J. Chem. Ecol. 1976, Vol. 2, No. 3, pp. 307–331

DETERMINATION OF THE ENANTIOMERIC COMPOSITION OF SEVERAL INSECT PHEROMONE ALCOHOLS

E.L. PLUMMER,[1] T.E. STEWART,[2], K. BYRNE,[3] G.T. PEARCE,[4] and R.M. SILVERSTEIN

College of Environmental Science and Forestry
Syracuse, New York 13210

(*Received November 4, 1975; revised January 15, 1976*)

Abstract—Details are given for the determination of the enantiomeric composition of several insect pheromone alcohols. The two methods used in the determination were: formation of the derivative with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride and the use of chiral lanthanide shift reagents. The five alcohols studied and their enantiomeric compositions are: sulcatol from *Gnathotrichus sulcatus* 65:35 (+)/(−), *trans*-verbenol from *Dendroctonus frontalis* 60:40 (+)/(−), 4-methyl-3-heptanol from *Scolytus multistriatus* 100% (−), seudenol from *Dendroctonus pseudotsugae* 50:50 (+)/(−), and ipsdienol from *Ips pini* (Idaho) 100% (−). Determinations were done on 50–500 μg substrate.

Key Words—pheromone, *Gnathotrichus sulcatus*, *Scolytus multistriatus*, *Dendroctonus frontalis*, *Dendroctonus pseudotsugae*, *Ips pini* Idaho, enantiomeric composition, seudenol, sulcatol, *trans*-verbenol, chiral shift reagents, 4-methyl-3-heptonal.

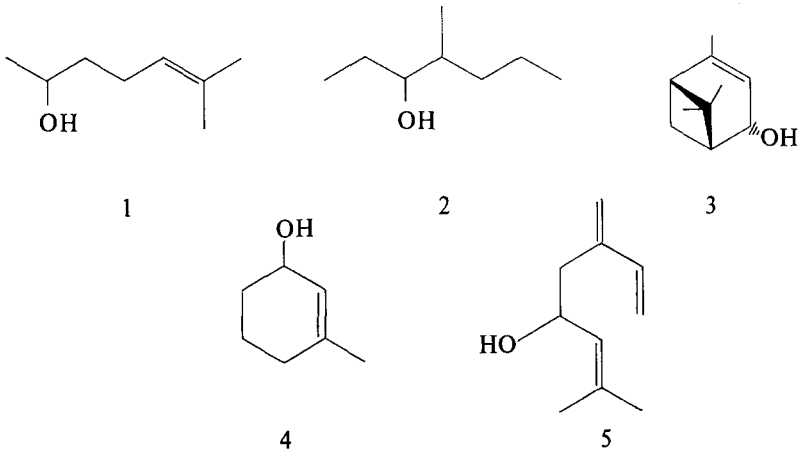
INTRODUCTION

Since "odor" receptors in man and in insects can discriminate between enantiomers (Friedman and Miller 1971, Riley et al. 1974*a*, Riley et al. 1974*b*, Iwaki et al. 1974, Kafka et al. 1973, Lensky and Blum 1974, Staedler 1974), it is

[1] E.L. Plummer's present address is FMC Corp., Princeton, New Jersey 08540.
[2] T.E. Stewart's present address is Thompson–Hayward Co., Kansas City, Kansas 66106.
[3] K. Byrne's present address is Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104.
[4] G.T. Pearce's present address is Eastman Kodak, Rochester, New York 14650.

important to describe the enantiomeric composition of chiral molecules that impinge on these receptors. It has also been shown that one enantiomer can synergize the other (John Borden, private communication).

Usually, pheromone components are isolated in such small amounts that an accurate optical rotation cannot be obtained. Recourse may be had to two recently developed procedures for determining enantiomeric composition: the use of Mosher's reagent or of chiral shift reagents. We report here details of the application of these reagents to several chiral alcohols that are components of insect pheromones. The alcohols previously isolated and identified are: (1) sulcatol, 6-methyl-5-hepten-2-ol, from *Gnathotrichus sulcatus* (Byrne et al. 1974); (2) 4-methyl-3-heptanol, from *Scolytus multistriatus* (Pearce et al. 1975); (3) *trans*-verbenol, from *Dendroctonus frontalis* (Renwick 1967); (4) seudenol, 3-methyl-2-cyclohexen-1-ol, from *Dendroctonus pseudotsugae* (Vité et al. 1972*a*); and (5) ipsdienol, 2-methyl-6-methylene-2,7-octadien-4-ol from *Ips pini* Idaho (Vité et al. 1972*b*).



METHODS AND MATERIALS

Synthetic sulcatol (structure 1) was obtained by lithium aluminum hydride reduction of the commercially available ketone and was resolved as the brucine salt of the half-acid phthalate. The oily half-acid phthalate (Vogel 1962) was extracted with benzene–ether, and the extracts were taken to dryness in vacuo and used without further purification. A hot saturated acetone solution of this product (13.8 g) and 21.5 g brucine gave 13.3 g crystals on cooling to room temperature. Three recrystallizations gave 6.9 g brucine salt. The alcohol was recovered as described by Vogel (1962). The

gas-liquid chromatography (GLC) purified alcohol had a specific rotation of $[\alpha]_D^{20} = +14.8°$ (hexane). Natural sulcatol (structure 1) was obtained from the frass of the ambrosia beetle, *Gnathotrichus sulcatus* (Byrne et al. 1974).

Synthetic 4-methyl-3-heptanol (structure 2) was obtained by reduction of the commercially available ketone with lithium aluminum hydride; the diastereomers were separated by gas chromatography. The natural 4-methyl-3-heptanol (structure 2) isolated from the elm bark beetle, *Scolytus multistriatus* (Pearce et al. 1975), was one diastereomer.

One sample of synthetic *trans*-verbenol (structure 3) ($[\alpha]_D^{20} = +6.25°$, c = 9.9, $CHCl_3$) was obtained from Chemical Samples Co., Inc., and another ($[\alpha]_D^{20} = +165°$, c = 0.5, $CHCl_3$) was prepared from (+) α-pinene ($[\alpha]_D^{20} = 46.5°$, neat, by the method of Cooper et al. (1967). Natural *trans*-verbenol (structure 3) was obtained by collection for 96 h on Porapak Q (Byrne et al. 1975) of volatiles from 16 short leaf pine logs infested with 800 female *Dendroctonus frontalis*. The Porapak Q was extracted with pentane in a Soxhlet extractor and concentrated by distillation of the solvent through a glass bead packed column at atmospheric pressure. The concentrated sample was subjected to fractionation by gas chromatography as follows: Column A glass, 10% Carbowax 20M on Chromosorb W 60/80 mesh, 1·5 m × 10.3 mm (OD), 120 cm^3/min He flow rate, 130°C isothermal, collect fraction 24–30 min; Column B glass, 5% Apiezon L on Chromosorb G 60/80 mesh, 2.4 m × 6.3 mm (OD), 60 cm^3/min He flow rate, 110°C isothermal, collect fraction 14–18 min; Column C glass, 5% FFAP on Varaport 30, 1.5 m × 6.3 mm (OD), 50 cm^3/min He flow rate, 100°C isothermal, collect fraction 14–17 min.

Synthetic seudenol (structure 4) was obtained by lithium aluminum hydride reduction of 3-methyl-2-cyclohexen-1-one. Natural seudenol (structure 4) was obtained by homogenization in hexane in a Waring blender of about 400 g frass from female Douglas fir beetles, *Dendroctonus pseudotsugae*. The filtered extract was concentrated by distillation at atmospheric pressure of the solvent through a 250-mm column packed with glass helices. A crude concentrate, obtained by short-path vacuum distillation at 80°C at 0.005 mm, was fractionated by gas chromatography on the following glass columns: Column A, 10% Carbowax 20M on Chromosorb W 60/80 mesh, 1.5 m × 10.3 mm (OD), 100 cm^3/min He flow rate, 120°C isothermal, collect fraction 16–27 min; Column B, 5% SE-30 on Chromosorb G 60/80 mesh, 3 m × 6.3 mm (OD), 50 cm^3/min He flow rate, 75°C isothermal, collect fraction 13–18 min; Column C, 4% Carbowax 20M on Chromosorb G 60/80 mesh, 5.5 × 6.3 mm (OD), 60 cm^3/min He flow rate, 130°C isothermal, collect fraction 24–27 min.

Synthetic ipsdienol (structure 5) was prepared as reported by Riley et al. (1974*c*). Natural ipsdienol (structure 5) was obtained by Porapak Q aeration

of 1000 males of *Ips pini*, Idaho. The Porapak Q was Soxhlet extracted with pentane, and the pentane extracts were concentrated in the same manner as above. The ipsdienol (structure 5) was fractionated by gas chromatography as follows: Column A glass, 4% Carbowax 20M on Chromosorb G 60/80 mesh, 5.5 m × 6.3 mm (OD), 45 cm^3/min He flow rate, 135°C isothermal, collect fraction 38–47 minutes. Column A, temperature program starting at 100°C by 0.5°/min, 80 cm^3/min He flow rate, collect fraction 61–65 min. Column A, 155°C isothermal, 90 cm^3/min He flow rate, collect fraction 15–16 min.

A resolution of the ipsdienol alcohol was attempted by the method of Vogel (1962). The brucine salt of the ipsdienol–hydrogen phthalate was recrystallized to constant rotation $[\alpha]_D = 39.6°$. Decomposition of the brucine salt and subsequent reduction of the ipsdienol–hydrogen phthalate with lithium aluminum hydride gave a very poor yield of the desired allylic alcohol; however, a source of (+) ipsdienol was available from the species *Ips paraconfusus* Lanier ($[\alpha]_D^{20} = +10 \pm 0.9°$, Silverstein et al. 1966). The volatiles released by the males of *I. paraconfusus* were cold-trapped (Browne et al. 1974). The cold trap was rinsed with ether, and the condensate was continuously extracted with ether for 24 h. The combined ether solutions were dried over sodium sulfate and concentrated as described previously. The ipsdienol was fractionated from the concentrated ether solution in the same manner as was ipsdienol from *I. pini*, except that an additional GLC column was used: Column D, 5% Apiezon L on Chromosorb G 60/80 mesh, 2.0 m × 6.3 mm (OD), 60 cm^3/min He flow rate, 125°C isothermal, collect fraction 9–10 min.

