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Myrrh from Yemen: Chemistry of Commiphora Oleo-Gum-Resin and the Roles of NMR Spectroscopy and Isotope Ratio Mass Spectrometry in Authentication and Geographical Origin Assignment

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Abstract

Myrrh, the oleo-gum-resin of *Commiphora myrrha* (T. Nees) Engl. and related species, is traded under origin names that frequently describe a port or market rather than a place of growth, and the question of what can be proven about the authenticity and geographical origin of a resin sold as Yemeni myrrh is therefore an analytical one. In this white paper the chemistry of the three fractions of the exudate is reviewed: the furanosesquiterpene-rich volatile oil, the terpenoid resin, and the proteoglycan gum, whose sugar composition is species-specific. The evidence base for nuclear magnetic resonance (NMR) spectroscopy is then examined. Every accepted marker of true myrrh has been characterized by ^{1}H and ^{13}C NMR, and NMR performed at ambient temperature is shown to be the only routine technique that reports the native germacrane–elemane balance of the resin, because the furanodiene-type germacranes rearrange to curzerene-type elemanes in hot gas-chromatographic inlets, making most published GC compositions method-defined. Solid-state ^{13}C NMR of the intact tear, ^{1}H and ^{13}C NMR of solvent fractions, quantitative NMR of marker compounds, and NMR-based chemometrics are assembled into a tiered protocol for botanical identity, adulteration, and grade. Isotope ratio mass spectrometry (IRMS) is examined as the complementary technique for provenance. The carbon, hydrogen, oxygen, nitrogen and strontium isotope systems are related to the biosynthetic compartments of the exudate, and it is shown that bulk measurements on an unfractionated gum-resin are confounded by the variable gum-to-resin proportion, so that fraction-specific and compound-specific measurements are required. No published isotopic reference dataset for *C. myrrha* of documented origin was found, and the only resin isotope study designed to test provenance could not resolve manufacturing sites. Site-specific natural isotope fractionation NMR (SNIF-NMR) and ^{13}C isotopomics by NMR are identified as the bridge between the two techniques. A reference program combining tree-collected, DNA-verified material, NMR fingerprints, fraction-specific IRMS and ^{87}Sr/^{86}Sr is proposed, together with a candid assessment of what an opposing expert would challenge in any present-day claim of Yemeni origin.

Keywords: myrrh; Commiphora myrrha; Yemen; oleo-gum-resin; furanosesquiterpenes; furanodiene; curzerene; Cope rearrangement; ^{1}H NMR; ^{13}C NMR; solid-state NMR; qNMR; chemometrics; isotope ratio mass spectrometry; δ^{2}H; δ^{13}C; δ^{18}O; ^{87}Sr/^{86}Sr; SNIF-NMR; authentication; geographical origin.

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1. Introduction and Scope

Myrrh is the air-hardened oleo-gum-resin that exudes from wounds in the bark of small trees of the genus *Commiphora* (Burseraceae), and the pharmaceutical and perfumery material of commerce is obtained principally from *Commiphora myrrha* (T. Nees) Engl., for which *Commiphora molmol* (Engl.) Engl. ex Tschirch is a synonym, growing in the arid scrub of the Horn of Africa and the southern Arabian Peninsula[1–3]. The exudate is a physical mixture of three chemically unrelated fractions: a volatile oil dominated by furanosesquiterpenes, an alcohol-soluble resin of sesquiterpenoids and triterpenoids, and a water-soluble gum composed of acidic proteoglycans. In the classical description the volatile oil accounts for 2–10 %, the resin for 25–40 % and the gum for 30–60 % of the material[1]. Each fraction carries a different kind of information about the plant that produced it, and it is this partition of information that determines how NMR spectroscopy and isotope ratio mass spectrometry (IRMS) relate to one another when authenticity and origin are in question.

The request that prompted this document concerned material described as Yemeni myrrh, and the two words in that description are worth separating. Authenticity is a statement about botanical identity, about the absence of substitution or dilution, and about compliance with a pharmacopoeial definition. Geographical origin is a statement about where the tree grew. The first is a compositional question that NMR addresses well; the second is a question about environmental history, which composition alone cannot answer for a species that grows on both shores of the Gulf of Aden, and which is properly the domain of stable and radiogenic isotope measurements interpreted against a reference database. Throughout this paper the two questions are kept distinct, because the literature on myrrh routinely conflates them and because an adversarial reader would seize on the conflation.

The scope of the paper is as follows. The botanical, commercial and regulatory context of Arabian and Yemeni myrrh is summarized first, because the meaning of the trade name governs what any analysis can be expected to demonstrate. The chemistry of the three fractions is then reviewed with emphasis on the compounds that serve as markers. The NMR evidence is examined in depth, including a systematic artifact of gas chromatography that NMR alone avoids, and a tiered protocol suited to the instrumentation of Process NMR Associates (PNA) is proposed. The isotope methods are then treated, with attention to the biosynthetic compartmentalization that makes a gum-resin an unusually awkward matrix for bulk isotope work, and the two techniques are placed in an integrated evidence framework. A reference program for Yemeni material is outlined, and the paper closes with an assessment of the weaknesses that a reviewer or an opposing expert would identify. All references were verified against the Crossref registry before drafting, and only verified sources are cited.

2. Botanical, Commercial and Regulatory Context

2.1 The Commiphora species behind Arabian myrrh

The genus *Commiphora* comprises roughly two hundred species of shrubs and small trees distributed across the drylands of Africa, Arabia, Madagascar and the Indian subcontinent, and a considerable number of them yield exudates that have been sold, deliberately or otherwise, as myrrh[1,2]. The species of primary pharmaceutical interest, *C. myrrha*, is native to Ethiopia, Djibouti, Eritrea, Somalia and northeastern Kenya and to the southwestern and southern Arabian Peninsula, so that the same taxon grows in Yemen, in southern Oman and in the Somali and Ethiopian lowlands across the Gulf of Aden[1,2], and a 2025 review from Sana'a University compiles the distribution, phytochemistry and traditional uses of Yemeni *C. myrrha* and its congeners across the southern and eastern governorates and the Tihama foothills[4]. This single fact

constrains every provenance argument that follows: there is no botanical difference to exploit between Yemeni and Somali myrrh, and any chemical or isotopic distinction must arise from environment, harvest practice or post-harvest handling rather than from taxonomy.

Several other Arabian species complicate the picture. *Commiphora kataf* (Forssk.) Engl. was described from Yemen by Forsskål in the eighteenth century and, in current treatments, absorbs the East African taxa formerly separated as *C. erythraea* and *C. holtziana*; its resin is the bisabol or hagar type rather than true myrrh, and Dekebo and co-workers listed *C. kataf*, *C. holtziana* and *C. sphaerocarpa* among the adulterants of true myrrh whose hydrodistillates they characterized by NMR and GC–MS[5]. Scented myrrh, opopanax or bissabol, was shown by Thulin and Claeson to derive from *C. guidottii* of Somalia rather than from *C. erythraea* as long assumed, a correction that illustrates how unreliable the trade nomenclature has been[6]. *Commiphora gileadensis* (L.) C.Chr., the balsam of Mecca or besham, grows in the mountains of the western Arabian Peninsula including Yemen and has its own history of confusion with the biblical balm of Gilead[7]. *Commiphora habessinica* (O. Berg) Engl. also grows in Yemen, and the volatile oil of its resin collected in the Taiz governorate was dominated by sesquiterpene hydrocarbons, with β-elemene as the principal constituent at about one-third of the oil and α-selinene next, and lacked the furanoeudesmane signature of true myrrh; such a resin is easy to distinguish from *C. myrrha* by NMR and, for the same reason, is a plausible bulking agent for Yemeni consignments[8]. On Socotra, politically Yemeni but biogeographically distinct, the endemics *C. ornifolia* and *C. parvifolia* produce oleo-gum-resins whose volatile oils are dominated by oxygenated monoterpenes such as camphor and fenchol rather than by furanosesquiterpenes[9], and whose non-volatile fraction contains the furofuran lignan yangambin at 3.5–9.1 % of the ethanol extract and a gum built from arabinose, rhamnose, galactose and galacturonic acid[10]. Socotran material is therefore chemically remote from mainland myrrh even though it may legitimately be labeled Yemeni.

2.2 Trade geography and the meaning of a Yemeni origin label

Southern Arabia has been an entrepôt for aromatic resins for at least three millennia, and the incense routes through the Hadramaut, Shabwa and Marib carried frankincense and myrrh from both African and Arabian sources toward the Mediterranean[7]. In the modern period the balance of production shifted decisively to Africa; Somalia alone was estimated to have produced on the order of 1,500 tonnes of myrrh in 1987, while Arabian production remained artisanal[1]. A consequence of this history is that Yemeni ports, Aden above all, have long handled African exudates, and the archaeometric record supports the point directly. When resin-like objects from the medieval warehouse of Sharma on the Hadramaut coast were examined by infrared spectroscopy, direct-inlet mass spectrometry and pyrolysis-GC–MS, 84 % of them proved to be copal from *Hymenaea* trees growing in Madagascar and East Africa, and frankincense was identified with certainty in only two samples, overturning the assumption that the site had traded local resins[11]. A resin sold in a Yemeni market, or exported through a Yemeni port, may therefore have grown anywhere along the Gulf of Aden, and the origin label attached at the point of sale should be treated as a hypothesis to be tested rather than a fact to be confirmed.

2.3 Pharmacopoeial definitions

The European Pharmacopoeia defines Myrrh (monograph 1349) as the gum-resin obtained from *Commiphora myrrha* (Nees) Engl. and/or other species of *Commiphora*, and the European Medicines Agency notes in its herbal assessment that *C. molmol* is listed there as a synonym[3,12]. The permissive phrase and/or other species is analytically consequential, because it means that a pharmacopoeial identity test is not, by itself, a test of species. The Chinese Pharmacopoeia recognizes two commercial forms, natural

myrrh and colloidal myrrh, attributed to *C. myrrha* and *C. molmol* respectively, and sets minimum volatile oil contents of 4 % (mL/g) and 2 % (mL/g) for the two forms; the United States Pharmacopeia specifies an alcohol-soluble extractive of 40–70 %, a water-soluble extractive of not less than 50 % and a volatile oil content of not less than 6.0 %[13]. None of these compendia specifies a geographical origin, and none prescribes a quantitative marker assay comparable to those used for other botanicals, so that origin claims for myrrh are unregulated in the pharmacopoeial sense and rest entirely on whatever evidence the seller and the analyst can produce.

2.4 What the market sells

Two recent studies define the analytical problem with unusual clarity. In the first, twenty-five commercial myrrh samples taken from customs districts in China were sequenced at the chloroplast psbA–trnH locus; three species were identified, *C. gileadensis*, *C. myrrha* and *C. edulis*, only 8 % of the samples aligned with a pharmacopoeia-specified species, and one sample proved to be *Liquidambar formosana*, a storax-producing tree of an unrelated family[14]. In the second, seventeen retail samples purchased in Sana'a, Yemen and in Riyadh and Al-Baha, Saudi Arabia were assayed by validated high-performance thin-layer chromatography for two furanosesquiterpenoids, 2-methoxyfuranodiene and 2-acetoxyfuranodiene[15]. Among the five Sana'a samples alone the 2-methoxyfuranodiene content ranged from 15.6 to 141 µg per mg of dried extract and the 2-acetoxyfuranodiene content from 35 to 674 µg per mg, an order-of-magnitude spread within a single city's spice markets. The conclusion is unavoidable: material sold as Yemeni myrrh is chemically heterogeneous and, on the genetic evidence from the wider market, is often not *C. myrrha* at all. Any analytical scheme that assumes a single Yemeni chemotype is built on a premise that the market itself refutes, and the first duty of an analyst is to establish species and integrity before any question of origin is entertained.

3. Chemistry of the Oleo-Gum-Resin

3.1 Fraction structure

The three fractions of myrrh are separable by their solubility, and this physical partition is the basis of the pharmacopoeial extractive limits cited above as well as of the analytical strategy developed later in this paper. The volatile oil, obtained by hydrodistillation, steam distillation or supercritical carbon dioxide extraction, contains the furanosesquiterpenes responsible for the characteristic odor and for much of the reported bioactivity[1,16]. The resin proper dissolves in ethanol and chloroform and consists of the less volatile sesquiterpenoids together with triterpenoids, steroids and, in some species, lignans[17,18]. The gum remains after alcohol extraction and dissolves or swells in water; it is a mixture of proteoglycans in which uronic-acid-rich polysaccharide chains are covalently associated with protein[19]. Supercritical extraction of a myrrh sample gave a volatile oil yield of 3.2 % compared with 2.8 % by hydrodistillation and 0.4 % by steam distillation from the same starting material, which shows that the volatile oil content of a given lot is itself method-dependent[20].

3.2 Volatile and resin terpenoids: the marker compounds

The odor principle of myrrh was placed on a structural footing by Brieskorn and Noble, who isolated lindestrene, furanoeudesma-1,3-diene and furanoeudesma-1,4-diene-6-one from the essential oil and established their structures by spectroscopic methods, and who then extended the series to further furanosesquiterpenes including furanodiene derivatives[21,22]. Maradufu and Warthen separated the furanosesquiterpenoids of myrrh oil by high-performance liquid chromatography and identified

isofuranogermacrene (curzerene), lindestrene, furanoeudesma-1,3-diene and furanodiene by combined NMR, ultraviolet and mass spectrometry, a non-thermal separation that is significant for reasons developed in Section 5.2[23]. Zhu and co-workers added new furanosesquiterpenoids and a series of aromatic sesquiterpenes whose structures rested on NMR evidence[24], and Dekebo, Dagne and Sterner characterized curzerenone, furanodienone and a new 8,12-epoxygermacratetraenone from *C. sphaerocarpa* while using NMR alongside GC–MS to compare the hydrodistillates of *C. sphaerocarpa*, *C. holtziana*, *C. kataf* and *C. myrrha*[5]. Two oxygenated germacranes, 2-methoxyfuranodiene and 2-acetoxyfuranodiene, were isolated from Arabian resin and identified by one- and two-dimensional NMR, and these two compounds have since served as quantitative markers in the regional survey discussed in Section 2.4[15,25].

