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BOTANICAL AND CHEMICAL CHARACTERIZATION OF FRANKINCENSE RESIN FROM DHOFAR

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INTRODUCTION

In Dhofar the species producing frankincense is *Boswellia sacra* Flueck. (Burseraceae), growing also in Yemen (Ḥaḍramawt and the Mahra region) and in Northern Somalia.
Regarding Dhofar's frankincense, today there is a strong reduction, respect to the past, of the natural *Boswellia sacra* coenosis, both in the distribution in the territory and in the number of individuals forming them. The causes of this reduction are partially related to the general aridification process due to the temperature increase and to scarce precipitation, and partially tied to human action that has impoverished the consistency and natural composition of the *Boswellia* coenoses[1].
The quality of *Boswellia sacra* frankincense is excellent, even if it can vary according to the climatic conditions, especially temperature and humidity of the areas in which the trees grow. According to many authors[2], the best frankincense comes from the pre-desert arid areas that are not under the influence of the monsoon moisture, while the incense coming from plants growing on the coastal mountain slopes facing the Arabian Sea, and therefore exposed to humidity from the SW monsoon, is considered of lower quality.
The aim of this research was to chemically study the frankincense from *Boswellia sacra* in the various localities of Dhofar where the plant is still abundant, in order to verify the presence of chemical differences in the resins. In particular, this study deals with the chemical characterization of the tritepenoid fraction of frankincense resins collected in Dhofar by means of an analytical procedure based on gas chromatography coupled with mass spectrometry (GC/MS).

LOCALITIES OF RESIN COLLECTION

Areas under monsoon influence

al Mughsayl
This is a mountain area along the coast, located 50 km west of Salalah that stretches inland for 20-30 km, and characterized by steep mountains deeply engraved by the action of the wadis forming gorges and deep canyons. *Boswellia sacra* is abundant on the mountain slopes and in the narrow valleys cut by the wadis (fig. 1: a).

[1] Raffaelli, Mosti, Tardelli 2003; Raffaelli, Tardelli, Mosti 2003; Raffaelli, Mosti, Tardelli 2006.

[2] El Qassani 1984; Miller, Morris 2004.

Figure 1 – Frankincense areas in Dhofar: a) al Mughsayl; b) wadi Adonib; c) wadi Doka; d) An old tree of Boswellia sacra *at wadi Doka; e) Hasik mountains; f) Hasik: frankincense oozing from the bark of a* Boswellia sacra *tree.*

wadi Adonib
Area formed by rocky hills and depressions carved by the numerous tributary wadis of the big wadi Adonib. It is located 30 km west of Salalah, 7-10 km inland from the Arabian sea, and therefore under the influence of the humid monsoon climate. *Boswellia sacra* grows on the hills and in the wadi beds in isolated individuals or gathered in small groups (fig. 1: b).

Predesert areas with little or no monsoon influence

wadi Doka
The area of wadi Doka is located ca. 42 km N of Salalah, along the Salalah-Thumrayt road. The predesert environment is characterized by low, roundish, rocky hills and shallow depressions engraved by the wadis. Being beyond the coastal mountains, this area is not affected, or is weakly affected by the moisture transported by the monsoon winds. *Boswellia sacra* is the predominant tree in the landscape; it mainly grows on the gravely and rocky wadi beds, and forms scattered scrubs and trees with some tall individuals characterized by massive branching systems (fig. 1: c, d). The area of wadi Doka in included in the UNESCO natural heritage sites of Oman.

Hasik
The area of Hasik, located at the eastern margin of Oman, is characterized by inaccessible mountains with steep slopes and deep canyons. *Boswellia sacra* is present starting from the hill slopes facing the Arabic Sea (fig. 1: e, f), but it becomes abundant in the inland mountains behind Hasik. Due to its location at the eastern margin of Dhofar, this area is scarcely influenced by the moisture brought by the monsoon and, traditionally, it is known for the highest frankincense production. The resin was transported from the gathering areas to Hasik, and then by sea to Sadh, or by land directly to Sadh, or towards other gathering areas in the North, along the wadi Andur, behind Jebel Samhan.

Salalah marketplace
Besides the resin samples coming from natural *Boswellia sacra* coenoses, also resin purchased in the marketplace of Salalah was analysed.

