FULL PAPER

Frankincense Revisited, Part II: Volatiles in Rare *Boswellia* Species and Hybrids

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In this second part of the investigation of volatiles and semivolatiles in *Boswellia* gum resins, an additional five less common species were analyzed by (SPME-)GC/MS, namely *B. ameero*, *B. elongata*, *B. neglecta*, *B. popoviana*, and *B. rivae*. Moreover, the results of hybridization experiments are reported in combination with the volatile composition of their gum resins. Our study shows that *B. sacra* benefits from an intraspecific cross-pollination, as the resulting hybrid *B. sacra* var. *supersacra* has a far higher seed germination rate and viability.

Keywords: *Boswellia*, Burseraceae, Hybrid, Frankincense, Olibanum, SPME-GC/MS.

Introduction

Frankincense has been a valued commodity for millennia, prized for its pleasant scent and burnt as an offering to the gods of many different religions. From Ancient Egypt to Judaism, from the Roman Empire to the modern Catholic Church, but also in China, Japan, and India, frankincense was and is a traditional incense material and is also used as a natural remedy for various ailments [1 – 4]. The material is a gum resin sourced from the genus *Boswellia* and traded as such in various quality grades as well as in form of the distilled essential oil.

In part I of this study, the volatile and semivolatile composition of the gum resins originating from the four commercially most important *Boswellia* species, namely *B. sacra*, *B. serrata*, *B. papyrifera*, and *B. frereana,* were analyzed and discussed. Part II continues this investigation with a focus on less common *Boswellia* species and two samples from hybridization experiments.

The Island of Socotra, located at the entrance to the Gulf of Aden between Somalia and Yemen, harbors seven endemic *Boswellia* species [5]: *B. ameero* BALF.F., *B. bullata* THULIN, *B. dioscoridis* THULIN, *B. elongata* BALF.F., *B. nana* HEPPER, *B. popoviana* HEPPER, and *B. socotrana* BALF.F., three of which are treated in this study and have not or only rarely been investigated. In addition to these, *B. rivae* ENGL. and *B. neglecta* S. MOORE from Africa were analyzed.

The bisexual flowers of *Boswellia* species are able to self-pollinate and produce a capsule with 3 – 5 winged seeds. Frequent self-pollination appears to be a problem in particular for *B. sacra* and leads to a loss of genetic diversity. By contrast, interspecific hybrids of *Boswellia* species are also known to occur naturally and artificially [6][7]. The botanical and chemotaxonomical investigation of these hybrids is of particular interest with regard to the identification of plant and gum resin specimens as well as for preservation strategies.

For the first time, the volatile composition of *Boswellia* resins grown outside their natural habitat as well as data on the gum resin of hybrids is reported in this study. The extensive investigation of compounds from these samples is intended to help clarify the confusions surrounding the volatile composition of *Boswellia* material.

Results and Discussion

Sample Set, General Results, and Preliminary Remarks

The total sample set from part I and II comprised of 55 samples, with 38 gum resins and 17 essential oils from nine different species and two hybrids. The second part of this study treats the remaining four essential oils and five gum resins. These will be discussed in two groups: one hand, samples from those species that are rarely traded on the international market, comprising *B. neglecta* and *B. rivae* in the form of essential oil, and *B. ameero*, *B. elongata*, and *B. popoviana* as gum resin material. The latter three samples were donated by *Jason Eslamieh* [8] and obtained by tapping authentic specimens grown in his research facility in Arizona, U.S.A.. On the other hand, *Jason Eslamieh* also contributed botanical descriptions of two of his hybrids and supplied gum resin samples from these plants for chemical analyses.

These samples were investigated by gas chromatography/mass spectrometry (GC/MS), with two different

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sample workup procedures adapted to the corresponding sample material. In case of the gum resin samples, a solid-phase microextraction (SPME) method was applied. This is a common procedure for solid samples, such as frankincense [9], and was specifically adapted to broad-range sampling. However, it has to be kept in mind that the data reported here is not a true quantification, but at best a semiquantitative estimate. A correct quantification would require in each case a calibration with the respective reference compound, which was beyond the scope of this investigation. In essential oils, the samples were diluted in CH_2Cl_2 and injected via an on-column injector into the GC/MS.

Overall, 268 distinct peaks are reported in both parts of this publication, and 230 compounds were tentatively identified (by retention index (*RI*) and mass spectrum (MS)) or identified (using *RI* and MS of reference compounds). In this second part, of a total of 157 detected compounds, 47 compounds were identified and 84 tentatively identified. Detailed information on identification procedures for each compound can be found in *Table 1*. Missing compound numbers in the tables account for compounds found in part I of the study, and are intended to maintain consistent compound numbering in both parts. For a full list of retention indices as well as a list of unidentified compounds and their mass spectral data, please refer to the supporting information of part I [10].

The identification rates for the samples are given in *Table 2*. Overall, 92.1% of the total peak area was (tentatively) identified, with the rate for individual samples varying from 81.2% to 99.4%. Thereby, the majority of peaks are accounted for in this investigation. *Fig. 1* shows a chromatogram of each of the five species investigated with the most abundant compounds labeled.

Boswellia neglecta S. Moore

Boswellia neglecta is native to Uganda, Tanzania, Kenya, southern Ethiopia, Sudan, and, to a small extent, also Somalia. The shrub-like trees exude a black and a white resin during the dry season, with the white one reported as more aromatic [11]. It is commonly traded as 'Borena-type' olibanum. Internationally, it appears to be available solely as an essential oil. According to an investigation on the economic role of frankincense trade in Ethiopia [12], *B. neglecta* gum resin from the region Liban in southern Ethiopia is usually mixed with material of *B. ogadensis* before being sold on the local and international markets. Thus, it could well be that the essential oil samples available on the market are of mixed botanical origin.

Three essential oil samples were investigated in this study and showed a very similar composition. In total, 67 constituents were (tentatively) identified at an identification rate of 98.4 – 99.4%. The essential oil was composed of 97.1% monoterpenes. This high monoterpene content appears to be characteristic for *B. neglecta*. Two sesquiterpenes, namely α-copaene **157** (0.4%) and β-elemene **161** (0.4%) were the only ones detected above trace level (< 0.1%). The most abundant compounds and their range of % RPA (for mean values see *Table 1*) were: α-thujene **16** (14.5 – 18.4%), α-pinene **17** (25.2 – 34.1%), camphene **22** (1.8 – 2.2%), β-pinene **28** (3.1 – 4.4%), δ-3-carene **39** (3.5 – 10.1%), *p*-cymene **43** (3.3 – 8.0%), limonene **46** (2.2 – 3.9%), 4-terpineol **98** (9.4 – 10.8%), and α-terpineol **104** (1.8 – 5.5%). The latter two compounds were observed at the highest levels of all samples.

Boswellia neglecta was the smallest sample set ($n = 3$), thus the identification of markers is to be considered tentative. In general, *B. neglecta* is characterized by a lack of sesqui- and diterpenes, which appear only as traces. The large monoterpenoid fraction shows several characteristic features: the presence of α-fenchene **21** as a peak which is coeluting with α-camphene, as well as high amounts (± SD) of β-pinene **28** (3.9% ± 0.58), δ-3-carene **39** (7.3% ± 2.8), γ-terpinene **55** (1.0% ± 0.1), 4-terpineol **98** (10.0% ± 0.58), and α-terpineol **104** (3.3% ± 1.6). Borneol **94** (0.53% ± 0.09) was exclusively found in *B. neglecta*. Together with the high levels of otherwise rather unspecific monoterpenes, this could serve to identify *B. neglecta* essential oil.

