Tritium-Labeled Enantiomers of Disparlure. Synthesis and in Vitro Metabolism

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Abstract: Both of the enantiomers of disparlure, the gypsy moth sex attractant, have been prepared at high specific activity (58 Ci/mmol) by homogeneous tritiation of the optically active alkenyl oxiranes. An improved preparation of the racemic disparlure is also described. The radiolabeled epoxides are cleanly converted to a single product by enzymes isolated from adult gypsy moths (*Lymantria dispar*). Enzymatic activity obtained from male antennae showed the highest conversion; enzymes isolated from female antennae and from male and female legs showed lower activity. The identification of the metabolite as the *threo*-7,8-diol is established by independent synthesis and by chemical derivatizations to the diacetate, *n*-butylboronate, and bis(trimethylsilyl) ether. A symmetrical epoxide analogous to disparlure is converted to a diol product at a significantly lower rate.

The chemistry of pheromonal communication and the behaviors released by pheromones have been extensively investigated in the last two decades. Until the last five years, surprisingly little attention had been paid to the biochemistry of pheromone production, catabolism, and reception. Many of the exciting results in this new discipline have been recently summarized,[1] and the opportunity of understanding pheromone perception on a molecular level is now at hand. Herein, we describe results on the metabolism of an epoxide pheromone by the gypsy moth.

The female gypsy moth, *Lymantria dispar*, produces a single major pheromone, (+)-disparlure or (7*R*,8*S*)-7,8-epoxy-2-methyloctadecane. It was first identified[2] without reference to its absolute configuration. Synthesis of the enantiomerically enriched (+)- and (−)-disparlure antipodes has now been accomplished by several routes.[3] The availability of the separate enantiomers led to two key biological results: (1) *L. dispar* males possess separate receptor cells for (+)- and (−)-disparlure[4] and (2) the (+)-enantiomer is attractive and the (−)-enantiomer is inhibitory in field situations,[5] in wind-tunnel experiments,[6] in tethered flight,[7] and in electroantennogram assays.[4b,8]

Tritium-labeled racemic disparlure[9] was first reported to be converted to unidentified polar metabolites in whole antennae with a half-life of 1.4 min at a loading of 5×10^{11} molecules/antenna.[10] Unfortunately, no attempts were reported to chemically characterize the metabolites or biochemically characterize the responsible enzymes. We wished to determine the identity of the metabolites produced by these antennal enzymes and to determine the tissue specificity of the enzymatic activity. To this end, we now describe the first preparation of the radiolabeled enantiomers of disparlure. High specific activity (58 Ci/mmol) is attained by reduction of an alkenyl oxirane with carrier-free tritium gas, giving tritium atoms at C-5 and C-6. A modified synthesis[9] of the racemic disparlure is also presented with tritiums at C-7 and C-8. The metabolism of the racemic and >95% enantiomerically enriched disparlure compounds by antennal proteins of male and female gypsy moths is described. A single product, the *threo*-7,8-diol, is obtained in the rapid biochemical conversion catalyzed by male antennal enzymes. The structure of the diol product has been confirmed by chromatographic comparisons of synthetic diol and its derivatives with labeled and unlabeled diol obtained biochemically.

Results and Discussion

Synthesis of Substrates. A supply of racemic, tritium-labeled disparlure was necessary in order to have tritium placed where it would be metabolically stable but chemically labile. Thus, the semitritiation[9] of alkyne **1** was carried out at the National Tritium Labeling Facility (NTLF) in Berkeley, CA (Figure 1). The use of tetrahydrofuran (THF) as the hydrogenation solvent[1d] instead of methanol[9] reduced proton–triton exchange and enabled us to obtain alkene **2** with a specific activity of 49 Ci/mmol, close to the theoretical maximum of 58 Ci/mmol for two tritons. Epoxidation of the tritiated (*Z*)-alkene **2** gave racemic disparlure **3**.

The radiolabeled disparlure enantiomers were prepared following the Sharpless procedure[3a] as modified by Zhou and coworkers[11] (Figure 2). Thus, (*Z*)-2-tridecen-1-ol (**5**), obtained by semihydrogenation of the alkynol **4**, was converted to the enantiomerically enriched epoxy alcohols with $Ti(OiPr)_4$, *tert*-butyl hydroperoxide, 10 mol % calcium hydride, 10 mol % silica gel, and either (−)- or (+)-diethyl tartrate. The products, (−)-**6** and (+)-**6**, were both obtained in >95% ee, based on the integration of the C-1 ^1H NMR signals of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters (Figure 3). Oxidation with chromium trioxide–pyridine complex in CH_2Cl_2 afforded the epoxy aldehydes **7** in 61% yield, and condensation with 4-methylpentylidene triphenylphosphorane in THF afforded the enantiomeric alkenyl oxiranes **8** in 82% yield (*E*:*Z* = 20:80). The reduction of the double bonds was performed in dry benzene at 740 Torr of *either* hydrogen or carrier-free tritium gas with a stoichiometric amount of Wilkinson's catalyst, $RhCl(PPh_3)_3$, which had been presaturated with hydrogen (tritium) for 2 h prior to the addition of substrate. This modification was optimized[12]

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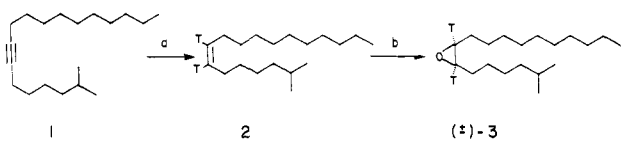

Figure 1. Synthesis of racemic, labeled disparlure. Reagents: (a) $^3H_2$, 5% $Pd/BaSO_4$, quinoline, THF; or $^1H_2$, 5% $Pd/CaCO_3$, pyridine; (b) *m*-CPBA, CH_2Cl_2.

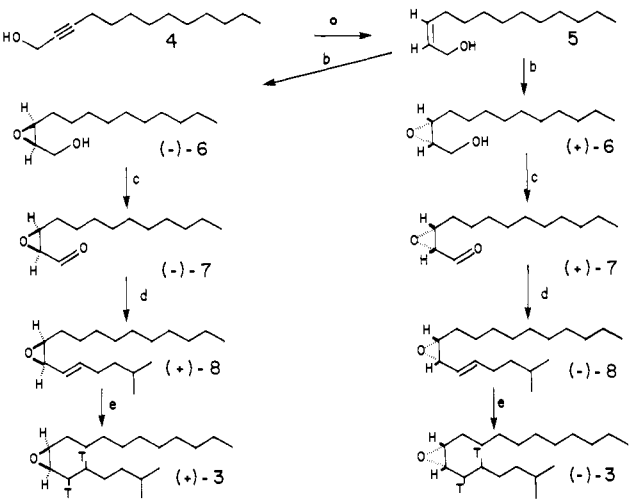

Figure 2. Synthesis of enantiomers of labeled disparlure. Reagents: (a) H_2, 5% $Pd/CaCO_3$, pyridine; (b) *t*-BuOOH, $Ti(OiPr)_4$, CH_2Cl_2, CaH_2, SiO_2, (+)-DET or (−)-DET; (c) $CrO_3{\cdot}(Py)_2$, CH_2Cl_2; (d) 4-methylpentylidene triphenylphosphorane, THF; (e) $^nH_2$ (n = 1,3), $Rh(Ph_3P)_3Cl$, C_6H_6.

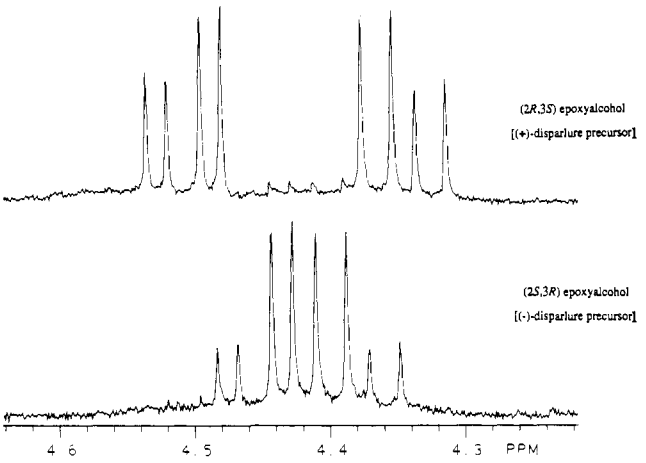

Figure 3. 300 MHz ^1H NMR of the C-1 protons of the (*R*)-(+)-MPTA esters of (−)-**6** (top, the precursor to (+)-**3**) and (+)-**6** (bottom, the precursor to (−)-**3**).

