

Analytical characterization of diterpenoid resins present in pictorial varnishes using pyrolysis–gas chromatography–mass spectrometry with on line trimethylsilylation

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Abstract

A procedure based on the technique of the pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) has been applied, in this work, in order to determine the composition of diterpenoid resin employed in art works. The method is based on the on line derivatization of these resins using hexamethyldisilazane (HMDS). Results obtained were compared with those previously reported in literature from Venice turpentine, Strasbourg turpentine, colophony, sandarac and Manila copal using this same method and with those others from in situ thermally assisted hydrolysis and methylation with tetramethylammonium hydroxide (TMAH). Canada balsam, copper resinate and Copaiba balsam have been also analyzed extending the scope of this method in the field of the analysis of artwork materials. Several non-reported trimethylsilylated derivatives of compounds present in the diterpenoid resins have been identified. An improvement in sensitivity has been obtained by using HMDS as derivatizing reagent, together with a better resolution of the most representative peaks. Additionally, this method reduces the number of pyrolytic fragmentation, recombination, dehydration and isomerization products formed during the pyrolysis process and, in consequence, more simplified chromatograms are obtained. Finally, the reported procedure has been successfully used for characterizing two diterpenoid resin-based varnishes present in the canvas painting “The Betrothal of the Virgin” (Anonymous, 17th century) which is included in the pictorial collection of Saint Joseph Church in Taormina (Italy) and the Magdalena Tryptich (Master of Alzira, 16th century, Valencia, Spain).

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1. Introduction

Terpenoid resins have been used for artistic purposes since very ancient times. Thus, Gettens and Stout [1] have reported the use of them as coatings of a coffin of the XIX Egyptian Dynasty. Similar findings were made from Carthaginian, Chinese and Japanese ancient artefacts. Sabin [2] mentions the use of resin-based varnishes in Persian, Indian, Chinese and Greek ancient cultures. History of manufacture of art materials and, in particular, of painting varnishes shows a close relationship with changes in preparation procedures. Oil varnishes were the earliest coating of paintings. The

vernice liquida, which consisted of sandarac or mastic and linseed or walnut oil and, next to that, the *vernice comune*, which was made of colophony or oleoresin and linseed oil, were commonly used in the Middle Ages [3]. Cennino Cennini [4] and latter, Leonardo da Vinci [5], among other artists [1], describe the process of varnish preparation in which the resin was melted to a clear liquid and then poured into hot drying oil.

Spirit varnishes were introduced about the 16th century. Spirits of wine and turpentine were the solvents mainly used. During the 17th century the manufacture of readymade materials became important and this fact is connected with the technological innovations and the development of Academies of Fine Arts. Since that time until nowadays, many art treatises and related documents have described

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varnish formulations in which diterpenoid resins, from Pinaceae, Cupressaceae, Araucariaceae families of the Coniferae group and some species of Leguminosae, are included. Palomino in his art treatise “The Pictorial Museum and Optical Scale” [6], describes a varnish in which pine resin (*Pinus* spp.) is the main component together with small amounts of sandarac and aloe which is added as coloring compound. Different formulations for musical instrument varnishes from 18th century have been reported in which pine resin is the main component [7]. Several art and crafts treatises from 19th century include recipes for turpentine spirit varnishes and lacquers based on colophony resin [8,9].

Venice turpentine oil varnish (*Larix* spp.) has been frequently used since 16th century, in particular, in Flandres. Venice turpentine has been often reported in varnish recipes as main component of oil varnishes [1]. Venice turpentine was used as plasticizer in turpentine spirit varnishes alone or together with oil of spike [1,7].

The European *Abies* species yield the oleoresin known as Strasbourg turpentine whereas the North American species yield the Canada balsam whose use as main component of varnishes or paint medium has been reported by Mills and White [7]. This Pinaceae resin has been also used as raw material for the preparation of the green pigment copper resinate [10].

Formulations based on the use of the Cupressaceae sandarac resin (*Tetraclinis articulata*) have been widely reported referred to both types of oil and spirit varnishes [1,2,6,8,9,11,12]. In some cases, sandarac is employed as additional ingredient for enhancing the hardness of the coating film [13].

Oil varnishes based on the Araucariaceae (*Agathis* genus) copal resins (i.e. Manila copal, *Agathis dammara*) have been widely used not only as painting varnishes [6,7,9] but also as paint media (i.e. Roberson’s medium [14]), and as materials for coating woodwork [15].

Leguminosae family includes a large variety of copal resins from the Caesalpinioideae subfamily. The Copaiba balsam (*Copaifera* genus) has been used as additive in paint media owing to the inhibitory effect on the drying of the oil paint, in lining conservation treatments of paintings, as cleaning product of varnishes by forming a soap with ammonia and as one of the variants of the Pettenkofer treatment applied to regenerate blanched varnishes [16].

Pyrolysis has been proposed for the analysis of diterpenoid compounds, present in art and archaeological objects, directly coupled to mass spectrometry (Py–MS) [17] or gas chromatography (Py–GC) [18]. Characterization of diterpenoids used as painting materials has been also carried out using the most powerful combination of pyrolysis, gas chromatography and mass spectrometry (Py–GC–MS) [19]. More recently, derivatization associated with the pyrolytic process has been proposed to improve the behavior of the analyte in the chromatographic column and to enhance its detectability. In situ thermally assisted hydrolysis and methylation (THM–GC–MS) performed with tetramethylammonium

hydroxide (TMAH) has been preferably used in the analysis of diterpenoid resins [16,20–25]. This reagent transmethylates the esters and methylates the free acids and hydroxyl groups, these latter depending on the pK_a [26,27].

Nevertheless, other derivatization techniques such as trimethylsilylation have probed their efficacy in the analysis of natural polymeric materials used in art works by means of Py–GC–MS. Among other derivatizing reagents, hexamethyldisilazane (HMDS) has been proposed for the analysis of natural products formed by polar molecules as in the case of proteins [28], lipids [29] and carbohydrates [30] or bis(trimethylsilyl)trifluoroacetamide (BSTFA) successfully used in the analysis of lignin [31]. More recently, this reagent has been applied to the analysis of Venice turpentine, Strasbourg turpentine, colophony, sandarac and Manila copal, all of them, diterpenoid resins commonly employed in the preparation of varnishes, binding media and pigments, among other artists’ materials [32].

In this work on line trimethylsilylation using HMDS with Py–GC–MS has been applied for the recognition of the main diterpenoid components of natural resins present in art works by means of Py–GC–MS. For this purpose, different diterpenoid resins, traditionally used in artworks as varnishes, lining materials, binding media and pigments were analyzed by means of Py–GC–MS combined with HMDS and the results obtained were compared with those from THM–GC–MS with TMAH [33]. In addition to Venice turpentine, Strasbourg turpentine, colophony, sandarac and Manila copal, previously analyzed by Chiavari et al. [32] by means of this technique, Canada Balsam, copper resinate and Copaiba balsam have been analyzed extending the scope of this method in the field of the analysis of artwork materials. Non-reported trimethylsilylated derivatives of compounds present in the studied diterpenoid resins have been identified.

Finally, the on line trimethylsilylation using HMDS with Py–GC–MS has been used for characterizing two diterpenoid resin-based varnishes found in the canvas painting “The Betrothal of the Virgin” (Anonymous, 17th century), which is included in the pictorial collection of Saint Joseph Church in Taormina (Italy) and the “Magdalena Tryptich” (Master of Alzira, 16th century, Valencia, Spain).

2. Experimental

2.1. Solvents and reagents

2.1.1. Analytical reagents

The following reagents were used to treat the samples: HMDS and TMAH pentahydrate (97%) (Sigma, Steinheim, Germany). Absolute ethanol for analysis was purchased from Panreac Quimica (Barcelona, Spain).

2.1.2. Diterpenoid standards

Strasbourg turpentine, Canada Balsam, copper resinate, Manila copal, sandarac and linseed oil (Kremer, Farbmühle,

Aichstten/Allgäu, Germany) were supplied by AP Fitzpatrick (London, UK). Colophony and Venice turpentine (Talens), were supplied by RCM (Productos de Conservación, Barcelona, Spain). Abietic acid (85%) was purchased from Sigma.

