

Stereochemistry of 1,6-Germacradien-5-ol, a Constituent of the Needles of Scots Pine (*Pinus sylvestris*) and of the Defence Secretion from Larvae of the Pine Sawfly *Neodiprion sertifer*

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(–)-1,6-Germacradien-5-ol [(*E,E*)-1,5-dimethyl-8-isopropylcyclodeca-1,6-dien-5-ol, **1**] has been isolated from the defence secretion from larvae of the pine sawfly *Neodiprion sertifer* and from needles of Scots pine (*Pinus sylvestris*). It was characterised by means of spectroscopic methods and by its optical rotation. Acid-promoted transannular cyclisation isomerised **1** to the known (–)- α -cadinol (**4**), whereby the configuration at position 8 was established as *S*. The configuration at the second stereocentre, position 5, carrying the alcohol group is discussed on the basis of molecular mechanics calculations (MM2), NMR shift values, coupling constants and NOESY experiments. 1-*endo*-Bourbonanol (**7a**) and (–)-5-methoxy-1,6-germacradiene (**8**) were prepared from **1** and investigated by NMR spectroscopy. The evidence collected indicated that the stereoisomer isolated was (5*S*,8*S*)-1,6-germacradien-5-ol.

The pine sawfly *Neodiprion sertifer* is a serious pest of Scots pine (*Pinus sylvestris*). The larvae form colonies and feed on pine needles, sometimes resulting in serious defoliation. The larvae sequester the resin from the needles in pouches in the foregut and use this as a defence against predators such as wasps, ants, spiders and birds.¹ The viscous defence secretion of *Neodiprion sertifer* larvae has been shown to contain monoterpenes, sesquiterpenes and resin acids.^{2–4} GC–MS analysis of the neutral fraction of the secretion recently identified its major sesquiterpene constituent as 1,6-germacradien-5-ol (**1**).⁴ This compound has also been isolated from other natural sources.^{5–16} However, the relative and absolute configurations assigned to the various samples of **1** vary and the arguments used by different authors are not consistent with each other.^{5–16} These assignments have been made on the basis of optical rotations and NMR-spectral data (e.g. NOE^{6,7,10,11}) and are briefly summarised in Table 1. Both of the possible relative configurations [(5*R**,8*R**) and (5*R**,8*S**)] have been proposed for **1** isolated from different sources. When the optical rotation values available are compared, no significant difference can be found between the two diastereomers. The reported values for the various (–) and (+) samples could perhaps be the result of a natural variation of the

Table 1. Some literature data for 1,6-germacradien-5-ol (**1**).

$[\alpha]_D$ (CHCl ₃)	δ_{H6} d (CDCl ₃)	δ_{H7} dd (CDCl ₃)	Configurations at C5 and C8	Refs.
–117, <i>c</i> 1.0	5.17	5.25	5 <i>R</i> ,8 <i>S</i>	5, 6
+122, <i>c</i> 1.22	5.24	5.19	5 <i>R</i> ,8 <i>S</i> (relative)	7
–201, <i>c</i> 1.095			5 <i>R</i> ,8 <i>S</i>	8
N.r.	Ref. to 5	Ref. to 5	5 <i>R</i> ,8 <i>S</i> (relative)	9
+184, <i>c</i> 3.1	5.25	5.18	5 <i>R</i> ,8 <i>R</i>	10
–109, <i>c</i> 0.62	5.25	5.17	5 <i>S</i> ,8 <i>S</i>	6
–112, <i>c</i> 0.25	5.25	5.17	5 <i>S</i> ,8 <i>S</i>	11
–177, <i>c</i> 12.7			5 <i>S</i> ,8 <i>S</i>	12
–130, <i>c</i> 1.46	Ref. to 4	Ref. to 4	5 <i>S</i> ,8 <i>S</i>	13
–64, <i>c</i> 1.0	Ref. to 4	Ref. to 4	N.r.	14
N.r.	5.24	5.17	N.r.	15
N.r.	Ref. to 4	Ref. to 4	5 <i>S</i> ,8 <i>S</i>	16
–185, <i>c</i> 1.02	5.25	5.18	5 <i>S</i> / <i>R</i> ,8 <i>S</i>	This work

N.r. not reported.

optical purity of **1**. According to Bohlmann *et al.* the only significant difference observed in the proton NMR spectra of the two diastereomers (5*R*,8*S*)-**1** and (5*S*,8*S*)-**1** is that the chemical shifts of the olefinic protons H6 and H7 seem to have changed places in the two spectra.⁶ However, this switch was not noted by Izak *et al.*⁷ with their sample of (+)-**1**, to which they assigned the relative (5*R**,8*S**)-configuration.

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Attempts have been made to deduce the absolute configurations by chemical transformation of **1** followed by NMR studies. Thus, oxidation of (+)-**1**, nephtenol, a *Nepthea* coral constituent,¹⁰ with VO(acac)₂ – *tert*-BuOOH furnishes a diol, to which the structure **2** has been assigned on the basis of its NOE and CD spectra. This provides evidence for the (5*R*,8*R*) configuration of the starting material (Fig. 1).¹⁰ In addition, MCPBA oxidation of (–)-**1** followed by NOE studies on the major product **3** and NMR solvent shift studies on the ketone obtained on oxidation of **3** supported the (5*S*,8*S*) configuration of this sample of **1** (Fig. 1).¹¹

Results and discussion

We have long been interested in the ecological chemistry of pine sawflies.^{17,18} The defence secretion of the larvae is probably of vital importance to the survival of the species in the ecosystem in which they live,¹ and the major sesquiterpene component of the secretion,⁴ (–)-germacradienol, **1**, may play an important role in the larval defence. For that reason, this compound attracted our attention as a potential semiochemical.