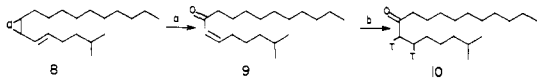

Figure 4. Byproducts of homogeneous hydrogenation. Reagents: (a) $Rh(Ph_3P)_3Cl$; (b) $T_2Rh(Ph_3P)Cl$ (T = ^1H or ^3H).

for rapid transfer of tritons to an alkenyl oxirane precursor to the juvenile hormone JH I and allowed reduction in 2–3 h in contrast to the 2 days at 4 atm reported previously.[3a] Furthermore, as found by Rossiter et al.,[3a] the rearranged enone **9** and its labeled reduction product, 2-methyloctadecan-8-one **10**, are present as 15–20% of the mixture and cochromatographed on silica gel with the desired epoxide (Figure 4). The ketonic impurities were removed by stirring either labeled or unlabeled oxiranes with excess

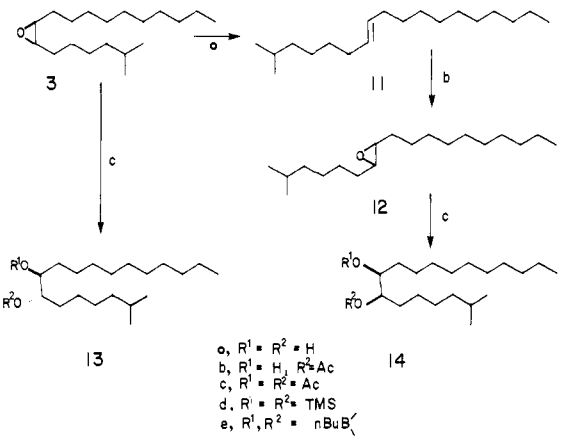

Figure 5. Preparation of inverted epoxides and diastereomeric diols and their derivatives. Reagents: (a) LiCl, DMF, $(CF_3CO)_2O$, and then NaI; (b) *m*-CPBA, CH_2Cl_2; (c) HOAc, Al_2O_3; or 3% aqueous $HClO_4$.

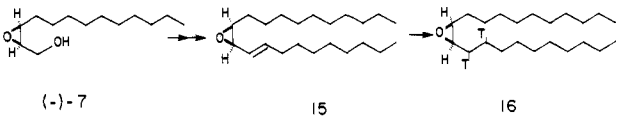

Figure 6. Synthesis of labeled *meso*-diol **16**.

sodium borohydride in ethanol for 6 h at room temperature, followed by flash chromatography to remove the hydroxyl-containing byproducts.

Thus, the tritium-labeled disparlure enantiomers (+)-**3** and (−)-**3** were obtained at a nominal specific activity of 58 Ci/mmol in ca. 50% radiochemical yield. High specific activity pheromones and pheromone precursors are best stored at −20 to −80 °C in degassed and distilled 1:1 heptane–toluene at concentrations of 10–500 mCi/mL.[1d,13]

Synthesis of Metabolites. We expected that pheromone-sensitive tissues would deactivate the epoxide by hydration to a diol via a tissue- and substrate-specific epoxide hydrolase. Moreover, we anticipated that only the *threo*-diol, resulting from the S_N2 type opening of the *cis*-oxirane ring, would be produced.[14] Thus, we prepared both the racemic threo (**13a**) and erythro (**14a**) diols[15] from the *cis*- and *trans*-2-methyl-7,8-epoxyoctadecanes (**3** and **12**, respectively) (Figure 5). The *trans*-epoxide **12** was obtained by conversion of the *cis*-epoxide **3** to the (*E*)-olefin **11** with trifluoroacetic anhydride–lithium chloride in dimethylformamide (DMF) followed by sodium iodide[16] and then epoxidation with *m*-chloroperbenzoic acid (*m*-CPBA).

The oxirane ring was unexpectedly resistant to opening with base. Only a 10% yield of diol was obtained after heating a solution of *cis*-epoxide with 50% aqueous NaOH in 1-butanol at 120 °C for 3 days. Conversion of either epoxide to its diol was conveniently achieved by hydration with 3% aqueous perchloric acid in 1,4-dioxane (room temperature, 24 h). Diols prepared in this manner were used to prepare the diacetates **13c** and **14c**, the bis(trimethylsilyl) ether **13d** and **14d**, and the *n*-butylboronates **13e** and **14e** for capillary gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) analyses. While the threo and erythro isomers of all three derivatives (and even the underivatized diol) could all be separated on the capillary GC, base-line separation was achieved most readily with the *n*-bu-

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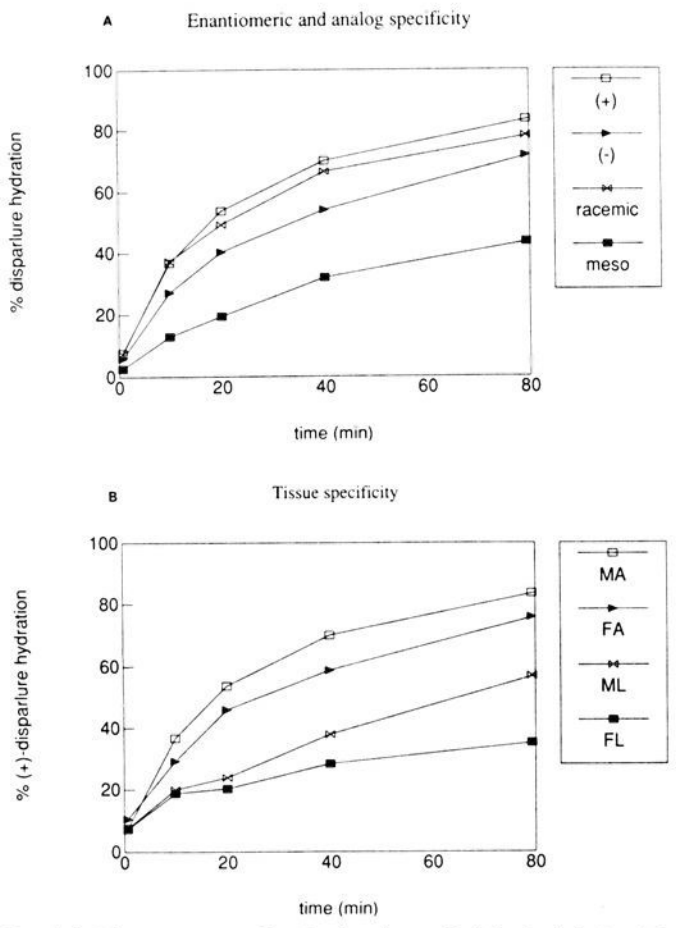

Figure 7. (a) Time course for hydration of (+)-**3**, (−)-**3**, (±)-**3**, and *meso*-**16** by antennal enzymes of the male gypsy moth. (b) Time course for hydration of (+)-**3** as a function of time and of the source of the enzyme: MA = male antennae; FA = female antennae; ML = male legs; FL = female legs.

tylboronates. In addition, only the *n*-butylboronate derivative gave a molecular ion in the mass spectrum.

When treated with activated alumina and acetic acid in ether solution,[17] the *cis*-epoxide (90:10 cis–trans) was found to give only the *threo*-monoacetate **13b**. Methanolysis with potassium carbonate–methanol afforded only *threo*-diol **13a**, and treatment of diol **13a** or monoacetate **13b** with Ac_2O–pyridine–4-(dimethylamino)pyridine (DMAP) gave the *threo*-diacetate **13c**. None of the erythro isomer **14b** could be detected when the epoxides were treated with alumina–acetic acid. Indeed, the *trans*-epoxide was not opened at an appreciable rate under these conditions.

Synthesis of a Symmetrical Analogue. To test the substrate specificity of the epoxide hydrolase (EH), we prepared an unbranched, symmetrical epoxide, 11,12-epoxydocosane **16**, in such a fashion that the label would reside in the prochiral "arm", which corresponded to the branched chain in disparlure (Figure 6). This was readily accomplished by olefination of epoxy aldehyde (−)-**7** with nonylidene triphenylphosphorane in THF. Reduction of the alkenyl oxirane **15** to the saturated epoxide **16** was carried out under homogeneous catalysis as for alkenyl oxirane **8**, using either hydrogen or tritium gas.

Metabolic Experiments. Gypsy moth (*L. dispar*) pupae were sexed and placed in separate plastic boxes at 26 °C in a long-day photoperiod to allow adult emergence. Virgin moths were collected on day 0 or day 1 after eclosion and cooled to 4 °C, and their tissues (legs, antennae) were clipped into ice-chilled plastic tubes. Fresh materials were generally used, although storage at −80 °C did not significantly affect the enzymic activity. A full account of the tissue, sex, substrate, and posteclosion time specificity of the enzymatic activity will be described elsewhere. For these assays, crude tissue homogenates were used after removal of cuticle and cell fragments by centrifugation for 10 min at 12000*g*.

