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RESEARCH NOTE

Essential Leaf Oil of *Amyris diatrypa* Sprengel from the Dominican Republic

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Abstract

The essential oil from the leaves of *Amyris diatrypa* Sprengel (Rutaceae) from the Dominican Republic was analyzed by GC/MS. The oil contains over 80 components. The oil was found to contain a large amount of pregeijerene (17-21%), with moderate amounts of 1,8-cineole (2-21%), linalool (8-10%), geijerene (3-7.7%), sabinene (1.0-9.3%), and (E)-caryophyllene (3.2-5.9%).

Key Word Index

Amyris diatrypa Sprengel, Rutaceae, essential oil composition, linalool, 1,8-cineole, sesquisabinene, pregeijerene, geijerene.

Plant Name

Amyris diatrypa, common name: guaconejo, Rutaceae.

Source

The leaves of *Amyris diatrypa*, a species which occurs in Cuba and Hispanola, were collected in the Dominican Republic and voucher specimens for samples 5795 (Zanoni and Pimentel 40347) and 7251 (R. Garcia & R. Marco 5564) are deposited in the herbarium at Jardin Botanico Nacional, Santo Domingo (JBSD).

Plant Part

The volatile leaf oils were isolated by steam distillation (200 g fresh foliage, FW) using a circulatory Clevenger apparatus (1) for 2 h. Oil yield was 0.88% (oil wt./extracted foliage, dried 48 h, 100°C). The oil samples were concentrated (diethyl ether trap evaporated) with nitrogen and stored at –20°C until analyzed.

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Table I. Variation in the composition of the volatile leaf oil of *Amyris diatrypa* from trees 5795 and 7251

	RI	Compound	5795	7251
1	931	α-thujene	0.2	-
2	939	α-pinene	0.6	t
3	976	sabinene	9.3	1.1
4	980	β-pinene	0.5	-
5	981	3-methyl pentyl acetate*	t	0.8
6	991	myrcene	1.0	0.2
7	991	dehydro-1,8-cineole	t	0.2
8	1004	2-methyl propyl 3-methyl butanoate	t	t
9	1011	δ-3-carene	0.1	t
10	1018	α-terpinene	0.3	t
11	1026	p-cymene	0.1	0.2
12	1031	limonene	0.5	0.2
13	1033	1,8-cineole	21.7	2.0
14	1040	(Z)-β-ocimene	0.3	0.1
15	1043	2-heptyl acetate	0.1	0.3
16	1050	(E)-β-ocimene	1.7	0.8
17	1062	γ-terpinene	0.6	t
18	1068	cis-sabinene hydrate	0.4	t
19	1074	cis-linalyl oxide	0.2	1.3
20	1088	terpinolene	0.2	0.2
21	1097	trans-sabinene hydrate	0.4	-
22	1098	linalool	10.0	8.3
23	1103	isopentyl isovalerate	0.1	-
24	1116	3-methyl-3-butenyl 3-methyl butanoate	0.1	-
25	1121	cis-pinene hydrate	0.1	-
26	1137	geijerene isomer	1.4	4.0
27	1144	geijerene	3.3	7.7
28	1159	p-mentha-1,5-dien-8-ol	t	-
29	1166	δ-terpineol	0.2	t
30	1177	terpinen-4-ol	1.1	0.1
31	1183	p-cymen-8-ol	t	t
32	1189	α-terpineol	6.8	3.3
33	1243	hexyl 3-methyl butanoate	t	-
34	1250	isogeijerene C	1.3	3.4
35	1256	2-phenyl ethyl acetate	t	t
36	1288	pregeijerene	17.3	21.3
37	1288	indole	t	-
38	1350	α-terpinyl acetate	0.2	t
39	1365	neryl acetate	t	-
40	1378	cis-dictamnol	0.2	0.2
41	1384	β-bourbonene	t	0.3
42	1391	β-elemene	t	0.3
43	1418	β-caryophyllene	3.2	5.9
44	1425	trans-dictamnol	1.3	1.9
45	1454	α-humulene	0.4	0.7
46	1458	sesquisabinene	0.7	3.2
47	1480	germacrene D	1.1	2.4
48	1485	β-selinene	0.3	1.6
49	1489	2-phenylethyl 3-methyl butyrate	t	-
50	1493	epi-cubebol	0.2	t
51	1494	α-selinene	t	2.3
52	1494	α-zingiberene	t	t
53	1494	bicyclogermacrene	0.5	t
54	1499	α-muurolene	t	-
55	1503	germacrene A	t	0.1
56	1509	β-bisabolene	t	0.9
57	1512	β-curcumene	t	0.4
58	1514	cubebol	t	t
59	1524	β-sesquisphellandrene	1.0	4.1
60	1531	liguloxide	t	-
61	1533	(E)-bisabolene	-	0.6
62	1534	(Z)-nerolidol	t	-
63	1545	cis-sesquisabinene hydrate	t	-
64	1549	elemol	0.8	1.5
65	1554	sesquiterpene alcohol	0.3	0.7
66	1564	(E)-nerolidol	t	0.2
67	1574	germacrene D-4-ol	0.1	-
68	1576	spathulenol	0.3	-
69	1581	caryophyllene oxide	0.8	0.4
70	1595	guaiol	t	-
71	1600	sesquiterpene alcohol	0.4	0.8
72	1611	sesquiterpene alcohol	0.4	0.9
73	1630	α-acorenol	t	t
74	1640	epi-α-cadinol (=T-cadinol)	t	t
75	1641	epi-α-muurolol (=T-muurolol)	t	-
76	1649	β-eudesmol	2.1	1.8
77	1652	α-eudesmol	0.9	0.8
78	1658	7-epi-α-eudesmol	t	t
79	1666	bulnesol	-	0.2
80	1671	β-bisabolol	0.2	0.6
81	1676	sesquiterpene alcohol	0.2	0.8
82	1786	β-eudesmol acetate	0.2	0.1
83	1877	sesquiterpene acetate	t	0.6
84	1930	dictamnine	0.1	0.5
85	1992	seselin	-	2.6
		Total %	98.7	94.6