Initially we isolated (–)-1,6-germacradienol (**1**, [α]_D²⁵ –181) preparatively from the defence secretion of *Neodiprion sertifer* larvae. A more practical source of the same compound appeared to be the needles of *Pinus sylvestris* on which the larvae feed. Thus, solvent extraction of 1 kg of Scots pine needles followed by chromatographic purification furnished 1.3 g of pure germacradienol (**1**, [α]_D²⁵ –185). Before initiating synthetic work and studies on the biological activity of **1** we wanted to establish its stereochemistry firmly.

In the light of the discussion presented above we found it difficult to assign unambiguously the correct config-

uration of our isolated 1,6-germacradien-5-ol sample on the basis of previous work^{5–16} only.

Germacradienol **1** is quite an unstable compound. It is sensitive to light as well as to traces of acid. Thus, the (–)-germacradienol **1** that we had isolated from pine needles was readily converted, as previously described,¹² into a mixture of (–)- α - and T-cadinol (**4**, 64% and **5**, 22%, respectively) on treatment with acid (Fig. 2). The absolute configuration of (–)- α -cadinol is known.¹⁹ Since the stereogenic centre at position 5 in **1** was destroyed in this reaction, but the one at position 8 was preserved, it followed that the (–)-1,6-germacradienol sample that we had isolated had the (8*S*) configuration. However, the relative configuration was still ambiguous. In an attempt to clarify this point we decided to study our sample of (–)-**1** using various NMR techniques. In order to interpret the results correctly, the preferred conformation of the relatively flexible 10-membered ring in **1** must first be established.

Previous molecular mechanics studies (MM2) on the (*E,E*)-cyclodeca-1,6-diene systems **6a–c** show that the 10-membered ring preferentially adopts a conformation with parallel double bonds and that the ‘chair–chair’ conformation has the lowest energy (Fig. 3).²⁰ In **6a,b** the parallel ‘boat–chair’ conformation is 10 and 6 kJ mol^{–1} higher in energy, respectively, while conformations in which the double bonds are crossed are even higher in energy, 14 and 11 kJ mol^{–1}, respectively (Fig. 3a). For germacrene-D (**6c**) the order between the ‘boat–chair’ and the ‘crossed’ conformers is changed so that the ‘crossed’ and ‘boat–chair’ conformations are 6 and 13 kJ mol^{–1} higher, respectively, than the lowest parallel ‘chair–chair’ conformation of **6c** (Fig. 3b).²⁰

MM2 calculations on germacradienol **1** showed that the isopropyl group effectively locks the ring half to which it is attached in a conformation where the isopropyl group is pseudoequatorial. Conformations where the isopropyl group is pseudoaxial were found to be 13–20 kJ mol^{–1} higher in energy. The hydroxy and methyl groups attached to carbon 5 in **1** in the other half of the 10-membered ring can be expected to stabilise or destabilise the preferred parallel ‘chair–chair’ conformation of the ring depending on the relative configurations of positions 5 and 8. The eight minimised ring conformers of **1**, judged to be the most stable ones, are shown in Fig. 4.

It is known that 1-methylcyclohexanol prefers the conformation with an axial hydroxy group, which is 1.5 kJ mol^{–1} lower in energy than that with an equatorial one.²¹ In 3-hydroxycyclohexene, the allylic hydroxy group is also stabilised in the pseudoaxial position, which has been interpreted as being due to an anomeric effect.²² In 4-hydroxycyclohexene, an axial orientation of the hydroxy group is favoured, probably due to an intermolecular interaction with the double bond.²³ In contrast, the methyl groups in methylcyclohexane and in the methylcyclohexenes prefer the equatorial and pseudo-equatorial positions, respectively.^{22,23} From these known

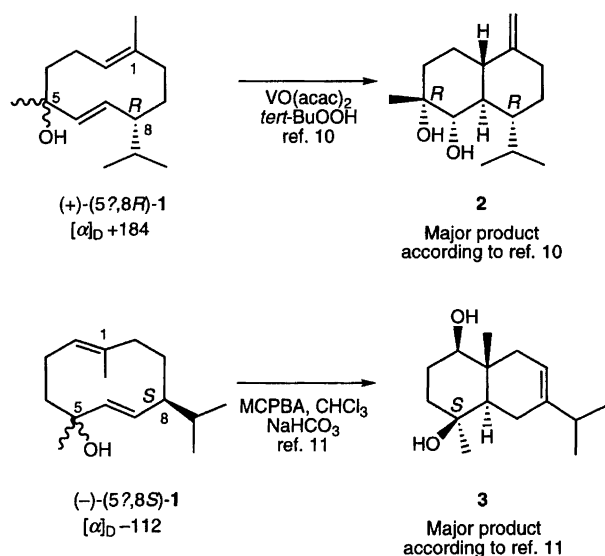


Fig. 1. Previously published transformations of (+)- and (–)-**1** into compounds, the structures of which support the relative (5*R**,8*R**)-configuration of **1**.

