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CHIRAL SPECIFICITY OF THE SEX PHEROMONE OF THE RED-HEADED PINE SAWFLY, *Neodiprion lecontei*

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Abstract—The stereospecificity of the sawfly pheromone 3,7-dimethyl-2-pentadecanol acetate against *Neodiprion lecontei* was studied. Two *erythro* and a 1:1 mixture of *threo* isomers (C-2 and C-3) were synthesized for this purpose. It was found that only one isomer with (−)-*erythro* configuration (2*S*, 3*S*) had biological activity. The potency of this synthetic pheromone was roughly identical to the one shown by the naturally occurring pheromone in this species.

Key Words—Sawflies, pheromone, stereospecificity, (−)-*erythro* configuration, enantiomers, chirality, optical isomers, *Neodiprion lecontei.*

INTRODUCTION

Earlier we have reported (Jewett et al., 1976) the identification of the chemical structure of the sex attractant of diprionid sawflies. In brief, females of many species of sawflies use either the acetic or propionic ester of 3,7-dimethyl-2-pentadecanol as the pheromone to attract the males. The pheromone is said (Casida et al., 1963) to be one of the most potent chemical attractants, being able to attract males from a few hundred meters away. During the process of identification, a racemic mixture of the above alcohol was synthesized by our group (Jewett et al., 1976). Soon three other groups (Kocienski and Ansell, 1977; Magnusson, 1977; Place et al., 1978) also independently synthesized it.

We have since tested the synthetic pheromone in the field against four different species and found (Jewett et al., 1978) that it is not as active as the naturally occurring pheromone in every case, although it did attract males. For instance, in a test against *Neodiprion sertifer*, traps baited by the racemic synthetic pheromone sporadically caught one or two males, while those baited by

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the natural pheromone constantly collected large numbers of males. Furthermore, when the synthetic pheromone was added adjacent to the natural pheromone in a trap, the total catch was drastically reduced.

We have interpreted the above information to mean that the naturally occurring pheromone has a rigid optical requirement and that some of the optical isomers in the synthetic racemic mixture were actually inhibitory to the attractiveness of the pheromone (Jewett et al., 1976). There is some supporting evidence. First, the natural pheromone of *N. lecontei* that we isolated showed a proton magnetic resonance pattern which is compatible with an *erythro* arrangement of two hydrogens on carbon 2 and carbon 3. Second, naturally occurring pheromones from different species, despite being identical in the overall chemical structure, and even after they were highly purified, elicited varying degrees of responses from males of other species.

To prove the above hypothesis, we have synthesized three stereochemically pure pheromones with respect to carbon 2 and 3 positions. These two positions were chosen because of the known *erythro* configuration in *N. lecontei* and the prediction that the chirality at the sites close to the functional group (in this case the ester moiety) is likely more important than that far removed from it.

METHODS AND MATERIALS

Bioassay. To test the efficacy of the synthetic pheromones against *Neodiprion lecontei* males, a field experiment was conducted in Sault Ste. Marie, Ontario, in a jack pine stand during the summer months of 1977. The trap used was a 3M Co. trap, 20 × 20 cm, with sticky inner surfaces. A known quantity of synthetic pheromone in 2 ml of ether was added to a 5-cm piece of cotton dental wick. After evaporation of the solvent, the wick was placed at the lower center of one side of the sticky surface. The traps were hung from appropriate branches at the approximate height of 1.5 m above the ground and 4 to 5 m apart from each other. The catches were recorded at one week intervals until the adult populations in the field disappeared (after 6 weeks). Two lots separated by a road but adjacent to each other, were chosen. Each lot was used for one series of each dilution for those three compounds.

Synthesis of Optical Isomers of Pheromone. The overall scheme of synthesis is shown in Figure 1.

Preparation of C_{12} Unit. For 2-bromodecane (I): to 127 g of 2-decanol, obtained by the reaction of nonanal and CH_3MgI, 240 g PBr_3 was added with stirring so that the temperature was kept under 5° C. This required about 2 hr. After the solution was allowed to stand at room temperature overnight, it was heated at 90° C for 1.5 hr, cooled to room temperature, and poured into 300 g crushed ice. The oily layer was separated, and the aqueous layer was extracted with two 100-ml portions of ether. The extract combined with oil was washed

successively with 30 ml conc. H_2SO_4 and 100 ml of water, then dried over $CaCl_2$. After the ether was removed, the residue was distilled to yield 154 g of (I), bp 110–115°C/20 mm Hg. Found: C, 54.03; H, 9.169%. Calc: C, 54.30; H, 9.57%.

