

Sesquiterpenes from *Cupressus macrocarpa* foliage

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Abstract

Ten sesquiterpenes, many with unusual carbon skeletons, were identified in foliage of *Cupressus macrocarpa*. These are (–)-10-*epi*- β -acoradiene; *ent*-widdra-2,4(14)-diene; (*E*)-iso- γ -bisabolene, i.e., (4*E*)-4-(1,5-dimethylhex-5-enylidene)-1-methylcyclohexene; (–)-cumacrene, i.e., (4*S*)-4-[(1*R*,2*S*)-2-isopropenyl-1-methylcyclobutyl]-1-methylcyclohexene; (–)- α -chamipinene, i.e., (1*S*,6*S*,7*S*)-2,2,6,8-tetramethyltricyclo[5.3.1.0^{1,6}]undec-8-ene; and five sesquiterpenes with a 3,3,4'-trimethyl-1,1'-bi(cyclohexyl) skeleton for which the trivial name macrocarpane is proposed. The possible single-enzyme biogenesis of these sesquiterpenes is discussed.

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1. Introduction

Cupressus macrocarpa Hartw. ex Gord. (Monterey cypress) is a narrow endemic native only on Point Lobos and Cypress Point, Monterey Co., California. It is a large and picturesque tree, and despite its limited natural range it is one of the best-known and most widely planted conifers in the state.

Within *Cupressus*, Monterey cypress has been considered most closely related to four other coastal Californian cypress taxa with non-glandular foliage: *C. abramsiana* C.B. Wolf, *C. goveniana* ssp. *goveniana* Gord., *C. goveniana* ssp. *pygmaea* (Lemm.) Bartel, and *C. sargentii* Jeps., although Wolf (1948) saw it as morphologically somewhat distant from the others. The distinction of *C. macrocarpa* within this north-coastal group of cypresses was supported by analysis of their foliar monoterpene composition (Zavarin et al., 1971).

All of the north-coastal *Cupressus* species produce an essential oil that is low in sesquiterpenoids (Zavarin et al., 1971). Briggs and Sutherland (1942) analyzed *C. macrocarpa* foliar oil by fractional distillation and reported only ca. 0.7% for the sesquiterpene fraction of the oil. Our examination of 40 *C. macrocarpa* specimens (20 planted and 20 from the Point Lobos population) by gas chromatography–mass spectrometry (GC–MS) confirmed that sesquiterpenes are generally a minor component of the volatile oil. However, some individuals produced oil with up to 9% sesquiterpenoids, which included a number of apparently novel hydrocarbons. The identity of ten of these sesquiterpenes is the subject of this report.

2. Results and discussion

A number of sesquiterpenoids were ubiquitous in *C. macrocarpa* foliage, though in widely varying amounts: α -cubebene, β -funebrene, caryophyllene, germacrene A and B, cedrol, α -bisabolol, nerolidol, and farnesol were detected by GC–MS in nearly all trees.

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There was also a distinct chemophenotype, represented in three of the 40 trees, which produced sizable amounts of germacrene C (detected as the Cope rearrangement product δ -elemene), (+)-guaia-6,9-diene **1**, and an unknown that is tentatively identified as (1*R*,4*S*,5*R*)-guaia-6,10(15)-diene **2** (see Section 3). The chirality of **1** was determined from its retention time on enantioselective GC (EGC hereinafter), which was different from that of authentic (–)-guaia-6,9-diene. (+)-Guaia-6,10(15)-diene has been reported from the coral *Nephthea chabrolii* (Rao et al., 2000), though the absolute stereochemistry was not determined. The absolute stereochemistry of putative **2** from *C. macrocarpa* is assumed to be the same as **1**.

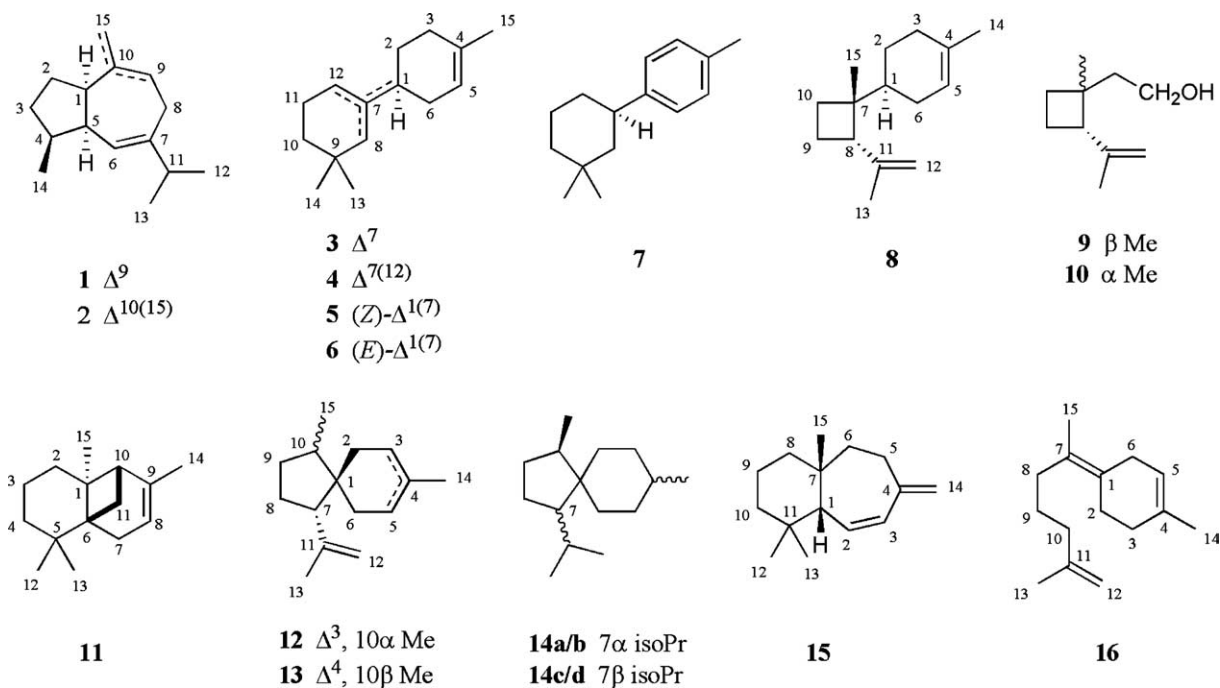
A second, and more common, *C. macrocarpa* chemotype (29 of 40 trees) was typified by presence of over 25 unknown sesquiterpenes, ten of which were in sufficiently high concentration for isolation and characterization. Known compounds (–)- β -sesquiphellandrene, δ -cuprenene, acora-3,7- and -4,7-diene (Kaiser and Naegele, 1972; Marx and Norman, 1975), (–)-dauca-8,11-diene (Cool, 2001), and the rare (–)-cuparene and (–)- α -cuprenene, were also detected in small amounts during isolation of the major sesquiterpenes in the oil.

fourth having presumably been incorporated during biogenesis of the dimethylcyclohexane ring.

The structures of α - and β -macrocarpene (**3** and **4**), which were the major sesquiterpenes in this chemotype, were deduced from the 1D NMR data (see Section 3). The ^1H and ^{13}C spectra of both included seven signals almost identical to those of the corresponding non-isopropenyl atoms of limonene, while other ^1H signals of **3** and **4** closely matched data for the ring protons of 1,3,3- and 1,5,5-trimethylcyclohexene, respectively (Snowdon et al., 1987). HMQC experiments permitted assignment of all ^1H and ^{13}C signals, and an HMBC experiment on **4** confirmed the macrocarpane carbon skeleton (correlations in Table 1).

Both **3** and **4** are levorotatory, and based on studies on the optical rotation of 4- substituted 1-methylcyclohexenes (Mills, 1952), their absolute stereochemistry can be assumed to be that of (–)-limonene, i.e., 1*S*.

