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Diterpenoids from *Calceolaria inamoena*☆

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Abstract

Four 9-*epi*-*ent*-labdanes were isolated from the aerial parts of *Calceolaria inamoena*. Their structures, 2β-hydroxy-9-*epi*-*ent*-labda8(17)-13(*E*)dien-15-oic acid, 2β-hydroxy-9-*epi*-*ent*-labda8(17)-13(*Z*)dien-15-oic acid, 2β-hydroxy-9-*epi*-*ent*-labda8(17)-13(*E*)dien-15-al and 2β-hydroxy-9-*epi*-*ent*-labda-8(17)-13(*Z*)dien-15-al, were established by spectroscopic methods including by analysis of 2 dimensional heteronuclear correlation experiments $^{1}H/^{13}C$ (normal and long range), and NOE and gs-sel-1D-NOESY and TOCSY of their methyl ester or acetyl derivatives.

Keywords: *Calceolaria inamoena*; Scrophulariaceae; Diterpene; *9-epi-ent*-labdanes

1. Introduction

Plants of the genus *Calceolaria* (Scrophulariaceae) are found distributed throughout Central and South America and New Zealand, and in Chile where there are 85 species (Marticorena and Quezada, 1985). Traditionally, various species belonging to the genus *Calceolaria* are used as bactericidal agents, for treating stomach ache, and as sweeteners. Continuing our studies on Chilean species belonging to the *Calceolaria* genus (Chamy et al., 1991,1998, 2001), we have now examined a petrol ether extract of the aerial parts of *Calceolaria inamoena*. Four labdane diterpenes **1**–**9** were isolated whose structures are proposed herein on the basis of spectroscopic analysis.

Calceolaria inamoena is a shrub, common to the northern subandinean part of Chile. Previous studies on species of this genus have resulted so far in the isolation of diterpenes having labdane (Garbarino and Molinari, 1992), pimarane (Piovano et al., 1988; Chamy et al., 1998), abietane (Chamy et al., 1987) stemodane (Chamy et al., 1995a,b) and stemarane (Garbarino and Molinari, 1990; Chamy et al., 1995a,b) skeleta. From a biogenetic point of view, the formation of diterpenes found in *Calceolaria* species thus far involves an enzymatic activity leading towards a "chair–boat" cyclization of geranyl–geranyl pyrophosphate, as oppossed to the normal "chair–chair" cyclization in the construction of the bicyclic intermediate (Garbarino et al., 2001). When the atypical "chair–boat" cyclization occurs, H-9 and C-20 adopt a "cis" relation and C-11 becomes "trans" with respect to C-20. This fact can explain the formation of the 9-epimeric labdanes and 9-*epi*-pimaranes and also formation of other skeletal types found in this genus, i.e. stemaranes (Garbarino and Molinari, 1990; Chamy et al., 1995a,b), thyrsifloranes (Chamy et al., 1991) and stemodanes (Chamy et al., 1995a,b).

In the course of our current research on the chemistry of the genus *Calceolaria*, we report here the isolation and structural determination of some constituents from the aerial parts of *C. inamoena* collected between Belen and Pachama, in the subandinean level of Arica, I Región, Chile. Their structures were established by spectroscopic analysis.

2. Results and discussion

The petroleum ether (60–80 °C) extract of the aerial parts of *C. inamoena*, after successive cc purifications afforded compounds **1**–**4**.

☆ Part 21 in the series "Diterpenoids of *Calceolaria* species". For part 20 see Chamy et al. (2001).

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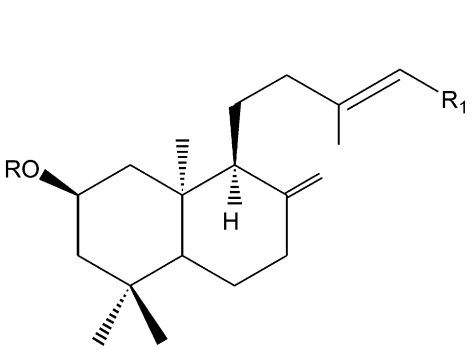
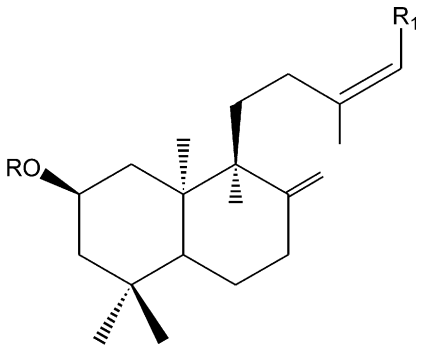


