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Analytica Chimica Acta 447 (2001) 113–123

ANALYTICA CHIMICA ACTA

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Spectrometric identifications of sesquiterpene alcohols from niaouli (*Melaleuca quinquenervia*) essential oil

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Received 20 March 2001; accepted 25 July 2001

Abstract

Oxidation and reduction reactions on alloaromadendrene and aromadendrene sesquiterpene hydrocarbons have been investigated in order to produce alcohols with an aromadendrene skeleton for checking the chemical structure and therefore the identity of one main alcohol present in niaouli (*Melaleuca quinquenervia*) essential oil. Oxidation using *m*-chloroperbenzoic acid was carried out to produce two diastereoisomer epoxides and two corresponding aldehyde isomers for each sesquiterpene. Epoxide reductions yielded two alcohols ledol and viridiflorol, from alloaromadendrene and globulol and epiglobulol from aromadendrene. The structure determination of all compounds, i.e. epoxides, aldehydes, and alcohols, was achieved using spectrometric methods: 2D-NMR and mass spectroscopy. The stereochemistry of known sesquiterpenic alcohols, viridiflorol and ledol, has been unambiguously established.

Keywords: Alloaromadendrene; Sesquiterpenol; Ledol; Viridiflorol; Sesquiterpene aldehydes; Sesquiterpene epoxides; Oxidation; Reduction; 2D-NMR; MS

1. Introduction

Niaouli essential oil contains a high level of monoterpenes and sesquiterpenes [1,2]. Among the various niaouli essential oils described, investigations for compound contents reveal the occurrence of several chemotypes, including a chemotype from Madagascar rich in viridiflorol (48%) [1,2]. Viridiflorol was tentatively identified on the basis of its retention indices and mass spectral data and its structure given using ^{1}H and ^{13}C NMR spectroscopy [3].

Viridiflorol was reported to be the major sesquiterpenol in niaouli essential oils [4,5], result confirmed by Guenther [6]. According to Ekundayo et al. [7], the major sesquiterpenol would be globulol, and guaiol for Motl et al. [8]. Following the literature data, the identification of the two isomers viridiflorol **6** and ledol **7** (Scheme 1) is difficult because their spectral data are very similar. Taking into account the NMR studies on ^{1}H and ^{13}C of ledol and viridiflorol [9–17], and more recently a paper of Wu et al. [18] the NMR assignments for ledol and viridiflorol

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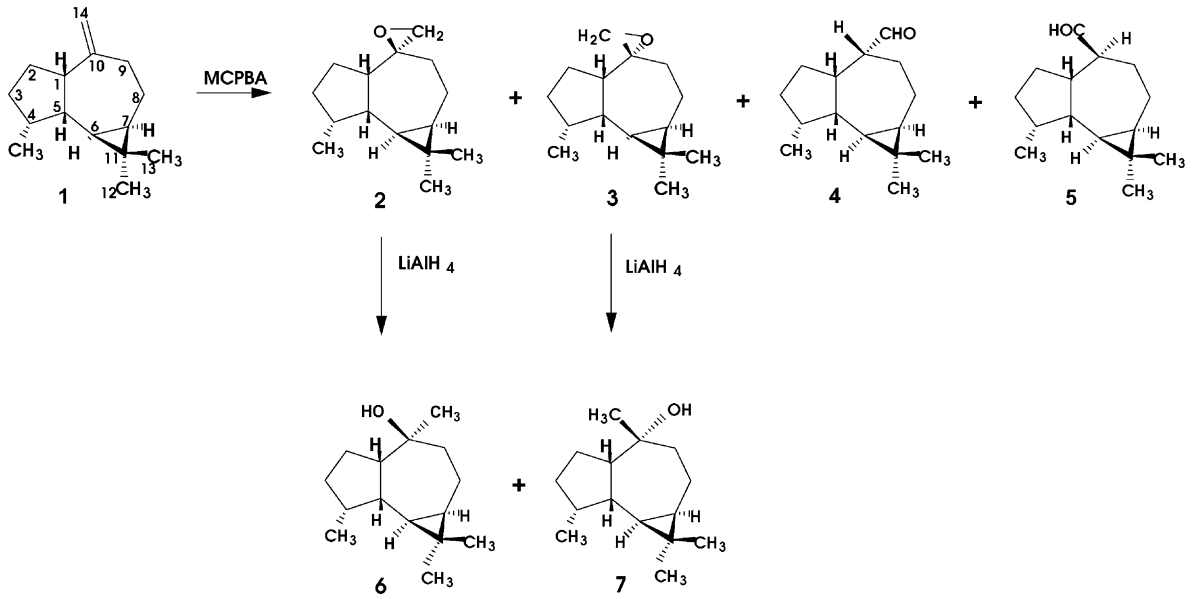
0003-2670/01/$ – see front matter
PII: S0003-2670(01)01307-1