The gas-chromatographic composition of the oil has been reported many times and the reported values disagree in ways that are instructive. Furanoeudesma-1,3-diene was the principal component at 34 % in the oil examined by Başer and colleagues, who contrasted it with opopanax from *C. guidottii*, in which (E)-β-ocimene dominated at 33 %[26]; it was 34.9 % in the supercritical extract of Marongiu and co-workers, accompanied by lindestrene (12.9 %), curzerene (8.5 %) and germacrone (5.8 %)[20]. Morteza-Semnani and Saeedi, by contrast, reported curzerene as the major constituent at 40.1 % with furanoeudesma-1,3-diene at 15.0 % and β-elemene at 8.4 %[27], and a recent GC–MS study of resin extracts likewise listed curzerene first at 33.6 % together with β-elemene (5.8 %), 2-methoxyfuranodiene (5.4 %) and germacrene B (4.4 %)[28]. An ethanolic extract analyzed by GC–MS gave curzerene at only 7 % of the integrated area alongside limonene, germacrene B, β-selinene and spathulenol[29]. A commercial extract standardized for its furanodiene content contained curzerene, furanoeudesma-1,3-diene and lindestrene at 12.3, 18.8 and 6.2 g per kg with a total furanodiene content of 40.9 g per kg[16]. Part of this spread reflects real variation between lots and extraction methods, but a substantial part of it is an artifact of analytical temperature, as Section 5.2 explains, and the markers, their structural classes and their diagnostic NMR features are collected in Table 1. The structures of these compounds, with the carbon numbering used in the literature, are drawn in Appendix A (Figures A1 and A2), and the complete ^{1}H and ^{13}C assignments that could be verified from open-access sources are tabulated there (Tables A2–A11).

Table 1. Principal marker compounds of Commiphora myrrha oleo-gum-resin, their occurrence and their approximate diagnostic NMR features ($CDCl_3$ unless stated). Ranges are approximate and drawn from the cited structural papers; a validated method would use spectra of isolated standards acquired on the PNA instruments.

Compound	Skeleton / class	Occurrence reported	Diagnostic NMR features (approximate)	Key references
Furanoeudesma-1,3-diene	Furanoeudesmane (bicyclic)	Major component; 15–39 % of oil by GC	α-Furan H δ ≈ 7.0–7.1 (br s); furan-CH_3 δ ≈ 1.9–2.0 (s); H-1/H-2/H-3 olefinic multiplets δ 5.5–6.1; angular CH_3 δ ≈ 1.0 (s)	20,21,23,26
Lindestrene	Furanoeudesmane with exocyclic methylene	6–14 % of oil by GC	α-Furan H δ ≈ 7.0; exocyclic =CH_2 δ ≈ 4.6–4.9 (2 × br s); ring olefinic H δ ≈ 5.6	20,21,23
Curzerene (isofuranogermacrene)	Furanoelemane (monocyclic, divinyl)	8–40 % by GC; part is an inlet artifact of furanodiene	Vinyl ABX δ ≈ 5.8 (dd) and 4.9–5.0 (2H); isopropenyl =CH_2 δ ≈ 4.6–4.8; α-furan H δ ≈ 7.0	23,27,30
Furanodiene	Furanogermacrane (ten-membered ring)	Present in unheated oil and extracts; rearranges to curzerene on GC	Broadened ring olefinic methines δ ≈ 4.7–5.3 (conformational exchange); α-furan H δ ≈ 7.0; quantified by ^{13}C NMR	23,30,31
2-Methoxyfuranodiene	Oxygenated furanogermacrane	≈ 5 % by GC; HPTLC marker in Arabian and Yemeni retail samples	OCH_3 singlet δ ≈ 3.3–3.5 (^{13}C ≈ 55–57); oxymethine multiplet; α-furan H δ ≈ 7.0	15,25,28

Compound	Skeleton / class	Occurrence reported	Diagnostic NMR features (approximate)	Key references
2-Acetoxyfuranodiene	Oxygenated furanogermacrane	HPTLC marker; 35–674 µg/mg extract in Sana'a retail samples	Acetate CH_3 singlet δ ≈ 2.0–2.1; oxymethine δ ≈ 5.2–5.5; ester C=O ^{13}C ≈ 170	15,25
Furanodienone; curzerenone	Furanogermacrenone; furanoelemenone (conjugated ketones)	Characteristic of C. sphaerocarpa, C. holtziana and C. kataf; minor in C. myrrha	Conjugated ketone ^{13}C ≈ 185–195; deshielded α-furan H; curzerenone used as species marker by HPLC–MS/MS	5,32
Germacrene B / γ-elemene	Germacrane / elemane hydrocarbons	Germacrene B 1–4 % by GC; the pair reports thermal history	Germacrene B: broadened olefinic H, vinylic CH_3 δ 1.5–1.7; γ-elemene: vinyl and isopropenyl $=CH_2$ signals	28,29,31
β-Elemene	Elemane hydrocarbon	6–8 % by GC; may partly derive from germacrene A in the inlet	Vinyl δ ≈ 5.8 / 4.9; two isopropenyl $=CH_2$ δ ≈ 4.6–4.8; isolated from the resin	27,31,33
Mansumbinone; 3,4-seco-mansumbinoic acid	Octanordammarane triterpenoids	Resin fraction; full $^{1}H/^{13}C$ assignment published	Ketone ^{13}C ≈ 200–215; COOH ^{13}C ≈ 180; methyl singlets δ 0.8–1.3	33
(E)-/(Z)-Guggulsterone	Pregna-4,17(20)-diene-3,16-dione steroids	Characteristic of C. mukul; traces in C. myrrha; 446 ng/mg in an Ogaden resin	H-4 vinyl singlet δ ≈ 5.7; conjugated ketones ^{13}C ≈ 199 and 209; H-21 CH_3 doublet δ ≈ 1.9	18,32
Gum: 4-O-methylglucuronogalactan and arabino-3,6-galactan-protein	Proteoglycan (D_2O)	30–60 % of the tear; protein 16–31 % of AGP fractions	Anomeric H δ 4.4–5.4; OCH_3 δ ≈ 3.4–3.5 (^{13}C ≈ 60); uronic C-6 ^{13}C ≈ 175; rhamnose CH_3 doublet δ ≈ 1.25 absent or weak	1,19

3.3 Triterpenoids, steroids and dimeric sesquiterpenoids

The non-volatile resin has been re-examined intensively in the last decade with NMR as the primary structural tool. Rahman and co-workers isolated the octanordammaranes mansumbinone and 3,4-seco-mansumbinoic acid together with β-elemene and T-cadinol from the oleo-resin of *C. molmol*, assigned all ^{1}H and ^{13}C resonances of the seco-acid unambiguously by one- and two-dimensional experiments and corrected earlier ^{13}C data[33]. Kuck and colleagues isolated thirty-four secondary metabolites from myrrh using flash chromatography, centrifugal partition chromatography and preparative HPLC, among them seven new sesquiterpenes, a dimeric sesquiterpene and two new triterpenes, all characterized by NMR, high-resolution mass spectrometry and circular dichroism[17]. The same group subsequently reported an unprecedented 4,8-cycloeudesmane skeleton together with further sesquiterpenoids, a triterpene, several steroids including 7-ketostigmasterol, and a lignan from the resin[18], and a Chinese group described six cadinane, two guaiane and one germacrane sesquiterpenoid whose structures were secured by one- and two-dimensional NMR supported by quantum-chemical NMR and electronic circular dichroism calculations[34]. Steroids are characteristic of *C. mukul* (*C. wightii*), the Indian guggul, but guggulsterones have also been detected in *C. myrrha* at low levels, and an HPLC–MS/MS method quantifying (E)- and (Z)-guggulsterone, curzerenone, furanoeudesma-1,3-diene and myrrhone permitted resins of *C. myrrha*, *C. erythraea*, *C. mukul*, *C. holtziana*, *C. confusa* and *C. kua* to be classified, with the surprising finding that an Ethiopian resin from the Ogaden contained 446 ng per mg of guggulsterones[32]. The chemotaxonomy of the genus remains unsettled in places: the tirucallane triterpenoid myrrhasin, once reported from *C. myrrha*, was re-isolated from *C. oddurensis* and shown by NMR examination of authentic myrrh to be absent from *C.*

myrrha gum-resin[35]. For an analyst, the practical lesson of this literature is that the resin fraction carries species-level information in its triterpenoid and steroid content that the volatile oil does not.

3.4 The gum: a proteoglycan with a species-specific sugar signature

The water-soluble fraction of myrrh was characterized in detail by Wiendl, Müller and Franz, who found it to consist of proteoglycans dominated by uronic acid polymers. Hydrolysis and degradation revealed chains of galactose, arabinose and 4-O-methylglucuronic acid, and the specific arrangement in which galactose is substituted at C-4 by 4-O-methylglucuronic acid, itself substituted at C-2 by galactose, led the authors to propose the term 4-O-methyl-glucurono-galactan for a structure that had not been reported in any other gum. Arabino-3,6-galactan-protein fractions of average molecular weight about 70,000 with protein contents of 31 % and 16 % were also isolated[19]. Earlier work summarized by Hanuš and co-workers had identified D-galactose, L-arabinose and 4-O-methyl-D-glucuronic acid as components of the gum together with xylose, and at least fifteen amino acids were detected after hydrolysis[1]. Two points follow. First, the gum is not an inert diluent; its methylated uronic acid and its protein are compositionally distinctive and are observable by NMR. Second, the gum is species-specific: the Socotran *C. ornifolia* gum contains rhamnose and galacturonic acid[10], neither of which features in the classical description of *C. myrrha* gum, and the arabinogalactan-protein gums of *Acacia* that might be used as diluents are rhamnose-rich. The gum therefore offers authentication handles that have been almost entirely neglected because the pharmacological interest lies in the terpenoids.

3.5 Inorganic constituents

The uronic acid residues of the gum exist largely as salts of calcium, magnesium and potassium, and the mineral load of a tear reflects the soil solution from which the tree drew its cations. Ahamad and co-workers reported sixty-two elements by ICP-MS in an Arabian myrrh resin, with calcium among the most abundant[29]. A 2025 data note compared single samples of Somali, Omani and Yemeni myrrh by ICP and found fourteen elements, including strontium, with potassium the most concentrated in all three and with element concentrations that differed between the three sources[36]. That study examined one specimen per origin, did not verify species, and cannot support any origin inference, but it does establish two facts that matter for Section 6: strontium is present in the gum at measurable levels, and the elemental profile of the gum is a variable that a provenance model could exploit.

4. Adulteration, Substitution and Grade

The modes by which a lot of myrrh may fail to be what it is sold as fall into four groups, and the analytical response to each is different. The first and most common is substitution by, or admixture with, the exudate of another *Commiphora* species. The bisabol-type resins of *C. kataf* and its allies, the scented myrrh of *C. guidottii*, the resins of *C. sphaerocarpa*, *C. holtziana* and *C. confusa* from the Horn of Africa, the Arabian balsam of *C. gileadensis*, the Yemeni *C. habessinica* and the Indian guggul of *C. wightii* have all appeared in, or are available to, the myrrh trade[5,6,8,14,32]. These resins share the furanosesquiterpene chemistry of true myrrh to varying degrees, so that detection relies on quantitative marker ratios rather than on presence or absence: opopanax is dominated by (E)-β-ocimene where myrrh is dominated by furanoeudesma-1,3-diene[26]; the African adulterants carry furanodienone, curzerenone and methoxylated germacrenones in proportions that differ from those of *C. myrrha*[5]; and guggul carries pregnane-dione steroids that true myrrh contains only in traces[32].

The second mode is substitution by exudates from outside the genus. The identification of *Liquidambar formosana* among commercial myrrh samples[14] shows that even distant substitutions occur, and the long history of the resin trade, in which African copal moved through Yemeni ports under other names[11], makes colophony from *Pinus*, dammar and various gums the obvious candidates for bulking or imitating a resin of higher value. Such substitutions are chemically gross and are easily detected by NMR, as discussed in Section 5.5. The third mode is dilution of the gum fraction with cheaper gums, of which gum arabic is the obvious candidate, or with mineral matter and moisture; the pharmacopoeial extractive limits are the historical response to this practice[13]. The fourth mode is not adulteration at all but the confusion of grades: the natural and colloidal forms of the Chinese Pharmacopoeia differ in volatile oil content by a factor of two[13], and the volatile oil content of a tear falls with age and exposure, so that a low oil content is not in itself evidence of fraud. A defensible analytical report separates these four possibilities explicitly, states which of them the data exclude, and does not describe a compositional anomaly as adulteration unless the alternative explanations of species, grade and aging have been eliminated.

