

# Sesquiterpene Hydrocarbons in Glandular Trichome Exudate of *Rosa rugosa* Leaves

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From glandular trichome of *Rosa rugosa*, three major sesquiterpene hydrocarbons [acora-3(4),7(15)-diene, carota-4(5),11(12)-diene (isodaucene) and carota-1,4-diene], and two minor [daucene and acora-3(4),7(8)-diene] were identified. The presence of these carotadienes, which are possibly precursors of the corresponding C-14-oxygenated carotanoids, suggested that the formation of the carotane skeleton followed by the regio-specific oxygenation at C-14 occur in the biosynthesis of *R. rugosa* carotanoids.

## Introduction

As reported in our previous paper [1], a carotane peroxide rugosal A and its related compounds, carota-1,4-dienaldehyde (7) and rugosic acid A, were found in the glandular trichome exudate of *Rosa rugosa* leaves in high concentration. The presence of rugosal A in the exudate suggested a defensive role of the glandular trichome, because of marked fungitoxicity of this sesquiterpene peroxide [2]. In addition to these major carotane sesquiterpenes, some other compounds, minor carotanes, non-carotane (bisabolane and acorane) sesquiterpenes and 2-phenoxychromones were also identified as constituents of the exudate. Since it was suggested that biosynthesis of the carotane sesquiterpenes is highly active in the tip cells of the glandular trichomes, precursor sesquiterpene hydrocarbons were expected to be present in the exudate. In the present investigation of less polar fractions, some sesquiterpene hydrocarbons were detected, and three major and two minor compounds were identified by means of spectroscopic analyses or direct comparison with chemically derivatized authentic compounds. The presence of these hydrocarbons may prove the occurrence of regio-specific oxygenation at C-14 of carotane sesquiterpene after formation of the bicyclic skeleton. The skeleton-specific oxygenation at the methyl carbon

originated from C-13 of farnesyl pyrophosphate is discussed.

## Results and Discussion

When leaf rinses were chromatographed in a silica gel column, a non-quenching spot less polar than carotane C-14-aldehydes [3, 4] and showing a reddish purple coloration with vanillin-sulphate reagent was detected on TLC. By gas-liquid chromatography (GLC: Chrompac CP-Wax 52 CB, capillary column 10 m), a column fraction eluted with chloroform (7.3 mg from methanol rinses of 200 g leaves, collected in May) was found to be a mixture of sesquiterpene hydrocarbons. More amounts of the sesquiterpene hydrocarbon mixture were obtained from methanol extracts from 6.0 kg of *R. rugosa* leaves, and each constituent in the fraction was separated by prep. TLC (Kieselgel 60 F<sub>254</sub> impregnated with AgNO<sub>3</sub>, in *n*-hexane-Et<sub>2</sub>O = 25:1) with a guide of vanillin-sulphonic acid spray reagent.

Compound **1** appearing as the largest peak (32%, *t*<sub>R</sub> 7.8 min) showed an M<sup>+</sup> at *m/z* 204.193 (C<sub>15</sub>H<sub>24</sub>, calc. 204.188) in GC mass spectrometry. The <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> [an isopropyl (δ<sub>H</sub> 0.94 and 0.85, each d, *J* = 7 Hz), an allylic methyl (δ<sub>H</sub> 1.65, br. s), and an exomethylene groups (δ<sub>H</sub> 4.76, dd, *J* = 2 and 2 Hz, and 4.72, d, *J* = 2 Hz)] and <sup>13</sup>C NMR data, both similar to those of rosacorenone (**2**) previously isolated from *R. rugosa* leaves [4], were attributable to acora-3(4), 7(15)-diene. The relative configuration of **1**

