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Sex pheromone of the pine sawfly *Diprion pini* (Hymenoptera: Diprionidae): chemical identification, synthesis and biological activity

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Abstract. The main component of the sex pheromone secretion of female *Diprion pini* L. (Hymenoptera: Diprionidae) from insects collected both in Finland and in France has been identified as a *threo*-3,7-dimethyl-2-tridecanol (8 ng per female) stereoisomer by GC-MS and synthesis. The secretion also contains lower and higher homologues in small amounts (1–4% of the main component). Combined gas chromatographic-electroantennographic detection showed activity in both natural and esterified extracts (acetates and propionates); the esters of the main component gave the largest responses. The acetates and propionates of the eight stereoisomers of 3,7-dimethyl-2-tridecanol were synthesized from enantiomerically highly enriched (>99% ee) building blocks. The stereochemistry of the main component was established to be (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol by GC analysis of the natural material. It was purified by liquid chromatography prior to the GC analysis of both its pentafluorobenzoates and its isopropylcarbamates on a non-chiral polar column (ECD) and a chiral column (NPD), respectively. Field tests demonstrated that both the acetate and propionate of the main component (100 µg of each applied on cotton roll dispensers) were active in attracting males, with or without the presence of several of the minor compounds. Experiments with smaller amounts of the acetate and the propionate (1 µg in France and 50 µg in Finland) demonstrated that the propionate was more active than the acetate, and that it also caught more males than a blend of the two compounds.
Key words. *Diprion pini*; semiochemicals; sex attractant; 3,7-dimethyl-2-tridecanol; chiral synthesis; lipase; chiral analysis.

The mainly holarctic sawfly family Diprionidae consists of about 125 species, including several which are considered to be severe pests of conifers, especially pine trees[1]. Females oviposit on the needles, which are then consumed by the larvae. The larval feeding results in reduced growth, and in some cases even death, of the trees. This led to attempts, beginning as early as 1960, to identify the female-produced sex pheromones of some pine sawflies[2–4]. However, it took more than one and a half decades before the first pine sawfly pheromone was identified. In 1976, the acetate and the propionate of the alcohol 3,7-dimethyl-2-pentadecanol (diprionol) were identified as behaviourally active compounds in North American *Neodiprion lecontei* (Fitch) and *Diprion similis* (Hartig), respectively[5]. Subsequently, the pheromones of several diprionids that occur in eastern North America were identified, either by analyses of the pheromone content of females or by screening various esters and isomers of diprionol in field trapping experiments[6]. All these species, which include several *Neodiprion* species native to North America as well as species introduced from Europe, namely *N. sertifer* (Geoffr.), *D. similis* and *Gilpinia frutetorum* F., use the acetate and/or propionate of different diprionol stereoisomers as their sex pheromone[5]. Within Europe, pheromonal studies have been restricted to *N. sertifer*[7–9], which seems to differ from the other species only in its response to a minor component of its pheromone[6]. Female sawflies in general contain chiefly diprionol itself; they have only very small amounts of its behaviourally active ester[6].

The relative stereochemistry of the *N. lecontei* pheromone at carbons 2 and 3 was identified as (2*R**,3*R**)- and it was defined as an *erythro*-isomer [the *threo*-isomers hence have the (2*R**,3*S**)- stereochemistry, and these definitions are used here].

Diprion pini L. is common in most parts of Europe and southwestern Siberia, and has been found in North Africa[10,11]. Mass outbreaks occur frequently and result in heavy defoliation of pine trees. Chemical insecticides are normally used in attempts to control outbreaks, and tests involving the release of natural enemies have been done on only a small scale. No virus potentially useful

in biological control is known for this species[10]. When we initiated our investigations of the sex pheromone of *D. pini*, the available evidence suggested that all diprionid sex pheromones consisted of stereoisomers of diprionyl esters. However, findings from electrophysiological studies, examining the responses of *D. pini* to such stereoisomers, and from preliminary behavioural field tests[12], made it clear that a more thorough investigation needed to be performed, the results of which are presented below.

Materials

Insects

In the autumns of 1991 and 1992, cocoons were collected from the soil in an outbreak area near Kauhajoki, southern Finland, and stored outdoors over the winter; insects emerging the following spring were collected and taken to the laboratory. In 1992 and 1993, a second batch of cocoons were obtained from a culture originating from Briançon in the French Alps, and bred outdoors in a Scots pine, *Pinus sylvestris* L., nursery at the Centre de Recherche d'Orleans in Olivet, France. Females used for chemical analyses were collected within a few hours after emergence and stored in a freezer (−20 °C) until extraction. Males used in electrophysiological tests were collected shortly after emergence and stored at 5 °C for at most five days before testing.

Chemicals

The ethyl acetate and pentane were of high purities (American Burdick & Jackson, Fluka, Switzerland), as were also pyridine (99.8%), methanol (99.5%), isopropyl isocyanate (98%) and 2,3,4,5,6-pentafluorobenzoyl chloride (highest purity available) (Fluka). Acetyl and propionyl chlorides were of pro analysi quality (Merck, Chimica, Geel, Belgium). The synthetic internal standard, (2*S*,3*S*,7*S*)-3,7-dimethyl-2-hexadecanol, and its lower homologues, (2*S*,3*S*,7*S*)-3,7-dimethyl-2-tridecanol and (2*S*,3*S*,7*S*)-3,7-dimethyl-2-tetradecanol, were synthesized according to Hedenström et al.[13]. The Chromabond cartridges contained either 100 mg or 500 mg sorbent weight with the SiOH phase (Skandinaviska GeneTec AB, Kungsbacka, Sweden).

Chemical analyses

Extraction. Potential pheromone candidates were isolated by solvent extraction. The whole bodies of 20 females were extracted in about 1 ml ethyl acetate for 72 h at room temperature.

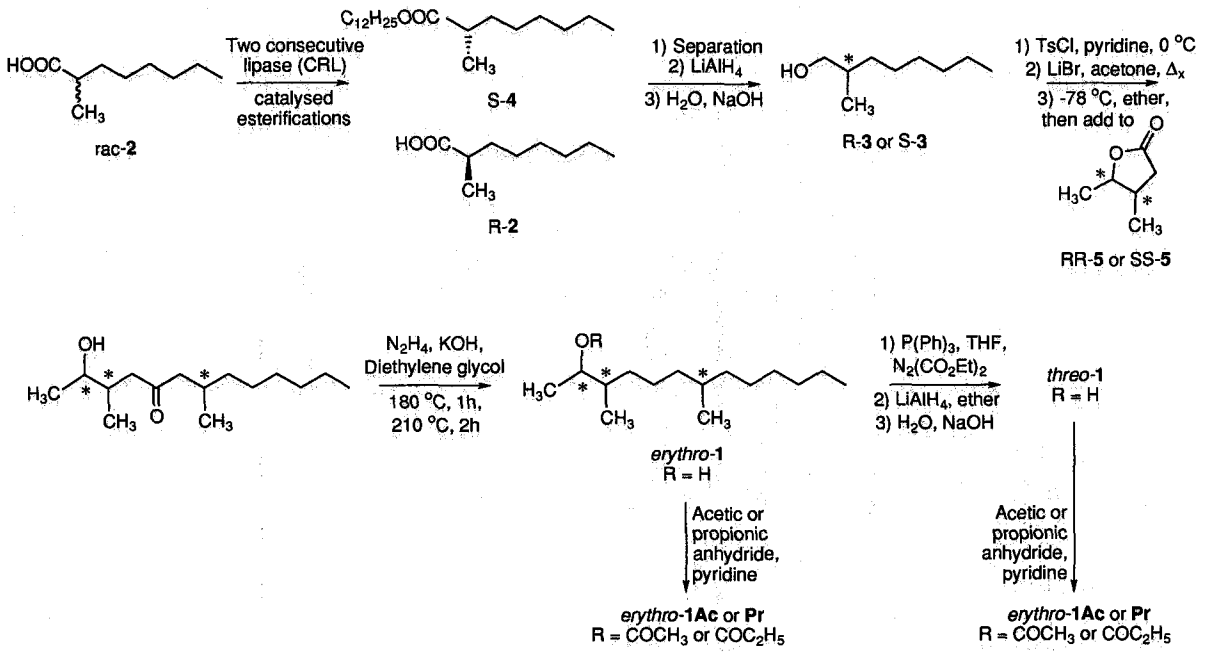
Liquid chromatography. Selective sample preparation was performed by solid phase chromatography (SiOH). Selection of the sorbent amount depended on the number of females from which the extract was made: 100 mg sorbent was suitable for 1 to 10 females and for a gradient elution from 1% to 10% ethyl acetate in pentane with a 150 µl eluation volume in each fraction; larger extracts were cleaned up on 500 mg and with a 450 µl elution volume. Recovery of 3,7-dimethyl-2-tridecanol and homologues, following sample preparation on Chromabond columns, was 73%. Fractions 6, 7 and 8, which contained 3,7-dimethyl-2-tridecanol and homologues, were combined and washed with 0.2 M KOH. For extracts containing more than 20 female equivalents, fractions 6–8 were chromatographed one additional time on a 100 mg Chromabond column before washing with KOH.