(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride [(+)-MTPA-Cl] was prepared from (+)-α-methoxy-α-trifluoromethylphenylacetic acid (Aldrich lot 043017) by reaction with thionyl chloride (Dale et al. 1969). (+)-MTPA esters of synthetic and natural alcohols were prepared in the following way: GLC-purified samples were washed from collection tubes into 4 mm OD glass tubes with 50 μl dry carbon tetrachloride, and 5 μl (+)-MTPA-Cl was added along with 50 μl dry pyridine. The glass tube was purged with nitrogen, sealed, shaken, and left with occasional shaking for 24 h. The esters of the two secondary alcohols, 4-methyl-3-heptanol (structure 2) and sulcatol (structure 1), were isolated in pure form by GLC fractionation, but the thermally labile esters of the two allylic alcohols were purified by extraction–liquid chromatography: The reaction product was treated with 5 μl 3-dimethylamino-1-propylamine (Dale and Mosher 1973), 1 ml carbon tetrachloride was added, and the solution was sequentially washed with 1 ml 6 N hydrochloric acid, 1 ml water, 1 ml saturated sodium carbonate, and 1 ml water. The product was washed into a silica gel column (1.5 cm × 6.0 mm ID), washed with 3 portions of carbon tetrachloride, and eluted with benzene.

This solution was evaporated to dryness, and the residue was taken up in the appropriate solvent for NMR studies.

The following shift reagents were obtained and sublimed immediately before use: *tris*(3-heptafluorobutyryl-d-camphorato)europium III, $Eu(hfbc)_3$ (Norell Chemical Co., Inc., 170–180°/0.005 Torr; Aldrich Chemical Co., Inc., 170–180°/0.005 Torr); *tris*(3-trifluoromethylhydroxymethylene-d-camphorato)europium III [Alfa Inorganics, Inc., Eu-Opt, lot no. 111772, $Eu(facam)_3$, 200°/0.010 Torr]; and the achiral shift reagent *tris*(6,6,7,7,8,8,8, heptafluoro-2,2-dimethyl-3,5-octanedionato)europium III, $Eu(fod)_3$ (Alfa, 135–140°/0.010 Torr).

Coaxial tubes (inside sample capacity, 50 μl) in thin-wall (5 mm OD) NMR tubes (Wilmad Glass Co., Inc.) were used in all lanthanide shift reagent studies and most Mosher derivative studies. The remaining Mosher derivatives were studied in 100-μl capacity NMR tubes also obtained from Wilmad Glass Co.

Benzene-d_6 and chloroform-d, 100% deuterated, were obtained from Stoler Isotopes, Inc. Carbon tetrachloride was spectranalyzed grade from Fisher Scientific, predried for at least 1 week over 4A molecular sieves. In the coaxial tube experiments, tetramethylsilane and trifluorotrichloroethane in deuterated chloroform (100%) were placed in the outer tube for ^1H (PMR) and ^{19}F (FMR) studies, respectively.

Lanthanide shift reagent studies were performed in the following way: All operations that would involve exposure of the samples to the atmosphere were performed under an argon atmosphere in a dry box. A weighed quantity of freshly sublimed lanthanide shift reagent was dissolved in a known volume of dry carbon tetrachloride. This stock solution of lanthanide shift reagent was stored and used under argon, but was discarded after 72 h. A stock solution of GLC-purified synthetic substrate was prepared in carbon tetrachloride, and its concentration was determined by GLC integration both before and after shift studies. The substrate and shift reagent stock solutions were mixed, then filtered through a glass-wool plug into the NMR tube. Total sample volume was 40–50 μl and was measured exactly. Except where noted in the text, substrate concentration was held constant and was approximately 0.05–0.08 M (500 μg in 50 μl solvent). Separate samples were prepared for each lanthanide-to-substrate ratio tested.

Gas-liquid chromatography was performed on a Varian Model 2740 gas chromatograph fitted with flame ionization detectors and a splitter with a split ratio of 100:1. A thermal gradient collector (Brownlee and Silverstein 1968) was used to collect fractions of column effluent in 30.5-cm glass capillary tubes. Optical rotations were obtained on either a Durrum Jasco Model ORD/UV/CD-5 recording spectropolarimeter or a Perkin–Elmer Model 531 polarimeter. NMR spectra were obtained on a Varian XL-100 Fourier Transform spectrometer with an internal deuterium lock.

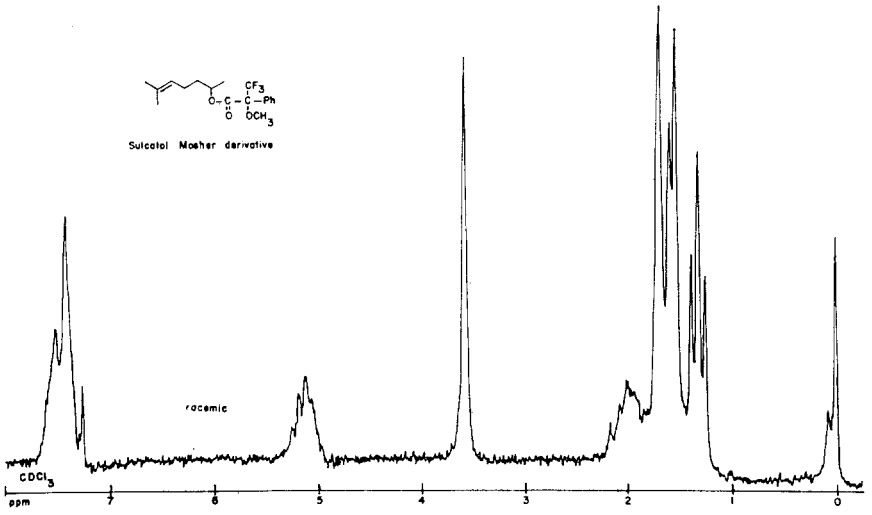
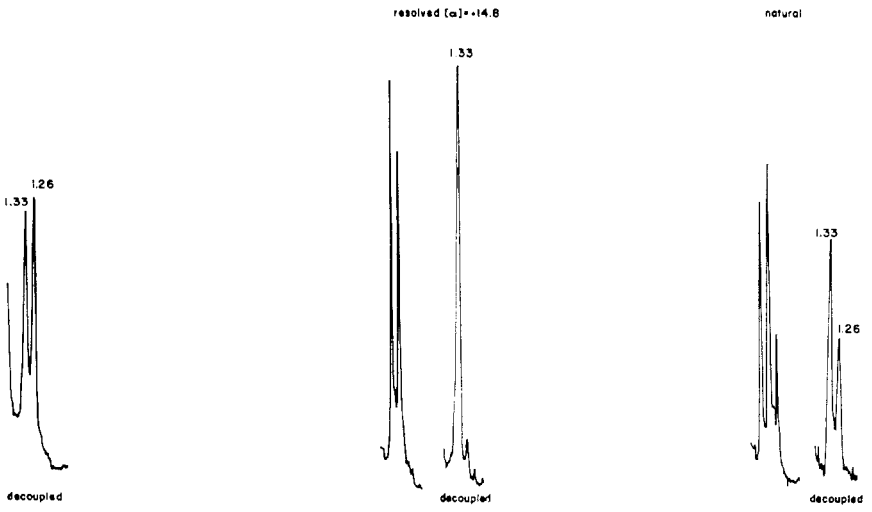


FIG. 1a. NMR spectrum of the Mosher derivative of racemic sulcatol.



(b) (c) (d)

FIG. 1b–d. The C-1 methyl signal from the decoupled spectra of (b) racemic, (c) resolved, (d) and natural sulcatol.

RESULTS AND DISCUSSION

Mosher Derivative Studies

The PMR spectrum of the (+)-MTPA ester of synthetic racemic sulcatol is shown in Fig. 1a. The only signal that reflected the enantiomeric composition was that of the C-1 methyl protons. Decoupling by irradiation of the C-2 methine proton (Fig. 1b) collapsed the C-1 methyl triplet to a pair of singlets, at 1.33 and 1.26 ppm, indicating that the original triplet was actually two overlapping doublets, each representing an enantiomer. In the PMR spectrum of the (+)-MTPA ester of (+)-sulcatol, the C-1 methyl signal was a doublet (Fig. 1c), which collapsed to a singlet at 1.33 ppm when the C-2 methine proton was irradiated. The (+)-MTPA ester of sulcatol, isolated from the insect (120 μg), gave a PMR spectrum in which the C-1 methyl proton signal appeared as an unsymmetrical triplet (Fig. 1d). This signal collapsed to two singlets of unequal intensity, the dominant singlet at 1.33 ppm and the smaller singlet at 1.26 ppm. The natural sample thus consists of a 65(+)/35(−) mixture of enantiomers. The FMR spectrum of each of the sulcatol derivatives showed only a singlet for the trifluoromethyl fluorine resonance.

Of the two diastereomeric pairs present in synthetic 4-methyl-3-heptanol (structure 2), only one was observed in the natural product, as indicated by GLC analysis. The (+)-MTPA ester of the synthetic diastereomer corresponding to that isolated gave the PMR and FMR spectra reproduced in Fig. 2. The carbinyl proton signal (5.0 ppm) appeared as two overlapping quartets, reflecting the racemic composition of the alcohol. The same composition gave rise to two apparent singlets for the trifluoromethyl fluorine resonance in the FMR spectrum. The (+)-MTPA ester of the natural alcohol gave the PMR and FMR spectra shown in Fig. 3. Clearly, only one enantiomer was present in the isolated alcohol, which is responsible for the upfield apparent singlet in the FMR spectrum of the racemic ester. The natural product has an optical rotation of $[\alpha]_D^{20} = -15°$, and it can be concluded from these data that it is enantiomerically pure. The OCH_3 absorption ($\sim$ 3.56 ppm) reflects small long-range coupling to the CF_3 group.

