187. Absolute Configuration of Multifidene and Viridiene, the Sperm Releasing and Attracting Pheromones of Brown Algae[1])

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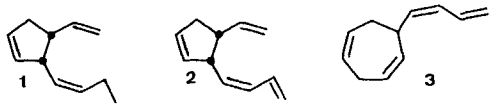
Summary

The absolute configurations of the two algal pheromones multifidene **1** and viridiene **2** were determined as (+)-(3*S*,4*S*)-3-[(*Z*)-1-butenyl]-4-vinylcyclopentene and (+)-(3*R*,4*S*)-3-[(1*Z*)-1,3-butadienyl]-4-vinylcyclopentene, respectively. The strategy involves enzyme-initiated asymmetric synthesis of the ring-saturated pheromone analogues (+)-**8a** and (−)-**8b** and their subsequent catalytic hydrogenation to the chiral cycloalkanes **9a** and **9b**, only the letter of which is also obtained from the two natural messengers (+)-**1** or (+)-**2**. Biological activity assays proved these enantiomers of **1** or **2** to be the characteristic pheromones for male gametes of the seaweeds *Syringoderma, Cutleria multifida* and *Chorda tomentosa*.

1. Introduction. – It is now well-established that sexual reproduction of brown algae is mediated by signal substances which are given off by mature gynogametes and act on the freely swimming male gametes or the gametangia [2] [3]. The signal substances are highly unsaturated aliphatic or cyclic hydrocarbons with olefinic side chains (general formula, *e.g.* $C_{11}H_{14}$, $C_{11}H_{16}$, $C_{11}H_{18}$).

Among the various alicyclic C_{11}-compounds cyclopentene- and cycloheptadiene systems display a special feature since some of them can trigger also mass discharge of androgametes from the antheridia possibly by an enzyme-activating mechanism. Only then their known function as luring substances comes into effect [4]. The most widespread representatives of this type of gamete attractants in phaeophytes are multifidene **1**, viridiene **2** and desmarestene **3**.



Multifidene **1** was first discovered as the active component of the pheromone bouquet of *Cutleria multifida* [5] [6]. Recently, its additional activity as a specific

[1]) Part V of a series; Part IV see [1].

and efficient release factor for *Chorda tomentosa* androgametes was recognized [7]. Viridiene **2** and desmarestene **3** are produced by mature gametophytes of the two North Atlantic species *Desmarestia aculeata* and *Desmarestia viridis* to liberate and attract their corresponding androgametes [8] [9].

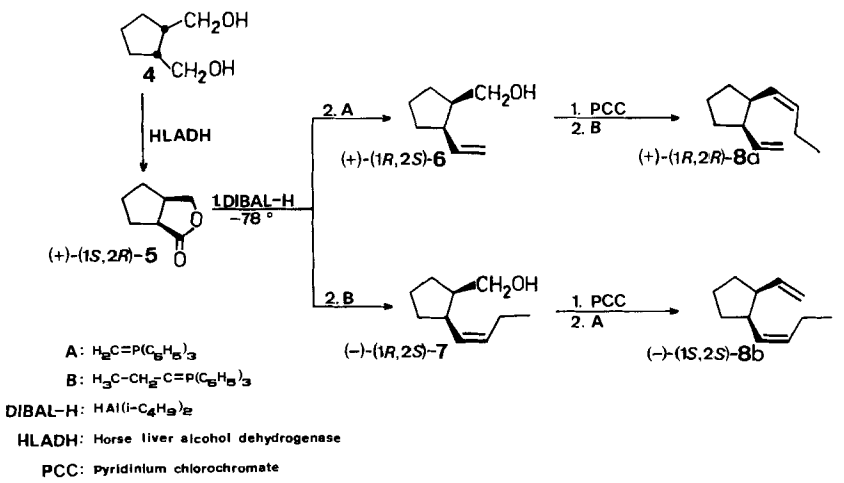
Viridiene **2** is also excreted by gynogametes of *Syringoderma* [10], a brown alga of still uncertain taxonomic position. Quantitation of such biological events under carefully controlled laboratory conditions gave concentrations between 1 and 90 picomoles per liter as the threshold to elicit a statistically significant biological response [7] [8] [10].

