Highly Diastereoselective, Biogenetically Patterned Synthesis of (+)-(1*S*,6*R*)-Volvatellin (=(+)-(4*R*,5*S*)-5-Hydroxy-4-(5-methyl-1-methylenehex-4-en-2-ynyl)cyclohex-1-ene-1-carbaldehyde)

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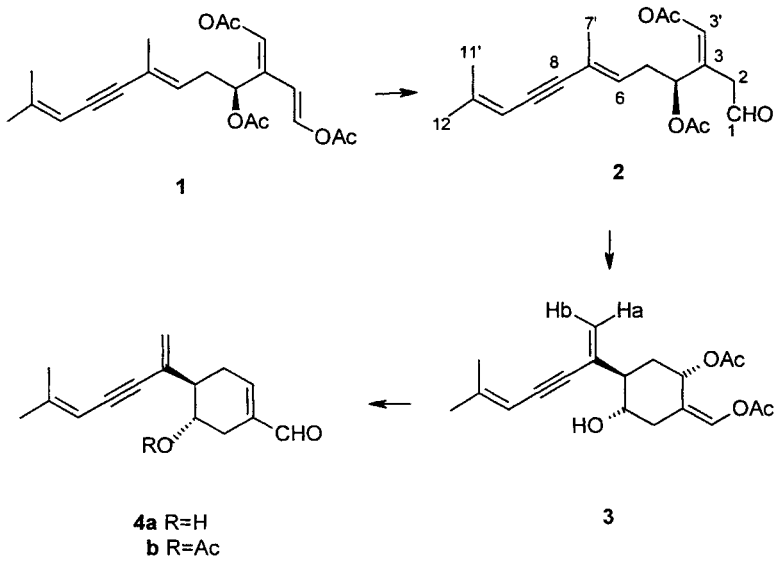
The synthesis of volvatellin (**4a**), previously isolated from a herbivorous marine mollusk, was achieved with high diastereoselectivity from putative dietary oxytoxin-1 (**2**). A biogenetically patterned carbonyl-ene route was chosen, proceeding from **2** predominantly *via* the *trans* cyclization product **3** without the use of enzymes. This challenges the involvement of enzymes in the formation of **4a** in nature. The optical purity and absolute configuration (1*S*,4*S*,6*R*), assigned to **3** from high-field ^{1}H-NMR examination of its *Mosher* (MTPA) esters **6**, was retained on its chemical conversion to (+)-(1*S*,6*R*)-configured **4a** and is consistent with the (4*S*) configuration previously established for caulerpenyne (**1**).

1. Introduction. – Coevolution of seaweeds which produce distasteful metabolites as a protection against herbivorous grazers, and shell-less opisthobranch mollusks which use such algal products for their own defense, finds experimental support [1]. However, apart from clear-cut cases of uptake of algal metabolites, the role of these mollusks as chemical machineries, either in transforming the algal products to tune up their effects, or in *de novo* synthesis of distasteful metabolites, is far from being clear [2].

Our work presented here challenges the hypothesis of the evolution of the enzyme system of these mollusks in a specific, representative instance. This concerns the sesquiterpenoid volvatellin (**4a**), which co-occur with caulerpenyne (**1**), which is presumably taken up by the opisthobranch mollusk *Volvatella* sp. from the Indian coast of the Bay of Bengal from the algal diet, and which was proposed to be formed enzymatically from caulerpenyne-derived oxytoxin-1 (**2**) *via* the elusive intermediate **3** (*Scheme 1*) [3].

2. Results and Discussion. – For the projected synthesis of volvatellin (**4a**), we had at hand both caulerpenyne (**1**) and oxytoxin-1 (**2**), recently isolated alongside glycoglycerolipids, and α-keto esters from a Mediterranean sample of the green alga *Caulerpa taxifolia* [4a]. On the other hand, it has already been shown that **2** may be obtained by the nonenzymatic transformation of **1** [4b]. Thus, oxytoxin-1 (**2**) was treated with *Lewis* acids to give the *trans*-cyclization product **3** and its *cis*-diastereoisomer **5** (*Scheme 2*) in good overall yields (*ca.* 70%, based on isolated products after HPLC purification) and relative ratios that depended on the acidic system used. In any case, the **3**/**5** ratio was always in favor of the *trans* product, as was preliminarily established by integration of the H–C(1) NMR signals of the crude

