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Synthesis and Absolute Configuration of Desmarestene, the Gamete-Releasing and Gamete-Attracting Pheromone of the Brown Algae *Desmarestia aculeata* and *D. firma* (Phaeophyceae).

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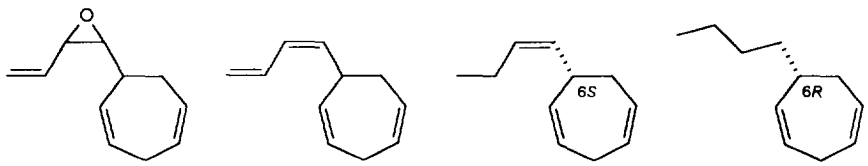
Abstract: The absolute configuration of the gamete-releasing and gamete-attracting pheromone desmarestene **2** from the North Atlantic brown alga *Desmarestia aculeata* is determined as (6*R*) by synthesis and biological activity tests. Utilising the *cis-endo* transition state geometry of the Cope rearrangement of 1,2-*cis*-vinylalkenylcyclopropanes, a non-stereoselective Wittig approach with a single chiral precursor providing (*E*)- and (*Z*)-**12**, gives access to both enantiomers of **2**. According to GLC on a chiral stationary phase calling females of *Desmarestia aculeata* and *Cladostephus spongiosus* release (6*R*)-**2** with high enantiomeric purity (87% *e.e.* and 96% *e.e.* respectively) while female gametes of the South American *D. firma* secrete (6*R*)-**2** with only 28% *e.e.*.

Introduction. Fertile female gametes of many marine brown algae attract conspecific males by chemical signals.[1,2] In the highly evolved orders Laminariales, Desmarestiales, and Sporochnales the sexual pheromones first induce spermatozoid release from antheridia prior to attraction and fertilisation.[3,4] This release „on demand" gives rise to perfect synchronisation between the dioecious female and male plants, and has the additional advantage of providing an immediate pheromone gradient leading to the signalling female. The mass release is an impressive phenomenon and represents one of the fastest signal/response actions known in the plant kingdom. For example, the release of male gametes from the macroalga *Laminaria digitata* is triggered by lamoxirene **1** down to a threshold of *ca.* 50 pmol and occurs within 8-12 seconds after application of the signal.[5]



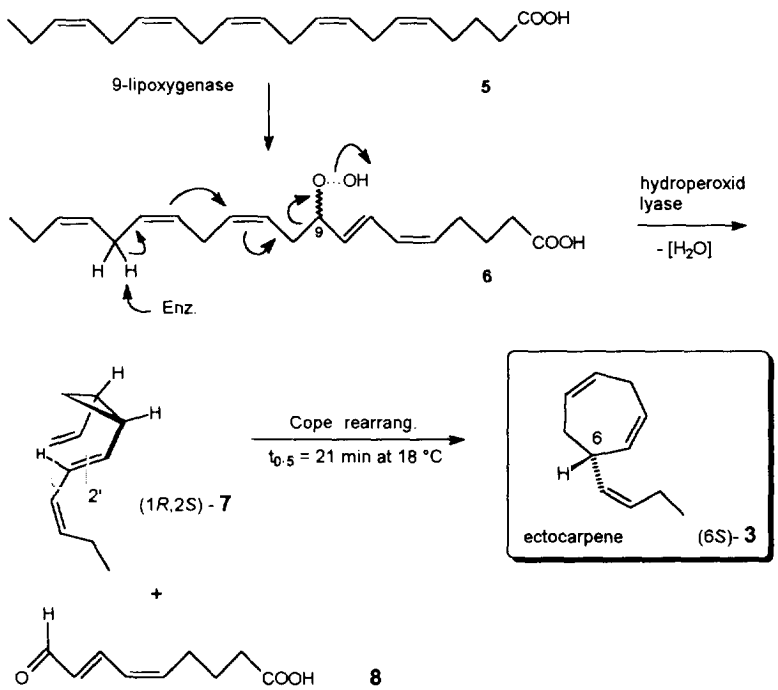
lamoxirene **1** desmarestene **2** ectocarpene **3** dictyotene **4**

Male gametes of the North Atlantic algae *Desmarestia aculeata* and *D. viridis* and *D. firma* from South America (Chile) respond in a similar fashion to the non-oxygen-functionalised hydrocarbon desmarestene **2**.[6] While the configuration of the related chemoattractants ectocarpene **3** and dictyotene **4** is known,[3,4] to date no at-

tempt has been made to synthesise and characterise the biological activity of the configurational isomers of desmarestene **2** and lamoxirene **1**.