The structures of non-chiral double bond isomers (*Z*)- and (*E*)- γ -macrocarpene (**5** and **6**, respectively), which occur in ca. 1:5 ratio in the oil, were inferred from their ^1H , ^{13}C , ^1H – ^1H NOESY, and (for **5**) HMBC NMR data (see Table 1). The spectra of **5** and **6** were very similar and absence of a chiral center in these compounds



Compounds **3**–**7** proved to be irregular sesquiterpenes with a 3,3,4'-trimethyl-1,1'-(bicyclohexyl) skeleton, for which the trivial name macrocarpane is proposed. Greek letters and *ar*-, *E*- and *Z*-prefixes indicate the various double-bond and geometric macrocarpene isomers (see Section 3). These sesquiterpenes are remarkable in having only three terminal carbons, the

was evident from magnetic equivalence of the ^1H or ^{13}C signals from both atoms of each geminal pair. NMR data of terpinolene (^1H and ^{13}C) and 3,3-dimethylmethylenecyclohexane (^1H ; Bernard et al., 1974) had the pertinent methyl and ring signals very close to those of **5** and **6**. The macrocarpane carbon skeleton of the predominant *E* isomer **6** was confirmed by hydrogenation

Table 1
¹³C and ¹H NMR spectroscopic data^a of macrocarpenes **3–6**, δ from TMS^b

C. No.	3 (CDCl ₃)		4 (CDCl ₃)			5 (C ₆ D ₆)				6 (C ₆ D ₆)		
	¹³ C δ	¹ H δ	¹³ C δ	¹ H δ	HMBC (C. No.)	¹³ C δ	¹ H δ	NOESY ^c	HMBC (C. No.)	¹³ C δ	¹ H δ	NOESY ^c
1	41.1	1.94 <i>m</i>	41.3	1.96 <i>m</i>	3/6,7,8,12	~127.6	—	—	—	126.7	—	—
2	27.9	1.42 <i>dddd</i> (6,12,12,13)	27.7	1.44 <i>dddd</i> (6,12,12,13)	1,3/6,4	26.9	2.39 <i>t</i> (6)	12	1,3,4,6?	26.8	2.34 <i>t</i> (6)	8
		1.70 <i>m</i>		1.72 <i>m</i>	1,3/6,4,7?							
3	30.7 ^d	1.86 <i>m</i> , 1.98 <i>m</i>	30.7	1.89 <i>m</i> , 2.05 <i>m</i>	1,2,4,5	32.4	1.96 <i>br t</i> (5)		2?,4,5	32.3	1.97 <i>br t</i> (5)	
4	133.7	—	133.7	—	—	134.0	—	—	—	133.9	—	—
5	121.0	5.37 <i>br m</i>	120.9	5.39 <i>br m</i>	1,6	121.4	5.40 <i>br m</i>	—	—	121.5	5.39 <i>br m</i>	—
6	30.9 ^d	2H, 1.98 <i>m</i>	30.7	2H, 1.96 <i>m</i>	1,4,5	29.8 ^d	2.79 <i>br s</i>	8	—	29.7 ^d	2.83 <i>br s</i>	12
7	139.4	—	140.8	—	—	128.6	—	—	—	128.5	—	—
8	130.1	5.09 <i>s</i>	40.5	1.705 <i>s</i> , 1.709 <i>s</i>	1?,7,9?,10,12,13/14	43.8	1.97 <i>s</i>	6,13/14	1,7,9,10,13/14	43.5	2.01 <i>s</i>	2,13/14
9	31.4	—	29.0	—	—	~33.5	—	—	—	33.3	—	—
10	37.5	2H, 1.35 <i>t</i> (7)	35.3	2H, 1.30 <i>t</i> (7)	8,9,11,12,13,14	40.4	1.30 <i>m</i>	13/14	8,9,11,12?,13/14	40.3	1.30 <i>m</i>	13/14
11	20.2	2H, 1.58 <i>m</i>	23.1	2H, 2.01 <i>m</i>	7,9,10,12	24.7	1.50 <i>m</i>	—	7,9,12	24.3	1.46 <i>m</i>	—
12	26.6	1.846 <i>t</i> , 1.850 <i>t</i>	117.8	5.36 <i>br m</i>	1/8,10,11	30.0 ^d	2.15 <i>br t</i> (6)	2	1,7,8,10,11	30.4 ^d	2.09 <i>br t</i> (6)	6
13	30.3 ^c	0.912 <i>s</i>	28.0 ^d	0.890 <i>s</i>	8,9/14,10	28.6	0.88 <i>s</i>	8,10	8,9,10,14	28.7	0.89 <i>s</i>	8,10
14	30.4 ^c	0.915 <i>s</i>	28.4 ^d	0.882 <i>s</i>	8,9/13,10	28.6	0.88 <i>s</i>	8,10	8,9,10,13	28.7	0.89 <i>s</i>	8,10
15	23.5	1.62 <i>br s</i>	23.5	1.64 <i>br s</i>	3,4,5	23.6	1.63 <i>br m</i>	3,5	3,4,5	23.6	1.64 <i>br m</i>	3,5

^a ¹H–¹H coupling constants (Hz) in parentheses; query (?) = weak or indistinct 2D signal; slash (/) = and/or.

^b Solvent references, δ from TMS: CDCl₃ ¹H 7.26, ¹³C 77.0; C₆D₆ ¹H 7.15, ¹³C 128.0.

^c Two- and three-bond correlations not listed.

^{d,e} Column entries with the same superscript may be reversed.

tion, which yielded the same two isomeric $M_r = 208$ compounds as were obtained from hydrogenation of **4**. The geometry of the tetrasubstituted double bond was determined by NOESY experiments, in which the decisive cross-peaks were from protons $2 \rightarrow 12$ and $6 \rightarrow 8$ in **5** vs. from $2 \rightarrow 8$ and $6 \rightarrow 12$ in **6** (Table 1).

It should be noted that **5** and **6** were very unstable when purified, being oxidized and isomerised within minutes unless preserved with an antioxidant.

Minor component *ar*-macrocarpene **7** ($M_r = 202$) had ^1H NMR signals (see Section 3) that indicated its basic structural features: a bicyclic sesquiterpene consisting of a 1,4-disubstituted benzene ring (4H, δ 7.06) with a methyl group (3H, δ 2.18) and attached to a saturated cycloalkyl ring bearing a geminal methyl pair (two singlets, δ 0.91 and 0.93, 3H each). The macrocarpane structure suggested by these features was confirmed by GC comparison of the hydrogenation products of **7** with those of **4** and **6**. All three starting materials produced the same two isomeric macrocarpanes, though in different ratios (see Section 3). Since **7** is chiral and was found to be dextrorotatory ($\alpha_D = +7.2^\circ$), its enantiomeric purity was investigated by EGC. Neither **7** itself nor its hydrogenation products showed evidence of separable enantiomers. However, EGC analysis of the hydrogenation product mix from **6**, which must contain both enantiomers of each diastereomer, also showed no separation of the enantiomeric pairs. Thus there is no assurance that **7** is optically pure. However, the fact that it is not racemic suggests that its biosynthesis involves a stereospecific enzyme-mediated hydride shift from C-1 to C-7 before aromatization. The absolute stereochemistry shown is based on the fact that 3,3-dimethylcyclohexanes with achiral 1*R* substituents are reported to be dextrorotatory (Ansari, 1973).

The structure of cyclobutanoid sesquiterpene **8**, which is given the trivial name cumacrene, was determined by a series of 2D NMR experiments (data in Table 2). ^1H – ^1H TOCSY defined the coupled proton system $\text{H-8} \rightarrow \text{H-9}\alpha\beta \rightarrow \text{H-10}\alpha\beta$, and HMQC gave the proton-carbon assignments. HMBC gave 2- and 3-bond heteronuclear correlations that were only consistent with a cyclobutane ring substituted as shown. The *trans* relative stereochemistry at C-7 and C-8 was clear from a strong NOESY peak between H-8 and H-15. The attachment of the 4-methylcyclohexene ring at C-1 was required by the TOCSY and HMBC data.