Scheme 1. *Hypothetical Biosynthesis of Volvatellin* (**4a**) *from Dietary Caulerpenyne* (**1**) *in a Herbivorous Mollusk*



mixture and then confirmed by analysis of the isolated products. Thus, a **3/5** selectivity ratio of 85:15 was obtained with either $ZnBr_2$ at room temperature or $EtAlCl_2$ at $-78°$. That the latter reaction is kinetically controlled was shown by comparative data obtained at room temperature (see *Exper. Part*)[1]); in agreement, **5** remained unchanged in the presence of $ZnBr_2$ under stirring at r.t. for 8 h.

trans-Configuration of the substituents at the newly formed C(1)–C(6) bond of **3**, and *cis*-configuration for **5**, as well as the axial position for AcO–C(4), firmly rest on the *J* values for H–C(1), H–C(6), and H–C(4) (see *Exper. Part*)[2]). The (*Z*)-configuration at the enol acetate double bond was established by a NOE enhancement observed between H–C(3′) and H_{eq}–C(2). The side-chain conformation (see derivative **6** in *Scheme 2*) is supported by a NOE enhancement observed between the signals at 5.40 and 2.55 ppm (H–C(7′) and H–C(6), resp.; see *Exper. Part*). MM Calculations correctly simulated this situation.

While volvatellin (**4a**) proved unstable [3], compound **3** – being stable and in the middle of the synthetic sequence (*vide infra*) – looked promising for assignment of the absolute configuration for all the compounds involved. Thus, the α-methoxy-α-(trifluoromethyl)benzeneacetates (MTPA) **6a** and **6b** were prepared from **3** and either (–)-(*R*)-MTPA-Cl or (+)-(*S*)-MTPA-Cl, respectively, as single diastereoisomers (*Scheme 2* and *Exper. Part*), thus establishing the enantiomer purity of **3**. Examination of **6a** and **6b** according to the high-field ^{1}H-NMR *Mosher* method [5] allowed us to

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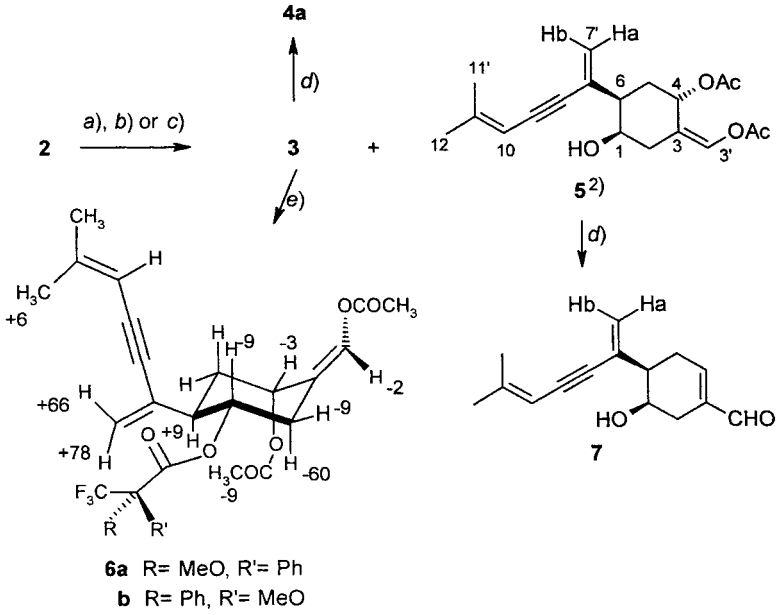
[1]) Using the protic acid *p*-toluenesulfonic acid monohydrate, the selectivity ratio **3/5** was 55:45 after ½ h at room temperature.

[2]) Arbitrary numbering; for systematic names, see *Exper. Part*.

Scheme 2. *Carbonyl-Ene Reaction of Oxytoxin-1* (**2**) *to* **3** *and* **5**



a) $ZnBr_2$, $CHCl_3$, r.t., 8 h; 70%, **3**/**5** 85 : 15. *b*) $EtAlCl_2$, CH_2Cl_2, −78°, 1 h; 74%, **3**/**5** 85 : 15. *c*) $EtAlCl_2$, CH_2Cl_2, r.t., 5 min, 72%, **3**/**5** 7 : 3. *d*) K_2CO_3, MeOH, r.t., 5 min; 90%. *e*) (−)-(*R*)-MTPA-Cl or (+)-(*S*)-MTPACl, DMAP, pyridine, r.t., overnight; 88%.

assign the absolute configuration (1*S*,4*S*,6*R*)[2]) to **3**[3]), in agreement with the configuration (4*S*) attributed to caulerpenyne (**1**) [6].