Scheme 1. Alloaromadendrene **1**, oxidation and reduction products. Carbon numbering used for NMR assignments.

are quite confusing. Since contradictory results on the ledol and viridiflorol appeared, we decided to reinvestigate the structure determination of the main sesquiterpenic alcohol contained in niaouli essential oil. In this way, we have synthesized sesquiterpenols with an aromadendrene skeleton and have characterized aldehydic and epoxydic intermediates. Although some of these compounds are already known, the complete ^{1}H and ^{13}C NMR chemical shifts assignments have not yet been reported. From these results, we should be able to establish unambiguously the chemical composition of *Melaleuca quinquenervia* niaouli essential oil "rich" in viridiflorol.

2. Materials and methods

2.1. Gas chromatography (GC)

A Delsi 300 gas chromatograph equipped with a flame ionization detector (FID) was used for compound separations with a fused silica capillary column (25 m × 0.32 mm i.d.) coated with Carbowax 20 M (phase thickness = 0.20 μm; column temperature = 160 or 70–220°C, 3°C min^{-1}). Detector and inlet temperatures were 250°C. Helium was used as carrier gas at an inner pressure of 0.6 bar. The injections averaged 1 μl of a 2% solution of crude mixtures in pentane. The retention indices were calculated according to NFT 75-401 norms [19].

2.2. Gas chromatography–mass spectrometry (GC–MS)

Combined GC–MS was performed on a 5890 Hewlett-Packard (HP) series II chromatograph linked to a 5970 HP mass spectrometer coupled with a Vectra HP computer using HPChem software. The GC column was a HP-5 (5% methyl phenyl silicone) fused silica capillary column (25 m × 0.25 mm, 0.25 μm phase thickness). The column temperature was 60–200°C, 4°C min^{-1}; carrier gas helium (0.2 bar); ion source, 250°C; ionizing voltage, 70 eV.

2.3. Thin layer chromatography (TLC)

Analytical TLC was performed on precoated plates (5 cm × 10 cm, silica gel 60 F_{254}, 0.25 mm, Merck). Spots were visualized by examination under ultraviolet radiation (254 and 366 nm) and/or sulfuric acid spray reagent (solution of 5% sulfuric acid in diethyl ether) followed by brief heating at 100°C.

2.4. Liquid chromatography (LC)

For semi-preparative LC, a 1100 HP pump, a 1047 A HP refractive index detector, a 3396 A HP integrator, and a column packed with Nucleosil 100 Angstrom (250 mm × 10.5 mm i.d., 5 μm) were used. Isocratic conditions using a mixture of isooctane/ethyl acetate (90:10, v/v) were performed for elutions.

2.5. Nuclear magnetic resonance (NMR) spectroscopy

All spectra were recorded on Bruker AMX-400 and Bruker Advance 600 MHz spectrometers. The NMR spectra were measured as solutions in chloroform-*d* in 5 mm o.d. tubes for ^{13}C and ^{1}H. Tetramethylsilane was used as internal standard in both measurements. Proton–proton coupling constants were extracted from high-field resolution-enhanced ^{1}H spectra using the Gaussian multiplication technique [20]. Standard Bruker pulse sequences were used for homonuclear and heteronuclear correlation experiments. For other experimental details see Hanoun et al. [21].

2.6. General oxidation procedure [22]

m-Chloroperbenzoic acid (MCPBA; Fluka, 55% purity) was used as the oxidant. In the different experiments a solution of sesquiterpene (Fluka) in methylene chloride was stirred at ambient temperature during the addition of small portions of MCPBA (1:1 with regard to MCPBA purity) in methylene chloride. The reaction mixture was allowed to stand for 1 h. Unreacted MCPBA and by-product *m*-chlorobenzoic acid were removed using first an aqueous solution containing 10% sodium sulfite and then 10% sodium hydrogen carbonate. The organic layer was dried over magnesium sulfate and concentrated under vacuum on a Rotavapor.

2.7. General reduction procedure

Sesquiterpene epoxides (220 mg, 1 mmol) were reduced with lithium aluminum hydride (Fluka), (76 mg, 2 eq.), in THF (10 ml) at reflux during a 12 h period. The complex LiAl alcoholate was hydrolyzed with 10% sulfuric acid and reduction products were isolated by diethyl ether extraction.