The (+)-MTPA esters of *trans*-verbenol (structure 3), seudenol (structure 4), and ipsdienol (structure 5) gave PMR and FMR spectra that showed incomplete separation of the enantiomeric nuclei. The separation of enantiomeric nuclei observed in (+)-MTPA ester of 4-methyl-3-heptanol may be the result of the steric hindrance by the 4-methyl group. In addition to the small enantiomeric shift differences noted for the esters of these alcohols, it was found that the allylic esters were totally decomposed on attempts at GLC purification. This difficulty was avoided by use of the extraction–liquid chromatography procedure, but large losses were incurred in handling

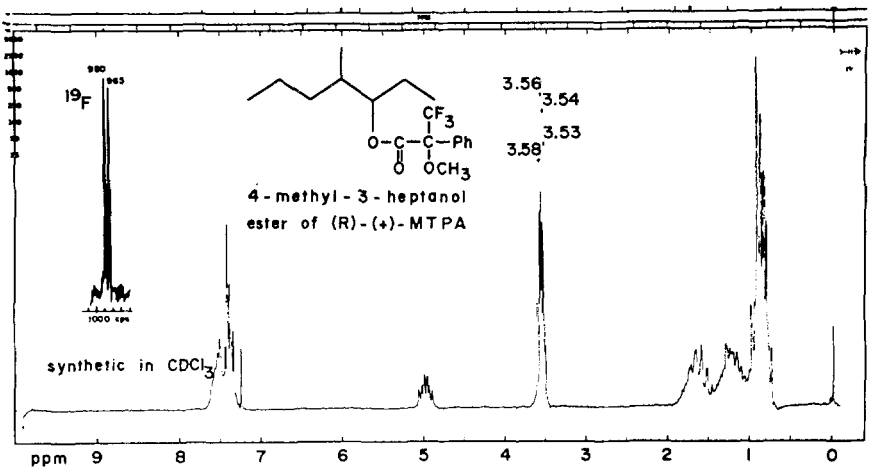


FIG. 2. PMR and FMR spectra of the (+)-MTPA ester of one of the diastereomers (racemic) of 4-methyl-3-heptanol.

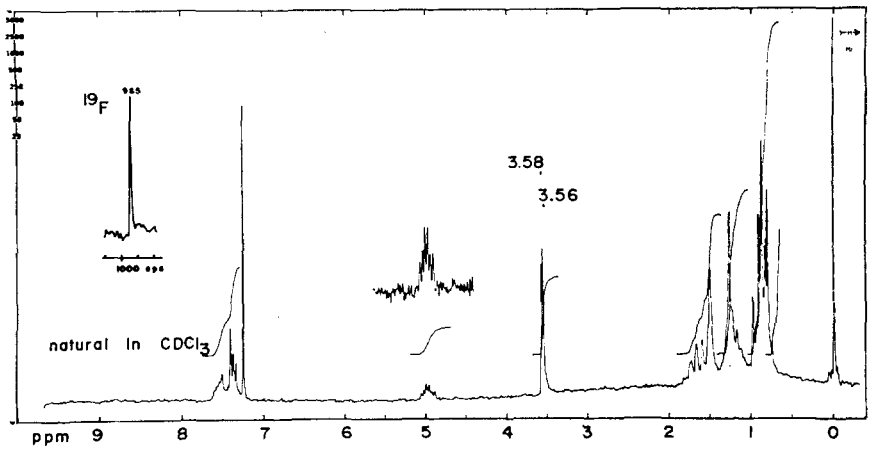


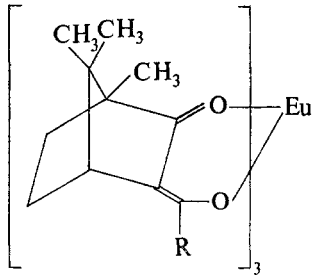
FIG. 3. PMR and FMR spectra of the (+)-MTPA ester of the natural (isolated) 4-methyl-3-heptanol.

small samples. Larger enantiomeric shift differences and thus applicability to a larger spectrum of alcohols were found with the use of chiral lanthanide shift reagents.

Lanthanide Shift Reagent Studies

Two chiral europium (III) shift reagents are commercially available and

have been extensively evaluated (Goering et al. 1974 and references cited therein). These are *tris*(3-trifluoroacetyl-d-camphorato)europium (III), $Eu(facam)_3$, and *tris*(3-heptafluorobutyryl-d-camphorato)europium (III) $Eu(hfbc)_3$, structures 6 and 7, respectively.



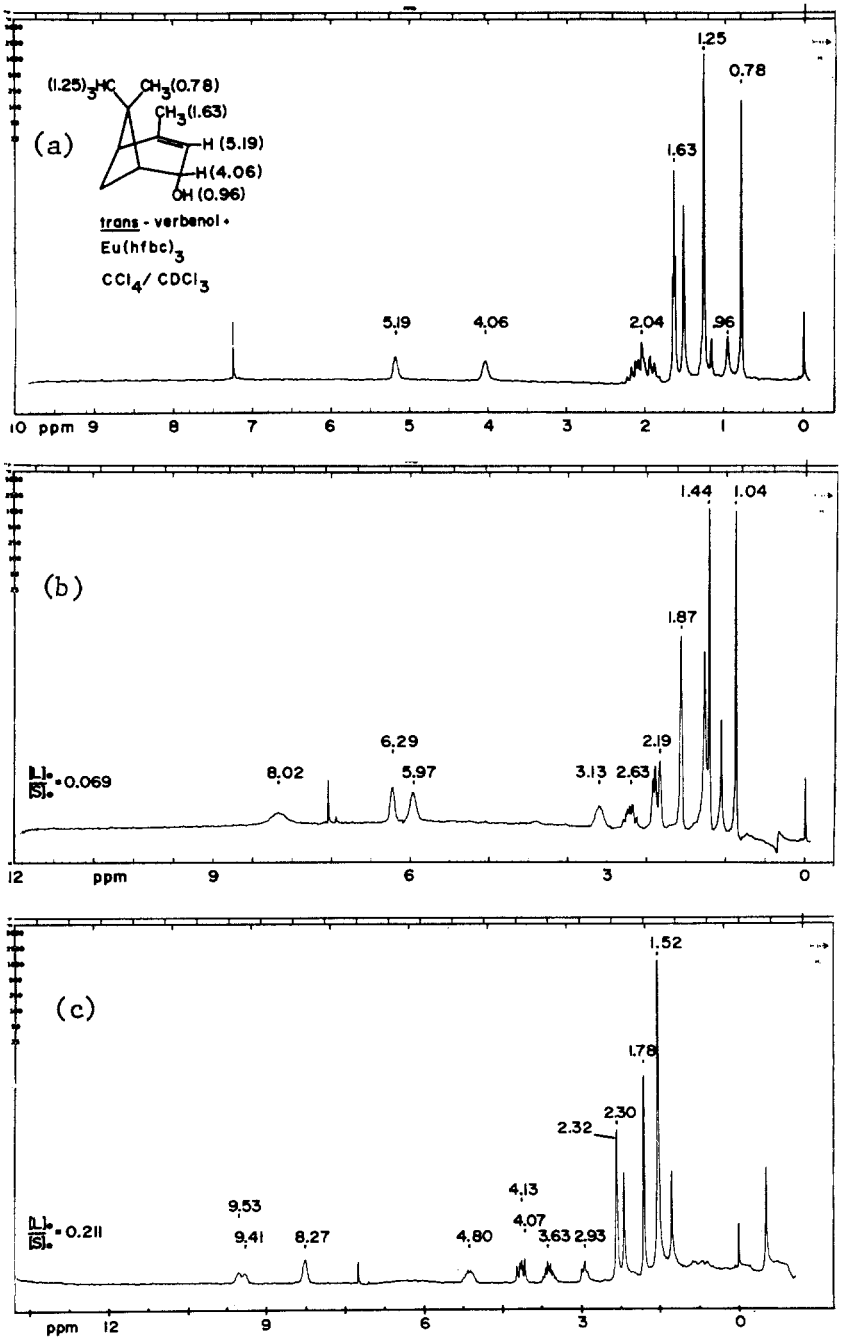
6. R = CF_3
7. R = C_3F_7

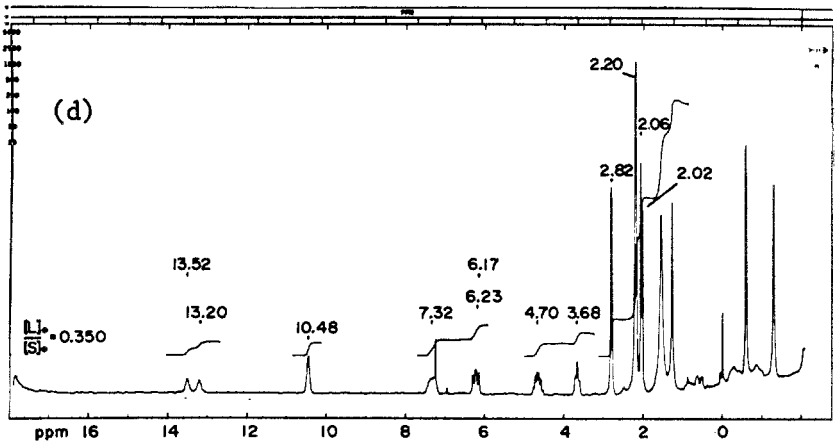
Goering et al. (1974) have noted that the magnitude of induced shifts ($\Delta\delta$) and of enantiomeric shift differences ($\Delta\Delta\delta$) for substrates in the presence of $Eu(facam)_3$ and $Eu(hfbc)_3$ are greater in carbon tetrachloride than in deuterobenzene or deuterochloroform. Our experience confirmed this finding. It is also easier to remove trace quantities of water from carbon tetrachloride than from either benzene or chloroform; it is the least hygroscopic of the three solvents and, with reasonable care in handling, can be used without serious interference from water.

The 50-μl capacity coaxial NMR tubes are suitable for 100–500-μg samples, since both induced shifts and enantiomeric shift differences dropped off severely at lower molar concentrations of substrate even when the lanthanide-to-substrate ratio was maintained. It was found that solutions of 50–100-μg samples could be used with these tubes if the solvent volume was reduced to less than 50 μl to maintain the concentration of the substrate in the 0.02–0.08 M region.

Commercial shift reagent samples were sublimed before use to eliminate impurities that caused serious broadening of spectra. Even with this step and the minimization of sample exposure to water, it was found necessary, especially at very high shift reagent–to–substrate ratios, to filter prepared solutions through a glass-wool plug in a constricted glass tube (McCreary et al. 1974).