The labeled racemic disparlure (±)-**3** and both enantiomers were converted to a single radioactive metabolite, as confirmed by X-ray autoradiography of 2,5-diphenyloxazole-sprayed thin-layer chromatography (TLC) plates, by cutting and liquid scintillation counting (LSC) of TLC plates, and by multichannel imaging scanner analysis of TLC-separated compounds. The time

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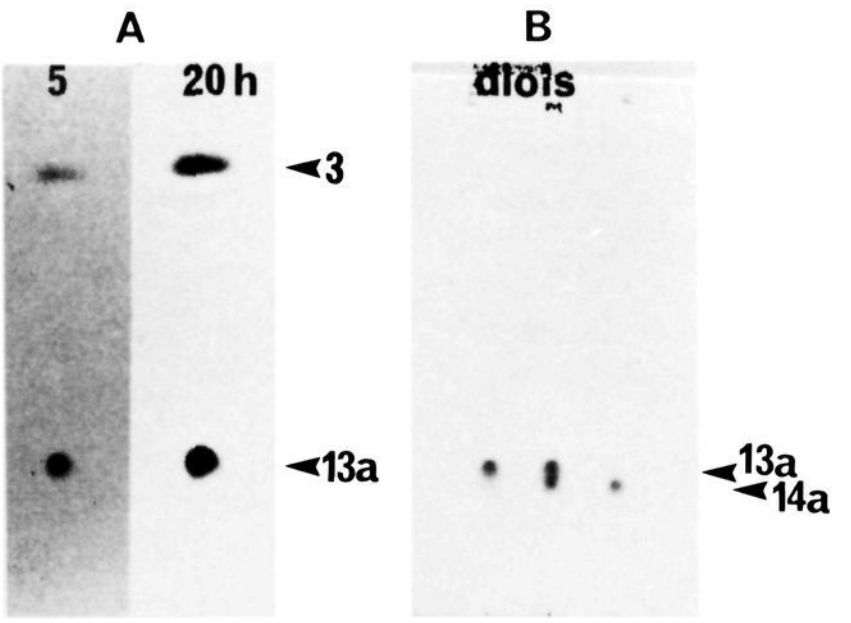

Figure 8. TLC (80:20 H–EA) of epoxide hydration: (A) fluorescence autoradiogram showing epoxide **3** (upper spot) and enzymatically produced *threo*-diol **13a**, with 5-h and 20-h exposure to X-ray film; (B) vanillin-stained *threo*-diol **13a** (top spot), mixed diols, and *erythro*-diol **14a** (bottom spot).

course, substrate selectivity, and structure of the product were verified as described below.

Figure 7A shows the results of a time course determination for the four labeled substrates. Each assay contained 3 μCi (52 pmol) of the labeled pheromone or analogue in 300 μL of buffer containing six male antennal equivalents. At 0-, 10-, 20-, 40-, and 80-min intervals, aliquots were withdrawn and quenched with ethyl acetate, and the product distribution was analyzed by TLC–LSC. There was a significantly greater rate of hydration of the natural attractive enantiomer (+)-**3**, as compared to the unnatural (−)-**3** enantiomer. Initially, the racemic (±)-**3** appears to by hydrated at virtually the same rate as the (+)-enantiomer; after the (+) is consumed, the curve parallels that of the (−)-enantiomer. Furthermore, the unnatural meso analogue **16** is hydrated to diol at approximately half of the rate of the natural enantiomer.

Figure 7B shows the results of a time course determination for four possible tissue sources. Each assay contained 3 μCi of labeled (+)-**3** in 300 μL of buffer containing six male or female antennal or leg equivalents. Aliquots were withdrawn, quenched, and analyzed as described for the time course experiment. Male antennae showed the greatest rate of hydration; female antennae were only slightly less active. Male and female legs showed a significantly lower rate of hydration, with female legs showing the least activity. Importantly, boiled enzyme solutions showed no conversion of [^{3}H]disparlure to labeled products. It is expected that general epoxide hydrolase activity would be present in other tissues to degrade adsorbed pheromone.[1d]

The labeled metabolite cochromatographed with authentic *threo*-diol **13a** as determined by TLC–autoradiography and radio TLC scanning (RTLCS), but initially the techniques were insufficient to detect low levels (<5–10%) of the *erythro*-diol **14a**, if present. Subsequent experiments using TLC–autoradiography have shown the amount of *erythro*-diol to be undetectable, even in overexposed X-ray films where visualization is possible below 0.5% conversion (Figure 8). Also, derivatization with acetic anhydride–DMAP–pyridine gave a labeled material indistinguishable by TLC from the authentic *threo*-diacetate **13c**.

Further assignment of the identity and stereochemistry of the diol metabolite was obtained as follows without radioisotopic tracer. A large-scale metabolism experiment was performed with 100 male antennal equivalents in 100 mL of 10 mM tris buffer (pH = 7.0) with 8 mg (28 μmol) of unlabeled racemic disparlure (cis:trans = 90:10). After flash chromatography, saponification of contaminating lipids, and rechromatography, a single diol metabolite was isolated. This diol was converted to the bis(trimethylsilyl) ether **13d** and analyzed by capillary GC and GC–MS. The retention time on a 30-m DB-1701 capillary column clearly showed the *threo*-bis(trimethylsilyl) ether **13d** as the single largest peak, with less than 10% of the integrated area in the region of

erythro isomer **14d**. The electron impact mass spectrum of the bis(trimethylsilyl) ether **13d** derived from the enzymatically produced diol **13a** had characteristic peaks at m/z 243 and 201, corresponding to scission of the C-7–C-8 bond.

Consistent with other known epoxide hydrolases, therefore, the primary mechanism for the hydration is an S_N2-type opening. It is highly likely that electrophilic activation of the oxiranyl oxygen and nucleophilic activation of the incoming water molecule are involved. Further studies using potential epoxide hydrolase inhibitors, ^{18}O-labeled materials, and chiral phase capillary GC are expected to clarify the mechanism, regiochemistry, and absolute stereochemistry of this hydration reaction.

Conclusion. The antennae of male gypsy moths contain high epoxide hydrolase activity, which converts the pheromone to a diol that is behaviorally inactive.[15b] This clearance mechanism is crucial to the processing of olfactory stimulants in insect sensory hairs, and understanding the chemical basis of pheromone processing and binding to antennal proteins forms the foundation for further studies on the molecular mechanisms in olfaction. Additional experiments on the tissue and sex specificity as well as the kinetics and absolute stereochemistry of this antennal EH for each enantiomer will be described in due course.[18]

Experimental Methods

General Procedures. 2-Methyl-7-octadecyne was a gift from R. T. Carde. Ether, THF, and benzene were distilled from sodium benzophenone; methylene chloride was distilled from calcium hydride. Dimethylformamide (DMF), acetonitrile, and ethanol were dried over molecular sieves. Pyridine was distilled from potassium hydroxide; dioxane was filtered through activated alumina. Other reagents were purified as necessary or were used as obtained from the supplier.

Flash chromatography was performed as by Still.[19] TLC was performed with MN Polygram Sil G/UV 254 (4 × 8 cm) TLC plates. Gas chromatography was performed on a Varian 3700 GC with 30 m × 0.25 mm (i.d.) DB-1, DB-5, or DB-1701 columns. The column film thickness was 0.25 μm unless otherwise stated. The GC injector (280 °C) had a split-type glass insert and a flame ionization detector (320 °C). Column conditions are reported as follows: initial temperature in degrees Celsius, initial time/program rate in degrees Celcius per minute/final temperature in degrees Celsius. 80-MHz ^1H NMR spectra were obtained on a Varian CFT-20 spectrometer; 300-MHz ^1H NMR and 75-MHz ^{13}C NMR spectra were obtained on a Nicolet NT-300 or GE QE-300 spectrometer. Electron impact mass spectra were obtained on a Spectros MS 30 spectrometer at 70 eV.

Tritiations were performed with carrier-free tritium gas at the National Tritium Labeling Facility at the Lawrence Berkeley Laboratory. Autoradiography (fluorography) was performed with Kodak XAR-5 film after spraying with EnHance (Du Pont–NEN). Radio TLC scanning (RTLCS) was performed on a Bioscan System 500 imaging scanner. Radioactive samples were counted in an LKB 1218 Rackbeta liquid scintillation counter using Fisher Scintiverse II scintillation cocktail. Counting efficiency was 57–61% for tritium and counts per minute data was corrected by the external standard ratio method.

Synthesis of Unlabeled Compounds. (*Z*)-2-Methyl-7-octadecene (2). A mixture of 2-methyl-7-octadecyne (**1**) (1026.2 mg, 3.880 mmol) and 5% palladium on $BaSO_4$ (206.4 mg) in 10 mL of dry pyridine was stirred vigorously under a continuous positive pressure atmosphere of H_2 for 12 h. The reaction was monitored by GC and TLC; the alkyne (R_f = 0.37) and alkene (R_f = 0.66) were easily separated (ΔR_f = 0.3) on silica gel when hexane was the eluant. The pyridine was removed by evaporation; the crude product was dissolved in hexane, filtered, and flash-chromatographed (hexane) to give 882.2 mg (3.310 mmol, 85%) of the desired alkene **2**, a clear liquid, as a 90:10 *Z*–*E* mixture (GC, *Z* = 38.37 min, *E* = 38.49 min, 50, 2/5/250, DB-5, 1-μm film). ^1H NMR (300 MHz, $CDCl_3$): δ 5.331 (m, 2 H), 1.999 (m, 4 H), 1.503 (m, 1 H), 1.245 (br s, 22 H), 0.86–0.84 (m, 9 H).

