-dien-3-one (27), olean-9(11),12-dien-3-ol (29), urs-9(11),12-dien-3-ol (30) and  $\beta$ -amyron (31) were found in sample C.1. It is interesting to note that peak intensity of  $\beta$ -amyrin drastically decreased from the raw resin to the light aged whereas  $\beta$ -amyron and olean-9(11),12-dien-3-ol, among other compounds, appeared which supports the hypothesis that  $\beta$ -amyrin can undergo an oxidation reaction during the accelerated ageing process. Finally, the comparison of the results obtained from samples C.1 and C.2 suggests that thermal treatment applied to sample C.2 affects the composition of Mexican copal C in a lesser extent than light ageing applied to sample C.1.

### 3.2.2. Py-GC–MS

Compounds identified by means of Py-GC–MS in samples A.1, C.1 and C.2 are listed in Table 3. Results concerning sample A.1 are quite similar to those from GC–MS in the sesquiterpenoid fraction whereas only the TMS derivatives

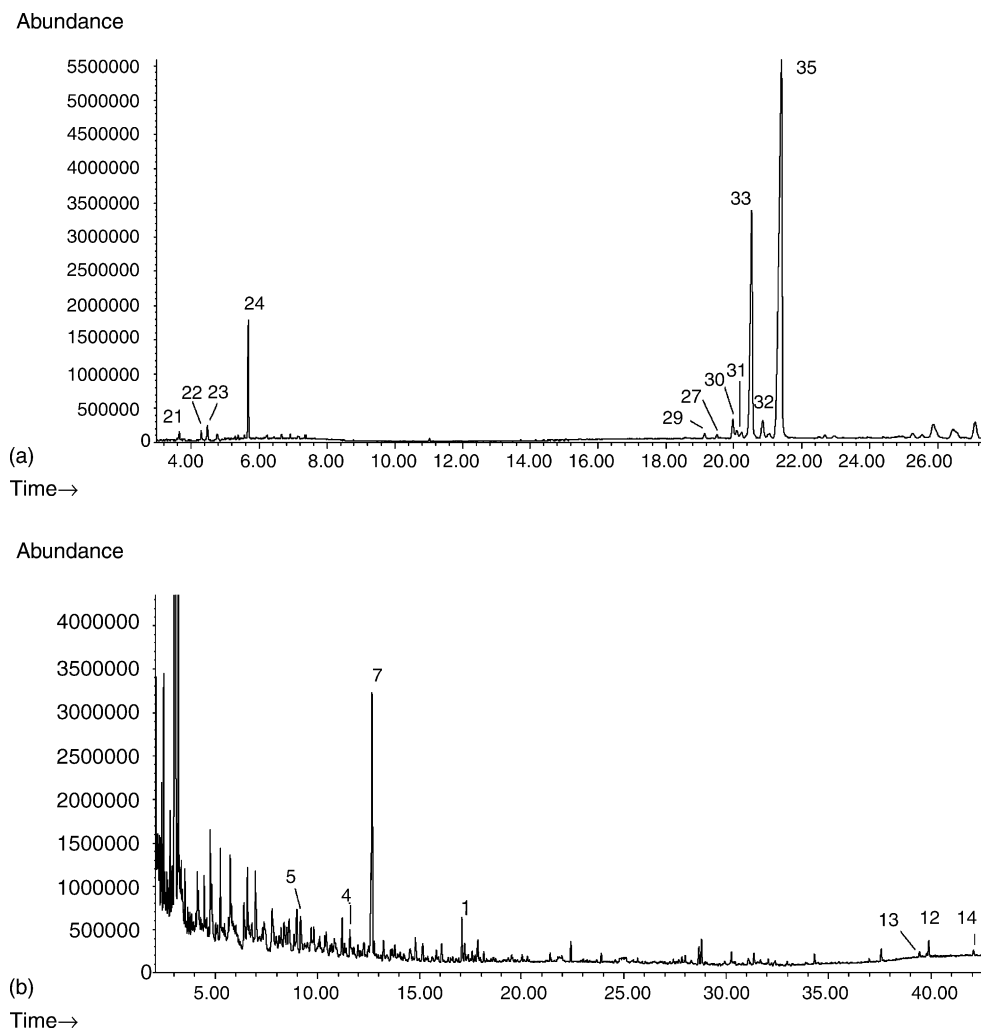


Fig. 6. (a) TIC chromatogram of light aged Manila elemi A.1 and (b) pyrogram of the TMS derivatives corresponding to the thermally treated Mexican copal C.2.