2.8. Oxidation reaction of alloaromadendrene **1**

The reaction was realized with 204 mg (1 mmol) of alloaromadendrene and 345 mg (1 mmol) of MCPBA. The crude mixture (210 mg) contained 68% of epoxide **2** {(1*S*,4*R*,5*S*,6*R*,7*R*)-10β,14-epoxy-4,11,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane}, 22% of epoxide **3** {(1*S*,4*R*,5*S*,6*R*,7*R*)-10α,14-epoxy-4,11,11 - trimethyltricyclo[6.3.0.0^{6,7}]undecane}, and 4.8% of a mixture of aldehydes **4** {(1*S*,4*R*,5*S*,6*R*,7*R*)-10α-carbaldehyde - 4,11,11 - trimethyltricyclo[6.3.0.0^{6,7}]undecane} and **5** {(1*S*,4*R*,5*S*,6*R*,7*R*)-10β-carbaldehyde-4,11,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane}. After 15 days at ambient temperature, we note the evolution of a mixture of composition 63% of **2**, 14% of **3** and 14% of a mixture of aldehydes **4** and **5**.

Purification using column chromatography (CC) over SiO_2, eluent pentane/diethyl ether (85:15, v/v, 300 ml) allowed one to isolate in tubes 22–24, 47 mg (27 mass% of the crude mixture) of a mixture of aldehydes **4** and **5** in a ratio 30:70 (from NMR) ($R_f = 0.52$, $I_R = 2117$, GC purity = 95%), and in tubes 28–34, 48 mg of **2** ($R_f = 0.47$, $I_R = 2004$, GC purity = 91%). Epoxide **3** cannot be isolated as it isomerized completely under CC ($I_R = 1967$), but it was isolated by preparative LC. A mixture of epoxides **2** and **3** (20 mg, 64 and 31%, respectively) was submitted to preparative LC analysis (see above). Epoxides **2** and **3** were obtained with 97 and 94% GC purity, respectively.

2.9. Reduction of epoxides **2** and **3**

Reduction of epoxides **2** (17 mg) and **3** (9 mg) gave ledol **6** (14 mg, 96% purity CG, $I_R = 2071$) {(1*S*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricyclo[6.3.0.0^{6,7}]undecan-10β-ol} and viridiflorol **7** (6 mg, 95% purity CG, $I_R = 2009$) {(1*S*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricyclo[6.3.0.0^{6,7}]undecan-10α-ol}, respectively.

2.10. Oxidation reaction of aromadendrene **8**

Reaction was realized with 204 mg (1 mmol) of aromadendrene **8** {(1*R*,4*R*,5*S*,6*R*,7*R*)-10-methylene-4,11,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane} and 345 mg (1 mmol) of MCPBA. The crude mixture contained 49.6% of epoxide **9** {(1*R*,4*R*,5*S*,6*R*,7*R*)-10β,14-epoxy-

4,11,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane}, 44.1% of epoxide **10** {(1*R*,4*R*,5*S*,6*R*,7*R*)-10α,14-epoxy-3,3,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane}, 3.6% of aldehyde **12** {(1*R*,4*R*,5*S*,6*R*,7*R*)-10β-carbaldehyde-4,11,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane} and 1.1% of aldehyde **11** {(1*R*,4*R*,5*S*,6*R*,7*R*)-10α-carbaldehyde-4,11,11-trimethyltricyclo[6.3.0.0^{6,7}]undecane} (the percentages given are a mean of three experiments).

Epoxides **9** and **10** were purified from 417 mg of a crude mixture containing 48.9% of **9** and 43.9% of **10** by CC over silica gel 60 (F_{254}, 230–400 mesh, Merck), eluent pentane-diethyl ether (85:15, v/v, 300 ml). Tubes 28–30, contained 121.7 mg of epoxide **9** ($R_f = 0.67$, $I_R = 1906$, GC purity = 94%) and tubes 31–34, contained 85.3 mg of **10** ($R_f = 0.56$, $I_R = 1965$, GC purity = 92%).

Purification of aldehydes **11** and **12**. A crude epoxidation of aromadendrene mixture (407 mg) containing 48.5% of epoxide **9**, 44.1% of epoxide **10**, 2.8% of aldehyde **12** and 1.2% of **11** was submitted to CC, eluent pentane-diethyl ether (85:15, v/v, 500 ml). Tubes 21–23, contained 65 mg of **12** ($R_f = 0.75$, $I_R = 1921$, GC purity = 95%) which represented 16 mass% of the crude mixture. Tubes 24–26, containing 91.2 mg of a mixture of epoxide **9** (54%), aldehyde **12** (13.4%) and aldehyde **11** (26.8%) was also submitted to CC, eluent pentane-diethyl ether (95:5, v/v, 600 ml). Tubes 49–51 contained 16 mg of **11** ($R_f = 0.55$, $I_R = 1999$, GC purity = 82%) which represented 4% of the starting crude mixture.

