Product Stereospecificity in the Microbial Reductions of Hydroaromatic Ketones

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A series of benzo-α-tetralone derivatives and related phenyl conjugated ketones were reduced by *Sporobolomycetes pararoseus*. The absolute stereochemistries of the alcohols obtained were determined as *S* by degradation to dimethyl (−)-α-acetoxyadipate and dimethyl (−)-α-acetoxyglutarate. Additional studies of the effects of substituents on the carbon atom α to the carbonyl and of ring size on the stereochemistries of the products are described. These results are discussed with reference to Prelog's suggestion that some enzymes exhibit "product stereospecificity".

Although considerable progress has been made in increasing the enantiomeric excesses obtained in the asymmetric reductions of ketones,[1] and in developing resolving agents that simplify the resolution of a series of alcohols, comparatively little work has been devoted to the use of microorganisms as reducing agents for the preparation of alcohols with a predictable absolute stereochemistry. If one could prepare optically active hydroaromatic alcohols of a predictable configuration, then studies similar to ones we have carried out on the absolute stereochemistry of a series of metabolites[2] (cis and trans dihydrodiols) would be greatly simplified. Our interest in metabolites of aromatic substrates prompted us to examine first the reduction of a series of α-tetralone derivatives. We wish to report on: (1) the absolute stereochemistry of the tetralols obtained in these microbiological reductions; (2) the effect on the reduction due to substituents on the methylene groups adjacent to the carbonyl in α-tetralone; (3) a solution to the problem of preparing the enantiomer of the alcohol formed in these reductions.

Prelog and his co-workers[3] have determined the stereochemistry of a series of aliphatic alcohols obtained by reduction of the corresponding ketones using a purified oxidoreductase isolated from *Curvularia falcata*. From these studies, Prelog formulated the rule shown in Figure 1 to account for the observed stereochemistry: if the ketone is placed with the larger group on the observer's left, the hydroxyl group formed is closer to the observer.

In addition, Prelog et al.[3a,b] investigated the effect of a variety of substituents on the cyclohexanone ring on the rate of reduction. Their analysis described the topological surface of the ternary complex formed by the enzyme, substrate, and coenzyme, in which the chair conformation of cyclohexanol formed the center of a polycyclohexane diamond-like arrangement (lattice) of carbon atoms. The hydrogen delivered during the reduction is that resulting from an equatorial approach.[3b] Prelog et al. and other groups noted that the effect of substituents at several positions varies from slightly decreasing the rate of reduction to completely stopping it. In the latter, substituents occupied what were called "forbidden positions".

The microbiological reductions of several hydroaromatic ketones have been previously reported. Cervinka and Hub[4] have described the reduction of α-tetralone in low yield (1.4%), to (+)-(1*S*)-tetralol (**1**), using *Saccharomyces cerevesiae*. Siewinski[5] reported in the reduction of several polycyclic aromatic ketones (precursors of **2** and **3**) using *Rhodotorula mucilaginosa*. However, while **2** was assigned a *S* configuration, consistent with Prelog's rule shown in Figure 1, Siewinski assigned **3** an *R* configuration, thus greatly complicating the projected use of these microbial reductions to prepare alcohols of a predictable stereochemistry. It is possible to rationalize the *R* configuration for **3** by assuming in this case that for steric considerations the enzyme treats the methylene adjacent to the carbonyl as relatively larger than the aromatic group. However, estimates of the relative sizes of groups flanking the carbonyl must then be made on the basis of empirical observations. A second explanation of Siewinski's results postulates the existence of more than one oxidoreductase and assumes that different enzymes are responsible for the formation of **2** and **3**. A third explanation challenges the assigned absolute stereochemistry of **3**. The reported assignment was made by the method of Horeau and Kagan[7] in which *dl*-α-phenylbutyric anhydride is reacted with an optically active alcohol. The absolute stereochemistry of the alcohol is assigned empirically from the sign of $[\alpha]_D$ of the α-phenylbutyric acid formed. Although the method has been successful in numerous cases, Horeau et al.[8] also have reported several examples where the absolute stereochemistry assigned by this procedure differed from that determined by chemical degradation. Therefore, before embarking on a lengthy study defining the relative size of a substituent or attempting to isolate and purify a single enzyme from a microorganism, we reinvestigated the absolute stereochemistry of (−)-**3**.