CHEMICAL INFORMATION ON FRANKINCENSE RESIN

Natural resins are substances with a high viscosity, semisolids or solid and insoluble in water. They are formed in the so-called "resiniferous canals" of several trees. Many varieties of plants spontaneously exude resins as a product of their metabolism, to protect themselves against excessive loss of water and attacks by micro-organisms.
From a chemical point of view, vegetable resins are a complex mixture of mono-, sesqui-, di- and triterpenes, which have respectively 10, 15, 20, and 30 carbon atoms per molecule. The mono- and sesquiterpenes are both present in most resins. The di- and triterpenes are rarely found together in the same resin, which means that terpenic resins can be divided into two main classes: these compounds enable us to identify resins thereby identifying their botanical origin.
Among triterpenoid resins, there are gum-resins from the *Burseraceae* family including myrrh and frankincense. Frankincense, also known as olibanum, has been obtained since ancient times from trees belonging to the genus *Boswellia* (family *Burseraceae*). It is one of the best-known ancient plant resins. It has been used as incense in embalming practises and in the preparation of medicines, cosmetics and perfumes since the ancient Egyptians, and nowadays it is still used therapeutically.
The majority of the papers referring to the chemical composition of frankincense agrees with the presence of pentacyclic triterpenoids belonging to oleanane, ursane or lupane type molecules and in particular with the presence of α- and β-boswellic acids, and of their *O*-acetates[3] (fig. 2). 11-oxo-β-boswellic acid and its acetyl derivative (fig. 2), identified in several *Boswellia* species, are also diagnostic for frankincense[4].
However, some papers report that important constituents from the non-volatile fraction of the resin also include cembrane and verticillane diterpenes[5], and tetracyclic triterpenes with dammarane skeleton[6]. The gum component (5-10%) of frankincense is basically made up of D-galactose and L-arabinose with trace amounts of fucose and rhamnose[7].

[3] Evershed, van Bergen, Peakman, Leigh-Firbank, Horton, Edwards, Biddle, Kjølbye-Biddle, Rowley-Conwy 1997; Mahajan, Taneja, Sethi, Dhar 1995; Hairfield, Hairfield Jr., McNair 1989; Mathe, Culioli, Archier, Vieillescazes 2004 and 2004a; Buchele, Simmet 2003; Buchele, Zugmaier, Simmet 2003; Culioli, Mathe, Archier, Vieillescazes 2003; Archier, Vieillescazes 2000.

[4] Pardhy, Bhattacharyya 1978.

[5] Hamm, Lesellier, Bleton, Tchapla 2003.

[6] Pardhy, Bhattacharyya 1978a; Fattorusso, Santacroce, Xaasan Cabdi 1985.

[7] Jones, Nunn 1955.