(±)-*cis*-7,8-Epoxy-2-methyloctadecane [(±)-Disparlure] [(±)-3]. To a stirred solution of *m*-chloroperoxybenzoic acid (*m*-CPBA; 811 mg, Aldrich 80–85% technical grade, 3.75–3.99 mmol) in 13 mL CH_2Cl_2 was added (*Z*)-2-methyl-7-octadecene (**2**) (880 mg, 3.30 mmol) in 8 mL of dry CH_2Cl_2. Both TLC and GC showed the reaction to be complete in <75 min. The reaction was stirred with 10 mL of 10% Na_2SO_3 for 15 min and then washed with 10 mL of 5% NaOH and 20 mL of water, and the CH_2Cl_2 solution was filtered through Florisil. The solution was concentrated, redissolved in hexane, and flash-chromatographed [hexane; 95:5, 90:10 hexane–ethyl acetate (H–EA)] to give 775.4 mg (2.74 mmol, 83%) of the desired epoxide (±)-**3**, a clear liquid, as a 90:10 cis–trans mixture (GC, trans = 19.94 min, cis = 20.25 min, 180, 2/5/280, DB-5, 1-μm film). ^1H NMR (300 MHz, $CDCl_3$): δ 2.884 (m, 2 H), 1.6–1.4 (m, 5 H), 1.4–1.2 (br s, 22 H), 0.86–0.84 (m, 9 H). For the metabolic studies, we required "100% cis" (±)-**3** in order to avoid any ambiguities in the origin of any *erythro*-diol. Thus, 32 mg of alkene **2** was flash-chromatographed on 10% (w/w) $AgNO_3$ silica gel (hexane; 99:1, 98:2, 95:5 H–EA) to give 21.5 mg of alkene **2**, >99.6% *Z* by GC (27.77 min, 180, 0/1/250, DB-5, 1-μm film). A portion of this 100% cis alkene was epoxidized as described above to give (±)-**3**, also >99.6% cis by GC (18.41 min, 180, 0/5/270, DB-5, 1-μm film).

(*E*)-2-Methyl-7-octadecene (11). A dry, argon-flushed screw-cap vial was charged with dry LiCl (21.4 mg, 0.505 mmol), 1.0 mL of dry DMF, and 60 μL (0.43 mmol) of trifluoroacetic anhydride. The mixture was stirred 15 min, and (±)-*cis*-2-methyl-7,8-epoxyoctadecane [(±)-**3**] (102.0 mg, 0.361 mmol, 90:10 cis–trans by GC (DB-5 capillary) and ^1H NMR) was added (neat, using a glass micropipet). The reaction was stirred at room temperature for 6.5 h (TLC showed complete loss of starting epoxide), followed by addition of dry NaI (234.6 mg, 1.565 mmol) and heating at 130 °C for 24 h. The reaction was cooled to room temperature, diluted with 1 mL of 10% $NaHSO_3$, and extracted with 3 × 10 mL portions of hexane. The combined hexane fractions were washed with 3 × 10 mL portions of water, dried ($MgSO_4$), and flash-chromatographed (hexane; 98:2, 95:5 H–EA) to give 65.5 mg (0.246 mmol, 68%) of alkene **11**, a clear liquid, as a 8:92 *Z*–*E* mixture (GC, *Z* = 29.83 min, *E* = 30.10 min, 50, 2/5/175, DB-5). ^1H NMR (300 MHz, $CDCl_3$): δ 5.365 (m, 2 H), 1.96–1.94 (m, 4 H), 1.50 (m, 1 H), 1.4–1.1 (br s, 22 H), 0.86–0.84 (m, 9 H). IR: 975 cm^{-1} (*trans*-HC=CH).

(±)-*trans*-7,8-Epoxy-2-methyloctadecane [(±)-12]. The (*E*)-alkene **11** (21.0 mg) was epoxidized with *m*-CPBA as described for the (*Z*)-alkene to give 18.9 mg (85%) of epoxide (±)-**12**, a clear liquid, as an 88:12 trans–cis mixture (GC, trans = 19.94 min, cis = 20.24 min, 180, 2/5/280, DB-5, 1-μm film). ^1H NMR (300 MHz, $CDCl_3$): δ 2.882 (due to the cis isomer), 2.632 (m, 2 H), 1.6–1.4 (m, 5 H), 1.4–1.1 (br s, 22 H), 0.86–0.84 (m, 9 H).

4-Methylpent-1-yl Tosylate. A solution of 4-methyl-1-pentanol (5.24 g, 51.3 mmol) and *p*-toluenesulfonyl chloride (TsCl; 11.83 g, 62.05 mmol) in 50 mL of dry pyridine was stirred under N_2 at 0 °C for 7 h. The solution was poured over 200–300 g of ice and extracted with Et_2O (3 × 100 mL). The combined organic layers were washed successively with 1:1 concentrated HCl–H_2O (2 × 100 mL), H_2O (1 × 100 mL), and saturated NaCl (1 × 100 mL). The solution was dried ($MgSO_4$) and solvent evaporated to give 11.27 g (43.96 mmol, 86%) of the desired tosylate, a clear liquid. ^1H NMR (80 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8 Hz, 2 H), 7.34 (d, *J* = 8 Hz, 2 H), 4.01 (t, *J* = 7.2 Hz, 2 H), 2.44 (s, 3 H), 1.9–1.0 (m, 5 H), 0.84 (d, *J* = 6.4 Hz, 6 H).

1-Iodo-4-methylpentane. A mixture of 4-methylpent-1-yl tosylate (8.19 g, 31.9 mmol), dry NaI (5.50 g, 36.7 mmol), and 50 mL of acetone was stirred at room temperature under N_2 for 24 h. The initial workup (Et_2O–H_2O extraction followed by flash chromatography) led to violet colored solutions of product in H–EA. Fractions were combined, the solvent was evaporated, and the product was dissolved in hexane and washed with 10% aqueous Na_2SO_3 to remove the violet color. The hexane solution was concentrated and rechromatographed to give 3.05 g (14.4 mmol, 45%) of the desired alkyl iodide. ^1H NMR (80 MHz, $CDCl_3$): δ 3.17 (t, *J* = 7.2 Hz, 2 H), 2.0–1.0 (m, 5 H), 0.89 (d, *J* = 6.4 Hz, 6 H).

(4-Methylpent-1-yl)triphenylphosphonium Iodide. A solution of 1-iodo-4-methylpentane (717.5 mg, 3.383 mmol) and triphenylphosphine (Ph_3P; 888.6 mg, 3.389 mmol) in 2.5 mL of dry acetonitrile was stirred at reflux under N_2 for 16 h. The reaction was cooled to room temperature, and ca. 3 mL of Et_2O (dried over $MgSO_4$) was added. After stirring briefly, the top layer (Et_2O) was removed with a pipet. Three additional Et_2O washes removed the remaining Ph_3P, leaving a viscous residue that hardened and became microcrystalline upon the addition of several milliliters of CH_2Cl_2. Upon heating, the solid dissolves. Slow cooling to 0 °C, followed by the addition of Et_2O (previously dried over $MgSO_4$) gave 875 mg (1.85 mmol, 55%) of white crystals, mp 198–200 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 7.83–7.65 (m, 15 H), 3.616 (dt, *J* = 12.7 ($^2J_{PCH}$), 7.8 Hz, 2 H), 1.7–1.4 (m, 5 H), 0.760 (d, *J* = 6.1 Hz, 6 H).

(*Z*)-2-Tridecen-1-ol (5). A mixture of 2-tridecyn-1-ol (**4**) (1.84 g, 9.41 mmol) and 5% palladium on $CaCO_3$ (220 mg) in 15 mL of dry pyridine was stirred vigorously under a continuous positive pressure atmosphere of H_2 until conversion of alkyne to alkene was complete (approximately 24 h as monitored by TLC using 10% $AgNO_3$ silica gel

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plates with 8:2 H–EA as eluant; alkynol, R_f = 0.40; alkenol, R_f = 0.34). The mixture was diluted with 50 mL of Et_2O, filtered through Florisil, and solvent evaporated, with residual pyridine removed on the vacuum pump to give crude (*Z*)-2-tridecen-1-ol (**5**), a clear liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.559 (dtt, *J* = 11.0, 6.3, 1.0 Hz, H-2), 5.507 (dtt, *J* = 11.0, 7.0, 1.0 Hz, H-3), 4.156 (d, *J* = 6.0 Hz, H-1), 2.035 (q, *J* = 6.9 Hz, H-4), 1.532 (br s, 1 H, OH), 1.232 (br s, 16 H), 0.851 (t, *J* = 6.8 Hz, 3 H). ^{13}C NMR ($CDCl_3$): δ 132.3 (C-3), 128.4 (C-2), 58.1 (C-1), 31.8 (C-11), 29.5 (C-7, C-8, C-9), 29.2 (C-5, C-6, C-10), 27.3 (C-4), 22.6 (C-12), 13.9 (C-13). The crude product was flash-chromatographed on silica gel with 8:2 H–EA to give 1.33 g (6.75 mmol, 71%) of product as a clear liquid, >99% pure by GC (10.57 min, 100, 2/10/250, DB-1701.