2.2. Instrumentation and procedures

2.2.1. Py–GC–MS

Experiments were carried out with an integrated system composed of a CDS Pyroprobe 1000 heated filament pyrolyser (CDS, Analytical Inc., USA), an Agilent 6890N gas chromatograph (Agilent Instruments, USA) coupled with an Agilent 5973N mass spectrometer (Agilent Instruments, USA) and equipped with both on-column and pyrolysis injection systems. Capillary column HP-5MS (stationary phase 5% phenyl–95% methylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness) was used in order to provide a suitable separation of pyrolysate components.

Pyrolysis was performed at 600 °C for 10 s. Pyrolysis was carried out using precalibrated Pt coil type pyrolyzer (CDS pyroprobe). The pyrolyser interface and the inlet were set at 250 °C. The samples were injected in split mode (split ratio 1:80). The chromatographic conditions were as follows: initial temperature of 100 °C increased at 5 °C min⁻¹ up to 295 °C held for 8 min. Helium gas flow was set at 1.6 mL min⁻¹. The inlet pressure of the carrier gas was 72.5 kPa. The electronic pressure control was set to constant flow mode with vacuum compensation.

Ions were generated by electron ionization (70 eV) in the ionization chamber of the mass spectrometer. The mass spectrometer was scanned from *m/z* 20 to 800, with a cycle time of 1 s. Agilent Chemstation software (MSD) was used for the integration of peaks and for the mass spectra evaluation. EI mass spectra were measured in the total ion-monitoring mode and the peak area (TIC) data was used for quantitative analysis.

Samples were placed in a micro quartz pyrolysis tube and then two small portions of quartz wool were introduced in both sides of the quartz tube in order to avoid undesirable displacements of the sample and 10 μL of 25% aqueous TMAH solution or 5–10 μL of HMDS were added. Afterwards, the sample was placed in the pyrolysis coil and introduced in the pyrolysis interface, which was kept at 250 °C.

2.3. Description of ancient varnishes studied

Analyses were performed on samples taken from panel and canvas paintings from different provenance:

- (1) The Magdalene Triptych (Master of Alzira, 16th century) (sample MA-1). The panels conserved represent the scene of the Magdalene's conversion and the scene of the Magdalene's offering to Jesus Christ.
- (2) The painting analyzed represents the scene of the Betrothal of the Virgin (sample T-4.1) and is included in the

canvas painting collection (anonymous, 17th century) of the Saint Joseph Church in Taormina (Italy).

2.4. Preparation of samples from paintings

A small fragment of the coating was scraped with the help of a scalpel in order to identify the diterpenoid compounds from the varnish and subsequently examined by on line trimethylsilylation by using Py–GC–MS and THM–GC–MS. The samples were prepared for analysis as was mentioned in Section 2.2.

3. Results and discussion

3.1. Analysis of diterpenoid resins

The potentiality of the pyrolysis in combination with *in situ* trimethylsilylation method proposed by Chiavari et al. [32] for the analysis of diterpenoid resins has been investigated in this work. The study carried out here is focused on the analysis of the diterpenoid fraction due to its major analytical interest for characterizing diterpenic resins.

A first experience was carried out in order to compare the sensitivity of both the HMDS trimethylsilylating reagent and the more currently used TMAH methylating reagent. Quantitative measurements were obtained from a 0.1 M standard solution of abietic acid in ethanol. One microliter of the abietic acid solution was directly placed in the quartz tubes used for pyrolysis. Then, the solution was evaporated to dryness in the quartz tube before adding the derivatizing reagent HMDS or TMAH. Values of peak areas of the dehydroabietic derivative obtained from three replicates of the standard solution derivatized with HMDS were 1.20 ± 0.03 times those from the methyl dehydroabietate peak derivatized using TMAH.

Influence of temperature on the distribution of pyrolysates was also considered in these previous experiences. Trials at different temperatures ranging from 400 to 800 °C on all the studied resins were performed in which the distribution of products observed was closely similar concluding that pyrolysis temperature within the studied range does not significantly affect the relative abundances of compounds obtained. Fig. 1 shows the total ion chromatograms corresponding to Manila copal for illustrating this trial. At 300 °C, a high level of residual materials were observed after pyrolysis in the quartz tube. Analyses performed at 900 °C revealed a significant effect on the observable distribution of some hydroxylated compounds as was evidenced by the slight decreasing of peaks corresponding trimethylsilyl 3-hydroxydehydroabietate and trimethylsilyl 15-hydroxydehydroabietate, which are characteristic compounds of Pinaceae resins, since dehydration reactions are promoted in those conditions. Above 800 °C increasing of secondary products of pyrolysis was also observed.

Chromatograms obtained from samples of copper resinate, in which both derivatization procedures using

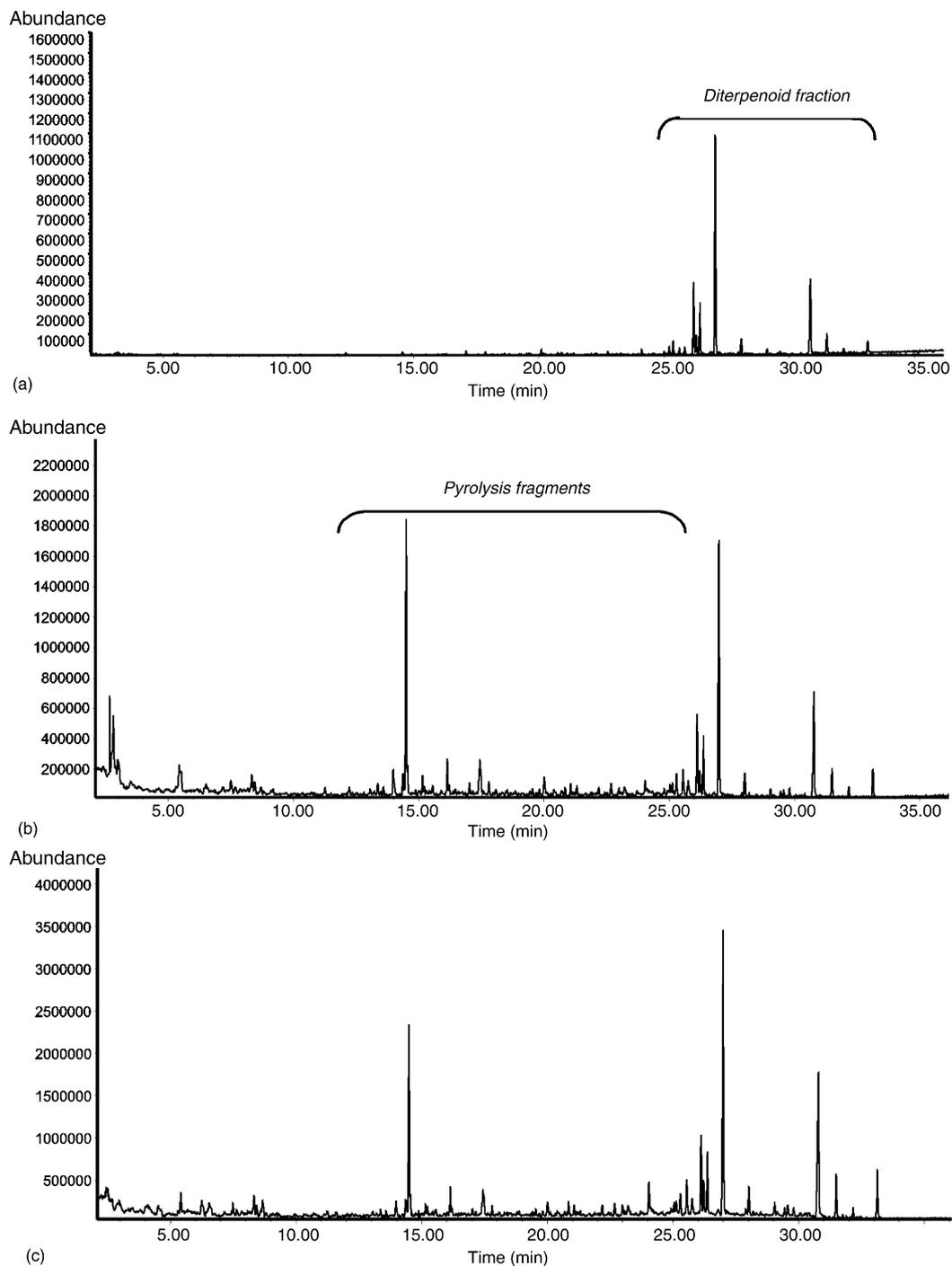


Fig. 1. Chromatogram obtained from on line trimethylsilylation Py-GC-MS of Manila copal using HMDS: (a) 400 °C, (b) 600 °C and (c) 800 °C.