Formation of **3** and **5** only, out of four possible diastereoisomers in the carbonyl-ene reactions [7] of **2** (*Scheme 3*), with a bias towards **3**, warrants some comment. The 'axial' position of AcO−C(4)[2]) in folded conformations[4]) of **2** – which alleviates the 1,3-allylic strain [8][9] of the 'exo'-methylene and acetoxy groups – leads to four unequally populated conformations **2a**–**d**. Treating the carbonyl-ene reaction as a concerted process [7], it is only from **2a** and **2b** that formation of an incipient chair-like cyclohexene ring can be imagined (rate-limiting formation [7] of transition states **2a**[≠]

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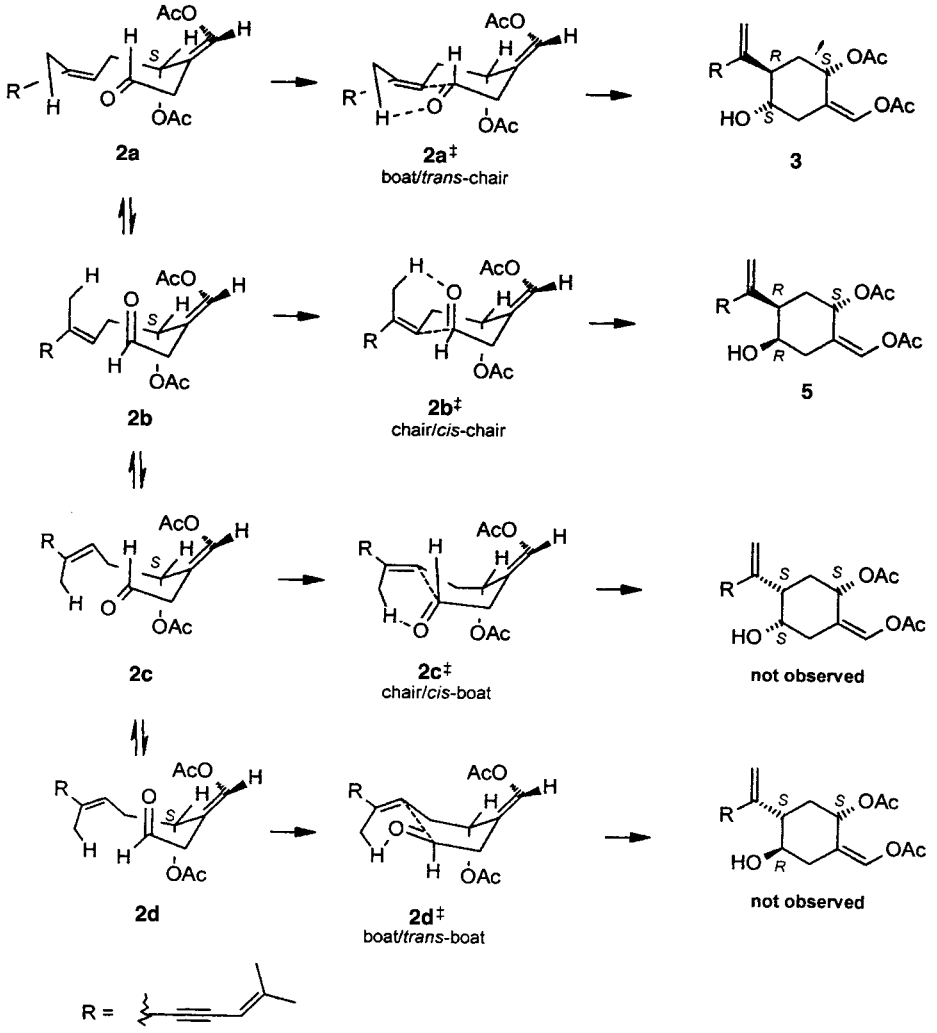
[3]) A conformational analysis with the aid of MM calculations was carried out for the conformers derived by rotation around the relevant bonds. Thus, in the least-strain-energy conformation of **6a**, approximately depicted in *Scheme 2*, the C=O and C−CF_3 bonds deviate by +12 and −64°, respectively, from the ideal *Mosher* plane that should align them with the H−C(1) bond [5]. For **6b**, corresponding values of −16° and +60° were calculated. Although noticeable, this deviation of the C−CF_3 bond from the ideal plane does not invalidate the *Mosher* analysis which, according to widely established models [5], could be carried out with confidence in this case. It should also be mentioned that no splitting or broadening of the ^{1}H-NMR signals of the diastereoisomer **5** could be noticed on addition of the chiral shift reagent [Yb(hfbc)$_3$] (see *Exper. Part*). Even though not a proof *per se*, this is in line with the conclusions from the *Mosher* experiment as to the enantiomer purity of **3**.