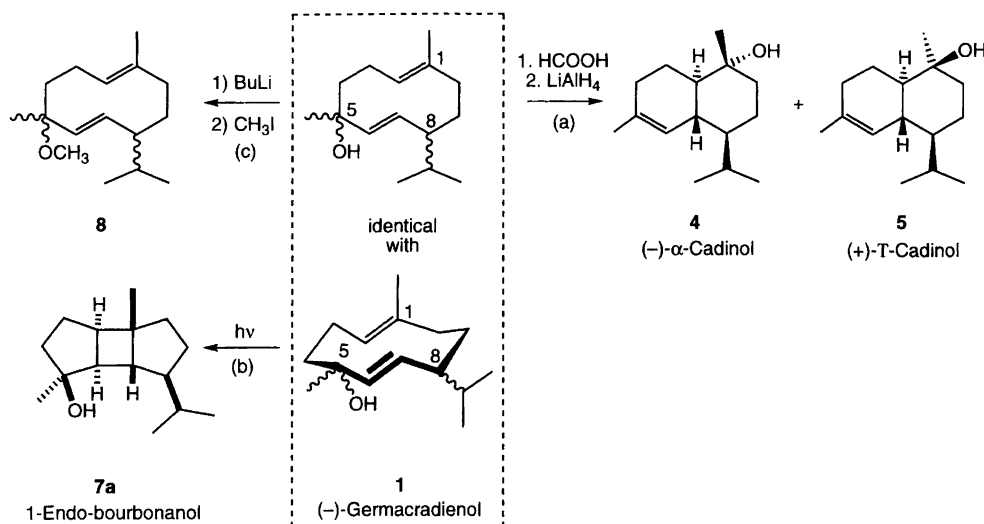


Fig. 2. (a) Isomerisation of (-)-1,6-germacradien-5-ol (**1**) in formic acid giving (-)-α-cadinol (**4**) and T-cadinol (**5**), revealing the configuration (8*S*) in **1**; (b) UV irradiation of **1** to give 1-endo-bourbonanol (**7a**) providing evidence for the relative configuration (5*R**,8*R**) in **1**; (c) 5-methoxy-1,6-germacradiene (**8**) was prepared from **1** by deprotonation with BuLi followed by nucleophilic substitution on CH₃I.

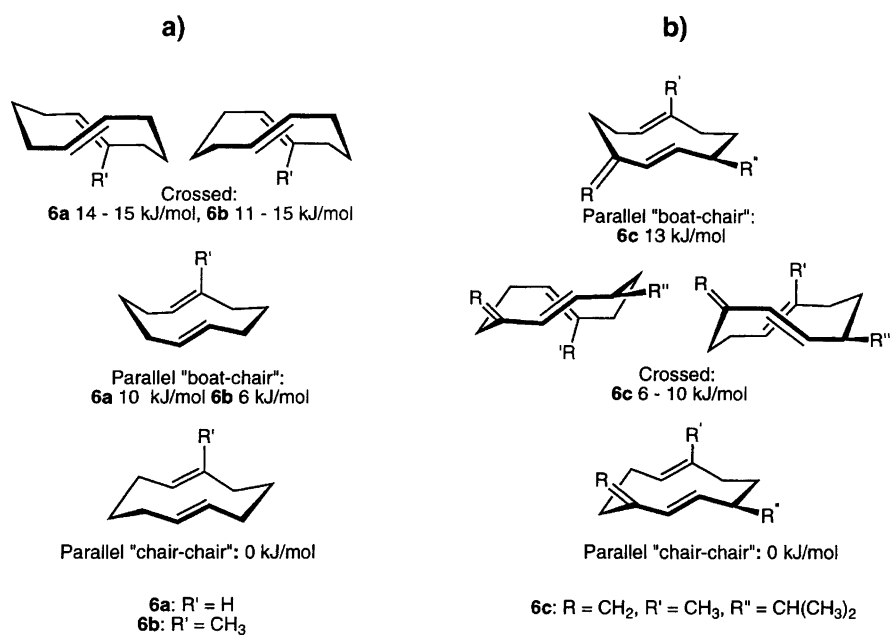


Fig. 3. (a) Relative energies of various conformations of (*E,E*)-cyclodeca-1,6-diene (**6a**) and (*E,E*)-1-methylcyclodeca-1,6-diene (**6b**) according to Ref. 20; (b) relative energies of some conformations of (*E,E*)-5-methylenecyclodeca-1,6-diene (germacrene-D, **6c**) according to Ref. 20.

conformational preferences one can assume that (5*S*,8*S*)-**1** will adopt a 'chair-chair' conformation, P1cc (see Fig. 4), with the hydroxy group in a pseudoaxial position. MM2 calculations on (5*S*,8*S*)-**1** gives an energy difference of 4 kJ mol⁻¹ in favour of the 'chair-chair' conformation P1cc over the 'boat-chair' conformation, P1bc. It was not equally simple to predict which conformation should be the favoured one in the (5*R*,8*S*)-diastereomer of **1**. On the one hand the 10-membered ring prefers the 'chair-chair' conformation, but on the other, the methyl and hydroxy groups prefer the pseudo-

equatorial and pseudoaxial positions, respectively, which may force the ring into the 'boat-chair' conformation, P1bc. MM2 calculations on the (5*R*,8*S*)-diastereomer of **1** indeed showed that the 'boat-chair' conformation P1bc is 3 kJ mol⁻¹ lower in energy than the 'chair-boat' conformation, P2cb (Fig. 4), in which the double bonds are oriented in the opposite direction, compared with P1. The 'chair-chair' conformer P1cc was found to be 5 kJ mol⁻¹ higher than the lowest one, P1bc.

The ¹H and ¹³C NMR data of our germacradienol sample are presented in Tables 2 and 3, respectively.

