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CHEMICAL DIFFERENCES BETWEEN ALPINE FIRS OF BRITISH COLUMBIA

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Key Word Index—*Abies lasiocarpa*, *A. bifolia*, A. *balsamea*, Pinaceae; juvabione; epijuvabione, 1′*E* and 1′*Z*-dehydrojuvabione, 4′-dehydrojuvabione; 4′-dehydroepijuvabione; juvabiol, epijuvabiol, 1′*E*-dehydrojuvabiol, chemotaxonomy.

Abstract—Alpine fir trees from Vancouver Island contained the sesquiterpenoids *Z*-α-atlantone, *E*-α-atlantone, lasiocarpenone (4*R*,1′*S*), lasiocarpenonol (4*R*,5*R*,1′*S*), juvabione (4*R*,1′*R*), epijuvabione (4*R*,1′*S*), 1′*Z*-dehydrojuvabione (4*R*), 1′*E*-dehydrojuvabione (4*R*), juvabiol (4*R*,1′*R*,3′*S*), epijuvabiol (4*R*,1′*S*,3′*S*), 1′*E*-dehydrojuvabiol (4*R*,3′*S*), 4′-dehydrojuvabione (4*R*,1′*R*), and 4′-dehydroepijuvabione (4*R*,1′*S*). The presence of epijuvabione, 4′-dehydrojuvabione, and 4′-dehydroepijuvabione in coastal alpine fir provides a strong qualitative chemical basis for differentiating coastal alpine fir (*Abies lasiocarpa*) from Rocky Mountain alpine fir of central British Columbia (*A bifolia*).

INTRODUCTION

Alpine fir ranges from the Pacific coast east to central Alberta, and from the Yukon south to New Mexico. Quantitative differences in the amounts of terpenes have formed a basis for differentiating among alpine firs (*Abies* spp.) [1–6] and between alpine and balsam fir [*A. balsamea* (L.) Mill.] of Canada's boreal forest. More recently, Hunt and von Rudloff [2, 3] provided evidence for the separation of Western populations of alpine fir into coastal [*A. lasiocarpa* (Hook.) Nutt.] and Rocky Mountain (*A. bifolia* A Murr) species, with extensive introgression between them The coastal populations on Vancouver Island, British Columbia, are morphologically identical to the *A. lasiocarpa*, collected by David Douglas in coastal Oregon and described by Hooker, according to Hunt and von Rudloff [2, 3]. This position is supported by the recent work of Cope [4].

While this recent finding of Hunt and von Rudloff [2, 3] and Cope [4] is not universally accepted [7], we agree with and accept the separation of these alpine firs growing in British Columbia. Thus the differences reported between the coastal and the Rocky Mountain populations of alpine fir [2–4], earlier reports by Fraser and Swan [8–11], Manville and Kriz [12], and Leach and Thakore [13] on alpine fir extractives of *A. lasiocarpa*, actually describe work on *A. bifolia*.

Fraser and Swan [11] showed that epimeric 4 (*R*)-[1,5′-dimethyl-1′(*R*/*S*)-hydroxy-2′-hexenyl]-1-cyclohexene-1-carboxylic-acid methyl esters (**13**) isolated from Rocky Mountain alpine fir were artifacts of their isolation procedure. These artifacts had been previously reported by Manville and Kriz [12] and Leach and Thakore [13] as extractives of alpine fir wood obtained from interior British Columbia trees and pulp mill effluent, respectively. None of these studies reported the isolation of the postulated precursor dehydroalcohol [12].

