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Article

Two Woody Scented Oils from the Amazonian Forest

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The essential oils from *Aniba parviflora* and the balsam from *Protium rubrum*, which are two woody scented Amazonian species, were analyzed by gas chromatography-mass spectrometry (GC-MS), ^{1}H and ^{13}C nuclear magnetic resonance (NMR). GC-MS analysis revealed the presence of unknown constituents. These compounds were then isolated, analyzed and characterized by NMR. Both substances presented distinct woody notes that contributed to the bulk woody character of the crude oils.

Keywords: *Aniba parviflora*, *Protium rubrum*, β-phellandrene, $\Delta^{4(4a)}$-(2*R**,8*R**,8a*S**)-eremophilen-9-ol, thymol derivatives

Introduction

Brazil is one of world's richest countries in terms of biodiversity, but it has made only a small contribution of new natural raw materials to the flavors and fragrances (F&F) industry. Despite the growing number of published articles, the aromatic ingredients available from Brazilian plants in the F&F market are almost the same as 50 years ago: rosewood, copaiba and tonka beans.[1]

With the aim of improving this situation, the chemical composition and potential application in the F&F market were examined for an essential oil and a balsam from the Amazon region after an evaluation by a perfumer.

The essential oil of *Aniba parviflora* (Meissner) Mez is produced by a species belonging to Lauraceae, a family possessing approximately 52 genera and 1900 species that are well distributed in the tropics.[2] Examples of Lauraceae essential oils that are consumed worldwide include bay (*Laurus nobilis*), cinnamon (*Cinnamomum zeylanicum* and *Cinnamomum cassia*), and camphor (*Cinnamomum camphora*). The genus *Aniba* is restricted to the Neotropical region, and rosewood (*Aniba rosaeodora*) is its most famous species.

Aniba parviflora is a small tree similar to rosewood, and it is often misclassified as a young *A. rosaeodora*; it is native to the central eastern Brazilian Amazon and occurs naturally near small rivers in non-flooded areas. The wounded bark is yellow in *A. parviflora* and reddish in rosewood, and there is a remarkable olfactory difference between them. Two *A. parviflora* experimental plantations that exist for commercial purposes can be found near the city of Belém, and these were made by mistake 15 years ago when the researchers thought they were planting rosewood. A third plantation is located between the Amazon and Tapajos rivers at the experimental campus of Amazon Federal Rural University (UFRA), and it is approximately 30 years old and occupies 5 hectares of a forest regeneration experimental station founded by FAO (Food and Agriculture Organization of United Nation). Plant material from this 30-year-old plantation was used for this study.

The other woody scent comes from *Protium rubrum*, known as "Cuatrec", which is a rare Burseraceae distributed in Brazil, Peru and Colombia.[3] The Burseraceae family has approximately 20 genera and 600 species that are well distributed in the tropics.[4] This family is well known for its triterpenic resins, a bark exudate, such as myrrh, olibanum

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and frankincense. In Brazil, this kind of resin is very common in the Amazon region and on the Bahia coast, where people use it to add perfume to their homes by burning it, or to seal boat hull cracks. Unlike other species of *Protium* trees, this *P. rubrum* has a balsam instead of a resin. Similar to Copaiba trees, the balsam is collected by utilizing a deep hole in the tree bark. This balsam does not have a traditional use and was discovered when farmers were cutting down an area for cassava cultivation in Silves, AM.

Experimental

Reagents and solvents

Unless stated otherwise, all chemicals were purchased from Sigma-Aldrich (São Paulo, Brazil), and the solvents were of high-performance liquid chromatography (HPLC) grade (São Paulo, Brazil).

