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EUDESMANE SESQUITERPENES FROM *LAGGERA ALATA*

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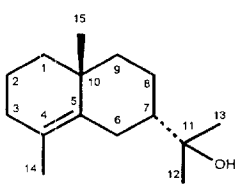
Key Word Index—*Laggera alata* var. *alata*; Compositae; eudesmane-type sesquiterpenoids; 7-*epi*-γ-eudesmol; 7-*epi*-β-eudesmol.

Abstract—Two new eudesmane-type sesquiterpenoids (7-*epi*-γ-eudesmol and 7-*epi*-β-eudesmol) were isolated and characterized along with five other sesquiterpene compounds (β-selinene, 7-*epi*-α-eudesmol, isointermedeol, juniper camphor and β-dihydroagarofuran) from the essential oil of *Laggera alata* var. *alata* grown in Madagascar. The assigned structures were based on their spectral data. © 1998 Published by Elsevier Science Ltd

INTRODUCTION

The herbaceous plant *Laggera alata* var. *alata* Sch. Bip. ex oliv. is widespread in the Highlands and East coast part of Madagascar and is locally called "Ariandro" [1]. It has some traditional medicinal applications including the use of its volatile components as an antiseptic [2]. The aerial parts of this naturally grown plant are odoriferous, having a persistent fragrance. So far, little is known about the composition of its essential oil.

In previous papers, we reported on the eudesmane-type sesquiterpene compounds contained in *Neocallitropsis pancheri* oil [3–5]. In this paper, we report on the major components of the essential oil of *L. alata* var. *alata* grown in Madagascar which are mainly eudesmane-type sesquiterpenes. Seven sesquiterpene constitutents were isolated from this essential oil. Two were new compounds, identified as 7-*epi*-γ-eudesmol (**1**) and 7-*epi*-β-eudesmol (**2**) on the basis of their spectral data. The five known sesquiterpene compounds isolated were β-selinene (**3**), 7-*epi*-α-eudesmol (**4**), isointermedeol (**5**), juniper camphor (**6**) and β-dihydroagarofuran (**7**).



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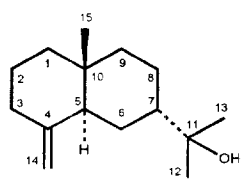

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RESULTS AND DISCUSSION

The EImass spectrum of compound **1** showed the molecular ion at *m/z* 222 in agreement with the molecular formula $C_{15}H_{26}O$. Its IR spectrum displayed a hydroxyl group band (3360 cm^{-1}) and carbon-carbon double bond band (1645 cm^{-1}). In the ^1H NMR spectrum of compound **1**, four singlets due to methyl groups were identified (δ 1.06, 1.16, 1.22 and 1.66). The signal resonating at δ 1.66 was attributable to an olefinic methyl group.

Inspection of its ^{13}C NMR spectrum showed that compound **1** contained 15 carbon atoms, the chemical shift values of which were very close to those of γ-eudesmol [4], except for one carbon which was shifted upfield. The DEPT pulse sequence [6] spectra indicated that compound **1** contained four methyl groups, six methylene groups, four quaternary carbons and one methine group. This latter group corresponded to the upfield signal. These results suggested that **1** was a bicyclic sesquiterpenol.

The above four methyl groups were used as starting points to assign the other proton and carbon atoms of compound **1** by examination of the long-range connectivities given in the HMBC [7] diagram and the heteronuclear correlations from the cross-sections in the HMQC [8] diagram (Fig. 1). This network [9] led

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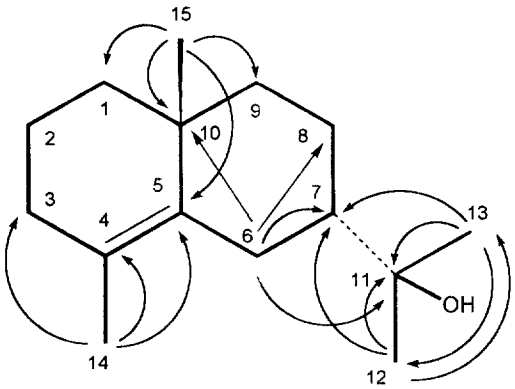

Fig. 1. Long range proton-carbon (→) connectivities observed in the HMBC plot of 7-*epi*-γ-eudesmol.