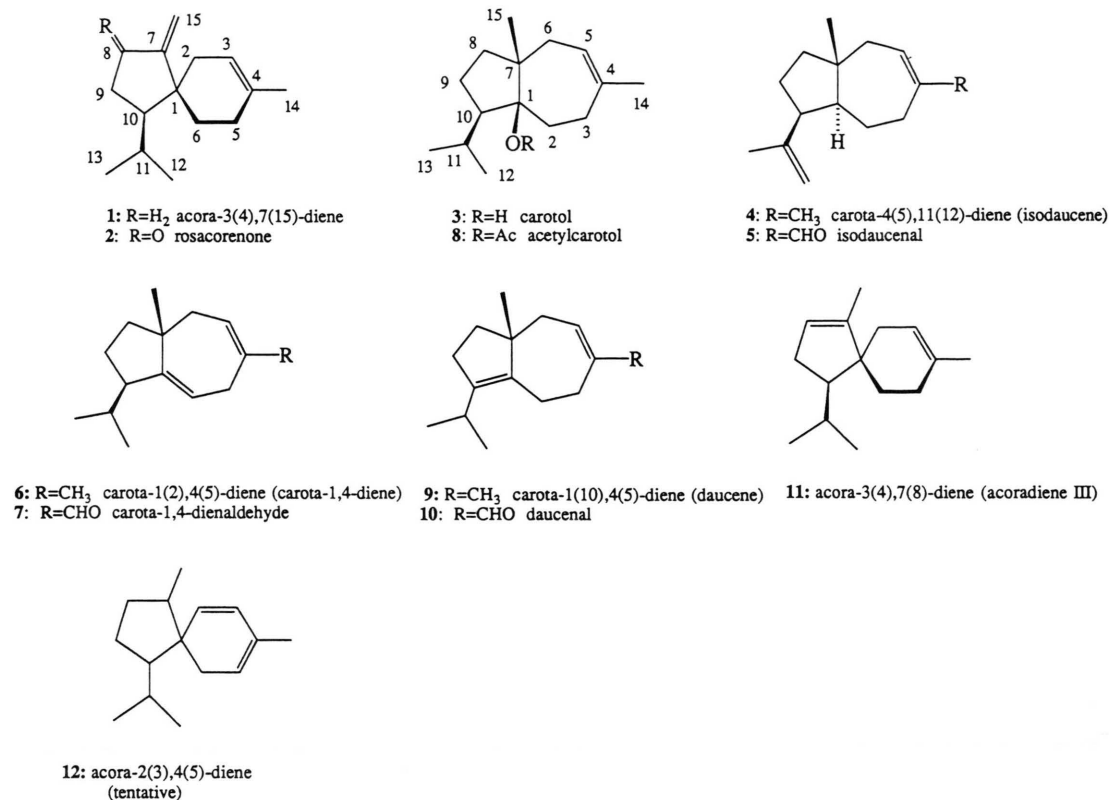


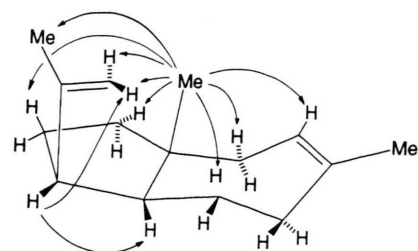
Fig. 1. Sesquiterpene hydrocarbons from glandular trichome exudates of *Rosa rugosa* and their related compounds.

was expected to be the same with that of **2**. Acora-3(4),7(15)-diene has already been reported from *Calea prunifolia* (Compositae) by Castro *et al.* [5], and its stereostructure was unambiguously elucidated by chemical derivatization from carotol (**3**) [6]. Because the spectroscopic data ( $[\alpha]_D^{25}$ ,  $^1\text{H}$  NMR and EI mass spectrometry) of isolated **1** showed a good agreement with those of the derivative from **3**, the expected stereostructure of **1** seemed reasonable.

Carota-4(5),11(12)-diene (isodaucene, **4**) was isolated as the second major compound (11%, *t**R* 9.0 min) in the mixture. In the  $^1\text{H}$  NMR spectrum, an isopropenyl ( $\delta_{\text{H}}$  1.74 for allylic methyl proton, and  $\delta_{\text{H}}$  4.78 and 4.70 for exomethylene protons), C-14 allylic methyl ( $\delta_{\text{H}}$  1.70, br. s) and C-15 bridgehead methyl ( $\delta_{\text{H}}$  0.81, s) groups were detected to formulate a carotane skeleton corresponding to that of isodaucenal (**5**) [4]. The C-10 methine proton resonated downfield at  $\delta_{\text{H}}$  2.90 (showing a cross peak with a methinic  $\delta_{\text{C}}$  50.3, in CH-COSY).

This characteristic downfield shift due to its allylic position was also observed in **5** having the C12/C13-double bond. It was expected that compound **4** has the same relative configuration with that of **5** whose relative stereostructure has already been determined by using NOE experiment [4]. The NOE experiments on **4** were therefore carried out. When compound **4** was irradiated at C-10 proton ( $\delta_{\text{H}}$  2.95), 6% NOE on C-1 proton ( $\delta_{\text{H}}$  1.778, br. dd,  $J = 11$  and 11 Hz, cross peak with methinic  $\delta_{\text{C}}$  56.8) was observed to prove the *cis*-orientation regarding C-1 and C-10 protons. The other NOEs observed by irradiation at C-15 methyl protons (Fig. 2) was indicative of its relative configuration being the same as that of **5**.