Results and Discussion

Although we were unable to obtain a culture of *R. mucilaginosa* (used by Siewinski), a related organism, *Sporobolomycetes pararoseus* (ATCC No. 11386), was found to reduce the ketone precursors of **1**, **2**, and **3**, yielding optically active alcohols of the same sign as reported by Siewinski. The specific rotation observed for microbiologically produced **1** was 26.8°, reported 26.5°;[6] thus it is optically pure within experimental error. The rotations and yields for **2** and **3** obtained by reduction of the ketones are listed in Table I.

To verify the configurations assigned to **2** and **3** we have developed a general procedure to determine the configuration of hydroxyhydroaromatic compounds. The procedure is shown for (1*S*)-tetralol (see Scheme I) and involves acetylating the alcohol, followed by exhaustive ozonolysis to produce a mixture of α-acetoxyadipic and α-acetoxyglutaric acids. The dimethyl esters (**4** and **5**) were prepared, using diazomethane, and separated by preparative GLC. Dimethyl α-acetoxyadipate (**4**) was identified by a comparison of its NMR spectrum, mass spectrum, and GLC retention time with that of authentic **4** prepared from ozonolysis of 3-acetoxycyclohexene. The structure of dimethyl α-acetoxyglutarate (**5**) was assigned

Scheme I

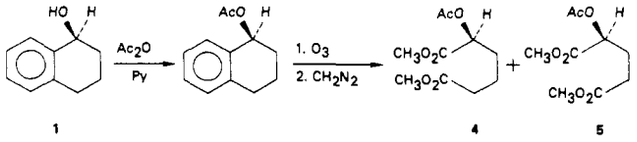

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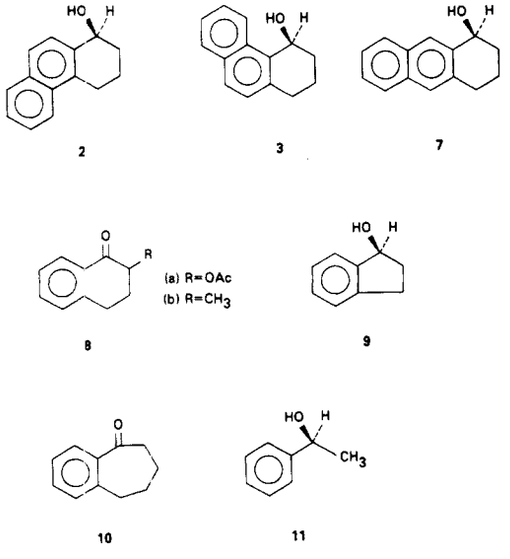
Table I. Microbiological Reductions

Alcohol or ketone precursor	Registry no.	Yield of alcohol, %	Registry no. of alcohol	Recovered ketone, %	Absolute stereochemistry	Alcohol $[\alpha]^{25}_D$ Obsd	Alcohol $[\alpha]^{25}_D$ Reported [ref]
1[b]	529-34-0	15	53732-47-1	80	1*S*	+26.8° ($CHCl_3$)	+26.5° ($CHCl_3$) [6]
2[a]	573-22-8	6	27544-17-8	77	1*S*	−59° (acetone)	−72° (acetone) [5]
3[a]	778-48-3	41	27549-85-5	59	4*S*	−19.5° (acetone)	−5° (acetone) [5]
7[a]	54784-07-5	13	65915-71-1	36	1*S*	+138° ($CHCl_3$)	
8a[a]	65915-70-0	23	65915-61-9		1*R*,2*R* (trans)	+87.9° ($CHCl_3$)	−110° [9]
		23	65915-62-0		1*R*,2*S* (cis)	−43° ($CHCl_3$)	−38° [9]
8b[a]	65941-82-4	2	65941-81-3	79	1*S*,2*S* (cis)	−61° (C_6H_{12})	+33° (benzene) [11]
		5	38157-10-7		1*S*,2*R* (trans)	+79° (C_6H_{12})	+65° (benzene) [11]
9[a]	83-33-0	9	25501-32-0	90	1*S*	+22.6°	+17° [3]
10[b]	826-73-3	27	65915-63-1	73	1*S*	−26.6° (*c* 4, $CHCl_3$)	
11[b]	98-86-2	90	1445-91-6		1*S*	−57° ($CHCl_3$)	+54° [14]

[a] These reductions were carried out using *Sporobolomycetes pararoseus* ATCC No. 11386. [b] These reductions were carried out using *Cryptococcus macerans* obtained from Dr. D. Perlman of the University of Wisconsin.