Gas chromatography. The amount of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol in fractions 6–8 was determined by gas chromatographic analysis with the addition of (2*S*,3*S*,7*S*)-3,7-dimethyl-hexadecanol as an internal standard[13]. The analyses were performed on an HP-5880 gas chromatograph equipped with a flame ionisation detector, and with a fused silica column (25 m × 0.25 mm I.D.) coated with CP-Wax-52 CB.

Chiral analysis. Determination of the stereochemical composition of 3,7-dimethyl-2-tridecanol was performed by two gas chromatographic methods. First the *threo*-isomers of 3,7-dimethyl-2-tridecanol were separated as isopropyl carbamate derivatives on a XE-60-(*S*)-valine-(*S*)-2-phenylethylamide column (50 m × 0.23 mm I.D.) equipped with a N/P detector[15]. Second, the diastereomers were separated as halogenated derivatives by electron capture detection, described in Wassgren and Bergström[14], using a Chrompack CP 9000 gas chromatograph fitted with CP-SIL-88 columns and a ^{63}Ni electron capture detector (ECD), Model 902. These methods were used for the analysis of natural materials and also for the purity determination of synthetic materials.

Gas chromatography-mass spectrometry (GC-MS). Compounds were identified with the use of a HP-5890 gas chromatograph coupled to a Finnigan TSQ 700 quadrupole mass spectrometer in the electron impact mode (EI). Chemical ionization (CI) at 70 eV, with methane as the reaction gas, was performed on the same instrument. The separation column was a polar DB-Wax (30 m × 0.25 mm I.D.). Amounts of homologues were calculated from the mass chromatograms of the ion m/z = 45.

Gas chromatographic-electroantennographic detection (GC-EAD). Fractions 7 and 8 of an extract (from 39 females) purified by liquid chromatography were combined and then divided into three equal parts. One part was acetylated with acetyl chloride, the second was transformed to propionate with propionyl chloride, and the third part was left unmodified (i.e. it contained natural untreated material). GC-EAD recordings were made both with esterified and non-esterified extracts. Preparation of the male antennae and recording of their responses were conducted as in previous studies on



Scheme. The synthetic sequence used for the preparation of the eight stereoisomers of 3,7-dimethyl-2-tridecanyl esters (**1Ac** and **1Pr**). * Symbolizes a stereogenic centre with a single configuration *S* or *R*.

N. sertifer[7,16]. About 2 female equivalents of the extract were injected into the GC (Hewlett-Packard 5830A) and passed through a fused silica column, coated with methyl silicone (DB-1), after which the extract was split into a 1:1 ratio by a T-tube. Half of the extract was led to the flame ionisation detector. The other half passed through an uncoated section of silica column, followed by a heated outlet, and then into a purified and moistened airstream, which carried the molecules to the antenna at an air speed of about 0.5 ms^{-1}.

Chemical syntheses of natural products

Compounds used as potential pheromone components in the biological tests were prepared employing essentially the same methodology as previously used by us for the syntheses of the eight individual stereoisomers of 3,7-dimethyl-2-pentadecanyl acetate (diprionyl acetate)[15]. Accordingly, the acetates and propionates of the eight pure stereoisomers of 3,7-dimethyl-2-tridecanol (**1**) were prepared as shown in the scheme. Racemic 2-methyloctanoic acid (rac-**2**) was subjected to enzyme catalysed esterification using commercial lipase from *Candida rugosa* (CRL). After two esterification cycles and subsequent lithium aluminium hydride ($LiAlH_4$) reductions, the remaining R-acid furnished (*R*)-2-methyl-1-decanol (R-**3**, >99.6% ee), and the S-ester produced (S-**4**) gave (*S*)-2-methyl-1-decanol (S-**3**, >98.8% ee); ee meaning enantiomeric excess. The appropriate enantiomer of the alcohol **3** was converted via the bromide into the alkyllithium, which was reacted with the appropriate enantiomer of *cis*-3,4-dimethyl-γ-butyrolactone (**5**) to give a ketoalcohol, which, when subjected to the Huang-Minlon reduction, gave the desired stereoisomer of *erythro*-3,7-dimethyl-2-tridecanol (**1**). A Mitsonubu reaction with benzoic acid furnished a benzoate with inverted configuration at carbon 2. $LiAlH_4$ reduction of the benzoate provided *threo*-3,7-dimethyl-2-tridecanol (**1**). Subsequent acylation furnished the desired acetate or propionate. Acetates and propionates of a mixture of the eight stereoisomers of 3,7-dimethyl-2-dodecanol were prepared in the same way from a racemic mixture of diastereomers of 3,4-dimethyl-γ-butyrolactone and racemic 2-methyl-1-heptanol.

Commercially available chemicals were used as received, unless otherwise stated. Dry diethyl ether ($LiAlH_4$), pyridine (CaH_2) and acetone (Na_2CO_3) were distilled from the indicated drying agents. Reactions sensitive to moisture and/or oxygen were carried out under argon. Preparative liquid chromatography was performed on straight phase silica gel (Merck 60, 230–400 mesh, 0.040–0.063 mm, 10–50 g/g of mixture) using gradient elution with an increasing concentration of distilled ethyl acetate in distilled cyclohexane[17]. Thin layer chromatography (TLC) was performed on silica gel plates (Merck 60, pre-coated aluminium foil) using ethyl acetate (20 or 40%) in hexane as an eluent, and developed by means of ultraviolet irradiation and/or by spraying with vanillin in sulfuric acid and heating at 120 °C. Unless otherwise stated, GC analyses were carried out using a capillary column [J & W DB-Wax, 30 m × 0.32 mm I.D., $d_f = 0.25$ μm, carrier gas He

Table 1. Catch of male *D. pini* in traps baited with 100 µg of the acetate or propionate of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol alone or in combination with minor compounds*, and released from two different kinds of dispensers. Test 1, Fontainebleau Forest, France, April 20, 1993.