The C-1/C-7 relative stereochemistry was investigated by comparing results of NOESY experiments (Table 2) with minimum-energy (MM2 force field) conformation calculations for **8** and its C-1 diastereomer. The calculations for **8** found two low-energy conformations differing by just $0.18 \text{ kcal mol}^{-1}$, while for the C-1 epimer the steric energy difference between the two lowest-energy conformations was 1 kcal mol^{-1} . Potentially informative close contacts for **8** would be from quasi-

equatorial H-6 α to H-13 in one conformation or to H-15 in the other, while for the C-1 epimer neither of these interactions would be expected. The NOESY experiment in CDCl_3 was not definitive due to overlap of key signals, but in C_6D_6 , where the broad H-6 α multiplet as well resolved, there was a cross-peak indicating an H-13/H-6 α correlation. Furthermore, cross-peaks from H-15 to H-6 β and -6 α appeared which suggested the participation of both conformations of **8** in the observed NOEs.

The depicted absolute stereochemistry of **8** is based on the assumption that the compound derives from the same (*S*)-bisabolyol precursor as congeners **3** and **4** (see discussion below).

A sesquiterpenoid carboxylic acid with the 1-(2-isopropyl-1-methyl-cyclobutyl)-4-methylcyclohexane (“dunniane”) carbon skeleton has been described from *Illicium dunnianum* (Sy and Brown, 1998). Besides the C-14 carboxyl functionality, this compound differs from **8** in having *cis* relative stereochemistry at C-7 and -8. Thus the cumacrane and dunniane carbon skeletons bear the same stereochemical relationship as the cyclobutane monoterpenoids grandisol **9** and fragranol **10**, respectively. In fact, co-occurrence of **8** with the macrocarpenes in *C. macrocarpa* suggests a biosynthetic parallel between sesquiterpene **8** and monoterpenoid **9**, in that the latter also occurs naturally with corresponding monoterpenoid dimethylcyclohexane congeners, e.g., 2-(3,3-dimethylcyclohexylidene)ethanol (Tumlinson et al., 1971).

Compound **11**, another minor component ($\sim 0.5\%$ of total sesquiterpenes), had a MS quite similar to that of thujopsene. 1D NMR of the purified compound showed that it is a tricyclic sesquiterpene with four methyls (one of them vinyl) on quaternary carbons. Unlike thujopsene, there were no high-field signals indicative of a cyclopropane ring. The NMR spectroscopic data (in C_6D_6 , see Section 3) were very similar to the literature values (in CDCl_3) for one candidate structure, 2,2,6,8-tetramethyltricyclo[5.3.1.0^{1,6}]undec-8-ene (Naegeli and Wetli, 1981). A ^1H NMR experiment with the *C. macrocarpa* unknown in CDCl_3 gave spectral data identical to the literature values, confirming its identification.

The compound reported by Naegeli and Wetli (1981) was a by-product in a synthesis of the 1,1,5,9-tetramethylspiro[5.5]undecane (chamigrane) skeleton. The authors note that acid-catalyzed scission of the C-1/C-10 bond of **11** leads directly to the chamigrenes. This relationship and the presence of a pinene substructure prompt the proposal of α -chamipinene as the trivial name for **11**. None of the related compounds found by Naegeli and Wetli (which by analogy would be called β -chamipinene, chamicamphene, and α -chamifenchene) was detected in *C. macrocarpa*.

Naegeli and Wetli (1981) synthesized racemic mixtures in their work, so there was no information on

Table 2
¹³C and ¹H NMR spectroscopic data^a of sesquiterpenes **8** and **12**, δ from TMS^b

C. No.	8 (CDCl ₃)					8 (C ₆ D ₆)			12 (CDCl ₃)			
	¹³ C δ	¹ H δ	TOCSY ^c	NOESY ^d	HMBC (C. No.)	¹³ C δ	¹ H δ	NOESY ^d	¹³ C δ	¹ H δ	COSY ^e	NOESY ^{d,e}
1	37.8	1.69 <i>m</i>	2 β	15?	2,3,7,15	38.2	1.79 <i>m</i>	15?	44.6	–	–	–
2	24.3	β 1.20 <i>m</i> α 1.73 <i>m</i>	1,3 $\alpha\beta$ 3 $\alpha\beta$?	15	1,3,6 3,4	24.7	β 1.20 <i>m</i> α 1.70 <i>m</i>	15	39.1	<i>eq</i> 1.93 <i>br dm</i> <i>ax</i> 2.01 <i>br dm</i>	3,14 3,14	7
3	31.3	β 1.91 <i>br m</i> α 2.00 <i>br m</i>	2 α ? β 2 β	14 14	4 4?	31.6	1.86 <i>br dm</i> 1.98 <i>br m</i>	14 14	120.9	5.37 <i>br m</i>	2 α , <i>eq</i> ;14	
4	133.7	–	–	–	–	133.3	–	–	133.9	–	–	–
5	121.4	5.33 <i>br d</i> (4)	6 $\alpha\beta$	14	1,3,6,14	121.9	5.43 <i>br d</i> (4)	14	28.8	<i>ax</i> 1.93 <i>br dm</i> <i>eq</i> 2.01 <i>br dm</i>	6 6	15
6	26.5	β 1.69 <i>m</i> α 1.93 <i>m</i>	5? 5	15? 15?	–	27.0	β 1.77 <i>m</i> α 2.05 <i>br dm</i> (14)	15 13,15	24.2	2H, 1.46 <i>m</i>	5 α , <i>eq</i>	12Z,13,15
7	46.2	–	–	–	–	46.4	–	–	58.0	2.18 <i>m</i>	8 $\alpha\beta$	2 α ,13?
8	52.5	2.56 <i>t</i> (9)	9 $\alpha\beta$	13,15	1,7,9,10,11,12,13,15	52.8	2.48 <i>t</i> (8.9)	13,15	27.9	β 1.68 <i>m</i> α 1.79 <i>m</i>	7,9 $\alpha\beta$ 7,9 $\alpha\beta$	12Z,15?
9	19.9	β 1.86 <i>m</i> α 1.94 <i>m</i>	8,10 β 8,10 β	12Z	7,8,10 7,8,10,11	20.2	β 1.83 <i>m</i> α 1.94 <i>m</i>	12Z	31.4	α 1.35 <i>m</i> β 1.73 <i>m</i>	8 $\alpha\beta$,10 8 $\alpha\beta$,10	15
10	29.6	β 1.55 <i>dt</i> (9,11) α 1.79 <i>m</i>	9 $\alpha\beta$	15	1,7,8,9,15 1,7,8,9	29.9	β 1.46 <i>dt</i> (9,11) α 1.76 <i>m</i>	15	44.0	1.75 <i>m</i>	9 α ,15	
11	146.8	–	–	–	–	146.6	–	–	147.1	–	–	–
12	110.0	(<i>E</i>) 4.80 <i>br s</i> (<i>Z</i>) 4.71 <i>br s</i>	13 9 α		8,13	110.5	(<i>E</i>) 4.86 <i>br s</i> (<i>Z</i>) 4.82 <i>br s</i>	13 9 α	111.8	(<i>Z</i>) 4.72 <i>br s</i> (<i>E</i>) 4.83 <i>br m</i>	7? 13	6,8 α ,15 13
13	23.8	1.77 <i>br s</i>		8,12 <i>E</i> ,15	8,12	23.9	1.70 <i>br s</i>	6 α ,8,12 <i>E</i> , 15	24.1	1.77 <i>s</i>		6,7?,12 <i>E</i>
14	23.4	1.63 <i>br s</i>		3 $\alpha\beta$,5	3,4,5	23.6	1.64 <i>br s</i>	3 $\alpha\beta$,5	23.3	1.61 <i>br s</i>	2 α , <i>eq</i>	–
15	25.2	1.11 <i>s</i>		1?,2 β ,6 α ? β ?, 8,10 β ,13	1,7,8,10	25.4	1.03 <i>s</i>	1?,2 β ,6 $\alpha\beta$,8,10 β ,13	15.8	0.94 <i>d</i> (7)	10	8 α ?,9 α ,5 α , 6,12Z

^a ¹H–¹H coupling constants (Hz) in parentheses; query (?) = weak or indistinct 2D signal.

^b Solvent references, δ from TMS: CDCl₃ ¹H 7.26, ¹³C 77.0; C₆D₆ ¹H 7.15, ¹³C 128.0.

^c 24 ms mixing time used to limit correlations to 2 and 3 bonds; two-bond correlations not listed.