2.11. Reduction of epoxides *9* and *10*

Reduction of epoxides **9** and **10** give epiglobulol **13** {(1*R*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricyclo[6.3.0.0^{6,7}]undecan-10β-ol}, with 82% yield (I_R = 1995) and globulol **14** {(1*R*,4*R*,5*S*,6*R*,7*R*)-4,10,11,11-tetramethyltricyclo[6.3.0.0^{6,7}]undecan-10α-ol} with 90% yield ($I_R = 2063$), respectively.

3. Results and discussion

Epoxidation reaction of alloaromadendrene **1** afforded a mixture of epoxides **2** and **3** (68 and 22%, respectively) and aldehydes **4** and **5** which represented 4.8% of the crude mixture (Scheme 1). Alloaromaden-

Table 1
^{1}H and ^{13}C NMR chemical shifts of alloaromadendrene epoxides **2** and **3**

Assignment[a]	Epoxide **2**			Epoxide **3**		
	$\delta^{13}C$[b]	Multiplicity[c]	δ^1H[b]	$\delta^{13}C$[b]	Multiplicity[c]	δ^1H[b]
C-1	48.19	CH	2.16, q, $J = 7.6$ Hz	48.02	CH	2.33, dt, $J = 9.3$, 6.8 Hz
C-2	25.59	CH_2	1.45	23.59	CH_2	1.61; 1.48
C-3	31.44	CH_2	1.59; 1.16	30.78	CH_2	1.51; 1.25
C-4	38.67	CH	1.94	38.32	CH	1.98
C-5	40.54	CH	1.94	41.50	CH	1.88, dt, $J = 10.9$, 6.5 Hz
C-6	23.97	CH	0.21, t, $J = 9.6$ Hz	23.50	CH	0.40, dd, $J = 10.9$, 9.4 Hz
C-7	26.46	CH	0.64, ddd, $J = 12.0$, 9.3, 4.9 Hz	24.98	CH	0.78, ddd, $J = 11.9$, 9.4, 5.6 Hz
C-8	20.26	CH_2	1.78; 1.26	20.45	CH_2	1.80, ddd, $J = 14.5$, 6.8, 5.6, 2.8 Hz; 1.28
C-9	35.79	CH_2	1.80; 1.59	35.06	CH_2	1.95; 1.56
C-10	59.66	C	–	58.75	C	–
C-11	18.37	C	–	18.16	C	–
C-12	28.70	CH_3	0.99, s	28.64	CH_3	1.02, s
C-13	15.50	CH_3	0.97, s	15.68	CH_3	0.97, s
C-14	53.73	CH_2	2.62, d, $J = 4.5$ Hz; 2.54, d, $J = 4.6$ Hz	50.46	CH_2	2.43; 2.31
C-15	15.56	CH_3	0.90, d, $J = 6.3$ Hz	16.10	CH_3	0.92, d, $J = 6.7$ Hz

[a] Determined from 2D-NMR experiments. For carbon numbering, see Scheme 1.
[b] In ppm with regard to TMS.
[c] Determined from DEPT experiments.

Table 2
^{1}H and ^{13}C NMR chemical shifts of alloaromadendrene aldehyde **5**

Assignment[a]	Aldehyde **5**		
	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]
C-1	42.76	CH	2.22
C-2	30.41	CH_2	1.90; 1.38
C-3	31.42	CH_2	1.68; 1.33
C-4	37.93	CH	1.97
C-5	41.77	CH	1.82
C-6	22.89	CH	0.27, dd, $J = 11.4$, 9.1 Hz
C-7	22.89	CH	0.56, ddd, $J = 12.1$, 9.07, 5.0 Hz
C-8	19.92	CH_2	1.80; 1.25
C-9	25.04	CH_2	1.88; 1.51
C-10	54.58	CH	2.69, tt, $J = 11.4$, 3.0 Hz
C-11	17.73	C	–
C-12	28.80	CH_3	1.00, s
C-13	15.38	CH_3	0.95, s
C-14	205.91	CH	9.58, d, $J = 2.8$ Hz
C-15	15.83	CH_3	0.92, d, $J = 6.8$ Hz

[a] Determined from 2D-NMR experiments. For carbon numbering, see Scheme 1.
[b] In ppm with regard to TMS.
[c] Determined from DEPT experiments.

drene epoxides are labile and isomerized into aldehyde compounds. Isomerization was observed at ambient temperature and the yield of aldehydes increased from 4 to 14% after 15 days and from 4 to 27% after column chromatography over SiO_2. Isomerization of epoxides into carbonyl compounds has been observed previously [23,24] and the mechanism is well known [25]. Although epoxides **2** and **3** had already been obtained [22] by synthesis as intermediates in ledol and viridiflorol synthesis, they had not been isolated in such experiments [11,13,17]. Aldehydes were not separated by GC and were isolated as a mixture in a ratio 30:70 (^{1}H NMR determination). The minor epoxide **3** could not be isolated by CC since it isomerizes completely into aldehydes during the attempt of purification. Therefore, epoxides **2** and **3** can be obtained with 97 and 94% GC purity, respectively, by semi-preparative LC. The ^{1}H and ^{13}C chemical shifts of epoxides **2** and **3**, and aldehyde **5** are given in Tables 1 and 2, respectively, and the mass spectral data of **2** and **3** in Table 3.