The whole series of known algal pheromones are derived from unsaturated eicosanoids.[7,8] In the case of ectocarpene (6*S*)-**3**, *cis*-eicosa-5,8,11,14,17-pentaenoic acid **5** is oxidatively degraded, presumably via the 9-hydroperoxy acid **6**, to the unstable vinylalkenylcyclopropane (1*R*,2*S*)-**7** which spontaneously rearranges to (6*S*)-**3**.[9] Since the reaction proceeds exclusively via *cis-endo* transition state geometry as shown in Scheme 1, it is the stereochemistry at C-2′ of the hexadienyl side chain which determines the absolute configuration of ectocarpene (6*S*)-**3**.

Scheme 1.

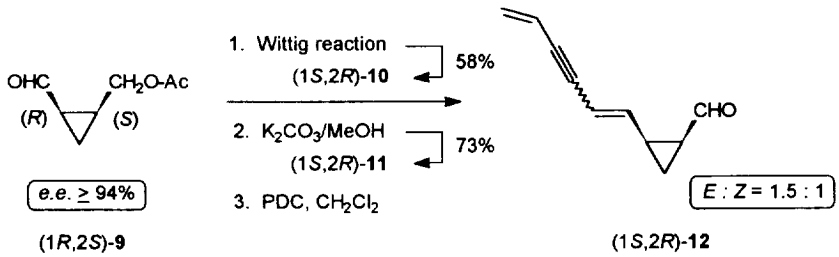


Here we describe a brief and efficient synthetic route to both enantiomers of desmarestene **2** which utilises the peculiar feature of the Cope rearrangement to convert the C-2′ stereoisomers of a disubstituted *cis*-vinylalkenylcyclopropane of type **7** specifically into the corresponding enantiomer of a substituted cyclohepta-1,4-diene.[10,11]

Synthesis. A useful precursor of *cis*-vinylalkenylcyclopropanes is the aldehyde (1*R*,2*S*)-**9** (Scheme 2), which is readily available in large quantities, and high *e.e.* (>94%), from *meso*-cyclopropanedimethanol[12] by enzymatic esterification with porcine pancreas lipase (PPL) in vinyl acetate.[13,14] Swern oxidation of the resulting monoacetate provides (1*R*,2*S*)-**9** in an overall yield of 80%. If required, the antipode, (1*S*,2*R*)-**9** (>98% e.e.), can be prepared from *meso cis*-1,2-bis(butyryloxy)cyclopropane using PPL in the hydrolytic mode.[11] Wittig

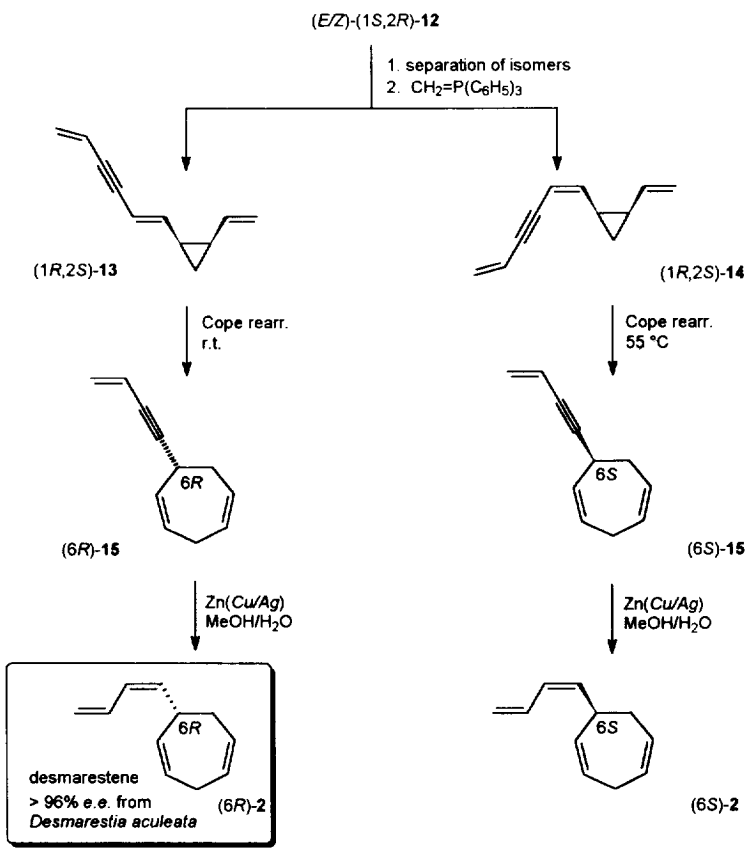
reaction of (1*R*,2*S*)-**9** with pent-4-en-2-ynylidenetriphenylphosphorane[15] completes the C_6 side chain in a single operation (58% yield) and introduces the new double bond, as intended, in a non-stereoselective fashion (*E*:*Z* = 1.5:1). Removal of the acetate is readily achieved by stirring with MeOH/K_2CO_3.