Goering et al. (1974) have observed that, in general, both induced shifts ($\Delta\delta$) and enantiomeric shift differences are greater in the presence of $Eu(hfbc)_3$ than in the presence of $Eu(facam)_3$. In preliminary studies on ipsdienol (see below), the same results were obtained. In general, it was found that with substrate concentrations of from 0.02 to 0.08 M, signal broadening was experienced when shift reagent–to–substrate ratios exceeded about


(a)
(1.25)3HC
CH3(0.78)
CH3(1.63)
H (5.19)
H(4.06)
OH (0.96)
trans - verbenol -
Eu(hfbc)3
CCl4/ CDCl3
1.25
0.78
1.63
5.19
4.06
2.04
.96
10 ppm 9 8 7 6 5 4 3 2 1 0
(b)
1.44
1.04
1.87
6.29
2.19
[L]o/[S]o = 0.069
8.02
5.97
3.13
2.63
12 ppm 9 6 3 0
(c)
1.52
1.78
2.32
2.30
4.13
4.07
9.53
9.41
8.27
4.80
3.63
2.93
[L]o/[S]o = 0.211
ppm 12 9 6 3 0



FIGS. 4a–d. NMR spectrum of *trans*-verbenol and the chemical shifts caused by increasing L_o/S_o.

0.5:1. In two cases tested, this broadening was reversed when substrate was added to decrease the shift reagent–to–substrate ratio.

One important note should be stressed in the interpretation of spectra involving either chiral or achiral shift reagents. The two protons of a CH_2 group in an enantiomer may be diastereotopic, but they may appear to be a sharp singlet (fortuitous chemical shift equivalence). In the presence of a chiral or achiral shift reagent, they may pull apart into two signals. This separation can lead to confusion in the determination of enantiomeric composition. The determination should be based on the signals arising from CH or CH_3 groups.

Figure 4 shows NMR spectra obtained on a synthetic sample of *trans*-verbenol (structure 3) ($[\alpha]_D = +6°$) in the presence of Eu(hfbc)$_3$. The carbinyl proton, observed at 4.06 ppm in the unshifted spectrum, moves past the olefinic proton, and at a lanthanide–to–substrate molar ratio of 0.211 ($[L]_o/[S]_o$) separates into two signals. At a lanthanide–to–substrate ratio of 0.350 ppm, the separation of the enantiomeric proton signals reaches baseline (13.20, 13.52 ppm). None of the other peaks showed useful separation.

It has been reported (McCreary et al. 1974) that when the probe temperature is lowered, both $\Delta\delta$ and $\Delta\Delta\delta$ increase. Figure 5 shows that, for the carbinyl proton of *trans*-verbenol (structure 3) ($[\alpha]_D = +6°$), $\Delta\Delta\delta$ is 32 Hz at 38°C (13.20, 13.52 ppm), 57 Hz at 10°C (14.41, 14.98 ppm), and 60 Hz at 0°C (14.72, 15.32 ppm). In addition, at 0°C, the enantiomeric olefinic protons (11.71, 11.99 ppm) reached baseline separation ($\Delta\Delta\delta = 28$ Hz), and the methyl signals showed significant enantiomeric shift differences.

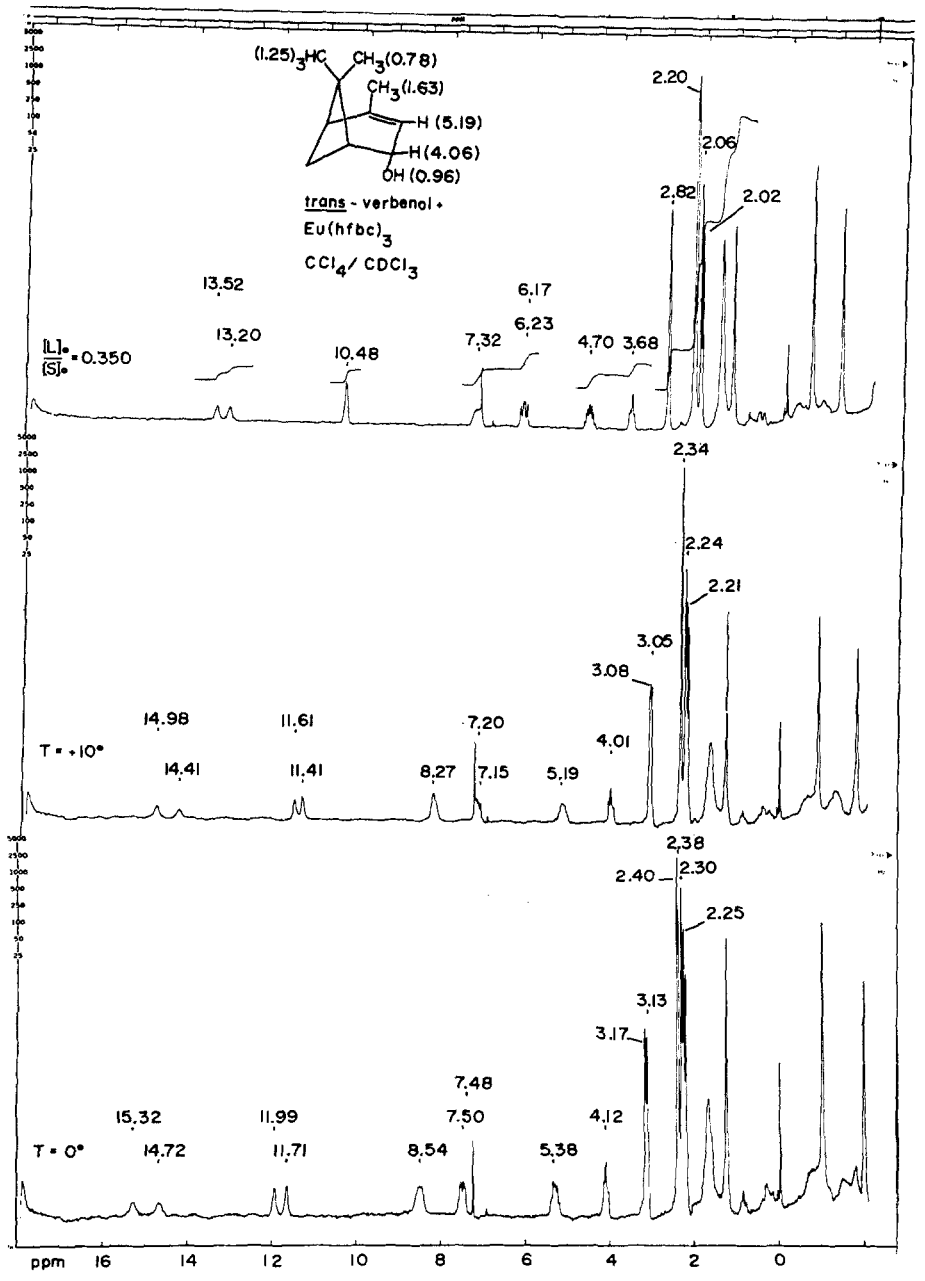


FIG. 5. Temperature dependence of enantiomeric shift differences of *trans*-verbenol induced by $Eu(hfbc)_3$.

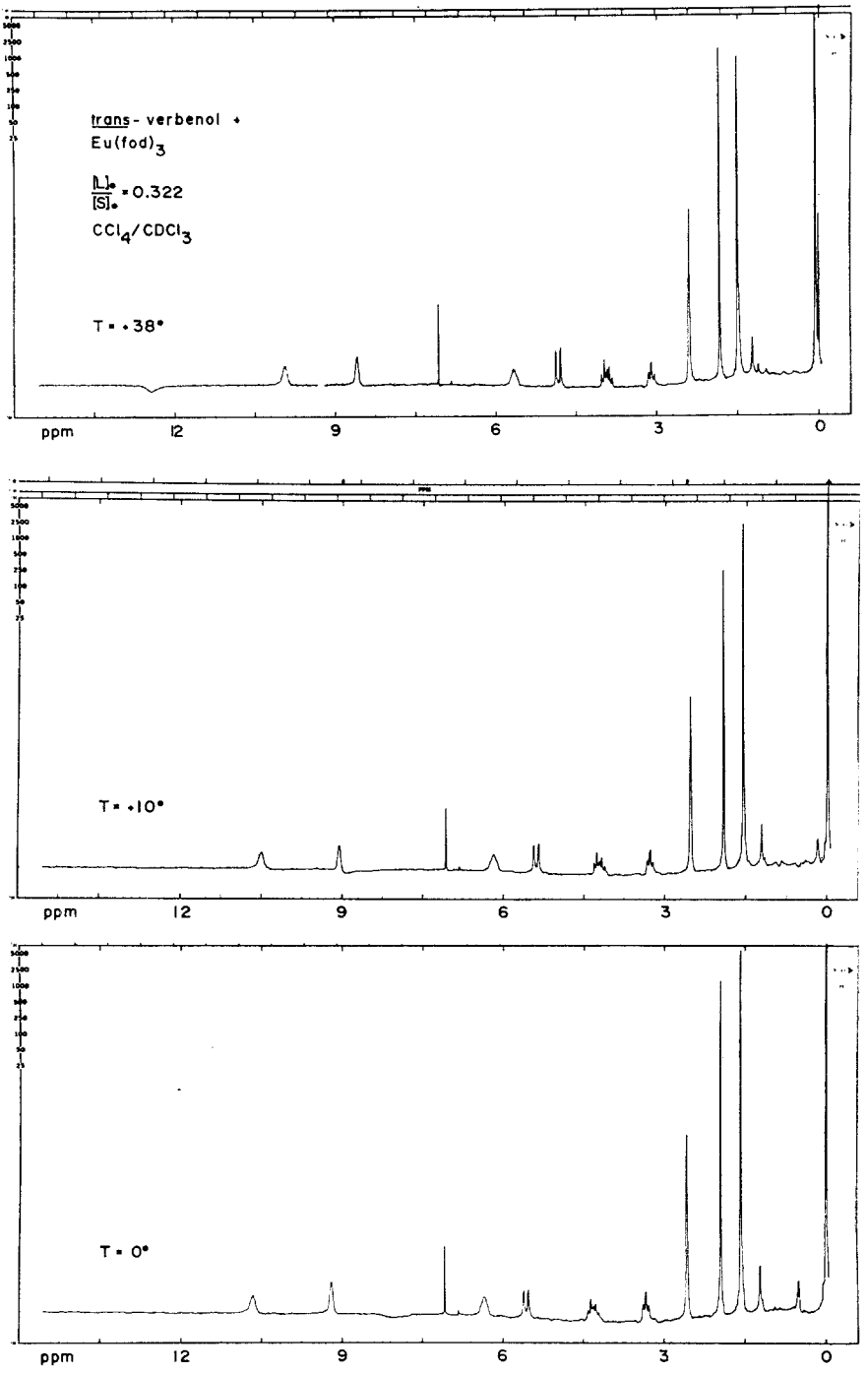


FIG. 6. Temperature dependence of enantiomeric shift differences of *trans*-verbenol induced by $Eu(fod)_3$.