Lithium aluminum hydride is commonly used for reduction of sesquiterpene epoxides [22–24,26]. Reduction of alloaromadendrene epoxides **2** and **3** with

Table 3
Mass spectra of oxidation–reduction products of alloaromadendrene

Compound[a]	Mass spectral data[b]
2	M^+ 220(7), 41(100), 91(66), 55(52), 81(52), 67(49), 105(47), 107(35), 159(26), 133(25), 147(24), 119(21), 177(15), 189(14), 202(12)
3	M^+ 220(7), 41(100), 91(64), 81(61), 105(59), 55(56), 67(54), 121(28), 133(26), 177(23), 159(22), 149(21), 202(19), 189(16)
6	M^+ 222(4), 43(100), 43(100), 41(78), 161(53), 69(45), 109(44), 93(43), 105(43), 67(38), 55(38), 81(38), 91(37), 119(29), 189(28), 95(27), 204(26)
7	M^+ 222(5), 43(100), 41(75), 69(48), 109(45), 81(44), 55(41), 122(40), 107(40), 167(40), 161(37), 93(36), 105(33), 79(33), 95(32), 204(19)

[a] See Scheme 1 for structural formula.
[b] m/z (relative intensity).

Table 4
^{1}H and ^{13}C NMR chemical shifts of viridiflorol **6** and ledol **7**

Assignment[a]	Viridiflorol **6**			Ledol **7**		
	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]
C-1	58.36	CH	1.80	53.89	CH	2.06
C-2	25.85	CH_2	1.63; 1.57	24.67	CH_2	1.88; 1.67
C-3	29.26	CH_2	1.78; 1.27	30.88	CH_2	1.68; 1.27
C-4	38.53	CH	1.98	38.48	CH	1.96
C-5	39.85	CH	1.84	40.86	CH	1.78
C-6	22.52	CH	0.11	23.51	CH	0.31
C-7	28.71	CH	0.61	25.10	CH	0.69
C-8	18.90	CH_2	1.60; 1.45	20.35	CH_2	1.80; 1.20
C-9	37.91	CH_2	1.66; 1.57	39.28	CH_2	1.81; 1.67
C-10	74.48	C	–	74.64	C	–
C-11	18.43	C	–	19.23	C	–
C-12	28.71	CH_3	1.03	28.70	CH_3	1.01
C-13	16.12	CH_3	1.00	15.43	CH_3	0.96
C-14	32.14	CH_3	1.14	30.57	CH_3	1.12
C-15	16.33	CH_3	0.93	16.02	CH_3	0.91

[a] Determined from 2D-NMR experiments. For carbon numbering, see Scheme 1.
[b] In ppm with regard to TMS.
[c] Determined from DEPT experiments.