5. NMR Spectroscopy: Composition, Identity and Adulteration

5.1 NMR as the structural basis of every myrrh marker

It is worth stating plainly that the entire marker chemistry of myrrh rests on NMR. The furanoeudesmanes of Brieskorn and Noble, the furanogermacranes of Maradufu and Warthen, the oxygenated germacranes of the Arabian resin, the octanordammaranes of Rahman and co-workers, and the thirty-four metabolites of Kuck and colleagues were all identified or confirmed by ^{1}H and ^{13}C NMR, increasingly supported by two-dimensional correlation experiments and, most recently, by quantum-chemical shift prediction[17,18,21–23,25,33,34]. Gas chromatography with mass spectrometry, the workhorse of essential oil analysis, identifies these compounds by comparison with libraries that were themselves populated from NMR-characterized isolates. When a chromatographic peak is labeled furanoeudesma-1,3-diene, that label is an NMR result at one remove. The consequence for authentication is that NMR applied directly to a resin extract does not merely complement GC–MS; it reports the same compounds by the technique that defined them, with the additional advantages that the measurement is quantitative without response factors, that it is non-destructive, and that it does not require the analyte to survive a hot injector.

5.2 The furanodiene–curzerene problem: why GC-derived compositions are method-defined

Germacrane sesquiterpenes containing a cyclodeca-1,5-diene system undergo a thermal [3,3]-sigmatropic (Cope) rearrangement to the corresponding elemanes, and the reaction proceeds readily at the temperatures of gas-chromatographic injectors and columns. The phenomenon is general and has been reviewed comprehensively: germacrene A gives β-elemene, germacrene B gives γ-elemene, germacrene C gives δ-elemene, germacrone gives elemenone, and the conversion increases with injector temperature, so that the co-occurrence of a germacrane and its elemane in a chromatogram is frequently an artifact of the analysis rather than a property of the plant[31]. Furanodiene, the germacrane that bears the furan ring, rearranges in exactly this way to curzerene, its elemane isomer[30,31].

The implication for myrrh is direct. Curzerene is reported as the major constituent of myrrh oil in several GC studies, at 40.1 % and 33.6 % in two of the analyses cited in Section 3.2[27,28], while furanoeudesma-1,3-diene leads in others[20,26], and a standardized extract is described in terms of its total furanodiene content[16]. Some of the curzerene in these reports is native, since curzerene was among the compounds isolated by HPLC at

ambient temperature[23]; but an unknown and probably large fraction of it is furanodiene that rearranged in the inlet. The point was made explicitly for another furanodiene-rich oil, that of *Eugenia uniflora*, in which NMR of the raw oil revealed germacrene-type compounds with only a small amount of curzerene, whereas the same oil heated to 240 °C, and its GC–MS trace, showed the elemanes; the authors concluded that the bioactivities attributed to curzerene over many years may belong to furanodiene[37]. Baldovini, Tomi and Casanova had already demonstrated the remedy two decades earlier by identifying and quantifying furanodiene and its rearrangement product furanoelemene in an essential oil directly by ^{13}C NMR, using the computer-aided component identification method developed in Corsica for whole essential oils[30], and the same laboratory applied ^{13}C NMR directly to a coniferous oleoresin to identify and quantify its acidic and neutral diterpenes without derivatization[38].

Three consequences follow for the authentication of myrrh. First, the marker ratios published from GC–MS are method-defined: two laboratories with different inlet temperatures will report different curzerene-to-furanodiene ratios for the same lot, and neither will report the native ratio. Second, any pharmacopoeial or contractual specification that fixes a curzerene content is, in effect, specifying the thermal history of the analysis. Third, NMR of the resin extract at ambient temperature is the only routine technique that reports the germacrane–elemane balance as it exists in the tear, and the native ratio of furanodiene to curzerene, together with that of germacrene B to γ-elemene, becomes available as an authentication and freshness variable that GC cannot supply. Germacrane ring protons in solution, moreover, show the broadened and temperature-dependent line shapes that arise from conformational exchange in the ten-membered ring, so that the germacrane and elemane forms are recognizable by their line shapes as well as by their chemical shifts[31]. Table 2 collects the GC-derived compositions from the literature so that the spread can be read against this explanation.

Table 2. Gas-chromatographic compositions of myrrh volatile fractions reported in the literature. Curzerene values include an unquantified contribution from furanodiene rearranged in the GC inlet, and the spread between studies is therefore partly analytical rather than botanical.

Study	Material and method	Curzerene	Furanoeudesma-1,3-diene	Lindestrene	Other major components / remarks
Başer et al. 2003[26]	Hydrodistilled resin oil; GC–MS	—	34 %	—	Opopanax (C. guidottii) compared: (E)-β-ocimene 33 %
Marongiu et al. 2005[20]	Supercritical CO_2 extract (9.0 MPa, 50 °C); GC–MS	8.5 %	34.9 %	12.9 %	Germacrone 5.8 %; yields SFE 3.2 %, hydrodistillation 2.8 %, steam distillation 0.4 %
Morteza-Semnani and Saeedi 2003[27]	Hydrodistilled oil (var. molmol); GC–MS	40.1 %	15.0 %	—	β-Elemene 8.4 %; 32 components identified
Alanazi et al. 2024[28]	Resin extracts; GC–MS	33.6 %	—	—	β-Elemene 5.8 %, 2-methoxyfuranodiene 5.4 %, germacrene B 4.4 %; 50 components
Ahamad et al. 2017[29]	Ethanolic extract of Arabian resin; GC–MS	7.1 % (area)	—	—	Limonene, germacrene B, β-selinene, spathulenol; 62 elements by ICP-MS
Germano et al. 2017[16]	Standardized extract (MyrLiq); GC	12.3 g/kg	18.8 g/kg	6.2 g/kg	Total furanodienes 40.9 g/kg

5.3 Whole-tear classification by solid-state ^{13}C NMR

The intact tear can be examined without any sample preparation beyond grinding. Lambert, Santiago-Blay and their collaborators have shown over two decades that the solid-state ^{13}C NMR spectrum of a plant exudate assigns it unambiguously to one of a small number of molecular classes. Resins are terpenoid and show saturated carbon envelopes between about 10 and 50 ppm with olefinic and, where present, aromatic carbons above 100 ppm; gums are polysaccharide and show the carbohydrate envelope between about 60 and 110 ppm; gum resins superimpose the two; and kinos and other phenolic exudates are recognized by a dominant phenolic carbon near 150 ppm[39]. Diagnostic criteria developed from sixty-five species allowed resins, gums and gum resins to be distinguished, and the typology that emerged followed taxonomy at the family level and in some cases at the genus or species level[39]. Principal-component and cluster analysis of NMR variables separated resins of the Araucariaceae, Cupressaceae, Pinaceae and Fabaceae from one another and from gums, gum resins and kinos[40], and a survey of 554 rosid exudates, the clade that contains the Burseraceae, classified about two-fifths as resins, a third as gums, one-ninth as gum resins and one-twelfth as kinos[41].

Myrrh is a gum resin in this classification, and its solid-state spectrum is therefore expected to display the carbohydrate and terpenoid envelopes side by side. The relative intensity of the two envelopes is a direct measure of the gum-to-resin proportion of the tear, which is precisely the quantity that the pharmacopoeial extractive limits address by gravimetry, and which varies with species, grade, age and adulteration. A gum-arabic-diluted lot would show an enhanced carbohydrate envelope; a colophony-adulterated lot would show the carboxyl carbon of the abietane resin acids near 185 ppm together with an enhanced saturated envelope; and a *Liquidambar* substitution would show the aromatic carbons of cinnamate esters. Because cross-polarization is not intrinsically quantitative, a direct-polarization magic-angle-spinning experiment with an adequate recycle delay, or a calibration of the cross-polarization response against gravimetrically fractionated reference tears, would be required before the envelope ratio could be reported as a gum-to-resin value. The same classification logic has been applied in solution: a recent study of *C. oddurensis* recorded ^{13}C spectra of the methanol-soluble and chloroform-soluble portions of the crude extract and interpreted them explicitly in terms of the resin, gum and gum-resin chemical-shift profiles[35]. PNA's solid-state capability makes the whole-tear experiment an inexpensive first screen, and it is the only measurement in the proposed protocol that examines the sample before any fraction has been discarded.

5.4 Solution-state fingerprinting and metabolomics of Commiphora resins

The first NMR-based metabolomic profiling of *Commiphora* oleo-gum-resins was reported in 2025 for four Arabian taxa, *C. gileadensis*, *C. quadricincta* and male and female *C. kataf*. Methanol extracts of resins and cell saps were examined by one- and two-dimensional NMR, thirty-six metabolites were identified comprising cycloartane triterpenoids, twelve amino acids and derivatives, seven organic acids, a phenolic acid, six sugars and sugar alcohols, six terpenoids and a nitrogenous compound, unsupervised and supervised multivariate models classified the samples by species, nine markers were quantified by qNMR, and terpenoids emerged as the discriminant compound class, with cycloartane derivatives enriched in *C. quadricincta* resin and *C. gileadensis* sap and trehalose more abundant in the other species[42]. The study demonstrates in the genus itself what plant NMR metabolomics had long promised: that a single ^{1}H NMR spectrum of a polar extract reports primary and secondary metabolites simultaneously, that the resulting fingerprint classifies species without prior selection of markers, and that the same spectrum yields absolute quantities for the markers that are selected. It does not yet include *C. myrrha*, and the extension of the approach to true myrrh, its African congeners and its documented adulterants is an obvious and unexploited opportunity.

The vetiver oil study of Schripsema and co-workers is the closest published model for the adulteration problem. Eight commercial oils declared pure were examined by GC–MS and by ^{1}H and ^{13}C NMR combined with spectral similarity calculations against registered reference spectra and with differential spectroscopy. NMR detected all six adulterants present in four of the samples, whereas GC–MS failed to detect adulteration with vegetable oils; ^{1}H NMR gave results within minutes, ^{13}C NMR gave unequivocal identification of the adulterants through difference spectra, and, because ^{13}C shifts are field-independent, a ^{13}C reference spectrum acquired on one instrument could be used with data from another[43]. Every element of this design transfers to myrrh: a registered reference spectrum of authenticated *C. myrrha* extract, a similarity score for each unknown, and a difference spectrum that exposes whatever the unknown contains that the reference does not. It is the difference spectrum, rather than any single marker, that would reveal a colophony, a foreign gum, a guggul or a Socotran lignan-rich resin in a lot sold as Yemeni myrrh.

5.5 A tiered NMR protocol for PNA

The protocol proposed in Table 3 is organized so that each tier answers a defined question and so that a lot can be released or rejected at the earliest tier that decides the matter. The whole-tear solid-state experiment of Tier 0 classifies the material and estimates the gum-to-resin proportion. Tier 1 is a ^{1}H spectrum of a chloroform-d extract of the powdered tear at 300 MHz. The furanosesquiterpenes present their α-furan proton as a sharp signal between δ 6.9 and 7.1, with the two compounds of Dekebo and co-workers, for example, resonating at δ 6.97 (d, J ≈ 1 Hz) and δ 7.07 (br s)[5]; the furan-bound methyl group appears as a singlet near δ 1.9–2.0; the eudesma-1,3-diene system of furanoeudesma-1,3-diene contributes olefinic methine multiplets between δ 5.5 and 6.1; lindestrene contributes exocyclic methylene protons near δ 4.6–4.9; curzerene contributes the vinyl ABX pattern of an elemane between δ 4.9 and 5.9; the methoxy and acetoxy substituents of 2-methoxyfuranodiene and 2-acetoxyfuranodiene appear as singlets near δ 3.3–3.5 and δ 2.0–2.1 respectively; and the germacrane ring methines of furanodiene and germacrene B appear as broadened signals between δ 4.7 and 5.3 whose breadth is itself diagnostic. These regions are cited from the primary structural papers as approximate ranges, and the exact assignments for a validated PNA method would be taken from spectra of isolated reference compounds run on the PNA instrument rather than from the literature. The literature assignments that could be verified in full are reproduced in Appendix A, where two practical points are also recorded: 2-acetoxyfuranodiene rearranges in chloroform-d that contains traces of acid, so that methanol-d_4 or freshly neutralized chloroform-d should be specified for the acetoxylated germacranes[44], and the furanodiene ring protons are broadened by conformational exchange, so that the ^{13}C experiment of Tier 2 rather than ^{1}H integration should carry the furanodiene quantitation[37].

Quantitation in Tier 1 follows ordinary qNMR practice: a weighed internal standard resonating in a vacant region of the spectrum, such as 1,4-dinitrobenzene at δ 8.41 or dimethyl terephthalate at δ 8.10; a 90° excitation pulse; a relaxation delay of at least five times the longest relevant ^{1}H T_1, which for the furan protons in a viscous resin solution is likely to require 20–30 s; and integration of the α-furan singlets, which are the cleanest handles in the spectrum. The sum of the α-furan integrals gives the total furanosesquiterpene content of the extract in a single measurement, and the resolved singlets give the individual markers. Tier 2 is the quantitative ^{13}C experiment, inverse-gated to suppress the nuclear Overhauser enhancement, which is the tier that adjudicates the furanodiene–curzerene and germacrene B–γ-elemene questions along the lines established by Baldovini, Tomi and Casanova[30], and which identifies triterpenoid and steroid substitutions through their carbonyl, olefinic and methyl carbons. The Corsican method identifies individual components in whole oils at contents as low as a few tenths of a percent from their ^{13}C shifts and relative intensities[30,38], and a chemical-shift library for the myrrh markers would need to be assembled from the primary literature and from isolated standards before the method could be applied routinely. Tier 3 examines the gum in

deuterium oxide: the anomeric region between δ 4.4 and 5.4, the 4-O-methyl singlet of the methylglucuronic acid near δ 3.4–3.5 with its carbon near 60 ppm, the uronic carboxyl carbon near 175 ppm, the amino-acid signals of the protein, and, as a flag for foreign gums, the rhamnose methyl doublet near δ 1.2–1.3, which is expected to be absent or weak in *C. myrrha* gum on the basis of the published sugar composition[1,19] but is prominent in arabinogalactan-protein gums of *Acacia* and was found in the gum of the Socotran *C. ornifolia*[10]. Tier 4 subjects the Tier 1 and Tier 3 spectra to chemometric classification against an authenticated library, using the similarity and difference-spectrum logic of the vetiver study[43] and the supervised models of the *Commiphora* metabolomics study[42].