(+)-(2*S*,3*R*)-Epoxytridecan-1-ol [(+)-6].[3a] A flask containing 8 mL of CH_2Cl_2 was cooled to ca. −25 °C under argon and charged with titanium tetraisopropoxide (0.40 mL, 1.3 mmol), CaH_2 (4.90 mg, 0.116 mmol), silica gel (SiO_2; 7.53 mg, 0.125 mmol), and L-(+)-diethyl tartrate (DET; 0.26 mL, 1.5 mmol).[11] After the mixture was stirred for 10 min, (*Z*)-2-tridecen-1-ol (**5**) (251.0 mg, 1.265 mmol) was added, and the mixture was stirred an additional 10 min while it cooled to −40 °C. *tert*-Butyl hydroperoxide (TBHP) (0.9 M in CH_2Cl_2, 2.8 mL, 2.5 mmol, dried as a CH_2Cl_2 solution over $MgSO_4$) was added, and the reaction was stirred at −40 °C for 21 h. Aqueous 10% L-tartaric acid (3 mL) was added and the reaction stirred at −25 °C for 30 min and at room temperature for 15 min. The yellow aqueous layer was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), and solvent was removed to give a white solid. Et_2O (8 mL), and then 3 mL of 1 M LiOH, was added with stirring at 0 °C for 30 min. The aqueous layer was extracted with Et_2O (4 × 20 mL), and the combined organic layers were washed with saturated NaCl (3 × 20 mL), dried ($MgSO_4$), and solvent evaporated. The crude product so obtained was twice recrystallized from high-boiling ligroine to give 116 mg (0.541 mmol, 43%) of the desired epoxy alcohol (+)-**6** as a white solid, mp 61–63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.861 (ddd, *J* = 12.1, 7.4, 4.1 Hz, H-1a), 3.679 (ddd, *J* = 12.1, 6.9, 5.0 Hz, H-1b), 3.156 (dt, *J* = 6.9, 4.1 Hz, H-2), 3.034 (m, H-3), 1.7–1.5 (m, 3 H, OH and H-4), 1.4–1.2 (br s, 16 H), 0.87 (t, *J* = 6.7 Hz, 3 H). $[\alpha]^{24}_D$ = +7.8° (*c* = 1.28, EtOH). The epoxy alcohol (5 mg) was allowed to react with dry pyridine (8.6 mg) and an excess of the acid chloride of (*R*)-(+)-MTPA in 300 μL of CCl_4 overnight at room temperature to give the MTPA ester of the epoxy alcohol. After purification by flash chromatography, integration of the diastereomeric C-1 methylene proton resonances indicated that the product had ≥95% ee (cf. Figure 3).

(+)-(2*S*,3*R*)-Epoxytridecan-1-al [(+)-7].[3a] To a stirred solution of dry pyridine (303 mg, 3.83 mmol) in dry CH_2Cl_2 (4.7 mL) under N_2 was added CrO_3 (189 mg, 1.89 mmol). The solution was stirred at 0 °C for 1 h and then warmed to room temperature over 40 min, with the solution turning deep red during this time. A solution of the epoxy alcohol (+)-**6** (72.3 mg, 0.337 mmol in 0.5 mL of CH_2Cl_2) was added, and the solution immediately became black. The reaction was stirred 4.75 h at room temperature. The solution was decanted and the residue washed with dry Et_2O (3 × 10 mL). The combined organic layers were washed successively with ice-cold 5% NaOH (3 × 30 mL), ice-cold saturated $CuSO_4$ (1 × 30 mL), and saturated NaCl (1 × 30 mL) and dried ($MgSO_4$). Flash chromatography (8:2 H–EA) gave 43.3 mg (0.204 mmol, 61%) of the desired epoxy aldehyde (+)-**7**, a clear liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.479 (d, *J* = 5.0 Hz, H-1), 3.356 (t, *J* = 5.0 Hz, H-2), 3.278 (m, H-3), 1.8–1.4 (m, H-4), 1.4–1.2 (br s, 16 H), 0.896 (t, *J* = 6.6 Hz, 3 H).

2-Methyl-(7*R*,8*S*)-epoxyoctadec-5-ene [(−)-8]. A dry flask was charged with (4-methylpent-1-yl)triphenylphosphonium iodide (160 mg, 0.337 mmol), *gently* warmed under vacuum, and cooled under argon. Dry THF (3 mL) was added; the mixture was stirred at −78 °C for several minutes, and then 0.12 mL of *n*-BuLi in hexanes (1.87 M, 0.22 mmol) was added. The mixture was stirred at −78 °C for 45 min to 2 h, during which time the color of the solution went from pale yellow to deep orange. A solution of the (2*S*,3*R*)-epoxy aldehyde (+)-**7** (35.0 mg, 0.165 mmol) in 1 mL of dry THF was added and the reaction stirred 3 h, after which it was quenched by the addition of several milliliters of moist Et_2O and 1 mL of H_2O. The aqueous layer was extracted once with 3 mL of Et_2O, and the combined organic layers were dried ($MgSO_4$), concentrated, and flash-chromatographed (95:5 H–EA) to give 38.0 mg (0.135 mmol, 82%) of the desired epoxy alkene (−)-**8** (GC, 5*E* = 14.48 min, 19%; 5*Z* = 15.33 min, 81%, 120, 4/10/250, DB-1701) as a clear liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.730 (dtd, *J* = 11.1, 7.6, 0.8 Hz, H-5), 5.176 (ddt, *J* = 11.0, 8.4, 1.5 Hz, H-6), 3.618 (ddd, *J* = 8.4, 4.3, 0.8 Hz, H-7), 3.053 (m, H-8), 2.186 (m, H-4), 1.7–1.4 (m, 3 H), 1.4–1.2 (br s, 18 H), 0.90–0.85 (m, 9 H).

(−)-2-Methyl-(7*R*,8*S*)-epoxyoctadecane [(−)-Disparlure] [(−)-3]. A vigorously stirred solution of Wilkinson's catalyst [$(Ph_3P)_3RhCl$; 21.1 mg, 22.8 μmol) in 1.0 mL of benzene was degassed as follows. The flask was evacuated (vacuum pump) until the initial vigorous bubbling slowed down; H_2 at atmospheric pressure was then admitted. This procedure was repated twice more, and then the solution was stirred under a H_2 atmosphere until the solution color, originally deep red, changed to yellow-orange (approximately 60–90 min). The color change indicated the successful formation of reduced Wilkinson's catalyst, $(Ph_3P)_3RhCl{\cdot}H_2$. (When the solution was not degassed, the color change was not observed, even after 3–6 h.) After the color change (30 min), the (7*R*,8*S*)-epoxy alkene (−)-**8** (4.9 mg, 18 μmol) in 0.8 mL of benzene was added and the reaction was stirred (still under H_2) for 3 h. Hexane (5 mL) was added to precipitate the rhodium reagent. The solid was resuspended and the reaction mixture filtered through Florisil to give a clear solution. Flash chromatography (hexane; 95:5 H–EA) gave 2.5 mg (50%) of (−)-disparlure (−)-**3**, spectroscopically identical with the (±)-**3** material obtained above, except for the presence of a small peak at δ 2.36 in the ^{1}H NMR. This peak was later determined to be 2-methyloctadecan-8-one (**10**), arising from the rhodium-induced rearrangement of the alkenyl oxirane **7** to 2-methyloctadec-6-en-8-one (**9**).[3a,20] Under the reaction conditions, this material was also hydrogenated, and it comigrated on TLC and flash columns with the (−)-disparlure. This material was found to be present in both the (+)-**3** and the (−)-**3** isomers in ca. 10–20 mol %. It was easily removed by reduction with 2–3 mg of sodium borohydride in 1 mL of ethanol at room temperature, followed by flash chromatography.

(+)-2-Methyl-(7*S*,8*R*)-epoxyoctadecane [(+)-Disparlure] [(+)-3]. Starting with the allylic alcohol **5**, the asymmetric epoxidation was conducted with (−)-DET to give epoxy alcohol (−)-**6**, which was oxidized and olefinated to give alkenyl oxirane (+)-**8** following the procedures detailed above. Hydrogenation and borohydride reduction as described above gave (+)-**3**.