of  $\alpha$ -amyrin (14) and  $\beta$ -amyrin (13) were found in the triterpenoid fraction. However, a lesser number of compounds could be identified in samples C.1 and C.2 when Py-GC-MS was used. Sample C.1 exhibited peaks corresponding to the TMS derivatives of the three main triterpenoids present in this resin, namely,  $\alpha$ - and  $\beta$ -amyrin and hop-22(29)-en-3 $\beta$ -ol (12). Pyrogram of sample C.2 (Fig. 6b) exhibits peaks corresponding to the monoterpenoid  $\alpha$ -phellandrene (1) and the sesquiterpenoids *cis*-calamenene (4), *trans*-caryophyllene (5) and caryophyllene oxide (7) together with the triterpenoids  $\alpha$ - and  $\beta$ -amyrin and hop-22(29)-en-3 $\beta$ -ol. These results and those obtained by GC-MS suggest that thermal treatment applied in sample C.2 affects in a lesser extent to the composition of Mexican copal C than light ageing.

### 3.2.3. *Burseraceae* markers

Finding of significant amounts of  $\alpha$ - and  $\beta$ -amyrin in fossil resins dated on many millions of years old, previously reported in literature [7,20], suggests that these compounds have enough stability to remain unchanged during long peri-

ods of time and, therefore, they could be used as markers. Thus, the possible use of the triterpenoids  $\alpha$ -amyrin,  $\beta$ -amyrin and hop-22(29)-en-3 $\beta$ -ol as markers of the resins from *Burseraceae* family used in art works is considered in this section.

The ageing experiments performed in the present study on the two studied *Burseraceae* resins prepared as thin films demonstrate that some changes take place in such instances due to the readiness of the above mentioned triterpenoids to undergo oxidation processes yielding oxidation products such as  $\alpha$ - and  $\beta$ -amyrone and A-neooleana-3(5),12-diene. These processes have been observed not only in the aged thin films but also in the pieces of resin B stored for 12 years in the laboratory. A tentative assessment of the behaviour of such compounds can be based on the comparison of  $\beta$ -amyrin/ $\alpha$ -amyrin peak area ratios found for these compounds in the different samples analysed by using the GC-MS method. Average values of such parameters are listed in Table 4 together with the standard deviation corresponding to ten replicates. Samples A and B of untreated Manila elemi exhib-

Table 4

Values of hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin, hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin and  $\beta$ -amyrin/ $\alpha$ -amyrin peak area ratios corresponding to 10 replicates from Manila elemi and Mexican copal analysed by GC-MS

Sample	$\beta$ -Amyrin/ $\alpha$ -amyrin		Hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin		Hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin	
	Mean value	s	Mean value	s	Mean value	s
A	0.46	0.04	–	–	–	–
A.1	0.38	0.03	–	–	–	–
B	0.45	0.03	–	–	–	–
C	0.41	0.02	2.07	0.15	5.1	0.2
C.1	0.15	0.01	4.3	0.2	28.7	0.4
C.2	0.36	0.05	1.8	0.2	5.0	0.2
D	0.41	0.03	1.87	0.18	4.6	0.2
E.1 (chrome orange + chalk)	0.34	0.03	2.7	0.2	8.0	0.3
E.2 (Prussian blue)	0.30	0.02	2.6	0.2	8.6	0.3
E.3 (Mars black)	0.27	0.02	3.5	0.2	13.0	0.3
E.4 (titanium white + lead white)	0.32	0.03	3.6	0.2	11.1	0.3
E.5 (iron oxide red)	0.35	0.03	2.7	0.2	7.7	0.3
E.6 (chrome yellow)	0.26	0.02	2.6	0.2	10.2	0.3