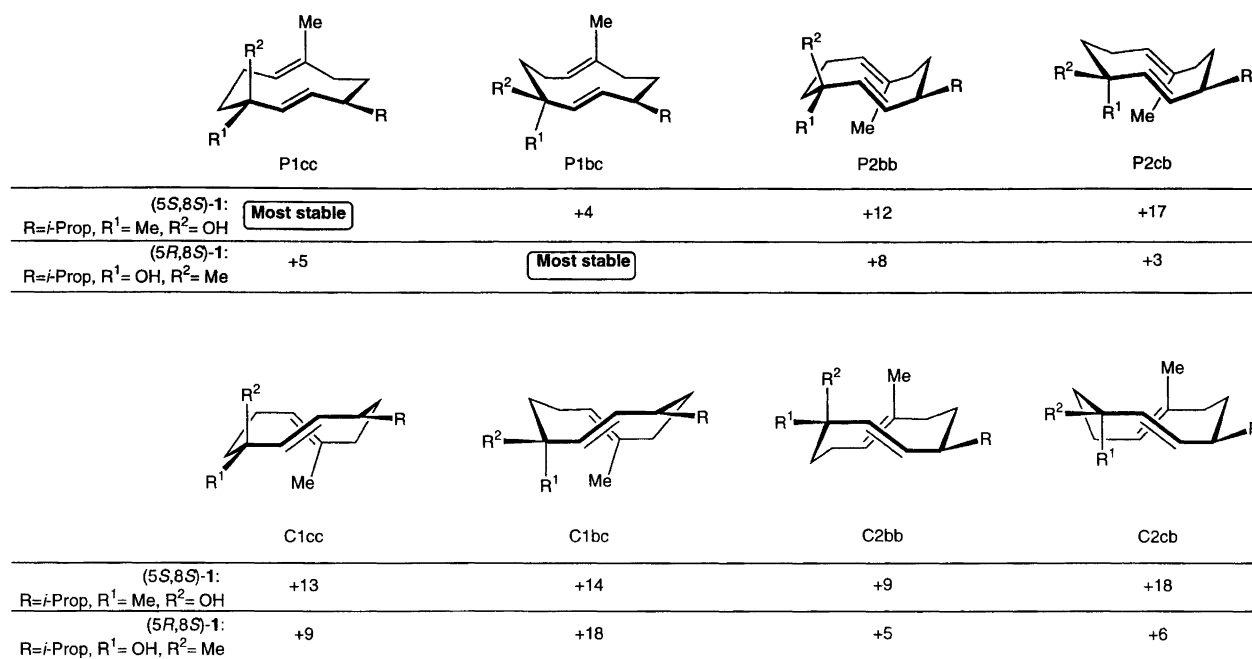


Fig. 4. The eight ring conformations of **1** with pseudoequatorial isopropyl groups and their relative MM2-energies (kJ mol⁻¹). P means parallel, C means crossed double bonds and cb means 'chair-boat' conformation, i.e., pseudochair in the left half and pseudoboat in the right half of the ring. In the crossed C-conformers the notation relates to the double bonds in the front of the drawings.

Table 2. ¹H NMR data for (–)-1,6-germacradien-5-ol (**1**).

¹ H	δ (C ₆ D ₆) ^a	δ (CDCl ₃) ^b	J/Hz	NOESY correlations ^c
H2	5.06 app d br ^d	4.95 app d br ^d	11.5	H10
H3'	2.76 dddd	2.50 dddd	15, 12.7, 11.6, 4.3	H14
H3	2.03 br d	1.95 br d	15	
H4'	1.59 m	1.64 m		
H4	1.44 m	1.54 m		
H6	5.16 d	5.25 d	15.8	H4', 8, 12, ^e 15
H7	5.39 dd	5.18 dd	15.5, 9.8	H13, 14
H8	2.05 m	2.01 m		
H9	1.44 m	1.39 m		
H10	2.38 m	2.25 m		H14
H11	1.48 m	1.39 m		
H12	1.04 d	0.83 d	6.6	
H13	0.99 d	0.79 d	6.9	
H14	1.68 br s	1.54 br s		
H15	1.21 s	1.19 s		

^aReference peak solvent 7.3 ppm. ^bReference peak TMS 0.0 ppm. ^cCross-peaks between signals from protons closer than 4 bonds are not included. ^dThe apparent broad doublet is a ddtq with ³J=11.5 Hz and 3 Hz and ⁴J≈1,1 Hz to the methyl group H14 and methylene group H10. ^eWeak.

Assignments were made on the basis of DEPT and 2D-techniques (COSY, r-COSY, DQF-COSY, NOESY and ¹H-¹³C HETCOR). Our results and assignments are in good agreement with those reported by Park *et al.*¹⁵ However, others have reported slightly different ¹³C-assignments.^{7,9,10} NOESY data on **1** are presented in Table 2 and Fig. 5.

The MM2 calculations on **1** described above led us to expect that the double bonds should prefer a parallel orientation. This was indeed supported by the NOE observed between H7 and H14 (Fig. 5). The parallel

orientation of the double bonds in **6b** (Fig. 3) gives a tailing towards the red in the UV spectrum.²⁴ A similar tailing towards the red was observed for **1**, which further supported the structure with parallel double bonds proposed for **1**. The formation of **4** and **5**, as described above, indicated that the double bonds were oriented as in conformers P1 not as in P2 (Fig. 4).

Further support for the P1 conformers was given by the large coupling constant between H7 and H8, ³J=9.8 Hz, which indicates a *trans* orientation of the two protons which is possible only in the P1 and C1 con-

Table 3. ^{13}C NMR data and DEPT of (–)-1,6-germacradien-5-ol (**1**).

^{13}C	δ (C_6D_6) ^a	δ (CDCl_3) ^b	DEPT multiplicity
C1	132.33	132.58	C
C2	129.31	128.88	CH
C3	24.14	23.67	CH ₂
C4	39.95	39.62	CH ₂
C5	72.66	73.12	C
C6	140.49	140.09	CH
C7	126.01	125.73	CH
C8	53.05	52.83	CH
C9	26.42	25.95	CH ₂
C10	41.66	41.28	CH ₂
C11	33.34	32.99	CH
C12	20.87	20.60	CH ₃
C13	19.22	18.96	CH ₃
C14	16.77	16.70	CH ₃
C15	31.24	30.69	CH ₃

^aReference peak solvent 128.0 ppm. ^bReference peak solvent 77.0 ppm.