A carotadiene being postulated to have the same planar structure as that of **4** but epimeric at C-10 has been reported by Zdero and Bohlmann from *Vernonia galpinii* (Compositae) [7]. Some spectroscopic data of this Compositae carotadiene are slightly different (*e.g.* separated C-9 and C-13 car-



Irradiated proton	NOE-observed proton	(NOE %)
0.81 (C-15-H <sub>3</sub> )	5.38 (C-5-H)	0.5
	4.71 (C-12-Hb)	0.5
	2.07 (C-6-Ha)	1
	1.76 (C-9-Ha)	0.5
	1.70 (C-13-H <sub>3</sub> )	2
	1.49 (C-8-Ha)	1
	1.24 (C-2-Hb)	1
2.95 (C-10-H)	4.71 (C-12-Hb)	2
	1.78 (C-1-H)	6

Fig. 2. NOEs observed on compound **4** (500 MHz, in CDCl<sub>3</sub>).

bons in <sup>13</sup>C NMR overlapped each other in **4**); however, most of the physicochemical data showed good agreement (e.g. <sup>1</sup>H NMR spectrum and ORD curve) with those of **4** (see Experimental and ref. 7). Accordingly, the carotadiene of *V. galpinii*-origin should have the same stereostructure as that of **4** due to the agreement in the <sup>1</sup>H NMR data, otherwise the epimeric compound may show some clearly distinguishable proton signals from those of **4** because of an olefinic bond in the isopropenyl side chain attached to C-10. Indeed, stereostructure of the Compositae carotadiene is not reliable because it was only based on coupling constants (e.g.  $J_{1-H:10-H} = 10$  Hz). From our observation on carotane sesquiterpenes [2–4], *trans*-coupling constant on a five-membered ring system is almost indistinguishable from that of *cis*-coupling. However, the revised structure for the carotadiene of *V. galpinii*-origin is still a matter of conjecture. The fact that acoradiene (**1**) and carotadiene (**4** or its epimer) found in Compositae [5] also coexisted in *R. rugosa* indicated a close correlation between **1** (acorane) and **4** (carotane) from a biosynthetic viewpoints [8].

The third compound **6**, showing a similar EIMS fragmentation and <sup>1</sup>H NMR signals to those of

carota-1,4-dienaldehyde (**7**) (e.g.,  $m/z$  161 and 105 in GCMS, and two olefinic protons at  $\delta_H$  5.42 and 5.18, and bisallyl methylene protons at  $\delta_H$  2.90 and 2.75 in <sup>1</sup>H NMR), was identified to be carota-1,4-diene in spectroscopic comparison with that chemically derived from carotol acetate (**8**) by pyrolysis *via cis*-elimination of acetic acid [9]. The *tR* in GLC (8.2 min, Chrompac CP-Wax 52CB), GC mass spectrometry and <sup>1</sup>H NMR spectrum of the derivatized **6** showed good accordance with those of the isolate from *R. rugosa*. The stereostructure of natural and synthesized **6** both showing the same ORD curve (laevorotatory 650 nm–300 nm) was confirmed as 7*R*:10*R* [9]. Stereochemistry of **6** was thus found to be the same with that of **6** at the C-7 and C-10 chiral carbons [3, 10], suggesting that **6** was the precursor of **7**. The presence of **7** may indicate that carota-1,4-diene skeleton is produced before the regio-specific oxygenation at C-14 in the main biosynthetic route to rugosal A. This is the first isolation of compound **6** as a naturally occurring sesquiterpene hydrocarbon.

Compound **9** (*tR* 4.9 min, the first peak) was identified as daucene in comparison of *tR* in GLC and GC mass spectrometry with authentic daucene prepared as described in Experimental, although the isolation of this compound was unsuccessful because of overlapping with a large amount of **1**. Thus, daucene (**9**) was confirmed to be contained in the glandular trichome exudate of *R. rugosa* leaves. The presence of **9**, originally found as a constituent of carrot seed oil [11], was reasonable because the corresponding C-14-oxocarotane, daucenal (**10**) has already been isolated [4].

Compound **11** (*tR* 7.4 min) in the hydrocarbon fraction agreed in GC mass spectrometry ( $M^+$  204.183, C<sub>15</sub>H<sub>24</sub>) with authentic acora-3(4), 7(8)-diene prepared from **8**. The mass and NMR spectra showed good accordance also with the reported data of **11** which has been isolated from *Vetiveria zizanioides* (Gramineae) [12]. On the other hand, the constituent appearing as forth peak (*tR* 6.7 min) also showed an  $M^+$  at  $m/z$  204.187, C<sub>15</sub>H<sub>24</sub>). This compound detected as a spot with  $R_f$  0.79 on AgNO<sub>3</sub>-impregnated thin-layer plates was tentatively identified as **12**, because <sup>1</sup>H NMR spectrum of the isolate well agreed with acora-2(3), 4(5)-diene reported from *C. prunifolia* [5]. However, stereochemistry of the isolate has not been characterized yet. The presence of these minor