Assuming only weak dipole/dipole interactions between messenger and binding macromolecule [11] [12], the receptor has to be an accurate imprint of the pheromone, which includes effective differentiation between its enantiomers. This was first corroborated by chemical synthesis and subsequent bioassay of (+)- and (−)-multifidene **1** [13]. However, the absolute configuration of **1** and **2** remained open.

Starting from an enzymatically prepared intermediate of known absolute configuration, a synthetic sequence is presented which allows correct and unambigous assignment of configuration for this class of natural products.

2. Enantioselective Synthesis of 1,2-Dihydromultifidenes. – Recently, horse liver alcohol dehydrogenase (HLADH) was introduced by *Jakovac et al.* [14] into organic chemistry as a versatile tool for the enantiospecific preparation of lactone-synthons from easily available meso-diols. These authors described already the oxidation of the diol **4** to the lactone **5** with given absolute configuration *(cf. Scheme 1)*.

Scheme 1



To obtain the C-skeleton of **1** or **2** only the two olefinic side chains had to be introduced without touching the chiral centers once installed. This was easily achieved by reductive alkenylation [15] of **5** with either methylidene- or propylidene-

phosphorane as alkenylating agents. The resulting alcohols **6** and **7** were oxidized and immediately converted into the two enantiomers of **8** using the appropriate *Wittig*-reagents.

3. Determination of Absolute Configurations. – As highly bioactive messengers the pheromones **1** or **2** are secreted in minute amounts only[2]).

In consequence, any information on their configuration can be obtained only by synthesizing the pure enantiomers and testing their biological activity. In fact, of the synthetic (+)- and (−)-multifidenes **1** (>97% ee. each) the (+)-enantiomer was found to be 100 times more effective for *Cutleria androgametes* (20 *vs.* 2000 picomoles per liter sea water for the two enantiomers) [13].

Also, on exposing *Cutleria* males to the related *Syringoderma* pheromone viridiene, (+)- or (−)-**2**, obtained as described, the (+)-enantiomer was clearly preferred, not withstanding a changed behaviour towards the unfamiliar compound. The luring threshold for (+)-viridiene was found at 6.5 nmol per liter sea water, whereas the (−)-antipode required a remarkably higher concentration to cause a comparable biological effect *(Fig. 1)*.

In view of these results together with the above-mentioned precise mutual interaction of pheromone molecules and their specific receptors we conclude that both, (+)-multifidene and (+)-viridiene, will possess the same spatial arrangement.

Although the bioassay offers a convenient method to test chirality at a nanogram level or even beyond, up to here no statement on absolute configurations can be