Bait	Ester	Minor compounds* present	Dispenser type	Mean catch ± SD (n = 9)**
A	unbaited	-	-	0.1 ± 0.3^{a}
B	acetate	yes	rubber septa	0.3 ± 0.5^{a}
C	propionate	yes	rubber septa	0.6 ± 1.0^{a}
D	acetate	yes	cotton roll	10.0 ± 9.2^{c}
E	propionate	yes	cotton roll	4.8 ± 3.8^{bc}
F	acetate	no	rubber septa	0.2 ± 0.4^{a}
G	propionate	no	rubber septa	1.0 ± 1.7^{ab}
H	acetate	no	cotton roll	5.8 ± 5.3^{c}
I	propionate	no	cotton roll	10.3 ± 8.1^{c}

*Minor compounds in baits B and D consisted of: 3 µg 3,7-dimethyl-2-dodecanyl acetate (*threo*:*erythro* = 30:70), 2 µg (2*S*,3*S*,7*S*)-3,7-dimethyl-2-tetradecanyl acetate, 0.75 µg of each of the eight isomers of 3,7-dimethyl-2-pentadecanyl acetate, 1 µg (2*S*,3*S*,7*S*)-3,7-dimethyl-2-hexadecanyl acetate. Minor compounds in baits C and E consisted of: 3 µg 3,7-dimethyl-2-dodecanyl propionate (*threo*:*erythro* = 30:70), 0.75 µg of each of the eight isomers of 3,7-dimethyl-2-pentadecanyl acetate.
**Means followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test performed on log(catch + 1) transformed data.

(15 psi), split ratio 1/30]. Melting and boiling points are uncorrected and, unless otherwise stated, the latter are given as air bath temperatures (bath temp./mm Hg) in a bulb to bulb (Büchi GKR-51) apparatus. Optical rotations were measured in a 1 cm cell using a Perkin Elmer 241 polarimeter. IR spectra were recorded between NaCl plates using a Perkin Elmer 782 infrared spectrometer. NMR spectra were recorded with tetramethylsilane as an internal standard and using either a Jeol EX270 (270 MHz ^{1}H and 67.8 MHz ^{13}C) or a Jeol PMX60SI (60 MHz ^{1}H) instrument. Elemental analysis was performed by Mikrokemi AB, Uppsala, Sweden. Details of the synthetic methods can be found in the appendix.

Field tests

Attraction of males to pheromone candidates was tested on a bivoltine population in April (test 1) and May (test 2) 1993, during the flight of the first generation, and in August 1993 (test 3), during the flight of the second generation. These 3 field tests were conducted in a Scots pine stand in the Fontainebleau Forest about 100 km S.E. of Paris, France; Test 3 was also carried out in a Scots pine stand near Harjavalta, southern Finland in July, 1993. Compounds were applied to Lund-I sticky traps[26], which were placed in pine trees 1.5–2 m above the ground and at least 30 m apart. In order to reduce positional effects, the traps were moved to a new random position after each control. Tests 1 and 2 followed a Latin square design, with each bait being placed exactly once at each position; low flight intensity did not allow as many replicates for test 3. In test 1, the activity of the main component was compared with that of a synthetic mixture corresponding as closely as possible to the natural extract. Relevant isomers of the homologues detected in the female extract were added to the main component in approximately the same ratio as they occurred in the female. Not all identified substances were available and the exact composition of the 'synthetic full blend' is given in table 1. All test substances were applied to the dispenser as heptane solutions. In addition, the acetates and propionates of both the main component and the full blend were compared. Two dispenser types, rubber septa (red, 5 × 9 mm, A. Thomas Sci., New Jersey, USA) and cotton rolls (4 × 1 cm, Celluron No. 2, Paul Hartmann S.A., France) were also compared in this test to see which would be most useful in future experiments. In test 2, esterified raw extracts (not cleaned) were compared to the main component and to the full blend at the same concentration, using cotton rolls as dispensers. The acetate was obtained by adding acetyl chloride to the raw extract (about equal volumes), and then shaking the vial and keeping it at room temperature for 24 h. Surplus acetyl chloride was removed under nitrogen. The propionate was obtained similarly by adding propionyl chloride. In test 3, the possible synergistic effect between the acetate and propionate esters of the main component were tested, using cotton rolls as dispensers.

Results

Pheromone components and related compounds in whole body extracts of both Finnish and French *D. pini* were successfully identified by GC-MS analysis. In the first mass spectrometric analysis, carried out on an extract containing Finnish *D. pini*, the main compound could be identified as *threo*-3,7-dimethyl-2-tridecanol through comparison with a reference mixture containing *erythro*-3,7-dimethyl-2-tridecanol, *erythro*-3,7-dimethyl-2-tetradecanol and *erythro*- and *threo*-3,7-dimethyl-2-pentadecanol. Mass spectra of *erythro*- and

Table 2. Approximate amounts of homologues, calculated as percentage of the main sex pheromone component (100%) (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol in extracts of Finnish and French females. When no value is given, if a compound is present, it is below the detection limit.

Compound	Finnish[a] (1992)	Finnish[b] (1993)	French[c] (1992)	French[d] (1993)
threo-3,7-dimethyl-2-dodecanol	2,0	1,0	2,0	1,0
threo-3,7-dimethyl-2-tridecanol	100	100	100	100
erythro-3,7-dimethyl-2-tetradecanol	<0,5	1,0	-	-
threo-3,7-dimethyl-2-tetradecanol	3,5	4,0	3,0	3,0
erythro-3,7-dimethyl-2-pentadecanol	2,0	1,5	4,0	2,0
threo-3,7-dimethyl-2-pentadecanol	3,0	2,0	3,0	2,0
threo-3,7-dimethyl-2-hexadecanol	-	-	<0,5	-

[a]extract of 80 females; [b]extract of 70 females; [c]extract of 35 females; [d]extract of 24 females.

threo-isomers are similar in general appearance and the compounds differ only in their GC retention times. The main compound was identified by comparison with *erythro*- and *threo*-3,7-dimethyl-2-pentadecanol, which gave baseline separation. No traces of *erythro*-3,7-dimethyl-2-tridecanol were found. *Erythro*- and *threo*-isomers of homologues were identified in small quantities. Their identification, not possible by MS in EI (electron impact) mode, required the chemical ionization (CI) technique (which gave the molecular weight M-1). The *erythro*-isomer of 3,7-dimethyl-2-tetradecanol was found in extracts from Finnish *D. pini* females (table 2), while all extracts of both Finnish and French females contained *erythro*-3,7-dimethyl-2-pentadecanol in the same proportion as the *threo*-isomer. In addition, some compounds of high volatility were identified: (*E*,*E*)-2,4-heptadienal and (*E*,*E*)-2,4-decadienal. The known volatile *trans*-perillenal, which has been identified in *N. sertifer*[7], was not found in *D. pini* females, and no traces of any 3,7-dimethyl-2-tridecanyl esters were found in *D. pini* females.