Juvabione-type compounds in the wood of *Abies* spp. appear to have chemotaxonomic significance. Several analogues of these insect juvenile hormones have been isolated from European Silver fir (*A alba* Mill.) [14], balsam fir [15–17], Rocky Mountain alpine fir [12], and coastal alpine fir [18] Many sesquiterpenoids isolated from North American grown *Abies* spp. are listed in Table 1 which includes those compounds reported in this study

The separation of balsam fir from the alpine firs is relatively straightforward on the basis of geometric or positional isomers. The double bond of the side chain in diene sesquiterpenoids from *A balsamea* has been shown to be distal (4′–5′), and in *A. bifolia*, proximal (1′–2′). While geometric or positional isomers can be used to differentiate some species, the stereochemical compositions of co-isolates can provide key chemotaxonomic information. *Abies balsamea* and *A. alba* contain sesquiterpene alcohols that have either the *R* or *S* configuration at C-3′ (**9** and **10**) [17, 14], while *A. bifolia* has been shown to contain only alcohols with the *S* configuration at C-3′ [12] *Abies balsamea*, *A bifolia* and *A. alba* contain juvabione (**5**) with *R* chirality at C-1′, they do not contain any epijuvabione (**6**).

We report here that structural differences of specific sesquiterpenoids found in *A bifolia* and *A. lasiocarpa* can differentiate these two true firs.

RESULTS AND DISCUSSION

Sesquiterpenoid components and amounts isolated from each of three coastal alpine firs are listed in Table 2. Numerical assignments are in accordance with their GC elution order on DB-1. Many of these compounds have been previously isolated from alpine fir and other species [8–12, 14–21] The structural assignments were based on chromatographic behaviour, on the analysis of the mass, IR, ^{1}H, and especially ^{13}C NMR spectra of each component (Table 3), and by comparison with previously described compounds [8–12, 14–26].

Table 1. Juvabione-type compounds isolated from North American *Abies* spp

Compound	Configurations	*Abies*		
		*balsamea**	*bifolia*†	*lasiocarpa*‡
Z-α-Atlantone (**1**)	(4*R*)	√	√	√
E-α-Atlantone (**2**)	(4*R*)	√	√	√
Lasiocarpenone (**3**)	(4*R*,1′*S*)	√	√	√
Lasiocarpenonol (**4**)	(4*R*,5*R*,1′*S*)	n	n	√
Juvabione (**5**)	(4*R*,1′*R*)	√	√	√
Epijuvabione (**6**)	(4*R*,1′*S*)		–	√
1′*Z*-Dehydrojuvabione (**7**)	(4*R*)	—	√	√
1′*E*-Dehydrojuvabione (**8**)	(4*R*)	–	√	√
Juvabiol (**9**)	(4*R*,1′*R*,3′*S*)	√	√	√
Isojuvabiol (**10**)	(4*R*,1′*R*,3′*R*)	√		-
Epijuvabiol (**11**)	(4*R*,1′*S*,3′*S*)		√	√
1′*E*-Dehydrojuvabi-3′-ol (**12**)	(4*R*,3′*S*)		-	√
2′-Dehydrojuvabi-1′-ols (**13**)§	(4*R*,1′*R*/*S*)	–	√*	
4′-Dehydrojuvabione (**14**)	(4*R*,1′*R*)	√	–	√
4′-Dehydroepijuvabione (**15**)	(4*R*,1′*S*)	–	-	√
4′-Dehydrojuvabi-3′-ol (**16**)	(4*R*,1′*R*,3′*S*)	—		
3′-Dehydrojuvabi-5′-ol (**17**)§	(4*R*,1′*R*)	√*	–	-

A. balsamea (L.) Mill., balsam fir
†*A. bifolia* A. Murr., Rocky Mountan alpine fir
‡*A. lasiocarpa* (Hook.) Nutt., coastal alpine fir
√ Compound present in the wood.
nCompound not looked for
§These compounds are artifacts, the preceding compound is the extractive present in the wood
— Not present in detectable amounts