(11*R*,12*S*)-Epoxydocos-9-ene (15). A sample of epoxy aldehyde (−)-**7** with 65% ee was converted to an unbranched analogue of disparlure by olefination with nonylidene triphenylphosphorane following the procedures above. Thus, a solution of 75.0 mg of (−)-**7** (0.353 mmol) in THF was added to the ylid formed from 507 mg (0.98 mmol) of nonyl triphenylphosphonium iodide and 0.35 mL of 2 M *n*-BuLi (0.7 mmol) in THF. After 2 h at −78 °C, workup as described above afforded 85.6 mg (0.265 mmol, 75%) of the desired predominantly (*Z*)-alkenyl oxirane **15**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.751 (dtd, *J* = 11.2, 7.6, 0.9 Hz, H-9), 5.204 (ddt, *J* = 11.2, 8.3, 1.5 Hz, H-10), 3.620 (ddd, *J* = 8.3, 4.3, 0.9 Hz, H-11), 3.051 (m, H-12), 2.196 (m, H-8), 1.5–2.2 (m, br s, 30 H), 0.88 (br t, 6 H).

***meso*-11,12-Epoxydocosane (16).** Homogeneous hydrogenation of epoxyalkene **15** (6.7 mg, 21 μmol) with prereduced Wilkinson's catalyst (26.9 mg, 29.1 μmol) in benzene (1 mL) and workup as usual afforded the *meso*-epoxide **16** in 63% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.883 (m, H-11, H-12), 1.48 (br s, H-10, H-13), 1.24 (br s, 32 H), 0.862 (t, *J* = 6.6 Hz, 6 H). As with the (+)-**3** and (−)-**3** cases, the rhodium-catalyzed hydrogenation of (+)-**15** led to the formation of a small amount of the corresponding ketone, 11-docosanone (δ 2.359, t, *J* =7.4 Hz, methylene protons α to carbonyl), which could be removed by borohydride reduction and flash chromatography as described above.

Epoxide Ring Opening Reactions. *threo*-(±)-2-Methyl-7,8-octadecanediol (13a) (by Basic Hydration). A stirred solution of *cis*-(±)-2-methyl-7,8-epoxyoctadecane [3.5 mg, 12.3 μmol, 98:2 cis–trans by GC (DB-5 capillary)] and aqueous 50% (w/w) NaOH (50 μL, ca. 15 M, 750 μmol) in 0.5 mL of 1-butanol in a thick-walled screw-cap vial was heated at 120 °C for 3 days. The reaction mixture was diluted with 1 mL of H_2O and extracted with 9:1 H–EA (4 × 1 mL), and the combined H–EA extracts were flash-chromatographed to give a 10% yield of the desired *threo*-diol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.382 (br, H-7, H-8), 1.82 (br, 2 H, OH), 1.5–1.4 (m, 5 H), 1.24 (br s, 22 H), 0.88–0.84 (m, 9 H). No erythro isomer (δ 3.58) was detected by ^{1}H NMR. This was the only successful attempt at opening the epoxide ring under basic conditions. The epoxide ring remained intact when milder conditions (i.e., ethanol or ethylene glycol as solvent at lower temperatures and lower hydroxide concentrations) were employed.

Mixture of *threo*-(±)-7-Hydroxy-2-methyloctadec-8-yl Acetate, *threo*-(±)-8-Hydroxy-2-methyloctadec-7-yl Acetate (13b), and *threo*-(±)-2-Methyl-7,8-octadecanediol (13a) (Lewis Acid Catalyzed Hydration). In a glovebag, ca. 700 mg of alumina (Activity super I) was transferred to a clean, dry screw-cap vial, after which 0.8 mL of dry Et_2O was added and the slurry stirred for 5 min. Dry acetic acid (40 μL, 42 mg, 0.7 mmol, 5% (w/w) based on Al_2O_3) was added, the slurry was stirred for 5 min, and a solution of 4.0 mg (18 μmol) of *cis*-(±)-2-methyl-7,8-epoxyoctadecane (98:2 cis–trans) in 0.5 mL of dry Et_2O was added. After stirring for 3 days, the reaction was diluted with Et_2O and

(20) Sato, S.; Matsuda, I.; Izumi, Y. *Tetrahedron Lett.* **1985**, *26*, 1527.

the solution filtered through a small plug of silica gel. The remaining slurry was washed with Et_2O (3 × 2 mL) and each wash filtered as above. The combined fitlrates were evaporated and flash-chromatographed (95:5, 90:10, 80:20, 60:40 H:EA) to give 0.7 mg (2.3 μmol, 16%) of the *threo*-(±)-diol (**13a**), 1.7 mg (5 μmol, 35%) of the desired *threo*-(±)- C-7 and C-8 monoacetates (**13b**), and 1.4 mg (5 μmol, 35%) of recovered starting material. For *threo*-(±)-diol (**13a**), ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.38 (br, H-7, H-8), 1.93 (br, 2 H, OH), 1.514 (m, 5 H), 1.4–1.1 (br s, 22 H), 0.88–0.84 (m, 9 H). For *threo*-(±)- C-7 and C-8 monoacetates (**13b**), ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.804 (td, J = 6.6, 3.9 Hz, 1 H, $RCH_2CH(OH)$–$CH(OAc)CH_2R$), 3.561 (m, 1 H, $RCH_2CH(OH)CH(OAc)CH_2R$), 2.074 (s, 3 H, acetate CH_3), 1.7–1.5 (m, 3 H, $RCH_2CH(OH)CH(OAc)CH_2R$ and OH), 1.5–1.3 (m, 3 H, $RCH_2CH(OH)CH(OAc)CH_2R$ and H-2), 1.3–1.2 (br s, 22H), 0.88–0.83 (m, 9 H). Neither the 300 MHz ^{1}H NMR nor GC (DB-5 capillary) were able to resolve the C-7 and C-8 acetate regioisomers. The regioisomeric monoacetates could be saponified (aqueous 1.2 M NaOH in EtOH) to give *threo*-diol identical with that mentioned above.

***erythro*-(±)-7-Hydroxy-2-methyloctadec-8-yl Acetate and *erythro*-(±)-8-Hydroxy-2-methyloctadec-7-yl Acetate (14b) (Lewis Acid Catalyzed Hydration).** To determine whether *trans*-(±)-2-methyl-7,8-epoxyoctadecane (**12**) could be converted to the *erythro*-monoacetate **14b**, the following experiments were performed. Alumina (730 mg), Et_2O (1.2 mL), acetic acid (40 μL, 42 mg, 0.7 mmol), and *trans*-epoxide **12** (5.3 mg, 19 μmol, 12:88 cis–trans; added in 0.6 mL of Et_2O) were combined as described for the *threo*-monoacetate **13b**. After the mixture was stirred for 29 h at room temperature, neither monoacetate **13b** nor **14b** was detected by capillary GC. That the *trans*-epoxide was resistant to ring opening by the acetic acid–alumina system was demonstrated by adding (±)-*cis*-epoxide **3** (4.5 mg, 16 μmol, 90:10 cis–trans, added neat using a glass micropipet) directly to the *trans*-epoxide-containing reaction mixture. Only after the addition of the *cis*-epoxide was any monoacetate detected [3% monoacetate **13b** detectable 21 h after the *cis*-epoxide addition, as determined by capillary GC (*trans*-epoxide **12** 19.29 min; *cis*-epoxide **3**, 19.43 min; *threo*-monoacetate **13b**, 23.47 min; 50, 1/10/220), DB-1]. This result indicated that while the acetic acid–alumina system did not open the oxirane of *trans*-epoxide **12** at a measurable rate, it could open the oxirane ring of *cis*-epoxide **3**. Additional confirmation of this result was obtained from a repeat of *cis*-epoxide **3** to *threo*-monoacetate **13b** experiment. The monoacetates thus obtained were converted to diacetates as described below. Only one peak was observed in the GC (16.00 min, 180, 1/5/300, DB-5), which corresponded to *threo*-diacetate **13c** (the retention time of *erythro*-diacetate **14c** was 15.75 min under these conditions). Also, the starting epoxide, originally a 90:10 cis–trans mixture, was found to be 86:14 cis–trans (GC, *trans*-epoxide **12**, 11.16 min; *cis*-epoxide **3**, 11.43 min; 180, 1/5/300, DB-5) at the end of the reaction. This selective depletion of the *cis*-epoxide (or enrichment of the trans) again indicated that the *cis*-epoxide was opened by the acetic acid–alumina system but that the *trans*-epoxide was not.