Scheme 2.



Oxidation of the resulting alcohol (1*S*,2*R*)-**11** with pyridinium dichromate yields the mixture of the two isomeric aldehydes (1'*E*)- and (1'*Z*)-(1*S*,2*R*)-**12** which are readily separated on silica gel using a pentane/ether gradient. The isomeric acetates (1*S*,2*R*)-**10** and the alcohols (1*S*,2*R*)-**11** fail to separate by chromatography on silica gel.

Scheme 3.



Following separation, the two isomeric aldehydes (2'*E*)- and (2'*Z*)-**12** were treated with methylidenetriphenylphosphorane. The vinylalkenylcyclopropane (1*R*,2*S*)-**13**, resulting from (1'*E*)-**12** is unstable at r.t. and immediately rearranges to (6*R*)-**15**. In the case of (1*R*,2S)-**14** the *cis-endo* transition state suffers from unfavorite steric interactions between the methylene hydrogens of the cyclopropane and the acetylenic moiety, however, increasing the temp. to 55 °C completes the Cope rearrangement of (1*R*,2*S*)-**14** within 1 hour. According to gas chromatography on 6-*O*-methyl-2,3-*O*-pentyl-γ-cyclodextrin and optical rotation data, both, (6*R*)-**15** and (6*S*)-**15**, are of high enantiomeric purity (≥ 94% *e.e.*) suggesting that in both cases the rearrangement proceeds in a highly concerted manner. Final reduction of the triple bond is achieved without difficulty by stirring of (6*S*)- and (6*R*)-**15** with Zn(*Cu/Ag*)[16,17] in aq. methanol.

Kinetics. The Cope rearrangement of (1*R*,2*S*)-**14** to (6*S*)-**15** was studied at different temperatures in a thermostatized cuvette by UV-spectroscopy. Owing to the irreversible formation of the cycloheptadiene (6*S*)-**15** the [3.3]-sigmatropic rearrangement is easily followed by the disappearance of the dienyne absorption at 254 nm. The kinetic measurements were performed as solutions in *n*-undecane, and the data were analysed according to a first-order kinetics. Figure 1 shows the *Arrhenius* plot for the rearrangement (1*R*,2*S*)-**14** → (6*S*)-**15** in the range of 20 °C to 70 °C.

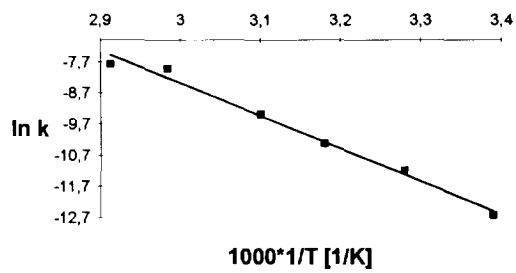


Figure 1. *Arrhenius* plot for the [3.3]-sigmatropic rearrangement (1*R*,2*S*)-**14** to (6*S*)-**15** in the range of 20 °C to 70 °C.

The activation parameters (Ea = 87.2 ± 1.2 KJ mol^{-1}, $\Delta S^{\neq}$ = -52.9 ± 4.1 J mol^{-1} K^{-1}) calculated from the *Arrhenius* plot compare well to those for the previously reported rearrangement of divinylcyclopropane to cyclohepta-1,4-diene.[18,19]

Absolute configuration and enantiomeric excess of the signal compounds. Gas chromatographic analysis of the hydrocarbons from fertile gynogametes of the genus Desmarestia and Cladostephus was performed on a fused silica column coated with 6-*O*-Me-2,3-di-*O*-pentyl-γ-cyclodextrin[20] as the chiral stationary phase. Synthetic (6*R*)- and (6*S*)-**2** were used as references to establish the order of elution of the two enantiomers. For details regarding other compounds from the pheromone blend of the following algae see ref. 1. *Desmarestia aculeata*:[6] (3*R*,4*R*)-viridiene (12.6%; ≥97% *e.e.*), (6*S*)-ectocarpene **3** (13%; ≥96% *e.e.*), (6*R*)-desmarestene **2**

(74.4%, 87.2% *e.e.*). *Desmarestia firma*: (6*S*)-ectocarpene **3**, (18%), (6*R*)-desmarestene **2** (82%, 28% *e.e.*). *Cladostephus spongiosus*[21] (collected in the intertidal at Flinders, Victoria, Australia): (6*R*)-desmarestene **2** (≥ 96% *e.e.*).