These results are in good agreement with data from five literature sources, namely *Basar* [13], *Başer et al.* [14], *Bekana et al.* [15], *Provan et al.* [16], and *Van Vuuren et al.* [17]. By contrast, the compounds identified by *Fanta et al.* [18] in black *B. neglecta* frankincense differ completely from the other sources and show some typical characteristics of *B. papyrifera* samples, such as high amounts of octyl acetate and incensole acetate. Another reported compound, nerolidyl propionate, was not found in any other study to be a constituent of any *Boswellia* material. *Dekebo et al.* [11] reported some triterpenes from *B. neglecta*, but so far no boswellic acids were detected for this species.

Boswellia ameero Balf.f

Boswellia ameero is one of the species endemic to the Island of Socotra. The sample investigated in this study was obtained by tapping a specimen from the collection of *Jason Eslamieh*. Thus it cannot be excluded that notable differences in the volatile profile might arise from the differences in habitat, specimen age, and growing and tapping conditions.

The resin examined here was exclusively composed of mono- (56%), sesqui- (31%), and diterpenoids (2%), with the only exception of traces of acetic and formic acid. A total of 53 different compounds were detected and 47 (tentatively) identified. The most abundant compounds were α-thujene **16** (25.6%), caryophyllene oxide **224** (8.8%), β-caryophyllene **172** (6.6%), α-pinene **17** (6.1%), and *p*-cymene **43** (5.7%). The remarkable characteristic is the high abundance of the thujane skeleton, accounting for over 35% of the total peak area, also with oxygenated

Table 1. Overview of resin and essential oil data of five *Boswellia* species and two hybrids. The (semi)quantitative data and compound identification is reported for individual samples except for *B. neglecta*, *B. rivae*, where mean values of a small set (n = 3) are given

Nr.	Compound	Identification	Retention indices (DB-5)				Sample set		Individual samples from other species				Hybrids	
			Literature values		Measured values		*Boswellia neglecta*		*B. ameero*	*B. elongata*	*B. popoviana*	*B. rivae*	*B. 'supersacra'*	*B. sacra x nana ('sacrana')*
		Rating of identification	RI_{Lit}	References	RI_{exp} (by SPME-GC/MS)	RI_{exp} (by GC/MS)	Mean % RPA	Count (n = 3)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)
	Sample type				Gum resin	Essential oil	Essential oil		Gum resin	Gum resin	Gum resin	Essential oil	Gum resin	Gum resin
2	Acetone (lab air impurity, [a]))	A[b])	< 800		[c])	[c])			0.03	0.03	0.10		0.06	0.15
4	Formic acid	A	< 800						0.08					
5	Acetic acid	A	< 800						0.21		0.31		0.09	0.24
8	Toluene	A	< 800				0.13	3				0.22	0.05	0.10
9	3-Methyl-2-butenal	B	< 800										0.02	
14	Hashishene (5,5-Dimethyl-1-vinylbicyclo [2.1.1]hexane)	B	924	[30]	922	921				0.59		0.08	0.01	
15	Tricyclene	B	921	[31]	926	923	0.26	3				0.23		0.08
16	α-Thujene	B	924	[31]	932	930	16.8	3	25.6	22.0	1.1	3.5	0.04	8.0
17	α-Pinene	A	942	[32]	939	937	28.4	3	6.1	0.32		26.8	5.1	10.5
19	Thuja-2,4(10)-diene-Isomer?	C	946	[32]	948	945	1.4	3	1.2	0.26	0.22	0.06		
21	α-Fenchene	B	945	[31]		950	0.78	3				coel.[d])		
22	Camphene	A	946	[31]	953	951	2.0	3				1.9	0.25	1.3
24	Thuja-2,4(10)-diene	B	953	[31]	958	954	0.67	3	0.31			0.41	0.08	0.22
26	3,7,7-Trimethyl-1,3,5-cycloheptatriene	B	973	[33]	974	972	0.77	2				2.3	0.35	
27	Sabinene	A	969	[31]	977	974	1.4	3	5.7	2.8	0.11	2.8	0.10	
28	*β*-Pinene	A	974	[31]	980	979	3.9	3	coel.	coel.		9.8	0.19	13.2
29	6-Methyl-5-hepten-2-one	B	981	[31]	987	985	0.04	3				0.05		
31	*β*-Myrcene	A	988	[31]	993	990	1.1	3	0.69	18.2		0.22	0.29	0.21
32	6-Methyl-5-hepten-2-ol	B	989	[31]		993	0.08	3						
34	*δ*-2-Carene	B	1001	[31]	1001	999						0.23		
36	α-Phellandrene	A	1002	[31]	1006	1007	coel.	1	0.05	0.11			2.0	1.3
37	*o*-Methylanisole	A	1005	[31]	1011	1007	coel.	1						0.07
39	*δ*-3-Carene	B	1008	[31]	1013	1009	7.3	3		2.3		7.9	10.2	
40	1,4-Cineol (Isocineol)	B	1012	[31]		1015	0.09	3						
41	α-Terpinene	A	1014	[31]	1019	1017	0.48	3	0.11	0.40		0.13	coel.	0.06
42	*m*-Cymene	A	1021	[34]	1025	1020	0.13	3				0.64		
43	*p*-Cymene	A	1020	[31]	1028	1026	6.1	3	5.7	1.1	1.6	4.5	1.3	4.7
44	Oxygenated Monoterpene 1	C	–		1031	1028			coel.	coel.	coel.			
46	Limonene	A	1024	[31]	1034	1031	2.9	3		0.86		4.9	0.80	1.6
47	*β*-Phellandrene	B	1025	[31]		1032	0.85	3				coel.		
48	Eucalyptole (1,8-Cineol)	A	1026	[31]	1036	1034	coel.	3				0.13	coel.	
50	*cis*-*β*-Ocimene	A	1032	[31]	1041	1036	0.08	3		0.05		0.28	0.41	0.15
51	*o*-Cymene	A	1022	[31]		1042						0.08		
52	*trans*-*β*-Ocimene	A	1044	[31]	1052	1046	0.04	3	0.68	0.18		0.23	1.5	0.31

Table 1. (cont.)