This paper reports the first isolation of a 1′*E*-dehydrojuvabiol (**12**), the precursor which has been postulated [12] for the isolated tertiary alcohols (**13**). Compound **12** was obtained from the whole wood of each of three coastal alpine fir trees growing on Vancouver Island. This compound was isolated in a manner similar to that used previously in isolating the 1′-(**13**) [11, 12] and 5′-(**17**) [17] dehydro-tertiary alcohols, except that no acid-washed glassware was used and only HPLC grade solvents were used. Dehydro-alcohol **12** proved to be most stable and showed no tendency to rearrange to the tertiary alcohol mixture, 4(*R*)-[1′,5′-dimethyl-1′(*R*/*S*)-hydroxy-2′-hexenyl]-1-cyclohexene-1-carboxylic acid methyl esters (**13**) [11, 12]. No evidence of the epimeric *E*- or *Z*-dehydro-alcohols was noted. The basic structure of **12** and its acetate (**12Ac**) was determined by extensive ^{1}H and ^{13}C NMR studies (Table 3). It was confirmed by reducing a sample of 1′*E*-dehydrojuvabione (**8**) with sodium borohydride to a mixture of two (1:1) 1′*E*-dehydro-alcohols (**12** and **12b**), one of which was identical to the isolated dehydro-alcohol (**12**) as evidenced by their ^{13}C NMR spectra (Table 3). This isolation provides strong evidence that the tertiary alcohols (**13** and **17**) (Table 1) found in the isolates from *A. bifolia* [11, 12] and *A. balsamea* [17] are artifacts of the isolation/purification procedures.

The occurrence of only one dehydro-alcohol in the wood of *A. lasiocarpa* and the absence of any asymmetric centre at C-1′ precludes the possibility of assigning the stereo-configuration at C-3′ by ^{13}C NMR. However, the isolation of juvabiol (**9**) and epijuvabiol (**11**) from the same wood, each with the *S* configuration at C-3′, indicates (Fig. 1) that for this isolated precursor dehydroalcohol (**12**), the configuration about C-3′ is *S*. We therefore assign the structure of **12** as 4(*R*)-[1′,5′-dimethyl-3′(*S*)-hydroxy-2′-hexenyl]-1-cyclohexene-1-carboxylic acid methyl ester.

Rocky Mountain alpine fir was known to contain varying quantities of epimeric juvabiols—juvabiol (**9**) and epijuvabiol (**11**) in a 10:1 ratio [17]. Coastal alpine fir (Table 1) contains minor amounts of the same two alcohols with the amount of **9** varying from *ca* 7% to less than 2% of the amount of **11**. Each alcohol has the same *S* configuration at C-3′, but differs in stereochemistry at C-1′. The absence of alcohols with the *R* configuration at C-3′ confirms the species differentiation from *A. balsamea*.

The *S* configuration at C-3′ in the alcohols (**9** and **11**, Table 1) supports the postulated biosynthetic pathway (Fig. 1) and is in general agreement with the biosynthetic pathway previously presented by Manville and Kriz [12] for Rocky Mountain alpine fir. The notable difference in the current biosynthetic pathway is the presence of significant quantities of a naturally occurring epimer of juvabione (**5**), namely epijuvabione (**6**) and the presence of dehydrojuvabione (**14**) and 4′-dehydroepijuvabione (**15**).

The isolation of **7** in admixture with **5** and **6** afforded the opportunity to assign the ^{13}C NMR chemical shifts of all carbons (Table 3). Comparison of the shifts of C-3, C-4, C-5 and C-6 of the *E* and *Z* pair of 1′-dehydrojuvabiones (**7** and **8**) and the corresponding pair of α-atlantones (**1** and **2**) [18] indicates that the preferred conformation for **7** is similar to that of **1**, as shown in Fig. 1.

Epijuvabione (**6**) and 4′-dehydroepijuvabione (**15**) were not previously known to occur in the North American-grown firs [12, 15, 16]. Cerny *et al.* [21], reported the occurrence of **6** and **15** in balsam fir, but the work was conducted on a putative 4 *balsamea* cross growing in a

Table 2. *Abies lasiocarpa*, lipophilic extractives.