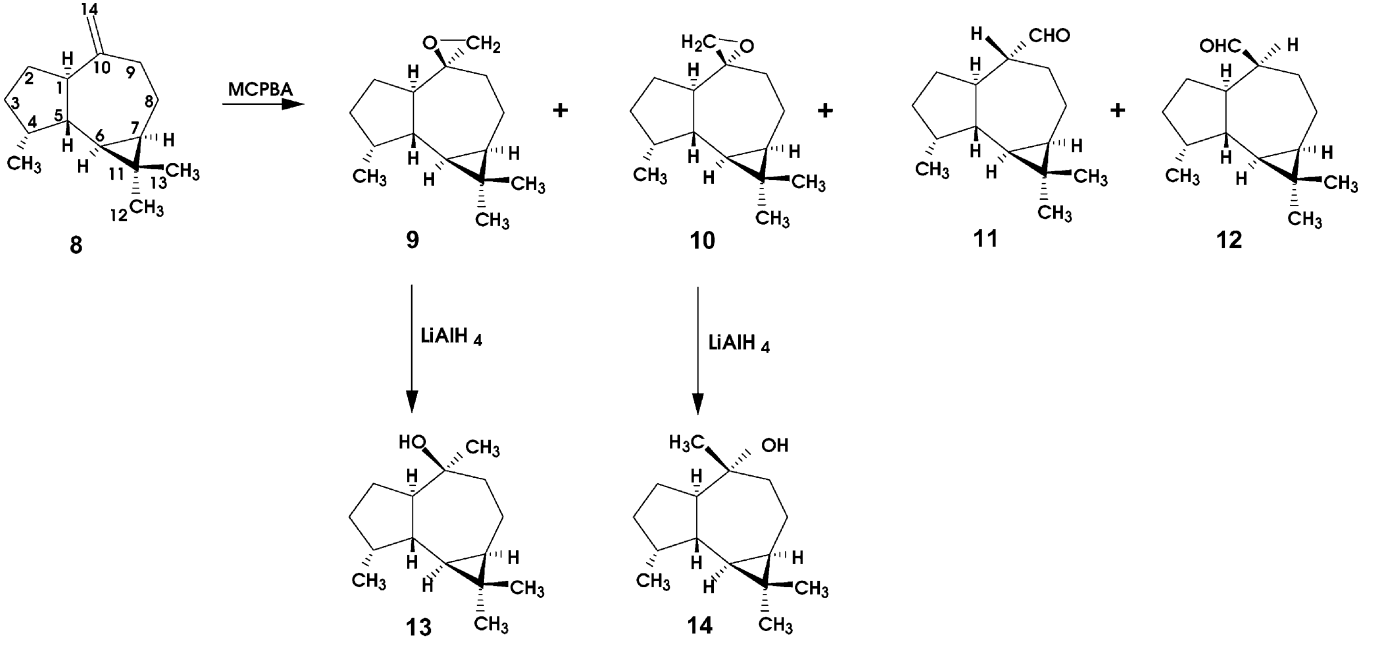
$LiAlH_4$ afforded viridiflorol **6** and ledol **7**, respectively (Scheme 1). The corresponding ^{1}H and ^{13}C chemical shifts are given in Table 4. Ledol and viridiflorol were already obtained in a ratio 1:4 following the same procedure (treatment of alloaromadendrene with MCPBA followed by $LiAlH_4$ reduction), and the structures were established by Gijsen et al. [13] by comparison of literature spectral data [10,11] and from nuclear overhouser effect (NOE) experiments [17]. These results are in agreement with our results.

Epoxidation of aromadendrene **8** (Scheme 2) led to four compounds, including two epoxides **9** and **10** and two epimer aldehydes **11** and **12**. Epoxides **9** and **10** were obtained in 49.6 and 44.1% yield, respectively. Aldehydes **11** and **12** were obtained in lower amounts (1.1 and 3.6% yields, respectively). Increasing amounts of aldehydes **11** and **12** after CC fractionation (4 and 16 mass% yield from the crude mixture, respectively) is explained by isomerization of the corresponding epoxides and therefore their contents decreased by the same amount. Although epoxides **9** and **10** had already been obtained [22] by synthesis as intermediates in globulol and epiglobulol synthesis, they had not been isolated in such experiments [13]. Epoxide **9** is a commercial product (Fluka) and epoxide **10** has been obtained as a by-product [27] but ^{13}C NMR data were not reported and ^{1}H NMR chemical assignments were not complete. The ^{1}H and ^{13}C chemical shifts are given in Tables 5 and 6.

Reduction of the β- and α-epoxides of aromadendrene **9** and **10** with $LiAlH_4$ afforded epiglobulol **13** and globulol **14**, in 82 and 90% yields, respectively (Scheme 2). Although these alcohols had already been obtained by following the same procedure [13,28], the ^{1}H and ^{13}C data were incomplete. The corresponding ^{1}H and ^{13}C chemical shifts are given in Table 7.

3.1. Structures, configurations and conformations

The structures of compounds **2**–**14** and their ^{1}H and ^{13}C NMR spectral parameters were deduced from concerted application of homonuclear and both direct and long-range heteronuclear chemical shift correlation experiments. Firstly, the establishment of proton connectivities was deduced from the gs-COSY spectrum [29]. Then, one-bond proton–carbon chemical shift correlations were achieved by using proton-detected C, H-correlation experiments (gs-HMQC sequence) [30], while assignments of the CH_n groups were obtained from analysis of the long-range correlation responses over two or three bonds (^{2}J or ^{3}J couplings) by using the gs-HMBC techniques [31]. To illustrate



Scheme 2. Aromadendrene **8**, oxidation and reduction products. Carbon numbering used for NMR assignments.

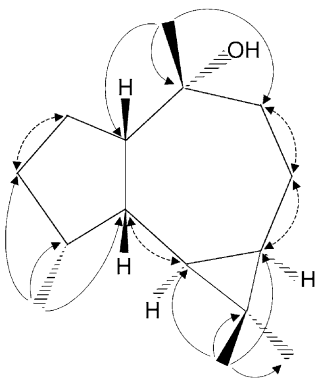
Table 5
^{1}H and ^{13}C NMR chemical shifts of aromadendrene epoxides **9** and **10**