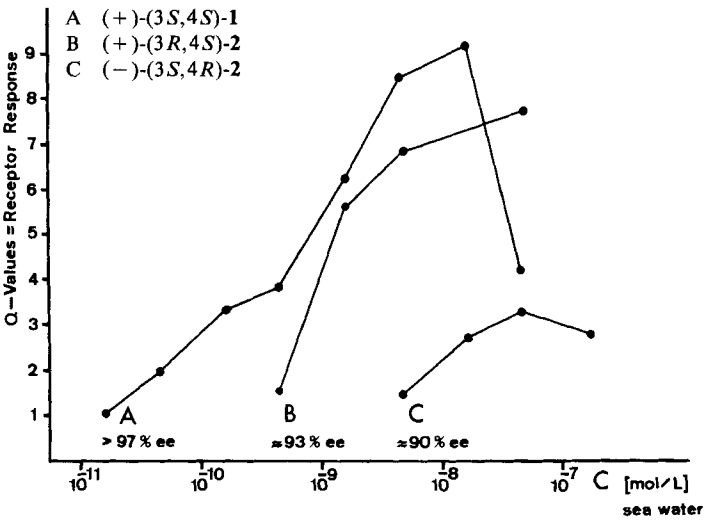


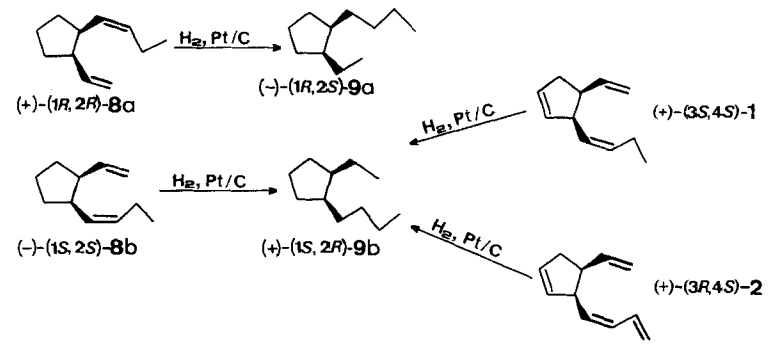
Fig. 1. *Concentration/response behaviour of the* Cutleria *receptor system towards its natural signal (+)-multifidene* **1** (A) *and the two enantiomeric viridienes (+)-***2** (B) *and (−)-***2** (C). Plotted are relative receptor responses (Q-values) against the pheromone concentration in the aqueous phase. The assay technique is described in [1].

[2]) To give an impression of the operating dimensions: a gynogamete of the large sublitoral brown alga *Laminaria digitata* produces per min 10,000 molecules of the specific hormone, 15,000 molecules ectocarpene, and 30,000 molecules of pentadecane [4].

Table. *Isomer Distribution (cis/trans-ratios) of Hydrogenation Experiments* (Error limit ± 1%)

Compound	Reaction temperatures [°C]				
	20°	2°	−10°	−20°	−30°
1	88:12	89:11	91:9	93:7	94:6
2	73:27	78:22	83:17	85:15	87:13
8	86:14	90:10	95:5	96:4	96:4

Scheme 2



made. To do this, we prepared the enantiomerically pure hydrocarbons (+)-**8a** and (−)-**8b** and subsequently transformed all compounds of interest into a common intermediate with known absolute configuration by the reaction sequence outlined in *Scheme 2*.

There are several reports on successful transformations of chiral olefines into alkanes without significant loss of optical activity using Pt/C as hydrogenation catalyst [16]. However, in our first attempts at ambient temperature massive scrambling of double bonds led to large amounts of *trans*-disubstituted products (*cf.* the *Table*). Since other catalysts (Pd/C, Pd/$BaSO_4$, nickelboride or tris(triphenylphosphine)rhodium chloride) resulted in even worse results, we returned to Pt/C as the standard catalyst and tried to minimize isomerization by lowering the temperature. In fact, satisfactory results were obtained at $< -20°$.

If the reductions of (+)-**8a** or (−)-**8b** were carried out keeping the reaction temperature at −30°, the alkanes (+)-**9b** or (−)-**9a** were obtained in 95% isomeric purity. Last traces of *trans*-admixtures were removed by preparative GC using *Apiezon L* as the stationary phase. When the two bioactive molecules, (+)-multifidene **1** and (+)-viridiene **2**, were so treated, the dextrorotatory alkane (+)-**9b** was obtained in both cases thus indicating correspondence of their three-dimensional arrangement.

In *Scheme 2* the two natural products (+)-**1** and (+)-**2** can be traced back to the olefine (−)-**8b**, the absolute configuration of which has to be (1*S*,2*S*) with respect to the starting material (+)-**5**. Thus, our original, but then tentative assignment of (+)-**1** as (+)-(3*S*,4*S*)-3-[(*Z*)-1-butenyl]-4-vinylcyclopentene [13] is experimentally confirmed by an unambigous reaction sequence. Together with this the absolute configuration of (+)-viridiene **2** is determined as (+)-(3*R*,4*S*)-3-

[(1*Z*)-1,3-butadienyl]-4-vinylcyclopentene and corroborates the close structural and biogenetic relationship between both messengers.

4. Chiral Recognition of Algal Receptor Systems. – Usually enantiomeric, but nonpolar molecules, do not have a preferred orientation in their contact with hydrophobic surfaces. The only relevant criterion for the occurence of the 'necessarily' weak and unspecific interaction is rotational and conformational adaption to the host site. If, however, as suggested [11] [12], mutual dipolar interaction between a pheromone's double bonds and the receptor matrix governs the primary events of the signal recognition, only one and a single possibility to superimpose signal and receptor will be expected. Significant differences in response qualities of enantiomeric pheromone pairs are therefore a direct proof of this type of weak dispersion forces.