^d Two- and three-bond correlations not listed.

^e Two-bond correlations not listed; assignments for H-2 and -5 confirmed by COSY (or NOESY) experiment in C₆D₆.

the optical rotation of the enantiomers of these compounds. But since the absolute stereochemistry of α - and β -chamigrene is known, it seemed possible to determine that of **11** ($\alpha_D -4.3^\circ$) by acid-catalyzed rearrangement followed by enantioselective analysis of any chamigrenes produced. When this was done, **11** completely rearranged to two major products that were identical to (–)- α - and (–)- β -chamigrene when analyzed by GC–MS and dual-column GC–FID, but differed in retention time from the (–) enantiomers on EGC. Although (+)- and (–)- α -chamigrene were only slightly separated, the β -chamigrene enantiomers were resolved to the baseline, showing that the enantiomeric purity of the (+)- β -chamigrene, and therefore of **11** itself, is very high.

Acoradiene **12** is the C-10 epimer of (+)- β -acoradiene. Its GC retention time was slightly longer than that of β -acoradiene, while its mass spectrum was very similar, the only significant difference being the somewhat larger ratio of ion m/z 108 to ion m/z 107. The structure and absolute stereochemistry of **12** were elucidated by a combination of 2D NMR, molecular mechanics (MM2) calculation, hydrogenation experiments, and EGC.

^1H – ^1H COSY and ^1H – ^{13}C HSQC experiments indicated the acoradiene structure and allowed assignment of the ^1H and ^{13}C signals (Table 2). Position of the endocyclic double bond at C-3¹ was inferred from the 39 ppm ^{13}C signal for C-2, which is significantly different from that of the corresponding allylic carbon of α - and β -acoradiene ($\delta \sim 31$ ppm). This downfield chemical shift can be explained by absence of a γ substituent interaction between Me-15 and C-2. Examination of the MM2 minimum-energy conformations of all four diastereomers of acora-3,11- and -4,11-diene shows that in three of them H-15 is within 2.45 Å of the nearest allylic proton (H-2 or -6). Only in **12** is this distance large enough (ca. 4.7 Å) to preclude the upfield shift typical for this allylic carbon.

GC–MS, dual-column GC–FID, and EGC comparison of the hydrogenation products of **12** and authentic (–)- α -acoradiene **13** gave final proof of the relative and absolute stereochemistry at C-7 and C-10. Hydrogenation of **13** gave two major saturated ($M_r = 208$) compounds **14a/b**, along with small amounts of isopropyl epimers **14c/d**. Compound **12** gave nearly equal amounts of *ent*-**14c/d** and *ent*-**14a/b**. The assumption that *ent*-**14a/b** were artefacts due to acid-catalysed isomerization at C-7 was tested by repeating the hydrogenation of **12** with addition of a small amount of pyridine. Though hydrogenation was less complete (there were now sizable amounts of monounsaturated $M_r = 206$ compounds), the amount of *ent*-**14a/b** was greatly reduced compared

to *ent*-**14c/d** (see Section 3). An unexpected finding in this experiment was that although no unreacted **12** remained, a large peak corresponding to *ent*- γ -acoradiene [acora-4,7(11)-diene] was seen.

A search through our in-house library of plant terpenoid GC–MS analyses showed that the mass spectrum of a sesquiterpene from the liverwort *Pellia epiphylla* (L.) Corda, collected in Humboldt Co., CA, very closely matched that of **12**. GC–MS, dual-column GC–FID, and EGC comparisons under identical conditions showed that the *P. epiphylla* component, present at a concentration of ca. 2% in the oil, had MS and retention data exactly the same as that of **12** from *C. macrocarpa*. Thus the former is probably also (–)-10-*epi*- β -acoradiene.

A compound with the same structure as **12** (or its enantiomer) has been reported as an aggregation pheromone in the flour beetle *Gnathocerus cornutus* (Tebayashi et al., 1998). However, the published NMR and MS data are clearly different from those of **12**. Since no conformational analysis was done, the authors' interpretation of the NOESY data for the beetle pheromone may be open to question. Of the four acora-3,11- and -4,11-diene diastereomers, only **12** and α -acoradiene are consistent with the reported NOESY cross-peak from H-15 to non-allylic H-6 or -2. But since the α -acoradiene NMR spectroscopic data referred to in the paper was from a low-resolution experiment run in CCl_4 rather than CDCl_3 , the possibility that the unknown (reported $\alpha_D = +37.1^\circ$) might actually be the enantiomer of (–)- α -acoradiene **13** was missed.

To check this, a high-resolution (400 MHz) ^1H NMR spectrum was run on authentic **13** in CDCl_3 . The resulting signals were identical to those reported by Tebayashi et al. (1998), with two exceptions: vinyl H-5 is at δ 5.30, not 5.60 as reported; and vinyl Me-14 is at δ 1.63, not 1.67. Both of these discrepancies are presumably due to typographical errors: the depicted COSY spectrum clearly shows Me-14 at δ 1.63, and the authors characterize the ^1H signals of the *G. cornutus* acoradiene as “similar to those in the literature” for α - and β -acoradiene, both of which have the vinyl methyl at δ 5.25 (CCl_4 data). Thus the identity of the *G. cornutus* aggregation pheromone is revised to (+)- α -acoradiene.

A small amount of compound **15**, with a widdrane carbon skeleton, was isolated and identified by 1D and 2D NMR spectroscopic experiments (Table 3). The essential features of the molecule were suggested by the 1D spectrum: a bicyclic sesquiterpene with three methyl singlets and a disubstituted double bond flanked by (a) methine proton H-1 (δ 1.75, doublet) at a bridgehead carbon; and (b) *exo*-methylene-bearing quaternary carbon C-4. A TOCSY experiment confirmed three correlated proton systems: the allylic protons at C-5 were coupled to H-6 α , β ; three methylenes at C-8, -9, and -10 formed a second coupled system;

¹ In this discussion, numbering of the acoradienes begins at spiro C-1, with C-2 being the adjacent carbon in the cyclohexene ring that is *trans* to the isopropenyl group.

Table 3
¹³C and ¹H NMR spectroscopic data^a of sesquiterpenes **15** and **16**, δ from TMS^b

C. No.	15 (C ₆ D ₆)					16 (C ₆ D ₆)				
	¹³ C δ	¹ H δ	TOCSY ^c	NOESY ^d	HMBC (C. No.)	¹³ C δ	¹ H δ	TOCSY ^c	NOESY ^d	HMBC (C. No.)
1	58.2	1.75 <i>d</i> (8)	2	10 β ,13,15	2,6/7,8?,11,12,13,15	128.7	—	—	—	—
2	131.1	5.55 <i>dd</i> (13,8)	1,3	13	1?,4,7/11	26.9	2.34 <i>br t</i> (6)	3	8,9	1,3,4,6,7
3	132.6	6.28 <i>br d</i> (13)	2	14Z	1,5,14	32.1	1.95 <i>br m</i>	2	14	1,2,4,5,14?
4	148.0	—	—	—	—	133.9	—	—	—	—
5	32.2	β 2.48 <i>tm</i> (14) α 2.30 <i>ddd</i> (15,5,4)	6 $\alpha\beta$ 6 $\alpha\beta$	15 14E	6/7	121.3	5.39 <i>br s</i>	6	14	—
6	35.7	β 1.08 <i>m</i> α 2.16 <i>ddd</i> (14,12,4)	5 $\alpha\beta$ 5 $\alpha\beta$	15? 9 α ,12	1,7?,8?,5/15 5?,7,15	30.2	2.75 <i>br s</i>	5,14?	15	1,4,5,7
7	~36	—	—	—	—	126.0	—	—	—	—
8	44.6	β 1.11 <i>ddd</i> (13,13,4) α 1.37 <i>ddd</i> (13,3,3)	9 $\alpha\beta$ 9 $\alpha\beta$	15? 15	6/7,9?,15	33.9	2.07 <i>br t</i> (8)	9	2,10,15	1,6,9,10,15
9	18.6	β 1.29 <i>m</i> α 1.56 <i>qt</i> (14,3)	8 α /10 α ,8 β /10 β 8 α /10 α ,8 β /10 β	6 α ,12	10?	27.0	1.53 <i>m</i>	8,10	2	7,8,10,11
10	42.0	β 1.11 <i>ddd</i> (13,13,4) α 1.37 <i>ddd</i> (13,3,3)	9 $\alpha\beta$ 9 $\alpha\beta$	1 12	8,9?,12?,13? 1,8?,12,13?	38.0	1.97 <i>br m</i>	9	8,12	8,9,11,12,13
11	36.7	—	—	—	—	145.8	—	—	—	—
12	23.3	α 0.93 <i>s</i>	—	6 α ,9 α ,10 α	1,10,11,13	110.3	4.82 <i>br s</i>	—	10,13	10,11,13
13	33.3	β 0.91 <i>s</i>	—	1,2	1,10,11,12	22.5	1.66 <i>br s</i>	—	12	10,11,12
14	114.2	(<i>Z</i>) 4.83 <i>br s</i> (<i>E</i>) 4.92 <i>br s</i>	—	3 5 α	3,5 3,5	23.6	1.63 <i>br s</i>	6?	3,5	3,4,5
15	30.7	1.01 <i>s</i>	—	1,5 β ,6 β ?,8 $\alpha\beta$?	1,6/7,8	18.5	1.61 <i>br s</i>	—	6,8	1,7,8