	R	R_1
1	H	COOH
1a	H	COOMe
1b	Ac	COOMe
3	H	CHO
3a	Ac	CHO



	R	R_1
2	H	COOH
2a	H	COOMe
4	H	CHO

Joint examination of spectroscopic data showed that the four compounds were closely related and differed only in the level of oxidation and stereochemistry of their side-chains. The main structural features shown by ^{1}H and ^{13}C NMR spectroscopy (see Section 3 and Table 1) were consistent with a diterpenic bicyclic structure (labdane), all having a $\Delta^{8(17)}$ double bond and a 2β-hydroxyl group as common characteristics.

Compound **1** was purified and characterized as its methyl ester derivative **1a**, which was obtained as an oil, with a molecular formula $C_{21}H_{34}O_3$ ([M]$^+$ at 334). Its ^{1}H NMR spectrum indicated the existence of three tertiary methyl groups at δ 0.92, 0.85 and 0.72, corresponding to Me-18, Me-19 and Me-20, respectively, as well as two broad singlets at δ 4.82 and 4.46 for vinylidenic protons H-17, and another signal at δ 3.62 for the methyl ester. A double double triplet at δ 3.82 was also assignable to the hydrogen geminal to an hydroxyl group, with the aid of a gs-sel-1D-TOCSY experiment, it was unequivocally determined to be located between C-1 and C-3. Lack of a NOE cross peak between H-5 and Me-20 suggested that the latter is oriented *trans* to H-5. The relative stereochemistry of **1a** was assigned on the basis of both a NOESY correlation and coupling constant data. H-1α/Me-20, H-1α/Me-19, H-2α/Me-20, H-2α/Me-19, Me-20/H-6α, Me-20/H-9 indicated that rings A and B were *trans*-fused, and with the chair–boat configuration, H-2 was α axial oriented. The NOESY correlation between Me-20/H-9 indicated a 9-*epi*-labdane. On the other hand, an axial–axial (J_{aa} = 11.8 Hz) and axial–equatorial (J_{ae} = 4.2 Hz) coupling constant for H-1, H-2α and H-3 indicated that the OH substituent in C-2 was β equatorial (see Fig. 1). Furthermore, an allylic methyl group as indicated by a signal in the ^{1}H NMR spectrum at δ 2.18 ($\delta_{C\text{-}16}$ 19.3) and long range coupled to a broad singlet corresponding to a vinylic proton at δ 5.68 ppm, was characteristic of an unsaturated side chain of a bicyclic diterpenoid having an *E* configuration for the olefinic bond (San Feliciano et al., 1988).

To confirm the position of the hydroxyl group, **1a** was treated with acetic anhydride–pyridine to afford **1b**. As expected, in the ^{1}H NMR spectrum of **1b** a singlet assigned to an acetoxyl group appeared at δ 2.03 (3H, *s*), and the signal corresponding to the H geminal to the OH group in **1a** was shifted downfield to δ 5.02 (1H,

Table 1
^{13}C NMR spectroscopic data of compounds **1a**, **1b**, **2a**, **3**, **3a** and **4** measured in $CDCl_3$

C	**1a**	**1b**	**2a**	**3**	**3a**	**4**
1	48.1	43.9	48.4	48.1	44.0	48.0
2	65.5	68.5	65.9	65.5	69.1	65.5
3	51.0	46.6	51.1	51.0	46.6	51.1
4	35.0	34.8	35.0	35.0	34.8	35.0
5	54.8	54.8	54.8	54.9	54.8	54.9
6	23.9	23.7	23.9	23.9	23.8	23.9
7	37.9	37.8	38.0	38.0	37.8	37.9
8	147.3	146.9	147.4	147.2	146.9	147,3
9	56.0	55.9	57.0	56.0	56.0	55,7
10	41.0	40.8	41.1	41.1	40.9	41.0
11	21.7	21.6	22.2	21.5	21.4	22,3
12	39.7	39.5	32.6	39.4	39.3	31.0
13	160.8	160.6	160.8	164.7	164.5	164,6
14	115.0	114.9	115.7	127.2	127.1	129,2
15	167.6	167.1	166.7	191.4	191.3	191,1
16	18.9	18.7	25.3	17.7	17.6	24,8
17	107.2	107.3	107.3	107.3	107.4	107,5
18	33.6	33.4	33.6	33.7	33.4	33,6
19	22.6	22.3	22.6	22.6	22.3	22,6
20	15.3	15.0	15.3	15.4	15.1	15,5
$O\underline{C}OCH_3$		170.5			170.6	
$OCO\underline{C}H_3$		21.5			21.4	
$COO\underline{C}H_3$	51.2	50.6	51.2			