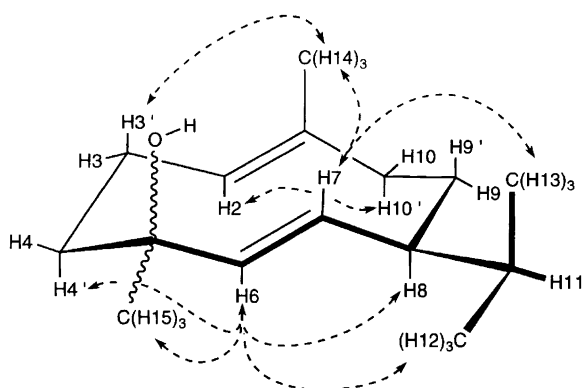


Fig. 5. The observed ^1H - ^1H NOEs for the stereoisomer of 1,6-germacradien-5-ol (**1**) isolated from pine sawfly larvae and Scots pine.

formers. The latter can be ruled out, since the double bonds should be parallel in the stereoisomer of **1** studied.

NOE between H6 and H4', H6 and H8, H3' and H14, H2 and H10' indicates a 'chair-chair' conformation, P1cc, of the 10-membered ring (Fig. 5). NOE between H7 and H13, H6 and H12 (weak) suggests that the major isopropyl rotamer is oriented as shown in Fig. 5.

Further support for the 'chair-chair' conformation (P1cc) suggested for **1** is given by the coupling patterns of H2 and H3'. The broad apparent doublet of H2 (Fig. 6c) is actually a *ddtq* with $^3J_{\text{H2-H3}'} = 11.5$ Hz, $^3J_{\text{H2-H3}} = 3$ Hz and $^4J \approx 1.1$ Hz (allylic couplings from H2 to H10, H10' and H14). One large and one small 3J for H2 is expected for a 'chair' conformation in that half of the ring (Fig. 6b). A 'boat' conformation (P1bc) in the same half would give a broad triplet with both $^3J \approx 7$ Hz according to simple Karplus relations (Fig. 6a). The coupling constants calculated using MacMimic[®] (Karplus eqn.) for H3' gives the observed *dddd* coupling pattern only in the 'chair' conformation, (Figs. 6a–c). Thus, the NOEs and the coupling constants clearly show

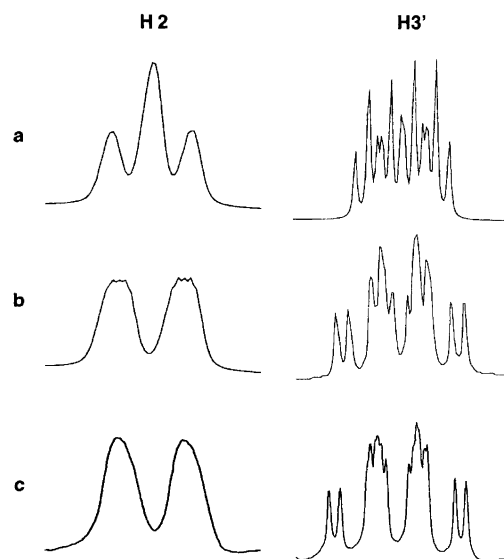


Fig. 6. (a) Simulated ^1H NMR spectrum of **1** in the 'boat-chair' conformation, P1bc, showing H2 and H3'; (b) simulated ^1H NMR spectrum of **1** in the 'chair-chair' conformation, P1cc, showing H2 and H3'; (c) part of 270 MHz ^1H NMR spectrum of **1** in CDCl_3 showing H2 and H3'.

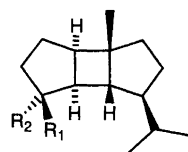
that the preferred conformation of the ring is 'chair-chair' (P1cc) in our sample of (–)-**1**.

NOE between H6 and H15 indicates that the methyl group at C5 is equatorial in the 'chair-chair' conformation. From this, the configuration at C5 is suggested to be *S* with the hydroxy group in an axial position (Fig. 5). Using MacMimic[®], the NOEs between the methyl protons H15 and the olefin proton H6 were estimated for both diastereomers of **1**. Theoretical NOEs $\approx 6\%$ were obtained for the (5*S*,8*S*)-diastereomer both in the 'chair-chair' (P1cc) and in the 'boat-chair' (P1bc) conformation. For 5*R*,8*S* the estimated NOEs were $\approx 2\%$ and 4% in the 'boat-chair' (P1bc) and 'chair-boat' (P2cb) conformers, respectively, and ≈ 0 for the 'chair-chair' (P1cc) conformer. Clearly the NOE between H6 and H15 can be explained by an (*R*) configuration at C5, but in that case the ring must have either a 'boat-chair' conformation (P1bc) or a 'chair-boat' conformation (P2cb) with the methyl groups in quasi-equatorial orientations (Fig. 4). This is contradictory to the conclusion drawn above, where the ring conformation was established as 'chair-chair'.

As regards protons situated in similar allylic positions, the chemical shift of the axial H3' is at a downfield position in relation to the chemical shifts of protons H3, H8 and H10 (Table 2, Fig. 5). The axial orientation of the hydroxy group in the suggested 'chair-chair' conformation P1cc for the (5*S*,8*S*)-diastereomer of **1** (Fig. 5) could be responsible for this by steric interactions with the axial H3'. In CDCl_3 the chemical shift of H3' was found 0.56, 0.50 and 0.26 ppm downfield compared with those of H3, H8, H10 and H10' respectively. In benzene-*d*₆, the chemical shift of H3' is 0.73, 0.71 and 0.38 ppm

downfield compared with those of H3, H8, H10 and H10', respectively (see Table 2).