Biological activity of the Pheromones. According to the standard assay for gamete release,[6] the threshold concentration of (6*R*)-**2** was found for *Desmarestia aculeata* at 0.6 nM while for (6*S*)-**2** ca. 130 nM were required to trigger gamete release. The large difference between the biological activities of (6*R*)- and (6*S*)-**2** is remarkable (factor 160) and significantly exceeds the previously reported activity differences of other cycloheptadiene based pheromone systems.[5,23] Unlike our recent finding that the unstable cyclopropane (1*R*,2*S*)-**7** instead of the long believed (6*S*)-**3** is the genuine pheromone[9] of *Ectocarpus siliculosus* males, assays with male gametes of *D. aculeata* proved the cycloheptadiene (6*R*)-**2** as the superior attractant. With respect to their absolute configurations the three C_{11} hydrocarbons (6*R*)-**2**, (6*S*)-**3** and (6*R*)-**4** belong to the same series and, hence, reflect a common enzymatic transformation of their eicosanoid precursors.

EXPERIMENTAL

General: Reactions were conducted under Ar; solvents were dried according to standard methods. IR spectra were recorded on a Perkin-Elmer Infrared Spectrophotometer 882. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a Bruker Cryospec WM 250 or a Bruker AM 400. Chemical shifts of ^1H and ^{13}C NMR are given in ppm (δ) downfield relative to TMS as an internal standard. GC-MS and EI-HR-MS (70eV) were recorded with a Finnigan MAT 90 and a Finnigan ITD 800 for routine spectra. Optical rotations were determined on a Perkin-Elmer 241 polarimeter at the sodium D line, with CH_2Cl_2 as the solvent. Kinetical measurements were perfomed on a Perkin Elmer Lambda 2 spectrophotometer. Gas chromatography: Carlo Erba, Series 4100, equipped with a fused silica capillary columns coated with either SE30 (10m x 0.31 mm) or 6-methyl-2,3-di-*O*-pentyl-γ-cyclodextrin (50m x 0.31mm) from Macherey & Nagel (Düren, Germany). Silica gel, Si 60 (0.200-0.063, *E. Merck*, Darmstadt, Germany) was used for chromatography. Thin layer chromatography was performed with silica gel plates Polygram Sil G_{F254}, from Macherey & Nagel.

(1*S*,2*R*)-1-Acetic acid-2-(hexa-1′*E*/*Z*,5′-dien-3′-ynyl)cyclopropyl ester ((1*S*,2*R*)- 10)

A suspension of pent-4-en-2-ynyltriphenylphosphonium bromide[15] (2.9 g, 7.125 mmol) in THF (30 ml) at 0 °C is gradually treated under stirring with *n*-BuLi (2.75 ml, 6.9 mmol, 2.5 M in hexane). Stirring is continued for 1 h prior to addition of a soln. of the aldehyde (1*R*,2*S*)-**9** (1.0 g, 7.025 mmol in THF (10 ml)). The mixture is stirred for 90 min and, then, poured onto ice/dil. HCl. Extractive work-up with Et_2O and chromatography on silica gel using pentane/Et_2O (70/30) for elution yields the acetate (1*S*,2*R*)-**10** as a mixture of isomers. Yield: 0.77 g (58%; *E*:*Z* = 1.15:1). $[\alpha]_D^{21}$ = 82.6 (c = 5.490, CH_2Cl_2). IR (film, cm^{-1}): 3079, 3009, 2962, 2898,