HMDS and THM-TMAH were applied, are shown in Figs. 2 and 3, respectively. In addition to the higher sensitivity achieved from the derivatization with HMDS, a best resolution of peaks is also obtained. Peaks corresponding to methyl dehydro-dehydroabietate (**5***) and methyl dehydroabietate (**8***) are overlapped whereas the equivalent peaks of the trimethylsilylated derivatives are found at differentiated retention time. Similar results were obtained for the other resins studied and they suggest that a satisfactory

separation of the components from diterpenoid resins-based varnishes can be obtained from the trimethylsilylated derivatives.

3.1.1. Pinaceae resin-based varnishes

Table 1 summarizes the trimethylsilylated derivatives identified, using HMDS with Py-GC-MS, in samples of Canada balsam, copper resinates and also, colophony, Venice turpentine and Strasbourg turpentine. The most important

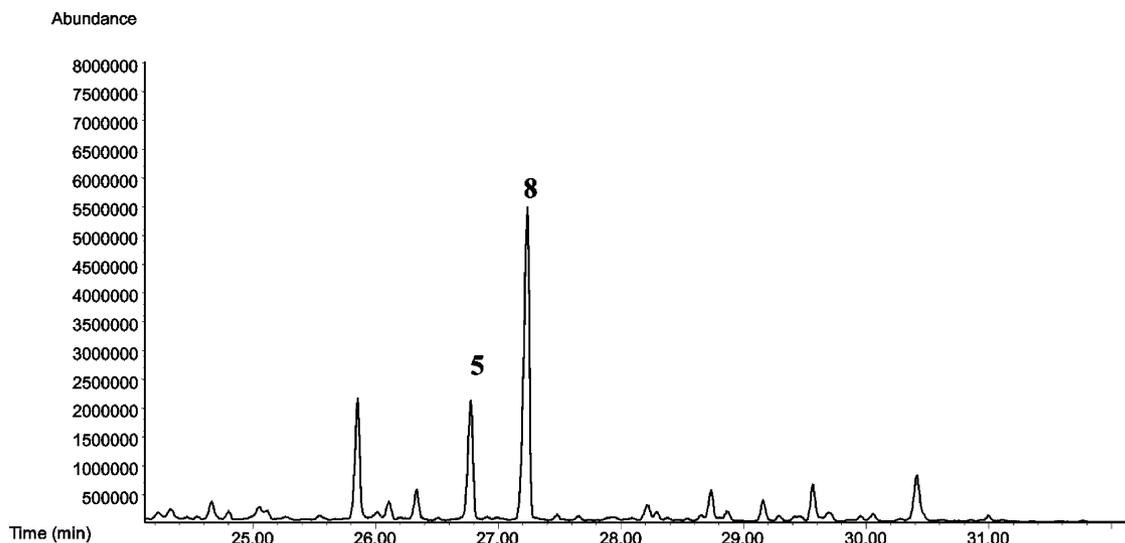


Fig. 2. Chromatogram obtained from on line trimethylsilylation Py-GC-MS of copper resinat using HMDS. Peak (5): TMS derivative of dehydro-dehydroabietic acid and peak (8): TMS derivative of dehydroabietic acid (see Table 1).

free diterpenoids have been recognized in the different resins analyzed according to the literature [24,34].

Pimarane and abietane are the two main diterpenoid skeletal types contained in the Pinaceae resin. In general, quantitative composition of abietane diterpenoid acids is variable owing to the isomerization of double bonds as in the case of laevopimaric acid/palustric acid, abietic acid and neoabietic acid. The proposed method of analysis leads to a proper identification of the overall free diterpenoid fraction present in the Pinaceae resins including pimaric acid (1), sandaracopimaric acid (2), isopimaric acid (3), palustric acid (4), isopimara-8,15-dienoic acid (7), abietic acid (9)

and related ester compounds such as larixyl acetate (6) only found in Venice turpentine.

It should be noted that derivatization with TMAH was successful in methylating carboxylic acids, but it resulted to be ineffective on hydroxyl groups, as reported previously in literature [25,35]. Derivatization with HMDS was effective on the hydroxyl group placed in 15-position of some oxidized acids such as 15-hydroxydehydroabietic acid and 15-hydroxy-7-oxodehydroabietic acid. Likewise, HMDS was effective in trimethylsilylating other compounds, including hydroxyl groups, from the drying oils present in the old varnishes tested as it will be described in Section

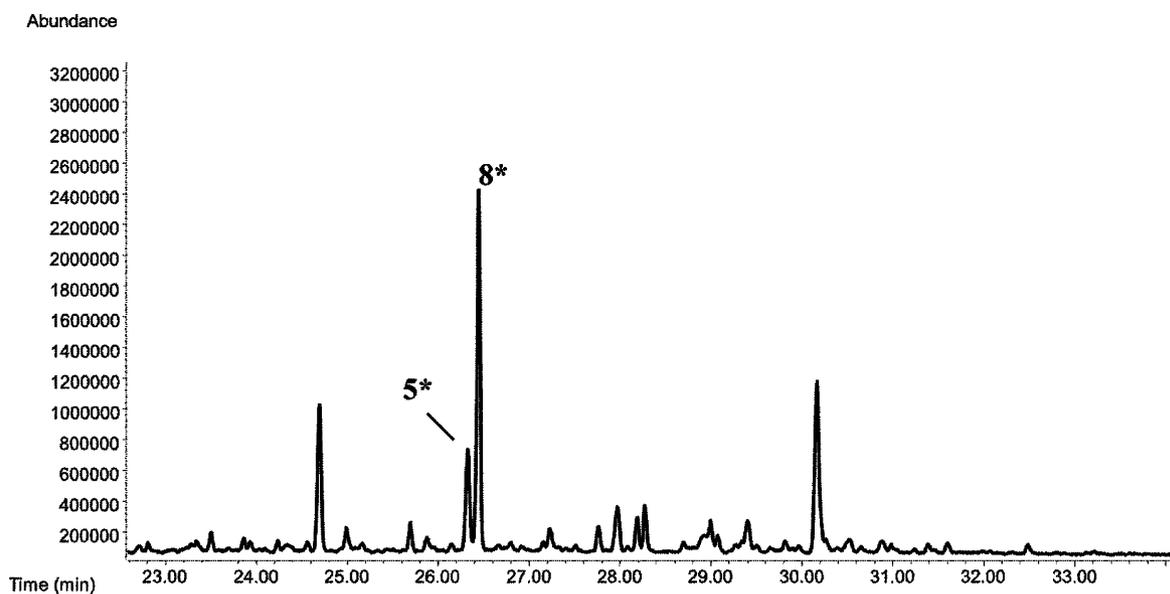


Fig. 3. Chromatogram obtained from on line methylation Py-GC-MS of copper resinat using TMAH. Peak (5*): methyl ester of dehydro-dehydroabietic acid and peak (8*): methyl ester of dehydroabietic acid.