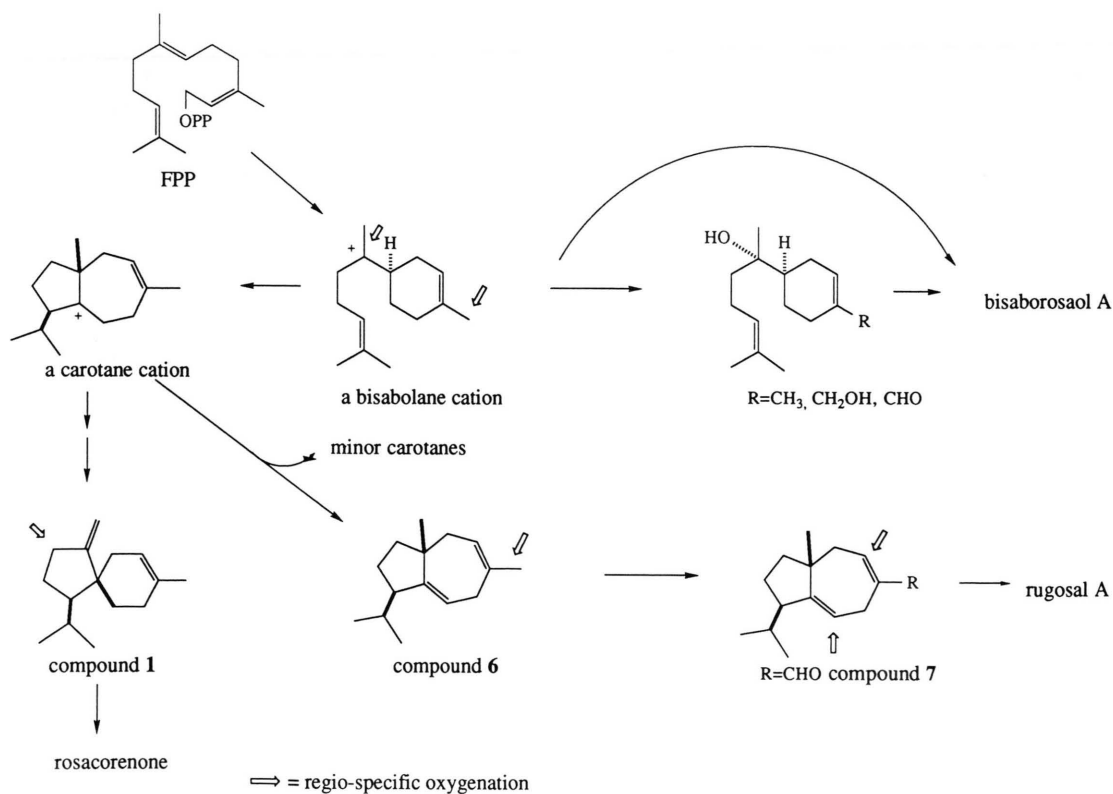
acoradienes also supported the biosynthetic correlation between acoranoids and carotanoids of *R. rugosa* leaves.

The presence of these sesquiterpene hydrocarbons in the trichome exudate suggested that the cyclization of *cis*, *trans*-farnesyl pyrophosphate (FPP) actively occurred in the multicellular tip of the glandular trichomes. As we have reported in our earlier papers, carotanoid, bisabolanoïd and acoranoid composed sesquiterpenes in the trichome exudate of *R. rugosa* [1–4, 10, 13]. Although these three sesquiterpenoids have the same precursor FPP, oxygenation and modification at the allylic methyl group (C-14 in carotane and acorane, and C-7 in bisabolane; all originated from C-13 methyl group of FPP) are different in each skeleton. In the bisabolanoïd, the C-7 is mostly oxygenated to a carboxylic carbon and further modified into a methoxy carbonyl carbon [13]. On the contrary, acoranoids of *R. rugosa* found so far all remained unmodified at the methyl group [4]. The carotanoids show several varia-

tions, methyl, hydroxylmethyl, formyl, carboxylic and methoxy carbonyl carbons at C-14, and formyl and carboxylic forms are major in this sesquiterpenoid. In the sesquiterpene hydrocarbons from the trichome exudate, acoranoid **1** was the most major component, while bisabolane hydrocarbons in the fraction were only a trace amount in GLC analysis. This fact may suggest that bisabolanoïds and some carotanoids provide good substrates for enzymatic oxygenation at the allylic methyl group, while the enzyme system may be inactive on acoranoids. Formation of these sesquiterpenoids is shown in Scheme 1.

