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ORIGINAL PAPER

Identification and differentiation of dragon's blood in works of art using gas chromatography/mass spectrometry

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Abstract Dragon's blood is a common but non-specific name for red-coloured resins that are produced by various plants, particularly exudations from plant species belonging to the genera *Dracaena* and *Daemonorops*. Although dragon's blood is mentioned in historic sources as a colourant, it has hardly ever been identified in real artworks. This paper reports the identification and discrimination of dragon's blood produced by *Dracaena cinnabari*, *Dracaena draco* as well as *Daemonorops draco* and *Daemonorops micracantha* by means of gas chromatography/mass spectrometry (GC/MS) within the context of a routine analysis of binding media used in works of art. The detection of specific flavonoid marker compounds in both underivatised and methylated methanol extracts provided the first evidence for the use of dragon's blood from all four species in various works of art from the fifteenth to nineteenth centuries. Dragon's blood was mainly used as a red colourant in gold lacquers as well as translucent glazes and paints, e.g. in reverse-glass paintings (*Hinterglasmalerei*).

Keywords Dragon's blood · *Dracaena* · *Daemonorops* · Flavonoids · Reverse-glass paintings · Binding medium analysis

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Introduction

Dragon's blood is a non-specific name for red resinous exudations from quite different plant species endemic to various regions around the globe that belong to the genera *Dracaena* (Africa) and *Daemonorops* (South-East Asia), more rarely also to the genera *Pterocarpus* and *Croton* (both South America) [1]. The name traces back to the myth of a struggle between a dragon and an elephant that, at its climax, led to the mixing of blood from the two creatures, resulting in a magical substance: dragon's blood [2]. Dragon's blood is used for medicinal purposes where it is endemic [1], and has also been traded as a colourant for use in works of art for centuries [3, 4]. The earliest historical source referring to dragon's blood as a red colour to paint blood is given by Pliny (~23–79 AD) [5]. Before the seventeenth century, only a few sources mention the use of dragon's blood, e.g. Heraclius (Chapters 52, 56 and 58 [6]), Cennini (Chapter 43 [7]) and Boltz von Ruffach ([8], p. 68). In later centuries, the deep red resin is mentioned more often as a colourant as well as a lacquer component [3, 9]. It has always been highly prized and expensive. Nowadays, dragon's blood is still used as a varnish component in red lacquers, e.g. for violins; however, due to its tendency to fade, more stable (synthetic) dyestuffs are used instead.

To date, dragon's blood has very rarely been identified in art objects [10]. Previous work focused on spectroscopic methods, such as infrared and Raman spectroscopy [11, 12] or nuclear magnetic resonance spectroscopy [13]. However, because dragon's blood is usually mixed with a broad range of other materials for use in works of art and has undergone considerable ageing and degradation, chromatographic methods are necessary for its unequivocal identification. This can be achieved by liquid chromatography with UV–VIS diode

array detection (HPLC-DAD), which even allows discrimination of *Dracaena* and *Daemonorops* resins [3, 14]. Unfortunately, this method is limited to chromophoric compounds which are strongly affected by ageing and fading [9, 15]. This paper describes the identification of dragon's blood by means of very specific and mainly non-chromophoric flavonoids that are detectable by GC/MS and which are preserved even in considerably aged and degraded artwork samples. This method can be used to discriminate dragon's blood from individual species, *Dracaena cinnabari*, *Dracaena draco* as well as *Daemonorops draco* and *Daemonorops micracantha*.

It goes without saying that sampling of a precious work of art has to be kept to a minimum and as much information as possible has to be obtained from a sample once it has been taken. For this purpose, a special procedure for the analysis of complex and aged binding media mixtures in artworks has been developed at our institute [10, 16, 17]. This analysis procedure includes stepwise extraction of the samples with isooctane, methanol, chloroform and methanol with anhydrous oxalic acid. One advantage of this successive extraction is the separation of chemically different binding media, such as oils, waxes, resins, proteins and polysaccharides. As a consequence, the complexity of the gas chromatograms is reduced, and minor components can be detected more sensitively and reliably. Resins such as dragon's blood appear in the methanol extract, which is analysed twice with GC/MS, firstly without any derivatisation, and secondly, after simultaneous hydrolysis and methylation with trimethyl sulfonium hydroxide (TMSH) [18]. The identification and differentiation of dragon's blood is thus achieved by recognising marker substances in both the underivatised and the methylated methanol extracts. The aim is not a complete chemical characterisation of the resins but their identification within the context of a routine binding medium analysis. The parallel analysis of derivatised and underivatised extracts is advantageous because specific markers can sometimes only be detected in either one or the other chromatogram [10].

Experimental

Reference materials

Reference materials were purchased from Kremer Farbmühle (88317 Aichstetten, Germany) and GED Gerhard Eggebrecht (25361 Süderau, Germany). Currently available commercial plant material has only limited botanic variety because mainly *Dae. draco* resin is still being traded. In addition, the relatively expensive dragon's blood may be adulterated with cheaper red colourants or natural resins. It is thus important to obtain reference materials with a verified botanical origin. Such samples were obtained from Katja Lewerentz during her diploma thesis on dragon's blood at the Cologne University of Applied Sciences [9], and included plant material from Tenerife, Socotra (Dr. Krekel, Stuttgart State Academy of Art and Design and Cologne University of Applied Sciences, Germany), Malaysia (Forest Research Institute Malaysia FRIM, 52109 Kepong, Malaysia), and Sumatra (Dr. Psota, Historisches Museum Bern, Switzerland). Additional resins were obtained from botanic collections in Germany (Dr. Esser, Botanischer Garten München and Dr. Vogt, Botanisches Museum Berlin–Dahlem) and from Kew Gardens (Dr. Nesbitt, Economic Botany Collection, United Kingdom) [19]. The Doerner Institut also houses a large collection of natural resins and colourants from the Martius Pharmacognosy Collection (dating around 1825–63) [20, 21], which was included in the study. A complete list of samples is given in the Electronic Supplementary Material (Table S1).

Analytical procedure

Samples (0.1–0.2 mg) of the dragon's blood reference materials were dissolved in methanol. In contrast, approx. 0.2–0.5 mg of historic samples from artworks (paintings, frames, reverse-glass paintings, lacquer cabinets) were pretreated by stepwise extraction with different solvents of increasing polarity (isooctane, methanol, chloroform/methanol [7:3 *v*/*v*], anhydrous oxalic acid in methanol [10% *w*/*v*]), all chromatographic grade (Merck Chemicals, 64293 Darmstadt). Reference materials as well as sample extracts were first injected into the GC/MS system without derivatisation. For the methylation/transesterification step, a solution of TMSH in methanol (0.2 M, Macherey-Nagel, 52355 Düren) was added to the dried extracts, and the mixture was heated for approximately 30 min at 50 °C in a closed sample vial [18].