It becomes evident from *Fig. 1* that the overall shape (absolute configuration) of (+)-viridiene **2**, and the similar topology of its π-electron density causes the perfect fitting with the *Cutleria* system. Comparing the two enantiomeric pairs of (+)- and (−)-**1** as well as (+)-**8a** and (−)-**8b** on *Cutleria* males is even more instructive *(Fig. 2)*.

The related configurations (+)-**1** and (−)-**8b** are clearly preferred. This means, that now only the double bonds of the side chains are involved in the strong interaction and that the cyclopentane ring rather codetermines the efficiency of interaction in a nonspecific, morphological way by influencing accessibility of the host site.

It is evident from the sum of all these observations, that the bioassay of a given receptor type with both enantiomers of its specific ligand allows a convenient and reliable determination of the chirality of the latter.

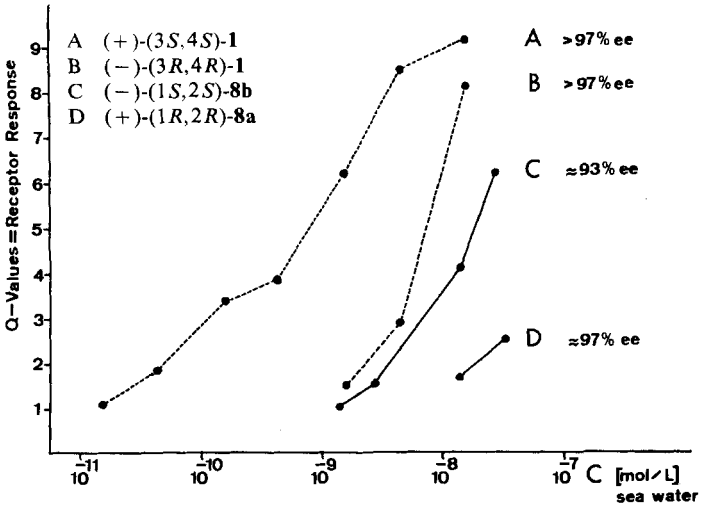


Fig. 2. *Comparative bioassay of (+)- and (−)-multifidene* **1** *and the two ring-saturated derivatives (+)-***8a** *and (−)-***8b**.

(A)=(+)-**1**; (B)=(−)-**1**; (C)=(−)-**8b**; (D)=(+)-**8a**. The enantiomer excess given for (A) and (B) is taken from [13]; the values for (C) and (D) are calculated from the purity of the starting lactone (+)-(1*S*,2*R*)-**5** (see *Exper. Part*).

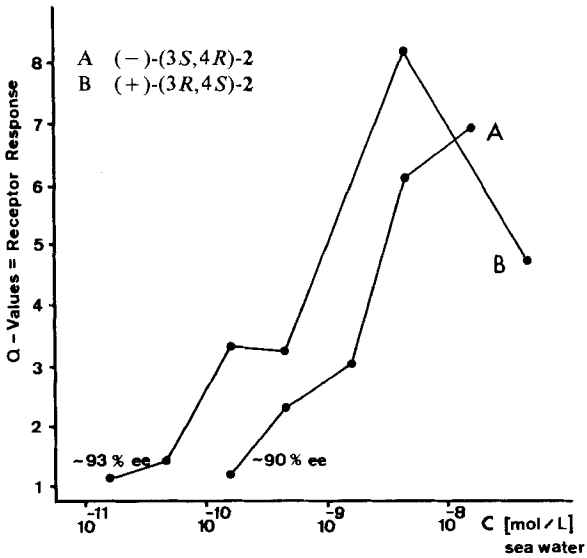


Fig. 3. *Experimental evaluation of the absolute configuration of the* Syringoderma *pheromone using the biological assay technique.*
(A) = (−)-viridiene **2**, (B) = (+)-viridiene **2**.

For example, testing the response of *Syringoderma* spermatozoids towards (+)- and (−)-viridiene **2**, a definite preference for the (+)-enantiomer can be stated *(cf. Fig. 3)*.

Similarly, a threshold concentration of 1 picomole (+)-multifidene **1** was sufficient to release gametes from antheridia of *Chorda tomentosa* [7]; to cause the same effect, the concentration of (−)-**1** had to be increased 20 fold which may be even higher considering the contamination with traces of its levorotatory antipode (−)-**1**.