^a ¹H–¹H coupling constants (Hz) in parentheses; query (?) = weak or indistinct signal; slash (/) = and/or.

^b Solvent references, δ from TMS: CDCl₃ ¹H 7.26, ¹³C 77.0; C₆D₆ ¹H 7.15, ¹³C 128.0.

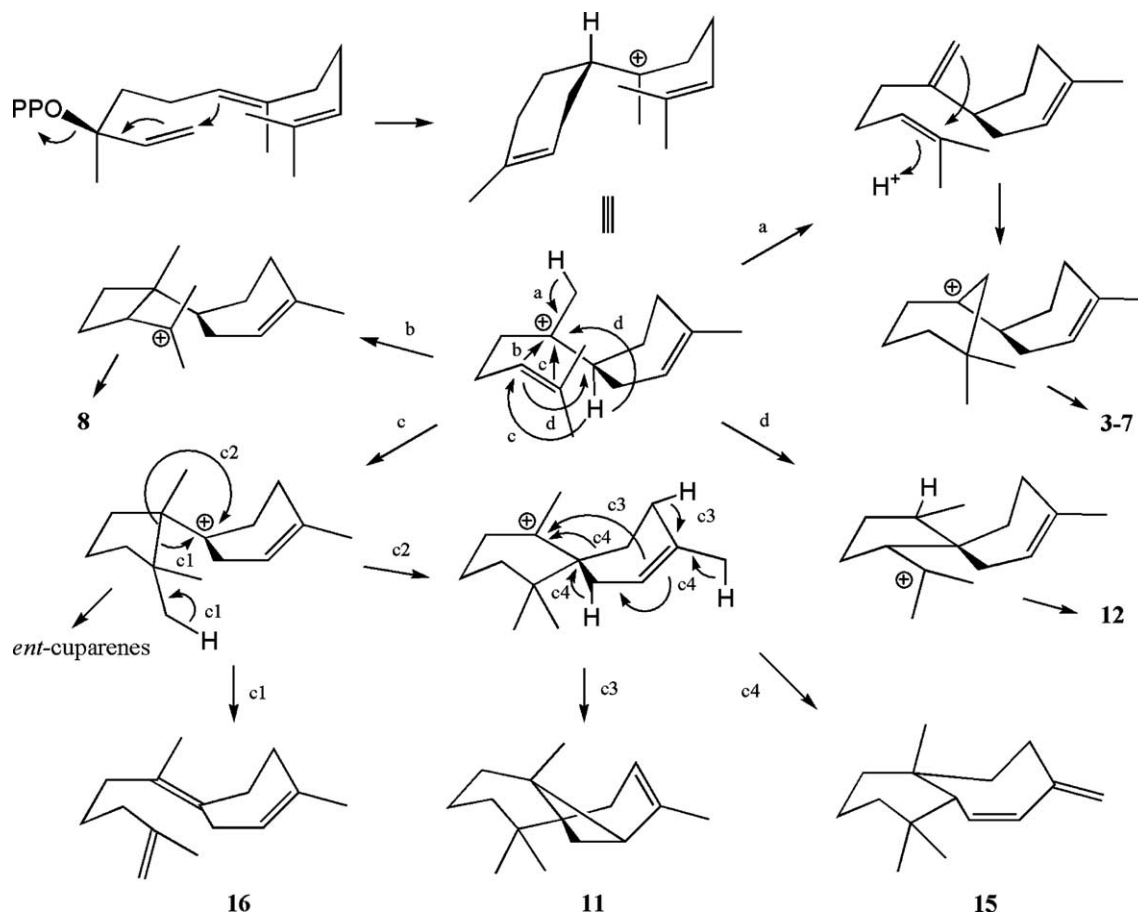
^c 24 ms mixing time used to limit correlations to 2 and 3 bonds; two-bond correlations not listed.

^d Two- and three-bond correlations not listed.

and vinyl H-2 (δ 5.55, *dd*) was coupled to H-3 (δ 6.30, *d*) and H-1. Since H-1 was coupled only to H-2, the other two carbon atoms adjacent to C-1 must be quaternary. HMQC accounted for 12 protonated carbons, so the three methyl singlets were obviously attached to these two quaternary carbons as a geminal pair and an angular methyl at the other bridgehead. The widdrane skeleton suggested by these data was confirmed by an HMBC experiment, which showed key correlations from H-12 and -13 to C-1, -10, and -11; H-6 to C-15; and H-15 to C-1, -6/7, and -8. A *cis* ring fusion was evident from a ¹H–¹H NOESY experiment, which gave a clear cross-peak from H-15 to H-1. The absolute stereochemistry was determined by EGC comparison of the two epimeric saturated hydrogenation products of **15** with those derived from authentic (+)-widdrol (7*S* absolute stereochemistry). Generation of reference (7*S*)-widdranes required a somewhat roundabout procedure, since dehydration of widdrol leads to the methyl migration product pseudowiddrene rather than widdradienes (Ito et al., 1974). (+)-Widdrol was first hydrogenated to dihydrowiddrol, which

was found to be the desired *cis*-fused stereoisomer (see Section 3). This was then dehydrated to two monoenes, which mixture was in turn hydrogenated to produce the *cis*-widdrane epimers. These had retention times identical to those from **15** on dual-column capillary GC but different on EGC, which established the 7*R* absolute stereochemistry of **15**.

1D NMR of compound **16** showed it to be an achiral monocyclic sesquiterpene with three double bonds – one tetrasubstituted, one trisubstituted, and one disubstituted terminal methylene – and three vinyl methyl singlets. HMQC and HMBC experiments (Table 3) allowed all signals to be unambiguously assigned, proving the iso- γ -bisabolene structure. The 1*E* geometry was determined by a ¹H–¹H NOESY experiment, which showed a cross peak from H-15 to *bis*-allylic H-6. Though it is a new natural product, this compound has been synthesized in a 60:40 mixture with the 1*Z* isomer, and the reported NMR data (in CDCl₃) and MS data are very close to that found for **16** (Braun et al., 2003).



Scheme 1. Proposed mechanism for biosynthesis of novel *C. macrocarpa* sesquiterpenes via an (*S*)-bisabolyl carbocation.

Production of very complex mixtures of constitutive sesquiterpenes by a single enzyme catalyst has been demonstrated in *Abies grandis* stem tissue (Steele et al., 1998). It is proposed that biosynthesis of the novel sesquiterpenes in *C. macrocarpa* foliage likewise involves a single “macrocarpene synthase” and can be rationalized as in Scheme 1. The first step (not shown) is cyclase-catalyzed isomerization of (*E,E*)-farnesyl diphosphate to (3*S*)-nerolidyl diphosphate (NPP) in the preferred *anti-endo* conformation (Cane, 1985, 1999). Ionization and C-1, C-6² closure then generates an (*S*)-bisabolyl carbocation that is the proposed precursor of all product sesquiterpenes.