Table 1
Abietadienes and pimaradienes identified in the Pinaceae resins by Py–GC–MS

Compound	t_R	M_r	Colophony	Strasbourg turpentine	Venetian turpentine	Copper resinates	Canada balsam	ID ^a
1 Pimaric acid TMS ester	25.35	374	✓		✓	✓	✓	L
2 Sandaracopimaric acid TMS ester	25.60	374	✓	✓	✓	✓	✓	L
3 Isopimaric acid TMS ester	25.83	374	✓	✓	✓	✓	✓	L
4 Palustric acid TMS ester	26.21	374		✓				P, I
5 Dehydro-dehydroabietic acid TMS ester ^b	26.27	370	✓	✓	✓	✓	✓	I
6 Larixyl acetate	26.45	348			✓			P
7 Isopimara-8,15-dienoic acid TMS ester	26.54	374		✓				P, I
8 Dehydroabietic acid TMS ester	26.75	372	✓	✓	✓	✓	✓	L
9 Abietic acid TMS ester	27.18	374	✓	✓	✓	✓	✓	L
10 Abieta-2,8,11,13,15-pentenoic acid TMS ester	27.74	368	✓	✓	✓	✓	✓	P
11 Abieta-8,11,13,15-tetraenoic acid TMS ester	28.26	366	✓	✓	✓	✓	✓	P
12 Neoabietic acid TMS ester	28.63	374	✓	✓	✓	✓	✓	L
13 7-Hydroxydehydroabietic acid TMS ether, TMS ester	28.70	460	✓	✓	✓	✓	✓	P
14 15-Hydroxydehydroabietic acid TMS ether, TMS ester	29.09	460	✓	✓	✓	✓	✓	P
15 Abieta-2,7,13-trienoic acid TMS ester	29.61	372	✓	✓	✓	✓	✓	P
16 7-Oxo-dehydroabietic acid TMS ester	29.97	386	✓	✓	✓	✓	✓	P
17 15-Hydroxy-7-oxo-dehydroabietic acid TMS ester	32.11	402	✓	✓	✓	✓	✓	P

^a Identification: L, Wiley Mass Spectra Library; P, published mass spectrum [21,34,35]; I, interpretation of MS fragmentation pattern.

^b Dehydro-dehydroabietic acid: 1,2,3,4,4a,10a-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-phenantrenecarboxylic acid [24].

3.3. These results suggest that the proposed trimethylsilylating method can be used for studying more complex systems composed by drying oils and diterpenoid resins, which are commonly used in the preparation of traditional varnishes.

Natural degradation processes occurring on abietane diterpenoid acids as consequence of natural ageing of the Pinaceae resins have been extensively described. Formation of 15-hydroxyabietic acid and 3 α -hydroxyabietic acid as result of a degradation process undergone by abietic acid has been reported [36,37]. Similarly, dehydroabietic acid occurs naturally and can be also formed from abietane diterpenoid acids by a dehydrogenation process [38]. This compound can undergo a dehydrogenation process to dehydro-dehydroabietic acid [24,39], can be oxidized to 7-oxodehydroabietic acid by formation of a peroxide next to a double bond and further loss of water [20,38], or can form a hydroxydehydroabietic acid after reduction of the intermediate peroxide formed [20,40]. In addition to those, different reaction pathways have been described in which dehydroabietic acid gives rise to several compounds, occurring in two higher stages of oxidation, such as 3,15-dihydroxydehydroabietic acid and 7-oxo-15-hydroxydehydroabietic acid [20]. Fig. 4 schematizes the different reaction pathways proposed to describe the degradation and oxidation products present in Pinaceae resins and the abietane compounds involved.

Studies focused on the effect of the pyrolysis process and experimental conditions involved on the nature and distribution of pyrolysates obtained in the analysis of Pinaceae resins have been also carried out. Particularly remarkable is the study carried out by Anderson and Winans [35] in which several compounds are obtained from hydroxyabietic and hydroxydehydroabietic acids as result of pyrolytic dehydration reactions involving the loss of water and formation of

a double bond. These same authors have also reported the occurrence of a significant number of N-containing products as consequence of reaction of the abietane compounds with the methylating reagent TMAH. Appearance of these undesirable byproducts considerably hinders the analysis and characterization of the highly oxidized abietane compounds. In a similar way, formation of three methylation products of 7-oxo-dehydroabietic acid and seven methylation products of 7-oxo-15-hydroxydehydroabietic acid, as consequence of side reactions occurring upon methylation with TMAH under Py–GC–MS conditions, have been reported by Pastorova et al. [21].

According to Anderson and Winans [35] trimethylsilyl abieta-8,11,13,15-tetraenoate formed by pyrolytic dehydration of 15-hydroxydehydroabietic acid has been found (11) in the samples analyzed. In a similar way, trimethylsilyl abieta-2,8,11,13,15-pentenoate and trimethylsilyl abieta-2,7,13-trienoate are derived from 3,15-dihydroxydehydroabietic acid and 2 α -dihydroxyabietic acid (10,15). Data obtained from GC–MS analysis confirmed that these products derive from pyrolysis–trimethylsilylation process. Fig. 4 schematizes, as an example, the different compounds found in the copper resinates using the proposed method.

It is of interest to note that derivatization with HMDS allows a reduction in the number of side reactions such as incorporation of nitrogen that complicates the interpretation of the results, as has been previously reported to occur using the in situ thermally assisted methylation with TMAH. Additionally, most of side reactions occurring upon methylation of the higher oxidized diterpenoids are avoided by using the proposed trimethylsilylation method. Therefore, a more simplified chromatogram is obtained, when derivatization with HMDS is performed, in which only a trimethylsilylation product is obtained from each abietadiene acid or

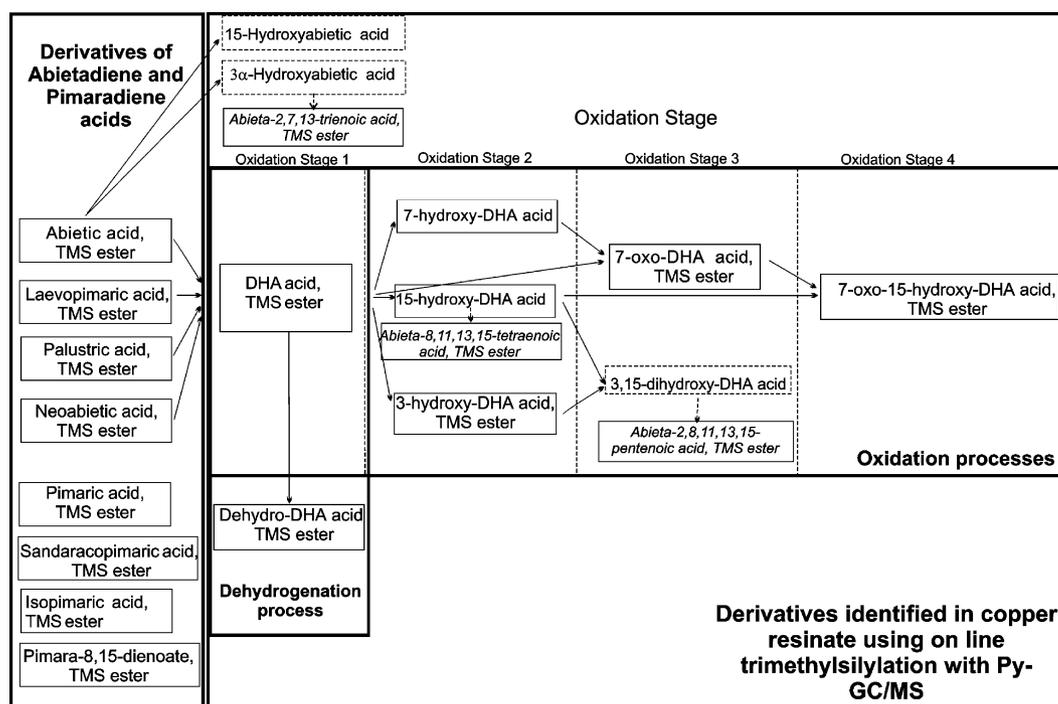


Fig. 4. Derivatives identified in copper resinates varnish using HMDS trimethylsilylation and Py-GC-MS.

oxidized diterpenoid acid. Likewise, three pyrolytic dehydration products (trimethylsilyl abieta-2,8,11,13,15-pentenoate, trimethylsilyl abieta-8,11,13,15-tetraenoate and trimethylsilyl abieta-2,7,13-trienoate) are only formed due to dehydration reactions. Thus, uncertainties in the calculations leading to the determination of the oxidation state of the diterpenoid material are notably reduced when trimethylsilylation is carried out. These calculations are based on the integration of the peak areas corresponding to each oxidized form present in the resin. In particular, summation of the peak areas corresponding to different pyrolysis products obtained from the same oxidized diterpenoid acid, when HMDS is used, is avoided for the majority of the compounds.