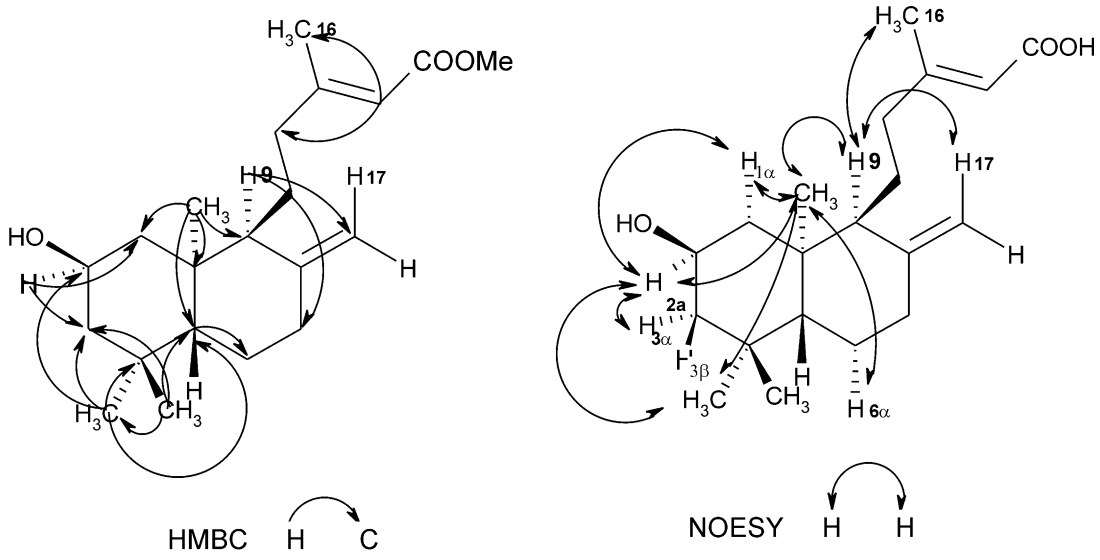


Fig. 1. The HMBC and NOESY correlations of **1a**.

ddt, $J = 4.2$, 11.8,11.8 Hz, H-2), whereas one olefin proton at δ 5.65 was assigned to the double bond of the side chain. In addition, two olefinic proton signals at δ 4.89 and 4.53 revealed the presence of an exomethylene group (1H each, *br d*, H-17 and H-17′), with a singlet at 2.16 (3H, *s*, Me-16) indicating that the stereochemistry of the double bond of the sidechain was *E*. In the case of the Z isomer, the Me-16 appeared at δ_H 1.9 and δ_C 25.7.

These assumptions were also supported by analysis of the ^{13}C NMR spectral data. Comparison with the spectral data of similar labdane diterpenoids (Buckwalter et al., 1975; Bohlmann et al., 1984; Garbarino et al., 1986; Zdero et al., 1992), showed unambiguosly that **1b** was *methyl-2β-acetoxy-9-epi-ent-labdan-8(17),13(E)-dien-15-oate* and therefore, **1** must be *2β-hydroxy-9-epi-ent-labda-8(17),13(E)-dien-15-oic acid*.