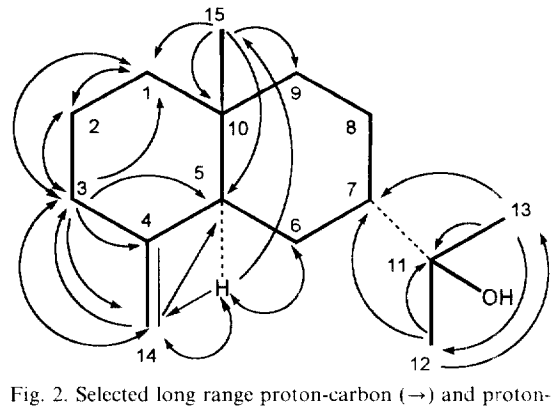

Fig. 2. Selected long range proton-carbon (→) and proton-proton (↔) connectivities observed respectively in the HMBC and COSY plots of 7-*epi*-β-eudesmol.

to the complete assignment of the ^1H and ^{13}C NMR data of compound **1** (Table 1).

The coupling constant values observed for the H-6 protons ($^3J = 14.9$ Hz and 15.2 Hz) suggested that the H-7 methine proton was in a pseudo-equatorial position. Thus, the isopropanol group attached to C-7 was deduced to be α-positioned. The C-7 *epi* stereochemistry was also confirmed by the characteristically upfield ^{13}C chemical shift of C-7 in compound **1** with respect to that of γ-eudesmol. Consequently, the structure of compound **1** was established as 7-*epi*-γ-eudesmol and constituted a newly characterized eudesmane sesquiterpenoid.

The EImass spectrum of compound **2** contained the $[M]^+$ peak at *m/z* 222 consistent with a molecular formula $C_{15}H_{26}O$. The occurence of the alcohol function and carbon-carbon double bond were shown by IR bands (3358 cm^{-1} and 1640 cm^{-1}). At first sight, the ^1H NMR data established the presence of an exocyclic methylene group (two signals at δ 4.70 and 4.44) and three methyl groups (at δ 0.72, 1.23 and 1.24). The ^{13}C NMR data of compound **2** showed great similarities to that of β-eudesmol [4], except for one chemical shift value which was more shielded for compound **2**. The multiplicities of the individual ^{13}C signals determined by the use of DEPT pulse sequence indicated three methyl groups, seven methylene groups, two methine groups and three quaternary carbon atoms. It was concluded from this data that compound **2** must contain two rings and an alcohol function.

Starting the ^1H and ^{13}C assignments using the above ^1H signals, concerted use of two dimensional homonuclear and heteronuclear diagrams (HMBC and HMQC) allowed deduction of the interconnections between proton and carbon atoms bond after bond (Fig. 2). Following the same investigational method described above for compound **1**, the complete assignment of the ^1H and ^{13}C NMR data of compound **2** was achieved (Table 2). As for 7-*epi*-γ-eudesmol (compound **1**), the epi C-7 stereochemistry was evident by noting the upfield ^{13}C chemical shift of C-7

Table 1. ^1H and ^{13}C NMR data for compound **1**

C/H	^{13}C	^1H[#]
1	38.20	1.65 and 1.28
2	19	1.52
3	32.80	1.96 and 1.89
4	125.98	—
5	135.07	
6	26.99	2.68 *dm* (14.9) and 2.09 *dm* (15.2)
7	44.21	1.66
8	22.66	1.69 and 1.60
9	39.53	1.40 *dt* (12.5, 4.2) and 1.15 *dt* (12.3, 3.5)
10	34.51	—
11	74.61	—
12	29.89	1.22 *s*
13	27.95	1.16 *s*
14	19.71	1.66 *s*
15	26.02	1.06 *s*

[#] *J* (Hz) in parentheses.