3.1.2. Cupressaceae and Araucariaceae resin-based varnishes

Composition of these two families within the Coniferae group is markedly different to that from the Pinaceae. Pimaradiene and labdane compounds are found in these families of diterpenoid resins together with polymerizable labdane compounds. Sandaracopimaric acid is the most abundant pimaradiene present in the sandarac Cupressaceae resin studied in this work. Agathic acid and related labdane compounds are predominant in the Manila copal. In addition to the free diterpenoids, polycommunic acid, formed from polymerization of communic acid, is also extensively contributing to the chemical composition of these two groups of resins.

Analysis of sandarac and Manila copal by THM-Py-GC-MS has been carried out in which free diterpenoids and pyrolysis compounds from the polymer fraction have been identified [25]. These authors also

identified a large number of compounds formed as result of a variety of secondary pyrolysis reactions such as fragmentation, isomerization and recombination reactions undergone by the free diterpenoid present in the resins.

According to the previous studies reported in literature [32] pyrolysis using HMDS as derivatizing reagent enables the simultaneous inspection of free diterpenoids and pyrolysis fragments of the polymeric fraction, mainly acid and ester monomers.

Fig. 5 shows the chromatogram obtained from on line trimethylsilylation with Py-GC-MS of Manila copal using HMDS and the trimethylsilyl derivatives obtained are listed in Table 2. *cis*-Communic acid (9), sandaracopimaric acid (10), *trans*-communic acid (11), agathalic acid (16), agathic acid (19) and its mono methyl ester (17), agatholic acid (18) and acetoxy agatholic acid (20) trimethylsilyl derivatives have been the free diterpenoids identified by means of the proposed method. Mono methyl ester of agathic acid occurs in the natural resin together with the agathic acid. It should be noted that HMDS allow the proper identification of both compounds and improves the results obtained with TMAH, which only affords a derivative and, therefore, it results unable to discriminate both compounds. Thus, the proposed method usable for estimates of the different extent in which each compound is present in the resin. Fig. 6a and b shows the mass spectra of the mono TMS ester of methyl agathic acid and the di-TMS ester of agathic acid. Characteristic fragment ions formed from these labdadiene trimethylsilyl derivatives, according to the fragmentation pathway proposed by Scalzone et al. [25] have been depicted in the bar diagram and are also summarized in Table 3. The communic acids can occur as

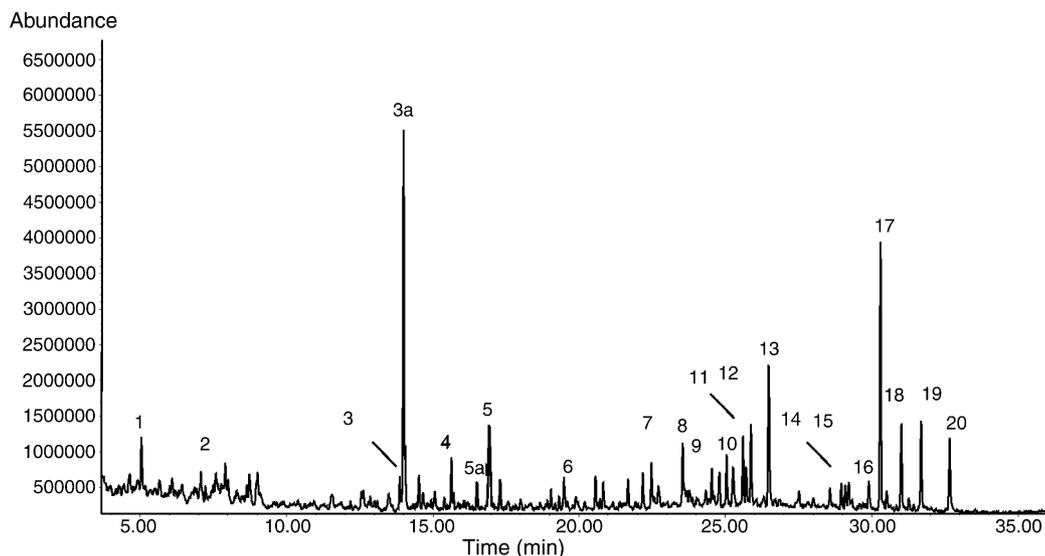


Fig. 5. Chromatogram obtained from on line trimethylsilylation Py-GC-MS of Manila copal using HMDS.

free acids but also can be formed from the pyrolysis of polycommunic acid. The compounds in the range of retention time from 10 to 23 min have been identified as pyrolysis products of the polymeric fraction, namely, 1,3-dimethyl-cyclohexa-2-en-carboxylic acid (**1**), 1,2,3-trimethyl-cyclohexa-2-en-carboxylic acid (**2**), 1,4a,6-trimethyl-1,2,3,4,4a,7,8,8a-octahydro-naphthalen-carboxylic acid (**3**), 1,4a,6-trimethyl-5-methylene-1,2,3,4,4a,5,8,8a-octahydro-naphthalen-carbo-

xylic acid (**4**), 1,4a,5,6-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-naphthalen-carboxylic acid (**5**), 5-ethyl-1,4a,6-trimethyl-1,2,3,4-tetrahydro-naphthalen-carboxylic acid (**6**), 5-(3-methyl-but-2-enyl)-6-methylene-1,4a,6-trimethyl-decahydro-naphthalen-carboxylic acid (**7**) and 3-methyl-(5,5,8a-trimethyl-2-methylene-decahydro-naphthalen-1-yl)-3-methyl-pent-2-enoic acid (**8**) trimethylsilyl derivatives, 19-norlabda-8(20),13-dien-15-oic acid (**12**) and 19-

Table 2

Pimaradienes, labdanes and related compounds identified in the Manila copal resin by Py-GC-MS

No.	Compound name	t_R	M_W	Resin		ID ^a
				Manila copal	Sandarac	
1	1,3-Dimethyl-cyclohexa-2-en-carboxylic acid TMS ester	5.05	226	✓	✓	P
2	1,2,3-Trimethyl-cyclohexa-2-en-carboxylic acid TMS ester	7.07	240	✓	✓	P
3	1,4a,6-Trimethyl-1,2,3,4,4a,7,8,8a-octahydro-naphthalen-carboxylic acid TMS ester	14.06	294	✓	✓	P
3a	Isomer of compound 3	14.10	294	✓	✓	P
4	1,4a,6-Trimethyl-5-methylene-1,2,3,4,4a,7,8,8a-octahydro-naphthalen-carboxylic acid TMS ester	15.65	306	✓	✓	P
5	1,4a,5,6-Tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-naphthalen-carboxylic acid TMS ester	16.95	308	✓	✓	P
5a	Isomer of compound 5	16.53	308	✓	✓	P
6	5-Ethyl-1,4a,6-trimethyl-1,2,3,4-tetrahydro-naphthalen-carboxylic acid TMS ester	19.51	318	✓	✓	P
7	5-(3-Methyl-but-2-enyl)-6-methylene-1,4a,6-trimethyl-decahydro-naphthalen-carboxylate	22.50	362	✓	✓	P
8	3-Methyl-(5,5,8a-trimethyl-2-methylene-decahydro-naphthalen-1-yl)-3-methyl-pent-2-enoic acid TMS ester	23.55	376	✓	✓	P
9	<i>cis</i> -Communic acid TMS ester	25.07	374	✓	✓	L
10	Sandaracopimaric acid TMS ester	25.64	374	✓	✓	L
11	<i>trans</i> -Communic acid TMS ester	25.73	374	✓	✓	L
12	19-Norlabda-8(20),13-dien-15-oate	25.89	362	✓	✓	P
13	Isopimaric acid TMS ester	26.49	374	✓	✓	L
14	5(5-Methoxymethyl-5,8a-dimethyl-2-methylene-decahydro-naphthalen-1-yl)-3-methyl-pent-2-enoic acid TMS ester	28.96	406	✓	✓	P
15	19-Norlabda-4,8(20),13-trien-15-oate	29.10	360	✓	✓	P
16	Agathalic acid TMS ester	29.91	390	✓	✓	P
17	Methyl agathalic acid TMS ester	30.30	420	✓	✓	I
18	Agatholic acid TMS ether, TMS ester	31.01	464	✓	✓	P
19	Agathic acid di-TMS ester	31.69	478	✓	✓	I
20	Acetoxy agatholic acid TMS ether, TMS ester	32.67	434	✓	✓	P

^a Identification: L, Wiley Mass Spectra Library; P, published mass spectrum [25,39]; I, interpretation of MS fragmentation pattern.