ited values of  $\beta$ -amyrin/ $\alpha$ -amyrin of 0.45 and 0.46 whereas the value obtained from the sample of artificially aged Manila elemi A.1 was slightly lesser. Similar behaviour is observed in the Mexican copal. Untreated resins C and D exhibited values of 0.41 whereas values obtained in the light and thermally aged samples C.1 and C.2 were 0.15 and 0.36. Thus, the decrease of the  $\beta$ -amyrin/ $\alpha$ -amyrin ratio, specially in the light aged specimen, is consistent with the finding of oxidation products and suggests that these processes affect  $\beta$ -amyrin to a greater extent than  $\alpha$ -amyrin.

Values of hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin and hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin found in un-aged samples C and D and in the thermally aged sample C.2 are close, suggesting that changes undergone by the resin owing to the heating treatment do not significantly affect these molecules. However, values obtained in light aged sample C.1 are notably higher than those from raw materials or the thermally treated sample, in particular, the 28.7 value obtained for the hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin ratio. These results indicate that hop-22(29)-en-3 $\beta$ -ol is altered to a lesser extent than  $\alpha$ - or  $\beta$ -amyrin and this last compound mostly undergoes chemical transformation.

Apart from stability, univocal assignment of these molecules to the *Burseraceae* resins should consider other requirement necessarily exhibited for them in order to establish their possible use as markers when employed in artworks. In this context, some authors have reported the presence of  $\beta$ -amyrin in mastic resins. Marner et al. [52] and Assimopoulou and Papageorgiou [83] identified this compound in fresh mastic whereas other authors mentioned the possible presence of this compound in fresh resin despite they did not detect it [84]. In particular, Stern et al. [53] suggested co-elution of this compound together with other major components of mastic as the cause of the undetectable levels of  $\beta$ -amyrin found in their analyses. Concerning  $\alpha$ -amyrin, it must be noted that no report has been found in the literature with regard to the occurrence of this compound in mastic or dammar resins apart from a mention made by van der Doelen [84] to the finding of small

amounts of  $\alpha$ -amyrin and  $\beta$ -amyrin in fresh resin samples by other authors. Thus, presence of a significant amount of this compound in paint samples could be reasonably associated to resins from the *Burseraceae* family, at least, to the two ones studied. Most interestingly, occurrence of hop-22(29)-en-3 $\beta$ -ol in paint samples could be used to identify Mexican copal from *Bursera cuneata*. Nevertheless, discrimination of both studied products when used together seems difficult. This is the case of D. Rivera paintings, where, as previously mentioned, the author combined elemi resin and Mexican copal for preparing wall painting binders [8].

### 3.3. Analysis of paint samples containing Mexican copal as component of binding media

A series of experiments was performed on real paint samples in order to assess the efficiency of the two proposed methods for identifying *Burseraceae* resins used in paintings. Samples were taken from zones of the paintings where different pigments had been applied. The experiments comprised the analysis of ten replicates for each one of the six coloured zones chosen. A previous analysis attempting to identify the pigments used and to determine the purity of them was carried out using a X-ray microanalysis system coupled to a scanning electron microscope (SEM/EDX). The following pigments were identified: Chrome orange, a lead chromate ( $\text{PbCrO}_4$ ) [4] or lead chromate oxide ( $\text{Pb}_2(\text{CrO}_4)\text{O}$ ) [85,86], in sample E.1. Prussian blue, a ferric ferrocyanide ( $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$ ) [1,2] or  $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3 \cdot x \text{H}_2\text{O}$  ( $x = 14-16$ ) [85] or a potassium-ferric ferrocyanide,  $\text{KFe}_3[\text{Fe}(\text{CN})_6]$  [4], in sample E.2. Mars black,  $\approx \text{FeO} \cdot 3\text{Fe}_2\text{O}_3$  [1], in sample E.3. Mixture of titanium dioxide white, a titanium (IV) oxide ( $\text{TiO}_2$ ) [1,2,4,85], and lead white, a lead basic carbonate ( $2\text{PbCO}_3 \cdot \text{Pb}(\text{OH})_2$ ) [1,2,4,86], in sample E.4. Iron oxide red, an iron (III) oxide ( $\text{Fe}_2\text{O}_3$ ) [1,2,4,85], in sample E.5. Chrome yellow, a lead chromate ( $\text{PbCrO}_4$ ) [1,2,4,85] or ( $\text{PbCrO}_4 \cdot \text{PbSO}_4$  and  $\text{PbCrO}_4$ ) [86], in sample E.6. In addition to the mentioned pigments, Ca was found in all cases,