For 3-methylundecanoic acid (II): to a solution of sodium methoxide in methanol, which was prepared from 15.2 g Na and 500 ml methanol, was added 88 g dimethyl malonate in one portion. To the mixture, 133 g of (I) was added at such a rate as to keep the temperature at 60–65°C. After the addition was completed, the reaction mixture was refluxed for 24 hr. Upon removal of methanol under reduced pressure, 150 ml water was added and the mixture was extracted with 200 ml ether. After the extract was dried over $CaCl_2$, the ether was removed by distillation, leaving 98 g crude dimethyl 1-methylnonylmalonate (bp 115–120°C/0.5 mm Hg). This was added to a mixture of 70 g KOH, 30 ml water, and 300 ml methanol. The reaction mixture was refluxed overnight and then concentrated to dryness under reduced pressure. The residue was acidified with 200 ml of 12 N HCl and refluxed overnight in an oil bath. The product was extracted with three 100-ml portions of ether. The combined extract was dried over anhydrous $MgSO_4$ and then concentrated under reduced pressure to give an oil which was distilled to yield 59 g (II), bp 131°C/1.2 mm Hg. Found: C, 71.60; H, 12.22. Calc: C, 71.95; H, 12.08%.

For 3-methyl-1-undecanol (III): to the solution of 22 g $LiAlH_4$ in 500 ml ether, 58 g (II) was added dropwise. After the addition was completed, the solution was refluxed for 1 hr, cooled to room temperature, hydrolyzed with 50 ml water, and acidified with 150 ml 6 N HCl. The alcohol in the aqueous layer was extracted with 100 ml ether.

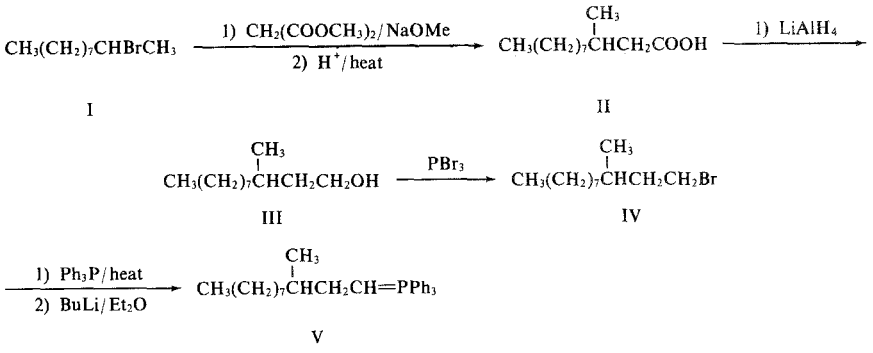
The extract was concentrated to 150 ml and dried over $MgSO_4$. After the ether was removed, the residue was distilled to yield 53 g (III), bp 97–98°C/0.8 mm Hg. Found: C, 77.01; H, 14.19%. Calc: C, 77.35; H, 14.07%.

For 1-bromo-3-methylundecane (IV): bromination of (III) with PBr_3 was carried out under the same procedure as that applied for the preparation of (I). From 52.5 g (III), 55.4 g (IV) was obtained; bp 95–96/1 mm Hg. Found: C, 57.62; H, 10.46. Calc: C, 57.90; H, 10.00%.

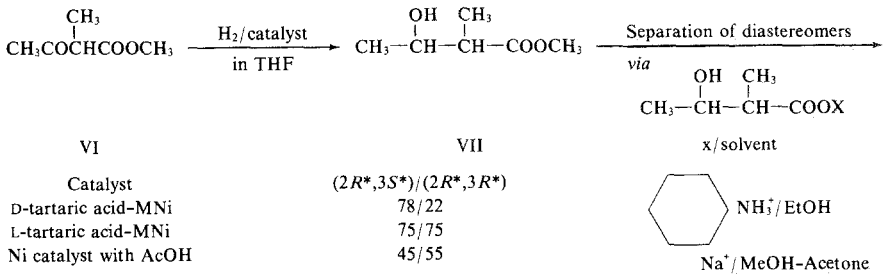
NMR δ = 0.90 and 0.91 (6H, overlapped distorted d and t, $-\underset{\underline{C}H_3}{\underset{|}{C}}H-$ and $C\underline{H}_3-CH_2-$) 1.25 (14H, m, $-(C\underline{H}_2)_n-$), 1.75 (4H, overlapped m, $-C\underline{H}_2-CH_2-Br$ and $-\underset{CH_3}{\underset{|}{C}}\underline{H}-$) 3.75 (2H, t, $-CH_2-C\underline{H}_2$ Br). IR, 2950, 2900, 2810, 1460 cm^{-1}.