Instrumentation

This paper presents analysis results collected over a period of 10 years. During that time, two GC/MS systems were employed that were equipped with comparable GC columns from several manufacturers. Hence, retention times of specific compounds may differ. These two GC/MS systems were, firstly, an HP 5890 series II gas chromatograph (Hewlett-Packard/Agilent) coupled to a Hewlett-Packard quadrupole mass spectrometer type 5989B (MS Engine) and secondly, a GC 6890 N coupled to a MSD 5975 (Agilent). Detection of mass spectra: EI mode, 70 eV, scan range *m*/*z* 40–500. The chromatographs were usually equipped with DB-5ht columns (J&W), 30 m, 0.25 mm ID, 0.1 μm film thickness. Measurement conditions: carrier

gas helium 5.0 (purified), constant-flow mode, 1.7 ml/min; split/splitless injector: injection temp. = 250°C, splitless mode, 1–2 µl injection volume, 0.5 min splitless; purge flow 36 ml/min; oven programme: T1=55°C, t1=1 min, R1=15°C/min, T2=150°C, R2=10°C/min, T3=360°C. The identification of small peaks was sometimes enhanced with selected ion extraction in the Agilent data analysis software (MSD ChemStation, Rev. D.02).

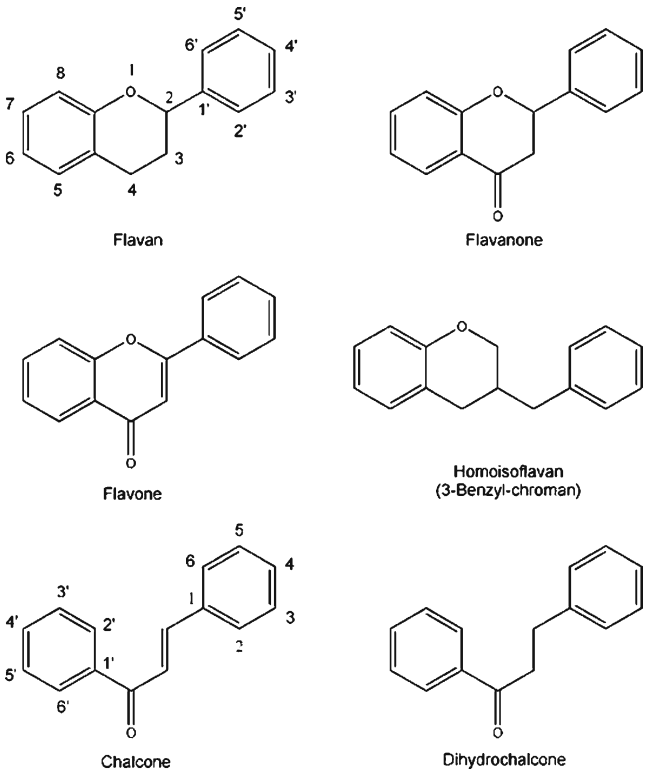
Analysis of reference materials

Dragon's blood is a phenolic resin that is mainly produced by monocots of the genera *Dracaena* and *Daemonorops* [22]. A clear botanical differentiation between several subspecies of *Dracaena* was first made by Balfour [23]. Nowadays the *Dracaena* genus comprises approximately 60 species that are mainly found in tropical and subtropical Africa. The *Daemonorops* genus comprises about 115 known species, most of which are native to India, South China and the Malay Archipelago. However, only a few species deliver the red resin that is traded as dragon's blood [1, 19, 24–26], and only some of these are considered to be the main sources of dragon's blood traded in recent centuries: *Dae. draco* (Willd.) Blume, as well as *Dr. draco* (L.) L. and *Dr. cinnabari* Balf. fil. [9, 26, 27]. It is unclear to what extent other species constitute underestimated sources for dragon's blood. In addition to these three species, dragon's blood from *Dae. micracantha* (Griff.) Becc., a species not normally associated with being a commercial source, was analysed.

Several other plants deliver red resins or red wood that are sometimes called dragon's blood and which have been traded for centuries. It is not entirely clear whether these materials were not distinguished or maybe even confused with dragon's blood from *Dracaena* and *Daemonorops* sources. Such exudates or wood extracts from *Pterocarpus* or *Croton* genera [1] will only be briefly discussed here.

The chemical composition of the different dragon's blood resins has been studied by various authors in the past. Despite significant differences, they were all found to consist of mainly flavonoids. An overview of their basic structures is given in Table 1. Representative gas chromatograms and mass spectrometric data for the different resins are given in the following sections and the Electronic Supplementary Material (Tables S3, S4, S5 and S6). The relative amounts of individual compounds vary to some extent. A tentative assignment of the peaks to compounds identified in dragon's blood is given on the basis of mass spectrometric reference values in the respective cited literature as well as general mass spectrometric data of flavonoids [28–31]. Flavans, flavanones and flavones generally fragment by means of retro-Diels–Alder processes, thus giving rise to characteristic fragments. In the case

Table 1 Basic chemical structures of flavonoids identified in dragon's blood



of homoisoflavones, the benzyl side-group is usually a dominant fragment. Chalcones can be cleaved on either side of the carbonyl group, but isomerisation to flavanones or aurones in the spectrometer with subsequent fragmentation clearly complicates the situation. It has to be stressed that many compounds identified in dragon's blood have no correlation to peaks in the chromatograms reported here and vice versa. An unambiguous assignment of individual compounds is beyond the scope of this work because there are too many possible isomers and fragmentation in the mass spectrometer is greatly influenced by the substitution pattern of the flavonoids [29]. Co-elution of different compounds further confuses the situation. As already stated, it is not the aim of this study to achieve a complete analysis of dragon's blood, but rather its identification and differentiation by marker compounds within a more comprehensive analysis routine for binding media.

It is important to note that flavonoids are ubiquitous in plants. In addition to the dragon's blood of the sources discussed here, other plant exudates or parts of plants used in works of art also contain flavonoids. This is especially true for many yellow dyes. Therefore, the identification of flavonoids in an artwork sample does not necessary mean that dragon's blood is present. To interpret such a finding, the context of the sample has to be taken into account. This paper deals with plant sources for Western European art (see the "Dragon's blood in works of art" section). Other sources of flavonoids might be relevant for Asian or ethnographic art.