2187, 1737, 1648, 1598, 1451, 1426, 1369, 1238, 973, 952, 919. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.93–5.78 (m, 2H-C(5'), H-C(2'), J=9), 5.68–5.64 (dd, H-C(2'), J=15.5, 2.1), 5.59–5.48 (m, 2H-C(6'), H-C(1')), 5.42–5.36 (dt, 2H-C(6'), J=11.2, 2.1), 4.17–4.11 (m, –C$\underline{H}_2$–OAc), 3.96–3.83 (m, –C$\underline{H}_2$–OAc); 2.18–2.04 (dquart., 1H-C(2), J=5.6, 2.8), 1.99 (s, –CO–C$\underline{H}_3$), 1.71–1.63 (dq, 1H-C(2), J=5.7, 2.8), 1.55–1.46 (m, H-C(1)), 1.45–1.37 (m, H-C(1)), 1.15–1.09 (dt, 1H-C(3), J=5, 3.2), 1.06–1.0 (dt, H-C(3), J=5, 3.2); 0.56–0.5 (m, H-C(3)). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 170.92 (C=O), 142.69/142.08 (C(1')), 126.13/126.02 (C(6')), 117.29/117.23 (C(5')), 110.02/109.10 (C(2')), 92.43/88.74 (C(4')), 87.50/87.09 (C(3')), 64.71/64.56 (–$\underline{C}H_2$–OAc), 20.87 (–CO–$\underline{C}H_3$), 19.59/18.31 (C(1)), 18.13/17.81 (C(2)); 13.15/12.08 (C(3)). MS (70 eV): 190 ($M^{+\cdot}$, 3), 147(8), 130 (8), 129(27), 128(13), 127(5), 116(7), 115(38), 103(7), 91(28), 78(11), 77(21), 73(8), 65(7), 63(11), 53(7), 52(5), 51(13), 50(14), 45(9), 44(6), 43(100), 39(20). HR-MS: *m/z* calcd. for $C_{12}H_{14}O_2$: 190.0994, found: 190.0974.

(1*S*,2*R*)-2-(Hexa-1'*E*/*Z*,5'-dien-3'-ynyl)-cyclopropyl-1-methanol ((1*S*,2*R*)-11)

The acetate (1*S*,2*R*)-**10** (0.774 g, 4.067 mmol) is added at r.t. to a well stirred suspension of K_2CO_3 (2.3 g, 16.6 mmol) in MeOH (8 ml). Stirring is continued for 30 min and aq. NH_4Cl is added. Extractive work-up with Et_2O and chromatography on silica gel using pentane/Et_2O (1:1, v:v) for elution affords (1*S*,2*R*)-**11** as a mixture of 2'*E*/*Z*-isomers (1.15:1). Yield: 0.44 g (73%). $[\alpha]_D^{22}$ = 88.9 (c = 5.21, CH_2Cl_2). IR (film, cm^{-1}): 3362*br.*, 3102, 3075, 3008, 2978, 2932, 2878, 2186, 1837, 1770, 1623, 1598, 1467, 1446, 1410, 1381, 1301, 1245, 1212, 1183, 1150, 1115, 1039, 1017, 972, 950, 917, 839, 773, 756, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.97–5.83 (m, 2H-C(5'), 1H-C(2')), 5.71 (d, 1H-C(2'), J=15.7), 5.62–5.54 (m, H-C(1'), H-C(6')), 5.46–5.39 (dt, 2H-C(6'), J=12.2, 1.6), 3.78–3.72 (m,–C$\underline{H}_2$–O), 3.48 (t, –C$\underline{H}_2$–O, J=12.2, 1.6), 2.18–2.10 (m, H-C(1)), 1.86 (s, br., H-O$\underline{H}$), 1.71–1.64 (m, H-C(1)), 1.54–1.46 (m, H-C(2)), 1.45–1.39 (m, H-C(2)), 1.16–1.11 (m, H-C(3)), 1.07–1.02 (m, H-C(3)), 0.57–0.5 (m, 2H-C(3)). ^{13}C-NMR ($CDCl_3$, 400 MHz): δ 143.48/142.70 (C(1')), 126.28/126.08 (C(6')), 117.27/117.23 (C(5')), 109.71/109.10 (C(2')), 92.68/88.88 (C(4')), 87.50/87.10 (C(3')), 63.24/62.85 (–$\underline{C}H_2$–OH), 22.43/22.28 (C(1)), 19.91/17.66 (C(2)), 13.04/12.14 (C(3)). MS (70eV): 148($M^{+\cdot}$, 13), 133(5), 129(8), 128(8), 120(22), 117(10), 116(15), 115(71), 105(21), 104(30), 103(58), 92(13), 91(100), 89(15), 79(8), 78(89), 77(52), 74(11), 65(37), 63(48), 62(16), 55(17), 52(16), 51(42), 50(28), 44(24), 43(29), 41(15), 39(86). HR-MS: *m/z* calcd. for $C_{10}H_{12}O$: 148.0888, found: 148.0895.