					% composition (GC) of sesquiterpenoid hexane solubles									
			Yield (% o.d. wood)		Un†	1	2	3	4	5,6,7	8	9,11	12	14,15
										R_t(min)				
Tree	Site*	Age	C_6H_6/EtOH	Hexane	7.3	7.4	7 9	8.3	8.8	9.8	10.0	10.1	10.2	10.3
1	1	73	2.52	0.72	4.0	4 3	35.7	8.0	4.0	11.5	6.2	3 1	8.1	0.8
2	2	36	2.60	0.78	2 7	4.8	32.9	3.6	2.0	8.3	19.7	5 8	10.6	0.9
3	2	56	2.38	0 93	1.5	2.4	15 9	29 5	10.2	6.3	6.9	3.1	8 2	5.7

*Site locations· (1) Mount Washington, west of Courtenay, B.C., (2) Green Mountain, southwest of Nanaimo, B.C.
†Un, unknown compound.

Table 3 ^{13}C NMR chemical shifts (δ) in $CDCl_3$

	C*														
Compound†	1	2	3	4	5	6	7	1′	2′	3′	4′	5′	6′	7′	8′
Juvabione (**5**)	129 9	138.8	29.4	37.5	24 5	24 5	167.2	32.4	47 4	209 7	52 2	24 2	22.3	22.3	16.2
Epijuvabione (**6**)	129 9	138 8	28.2	37.4	25.8	24 6	167.2	32.6	47.5	209.7	52.1	24.2	22 2	22.2	16.1
Z-1′-Dehydrojuvabione (**7**)	130 1	138.7	29 6	35.1	26.4	24 9	167.5	160.2	124.9	201 2	53.3	24.3	22.4	22.4	20.2
E-1′-Dehydrojuvabione (**8**)	130 0	138 0	30 4	43 2	26.5	25.0	167 4	159.9	122.6	201.2	53.4	24 2	22.4	22.4	17.2
E-1′-Dehydrojuvabiol (**12**)	130 1	139.0	31.0	41 8	31.0	29 7	167 8	141 1	127 9	66.9	47 0	24 7	22.6	24.7	14 7
E-1′-Dehydroisojuvabiol (**12b**)	130.1	139 0	31.0	41 7	31 0	29.7	167 8	141 0	127 8	66.8	47.0	24 6	23 0	24 6	14.6
E-1′-Dehydrojuvabiol acetate (**12Ac**)‡	129 9	138 8	30 8	41 5	26.8	24.4	167 7	142.7	123.4	70 0	44.0	24.5	22 7	22.5	14.8
4′-Dehydrojuvabione ((**14**)	130.0	139.1	29 5	37 5	24.6	24.6	167.7	33.1	48.5	200.6	123 8	155 0	20.4	27.4	16.3
4′-Dehydroepijuvabione(**15**)	130.0	139 1	28.2	37 5	25 9	24.7	167 7	33 3	48.7	200.6	123.8	155 0	20.4	27 4	16 1

*See **1–17** for position numbering.
†The methyl ester carbon resonates at δ51.3 $\pm$0.2 ppm.
‡The acetate carbons are found at δ169.9 and 21 0 $\pm$0.2 ppm

