95. Structure and Synthesis of (±)-Caudoxirene, a New Spermatozoid-Releasing and -Attracting Pheromone from the Marine Brown Alga *Perithalia caudata* (Phaeophyceae, Sporochnales)

Part V[1])

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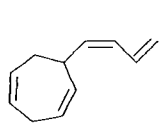
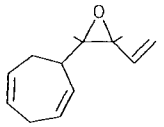
cis-*3-(*trans-*1,2-Epoxybut-3-enyl)-4-vinylcyclopentene* ((±)-**4**, caudoxirene) is a new gamete-releasing and gamete-attracting pheromone from the marine brown alga *Perithalia caudata* (Sporochnales). The key step of its synthesis is the diastereoselective alkylation of the aldehyde **8** with the [(phenylthio)allyl]titanium reagent **9** to yield the *erythro*-β-hydroxy sulfide **10** which affords **4** on sequential treatment with $Me_3O \cdot BF_4$ and aq. NaOH. The lowest effective concentration of (±)-**4** for gamete-release is found at 1.4×10^{-11} mol/l seawater.

Introduction. – Low-molecular olefinic hydrocarbons play a well recognized role as chemical messengers during the sexual fertilization of many marine brown algae. In particular, C_8- and C_{11}-hydrocarbons are released from female gametes into the aqueous environment to attract their conspecific males [2]. An even more sophisticated signal system has developed in the highly advanced Laminariales and some other brown algae. Presumably governed by the increasing light intensity of the beginning year [3], the female gametes leave their oogonia and start to settle on appropriate surfaces. After settlement, they secrete a chemical message which in turn liberates the males from their antheridia within a few seconds after perception of the signal. Then, the pheromonal activity of the compounds guides the male gametes to the 'calling' female [4] [2b]. The advantage of this coupled system is obvious: *i*) perfect synchronization of emitting and receiving cells, *ii*) the liberated male gamete can follow a preexisting signal trace. Up to now, this pheromone-induced spermatozoid liberation has been observed within the orders Desmarestiales, Laminariales, and Sporochnales [2b].

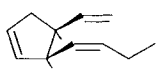
Irrespective of the relatively large number of the hitherto examined species, only a few general structures acting as gamete-releasing and/or gamete-attracting factors have been



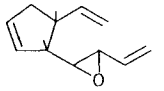
1 desmarestene



2 lamoxirene



3 (3*S*,4*S*)-multifidene



4 caudoxirene

[1]) Part IV: [1].

found: desmarestene (**1**) in two *Desmarestia* spp. [5]; the structurally related lamoxirene (**2**) in 11 species belonging to the families Laminariaceae, Alariaceae, and Lessoniaceae [6], multifidene (**3**) in *Chorda tomentosa* (Chordaceae) [7], and caudoxirene (**4**) in *Perithalia caudata* (Sporochnales) [8]. We now report the details of the identification of **4** and the confirmation of the previously assigned structure by synthesis of (±)-caudoxirene (**4**).

Identification of Caudoxirene. – Since all of the hitherto known pheromones of marine brown algae are volatile hydrocarbons or volatile derivatives thereof, fertile gametophyte cultures of *Perithalia caudata* (Victoria, Australia, and Tasmania) with freshly released eggs are extracted by the 'Closed-Loop-Stripping' technique as described in [2]. Desorption of the carbon filters (1.5 mg) with 30 µl CH_2Cl_2 results in a solution containing about 5–10 µg of the enriched volatiles. The exact details of the culture conditions, collection procedure, together with a tentative identification of **4** are given in [8]. GC Analysis (*Fig. 1*) shows the presence of three early eluting minor compounds **A** (8%), **B** (2%), **C** (4%), a major product **D** (84%) and, a closely eluting by-product **E** (2%).