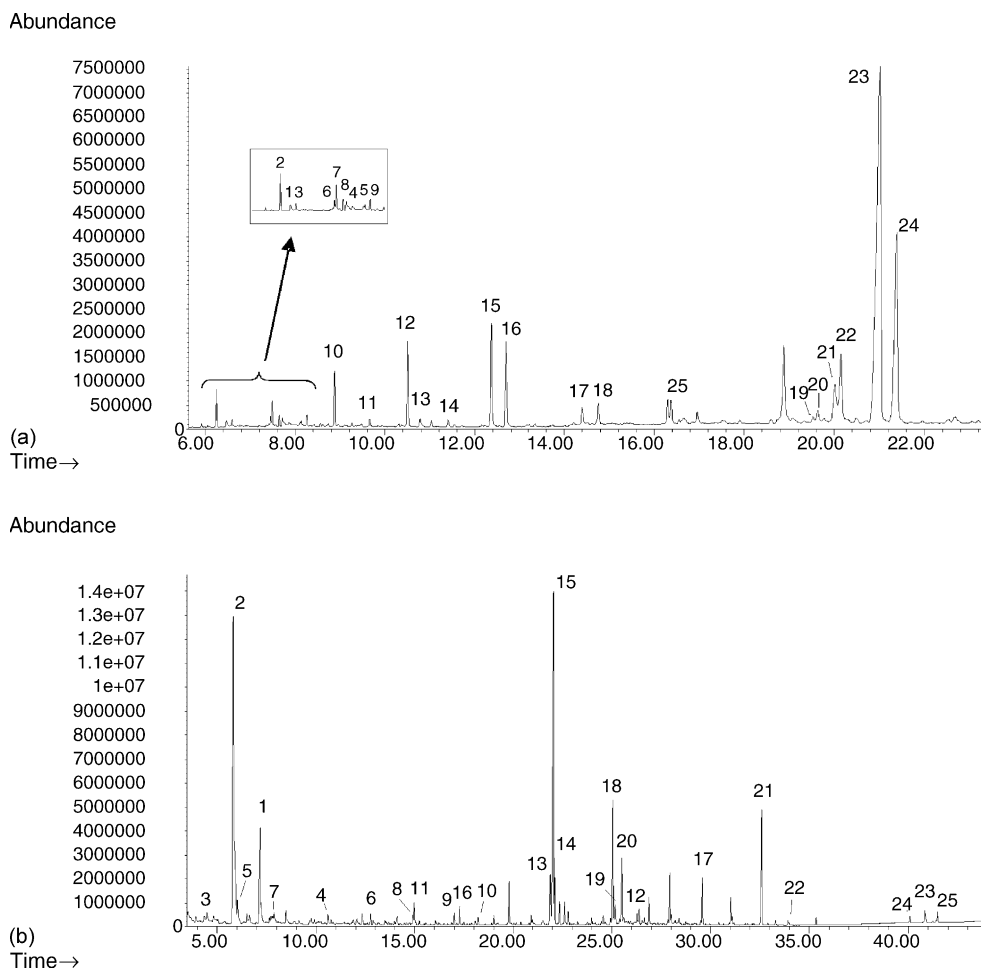


Fig. 7. (a) TIC chromatogram of paint sample E.2 and (b) pyrogram of the TMS derivatives corresponding to sample E.2.

associated to calcium carbonate ( $\text{CaCO}_3$ ), probably added to the colouring materials as an adulteration.