Nr.	Compound	Identification	Retention indices (DB-5)				Sample set		Individual samples from other species				Hybrids	
			Literature values		Measured values		*Boswellia neglecta*		*B. ameero*	*B. elongata*	*B. popoviana*	*B. rivae*	*B. 'supersacra'*	*B. sacra x nana ('sacrana')*
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	Sample type				Gum resin	Essential oil	Essential oil		Gum resin	Gum resin	Gum resin	Essential oil	Gum resin	Gum resin
53	Oxygenated Monoterpene 2	C	1049	[32]	1053	1050	0.05	3			0.33	0.07		
55	*γ*-Terpinene	A	1054	[31]	1062	1058	1.0	3	0.23	0.55		0.26	0.08	0.14
57	*cis*-Sabinene hydrate	B	1065	[31]	1071	1071	0.08	2	0.15	0.02		0.21		
58	*cis*-Linalool oxide (furanoid)	A	1073	[32]	1074	1071	0.05	1						
61	Oxygenated Monoterpene 3	C	–		1087	1087						0.44		0.07
63	Terpinolene	A	1086	[31]	1089	1085	0.54	3		0.20		0.13	0.92	
64	*p*-Cymenene (*p*,*α*-Dimethylstyrene)	A	1089	[31]	1090	1089	0.54	3	0.21		0.15	0.31	0.13	0.32
66	Perillene	B	1102	[31]	1101	1097				0.28				
67	Linalool	A	1095	[31]	1101	1099	0.56	3						
68	*α*-Pinene oxide	A	1099	[31]	1098	1100			0.37			0.27	0.11	0.19
69	*trans*-Sabinene hydrate	B	1098	[31]	1101	1101						0.27		
71	*cis*-Thujone (*α*-Thujone)	B	1101	[31]	1106	1107	0.10	2						
72	Oxygenated Monoterpene 4	C	1108	[32]	1110	1111	0.10	3	0.40			0.40	0.19	
73	*trans*-Thujone (*β*-Thujone)	B	1112	[31]	1120	1119	0.46	3	2.8	0.66	1.1	0.17		0.56
74	*exo*-Fenchol	B	1118	[31]		1120	0.19	3				0.19		
76	*trans*-*p*-Mentha-2,8-dienol	B	1119	[31]	1124	1122						0.11		
78	Unidentified peak 3	C	–		1133	1125	0.36	3						
80	*α*-Campholenal	B	1122	[31]	1129	1128	0.11	2	0.21			0.42	0.14	0.54
81	Oxygenated Monoterpene 6	C	–			1133	0.16	2				0.54		
83	*cis*-*p*-Mentha-2,8-dien-1-ol	B	1133	[31]	1140	1137						0.09		
85	*trans*-Sabinol	B	1137	[31]	1145	1140				0.13				
86	*trans*-Pinocarveol	A	1135	[31]	1145	1142	0.80	3	0.20			3.0	0.13	1.2
87	*trans*-Verbenol	A	1137	[31]	1151	1147	0.37	3	0.40			4.1	0.22	1.0
88	Camphor	B	1141	[31]		1149	0.23	3						
89	Unidentified peak 4	C	–			1152	0.18	3				0.25		
92	*trans*-Pinocamphone	B	1158	[31]	1165	1161	0.15	3				0.52		
93	Pinocarvone (2(10)-Pinen-3-one)	A	1160	[31]	1167	1163	0.08	1						0.91
94	Borneol	B	1165	[31]	1167	1174	0.53	3						
95	*cis*-Sabinol	B	1170	[32]	1174	1170				0.33	0.50	0.09		
96	3-Thujen-2-one (Umbellone)	B	1167	[31]	1176	1171	0.17	3	1.3		0.50			0.65
97	Ethyl benzoate	B	1169	[31]	1174									coel.

Table 1. (cont.)

Nr.	Compound	Identification	Retention indices (DB-5)				Sample set		Individual samples from other species				Hybrids	
			Literature values		Measured values		*Boswellia neglecta*		*B. ameero*	*B. elongata*	*B. popoviana*	*B. rivae*	*B. 'supersacra'*	*B. sacra x nana ('sacrana')*
		Rating of identification	RI_{Lit}	References	RI_{exp} (by SPME-GC/MS)	RI_{exp} (by GC/MS)	Mean % RPA	Count (*n* = 3)	% RPA (*n* = 1)	% RPA (*n* = 1)	% RPA (*n* = 1)	% RPA (*n* = 1)	% RPA (*n* = 1)	% RPA (*n* = 1)
	Sample type				Gum resin	Essential oil	Essential oil		Gum resin	Gum resin	Gum resin	Essential oil	Gum resin	Gum resin
98	4-Terpineol	A	1182	[32]	1182	1182	10.0	3	1.1	0.24		2.5		
100	*p*-Methylacetophenone	A	1186	[32]	1187	1186					0.37	0.12		
101	Crypton	B	1183	[31]	1187									0.20
103	*p*-Cymen-8-ol	A	1187	[32]	1189	1188	0.49	3	0.53	0.16	0.73	0.85	0.41	0.35
104	α-Terpineol	A	1193	[32]	1195	1196	3.3	3				coel.		0.40
105	Myrtenal	A	1195	[31]	1198	1198						3.2		1.1
107	Myrtenol	A	1198	[32]	1201	1195			0.32			coel.	0.05	1.0
110	Eucarvone	B	1222	[35]	1213	1212	0.14	2			0.07	0.46	0.16	
112	Verbenone	A	1204	[31]	1213	1208	0.49	3	0.34			1.7	0.42	0.76
113	*trans*-Carveol	A	1215	[31]	1224	1220	0.14	3				0.69		0.44
118	Cumaldehyde	A	1238	[31]	1243	1244	0.15	1	0.32		0.68		0.05	0.16
119	Thymol methyl ether	B	1232	[31]	1236	1238	0.05	1		0.13				
120	Carvone	A	1239	[31]	1247	1245	0.16	2				0.36	0.03	0.12
121	*p*-Menth-6-en-2-one (Carvotanacetone)	B	1244	[31]	1252	1248					0.19			
125	Unidentified stereoisomer of dimethoxytoluene + coeluting linalyl acetate	C	–			1248	0.63	3						
128	Piperitone	B	1249	[31]		1255	0.14	2						
131	3,5-Dimethoxytoluene	B	1264	[32]	1270	1265	0.07	3						
135	Perillaldehyde	A	1269	[31]	1279	1277						0.09		
137	Bornyl acetate	B	1285	[31]	1287	1285	0.52	3	0.44			0.19	0.12	4.7
139	Thymol	A	1289	[31]	1302	1298	0.11	2		0.11	0.45	0.04		
140	*p*-Cymen-7-ol (Cuminol)	B	1289	[31]	1295				0.24					
142	Carvacrol	A	1298	[31]	1310	1297			0.50		0.37			0.49
143	Unidentified peak 10	C	–		1312	1305	0.02	1		0.21			0.63	
144	δ-Elemene	B	1335	[31]	1342	1335			0.66	0.25	0.29	0.05	0.41	1.4
147	Terpinyl acetate	B	1346	[31]	1354	1346	0.28	3				0.07		
148	α-Cubebene	B	1348	[31]	1357	1347							0.20	
149	α-Longipinene [a])	B	1352	[31]	1358	1353						0.45		
153	Cyclosativene	B	1369	[31]	1375								0.36	0.35
154	α-Ylangene	B	1373	[31]	1379	1370				0.13	0.66	0.11		0.48
155	Longicyclene	B	1371	[31]	1379	1378						0.38		
157	α-Copaene	B	1383	[32]	1384	1376	0.38	3					0.90	
159	β-Bourbonene	B	1387	[31]	1392	1385			0.21	0.08	0.38	0.05	1.9	3.0
161	β-Elemene	B	1393	[32]	1398	1390	0.42	3	0.64	0.14		2.1	4.4	0.70
168	Isocaryophyllene	B	1408	[31]	1413					0.12				0.16
169	α-Gurjunene	B	1409	[31]	1419	1408	0.04	2				0.10	0.21	
171	Longifolene [a])	B	1407	[31]	1419	1413						0.13		
172	β-Caryophyllene	A	1426	[32]	1430	1422	0.07	3	6.6	16.2	6.6	0.03	1.7	1.4
175	β-Copaene	B	1430	[31]	1437	1431			0.05		0.18		0.39	0.50

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Table 1. (cont.)