To confirm that the observed shift differences reflected enantiomeric composition, a 500-μg sample of *trans*-verbenol (structure 3) ($[\alpha]_D = +6°$) was observed in the presence of the achiral shift reagent Eu(fod)$_3$. Comparison of Figs. 5 and 6 shows that at the same lanthanide–to–substrate ratio, the carbinyl proton is a broad singlet in the presence of Eu(fod)$_3$ (10.00 ppm) (Fig. 6), whereas in the presence of Eu(hfbc)$_3$, two signals are observed (Fig. 5). On lowering the temperature to +10°C and 0°C, no separation of the signals was observed with the achiral shift reagent.

Spectra of a sample of about 200 μg *trans*-verbenol (structure 3) (isolated from female southern pine beetles) in the presence of Eu(hfbc)$_3$ at an approximate $[L]_o/[S]_o = 0.349$ are reproduced in Fig. 7. Clearly, this sample was a mixture of enantiomers, and this was confirmed when the probe temperature was reduced to −15°C; there is a 102-Hz shift difference for the enantiomeric carbinyl proton signals (17.95, 18.97 ppm), a 33-Hz shift difference for the enantiomeric olefinic protons (14.18, 13.85 ppm), and a 25-Hz shift difference for the bridghead proton adjacent to the hydroxy bearing carbon (10.51, 10.76 ppm). It should be noted that the relative shifts of the enantiomeric peaks may change for each of the enantiomeric protons observed. The isolated *trans*-verbenol was a 60:40 mixture of enantiomers.

To assign peaks to the enantiomers, we spiked the synthetic (+) *trans*-verbenol (structure 3) ($[\alpha]_D = +165°$) with synthetic *trans*-verbenol (structure 3) ($[\alpha]_D = +6°$) and determined the NMR of this sample in the presence of Eu(hfbc)$_3$ (Fig. 8). It can be seen that the (+) enantiomer is represented by the downfield signal of the two signals arising from the enantiomeric carbinyl protons (average position ~ 12.0 ppm) and by the upfield signal of the two signals from the olefinic proton (average position ~ 10.3 ppm). If only one enantiomer were present, a single signal would appear, and the shift would depend on the lanthanide–to–substrate ratio, the temperature, and other factors. Spiking eliminates errors due to changes in the various factors controlling the downfield position of the singlet by showing both enantiomers with one enhanced.

Figure 9 shows spectra of 500 μg synthetic seudenol (structure 4) in the presence of varying amounts of Eu(hfbc)$_3$. The olefinic proton absorption that appears at 5.33 ppm in the unshifted spectrum pulls apart at $[L]_o/[S]_o = 0.102$, reaches baseline separation at $[L]_o/[S]_o = 0.205$, and gives $\Delta\Delta\delta$ of 18 Hz at $[L]_o/[S]_o = 0.255$ (8.93, 9.11 ppm). Although the 3-methyl singlet also separates, it does not reach baseline separation, and the olefinic proton is the only signal that can be used to determine quantitatively enantiomeric composition. In Fig. 10, a low-temperature study of the $[L]_o/[S]_o = 0.205$ sample shows enhancement of $\Delta\Delta\delta$ for the olefinic proton (8.82, 9.14 ppm), but an enantiomeric shift difference large enough to determine enantiomeric composition quantitatively is not observed for any other signal.

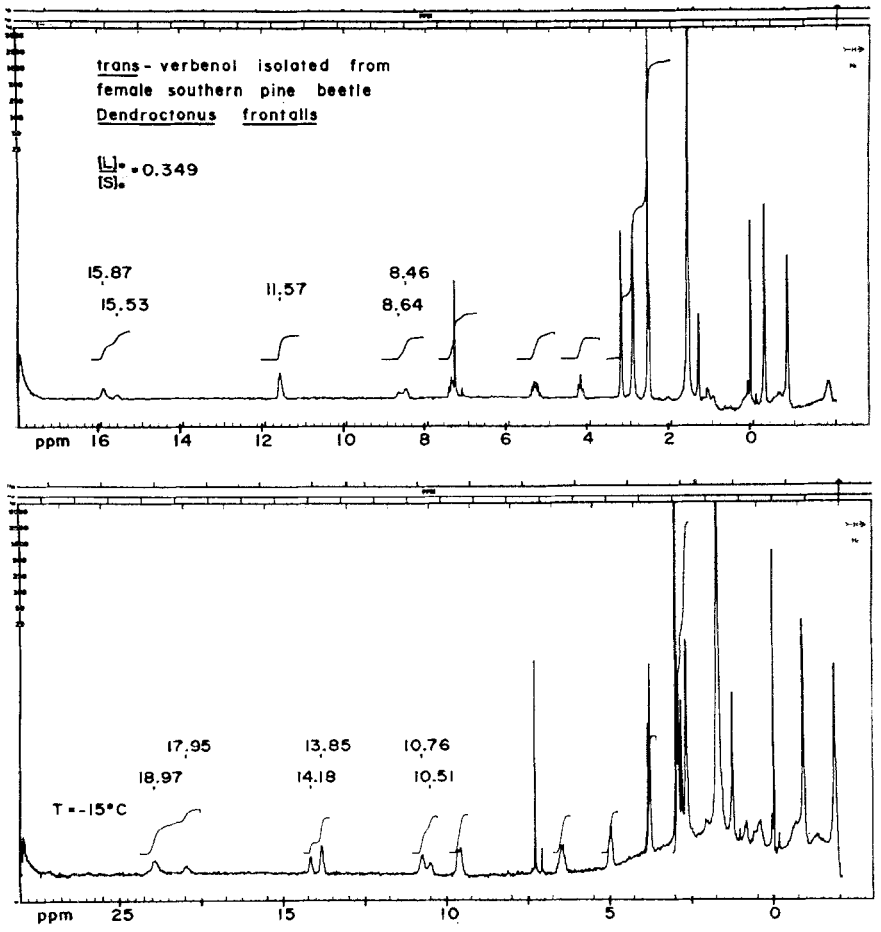


FIG. 7. NMR spectrum of natural *trans*-verbenol at +38°C and −15°C.