Assignment[a]	Epoxide **9**			Epoxide **10**		
	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]
C-1	50.14	CH	2.11, dt, $J = 7.0$, 10.3 Hz	49.42	CH	2.22, q, $J = 8.5$ Hz
C-2	25.68	CH_2	1.35; 1.03	24.39	CH_2	1.60; 0.98
C-3	34.41	CH_2	1.72; 1.07	34.50	CH_2	1.57; 1.55
C-4	34.55	CH	2.01	36.13	CH	2.00
C-5	40.23	CH	1.78	42.57	CH	1.43, ddd, $J = 10.7$, 9.9, 8.3 Hz
C-6	28.85	CH	0.54, dd, $J = 11.0$, 9.5 Hz	27.61	CH	0.57, dd, $J = 10.8$, 9.4 Hz
C-7	26.89	CH	0.60, ddd, $J = 10.8$, 9.4, 5.6 Hz	26.32	CH	0.64, ddd, $J = 10.8$, 9.4, 6.2 Hz
C-8	20.99	CH_2	1.78	21.20	CH_2	1.89, dddd, $J = 14.5$, 12.8, 6.4, 1.6 Hz
			1.39, dddd, $J = 14.3$, 12.5, 11.1, 1.3 Hz			1.08, dddd, $J = 14.3$, 12.8, 11.1, 1.5 Hz
C-9	37.31	CH_2	1.91, ddd, $J = 14.2$, 12.7, 1.7 Hz	38.13	CH_2	1.95, ddd, $J = 13.1$, 11.1, 2.0 Hz
			1.25, ddd, $J = 14.0$, 6.0, 1.1 Hz			1.25, dddt, $J = 13.1$, 8.0, 2.0, 1.1 Hz
C-10	60.77	C	–	62.83	C	–
C-11	20.57	C	–	19.20	C	–
C-12	28.78	CH_3	1.00, s	28.79	CH_3	0.98, s
C-13	15.75	CH_3	1.01, s	15.83	CH_3	1.01, s
C-14	54.40	CH_2	2.69, d, $J = 4.9$ Hz	49.23	CH_2	2.64, dd, $J = 4.4$, 2.3 Hz
			2.47, d, $J = 4.9$ Hz			2.41, dd, $J = 4.5$, 1.0 Hz
C-15	16.91	CH_3	0.89, d, $J = 7.1$ Hz	15.64	CH_3	0.89, d, $J = 7.1$ Hz

[a] Determined from 2D-NMR experiments. For carbon numbering, see Scheme 2.
[b] In ppm with regard to TMS.
[c] Determined from DEPT experiments.

this strategy, significant connectivities observed in COSY and HMBC diagrams for ledol **7** are presented in Scheme 3.

Alcohols **6** and **7** had been isolated when reduction was achieved with pure compounds **2** and **3**. The Structure of the alcohols has been specified on the basis of the structure determination of the corresponding epoxides and confirmed by 2D-NMR analyses. The stereochemistry of alcohols **6** and **7** and epoxide **2** were unambiguously established from the analysis of the phase-sensitive NOESY spectrum [32]. For compound **2** methylene protons of the epoxide group showed NOE cross-peaks with cyclopropane resonances (Scheme 4). In the case of viridiflorol **6** and ledol **7**, to suppress overlapping of significant signals, NOESY experiments were recorded at 600 MHz. The CH_3-14 signal in **7** indicated a NOE interaction with H-1 and H-5 while in



Scheme 3. Proton–proton (◀----▶) and long-range proton–carbon (——▶) connectivities derived, respectively, from COSY and HMBC experiments for ledol **7**.

Table 6
^{1}H and ^{13}C NMR chemical shifts of aromadendrene aldehydes **11** and **12**