Table 3. Tiered NMR protocol proposed for the authentication and grading of myrrh at PNA. Each tier answers a defined question, and a lot is released or rejected at the earliest tier that decides the matter.

Tier	Sample and experiment	Question answered	Key observables	Instrument
0	Ground intact tear; ^{13}C CP/MAS survey and DP/MAS for quantitation	Exudate class; gum-to-resin proportion; gross substitution	Carbohydrate envelope 60–110 ppm against terpenoid envelopes 10–50 and 105–150 ppm; resin-acid COOH ≈ 185 ppm; cinnamate aromatics	300 MHz solids or 200 MHz MAS
1	$CDCl_3$ extract (≈ 50 mg in 0.6 mL) with weighed internal standard; quantitative ^1H (90° pulse, $D_1 \geq 5\ T_1$)	Total and individual furanosesquiterpenes; methoxy and acetoxy markers; foreign terpenoids	α-Furan singlets δ 6.9–7.1; OCH_3 δ 3.3–3.5; OAc δ 2.0–2.1; olefinic region δ 4.6–6.1	300 MHz; totals feasible at 60 MHz
2	Same extract; inverse-gated quantitative ^{13}C	Native furanodiene : curzerene and germacrene B : γ-elemene; triterpenoid and steroid identity; colophony	Component identification from ^{13}C shifts and intensities; carbonyls 170–215 ppm; abietane resin-acid signals	300 MHz (^{13}C 75 MHz)
3	D_2O solution of the gum remaining after ethanol extraction; ^1H (and ^{13}C) at elevated temperature	Gum identity; foreign gums; protein content	Anomeric region δ 4.4–5.4; 4-O-methyl singlet δ ≈ 3.4–3.5; rhamnose CH_3 doublet δ ≈ 1.25; amino-acid signals	300 MHz
4	Chemometric evaluation of Tier 1 and Tier 3 spectra	Membership of the authenticated envelope; anomaly detection	PCA, SIMCA or OPLS-DA on bucketed spectra; similarity score and difference spectrum against a registered reference	Software

5.6 Benchtop feasibility at 60 MHz

Low-field benchtop spectrometers have advanced to the point where quantitative work on moderately complex mixtures is routine, and the limitations are those of dispersion rather than of sensitivity for a sample as concentrated as a resin extract[45]. At 60 MHz the α-furan singlets remain resolved from the olefinic multiplets because they lie more than 0.8 ppm downfield of them, and the furan-methyl, methoxy and acetoxy singlets are similarly isolated, so that a total-furanosesquiterpene assay and a 2-methoxyfuranodiene assay are feasible on PNA's 4IR AI-60 systems. The olefinic region between δ 4.6 and 6.1, which carries the information that distinguishes furanoeudesma-1,3-diene, lindestrene, curzerene and furanodiene from one another, collapses into overlapping multiplets at 60 MHz, and the individual-marker assay and the germacrane–elemane ratio therefore remain a 300 MHz task. The gum spectrum in deuterium oxide is a broad-line spectrum at any field and is not a benchtop candidate. A screening scheme in which the benchtop instrument performs the total-furanosesquiterpene assay and the gum-arabic flag on every lot, and the 300 MHz instrument performs the marker profile on lots that fail or that require a certificate, is the economically sensible division of labor.

5.7 What NMR cannot do

NMR reports composition, and composition is a function of species, tissue, harvest, age and adulteration, all of which it addresses well, but it is not a function of longitude. Two tears of *C. myrrha* of identical composition may have grown on opposite shores of the Gulf of Aden, and nothing in a ^{1}H or ^{13}C spectrum at natural abundance will distinguish them. Where compositional differences between regions do exist, they arise from the chemotype distribution of the populations, from harvest timing and from handling, and a chemometric model trained on such differences would be a model of the present-day trade rather than of geography, liable to fail as soon as harvest practice changed. The claims that NMR can support are therefore that a lot is or is not *C. myrrha*, that it does or does not contain identifiable foreign material, that its marker profile does or does not fall within the envelope of an authenticated reference set, and that its germacrane–elemane balance is or is not consistent with unheated resin. The claim that it grew in Yemen requires a different kind of evidence, to which the discussion now turns.

6. Isotope Ratio Mass Spectrometry and the Provenance Question

6.1 Principles

Stable isotope ratios of the light elements record the environment in which a plant fixed its carbon and took up its water and nutrients, and they do so through mechanisms that are understood well enough to be modeled. The carbon isotope composition of a C_3 plant is set by the discrimination of ribulose-1,5-bisphosphate carboxylase against $^{13}CO_2$, modulated by the ratio of intercellular to ambient CO_2, so that drought-stressed plants with restricted stomatal conductance are enriched in ^{13}C relative to well-watered plants of the same species[46]. The hydrogen and oxygen isotope ratios of plant organic matter derive from the local meteoric water, whose composition varies systematically with latitude, altitude, distance from the coast and rainfall amount and can be interpolated globally[47]; the oxygen and hydrogen of carbohydrates additionally record the evaporative enrichment of leaf water and a biochemical fractionation of approximately +27 ‰ for oxygen, as described by the mechanistic model of Roden, Lin and Ehleringer[48]. Plant nitrogen isotope ratios follow those of the soil, which become progressively enriched in ^{15}N as mean annual precipitation decreases and temperature increases, so that arid-zone vegetation tends toward high $\delta^{15}N$[49]. The radiogenic ratio $^{87}Sr/^{86}Sr$ is inherited without biological fractionation from the bioavailable strontium of the soil, and therefore from the age and lithology of the bedrock, for which predictive maps have been constructed[50]. Multi-element and multi-isotope analysis has become the standard approach to the geographical origin of foods, and its use in legal cases has been reviewed with attention to the reference databases and statistical validation that a court requires[51,52].

6.2 Isotope biochemistry of a three-fraction exudate

The difficulty that a gum-resin presents is that its three fractions are the products of three different biosynthetic compartments, and the isotope ratios of the whole tear are a weighted average of three different signals. The magnitude of the offsets is known from compound-specific studies of plant lipids. In C_3 angiosperms the n-alkanoic acids are depleted in deuterium by about 95 ‰ relative to ambient water and in ^{13}C by about 4 ‰ relative to bulk tissue, whereas the isoprenoids are depleted in deuterium far more strongly, by about 306 ‰ for phytol, which is synthesized through the plastidial methylerythritol-phosphate pathway, and by about 211 ‰ for sterols, which are synthesized through the cytosolic mevalonate pathway, with only small ^{13}C depletions of about 4 ‰ and 1 ‰ respectively[53]. The sesquiterpenoids and triterpenoids of the resin and oil are isoprenoids, and by analogy with the sterols they would be expected to lie some 200

‰ below source water in $\delta^{2}H$ and only 1–2 ‰ below bulk tissue in $\delta^{13}C$, although no compound-specific values for furanosesquiterpenes have been published and the pathway from which *Commiphora* draws its sesquiterpene precursors would need to be confirmed. The gum, by contrast, is a carbohydrate and carries the canonical carbohydrate signals: an oxygen isotope ratio tied to leaf and source water through the +27 ‰ fractionation, a hydrogen isotope ratio that includes exchangeable hydroxyl hydrogens which must be equilibrated or removed before measurement, and, through its protein, a nitrogen isotope ratio.

Two consequences follow. First, a bulk $\delta^{2}H$ or $\delta^{18}O$ value of an unfractionated tear is not comparable with the plant isoscapes and food databases that have been built almost entirely on carbohydrate-dominated tissues, and a bulk $\delta^{13}C$ value moves with the gum-to-resin ratio by several parts per thousand independently of any environmental effect. Second, the three fractions can be separated cleanly by solvent, and each then reports on its own compartment: the gum on source water, leaf evaporative environment and soil nitrogen; the resin and oil on the same source water through a large but systematic biosynthetic offset; and the ash of the gum on the strontium of the soil. A provenance study of myrrh must therefore be designed as three parallel studies on three fractions, or as a compound-specific study, and any bulk isotope value quoted for a myrrh sample should be treated with suspicion unless the fraction analyzed is stated.

6.3 Published isotope data on resins and their lessons

Only one study was found that set out to test whether isotope ratios could locate the origin of a plant resin, and its outcome is sobering. Stern, Lampert Moore, Heron and Pollard refined methods for bulk hydrogen, carbon and oxygen isotope analysis of modern and archaeological resins and found that modern resins vary within a single tree, between trees, with species and with location. All three isotopes showed increasing depletion of the heavy isotope with altitude, and δD and $\delta^{13}C$ decreased linearly with latitude, but when the method was applied to *Pistacia* resin from Canaanite amphorae imported into Egypt and to *Pinus* resin lining Roman amphorae from Britain to the eastern Mediterranean, the resin types and the modern and archaeological groups separated, some modern resins showed regional differences, and yet no clear pattern related the samples to the amphora fabrics or to the suggested manufacturing sites[54]. The study demonstrates the environmental sensitivity that makes isotope provenance possible in principle and the within-source variance that makes it difficult in practice, and it did so on pure resins that are not complicated by a gum fraction.

The other published measurement on a Burseraceae resin makes a complementary point. In a survey of 119 herbs and spices from the Italian market, the resin of Indian frankincense, *Boswellia serrata*, showed the lowest $\delta^{18}O$ of the entire set at 14.7 ‰ and the lowest $\delta^{2}H$ at −158 ‰, values that the authors attributed to the altitude of its mountainous origin[55]. A more parsimonious explanation is available from Section 6.2: the material was a terpenoid resin measured alongside carbohydrate-dominated leaves, flowers and seeds, and the biosynthetic depletion of isoprenoids in deuterium, together with the non-carbohydrate origin of the resin's oxygen, would produce exactly such an offset without any altitude effect at all. The example is instructive because it shows how readily a bulk resin value can be misread when it is placed in a database of unlike matrices, and it underlines the requirement for a resin-specific, and fraction-specific, reference set. No isotopic data of any kind were found in this search for *C. myrrha* resin of documented geographical origin, and the provenance case for Yemeni myrrh therefore begins from an empty database.

6.4 Compound-specific isotope analysis of the furanosesquiterpenes

Gas chromatography coupled through a combustion or pyrolysis interface to an isotope ratio mass spectrometer (GC–C/P-IRMS) measures $\delta^{13}C$ and $\delta^{2}H$ of individual volatile compounds, and it has been the

decisive technique for the authenticity of essential oils since the linalool and linalyl acetate work of Hanneguelle and co-workers, which combined site-specific ^{2}H NMR with IRMS to characterize compounds of defined botanical and geographical origin[56]. Multi-element, multi-component GC–IRMS combined with enantioselective GC has since been applied to the authenticity assessment of citrus oils, in which the isotope ratios of individual terpenes distinguished natural from reconstituted material[57]. The requirements of the technique are modest in mass and severe in chromatography: the compound must be baseline-resolved and must enter the combustion or pyrolysis reactor unchanged.

The second requirement collides with Section 5.2. In a GC–IRMS injector furanodiene rearranges to curzerene exactly as it does in a GC–MS injector, so that the isotope ratio measured on the curzerene peak is that of a merged pool of native curzerene and rearranged furanodiene, and the same applies to the germacrene B–γ-elemene pair. Because a sigmatropic rearrangement conserves the atoms of the molecule, the merged value is not wrong as a description of the pool, but it is not the isotope ratio of either compound and it will vary with injector temperature. Furanoeudesma-1,3-diene and lindestrene, which are already bicyclic eudesmanes and have no cyclodecadiene to rearrange, are the thermally stable candidates for compound-specific work, and cool on-column or programmed-temperature vaporization injection would be required if the germacranes were to be measured at all. With these precautions, δ^{13}C and δ^{2}H of furanoeudesma-1,3-diene and lindestrene would provide the resin-side provenance variables, and δ^{2}H in particular, being tied to source water through a large but reproducible biosynthetic offset, is the parameter most likely to separate a Yemeni highland-fringe source from a Somali lowland source if such a separation exists.

6.5 Strontium and multi-element provenance

The gum fraction is the natural target for ^{87}Sr/^{86}Sr, because its uronic acid residues bind the alkaline-earth cations of the soil solution and because strontium has been shown to be present in Somali, Omani and Yemeni myrrh alike[36]. The ratio inherited by the tree reflects the weathering of the local bedrock: young mantle-derived volcanic rocks give ratios near 0.703–0.706, marine carbonates track the seawater curve of their depositional age near 0.707–0.709, and old crystalline basement gives ratios above 0.710, so that predictive isoscapes can be constructed from geological maps and validated with water and plant measurements[50]. The Yemeni highlands are dominated by Oligocene–Miocene flood basalts resting on Precambrian basement, while the Hadramaut plateau and much of the Somali myrrh country are underlain by Mesozoic and Paleogene marine sediments with basement inliers; the lithologies of the two shores therefore differ in places and overlap in others, and a strontium-based discrimination would succeed for some pairs of source areas and fail for others. The elemental profile of the gum, which the 2025 data note found to differ among its three single specimens[36], would add further variables, and the multi-element approach that has become standard for foods[51] would be applied to the ash of the gum rather than to the whole tear, since the resin contributes almost nothing to the mineral load. As with every provenance parameter, the value of ^{87}Sr/^{86}Sr lies entirely in the reference set against which it is read.