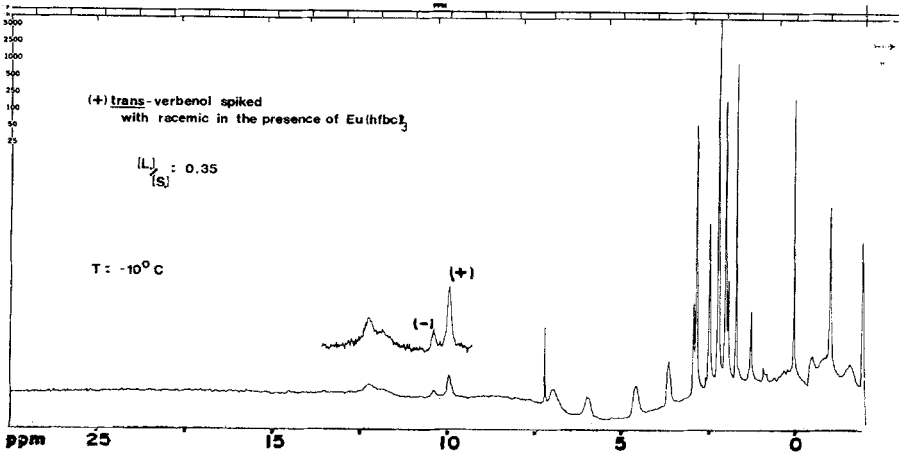
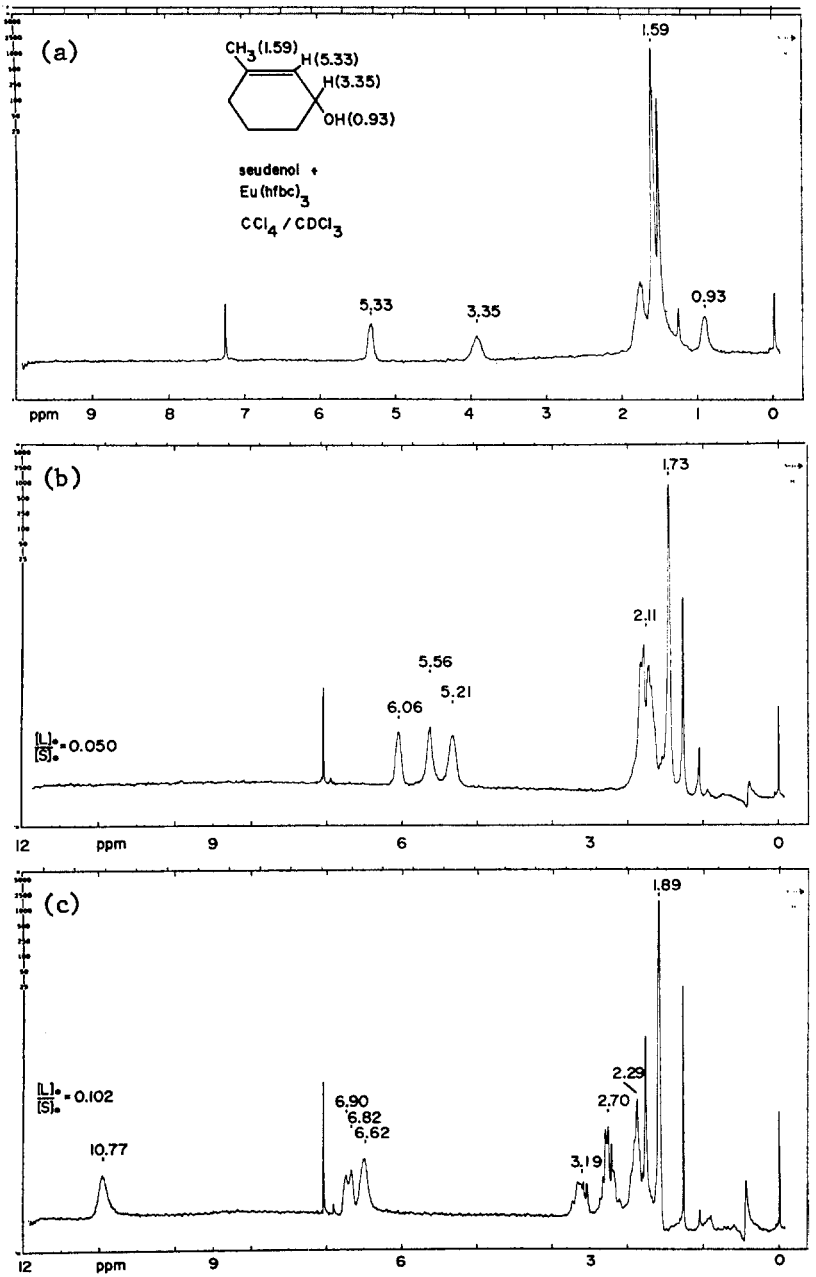
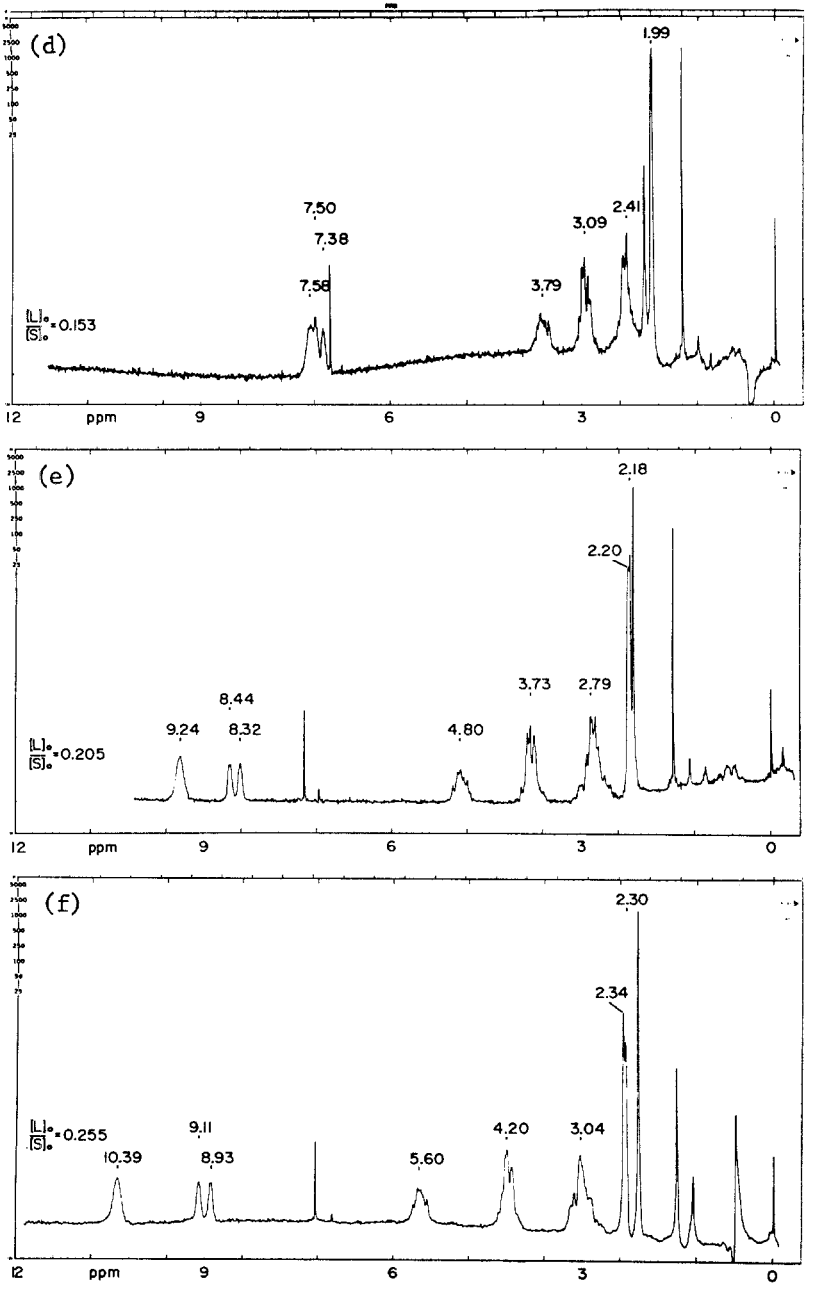
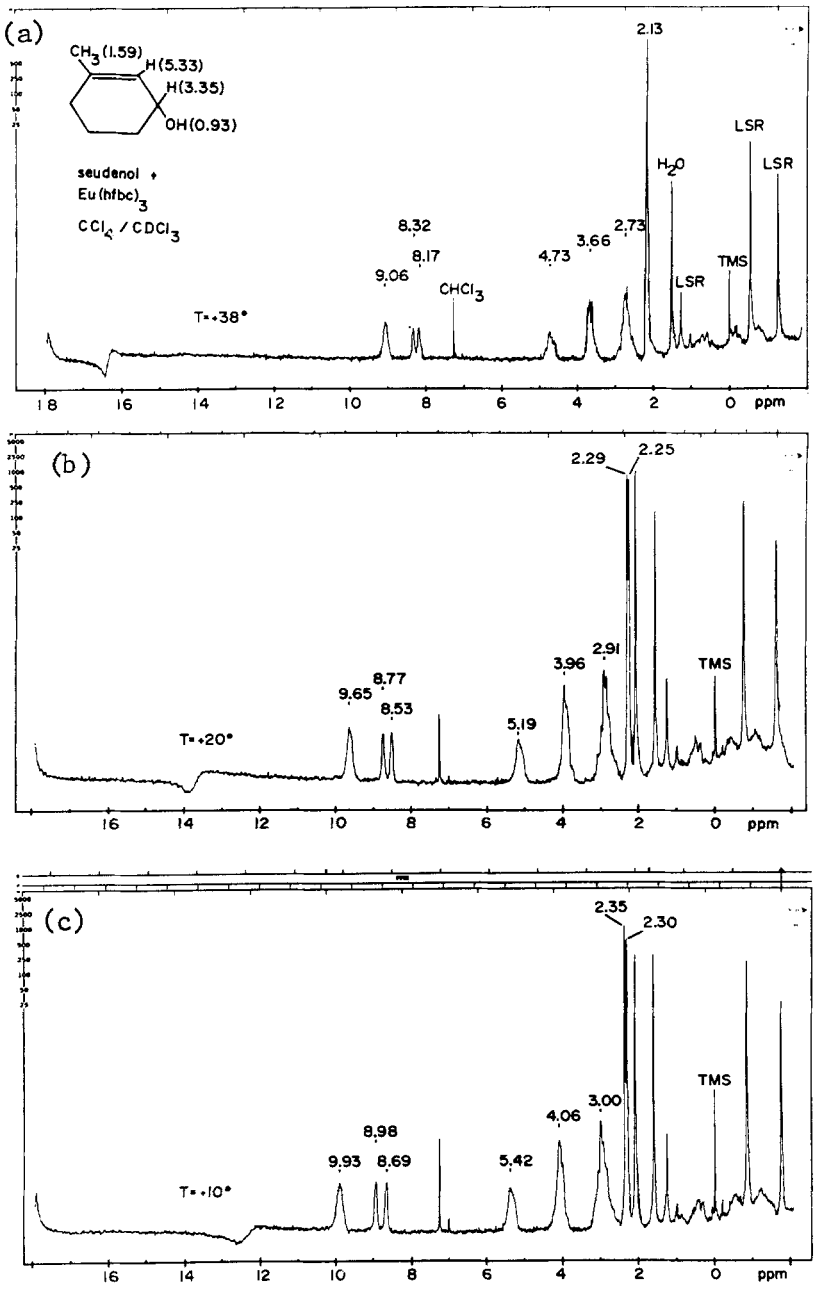


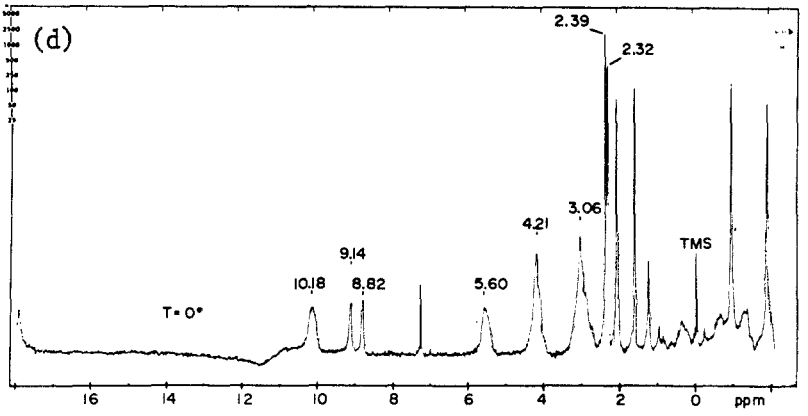
FIG. 8. Synthetic (+) *trans*-verbenol spiked with racemic in the presence of Eu(hfbc)$_3$ at −10°C.



FIGS. 9. (a) NMR spectrum of seudenol; (b–f). Chemical shifts of enantiomeric signals as a result of increasing $[L]_o/[S]_o$.


(d)
1.99
7.50
7.38
7.58
3.79
3.09
2.41
[L]o/[S]o=0.153
12
ppm
9
6
3
0
(e)
2.18
2.20
8.44
9.24
8.32
4.80
3.73
2.79
[L]o/[S]o=0.205
12
ppm
9
6
3
0
(f)
2.30
2.34
[L]o/[S]o=0.255
9.11
10.39
8.93
5.60
4.20
3.04
12
ppm
9
6
3
0


(a)
CH3 (1.59)
H(5.33)
H(3.35)
OH(0.93)
seudenol +
Eu(hfbc)3
CCl4 / CDCl3
T=+38°
9.06
8.32
8.17
CHCl3
4.73
3.66
2.73
2.13
H2O
LSR
TMS
LSR
LSR
18 16 14 12 10 8 6 4 2 0 ppm
(b)
T=+20°
9.65
8.77
8.53
5.19
3.96
2.91
2.29
2.25
TMS
16 14 12 10 8 6 4 2 0 ppm
(c)
T=+10°
9.93
8.98
8.69
5.42
4.06
3.00
2.35
2.30
TMS
16 14 12 10 8 6 4 2 0 ppm



FIGS. 10a–d. Temperature dependence of enantiomeric shift differences of seudenol in the presence of Eu(hfbc)$_3$.