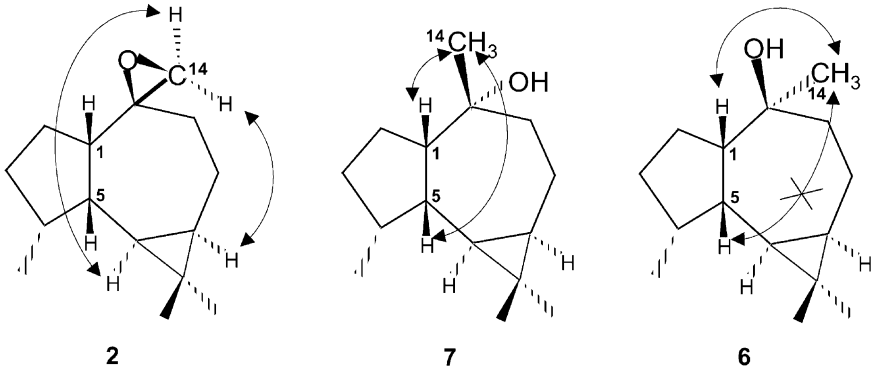
Assignment[a]	Aldehyde **11**			Aldehyde **12**		
	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]
C-1	46.18	CH		47.95	CH	2.08, dq, $J = 4.1$, 9.2 Hz
C-2	30.67	CH_2		29.64	CH_2	1.79, 1.61
C-3	35.33	CH_2		35.23	CH_2	1.83, 1.26
C-4	34.71	CH		36.75	CH	2.08
C-5	44.57	CH		41.53	CH	1.63
C-6	28.17	CH		29.15	CH	0.47, dd, $J = 10.5$, 9.5 Hz
C-7	27.71	CH		27.16	CH	0.54, dt, $J = 6.0$, 10.5 Hz
C-8	23.77	CH_2		21.55	CH_2	1.04 (α); 1.81 (β)
C-9	28.04	CH_2		28.37	CH_2	2.17 (α), ddd, $J = 14.1$, 3.1, 6.0 Hz; 1.47 (β), dt, $J = 4.5$, 13.5 Hz
C-10	60.77	CH		52.50	CH	2.67, q, $J = 3.8$ Hz
C-11	21.31	C	–	21.62	C	–
C-12	28.88	CH_3	1.01, s	28.81	CH_3	0.97, s
C-13	15.75	CH_3	1.01, s	15.84	CH_3	0.92, s
C-14	204.75	CH	9.52, d, $J = 3.5$ Hz	206.33	CH	9.83, s
C-15	16.86	CH_3	0.90, d, $J = 7.1$ Hz	16.01	CH_3	0.89, d, $J = 7.1$ Hz

[a] Determined from 2D-NMR experiments. For carbon numbering, see Scheme 2.
[b] In ppm with regard to TMS.
[c] Determined from DEPT experiments.

Table 7
^{1}H and ^{13}C NMR chemical shifts of epiglobulol **13** and globulol **14**

Assignment[a]	Epiglobulol **13**			Globulol **14**		
	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]	$\delta^{13}C$[b]	Multiplicity[c]	$\delta^{1}H$[b]
C-1	55.96	CH	1.70	57.16	CH	1.91
C-2	26.64	CH_2	1.71; 1.36	26.29	CH_2	1.78; 1.43
C-3	34.68	CH_2	1.72; 1.15	34.69	CH_2	1.67 (β); 1.24 (α)
C-4	35.82	CH	1.99	36.42	CH	2.02
C-5	37.59	CH	1.74	39.68	CH	1.23
C-6	28.91	CH	0.46	28.55	CH	0.51
C-7	27.12	CH	0.52	26.93	CH	0.59
C-8	19.20	CH_2	1.63; 1.37	20.24	CH_2	1.79 (α); 0.91 (β)
C-9	42.92	CH_2	1.65; 1.49	44.73	CH_2	1.73; 1.53
C-10	72.39	C	–	75.06	C	–
C-11	20.64	C	–	19.33	C	–
C-12	28.82	CH_3	0.98	28.71	CH_3	1.01
C-13	15.91	CH_3	1.01	15.80	CH_3	0.98
C-14	31.19	CH_3	1.18	20.24	CH_3	1.09
C-15	16.65	CH_3	0.89	16.09	CH_3	0.92

[a] Determined from 2D-NMR experiments. For carbon numbering, see Scheme 2.
[b] In ppm with regard to TMS.
[c] Determined from DEPT experiments.



Scheme 4. Significant cross-peaks observed in the NOESY diagram for compounds **2**, **6**, and **7**.

viridiflorol **6** only correlation of peaks between H-14 and H-1 was observed. These results are presented in Scheme 4.

Spectral data of ledol are in contradiction to those published by Miyazawa et al. [15] for an authentic sample of ledol purchased from Taiyo koryo and with those published by Wu et al. [18] for (−)-ledol from *Cephaloziella recurvifolia*. But they are in agreement with the data published for an authentic ledol sample from QUEST International [12]. Gwaltney et al. [16] have proposed a total synthesis of ledol which structure was characterized based on comparison with Gijsen's NMR data [13] which are in agreement with our results.

4. Conclusion

Viridiflorol has been often referred as a component of various niaouli essential oils analyzed by GC, GC–MS, and NMR spectroscopy. To distinguish between ledol and viridiflorol NMR data, we have unambiguously synthesized the sesquiterpenols ledol and viridiflorol as standards for characterization of the natural sesquiterpenols occurring in niaouli essential oils. From 2D-NMR experiments and GC analysis (I_R on CW 20 M, 2009 and 2071 for synthetic ledol and viridiflorol, respectively), it appears that the product identified as viridiflorol in *M. quinquenervia* (I_R on SPX-4, 1978 and 2036, for ledol and viridiflorol, respectively) [2] is viridiflorol, which confirms our results published earlier [3].

Acknowledgements

The authors are grateful to Dr. E. Guillet, from Institute of Natural Substances (Gif sur Yvette) for recording the spectrum at 600 MHz.

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