Compound **2** was also purified and characterized as its methyl ester derivative **2a**. Comparison of the ^1H NMR spectrum of **2a** with that of **1a** showed only differences in the signals due to the side-chain and not in the skeletal protons. As deduced from the ^1H NMR spectrum, **2a** was a labdane with a 13 *Z*-configuration (δ 5.58, H-14; 4.85 and 4.64, H-17 and 1.83 ppm, Me-16) (Garbarino et al., 1988). The ^{13}C NMR spectrum of **2a** (Table 1) confirmed this point. The resonances due to the decalin part remained almost identical to those of **1a**, and the only differences in their spectra are the shifts for Me-16 (δ 25.7 in **2a** and 19.1 in **1a**) and the methylene (δ 33.0 in **2a** and 40.1 in **1a**). The other signals due to the side-chain appeared at very similar positions to those of methyl labda-8(17),13(*Z*)-dien-oate (Bastard et al., 1984). So compound **2a** must be *methyl-2β-hydroxy-9-epi-ent-labdan-8(17),13(Z)-dien-15-oate*.

Compound **3** had a molecular formula of $C_{20}H_{32}O_2$. Comparison of the ^1H NMR spectrum of **3** and **1a**, showed only minor differences for the decalin part signals and differed only in the signals due to the side chain. The main difference between compound **3** and **1a** is the level of oxidation at C-15. As deduced from the ^1H NMR spectrum, a doublet at δ 9.98 (1H, $J = 8$ Hz, H-15) accounted for an aldehyde group at C-15 (δ_C 191.3 ppm), and a vinylic methyl group, as evidenced by a signal at δ_H 2.16 ($\delta_{C\text{-}16}$ 19.1), indicated that the stereochemistry of the double bond of the side chain was *E*. These features were also supported by analysis in the ^{13}C NMR spectrum (see Table 1). So compound **3** is determined to be *2β-hydroxy-9-epi-ent-labda-8(17),13(E)-dien-15-al*.

Compound **3** was treated with Ac_2O/Py to afford **3a**. As expected the signal corresponding to the H geminal to the OH group in **3** was shifted from δ 3.89 to 5.02 (*ddt*). So **3a** is *2β-acetoxy-9-epi-ent-labda-8(17),13(E)-dien-15-al*.

Compound **4** had a molecular formula of $C_{20}H_{32}O_2$. Comparison of the ^1H NMR spectrum of **4** and **3** showed as their only differences the values for the aldehyde group at C-15 and the Me-16 ($\delta_{H\text{-}15}$ 9.81, $\delta_{Me\text{-}16}$ 1.92 and $\delta_{H\text{-}15}$ 9.98, $\delta_{Me\text{-}16}$ 2.19, respectively). In their ^{13}C NMR spectra, the main differences are for Me-16 and the methylene bonded to the double bond of the side chain (see Section 3). Therefore, compound **4** is *2β-hydroxy-9-epi-ent-labda-8(17),13(Z)-dien-15-al*.

The relative stereochemistry of compounds **1**–**4** was determined as shown in Fig. 1 after a consideration of their NOESY correlations. The absolute configurations were not established and the *ent* configuration of compounds **1**–**4** is proposed considering that only compounds with an *ent* configuration have been isolated so far from *Calceolaria* species (Garbarino et al., 2001). The first diterpene isolated by us from *Calceolaria*

glandulosa was 18-hydroxy-9-*epi-ent*-isopimara-7,15-diene whose relative stereostructure was confirmed by X-ray crystallographic analysis (Piovano et al., 1988). The formation of the 9-*epi-ent*-pimarane involves as a bicyclic intermediate a 9-*epi-ent*-labdane.

From all the chilean *Calceolaria* species studied until today (50), only two species *C. densifolia* (Gabarino and Molinari, 1992) and *C. inamoena* accumulate 9-*epi-ent*-labdane, both of them recollected in the subandinean hills of the north part of Chile.

Some evolutionary implications could be based on the knowledge of the diterpenes biosynthesis; the presence of the 9-*epi-ent*-labdanes corresponds to an earlier biogenetic stage of these species than those that produces pimarane or stemarane types of diterpenes.

3. Experimental

3.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Nicolet Impact 420 spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ solutions, were all spectra obtained using an Avance 400 Digital NMR Bruker Spectrometer, equipped with a 5.00 mm Inverse Multinuclear Detection Pulsed Field Gradients probe heat (^{1}H-BB1, PFG-ZGRD, Z8202/0253), operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, located at Universidad Técnica Federico Santa Maria. Mass spectra were taken at 70 eV (probe) in a Shimadzu QP-2000 and high resolution spectra in a Micromass Autospectrometer. Silica gel (200–300 mesh) was used for CC and silica gel plates HF-254 for TLC. Spots were detected on TLC by heating after spraying with 25% H_2SO_4 in H_2O.