[4]) MM Calculations suggested that elongated conformations entail less strain energy than folded conformations, **2c** and **2d** turning out to be less strained than **2a** and **2b**. This implies that the product distribution is not controlled by the relative strain of the least-strained conformers and, therefore, **2a** and **2b** may be termed reactive conformations.

Scheme 3. *Suggested Pathways for the Carbonyl-Ene Reaction of Oxytoxin-1* (**2**)



and **2b**‡). The corresponding transition states from **2c** and **2d** (**2c**‡ and **2d**‡) are in boat-like incipient cyclohexene forms, which may explain why these two routes are disfavored[5]). That formation of **3** is favored with respect to **5** is compatible with the formal boat-like H-transfer in **2a**‡ and chair-like-transfer in **2b**‡: the cases may be reconciled by viewing the incipient carbocyclic ring as intermediate between a cyclohexene and a cyclohexadiene form, where the classical energy values for chair/

[5]) Chair-like incipient cyclohexene rings for transition states **2c**‡ and **2d**‡ could also be drawn. However, they would be very highly strained because of 1,3 repulsive interactions between the Me–C(7) and pseudo-axial AcO–C(4) groups.

boat situations largely lack validity. Moreover, the route from **2a** goes through a *trans*-decaline structure, which is favored with respect to the *cis*-decaline structure of the route from **2b**[6]), in agreement with *a*) the preference for *trans* products in *ab initio* calculations for the carbonyl-ene reaction of formaldehyde with propene [7c] and *b*) observations for similar disubstituted (*E*)-C(6)=C(7) substrates [7d][7]).

On treatment with K_2CO_3 in MeOH at room temperature, **3** gave immediately dextrorotatory **4a**, which is thus assigned the absolute configuration (1*S*,6*R*)[2]). ^{1}H-NMR Spectra that are nicely superimposable with those of the natural volvatellin [3] were observed for the semisynthetic **4a**. The transformation **3** → **4a** was amazingly clean[8]), provided that evaporation to dryness was avoided in the presence of K_2CO_3, which would have triggered degradation of **4a**.

It should be noted that optical rotations previously reported for both natural volvatellin (**4a**) and its derivative **4b** [3] are opposite in sign and larger in absolute value with respect to our values (see *Exper. Part*). Our products are enantiomerically pure, and the discordance of the chiroptical data for **4a** can not be imputed to the different solvents used since the discordance of chiroptical data is similar for **4b**, where the same solvent was used. Moreover, our data relate to a sequence of products that, starting from (4*S*)-caulerpenyne (**1**), yielded pure **3**, the absolute configuration of which was independently determined. Therefore, we suggest that previous chiroptical data [3] might have suffered from difficulties in the separation of the natural product from highly optically active contaminants and in weighing the noncrystalline compounds available in minute amounts. In any event, no matter what we have obtained – either naturally occurring volvatellin or its enantiomer, a question that the published data [3] do not answer – our conclusion remains that **4a** is formed from **2** *via* a highly

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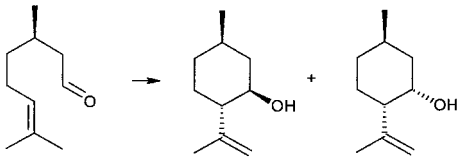
6) MM Calculations indicated that **3** is definitely less strained (by 0.8 or 1.5 kcal mol^{-1} according to the force field used, MM2 or MM3, resp.) than **5**.