Oxidation of (1*S*,2*R*)-(11): A soln. of the alcohol (1*S*,2*R*)-**11** (0.44 g, 2.969 mmol) in dry CH_2Cl_2 (10 ml) is added at 0 °C to a well stirred suspension of pyridinium dichromate (1.7 g, 4.46 mmol), $MgSO_4$ (0.1 g) and activated molecular sieves (4Å, 0.1 g) in dry CH_2Cl_2 (20 ml). Stirring is continued overnight. Then, the mixture is poured into a tenfold volume of pentane/Et_2O (1:1, v:v). The precipitate is removed, and last admixtures of chromium salts are removed by adsorption to $MgSO_4$. The soln. is flash-evaporated at 0 °C. The

crude residue is purified by chromatography on silica gel (column: 150 cm x 3 cm) using a pentane/Et_2O gradient for elution. The first eluting isomer is (2'*Z*)-(1*S*,2*R*)-**12**. Yield: 113.7 mg (26.2%). (2'*E*)-(1*S*,2*S*)-**12** elutes as the second isomer. Yield: 165 mg (38 %).

(1*S*,2*R*) 2-(Hexa-1'*Z*,5'-dien-3'-ynyl)cyclopropane-1-carbaldehyde ((1*S*,2*R*,2'*Z*)-12)

$[\alpha]_D^{22}$ = 745.1 (c = 1.137, CH_2Cl_2). IR (film, cm^{-1}): 3102, 3041, 3008, 2977, 2932, 2738, 2252, 2180, 1705, 1590, 1419, 1396, 1365, 1192, 1168, 1113, 969, 911, 773, 732, 672, 648. ^1H NMR ($CDCl_3$, 400 MHz): δ 9.52 (d, –CHO), 5.84 (m, H-C(5')), 5.83 (m, H-C(1')), 5.62 (m, H-C(2'), H-C(6')), 5.48 (m, H-C(6')), 2.70 (m, H-C(2)), 2.35 (m, H-C(1)), 1.59–1.48 (m, 2H-C(3)). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 199.82 (C=O), 139.14 (C(1')), 126.74 (C(6')), 117.09 (C(5')), 110.69 (C(2')), 93.33 (C(4')), 86.47 (C(3')), 30.58 (C(1)), 25.16 (C(2)), 15.95 (C(3)). MS (70 eV): 146 (M^+, 4), 145(38), 131(5), 118(8), 117(49), 116(20), 115(100), 103(9), 102(10), 92(6), 91(92), 89(21), 87(7), 81(7), 79(5), 78(20), 77(25), 76(7), 75(8), 74(12), 68(9), 65(44), 64(9), 63(65), 62(22), 61(11), 55(44), 53(10), 52(15), 51(42), 50(36), 43(7), 41(10), 40(14), 39(94). HR-MS: *m/z* calcd. for $C_{10}H_9O$ ($M^{+\bullet}$-1): 145.0653, found: 145.0624.

(1*S*,2*R*) 2-(Hexa-1'*E*,5'-dien-3'-ynyl)cyclopropane-1-carbaldehyde ((1*S*,2*R*,2'*E*)-12)

$[\alpha]_D^{24}$ = –110.1 (c = 2.054, CH_2Cl_2). IR (film, cm^{-1}): 3100, 3043, 3008, 2840, 2761, 2734, 2252, 2186, 1706, 1597, 1410, 1397, 1372, 1295, 1251, 1205, 1168, 1080, 1058, 955, 915, 873, 792, 732. ^1H NMR ($CDCl_3$, 400 MHz): δ 9.44 (d, –CHO), 6.06 (dd, H-C(1'), J=15.7, 8.7), 5.93–5.85 (ddd, H-C(5'), J=11.1, 6.4, 2.2), 5.80 (dd, H-C(2'), J=15.7, 2.1), 5.62 (dd, H-C(6'), J=17.5, 2), 5.46 (dd, H-C(6'), J=11.1, 2.1), 2.22 (m, H-C(1)), H-C(2)), 1.57 (m, H-C(3)), 1.48 (m, H-C(3)). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 199.65 (C=O), 139.95 (C(1')), 126.63 (C(6')), 117.02 (C(5')), 111.55 (C(2')), 88.47 (C(4')), 88.04 (C(3')), 30.52 (C(1)), 26.80 (C(2)), 15.23 (C(3)). MS (70 eV): 146 (M^+, 5), 145(38), 118(6), 117(49), 116(25), 115(100), 103(11), 102(8), 92(7), 91(85), 89(19), 78(22), 77(27), 75(17), 74(11), 68(9), 66(8), 65(41), 64(8), 63(52), 62(22), 61(11), 55(35), 53(10), 51(41), 50(29), 43(27), 39 (83). HR-MS: *m/z* calcd. for $C_{10}H_9O$ ($M^{+\bullet}$-1) : 145.0653, found: 145.0635.