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		Rating of identification	RI_{Lit}	References	RI_{exp} (by SPME-GC/MS)	RI_{exp} (by GC/MS)	Mean % RPA	Count (n = 3)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)	% RPA (n = 1)
	Sample type				Gum resin	Essential oil	Essential oil		Gum resin	Gum resin	Gum resin	Essential oil	Gum resin	Gum resin
176	(*Z*)-α-*trans*-Bergamotene	B	1432	[31]	1442	1432			1.0	0.14	1.1		0.08	
179	Isogermacrene D	B	1451	[33]	1453	1446							0.34	
181	β-Farnesene	B	1454	[31]	1459				2.6	7.0				0.73
182	α-Humulene	A	1452	[31]	1464	1458	0.03	1			0.81		1.0	
183	allo-Aromadendrene	B	1458	[31]	1471	1462						0.12	0.41	
184	Unidentified Sesquiterpene 3	C	–		1453				0.91	0.60	2.1			5.3
185	γ-Muurolene	B	1478	[31]	1484	1475				0.39	0.60		2.2	
186	Germacrene D [e])	B	1480	[31]	1489	1483			0.48	0.18	0.64		1.3	0.94
189	β-Eudesmene	B	1483	[32]	1497	1491	0.05	2					2.6	
190	δ-Selinene	B	1492	[31]	1495	1488							2.0	
191	4-epi-Cubebol	B	1493	[31]	1498	1494								0.58
192	α-Selinene	B	1498	[31]	1505	1497	0.05	1		0.26		0.07	2.9	
193	α-Muurolene	B	1500	[31]	1508	1498					0.28			
194	β-Himachlene	B	1500	[31]	1505	1503						0.05		
195	β-Bisabolene	B	1505	[31]	1514				0.22					
196	δ-Guaiene (α-Bulnesene)	B	1509	[31]	1513	1502					0.33		0.92	
197	Unidentified Sesquiterpene 4	C	–		1509					0.22	1.1			0.98
198	Unidentified peak 11	C	–		1520				1.6	1.3	9.8			
199	γ-Cadinene	B	1513	[31]	1523	1514				0.17	coel	0.46	0.90	0.18
200	δ-Amorphene	B	1511	[31]	1524	1518	0.05	3					2.0	
203	δ-Cadinene	B	1522	[31]	1532	1518					coel			0.15
205	Unidentified Sesquiterpene 5	C	–		1533				0.48	0.36	5.2			2.8
206	Kessane	B	1529	[31]	1541	1532							1.0	
210	Elemol	B	1548	[31]	1557	1549			1.0				2.7	0.34
211	Oxygenated sesquiterpene 1	C	–		1556	1553				0.18	1.4			
212	Oxygenated sesquiterpene 2	C	–		1565	1564							6.3	
215	*trans*-Nerolidol	B	1561	[31]	1568				0.38	0.21	4.4			
216	Dendrolasin	B	1570	[31]	1575					0.12				
222	Spatulenol	B	1577	[31]	1589	1579								coel.
223	Oxygenated sesquiterpene 3	C	–		1594						1.3			
224	Caryophyllene oxide	A	1582	[31]	1594	1585	0.03	1	8.8	4.1	21.4	0.04		5.1
225	Viridiflorol	B	1592	[31]	1592	1596				coel.				coel.
229	Guaiol	A	1600	[31]	1614	1596			1.9				0.29	
230	Humulene epoxide II	B	1608	[31]	1620	1612			1.1	0.22	2.3			
231	γ-Eudesmol	B	1630	[31]	1626	1615	0.12	1					12.3	0.72

Table 1. (cont.)

Nr.	Compound	Identification	Retention indices (DB-5)				Sample set		Individual samples from other species				Hybrids	
			Literature values		Measured values		*Boswellia neglecta*		*B. ameero*	*B. elongata*	*B. popoviana*	*B. rivae*	*B. 'supersacra'*	*B. sacra x nana ('sacrana')*
		Rating of identification	RI_{Lit}	References	RI_{exp} (by SPME-GC/MS)	RI_{exp} (by GC/MS)	Mean % RPA	Count ($n = 3$)	% RPA ($n = 1$)	% RPA ($n = 1$)	% RPA ($n = 1$)	% RPA ($n = 1$)	% RPA ($n = 1$)	% RPA ($n = 1$)
	Sample type				Gum resin	Essential oil	Essential oil		Gum resin	Gum resin	Gum resin	Essential oil	Gum resin	Gum resin
233	τ-Cadinol (epi-α-Cadinol)	B	1638	[31]	1651	1644					2.5	0.39		
236	α-Eudesmol [f])	B	1652	[31]	1664	1657							7.3	0.38
237	α-Cadinol	B	1652	[31]	1666				1.6					
238	Bulnesol (Guai-1(10)-en-11-ol)	B	1670	[31]	1672	1666			0.71					
241	Dimer of phellandrene 1[g])	C	1732	[32]	1743	1735							0.48	0.17
244	Dimer of phellandrene 2	C	1795	[32]	1788	1795						0.03	1.1	2.5
245	Dimer of phellandrene 3	C	1810	[32]	1806	1803							traces	0.44
247	Dimer of phellandrene 4	C	1815	[32]	1820	1825							0.55	0.09
248	Dimer of phellandrene 5	C	1837	[32]	1833	1830							0.41	0.54
249	Dimer of phellandrene 8[h])	C	–		1847	1839							0.11	
253	Unidentified diterpene 2	C	–		1926					0.31				
255	*m*-Camphorene	B	1945	[32]	1959	1946				1.7				
256	Cembrene A	B	1965	[31]	1975	1955			0.76	2.9	1.7			0.79
257	Unidentified diterpene 3	C	–		1985						4.0			
258	*p*-Camphorene	B	1981	[32]	1983					0.61				
260	Cembrene C	B	2002	[32]	2022	2000				0.15				
263	Unidentified diterpene 5	C	2141	[32]	2158	2138			0.79	3.2	0.81			0.62
264	Incensole	A	2158	[31]	2170				0.56	3.5	1.7			
265	Incensole + Serratol (coelution [i]))	A	2152	[32]	2174	2151	0.04	2						1.7

[a]) Compound is presumed to be a contamination and not a constituent of the resin or essential oil. [b]) A: identified, data compared to reference substance by retention index (*RI*) and mass spectrum (MS); B: tentative identification based on *RI*, MS; C: unidentified, structure hypothesis based on MS. [c]) Retention indices below 800 were not determined. [d]) coel.: coeluting with a preceding or following peak. [e]) Compound **186** might also be bicyclosesquiphellandrene (*cis*-muurola-4(15),5-diene) or epi-bicyclosesquiphellandrene (*trans*-...), as their mass spectra are almost identical to germacrene D and their *RI* difference is only about 10. [f]) Compounds **235** and **236** *β*- and *α*-eudesmol elute most closely (*RI* difference approx. 2 – 4) and were assigned solely based on the elution order taken from *Adams* [31]. [g]) Spectra for the dimers of phellandrene look very similar (main signals 77, 93, 136) and were depicted by *Hamm et al.* [32]. Structural elucidation of an exemplary compound was performed by *Basar* [13]. [h]) Dimer of phellandrene 8 (**249**) was not listed in the study by *Hamm et al.* [32], but shares an almost identical mass spectrum with the other dimers. [i]) Coelution on a DB-5 and resolution of peaks of incensole and serratol **265** on a polar column were demonstrated by *Niebler* and *Buettner* [29].