Macrocarpenes 3–7 can be considered to arise via path (a), the first step of which is deprotonation of the bisabolyl carbocation to (*S*)- β -bisabolene. This neutral intermediate is not released from the enzyme active site but is immediately re protonated at C-10 with ring closure from the C-7 methylene to C-11. (A similar mechanism has been adduced by Erman (1985) in the biogenesis of the dimethylcyclohexylidene

monoterpenoid pheromones in *Anthonomus grandis*.) Deprotonation of the resulting macrocarpene carbocation at C-6, C-8, or at the newly incorporated methylene (C-8 in macrocarpene numbering) gives 3–6, while a C-6, C-7 hydride shift followed by aromatization accounts for *ar*-macrocarpene 7.

Path (b), involving C-7, C-10 cyclization of the original (*S*)-bisabolyl carbocation with proton loss from C-12, leads to 8. Alternatively, path (c), C-7, C-11 cyclization with a well-attested C-6, C-10 hydride shift (Cane, 1981, and references therein; Nabeta et al., 1993), generates an *ent*-cuparenyl carbocation. Deprotonation of this cation accounts for the rare (7*S*)-cuparenes observed, while C-7, C-11 bond scission with deprotonation at C-12 [path (c1)] is a plausible mechanism leading to bisabolene 16, with its unusual C-12 unsaturation.

An alternative reaction of the *ent*-cuparenyl carbocation is C-7, C-6 skeletal rearrangement (c2), which generates an *ent*-chamigrenyl carbocation. This opens the way to 11 via path (c3), C-2, C-7 closure with C-4 deprotonation; and to 15 by path (c4), C-6, C-7 skeletal rearrangement and a C-1, C-6 hydride and double bond shift, with deprotonation at the C-3 methyl.

² NPP numbering is used throughout this discussion.

Finally, biosynthesis of acoradiene **12** [route (d)] requires a 1,2 hydride shift from C-6 to C-7 of the bisab-olyl carbocation (Nabeta et al., 1990) followed by C-6, C10 cyclization and proton loss from C-12.

3. Experimental

3.1. General experimental procedures

Survey analyses of *C. macrocarpa* trees were done by grinding ca. 2 g of liq. N₂-frozen foliage from each tree, extracting overnight with *n*-pentane, and analyzing the extracts by GC–MS (SE-54 WCOT column) and dual-column GC-FID (SE-54 and OV-17 WCOT columns) under these conditions: splitless (0.7 min) injection, injector 220 °C, FID detector 250 °C, column(s) 35 °C (0.7 min), 6 °C min⁻¹ program to 250 °C. The same GC conditions were used for retention time determinations of purified sesquiterpenes.

Hydrodistillation of bulk samples of liq. N₂-ground foliage was from satd NaCl with NaHCO₃ as previously described (Kim et al., 1994).

LC of hydrodistilled oil (silica gel; hexane–EtOAc eluents) and FTIR of purified compounds were described before (Kim et al., 1994), as was EGC on a 10% permethyl- β -cyclodextrin column (Cool, 2001).

3.2. Plant material

Small survey samples of foliage were taken from 20 widely-separated trees in the Point Lobos population of *C. macrocarpa* in March 1996. Planted specimens (20 trees) were similarly sampled from the University of California Richmond Field Station in July 2001, and the horticultural tree with the highest yield of macrocarpenes was used for compound isolation.

3.3. Sesquiterpene isolation and identification

The *n*-pentane extract (ca. 2 ml) of foliage from a tree containing germacrene C and the guaiaadienes was concentrated 10-fold under N₂. This was applied to a column of silica gel in a Pasteur pipette and the hydrocarbon fraction eluted with 2 column volumes of *n*-hexane. (+)-Guaia-6,9-diene **1** was identified by GC–MS, dual-column GC-FID, and EGC comparison of the hydrocarbon fraction with authentic (–)-guaia-6,9-diene from commercial rose geranium (*Pelargonium graveolens*) oil. The MS of the standard and the *C. macrocarpa* component were identical, as were the GC retention times under all conditions except on EGC.

Guaia-6,10(15)-diene **2** was tentatively identified by the similarity of its MS with that of the compound from *N. chabrolii* (Venkateswarlu, personal communication). GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (57), 189 [M–

Me]⁺ (31), 161 (100), 147 (20), 133 (48), 119 (61), 105 (87), 91 (92), 81 (55), 67 (38), 55 (37), 41 (59).

For isolation of the remaining sesquiterpenes, ca. 4000 g of liq. N₂-ground foliage from the chosen horticultural tree was hydrodistilled in 800 g batches, yielding ca. 20 g oil. Additional oil was obtained by 9× batchwise hexane extraction of –20 mesh ground air-dried foliage (1700 g), extract solvent removal, and subsequent hydrodistillation of the dark green residue (89 g) with 400 ml satd NaCl and 10 g NaHCO₃, yielding another 20 g of oil. The combined oils (40 g) consisted of ca. 86% monoterpenoids, 9% sesquiterpenoids, and 5% diterpenoids. Before silica gel LC, most of the monoterpenes were removed by evaporation under N₂ flow at ca. 120 °C, giving 13 g of monoterpene-depleted oil.

The first LC fraction, eluted with *n*-hexane, consisted solely of hydrocarbons. This fraction (8 g) was separated by repeated complexation LC of 1.5 g portions on 50 g of 20% AgNO₃/silica gel using hexane–toluene step gradients, giving 11 fractions. Minor fractions 1–3, 8 and 10 (each <0.1 g, complex mixtures of trace compounds) and major fraction 5 (1.6 g, mostly **3**), were not further analyzed. Part of major fraction 6 (3 g), consisting mainly of **3** and **4**, was subjected to prep. GC (see below) to give analytical samples (ca. 20 mg each) of **3** and **4**. Fraction 4 (0.6 g), consisting of ca. 100:3:1:1 **3**, **11**, **7** and **15** was re-chromatographed with 1% toluene/hexane to give a sub-fraction (ca. 0.1 g) enriched in **7**, **11** and **15**. In the case of **5**–**8**, **11**, **12** and **15**, further separation was by isocratic HPLC (hexane–toluene eluents; RI detection) of portions of fractions rich in these compounds on a 10 × 250 mm column packed with 15% AgNO₃/silica gel (5 μ), using the following toluene concentrations: 1.8% for fraction 4 sub-fraction (**7**, **11** and **15**), 3.5% for fraction 7 (1.3 g) (**5** and **6**), 7% for fraction 9 (0.2 g) (**8** and **12**). Final purification of all except **16** was by prep. GC on 4 mm i.d. packed columns (OV-17 column, 170 °C for **3**, **4**, **8** and **12**; SE-30 column, 185 °C for **7**, **11** and **15**; Carbowax 20M column, 165 °C for **5** and **6**). Compounds **5** and **6** were very prone to rearrangement and oxidation. Therefore, they were collected in capped 3 mm OD glass U-tubes which were treated with a solution of 100 μ g each of antioxidant (Iono-330, Ethyl Corp.) and pyridine-d₅ in 5 μ l of pentane, applied in the condensation zone of the U-tubes just before use. β -Sesquiphellandrene and **16**, in argentation LC fraction 11 (0.9 g; predominantly diterpenes), were inseparable by GC or AgNO₃/silica gel HPLC, but normal-phase silica gel HPLC (5 μ particle size; 10 × 250 mm column; RI detection) with *n*-hexane eluent partially resolved them, yielding >97% pure samples of **16** (12 mg) and β -sesquiphellandrene (9 mg; $[\alpha]_D^{22} \approx -15^\circ$, *c*5; CDCl₃).

Chirality of (–)-cuparene and (–)- α -cuprenene was determined by EGC comparison of prep. GC or HPLC

fractions rich in the appropriate compound with cedarwood (*Juniperus* sp.) oil hydrocarbon fractions enriched in (+) enantiomers of these compounds.

Hydrogenation experiments on **4**, **6**, and **7** were done under 70 atm H₂ with ca. 2 mg sesquiterpene and catalytic amounts of PtO₂ in 200 µl hexane. The same two *M_r* = 208 products, inseparable on a 5% phenyl methylpolysiloxane WCOT column but separable on 50% phenyl methylpolysiloxane, were produced from each starting material, in the following ratios (earlier-eluting to later): 43:57 for **4**, 32:68 for **6**, and 20:80 for **7**.