Further support for the (5*S*,8*S*)-configuration was gained from additional chemical transformations. Photochemically induced intramolecular cyclobutane formation of (*E,E*)-cyclodeca-1,6-diene systems **6a–c** is well known.^{20,24} Thus, the parallel orientation of the double bonds in **1** should give the expected cyclobutane structures in the forecasted products 1-*endo*-bourbonanol (**7a**) or 1-*exo*-bourbonanol (**7b**) depending on the relative stereochemistry of **1**. Surburg *et al.*²⁵ have prepared **7a** from norbourbonanone (**7c**) and **7b** from β -bourbonene (**7d**). Our sample of (–)-**1** was transformed into a bourbonanol **7** by UV irradiation (Fig. 2), using a procedure described in the literature.¹² The ¹³C NMR data of the bourbonanol **7** obtained are presented in Table 4. Assignments were made with DEPT and ¹³C–¹³C 2D-INADEQUATE techniques and most of the ¹³C data corresponded well with the data reported²⁵ for β -bourbonene (**7d**). When we compared the signals from the methyl groups H11, H12, H14 and H15 (Fig. 7) in the proton NMR spectra of **7a** and **7b** as described by Surburg,²⁵ it was obvious that our proton NMR-data matched the data given for 1-*endo*-bourbonanol (**7a**) and not those given for the *exo*-compound **7b**. This confirmed the suggested (5*S*,8*S*)-configuration of our sample of



7a: R¹ = OH, R² = CH₃
7b: R¹ = CH₃, R² = OH
7c: R¹, R² = O
7d: R¹, R² = CH₂

Table 4. ¹³C NMR shifts, DEPT and 2D-INADEQUATE for 1-*endo*-bourbonanol (**7a**).

¹³ C	δ^a	DEPT multiplicity	2D-INADEQUATE ^b correlations
C1	80.13	C	C2, C9, C11
C2	39.97	CH ₂	C1, C3
C3	25.30	CH ₂	C2, C10
C4	43.00	C	C5, C8, C10, C12
C5	42.07	CH ₂	C4, C6
C6	30.14	CH ₂	C5, C7
C7	56.73	CH	C6, C8, C13
C8	47.55	CH	C4, C7, C9
C9	51.97	CH	C1, C8, C10
C10	43.88	CH	C3, C4, C9
C11	28.56	CH ₃	C1
C12	21.54	CH ₃	C4
C13	31.44	CH	C7, C14, C15
C14	21.81	CH ₃	C13
C15	22.32	CH ₃	C13

^aCDCl₃ as solvent and TMS as reference. ^b250 mg of **7a** in CDCl₃ as solvent and Cr(acac)₃ as relaxation agent.

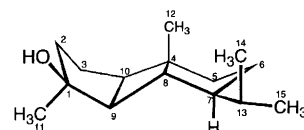


Fig. 7. (1*S*,7*S*)-1-*endo*-Bourbonanol (**7a**) in the conformation suggested by MM2 calculations and NMR spectroscopy.

(–)-**1**. The apparent triplet for H10 at 2.1 ppm suggests that the conformation of the five-membered ring is as shown in Fig. 7 where the coupling constant to one of the H3 protons becomes very small because the dihedral angle is close to 90°. NOE measurements were difficult to interpret because of the overlapping ¹H spectrum and no additional information on the configuration and conformation of **7a** was gained from those experiments.

In order to see whether further information about the conformation and configuration of compound **1** could be obtained, (–)-5-methoxy-1,6-germacradiene (**8**, [α]_D²⁵ –159) was prepared from (–)-germacradienol **1** by treatment of the latter with butyllithium and methyl iodide. The NMR data of (–)-**8** were rather similar to those obtained from **1**, with some exceptions. A shielding effect of the methoxy group could explain why the chemical shift of H6 in **8** is located 0.30 ppm upfield compared with that of H6 in **1**. The coupling pattern of H2 and H3' is similar though not identical to that observed for **1**. However, the conformation still appears to be 'chair–chair'. Irradiation of the singlet from the methoxy protons at 3.25 ppm gave only one significant NOE at the methyl protons H15. Unfortunately no NOESY cross-peaks from the methoxy group could be observed. The reason for this might be that the methyl group of the methoxy group points away from the ring and weak NOESY cross-peaks may be hidden in the T₁-noise of the intense signal from the methoxy protons.

The data and arguments presented above strongly indicate that the (–)-germacradienol isomer, which we have isolated from the larval secretion of *N. sertifer* and from needles of Scots pine, is (5*S*,8*S*)-**1**. However, in order to confirm this conclusion, it would be of great value to have access to both diastereomers of (–)-germacradienol **1**. At present we are working on a total synthesis, which hopefully will provide both these diastereomers.

Experimental

Materials and methods. Commercially available chemicals were used without further purification unless otherwise stated. Pentane and diethyl ether were distilled before use. Dry THF was distilled from a mixture of potassium and benzophenone before use. Defence secretion was collected in June 1995 from pine sawfly larvae, *Neodiprion sertifer*, feeding on needles of Scots pine (*Pinus sylvestris*) in a plantation in the province of Östergötland, Sweden. Needles were collected from Scots pine in Granlo, 3 km west of Sundsvall, Sweden. Preparative liquid chromatography

graphy (MPLC) was performed on straight-phase silica gel (Merck 60, 230–400 mesh) employing a gradient of an increasing concentration of diethyl ether in pentane as the eluent.²⁶ Air-sensitive reagents were handled with gas-tight syringes and the reactions were performed under argon. Melting points and boiling points are not corrected. ¹H and ¹³C NMR spectra were recorded on a Jeol EX 270 spectrometer and a Bruker DMX 500. FT-IR spectra were recorded using a Nicolet 55XC spectrometer. Optical rotation was measured on a Perkin Elmer 241 polarimeter. Mass spectra were recorded using GC-MS (Varian 3400 and an ion trap detector, Varian Saturn 2). GC analyses were carried out on a Carbowax[®] coated capillary column (20 m, $d_f=0.25\ \mu\text{m}$) with He as the carrier gas, and on a DB-5 coated capillary column (20 m, $d_f=0.25\ \mu\text{m}$) with N₂ as the carrier gas. HPLC analyses were carried out isocratically (90 and 100% methanol in water) on a Waters 600E equipped with a Spherisorb[®] 5 μ column and a refractive index detector. Elemental analyses were carried out by Mikrokemi, Uppsala, Sweden.