6.6 Isotopic NMR: SNIF-NMR and ^{13}C isotopomics as the bridge between the techniques

The relationship between NMR and IRMS is closer than the preceding sections suggest, because NMR is itself an isotope-ratio technique when it is used to measure natural-abundance deuterium or the site-specific distribution of ^{13}C. Martin and Martin showed in 1981 that quantitative ^{2}H NMR at high field could measure the natural deuterium distribution within a molecule[58], and the resulting method, site-specific natural isotope fractionation studied by NMR (SNIF-NMR), was applied to plant products to reveal that the deuterium content of each hydrogen site records the biosynthetic pathway and the environmental water of the source

plant in a way that a molecular average from IRMS cannot[59]. It was exactly this combination of site-specific ^{2}H NMR and IRMS that Hanneguelle and co-workers used to characterize linalool of known botanical and geographical origin[56]. The corresponding ^{13}C method, in which a highly precise quantitative ^{13}C spectrum yields the isotope ratio at every carbon of a pure compound, was established by the Nantes group with a demonstrated repeatability suitable for authenticity work[60] and has been reviewed under the name isotopic NMR at natural abundance[61].

For myrrh the application would be to furanoeudesma-1,3-diene, which is the most abundant single component of the oil and is isolable by chromatography. The mass requirement is the limiting factor: SNIF-NMR and ^{13}C isotopomics require of the order of one to two hundred milligrams of pure compound per determination. At a volatile oil yield of about 3 % and a furanoeudesma-1,3-diene content of about 35 % of the oil[20], one hundred grams of resin would furnish on the order of a gram of the compound before chromatographic losses, so that the requirement is met with a modest quantity of a low-cost raw material, and the corresponding arithmetic is recorded in the companion workbook. What such a measurement would provide is a multi-site isotopic fingerprint of a single, thermally stable, species-characteristic molecule, immune to the injector artifact of Section 6.4 and independent of the gum-to-resin ratio of Section 6.2, and capable in principle of distinguishing a natural furanosesquiterpene from a synthetic or biotechnological one should reconstituted myrrh oils ever appear. PNA's 300 MHz system lies within the field range used for SNIF-NMR of ethanol under the official wine method, but a ^{2}H observation channel with a ^{19}F lock and the associated reference materials would be required, and site-specific ^{13}C work at the precision of the Nantes group has been performed at fields of 9.4 T and above. The technique is therefore presented as a prospective capability and as the conceptual link between the two halves of this paper rather than as a current PNA service.

6.7 Expectations for Yemeni material and the reference-database requirement

It is possible to state qualitatively what isotopic character Yemeni-grown *C. myrrha* should have, provided that the statements are recognized as predictions rather than measurements. As a C_3 shrub of arid scrub the plant should carry $\delta^{13}C$ values toward the ^{13}C-enriched end of the C_3 range, reflecting the stomatal limitation of drought[46], and the same should be true of its Somali counterpart, so that carbon is unlikely to be the discriminating element. Its gum $\delta^{15}N$ should be high, reflecting arid soils[49], again on both shores. The hydrogen and oxygen isotope ratios of the gum, and the hydrogen isotope ratios of the furanosesquiterpenes, should reflect the local precipitation; the Yemeni western highlands receive orographic and monsoonal rainfall that is depleted by altitude relative to the coastal lowlands and to the Somali plains, but *C. myrrha* occupies low to moderate elevations on both sides of the Gulf of Aden, so that the altitude lever available to the analyst is smaller than the topography of Yemen might suggest. Strontium offers the most categorical contrast where basalt-derived soils are involved, and no contrast where carbonate-derived soils face one another across the Gulf. In short, the isotope systems that are most likely to separate a Yemeni from a Somali source are $\delta^{2}H$ and $\delta^{18}O$ of the gum, $\delta^{2}H$ of the furanosesquiterpenes and $^{87}Sr/^{86}Sr$ of the gum ash, and whether they actually do so can only be learned from a reference set.

The requirements of that reference set are those that have been articulated for food provenance in legal contexts[52]: samples collected from identified trees at known coordinates rather than purchased in markets, species confirmed by a genetic method such as the psbA–trnH barcode that has already been validated for commercial myrrh[14], replication across trees, sites and harvest years sufficient to estimate the within-source variance that Stern and co-workers found to be large[54], fraction-specific measurement, classification models validated on held-out samples, and a statement of the probability of misassignment rather than a categorical origin. Until such a set exists, the honest position is that a Yemeni origin for a myrrh lot can be made

consistent with isotopic data but cannot be proven by them, and that the same lot can be shown by NMR to be, or not to be, authentic *C. myrrha* of good grade regardless of where it grew.

7. How NMR and IRMS Relate: an Integrated Evidence Framework

The two techniques answer different questions, and the relationship between them is sequential rather than parallel. NMR establishes what the material is; IRMS, read against a reference set, establishes where a material of that identity is likely to have grown; and isotopic NMR, when it becomes available, would report the isotopic character of a single named molecule with a site resolution that IRMS cannot reach. Table 4 summarizes the isotope systems and the fraction on which each must be measured, and Table 5 sets the questions of authenticity and origin against the two techniques. The logic of Figure 1 follows from these tables. A lot is first classified and screened by whole-tear ^{13}C NMR, then profiled by ^{1}H and ^{13}C NMR of its solvent fractions and classified against the authenticated library; only a lot that passes as *C. myrrha* free of foreign material proceeds to fraction-specific isotope analysis, because an isotope value measured on a substituted or diluted lot describes a mixture and cannot be assigned to any single source. The order matters for cost as well as for logic: the NMR tiers are performed on PNA instruments in hours, whereas GC–C/P-IRMS and multi-collector ICP-MS for strontium are subcontracted measurements that should be reserved for lots whose identity has been secured.

Table 4. Isotope systems available for the provenance of myrrh, the fraction on which each must be measured, the controlling variables, and the expected power to separate Yemeni from Somali sources. All expectations are predictions; no reference data exist for C. myrrha.

Isotope system	Fraction measured	Controlling variables	Expected discriminating power	Key references
$\delta^{13}C$ (EA-IRMS; GC–C-IRMS)	Gum; furanoeudesma-1,3-diene and lindestrene	C_3 discrimination and stomatal limitation under drought; small isoprenoid offset	Low: both source regions are arid C_3 scrub	[46,53]
$\delta^{2}H$ (EA-IRMS after exchangeable-H control; GC–P-IRMS)	Gum; furanosesquiterpenes	Source water and leaf evaporation; isoprenoid depletion of ≈ 200 ‰	Moderate: altitude and continentality contrasts, limited by the low-elevation habitat	[47,48,53]
$\delta^{18}O$ (EA-IRMS)	Gum	Source water and leaf enrichment; +27 ‰ carbohydrate fractionation	Moderate	[47,48]
$\delta^{15}N$ (EA-IRMS)	Gum protein	Soil nitrogen and aridity	Low	[49]
$^{87}Sr/^{86}Sr$ (MC-ICP-MS)	Gum ash	Bedrock lithology through bioavailable soil strontium	High where basalt-derived soils face carbonate-derived soils; nil where lithologies coincide	[36,50]
Multi-element (ICP-MS)	Gum ash	Soil chemistry and dust	Unknown; only single-sample data published	[36,51]
Site-specific ^{2}H and ^{13}C (isotopic NMR)	Isolated furanoeudesma-1,3-diene (≥ 100–200 mg)	Biosynthetic and water signals resolved per site	Prospective; also natural-versus-synthetic discrimination	[56,59–61]

Table 5. Questions of authenticity and origin for a myrrh lot set against the evidence that NMR spectroscopy and isotope ratio mass spectrometry can supply.

Question	NMR	IRMS	Decisive evidence	Principal limitation
Species identity (C. myrrha versus congeners)	Yes: marker profile, triterpenoid and steroid content, gum sugars	No	NMR tiers 1–3 supported by DNA barcoding	Chemotype overlap among furanosesquiterpene-bearing congeners
Foreign material (colophony, gum arabic, Liquidambar, guggul)	Yes: whole-tear spectrum, ^{13}C difference spectra, gum spectrum	Only for C_4-derived diluents through $\delta^{13}C$	NMR	Detection limit of the order of 1–2 % of the extract
Native germacrane–elemane balance and thermal history	Yes, uniquely	No; GC–IRMS shares the inlet artifact	Quantitative ^{13}C NMR	Requires a ^{13}C shift library for myrrh
Grade, volatile content and age	Yes, semi-quantitatively, with gravimetry	No	Tiers 0 and 1	Aging and dilution can produce similar gross composition
Natural versus synthetic marker compound	Prospective through isotopic NMR	Yes: δ^2H and $\delta^{13}C$ of the compound	IRMS now; SNIF-NMR later	No reference values for synthetic furanosesquiterpenes
Geographical origin	No	Yes, conditional on a reference database	Fraction-specific IRMS and $^{87}Sr/^{86}Sr$	No C. myrrha database exists; the trade name is unreliable

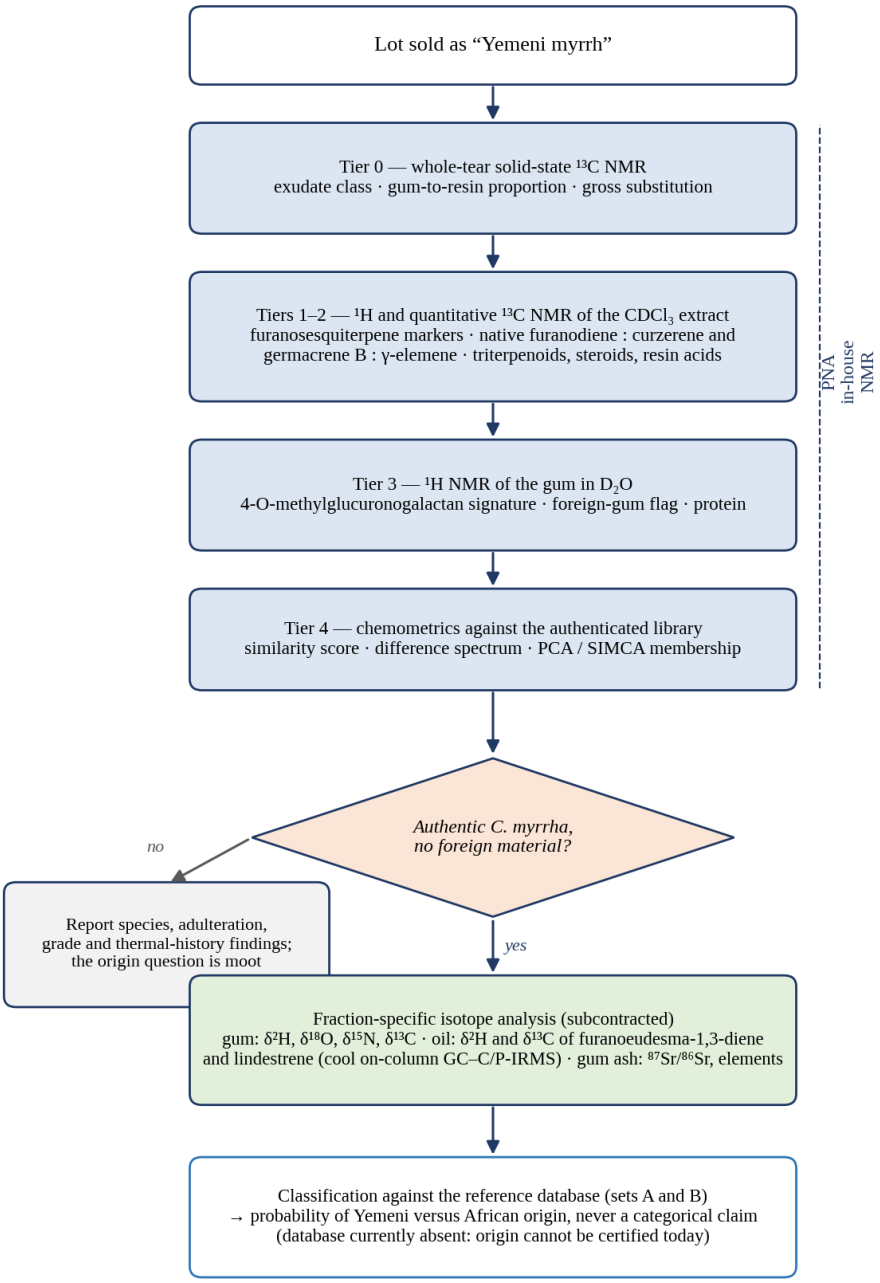


Figure 1. Sequential evidence framework for a lot sold as Yemeni myrrh. NMR establishes identity, integrity, grade and thermal history in-house; only lots that pass proceed to fraction-specific isotope analysis, whose interpretation is conditional on a reference database that does not yet exist for C. myrrha.

The framework also fixes the limits of what may be claimed. An NMR report can state that a lot is authentic *C. myrrha* of a stated grade, that it contains no detectable foreign resin, gum or steroid, and that its germacrane–elemane balance is consistent with unheated material. An isotope report can state that the fraction-specific values fall within, or outside, the envelope of a named reference set and can attach a classification probability to the assignment. Neither report can state that a lot grew in Yemen unless a Yemeni reference set exists, and the second report cannot be written at all until one does. The framework

therefore converts a question that is currently unanswerable, whether a given resin is Yemeni, into two questions that are answerable now and one that becomes answerable once a defined program of sampling and measurement has been completed.