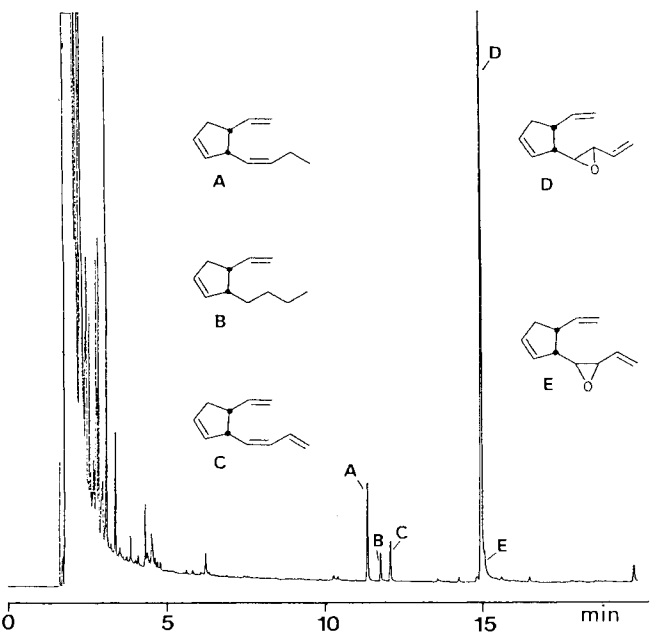


Fig. 1. *GC Separation of the trapped volatiles from* Perithalia caudata. Conditions: fused silica column *SE 30* (50 m × 0.25 mm). Temp. program: 50–200° with 4°/min. On-column injection, sample size 1 µl.

Further information is gained from MS which indicates molecular formulas of $C_{11}H_{16}$ (M^+ 148 Da) for compound **A**, $C_{11}H_{18}$ (M^+ 150 Da) for **B**, and $C_{11}H_{14}$ (M^+ 146 Da) for **C**. Compounds **D** and **E** display identical MS consistent with a molecular formula of $C_{11}H_{14}O$ (M^+ 162; *cf. Fig. 2*). Compounds **A**–**C** are readily identified as multifidene (**3** = **A**) [2], *cis*-3-butyl-4-vinylcyclopentene (=**B**) [7], and viridiene (**6** = **C**), the pheromone of *Syringoderma phinneyi* [9] and *Desmarestia viridis* [5]. The MS of **D** and **E**

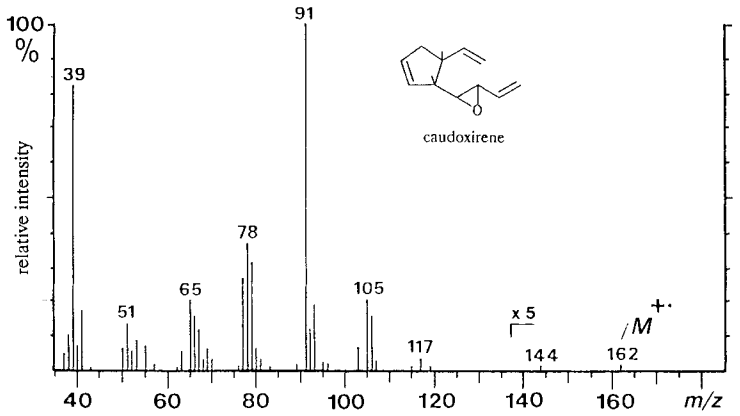
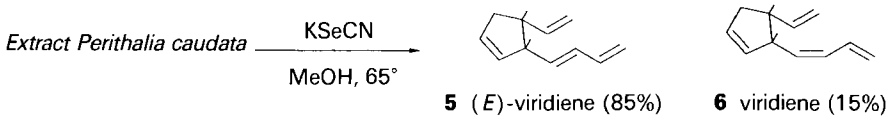


Fig. 2. *MS of (±)-4.* The fragmentation pattern is representative for other $C_{11}H_{14}O$ pheromones of marine brown algae. Conditions: *Finnigan Ion Trap, ITD 800* coupled with a *Carlo Erba Vega*, Series *2*, gas chromatograph. Transfer line at 270°; electron impact (70 eV); scan range: 35–250 Da/s.

(*Fig. 2*) are strongly reminiscent of lamoxirene (**2**) [10], and, hence, an epoxide moiety might be also present within these molecules. To obtain more information about the C-framework of **D** and **E**, the CH_2Cl_2 extract is concentrated under gentle stream of N_2 at 0° and treated for 2 h at 65° with a solution of KSeCN in MeOH (50 μg KSeCN in 20 μl MeOH).

In fact, **C** and **D** are smoothly deoxygenated and yield a mixture of 85% (*E*)-viridiene (**5**) and 15% of its (*Z*)-isomer **6** (= viridiene) [11]. Knowing the C-backbone and taking into account the virtually identical MS-fragmentation pattern of lamoxirene **2**, the epoxy group should be also located in the 1,2-position of the C_4-side chain (*Scheme 1*). Since