The present study of enantiomeric response differences in chemoreception of brown algae offers proof for the previously discussed binding mechanism [11] [12]; however, more and detailed information is necessary to understand the specific interactions of olefinic pheromones with their macromolecular receptors.

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Experimental Part

General. Specific rotations were determined at 20° with a *Carl Zeiss* Präzisionspolarimeter (±0.005°). IR spectra (cm^{-1}) were recorded with a *Pye Unicam SP3-200* spectrophotometer. The ^{1}H-NMR spectra were obtained with a *Varian EM-390* 90-MHz-spectrometer in CCl_4 with TMS as internal standard. MS (*m/z*) were run on a *Finnigan 4510* GC/MS system. Analytical GC: *Carlo Erba* gaschromatograph, *Series 4200* equipped with *Duran* glass capillaries 50 m × 0.32 mm coated with *OV 73*. Preparative GC: stainless steel column 2 m × 4 mm filled with 15% *Apiezon L* on *Chromosorb W*, 40–60 mesh, *AW*, DMCS-treated. Elemental analyses were performed by *I. Beetz*, Kronach.

Horse liver alcohol dehydrogenase (HLADH) was purchased from *Boehringer*, Mannheim and flavine mononucleotide (FMN), commercial grade, was obtained from *Sigma Chemie*, München.

All solvents and reagents were purified prior to use. Reactions, except the enzymatic oxidation, were carried out under an inert atmosphere if not stated otherwise.

(+)-(1R,5S)-3-Oxobicyclo[3.3.0]octane-2-one (**5**) [14]. *cis*-1,2-Bis(hydroxymethyl)cyclopentane (**4**) (1.95 g, 15 mmol) [17] was dissolved in 600 ml 0.1M glycine-NaOH buffer (pH 9.0) and 720 mg NAD^+ (1.1 mmol) and 9.72 g FMN (20.3 mmol) were added with stirring. The pH was readjusted to 9.0 followed by addition of 50 mg HLADH. After *ca.* 30 min the solution becomes dark brown. Stirring was continued for 48 h while the progress of the oxidation was monitored by GC. To isolate the lactone formed the mixture was acidified to pH 3.0 and continuously extracted with Et_2O over night. After drying and evaporation of solvent *in vacuo* the crude pale yellow residue could be purified by CC on silica gel with hexane/Et_2O (60:40). Removal of solvent yielded 1.57 g (+)-**5** (89.6%); $[\alpha]_{578}^{20} = +97.1°$, $[\alpha]_D^{20} = +92.1°$ ($c = 3.34$, $CHCl_3$), ([14]: 97.5% ee., $[\alpha]_D^{20} = +96.9°$).

A second sample using 2% MeOH as co-solvent gave a less pure product; $[\alpha]_{578}^{20} = +88.7°$ corresponding to 93.4% ee.

(−)-(1R,2S)-{2-[(1Z)-1-Butenyl]-1-cyclopentyl}methanol (**7**) [18]. A 1.76M solution (5.6 ml) of diisobutylaluminium in toluene (9.9 mmol) was added dropwise at −78° to a well-stirred solution of 1.0 g (+)-**5** (7.9 mmol), $[\alpha]_{578}^{20} = +88.7°$, in 20 ml dry toluene. Stirring was continued at −78°, until GC control indicated complete reduction of starting material (*ca.* 30 min). Excess of diisobutylaliminium hydride was decomposed by injection of 0.2 ml abs. MeOH, and the whole solution was then quickly poured into a freshly prepared solution of propylidenetriphenylphosphorane (12 mmol) in 60 ml of dry THF (BuLi as base). After 1 h stirring at 0° the mixture was hydrolyzed by addition of 50 ml 2.0N HCl. The org. layer was separated and the aq. phase was extracted with Et_2O (3 × 100 ml). The combined org. phases were successively washed with 2.0N HCl, sat. $NaHCO_3$-solution and H_2O. After removing the solvent under reduced pressure the crude residue was triturated with pentane, in which the triphenylphosphine oxide is insoluble. The filtered pentane extract was concentrated *in vacuo* and the product purified by CC on silica gel with hexane/Et_2O (80:20). After vacuum evaporation of solvent 0.67 g (55%) of the levorotatory alcohol (−)-**7** were obtained; $[\alpha]_{578}^{20} = -8.1°$ ($c = 5.96$, CH_2Cl_2). IR (neat): 3340, 3008, 2960, 2880, 1460, 1040, 800 and 750. ^{1}H-NMR (CCl_4): 0.98 (*t*, 3 H); 1.15–2.30 (*m*, 9 H); 2.65 (br. *s*, 1 H); 2.88 (*m*, 1 H); 3.44 (*m*, *AB*-system, 2 H); 5.10–5.55 (*m*, 2 H). MS (70 eV): 154 (3, M^+), 136 (15), 123 (30), 107 (54), 97 (24), 93/94 (32), 81 (100), 79 (75), 67 (100), 57 (35), 55 (68), 41 (78), 39 (37).