8. Proposed PNA Reference Program for Yemeni Myrrh

The program outlined in Table 6 is designed to build the two libraries that the framework requires: an authenticity library of NMR fingerprints covering *C. myrrha*, its congeners and its documented adulterants, and a provenance library of fraction-specific isotope values for tree-collected *C. myrrha* from both shores of the Gulf of Aden. The authenticity library is the more tractable, because the congeners and adulterants can be obtained from botanical gardens, from reference collections and from the trade, and because the NMR measurements are performed in-house. The provenance library is the more demanding, because it requires field collection in Yemen and in Somalia, Ethiopia or Kenya under conditions that are logistically and politically difficult, and because the within-source variance reported for other resins[54] implies that dozens of trees per region, sampled across at least two harvest seasons, will be needed before a classification model can be validated on held-out samples. Collection protocols would record the tree, its coordinates and elevation, the date and method of tapping, the age of the tear at collection and the storage history, and each collection would be vouchered and sequenced at the psbA–trnH locus so that species identity rests on genetic rather than morphological evidence[14].

The measurement plan applies every NMR tier of Section 5.5 to every sample and adds, for sets A, B and D, elemental-analyzer IRMS of the gum for $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$ after equilibration or nitration to control exchangeable hydrogen, GC–C/P-IRMS of furanoeudesma-1,3-diene and lindestrene in the volatile oil with cool on-column injection, and $^{87}Sr/^{86}Sr$ by multi-collector ICP-MS on the ash of the gum. Every isotope value is reported with the fraction on which it was measured. The statistical treatment follows the practice recommended for legal food-origin work[52]: exploratory principal-component analysis of the combined NMR and isotope variables, supervised classification by partial-least-squares discriminant analysis or soft independent modeling of class analogy trained on sets A and B, external validation on the retail set D, and reporting of misclassification rates and of the variables that drive each separation. The deliverables would be an NMR reference-spectrum library with registered similarity thresholds, a fraction-specific isotope database with its metadata, a validated classification model with its performance statistics, and a written method whose scope is limited to what the data support. The same discipline that has served PNA in litigation, namely restricting an expert opinion to the methodology and evidence actually in hand, applies here: an origin claim would be reported as a classification probability against a named reference set and never as a categorical fact.

Table 6. Sample sets and measurements of the proposed PNA reference program for Yemeni myrrh.

Set	Composition	Purpose	Target size	Measurements
A	Tree-collected C. myrrha from Yemeni source areas (Hadramaut, Shabwa, Abyan, Lahij and the Tihama foothills), GPS-referenced, vouchered and psbA–trnH-verified	Yemeni provenance reference	≥ 30 trees over ≥ 2 harvest seasons	All NMR tiers; fraction-specific EA-IRMS; GC–C/P-IRMS of furanoeudesma-1,3-diene and lindestrene; $^{87}Sr/^{86}Sr$ and multi-element on gum ash
B	Tree-collected C. myrrha from Somaliland, Puntland, the Ogaden and northeastern Kenya, documented as for set A	African comparison class	≥ 30 trees over ≥ 2 harvest seasons	As for set A

Set	Composition	Purpose	Target size	Measurements
C	Congeners and adulterants: C. kataf, C. guidottii, C. gileadensis, C. wightii, C. sphaerocarpa, Socotran C. ornifolia; gum arabic; colophony; Liquidambar storax	Authenticity library	≥ 5 lots each	All NMR tiers
D	Commercial lots sold as Yemeni myrrh (Sana'a, Aden, Mukalla; export lots)	External test set	≥ 20 lots	All NMR tiers; isotopes on lots passing the NMR tiers
E	Aged and deliberately heated subsets of set A	Freshness and thermal-history variables	Subset	Tiers 1 and 2 (germacrane–elemane ratios)

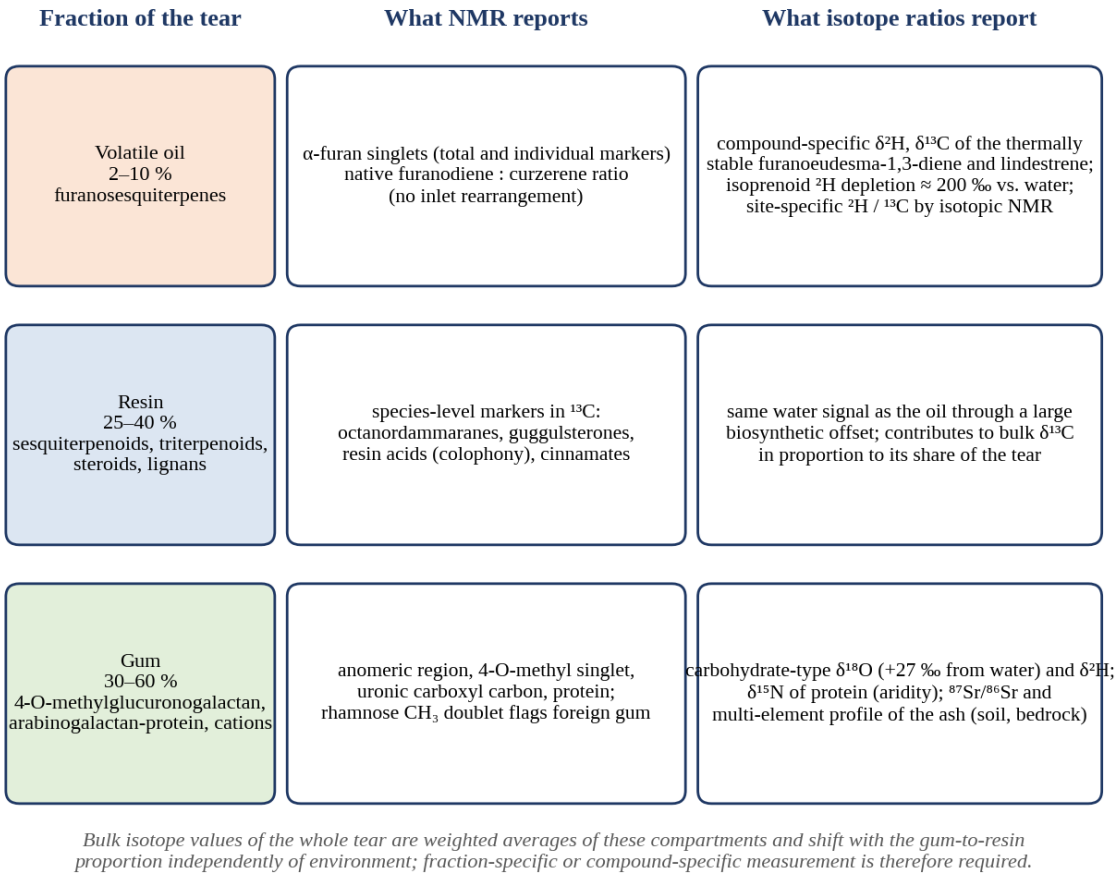


Figure 2. Schematic partition of chemical and isotopic information among the three fractions of a myrrh tear. Bulk isotope values of the whole tear are weighted averages of compartments with different biosynthetic offsets, which is why fraction-specific measurement is required.

9. Critical Assessment

A reviewer or an opposing expert asked to evaluate a present-day claim of Yemeni origin for a myrrh lot would have no difficulty identifying its weaknesses, and it is better that they be stated here. The first is the trade name itself: as Section 2.2 showed, Yemeni ports have handled African exudates for a thousand

years[11], so that a Yemeni market provenance is evidence of sale rather than of growth. The second is the heterogeneity of the retail material, demonstrated by an order-of-magnitude spread in marker content among five Sana'a samples[15] and by the finding that only 8 % of commercial myrrh samples from a major import market matched a pharmacopoeial species[14]; a model of Yemeni myrrh trained on market samples would be a model of the market. The third is the absence of any isotopic reference dataset for *C. myrrha* of documented origin, so that every isotopic expectation in Section 6.7 is a prediction from first principles rather than an observation. The fourth is the outcome of the only resin provenance study designed to test the approach, which could not resolve manufacturing sites despite clear environmental trends[54]. The fifth is the confounding of bulk isotope values by the gum-to-resin proportion, which invalidates any whole-tear isotope comparison and which has already led to a questionable altitude interpretation of a Burseraceae resin in the literature[55].

On the NMR side the weaknesses are of a different kind. The chemical-shift assignments assembled in Table 1 are drawn from structural papers on isolated compounds and from a small number of mixture studies, and a validated PNA method would require spectra of isolated standards on the PNA instruments and a ^{13}C shift library of the kind that the Corsican group built for essential oils[30,38]; until that library exists, the identification of minor components in a whole extract rests on the literature and should be described as tentative. The benchtop assay is limited to totals and to the isolated singlets, and any claim to profile individual markers at 60 MHz would be contested. The published GC compositions that a reviewer would cite as the accepted chemistry of myrrh are themselves compromised by the inlet rearrangement described in Section 5.2, and a laboratory that quantifies furanodiene by NMR must be prepared to explain why its curzerene values are lower than those in the literature, with the Corsican and Brazilian studies as its authority[30,37]. Finally, the gum-based markers proposed in Section 5.5, in particular the rhamnose flag, rest on a sugar composition reported in 1995 for an unspecified number of samples[19] and contradicted, for a Socotran congener, by a 2025 study[10]; the variability of the gum composition within *C. myrrha* itself is unknown and would have to be established before the flag could be used against a lot.

None of these weaknesses is fatal, but each dictates the wording of any report. The compositional claims of Section 7 can be made now, with the stated caveats on shift libraries and detection limits. The provenance claims cannot be made now, and a client who asks for a certificate of Yemeni origin on the basis of composition or of a single isotope value should be told that no such certificate is scientifically defensible, that the reference program of Section 8 is the route to one, and that even a completed program would deliver a probability rather than a proof.

10. Conclusions

Myrrh from Yemen presents the general problem of resin authentication in a particularly clear form. The producing species grows on both shores of the Gulf of Aden, the trade has moved African resin through Yemeni ports for a millennium, the pharmacopoeias define the material permissively and without reference to origin, and the retail material is chemically and genetically heterogeneous. Against this background NMR spectroscopy is the technique of choice for the compositional half of the problem. Every marker of true myrrh was defined by NMR; NMR of the resin extract at ambient temperature reports the native germacrane–elemane balance that gas chromatography destroys in its inlet; solid-state ^{13}C NMR classifies the intact tear and measures its gum-to-resin proportion; and ^{1}H and ^{13}C NMR of the gum expose a proteoglycan chemistry that is species-specific and has been neglected. A tiered protocol on PNA's instruments can therefore establish species, grade, thermal history and the presence of foreign material for any lot. Isotope ratio mass spectrometry is the technique for the geographical half, but only when it is

applied to separated fractions or to individual thermally stable compounds, only when the analytes are interpreted with the biosynthetic offsets of a gum-resin in mind, and only against a reference set that does not yet exist for *C. myrrha*. Isotopic NMR on isolated furanoeudesma-1,3-diene is the natural bridge between the two techniques and a realistic future capability. The reference program proposed here would convert the question of Yemeni origin from an unanswerable one into a statistically bounded one, and the critical assessment records the limits within which any report on this material should be written.

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Summary

Myrrh is a three-fraction exudate whose volatile oil, resin and gum carry different chemical and isotopic information. *Commiphora myrrha* grows in both Yemen and the Horn of Africa, Yemeni ports have historically re-exported African resins, the pharmacopoeias admit other *Commiphora* species and set no origin requirement, and retail material sold as Yemeni myrrh varies by an order of magnitude in marker content and is frequently not *C. myrrha*. Authenticity and origin are therefore separate questions requiring separate evidence.

NMR spectroscopy is the foundation of the marker chemistry of myrrh and is uniquely able to report the native furanodiene-to-curzerene and germacrene B-to-γ-elemene ratios, because these germacranes undergo Cope rearrangement in gas-chromatographic inlets and most published GC compositions are consequently method-defined. Solid-state ^{13}C NMR of the intact tear classifies the exudate and estimates the

gum-to-resin proportion; quantitative ^{1}H NMR of the chloroform extract yields total and individual furanosesquiterpene contents from the isolated α-furan singlets; inverse-gated ^{13}C NMR identifies triterpenoid, steroid and resin-acid substitutions; ^{1}H NMR of the gum in deuterium oxide exposes the 4-O-methylglucuronogalactan signature and flags foreign gums; and chemometric comparison against an authenticated library detects anomalies. A benchtop assay at 60 MHz can perform the total-furanosesquiterpene and foreign-gum screens.

Isotope ratio mass spectrometry addresses origin through δ^{13}C, δ^{2}H, δ^{18}O, δ^{15}N and ^{87}Sr/^{86}Sr, but the isoprenoid fractions of myrrh are depleted in deuterium by roughly 200 ‰ relative to source water while the gum carries carbohydrate-type signals, so that bulk values on an unfractionated tear are confounded by composition. Fraction-specific measurements on the gum, compound-specific measurements on the thermally stable furanoeudesma-1,3-diene and lindestrene, and strontium isotope measurements on the gum ash are the defensible design. No isotopic reference data exist for *C. myrrha* of documented origin, the only provenance study on resins could not resolve sites, and a Yemeni origin can at present be made consistent with data but not proven.

SNIF-NMR and ^{13}C isotopomics on isolated furanoeudesma-1,3-diene, feasible in mass terms from a hundred grams of resin, are the bridge between NMR and IRMS and a prospective PNA capability. A reference program of tree-collected, DNA-verified samples from Yemen and East Africa, analyzed by the full NMR protocol and by fraction-specific IRMS with external validation, is proposed as the only route to a scientifically defensible origin claim, which would be reported as a classification probability against a named reference set rather than as a categorical fact.