Production of dragon's blood, historic products and adulterations

Dragon's blood from *Daemonorops* spp. is obtained from the immature fruits. The dried fruits are shaken in sacks or baskets to detach the resinous layer on the outside of the fruits. The resulting powder is softened by heat and moulded into cakes or sticks, sometimes with the aid of water or steam [9, 24, 26]. In contrast, the *Dracaena* resins are exuded from the wounded trunk or branches of the tree [19, 22]. They are sold as small pieces or cakes and sticks after melting and purification [9].

The reference materials in historic collections are often inscribed with Latin names, such as *resina sangui[ni]s draconis*. In addition, descriptions of the form are given, e. g. in granis, in lacrimis, in massis (Electronic Supplementary Material, Table S2). These names correspond to the form and size of the pieces of commercial resin; however, they provide no information on the origin of the dragon's blood. This is confirmed by the literature [32–34] as well as by our analyses; indeed, the dragon's blood in the Martius collection was obtained from diverse plant sources.

Adulterations of valuable dragon's blood with cheaper red colourants or natural resins are regularly detected. In our studies, one specimen was mixed with shellac (Electronic Supplementary Material, Table S1). Diterpenoids with the typical pattern of colophony are sometimes detected in commercial resins [35], but not in genuine reference materials. The large amounts of abietic and pimaric acids and their isomers certainly derive from adulterations with colophony, as previously suspected [36]. However, the situation is more complex with dragon's blood from *Dae. draco*, which fairly often contains considerable amounts of triterpenoids, even in reliable reference samples [37, 38]. It is not entirely clear whether these derive from adulterations with other resins, such as dammar, upon collection of the dragon's blood or during processing for trade. Another possibility might be that these compounds are common components in other parts of the plant and may constitute minor components of the resins [39]. The role of triterpenoids will be discussed in more detail in the "Dragon's blood from *Dae. draco* (Willd.) Blume" section.

Dragon's blood from *Dracaena cinnabari* Balf. fil.

In contrast to other *Dracaena* species growing in Macaronesia, East Africa and Arabia, *Dr. cinnabari* is endemic to the island of Socotra (Yemen). It is a distinct species and thus has several unique morphological as well as chemical features [40]. Dragon's blood from the botanical collection in Berlin–Dahlem as well as plant material collected in Socotra was used for reference analyses.

According to the literature, dragon's blood of *Dr. cinnabari* mainly consists of homoisoflavans, along with flavans, flavanones, flavones, chalcones, dihydrochalcones, di- and triflavonoids and other compounds [41–45]. This is in agreement with our analyses. Representative gas chromatograms of underivatised and methylated methanol extracts are given in Figs. 1 and 2. Peak labels correspond to those in Tables 2 and 3. The dominant compound in the extracts of fresh material was 7-hydroxy-3-(4-hydroxybenzyl)-8-methoxy-chroman (DrC11), and many other compounds are detectable as minor compounds in varying amounts. After derivatisation, some of the resulting peaks were assigned to permethylated compounds from the methanol extract, but no assignments could be made for the other compounds even though they deliver quite characteristic peaks (Fig. 2). The reason for this is unclear, but as previously mentioned, the aim of this work is to characterise marker compounds and not to unambiguously identify all components, which would require much more in-depth mass spectral analysis. For the same reason, it is not always clear whether some unassigned compounds from different species with similar mass spectra are identical or not.