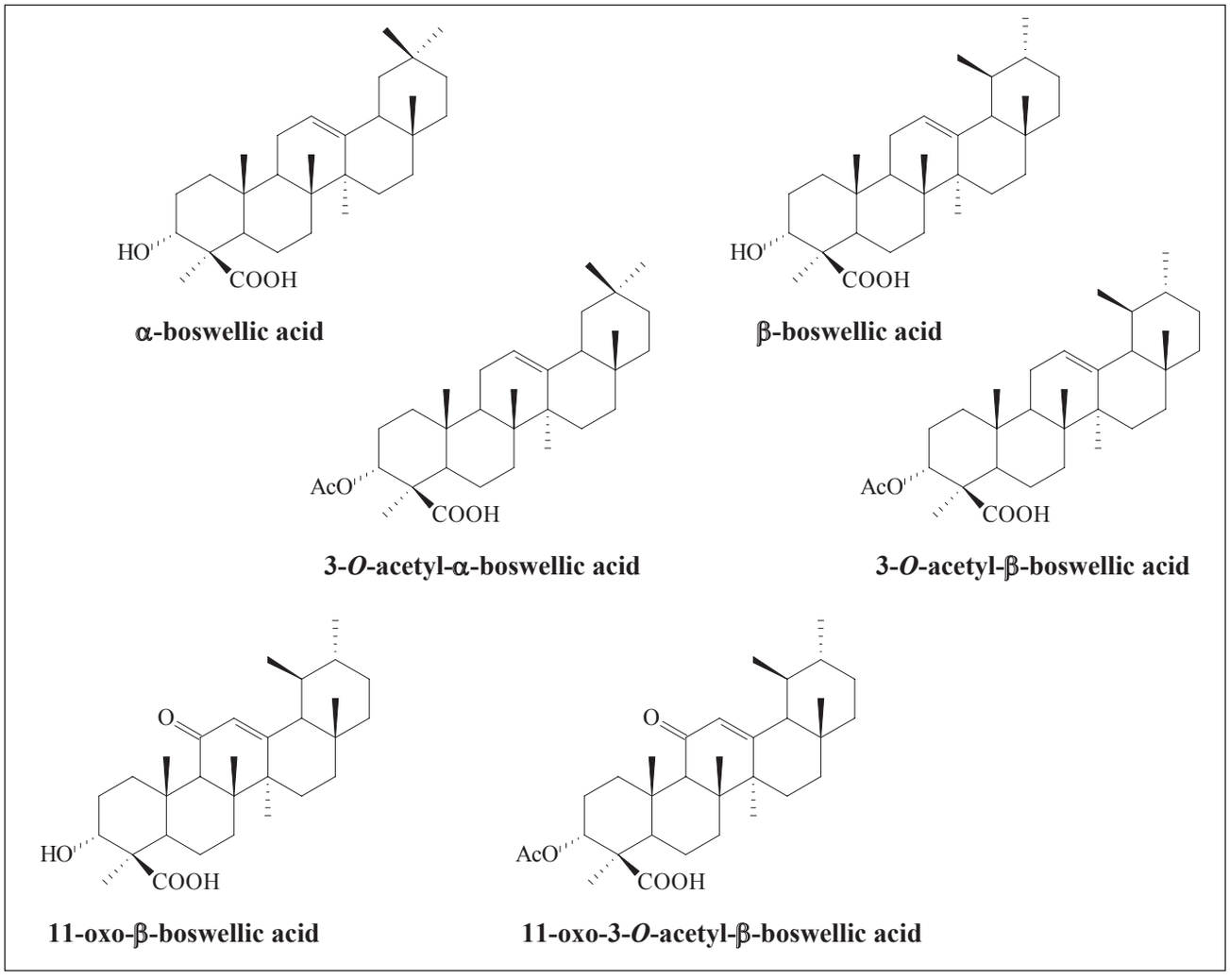

Figure 2 – Typical triterpenoid molecules of frankincense resin.

METHODS AND MATERIALS

GC/MS analytical procedure

The analytical procedure, already reported in the literature[8], used to chemically characterise the frankincense samples is based on gas chromatography/mass spectrometry (GC/MS). A brief summary of the sample preparation and GC/MS operating conditions follows. Samples were hydrolysed using 10% hydroalcoholic KOH (3h, 60°C, with sonication). Neutral organic components were extracted with *n*-hexane (the combined extracts made up the neutral fraction) and, after acidification, the acidic organic components were extracted from the residual solution with diethyl ether (the combined extracts made up the acidic fraction).

Aliquots of both fractions were derivatised with N,O-bis(trimethyl)silyltrifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane, using *iso*octane as the solvent; 2µl of the resulting solution were analysed by GC/MS (Trace GC gas chromatograph-Thermo Electron Corporation coupled with a mass spectrometric detector based on an ion trap analyser–Thermo Electron Corporation Polaris Q) using hexadecane and tridecanoic acid as internal standards.

[8] Colombini, Modugno, Ribechini 2006.