Scheme 1

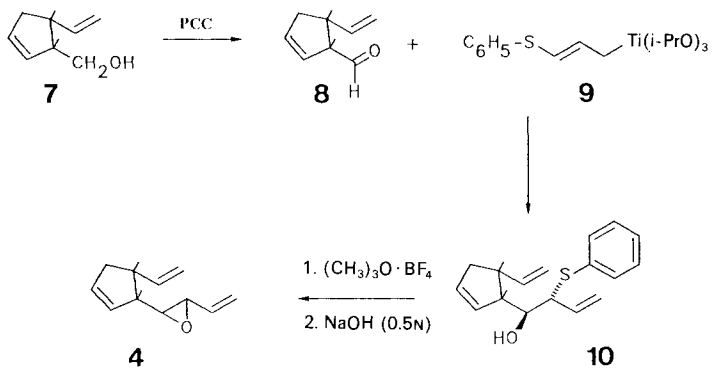


KSeCN is known to convert epoxides into olefines with net retention of the original configuration [12], the (*E*)-configuration of the viridiene isomer **5** is indicative for a *trans*-epoxide moiety in **4**. Due to the 85:15 composition of the deoxygenation products, compound **E** could represent the corresponding *cis*-epoxide (*vide infra*). The assigned structure of **D** is finally confirmed by synthesis as described.

2. Synthesis of (±)-Caudoxirene (4). – The C-framework of (±)-caudoxirene (**4**) is readily assembled from the cyclopentenylmethanol **7** [11] as outlined in *Scheme 2*. Oxidation of **7** with pyridinium chlorochromate yields the very sensitive aldehyde **8** which is immediately alkylated at – 78° with the [(phenylthio)allyl]titanium reagent **9** [13].

In agreement with a high *Cram*-selectivity, the bulky reagent **9** adds to the least hindered π-face of the carbonyl compound, and the *erythro*-β-hydroxy sulfide **10** is formed in excess (*ca.* 97% d.e., *ul*-addition). Medium-pressure chromatography on silica gel affords pure **10**. After conversion of **10** into the corresponding sulfonium salt by

Scheme 2



addition of 2.2 equiv. of $(CH_3)_3O \cdot BF_4$ in dry CH_2Cl_2, (±)-caudoxirene (**4**) is obtained after 1 h stirring of the solution with dil. NaOH (0.5N). Chromatography on SiO_2 (pentane/Et_2O 98:2) gives the new pheromone (±)-**4** in 74% overall yield from **10**. The analytical data (GLC and GLC/MS) of synthetic (±)-**4** are in complete agreement with those of the natural product.

To check the stereochemical course of the deoxygenation reaction (*vide supra*) with a sample of defined configuration, the pure synthetic pheromone (±)-**4** ($\geq 98\%$ *trans*-epoxide) is treated with KSeCN in MeOH in the same way as decribed for the natural products. Subsequent GC analysis of the reaction products indicates the presence of (*E*)- and (*Z*)-viridiene in the ratio of 90:10. Comparing these data with that of the deoxygenation of the natural products (85:15), it follows immediately that compound **E** has to be the (*Z*)-isomer of caudoxirene.

3. Biological Activity of (±)-Caudoxirene (4). – The biological activity of (±)-**4** is determined as described previously [8]. For gamete release, a decadic dilution series (1 mmol → 1 pmol) of synthetic (±)-**4** is prepared in *FC 72* (fluorocarbon, *3M Company*, Düsseldorf, FRG). Microdroplets (0.1 µl) are placed into the close vicinity to fertile male antheridia of *Perithalia caudata*. At the highest concentrations (mmol → µmol), immediate mass release of male gametes occurs within *ca.* 20 s. The lowest effective concentration of (±)-**4** in *FC 72* is found at 0.3 nmol/l. Based on the experimentally determined partition coefficient of (±)-**4** for the system *FC 72*/seawater ($K_{FC\ 72/seawater} = 21 \pm 2.5$), this corresponds to an effective threshold concentration of 1.4×10^{-11} mol/l in the aqueous interphase. This fits well with other communication systems of marine brown algae which are usually found in the range of $10^{-9} \rightarrow 10^{-12}$ mol/l seawater [2]. Data on the chemotatic activity of (±)-**4** and male gametes of *P. caudata* are as yet not available.

The absolute configuration and the enantiomeric excess of **4** are currently established by an asymmetric synthesis and will be reported in due course.