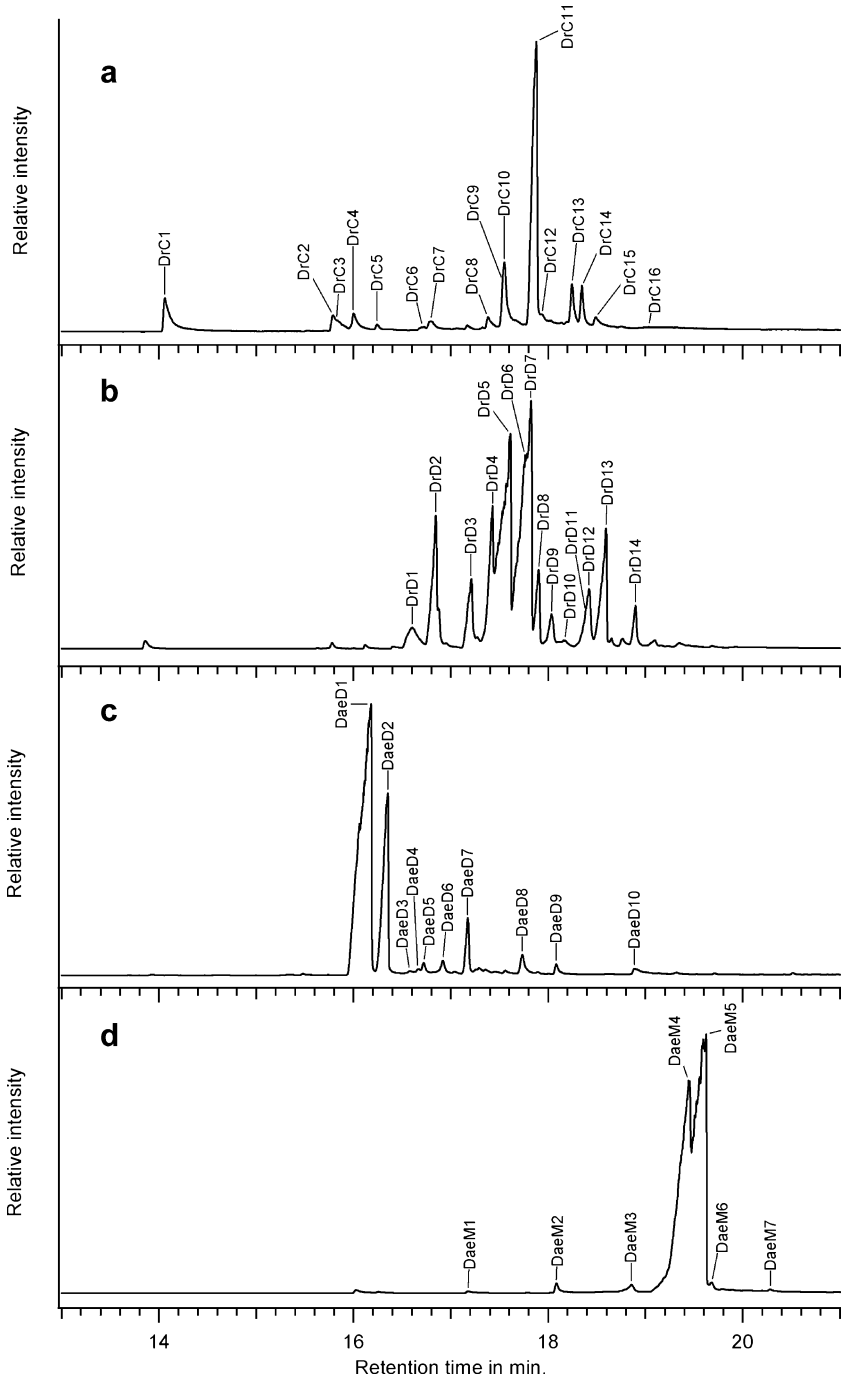


Fig. 1 Representative gas chromatograms of underivatised methanol extracts of resins from **a** *Dracaena cinnabari* (*DrC*), **b** *Dracaena draco* (*DrD*), **c** *Daemonorops draco* (*DaeD*) and **d** *Daemonorops micracantha* (*DaeM*). Peak labels correspond to those given in Table 2

Dragon's blood from *Dracaena draco*

Among the *Dracaena* species growing in West Africa, mainly *Dr. draco* is an important source of dragon's blood. There are two varieties: subspecies *draco* in the Madeira, Canary and Cape Verde archipelagos and subspecies *ajgal* in SW Morocco [40]. The latter was probably not used for harvesting resin [9]. Reference materials from the herbarium of the Botanic Garden Munich and fresh material from Gran Canaria provided reliable sources for the analyses presented here (Electronic Supplementary Material, Table S1).

The resin mainly consists of flavans and methylflavans, along with flavanones, homoisoflavans, homoisoflavones, chalcones, dihydrochalcones and others (e.g. dracaenone) [13, 46, 47]. Representative gas chromatograms of unde-

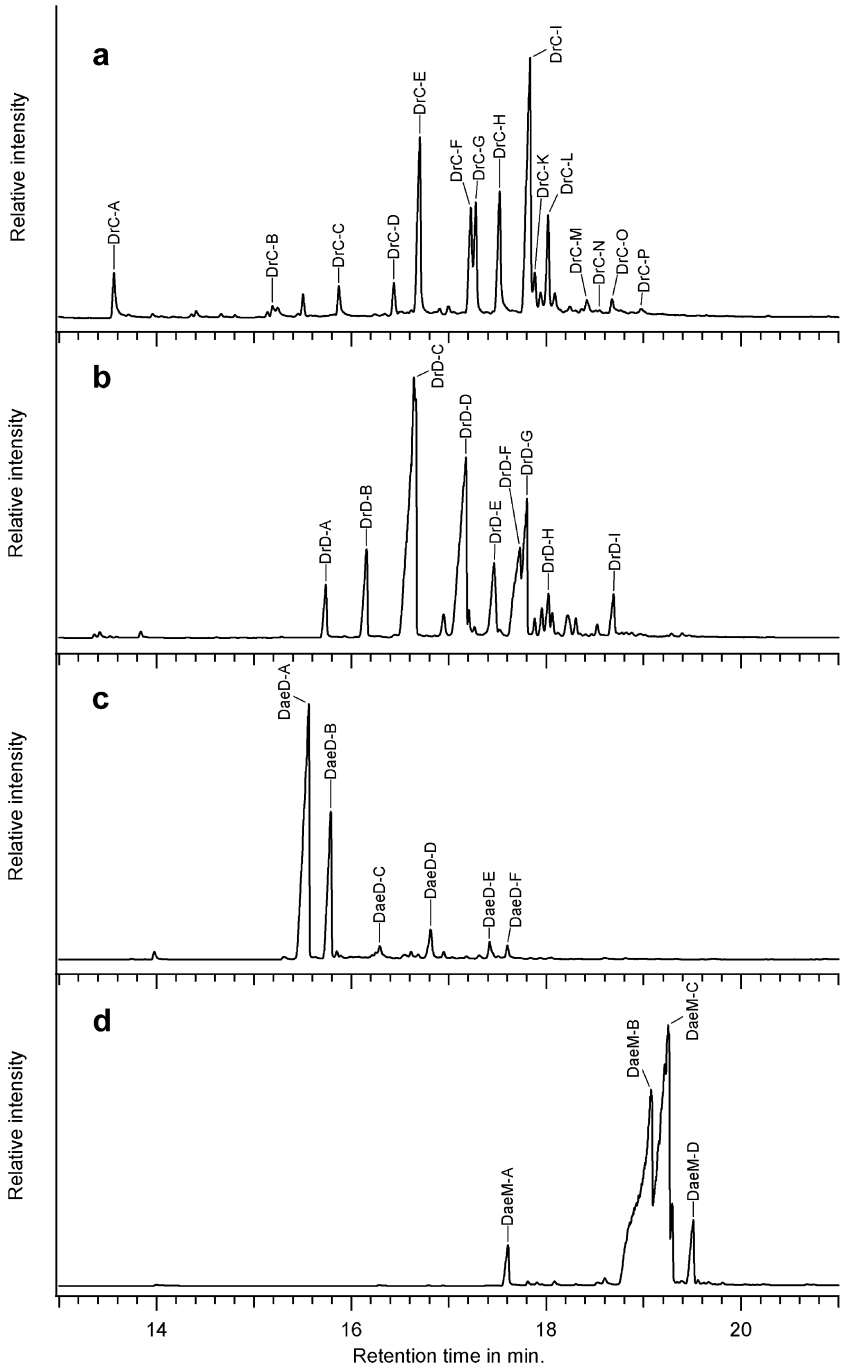


Fig. 2 Representative gas chromatograms of methylated methanol extracts of resins from **a** *Dracaena cinnabari* (*DrC*), **b** *Dracaena draco* (*DrD*), **c** *Daemonorops draco* (*DaeD*) and **d** *Daemonorops micracantha* (*DaeM*). *Peak labels* correspond to those given in Table 3

rivatised and methylated methanol extracts are given in Figs. 1 and 2, peak labels correspond to those given in Tables 2 and 3. In contrast to dragon's blood from *Dr. cinnabari*, the resin of *Dr. draco* shows about ten major peaks, and the dominant flavonoid DrC11 is missing. Therefore, a clear differentiation can be made between these two *Dracaena* species. No significant difference was detected between resins of subspecies *Dr. draco draco* and *Dr. draco ajgal*.