***threo*-(±)-2-Methyl-7,8-octadecanediol (13a) (Acid Catalyzed Hydration).** A solution of *cis*-(±)-2-methyl-7,8-epoxyoctadecane (6.4 mg, 22.6 μmol, 90:10 cis–trans, DB-5 capillary) and 3% (w/w) aqueous $HClO_4$ (50 μL) in 0.5 mL of 1,4-dioxane in a screw-cap vial was stirred at room temperature for 24 h. The reaction was neuralized with 2 drops of 1.2 M aqueous NaOH and extracted with 9:1 H–EA (3 × 1 mL), and the combined H–EA extracts were dried ($MgSO_4$) and flash-chromatographed to give 4.6 mg (15.3 μmol, 68%) of the diol, a white solid, in an approximate 85:15 threo–erythro mixture (based on ^{1}H NMR), mp 53–55 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.58 (m, H-7 and H-8, erythro isomer **14a**), 3.38 (m, 2 H, threo isomer **13a**, H-7 and H-8), 1.92 (br s, 2 H, exchangeable with D_2O), 1.6–1.4 (m, 5 H), 1.4–1.2 (br s, 22 H), 0.86–0.84 (m, 9 H).

***erythro*-(±)-2-Methyl-7,8-octadecanediol (14a) (Acid Catalyzed Hydration).** *trans*-(±)-2-Methyl-7,8-epoxyoctadecane (**12**) (6.2 mg, 88:12 trans–cis, DB-5 capillary) was hydrated as above to give 5.2 mg (79%) of the diol, a white solid, in an approximate 85:15 erythro–threo ratio (based on ^{1}H NMR), mp 82–84 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.58 (m, 2 H, erythro isomer **14a**, H-7 and H-8), 3.38 (m, 2 H, threo isomer **13a**), 1.94 (br s, threo isomer **13a**, exchangeable with D_2O), 1.775 (d, J = 4.6 Hz, 2 H, erythro isomer **14a**, exchangeable with D_2O), 1.6–1.4 (m, 5 H), 1.4–1.2 (br s, 22 H), 0.86–0.84 (m, 9 H). The diol GC trace (DB-5 column) showed nonsymmetric peaks and potentially inaccurate integration. In general, the exact erythro–threo ratio is best determined by the *n*-butylboronates **13e** and **14e** and the diacetates **13c** and **14c**.

Diacetate 13c from Monoacetate Mixture 13b. A solution of the *threo*-(±)- C-7 and C-8 monoacetates **13b** (4 mg, 12 μmol, produced from the 98:2 *cis*-epoxide–*trans*-epoxide mixture), acetic anhydride (162 μL, 1.7 μmol), and 4-(dimethylamino)pyridine (DMAP; 9 mg, 74 μmol) in 1 mL of dry pyridine in a screw-cap vial was stirred for 3 h at room temperature. The reaction was diluted with 1 mL of 8:2 H–EA and washed with saturated $CuSO_4$ (3 × 1 mL) to remove pyridine, and the H–EA was evaporated. The crude product so obtained was flash-chromatographed (95:5, 90:10, 80:20 H–EA) to give *threo*-(±)-2-methyl-7,8-octadecyl diacetate **13c** in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.975 (m, H-7, H-8), 2.060 (s, 6 H, acetate methyls); 1.483 (m, 5 H), 1.229 (br s, 22 H), 0.88–0.82 (t overlapped with d, J = 6.6 Hz, 9 H). GC analysis showed only the *threo*-diacetate **13c** (18.52 min, 120, 4/10/250, DB-1701).

***erythro*-Diacetate 14c from *erythro*-Diol 14a.** A solution of the *erythro*-diol **14a** (ca. 0.5 mg, produced from the 88:12 *trans*-epoxide–*cis*-epoxide **12**) was treated as described above for diol **13a**. Analysis by GC showed the two sharp diacetate peaks with base-line separation in an 88:12 erythro–threo ratio, which agrees with the 88:12 trans–cis ratio found in the starting epoxide **12**. GC (180, 1/5/300, DB-5): 15.76 min (88.4%, **14c**), 16.02 min (11.6%, **13c**).

***threo*-Diol 13a as Its Bis(trimethylsilyl) Ether 13d.** To *threo*-(±)-2-methyl-7,8-octadecanediol (**13a**; 0.54 mg, 1.8 μmol) in a screw-cap vial was added 0.5 mL of Pierce Tri-Sil BSA Formula D (2.5 mequiv/mL of *N,O*-bis(trimethylsilyl)acetamide in DMF). GC monitoring showed the reaction to be complete in <4 h at room temperature, giving *threo*-(±)-bis[(trimethylsilyl)oxy]-2-methyl-7,8-octadecane (**13d**). GC (120, 4/10/250, DB-1701): 15.86 min (100%). GC–MS (*m/z*, (relative abundance)): No M^+; 243 (59%, $Me_3SiO^+{=}CHC_{10}H_{21}$); 201 (45%, $Me_3SiO^+{=}CHC_7H_{15}$); 185 (9%); 147 (35%); 129 (15%); 111 (28%); 103 (25%); 97 (17%); 83 (17%); 83 (17%); 75 (19%); 73 (100%, Me_3Si^+); 69 (42%); 55 (10%); 41 (3%).

***threo*-Diol 13a as Its *n*-Butylboronate 13e.** A solution of (±)-*threo*-2-methyl-7,8-octadecanediol **13a** (1.0 mg, 3.3 μmol, an approximate 85:15 threo–erythro mix based on ^{1}H NMR) and *n*-butylboronic acid (0.3 mg, 3 μmol) in 100 μL of dry DMF was allowed to react for 12 h at room temperature, giving *threo*-(±)-2-methyl-7,8-octadecyl *n*-butylboronate (**13e**). GC (120, 4/10/250, DB-1701): 17.50 min (85.2%, **13e**), 17.73 min (14.8%, **14e**). GC–MS (*m/z*, (relative abundance)): 366 (0.6%, M^+); 309 (2.8%, $M - C_4H_9$); 267 (40%, $M - C_7H_{15}$); 225 (37%, $M - C_{10}H_{21}$).

***erythro*-Diol 14a as Its *n*-Butylboronate (14e).** A solution of (±)-*erythro*-2-methyl-7,8-octadecanediol **14a** (1.0 mg, 3.3 μmol, an approximate 15:85 threo–erythro mixture based on ^{1}H NMR) and *n*-butylboronic acid (0.3 mg, 3 μmol) in 100 μL of dry DMF was allowed to react for 12 h at room temperature, giving *erythro*-(±)-2-methyl-7,8-octadecyl *n*-butylboronate (**14e**). GC (120, 4/10/250, DB-1701): 17.54 min (15.2%, **13e**), 17.81 min (84.8%, **14e**).

Radiosynthetic Procedures. [7,8-$^3H_2$]-(*Z*)-2-Methyl-7-octadecene (2). Semitritiation of 2-methyl-7-octadecyne (**1**) was performed at the National Tritium Labeling Facility by modification of the procedure of Sheads and Beroza.[9] Thus, a solution of 20 mg (0.076 mmol) of alkyne **1** in 2 mL of dry THF containing 2.0 mg of quinoline and 8.5 mg of 5% $Pd/BaSO_4$ was freeze–thaw–degassed on the vacuum line three times with nitrogen flushing. After the final cycle, 740 Torr of carrier-free tritium gas was introduced, and the reaction was stirred vigorously at 20 °C for 1 h. The reaction was frozen with liquid N_2, reduced in volume in vacuo, filtered through a glass fiber filter, and lyophilized. The crude product was chromatographed with a disposable pipet half-filled with 37–75-μm silica gel and eluted with hexane to give homogeneous [7,8-$^3H_2$]alkene **2** (R_f = 0.69 for 95:5 H–EA) in quantitative chemical yield and a total of 3.69 Ci with calculated specific activity 49 Ci/mmol. The labeled material was transported to Stony Brook while frozen in benzene. However, to minimize radiolytic decomposition, this and all subsequently described radiolabeled materials were diluted to between 10 and 500 mCi/mL in a 1:1 solution of toluene and heptane.[1d] This can be stored below −20 °C without freezing.

[7,8-$^3H_2$]-(±)-*cis*-7,8-Epoxy-2-methyloctadecane ([7,8-$^3H_2$]-(±)-Disparlure, [7,8-$^3H_2$]-(±)-3). To a solution of 2.6 mg (222 mCi, 9.7 μmol) of [7,8-$^3H_2$]-**2** in 1 mL of dry CH_2Cl_2 was added 5.0 mg of technical grade *m*-CPBA. After the solution was stirred at room temperature for 2 h, the reaction was reduced to dryness, taken up in hexane, and chromatographed as described for labeled **2**. Homogeneous labeled disparlure [7,8-$^3H_2$]-(±)-**3** was eluted in two fractions with 2.5% ethyl acetate in hexane (R_f = 0.26 for 95:5 H–EA) and was stored at −20 °C in toluene–heptane at ca. 10 mCi/mL.