7) Parallel experiments were devised to become experimentally acquainted with the carbonyl-ene reaction. We chose the reaction of (+)-(*R*)-citronellal (**8**) since its carbonyl-ene conversion to (−)-isopulegol (**9**) is at the basis of the industrial preparation of (−)-menthol, and thus, data for various *Lewis* and protic acids [10] as well as superacids [11] as catalysts are available. Albeit more reactive than **2** in giving **3** and **5**, (+)-(*R*)-citronellal (**8**) showed, under similar conditions [7d][10], much the same diastereoselectivity of ring closure to **9** and **10** (*Scheme 4*; see *Exper. Part*). However, the similar behavior of **8** and **2** may be fortuitous since in **8**, the H-transfer takes place predominantly from the Me group in *trans* position to the C-chain.

Scheme 4. *Carbonyl-Ene Reaction of (+)-(*R*)-Citronellal* (**8**). Conditions similar to those in *Scheme 2.*



8 **9** **10**

8) Addition of solid K_2CO_3 to a solution of either **3** or **5** in CD_3OD in a NMR tube allowed the observation, within a few minutes, of the ^{1}H-NMR spectrum of **4a** or of the diastereoisomeric aldehyde **7**, respectively, each one not contaminated by the other one arising from complete hydrolysis of the corresponding precursor (see *Exper. Part*). This further established the enantiomer purity of the compounds used and obtained here, in agreement with the text and *Footnote 3*.

diastereoselective intramolecular carbonyl-ene cyclization. This challenges in this case the hypothesis of the evolution of the enzyme system to transform dietary products in the mollusk [3], where the presence of biological membranes and interfaces makes it difficult to imagine and to simulate the state of the acids presumptively involved in the carbonyl-ene reaction described here.

Experimental Part

1. *General*. Yields are given in terms of substrates reacted. All evaporations were carried out under reduced pressure. Flash chromatography (FC): *Merck Si-60* (15–25 μm). TLC: *Merck* silica gel *60 PF_{254}* and *Merck RP-18 F_{254}*. Prep. HPLC: *Merck LiChrospher Si60*, 25 × 1 cm columns; UV monitoring at λ 254 nm and solvent flux 5 ml min^{-1}, if not otherwise stated; t_R in min. Optical rotation: *JASCO-DP-181* polarimeter, the integrity of which was positively checked with known optically active compounds of high purity; $[\alpha]$ in deg cm^2 g^{-1}. NMR Spectra: *Varian-XL-300* spectrometer, ^1H at 299.94 and ^{13}C at 75.43 MHz; in $CDCl_3$, if not otherwise stated; δ in ppm rel. to $SiMe_4$ (=0 ppm) and J in Hz; multiplicities from DEPT; $^1H,^1H$ correlations from COSY60 and selective decoupling irradiations; $^1H,^{13}C$ assignments from $^1H,^{13}C$-COSY; NOE (=1D differential NOE) reported as 'irradiated proton → NOE observed on the proton(s)'. EI-MS: *Kratos-MS80* mass spectrometer equipped with a home-built computerized data system. MM Calculations: programs PCMODEL 4.0 from *Serena Software*, Bloomingtone, Indiana, and MM3(96) from *QCPE*, Indiana University.

2. *Isolation of Compounds.* Oxytoxin-1 (**2**) was obtained by HPLC purification of a 2:1 mixture with caulerpenyne (**1**), as contained in *Fr. 11* from FC (hexane/AcOEt/MeOH gradient) of the residue from evaporation of the EtOH extracts of *Caulerpa taxifolia* from Elba Island [4a]).

3. *Cyclizations of Oxytoxin-1* (**2**). 3.1. *With $ZnBr_2$*. To a soln. of **2** [4a] (20.0 mg, 0.06 mmol) in either $CHCl_3$ or CH_2Cl_2 (3 ml), $ZnBr_2$ (1 equiv.) was added at r.t., and stirring was continued for 8 h until all **2** had disappeared (TLC monitoring). The mixture was then evaporated and the residue submitted to FC (SiO_2, $CHCl_3/Et_2O$ 1:1; TLC monitoring). The eluate of interest was subjected to HPLC purification (hexane/AcOEt 64:36): **3** (t_R 8.3; 11.9 mg, 60%) and **5** (t_R 6.6; 2.1 mg, 10%).