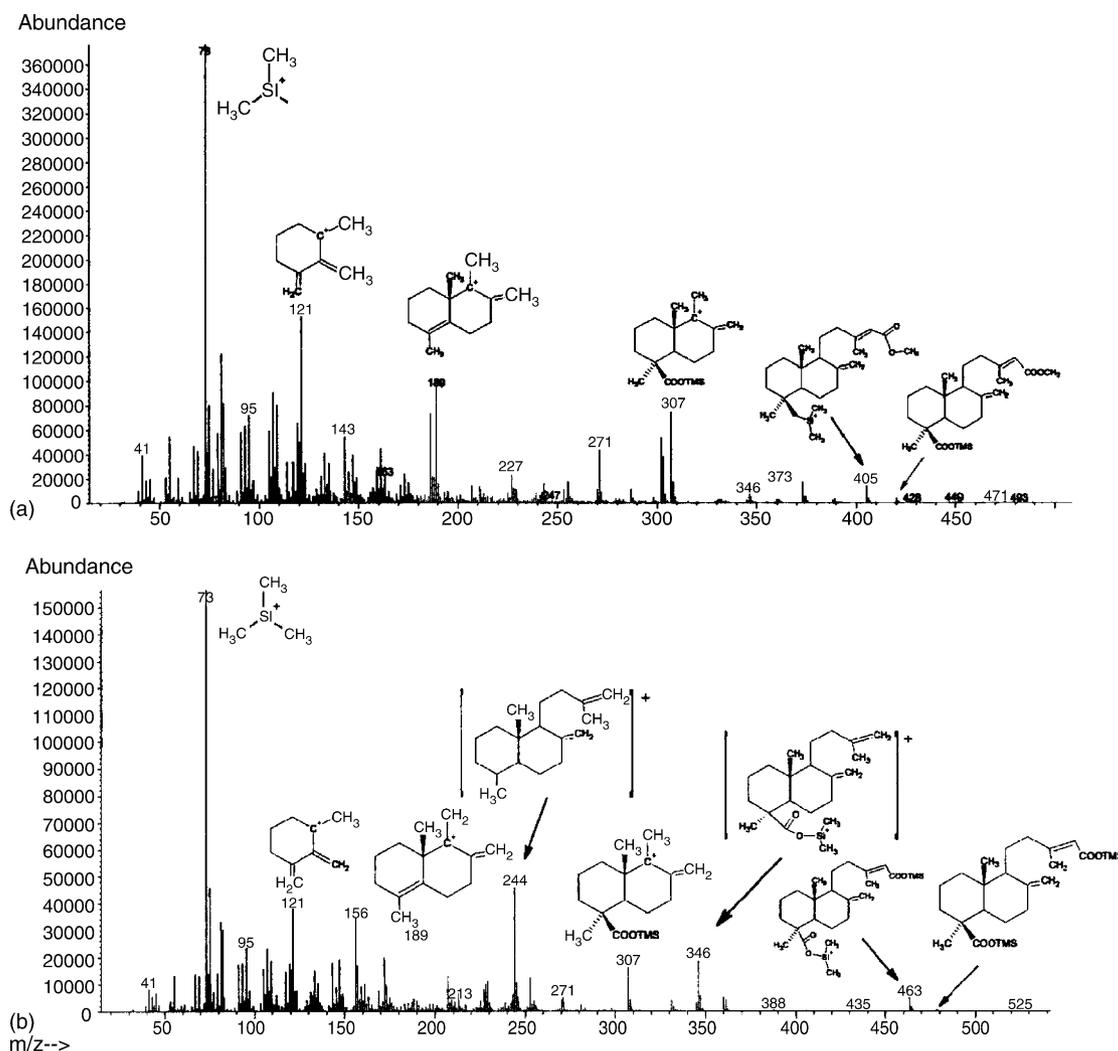


Fig. 6. Mass spectra of: (a) methyl agathic acid, TMS ester; (b) agathic acid, di-TMS.

Table 3
 Characteristic fragment ions and m/z values found in the mass spectra assigned to TMS methyl agathate and di-TMS agathate

	Methyl agathic acid, TMS ester	Agathic acid, di-TMS ester
$M^{+\bullet}$	420	478
$[M-CH_3]^+$	405	463
$[M-COOTMS]^+$	303	361
$[M-CH_3OH]^+$	388	–
$[M-TMS]^+$	347	405
$[M-2TMS]^+$	–	332
$[M-COOCH_3]^+$	361	–
$[M-COOTMS-CH_3]^+$	–	346
$[M-COOTMS-COOTMS]^+$	–	244
$[M-COOTMS-COOCH_3]^+$	244	–
a	249	307
b	189	189
c	181	241

norlabda-4,8(20),13-trien-15-oic acid (**15**) trimethylsilyl derivative.

Additionally, the proposed method of trimethylsilylation with Py–GC–MS differs markedly from the THM–GC–MS due to the absence of secondary pyrolysis products of labdane molecules. Therefore, the chromatogram appears significantly simplified.

3.1.3. Leguminosae resin-based varnishes

The Copaiba balsam studied in this work is a product commercially available from suppliers of artists' materials that consists primarily in a solution of non-polymerizing labdane compounds in sesquiterpenes. Fig. 7 shows the chromatograms obtained from on line trimethylsilylation with Py–GC–MS of Copaiba balsam using HMDS and the trimethylsilyl derivatives obtained are listed in Table 4. As can be seen, the diterpenoid fraction ranges from the 25 to the 32 min and includes a peak of the trimethylsilyl derivative of copalic acid (**6**). In addition to those mentioned,

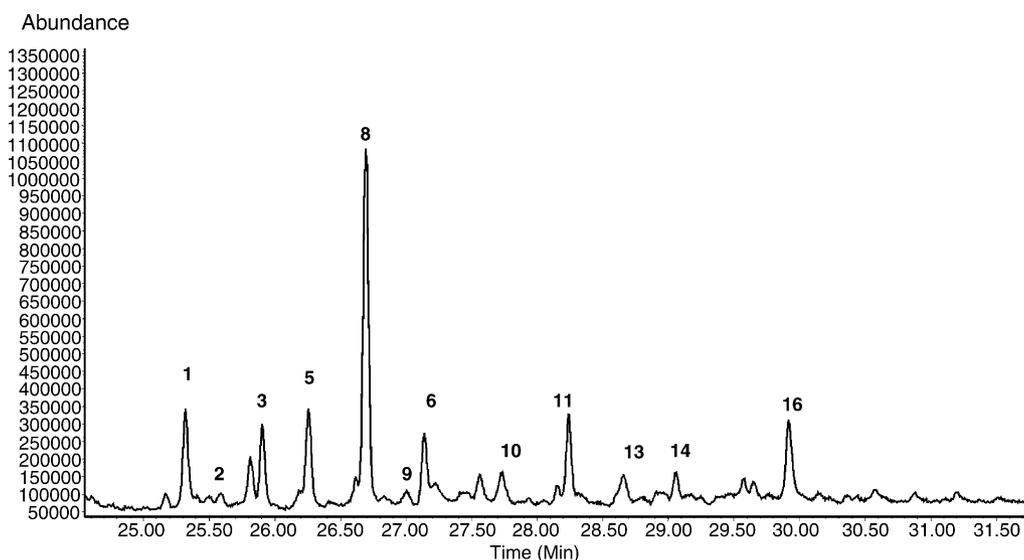


Fig. 7. Chromatogram obtained from on line trimethylsilylation Py–GC–MS of Copaiba balsam using HMDS.

several trimethylsilyl derivatives of abietadiene compounds have been found in a significant extent. Pimaric acid (**1**), sandaracopimaric acid (**2**), isopimaric acid (**3**), dehydrodehydroabietic acid (**4**), dehydroabietic acid (**5**), abietic acid (**7**) and related compounds. Finally, trimethylsilyl derivatives of fatty acids have also been identified. Presence of abietadiene compounds indicates that the commercial product analyzed was adulterated with other resins from Pinaceae family in order to improve its viscosity properties [16].