### 3.3.1. GC-MS

Fig. 7a shows the chromatogram corresponding to sample E.2. The chromatograms of paint samples were, in general, dominated by the resin peaks, which have been described earlier. A summary of the results is shown in Table 5. The most intense peaks were those from  $\alpha$ -amyrin (24) and hop-22(29)-en-3 $\beta$ -ol (23), although urs-9(11),12-dien-3-one (19), olean-9(11),12-dien-3-ol (20), urs-9(11),12-dien-3-ol (21) and  $\beta$ -amyrin (22) were also found. The rest of peaks were identified as components of the linseed oil and the wax used for preparing the oleoresin medium. Heneicosane (6), docosane (9), tricosane (10), tetracosane (11), pentacosane (12), hexacosane (14), heptacosane (15) and nonacosane (17), were the hydrocarbons found ascribed to the beeswax used by the artist for preparing the binding medium. Methyl esters of docosanoic acid (13), tetracosanoic acid (16), hexacosanoic acid (18), octacosanoic acid (25) and triacontanoic acid (26) were also ascribed to the beeswax used. Methyl ester of hexadecanoic (palmitic) acid (2), 9-octadecenoic (oleic) acid (7) and octadecanoic (stearic) acid (8) were associated to the

beeswax and linseed oil included in the medium formula. It must be noted that small amounts of underivatized palmitic acid (1), oleic acid (4) and stearic acid (5) were also present in the chromatogram.

Values of hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin, hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin and  $\beta$ -amyrin/ $\alpha$ -amyrin ratios from the series of paint samples are shown in Table 4. The quotients obtained from paint samples E.1 to E.6 have been compared with those from the pure Mexican copal C and D. In a similar way to the light aged sample C.1, the overall set of paint samples exhibited lower  $\beta$ -amyrin/ $\alpha$ -amyrin ratio than samples C and D from pure resin whereas higher values than those from pure resin were found for hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin and hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin ratios. Results indicate that Mars black produces the most significant change on the resin together with the sample of titanium white-lead white mixture. The two lead chromate pigments and the rest of pigments in which Fe was present exhibited values closer to these from the pure resins. The different extent in which each pigment affects the behaviour of the resin suggests that pigments could influence the oxidation processes taking place during the natural ageing of the resins. In addition to the pigment type, pigment-binding medium proportion used in the preparation



Table 5  
Main compounds identified by GC–MS in paint samples

Peak	Assignment <sup>a</sup>	$M_w$	Colour zone					
			E.1	E.2	E.3	E.4	E.5	E.6
1	Hexadecanoic acid	256	✓	✓	✓	✓	✓	✓
2	Hexadecanoic acid, methyl ester	270	✓	✓	✓	✓	✓	✓
3	Dibutyl phtalate	278		✓		✓	✓	
4	9-Octadecenoic acid	282	✓	✓	✓	✓	✓	✓
5	Octadecanoic acid	284	✓	✓		✓		✓
6	Heneicosane	296	✓	✓			✓	✓
7	9-Octadecenoic acid, methyl ester	296	✓	✓	✓	✓	✓	✓
8	Octadecanoic acid, methyl ester	298	✓	✓	✓	✓	✓	✓
9	Docosane	310		✓				
10	Tricosane	324	✓	✓	✓	✓	✓	✓
11	Tetracosane	338		✓	✓	✓		
12	Pentacosane	352	✓	✓	✓	✓	✓	✓
13	Docosanoic acid, methyl ester	354		✓	✓	✓		✓
14	Hexacosane	366		✓		✓		✓
15	Heptacosane	380	✓	✓	✓	✓	✓	✓
16	Tetracosanoic acid, methyl ester	382	✓	✓	✓	✓	✓	✓
17	Nonacosane	408	✓	✓	✓	✓	✓	✓
18	Hexacosanoic acid, methyl ester	410	✓	✓	✓	✓	✓	✓
19	Ursa-9(11),12-dien-3-one	422		✓	✓	✓	✓	✓
20	Olean-9(11),12-dien-3-ol	424	✓	✓	✓	✓	✓	✓
21	Ursa-9(11),12-dien-3-ol	424	✓	✓	✓	✓	✓	✓
22	β-Amyrin	426	✓	✓	✓	✓	✓	✓
23	Hop-22(29)-en-3-ol	426	✓	✓	✓	✓	✓	✓
24	α-Amyrin	426	✓	✓	✓	✓	✓	✓
25	Octacosanoic acid, methyl ester	438	✓	✓	✓	✓	✓	✓
26	Triacosanoic acid, methyl ester	466				✓		