Table 2. Average identification rate for the different species

Species	Samples	Average Identification Rate
B. neglecta	3	98.9%
B. ameero	1	90.4%
B. elongata	1	97.7%
B. popoviana	1	81.2%
B. rivae	1	94.2%
B. sacra var. *supersacra*	1	86.7%
B. sacra × *B. nana (xSacrana)*	1	90.6%
Overall average		92.1%

thujane-structures like *trans*-thujone **73** (2.8%) and 3-thujen-2-one **96** (1.3%). Additionally, the absence of the almost omnipresent limonene was only noted in one other sample in this set (*B. popoviana*). Other uncommon features include *β*-farnesene **181** (2.6%) and guaiol **229** (1.9%).

Boswellia ameero has hardly received any attention in research, the composition of the essential oil was to the best of our knowledge only reported by *Ali et al.* [19]. In this publication, the authors found the essential oil to be composed with 98.3% of only five constituents: α-campholenal (13.4%), (3*E*,5*E*)-2,6-dimethyl-1,3,5,7-octatetraene (34.9%), 3,4-dimethylstyrene (17.3%), α-terpineol (12.4%), and 1-(2,4-dimethylphenyl)ethanol (20.3%). Of these, only α-campholenal was found in trace amounts (0.2%) in our study, otherwise our results differ strongly from theirs. Potential explanations could include differences in raw sample material, distillation and workup procedures, and potentially also method parameters.

Although literature on its volatiles is scarce, *B. ameero* was also investigated with regard to the bioactivity of its bark extracts. The bark material, most probably, also contains some of the gum resin constituents. In a series of publications, *Mothana et al.* [20 – 22] reported triterpenoids and phytosterols in the bark of *B. ameero*, and showed an antiviral and antimicrobial activity of the MeOH extracts, but no anticancer activity.

Boswellia elongata Balf.f.

Just like *B. ameero*, *B. elongata* Balf.f. is an endemic species of Socotra. It is relatively easy to identify due to its long pinnate leaves that are usually colored purple to gray. The sample investigated in this study was supplied by *Jason Eslamieh* by tapping a seed grown specimen.

A total of 54 different compounds were detected in this sample, accounting for 97.7% of the total peak area. Of these, 44 were (tentatively) identified. The identified compounds exclusively comprised mono- (51.8%), sesqui- (30.1%), and diterpenes (12.5%). The most abundant compounds were α-thujene **16** (22.0%), *β*-myrcene **31** (18.2%), *β*-caryophyllene **172** (16.2%), *β*-farnesene **181** (7.0%), caryophyllene oxide **224** (4.1%), and incensole **264** (3.5%). It is interesting to note the detection of dendrolasin **216** (0.1%), which was not detected in any other species. *β*-Farnesene **181** was additionally only found in *B. ameero*. *Boswellia elongata* showed the highest *β*-myrcene **31** content compared to all other samples, including those from part I. Another interesting feature is the identification of *m*- (1.7%) and *p*-camphorene (0.6%), **255** and **258**, which are typically found in samples of *B. serrata* [10]. The analogy does, however, not extend any further, since *B. elongata* totally lacks the phenylpropanoid compounds of *B. serrata*. By contrast, the detected sesquiterpenes, such as **172** and **224**, are usually predominant in *B. sacra*.

Ali et al. [19] reported only three compounds as comprising 99.4% of the essential oil of *B. elongata*. *β*-Caryophyllene, reported with 39.1%, was also found in comparably high amounts (16.2%) in our study. Methylcycloundecanecarboxylate (7.9% in the study by *Ali et al.*) most probably refers to our unidentified peak 11 **198** (found here with 1.3%). However, we assume that compound **198** cannot be aligned with this structural proposition, due to a low NIST database match (< 85%). Moreover, no retention index has been reported in literature for methylcycloundecanecarboxylate, so that this parameter cannot be recruited for further clarification. The third compound reported by *Ali et al.*, verticiol (52.4%), might in fact relate to the compound assigned as incensole **264** in the present study and being found here with 3.5%.

In view of their physiological properties, the essential oil distilled from bark and bark extracts were investigated for their antimicrobial activity by *Mothana et al.* [23] and *Mothana* and *Lindequist* [20]. The authors reported a high diterpene content with incensole (14.8%) as the major compound, along with a variety of mono- and sesquiterpenoids. These results are in acceptable qualitative agreement with our findings, especially regarding most of the major compounds like **16**, **31**, **172**, **181**, and **264**. Quantitative deviations are quite plausible considering the different analytical methods (distillation and GC-FID by *Mothana et al.* compared to SPME-GC/MS in the present study). However, some major constituents were reported by *Mothana et al.* that are absent in our data, *e.g.*, *β*-eudesmol **235**, verticilla-4(20),7,11-triene **261,** and incensole acetate **266**. Other studies showed an anti-inflammatory, antinociceptive, antioxidant [24] as well as a peptidase inhibitory [25] and antiviral [21] effect of a MeOH bark extract of *B. elongata*.

Boswellia popoviana Hepper

The *Boswellia popoviana* sample is another representative of the collection of *Jason Eslamieh* and a species endemic to Socotra. A total of 43 compounds were detected and 32 (tentatively) identified, accounting for 81.2% of the total peak area. The identified compounds were almost