3.2. Analysis of Magdalene Triptych varnish

Results obtained on the varnish analyzed are shown in Table 5. Despite the absence of peak from derivatives of abietadiene acids (see chromatogram in Fig. 8), the varnish exhibited 46% (w/w) of dehydroabietic acid and 30% (w/w) of 7-oxodehydroabietic acid (see Table 6). These high

values suggest that the varnish analyzed had achieved a notable degree of oxidation as consequence of the natural ageing. Nevertheless, the significantly lesser content of 15-hydroxy-7-oxodehydroabietic acid found in the varnish indicates that the majority of the abietadienoid material have not still completed the oxidation process that results in the formation of the most highly oxidated 15-hydroxy-7-oxodehydroabietic acid.

Apart from the above mentioned diterpenoids, the sample exhibited small peaks corresponding to TMS derivatives of pimaric acid, isopimaric acid, dehydrodehydroabietic acid, abieta-2,8,11,13,15-pentenoic acid, abieta-8,11,13,15-tetraenoic acid, abieta-8,11,13-trienoic acid and abieta-2,7,13-trienoic acid, which confirm the ageing process undergone by this varnish. Likewise, a series of peaks belonging to a drying oil have appeared in the analyzed sample. Peaks due to trimethylsilyl esters of mono- and dicarboxylic acids in the range C₄–C₁₀ have been found

Table 4
Pimaradienes, labdanes and related compounds identified in the Copaiba balsam resin by Py–GC–MS

	Compound	<i>t_R</i>	<i>M_r</i>	ID ^a
1	Pimaric acid TMS ester	25.35	374	L
2	Sandaracopimaric acid TMS ester	25.60	374	L
3	Isopimaric acid TMS ester	25.83	374	L
4	Dehydro-dehydroabietic acid TMS ester	26.27	370	I
5	Dehydroabietic acid TMS ester	26.75	372	L
6	Copalic acid TMS ester	27.04	374	L
7	Abietic acid TMS ester	27.18	374	L
8	Abieta-2,8,11,13,15-pentenoic acid TMS ester	27.74	368	P
9	Abieta-8,11,13,15-tetraenoic acid TMS ester	28.26	366	P
10	7-Hydroxydehydroabietic acid TMS ether, TMS ester	28.70	460	P
11	15-Hydroxydehydroabietic acid TMS ether, TMS ester	29.09	460	P
12	7-Oxo-dehydroabietic acid TMS ester	29.97	386	P
13	15-Hydroxy-7-oxo-dehydroabietic acid TMS ester	32.11	402	P

^a Identification: L, Wiley Mass Spectra Library; P, published mass spectrum [21,34,35]; I, interpretation of MS fragmentation pattern.

Table 5

Abietadienes, pimaradienes, fatty acids and degradation products of them identified in the two varnishes analyzed from the Magdalena Triptych (MA-1) and “The Betrothal of the Virgin” (T-4.1)

No.	Compound name	M_r	t_R	MA-1	T-4.1
1	Benzoic acid, TMS ester	194	4.97	✓	
2	Heptanoic acid, TMS ester	202	3.78	✓	
3	Octanoic acid, TMS ester	204	4.50	✓	
4	Glycerol, 1,2,3-tris[(TMS)oxy]	308	5.49	✓	
5	Butanedioic acid, di-TMS ester	262	6.09	✓	✓
6	Nonanoic acid, TMS ester	230	6.91	✓	
7	Pentanedioic acid, di-TMS ester	276	7.87	✓	
8	Hexanedioic acid, di-TMS ester	290	9.99	✓	✓
9	Heptanedioic acid, TMS ester	304	12.09	✓	✓
10	4-[(TMS)oxy]benzoic acid, TMS ester	282	12.55	✓	
11	Dodecanoic acid, TMS ester	272	13.00	✓	✓
12	Octanedioic acid, TMS ester	318	14.09	✓	✓
13	Nonanedioic acid, TMS ester	332	16.31	✓	✓
14	Tetradecanoic acid, TMS ester	300	17.05	✓	✓
15	Decanedioic acid, TMS ester	346	18.06	✓	✓
16	Pentadecanoic acid, TMS ester	314	18.97	✓	
17	Undecanedioic acid, TMS ester	360	19.92	✓	
18	Hexadecanoic acid, TMS ester	328	20.83	✓	✓
19	Decanedioic acid, α -TMS ether, TMS ester	434	21.23	✓	
20	Heptadecanoic acid, TMS ester	342	22.62	✓	
21	Octadecan-1-ol, TMS ether	342	22.82	✓	
22	9,12-Octadecadienoic acid, TMS ester	352	23.41		✓
23	Isomer of compound 22	352	23.87		✓
24	9-Octadecenoic acid, TMS ester	354	24.53	✓	✓
25	Isomer of compound 24	354	25.55	✓	✓
26	Octadecanoic acid, TMS ester	356	24.52	✓	✓
27	Pimaric acid, TMS ester	377	25.32	✓	
28	Isopimaric acid, TMS ester	377	25.81	✓	
29	Dehydro-dehydroabietic acid, TMS ester	370	26.26	✓	✓
30	Dehydroabietic acid, TMS ester	372	26.70	✓	✓
31	Eicosanoic acid, TMS ester	384	27.76	✓	✓
32	Abieta-2,8,11,13,15-pentenoic acid, TMS ester	368	27.74	✓	✓
33	Abieta-8,11,13,15-tetraenoic acid, TMS ester	370	28.25	✓	✓
34	Abieta-8,11,13-trienoic acid, TMS ester	372	28.66	✓	
35	15-[(TMS)oxy]dehydroabietic acid, TMS ester	460	29.06	✓	
36	Abieta-2,7,13-trienoic acid, TMS ester	388	29.58	✓	
37	7-Oxo-dehydroabietic acid, TMS ester	386	29.92	✓	✓
38	Docosanoic acid, TMS ester	412	30.77		✓
39	15-[(TMS)oxy]-7-oxodehydroabietic acid, TMS ester	474	32.09	✓	✓

in the chromatogram. These acids are produced during oil ageing from the attack of atmospheric oxygen on the unsaturation of the oil components. Among them, nonanedioic acid is the most abundant product of oxidative degradation. The chromatogram also showed peaks from trimethylsilyl derivatives of carboxylic acids with number of C atoms between 12 and 22. In addition to the above mentioned compounds, more oxidized ones are observed owing to the incorporation of oxygen by formation of a peroxide next to a double bond and further loss of water or reduction of this peroxide that results in the formation of an oxo or hydroxy compound. This is the case with 2-hydroxydecanedioic acid (**19**) for which derivatization with HMDS was effective on the hydroxyl group. These results suggest that the proposed method can be used for studying more complex systems composed by drying oils and diterpenoid resins, which are commonly employed in the preparation of traditional paint varnishes.

3.3. Analysis of “The Betrothal of the Virgin” varnish

Fig. 9 and Table 5 show the chromatogram and the identified compounds obtained from the varnish of “The Betrothal of the Virgin” varnish corresponding to the Saint Joseph Church (Taormina) collection of paintings

Table 6

Normalized peak area values of abietic acid and their oxidation products in the two varnishes analyzed from the Magdalena Triptych (MA-1) and “The Betrothal of the Virgin” (T-4.1)

Compound	MA-1	T-4.1
Abietic acid, TMS ester	ND	ND
Dehydroabietic acid, TMS ester	46	74.8
7-Oxo-dehydroabietic acid, TMS ester	30	24.8
15-[(TMS)oxy]-7-oxodehydroabietic acid, TMS ester	24	0.4
IDOX	0.6	0.42

ND: not detected.