<sup>a</sup> Wiley Spectra Library was used for identifying compounds.

Table 6  
Main compounds identified by Py-GC–MS in paint samples

Peak	Assignment <sup>a</sup>	$M_w$	Colour zone					
			E.1	E.2	E.3	E.4	E.5	E.6
1	Phthalic anhydride	148	✓	✓	✓	✓	✓	✓
2	Benzoic acid TMS ester	194	✓	✓	✓	✓	✓	✓
3	Heptanoic acid TMS ester	202	✓	✓	✓	✓		✓
4	Pentadecane	212		✓	✓			
5	Octanoic acid TMS ester	216	✓	✓	✓	✓	✓	✓
6	Hexadecene	224	✓	✓	✓		✓	
7	Nonanoic acid TMS ester	230		✓	✓	✓		✓
8	Heptadecene	238		✓	✓			
9	Octadecene	252		✓	✓			
10	Nonadecene	266		✓	✓			
11	Dodecanoic acid TMS ester	272		✓	✓		✓	
12	Tricosane	324	✓	✓	✓		✓	
13	9-cis-Hexadecenoic acid TMS ester	326	✓	✓	✓	✓	✓	✓
14	9-trans-Hexadecenoic acid TMS ester	326	✓	✓	✓	✓	✓	✓
15	Hexadecanoic acid TMS ester	328	✓	✓	✓	✓	✓	✓
16	1,9-Nonanedioic acid bisTMS ester	332	✓	✓	✓	✓	✓	✓
17	Pentacosane	352	✓	✓	✓	✓	✓	✓
18	9-cis-Octadecenoic acid TMS ester	354	✓	✓	✓	✓	✓	✓
19	9-trans-Octadecenoic acid TMS ester	354	✓	✓	✓	✓	✓	✓
20	Octadecanoic acid TMS ester	356	✓	✓	✓	✓	✓	✓
21	Heptacosane	380	✓	✓	✓	✓	✓	✓
22	Nonacosane	408	✓	✓	✓	✓	✓	✓
23	Hop-22(29)-en-3β-ol TMS ether	498	✓	✓	✓	✓	✓	✓
24	β-Amyrin TMS ether	498	✓	✓	✓	✓	✓	✓
25	α-Amyrin TMS ether	498	✓	✓	✓	✓	✓	✓

<sup>a</sup> Wiley Spectra Library was used for identifying compounds.

of the paints must be considered as factor affecting the composition of the triterpenoid fraction of the resin and hence, the analysis of resin. Unfortunately, there was no disposal of accurate quantitative composition of the real samples analysed in this work and, as consequence, this hypothesis has not been still demonstrated. Nevertheless, experiments aimed at resolving this issue are currently in progress.