Appendix A. Molecular Structures and Reference NMR Data for the Marker Compounds

A.1 Scope, sources and limitations

The compounds discussed in Sections 3 and 5 are drawn in Figures A1 and A2 with the carbon numbering used in the NMR tables that follow, and their identifiers are collected in Table A1. Structures were generated from SMILES strings whose connectivity and stereodescriptors were verified against the PubChem records[62] listed in Table A1, with two exceptions: the PubChem record returned for the name curzerene describes a dihydro compound ($C_{15}H_{22}O$) rather than curzerene ($C_{15}H_{20}O$) and was not used, and no PubChem record exists for 2-acetoxyfuranodiene or 3,4-seco-mansumbinoic acid, whose structures were derived from the 2-methoxyfuranodiene and mansumbinone records respectively and checked against the published ^{13}C data. Double-bond geometries are drawn as recorded in PubChem; the relative configuration of curzerene is not depicted. InChIKeys were computed from the verified structures so that any of them can be matched against spectral libraries without ambiguity.

Only ^{1}H and ^{13}C data that could be transcribed from sources verified in full text during the preparation of this document are reproduced. Three such sources exist. The first is the NMR study of *Eugenia uniflora* essential oil by Schaffka and co-workers, in which furanodiene, germacrene B and germacrone were assigned in the unheated oil, and curzerene and γ-elemene in the oil after heating to 240 °C, by HSQC, selective HSQC and HMBC at 400 MHz in $CDCl_3$[37]; because these assignments were made on a mixture, the ^{1}H signals are listed as published, without position assignments except for H-12, and probable assignments added in this document are marked as such. The second is the dissertation of Unterholzner, from which the fully assigned 600 MHz data for 2-acetoxyfuranodiene, 1(10)E,4E-furanodienone, curzerenone and myrrhone isolated from *C. myrrha* resin are taken[44]; the corresponding isolations are reported in the associated journal article[18]. The third is the supplementary material of Kuck and co-workers, which tabulates the 400 MHz data of 3,4-seco-mansumbinoic acid isolated from myrrh[17]. Complete assignments for furanoeudesma-1,3-diene and lindestrene were published by Brieskorn and Noble and by Maradufu and Warthen[21,23], and data for mansumbinone by Rahman and co-workers[33]; those papers are not open access and their tables could not be verified in this session, so their data are not reproduced here and should be transcribed by the author from the original articles. No verified open-access assignment for the guggulsterones was located and none is reproduced.

Two observations in the sources bear directly on the PNA protocol of Section 5.5. Unterholzner reported that 2-acetoxyfuranodiene rearranged to an artifact during NMR measurement in $CDCl_3$ and that a second isolate of the same compound discolored in the same solvent, so that the reference data were recorded in methanol-d_4[44]; chloroform-d that has not been freed of traces of acid is therefore an unsafe solvent for quantitative work on the acetoxylated germacranes, and either methanol-d_4 or chloroform-d passed through basic alumina immediately before use should be specified. Schaffka and co-workers observed that the germacrane ring protons of furanodiene are broadened by conformational exchange and that curzerene could be assigned only after thermal treatment of the oil[37], which is the spectroscopic counterpart of the inlet artifact discussed in Section 5.2.

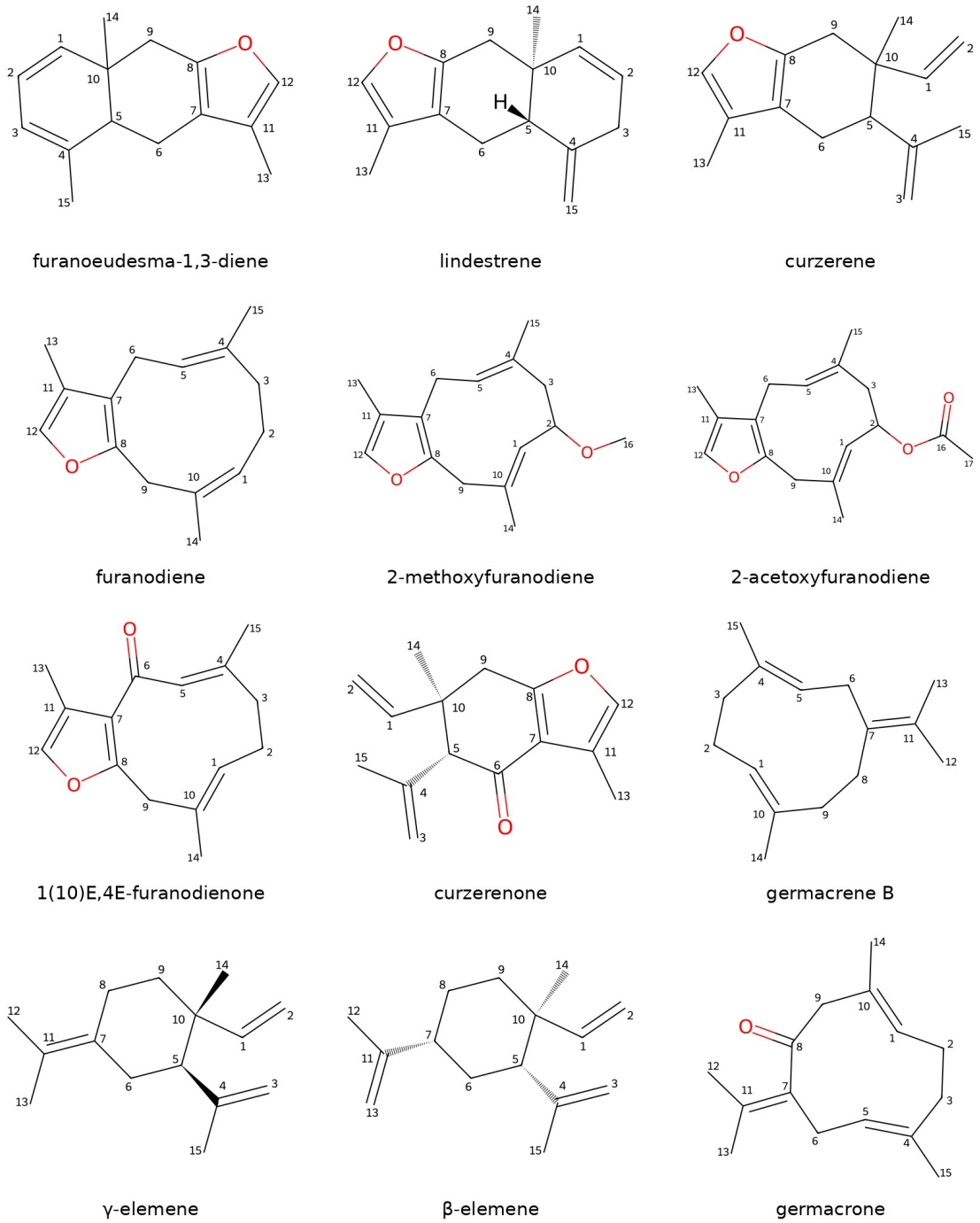


Figure A1. Furanosesquiterpene and hydrocarbon marker compounds of myrrh with the carbon numbering used in Tables A2–A9. Double-bond geometries as recorded in PubChem; the relative configuration of curzerene is not depicted.

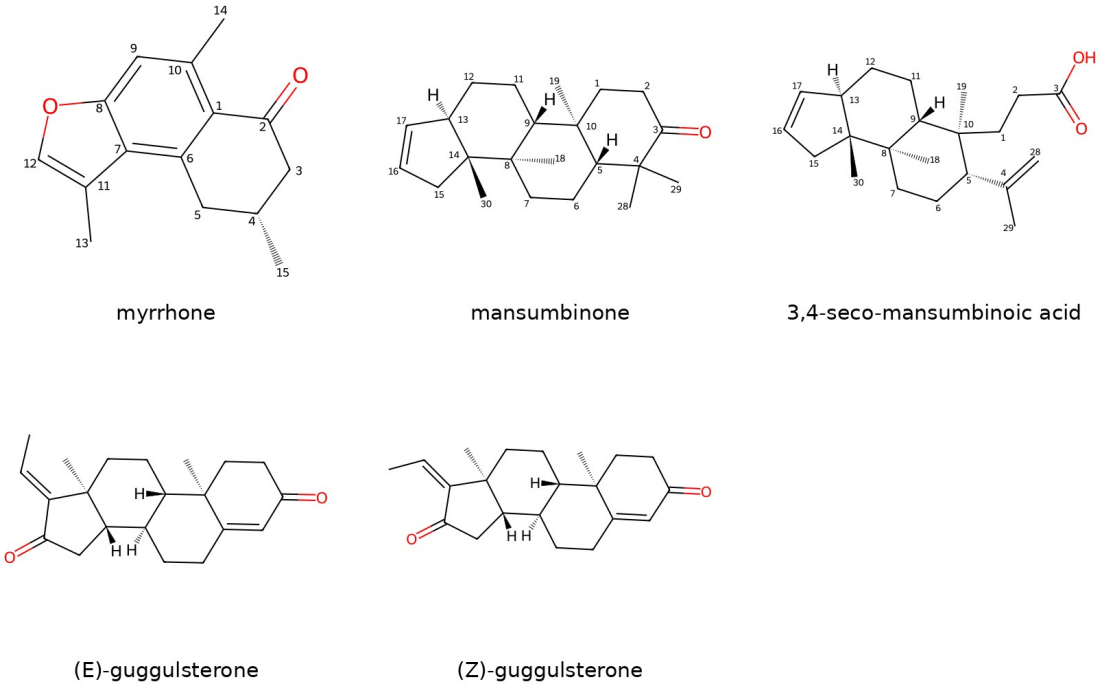


Figure A2. Myrrhone, the octanordammarane triterpenoids mansumbinone and 3,4-seco-mansumbinoic acid (numbering used in Table A11), and the (E)- and (Z)-guggulsterones that mark guggul (C. wightii) substitution.

A.2 Identifiers

Table A1. Identifiers of the compounds drawn in Figures A1 and A2. Molecular formulas, molecular weights and InChIKeys were computed from the verified structures; PubChem compound identifiers (CID) are given where the database record was checked[62].

Compound	Class	Formula	MW	InChIKey	PubChem CID	NMR data in this appendix
Furanoeudesma-1,3-diene	Furanoeudesmane	$C_{15}H_{18}O$	214.31	AUSAHAHDEVYCOC-UHFFFAOYSA-N	13874240	Not reproduced (refs.[21,23])
Lindestrene	Furanoeudesmane	$C_{15}H_{18}O$	214.31	BZQURGSQMBBPRU-DZGCQCFKSA-N	12311270	Not reproduced (refs.[21,23])
Curzerene	Furanoelemane	$C_{15}H_{20}O$	216.32	HICAMHOOTMOHPA-UHFFFAOYSA-N	—	Table A3[37]
Furanodiene	Furanogermacrane	$C_{15}H_{20}O$	216.32	VMDXHYHOJPKFEK-RZWYOFBOSA-N	9601230	Table A2[37]
2-methoxyfuranodiene	Oxygenated furanogermacrane	$C_{16}H_{22}O_2$	246.35	NWLNPDFDTSFGEU-QAZZFAKDSA-N	6325622	Not reproduced (ref.[22])
2-acetoxyfuranodiene	Oxygenated furanogermacrane	$C_{17}H_{22}O_3$	274.36	VIAGREYUICSQGX-QAZZFAKDSA-N	—	Table A7[44]
1(10)E,4E-Furanodienone	Furanogermacrenone	$C_{15}H_{18}O_2$	230.31	XVOHELPNOXGRBQ-NXAIOARDSA-N	6506548	Table A8[44]
Curzerenone	Furanoelemenone	$C_{15}H_{18}O_2$	230.31	ZVMJXSJCBLRAPD-ZFWWWQNUSA-N	3081930	Table A9[44]
Germacrene B	Germacrane hydrocarbon	$C_{15}H_{24}$	204.36	GXEGJTGWYVZSNR-SJRHNVSNSA-N	5281519	Table A4[37]

Compound	Class	Formula	MW	InChIKey	PubChem CID	NMR data in this appendix
γ-Elemene	Elemane hydrocarbon	$C_{15}H_{24}$	204.36	BQSLMQNYHVFRDT-CABCVRRESA-N	12309452	Table A5[37]
β-Elemene	Elemane hydrocarbon	$C_{15}H_{24}$	204.36	OPFTUNCRGUEPRZ-QLFBSQMISA-N	6918391	Not reproduced (ref.[33])
Germacrone	Germacranone	$C_{15}H_{22}O$	218.34	CAULGCQHVOVVRN-SWZPTJTJSA-N	6436348	Table A6[37]
Myrrhone	Aromatic cadinane-derived furanoketone	$C_{15}H_{16}O_2$	228.29	SHEOKDCVBGTHJG-MRVPVSSYSA-N	134715234	Table A10[44]
Mansumbinone	Octanordammarane triterpenoid	$C_{22}H_{34}O$	314.51	SCVQXPPZHIKYMJ-AQUCMXFHSA-N	12071382	Not reproduced (ref.[33])
3,4-seco-Mansumbinoic acid	3,4-seco-Octanordammarane acid	$C_{22}H_{34}O_2$	330.51	JHGHHJNAAWXVMF-QPOFKBRPSA-N	—	Table A11[17]
(E)-Guggulsterone	Pregnane-dione steroid	$C_{21}H_{28}O_2$	312.45	WDXRGPWQVHZTQJ-AUKWTSKRSA-N	6439929	Not reproduced
(Z)-Guggulsterone	Pregnane-dione steroid	$C_{21}H_{28}O_2$	312.45	WDXRGPWQVHZTQJ-OSJVMJFVSA-N	6450278	Not reproduced

A.3 Reference ^{1}H and ^{13}C NMR data

Chemical shifts are given in ppm relative to tetramethylsilane, multiplicities and coupling constants (Hz) as printed in the source, and the numbering follows Figures A1 and A2. Where a source lists ^{1}H signals without position assignments, the column headed probable assignment contains the assignment inferred in this document from the ^{13}C data, the multiplicities and the known spectra of the compound class, and should be treated as a working hypothesis until confirmed on an isolated standard.

