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Phytochemical Analysis and Comparison for Differentiation of *Boswellia carterii* and *B. serrata*

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Frankincense (*Boswellia carterii*) and olibanum (*B. serrata*) were chemically analyzed to determine compounds that would enable the differentiation of the two products. Typical compounds for both species were identified. Three new compounds, 24-noroleana-3,9(11),12-triene, 24-norursa-3,9(11),12-triene, and 24-norursa-3,12-diene-11-one, were identified in olibanum; these may be either naturally occuring or pyrolytic products.

Keywords: *Boswellia carterii*, *Boswellia serrata*, 24-noroleana-3,9(11),12-triene, 24-norursa-3,9(11),12-triene, 24-norursa-3,12-diene-11-one, GC-MS.

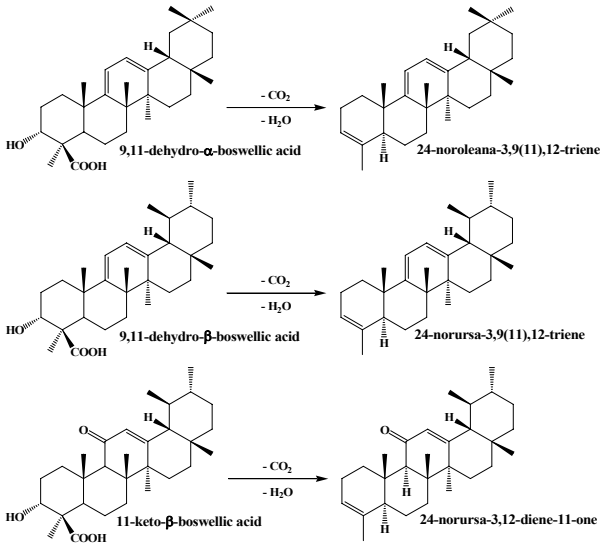
Frankincense (olibanum) is the dried aromatic sap that exudes from trees of the genus *Boswellia*. The sap exudes slowly and is allowed to harden by exposure to air. The resin attains the required degree of consistency in about three months, hardening into yellowish tear-like shapes. The hardened sap is collected and used as frankincense (olibanum). Frankincense, mixed with spices, seeds and roots, creates different aromas and, hence, is commonly used to produce incense. The smell of the frankincense (olibanum) smoke is due to some products produced upon pyrolysis. Although frankincense and olibanum are used as synonyms, olibanum actually refers to a different species of resin than frankincense. The principal geographical sources of olibanum and frankincense are India, Oman, Eritrea, Yemen, Somalia, Sudan, Kenya and Ethiopia.

Olibanum of the Old Testament is in fact *Boswellia serrata* (Roxb.) Colebr. (syn. *B. glabra* Roxb, *B. thurifera* Roxb.), a close relative of frankincense, which comes from a tree best known in India, but also grows elsewhere. The aroma of the various *B. serrata* resins is distinct from that of other species. Olibanum has a distinct dark and heavy, orange citrus aroma, as in the African variety, as well as a light and clear one, as in some of the Indian varieties. Most frankincense resins, starting with that from *B. sacra* Flückiger of Oman, and running down through *B. carterii* Birdw. (syn. *B. sacra* Flückiger) of Africa, have a distinct lemon or sometimes lemon-lime aroma. There is a definite difference in aroma and color between *B. serrata* and the other *Boswellia* species.

Cembrene was isolated for the first time from *Pinus albicaulis* [1] and later from the essential oil of *Boswellia frereana* [2]. Cembrene A (**1**) was isolated from the oleoresins of *Picea obovata* (spruce) and *Pinus koraiensis* (pine) [3] and, more recently, from *Commiphora mukul* (guggulu) [4]. From the oleoresin of *Pinus koraiensis* and *P. sibirica*, cembrene C (**2**) was isolated [5]. In the essential oil of *Boswellia carterii*, Basar [6] found verticilla-4(20),7,11-triene (**3**). A new diterpene alcohol, incensole (**4**), was isolated from frankincense [7], and incensole acetate (**5**) [8] was found in olibanum oil