Figure 11 shows spectra of 128 μg seudenol (structure 4), isolated from the frass of female *D. pseudotsugae*, in the presence of Eu(hfbc)$_3$ at $[L]_o/[S]_o$ = 0.195. The olefinic proton shows $\Delta\Delta\delta$ of 11 Hz at ambient probe temperature (7.85, 7.96 ppm), and 36 Hz at -15°C (8.61, 8.97 ppm). The material isolated is racemic.

Spectra of synthetic ipsdienol (structure 5) are shown in the presence of Eu(facam)$_3$ (Fig. 12) and of Eu(hfbc)$_3$ (Fig. 13). The spectra of ipsdienol in the presence of Eu(facam)$_3$ were obtained by incremental addition of the shift reagent to a 100-μl solution of 1 mg ipsdienol in carbon tetrachloride. The spectra in the presence of Eu(hfbc)$_3$ were obtained at constant lanthanide concentration by using separate samples of substrate varying from 152 μg/100 μl to 1.19 mg/100 μl. In practice, these techniques were inferior to that of using separate samples of constant substrate concentration, since extensive instrument retuning was not required for the latter technique.

At an Eu(hfbc)$_3$–to–substrate ratio of 0.400, enantiomeric composition is reflected by the separation of the methyl signals at 3.45 and 3.00 ppm. The double quartets for the C-7 conjugated olefinic proton also reflect the enantiomeric composition. These enantiomeric shift differences were enhanced at 0°C, but the separation was not baseline for both types of protons. It should be noted that the enantiomeric olefinic protons at C-3 were found to give baseline separation at $[L]_o/[S]_o$ = 0.40 (not shown in Fig. 9) with an enantiomeric shift difference of 40 Hz.

A sample of approximately 250 μg ipsdienol (structure 5) isolated from *Ips pini* Idaho was observed in the presence of Eu(hfbc)$_3$. The spectra obtained on this sample, both at ambient probe temperature and at 0°C, are compared

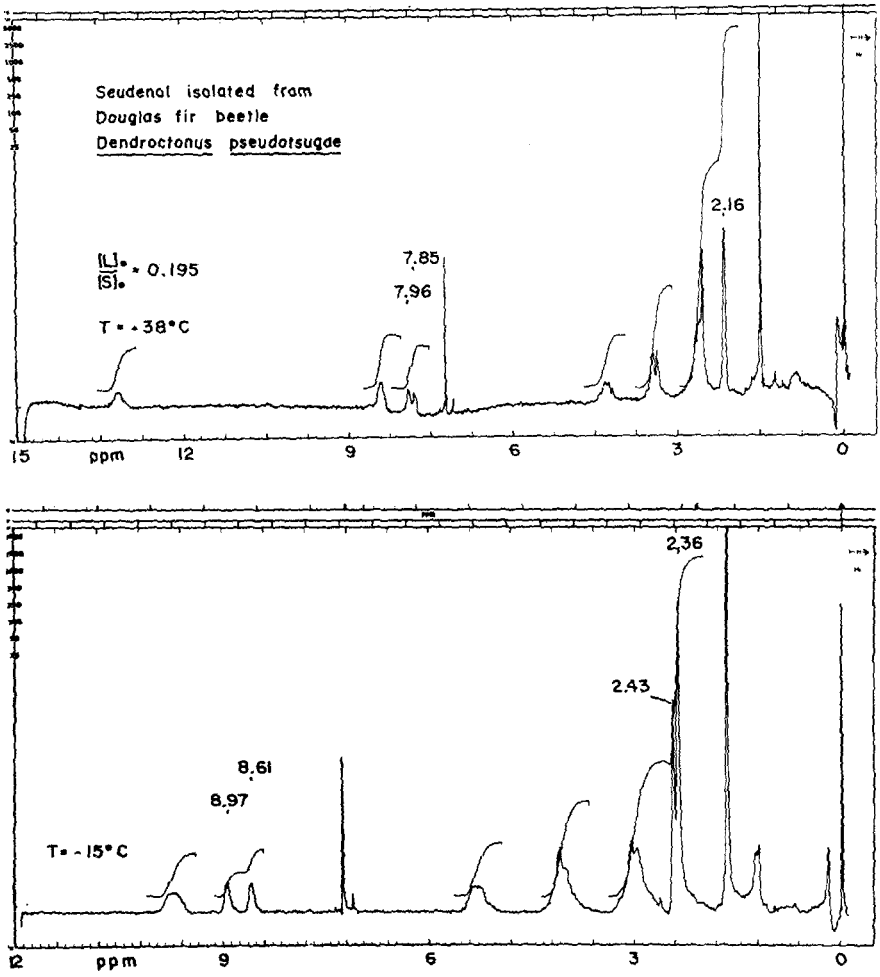


FIG. 11. NMR spectra of natural seudenol at +38°C and −15°C.

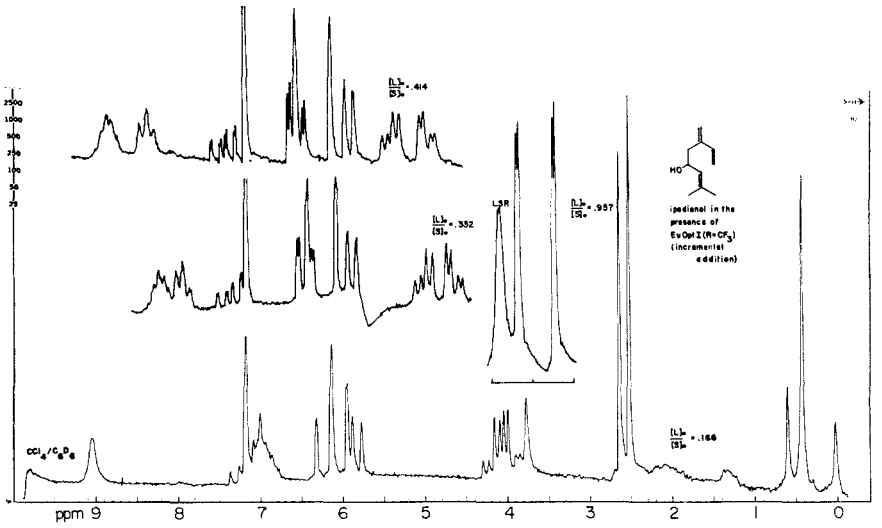


FIG. 12. NMR spectra of ipsdienol in the presence of Eu-Opt I (R = CF_3).

with the spectrum of synthetic racemic ipsdienol (structure 5) in Fig. 14. The calculated $[L]_o/[S]_o$ is the same for all spectra. It is apparent that the natural sample is a pure enantiomer—but which? Since the resolution of ipsdienol (structure 5) was unsuccessful, we turned to ipsdienol (+10°, Silverstein et al. 1966) isolated from the species *I. paraconfusus* Lanier to answer this question.

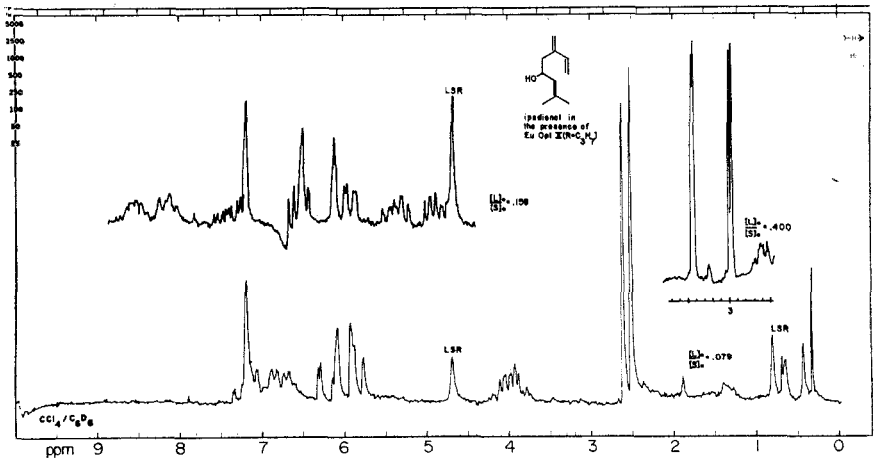


FIG. 13. NMR spectra of ipsdienol in the presence of $Eu(hfbc)_3$.

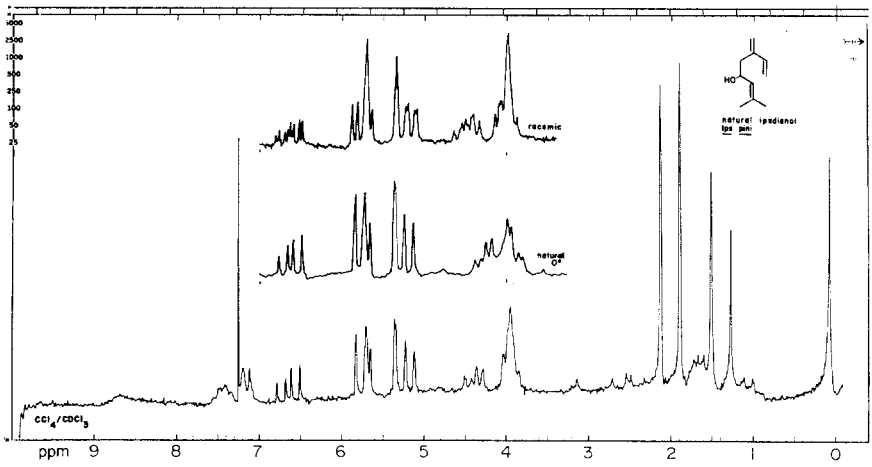


FIG. 14. NMR spectra of racemic and natural ipsdienol in the presence of Eu(hfbc)$_3$.