Plant material

Thin branches and leaves of *A. parviflora* were collected (year 2000) in the Curuá-una Experimental Field of the Wood Technology Center (CTM) of UFRA. The material (5200 kg) was collected for 3 days and was transported by river to the town of Santarem in a 24 h trip, where it was steam distilled in a 3000 L iron tank fed by an antique iron retort operating with a vapor flow of 30 kg h m^3. The distillation tank could process 700 kg with each operation; therefore 3 days were required to perform the 8 necessary operations. Water came from an artesian well and went directly to the retort. The plant material was ground before undergoing distillation, and it was distilled for four hours at atmospheric pressure. The oil was treated with sodium sulfate before chromatographic analyses.

The *Protium rubrum* balsam was collected at the São Pedro community, Silves, in the central Amazon (year 2000). A tree was cut while an area was cleared for a cassava plantation, and the balsam was released from it in abundance. It was collected in a polyethylene bottle, brought to the city and transferred to a glass bottle. Before analysis, the balsam was treated with sodium sulfate and filtered.

Compound isolation

Sesquiterpenoid AF105 (later identified as eremophilen-9-ol, **1**), which is responsible for some woody and floral notes, is present at 5% in *A. parviflora* essential crude oil. For isolation, 10 g of the essential oil was fractionally distilled with 10 g of paraffin (Synth, São Paulo, Brazil) to increase the thermal capacity of the system. The mixture was heated under a vacuum in an oil bath. The monoterpenes and nonoxygenated sesquiterpenes were distilled using up to 80% of the total oil. The cooled residue was then extracted 3 times with ethanol. This procedure increased the concentration of the woody scented target compound from 5 to 25%. Then, regular fractioning was performed using a silica column (Acros, São Paulo, Brazil, 0.035-0.070 mm particles with 6 nm pore diameter) followed by silica coated with 10% $AgNO_3$ (Acros, São Paulo, Brazil). Both columns were eluted with hexane that contained increasing amounts of ethyl acetate. This procedure yielded compound **1** with 97% purity. Previous attempts without the fractionation/distillation step resulted in a contamination of oxygenated monoterpene after two chromatographic separations.

Compounds **5** and **6**, isolated from *P. rubrum*, were obtained by single silica column fractionation.

Synthesis of the valerianol compound

To obtain the valerianol substrate, two synthetic steps were necessary.

(*i*) Trifluoroacetylation of valencene

Two grams of commercial Acros brand valencene (São Paulo, Brazil) were solubilized in 20 mL of hexane. The mixture was homogenized by adding 1.6 g of trifluoroacetic acid. Immediately the solution turned from colorless to violet.

The mixture remained under magnetic stirring at about 50 °C for 120 min. Due to the low yield verified by thin layer chromatography (TLC), the reaction was kept for another 24 h at room temperature. The mixture was washed with saturated sodium bicarbonate solution ($NaHCO_3$), extracted with ethyl acetate and dried over anhydrous sodium sulfate (Na_2SO_4). The solvent was initially evaporated in a rotary evaporator and then in a N_2 gas line. The product generated, valerianyl trifluoroacetate, was isolated by column chromatography.

(*ii*) Hydrolysis of valerianyl trifluoroacetate

Valerianyl trifluoroacetate (31.8 mg) and 4 mL of solvent were added as follows: 2.4 mL of tetrahydrofuran, 0.8 mL of methanol, 0.8 mL of water. After homogenization, 16.8 mg of hydrated lithium hydroxide ($LiOH{\cdot}H_2O$) was added. The mixture was kept under magnetic stirring in ambient temperature for 2 h.

It was then washed with 10% hydrochloric acid solution, extracted with ethyl acetate and dried over sodium sulfate. The solvent was removed in an analogous manner to the previous reaction. The alcohol formed, valerianol, was isolated by chromatography on column.