Table II. Ozonolysis of Hydrocarbon Acetates

Alcohol	Acetate $[\alpha]^{25}_D$ (*c* 2.95, $CHCl_3$)	Registry no. of acetate	Wt ozonized, mg	Wt of crude ester mixture, mg	$[\alpha]^{25}_{320}$ ($CHCl_3$) 4	$[\alpha]^{25}_{320}$ ($CHCl_3$) 5
(1*S*)-Tetralol	−97°	65915-64-2	390	135	−52°	−72°
2	−178° (*c* 3.3)	65915-65-3	250	61	−62°	−46°
3	−128° (*c* 1.0)	65915-66-4	250	73	−69°	−74°
7	−74° (*c* 4.1)	65915-67-5	200	90	−62°	−63°



from its NMR spectrum, mass spectrum, and a comparison of the mass spectrum's fragmentation pattern with that of one obtained from **4**. The relation between $[\alpha]^{20}_{320}$ and the absolute stereochemistry of **4** (and **5**) was thus established.[9] The tricyclic alcohols **2** and **3** were then acetylated and ozonized as described for **1**. The rotations of **4** and **5** obtained from **2** and **3** are listed in Table II. The results clearly show that the *configuration of both* **2** *and* **3** *are S*, and that the previously assigned configuration of **3** was incorrect. Both esters (**4** and **5**) were used to assign absolute stereochemistries because the quantities of each acid formed in the ozonolysis depend upon the structure of the starting acetate, i.e., one of the acids was not consistently the major ozonolysis product. In addition, **4** contained some dimethyl phthalate, which although optically inactive does affect the magnitude of the specific rotation.

For completeness and as a test of the "product stereospecificity" concept, we prepared the remaining tricyclic α-tetralone derivative, **6**, as shown in Scheme II. Microbiological reduction of this precursor by *S. pararoseus* produced the (+)-alcohol **7** which was converted to the (−)-acetate. On ozonolysis and purification (as described for **1**) the esters **4** and **5** were each found to be levorotatory (Table II), again demonstrating that the *S* alcohol was formed in the reduction. The evidence now strongly suggests that the reduction exhibits "product stereospecificity".[3]

The above experiments establish that microbiological reduction of benzo-α-tetralone derivatives yield the *S* alcohols. Since the enzyme appears to distinguish between substituents on either side of the carbonyl on the basis of size, the effect of introducing a substituent on the methylene group was uncertain. The presence of a substituent also permitted us to reduce α-tetralone derivatives which yield diols closely related to the metabolites from the mammalian[2c] and microbial oxidation[2a,b] of aromatic hydrocarbons. We chose to study the reduction of 2-acetoxytetral-1-one (**8a**) because the configurations of the *cis*- and *trans*-1,2-tetrahydrodiols are known.[10] As reduction of the carbonyl now can yield two geometric (cis and trans) isomers, we were therefore interested in determining whether one or both stereoisomers were formed and in establishing the absolute stereochemistry of the products. The configuration of the diols produced should eliminate the possibility that reduction occurred by another oxidoreductase with a different "product specificity". Microbiological reduction of **8a** yielded a mixture which was separated into three fractions: recovered starting material, a mixture of hydroxy acetates, and trace quantities of the cis and trans diols. The

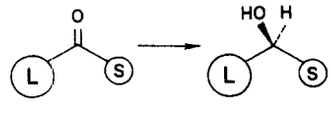
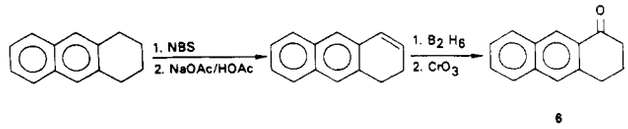


Figure 1.

Scheme II



fraction containing the mixture of hydroxy acetates was reductively ($LiAlH_4$) hydrolyzed, and the resulting mixture of cis and trans diols separated by thick-layer chromatography. The absolute stereochemistry of diols obtained is given in Table I. As the configuration at C-1 in each of the diols is *R*,[11] the presence of a substituent does *not* alter the course of the reduction, and the aromatic ring is always the "large group". These reductions can thus be employed to prepare optically active cis and trans diols. Furthermore, as the conversion of cis diols to epoxides has been established,[12] these diols can be used to prepare amino alcohols, mercapto alcohols, and a host of other products. These results, therefore, dramatically increase the potential value of these microbiological reductions.