### Experimental

Glandular trichome exudates (1.75 g, rinsed out by methanol) of *R. rugosa* leaves (200 g fr. wt.) were chromatographed in a silica gel column, and the fractions eluted with 10–20% EtOH/CHCl<sub>3</sub> (750 mg) were re-chromatographed as described in our previous paper [1]. Fraction FrB-3 showed a



Scheme 1. Hypothetical formation of *R. rugosa* sesquiterpenoids from FPP.

non-quenching and less polar spot than that of carota-1,4-dienaldehyde (**7**) on Kieselgel 60 F<sub>254</sub> plate ( $R_f$  0.99 in *n*-hexane-EtOAc = 4:1; *cf.* **7**:  $R_f$  0.88). This spot showing a pinkish red with vanillin-sulphate reagent was obtained by prep. TLC as a mixture of sesquiterpene hydrocarbons (7.3 mg). In GLC analyses (Chrompac CP-Wax 52 CB, 10 m × 0.25 mm i.d., 5 °C/min from 50 °C, initial time 1 min), **1** ( $t_R$  7.8 min, rel. int. 32%), **4** (9.0 min, 20%), **6** (8.2 min, 11%), **9** (4.9 min, 3%), **11** (7.4 min, 3%), **12** (6.7 min, 6%) and two unknown (5.2 min and 7 min; 5% and 8%, respectively) were detected as major peaks. More amounts of the mixture (*ca.* 200 mg) were obtained from an earlier fraction of hexane solubles prepared from methanol extracts of 6.0 kg leaves. These sesquiterpene hydrocarbons were eluted with 2% EtOAc/*n*-hexane from a silica gel column [3]. Each compound detected from the trichome exudate was then isolated by prep. TLC (Kieselgel 60 F<sub>254</sub> plates sprayed 5% AgNO<sub>3</sub> and dried at 120 °C for 30 min, developed in *n*-hexane-Et<sub>2</sub>O = 25:1). Major spots were scraped out and eluted with EtOAc, and the isolates were respectively correlated to GLC peaks. NMR data of compounds **9** and **11** were taken by the derivatized ones, respectively.

**Compound 1** [acora-3(4), 8(15)-diene]. An oil.  $R_f$  0.38 (Et<sub>2</sub>O-*n*-hexane = 25:1 on 5% AgNO<sub>3</sub> silica gel). Vanillin-sulphate: bluish purple.  $[\alpha]_D^{23} + 34^\circ$  (*c.* 0.15, in CHCl<sub>3</sub>). HRMS: 204.193 (C<sub>15</sub>H<sub>24</sub>, calc. 204.188). GCMS  $m/z$  (rel. int.): 204 (M<sup>+</sup>, 5.4), 189 (2.0), 176 (3.3), 162 (16), 161 (100), 133 (15), 119 (32), 105 (58), 94 (71), 93 (50), 91 (37), 81 (34), 79 (49), 77 (25), 67 (15), 55 (19), 41 (49). <sup>1</sup>H NMR δ(270 MHz, CDCl<sub>3</sub>): 5.36 (br. s, 3-H), 4.76 (dd,  $J = 2$  and 2 Hz, 15-Ha), 4.72 (d,  $J = 2$  Hz, 15-Hb), 1.65 (br. s, 14-H<sub>3</sub>), 0.94 (d,  $J = 7$  Hz, 12-H<sub>3</sub>), 0.85 (d,  $J = 7$  Hz, 13-H<sub>3</sub>). <sup>13</sup>C NMR δ(68 MHz, CDCl<sub>3</sub>, DEPT and CH-COSY): 160.5 (7-C), 133.7 (4-C), 120.5 (3-CH), 103.5 (15-CH<sub>2</sub>), 53.9 (10-CH), 46.3 (1-C), 36.6 (2-CH<sub>2</sub>), 30.3 (8-CH<sub>2</sub>), 27.8 (11-CH), 27.4 (5-CH<sub>2</sub>), 24.9 (6-CH<sub>2</sub>), 23.92 (9-CH<sub>2</sub>), 23.87 (14-CH<sub>3</sub>), 23.1 (12-CH<sub>3</sub>), 20.8 (13-CH<sub>3</sub>).

**Compound 4** [carota-4(5),11(12)-diene, isodaucene]. An oil.  $R_f$  0.27 (Et<sub>2</sub>O-*n*-hexane = 25:1 on 5% AgNO<sub>3</sub> silica gel). Vanillin-sulphate: pinkish purple.  $[\alpha]_D^{23} + 13^\circ$  (*c.* 0.16, in CHCl<sub>3</sub>). HRMS: 204.187 (C<sub>15</sub>H<sub>24</sub>, calc. 204.188). GCMS  $m/z$  (rel. int.): 204 (M<sup>+</sup>, 19), 189 (34), 175 (4.5), 163 (8.5), 161 (13), 147 (20), 134 (29), 133 (28), 121 (58), 119