3.2. Plant material

Aerial parts of *Calceolaria inamoena* Phil. were collected in Arica, I Region, Chile, in September 2000, and authenticated by Professor Eliana Belmonte, Universidad de Tarapacá. A voucher specimen is deposited in the Herbarium of the Natural Product Laboratory of Universidad Técnica Federico Santa María (# 98007).

3.3. Extraction, isolation and characterization

Aerial parts of *C. inamoena* (1.5 kg) were extracted at room temp. successively with petrol ether (60–80°) (15 L) and CH_2Cl_2 (15 L) for 48 h each. The solvents were removed in vacuo to yield 30 g (petrol extract) and 50 g (CH_2Cl_2 extract) of a syrupy residue. The extracts were each subjected separately to silica gel column chromatography (400 g) eluted with mixts of petrol and EtOAc of increasing polarity. Frs. (125 ml) were combined based on TLC and ^{1}H NMR (60 MHz) monitoring, well some of the combined frs. methylated (with ethereal CH_2N_2) and/or acetylated (acetic anhydride/pyr.) and purified by repeated CC on silica gel or silica gel impregnated with $AgNO_3$ (10%).

3.4. Methyl-2β-hydroxy-9-epi-ent-labda-8(17),13(E)-dien-15-oate (**1a**)

Oil; Rf = 0.73 (petrol:EtOAc 8:1) IR(KBr) ν_{max} (cm^{-1}): 3625, 3090, 2920–2880, 1720, 1650, 910. ^{1}H NMR δ: 5.68 (1H, *br s*, H-14), 4.92 (1H, *d*, J = 1.2 Hz, *H-17*), 4.54 (1H, *br s*, H-17′), 3.89 (1H, *ddt*, J = 4.2, 11.8, 11.8 Hz, H-2), 3.62 (3H, *s*, OMe), 2.18 (3H, *d*, J = 1.2 Hz, Me-16), 2.09 (1H, *dd*, J = 4.2, 13.4 Hz, H-1α), 1.78 (1H, *dd*, J = 4.2, 14.0 Hz, H-3α), 1.76 (1H, *dd*, J = 11.8, 13.4 Hz, H-1β), 0.97 (1H, *dd*, J = 11.8, 14.0 Hz, H-3β), 0.92 (3H, *s*, Me-18), 0.85 and 0.72 (3H each, *s*, Me-19 and Me-20). For ^{13}C NMR, see Table 1. EIMS *m*/*z* (rel.int): 334 [M$^+$, $C_{21}H_{34}O_3$] (47), 316 [M$^+$ −H_2O] (17), 301 [316-Me], (38), 273 (12), 242 (16), 203 (39), 201 (13), 187 (19), 175 (24), 173 (19), 161 (20), 159 (19), 147 (37), 145 (14), 144 (72), 137 (16), 135 (100), 134 (24), 133 (34), 131 (13), 123 (21), 122 (24), 121 (65), 120 (20), 119 (42), 109 (36), 107 (57), 105 (41), 95 (44), 93 (59), 91 (42), 83 (30), 82 (27), 81 (45), 79 (35), 77 (19), 69 (45), 67 (41), 57 (25), 55 (42), 53 (20). Found [M$^+$ at *m*/*z* 334.25227. $C_{21}H_{34}O_3$ requires 334.25079.