Gas chromatography-mass spectrometry (GC-MS) analysis

Both the *A. parviflora* oil and *P. rubrum* balsam were analyzed on a Hewlett-Packard gas chromatograph Model 6890 coupled to a Hewlett-Packard mass spectrometer (MS) Model 5973 (Agilent, São Paulo, Brazil) equipped with an HP5 column (30 m × 0.25 mm, 0.25 μm film thickness) programmed from 50 to 190 °C at 3 °C min^{-1} with a 5 min hold. The carrier gas was helium at 1 mL min^{-1}; split mode injection (1:30) and an injector temperature of 200 °C were used. The MS ran in electron impact mode at 70 eV, the electron multiplier was set to 1800 V and the ion source temperature was 280 °C. Mass spectral data were acquired in the scan mode from *m/z* 40 to 500 a.m.u.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) analysis

The isolated compounds underwent ^{1}H, ^{13}C, and 1 and 2D NMR experiments with Bruker Avance III equipment (Eisenhutweg, Germany). The spectra were acquired with a Varian INOVA-500 (B_0 = 11.7 T) operating at 499.88 MHz for ^{1}H and 125.71 MHz for ^{13}C, using 5 mm probes for direct and indirect detections. The material was dissolved in $CDCl_3$ at concentrations varying from 2 to 20 mg mL^{-1}. The residual chloroform present in deuterochloroform (7.27 ppm) was used as the internal reference.

Results and Discussion

The steam distillation of *A. parviflora* leaves yielded 0.10% of an essential oil, which was analyzed by GC-MS (Table 1), revealing a nonidentified compound named AF105 that was present at ca. 5% with a calculated retention index of 1626.

With the chemical composition analysis of essential oil, it was possible to observe that main compounds were

Table 1. GC-MS results of *A. parviflora* essential oil. The isolated compound AF 105 appears in bold

No.	Compound[5]	KI lit[a]	KI exp[b]	%[c]
1	α-thuyene	931	930	0.31
2	α-pinene	939	935	2.86
3	camphene	953	947	0.80
4	sabinene	976	971	0.21
5	β-pinene	980	973	1.52
6	myrcene	991	989	1.70
7	α-phellandrene	1005	1001	7.98
8	δ-3-carene	1011	1006	0.15
9	α-terpinene	1018	1012	0.18
10	*p*-cymene	1026	1020	4.02
11	β-phellandrene	1031	1024	15.12
12	eucalyptol	1033	1026	0.86
13	*Z*-beta-ocimene	1040	1034	0.16
14	*E*-beta-ocimene	1050	1044	3.81
15	γ-terpinene	1062	1053	0.31
16	terpinolene	1088	1082	0.34
17	linalool	1098	1097	14.60
18	borneol	1165	1160	0.16
19	terpine-4-ol	1177	1172	0.25
20	α-terpineol	1189	1186	0.90
21	α-cubebene	1351	1345	0.43
22	α-ylangene	1372	1366	0.14
23	isoledene	1373	1368	0.11
24	α-copaene	1376	1370	0.49
25	β-elemene	1391	1387	0.52
26	α-gurjunene	1409	1404	0.16
27	β-caryophyllene	1418	1413	6.05
28	β-gurjunene	1432	1423	0.20
29	not identified	–	1428	0.12
30	aromadendrene	1439	1433	1.79
31	not identified	–	1439	0.36
32	α-humulene	1454	1447	0.79
33	alloaromadendrene	1461	1455	0.60
34	γ-muurolene	1477	1472	0.70
35	germacrene D	1480	1475	0.97
36	β-selinene	1485	1480	1.23
37	not identified	–	1482	0.17
38	bicyclogermacrene	1494	1492	6.75
40	(*E*,*E*)-α-farnesene	1508	1507	0.53
41	γ-cadinene	1513	1509	0.28
42	δ-cadinene	1524	1520	0.80
43	elemol	1549	1546	0.39
44	germacrene B	1556	1551	0.81
45	not identified	–	1554	0.11
46	*E*-nerolidol	1564	1562	0.47
47	not identified	–	1566	0.14
48	spathulenol	1576	1573	4.13
49	caryophyllene oxide	1581	1577	2.16
50	viridiflorol	1590	1586	0.36
51	not identified	–	1588	0.37
52	guaiol	1595	1593	0.49
53	not identified	–	1596	0.45
54	not identified	–	1602	0.25
55	not identified	–	1607	0.14
56	not identified	–	1613	0.27
57	not identified	–	1617	0.33
58	**AF 105**	**1626**	**1626**	**5.05**
59	iso-spathulenol	1639	1634	0.59
60	not identified		1638	0.30
61	β-eudesmol	1649	1644	1.03
62	α-eudesmol	1652	1648	1.27
63	not identified	–	1653	0.16
64	not identified	–	1661	0.37
66	benzyl benzoate	1762	1761	0.12
Total recovered				99.19