(29), 107 (47), 105 (26), 95 (23), 94 (34), 93 (100), 91 (29), 81 (32), 79 (45), 68 (94), 67 (43), 55 (32), 53 (29), 41 (59). <sup>1</sup>H NMR δ(270 MHz, CDCl<sub>3</sub>): 5.38 (br. d,  $J = 9$  Hz, 5-H), 4.78 (dd,  $J = 2$  and 1 Hz, 12-Ha), 4.70 (dd,  $J = 2$  and 1 Hz, 12-Hb), 2.95 (ddd,  $J = 11$ , 9 and 9 Hz, 10-H), 2.08 (dd,  $J = 15$  and 9 Hz, 6-Ha), 1.74 (s, 14-H<sub>3</sub>), 1.70 (d,  $J = 1$  Hz, 13-H<sub>3</sub>), 0.81 (s, 15-H<sub>3</sub>). <sup>13</sup>C NMR δ(68 MHz, CDCl<sub>3</sub>, DEPT and CH-COSY): 148.1 (11-C), 138.8 (4-C), 122.7 (5-CH), 112.7 (12-CH<sub>2</sub>), 56.8 (1-CH), 50.4 (10-CH), 42.6 (7-C), 42.4 (6-CH<sub>2</sub>), 42.0 (8-CH<sub>2</sub>), 35.4 (3-CH<sub>2</sub>), 28.3 (2-CH<sub>2</sub>), 27.6 (14-CH<sub>3</sub>), 23.1 (13-CH<sub>3</sub>), 23.5 in C<sub>6</sub>D<sub>6</sub>), 23.1 (9-CH<sub>2</sub>), 23.2 in C<sub>6</sub>D<sub>6</sub>), 19.3 (15-CH<sub>3</sub>).

**Compound 6** [carota-1(10),4(5)-diene, carota-1,4-diene]. An oil.  $R_f$  0.55 (Et<sub>2</sub>O-*n*-hexane = 25:1 on 5% AgNO<sub>3</sub> silica gel). Vanillin-sulphate: pinkish purple.  $[\alpha]_D^{23} - 121^\circ$  (*c.* 0.34 in CHCl<sub>3</sub>). HRMS: 204.184 (C<sub>15</sub>H<sub>24</sub>, calc. 204.188). GCMS  $m/z$  (rel. int.): 204 (M<sup>+</sup>, 11), 189 (3.9), 183 (8.7), 162 (13), 161 (100), 133 (15), 119 (51), 105 (82), 93 (32), 91 (32), 81 (82), 79 (28), 77 (17), 67 (16), 55 (28), 41 (41). <sup>1</sup>H NMR δ(270 MHz, CDCl<sub>3</sub>): 5.42 (br. d,  $J = 7$  Hz, 5-H), 5.18 (ddd,  $J = 6$ , 3 and 3 Hz, 2-H), 2.90 (br. d,  $J = 22$  Hz, 3-Ha), 2.75 (br. dd,  $J = 22$  and 6 Hz, 3-Hb), 2.42 (br. m, 10-H), 2.25 (br. d,  $J = 16$  Hz, 6-Ha), 2.00 (dd,  $J = 16$  and 7 Hz, 6-Hb), 1.86 (sept d,  $J = 7$  and 6 Hz, 11-H), 1.69 (d,  $J = 0.5$  Hz, 14-H<sub>3</sub>), 1.03 (s, 15-H<sub>3</sub>), 0.93 (d,  $J = 7$  Hz, 12-H<sub>3</sub>), 0.78 (d,  $J = 7$  Hz, 13-H<sub>3</sub>). <sup>13</sup>C NMR δ(68 MHz, CDCl<sub>3</sub>, DEPT and CH-COSY): 152.9 (1-C), 133.6 (4-C), 122.1 (5-CH), 116.0 (2-CH), 52.5 (10-CH), 45.5 (7-C), 41.5 (8-CH<sub>2</sub>), 40.9 (6-CH<sub>2</sub>), 35.0 (3-CH<sub>2</sub>), 29.2 (11-CH), 26.7 (14-CH<sub>3</sub>), 23.7 (15-CH<sub>3</sub>), 23.4 (9-CH<sub>2</sub>), 21.8 (12-CH<sub>3</sub>), 17.0 (13-CH<sub>3</sub>).  $[\alpha]_D$  and <sup>13</sup>C NMR data were taken using derivatized one.