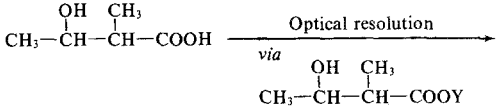
For 3-methylundecanyltriphenylphosphonium ion (V): the mixture of 40 g triphenylphosphine and 38 g (IV) was placed in a flask filled with dry nitro-

C_{12} unit



C_5 unit





VIII

Major enantiomer/
Optical purity
(+)-(2*S*,3*R*)/56%
(−)-(2*R*,3*S*)/60%
(2*R**,3*R**)

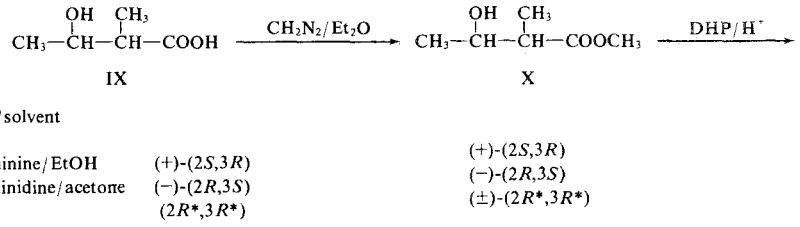


FIG. 1. Synthetic route of 3,7-dimethyl-2-pentadecanol (XV) and its acetic ester (XVI). *Mixture (1:1) of 2*R*,3*S* and 2*S*,3*R*.

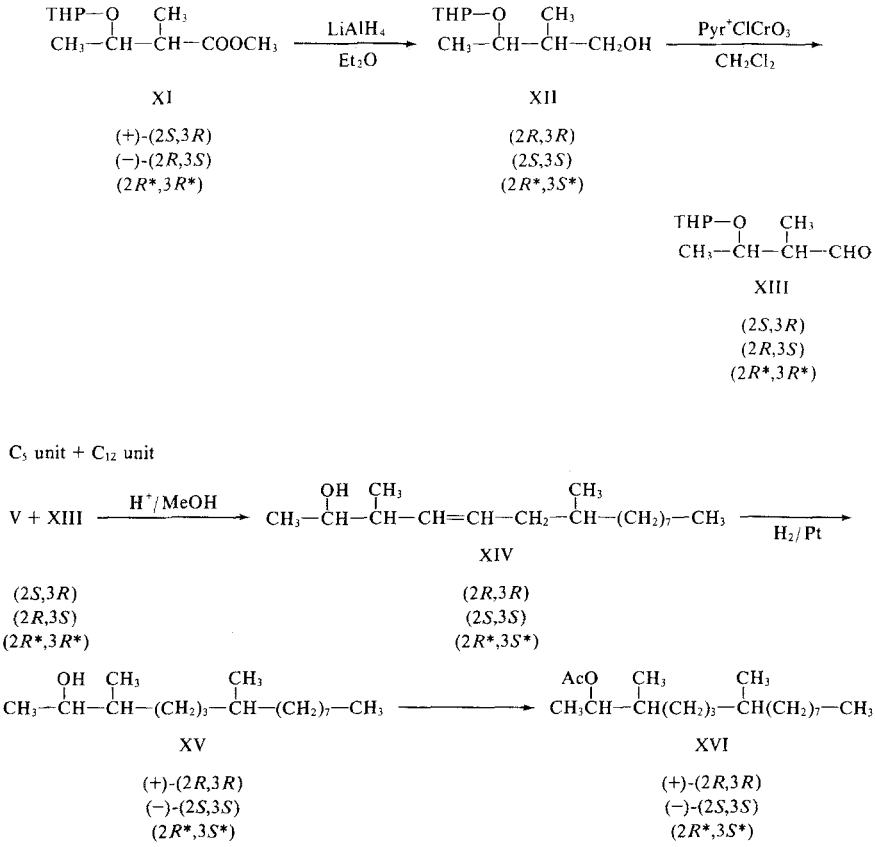


FIG. 1. (*continued*)

gen and heated at 180°C for 30 hr. The viscous mass was cooled to 50°C and mixed with 300 ml ether to form white crystals. The crystals were ground in the ether to make a slurry. The slurry was placed in a centrifuge tube and the crystals were precipitated by centrifugation. After the ether was removed by decantation, *n*-hexane was added to make a slurry again and centrifuged. This procedure was repeated until almost all the PPh_3 was removed (5 times). The crystals in the centrifuge tube were placed in a desiccator with P_2O_5 under 0.2 mm Hg for 2 days. Then the crystals were ground in a dry box, dried with P_2O_5, and again placed in the desiccator under the reduced pressure for a week to yield 73 g 3-methylundecanyltriphenylphosphonium bromide; mp 60–61°C. Addition of BuLi into a suspension of the phosphonium salt in ether gave a solution of (V).