As our confidence in applying Prelog's principle of "product stereospecificity" increased, we examined the reduction of 2-methyltetral-1-one (**8b**). At the time this work started, the configuration of the cis alcohol was unknown while that of the trans alcohol had been reported by Kagan et al.[13a] Microbiological reduction of 2-methyltetral-1-one yielded a mixture of alcohols which were separated by column chromatography to yield the (+)-trans and (−)-cis alcohols identified from their NMR spectra. From geometric considerations the (+)-trans alcohol was either (1*R*,2*S*) or (1*S*,2*R*), while the principle of "product stereospecificity" requires the *S* configuration at C-1. The absolute stereochemistry deduced for the trans alcohol is then (1*S*,2*R*), the opposite of that assigned by Kagan et al.[13a] This discrepancy has now been removed by Horeau et al.[13b] in a recent publication where they related the *cis*- and *trans*-2-methyltetralols to 2-aminotetralin of unequivocal stereochemistry. This group also established the absolute stereochemistry of the (−)-cis alcohol as (1*S*,2*S*), in agreement with that required using the product stereospecificity argument.

In attempted microbiological reductions of 2,2-dimethyltetral-1-one and 2,2-dimethylindan-1-one, we were unable to isolate any alcohol. Prelog and co-workers[3] have observed similar examples in their studies and suggested that substituents in "forbidden positions" interfered (steric effects) with the formation of a substrate coenzyme complex on the enzyme surface, which is necessary for reduction to occur.

Finally, the effect of varying the size of the cycloalkanone ring on the course of the reduction was examined. When 1-indanone was reduced under the usual conditions, (+)-(1*S*)-indanol (**9**) formed,[3] reduction occurring with the usual stereospecificity.

Although the high optical yields and defined configurations are important assets, the yields of alcohol in these reductions was lower than desired. We therefore devoted time searching for other microorganisms that exhibit stereospecificity while increasing the yields of alcohol produced. We found that *Cryptococcus macerans* increased the yield of α-tetralol two- to threefold and showed the same stereospecificity. This organism was then used to reduce the next higher homologue of α-tetralone, 1-benzsuberone (**10**) to yield the levorotatory alcohol ($[\alpha]^{25}_D$ −26.6°) whose stereochemistry was not otherwise examined, but is presumed to be *S* on the basis of the "product stereospecificity" concept. The same microorganism was used to reduce acetophenone, which produced the (−)-alcohol **11** known to be *S*.[15]

While these reductions allow one to prepare one enantiomer, in many studies in pharmacology and molecular biology it is important to have both enantiomers. We were therefore interested in determining if the stereochemistry of these benzylic alcohols could be inverted without extensive racemization. One attractive procedure employing triphenylphosphine, benzoic acid, and diethyl azodicarboxylate has been used to epimerize and esterify a variety of secondary alcohols.[16] When (−)-(1*S*)-phenylethanol was esterified in this manner, the (−)-(1*R*)-benzoate formed with an enantiomeric excess of approximately 95%. While some racemization has occurred, the optical purity is sufficiently high for many studies. It is also possible that additional work on reaction conditions could reduce the amount of racemization.

Conclusion

We have shown that microbiological reductions may be employed to prepare optically active hydroaromatic alcohols with a predictable configuration. Furthermore, it is not necessary to search for an organism to prepare the desired enantiomer, but in concert with standard chemical transformations either or both antipodes can be prepared. Substituents near the carbonyl group do not alter the configuration of the alcohols produced, but sometimes hinder reduction.

Experimental Section

Microbiological Reductions. A. α-Tetralone. A 1-L Erlenmeyer flask containing 600 mL of a solution of 6% glucose, 4% peptone, 4% yeast extract, and 4% malt extract was innoculated with a culture of *S. pararoseus*,[17] and the flask was shaken at 30 °C for 2 days. To the optically dense culture was then added 0.7 mL of α-tetralone and 600 mg of sodium desoxycholate, and shaking was continued for 5–7 days. The suspension was then extracted three times with 250-mL portions of ethyl acetate, the organic phase was concentrated in vacuo, and the dark residue was extracted into warm hexane. The hexane solution was again concentrated in vacuo and the desired alcohol separated by column chromatography on silica gel to yield 90 mg of (1*S*)-tetralol, $[\alpha]^{25}_D$ +26.8 (*c* 2.3, $CHCl_3$), and recovered α-tetralone (500 mg). The alcohol was acetylated with acetic anhydride in pyridine in the usual manner, and the resulting acetate was purified by thick-layer chromatography on silica gel.