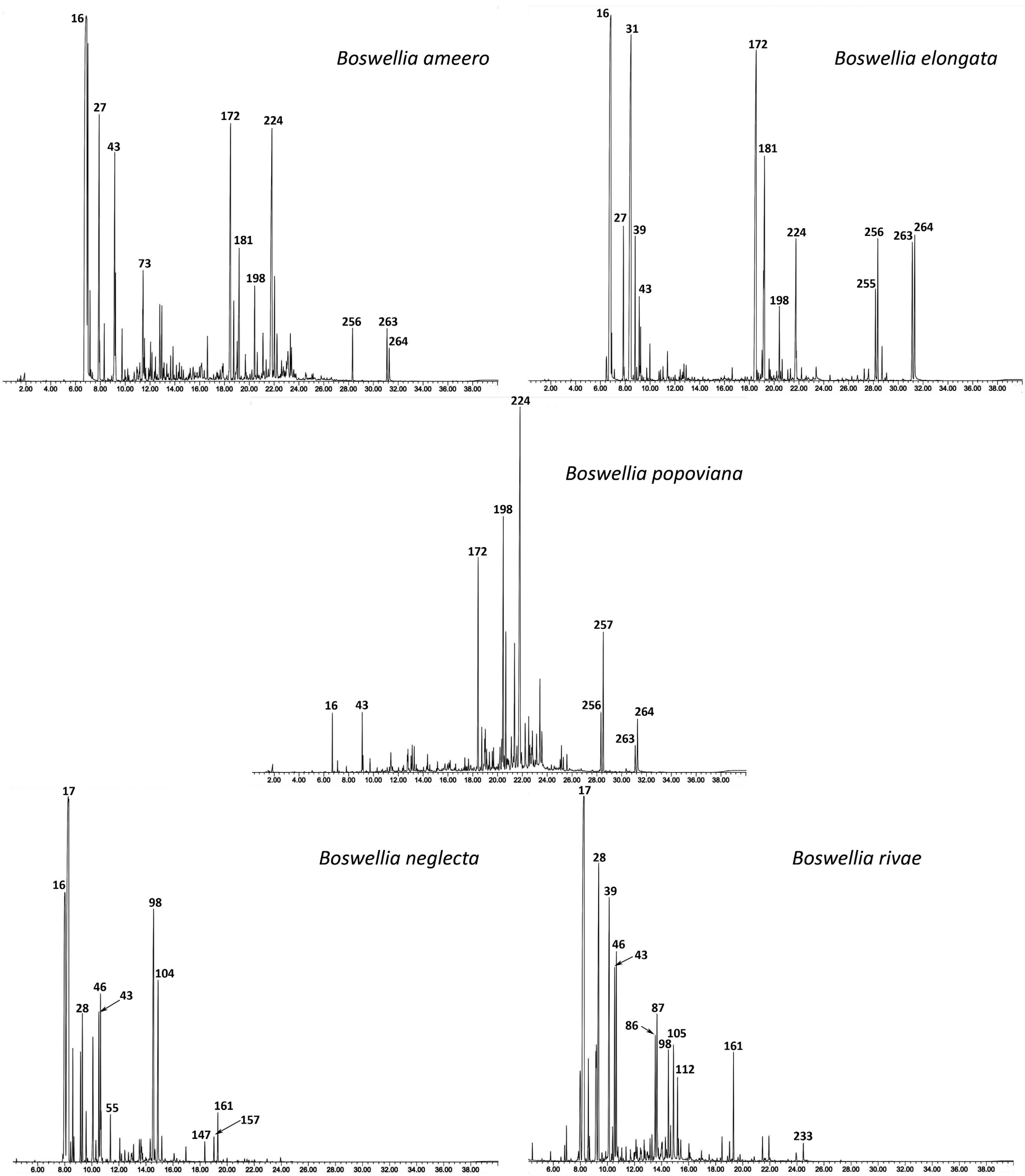

Fig. 1. Exemplary chromatograms of the five *Boswellia* species. Numbers refer to compounds from *Table 1*.

exclusively of terpenoid origin, with the sesquiterpenoids accounting for 63.3%, followed by mono- (8.5%) and diterpenoids (8.2%). The most abundant peaks were caryophyllene oxide **224** (21.4%), the unidentified peak 11 **198** (9.8%), *β*-caryophyllene **172** (6.6%), unidentified sesquiterpene 5 **205** (5.2%), and *trans*-nerolidol **215** (4.4%). With the exception of **172**, these were the highest abundances found in all samples for the respective substances. Among the monoterpenes, this was the only sample without a notable peak of *α*-pinene **17**, although traces below the integration threshold were detectable at the respective retention time. Among the monoterpenes, predominantly thujane-based (**16**, **27**, **73**, **95**, **96**) and aromatic (**43**, **64**, **103**, **118**, **139**, **142**) monoterpene structures were found. In this sample, many compounds remain to be structurally elucidated.

To the best of our knowledge, this is the first report of the volatile chemical composition of resin from *B. popoviana*. Aside from botanical descriptions and investigations, we could not find any literature reports on chemical components of *B. popoviana* or their effects.

Boswellia rivae Engl

Boswellia rivae is a large shrub or small tree native to Somalia, eastern Ethiopia, and northern Kenya. The dark resin, often traded as 'Ogaden-type' olibanum, is usually hydrodistilled to yield the fragrant essential oil.

A single essential oil sample of *B. rivae* was investigated. A total of 71 peaks were detected and 65 compounds were (tentatively) identified, with an identification rate of 94.2%. The sample was composed primarily of monoterpenoids (89.3%) and some minor proportion of sesquiterpenes (4.6%). No diterpenes were detected, except for the trace of a α-phellandrene dimer **244**. The most abundant compounds in order of their elution were: α-thujene **16** (3.5%), α-pinene **17** (26.8%), β-pinene **28** (9.8%), δ-3-carene **39** (7.9%), *p*-cymene **43** (4.5%), limonene **46** (4.9%), *trans*-pinocarveol **86** (3.0%), *trans*-verbenol **87** (4.1%), and myrtenal **105** (3.2%). For myrtenal, this percentage was the highest observed in any of the samples. Among the sesquiterpenes, the predominating compounds were β-elemene **161** (2.1%) and γ-cadinene **199** (0.5%). Several unusual sesquiterpenes were detected in this sample in traces, such as longifolene **171** (0.1%), longicyclene **154** (0.4%), and α-longipinene **149** (0.5%). These compounds have not been reported for *Boswellia* species, except as potential adulteration indicators [10], and are typically found in Pinaceae resins [26]. It is not possible to conclude from a single sample if these compounds are actually true constituents or just a contamination during processing, for example, from the distillation process. Disregarding the diterpenes, *B. rivae* resembles *B. neglecta* to a certain extent.

Available literature data on *Boswellia rivae* generally agrees with our findings. *Basar* [13], *Başer et al.* [14], *Bekana et al.* [15], *Camarda et al.* [27], and *Schillaci et al.* [28] reported a similar monoterpenoic composition, but no sesquiterpenes or just trace amounts (**161** in *Camarda et al.* [27]). In both parts of the present study, *o*-cymene **51** (0.1%) was exclusively detected in *B. rivae*, in agreement with reports from literature [13][15][27].

Hybrid Samples

Some hybrids of *Boswellia* species were previously reported [7][8] and were shown to possess different traits and properties than their parent plants. The purpose of this part of the study was on the one hand to discuss two hybrids that were not yet reported with detailed botanical descriptions, and on the other hand combine this botanical description with a chemical investigation of their gum resin by SPME-GC/MS. The corresponding chromatograms are shown in *Fig. 2*. The primary objective of creating a series of hybrids was to test and examine the possible manifestation of the heterosis (hybrid vigor) on the boswellic acids, incensole acetate, and other physiologically beneficial chemical compounds. The purpose was to discover the possibility of a new hybrid with a larger quantity of beneficial compounds. In the course of this study, a first screening of volatile metabolites was performed in order to evaluate changes in the metabolite profile and to screen for the presence of incensole and incensole acetate.

It is important to mention that the hybrids were bred in a controlled environment and a careful process was put into place with great attention to ensure that the hybrids resulted from a pollen-free environment and no presence of foreign pollen was possible (*cp. Experimental Part*).