Table 2. ^1H and ^{13}C NMR data for compound **2**

C/H	^{13}C	^1H[#]
1	42.56	1.40 *dtd* (12.9, 3.3, 1.7) and 1.26
2	21.12	1.55
3	37.28	2.27 *ddt* (13, 3.8, 1.7) and 1.98
4	151.54	—
5	44.47	2.11
6	24.09	1.67 and 1.53
7	42.37	1.68
8	23.76	1.70 and 1.65
9	38.33	1.52 *ddd* (12.5, 10.8, 1.4) and 1.65
10	35.39	—
11	74.70	—
12	29.73	1.24
13	29.28	1.23s
14	105.37	4.70 *q* (1.8) and 4.44 *q* (1.8)
15	17.17	0.72

[#] *J* (Hz) in parentheses.

for compound **2** compared to that of β-eudesmol and by consideration of the coupling constant pattern. Thus, compound **2**, determined as 7-*epi*-β-eudesmol, was found for the first time from a natural source.

The other isolated compounds were identified on the basis of their EIMS and ^{13}C NMR characteristics in respect to the literature data: compound **3** being β-selinene, compound **4** being 7-*epi*-α-eudesmol, compound **5** being isointermedeol, compound **6** being juniper camphor and finally compound **7** being β-dihydroagarofuran [10, 11].

It is a remarkable fact that all these compounds isolated from *L. alata* oil are eudesmane-type sesquiterpenes. The occurrence of other molecules having the eudesmane skeleton (cuanthemone derivatives) has been reported for *L. alata* grown in South Africa [12, 13]. Except for β-selinene and juniper camphor, all the sesquiterpenes isolated from this *L. alata* oil contain a C-7 α-positioned epimer substituent. This fact raises questions about a new biogenetic pathway for the secondary metabolites from this plant.

EXPERIMENTAL

The aerial part (420g) of *L. alata* var. *alata* harvested in Lazaina (Madagascar, Nov 1995) were submitted to steam distillation for 10hr using a Clavenger type apparatus giving a pale yellow essential oil (2g). A voucher specimen (N° AR 10-04) has been deposited in the Herbarium of the Ecole Supérieure des Sciences Agronomiques (Antananarivo, Madagascar).

The *L. alata* var. *alata* oil (1.5g) was separated on silica gel coated with 10% of $AgNO_3$ using a stepwise gradient solvent system from isooctane to EtOAc. Compounds **1**-**7** were obtained from the following frs: isooctane-EtOAc (97:3) gave **7**, (95:5) gave **3**, (93:7) gave **1**, (91:9) gave **4**, (89:11) gave **5**, (87:13) gave **6**, and (84:16) gave **2**. Repeated elution, when necessary, was used to purify every compound.

1D and 2D NMR: $CDCl_3$ solns with TMS as int. stand.; ^1H at 400MHz and ^{13}C at 100.61 MHz.

7-*epi*-γ-*Eudesmol* (**1**)

$C_{15}H_{26}O$. Oil, $[\alpha]_D^{25} = -23.3$ ($CHCl_3$, c 1.2). EIMS 70eV, *m/z* (rel.int.): 222 $[M]^+$ (7), 204 (35), 189 (71), 161 (69), 149 (25), 147 (18), 133 (56), 119 (20), 107 (30), 105 (43), 95 (23), 91 (60), 79 (37), 77 (29), 67 (26), 59 (100), 55 (37), 53 (18), 43 (84), 41 (59).

7-*epi*-β-*Eudesmol* (**2**)

$C_{15}H_{26}O$. Oil, $[\alpha]_D^{25} = -13.3$ ($CHCl_3$, c 0.85). EIMS 70eV, *m/z* (rel.int.): 222 $[M]^+$ (0), 204 (25), 189 (23), 161 (43), 149 (21), 147 (17), 133 (26), 119 (18), 109 (14), 108 (11), 107 (22), 105 (35), 95 (20), 93 (25), 91 (36), 81 (28), 79 (30), 77 (17), 67 (31), 59 (100), 55 (26), 53 (14), 43 (45), 41 (56).

β-*Selinene* (**3**)

$C_{15}H_{24}$. EIMS 70eV, *m/z* (rel.int.): 204 $[M]^+$ (28), 189 (23), 161 (29), 147 (30), 133 (29), 121 (38), 119 (30), 108 (33), 107 (58), 105 (66), 95 (32), 94 (23), 93 (61), 91 (55), 81 (55), 80 (23), 79 (70), 77 (33), 67 (61), 55 (48), 53 (37), 41 (100); ^{13}C NMR ($CDCl_3$): δ 151.1 (C-4), 150.9 (C-11), 108.2 (CH_2-12), 105.4 (CH_2-14), 50 (CH-5), 46 (CH-7), 42.1 (CH_2-1), 41.3 (CH_2-9), 37 (CH_2-3), 36.1 (C-10), 29.6 (CH_2-6), 26.9 (CH_2-8), 23.6 (CH_2-2), 21.1 (CH_3-13), 16.4 (CH_3-15).