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Experimental Part

General. Reactions are performed under Ar. Solvents and reagents are purified and dried prior to use. Anh. $MgSO_4$ is used for drying operations. Solns. are usually concentrated by flash evaporation under reduced pressure. Anal. GLC: *Carlo Erba* gas chromatograph, *HRGC 5300, Mega* series, equipped with fused-silica capillaries, *SE 30* (10 m × 0.31 mm); H_2 at 30 cm/s served as carrier gas. IR (cm^{-1}): *Perkin-Elmer 882* IR spectrophotometer. ^{1}H-NMR (250 MHz or 400 MHz, $CDCl_3$, TMS as internal standard): *Bruker Cryospec WM 250* and *Bruker WM 400*. MS (*m/z*): *Finnigan MAT 90* GLC/MS system and *Finnigan ITD 800* combined with a *Carlo Erba* gas chromatograph, model *Vega*, equipped with a fused silica capillary *OV 101* (10 m × 0.32); He at 30 cm/s as carrier gas. Scan range: 35–249 Da/s.

cis-*5-Vinylcyclopent-2-ene-1-carbaldehyde* (**8**). Pyridinium chlorochromate (2.7 g, 12.5 mmol) is added, under stirring at 0°, to a soln. of **7** (0.62 g, 5.0 mmol) in the same solvent (100 ml). The soln. turns dark, and, after 6 h, another portion of the oxidant (1.07 g, 5 mmol) is added. Stirring is continued for further 2 h followed by addition of pentane (80 ml) and $MgSO_4$ (*ca.* 1 g). The precipitated and adsorbed chromium salts are removed by filtration, and the soln. is evaporated *i.v.* A second addition of pentane and $MgSO_4$ removes last admixtures of the chromium salts, and, after evaporation of solvents *i.v.*, the crude aldehyde is ready for the alkylation. MS (70 eV): 122 (3, $M^{+\cdot}$), 107 (4), 105 (5), 93 (28), 91 (92), 79 (41), 77 (100), 66 (39), 65 (40), 51 (30), 39 (92).

cis-*1-(5-Vinylcyclopent-2-enyl)-2-(phenylthio)but-3-en-1-ol* (**10**). To a cooled soln. (– 78°) of allylphenyl sulfide (2.25 g, 15 mmol) in dry THF (100 ml) is added under stirring BuLi (6.0 ml, 15.0 mmol; 2.5M soln. in hexane). The soln. is allowed to come to r.t., at which the yellow color turns red. Then, the soln. is cooled (–78°), and $Ti(i\text{-}PrO)_4$ (4.5 ml, 15.0 mmol) is added. After 15 min, **8** (5 mmol) is injected, and stirring is continued for 10 min at – 78° and 1 h at 0°. The mixture is hydrolyzed with 2N HCl (50 ml). The aq. phase is saturated with NaCl and extracted with Et_2O (2 × 50 ml). The combined org. layer is washed with sat. $NaHCO_3$ and NaCl solns. (30 ml), dried, and evaporated *i.v.* The crude product is purified by CC on silica gel (pentane/Et_2O 95:5) and affords 0.723 g (53%) of 10 as a mixture of *erythro*- and *threo*-diastereoisomers (*ca.* 97:3). The pure *erythro*-β-hydroxysulfide **10** is obtained by MPLC on SiO_2 (pentane/Et_2O 9:1). IR (film): 3475*s* (br.), 3075*s*, 2979*m*, 2922*s*, 2849*m*, 1634*m*, 1538*m*, 1479*m*, 1438*s*, 1419*m*, 1066*s*, 1003*s*, 915*s*, 742*s*, 690*s*. ^{1}H-NMR ($CDCl_3$)[2]): 7.41 (*m*, 2 arom. H); 7.17 (*m*, 3 arom. H); 5.98–5.79 (*m*, H–C(2), H–C(5), H–C(6), H–C(10)); 5.14 (*dd*, ^3J = 10.5, ^2J = 1.4, 1 H–C(1 or 11)); 5.09 (*dd*, ^3J = 17.2, ^2J = 1.8 1 H–C(1 or 11)); 5.06 (*dd*, ^3J = 16.5, ^2J = 1.4 1 H–C(1 or 11)); 4.99 (*dd*, ^3J = 9.8, ^2J = 1.8 1 H–C(1 or 11)); 3.78–3.69 (*m*, H–C(8), H–C(9)); 3.09–3.04 (*m*, 1 H–C(7)); 2.97–2.89 (br. *quint.*, 1 H–C(3)); 2.55–2.45 (*m*, 1 H–C(4)); 2.25–2.19 (*m*, 1 H–C(4)); 2.13 (*d*, ^3J = 4.3, OH). ^{13}C-NMR ($CDCl_3$): 139.7 (C(2)); 134.7 (C(10)); 133.4 (arom. (C)); 133.1 (arom. (C)); 132.9, 129.6 (C(5), C(6)); 128.8 (arom. (C)); 127.5 (arom. (C)); 118.4 (C(11)); 115.5 (C(1)); 71.8 (C(8)); 57.3 (C(9)); 51.5 (C(7)); 45.3 (C(3)); 39.1 (C(4)). MS (70 eV): 272 (1, $M^{+\cdot}$), 258 (3), 179 (2), 161 (8), 150 (68), 149 (50), 145 (28), 135 (72), 117 (21), 116 (25), 115 (26), 109 (37), 105 (27), 93 (22), 91 (87), 79 (27), 77 (94), 69 (32), 65 (59), 51 (36), 41 (69), 39 (100). HR-MS: 272.1237 ($C_{17}H_{20}SO$, $M^{+\cdot}$, calc. 272.12348).