Preparation of C_5 Unit. For the modified nickel catalyst: well pulverized $NiCO_3$ (10 g) was reduced for 1 hr at 350°C under an 8-liter/hr hydrogen

stream. The resulting reduced nickel (6 g) was soaked for 1 hr at 85°C in 60 ml 1% tartaric acid solution adjusted to pH 4.1 with N sodium hydroxide.

After removal of the solution, the modified nickel catalyst was washed successively with a 60-ml portion of water, two 300-ml portions of methanol, and a 300-ml portion of THF.

For (+)-(2*S*,3*R*)-methyl-3-hydroxy-2-methylbutyrate [(2*S*,3*R*)-VII]: methyl-2-methyl-3-oxobutyrate (100 g) in 200 ml THF and 0.6 ml acetic acid was hydrogenated with 6 g (2*R*,3*R*)-tartaric acid-modified nickel under 110 kg/cm^2 of initial hydrogen pressure at 100°C for 30 hr.

After removal of the catalyst, fractional distillation gave 90 g of diastereomeric VII. GLC (90°C) showed 78% *erythro* isomer, retention time 14.1 min, and 22% of *threo* isomer, retention time 12.8 min. The isolation of (2*S*,3*R*)-VIII was carried out by the method reported before (Tai et al., 1978; Tai and Imada, 1978). The ester was saponified to 3-hydroxy-2-methylbutyric acid (VIII) which was then converted into the cyclohexylammonium salt. Three successive recrystallizations of the salt from ethanol gave the *erythro* isomer. Removal of base with ion exchange resin gave 41 g of *erythro*-VII whose optical purity was 56% [(2*S*,3*R*) enantiomer in excess].

Optical resolution of (+)-(2*S*,3*R*)-VIII was carried out via its quinine salt. Two recrystallizations from ethanol gave optically pure products. At this stage of recrystallization, the optical rotation of the sodium salt of (+)-VIII, derived from the quinine salt, reached a steady value and remained unchanged by three further recrystallizations. The mixed melting point determination of the quinine salts at each crystallization showed that the sample of this stage was homogeneous. After removing the quinine, the liberated acid (IX) was treated with ethereal diazomethane to give 23 g (+)-(2*S*,3*R*)-X, bp 75°C/15 mm Hg, $[\alpha]_D^{20} = 11.35$ (neat). GLC (90°) showed a single peak at 14.1 min. IR and NMR spectra were identical with those of the one prepared before. NMR taken in the presence of Eu(tfmc)$_3$ indicated that the sample was optically pure.

For (−)-(2*R*,3*S*)-methyl-3-hydroxy-2-methylbutyrate [(2*R*,3*S*)-X]: hydrogenation of methyl-2-methyl-3-oxobutyrate (100 g) with (2*S*,3*S*)-tartaric acid-modified nickel by the same procedure as before gave 93 g diastereomeric (VII) from which 42 g *erythro*-VII [optical purity 60%, (2*R*,3*S*) enantiomer in excess] was obtained.

Optical resolution with quinidine (two recrystallizations of the quinidine salt from methanol-acetone), followed by esterification with diazomethane, gave 27 g (−)-(2*S*,3*R*)-X, $[\alpha]_D^{20} = -11.36$ (neat). GLC (90°C) indicated 100% purity. IR and NMR were identical with those of (+)-(2*S*,3*R*)-X.

For (±)-(2*R**,3*R**)-methyl-3-hydroxy-2-methylbutyrate [(2*R**,3*R**)-X]: methyl-2-methyl-3-oxobutyrate (100 g) in 200 ml of THF and 0.8 ml of acetic acid was hydrogenated with unmodified reduced nickel catalyst to give 92 g of diastereomeric (VII) (*threo*/*erythro* = 55:45).