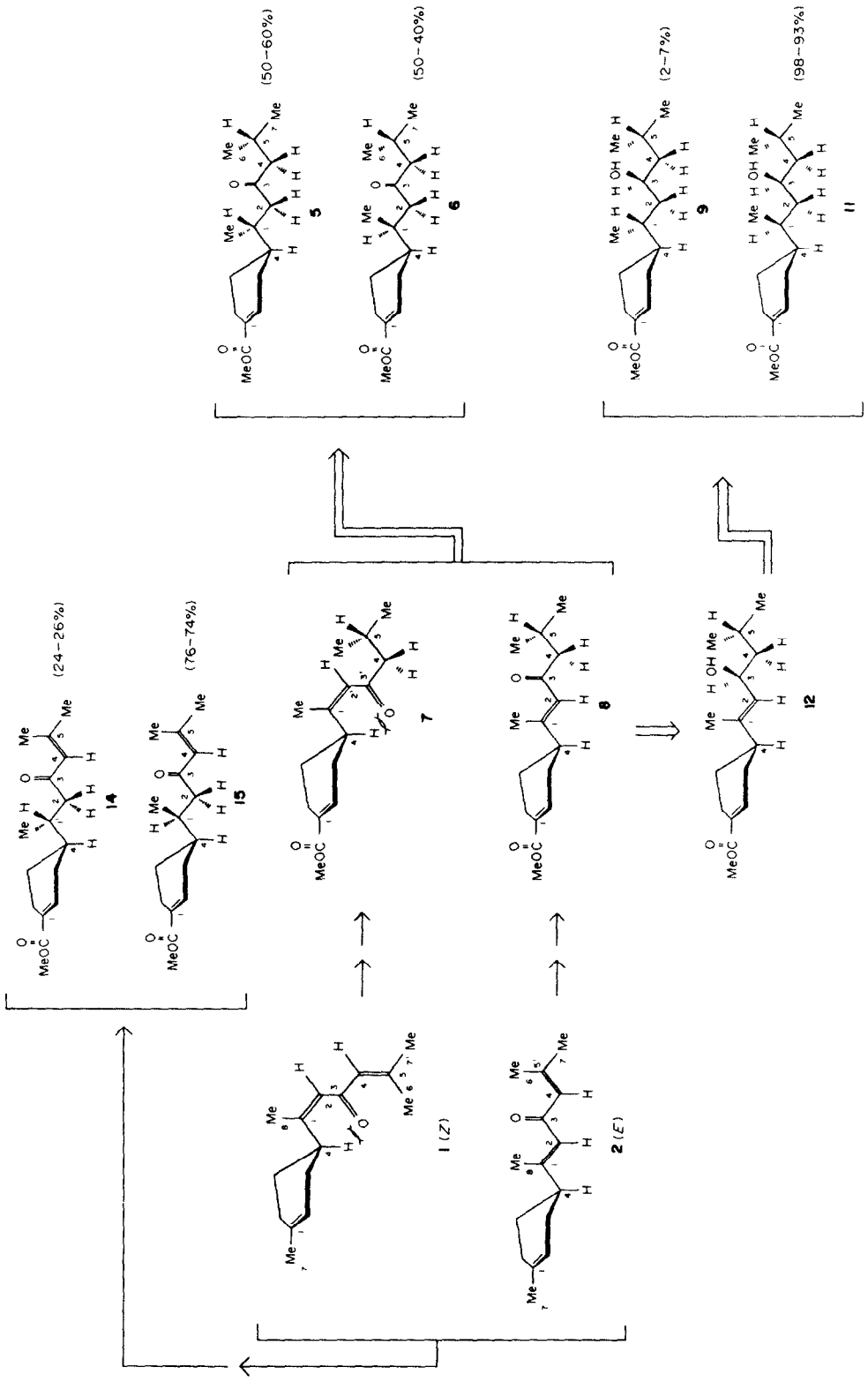
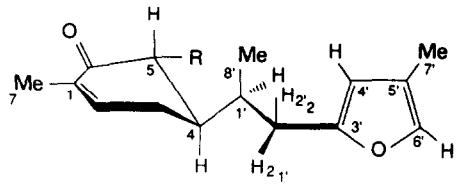
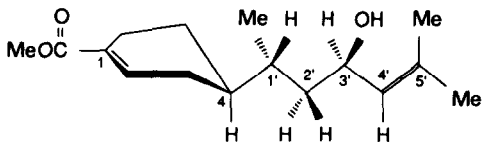


Fig 1 Postulated biosynthetic pathways for juvabione-type sesquiterpenoids of coastal alpine fir.