B. 1-Oxo-1,2,3,4-Tetrahydrophenanthrene. A solution of 1-oxo-1,2,3,4-tetrahydrophenanthrene (200 mg) in acetone was mixed with 600 mg of Celite and the acetone was allowed to evaporate. This powder was used in the reduction which was otherwise done as described for α-tetralone. The alcohol (22 mg) purified by thick-layer chromatography was crystallized from hexane–acetone to yield 9.8 mg, $[\alpha]^{25}_D$ −59° (*c* 4.9, acetone). The NMR spectrum of the alcohol **2** was identical with that of racemic material prepared from hydride reduction of the starting ketone. The acetate was prepared as described for α-tetralol, $[\alpha]^{25}_D$ −178° (*c* 3.3, $CHCl_3$).

C. 4-Oxo-1,2,3,4-tetrahydrophenanthrene. A solution of 4-oxo-1,2,3,4-tetrahydrophenanthrene (300 mg) in acetone was mixed with 1.0 g of Celite and the solvent was allowed to evaporate. The solid was then divided into three equal parts and added to three 1-L Erlenmeyer flasks as described for α-tetralone. The alcohol [162 mg, $[\alpha]^{25}_D$ −19.5° (*c* 2.17, acetone)] was separated by thick-layer chromatography and its NMR spectrum was identical with racemic material obtained by hydride reduction of the starting ketone. The acetate was prepared as described for α-tetralol, $[\alpha]^{25}_D$ −128° (*c* 1.0, $CHCl_3$).

D. 1-Oxo-1,2,3,4-tetrahydroanthracene. Synthesis of this ketone is described below in the Experimental Section. A solution of the ketone (101 mg) in acetone was mixed with 460 mg of Celite, and the acetone was allowed to evaporate. The powder was added to the culture as described for α-tetralone. The alcohol **7** [26 mg, $[\alpha]^{25}_D$ +138° (*c* 0.8, $CHCl_3$)] was obtained by thick-layer chromatography and its NMR spectrum was identical with that of racemic material prepared by hydride reduction of the ketone. The acetate was prepared as described for α-tetralol, $[\alpha]^{25}_D$ −74° (*c* 4.1, $CHCl_3$).

E. 2-Methyl-1-tetralone. To each of four 1-L cultures of *S. pararoseus* was added 0.5 mL of 2-methyl-1-tetralone but *no* sodium desoxycholate. The reduction was worked up as usual and the resulting mixture (2 g) was purified by column chromatography on silica gel. The NMR spectrum of the cis alcohol was identical with that of racemic material prepared by hydride reduction of the ketone and purified by column chromatography. The cis stereochemistry was assigned on the basis of the coupling constant at δ 4.52 with $J_{1,2}$ = 2 Hz and $[\alpha]^{25}_D$ −44° (*c* 1.1, $CHCl_3$). The trans isomer showed a doublet at δ 4.27 with $J_{1,2}$ = 10 Hz and $[\alpha]^{25}_D$ +24° (*c* 2.46, $CHCl_3$).

F. 2-Acetoxy-1-tetralone. 2-Acetoxy-1-tetralone was prepared as described by Gardner[18] from α-tetralone by oxidation (*m*-chloroperbenzoic acid) of the intermediate α-tetralone enol acetate. A solution of **8a** (507 mg) in acetone was mixed with 1.0 g of Celite and the solvent was evaporated. The solid was then divided into two and

added to two Erlenmeyer flasks as usual. The mixture of acetoxy alcohols obtained from thick-layer chromatography on silica gel was reduced with lithium aluminum hydride in ether, and the resulting diols were separated by thick-layer chromatography on silica gel. The cis alcohol, $[\alpha]^{25}_D$ −43° (c 3.35, methanol), reported[10b] −38°. The trans alcohol, $[\alpha]^{25}_D$ +87.9° (c 2.39, $CHCl_3$), had an optical purity of 80%.