(6*S*)-6-(But-3-en-1-ynyl)cyclohepta-1,4-diene ((6*S*)-15)

A well stirred suspension of methyltriphenylphosphonium bromide (0.413 g, 1.165 mmol) in Et_2O (25 ml) is treated slowly at 0 °C with *n*-BuLi (0.46 ml, 1.165 mmol, 2.5 M in *n*-hexane). After 1 h a soln. of the aldehyde (1*S*,2*R*,2'*Z*)-**12** (0.114 g, 0.8 mmol) in Et_2O (5 ml) is added, and stirring is continued for 1 h. The mixture is poured onto ice/dil. HCl, and the product is extracted with pentane (4 x 20 ml). Chromatography on silica gel affords (6*S*)-**15** together with the non-rearranged thermolabile cyclopropane (1*R*,2*S*,2'*Z*)-**14**. Yield:

0.052 g (47 %). Heating (55 °C) of a soln. of crude (1*R*,2*S*,2'Z)-**14** in $CHCl_3$ for 1 h affords (6*S*)-**15** in quantitative yield. $[\alpha]_D^{21}$ = -38.9 (c = 4.754, CH_2Cl_2). IR (film, cm^{-1}): 3098, 3017, 2907, 2856, 2222, 1830, 1647, 1605, 1429, 1382, 1319, 1281, 1176, 1156, 1082, 1041, 972, 915, 877, 853, 818, 799, 781, 734, 677, 648. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.83–5.76 (ddd, H-C(3'), J=11.1, 6.4, 2.1), 5.70 (m, H-C(1), H-C(2), H-(4), H-(5)), 5.57 (dd, H-C(4'), J=17.5, 2.2), 5.40 (dd, H-C(4'), J=11, 2.2), 3.59 (m, H-C(6)), 2.96–2.76 (m, 2H-C(3)), 2.49 (m, 2H-C(7)). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 132.06 (C(5)), 128.84 (C(2)), 128.83 (C(1)), 128.44 (C(4)), 125.86 (C(4')), 117.42 (C(3')), 93.15 (C(1')), 79.10 (C(2')), 33.16 (C(7)), 30.37 (C(6)), 27.93 (C(3)). MS (70 eV): 144 (M^+, 2), 143(14), 141(10), 130(7), 129(78), 128(100), 127(23), 117(13), 116(26), 115(67), 103(13), 102(10), 92(16), 91(50), 89(17), 79(26), 78(19), 77(41), 74(13), 66(48), 65(35), 63(50), 62(17), 52(16), 51(49), 50(33), 40(9), 39(93). HR-MS: *m/z* calcd. for $C_{11}H_{12}O$: 144.0939, found: 144.0883.

(6*R*)-6-(But-3-en-1-ynyl)cyclohepta-1,4-diene ((6*R*)-15)

Prepared from (1*R*,2*R*,2'*E*)-**12** (0.609 g, 1.71 mmol) as described for (6*S*)-**15**. The Cope rearrangement is complete at r.t..[9] Yield: 0.068 g (42.4%). $[\alpha]_D^{22}$= +48.1 (c = 0.542, CH_2Cl_2). For all other spectroscopic data refer to (6*S*)-**15**. HR-MS: *m/z* calcd. for $C_{11}H_{12}$: 144.0939, found: 144.0913.

(6*S*)-6-(Buta-1*Z*,3-dienyl)cyclohepta-1,4-diene ((6S)-2)