3 R = H

4 R = OH



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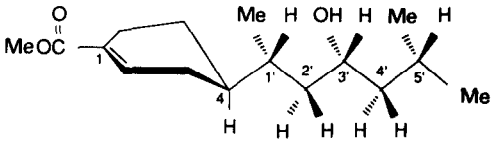

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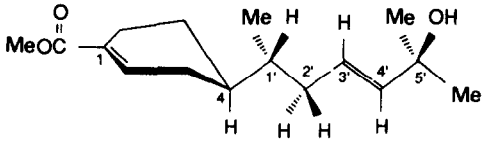

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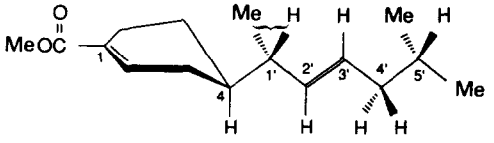

13

European arboretum [16, 19]. The presence of **5** in *A. bifolia* and **5**, **6**, **14** and **15** in the whole wood of Vancouver Island grown alpine firs provides the basis for a chemotaxonomic differentiation between *A. lasiocarpa* and *A. bifolia*.

EXPERIMENTAL

Isolation. The compounds used for this study were isolated from air-dried, debarked whole wood of branches from coastal alpine firs from Green Mountain and Mount Washington on Vancouver Island using methods described elsewhere [9, 12, 16, 17].

General. The ^1H and ^{13}C NMR spectra were determined on samples dissolved in $CDCl_3$ ($\delta^1H = 7.26$ at 400 MHz and $\delta^{13}C = 76.88$ at 100.6 MHz) at ambient temperatures (21°) using a Brucker 400 MHz NMR spectrometer. Samples for proton spectra were about a factor 20 more dilute than those for the carbon spectra Low resolution mass spectra were determined on a Finnigan GC/MS at the Institute of Ocean Sciences, Pat Bay and on a Finnigan mass spectrometric detector (ION-TRAP®). GC analyses were performed on a Spectra-Physics model 7100 GC equipped with a flame ionization detector (FID) and the ION-TRAP. The columns used were 30 m × 0.25 mm (i.d.) DB-1 fused silica columns The column oven was programmed as follows: 200° (1 min), 10° per min to 275° (15 min). The first split/splitless injector was maintained at 250°, the second was a J&W cool on-column injector, for the ION-TRAP detector. The FID detector was kept at 295°. The carrier gas was maintained at 150 psig in the split/splitless injector port and at 11 psig in the cool on-column injection port. Butane gas had a linear velocity of 22 cm^{-1}. Extractions and separations were similar to those reported earlier [9, 12, 16, 17], except for the purification of **4** which was accomplished by Al_2O_3 CC using 1% EtOH in benzene [see 18]

Z-α-Atlantone (**1**). Identification of **1** was made on the basis of its ^1H, ^{13}C NMR, IR and MS comparison with published information [12, 18, 22–25]

E-α-Atlantone (**2**). Identification of **2** was made on the basis of its ^1H, ^{13}C NMR, IR and MS and comparison with published information [12, 18, 22–25].

Lasiocarpenone (**3**). Identification of **3** was made on the basis of its ^1H, ^{13}C NMR, IR and MS and comparison with published information [10, 18].

Lasiocarpenonol (**4**). See ref. [18] for the details of the identification of **4** which was made on the basis of its ^1H, ^{13}C NMR, IR and MS and comparison with **3**.

Juvabione (**5**), *epijuvabione* (**6**) and *1'Z-dehydrojuvabione* (**7**) Identifications of **5–7** were made on the basis of their ^1H, ^{13}C NMR (Table 3), and comparison with published information [12–21].

1'E-Dehydrojuvabione (**8**). Identification of **8** was made on the basis of its ^1H and ^{13}C NMR, IR and MS and published information and comparison with other structurally related compounds [12–21] ^1H NMR: δ6.94 (1H, *m*, H-2), 6 01 (1H, *br s*, H-2'), 3 68 (3H, *s*, –OMe), 2.44 (1H, *br d*, J = 17.5 Hz,H-3e), 2.25 (2H, *d*, J = 7 1 Hz, H-4'), 2.07 (3H, *d*, J = 1 Hz, C-1' –Me), 2.05 (1H, *m*, H-5'), 1.83 (1H, *m*, H-5e), 1.49 (1H, *dtd*, J = 5 5, 11 and 13 Hz, H-5a), 0.87 [6H, C-5'-$(Me)_2$], 2.3–1.9 (6H); ^{13}C NMR: see Table 3; MS. no $[M]^+$, 232 (3), 207 (80), 204 (31), 175 (42), 147 (100), 137 (21), 119 (60), 105 (30), 95 (75), 91 (31), 85 (60), 79 (21), 77 (27), 69 (95), 67 (35), 57 (32) and 41 (30).