RI = Retention Index on DB-5(=SE54) column; * = Tentatively identified based only on MS data
Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.
Quantitation based on total ion count (TIC) by ion trap MS.

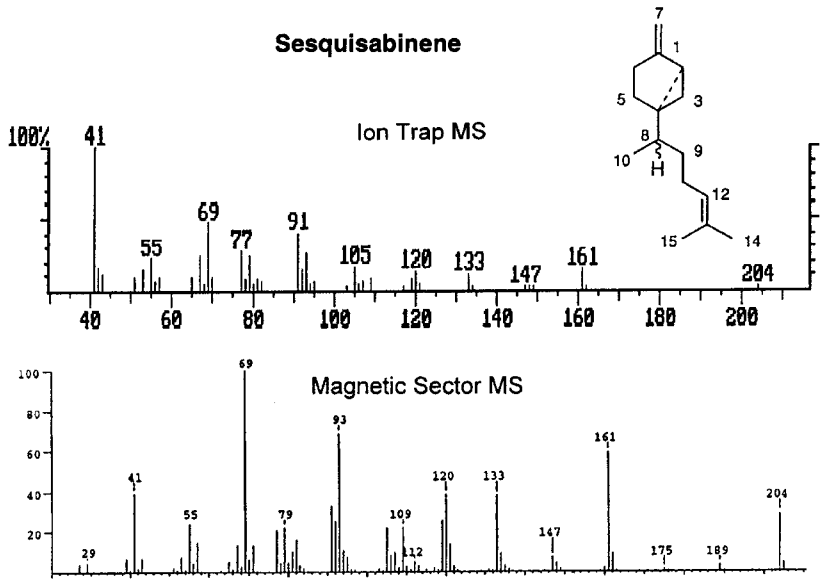

Figure 1. NMR spectra of sesquisabinene

Previous Work

There is a report on the alkaloids and coumarins from the leaves of *A. diatrypa* in Cuba (2), but there are no reports on the leaf oils.

Present Work

Mass spectra were recorded with a Finnigan Ion Trap (ITD) mass spectrometer, model 800, directly coupled to a Varian 6500 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 μm coating thickness, fused silica capillary column (see reference 3 for operating details). Identifications were made by library searches of our volatile oil library, LIBR(TP) (3) using the Finnigan library search routines based on fit and purity, coupled with retention time data of reference compounds. Mass spectra were also obtained with a Finnigan MAT 95 mass spectrometer (70 eV EI - ionization) and compared with the spectra in the Wageningen collection.

The major components in sample 5795 are 1,8-cineole (21.7%), pregeijerene (17.3%), linalool (10.0%) and sabinene (9.3%), whereas the other sample, 7251, has major amounts of pregeijerene (21.3%), linalool (8.3%) and geijerene (7.7%).

The leaf oil composition is shown in Table I for two oil sources. There appears to be a considerable amount of infraspecific variability, based on these limited samples. Characteristic for both oils is the relatively large content of the uncommon trinor-germacrene pregeijerene (Cpd. 36, Table I). Also found was a trinor-guaiane type sesquiterpene, dictamnol, which has been recently reported from the roots of *Dictamnus dasycarpus* (4,5). Dictamnol, or isomers thereof, had been reported in the oil of *Nepeta govaniana* (6.1%) but they were not identified at that time (6). Also detected in both oil samples was the rare sesquiterpene hydrocarbon, sesquisabinene. Although both the mass spectrum and the retention index for KI 1458 are nearly identical to those for (E)-β-farnesene, small but significant differences prompted us to trap this compound by repeated injections on a preparative GC. Subsequent NMR-analysis (van Beek et al. unpublished) identified this compound as sesquisabinene (Figure 1).