Quantification of the main compound in whole body extracts was carried out by standard GC methods with a standard series covering the range 50–300 ng using a constant amount of internal standard (200 ng 3,7-dimethyl-2-hexadecanol)[7]. The plotted standard curve for 3,7-dimethyl-2-tridecanol was essentially straight in the concentration range found in the insects and the main component occurred in amounts of about 8 ng per individual. All isomers were calculated as percentages of the main compound, *threo*-3,7-dimethyl-2-tridecanol. Stereochemical identification of the *threo*-isomer 3,7-dimethyl-2-tridecanol, derivatized both as isopropyl carbamates and pentafluorobenzoates, was performed on an extract of French females. The four synthetic *threo*-stereoisomers separated as isopropyl carbamates on a XE-60-(S)-Valine-2-phenylethylamide column (50 m × 0.23 mm I.D.) and were detected by a N/P detector. However, as the sensitivity was not high enough to detect trace amounts in the extracts, a transformation to pentafluorobenzoate gave a higher response according to Wassgren and Bergström's method[14]. Figure 1a shows a gas chromatogram from an injection of LC fractions 7 and 8, which were derivatized to pentafluorobenzoate and detected by ECD. The injection contains approximately 100 pg of the main compound; no more stereoisomers were found. The synthetic (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol reference, which contains 2.5% of the (2*S*,3*R*,7*S*)-isomer, is shown in figure 1b, and a co-injection of a + b, i.e. a mixture of natural derivatized extract and the synthetic reference, is shown in figure 1c. The stereochemical identity of the main compound was thereby also determined as (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol.

Both the natural and esterified (acetates and propionates) whole body extracts showed electrophysiological activity. GC-EAD recordings using the natural extract revealed one active peak, whereas esterified extracts usually contained several compounds that elicited antennal activity (fig. 2). Esters of the main compound gave the largest EAD response. The insects also responded to three other compounds, which were present in very small amounts. These electrophysiologically active minor compounds were *threo*-isomers of homologues: 3,7-dimethyl-2-dodecanol, 3,7-dimethyl-2-tetradecanol and 3,7-dimethyl-2-pentadecanol; traces of 3,7-dimethyl-2-hexadecanol were present as well.

Three field tests were carried out in France and in Finland. The main purpose was to find out the activities of the acetate and the propionate of the main compound, and the role of the minor components.

Test 1, using esterified material, showed that both the synthetic full blend and the main component alone were highly attractive to *D. pini* males at the beginning of their flight season. Analysis of variance using as factors the type of ester, dispenser and blend, showed a significant effect for the dispenser type only ($p < 0.001$). No interactions were significant. Rubber septa apparently released the substances at too low a rate, and catches were at blank trap levels. Addition of minor substances

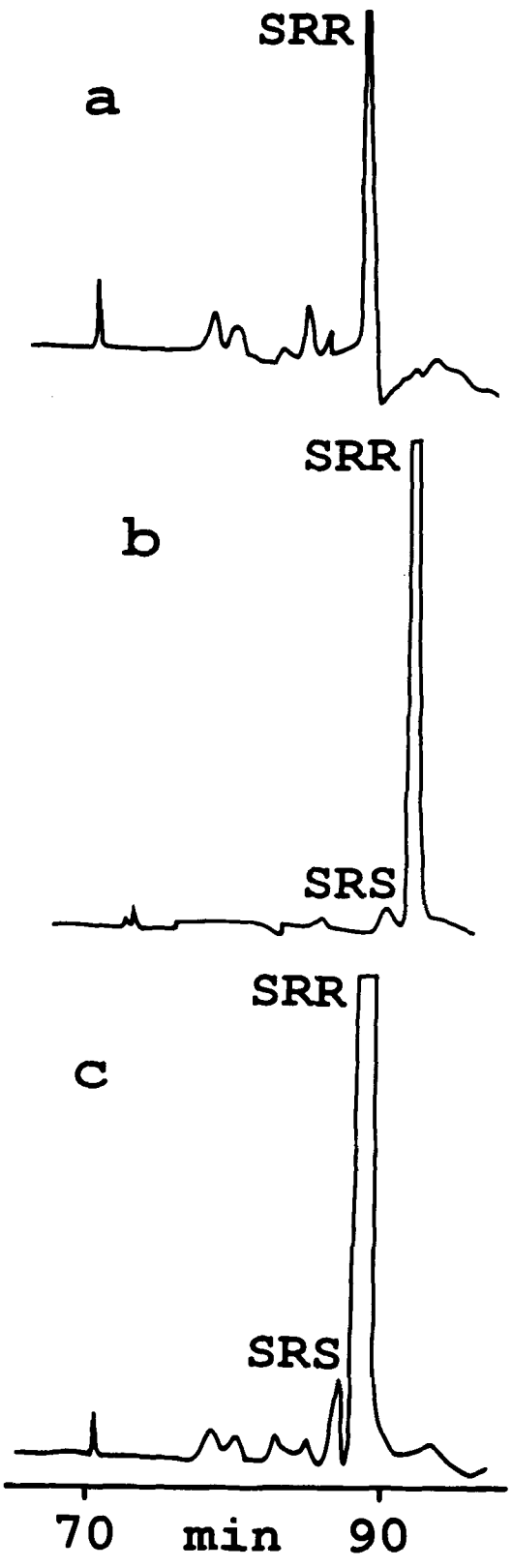


Figure 1. Diastereomeric analysis of perfluorobenzoyl esters. *a* An extract of French *D. pini* females, *b* synthetic (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol, *c* a co-injection of natural material and synthetic reference. Chromatographic conditions: columns, Sil-88, 50 m × 0.25 mm I.D. and 25 m × 0.25 mm I.D. coupled in series; 150 °C isothermal; carrier gas N_2 75 kPa.

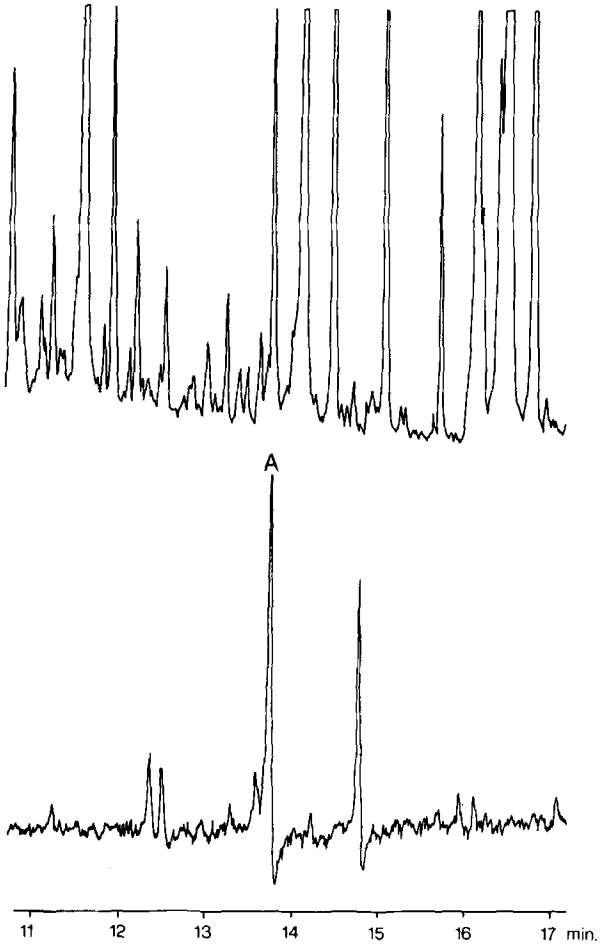


Figure 2. FID (upper trace) GC and GC-EAD (lower trace) recording of a male *D. pini* antenna stimulated with approximately two female equivalents of acetylated extract (liquid chromatography fractions 7–9), with the peak A corresponding to 3,7-dimethyl-2-tridecanyl acetate. Smaller peaks correspond to homologues. Insects were of Finnish origin.

to the main component did not increase the response levels and the acetate and propionate were equally attractive (table 1).

In test 2, made at the end of the first generation's flight season, the synthetic baits were 100 times weaker than in test 1, but the propionate baits were still attractive. Neither the synthetic acetate bait nor the esterified natural extracts of comparable concentration caught significantly more males than the unbaited trap (table 3).