obtained from Eritrea resin [9]. A new diterpene, incensole oxide (**6**) [10], was extracted in small amounts from frankincense, and supercritical fluid extraction led to the characterization of incensole oxide acetate (**7**) [11]. One of the first compounds isolated from olibanum was boswellic acid [12-15]. Other compounds identified in *Boswellia* include 3-*O*-acetyl-α- and 3-*O*-acetyl-β-boswellic acids (**19**, **20**) [16-18], 3-*O*-acetyl-11-methoxy-β-boswellic acid (**18**), 9,11-dehydro-β-boswellic acid (**14**) [19-21], 3-oxo-8,24-diene-tirucallic acid (**17**) [22-24], and 3-hydroxy-8,24-diene-tirucallic acid (**13**) [25,26].

As olibanum was the best known of the ancient plant resins, archeological resins were studied for their contents of boswellic acids and their derivatives [30,31]. The focus of our investigation was on the pyrolytic study of the insoluble residue of the ancient resins [27,28]. The pyrolysate was dominated by two products, 24-noroleana-3,12-diene (**10**) and 24-norursa-3,12-diene (**11**) [27]. In the resin, α- and β-boswellic acids (**15**, **16**) and the corresponding *O*-acetyl derivatives (**19**, **20**) were characterized.



Scheme 1: Biogenesis of new compounds from their boswellic acids precursors (also possible from appropriate 3-*O*-acetyl- compounds after loss of acetic acid).

[28]. In addition to these markers, small amounts of 3α-hydroxy-lup-20(29)-en-24-oic acid, its *O*-acetyl derivative and 24-norlupa-3,20(29)-diene were detected, resulting from chemical degradation of the sample [29]. 24-Noroleana-3,12-diene (**10**) and 24-norursa-3,12-diene (**11**) are known degradation products of α- and β-boswellic acids and their *O*-acetates. Accordingly, 24-norlupa-3,20(29)-diene, is a degradation product of 3α-hydroxy-lup-20(29)-en-24-oic acid and 3α-*O*-acetyl-lup-20(29)-en-24-oic acid [29].

Our target was to distinguish both resins with the help of the existence of these typical compounds. Analysis of the *n*-hexane extract without derivatization (see Table 1) revealed easy differentiation between these two resins. In *B. carterii* the main compounds were incensole (**4**; 21.5%) and incensole acetate (**5**; 19.9%), whereas in *B. serrata*, these were 24-norursa-3,12-diene (**11**; 50.8%) and 24-noroleana-3,12-diene (**10**; 14.2%). Analysis of three unknown peaks in the GC/MS spectrum revealed three new compounds: 24-noroleana-3,9(11),12-triene (**8**), 24-norursa-3,9(11),12-triene (**9**), and 24-norursa-3,12-diene-11-one (**12**). Scheme 1 shows the structures of the new compounds with their proposed biogenesis. We suppose that these three compounds originate from boswellic acid derivatives, similar to compounds **10** and **11** [27].

Table 1: Compounds identified in *n*-hexane extract of *B. carterii* and *B. serrata* resins.

Compound	RT (mins)	RI	% (B.c.)	% (B.s.)
octyl acetate	5.76	1210	8.9	-
cembrene	14.78	1624	0.1	-
1 cembrene A	15.08	1633	0.9	-
2 cembrene C	15.52	1645	0.6	-
3 verticilla-4(20),7,11-triene	15.65	1649	4.2	-
4 incensole	16.95	1684	21.5	-
5 incensole acetate	17.14	1689	19.9	-
6 incensole oxide	18.07	1712	3.15	-
7 incensole oxide acetate	18.51	1723	2.5	-
8 24-noroleana-3,9(11),12-triene	24.58	1847	0.1	1.3
9 24-norursa-3,9(11),12-triene	25.40	1861	0.6	5.0
10 24-noroleana-3,12-diene	25.63	1866	2.4	14.2
11 24-norursa-3,12-diene	26.40	1879	4.3	50.8
m/z 408 (base peak 255)*	29.87	1932	-	0.7
m/z 410 (base peak 218)**	30.66	1943	-	1.3
12 24-norursa-3,12-diene-11-one	31.87	1961	2.2	7.6