3.5. Methyl-2β-acetoxy-9-epi-ent-labdan-8(17),13(E)-diene (**1b**)

IR(KBr) ν_{max} (cm^{-1}): 2920–2880, 1720, 1630, 1460, 1420, 1380, 1230, 1130, 1010, 970, 895. ^{1}H NMR δ : 5.65 (1H, *br s*, H-14), 5.02 (1H, *ddd*, J = 4.2, 11.8, 11.8 Hz, H-2), 4.89 (1H, *br s*, *H-17*), 4.53 (1H, *br s*, H-17′), 3.69 (3H, *s*, OMe), 2.16 (3H, *s*, Me-16), 2.04 (1H, *dd*, J = 4.2, 13.2 Hz, H-1α), 2.03 (3H, *s*, OAc), 1.76 (1H, *dd*, J = 4.2, 13.5 Hz, H-3α), 1.75 (1H, *dd*, J = 11.9, 13.0 Hz, H-1β), 1.60 (1H, *dd*, J = 11.9, 13.0 Hz, H-3β), 0.94 (3H, *s*, Me-18), 0.89 and 0.77 (3H each, *s*, Me-19 and Me-20). For ^{13}C NMR, see Table 1. MS [70 eV] (%): 376 [M$^+$, $C_{23}H_{36}O_4$](0.1), 344 [M–MeOH]$^+$ (0.5), 318 (1), 316[M–HCOOMe]$^+$ (4), 303 (3), 301 (21), 203 (30), 201 (17), 189 (11), 187 (16), 175 (22), 173 (20), 161 (19), 145 (11), 135 (89), 133 (36), 123 (13), 121 (45), 120 (19), 119 (52), 114 (34), 107 (63), 105 (44), 95 (37), 93 (61), 91 (48), 85 (41), 83 (43), 81 (56), 79 (49), 69 (49), 67 (52), 55 (65), 53 (36), 43 (100), 41 (75).

3.6. Methyl-2β-hydroxy-9-epi-ent-labda-8(17),13(Z)-dien-15-oate (**2a**)

Rf = 0.73 (petrol:EtOAc 8:1) ^{1}H NMR δ: 5.58 (1H, *br s*, H-14), 4.85 (1H, *d*, J = 1.2 Hz, *H-17*), 4.64 (1H, *br s*,

H-17′), 3.81 (1H, *ddt*, $J=4.1$, 11.5, 11.5 Hz, H-2), 3.60 (3H, *s*, OMe), 1.83 (3H, *d*, $J=1.2$ Hz, Me-16), 0.89 (3H, *s*, Me-18), 0.76 and 0.65 (3H each, *s*, Me-19 and Me-20). For ^{13}C NMR, see Table 1. MS [70 eV] (%): 334 [M^+, $C_{21}H_{34}O_3$] (30), 316 [M^+–H_2O] (21), 301 [316–Me], (35), 273 (8), 203 (40), 201 (8), 187 (18), 175 (25), 173 (25), 161 (10), 145 (12), 144 (65), 137 (18), 135 (100), 123 (30), 120 (20), 119 (42), 109 (30), 107 (64), 105 (30), 95 (45), 93 (59), 84 (80), 81 (40), 79 (25), 69 (50). Found [M^+ at *m*/*z* 334.25219. $C_{21}H_{34}O_3$ requires 334.25079.

3.7. 2β-Hydroxy-9-epi-ent-labda-8(17),13(E)-dien-15-al (*3*)

IR(KBr) ν_{max} (cm^{-1}): 3400, 3090, 2920–2830, 1680, 1460, 1430, 1390, 1370, 1200, 1120, 1020, 895, 750. ^{1}H NMR δ: 9.98 (1H, *d*, $J=8.1$ Hz, H-15), 5.86 (1H, *d*, $J=8.1$ Hz, H-14), 4.90 (1H, *s*, H-17), 4.52 (1H, *s*, H-17′), 3.89 (1H, *ddt*, $J=4.1$, 11.4, 11.6 Hz, H-2), 2.18 (3H, *s*, Me-16), 2.08 (1H, *dd*, $J=4.1$, 13.8 Hz, H-1α) 1.77 (1H, *dd*, $J=4.1$, 13.8 Hz, H-3α), 1.16 (1H, *dd*, $J=11.6$, 13.8 Hz, H-1β), 0.98 (3H, *s*, Me-18), 0.97 (1H, *dd*, $J=11.4$, 13.8 Hz, H-3β), 0.88 and 0.72 (3H each, *s*, Me-19 and Me-20). For ^{13}C NMR, see Table 1. EIMS *m*/*z* (rel.Int) : 304 [M^+, $C_{20}H_{32}O_2$] (0.1), 289 [M–Me]$^+$ (3), 286 [M–H_2O]$^+$ (3), 271 [M–Me–H_2O]$^+$ (10), 243 (12), 227 (14), 203 [286–$CH_2C(CH_3)CHCHO$]$^+$ (9), 186 (11), 160 (11), 159 (15), 146 (17), 145 (23), 135 (100), 133 (45), 131 (9), 122 (20), 121 (35), 120 (42), 119 (32), 118 (39), 116 (11), 109 (24), 108 (18), 107 (87), 105 (21), 104 (38), 95 (54), 93 (23), 92 (45), 91 (70), 84 (24), 83 [$CH_2C(CH_3)CHCHO$] (62), 81 (21), 80 (36), 79 (20), 78 (40), 69 (18), 68 (30), 67 (20), 66 (27), 57 (13), 55 (30), 54 (42), 43 (64), 41 (86). Found [M^+ at *m*/*z* 304.23863. $C_{20}H_{32}O_2$ requires 304.24023.