$C_{11}H_{18}O$ (154.25) Calc. C 77.87 H 11.76% Found C 77.83 H 11.89%

(+)-(1R,2S)-2-Vinyl-1-cyclopentylmethanol (**6**). The lactone (+)-**5** (1.0 g, 7.9 mmol) was converted to **6** as described for the preparation of **7**. Removal of solvent yielded 0.57 g (57%) of pure product; $[\alpha]_{578}^{20} = +8.6°$ ($c = 1.85$, $CHCl_3$). IR (neat): 3340, 3080, 2960, 2880, 1640, 1450, 1420, 1040, 1000, 915 and 700. ^{1}H-NMR (CCl_4): 1.10–1.90 (*m*, 6 H); 2.1 (*m*, 1 H); 2.65 (*m*, 1 H); 3.20–3.65 (*AB*-system, 2 H); 3.35 (*s*, 1 H); 4.98 (*dd*, $J = 10.5$ and 3, 1 H); 5.02 (*dd*, $J = 16.5$ and 3, 1 H); 5.82 (*ddd*, $J = 16.5$, 10.5 and 2, 1 H). MS (70 eV): 126 (0.07, M^+), 108 (17), 95 (76), 93 (90), 79 (72), 67 (100), 57 (41), 54 (53), 41 (85), 39 (58).

$C_{11}H_{18}O$ (126.20) Calc. C 76.14 H 11.18% Found C 76.13 H 11.03%

(−)-(1S,2S)-1-[(1Z)-1-Butenyl]-2-vinylcyclopentane (**8b**) [18]. - *a) Oxidation.* To a suspension of 2.6 g (12 mmol) pyridinium chlorochromate in 20 ml CH_2Cl_2 were added with stirring 0.5 g (4 mmol) of (−)-**7**. When GC analysis indicated complete conversion of starting material, 50 ml pentane were added and the precipitated chromium salts removed by suction. Following evaporation of solvent under reduced pressure the crude mixture was redissolved in 20 ml pentane, dried ($MgSO_4$) and filtered. Vacuum concentration left pure aldehyde which was used without further purification.

b) Wittig-*Reaction.* A solution of 0.4 g crude aldehyde (3.2 mmol) in 5 ml abs. THF were added dropwise to a solution of 6 mmol methylidenetriphenylphosphorane in 20 ml dry THF (BuLi as base). Stirring was continued for 30 min at 0°, and excess of phosphorane was quenched by addition of 20 ml 2.0N HCl. Following usual workup (*cf.* compound (−)-**7**) 0.24 g (−)-(1*S*,2*S*)-**8b** (50%) were obtained. CC on $AgNO_3$-impregnated silica gel (10%) with hexane/Et_2O (97:3) removed the (1*E*)-1-butenyl-isomer deriving from introduction of the butenyl moiety by *Wittig* reaction ((*Z*)/(*E*) = 9:1). A stereochemically homogeneous sample was obtained after prep. GC using *Apiezon L* (15%) as stationary phase; $[\alpha]_{578}^{20} = -50.8°$ ($c = 0.906$, pentane). IR (neat): 3080, 3010, 2960, 2880, 1640, 1455, 1303, 995,

913 and 750. ^{1}H-NMR (CCl_4): 0.95 (*t*, 3 H); 1.20–2.20 (*m*, 8 H); 2.50 (br. *quint.*, 1 H); 2.85 (br. *quint.*, 1 H); 4.92 (*dd*, J = 15.6 and 2.5, 1 H); 4.90 (*dd*, J = 12.0 and 2.5, 1 H); 5.68 (*ddd*, J = 15.6, 12.0 and 9, 1 H); 5.10–5.40 (*m*, 2 H). MS (70 eV): 150 (2.5, M^+), 135 (5), 121 (72), 107 (23), 95 (34), 93 (46), 91 (19), 81 (69), 79 (88), 77 (24), 67 (100), 55 (51), 54 (50), 41 (73), 39 (49).