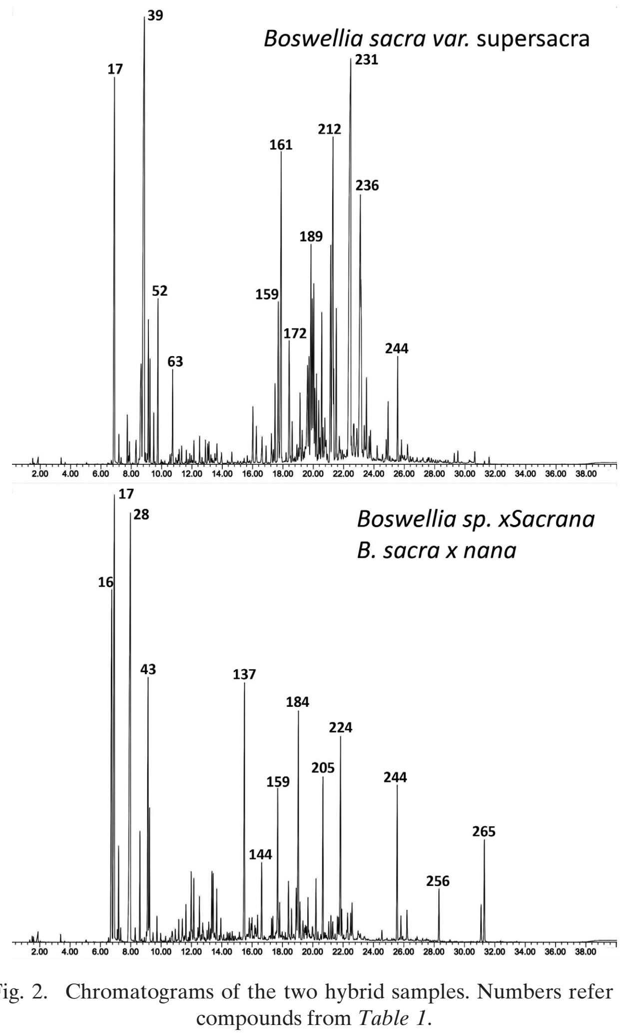

Fig. 2. Chromatograms of the two hybrid samples. Numbers refer to compounds from *Table 1*.

B. sacra *var.* 'supersacra'

This variety was created by cross-pollinating a *Boswellia sacra* specimen with different clones of the same species to avoid self-pollination. The attempt was made to increase the species' genetic makeup. This experiment was conducted due to genetic weaknesses in the species, such as poor seed germination rate, seedling fatality rate, etc., which is attributed to genetic bottlenecking of the species at one time in its history.

Botanical Description and Observations — The following botanical description is based on a variety resulting from three generations of preventing possible self-pollination: *Boswellia sacra* var. *supersacra*: *Eslamieh*, *J.* in "*Cultivation of Boswellia... Sacred Trees of Frankincense",* p. 150 (2011) [8]

It is a small tree or shrub 2 – 5 m tall, bark yellow exfoliating in large sheets or flacking papery outer layer over a thick resiniferous layer, base of trunk swollen at juvenile age, but then tapering to a conical form as the tree matures; twigs stout.

The leaves are oblanceolate in outline, 8 – 20 cm long, petiole 10 mm long, 7 – 15 foliolate; leaflets lanceolate, oblong, elliptic, or broadly ovate, up to 5 (–7.5) × 2 (–3.5) cm, obtuse to subacute at the apex, broadly cuneate or truncate and often asymmetric at the base, the margin often somewhat undulate, usually crenate, upper surface hirsute in young leaves then subglabrous. The color is dark green and appears glabrous due to the waxy surface. Lower surface is much paler, hirsute to densely tomentose with a prominent network of veins, terminal leaflet larger than the original species. The flowers are glabrous to sparsely pubescent racemes or little-branched panicles 6 – 20 cm long, including the 0.5 – 4 cm long peduncle; bracts 1 – 3 mm long; pedicels 2 – 10 mm long. The calyx 2 – 3 mm long, glabrous; petals cream or white, 5 × 2 (–3) mm; filaments glabrous, 2 – 3 mm long, linear; disk shortly tubular, yellow-orange, 1 – 2 mm deep. Its fruit 3 – 4, sometimes five-celled, 8 (–14) × 4 (–10) mm, narrowly to broadly pear-shaped, glabrous; pyrene 4 – 5 pointed with narrow apical and basal horns and broader lateral points, often surrounded by a persistent wing. The pyrenes are larger in both directions than the original species. The germination rate of this cultivated form is nearly 60%, in contrast to the original form, which produces seeds with < 10% germination. *Fig. 3* shows the flowers of a *B. sacra* var. *supersacra* specimen.

With these experiments, it was demonstrated that germination rate and seedling viability increased dramatically when *B. sacra* cross-pollinates. Usually, the seed germination rate of *B. sacra* is as low as 5 – 10%. In the hybrids of the second generation, the rate was nearly 60%. This is most probably to be attributed to the genetic bottlenecking, which occurred due to isolated stands, frequent self-pollination, and overtapping of *B. sacra* in habitat. This experiment proved that cross-pollination also results in much more vigorous seedlings and ultimately the trees seem to be more cold tolerant, more drought tolerant, faster growers, and produce much darker green leaves than the typical habitat trees.

Fig. 3. Flowers of *B. sacra* var. *supersacra*.

Chemical Analysis — The gum resin sample from tapping *B. sacra* var. *supersacra* showed a typical composition of *B. sacra* with the absence of specific diterpenes like **265**. Overall, the identification rate was 86.7% and 64 compounds were (tentatively) identified. The sesquiterpenes were the most abundant (57.1%), followed by monoterpenes (25.8%). Major compounds were α-pinene **17** (5.1%), δ-3-carene **39** (10.2%), β-elemene **161** (4.4%), β-eudesmene **189** (2.6%), α-selinene **192** (2.9%), elemol **210** (2.7%), oxygenated sesquiterpene 2 **212** (6.3%), γ-eudesmol **231** (12.3%), and α-eudesmol **236** (7.3%). It is interesting to note the occurrence of kessane **206** (1.0%), a sesquiterpene ether otherwise only detected in *B. serrata*. A large peak of an unidentified oxygenated sesquiterpene (**212**) was detected in just one other sample, a *B. sacra* essential oil (from part I). These two key features also distinguish this sample from the *B. sacra* sample by *Eslamieh* (*cp*. part I of this study), to which it otherwise bears the closest resemblance. *Boswellia*-specific diterpenes like incensole and serratol were not detected in this sample (see also [10]). The low content in monoterpenes is probably due to the low sample size (<< 1 g), which might lead to a quick evaporation of the more volatile compounds. In conclusion, it appears that this hybrid exhibits most of the typical traits of *B. sacra* in combination with a few unusual features.

Boswellia *sp.* xSacrana *(*B. sacra × B. nana*)*

Botanical Description and Observations — The second sample was a hybrid of *B. sacra* × *B. nana*, designated *B.* sp. *xSacrana*. This hybrid was first mentioned in

Eslamieh's book [8]. A more detailed botanical description of the hybrid is given here: *Boswellia* sp. *xSacrana (B. sacra* × *B. nana) hybrid*: *Eslamieh*, *J.* in '*Cultivation of Boswellia... Sacred Trees of Frankincense*', p. 180 (2011) [8]

This description is based on the hybrid at F-2 after it was planted in the ground for 3 years. It is a small tree or shrub, 1 – 3 m tall; bark yellow exfoliating in small sheets or flaking papery outer layer over a thick resiniferous layer, base of trunk swollen at juvenile age, but then tapering to a conical form as the tree matures; twigs stout. The leaves are oblanceolate in outline, 8 – 15 cm long, petiole 10 mm long, 7 – 9 foliolate; leaflets lanceolate, oblong, elliptic or broadly ovate, up to 5 (−7.5) × 2 (−3.5) cm, obtuse to subacute at the apex, broadly cuneate or truncate and often asymmetric at the base, the margin often somewhat undulate, usually crenate, upper surface hirsute in young leaves then subglabrous, lower surface much paler, hirsute to densely tomentose with a prominent network of veins, terminal leaflet much larger, new leaves reddish to pink, then dark green with a heavy rough surface. The flowers are glabrous to sparsely pubescent racemes or little-branched panicles 6 – 20 cm long, including the 0.5 – 4 cm long peduncle; bracts 1 – 2.5 mm long; pedicels 2 – 8 mm long. The calyx is 2 – 2.5 mm long, glabrous; petals red, pink, or sometimes cream, 4 (−5) × 2 (−2.5) mm; filaments glabrous, 2 – 2.5 mm long, linear; disk shortly tubular, yellow-orange, 1 – 1.5 mm deep. The fruit three- to four-celled, 8 (−12) × 4 (−9) mm, narrowly to broadly pear-shaped, glabrous; pyrene four-pointed with narrow apical and basal horns and broader lateral points, often surrounded by a persistent wing. The flowers of *B.* sp. *xSacrana* are depicted in *Fig. 4*.