[5,6-$^3H_2$]-(+)-(7*R*,8*S*)-7,8-Epoxy-2-methyloctadecane ([5,6-$^3H_2$]-(+)-Disparlure, [5,6-$^3H_2$]-(+)-3). The procedure of Prestwich and Wawrzenczyk[12] for the tritiation of a vinyl oxirane to give labeled juvenile hormones was followed. Thus, a solution of 24 mg (25.9 μmol) of $(Ph_3P)_3RhCl$ in 0.8 mL of dry benzene was freeze–thaw–degassed three times and then prereduced by stirring for 2 h at room temperature under 740 Torr of carrier-free tritium. A solution of 5.7 mg (20.4 μmol) of alkenyl oxirane (+)-**8** in 0.8 mL of benzene was added via syringe, and stirring was continued for 3 h. The reaction was quenched by freezing

and concentrated to near dryness in vacuo, and then hexane was added to precipitate the catalyst. After filtration and lyophilization, the crude product was flash-chromatographed with hexane and eluted with 2.5% ethyl acetate in hexane to give ca. 3.4 mg of [5,6-$^3H_2$]-(+)-**3** in two fractions at a nominal specific activity of 58 Ci/mmol (calculated at 62 ± 10 Ci/mmol). Prior to use in enzyme assays, the chromatographed product was stirred with an excess of $NaBH_4$ in ethanol for 24 h at room temperature to reduce ketonic rearrangement products. Evaporation to dryness and rechromatography of the residue gave homogeneous labeled epoxide and 10–20% of the label in a new spot that comigrated with 2-methyloctadecan-8-ol.

[5,6-$^3H_2$]-(−)-(7*S*,8*R*)-7,8-Epoxy-2-methyloctadecane ([5,6-$^3H_2$]-(−)-Disparlure, [5,6-$^3H_2$]-(−)-3). The alkenyl oxirane (−)-(**8**) (5 mg, 18 μmol) was tritiated as described above for its enantiomer to give ca. 3.0 mg of labeled [5,6-$^3H_2$]-(−)-**3**. Removal of the ketonic impurities was performed prior to use for enzyme assays.

[9,10-$^3H_2$]-(11*R*,12*S*)-11,12-Epoxydocosane ([9,10-$^3H_2$]-16). The alkenyl oxirane **15** was tritiated as described above for (+)-**8** to give labeled [9,10-$^3H_2$]-**16**. Removal of the ketonic impurities was performed prior to use for enzyme assays.

Metabolic Studies in Vitro. Enzyme Preparation. Enzyme used in this study was obtained from homogenization of male and female antennae and legs. Additional information of the tissue specificity, subcellular localization, and kinetics of this enzyme will be presented elsewhere. Gypsy moth larvae were obtained from Dr. C. Schwalbe, USDA, Otis Air Force Base, MA, and allowed to pupate in a long-day photoperiod at 26 °C. Pupae were sexed and allowed to emerge (ca. 2 weeks) in separate containers. In a typical time-course experiment, 24 antennae from newly emerged (day 0 to day 1) male gypsy moths (*L. dispar*) were homogenized in 1.2 mL of 10 mM Tris·HCl buffer (pH = 7.0) with a hand-operated, ground-glass tissue homogenizer cooled in an ice bath. The homogenate was centrifuged at 12000*g* for 10 min, and the supernatant was used immediately or stored at −80 °C prior to use. The final concentration in the time-course assay was 2 antennal equiv/100 μL of assay.

***threo*-[5,6-$^3H_2$]-2-Methyl-7,8-octadecanediol.** A homogenate of 50 male antennae in 2.5 mL of 10 mM Tris·HCl buffer (pH = 7.0) was prepared as described above. To this was added ca. 25–30 μCi of (+)-[5,6-$^3H_2$]-disparlure in 100 μL of ethanol. This mixture was stirred for 80 min and extracted with 8:2 H–EA (3 × 5 mL), and the combined extracts were evaporated under a stream of N_2. The crude extract was dissolved in 95:5 H–EA and flash-chromatographed (95:5, 90:10, 80:20, 60:40 H–EA) to give 14 μCi of recovered epoxide and 14 μCi of a lower R_f material than comigrated with standard *threo*-(±)-2-methyl-7,8-octadecanediol on TLC. The metabolite was homogeneous by RTLCS, although this technique was insufficient to detect low levels (<5–10%) of the *erythro*-diol, if present. Next, 3 μCi of the metabolite was acetylated with Ac_2O–DMAP–pyridine (162 μL:7.6 mg:1.0 mL) as described for *threo*-diol **13a** to give 3 μCi of RTLCS homogeneous material that comigrated with standard *threo*-(+)-2-methyl-7,8-octadecyl diacetate. On the basis of these results, the metabolite is the *threo*-diol of as yet unknown optical purity.

Hydration at High Disparlure Concentrations. Method A. Without ^3H-Labeled Disparlure as Tracer. A 100-mL sample containing 100 male *L. dispar* antennae homogenized in 10 mM Tris-HCl buffer (pH = 7.0) was treated with 0.5 mL of an ethanolic solution of 8.0 mg of racemic disparlure **3**, and the mixture was stirred for 24 h at 23 °C. The mixture was extracted with three 50-mL portions of EtOAc, and the combined extracts were concentrated in vacuo, redissolved in 95:5 H–EA, and flash-chromatographed. The diol-containing fractions (1.6 mg) were concentrated, and half the material was redissolved in 200 μL of ethanol and 100 μL of methanol, followed by treatment with 50 μL of 1.2 M NaOH (16 h, 23 °C). The hydrolysis mixture was diluted with water (250 μL) and extracted with three 1-mL portions of 8:2 H–EA. The organic layers were concentrated and rechromatographed (95:5–80:20 H–EA). The diol-containing fractions (0.39 mg) were treated with 0.5 mL of Pierce Tri-Sil BSA Formula D (3.4 h, 20 °C) and analyzed by capillary GC (120, +/10/250, DB-1701). The bis(trimethylsilyl) ether **13d** (retention time 15.88 min) of the enzymatically produced diol **13a** cochromatographed in the GC with the authentic **13d** (retention time 15.86 min) synthesized as described above. In addition, the characteristic *m/z* 243 and 201 ions were present in the GC–MS of the biologically derived sample.

Method B. With ^3H-Labeled Disparlure as Tracer. A 5-mL sample containing 100 antennae was cooled on ice while 100 μL of a 0.50 mM solution (50 nmol, 14000 ng) of 100% cis (±)-**3** was added, with vortex mixing, to give a final concentration of 10 μM in the incubation mixture. Then, 2 μL of a 1 μCi/μL solution of [7,8-$^3H_2$]-(±)-**3** was added as a tracer. The mixture was incubated at 23 °C for 4 h (ca. 60% conversion as determined by cutting and liquid scintillation counting of TLC plates), and the products were extracted with several portions of 80:20 H–EA. The combined organic extracts were dried over $MgSO_4$ and then *distilled* to dryness. The product was then dissolved in 100 μL of 95:5 H–EA, and 50 μL was spotted on a TLC plate. The plate was developed twice in 80:20 H–EA and autoradiography performed. Neither the 5-h nor the 20-h exposure showed any of the *erythro*-diol (Figure 8). On this basis the *erythro*-diol cannot constitute more than 0.5% of the diol metabolite.

Time Course of Hydration of Disparlure Enantiomers and the Meso Analogue. A 300-μL sample of homogenate (6 antennal equiv) was added to each of four test tubes in an ice bath. Then, a 3-μCi aliquot of each radiolabeled substrate at the same concentration of 1.0 μCi/μL in ethanol was added to each tube to give final concentrations of 10 μCi/mL. The molar concentrations were calculated with a specific activity of 49 Ci/mmol for the racemic disparlure and 58 Ci/mmol for the three homogeneously tritiated compounds, giving a final concentration of 200 nM in the incubation mixture for each of the four compounds. To avoid any ambiguities in relative rates of hydrolysis for compounds tritiated in different positions, the racemic disparlure used for time-course studies (Figure 7A) was prepared by mixing equal portions (determined by radio LSC) of the labeled (+)- and (−)-disparlure samples. A 50-μL aliquot (time 0) was removed after vortex mixing at 0 °C, and then the samples were each incubated at 26 °C. Aliquots (50 μL) were removed at t = 10, 20, 40, and 80 min. Each aliquot was vigorously mixed with 50 μL of ethyl acetate, and 3 μL of each organic layer was spotted in duplicate on a TLC plate prescored into three lanes and prespotted with unlabeled epoxide and diol as markers. After development in 80:20 H–EA, the epoxide and diol zones were cut out and counted on the liquid scintillation counter. Thus, the percent hydration of epoxide as a function of time was obtained, and these data are shown in Figure 7A.

Time Course of Hydration of (+)-Disparlure. Tissue Specificity. Tissue homogenates were prepared as described above with male antennae, female antennae, male front legs, and female front legs. A 300-μL sample of homogenate (6 antennal or leg equiv) was added to each of four test tubes in an ice bath, followed by 3 μL of 1 μCi/μL labeled (+)-**3** in ethanol. Aliquots were removed, quenched, and analyzed as in the time-course experiment, and these data are shown in Figure 7B.

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