Dragon's blood from *Daemonorops draco* (Willd.) Blume

Nowadays, *Dae. draco* is grown commercially for the production of dragon's blood. As early as 1950, the main colourants were demonstrated to be dracorubin and draco-rhodin [48], and these compounds are suitable for detecting the resin in artworks [27] and discriminating *Dae. draco* by means of HPLC [3, 14]. Dracorhodin (anhydro-7-hydroxy-5-methoxy-6-methyl-2-phenyl-benzopyranol, DaeD10) can

Table 2 Compounds identified in the methanol extract of dragon's blood species *Dracaena cinnabari* (DrC), *Dracaena draco* (DrD), *Daemonorops draco* (DaeD) and *Daemonorops micracantha* (DaeM)

No.	MW/BP	Tentative assignment	Ref.
DrC1	226/226	7-Hydroxyflavan	[42]
DrC2	258/240		
DrC3	256/137	4-Hydroxy-2-methoxy-dihydro-chalcone	[42]
DrC4	240/240	7-Hydroxyflavanone	[42]
DrC5	256/256		
DrC6	256/256	5,7-Dihydroxyflavanone	[41]
DrC7	242/242	Dihydroxyflavan	
DrC8	272/150	7,3′-Dihydroxy-4′-methoxyflavan	[42]
DrC9	256/256		
DrC10	256/256	7-Hydroxy-3-(4-hydroxybenzyl)chroman	[41–43]
DrC11	286/286	7-Hydroxy-3-(4-hydroxybenzyl) -8-methoxy-chroman	[41, 42]
DrC12	286/286		
DrC13	286/138	7-Hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman	[41, 42]
DrC14	284/284	3-(4-Hydroxybenzyl)-7,8-methylenedioxychroman	[41–43]
DrC15	316 ?/138		
DrC16	316/316		
DrD1	242/120	Dihydroxyflavan	
DrD2	256/120	4′,7-Dihydroxyflavanone or 4′,7-dihydroxy-8-methylflavan	[46] / [13, 46, 47]
DrD3	272/150	4′,7-Dihydroxy-3′-methoxyflavan	[13, 46, 47]
DrD4	286/150	4′,7-Dihydroxy-3′-methoxy-8-methylflavan	[13, 46, 47]
DrD5	256/107	7-Hydroxy-3-(4-hydroxybenzyl)chroman	[13, 46]
DrD6	272/150	3′,7-Dihydroxy-4′-methoxyflavan	
DrD7	286/107	5,7-Dihydroxy-3-(4-hydroxybenzyl)chroman-4-one	[13, 46]
DrD8	286/150	3′,7-Dihydroxy-4′-methoxy-8-methylflavan	[46, 47]
DrD9	284/284	10-Hydroxy-11-methoxy-dracaenone	[46]
DrD10	284/107	7,8-Methylenedioxy-3-(4-hydroxybenzyl)chroman	
DrD11	284/121		
DrD12	300/153	5-7-Dimethoxy-3-(4-hydroxybenzyl)chroman and other flavonoids	[46]
DrD13	286/166	4′,5-Dihydroxy-7-methoxy-8-methylflavan	[46, 47]
DrD14	286/286	7-Hydroxy-8-methoxy-3-(4-hydroxybenzyl)chroman	[13, 46]
DaeD1	256/256	7-Hydroxy-5-methoxyflavan	[38, 50]
DaeD2	270/270	7-Hydroxy-5-methoxy-6-methylflavan	[38, 50]
DaeD3	272/167		
DaeD4	256/256		
DaeD5	284/256		
DaeD6	286/253		
DaeD7	268/268		
DaeD8	286/154	4,6-Dihydroxy-2-methoxy-3-methyldihydrochalcone	[38]
DaeD9	286/134	7-Hydroxy-4′,5-dimethoxyflavan	
DaeD10	266/251	Dracorhodin	[38, 50]
DaeM1	268/268		
DaeM2	286/134		
DaeM3	272/272		
DaeM4	316/150		
DaeM5	316/316		
DaeM6	284/283		
DaeM7	316/316		

Table 3 Compounds identified in the methylated extracts of dragon's blood species *Dracaena cinnabari* (DrC), *Dracaena draco* (DrD), *Daemonorops draco* (DaeD) and *Daemonorops micracantha* (DaeM)

No.	MW/BP	Tentative assignment
DrC-A	240/240	DrC1+Me
DrC-B	272/151	DrC2+Me
DrC-C	270/134	DrC7+2 Me
DrC-D	?/151	
DrC-E	284 /121	DrC10+2 Me
DrC-F	300/164	DrC8+2 Me
DrC-G	300/151	
DrC-H	300/151	
DrC-I	314/314	DrC11+2 Me
DrC-K	298/121	
DrC-L	314/152	DrC13+2 Me
DrC-M	330/181	
DrC-N	330/151	DrC16+Me
DrC-O	298/121	DrC14+Me
DrC-P	344/164	
DrD-A	270/134	DrD1+2 Me
DrD-B	284/134	DrD2+2 Me
DrD-C	284/284	DrD5+2 Me
DrD-D	300/164	DrD3 / DrD6+2 Me
DrD-E	314/164	DrD4 / DrD8+2 Me
DrD-F	314/314	
DrD-G	314/180	DrD13+2 Me
DrD-H	314/121	DrD12+Me
DrD-I	298/121	DrD10+Me
DaeD-A	270/270	DaeD1+Me
DaeD-B	284/284	DaeD2+Me
DaeD-C	270/270	
DaeD-D	?/210	
DaeD-E	?/211	
DaeD-F	300/134	DaeD9+Me
DaeM-A	300/300	DaeM2+Me
DaeM-B	330/164	DaeM4+Me
DaeM-C	344/164	DaeM5+2 Me
DaeM-D	328 (?)/327	

also be identified by GC/MS [49], but the gas chromatogram of the methanol extract is dominated by two flavonoids, a flavan and a methylflavan (Figs. 1 and 2, Tables 2 and 3). The resin also contains chalcones, dihydrochalcones, bi- and triflavonoids, and other compounds, according to the literature [13, 38, 50–53].