cis-*3-(*trans-*1,2-Epoxybut-3-enyl)-4-vinylcyclopentene* ((±)-4; *caudoxirene*). A soln. of **10** (0.05 g, 0.18 mmol) and $Me_3O \cdot BF_4$ (0.06 g, 0.41 mmol) in dry CH_2Cl_2 (10 ml) is stirred for 2 h at r.t. Then, 0.5N NaOH (10 ml) is added, and efficient stirring is maintained for 1 h. The org. layer is separated, washed with brine, and dried. Removal of solvents and CC on silica gel (pentane/Et_2O 98:2) yields 22.1 mg (74%) of (±)-4 as a colorless liquid. IR (film): 3062*s*, 2982*s*, 2921*s*, 2849*s*, 1639*s*, 1612*m*, 1445*m*, 1421*m*, 1404*m*, 1233*m*, 987*s*, 915*s*, 882*s*, 805*m*, 733*s*, 663*m*. ^{1}H-NMR ($CDCl_3$)[2]): 5.95 (*ddd*, ^3J = 16.8, 10.5, 9.0, 1 H–C(2)); 5.88, 5.76 (*m*, 2 H–C(5,6)); 5.56 (*ddd*, ^3J = 17.2, 9.8, 7.5, 1 H–C(10)); 5.44 (*dd*, ^3J = 17.2, ^2J = 1.5, 1 H–C(11)); 5.24 (*dd*, ^3J = 9.8, ^2J = 1.5, 1 H–C(11)); 5.09 (*dd*, ^3J = 16.8, ^2J = 1.7, 1 H–C(1)); 5.03 (*dd*, ^3J = 10.5, ^2J = 1.7, 1 H–C(1)); 3.11 (*d*, *J* = 7.3, 1 H–C(9)); 3.06 (*quint.*, *J* = 8.4, 1 H–C(3)); 2.73 (*dd*, ^3J = 7.4, 2.2, 1 H–C(8)); 2.57 (*t*, *J* = 7.6 1 H–C(7)); 2.54–2.45 (*m*, 1 H–C(4)); 2.37–2.27 (*m*, 1 H–C(4)). ^{13}C-NMR ($CDCl_3$): 139.0 (C(2)); 135.6 (C(10)); 132.5, 130.3 (C(5), C(6)); 118.9 (C(11)); 115.3 (C(1)); 61.2 (C(8)); 58.1 (C(9)); 51.4 (C(7)); 45.5 (C(3)); 37.9 (C(4)). MS (70 eV): 162 (0.05, $M^{+\cdot}$), 144 (0.05), 131 (2), 129 (2), 117 (3), 105 (20), 93 (18), 91 (100), 79 (33), 78 (38), 77 (27), 67 (12), 66 (16), 65 (21), 55 (8), 51 (14), 41 (18), 39 (83). HR-MS: 162.1027 ($C_{11}H_{14}O$, $M^{+\cdot}$, calc. 162.10446).

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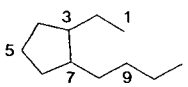
[2]) C-Atom numbering:



*Determination of the Partition Coefficient of (±)-***4** *between* FC 72 *and Seawater.* A stock soln. (400 µl) of (±)-**4** in *FC 72* (1.5–2.2 mg/1000 µl, *3M Company*, Düsseldorf, FRG) and seawater (6.0 ml), previously saturated with *FC 72*, are efficiently stirred for 3 h. The samples are centrifuged, and the lower *FC 72* phase is separated. The aq. layer (3.0 ml) is re-extracted by partitioning against CCl_4 (200 µl) as described above. The two org. layers are analyzed by GLC, and the partition coefficient ($K_{FC\ 72/seawater}$) for (±)-**4** is calculated from the relative peak areas, corrected for the dilution factors. The average of several runs results in $K_{FC\ 72/seawater} = 21 \pm 2.5$.

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