Figure 15 shows a sample of ipsdienol (structure 5) from *I. pini* spiked with racemic material. Note the shift and separation of signals from the methyl groups of both enantiomers (2.20, 3.00 ppm). The spectrum of 50 μg ipsdienol (structure 5) isolated from *I. paraconfusus* in the presence of Eu(hfbc)$_3$ is shown in Fig. 16. The enantiomeric peaks are reversed from those of Fig. 15 (2.20, 3.40 ppm). Since the ipsdienol (structure 5) from *I. paraconfusus* is (almost entirely) the (+) enantiomer, then ipsdienol (structure 5) from

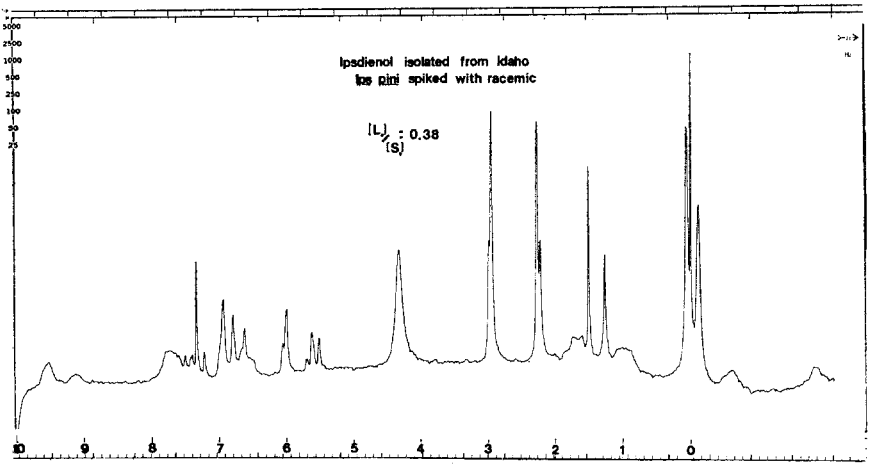


FIG. 15. NMR spectrum of natural ipsdeniol isolated from *I. pini* Idaho, spiked with racemic in the presence of Eu(hfbc)$_3$.

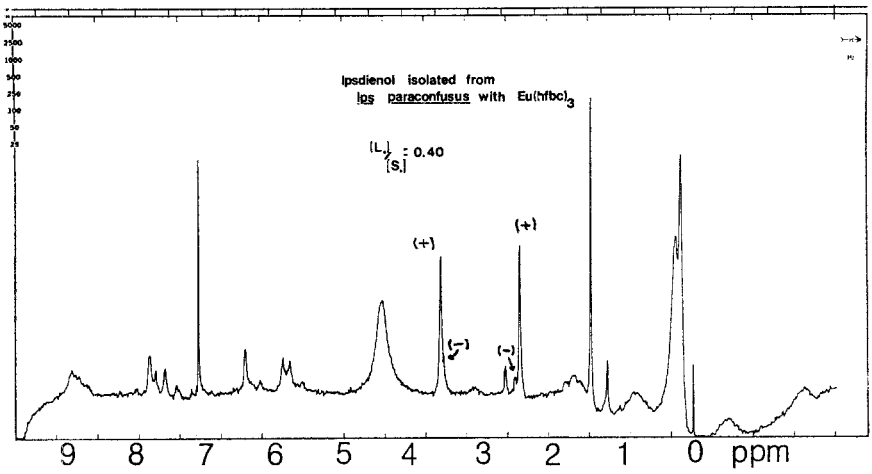


FIG. 16. NMR spectrum of natural ipsdienol isolated from *I. paraconfusus* in the presence of $Eu(hfbc)_3$.

I. pini Idaho is (−). Note that the sample of ipsdienol (structure 5) from *I. paraconfusus* was *not* spiked with a racemic mixture; it contained a small percentage of the (−) enantiomer.

The methylene protons on the C-5 carbon underwent a transformation from a doublet to a complex pattern when shift reagent, either $Eu(facam)_3$ or $Eu(hfbc)_3$, was added to the sample. This transformation merely reflects that these protons are at a prochiral center and are diastereotopic, but are fortuitously chemical shift equivalent at ambient probe temperatures.

SUMMARY

Both methods described in this paper can be successfully applied to the determination of enantiomeric composition in the submilligram range. The Mosher procedure requires more chemical manipulation of the sample and has been used to date only with alcohols; however, correlations have been made with absolute configurations (Dale and Mosher 1973). Chiral shift reagents have the following advantages: relative ease of experimental procedure and interpretation of the spectra, larger enantiomeric shift differences, smaller amounts of sample, and quantitative recovery of the samples by GLC. Disadvantages of this procedure are that the substrate must be a strong enough Lewis base to coordinate with the europium chelate, and that the experimentation to determine the best $[L]_o/[S]_o$ ratio is tedious.

Acknowledgments—We wish to thank Dr. William Gore for the samples of natural and synthetic 4-methyl-3-heptanol, Dr. Lew Edson for the aerated material from *D. frontalis*, Dr. John Borden for the frass from *D. pseudotsugae*, Dr. G.W. Lanier for the aerated material from *I. pini* Idaho, and Mr. Larry McCandless for NMR spectra. We gratefully acknowledge the support of the National Science Foundation, the Rockefeller Foundation, and the U.S. Forest Service.

REFERENCES

BEDARD, W.D., TILDEN, P.E., WOOD, D.L., SILVERSTEIN, R.M., BROWNLEE, R.G., and RODIN, J.O. 1969. Western pine beetle: field response to its sex pheromone and a synergistic host terpene. *Science Wash.* 164:1284–1285.

BROWNE, L.E., BIRCH, M.C., and WOOD, D.L. 1974. Novel trapping and delivery systems for airborne insect pheromones. *J. Insect Physiol.* 20:183–193.

BROWNLEE, R.G., and SILVERSTEIN, R.M. 1968. A micropreparative gas chromatograph and a modified carbon skeleton detector. *Analy. Chem.* 40:2077–2079.

BYRNE, K.J., SWIGAR, A.A., SILVERSTEIN, R.M., BORDEN, J.H., and STOKKINK, E. 1974. Sulcatol: Population aggregation pheromone in the scolytid beetle, *Gnathotrichus sulcatus. J. Insect Physiol.* 20:1895–1900.

BYRNE, K.J., GORE, W.E., PEARCE, G.T., and SILVERSTEIN, R.M., 1975. Porapak-Q collection of airborne organic compounds serving as models for insect pheromones. *J. Chem. Ecol.* 1:1–7.

COOPER, M.A., SALMON, J.R., WHITTAKER, D., and SCHEIDEGGER, X.X. 1967. Stereochemistry of the verbenols. *J. Chem. Soc. B*:1259–1261.

DALE, J.A., DULL, D.L., and MOSHER, H.S. 1969. α-Methoxy-α-trifluoromethyl-phenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. *J. Org. Chem.* 34:2543–2549.

DALE, J.A., and MOSHER, H.S. 1973. Nuclear magnetic resonance enantiomeric reagents. Configurational correlations via nuclear magnetic resonance chemical shifts of diastereomeric mandelate, O-methyl mandelate, and α-methoxy-α-trifluoromethylphenylacetate (MTPA) esters. *J. Am. Chem. Soc.* 95:512–519.

FRIEDMAN, L., and MILLER, J.G. 1971. Odor incongruity and chirality. *Science Wash.* 172:1044–1046.

GOERING, H.L., EIKENBERRY, J.N., KOERMER, G.S., and LATTIMER, C.J. 1974. Direct determination of enantiomeric compositions with optically active nuclear magnetic resonance lanthanide shift reagents. *J. Am. Chem. Soc.* 96:1493–1501.

IWAKI, S., MARUNO, S., SAITO, T., YAMADA, M., and KATAGIRI, K. 1974. Synthesis and activity of optically active disparlure. *J. Am. Chem. Soc.* 96:7842–7844.

KAFKA, W.A., OHLOFF, G., SCHNEIDER, D., and VARESCHI, E. 1973. Olfactory discrimination of two enantiomers of 4-methylhexanoic acid by the migratory locust and the honey bee. *J. Comp. Physiol.* 87:277–284.

LENSKY, Y., and BLUM, M.S. 1974. Chirality in insect chemoreceptors. *Life Sci.* 14:2045–2049.

MCCREARY, M.D., LEWIS, D.W., WARNICK, D.L., and WHITESIDES, G.M. 1974. The determination of enantiomeric purity using chiral lanthanide shift reagents. *J. Am. Chem. Soc.* 96:1038–1054.

PEARCE, G.T., GORE, W.E., SILVERSTEIN, R.M., PEACOCK, J.W., CUTHBERT, R.A., LANIER, G.N., and SIMEONE, J.B. 1975. Chemical attractants for the smaller european elm bark beetle *Scolytus multistriatus* (Coleoptera: Scolytidae). *J. Chem. Ecol.* 1:115–124.

RENWICK, J.A.A. 1967. Identification of two oxygenated terpenes from the bark beetles *Dendroctonus frontalis* and *Dendroctonus brevicomis*. *Contrib. Boyce Thompson Inst.* 23:355–360.

RILEY, R.G., SILVERSTEIN, R.M., and MOSER, J.C. 1974*a*. Biological responses of *Atta texana* to its alarm pheromone and the enantiomer of the pheromone. *Science Wash.* 183:760–762.

RILEY, R.G., SILVERSTEIN, R.M., and MOSER, J.C. 1974*b*. Isolation, identification, synthesis, and biological activity of volatile compounds from the heads of *Atta* ants. *J. Insect Physiol.* 20:1629–1637.

RILEY, R.G., SILVERSTEIN, R.M., KATZENELLENBOGEN, J.A., and LENOX, R.S. 1974*c*. Improved synthesis of 2-methyl-6-methylene-2,7-octadien-4-ol, a pheromone of *Ips paraconfusus*, and an alternative synthesis of the intermediate, 2-bromomethyl-1,3-butadiene. *J. Org. Chem.* 39:1957.

SILVERSTEIN, R.M., RODIN, J.O., and WOOD, D.L. 1966. Sex attractants in the frass produced by male *Ips confusus* in ponderosa pine. *Science Wash.* 154:509, 510.

STAEDLER, E. 1974. Host plant stimuli affecting oviposition behavior of the eastern spruce bud worm. *Entomol. Exp. Appl.* 17(12):176–188.

VITÉ, J.P., BAKKE, A., and RENWICK, J.A.A. 1972*a*. Pheromones in *Ips* (Coleoptera: Scolytidae): occurrence and production. *Can. Entomol.* 104:1967–1975.

VITÉ, J.P., PITMAN, G.B., FENTIMAN, A.F., JR., and KINZER, J.W. 1972*b*. 3-Methyl-2-cyclohexen-1-ol isolated from *Dendroctonus*. Naturwissenschaften 59:469.

VOGEL, A.I. 1962. Practical Organic Chemistry, 3rd ed. Wiley, New York. 503 pp.