G. Indan-1-one. A solution of indan-1-one (500 mg) was mixed with 1.0 g of Celite and the solvent was evaporated. The solid was added to one flask and continued as above. The alcohol (42 mg), $[\alpha]^{25}_D$ +22.6° (c 4.2, $CHCl_3$), was then isolated by thick-layer chromatography and vacuum distillation.

H. Benzsuber-1-one. Benzsuber-1-one was reduced with a different microorganism, *Cryptococcus macerans*, obtained from Professor D. Perlman at the University of Wisconsin. The same culture medium, etc., were used *except* each 1-L Erlenmeyer flask contained only 250 mL of medium and sodium desoxycholate was omitted. To each of two 1-L Erlenmeyer flask was added 0.15 mL of benzsuber-1-one, and the flasks were shaken for 5 days and worked up as usual. The alcohol (81 mg) was separated by thick-layer chromatography: $[\alpha]^{25}_D$ −26.6° (c 4.0, $CHCl_3$); mp 73–4 °C.

I. Acetophenone. To four 1-L Erlenmeyers containing growing *Cryptococcus macerans* was added 0.25 mL of acetophenone. The workup was simplified since no starting ketone remained. The alcohol was distilled at 115 °C (3.5 Torr) and had an $[\alpha]^{25}_D$ −57° (c 5.12, $CHCl_3$). The NMR spectrum was identical with that of racemic material perpared by hydride reduction of the starting ketone.

Ozonolysis of (1*S*)-Acetoxy-1,2,3-4-tetrahydronaphthalene. A stream of ozone (2–4%) from an Ozonator, Model O3V2, was passed through a solution of 1-acetoxy-1,2,3-4-tetrahydronaphthalene (390 mg, $[\alpha]^{25}_D$ −97°) in 4 mL of acetic acid. The volume of the acetic acid solution was maintained during the ozonolysis by addition of acetic acid as required. After 8 h on completion of the ozonolysis 1 mL of 30% hydrogen peroxide was added and the reaction mixture was allowed to stand overnight at room temperature. The solution was then warmed to 50 °C for 30 min and sodium sulfite was added to decompose any remaining hydrogen peroxide. The solvent was removed in vacuo, excess saturated aqueous sodium bicarbonate was added, and the solution was extracted with hexane. The aqueous layer was then acidified with hydrochloric acid, saturated with sodium chloride and extracted several times with ethyl acetate. The ethyl acetate extract was washed with saturated sodium chloride, dried over sodium sulfate and concentrated in vacuo. The residue was treated with a solution of diazomethane in ether. The solvent was then removed and distillation of the residue (bath temperature 100–120 °C/0.2 Torr) yielded a colorless oil (135 mg) from which pure samples of **4** and **5** were obtained by preparative GLC using a temperature programmed Bendix 2200. The column was a 10 ft 5% FFAP on 100–120 mesh ABS. The yields of **4** and **5** obtained in these ozonolyses varied from 2–10%. A portion of the crude mixture of dimethyl esters was purified by preparative GLC to yield 2–5 mg of **4** and **5**. The specific rotations at 320 nm of **4** and **5** obtained from each of the acetates is given in Table II and the NMR ($CDCl_3$, 220 MHz) spectrum of **4** showed resonances at δ 2.16 (s), 3.68 (s), 3.75 (s), and 5.02 (t) while the spectrum of **5** showed resonances at δ 2.14 (s), 3.68 (s), 3.75 (s), and 5.07 (q).

The mass spectrum (LKB) for **4** showed peaks at *m/e* values of: 233 (weak), 201 (weak), 190 (moderate), 173 (strong), 159 (strong), 141 and 140 (weak), 131 (strong), and 99 (strong). The mass spectrum for **5** showed peaks at: 219 (weak), 187 (moderate), 176 (strong), 159 (moderate), 145 (strong), 126 and 127 (weak), 117 (strong), and 85 (strong). For each strong peak in **4** there is in **5** a corresponding strong peak shifted by 14 mass units.

An authentic sample of **4** was prepared by ozonolysis of 3-acetoxycyclohexene, followed by esterification of the acid with diazomethane. The dimethyl ester was then distilled in vacuo.