Table A2. Furanodiene: ^{13}C assignments and ^{1}H signals recorded on the unheated essential oil of Eugenia uniflora ($CDCl_3$, 400/100 MHz)[37]. ^{1}H position assignments other than H-12 are inferred in this document.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	129.03	4.74 or 4.93 (m)	Ring olefinic methine, broadened by conformational exchange (probable)
C-2	26.80	1.79–2.24 / 2.10–2.14 (m)	Ring CH_2 (probable)
C-3	39.51	1.79–2.24 (m)	Ring CH_2 (probable)
C-4	128.82	—	
C-5	127.59	4.74 or 4.93 (m)	Ring olefinic methine (probable)
C-6	24.47	3.08 (m)	Bis-allylic CH_2 adjacent to the furan (probable)
C-7	118.86	—	Furan β-carbon
C-8	149.76	—	Furan carbon bonded to oxygen
C-9	40.92	3.43 (d, 15.71) / 3.54 (d, 15.71)	AB pair of the CH_2 between the furan and C-10 (probable)
C-10	134.36	—	
C-11	121.93	—	Furan carbon bearing CH_3-13

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-12	135.98	7.07 (q, 1.21 and 2.38)	α-Furan proton (assigned by the authors)
C-13	8.89	1.92 (d, 1.21)	Furan methyl, allylic coupling to H-12
C-14	16.49	1.27 or 1.60 (br s)	Vinylic methyl on C-10 (probable)
C-15	16.23	1.27 or 1.60 (br s)	Vinylic methyl on C-4 (probable)

Table A3. Curzerene: ^{13}C assignments and ^{1}H signals recorded on Eugenia uniflora essential oil after heating to 240 °C ($CDCl_3$, 400/100 MHz)[37]. ^{1}H position assignments other than H-12 are inferred in this document.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	147.75	5.87 (dd, 17.58 and 10.70)	Vinyl methine (probable)
C-2	110.97	4.88 / 4.96 (m)	Vinyl $=CH_2$; overlaps with H_2-3 (probable)
C-3	109.92	4.88 / 4.96 (m)	Isopropenyl $=CH_2$ (probable)
C-4	147.21	—	
C-5	50.00	2.29 (t, 7.37)	Ring methine (tentative)
C-6	24.19	2.41 (m)	CH_2 adjacent to the furan (tentative)
C-7	116.50	—	Furan β-carbon
C-8	149.50	—	Furan carbon bonded to oxygen
C-9	36.09	2.35 (br d, 16.49) / 2.67 (br d, 16.49)	AB pair of the CH_2 between the furan and C-10 (probable)
C-10	40.11	—	Quaternary carbon
C-11	119.35	—	Furan carbon bearing CH_3-13
C-12	137.18	7.05 (q, 1.05 and 2.26)	α-Furan proton (assigned by the authors)
C-13	8.13	1.91 (d, 1.05)	Furan methyl
C-14	19.54	1.06 (s)	Angular methyl on C-10 (probable)
C-15	24.46	1.72 (br s)	Isopropenyl methyl (probable)

Table A4. Germacrene B: ^{13}C assignments and ^{1}H signals recorded on the unheated essential oil of Eugenia uniflora ($CDCl_3$, 400/100 MHz)[37]. ^{1}H position assignments are inferred in this document.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	126.90	4.56 or 4.72 (m)	Ring olefinic methine (probable)
C-2	25.78	1.95–2.15 (m)	(probable)
C-3	38.89	1.95–2.15 (m)	(probable)
C-4	131.66	—	
C-5	128.20	4.56 or 4.72 (m)	Ring olefinic methine (probable)
C-6	38.89	2.54 (m)	Bis-allylic CH_2 (probable)
C-7	126.35	—	Ring carbon of the isopropylidene double bond
C-8	32.54	1.83 / 2.16 (m)	(probable)
C-9	32.54	2.04–2.29 (m)	(probable)

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-10	137.13	—	
C-11	133.56	—	Isopropylidene carbon
C-12	20.43	1.69 or 1.71 (s)	Isopropylidene methyl (probable)
C-13	20.80	1.69 or 1.71 (s)	Isopropylidene methyl (probable)
C-14	16.24	1.49 or 1.53 (s)	Vinylic methyl (probable)
C-15	16.23	1.49 or 1.53 (s)	Vinylic methyl (probable)

Table A5. γ-Elemene: ^{13}C assignments and ^{1}H signals recorded on Eugenia uniflora essential oil after heating to 240 °C ($CDCl_3$, 400/100 MHz)[37]. C-6, C-8 and C-9 were not reported; ^{1}H position assignments are inferred in this document.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	149.95	5.79 (dd, 17.62 and 10.72)	Vinyl methine (probable)
C-2	112.71	4.83 / 4.86 (m)	Vinyl $=CH_2$ (probable)
C-3	111.87	4.83 / 4.86 (m)	Isopropenyl $=CH_2$ (probable)
C-4	147.21	—	
C-5	53.03	1.95 (m)	Ring methine (tentative)
C-6	not reported	—	
C-7	130.94	—	Ring carbon of the isopropylidene double bond
C-8	not reported	—	
C-9	not reported	—	
C-10	39.94	—	Quaternary carbon
C-11	121.00	—	Isopropylidene carbon
C-12	19.92	1.66 (br s)	(probable)
C-13	20.04	1.66 (br s)	(probable)
C-14	16.81	1.06 (s)	Angular methyl (probable)
C-15	24.80	1.74 (br s)	Isopropenyl methyl (probable)

Table A6. Germacrone: ^{13}C assignments and ^{1}H signals recorded on the unheated essential oil of Eugenia uniflora ($CDCl_3$, 400/100 MHz)[37]. ^{1}H position assignments are inferred in this document.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	132.6	4.72 or 4.98 (m)	Ring olefinic methine (probable)
C-2	24.1	2.10–2.15 (m)	(probable)
C-3	38.18	2.10–2.15 / 2.38–2.40 (m)	(probable)
C-4	135.1	—	
C-5	125.5	4.72 or 4.98 (m)	Ring olefinic methine (probable)
C-6	29.25	2.87–2.93 (m)	Bis-allylic CH_2 (probable)
C-7	129.5	—	Ring carbon of the isopropylidene double bond
C-8	207.9	—	Ketone

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-9	56.01	2.94–3.42 (m)	CH_2 α to the ketone (probable)
C-10	126.7	—	
C-11	137.3	—	Isopropylidene carbon
C-12	19.92	1.72 or 1.77 (s)	(probable)
C-13	22.41	1.72 or 1.77 (s)	(probable)
C-14	16.73	1.44 (br s) or 1.62 (s)	(probable)
C-15	15.62	1.44 (br s) or 1.62 (s)	(probable)

Table A7. 2-Acetoxyfuranodiene isolated from C. myrrha resin (methanol-d_4, 600/151 MHz), fully assigned by 2D NMR[44]. The compound rearranged in $CDCl_3$ during measurement, which is why the data were recorded in methanol-d_4.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	129.1	5.04 (1H, d, 9.8)	
C-2	73.8	5.48 (1H, ddd, 4.6/10.3/10.3)	Oxymethine bearing the acetoxy group
C-3	45.9	2.00 (1H, dd, 11.1/11.1); 2.48 (1H, dd, 4.7/11.1)	
C-4	130.3	—	
C-5	131.8	5.11 (1H, br d, 11.0)	
C-6	25.3	3.05 (1H, br d, 17.1); 3.14 (1H, ddd, 2.1/11.3/17.3)	
C-7	120.4	—	Furan β-carbon
C-8	149.7	—	Furan carbon bonded to oxygen
C-9	41.5	3.43 (1H, d, 16.6); 3.50 (1H, d, 16.2)	
C-10	137.1	—	
C-11	123.1	—	
C-12	137.5	7.08 (1H, s)	α-Furan proton
C-13	8.8	1.90 (3H, d, 1.0)	Furan methyl
C-14	17.4	1.37 (3H, s)	
C-15	17.4	1.67 (3H, br s)	
C-16	172.6	—	Acetate carbonyl
C-17	21.2	2.04 (3H, s)	Acetate methyl

Table A8. 1(10)E,4E-Furanodienone isolated from C. myrrha resin ($CDCl_3$, 600/151 MHz)[44]. The isolate was of limited HPLC purity (47.6 % reported), which should be borne in mind when the ^1H data are used.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	130.5	5.18 (1H, dd, 4.4/11.6)	
C-2	26.4	2.18 (1H, m); 2.32 (1H, m)	
C-3	40.7	1.89 (1H, dt, 4.2/12.0); 2.47 (1H, dt, 3.6/11.6)	
C-4	145.8	—	

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-5	132.4	5.81 (1H, s)	Enone methine
C-6	189.8	—	Conjugated ketone
C-7	122.2	—	
C-8	156.5	—	Furan carbon bonded to oxygen
C-9	41.7	3.70 (1H, s); 3.71 (1H, s)	
C-10	135.4	—	
C-11	123.7	—	
C-12	138.1	7.08 (1H, s)	α-Furan proton
C-13	9.6	2.13 (3H, br s)	Furan methyl
C-14	15.8	1.31 (3H, s)	
C-15	19.0	2.00 (3H, br s)	

Table A9. Curzerenone isolated from C. myrrha resin ($CDCl_3$, 600/151 MHz)[44]. The relative configuration at C-5/C-10 was confirmed against literature ¹H data in the source.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	145.5	5.81 (1H, dd, 10.7/17.3)	Vinyl methine
C-2	113.0	4.96 (1H, d, 17.6); 4.96 (1H, d, 11.0)	Vinyl $=CH_2$
C-3	115.6	4.76 (1H, br s); 5.01 (1H, br s)	Isopropenyl $=CH_2$
C-4	141.1	—	
C-5	64.1	3.01 (1H, s)	Methine α to the ketone
C-6	194.8	—	Conjugated ketone
C-7	120.1	—	
C-8	165.5	—	Furan carbon bonded to oxygen
C-9	33.6	2.79 (1H, d, 17.6); 2.91 (1H, d, 17.6)	
C-10	42.8	—	Quaternary carbon
C-11	119.2	—	
C-12	139.5	7.09 (1H, s)	α-Furan proton
C-13	9.0	2.18 (3H, br s)	Furan methyl
C-14	24.8	1.19 (3H, s)	
C-15	24.9	1.83 (3H, s)	Isopropenyl methyl

Table A10. Myrrhone isolated from C. myrrha resin ($CDCl_3$, 400/101 MHz)[44]; a marker used for species classification by HPLC–MS/MS[32].

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	138.6	—	
C-2	199.8	—	Aryl ketone
C-3	48.8	2.37 (1H, m); 2.72 (1H, m)	Overlapped signals

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-4	29.9	2.35 (1H, m)	Overlapped signal
C-5	35.7	2.88 (1H, dd, 10.3/16.7); 3.55 (1H, dd, 3.8/16.5)	
C-6	140.6	—	
C-7	124.3	—	
C-8	157.2	—	Furan carbon bonded to oxygen
C-9	113.2	7.15 (1H, br s)	Aromatic methine
C-10	126.5	—	
C-11	116.7	—	
C-12	142.1	7.33 (1H, br s)	α-Furan proton
C-13	11.3	2.41 (3H, s)	Furan methyl
C-14	24.2	2.71 (3H, s)	Aromatic methyl peri to the ketone
C-15	21.5	1.18 (3H, d, 6.2)	Methyl on C-4

Table A11. 3,4-seco-Mansumbinoic acid isolated from myrrh ($CDCl_3$, 400/100 MHz), transcribed from the supplementary material of Kuck and co-workers[17]. The entries for positions 29 and 30 are reproduced as printed; the exocyclic methylene of a 3,4-seco-4(28)-ene requires two one-proton signals (δ 4.68 and 4.86) and a vinylic methyl singlet near δ 1.74, so the C-29/C-30 assignments should be confirmed against Rahman and co-workers[33] before use.

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-1	34.3	1.62 (2H, m)	
C-2	28.2	2.21 (1H, ddd, 7.3/10.5/15.2); 2.41 (1H, m)	CH_2 α to the carboxyl group
C-3	179.8	—	Carboxylic acid
C-4	147.5	—	Isopropenyl quaternary carbon
C-5	51.0	2.00 (1H, dd, 3.8/11.9)	
C-6	24.7	1.39 (1H, m); 1.87 (1H, ddd, 3.3/12.9/25.9)	J values as printed
C-7	34.1	1.25 (1H, ddd, 3.2/3.2/12.9); 1.62 (1H, m)	
C-8	39.6	—	
C-9	41.4	1.64 (1H, m)	
C-10	39.3	—	
C-11	22.4	1.34 (1H, m); 1.49 (1H, m)	
C-12	23.8	1.45 (1H, m); 1.74 (1H, m)	
C-13	47.7	2.72 (1H, m)	Allylic methine
C-14	53.4	—	
C-15	40.0	1.70 (1H, m); 2.35 (1H, br d)	
C-16	130.0	5.65 (1H, m)	Ring D olefinic methine
C-17	134.0	5.56 (1H, m)	Ring D olefinic methine
C-18	17.9	1.07 (3H, s)	
C-19	20.0	0.87 (3H, s)	

Position	δC (ppm)	δH (ppm), multiplicity, J (Hz)	Note
C-28	113.5	4.68 (1H, d, 1.5)	Exocyclic methylene (one proton listed)
C-29	23.2	4.86 (3H, br s) [as printed]	See caption
C-30	17.0	1.74 (3H, s) [as printed]	See caption

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