Molecular mechanics calculations. These were performed using MacMimic[®] employing the MM2(91) force field. Lone pairs were included in the calculations. Results obtained with the various ring conformers of (5*R**,8*R**)-1 and (5*R**,8*S**)-1 are given in Fig. 4. The hydroxy and isopropyl groups were rotated and minimised in all rotamers to find the global minimum for each conformer.

1,6-Germacradien-5-ol (1) from defence secretion. The secretion was collected on filter paper and extracted with pentane (2 × 300 ml). The solution was dried (MgSO₄) after which the solvent was evaporated off at reduced pressure, giving a colourless crude extract (0.76 g). The resin acids were removed by dissolving the extract in pentane (200 ml) and washing with 10% Na₂CO₃ (3 × 100 ml), 10% NaHCO₃ (100 ml) and saturated NaCl solution (100 ml). The clear colourless solution was dried (MgSO₄) and the solvent was evaporated off at reduced pressure to give a colourless oil (0.27 g), which contained 1,6-germacradien-5-ol (14% by GC). The components of the extract were separated by MPLC (40 g silica gel) giving 1,6-germacradien-5-ol 1 (8.7 mg, pure by GC and NMR), $[\alpha]_D^{25} -181$ (*c* 0.61 CHCl₃, lit. values see Table 1). NMR: see Tables 2 and 3. The mass spectrum was in good agreement with the literature reports.²⁷

1,6-Germacradien-5-ol (1) from pine needles. Fifty-gram portions of needles (1 kg) from *Pinus sylvestris* were cut in a mixer for 30 s into small pieces (1–5 mm). The needle pieces were extracted with distilled pentane (4.5 l) in an ultrasonic bath for 30 min, followed by further extraction at room temperature for 20 h. After filtration, the needle pieces collected were stirred with pentane (3 l) and then filtered off. The combined pentane extracts were washed with 10% Na₂CO₃ (2 × 600 ml), 10% NaHCO₃ (600 ml) and saturated NaCl (600 ml). The

clear green solution was dried (MgSO₄) and the solvent was evaporated off at reduced pressure, to give a dark green oil (14.6 g) which contained 1 (≈10% by GC). The components of the extract were separated by MPLC. The fractions containing 1 were combined and solvent evaporation left an orange mixture of crystals and an oil (3.4 g). The crystals were filtered off, washed with cold pentane, recrystallised from pentane, filtered off and washed again. The crystalline compound, m.p. 80–81.5 °C, $[\alpha]_D^{25} \approx 0$ (*c* 1.0 CHCl₃), was identified as 10-nonacosanol, which is the major component of the epicuticular wax of *P. sylvestris*.²⁸ The optical rotation for (*S*)-10-nonacosanol of high ee is reported to be $[\alpha]_D^{25} \approx 0$ (*c* 1.0, CHCl₃).²⁹ According to the literature, (*S*)-10-nonacosanol of high ee, isolated from *Picea pungens*, melts at 82 °C, while lower ee yields a lower m.p., e.g. *rac*-10-nonacosanol m.p. 76 °C. Thus the 10-nonacosanol isolated by us was probably of high enantiomeric purity but the configuration was not determined. The pentane fractions were combined (2.41 g) and chromatographed again, using silica gel (25 g) and a pentane–diethyl ether gradient as the eluent. The combined fractions containing 1 were evaporated and an orange oil remained (2.29 g, purity ≈90% by GC). Bulb-to-bulb distillation (113–114 °C/0.05 mbar) gave a clear colourless oil (1.27 g, pure by GC, HPLC and NMR). The compound isolated from the needles was identical with the compound isolated from the defence secretion as described above. $[\alpha]_D^{25} -184.6$ (*c* 1.02 CHCl₃), n_D^{20} 1.4964 (lit.¹² n_D^{22} 1.4961). UV (1.45 mM, hexane) extinction coefficients ϵ_{230} 1100, ϵ_{240} 518, ϵ_{260} 61. This tailing towards the red has also been found for (*E,E*)-1-methylcyclodeca-1,6-diene 6b.²⁴ IR (neat, NaCl): 3477, 2958, 2924, 2871, 1442, 1367, 1384, 1198, 982, 944 cm⁻¹. Anal. Found: C 81.3; H 11.65. Calc. for C₁₅H₂₆O: C 81.02; H 11.79. This compound rapidly decomposes at low pH and is sensitive to light.