Results from test 3 showed that the same main substance was also attractive to *D. pini* in Finland. However, in contrast to test 1 in France, only the baits containing the propionate were attractive, and no synergistic effect of the acetate was noted (table 4). The same experiment was tried in France, but due to low flight intensity of the second generation only 10 males were caught, eight with bait C (main component propionate) and two with bait D (1:1 mixture of main component acetate and propionate).

Table 3. Catch of male *D. pini* in traps baited with 1 µg (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanyl acetate or propionate, with or without minor compounds* added (baits B–E), and in traps baited with esterified raw female extracts (baits F and G). Test 2, Fontainebleau Forest, France, May 6, 1993.

Bait	Ester	Minor compounds* present	Mean catch ± SD (n = 7)**
A	unbaited	-	0.1 ± 0.4^a
B	acetate	yes	1.3 ± 1.5^a
C	propionate	yes	7.1 ± 2.9^b
D	acetate	no	1.3 ± 1.8^a
E	propionate	no	8.1 ± 3.8^b
F	female extract acetate***		0.3 ± 0.5^a
G	female extract propionate***		0.6 ± 0.5^a

*Minor compounds in bait B were identical to those in baits B and D of test 1 (table 1), but the amounts were only 1% of those in test 1. Minor compounds in bait C were identical to those in baits C and E of test 1.
**Means followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test performed on log(catch + 1) transformed data.
***Each extract was from 150 females each containing about 7 ng of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol. The intention was to make the extracts comparable in strength to the synthetic baits; they were, however, not GC-quantified.

Table 4. Catch of male *D. pini* in traps baited with (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanyl acetate or propionate or a combination of them. Test 3, Harjavalta, Finland, July 1–21, 1993. The results from two trap setups, with 2 and 4 replicates, were combined.

Bait	Ester, amounts	Mean catch ± SD (n = 6)*
A	unbaited	0.2 ± 0.4^a
B	acetate, 50 µg	0.0 ± 0.0^a
C	propionate, 50 µg	12.8 ± 9.5^c
D	acetate, 25 µg + propionate, 25 µg	3.2 ± 4.2^b

*Means followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's test performed on log(catch + 1) transformed data.

Discussion

This study demonstrates the existence of a pine sawfly sex pheromone that is not a diprionol ester. In the 12 sawfly species within the genera *Diprion*, *Neodiprion* and *Gilpinia* studied earlier, the attractive compound was found to be the acetate or propionate of a (2*S*)-diprionol isomer. In *Diprion pini*, however, the females contain about 8 ng of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol, which after acetylation or propionylation was highly attractive to conspecific males in field tests. This quantity of 3,7-dimethyl-2-tridecanol is similar to that of the active diprionol in females of *D. similis*[27], *Neodiprion pinetum* (Norton)[27] and *N. sertifer*[7,28,29]. In addition, several homologous alcohols, including diprionol, were found in amounts not exceeding 4% of this main compound. Several of these homologues were also found in *N. sertifer*[7], which suggests very similar pheromone biosyntheses among the different diprionid sawflies. The quantities of the main component and of the homologues were similar for females originating from Finland and France. The detection of *erythro*-3,7-dimethyl-tetradecanol only in Finnish females could be due to the fact that fewer French females were used to make the extracts.

The stereochemistry of the female pheromone precursors, determined earlier in four diprionid species, shows the main isomer in *D. similis* to be (2*S*,3*R*,7*R*)-diprionol and in *Neodiprion nanulus nanulus* Schedl, *N. pinetum* (Norton) and *N. sertifer* the (2*S*,3*S*,7*S*)-isomer. Field tests of male responses further suggest that several species use the (2*S*,3*R*,7*R*)-isomer, including *N. dubiosus* Schedl, possibly two or three other *Neodiprion* species, as well as *Gilpinia frutetorum* F. In the majority of *Neodiprion* species investigated to date males are attracted to the (2*S*,3*S*,7*S*)-isomer[6]. Thus, the stereochemistry of the *D. pini* pheromone is the same as that of the congeneric *D. similis*, but differs from that of most *Neodiprion* species.

The active ester has been detected in pine sawflies only once. Jewett et al.[5] found about 0.15 ng diprionyl acetate per female *N. lecontei* (Fitch) and about 1.85 ng diprionol per female, using about 30,000 individuals. None of the subsequent studies has reported the occurrence of the active ester in female extracts. Most likely the esterification takes place at the moment of release of the pheromone from the female. However, nothing is known about pheromone biosynthesis in pine sawflies. Both the acetate and propionate of (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol were attractive to male *D. pini*, at least in France. In Finland, only baits containing the propionate were active, but male catches were relatively low. The possibility that there is a geographical variation in pheromonal chemistry should be investigated in the future. Some of the previously investigated pine sawflies, e.g., *N. nanulus nanulus* and *N. sertifer* respond to both esters, whereas other species respond specifically to only one of the esters[6]. None of the homologues occurring in small quantities in the females of *D. pini* either enhanced or inhibited the activity when combined with the main compound (tables 1 and 3). We did not,

however, have access to all the isomers of homologues present in the females (table 2), and there is still a possibility that one or more of the minor compounds not yet tested have a synergistic or antagonistic effect. However, such activity has not been found in other diprionids, although in some species other stereoisomers of the diprionyl ester may influence the attractivity of the main isomer[6]. The possible existence of additional active compounds should be tested by comparing natural esterified extracts with synthetic (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanyl ester. The attempts made here (table 3) probably failed because raw (unpurified) whole body extracts were used. The low catches with the acetate and propionate female extract, baits F and G respectively in table 3, can be explained by limited (hampered) release of the pheromone from the cotton rolls because of the complexity of the extract. This material was not chromatographically purified prior to use in field tests.

Sex pheromones of pine sawflies are clearly more diverse than was earlier believed, and future studies of other European diprionids may reveal additional new pheromonal substances. A large diversity of diprionid pheromones will make it easier to understand how species within the early-flying group of pine sawflies in Europe find their conspecific mates, and avoid interspecific mating mistakes.

For future studies of *D. pini* mating behaviour, the cotton roll dispenser in combination with a sticky trap will be useful. Although the (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanyl ester is more volatile than the corresponding diprionyl ester, since it has two carbons fewer in the chain, it was released slowly from the rolls. However, the duration of activity has not yet been determined. Rubber septa, which are commonly used for release of moth pheromones, obviously released the compound at too low a rate to be useful.

The pheromone identification presented here provides scientists and foresters with a new tool for studying the occurrence and ecology of *D. pini*. The diprionids known as eruptive or outbreak species, e.g. *D. pini* and *N. sertifer*, are frequently studied during epidemic population phases, but to a much smaller extent during the latency period. During such endemic periods these species are often less common than the non-eruptive species[30], and pheromone traps could improve the effectiveness of investigations during such conditions. There also exists a possibility to develop pheromone based monitoring and control programs for *D. pini*.