**Compound 9** (daucene). An oil.  $R_f$  0.39 (Et<sub>2</sub>O-*n*-hexane = 25:1 on 5% AgNO<sub>3</sub> silica gel). Vanillin-sulphate: bluish purple. HRMS: 204.188 (C<sub>15</sub>H<sub>24</sub>, calc. 204.188). GCMS  $m/z$  (rel. int.): 204 (M<sup>+</sup>, 4.3), 189 (4.7), 175 (2.8), 162 (21), 161 (100), 147 (4.8), 136 (9.9), 135 (15), 134 (11), 133 (12), 121 (60), 119 (17), 107 (20), 105 (21), 93 (29), 91 (19), 81 (11), 79 (16), 77 (14), 67 (6.5), 55 (11), 41 (23). <sup>1</sup>H NMR δ(270 MHz, CDCl<sub>3</sub>): 5.43 (dd,  $J = 7$  and 6 Hz, 5-H), 2.66 (sept,  $J = 7$  Hz, 11-H), 2.39 (ddd,  $J = 15$ , 4 and 4 Hz, 2-Ha), 2.04 (br. t-like m, 9-H<sub>2</sub>), 1.62 (br. s, 14-H<sub>3</sub>), 0.98 (d,  $J = 7$  Hz, 12-H<sub>3</sub>), 0.92 (d,  $J = 7$  Hz, 13-H<sub>3</sub>), 0.91 (s, 15-H<sub>3</sub>). <sup>13</sup>C NMR

$\delta$ (68 MHz,  $\text{CDCl}_3$  DEPT and CH-COSY): 141.8 (1-C), 139.8 (10-C), 132.2 (4-C), 122.6 (5-CH), 49.5 (7-C), 40.3 (6- $\text{CH}_2$ ), 38.5 (8- $\text{CH}_2$ ), 33.5 (3- $\text{CH}_2$ ), 27.1 (9- $\text{CH}_2$ ), 26.4 (11-CH), 25.9 (14- $\text{CH}_3$ ), 23.5 (15- $\text{H}_3$ ), 22.5 (2- $\text{CH}_2$ ), 21.9 (12- $\text{CH}_3$ ), 21.2 (13- $\text{CH}_3$ ).

**Compound 11** [acora-3(4),7(8)-diene]. An oil.  $R_f$  0.87 ( $\text{Et}_2\text{O}$ -*n*-hexane = 25:1 on 5%  $\text{AgNO}_3$  silica gel). Vanillin-sulphate: bluish purple. HRMS: 204.183 ( $\text{C}_{15}\text{H}_{24}$ , calc. 204.188). GCMS  $m/z$  (rel. int.): 204 ( $\text{M}^+$ , 5.9), 189 (1.1), 161 (3.7), 136 (12), 121 (8.1), 105 (5.6), 95 (8.8), 94 (100), 93 (17), 91 (12), 79 (12), 77 (8.6), 65 (2.6), 55 (5.2), 40 (15).  $^1\text{H}$  NMR  $\delta$ (270 MHz,  $\text{CDCl}_3$ ): 5.38 (br. m, 3-H), 5.27 (br. s, 8-H), 2.18 (m, 5-Ha), 1.66 (br. s, 15- $\text{H}_3$ ), 1.61 (d,  $J = 2$  Hz, 14- $\text{H}_3$ ), 0.92 (d,  $J = 7$  Hz, 12- $\text{H}_3$ ), 0.81 (d,  $J = 7$  Hz, 13- $\text{H}_3$ ).  $^{13}\text{C}$  NMR  $\delta$ (68 MHz,  $\text{CDCl}_3$ , DEPT and CH-COSY): 148.6 (7-C), 133.7 (4-C), 123.5 (8-CH), 121.1 (3-CH), 55.0 (10-CH), 47.9 (1-C), 34.2 (2- $\text{CH}_2$ ), 32.2 (5- $\text{CH}_2$ ), 28.8 (9- $\text{CH}_2$ ), 28.1 (11-CH), 26.3 (6- $\text{CH}_2$ ), 23.6 (14- $\text{CH}_3$ ), 22.9 (12- $\text{CH}_3$ ), 21.1 (13- $\text{CH}_3$ ), 14.9 (15- $\text{CH}_3$ ).

**Compound 12** [acora-2(3),4(5)-diene (tentative)]. An oil.  $R_f$  0.79 ( $\text{Et}_2\text{O}$ -*n*-hexane = 25:1 on 5%  $\text{AgNO}_3$  silica gel). Vanillin-sulphate: reddish pink. HRMS: 204.187 ( $\text{C}_{15}\text{H}_{24}$ , calc. 204.188). GCMS  $m/z$  (rel. int.): 204 ( $\text{M}^+$ , 12), 161 (14), 120 (15), 119 (100), 105 (81), 92 (16), 91 (14), 55 (13), 41 (16).  $^1\text{H}$  NMR  $\delta$ (270 MHz,  $\text{CDCl}_3$ ): 5.63 (d,  $J = 10$  Hz, 3-H), 5.26 (br. m, 5-H), 5.24 (d,  $J = 10$  Hz, 2-H), 1.66 (br. s, 14- $\text{H}_3$ ), 0.96 (d,  $J = 7$  Hz, 15- $\text{H}_3$ ), 0.86 (d,  $J = 7$  Hz, 12- $\text{H}_3$ ), 0.83 (d,  $J = 7$  Hz, 13- $\text{H}_3$ ).