Preparation of 1-Oxo-1,2-3,4-tetrahydroanthracene (6). To a solution of 5.01 g of 1,2,3,4-tetrahydroanthracene in carbon tetrachloride (125 mL) was added *N*-bromosuccinimide (4.06 g) and benzoyl peroxide (20 mg). The solution was refluxed for 2 h and the solvent was removed in vacuo. The residue was treated with acetic acid (50 mL) and potassium acetate (5 g), heated for 1 h on a steam bath, and poured into water. The mixture was extracted with dichloromethane, and the organic phase was washed with saturated aqueous sodium bicarbonate, dried, and concentrated. The residue was dissolved in dry tetrahydrofuran, cooled in ice–water, and treated with excess diborane. The solution was slowly allowed to warm to room temperature and the borane was oxidized with 10% sodium hydroxide (10 mL) and 30% hydrogen peroxide (5 mL) at room temperature. Unreacted hydrogen peroxide was decomposed by stirring with Pt/charcoal and the tetrahydrofuran was removed in vacuo. Water was added and the mixture was extracted with chloroform. The chloroform solution was concentrated and the residue was chromatographed over silica gel to yield 1.465 g (27%) of 1-hydroxy-1,2,3,4-tetrahydroanthracene, mp 83–4 °C. Anal. Calcd for $C_{14}H_{14}O$: C, 84.79; H, 7.12. Found: C, 84.51; H, 7.07.

The NMR spectrum (220 MHz, $CDCl_3$) showed a broad singlet at δ 4.91 (1 H), 2.95 (m, 2 H), and 1.7–2.4 (complex, 4 H).

The alcohol was oxidized with Jones reagent to yield the ketone, mp 90–91 °C, in essentially quantitative yield. Anal. Calcd for $C_{14}H_{12}O$: C, 85.66; H, 6.17. Found: C, 85.75; H, 6.22.

The NMR spectrum of the ketone showed aromatic absorption and triplets at δ 3.11 and 2.73 and a multiplet at δ 2.18.

Epimerization of (−)-(1*S*)-Phenylethanol. A solution of (−)-(1*S*)-phenylethanol (122 mg, 1 mmol), triphenylphosphine (524 mg, 2 mmol), and benzoic acid (244 mg, 2 mmol) in dry THF (10 mL) was stirred under N_2 at room temperature. To this solution was added dropwise a solution of diethyl azodicarboxylate (348 mg, 2 mmol) in dry THF (5 mL). After stirring overnight the residue was dissolved in chloroform (20 mL), washed with 10% aqueous sodium bicarbonate and water, dried over sodium sulfate, filtered, and concentrated. Analysis of the crude reaction mixture by NMR indicated that 95% of the starting alcohol had been converted into the corresponding benzoate. The benzoate was purified by thick-layer chromatography (silica gel, ethyl acetate/hexane, 7:93) to yield 178 mg (91% yield) of (−)-(1*R*)-phenylethanol benzoate as a viscous oil, $[\alpha]^{25}_D$ −20.7° (c 2.28, EtOH). The NMR spectrum of this benzoate was identical with that of racemic material: δ 1.66 (3 H, d, J = 6.4 Hz), 6.13 (1 H, q, J = 6.4 Hz), ~7.27–7.55 (6 H, m), 7.33 (2 H, d, J = 7.1 Hz), 8.08 (2 H, d, J = 7.1 Hz).

The optical purity was 95% based on the rotation of (+)-(1*S*)-phenylethanol benzoate, $[\alpha]^{25}_D$ +21.9° (c 2.78, EtOH), prepared by reaction of (−)-(1*S*)-phenylethanol and benzoyl chloride in pyridine.

The (−)-(1*R*)-benzoate was then hydrolyzed to (+)-(1*R*)-phenylethanol. A solution of 178 mg (0.91 mmol) of (−)-(1*R*)-benzoate in 5 mL of methanol containing 1 mL of water and 200 mg of KOH was refluxed for 2 h. The solvent was removed in vacuo, the residue was extracted with ethyl acetate, washed with water, dried over sodium sulfate, and concentrated to yield an oil which was purified by thick-layer chromatography (silica gel, ethyl acetate/hexane, 3:7). The (+)-(1*R*)-phenylethanol (92 mg, 83% yield) was isolated, $[\alpha]^{25}_D$ +53.2° (c 5.41, $CHCl_3$), optical purity 98% based on the absolute rotation reported in the literature.[15] The NMR spectrum of this sample was identical with that of racemic material.