The isolation of *threo* isomer by the published method (Tai and Imada, 1978) gave 29 g of (2*R**,3*R**)-X: bp 75°C/15 mm Hg. GLC (90°C) showed a single peak at 12.8 min. IR and NMR spectra were identical with those of the one prepared before (Tai and Imada, 1978).

For methyl-2-methyl-3-tetrahydropyranoxybutyrate XI: to 15 g of (2*S*,3*R*)-X was added 16.2 g of dihydropyran. On adding a small amount of *p*-toluene-sulfonic acid, the reaction occurred exothermically. After the mixture was allowed to stand for 3 hr at room temperature, 100 ml of ether was added. The solution was washed with 50 ml of 2% aqueous solution of $NaHCO_3$ and dried over $CaCl_2$. After the ether was removed, the residue was distilled to yield 22.5 g of (2*S*,3*R*)-XI, bp 92°C/2 mm Hg.

Both (2*R*,3*S*)-X (15 g) and (2*R**,3*R**)-X (15 g) were converted into (2*R*,3*S*)-XI (22.0 g, bp 83°C/1.5 mm Hg) and (2*R**,3*R**)-XI (19.5 g, bp 95°C/3 mm Hg) by the method described above.

For 2-methyl-3-tetrahydropyranoxy-1-butanol (XII): to the cooled solution of 8 g $LiAlH_4$ in 500 ml ether, 22 g (2*S*,3*R*)-XI was added dropwise. After the addition was completed, the mixture was refluxed for 1 hr, cooled, and hydrolyzed with 5 ml water. The ether layer was concentrated to 50 ml and dried over K_2CO_3. After the ether was removed, the residue was distilled to yield 15.6 g (2*R*,3*R*)-XII, bp 105°C/3.5 mm Hg. Found: C, 63.48; H, 10.92. Calc: C, 63.79; H, 10.71%.

By the same method as above, (2*R*,3*S*)-XI (21 g) and (2*R**,3*R**)-XI (19.0 g) were converted to (2*S*,3*S*)-XII (15.1 g, bp, 93°C/2 mm Hg; found: C, 63.60; H, 10.82%) and (2*R**,3*S**)-XII (13.8 g, bp, 92°C/1.8 mm Hg; found: C, 64.01; H, 11.00).

For 2-methyl-3-tetrahydropyranoxybutanal (XIV): to the mixture of 21.5 g (100 mmol) pyridinium chlorochromate and 9.1 g anhydrous sodium acetate in 150 ml dry CH_2Cl_2, which was cooled at 2°C, was added 10.8 g (2*R*,3*R*)-XII in one portion. After the reaction mixture was stirred at 5°C for 4 hr, 200 ml ether was added. The mixture was passed through a column packed with 200 ml Florisil, and the eluate was concentrated under reduced pressure to give crude (2*S*,3*R*)-XIII.

This was used in the next reaction without further purification. By the same method mentioned above (2*R*,3*S*)-XIII and (2*R**,3*R**)-XIII were prepared from (2*S*,3*S*)-XII and (2*R**,3*S**)-XII, respectively.

Coupling of C_5 and C_{12} Units. For 3,7-dimethyl-2-hydroxy-4-pentadecene (XIV): an *n*-hexane solution of BuLi was dropped into the mixture of 20.5 g (40 mmol) V and 300 ml dry ether in an atmosphere of N_2 on ice bath. After stirring at room temperature for 4 hr, the reaction mixture was red and the white salt had disappeared. To this solution (2*S*,3*R*)-XIII was added dropwise until the red color of the mixture almost disappeared (7.4 g XIII was required). The mixture was stirred at room temperature overnight. The mixture was passed through a short Florisil column, and the eluate was concen-

trated under reduced pressure. The residue was chromatographed on a column (3 × 40 cm) packed with Florisil. The eluate with hexane-ether (95:5) yielded 6.6 g oil upon evaporation of solvent. The oil was suspended in 30 ml methanol containing a trace amount of *p*-toluene-sulfonic acid, and the mixture was stirred for 4 hr at room temperature to give a homogeneous solution. After removal of solvent under reduced pressure, the residue was dissolved in 30 ml ether and washed with 10 ml aqueous $NaHCO_3$ solution. After removal of ether, the residue was vacuum distilled to give 4.6 g (2*R*,3*R*)-XII, bp 115°C/0.1 mm Hg. Found: C, 79.80; H, 13.50%. Calc: C, 80.04; H, 13.47%.

Both (2*S*,3*S*)-XIV and (2*R**,3*S**)-XIV were prepared by the same procedure mentioned above, except for the use of (2*R*,3*S*)-XIII and (2*R*,3*R*)-XIII.