Some reference samples of *Dae. draco* were shown to contain additional triterpenes [37, 38] that reveal similarities with dammar, a well-known pale yellow resin produced in South-East Asia, mainly by trees of the *Dipterocarpaceae* family [54, 55]. Furthermore, greatly varying amounts of triterpenoids were identified in several *Dae. draco* resins studied here. In agreement with the literature, many of the identified compounds are typical of dammar, but some are not (Electronic Supplementary Material, Table S7). Triterpenoids with mass fragments of *m/z* 218 are usually typical for amyrins, which are very common in many plants, but not in dammar resin. Analysis of the *Dae. draco* reference material revealed that the resin adhering directly to the fruit contained no triterpenoids. Therefore, we checked whether these compounds might be extracted from parts of the fruit during the processing of dragon's blood (cf. "Production of dragon's blood, historic products and adulterations" section). Although analyses of the inner fruit parts obtained from the Economic Botany Collection in Kew did indeed reveal extractable triterpenoids, these were not identical to the compounds detected in the resins. Hence, it seems more likely that other resins from plants growing in the same areas as *Dae. draco* might be added during harvesting or incorporated during the production of the dragon's blood cakes. This might indeed be true for dammar resin [25]. Another explanation would be that resins with triterpenoids are derived from yet another *Daemonorops* species, although this seems rather unlikely.

Dragon's blood from *Daemonorops micracantha* (Griff.) *Becc.*

In contrast to the other types of dragon's blood, little is known about the composition of the *Dae. micracantha* resin. The precisely characterised botanical reference material from Malaysia reveals two main flavonoid components in the methanol extracts, similar to *Dae. draco* resin, but they clearly differ from the latter (Figs. 1 and 2, Tables 2 and 3). The molecular weights could not be unambiguously assigned because the chemical structures of the compounds are unknown. However, judging by the very similar infrared spectra and mass spectral patterns in both *Dae. draco* and *Dae. micracantha* resins, the main compounds DaeM4 and DaeM5 are probably flavonoids, but with a higher degree of methylation of the phenolic groups.

One sample of dragon's blood in the Martius collection revealed the same pattern as *Dae. micracantha*. We therefore conclude that resins from *Dae. micracantha* species — due to their similar appearance (Ref. [22], plate 47) to other *Daemonorops* resins of the Malay region — have been traded under the name of dragon's blood since historic times. This is further supported by nineteenth century samples of resinous fruit from the Economic Botany Collection Kew that are inscribed as *Dae. draco* Blume, but were found to originate from *Dae. micracantha* (Electronic Supplementary Material, Table S1).

Other sources of dragon's blood—*Croton* and *Pterocarpus*

Several Latin American species of the *Croton* genus (*C. draco*, *C. draconoides*, *C. lechleri*, *C. palanostigma*, *C.*

urucurana and *C. xalopensis*) yield red exudates commonly called dragon's blood [3, 22, 56, 57]. Similar to the Dracaena species, the dragon's blood is collected from incisions made in the stem. Four reference samples from *Croton* species were analysed (Electronic Supplementary Material, Table S1). In all cases, no flavonoids comparable to the *Dracaena* and *Daemonorops* resins were identified. This is in agreement with the literature, which reports the main components of these resins as being oligomeric proanthocyanidins [56]; these are too large and polar to be detected by GC/MS. Even if monomeric catechin, epicatechin, gallocatechin and epigallocatechin [58] were more abundant in degraded material, the pattern should be clearly distinguishable from the resins discussed above. However, more work is needed here.

The main dyes in red sandalwood (from *Pterocarpus santalinus L.*) [27], native to East India, Sri Lanka and the Philippines, are santalin A, B and C [59]. In addition, characteristic colourless isoflavonoids are detectable by GC/MS: pterocarpin $C_{17}H_{14}O_5$ (MW 298) and homoptero-

Table 4 Overview of dragon's blood identified in art objects

Object	Layer type	Dating	Main binding media	Dragon's blood species and other colourants
Effner frame, Bayerische Staatsgemäldesammlungen (BStGS) Inv.-no. R225	Gold lacquer over metal	1730	Shellac, sandarac, mastic	*Daemonorops micracantha* [DaeM4, DaeM5], Shellac
Neoclassical frame, BStGS Inv.-no. R247	Gold lacquer over metal	1767–1800	Shellac, sandarac, mastic	*Daemonorops micracantha,* [DaeM4, DaeM5], Shellac
St. Alto altar figure, Rococo church Altomünster, Germany	Red lacquer over metal	ca. 1770	Larch turpentine, sandarac and colophony	*Daemonorops draco,* [DaeD1, DaeD2], Gum benzoin
Pilgrim or powder bottle, Staatliche Kunstsammlungen Dresden, Rüstkammer, Inv.-no. RK X 751	Red lacquer on reverse-glass painting	1570–1600	Mastic	*Dracaena cinnabari,* [DrC-E, DrC-F, DrC-L]
Spanische Landkarte, Rijksmuseum Amsterdam, Inv.-no. RKB 17007	Red lacquer on reverse-glass painting	1549	Larch turpentine, (mastic)	*Dracaena draco*, [DrD2, DrD3, DrD5, DrD6, DrD7, DrD9], Gum benzoin
Corning House Altar, The Corning Museum of Glass, USA, Inv.-no. 59.3.39	Red lacquer on reverse-glass painting	ca. 1560–1580	Larch turpentine, colophony, mastic	*Daemonorops draco* [DaD10, Triterpenes], Cochineal
Folding game box decorated with amber, Staatliche Kunstsammlungen Dresden, Grünes Gewölbe, Inv.-no. RK P 358	Red lacquer on reverse-glass painting	ca. 1680	Larch turpentine	*Daemonorops micracantha,* [DaeM4, DaeM5]
St. Ansgar panel painting, St. Petri church, Hamburg	Glaze on red paint (false brocade)	1457	linseed oil	*Daemonorops draco* [DaeD10], unidentified red lake
Dagly-cabinet, Schloß Weilburg, Germany, Inv.-no. 2.5.110	Red glaze on aventurine lacquer	ca. 1700	Shellac, larch turpentine, boiled linseed oil (from aventurine lacquer)	*Daemonorops draco* [DaeD10], Gum benzoin, Shellac, Cinnabar pigment
Lecture hall, gynaecological hospital Munich	Dark-red wood lacquer	19th century	Shellac	Dragon's blood [DrC6] (and unspecified flavonoid markers)
Table with cherry wood veneer, Saxony	Dark-red wood polish	ca. 1810/15	Shellac	Sandal wood [homopterocarpin]

The detected dragon's blood markers are specified and correspond to Tables 2 and 3

Fig. 3 Neoclassical picture frame, dating 1767–1800. Gilding is imitated by the application of a reddish translucent lacquer on silvery metal. Due to ageing and fading, the lacquer shows a non-uniform appearance today. Photo: Johannes Engelhardt, © Bayerische Staatsgemäldesammlungen, Munich

carpin $C_{17}H_{16}O_4$ (MW 284) [27, 60]. These marker compounds were found in a sample of dragon's blood from the Collection Martius and *Pterocarpus* wood samples from Kew (Electronic Supplementary Material, Table S1).