Isolation of Sesquisabinene: Four mL of pure *Amyris diatrypa* oil (7251) was separated by means of preparative GC. This was repeated 13 times. Conditions: stationary phase: Carbowax high polymer

9.8% on Chromosorb 80-100; GC column: 2 m x 6.35 mm; temperature: column isothermal 120°C, injector 150°C, TCD 230°C; carrier gas: hydrogen, 25 mL/min. Trapping was carried out in glass tubes (1.6 mm x 30 cm) at room temperature. During the elution of sesquisabinene (21-23 min) the tube was directly connected to the exit of the TCD. Immediately after fraction collection, the trap was kept closed with a Teflon stopper. Approximately 1.5 mg of 90% pure sesquisabinene was collected. This was directly transferred into a NMR tube with 99.96% deuterated chloroform for ^{1}H- and ^{13}C-NMR measurements.

Identification of Sesquisabinene: Sesquisabinene was identified by its mass spectrum (see Figure 1), its retention indices on methyl silicone and PEG phases (1452 and 1663, respectively) and its 400 MHz ^{1}H- and 100 MHz ^{13}C-NMR data. A comparison with NMR data of sabinene was especially helpful. Sesquisabinene is a rare sesquiterpene hydrocarbon. From a comparison of our NMR data with those of Terhune et al. who first reported its occurrence in *Piper nigrum* oil (7), it is almost certain that our compound has the same stereochemistry. The configuration at C-8 remains unclear and will be reported on later (van Beek et al., in preparation).

^{13}C-NMR Data: 154.9 (C-1), 131.6 (C-13), 125.3 (C-12), 102.0 (C-7), 38.4 (C-8), 37.2 (C-4), 35.6 (C-9), 30.1 (C-2), 29.3^{+} (C-6), 26.7+ (C-5), 26.6^{+} (C-11), 26.1 (C-15), 18.7 (C-3), 18.1* (C-10), 17.9* (C-14)., $^{+}$, * = assignments may be interchanged.

Mass spectra for the unidentified compounds are [ITMS, m/z (rel. int.)]:

KI1554, 41(100), 55(35), 67(42), 82(28), 93(33), 107(12), 119(22), 137(4), 147(2), 161(8), 207(2), 222 (ca.)[M^{+}], sesquiterpene alcohol, KI1600, 43(100), 55(23), 67(32), 79(28), 91(30), 105(20), 119(18), 131(6), 145(10), 161(8), 189(1), 207(2), 222 (ca.)[M^{+}], sesquiterpene alcohol, KI1611, 41(100), 59(52), 67(37), 79(36), 91(41), 105(52), 119(48), 133(22), 147(12), 161(43), 175(8), 189(39), 204(25), 220 (ca.)[M^{+}], sesquiterpene alcohol, KI1676, 41(100), 59(69), 67(44), 79(59), 91(63), 105(82), 119(55), 133(30), 147(27), 161(59), 175(2), 189(51), 204(23), 222 (ca.)[M^{+}], sesquiterpene alcohol, KI1877, 43(100), 55(15), 67(18), 79(28), 91(31), 105(30), 119(12), 131(17), 145(19), 159(9), 173(8), 187(37), 202(16), 262 (ca.)[M^{+}], sesquiterpene acetate.

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References

1. R. P. Adams, *Cedar Wood Oil - Analysis and Properties.* In: *Modern Methods of Plant Analysis: Oils and Waxes.* Edits. H. F. Linskens and J. F. Jackson, pp 159-173, Springer-Verlag, Berlin (1991).
2. A. Laguna, *Alkaloids and coumarins from the leaves of Amyris diatrypa.* Planta Med., **50**, 112 (1984).
3. R. P. Adams, *Identification of Essential Oils Components by Gas Chromatography/ Mass Spectroscopy.* Allured Publ., Carol Stream, Illinois (1995).
4. N. Takeuchi, T. Fujita, K. Goto, N. Morisaki, N. Osone and S. Tobinaga, *Dictamnol, a new trinor-guiane type sesquiterpene from the roots of Dictamnus dasycarpus Turcz.* Chem. Pharm. Bull., **41**, 923 - 925 (1993).
5. D. P. Piet, R. V. A. Orru, L. H. D. Jenniskens, C. van de Haar, T. A. van Beek, M. C. R. Franssen, J. B. P. A. Wijnberg and Ae. de Groot, *Synthesis of (1α, 7α, 8β)-(+/-)-8-methyl-2-methylenebicyclo [5.3.0] dec-5-en-8-ol. Structure revision of natural dictamnol.* Chem. Pharm. Bull., **44**, 1400-1402 (1996).
6. C. S. Mathela, H. Kharkwal and R. Laurent, *Investigations on Himalayan Nepeta species. V. Essential oil of Nepeta govaniana Benth.* J. Essent. Oil Res., **6**, 425-428 (1994)
7. S. J. Terhune, J. W. Hogg, A. C. Bromstein and B. M. Lawrence, *Four new sesquiterpene analogs of common monoterpenes.* Can. J. Chem., **53**, 3285-3293 (1975).