7-*epi*-α-*Eudesmol* (**4**)

$C_{15}H_{26}O$. EIMS 70eV, *m/z* (rel.int.): 222 $[M]^+$ (0), 204 (20), 189 (15), 162 (13), 161 (80), 122 (54), 107 (30), 105 (15), 95 (16), 93 (18), 81 (25), 67 (12), 59 (100), 55 (32), 43 (45), 41 (56); ^{13}C NMR ($CDCl_3$): δ 135.6 (C-4), 121.1 (C-3), 74.3 (C-11), 42.7 (CH-5), 41.1 (CH-7), 38.8 (CH_2-8), 37.7 (CH_2-9), 31.4 (C-10), 29.2 (CH_3-13), 28.9 (CH_2-12), 21.1 (CH_3-14), 20.9 (CH_2-2), 18.6 (CH_3-15).

Isointermedeol (**5**)

$C_{15}H_{26}O$. EIMS 70eV, *m/z* (rel.int.): 222 $[M]^+$ (0), 204 (20), 189 (23), 161 (23), 135 (11), 125 (16), 123 (16), 122 (14), 121 (13), 109 (16), 107 (14), 105 (13), 95 (21), 93 (20), 82 (20), 81 (39), 79 (19), 71 (33), 69 (17), 67 (31), 55 (29), 43 (100), 41 (48); ^{13}C NMR ($CDCl_3$): δ 147 (C-11), 110.9 (CH_2-12), 72.2 (C-4), 49.3 (CH-5), 43.6 (CH_2-3), 41.5 (CH_2-1), 40.4 (CH_2-9), 39.4 (CH-7), 35.4 (C-10), 23.6 (CH_2-8), 22.9 (CH_3-13), 22.8 (CH_2-6), 22.4 (CH_3-14), 20.2 (CH_2-2), 18.5 (CH_3-15).

Juniper camphor (**6**)

$C_{15}H_{26}O$. EIMS 70eV, *m/z* (rel.int.): 222 $[M]^+$ (14), 204 (16), 189 (29), 161 (20), 135 (17), 133 (15), 121 (15), 109 (11), 107 (16), 105 (16), 95 (15), 93 (21), 91 (16), 81 (35), 79 (17), 71 (15), 55 (30), 53 (14), 43 (100), 41 (49); ^{13}C NMR ($CDCl_3$): δ 131.5 (C-7), 121 (C-11), 72.1 (C-4), 55.8 (CH-5), 45.3 (CH_2-9), 43.6 (CH_2-3), 41.1 (CH_2-1), 34.9 (C-10), 25.5 (CH_2-6), 24.7 (CH_2-8), 22.1 (CH-14), 20.3 (CH_2-2), 20.1 (CH-12), 20.1 (CH_3-13), 18.5 (CH_3-15).

β-*Dihydroagarofuran* (**7**)

$C_{15}H_{26}O$. EIMS 70eV, *m/z* (rel.int.): 222 $[M]^+$ (6), 208 (12), 207 (71), 189 (29), 151 (12), 149 (29), 137 (66), 125 (18), 123 (19), 109 (52), 108 (15), 107 (16), 95 (33), 93 (17), 91 (15), 83 (14), 81 (37), 77 (14), 69 (44), 67 (32), 57 (16), 55 (64), 53 (18), 43 (95), 41 (100); ^{13}C NMR ($CDCl_3$): δ 87.9 (C-5), 81.1 (C-11), 44.6 (CH-7), 40.5 (CH-4), 38.6 (C-10), 38.4 (CH_2-6), 38.4 (CH_2-1), 38.1 (CH_2-9), 37.5 (CH_2-3), 30.6 (CH_3-13), 29.5 (CH_2-6), 25 (CH_2-8), 23.6 (CH_3-12), 22.9 (CH_3-15), 17.8 (CH_3-14), 17 (CH_2-2).

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