RT = retention time, RI = retention indices; B. c. = *Boswellia carterii*; B. s. = *B. Serrata*; * 24-norolea-3,12-diene-11-one (not proved); ** ketone with one double bond (not proved)

The identification of 24-noroleana-3,9(11),12-triene (**8**), 24-norursa-3,9(11),12-triene (**9**), and 24-norursa-3,12-diene-11-one (**12**) was based on their mass spectra. These compounds were compared with boswellic acids and their derivatives. Compounds **8** and **9** showed, in their mass spectra, similarities and the same typical fragments. The fragmentation pattern of **8** exhibited a molecular ion at *m/z* 392 (10.3%) and a typical fragment at *m/z* 255 (8%). 24-Norursa-3,9(11),12-triene (**9**) revealed a molecular ion at *m/z* 392 (85.5%) and the ion at *m/z* 255 (91.2%) seen in the mass spectrum of **8**. The identity of 24-norursa-3,12-diene-11-one (**12**) was proved

also by EIMS analysis. The M^+ ion was observed at *m/z* 408 (18.2%) and the other ion formed in retro-Diels-Alder fragmentation at *m/z* 232 (100%).

For identification of boswellic acids and their derivatives, the *n*-hexane extract was silylated. We found in *B. serrata* significantly higher amount of these compounds, with β-boswellic acid (**16**; 25%) being the main one (see Table 2).

Table 2: Compounds identified in *n*-hexane extract of *B. carterii* and *B. serrata* resins after silylation with BSTFA.

	Compound	RT	RI	% (B.c.)	% (B.s.)
13	trimethylsilyl 3-*O*-trimethylsilyl-8,24-diene-tirucallate	34.15	2044	-	7.1
14	trimethylsilyl 3-*O*-trimethylsilyl-9,11-dehydro-β-boswellate	34.68	2052	-	2.7
15	trimethylsilyl 3-*O*-trimethylsilyl-α-boswellate	35.92	2069	0.2	6.9
16	trimethylsilyl 3-*O*-trimethylsilyl-β-boswellate	37.21	2085	0.5	25.0
	m/z 600 (base peak 73)	37.36	2087	-	1.3
17	trimethylsilyl 3-oxo-8,24-diene-tirucallate	39.06	2109	1.0	10.5
18	trimethylsilyl 3-*O*-acetyl-11-methoxy-β-boswellate	39.80	2117	0.5	2.6
19	trimethylsilyl 3-*O*-acetyl-α-boswellate	42.78	2152	0.5	1.2
20	trimethylsilyl 3-*O*-acetyl-β-boswellate	44.51	2171	0.7	3.0

RT = retention time, RI = retention indices
B. c. = *Boswellia carterii*; B. s. = *B. serrata.*

Almost 30 years ago it was found that the pyrolysis products of archeological samples of *Bowellia* resin are dominated by two compounds, **10** and **11** [27]. When α-boswellic acid (**15**) and β-boswellic acid (**16**) and their *O*-acetates (**19, 20**) were subjected to GC–MS analysis without TMS-derivatization, in order to carry out their thermal degradation in the injector of the GC, a single peak corresponding to the appropriate degradation product was observed [29]. We, surprisingly, found in the *n*-hexane extract not only these two compounds, but also another three (**8**, **9** and **12**). As there was the chance that these are products of pyrolysis in the injector, we analyzed this extract after silylation and even after that we found them. The question whether these compounds are natural products stays open. There is also the possibility that these compounds originate in the resin during long storage.

The resins of *B. carterii* and *B. serrata* were analyzed and compared. Each resin can be very easily determined from its constituents, which are typical of each species. In *B. serrata* three new compounds were identified, which may be either decomposition products produced in the injector of the GC-MS or a result of storing these resins for a long period.

Experimental

Plant material: Resins of *B. carterii* (from Somalia) and *B. serrata* (from India) were purchased from Pamir Ltd. (Bat Yam, Israel) and KCR Ltd. (Tel Aviv, Israel).