#### Preparation of **1**, **6**, **9** and **11** from carotol

Carotol (**3**) was obtained from a carot seed oil supplied from Takasago Koryo Co. Compound **3** was roughly separated by prep. TLC (*n*-hexane- $\text{Et}_2\text{O}$ - $\text{HCOOH}$  = 500:10:1,  $R_f$  0.7). The crude **3** was acetylated by acetyl chloride/*N,N*-dimethylaniline at 60 °C [9] to obtain pure carotol acetate (**8**, quantitative) by prep. TLC (*n*-hexane- $\text{EtOAc}$  = 25:1,  $R_f$  0.5). Compound **8** (187 mg) was heated at 170 °C in an excess amount of *N,N*-dimethylaniline (*ca.* 500 mg) for 8 h to yield 110 mg (76%) of almost pure **6** (recovered **8**, 45 mg). The product obtained by silica gel column chromatography

(eluted with *n*-hexane) was identical with natural **6** in *tR* in GLC (Chrompac CP-Wax 52CB, *tR* 7.8 min), GC mass spectrometry, ORD and  $^1\text{H}$  NMR spectrum.

Dehydration reaction of **3** with thionyl chloride in pyridine as reported by Zalkow and Clower, Jr. [7, 15] afforded compounds **9**, **1** and **11**. When acetate **8** was treated under the same reaction condition, no sesquiterpene hydrocarbon was produced. However, treatment of **8** (14.2 mg) with thionyl chloride in benzene at room temperature resulted in sesquiterpene hydrocarbons (9.1 mg as a mixture, 83% yield). The reaction products were passed through a silica gel column for GLC analysis. In the eluate, compounds **11** (37%) and **9** (24%) were major products associated with a trace amount of **1**. Two more unknown peaks (16% and 15%, *tR* 9.8 and 11.2 min, respectively) were also detected. GC mass spectrometries and *tR* of **11** and **9** each agreed with those of naturally occurring. The mixture was separated on  $\text{AgNO}_3$ -impregnated TL plates as described above. On the other hand, when **8** (28.9 mg) was treated with *p*-TsOH (in  $\text{EtOH}$ /benzene, for 2 h at room temperature), and the reaction mixture was partitioned between benzene and 5%  $\text{NaHCO}_3$  solution, the sesquiterpene hydrocarbon mixture (16.4 mg, 75%) was obtained. Compound **9** (65% of the total sesquiterpene hydrocarbons) was predominantly obtained, while **11** (19%) and **1** (13%) were the minor products.

**Compound 8** (carotol acetate). A syrup. Vanillin-sulphate: pinkish red. FIMS  $m/z$  (rel. int.): 264 ( $\text{M}^+$ , 57), 205 (40), 204 ( $\text{M}^+$ -AcOH, 100). EIMS  $m/z$  (rel. int.): 204 ( $\text{M}^+$ -AcOH, 15), 189 (7.9), 162 (16), 161 (97), 136 (45), 133 (17), 121 (65), 119 (42), 107 (30), 105 (63), 94 (100), 93 (60), 91 (46), 81 (50), 79 (40), 77 (30), 60 (29), 55 (36), 45 (48), 43 (53), 41 (69).  $^1\text{H}$  NMR  $\delta$ (270 MHz,  $\text{CDCl}_3$ ): 5.25 (ddd,  $J = 6, 5$  and 2 Hz, 5-H), 3.24 (ddd,  $J = 9, 6$  and 6 Hz, 2-Ha), 2.63 (br. d,  $J = 16$  Hz, 6-Ha), 2.10 (br., 3- $\text{H}_2$ ), 1.98 (s, 1'-OAc), 1.64 (br. s, 14- $\text{H}_3$ ), 0.99 (d,  $J = 6$  Hz, 12- $\text{H}_3$ ), 0.96 (s, 15- $\text{H}_3$ ), 0.93 (s, 13- $\text{H}_3$ ).  $^{13}\text{C}$  NMR  $\delta$ (68 MHz,  $\text{CDCl}_3$ , DEPT and CH-COSY): 170.6 (1'-OAc), 136.7 (4-C), 121.7 (5-CH), 97.8 (1-C), 53.5 (10-CH), 51.4 (7-C), 37.8 (8- $\text{CH}_2$ ), 37.1 (6- $\text{CH}_2$ ), 31.3 (2- $\text{CH}_2$ ), 31.1 (3- $\text{CH}_2$ ), 28.2 (11-CH), 25.2 (14- $\text{CH}_3$ ), 25.1 (15- $\text{CH}_3$ ), 25.0 (9- $\text{CH}_2$ ), 23.5 (12- $\text{CH}_3$ ), 22.4 (1'-OAc), 21.2 (13- $\text{CH}_3$ ).

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