This demonstrates that wood extracts of sandalwood have been traded together with dragon's blood from more common sources in past centuries, and sometimes they were confused or at least not distinguished from each other.

Dragon's blood in works of art

In recent years, dragon's blood from all the species discussed here was detected in a broad variety of art objects dating from the fifteenth to the nineteenth centuries (Table 4). According to these analyses, dragon's blood was predominantly used in gold lacquers and *Hinterglasmalerei* (reverse-glass paintings), and relatively rarely in glazes on conventional paintings or lacquers on furniture. In all these cases, the artists took advantage of the special properties of dragon's blood: a film-forming resin with a natural red colour that is nevertheless translucent. In lacquers, dragon's blood was mixed with other natural resins such as sandarac [61], larch turpentine [62] and mastic [63], and also with other red resins such as shellac or gum benzoin, which is a dark red balsamic resin obtained from several species of the genus *Styrax* [64].

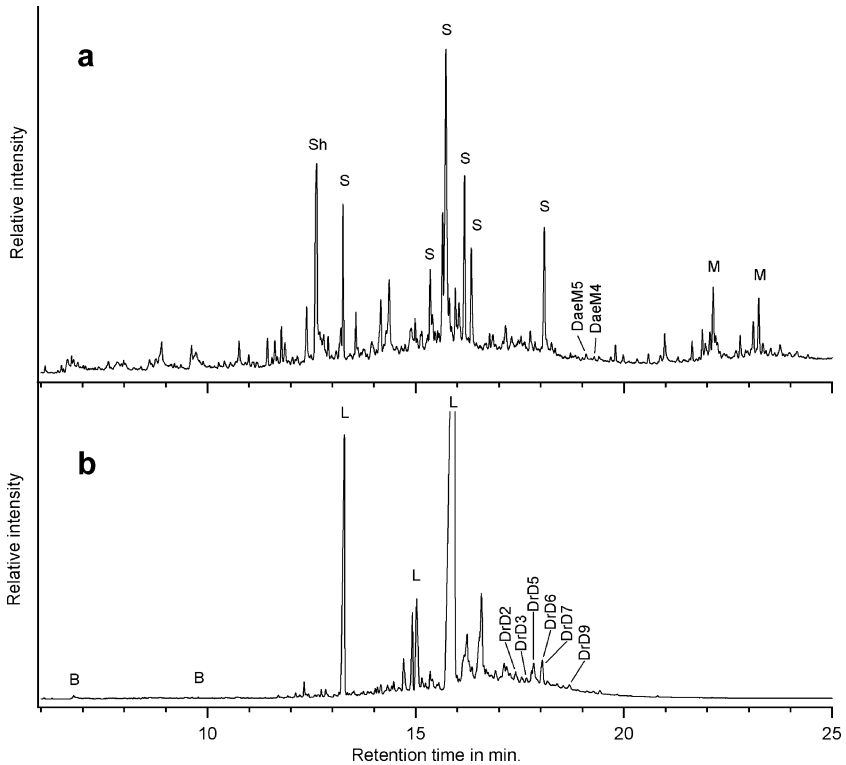


Fig. 4 Gas chromatograms of the methanol extracts of two artwork samples containing dragon's blood. *Above* gold lacquer from the neoclassical frame (Fig. 3). The main binding media are shellac (*Sh*), sandarac (*S*) and mastic (*M*). The reddish translucent colour was achieved by the addition of dragon's blood from *Dae. micracantha* and probably by the natural dyes of shellac. *Below* red lacquer from the 16th century reverse-glass painting known as the *Spanische Landkarte*. The binding medium is larch turpentine (*L*) with very little mastic (not visible in the gas chromatogram). Dragon's blood from *Dracaena draco* as well as gum benzoin (*B*) were used as red colourants

Dragon's blood in gold lacquers

So-called gold lacquers were used to imitate gold on altars, statues and frames: a foil of less expensive silvery metal, mostly tin or even silver, was applied to the object and covered with a yellowish to reddish translucent lacquer to achieve the illusion of gold.

Dragon's blood was detected in two gold lacquers on silver-coated frames within the collection of the Alte Pinakothek in Munich: on a neoclassical frame dating around 1767–1800 (Fig. 3) and on a frame created in 1730 by Johann Effner. The binding media in both cases were based on shellac, sandarac and mastic, but the golden colour was further intensified by the addition of dragon's blood. The markers of *Dae. micracantha* were detected in both samples (Fig. 4). The retention times of the DaeM4 and DaeM5 markers differ slightly from Fig. 1 because the chromatograms were recorded with different analytical systems (cf. "Instrumentation" section).

Dragon's blood was also identified on the altar figure of St. Alto in the Rococo church of the Birgitten cloister Altomünster, Bavaria, created around 1770. The methanol extract of two gold lacquer samples on metal leaf from the Saint's coat and shoe were shown to contain the flavans DaeD1 and DaeD2, typical of *Dae. draco*, as well as binding media based on larch turpentine, sandarac and colophony (Table 4). Additional typical components, such as dracorhodin or triterpenes, were missing in the St. Alto samples. The colour of the now faded, but originally reddish lacquer, was further intensified by the addition of gum benzoin. This resin was identified by small amounts of benzoyl and cinnamyl esters.

Dragon's blood in *Hinterglasmalerei*

Objects of *hinterglasmalerei* are painted on the backside of glass panels. Obviously, the paint layers have to be applied in reverse order, starting with the uppermost layer. The finished *hinterglas* painting is seen through the glass and thus reveals an impressive gloss and depth of colour. To intensify this impression, translucent paints were often applied in the sixteenth and seventeenth centuries. The *Amelierung* technique involves covering the reverse side of the glass with gold leaf, which is then etched and painted with colourful translucent lacquers. Metal foils were used as a third layer to reflect the light and intensify the luminosity of the paints [65]. As a consequence, the binding media of such reverse-glass paints are often resins, as prior analyses have shown [10]. Resins dry quickly, adhere well to the glass and can form translucent coloured lacquers with many dyes or pigments. In a research project studying *Hinterglasmalerei*, several of the investigated red glazes were found to contain dragon's blood, sometimes in combination with another red component, mainly gum benzoin.