(-)- α -Cadinol (4). The isomerisation of 1 to (-)- α -cadinol (4) was carried out as described in the literature.¹² Germacradienol (50 mg) was mixed with 85% formic acid (3 ml) and stirred at room temperature for 1 h. The mixture was extracted with pentane (3 × 1 ml) and the combined pentane fractions were washed with water (1 ml), dried (MgSO₄) and evaporated. The crude product was dissolved in dry diethyl ether (1 ml) and added to a suspension of LiAlH₄ (30 mg) in dry diethyl ether (3 ml) at room temperature. The mixture was stirred for 1 h after which THF–water 1:1 (60 μ l), 15% NaOH (30 μ l) and water (20 μ l) were added and the mixture was allowed to reflux for 10 min. After filtration, washing and drying (MgSO₄), the solvent was evaporated off to give a colourless oil (67 mg) consisting of a mixture of dehydrated products and two alcohols in the proportion 3:1 (no starting material). After separation by MPLC (13 g silica gel) the two alcohols were identified as (-)- α -cadinol (4) (32 mg, 64% yield) and T-cadinol (5) (11 mg, 22% yield). The spectral data were

in good agreement with the literature.¹⁹ (–)- α -Cadinol was further purified by bulb-to-bulb distillation (110 °C/0.15 mbar), m.p. 72–73.5 °C (lit.¹⁹ 73–74 °C) [α]_D²⁵ –34.8 (c 1.2 CHCl₃), {lit.¹⁹ [α]_D –37.1 (c 0.9 CHCl₃)}.

Bourbonanol (7a). The reaction was performed in a way slightly modified from that described.¹² Germacradienol (210 mg) in acetone (40 ml, p.a., deoxygenated) was irradiated under argon in a quartz vessel with a 300 W UV lamp for 24 h. After that time, less than 1% of the starting material remained (GC). The solvent was evaporated off and the residue was subjected to MPLC (25 g silica gel) to give the title compound (145 mg, 69% yield, >99% pure by GC), m.p. 62–64.5 °C. Sublimation at 42 °C and atmospheric pressure gave needle-shaped crystals, m.p. 66–67 °C (lit.¹² 69–70 °C). [α]_D²⁵ –1.4, lit.¹² +5.5 (c 3.7 CHCl₃), [α]₄₃₅²⁵ –1.0, [α]₄₃₅²⁵ +2.1, [α]₃₆₅²⁵ +12.0 (c 2.1 CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 0.88 (3 H, d, J =6 Hz, H14), 0.93 (3 H, d, J =6 Hz, H15), 0.99 (3 H, s, H12), 1.22 (3 H, s, H11), 1.35 (1 H, m, H13), 1.38 (1 H, m, H7), 1.53 (2 H, t, J =7 Hz, H5), 1.58 (1 H, dddd, J =14 Hz, 8 Hz, 8 Hz, 7 Hz, H3), 1.60 (1 H, s, –OH), 1.63 (1 H, m, H6'), 1.66 (1 H, dd, J =13 Hz, 7 Hz, H2), 1.69 (1 H, dd, J =14 Hz, 7 Hz, H3'), 1.77 (1 H, dd, J =7 Hz, 5 Hz, H9), 1.88 (1 H, ddd, J =13 Hz, 8 Hz, 5 Hz, H2'), 1.89 (1 H, ddd, J =14 Hz, 8 Hz, 7 Hz, H6), 1.93 (1 H, br d, J =6 Hz, H8), 2.13 (1 H, apparent t, J =7 Hz, H10). ¹³C NMR (125.8 MHz, CDCl₃): Table 4. The MS spectrum was in good agreement with the literature.²⁷ IR (KBr): 3328, 2943, 2857, 1448, 1369, 1167, 1144, 1104, 978, 931 cm^{–1}.

(–)-5-Methoxy-1,6-germacradiene (**8**). 1,6-Germacradien-5-ol (**1**) (0.250 g, 1.06 mmol) was stirred under argon in dry THF (3.0 ml) at –78 °C. Butyllithium in hexane (0.86 ml, 1.20 mmol) was added and the temperature was raised to room temperature (22 °C) over 1 h. The reaction was cooled to 0 °C, after which methyl iodide (0.47 g, 3.3 mmol) was added and the temperature was raised to room temperature overnight. No starting material **1** could be detected by GC. Methanol (40 mg, 1.25 mmol) was added and the solvent was evaporated off. The residue was dissolved in pentane (5 ml), washed with saturated NaCl (2 × 2 ml) and dried (MgSO₄). After filtration the product was purified by MPLC (9 g silica gel) and bulb-to-bulb distillation (109–110 °C/0.04 mbar) to give the title compound (0.231 g, 92% yield, >98% purity by GC), n_D^{20} 1.4865, [α]_D²⁵ –159.0 (c 1.8 CHCl₃). MS m/z (relative intensity): 236 (M^+ , 9), 221 (55), 205 (86), 204 (100), 189 (13), 161 (38), 123 (65), 121 (32), 105 (24), 85 (38), 81 (84). ¹H NMR (270 MHz, CDCl₃): δ 0.79 (3 H, d, J =6.9 Hz, H12), 0.84 (3 H, d, J =6.9 Hz, H13), 1.12 (3 H, s, H15), 1.35–1.48 (4 H, m, H4, H9, H11), 1.53 (3 H, s, H14), 1.78 (1 H, m, H4'), 1.85 (1 H, m, H3), 1.95 (1 H, m, H8), 2.22 (2 H, m, H10), 2.49 (1 H, dddd, H3'), 3.25 (3 H, s, CH₃–O), 4.94 (2 H, br d, J =15.8 Hz, H2, H6),

5.20 (1 H, dd, J =15.8 Hz, 9.9 Hz, H7). ¹³C NMR (67.8 MHz, CDCl₃): δ 16.57 (C14), 19.01 (C13), 20.70 (C12), 23.63 (C3), 24.62 (C15), 26.58 (C9), 33.15 (C11), 37.59 (C4), 41.22 (C10), 49.69 (CH₃–O), 52.83 (C8), 76.84 (C5), 129.58 (C2,C7), 132.36 (C1), 135.95 (C6). Anal. Found: C 81.0; H 11.8. Calc. for C₁₆H₂₈O: C 81.29; H 11.94.

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