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Appendix

Details of synthetic methods

(*S*)-(−)-2-Methyl-1-octanol (S-**3**). This compound was prepared using two consecutive lipase (CRL) catalysed esterification steps (similar to the one described in Berglund et al., which started with *rac*-2-methyldecanoic acid)[17] starting with *rac*-2-methyloctanoic acid (rac-**2**) (20.0 g, 126 mmol). The first esterification step gave 1-dodecyl (*S*)-2-methyloctanoate (S-**4**) (18.7 g, 46%, 87.3% ee) at a conversion (c) of 0.46. A sample of the S-acetate (10 mg) at c 0.46 was reduced (LAH) and oxidized (CrO_3) to the S-acid (see below), transformed into the acid chloride ($SOCl_2$), and reacted with (*R*)-1-phenylethylamine (100% ee). The resulting diastereomeric mixture of amides was analysed by GC and readily separated {[iso 190 °C, retention times: RR (minor) 21.00 min; RS (major) 22.81 min], 'amide'-method[18]}. The ester S-**4** was reduced to (*S*)-2-methyl-1-octanol (S-**3**) (6.75 g, 86%) with excess $LiAlH_4$ as described in ref. 18. Alcohol S-**3** (6.54 g, 46.9 mmol) was then oxidized to (*S*)-2-methyloctanoic acid (S-**2**) in acetone (1,0 l) for 2 h at room temperature by addition of Jones' reagent[19,20] (25 ml, 66.8 mmol, 2.67 M H_2CrO_4 in H_2SO_4/H_2O); saturated sodium bisulfite (500 ml) was carefully added to the reaction mixture. The mixture was extracted with pentane (3 × 500 ml), and the combined pentane phases were extracted with saturated sodium carbonate solution (5 × 200 ml). The pooled carbonate solution was acidified to pH 1 (conc HCl aq) and extracted with diethyl ether (5 × 300 ml). The ether phase was dried ($MgSO_4$) and concentrated to give S-**2** [6.0 g, 84% yield, >97.0% purity by GC and 87.3% ee (amide method[18])]. This S-acid sample (5.8 g, 36.7 mmol) was subjected to the second lipase catalysed esterification to give the ester S-**4** [98.8% ee (amide method[18]) at a conversion of 0.84], which was reduced to the alcohol S-**3** [4.2 g, 95% yield over the two steps, >98.0% pure by GC and 98.8% ee (amide method[18])], bp 100–104 °C/12 mm Hg and $[\alpha]_D^{20} - 10.62° \pm 0.03°$ (neat). Spectroscopic and physical data were identical to those described for R-**3**.

(*R*)-(−)-2-Methyloctanoic acid (R-**2**). The acid R-**2** [10.4 g (70.4% ee, amide method)[18]], obtained from the first esterification above, which gave S-**2**, was esterified once more to 0.36 conversion to give the title compound R-**2** pure by GC. [7.0 g and 99.6% ee (amide method)[18]]. $[\alpha]_D^{20} - 17.60° \pm 0.03°$ (neat). n_D^{20} 1.4300 ± 0.0002. IR 2955, 2925, 2857, 1707, 1465, 1415, 1237 cm^{-1}. ^1H 270 MHz NMR: δ 0.93 (3H, t, $J = 6.6$ Hz), 1.1 (3H, d, $J = 6.9$ Hz); 1.16–1.50 (8H, m), 1.43 (1H, m), 1.67 (1H, apparent sextet, $J = 7.0$ Hz), 2.45 (1H, apparent sextet, $J = 6.9$ Hz), 10.5 (1H, bs) ppm. ^{13}C NMR: δ 14.31, 17.07, 22.86, 27.37, 29.44, 31.95, 33.82, 39.59, 183.25 ppm.

Table 5. 270 MHz ^{1}H and 67.8 MHz ^{13}C NMR data of the eight stereoisomers of 3,7-dimethyl-2-tridecanol [see scheme (**1**)].

Compound **1**	^{1}H NMR (270 MHz)	^{13}C NMR (67.8 MHz)
RRR and SSS	δ 0.78 (3H, d, J = 6.4 Hz), 0.81 (3H, t, J = 6.3 Hz), 0.82 (3H, d, J = 6.7 Hz), 1.08 (3H, d, J = 6.3 Hz), 0.95–1.45 (19H, m), 3.63 (1H, d of q, J = 4.3 and 6.3 Hz)	δ 14.11, 14.18, 19.75, 20.25, 22.68, 24.76, 27.03, 29.67, 31.95, 32.76, 33.01, 37.00, 37.38, 39.79, 71.27
RSS and SRR	δ 0.84 (3H, d, J = 6.6 Hz), 0.87 (3H, d, J = 6.9 Hz), 0.88 (3H, t, J = 6.3 Hz), 1.12 (3H, d, J = 6.3 Hz), 1.00–1.55 (19H, m), 3.66 (1H, d of q, J = 5.7 and 6.3 Hz)	δ 14.40, 14.85, 19.61, 20.04, 22.97, 24.98, 27.30, 29.98, 32.24, 33.05, 33.19, 37.29, 37.68, 40.36, 72.06
SSR and RRS	δ 0.84 (3H, d, J = 6.5 Hz), 0.87 (3H, t, J = 6.0 Hz), 0.88 (3H, d, J = 6.7 Hz), 1.15 (3H, d, J = 6.3 Hz), 1.00–1.50 (19H, m), 3.69 (1H, d of q, J = 4.2 and 6.3 Hz)	δ 14.13, 14.30, 19.64, 20.31, 22.68, 24.75, 27.05, 29.67, 31.95, 32.72, 32.94, 37.18, 37.31, 38.81, 71.48
SRS and RSR	δ 0.84 (3H, d, J = 6.9 Hz), 0.87 (3H, d, J = 7.3 Hz), 0.88 (3H, t, J = 6.3 Hz), 1.12 (3H, d, J = 6.3 Hz), 1.00–1.60 (19H, m), 3.65 (1H, d of q, J = 5.6 and 6.3 Hz)	δ 14.09, 14.50, 19.28, 19.63, 22.68, 24.64, 27.05, 29.67, 31.95, 32.71, 32.77, 37.18, 37.31, 40.00, 71.70

(*R*)-(+)-2-Methyl-1-octanol (R-**3**). (*R*)-2-methyloctanoic acid (R-**2**) was reduced with $LiAlH_4$ using the preparative procedure described for (*R*)-2-methyl-1-decanol[21]. The title compound was obtained chemically pure by GC and 99.6% ee (amide method[18]). $[\alpha]_D^{20} + 11.83° \pm 0.03°$ (neat). n_D^{20} 1.4322 ± 0.0002. IR 3336, 2954, 2923, 2870, 2854, 1466, 1377, 1034 cm^{-1}. ^{1}H 270 MHz NMR: δ 0.8 (3H, t, J = 6.9 Hz), 0.90 (3H, d, J = 6.6 Hz), 1.00–1.50 (11H, m), 1.59 (1H, m), 3.39 (1H, d of d, J = 6.6 and 10.6 Hz), 3.49 (1H, d of d, J = 5.9 and 10.6 Hz) ppm. ^{13}C NMR: δ 14.36, 16.86, 22.93, 27.21, 29.87, 32.13, 33.44, 36.01, 68.56 ppm.

(*R*)-(−)-1-Bromo-2-methyloctane. The alcohol R-**3** was converted to the bromoalkane via bromide displacement of its tosylate as earlier described[21] for 2-methyldecanol. The (*R*)-1-bromo-2-methyloctane obtained was pure judged by GC. Bp 75 °C/1 mm Hg. n_D^{20} 1.4560 ± 0.0002. IR 2956, 2925, 2870, 2853, 1459, 1433, 1378, 1229, 652 cm^{-1}. ^{1}H 270 MHz NMR: δ 0.89 (3H, t, J = 6.6 Hz), 1.01 (3H, d, J = 6.9 Hz), 1.10–1.69 (10H, m), 1.78 (1H, m), 3.32 (1H, d of d, J = 6.0 and 9.9 Hz), 3.39 (1H, d of d, J = 5.0 and 9.9 Hz) ppm. ^{13}C NMR: δ 14.36, 19.09, 22.91, 27.12, 29.65, 32.08, 35.17, 35.51, 41.87 ppm.