All IUPAC names were obtained using the ACD/I-Lab Web service (ACD/IUPAC Name Free 7.06).

3.4. (–)- α -Macrocarpene, (1′S)-3,3,4′-trimethyl-1,1′-bi(cyclohexane)-1,3′-diene (3)

Oil. $[\alpha]_D^{22}$ –81° (*c* 3.9; *n*-hexane); IR ν_{\max}^{KBr} cm^{–1}: 2930, 1453, 1377, 1358, 1207, 1147, 1035, 939, 914, 858, 789; HREIMS: *m/z* found 204.1883, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (16), 189 (19), 176 (4), 162 (3), 148 (6), 136 (64), 121 (100), 107 (26), 95 (86), 93 (53), 92 (33), 79 (27), 67 (18), 55 (20), 41 (28).

3.5. (–)- β -Macrocarpene, (1′S)-4′,5,5-trimethyl-1,1′-bi(cyclohexane)-1,3′-diene (4)

Oil. $[\alpha]_D^{22}$ –72° (*c* 2.2; *n*-hexane); IR ν_{\max}^{KBr} cm^{–1}: 2911, 1471, 1363, 1217, 1146, 948, 914, 833, 796; HREIMS: *m/z* found 204.1883, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (33), 189 (22), 175 (7), 162 (6), 148 (13), 136 (53), 121 (56), 107 (53), 105 (18), 95 (20), 94 (33), 93 (100), 92 (40), 91 (23), 80 (41), 79 (50), 67 (17), 55 (19), 41 (38).

3.6. (Z)- γ -Macrocarpene, (1Z)-3′,3′,4-trimethyl-1,1′-bi(cyclohexane)-1(1′),3-diene (5)

Oil. HREIMS: *m/z* found 204.1883, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (59), 189 (10), 161 (19), 135 (25), 133 (18), 121 (20), 119 (26), 107 (36), 105 (43), 95 (27), 94 (100), 93 (79), 92 (28), 91 (50), 81 (19), 79 (57), 69 (31), 55 (31), 41 (53).

3.7. (E)- γ -Macrocarpene, (1E)-3′,3′,4-trimethyl-1,1′-bi(cyclohexane)-1(1′),3-diene (6)

Oil. HREIMS: *m/z* found 204.1883, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (59), 189 (10), 161 (13), 135 (26), 133 (22), 121 (30), 119 (35), 107 (38), 105 (43), 95 (30), 94 (100), 93 (86), 92 (30), 91 (51), 81 (19), 79 (58), 69 (29), 55 (35), 41 (51); ¹H NMR (400.13 MHz, CDCl₃, solvent ref. 7.26, δ from TMS): 0.86 (6H, *s*, H-13, -14), 1.34 (2H, *m*, H-10), 1.48 (2H, *m*, H-11), 1.66 (3H, *br s*, H-15), 1.98 (2H, *br t*,

H-3), 1.99 (2H, *s*, H-8), 2.10 (2H, *br t*, *J* = 6.1 Hz, H-12), 2.33 (2H, *t*, *J* = 6.3 Hz, H-2), 2.80 (2H, *br s*, H-6), 5.37 (1H, *br m*, H-5); ¹³C NMR (100.6 MHz, CDCl₃, solv. ref. 77.0, δ from TMS): 23.4 (*q*, C-15), 23.9 (*t*, C-11), 26.3 (*t*, C-2), 28.5 (2C, *q*, C-13, -14), 29.2 (*t*, C-3), 30.1 (*t*, C-12), 31.9 (*t*, C-6), 33.2 (*s*, C-9), 40.1 (*t*, C-10), 43.2 (*t*, C-8), 121.0 (*d*, C-5), 126.2 (*s*, C-1), 128.6 (*s*, C-7), 134.2 (*s*, C-4); multiplicities by DEPT.

3.8. (+)-Ar-macrocarpene, 1-[(1R)-3,3-dimethylcyclohexyl]-4-methylbenzene (7)

Oil. $[\alpha]_D^{22}$ +7.2° (*c* 5.1; *n*-hexane); HREIMS: *m/z* found 202.1728, Calc. for C₁₅H₂₂ 202.1722; GC–MS 70 eV, *m/z* (% rel. int.): 202 [M]⁺ (93), 187 (11), 159 (53), 131 (49), 118 (39), 117 (23), 105 (100), 91 (19), 57 (14), 41 (14). ¹H NMR (400.13 MHz, C₆D₆, solvent ref. 7.15, δ from TMS): 0.91 (3H, *s*), 0.93 (3H, *s*), 1.09 (1H, *ddd*, *J* = 4.5, 13.1, 13.1 Hz), 1.23 (1H, *dddd*, *J* = 4.5, 12.6, 12.6, 12.6 Hz), 1.26 (1H, *dd*, *J* = 12.8, 12.8 Hz), 1.33 (1H, *br d*, *J* = 12.9 Hz), 1.46 (1H, *dddd*, *J* = 3.5, 3.5, 13.2, 13.2, 13.2 Hz), 1.49–1.58 (2H, *m*), 1.83 (1H, *br d*, *J* = 12.8 Hz), 2.18 (3H, *s*), 2.62 (1H, *dddd*, *J* = 3.4, 3.4, 12.7, 12.7 Hz), 7.06 (4H, *s*); ¹³C NMR (100.6 MHz, C₆D₆, solvent ref. 128.0, δ from TMS): 21.0 (*q*), 23.1 (*t*), 24.7 (*q*), 31.3 (*s*), 33.6 (*q*), 34.6 (*t*), 39.2 (*t*), 39.9 (*d*), 48.0 (*t*), 127.2 (2C, *d*), 129.3 (2C, *d*), 135.2 (*s*), 145.0 (*s*); multiplicities by DEPT.

3.9. (–)-Cumacrene, (4S)-4-[(1R,2S)-2-isopropenyl-1-methylcyclobutyl]-1-methylcyclohexene (8)

Oil. $[\alpha]_D^{22}$ –77° (*c* 1.7; *n*-hexane); HREIMS: *m/z* found 204.1877, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (% rel. int.): 204 [M]⁺ (2), 189 (3), 175 (17), 161 (9), 147 (4), 136 (8), 121 (49), 109 (23), 107 (38), 105 (14), 94 (43), 93 (100), 79 (31), 68 (84), 67 (41), 55 (17), 53 (21), 41 (32).

3.10. (–)- α -Chamipinene, (1S,6S,7S)-2,2,6,8-tetramethyltricyclo[5.3.1.0^{1,6}]undec-8-ene (11)

Oil. $[\alpha]_D^{22}$ –4.3° (*c* 4.4, *n*-hexane); HREIMS: *m/z* found 204.1877, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (6), 189 (2), 161 (2), 147 (3), 133 (22), 123 (17), 121 (16), 119 (100), 111 (36), 105 (33), 94 (27), 93 (40), 92 (30), 91 (25), 81 (13), 69 (44), 55 (27), 41 (28); ¹H NMR (400.13 MHz, C₆D₆, solvent ref. 7.15, δ from TMS): 0.69 (3H, *s*), 0.79 (3H, *s*), 0.89 (3H, *s*), 1.16 (1H, *d*, *J* = 8.4 Hz), 1.18 (1H, *dm*, *J* ≈ 12 Hz), 1.67 (3H, *m*), 1.74 (1H, *dd*, *J* = 5.7, 1.6 Hz), 1.98 (1H, *dm*, *J* = 17.2 Hz), 2.07 (1H, *dm*, *J* = 17.0 Hz), 2.24 (1H, *dd*, *J* = 8.3, 5.7 Hz), 5.29 (1H, *br m*, *J* = 1.6 Hz); ¹³C NMR (100.6 MHz, C₆D₆, solvent ref. 128.0, δ from TMS): 18.2 (*t*), 18.7 (*q*), 21.9 (*q*), 22.5 (*q*), 24.0 (*q*), 30.8 (*t*), 31.4 (*s*), 31.5 (*t*), 34.8

(*t*), 38.1 (*t*), 38.2 (*s*), 44.4 (*d*), 47.2 (*s*), 117.0 (*d*), 142.0 (*s*); multiplicities by DEPT.