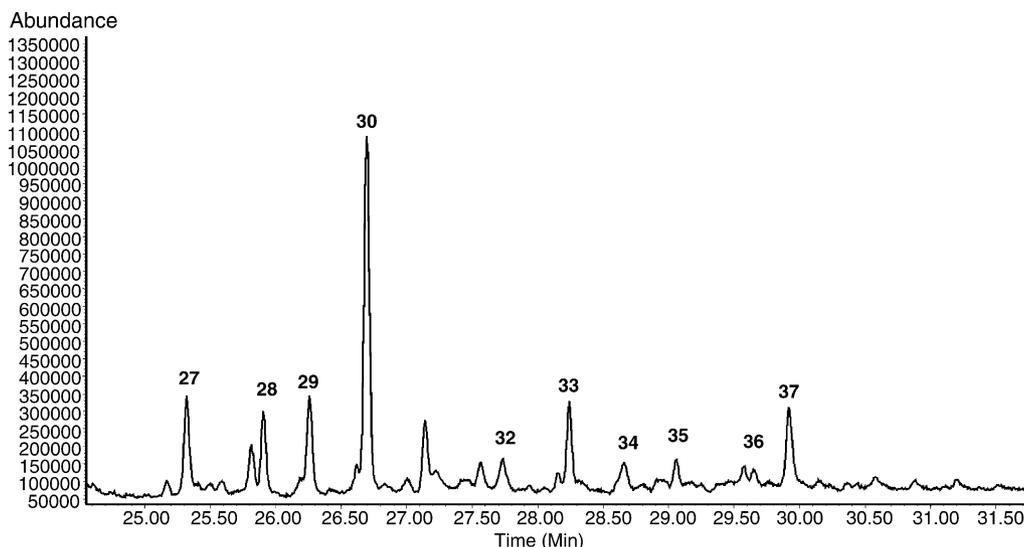


Fig. 8. Chromatogram obtained from sample MA-1 corresponding to the varnish of the Magdalena Triptych. On line trimethylsilylation Py–GC–MS using HMDS.

and the trimethylsilylated derivatives obtained. Trimethylsilyl dehydroabietate is the predominant derivative found in the chromatogram together with a less intense peak of trimethylsilyl 7-oxo-dehydroabietate. In addition to them, small peaks of trimethylsilyl dehydro-dehydroabietate, trimethylsilyl abieta-2,8,11,13,15-pentenoate trimethylsilyl abieta-8,11,13,15-tetraenoate have been found as the most significant products of the oxidative degradation occurring in the Pinaceae resin used in the varnish. The normalized values of peak area of oxidation products from derivatives of abietadiene acids show that oxidation stage 1 (see scheme in Fig. 4) is predominant in this varnish that exhibit a 74.8% (w/w) of dehydroabietic acid (see Table 6). 7-Oxo-dehydroabietic acid, arising from oxidation of dehydroabietic acid, is found in a lesser extent than the dehydroabietic acid (24.8% (w/w)). 15-Hydroxy-7-oxodehydroabietic acid was scarcely

found as indicated by the lowest area peak value found of 0.42% (w/w). These results suggest that the oxidation process undergone by this varnish has not reached its highest stages.

Assessment of the level of ageing has been established on the basis of the calculation of the index for the degree of oxidation (IDOX), defined by van den Berg [41] from the relative amounts of the abietadiene acids and their higher oxidized products:

$$\text{IDOX} = \left[(0 \times \text{AA}) + \left(\frac{1}{3} \times \text{DHA} \right) + \left(\frac{2}{3} \times \text{7-oxoDHA} \right) + (1 \times \text{15-OH-7-oxoDHA}) \right]$$

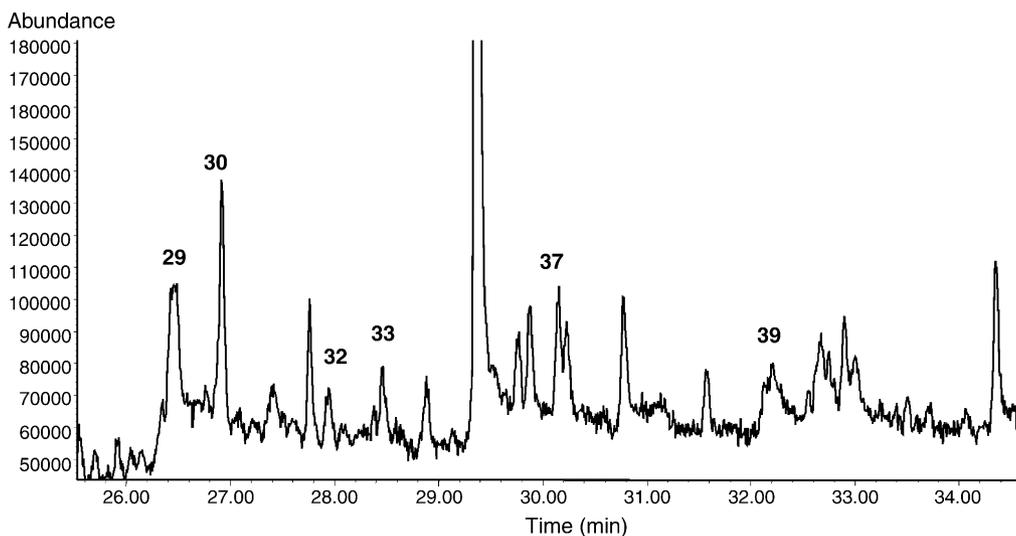


Fig. 9. Chromatogram obtained from sample T-4.1 corresponding to the “The Betrothal of the Virgin” of the Saint Joseph Church collection of paintings. On line trimethylsilylation Py–GC–MS using HMDS.

The calculated IDOX coefficient of 0.42 (see Table 6) confirms this lesser ageing occurring in this varnish.

Similarly to the previously described varnish, fatty acids and oxidative products related to them were found in this sample. Nevertheless, some interesting differences have been found that should be noted. Several lower molecular weight mono-acids (C_7 – C_9 , C_{15} , C_{17}) and dicarboxylic acids (C_5 , C_{11}) were not detected. On the contrary, peaks from derivatives of eicosanoic acid and docosanoic acid were found. Likewise, unsaturated fatty acids 9,12-octadecadienoic acid (linoleic acid) and 9-octadecenoic acid (oleic acid) were found in some extent. These two fatty acids not only react to form the cross-linked structure of the oil film but also they are decomposed by the atmospheric oxygen attack on the unsaturations present in the molecule over the ageing process of the varnish. Thus, presence of these last two compounds indicates that the drying oil present in this varnish is not at an advanced stage of drying [42] and this fact is in good agreement with the behavior exhibited by the diterpenoid resin also present in the varnish.

4. Conclusions

In this work, a comparative study has been carried out in which the proposed trimethylsilylating reagent HMDS has been compared with the methylating reagent TMAH more currently used in the analysis of diterpenoids. Copper resinate, Canada balsam and Copaiba balsam have been analyzed and the results obtained have been compared to those from colophony, Venice turpentine and Strasbourg turpentine. Sandarac and Manila copal have also analyzed in order to complete the study carried out on diterpenoid resins used for artistic purposes.

Characterization of Canada balsam and copper resinate has been carried out and the results obtained are in good agreement with those from the most currently used THM–Py–GC–MS. A more simplified chromatogram than for TMAH is obtained, when derivatization with HMDS is performed on Pinaceae resins combined with Py–GC–MS, in which only a trimethylsilylation product is obtained from each abietadiene acid or oxidized diterpenoid acid for the majority of the compounds. Thus, uncertainties in the calculations leading to the determination of the oxidation state of the diterpenoid material are notably reduced when trimethylsilylation is carried out.

According to the previous studies reported in literature of sandarac and Manila copal, pyrolysis using HMDS as derivatizing reagent enables the simultaneous characterization of free diterpenoids and pyrolysis fragments of the polymeric fraction. Mono methyl ester of agathic acid have been discriminate from agathic acid by means of their trimethylsilyl derivatives.

Copaiba balsam has been characterized. Additionally, presence of amounts of Pinaceae resin, added to improve the

viscosity properties of this artist's material has been detected in a commercial product analyzed.

The use of on line trimethylsilylation with Py–GC–MS leads to a successful characterization of samples of varnishes from painting of different provenances. Estimation of IDOX coefficient is of considerable interest for archaeometry and conservation studies in which assessment of the level of ageing of a varnish in order to decide the most suitable treatment for conservation the artwork. Values obtained in the two varnishes analyzed indicate that these varnishes are in a different oxidation stage due to the particular conditions in which each one were stored. The results obtained are in good agreement with the state of oxidation in which the drying oils also present in the samples were found.

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