[a]Kovats retention index in literature; [b]experimental Kovats retention index; [c]relative percentage of the compound identified.

terpenoids β-phellandrene (15.12%), linalool (14.60%), α-phellandrene (7.98%), bicyclogermacrene (6.75%) and β-caryophyllene (6.05%), corroborating with Xavier *et al.*[6] and Oliveira *et al.*,[7] who report the presence of these compounds in essential oil of *A. parviflora* with similar percentages.

To evaluate the contribution of AF 105, an unknown constituent, to the entire oil odor, a sniff test was set up with two columns of equal length attached to the same injector with one end linked to the mass detector and the other used for sniffing experiments. The odor of AF105 was therefore classified as having a woody-floral note with green touches.

The next step was to isolate the compound and determine its chemical structure by 1D and 2D NMR in a Varian probe (^{1}H NMR, ^{13}C NMR, heteronuclear single quantum correlation spectroscopy (HSQC), heteronuclear multiple bond correlation spectroscopy (HMBC), correlation spectroscopy (COSY)). By comparing the ^{13}C NMR chemical shifts with the other eremophilane,[8,9] we could conclude that this compound is identical to $\Delta^{4(4a)}$-(2*R**,8*R**,8a*S**)-eremophilen-9-ol or (2-[(2*R**,8*R**,8a*S**)-8,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl]-propan-2-ol), **1**, which was previously isolated by Itokawa *et al.*[9] (Figure 1). The carbon chemical shift differences between the jinkoheremol reported by Ishihara *et al.*[8] and **1** are probably due to diastereomers of these two compounds, which also display different optical rotations (jinkoheremol[5] ($[\alpha]_D^{29} = -74.5°$, $c = 1.33$, $CHCl_3$; eremophilen-9-ol[7] $[\alpha]_D^{29} = -14.9°$, $c = 0.45$, $CHCl_3$; isolated compound **1** $[\alpha]_D^{22} = -6°$, $c = 5$, $CHCl_3$) (Supplementary Information (SI) section).

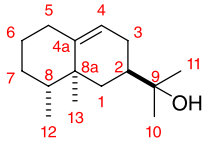

Figure 1. Structure of compound **1**.

Compound **1** was then sent to a perfumer for an olfactory evaluation, and its odor was classified as a floral balsamic, which was a bottom note in the entire oil odor.

The high structural similarity between eremophilen-9-ol **1** and valerianol **4**, a major constituent of *Valeriana officinalis* essential oil,[10] inspired us to produce **4** to perform an olfactory comparison between these two isomers. Therefore, valencene **2** was first derivatized to valerianyl trifluoroacetate **3** in 70% by applying Mattos *et al.*[11] methodology, which produced valerianol **4** upon hydrolysis (Figure 2).

Valerianol **4** was characterized by GC-MS, ^{1}H NMR and ^{13}C NMR and compared with data in the literature.[12]

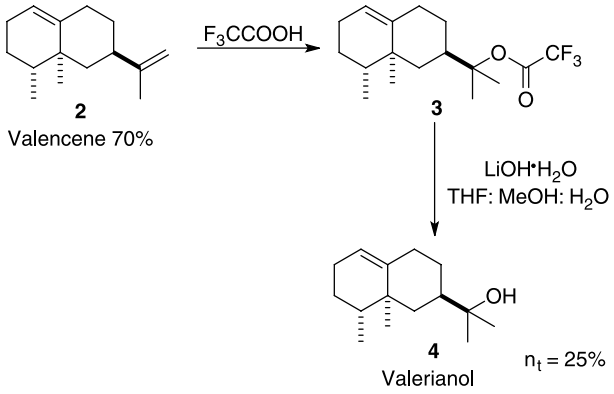

Figure 2. Synthetic route to produce valerianol from valencene.