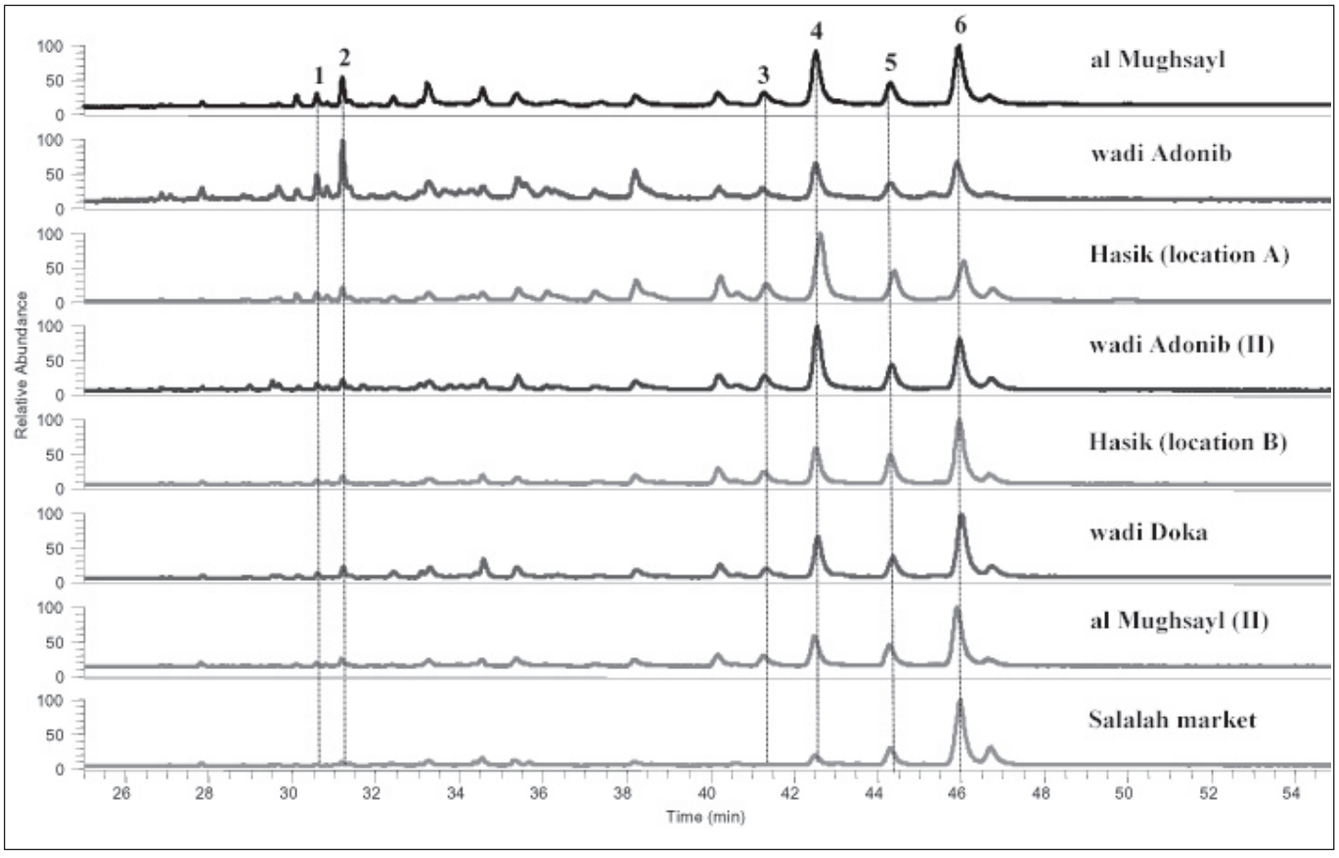

Figure 3 – Total ion current chromatograms of the acidic fractions of frankincense resins.

RESULTS AND DISCUSSION

Analysis by GC/MS

The results obtained by gas chromatography/mass spectrometry allowed the identification of the characteristic compounds of frankincense resin in all the samples analysed[9]. In particular, in the acidic fractions, whose chromatograms are reported in Figure 3, α-boswellic acid, β-boswellic acid, 3-*O*-acetyl-α-boswellic acid, 3-*O*-acetyl-β-boswellic acid, 11-keto-α-boswellic acid, 11-keto-β-boswellic acid and 11-keto-3-*O*-acetyl-α-boswellic acid were recognised. Table 1 reports the identified compounds in the acidic fraction of various resin samples. In the neutral fractions, several triterpenoids were observed, in particular α-amyrine was identified.

CONCLUSIONS

This study allowed us to chemically characterise the tritepenoid fraction of several samples of frankincense resin from Dhofar, revealing the occurrence of α-boswellic acid, β-boswellic acid, 3-*O*-acetyl-α-boswellic acid, 3-*O*-acetyl-β-boswellic acid, 11-keto-α-boswellic acid, 11-keto-β-boswellic acid and 11-keto-3-*O*-acetyl-α-boswellic in all the analysed samples. Moreover, this research pointed out that there are no significant differences in the chemical composition of the triterpenoid fraction of frankincense resin from the various localities of Dhofar and that the quantitavive dissimilarities could be addressed to the collection period rather than to the collection localities.

[9] Mathe, Culioli, Archier, Vieillescazes 2004; Evershed, van Bergen, Peakman, Leigh-Firbank, Horton, Edwards, Biddle, Kjølbye-Biddle, Rowley-Conwy 1997; van Bergen, Peakman, Leigh-Firbank, Evershed 1997.

Table 1 – Peak assignment in the chromatogram of acid fraction of frankincense resins shown in Figure 3; n.= peak number in the chromatograms.

n.	***Compound***
1	α-boswellic acid (3-hydroxy-olean-12-en-23-oic acid)
2	β-boswellic acid (3-hydroxy-urs-12-en-23-oic acid)
3	3-*O*-acetyl-α-boswellic acid (3-acetyloxy-olean-12-en-23-oic acid)
4	3-*O*-acetyl-β-boswellic acid (3-acetyloxy-urs-12-en-23-oic acid)
5	11-keto-α-boswellic acid (11-oxo-olean-12-en-23-oic acid)
6	11-keto-β-boswellic acid (11-oxo-urs-12-en-23-oic acid)

The qualitative chemical composition is the same for the all analysed samples, while by a quantitative point of view some differences should be noticed. In particular, samples Al-Mughsail and wadi Adownib, the first and the second chromatogram reported in fig. 3, present a higher amount of α- and β-boswellic acids. All the other samples show a high degree of oxidation by the presence of 11-keto-α-boswellic acid and 11-keto-β-boswellic acid. Nevertheless, these dissimilarities could not be correlated to the different place of sampling, as it emerge by analysing samples from the same places but collected in different moment of the year.

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