Extracts preparation: Samples of *Boswellia carterii* and *B. serrata* were extracted with *n*-hexane and filtered. The *n*-hexane extracts were concentrated and used for GC-MS analysis. For determination of acids, the samples were silylated with the help of BSTFA. Each sample (1 μL) was injected into the GC-MS for analysis.

GC-MS analysis: For qualitative analysis the samples were analyzed by GC-MS in a Hewlett-Packard G1800A GCD system that had a HP-5971 gas chromatograph with an electron ionization detector. An SPB-5 (30 m x 0.25 mm x 0.25 μm film thickness) column was used. For analysis, the following method was used: The column was held at 100°C for 2 min and after that the temperature was programmed from 100-280°C at 10°C/min (inlet - 250°C; detector - 280°C; splitless injection/purge time - 1.0 min; initial temperature - 100°C; initial time - 2.0 min), gas – helium (1 mL/min), solvent delay – 4 min. Total time of analysis - 40 min for *n*-hexane samples, 50 min for BSTFA silylated samples.

***Qualitative and quantitative analyses*:** For identification of the compounds, the NIST/EPA/NIH and Wiley 7th Mass Spectral Libraries were used, and comparisons made with published spectral data and retention indices. The constituents were also identified by comparison with pure standard compounds.

References

[1] Haagen-Smit AJ, Wang TH, Mirov NT. (**1951**) Composition of gum turpentines of pines. XIII. A report on *Pinus albicaulis*. *Journal of the American Pharmaceutical Association*, ***40***, 557-559.

[2] Strappaghetti G, Corsano S, Craveiro A, Proietti G. (**1982**) Constituents of essential oil of *Boswellia frereana*. *Phytochemistry,* ***21***, 2114-2115.

[3] Shmidt EN, Kashtanova NK, Pentegova VA. (**1970**) Neocembrene, a new diterpenic hydrocarbon from *Picea obovata* and *Pinus koraiensis*. *Khimiya Prirodnych Soedineii*, 694-698.

[4] Patil VD, Nayak UR, Dev S. (**1973**) Chemistry of ayurvedic crude drugs. II. Guggulu (resin from *Commiphora mukul*). 2. Diterpenoid constituents. *Tetrahedron*, ***29***, 341-348.

[5] Raldugin VA, Kashtanova NK, Pentegova VA. (**1971**) α, β and γ-Pinacenes , new diterpenoids from oleoresins of *Pinus koraiensis* and *Pinus sibirica*. *Khimiya Prirodnych Soedineii*, 604-608.

[6] Basar S, Koch A, Konig WA. (**2001**) A verticillane-type diterpene from *Boswellia carterii* essential oil. *Flavour and Fragrance Journal*, ***16***, 315-318.

[7] Corsano S, Nicoletti R. (**1967**) Structure of incensole. *Tetrahedron*, ***23***, 1977-1984.

[8] Forcellese ML, Nicoletti R, Santarelli C. (**1973**) Revised structure of isoincensole oxide. *Tetrahedron Letters*, 3783-3786.

[9] Obermann O. (**1977**) The chemical and aromatic differences of incense resins. *Dragoco Report*, ***24***, 260-265.

[10] Nicoletti R, Forcellese ML. (**1968**) Structure of incensole oxide. *Tetrahedron*, ***24***, 6519-6525.

[11] Ma X, Yu X, Zheng Z, Mao J. (**1991**) Analytical supercritical fluid extraction of Chinese herbal medicines. *Chromatographia*, ***32***, 40-44.

[12] Tschirch A, Halbey O. (**1898**) Untersuchungen über die Sekrete. 28: Über das Olibanum. *Archiv der Pharmazie*, ***236***, 487-502.

[13] Beaucourt K. (**1929**) Resin constituents. I. Boswellic acid from olibanum (incense). *Monatshefte für Chemie*, ***53-54***, 897-913.

[14] Winterstein A, Stein G. (**1932**) Saponin series. X. Monohydroxytriterpene acids. *Zeitschrift für Physiologische Chemie*, ***208***, 9-25.

[15] Allan GG. (**1968**) The stereochemistry of the boswellic acids. *Phytochemistry*, ***7***, 963-973.