Juvabiol (**9**) and *epijuvabiol* (**11**). Identifications of **9** and **11** and their acetates were made on the basis of their ^1H, ^{13}C NMR, and comparison with published information [12–14, 17, 26].

1'E-Dehydrojuvabi-3'-ol (**12**). ^1H NMR δ 7.00 (1H, *m*, H-2), 5.20 (1H, *br d*, J = 6.5 Hz, H-2'), 4.5 (1H, *dd*, J = 6 and 8 Hz, H-3'), 3 73 (3H, –OMe), 1.70 (1H, *d*, J = 2.1 Hz, 1' -Me), 0.93 (3H, *d*, J = 6.5 Hz, 5' -Me), 0 92 (3H, *d*, J = 6.5 Hz, 5' -Me) and 2.5–1.4

(11H), ^{13}C NMR see Table 3, MS. no $[M]^+$, 234 (5), 209 (7), 207 (6), 177 (66), 150 (28), 149 (40), 139 (43), 134 (21), 131 (17), 127 (19), 121 (21), 107 (39), 105 (28), 97 (32), 93 (50), 91 (35), 85 (100), 81 (47), 79 (70), 77 (33), 71 (95), 69 (42), 67 (38), 59 (34), 57 (99), 55 (39), 53 (48), 43 (77), and 41 (98)

Acetylation of **12** Compound **12** was dissolved in pyridine and Ac_2O and left at room temp overnight to afford 1′*E*-dehydrojuvabi-3′-ol acetate (**12**Ac) ^{1}H NMR δ 6 97 (1H, *m*, H-2), 5 58 (1H, *m*, H-3′), 5 08 (1H, *br d*, J = 9 0 Hz, H-2′), 3 72 (3H, *s*, –OMe), 2 43 (1H, *br d*, J = 18 Hz, H-3e), 2 27 (1H, *m*, H-6e), 2 22 (1H, *m*, H-3a), 2 11 (1H, *m*, H-4), 2 10 (1H, *m*, H-6a), 2 00 (3H, *s*, –Ac), 1 82 (1H, *m*, H-5e), 1 75 (3H, *d*, J = 2 1 Hz, 1′ -Me), 1 55 (2H, *m*, H-4′e and H-5a), 1.47 (1H, *m*, H-5′), 1 30 (1H, *m*, H-4′a), 0 90 (3H, *d*, J = 6 5 Hz, 5′ -Me), 0 88 (3H, *d*, J = 6 5 Hz, 5′ -Me); ^{13}C NMR see Table 3.

Reduction of **8** *with* $NaBH_4$ The 1′*E*-Dehydrojuvabiols, compounds **12** and **12b**, were obtained by $NaBH_4$ reduction of **8** as a 1 1 mixture ^{1}H NMR of **12** and **12b** was similar to that of **12**. ^{13}C NMR see Table 3, MS no $[M]^+$, 234 (5), 209 (8), 207 (7), 177 (65), 150 (30), 149 (37), 139 (41), 121 (21), 107 (40), 105 (25), 97 (30), 93 (50), 91 (30), 85 (100), 81 (45), 79 (70), 69 (42), 67 (38), 59 (34), 57 (98), 55 (38), 43 (77) and 41 (95)

4′-*Dehydrojuvabione* (**14**) and 4′-*dehydroepijuvabione* (**15**). Identification of **14** and **15** were made on the basis of MS, ^{1}H, ^{13}C NMR, and comparison with published information [12–14, 16, 19–21]

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