Fig. 5 Splendid and colourful *Hinterglasmalerei* in the *Amelierung* technique with gold foils on two panels of the Corning House Altar, ca. 1560–1580, before restoration. The red paints contain dragon's blood from *Dae. draco*. The width of the altar with closed wings is 19.5 cm. Photo: Simone Bretz, Oberau, © The Corning Museum of Glass, Corning (NY)

Six of the *Dr. draco* markers were identified in a small flake of red lacquer on a splendid sixteenth century rock crystal painted in *Hinterglasmalerei*, known as the *Spanische Landkarte* (Rijksmuseum Amsterdam). Larch turpentine and small amounts of mastic form the translucent binding media of the paint layer that was coloured with dragon's blood (Fig. 4, Table 4). The concentration of the aged *Dr. draco* is still high enough to allow detection of the markers in the methanol solution. The lacquer's colour was further intensified by the addition of gum benzoin.

In two samples of red lacquer from a South-German house altar (The Corning Museum of Glass, New York, cf. Fig. 5), dating probably around 1560–1580, dragon's blood was identified in addition to a cochineal dye [66]. The binding medium is based on larch turpentine, colophony and mastic. The methanol extract exhibited the typical mass spectrum of dracorhodin; the dragon's blood thus originates from *Dae. draco*. Additional triterpenoids with dammarane

structures seem to be derived from triterpenoid portions within the dragon's blood, as discussed above (Electronic Supplementary Material, Table S7).

A small pilgrim bottle with a medallion painted in the reverse-glass technique (late sixteenth century, Rüstkammer Dresden) was also analysed. The transparent red lacquer was completely soluble in methanol and the binding medium was based on mastic resin. Due to the very small sample size, the red colourant of the investigated paint flake was not distinguishable at first; however, the typical markers of *Dr. cinnabari* were identified after methylation.

As a last example, an amber game box dating around 1680 (Grünes Gewölbe Dresden) should be mentioned. The reverse side of the amber pieces decorating a folding box were ornamented by the *Amelierung* technique. During its conservation, it was possible to analyse two untouched samples of red lacquer [67]. The GC/MS results of the underivatised methanol extracts of both samples show larch turpentine as the main binder, and the marker compounds of *Dae. micracantha* were also identified (Table 4).

Dragon's blood in paintings and lacquers on furniture or wood surfaces

The use of dragon's blood on paintings, furniture or wood surfaces could only be demonstrated in isolated cases. More objects have to be studied to verify how often dragon's blood has been used in these kinds of applications.

The only painting on which we were able to detect dragon's blood is a votive panel dating from 1457 (Table 4). In a glaze on a red paint imitating brocade, it was possible to identify dracorhodin (DaeD10). This represents the oldest verified use of dragon's blood in this study, and also demonstrates a special application regarding the used binding media: the *Dae. draco* resin was applied with a drying oil (linseed oil). Another red lake pigment was present, but could not be identified by HPLC due to detection limits.

Similar to the use of gold lacquers or resin-based paints in *Hinterglasmalerei*, dragon's blood was also used in resinous lacquers on furniture. However, its use has only been demonstrated in one case so far: a cabinet inscribed Gérard and Jacques Dagly (1660–1728) from the castle of Weilburg (Germany). Parts of this cabinet are decorated with a so-called *aventurine* lacquer: copper filings bound in several layers of transparent lacquer placed on top of a black ground [68]. The appearance of the metal powder was modulated by a translucent red finishing lacquer composed of shellac, larch turpentine and dragon's blood of the species *Dae. draco* (Table 4).

Dragon's blood was presumably also used in a nineteenth century translucent lacquer on the wood panelling and furniture of the lecture hall in the Munich gynaecological hospital (Table 4). Again it was used to enhance the red colour of the coating based on shellac and thus produce a dark-red impression of the wood surface. Although the identified flavonoid markers were similar, they did not exactly match the pattern reported above. It is not clear at the moment whether this resin represents an additional, hitherto unspecified *Dracaena* species.

Another example of a dark-red wood polish based on shellac was found on a Saxonian table with a cherry wood veneer that dates around 1810/15 (Table 4). In this case, homopterocarpin was detected ("Other sources of dragon's blood—*Croton* and *Pterocarpus*" section), thus sandalwood was used as an additional colourant. Whether this red wood extract was consciously selected by the artist or accidentally used instead of true dragon's blood is not known.

Conclusions

Certainly, a wide variety of plant materials was used as red colourants in works of art. After the characterisation of carefully selected reference resins, the thus identified specific marker compounds allow differentiation between dragon's blood from the genera *Daemonorops* and *Dracaena* and even their species *Dae. draco*, *Dae. micracantha*, *Dr. draco* and *Dr. cinnabari*, in addition to dragon's blood derived from sandalwood. The red resins of all these species were indeed used in a broad range of artworks dating from the fifteenth to the nineteenth centuries.

This paper summarises the detection of dragon's blood in artworks investigated over the last 10 years in our institute. Although we were able to study a very broad variety of objects, the number of positive identifications is still rather small, and it is clear that it is difficult to draw certain conclusions at the moment. Nevertheless, it is quite obvious that dragon's blood has mainly been used in translucent red lacquers and glazes, predominantly in resinous binding media systems. This allows subtle colouring of artworks with lustrous, luminous paints to provide an impressive depth of colour. Accordingly, dragon's blood was often used as a red colourant for *Hinterglasmalerei* and in gold lacquers on metal foils to imitate gold on picture frames or altars in churches. Other applications such as glazes on paintings, lacquers on furniture or for the tinting of wood surfaces are more rare, but occasionally identified. The colour was often further intensified and modified with other red resins such as shellac or gum benzoin.

The analyses performed so far demonstrate that *Daemonorops* resins from South-East Asia have been used in European Art at least since the fifteenth century, and it seems they were used more frequently than dragon's blood from African *Dracaena* species. This is surprising because the

respective resins from Socotra or the Canaries, which have been known in Europe much longer, are always mentioned in historic sources and have definitely been traded during the last centuries because specimens are also included in botanic and pharmacognostic collections. However, the number of positive identifications in artworks is still rather small at the moment. The characterisation of marker compounds presented in this paper will hopefully result in further identifications of dragon's blood in the future. This will enable a more detailed and accurate perception of the use of different sorts of dragon's blood in art, leading to a clearer picture and a deeper understanding of ancient trading routes. Most of all, however, it demonstrates that the colourfulness of works of art that include organic colourants susceptible to fading were originally more vivid and impressive than can be seen today.

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