From 25 g (V) and 8.6 g (2*R*,3*S*)-XIII, 7.5 g (2*S*,3*S*)-XIV was obtained, bp 113°C/0.1 mm Hg. Found: C, 79.85; H, 13.71%.

From 9.5 g (V) and 3.2 g (2*R**,3*R**)-XIII, 2.4 g (2*R**,3*S**)-XIV was obtained, bp 100°C/0.05 mm Hg. Found: C, 79.88; H, 13.70%.

For 3,7-dimethyl-2-pentadecanol (XV): in an atmospheric hydrogenation flask filled with hydrogen, 100 mg Pt_2O and 20 ml methanol were shaken for 10 min. Into the mixture, 4.5 g (2*R*,3*R*)-XIV was added, and the mixture was shaken under hydrogen until no more hydrogen was absorbed. After removal of catalyst and solvent, the product was vacuum distilled to give 4.3 g (+)-(2*R*,3*R*)-XV, bp 108°C/0.05 mm Hg. Found: C, 79.11; H, 14.47. Calc: C, 79.61; H, 14.15% $[\alpha]_D^{20} = +10.97$ (neat). NMR (CCl_4, TMS) $\delta = 0.89$ (9H, envelope which can be resolved into two d and one t by the addition of Eu $(dpm)_3$, 3 $C\underline{H}_3$), 1.08 (3H, d, J = 7 Hz, $C\underline{H}_3$—CHOH—) 1.22 [22H, envelope, 10(CH_2) and 2(CH)], 3.55 [1H, two q $\underline{H}$—C(OH)CH_3]. NMR spectra taken in the presence of Eu$(dpm)_3$ and Eu $(hfmc)_3$ showed that the *erythro* isomer (major compound) was optically pure. IR (neat) 3370, 1465, 1380, 1100 cm^{-1}. GLC (180°C) indicated 98% purity, retention time 18.3 min, and 1% unidentified impurities, retention time at 7.9 and 9.1 min.

Both (−)-(2*S*,3*S*)-XV and (±)-(2*R**,3*S**)-XV were obtained by the same method as mentioned above, except for the use of (2*S*,3*S*)-XIV and (2*R**,3*S**)-XIV.

From 7.5 g (−)-(2*S*,3*S*)-XIV, 7.3 g (−)-(2*S*,3*S*)-XV was obtained. Found: C, 79.22; H, 14.41%. Calc: C, 79.61; H, 14.15%, $[\alpha]_D = -11.10$ (neat). GLC (180°C) indicated 98% purity. NMR and IR data were identical with those of (2*R*,3*R*)-XV. From 3.2 g (2*R**,3*S**)-XIV, 3.1 g (2*R**,3*S**)-XV was obtained. Found: C, 79.15; H, 14.45%. Calc: C, 79.61; H, 14.15%, NMR $\delta =$ 0.89 [9H, envelope which can be resolved into two d and one t by the addition of Eu$(dpm)_3$] 1.06 (3H, d, J = 6 Hz), 1.22 (22H, envelope), 3.56 (1H, two q); IR identical with (−)-(2*S*,3*S*)-XV. GLC (180°C) indicated 98% purity with the same impurities as (2*S*,3*S*)-XV.

Preparation of Pheromone. 2-Acetoxy-3,7-dimethylpentadecane (XVI) was prepared by treatment XV with acetic anhydryde and pyridine.

From 0.5 g (−)-(2*S*,3*S*)-XV, 0.45 g (2*S*,3*S*)-XVI was obtained, bp 108°C/0.05 mm Hg. Found: C, 75.71; H, 12.93%. Calc: C, 76.45; H, 12.83%, $[\alpha]_D^{20} = +5.4$ (*n*-hexane c = 17).

NMR (CCl_4TMS) $\delta = 0.87$ (9H, envelope, 3 × C$\underline{H}_3$—), $\delta = 1.10$ [3H, d, J = 6.1 Hz, C$\underline{H}_3$—CH(OH)—], 1.23 [22H, envelope, —$(CH_2)_n$— and —CH—], 1.91 (3H, S, C$\underline{H}_3$C—) 4.63 (m, —C$\underline{H}$OH—), IR (neat), 2950, 2940, 2850, 1735, 1465, 1372, 1240, 1015 cm^{-1}. GLC (170°C) indicated 98% purity, retention time 6.8 min.