[16] Ruzicka L, Wirz W. (**1939**) Triterpenes. L. Conversion of β-boswellic acid into β-amyrin. *Helvetica Chimica Acta*, ***22***, 948-951.

[17] Pardhy RS, Bhattacharyya SC. (**1978**) β-Boswellic acid, acetyl-β-boswellic acid, acetyl -11-keto-β-boswellic acid and 11-keto-β-boswellic acid, four pentacyclic triterpene acids from the resin of *Boswellia serrata* Roxb. *Indian Journal of Chemistry*, ***16B***, 176-178.

[18] Zhou J, Cui R. (**2002**) Chemical components of *Boswellia carterii*. *Yaoxue Xuebao*, ***37***, 633-635.

[19] Trost F. (**1937**) The triterpenic hydroxy acids in Somaliland incense. I. *Annali di Chimica Applicata*, ***27***, 178-188.

[20] Beton JL, Halsall .G, Jones ERH. (**1956**) Chemistry of triterpenes and related compounds. XXVIII. β-Boswellic acid. *Journal of the Chemical Society*, *Abstracts*, 2904-2909.

[21] Schweizer S, von Brocke AFW, Boden SE, Bayer E, Ammon HPT, Safayhi H. (**2000**) Workup-dependent formation of 5-lipoxygenase inhibitory boswellic acid analogues. *Journal of Natural Products*, ***63***, 1058-1061.

[22] Mladenovic M. (**1932**) Elemic acid from Manila elemi resin. III. Elemonic acid, its reduction product and bromohydroelemonic acid. *Monatshefte für Chemie*, ***59***, 7-15.

[23] Usubillaga A, Khouri N, Bahsas A, Abad A, Rojas LB. (**2004**) Triterpenes from the resin of *Protium crenatum* Sandwith. *Revista Latinoamericana de Quimica*, ***32***, 101-108.

[24] Boden SE, Schweizer S, Bertsche T, Dufer M, Drews G, Safayhi H. (**2001**) Stimulation of leukotriene synthesis in intact polymorphonuclear cells by the 5-lipoxygenase inhibitor 3-oxo-tirucallic acid. *Molecular Pharmacol*ogy, ***60***, 267-273.

[25] Cotterrell GP, Halsall TG, Wriglesworth MJ. (**1970**) Chemistry of triterpenes and related compounds. XLVII. Clarification of the nature of the tetracyclic triterpene acids of elemi resin. *Journal of the Chemical Society C*, 739-743.

[26] Badria FA, Mikhaeil BR, Maatooq GT, Amer MMA. (**2003**) Immunomodulatory triterpenoids from the oleogum resin of *Boswellia carterii* Birdwood. *Zeitschrift für Naturforschung C*, ***58***, 505-516.

[27] van Bergen PF, Peakman TM, Leigh-Firbank EC, Evershed RP. (**1997**) Chemical evidence for archaeological frankincense: boswellic acids and their derivatives in solvent soluble and insoluble fractions of resin-like materials. *Tetrahedron Letters*, ***38***, 8409-8412.

[28] Evershed RP, van Bergen PF, Peakman TM, Leigh-Firbank EC, Horton MC, Edwards D, Biddle M, Kjolbye-Biddle B, Rowley-Conwy PA. (**1997**) Archaeological frankincense. *Nature*, ***390***, 667-778.

[29] Mathe C, Culioli G, Archier P, Vieillescazes C. (**2004**) Characterization of archaeological frankincense by gas chromatography–mass spectrometry. *Journal of Chromatography A*, ***1023***, 277-285.

[30] Frank A, Unger M. (**2006**) Analysis of frankincense from various *Boswellia* species with inhibitory activity on human drug metabolizing cytochrome P450 enzymes using liquid chromatography mass spectrometry after automated on-line extraction. *Journal of Chromatography A*, ***1112***, 255-262.

[31] Modugno F, Ribechini E, Colombini MP. (**2006**) Chemical study of triterpenoid resinous material in archaeological findings by means of direct exposure electron ionization mass spectrometry and gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, ***20***, 1787-1800.