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Registry No.—**4**, 65915-68-6; **5**, 55095-00-6; 1,2,3,4-tetrahydroanthracene, 2141-42-6; (−)-(1*R*)-phenylethanol benzoate, 65915-69-7; (+)-(1*S*)-phenylethanol benzoate, 57473-79-7; (+)-(1*R*)-phenylethanol, 1517-69-7.

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Douglas Fir Tussock Moth Pheromone: Identification of a Diene Analogue of the Principal Attractant and Synthesis of Stereochemically Defined 1,6-, 2,6-, and 3,6-Heneicosadien-11-ones

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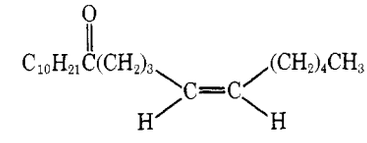
A diene analogue of the principal Douglas fir tussock moth sex pheromone (*Z*)-6-heneicosen-11-one has been isolated and identified as a 1,6-heneicosadien-11-one using mass spectrometry, microozonolysis, and gas chromatography. Five geometric and positional heneicosadien-11-one isomers were synthesized for chromatographic and spectroscopic comparison and for biological testing. Unambiguous structural assignments of the five isomers were established by capillary column gas chromatography, carbon magnetic resonance spectroscopy, infrared and laser Raman spectroscopy, and mass spectrometry.

The principal attractant of the sex pheromone system of the Douglas fir tussock moth (DFTM), *Orgyia pseudotsugata* (McDunnough), was identified as (*Z*)-6-heneicosen-11-one (**1**),[1] synthesized,[2-4] and successfully tested in laboratory and field bioassays.[5] We have now detected a closely related compound in attractive extracts of DFTM female abdominal tips and identified it as a 1,6-heneicosadien-11-one **2** or **3**. Both isomers (*Z*)-1,6-heneicosadien-11-one (**2**) and (*E*)-1,6-heneicosadien-11-one (**3**) have been synthesized for comparison with the natural material and for biological evaluation. In addition, the isomers (*E,Z*)-2,6-heneicosadien-11-one (**4**), (*Z,Z*)-3,6-heneicosadien-11-one (**5**), and (*E,Z*)-3,6-heneicosadien-11-one (**6**) were also synthesized, characterized, and evaluated for attractiveness to DFTM males.

Isolation and Structure Elucidation

The dienone was first observed by gas chromatography–mass spectrometry (GC/MS) studies of partially purified fractions obtained by dry column chromatography[1] of active DFTM extracts. Its mass spectrum is very similar to that of the principal attractant (**1**, Figure 1a) and corresponds to a diene analogue of **1**. Thus the molecular ion (*m/e* 306) established the probable empirical composition as $C_{21}H_{38}O$ and a cleavage ion at *m/e* 169 established the presence of a carbonyl at C-11 and a ten-carbon saturated alkyl chain. The other carbonyl α cleavage ion at *m/e* 165 confirmed assignment of both sites of unsaturation to the remaining ten-carbon alkyl chain. Furthermore, the appearance of an ion at *m/e* 122 (corresponding to the ion at *m/e* 124 in the spectrum of **1**, see Figure 1a), derived via a McLafferty rearrangement with charge retention on the hydrocarbon fragment, strongly suggested that one double bond was at position six[1] and the second double bond was contained in the five-carbon terminus of the alkyl chain (i.e., at positions 1, 2, or 3, see the spectra in Figures 1b, 1c, and 1d).

Isolation of the diene was undertaken from the dichloromethane extract of 1000 crushed DFTM female abdominal



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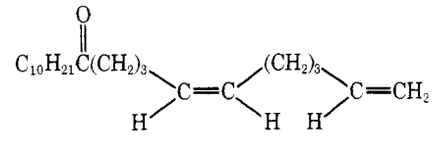

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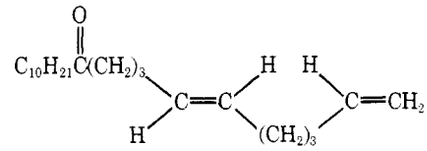

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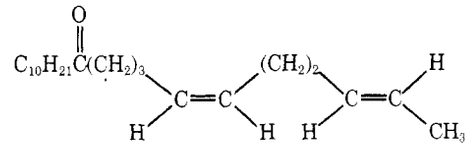

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