From 0.5 g (+)-(2*R*,3*R*)-XV, 0.46 g (2*R*,3*R*)-XVI was obtained, bp 109°C/0.05 mm Hg. $[\alpha]_D^{20} = -5.89$ (hexane c = 9.10). Found: C, 75.64; H, 12.90%.

NMR and IR data were same as that of (2*S*,3*S*)-XIV. GLC (170°C) indicated 98% purity.

From 0.5 g (2*R**,3*S**)-XV, 0.45 g (2*R**,3*S**)-XVI was obtained, bp 108°C/0.05 mm Hg. Found: C, 75.98; H, 13.44%.

NMR (CCl_4 TMS) $\delta = 0.87$ (9H, envelope 3 × C$\underline{H}_3$—) $\delta = 1.08$ (3H, d, J = 6.0 Hz, C$\underline{H}_3$—CHOH—), 1.23 [22H, envelope —$(CH_2)_n$— and —CH—], 1.91 (3H, s, C$\underline{H}_3$CO—), 4.63 (*m*—C$\underline{H}$OH—); IR (neat) same as that of (2*S*,3*S*) isomer. GLC (170°C) indicated 98% purity, retention time 6.8 min.

RESULTS AND DISCUSSION

The results of NMR analyses on 3,7-dimethylpentadecan-2-ol (Figure 2) show that each *erythro* isomer (2*R*,3*R* and 2*S*,3*S*) is free from contamination by the other *erythro* isomer. Thus they can be considered pure as far as the accuracy of the MNR assay method. On the other hand, both isomers contain approximately 5% each of a *threo* isomer. The reason for the introduction of *threo* isomers can be traced to the Wittig reaction (V and XIII; see Figure 1). In this reaction, a small amount of the enol form of the aldehyde is expected to form, and the resulting epimerization would yield a small amount of a *threo* isomer for each *erythro* isomer. Thus, the (+)-*erythro* pheromone (2*R*,3*R*) contains a trace of 2*R*,3*S* isomer, and the (−)-*erythro* pheromone (2*S*,3*S*) is similarly contaminated by 2*S*,3*R* isomer.

Bioassay samples were coded as follows: acetate of (2*R*,3*R*) as A-I; (2*S*,3*S*) as A-II; that of the *threo* mixture (2*R*,3*S* and 2*S*,3*R*) as A-III and corresponding propionate esters as P-I, P-II, and P-III, respectively.

The results of bioassays were summarized in Table 1 and include data only from A-II. Only the traps baited with A-II were active against *N. lecontei* males. The others (A-1, A-III, and controls) caught no males. The results