Acid-catalyzed rearrangement of (–)- α -chamipinene – 3 mg **11** and 1 mg Cu(OAc)₂ · H₂O were dissolved in 100 μ l of HOAc in a 1 ml vial, which was briefly flushed with argon then capped. After 18 h in a 110° oven, the vial was cooled and the contents diluted 20 \times with water and shaken 3 \times with 1 ml pentane. The combined pentane extracts were washed with dil. aq NaHCO₃, dried over Na₂SO₄, and analyzed by GC (2 columns), GC–MS, and EGC. Major products were (+)- α -chamigrene (44%) and (+)- β -chamigrene (38%), identical by GC and GC–MS (but differing in r.t. on EGC) with (–)- α - and (–)- β -chamigrene produced by acid-catalyzed rearrangement of authentic (–)-thujopsene under the same conditions (Daeniker et al., 1972).

3.11. (–)-10-*epi*- β -Acoradiene, (1*S*,4*S*,5*R*)-1-isopropenyl-4,8-dimethylspiro[4.5]dec-7-ene (**12**)

Oil. $[\alpha]_D^{22}$ –4.1° (*c* 1.8, *n*-hexane); HREIMS: *m/z* found 204.1877, Calc. for C₁₅H₂₄ 204.1878; GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (7), 189 (5), 175 (2), 161 (16), 148 (10), 147 (21), 134 (8), 133 (12), 121 (52), 119 (100), 108 (21), 107 (23), 105 (40), 93 (62), 91 (27), 79 (30), 68 (16), 67 (17), 55 (18), 53 (12), 41 (28); ¹H NMR (500.17 MHz, C₆D₆, solvent ref. 7.15, δ from TMS): 0.93 (3H, *d*, *J* = 6.7 Hz, H-15), 1.29 (1H, *m*, H-9 α), 1.44 (2H, *dd*, *J* = 6.8, 6.0 Hz, H-6), 1.62 (3H, *br s*, H-14), 1.64 (1H, *m*, H-8 β), 1.68 (1H, *m*, H-10), 1.69 (1H, *m*, H-9 β), 1.74 (3H, *s*, H-13), 1.78 (1H, *m*, H-8 α), 1.88 (2H, *br dm*, *J* \approx 17 Hz, H-2*eq*, H-5*ax*), 2.00 (2H, *br dm*, *J* \approx 17 Hz, H-2*ax*, H-5*eq*), 2.14 (1H, *dd*, *J* = 9.8, 8.3 Hz, H-7), 4.80 (1H, *s*, (Z)-H-12), 4.90 (1H, *s*, (E)-H-12), 5.38 (1H, *br s*, H-5); ¹³C NMR (125.8 MHz, C₆D₆, approx. δ from TMS, protonated carbons, indirect detection; multiplicities by HMQC): 15.8 (*q*, C-15), 23.3 (*q*, C-14), 24.0 (*q*, C-13), 24.0 (*t*, C-2), 27.8 (*t*, C-9), 28.9 (*t*, C-3), 31.5 (*t*, C-9), 39.1 (*t*, C-6), 44.1 (*d*, C-10), 58.1 (*d*, C-7), 112.2 (*t*, C-12), 121.2 (*d*, C-5).

Hydrogenation of **12 and (–)- α -acoradiene **13**** – ca. 1 mg of sesquiterpene in ca. 300 μ l hexane was hydrogenated over Pd/C catalyst at 70 atm H₂ pressure for (a) 1 h (**13**), giving *M_r* 208 products **14a–d** in ratios 23:69:2:6; (b) 12 h (**12**), giving *M_r* 208 products **ent-14a–d** in ratios 8:43:6:43; (c) 2 h (**12** with a trace of pyridine), giving *M_r* 208 products **ent-14a–d** in ratios 1:9:19:71.

3.12. (–)-Widdra-2,4(14)-diene, (4*aR*,9*aR*)-1,1,4*a*-trimethyl-7-methylene-2,3,4,4*a*,5,6,7,9*a*-octahydro-1*H*-benzo[7]annulene (**15**)

Oil. $[\alpha]_D^{22} \approx -18^\circ$ (*c* 0.27, benzene-*d*₆); UV $\lambda_{\max}^{\text{hexane}}$ nm (log ϵ): 233 (3.8); GC–MS 70 eV, *m/z* (rel. int.): 204 [M]⁺ (18), 189 (39), 161 (49), 148 (23), 133 (47), 123 (37), 121 (54), 119 (61), 111 (78), 105

(100), 94 (80), 93 (78), 91 (95), 79 (63), 69 (58), 55 (42), 41 (72).

Hydrogenation of (+)-widdrol and **15; determination of absolute stereochemistry:** 30 mg authentic (+)-widdrol (7*S* absolute stereochemistry), dissolved in 0.4 ml AcOH and with a catalytic amount of PtO₂, was hydrogenated for 18 h by bubbling in H₂ at atmospheric pressure (Enzell, 1962). Contaminating hydrocarbons were removed from the single oxygenated product by passing the mixture in hexane through a small silica gel column, then eluting the *cis*-dihydrowiddrol [(4*aS*,7*S*,9*aS*)-1,1,4*a*,7-tetramethyldecahydro-1*H*-benzo[7]annulen-7-ol] with 25% EtOAc/hexane (yield ca. 20 mg). NMR: ¹H (500.13 MHz, CDCl₃, solv. ref. 7.26, δ from TMS): 0.89 (3H, *s*, H-13), 1.00 (3H, *s*, H-12), 1.07 (1H, *d*, *J* = 10 Hz, H-1), 1.10 (3H, *s*, H-15), 1.12 (1H, *m*, H-2 β), 1.13 (1H, *m*, H-8*b*), 1.17 (1H, *m*, H-10*b*), 1.18 (1H, *m*, H-6 α), 1.24 (3H, *s*, H-14), 1.35 (2H, *m*, H-8*a*, H-10*a*), 1.36 (1H, *m*, H-6 β), 1.41 (1H, *m*, H-3 α), 1.46 (1H, *m*, H-9 β), 1.51 (1H, *m*, H-5 β), 1.53 (1H, *m*, H-2 α), 1.58 (1H, *qt*, *J* = 6.1, 1.8 Hz, H-9 α), 1.76 (1H, *dd*, *J* = 13.5, 11.7 Hz, H-5 α), 1.87 (1H, *ddd*, *J* = 13.1, 7.7, 2.4 Hz, H-3 β); ¹³C NMR (125.77 MHz, CDCl₃, solv. ref. 77.0, δ from TMS): 17.9 (*t*, C-9), 22.2 (*t*, C-2), 27.8 (*s*, C-14), 29.0 (*s*, C-13), 30.3 (*s*, C-15), 32.1 (*s*, C-12), 34.4 (*s*, C-7), 35.1 (*t*, C-8), 35.6 (*t*, C-10), 36.4 (*s*, C-11), 37.1 (*t*, C-5), 39.8 (*t*, C-6), 46.0 (*t*, C-3), 55.3 (*d*, C-1), 73.2 (*s*, C-4); assignments by HMQC and HMBC, *cis* ring fusion confirmed by NOESY cross-peaks from H-15 to H-12 (both α -oriented) and from H-12 to H-1, in reference to minimum-energy conformation determined by MM2 calculation. 10 mg of *cis*-dihydrowiddrol was dehydrated for 1 h with 8 drops POCl₃ in 0.3 ml pyridine, excess POCl₃ destroyed by adding ice, and the mixture of monoenes (2 isomers, ca. 4:1 ratio) extracted with pentane. About 2 mg of the product hydrocarbon mix was directly hydrogenated with a catalytic amount of PtO₂ in 0.4 ml MeOH for 2 h, yielding 2 fully saturated epimers in ca. 2:1 ratio, with some of the major monoene remaining unreacted. 1 mg **15** was similarly hydrogenated, yielding 2 fully saturated epimers in ca. 1:1 ratio. Retention times of the widdrol-derived widdranes were identical with those from **15** on conventional dual-column GC but longer on EGC.

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