A soln. of alkyne (6*S*)-**15** (0.0524 g, 0.363 mmol) in MeOH (4 ml) is added to a well stirred suspension of activated Zn(*Cu/Ag*)[16,17] in aq. MeOH/H_2O (2 ml, v:v = 1:1). Stirring is continued at r.t. until GLC indicates complete reduction (ca. 24 h). The solids are filtered off and carefully washed with MeOH (2 x 5 ml). The combined solns. are extracted with pentane (5 x 10 ml). Chromatography on silica gel and pentane yields (6S)-**2** as an intensively smelling, colourless liquid. Yield: 0.022 g (41.5%, 92% *e.e.* according to GLC). $[\alpha]_D^{22}$ = -153 (c = 1.136, CH_2Cl_2). IR (film, cm^{-1}): 3086, 3013, 2962, 2933, 2899, 2851, 1645, 1588, 1429, 1293, 1260, 1208, 1145, 1082, 1047, 996, 960, 904, 878, 841, 825, 788, 695, 659. ^1H–NMR ($CDCl_3$, 400 MHz): δ 6.70–6.63 (m, 1H-C(3'), J=11.1, 4.4, 2), 5.96 (dd, 1H-C(2'), J=11, 0.6)), 5.72 (m, 1H-C(1), 1H-C(2)), 5.65 (m, 1H-C(4)), 5.53 (m, 1H-C(5)), 5.49 (t, 1H-C(1'), J=10.4), 5.21 (dt, 1H-C(4'), J=16.9, 1.2), 5.11 (d, 1H-C(4'), J=10.2), 3.62 (br., 1H-C(6)), 2.97 (m, 1H-C(3)), 2.79 (m, 1H-C(3)), 2.27 (m, 2H-C(7)). ^{13}C–NMR ($CDCl_3$, 400 MHz): δ 136.30 (C(1')), 134.69 (C(5)), 132.49 (C(3')), 129.54 (C(2)), 129.31 (C(1)), 128.20 (C(2')), 127.89 (C(4)), 117.61 (C(4')), 36.78 (C(6)), 33.60 (C(3)), 28.63 (C(7)). MS (70 eV): 146(M^+, 9), 145(4), 131(28), 130(5), 129(10), 128(11), 118(13), 117(45), 115(22), 105(70), 104(26), 103(16), 92 (66), 91(100), 80(36), 79(70), 78(19), 77(38), 68(15), 66(13), 65(15), 51(30), 53(15). HR-MS: *m/z* calcd. for $C_{11}H_{14}$: 146.1095, found: 146.1085.

(6*R*)-6-(Buta-1*Z*,3-dienyl) cyclohepta-1,4-diene (6*R*)-(2)

From (6*R*)-**15** (0.068 g, 0.477 mmol) as described for (6*S*)-**2**. Yield: 0.027 g (38.7%, 93.3% *e.e.* according to GLC. $[\alpha]_D^{22} = 168$ (c = 1.468, CH_2Cl_2).

Kinetic Measurements. Dilute solutions of (1*R*,2*S*)-**14** (ca. 1% in *n*-undecane) in quartz cuvettes were placed into a water thermostated cell-holder of the spectrophotometer, connected to a *Haake D1* thermostat (± 0.1 °C). The temperature was continuously recorded as function of time using a NiCr-Ni thermocouple, type K, and a precision digital voltmeter. A typical run was followed over 2 to 3 half life times and repeated in triplicate. The isomerisation of (1*R*,2*S*)-**14** to (6*S*)-**15** was followed by the disappearance of the dienyne absorption at 254 nm. The rate constants were calculated from the *Arrhenius* plot (Fig. 1) and the raw data, recorded as a function of time. E_a = 87.2 kJ mol^{-1} ± 1.2 kJ mol^{-1}, $\Delta S^{\neq}$ = -52.9 J mol^{-1} K^{-1} ± 4.1 J mol^{-1} K^{-1} (confidence level).

Determination of the absolute configuration and the enantiomeric excess of the gamete-releasing and gamete-attracting pheromones by gas chromatography. Column: fused silica coated with 6-*O*-methyl-2,3-*O*-pentyl-γ-cyclodextrin[20] (50 m x 0.25 mm). Carrier gas: H_2 at 1 bar. Conditions: 60 °C for 2 h, then at 2 °C min^{-1} to 160 °C (2 min). Detection: FID. Elution order (e.g. *D. aculeata*): (3*R*,4*R*)-viridiene (60.3 min), (3*S*,4*S*)-viridiene (61.7 min, α = 1.024), (6*S*)-**3** (101 min), (6*R*)-**2** (132.7 min), (6*S*)-**2** (133.7 min; α = 1.007).

Biological activity test (Gamete-Release). Gametophytes of *Desmarestia aculeata* were vegetatively propagated at 17 °C as described.[22] To induce the formation of antheridia, the plants were kept for 20 days at 4 °C using a photophase of 14 h at 70 μE m^{-2} sec^{-1} photosynthetically active radiation. Biological activity tests were carried out at 4 °C by placing fertile male antheridia of *D. aculeata* into the close vicinity of graded dilutions of (6*S*)- and (6*R*)-**2** in a microdroplet of FC 72 (biologically inert, water immiscible fluorocarbon of high density). Gamete release occurred within 8 to 20 sec down to 0.5 μM for (6*R*)-**2** and down to 100 μM for (6*S*)-**2**. Around the threshold concentrations the experiments were generally repeated 15 times in order to obtain statistically relevant data. Following correction with the partition coefficient of **2** between FC 72 and water ($K_{FC\ 72/water}$ = 780 for **2**), the lowest effective concentration for (6*R*)-**2** in the water phase was calculated to be 0.6 nM.

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