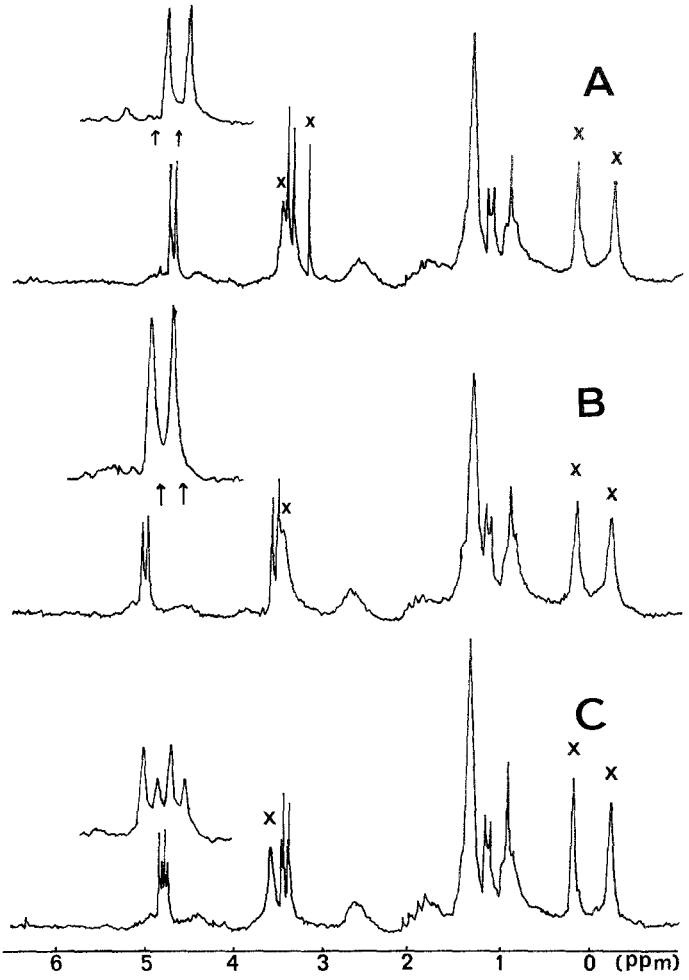


FIG. 2. NMR spectra of (A) 2*R*,3*R*; (B) 2*S*,3*S*, and (C) mixture of A and B of 3,7-dimethyl-2-pentadecanol. The doublets due to $C\underline{H}_3$—CH(OH)— clearly distinguishes 2*R*,3*R* from 2*S*,3*S*. In the case of (C), more 2*S*,3*S* than 2*R*,3*R* was added to distinguish these two isomers. NMR conditions are: sample 15 mg each and solvent $CDCl_3$ 500 μl with Eu(hfmc)$_3$ 50 mg. The measurement was made at 50°C by using JEOL Fx-100 with spectrum width 1000 Hz. The chemical shift values depend upon the concentration of shift agents. The cross-marks above some peaks indicate that they are due to the shift agent (in the case of A, there is one more contaminant peak due to acetone).

clearly indicate that the natural pheromone must have the configuration of (2*S*,3*S*)-*erythro* and that the chirality was the cause for the earlier problem of the inferior performance of the racemic synthetic pheromone. The fact that traps with other optical isomers did not catch any males must indicate the remarkable specificity of the antennae receptors for the pheromone. Also, since the threshold dose of A-II (i.e., 1 μg trap) was identical to the level obtained from the natural pheromone trap (Jewett et al., 1978), it is likely that the chirality at carbon 7 position does not play a significant role in this species.

In the case of gypsy moth pheromone (Cardé et al., 1977), the synthetic racemic mixture did not give a clear-cut dose-effect relationship, whereas optically pure preparations did. With *N. lecontei*, the racemic mixture and the optically pure preparation gave a dose-effect relationship (tested from 0.1 to 1000 μg/trap) (Jewett et al., 1978). The difference is that in the former the threshold concentration was on the order of 100 μg/trap in contrast to the latter case which was about 1 μg/trap. Since approximately 1/8 of the racemic mixture consists of the isomer of the right chirality, the above difference cannot be explained by the amount of the active ingredient alone. Rather, it must mean that other isomers do interact with the antennal receptors to reduce the field effectiveness of the pheromone by at least one order of magnitude.

The strictness of the requirement for pheromone chirality must be species specific. The degree and the nature of interference by optical isomers other

TABLE 1. NUMBER OF *N. Lecontei* MALES CAUGHT IN TRAPS BAITED WITH (−)-(2*S*,3*S*) ISOMER (A-II) OF 2-ACETOXY-3,7-DIMETHYLPENTADECANE[a]

Pheromone (μg)	Trap site	Examination date[b] 6/7	6/14	6/21	7/4	7/11	7/18	Cumulative total
300	A[c]	8	10	2	1	2	0	58
	B	12	12	7	1	1	2	
100	A	3	12	2	2	1	0	36
	B	2	8	4	2	0	0	
30	A	2	0	1	1	0	0	12
	B	3	3	2	0	0	0	
10	A	2	0	1	0	0	0	6
	B	1	1	1	0	0	0	
3	A	3	1	2	0	0	0	11
	B	4	1	0	0	0	0	
1	A	0	0	0	0	0	0	1
	B	1	0	0	0	0	0	

[a] Traps baited with other isomers caught no males.
[b] Only fresh catches have been recorded for each examination date.
[c] Two sites in a jack pine stand, A and B, are separated from each other by a road.

than the true pheromone are expected to vary from one species to another in analogy to the cases with geometric isomers: e.g., in some species the opposite isomer (i.e., *E* isomer to the insect utilizing the *Z* isomer) is inhibitory (Roelofs and Comeau, 1971), while in others it can be innocuous to stimulatory (Klun and Robinson, 1974). Many lepidopterous insects require precise ratios of mixtures of *E* and *Z* isomers for maximum activities (Roelofs and Cardé, 1974).

Research on effects of chiral isomers on antennal receptors lags far behind that of geometrical isomers, largely because of the difficulties in synthetic approaches. Our work here, along with that of others (e.g., Cardé et al., 1977; Chapman et al., 1978; Silverstein, 1978; Anderson et al., 1978; Mori, 1978), indicates that chirality of pheromone isomers plays a very important role. Thus it is likely that phenomenon of intricate isomer interaction should begin to unfold when scientists start using optically pure isomers for pheromone studies.

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