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ESSENTIAL OIL OF *AMYRIS BALSAMIFERA*

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Key Word Index—*Amyris balsamifera*; Rutaceae; essential oil; sesquiterpenes; 7-*epi*-α-eudesmol.

Abstract—The essential oil composition of *Amyris balsamifera* was investigated by GC-MS. Major constituents were separated by fractional distillation and various chromatographic techniques, and identified by mass, ^{1}H and ^{13}C NMR spectroscopy and/or chemical reactions. The oil consisted of 17.5% sesquiterpene hydrocarbons and 82.5% oxygenated sesquiterpenes. Major compounds were β-sesquiphellandrene, elemol, 10-*epi*-γ-eudesmol, γ-eudesmol, valerianol, α-eudesmol, 7-*epi*-α-eudesmol and β-eudesmol. 7-*epi*-α-Eudesmol is reported for the first time as a natural product.

INTRODUCTION

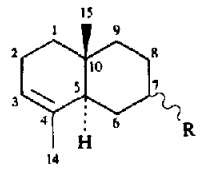
Recently we have developed a method for the preparative isolation of β-eudesmol from *Amyris* oil [unpublished results]. The β-eudesmol isolated will be used as a starting material for the synthesis of termite defence secretion compounds. In connection with this research the chemical composition of the total oil was also investigated.

Amyris oil is obtained from the wood of *Amyris balsamifera* (Rutaceae). Because this tree grows in the Caribbean area and has a sandal-wood odour it is sometimes incorrectly named 'West Indian sandalwood oil'. Of the two modern phytochemical investigations [1, 2] only the investigation by Rohmer *et al.* has yielded useful results. They reported the occurrence of α-agarofuran, 10-*epi*-γ-eudesmol, valerianol, β-eudesmol, elemol, 4-hydroxyagarofuran, an unknown sesquiterpene alcohol and less polar compounds. In the following we report on the total chemical composition of Amyris oil.

RESULTS AND DISCUSSION

The total oil was first investigated by GC on a 60 m polyethylene glycol column. Fifteen components occurring in a concentration of 1.0% or higher could be distinguished. Many were identified with GC-MS. When reference substances were available the MS identification was confirmed by means of the retention index of the compound. Major compounds that could not be identified were isolated by various forms of medium pressure LC (silica gel, Ag^+ impregnated silica gel, C18 modified silica gel with or without Ag^+ in the mobile phase) and subsequently identified by ^{1}H NMR, ^{13}C NMR, $[\alpha]_D$ and/or chemical conversion. Sometimes vacuum distilled fractions were used for chromatographic separation. The identified compounds are listed in Table 1; they were all sesquiterpenes.

All the compounds found by Rohmer *et al.* [1] were detected in the present study. Besides these, other major compounds were identified as α-zingiberene, β-sesquiphellandrene, *ar*-curcumene, selina-3,7(11)-diene, γ-eudesmol, α-eudesmol and 7-*epi*-α-eudesmol (**1**). Although



1 R= 

7-*epi*-α-eudesmol has been synthesized before [3], it has never been isolated from natural sources. Its structure was deduced from mass spectral, ^{1}H and ^{13}C NMR data. The mass and ^{1}H NMR spectra were identical to those reported by Schwartz and Willbrand for (±)-7-*epi*-α-eudesmol [3]. The ^{13}C NMR spectrum (decoupled and coupled) was in good agreement with the proposed structure; C-7 was characteristically shifted upfield. The optical rotation of **1** was positive from which it is concluded that the isolated compound is 7-*epi*-α-eudesmol and not 5-*epi*-10-*epi*-α-eudesmol which has identical mass and NMR data. When **1** was refluxed in methanolic hydrochloric acid it was converted mainly to selina-3,7(11)-diene and 7-*epi*-α-selinene. The 7-*epi* stereochemistry was deduced from the chemical shift of the two olefinic H-12 protons, respectively δ 4.85 and 4.90 (α-selinene: δ 4.69 and 4.69). In β-dictyopterol (δ 4.76 and 4.76) and 7-*epi*-β-dictyopterol (δ 4.82 and 4.91) a comparable situation exists [4]. Compounds with identical retention times and mass spectra as the two reaction products occurred in *Amyris* oil but were not isolated. Thus, no optical rotation was recorded and the absolute stereochemistry could not be determined. From a biogenetic point of view the same absolute stereochemistry at C-5 and C-10 as 7-*epi*-α-eudesmol seems most likely. (+)-7-*epi*-α-Selinene has been isolated by Klein *et al.* [5]. The enantiomer of this compound has to our knowledge never been described. The oil is an excellent source for the