The olfactory comparison revealed high similarity between **1** and **4**. Eremophilen-9-ol 1 has a slightly more pronounced floral character, while valerianol **4** has a more pronounced green note.

Protium rubrum

Compounds PR192 and PR210 were isolated and identified by ^{1}H NMR and ^{13}C NMR spectra (SI section). The suggested structures are 1-isopropenyl-2,5-dimethoxy-4-methylbenzene (**5**) and 1-isopropanol-2,5-dimethoxy-4-methylbenzene (**6**) (Figure 3).

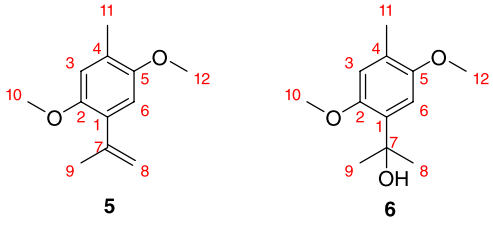

Figure 3. Structure of compounds 1-isopropenyl-2,5-dimethoxy-4-methylbenzene (**5**) and 1-isopropanol-2,5-dimethoxy-4-methylbenzene (**6**).

These molecules were also sent for an olfactory evaluation to determine their contribution to the whole oil fragrance and the olfactory difference caused by the presence of a hydroxyl group.

Compound **5** was classified as almost odorless with a very weak balsamic note, and although it is the main constituent, it has a very small contribution to the balsam fragrance. Compound **6** possesses a dry woody note that is easily identified in crude balsam. The GC-MS analysis of *P. rubrum* balsam is presented in Table 2.

Conclusions

The structure of compound AF105 was suggested to be 2-[(2*R**,8*R**,8a*S**)-8,8a-dimethyl-1,2,3,5,6,7,8,8a-octa-

Table 2. Compounds identified in *P. rubrum* balsam by GC-MS. Isolated molecules appearing in bold

No.	Compound[5]	KI lit[a]	KI exp[b]	%[c]
1	limonene	1031	1030	1.84
2	α-copaene	1376	1375	0.77
3	cyperene	1398	1401	0.32
5	*Z*-alpha-bergamotene	1415	1418	0.65
6	**PR 192**	**–**	**1434**	**84.18**
7	not identified	–	1440	2.17
8	not identified	–	1472	0.30
10	not identified	–	1503	0.69
11	β-bisabolene	1509	1506	2.16
12	*Z*-gamma-bisabolene	1515	1509	0.85
13	**PR 210**	**–**	**1557**	**6.07**
Total recovered				100.00

[a]Kovats retention index in literature; [b]experimental Kovats retention index, [c]relative percentage of compound identified.

hydronaphthalen-2-yl]-propan-2-ol based on GC-MS and ^{1}H and ^{13}C NMR data, which were identical to the eremophilenol previously isolated from *Alpinia japonica*. However, this is the first time eremophilenol **1** has been isolated from a Lauraceae.

The double bond position in 1 and in 4 did not change the olfactory characteristics of these compounds.

The same analytical tools were applied to **5** and **6**, revealing that for the first time these aromatic compounds were isolated from Burseraceae. Their olfactory properties were evaluated by a perfumer, and the authors believe that both essential oils have olfactory potential to be included in the perfumer's pallet.

Supplementary Information

Supplementary information (^{1}H NMR and ^{13}C NMR spectra for compounds **1**, **5**, and **6**) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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