B. sp. *xSacrana* has proven to be slightly more cold tolerant than either of the parents. It can withstand temperature as low as −4 °C (24 °F) with southern exposure *vs*. either of the parents that would be damaged at −1 °C (30 °F). Apparently in this hybrid, similar to *Boswellia* sp. *xTierney* (*B. nana* × *B. sacra* [7]), the hybrid vigor has manifested in their cold-hardiness. Another factor is that both *B. xSacrana* and *B. xTierney* produce seeds with 50% germination rate; this represents almost an average between *B. sacra* seed germination rate (5 – 10%) and *B. nana* seed germination rate (80 – 90%). Phenotypically speaking, it seems the hybrid has clearly been influenced by both parents. Just to mention a few of the similarities: the flowers are pink like *B. nana*, the leaves are pinnate like *B. sacra* but glabrous like *B. nana*, the size at full growth is an average between the two species and the gum resin is distinct from *B. sacra*. Unfortunately, a *B. nana* sample was not available for comparison of the chemical composition, and the volatiles of this species have not been reported in literature.

Chemical Analysis — In the gum resin sample from *B.* sp. *xSacrana*, 66 compounds were detected and 61 (tentatively) identified, covering 90.6% of the total peak

Fig. 4. Flowers of *Boswellia* sp. *xSacrana*.

area. The sample showed a higher monoterpene content (57.0%) than *B. sacra* var. *supersacra* and lower sesquiterpene content (26.1%). The diterpenes accounted for 6.9%, among them the coeluting incensole and serratol **265** (1.7%). The most abundant compounds were α-thujene **16** (8.0%), α-pinene **17** (10.5%), β-pinene **28** (13.2%), *p*-cymene **43** (4.7%), bornyl acetate **137** (4.7%), β-bourbonene **159** (3.0%), unidentified sesquiterpene 3 **184** (5.3%), unidentified sesquiterpene 5 **205** (2.8%), and caryophyllene oxide **224** (5.1%). The content in bornyl acetate **137** was the highest in all investigated samples. The coeluting diterpenes serratol and incensole **265** were found to be present in amounts comparable to *B. sacra* [29]. The unidentified sesquiterpenes 3, 4, and 5 (**184**, **197**, and **205**, respectively) along with β-farnesene **181** (0.7%) were otherwise only detected in the Socotrean species *B. ameero*, *B. popoviana*, and *B. elongata*. Since *B. nana* is also a species endemic to Socotra, it might well be that the *Boswellia* species from that isolated habitat share a common metabolic fingerprint. These compounds also form the main characteristics that cannot be attributed to *B. sacra*, and might thus constitute typical features either inherited from *B. nana* or entirely new in the hybrid.

Conclusions

In this second part of the investigation of *Boswellia* gum resins and essential oils, five additional, less common species were investigated. It was shown that the composition differs notably from that of the four commercially relevant species, yet not significantly enough to allow a clear distinction between several of the species reported here. In any case, larger sample sets are necessary to evaluate characteristic features of the respective species. The volatile composition of *B. popoviana* gum resin was reported here for the first time, likewise the composition of two samples from hybridization experiments with *B. sacra* and

B. nana. Additionally, the variety *B. sacra* var. *supersacra* and the hybrid *B.* sp. *xSacrana* were described in detail. Both hybrids exhibited a strong hybrid vigor, for example, with regard to seed germination rate, seedling viability and cold tolerance, while physiologically interesting compounds were absent (incensole acetate) or not notably increased (incensole).

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Experimental Part

Sample Material

Jason Eslamieh (*Miniatree Garden*, Tempe, AZ, USA) generously tapped some rare *Boswellia* species from his personal collection and supplied the samples of rare species and hybrids (five gum resin samples). The four essential oil samples were acquired through the following suppliers: *Maienfelser Naturkosmetik*, Maienfels, Germany; *Essence pur Ltd.*, Utting am Ammersee, Germany; *Senger Naturrohstoffe*, Dransfeld, Germany. Due to very low sample size in case of the gum resins, it was not possible to deposit voucher specimens in a herbarium. Small samples of the investigated essential oils can be accessed through the corresponding author.

Experimental Procedures, Instruments, and Methods

The instruments, methods, and procedures for the investigation of the gum resin and essential oil samples are identical to the ones reported in part I of this publication [10]. A brief summary is given here: powdered gum resin samples were extracted with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (DVB/CAR/PDMS, 1 cm fiber length, by *SUPELCO*, Bellefonte, PA, U.S.A.) at 80 °C for 30 min after a 30 min equilibration period. Desorption took place at 250 °C for 5 min at a split rate of 1:5. Essential oil samples were analyzed by on-column injection at a concentration of approx. 1 mg/ml in CH_2Cl_2. A GC/MS analysis was performed on a *DB-5* analytical capillary (30 m × 0.25 mm, film thickness 0.25 μm, *Agilent J&W Scientific*, Santa Clara, CA, USA) at 40 °C for 2 min, 6 °C/min to 200 °C, 40 °C/min to 300 °C, held for 5 – 10 min. Carrier gas was helium at a flow rate of 1.3 ml/min. A mass spectrometric analysis (*Agilent MSD 5975C*) was performed in full scan mode (*m/z* range 40 – 450 in essential oils, 29 – 450 in SPME analysis) at 70 eV ionization energy.

Hybridization Experiments: Means and Methods

With consideration to the bisexual nature of *Boswellia* species, hybridization demanded a process with precision. Once the flower of the species to receive pollen opened (usually early morning), a small straw-like tube was put on the entire stigma and rested on the base plate encapsulating the entire female part of the flower. Then all pistils were removed and with an air-can, the surface including the petals was cleaned. Pollen from the predetermined species had been already collected and was gently laid on the sticky stigma with a small brush. This method proved to result in accurate cross-breeding nearly 99% of the time.

Cultivation

The seeds of the hybrids ripened in 4 – 6 weeks and were collected by hand. They were cleaned, deshelled, and tagged for sowing. The seeds were sowed in a typical germination medium consisting of equal parts potting compost, perlite, and pumice. The seeds of the hybrids germinated in 4 – 14 days depending on the hybrid. The seedlings were transferred from flats to individual containers in 6 months and then repotted on an annual basis until they were transferred to the ground.

Growing Conditions

The hybrids were grown in containers for the first few years in potting medium and then grounded for size and full maturity. The gum resins collected for the testing referenced in this publication were from the plants grown in ground which consisted of natural earth (sandy loam) mixed with some organic compost.

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