(*S*)-(+)-1-Bromo-2-methyloctane. This compound was prepared as above, analysed by GC, and was obtained in >98.5% chemical purity, bp 75–85 °C/2 mm Hg. Spectroscopic and physical data were identical to those described for (*R*)-1-bromo-2-methyloctane.

(3*R*,4*R*)-(+)-*cis*-Dimethyl-(γ)-butyrolactone (RR-**5**) was prepared from (2*R*,3*R*)-epoxybutane using the same method as for SS-**5**[13]. GC analysis showed less than 0.03% *trans*-lactone. The enantiomeric excess of RR-**5** was 99.7% ee, as determined using a 50 m × 0.25 mm I.D. Chiraldex™ B-TA (β-cyclodextrin trifluoroacetyl) capillary column. The RR-**5** showed the following values: iso 140 °C, He 15 psi, split ratio 100:1, retention times: RR (major) 14.03 min; SS (minor) 17.91 min (99.7% ee).

(3*S*,4*S*)-(−)-*cis*-Dimethyl-(γ)-butyrolactone (SS-**5**). This compound came from the same batch described in ref. 13. The SS-**5** contained less than 0.04% *trans*-isomer according to GC, and the optical purity was >99.9% ee, analyzed as RR-**5** above.

(2*R*,3*R*,7*R*)-3,7-Dimethyl-2-tridecanol (RRR-**1**). This compound was prepared from (3*R*,4*R*)-(−)-*cis*-dimethyl-(γ)-butyrolactone (RR-**5**) and (*R*)-1-bromo-2-methyloctane using the synthetic methology previously described for the diprionol series[13,15,21]. (2*R*,3*R*,7*S*)-(2*S*,3*S*,7*S*)-, (2*S*,3*S*,7*R*)-3,7-dimethyl-2-tridecanol were obtained in a similar manner starting from the appropriate combinations of RR-**5** or SS-**5** and (*S*)-1-bromo-2-methyloctane or (*R*)-1-bromo-2-methyloctane. They were analysed by GC and each obtained in >99% chemical purity. 270 MHz ^{1}H and 67.8 MHz ^{13}C NMR data are shown in table 5.

(2*S*,3*R*,7*R*)-3,7-Dimethyl-2-tridecanol (SRR-**1**). This compound was synthesized from (2*R*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol (RRR-**1**) according to the experimental procedures used in the diprionol series[13,15,21]. Preparation of (2*R*,3*S*,7*R*)-, (2*S*,3*R*,7*S*)- and (2*R*,3*S*,7*S*)-3,7-dimethyl-2-tridecanol was carried out in the same manner, starting from (2*S*,3*S*,7*R*)-(2*R*,3*R*,7*S*)- and (2*S*,3*S*,7*S*)-3,7-dimethyl-2-tridecanol, respectively. They were analysed by GC and each obtained in >99% chemical purity. 270 MHz ^{1}H and 67.8 MHz ^{13}C NMR data are shown in table 5.

(2*S*,3*R*,7*R*)-3,7-Dimethyl-2-tridecanyl acetate (SRR-**1Ac**) **and propionate** (SRR-**1Pr**). The title compounds were prepared from (2*S*,3*R*,7*R*)-3,7-dimethyl-2-tridecanol (SRR-**1**) according to the experimental procedure used for the homologues in the diprionol series described earlier[13,15,21]. Preparation of the other seven 3,7-dimethyl-2-tridecanyl acetates and the seven 3,7-

Table 6. 270 MHz ^{1}H and 67.8 MHz ^{13}C NMR data of the eight stereoisomers of 3,7-dimethyl-2-tridecanyl acetate [see scheme (**1Ac**)].

Compound **1Ac**	^{1}H NMR (270 MHz)	^{13}C NMR (67.8 MHz)
RRR and SSS	δ 0.84 (3H, d, J = 6.3 Hz), 0.88 (3H, t, J = 6.3 Hz), 0.89 (3H, d, J = 6.6 Hz), 1.16 (3H, d, J = 6.6 Hz), 1.00–1.45 (17H, m), 1.50–1.65 (1H m), 2.03 (3H, s), 4.83 (1H, d of q, J = 4.9 and 6.5 Hz)	δ 14.12, 14.83, 16.95, 19.73, 21.33, 22.70, 24.53, 27.03, 29.69, 31.97, 32.74, 32.74, 37.03, 37.32, 37.63, 74.11, 170.80
RSS and SRR	δ 0.84 (3H, d, J = 6.3 Hz), 0.87 (3H, d, J = 6.9 Hz), 0.88 (3H, t, J = 6.0 Hz), 1.13 (3H, d, J = 6.6 Hz), 1.00–1.50 (17H, m), 1.55–1.75 (1H m), 2.03 (3H, s), 4.80 (1H, d of q, J = 5.6 and 6.3 Hz)	δ 14.09, 14.58, 15.78, 19.71, 21.37, 22.68, 24.53, 27.01, 29.67, 31.93, 32.72, 32.98, 36.98, 37.25, 37.31, 74.31, 170.73
RRS and SSR	δ 0.84 (3H, d, J = 6.3 Hz), 0.88 (3H, t, J = 6.4 Hz), 0.89 (3H, d, J = 6.6 Hz), 1.16 (3H, d, J = 6.3 Hz), 1.00–1.45 (17H, m), 1.50–1.65 (1H, m), 2.03 (3H, s), 4.83 (1H, d of q, J = 4.6 and 6.3 Hz)	δ 14.09, 14.76, 16.91, 19.62, 21.31, 22.68, 24.48, 27.03, 29.67, 31.93, 32.65, 32.69, 37.13, 37.22, 37.56, 74.14, 170.80
SRS and RSR	δ 0.84 (3H, d, J = 6.6 Hz), 0.87 (3H, d, J = 6.9 Hz), 0.88 (3H, t, J = 6.3 Hz), 1.13 (3H, d, J = 6.6 Hz), 1.00–1.50 (16H, m), 1.55–1.78 (2H, m), 2.03 (3H, s), 4.80 (1H, d of q, J = 5.7 and 6.3 Hz)	δ 14.11, 14.54, 15.85, 19.61, 21.37, 22.68, 24.49, 27.05, 29.67, 31.95, 32.69, 32.87, 37.14, 37.23, 37.23, 74.38, 170.75

Table 7. 270 MHz ^{1}H and 67.8 MHz ^{13}C NMR data of the eight stereoisomers of 3,7-dimethyl-2-tridecanyl propionate [see scheme (**1Pr**)].