Table 1. Composition of the essential oil from *A. balsamifera*

Peak no.	Component	Kovats index	% in oil	Methods of identification
1	unknown $C_{15}H_{24}$	1669	1.4	MS
2	α-Zingiberene	1728	2.4	GC, MS
3	β-Bisabolene	1734	0.8	GC, MS
4	β-Dihydro-agarofuran	1737	1.0	MS
5	7-*epi*-α-Selinene	1775	0.5	GC, MS
6	β-Sesquiphellandrene	1778	4.7	GC, MS
7	*ar*-Curcumene	1781	1.5	GC, MS
8	Selina-3,7(11)-diene	1791	2.5	GC, MS
9	α-Agarofuran	1907	0.5	MS
10	*E*-Nerolidol	2044	0.6	GC, MS
11	Elemol	2089	9.1	MS, ^{1}H NMR, ^{13}C NMR
12	10-*epi*-γ-Eudesmol	2121	9.7	MS, ^{1}H NMR, ^{13}C NMR, $[\alpha]_D$
13	Guai-9-en-11-ol isomer (tent.)	2147	1.0	MS, ^{1}H NMR
14	Guai-4(14)-en-11-ol isomer (tent.)	2161	0.9	MS, ^{1}H NMR
15	γ-Eudesmol	2182	6.6	MS, ^{1}H NMR, ^{13}C NMR
16	Jinkoh-eremol (tent.)	2215	0.7	MS
17	Valerianol	2231	21.5	MS, ^{1}H NMR, ^{13}C NMR
18	α-Eudesmol	2237	4.8	MS
19	7-*epi*-α-Eudesmol	2244	10.7	MS, ^{1}H NMR, ^{13}C NMR $[\alpha]_D$, reactions.
20	β-Eudesmol	2248	7.9	GC, MS, ^{1}H NMR, ^{13}C NMR, $[\alpha]_D$
21	4α-Hydroxydihydroagarofuran	2251	0.4	MS, ^{1}H NMR
22	6R,7R-Bisabolone	2306	0.3	MS, ^{1}H NMR
23	6S,7R-Bisabolone	2320	0.6	MS, ^{1}H NMR
24	Drimenol	2525	1.1	MS
			91.2	

Tent. = tentative identification.

preparative scale isolation of various sesquiterpene alcohols of the eudesmane type.

EXPERIMENTAL

The *Amyris* oil used in this investigation was obtained from Naarden Chemie International (now Quest) in 1986.

GC. A chromatograph equipped with a 60 m chemically bonded polyethylene glycol column (J&W DB-Wax), i.d. 0.25 mm with a film thickness of 0.25 μm was used; split ratio 1:100; carrier gas H_2, inlet pressure 20 psi, linear velocity 35 cm/sec; temp. prog. 50° to 238° (13 min hold) at 4°/min; inj. temp. 220°; det. temp. 260°; FID detection.

GC-MS. The same column as used for the GC analysis was employed; carrier gas He. Spectra were obtained at 70 eV.

NMR. 300 MHz ^{1}H NMR spectra and 75 MHz ^{13}C NMR spectra (both decoupled and coupled) were recorded in $CDCl_3$. No TMS was added, for ^{1}H NMR $CHCl_3$ = 7.26 ppm, for ^{13}C NMR $CDCl_3$ = 77.0 ppm.

Optical rotations were recorded in $CHCl_3$.

Fractional distillation was carried out at a pressure of 0.35 to 0.20 mm Hg and a temp. between 60 and 95°.

Chromatography. Medium pressure LC was carried out on (i) Jobin Yvon 50 × 2 cm or 50 × 4 cm Modulprep columns which were filled with 50 or 250 g of stationary phase, respectively. Stationary phase: Merck kieselgel 60 for prep. TLC with or without 10% $AgNO_3$ coating. Mobile phase: petrol (40–60°) with 5–10% MeOAc. Detection: on-line RI detection and/or off-line GC analysis of individual fractions. (ii) A Merck Lobar B column 31 × 2.5 cm. Stationary phase: LiChroprep RP-8 (40–63 μm). Mobile phase: MeOH with 10–35% H_2O with or without 0.01–0.03 M $AgClO_4$. Detection as in (i) above.

10-epi-γ-Eudesmol. MS *m/z* (rel. int.): 222 ($[M]^+$, 0), 204 (66), 189 (100), 163 (4), 162 (11), 161 (64), 149 (24), 147 (21), 133 (50), 107 (27), 105 (32), 95 (23), 93 (25), 91 (32), 81 (30), 59 (49), 55 (27), 41 (32). $[\alpha]_D$: negative. ^{1}H NMR δ: 2.71 (*dd*, *J* = 14.8 and 2.8 Hz, 1H), 1.68 (*br s*, 3H), 1.25 (*s*, 3H), 1.19 (*s*, 3H), 1.09 (*s*, 3H). ^{13}C NMR: see ref. [6].