### 3.3.2. Py-GC-MS

Fig. 7b shows the pyrogram corresponding to the sample E.2. It is interesting to note that weak signals of hop-22(29)-en-3 $\beta$ -ol (23),  $\beta$ -amyrin (24) and  $\alpha$ -amyrin (25) were obtained. The rest of peaks were identified as components of the linseed oil and the wax used for preparing the oleo-resin medium (see Table 6). It is interesting to note that a series of trimethylsilyl derivatives of short chain fatty mono- and di-acids could be detected by using Py-GC-MS. They are formed as result of degradation reactions, which involve binding of the atmospheric oxygen to the doubly or triply unsaturated fatty acids [87,88]. Thus, trimethylsilyl derivatives of heptanoic acid (3), octanoic acid (5), nonanoic acid (7), dodecanoic acid (11), and nonanedioic (azelaic) acid (16) were identified. In addition to the TMS derivatives of palmitic acid (15), oleic acid (18,19) and stearic acid (20) found by means of GC-MS, an unsaturated fatty acid, 9-hexadecenoic acid (palmitoleic acid) (13,14), from linseed oil, undetectable by GC-MS, was also found. Various hydrocarbons (peaks 4, 6, 8, 9, 10, 12, 17, 21 and 22) associated to the beeswax were also identified.

## 4. Conclusions

Analyses of samples of Manila elemi and Mexican copal have been performed using MCF as derivatisation reagent in GC-MS and on-line trimethylsilylation with HMDS combined with Py-GC-MS. GC-MS provides a more sensitive method for the mono-, sesqui- and triterpenoid fraction than Py-GC-MS and thus a larger number of compounds are identified by using this first technique. On the other hand, the derivatization reagent HMDS has been effective in the derivatisation of the major triterpenoid compounds present in the studied resins whereas MCF, especially active for carboxylic groups, has been not effective due to the absence of them in the identified compounds.

The GC-MS method enables the identification of a number of oxidation products formed during resin ageing. Comparison between samples of raw materials, artificially light aged and thermally treated resins has also enabled the study of the stability of the major triterpenoids  $\beta$ -amyrin,  $\alpha$ -amyrin, which occurred in the two types of resin and hop-22(29)-en-3 $\beta$ -ol, which only occurred in the Mexican copal. The thermal treatment undergone by the Mexican copal during the preparation of the binding medium does not significantly affect the composition of the triterpenoid fraction of the resin. Nevertheless, changes in composition due to natural and accelerated

ageing have been observed not only when the resins were as thin film but also when the resin was in pieces.

The values of  $\beta$ -amyrin/ $\alpha$ -amyrin, hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin and hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin peak area ratios have been calculated to evaluate the stability and the possible use of these three major triterpenoids as marker molecules of *Burseraceae* resins. Comparison of the triterpenoid ratio values for aged and un-aged resins indicates that  $\beta$ -amyrin decomposes faster than  $\alpha$ -amyrin in the Manila elemi and  $\beta$ - and  $\alpha$ -amyrin decomposes faster than hop-22(29)-en-3 $\beta$ -ol in the Mexican copal. Additionally, presence of small amounts of  $\beta$ -amyrin and  $\alpha$ -amyrin in fresh dammar and mastic has been previously reported in literature. Thus, identification of *Burseraceae* resins in paint samples based on the detection of these compounds should be considered after a rigorous study of the case. On the other hand, presence of a significant amount of hop-22(29)-en-3 $\beta$ -ol in paint samples could be reasonably associated to Mexican *Burseraceae* resins.

GC-MS analysis enables the identification of the Mexican copal, linseed oil and beeswax used for preparing the binding medium of a series of real paint samples. Fatty acids from beeswax and linseed oil, hydrocarbons from beeswax, and the three major triterpenoids from the Mexican copal have been identified. Therefore, this method can be considered suitable for the analysis of real paint samples in which Mexican copal has been used. Py-GC-MS was also successful in the identification of fatty acids from linseed oil and beeswax, and hydrocarbons from beeswax. Interestingly, short chain fatty mono acids and unsaturated fatty acids from the linseed oil were also detected. Nevertheless, the major triterpenoids from the Mexican copal were hardly recognized due to the weak signals appearing in the pyrograms. Finally, comparison of the  $\beta$ -amyrin/ $\alpha$ -amyrin, hop-22(29)-en-3 $\beta$ -ol/ $\alpha$ -amyrin and hop-22(29)-en-3 $\beta$ -ol/ $\beta$ -amyrin peak area ratio obtained by GC-MS from the real paint samples analysed suggests that pigments have a remarkable influence on the resin ageing.

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