Compound **1Pr**	^{1}H NMR (270 MHz)	^{13}C NMR (67.8 MHz)
RRR and SSS	δ 0.84 (3H, d, J = 6.3 Hz), 0.86 (3H, t, J = 6.0 Hz), 0.89 (3H, d, J = 6.9 Hz), 1.14 (3H, t, J = 7.6 Hz), 1.16 (3H, d, J = 6.3 Hz), 1.00–1.50 (17H, m), 1.55–1.70 (1H, m), 2.30 (2H, q, J = 7.6 Hz), 4.84 (1H, d of q, J = 5.0 and 6.4 Hz)	δ 9.29, 14.11, 14.81, 16.98, 19.70, 22.68, 24.51, 27.01, 28.02, 29.69, 31.95, 32.71, 32.81, 37.02, 37.31, 37.67, 73.78, 174.14
RSS and SRR	δ 0.84 (3H, d, J = 6.6 Hz), 0.87 (3H, d, J = 6.9 Hz), 0.88 (3H, t, J = 6.3 Hz), 1.13 (3H, d, J = 6.6 Hz), 1.14 (3H, t, J = 7.6 Hz), 1.00–1.45 (17H, m), 1.55–1.78 (1H, m), 2.30 (2H, q, J = 7.6 Hz), 4.82 (1H, d of q, J = 5.4 and 6.1 Hz)	δ 9.24, 14.09, 14.61, 15.83, 19.71, 22.68, 24.51, 27.01, 28.02, 29.67, 31.93, 32.71, 32.94, 36.98, 37.31, 37.31, 74.02, 174.07
RRS and SSR	δ 0.84 (3H, d, J = 6.3 Hz), 0.87 (3H, t, J = 6.1 Hz), 0.89 (3H, d, J = 6.6 Hz), 1.13 (3H, t, J = 7.6 Hz), 1.16 (3H, d, J = 6.6 Hz), 1.00–1.45 (17H, m), 1.50–1.65 (1H, m), 2.30 (2H, q, J = 7.6 Hz), 4.84 (1H, d of q, J = 5.0 and 6.6 Hz)	δ 9.27, 14.09, 14.76, 16.95, 19.62, 22.68, 24.48, 27.03, 28.00, 29.67, 31.93, 32.69, 32.72, 37.13, 37.22, 37.61, 73.84, 174.12
SRS and RSR	δ 0.84 (3H, d, J = 6.6 Hz), 0.87 (3H, d, J = 6.3 Hz), 0.88 (3H, t, J = 6.6 Hz), 1.13 (3H, d, J = 6.3 Hz), 1.14 (3H, t, J = 7.6 Hz), 1.00–1.50 (16H, m), 1.55–1.75 (2H, m), 2.30 (2H, q, J = 7.6 Hz), 4.81 (1H, d of q, J = 5.5 and 6.3 Hz)	δ 9.27, 14.12, 14.61, 15.92, 19.64, 22.70, 24.49, 27.06, 28.03, 29.69, 31.97, 32.07, 32.87, 37.16, 37.25, 37.32, 74.11, 174.12

dimethyl-2-tridecanyl propionates were made in the same way from the corresponding 3,7-dimethyl-2-tridecanols. They were analysed by GC and each obtained in >97% chemical purity. 270 MHz ^{1}H and 67.8 MHz ^{13}C NMR data are shown in table 6 for the acetates and table 7 for the propionates.

***rac*-2-Methyl-heptanoic acid.** The title compound was prepared according to the method of Karrer et al.[23]. The acid obtained was pure as judged by GC. Bp 120–122 °C/14 mm Hg (lit. 121–122 °C/13 mm Hg)[23]. n_D^{20} 1.4249 ± 0.0002 (lit. n_D^{20} 1.4235)[24]. IR 3033, 2957, 2926, 2872, 2858, 1702, 1466, 1292, 1238, 602 cm^{-1}. ^{1}H 60 MHz NMR: δ 0.9 (3H, t, $J \sim 6$ Hz), 1.2 (3H, d, $J \sim 7$ Hz), 1.0–1.8 (1H, broad), 2.5 (1H, m), 12.1 (1H, s) ppm.

***rac*-2-Methyl-1-heptanol.** This compound was obtained from the above acid via $LiAlH_4$ reduction (cf. ref. 15). The title compound obtained was pure as judged by GC. Bp 79–82 °C/11 mm Hg (lit. 85–89 °C/18 mm Hg)[24] n_D^{20} 1.4123 ± 0.0002 (lit. n_D^{20} 1.4256)[24]. IR 3334, 2954, 2922, 2870, 2853, 1466, 1377, 1038, 602. ^{1}H 60 MHz NMR was identical with that for (*S*)-2-methyl-1-heptanol in Högberg et al.[22].

***rac*-1-Bromo-2-methylheptane** was prepared from *rac*-2-methyl-1-heptanol (6.34 g, 48.7 mmol) which was added dropwise to a solution of triphenylphosphine (16.0 g,

104 mmol) and bromine (9.7 g, 60 mmol) in CH_2Cl_2 at 0 °C. The mixture was stirred overnight at ambient temperature. Successive removal of the solvent through a column, flash chromatography of the remaining oil and distillation [bp 70–72 °C/8 mm Hg, (lit. bp 70–78 °C/20 Torr)[25]] gave the pure product as judged by GC. n_D^{20} 1.4530 ± 0.0002. IR 2955, 2923, 2868, 2852, 1458, 1377, 653 cm^{-1}. ^1H 270 MHz NMR: δ 0.8 (3H, t, $J \sim 7$ Hz), 0.9 (3H, d, $J \sim 7$ Hz), 1.1–1.6 (9H, broad), 3.3 (2H, d, $J \sim 6$ Hz) ppm.

3,4-Dimethyl-(γ)-butyrolactone. The title compound was prepared as above but starting from 2,3-epoxybutane. The *trans*:*cis* ratio was 70:30 according to GC [iso 115 °C, retention times: *cis* (minor) 4.15 min; *trans* (major): 5.34 min). Bp 87–90 °C/10 mm Hg. n_D^{20} 1.4344 ± 0.0002. IR 2971, 1775, 1385, 1294, 1222, 1178, 1142, 1056, 948, 931 cm^{-1}. ^1H 270 MHz NMR: δ 1.03 (0.9H, d, $J = 6.9$ Hz), 1.14 (0.9H, d, $J = 6.3$ Hz), 1.29 (2.1H, d, $J = 6.3$ Hz), 1.40 (2.1H, d, $J = 6.3$ Hz), 2.10–2.27 (1.5H, m), 2.55 (1.5H, m), 4.14 (0.5H, d of q, $J = 7.6$ and $J = 6.3$ Hz), 4.66 (0.5H, d of q, $J = 6.3$ and $J = 6.6$ Hz) ppm. ^{13}C NMR: δ 14.36, 19.09, 22.91, 27.12, 29.65, 32.08, 35.17, 35.51, 41.87 ppm.

3,7-Dimethyl-2-dodecanol. The title compound was prepared using the same procedure as for SRR-**1** above, but starting from 3,4-dimethyl-(γ)-butyrolactone (*trans*:*cis* ratio 70:30) and *rac*-1-bromo-2-methylheptane. Bp 140–143 °C/0.5 mm Hg. n_D^{20} 1.4468 ± 0.0002. IR 3359, 2956, 2869, 2854, 1459, 1377, 1096, 1001, 602 cm^{-1}. ^1H 270 MHz NMR: δ 0.86 (3H, t, $J = 6.3$ Hz), 0.87 (3H, d, $J = 6.9$ Hz), 0.89 (3H, d, $J = 6.6$ Hz), 1.12 (3H, d, $J = 6.3$ Hz), 1.10–1.60 (17H, m), 3.68 (1H, d of q, $J = 6.3$ and 5.6 Hz) ppm. ^{13}C NMR: δ 14.36, 19.09, 22.91, 27.12, 29.65, 32.08, 35.17, 35.51, 41.87 ppm. Anal. calcd for $C_{14}H_{30}O$: C 78.4%, H 14.1%. Found: C 78.9%, H 13.8%.

3,7-Dimethyl-2-dodecanyl acetate and propionate. These compounds were prepared as described above for **SRR1-Ac** and **SSS1-Pr**, but from the mixture of diastereomeric 3,7-dimethyl-2-dodecanol. The compounds were analysed by GC and the *threo*:*erythro* ratio was 70:30 [iso 140 °C, retention times for the propionate: *erythro*-isomers (minor) 8.30 min; *threo*-isomers (major) 8.61 min].

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