γ-Eudesmol. MS *m/z* (rel. int.): 222 ($[M]^+$, 0), 204 (79), 189 (100), 163 (12), 162 (11), 161 (69), 147 (19), 133 (39), 107 (25), 105 (29), 95 (21), 93 (23), 91 (26), 81 (27), 79 (16), 59 (38), 55 (22), 41 (21). ^{1}H NMR δ: 2.62 (*ddd*, *J* = 13.8, 3.6 and 2.0 Hz, 1H), 1.60 (*br s*, 3H), 1.20 (*s*, 6H), 1.01 (*s*, 3H). ^{13}C NMR δ: 134.9 (C-5), 124.5 (C-4), 72.8 (C-11), 50.6 (C-7), 42.3 (C-9), 40.3 (C-1), 34.5 (C-10), 33.2 (C-3), 27.2 (C-12), 26.9 (C-13), 26.4 (C-6), 24.7 (C-14), 23.3 (C-8), 19.2 (C-2), 19.2 (C-15).

α-Eudesmol. MS *m/z* (rel. int.): 222 ($[M]^+$, 3), 204 (40), 189 (46), 161 (40), 149 (47), 122 (21), 109 (24), 107 (27), 105 (24), 95 (19), 93 (31), 91 (18), 81 (24), 59 (100), 41 (20).

7-epi-α-Eudesmol (**1**). MS *m/z* (rel. int.): 222 ($[M]^+$, 0), 204 (18), 189 (14), 162 (12), 161 (100), 122 (64), 107 (30), 105 (17), 95 (13), 93 (17), 91 (14), 81 (22), 67 (13), 59 (33), 55 (15), 41 (20). $[\alpha]_D$ + 10° (*c* 1.8). ^{1}H NMR δ: 5.29 *br s*, 1H), 2.18–2.12 *br d*, 2H), 1.62 (*br s*, 3H), 1.22 (*s*, 6H), 0.85 (*s*, 3H). ^{13}C NMR δ: 135.4 (C-4), 121.0 (C-3), 74.2 (C-11), 42.6 (C-5), 41.0 (C-7), 38.7 (C-1), 37.6 (C-9), 31.3 (C-10), 28.8 (C-12), 28.1 (C-13), 24.0 (C-6), 23.1 (C-8), 20.9 (C-14), 20.8 (C-2), 18.4 (C-15).

Conversion of **1** *into selina-3,7(11)-diene* (**2**) *and 7-epi-α-selinene* (**3**). Compound **1** (90 mg) was dissolved in 20 ml MeOH containing 1% 32% HCl soln and refluxed for 20 min. The volatile

extract obtained after pentane extraction and purification by silica gel CC with pentane as eluent consisted of 76.5% **2** and 13% **3**. Other compounds occurred in concentrations of 4% or lower and were not further identified. This fraction was analysed by GC-MS, ^{1}H and ^{13}C NMR. Minor component (*7-epi-α*-selinene, **3**). MS *m/z* (rel. int.): 204 ($[M]^+$, 16), 189 (10), 162 (11), 161 (100), 133 (10), 122 (86), 121 (14), 119 (12), 107 (45), 105 (24), 95 (13), 93 (22), 91 (18), 81 (27), 79 (14), 55 (16), 41 (27). ^{1}H NMR δ: 4.90 (*br s*, 1H), 4.85 (*br s*, 1H), 1.75 (*br s*, 3H), 0.84 (s, 3H). ^{13}C NMR: 110.8, 41.3, 38.3, 36.2, 32.9, 23.5, 22.9, 19.1, 15.5 (most signals coincide with those of the major component or are too small).

Major component (*selina*-3,7(11)-*diene*, **2**). MS *m/z* (rel. int.): 204 ($[M]^+$, 42), 189 (22), 162 (14), 161 (100), 149 (12), 135 (16), 133 (16), 122 (65), 121 (26), 119 (17), 107 (43), 105 (35), 95 (14), 93 (28), 91 (23), 81 (28), 79 (16), 67 (18), 55 (20), 41 (26). ^{1}H NMR δ: 5.33 (*br s*, 1H), 2.72 (*ddd*, J = 14, 3.5 and 2 Hz, 1H), 2.52 (*br d*, J = 15, 1H), 1.68 (*br s*, 6H), 1.65 (*br s*, 3H), 0.86 (*s*, 3H). ^{13}C NMR δ: 135.2 (C-3), 131.6 (C-7), 121.4 (C-11), 121.3 (C-3), 47.1 (C-4), 40.8 (C-1^a), 37.9 (C-9^a), 32.4 (C-10), 27.6 (C-8^b), 25.3 (C-6^b), 23.1 (C-2^b), 21.0 (C-14), 20.2 (C-12), 20.1 (C-13), 15.2 (C-15);^{a,b} may be interchanged.

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