$C_{11}H_{18}$ (150.27) Calc. C 87.93 H 12.0% Found C 87.86 H 12.12%

*(+)-(1*R,2R*)-1-[(1*Z*)-1-[Butenyl]-2-vinylcyclopentane* (**8a**). The alcohol (+)-**7** (0.5 g, 4 mmol) was converted into the olefine **8a** as described for **8b**; $[\alpha]_{578} = +55.8°$ (c = 2.557, pentane).

*(+)-(1*S,2R*)-1-Butyl-2-ethylcyclopentane* (**9b**). – *a) Temperature Dependence of Hydrogenation.* H_2 was bubbled through a suspension of 0.5 mg hydrocarbon and 10 mg Pt-catalyst (10% Pt/C) in 1 ml pentane while the temperature was kept constant until GC analysis indicated complete reduction of starting material. Each experiment was repeated twice and the average values of the produced isomer ratios are compiled in the *Table*. To establish the identity of the resulting *trans*-disubstituted cyclopentanes, a sample of *trans*-1-[(1*Z*)-1-butenyl]-2-vinylcyclopentane [18] was treated the same way. Only a single product was obtained and GC comparison (*Kováts*-Indices) on two columns of different polarity proved identity with the additional compound formed during hydrogenation of the *cis*-disubstituted cyclopentane derivatives.

b) Preparative Hydrogenations. For preparative purposes, 50 mg (0.3 mmol) (–)-**8b** were dissolved in 5 ml pentane and 50 mg catalyst (Pt/C) added. The reaction flask was immersed into a cooling bath (–30°) and hydrogenation was started. When all of the starting material had disappeared (GC analysis), the temperature was raised to –20° to allow complete reduction of the mono-olefine intermediate. The catalyst was removed by filtration and the solution concentrated *in vacuo*. A stereochemically homogeneous sample of (+)-**9b** was obtained using preparative GC with *Apiezon L* as stationary phase; $[\alpha]^{20}_{578} = +6.3°$ (c = 0.194, pentane). IR (neat): 2970, 2940, 2880, 2870, 1480, 1400. ^{1}H-NMR (CCl_4): 0.68–1.03 (*m*, 6 H); 1.03–1.46 (*m*, 10 H); 1.46–2.00 (*m*, 6 H). MS (70 eV): 154 (9, M^+), 125 (17), 111 (11), 97 (58), 83 (31), 69 (81), 55 (100), 43 (41), 41 (71).

$C_{11}H_{22}$ (154.30) Calc. C 85.63 H 14.37% Found C 85.59 H 14.39%

*(–)-(1*R,2S*)-1-Butyl-2-ethylcyclopentane* (**9a**). Compound (+)-**8a** (30 mg, 0.2 mmol) was treated as described for (+)-**9b**; $[\alpha]^{20}_{578} = -7.1°$ (c = 0.641, pentane).

Hydrogenation of (+)-Multifidene **1** [14]. Compound (+)-**1** (4.2 mg, 28.4 µmol; $[\alpha]^{20}_{578} = +261°$) was dissolved in 0.5 ml pentane and hydrogenated as described for (+)-**9b**. The catalyst was removed by filtration over a small pad of silica gel (2 cm) in a *Pasteur* pipette and the solution was concentrated *in vacuo* to about 100 µl. A final purification by preparative GC (*Apiezon L*) removed all *trans*-contaminations and gave 2.37 mg (54%) of the pure alkane (+)-**9b**; $[\alpha]^{20}_{578} = +6.7°$ (c = 0.119, pentane).

Hydrogenation of (+)-viridiene **2**[3]). Compound **2** (0.8 mg, 5.48 µmol, $[\alpha]^{20}_{578} = +255°$) was hydrogenated as described for **1**. After the final purification by GC 70 µg (+)-**9b** were obtained. Because of the very low concentration only the positive sign of rotation could be stated for certainty.

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