3.8. 2β-Acetoxy-9-epi-ent-labda-8(17),13(E)-dien-15-al (*3a*)

Oil, Rf = 0.63 (petrol:EtOAc, 8:1). IR(KBr) ν_{max} (cm^{-1}): 2980–2900, 2850, 1720, 1640, 1460, 1440, 1395, 1360, 1250, 1140, 1020, 960, 895. ^{1}H NMR δ: 9.98 (1H, *d*, $J=8$ Hz, H-15), 5.86 (1H, *d*, J = 8 Hz, H-14), 5.03 (1H, *ddt* $J=4.1$, 11.8, 12 Hz, H-2), 4.89 (1H, *br s*, H-17), 4.51 (1H, *br s*, H-17′), 2.16 (3H, *s*, Me-16), 2.03 (3H, *s*, OAc), 0.94 (3H, *s*, Me-18), 0.88 and 0.77 (3H each, *s*, Me-19 and Me-20). For ^{13}C NMR, see Table 1. MS *m*/*z* (rel.int): 346[M^+, $C_{22}H_{34}O_3$] (0.5), 289 (2), 286 [M–MeCOOH]$^+$ (5), 203 [286–OCHC = C-$MeCH_2$] (15), 201 (10), 189 (16), 187 (15), 175 (10), 159 (20), 157 (11), 149 (11), 147 (30), 145 (23), 136 (14), 135 (100), 133 (34), 121 (30), 120 (31), 119 (31), 118 (22), 109 (31), 97 (12), 95 (48), 93 (50), 91 (37), 85 (11), 83 (14), 81 (26), 79 (28), 69 (18), 67 (19), 55 (20), 43 (93), 41 (38).

3.9. 2β-Hydroxy-9-epi-ent-labda-8(17),13(Z)-dien-15-al(*4*)

IR(KBr) ν_{max} (cm^{-1}): 3400, 3090, 2920–2830, 1680, 1460, 1420, 1390, 1370, 1200, 1170, 1020, 895. ^{1}H NMR δ: 9.81(1H, *d*, $J=8.2$ Hz, H-15), 5.87 (1H, *d*, $J=8.2$ Hz, H-14), 4.90 (1H, *s*, H-17), 4.60 (1H, *s*, H-17′), 3.88 (1H, *ddt*, $J=4.2$, 11.8, 11.8 Hz, H-2), 2.06 (1H, *dd*, $J=4.2$, 13.7 Hz, H-1α) 1.77 (1H, *dd*, $J=4.2$, 14.5 Hz, H-3α), 1.15 (1H, *dd*, J = 11.8, 13.7 Hz, H-1β), 0.99 (3H, *s*, Me-18), 0.94 (1H, *dd*, $J=11.8$, 14.5 Hz, H-3β), 1.98 (3H, *s*, Me-16), 0.88 and 0.77 (3H each, *s*, Me-19 and Me-20). For ^{13}C NMR, see Table 1. MS [70 eV] (%): 305 [M + 1] (0.1), 289 [M–Me]$^+$ (3), 286 [M–H_2O]$^+$ (2), 271 [M–Me–H_2O]$^+$ (13), 253 (6), 203 [286-$CH_2C(CH_3)CHCHO$]$^+$ (10), 188 (6), 187 (96), 185 (6), 175 (10), 173 (7), 161 (8), 160 (7), 159 (11), 147 (23), 136 (11), 135 (85), 133 (34), 131 (16), 123 (12), 122 (15), 121 (36), 120 (21), 119 (43), 117 (12), 109 (22), 108 (14), 107 (21), 106 (38), 105 (16), 104 (22), 96 (18), 95 (17), 94 (35), 93 (19), 92 (37), 91 (29), 84 (100), 80 (34), 79 (47), 67 (36), 55 (46), 43 (21). Found [M + 1$^+$ at *m*/*z* 305.24741. $C_{20}H_{33}O_2$ requires 305.24806.

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