3.2. *With $EtAlCl_2$*. The reagents were mixed as described in *3.1*, except that $EtAlCl_2$ was used as *Lewis* acid and CH_2Cl_2 as solvent and stirring at −78° for 1 h. The crude mixture was subjected to HPLC to give **3** (12.6 mg, 63%) and **5** (2.2 mg, 11%). The latter reaction was also carried out at r.t. for 5 min, under otherwise identical conditions, to give **3** (10.1 mg, 51%) and **5** (4.3 mg, 21%).

(1S,2R,4S,5Z)-5-[(Acetyloxy)methylene]-2-(5-methyl-1-methylenehex-4-en-2-ynyl)cyclohexane-1,4-diol 4-Acetate (**3**): colorless oil. $[\alpha]_D^{22} = +14.6$ ($c = 0.7$, $CHCl_3$). ^{1}H-NMR[2]): 3.63 (*ddd*, $J = 5.2$, 9.9, 11.2, H−C(1)); 2.31 (*ddd*, $J = 2.2$, 11.2, 13.1, H_{ax}−C(2)); 2.44 (*dd*, $J = 4.4$, 13.1, H_{eq}−C(2)); 5.92 (*t*, $J = 2.8$, H−C(4)); 1.78 (*ddd*, $J = 2.8$, 12.9, 15.0, H_{ax}−C(5)); 2.00 (*ddd*, $J = 2.8$, 3.9, 15.0, H_{eq}−C(5)); 2.55 (*ddd*, $J = 3.9$, 9.9, 12.6, H−C(6)); 5.35 (*sept.*, $J = 1.3$, H−C(10)); 1.88 (*s*, 3 H−C(12)); 7.00 (*d*, $J = 2.1$, H−C(3′)); 5.40 (*d*, $J = 1.9$, H_a−C(7′)); 5.46 (*d*, $J = 1.9$, H_b−C(7′)); 1.81 (*s*, 3 H−C(11′)); 2.05 (*s*, AcO−C(4)); 2.16 (*s*, AcO−C(3′)). ^{13}C-NMR[2]): 71.08 (*d*, C(1); 33.44 or 34.24 (*t*, C(2)); 118.75 (*s*, C(3)); 64.97 (*d*, C(4)); 34.24 or 33.44 (*t*, C(5)); 48.38 (*d*, C(6)); 131.70 (*s*, C(7)); 90.03 (*s*, C(8)); 88.56 (*s*, C(9)); 104.83 (*d*, C(10)); 149.70 (*s*, C(11)); 24.88 (*q*, C(12)); 131.56 (*d*, C(3′)); 123.14 (*t*, C(7′)); 21.20 (*q*, C(11′)); 169.98 (*s*, Me*C*OO−C(4)); 167.89 (*s*, Me*C*OO−C(3′)); 20.07 (*q*, *Me*COO−C(4)); 21.27 (*q*, *Me*COO−C(3′)). NOE[2]): 3.63 (H−C(1)) → H_{eq}−C(2), H_{ax}−C(5), 5.92 (H−C(4)) → H_{ax}−C(5); 5.36 (H−C(10)) → 3 H−C(11′); 7.00 (H−C(3′)) → H_{eq}−C(2); 5.40 (H−C(7′)) → H−C(6). MS: 332 (3, $M^{+\cdot}$), 290 (2.6, $[M - CH_2CO]^{+\cdot}$), 272 (1.8, $[M - CH_3COOH]^{+\cdot}$), 230 (9.3, $[M - CH_2CO - CH_3COOH]^{+\cdot}$), 212 (9), 201 (7.2), 183 (10), 169 (8.4), 43 (100). HR-MS: 332.1621 ± 0.002 ($C_{19}H_{24}O_5^{+\cdot}$; calc. 332.1624).

(1R,2R,4S,5Z)-5-[(Acetyloxy)methylene]-2-(5-methyl-1-methylenehex-4-en-2-ynyl)cyclohexane-1,4-diol 4-Acetate (**5**): colorless oil. $[\alpha]_D^{22} = -5.3$ ($c = 0.2$, $CHCl_3$). ^{1}H-NMR[2]): 4.26 (*q*, $J = 2.2$, H−C(1)); 2.56 (*dd*, $J = 3.3$, 14.4, H_{ax}−C(2)); 2.28 (*dd*, $J = 3.3$, 14.4, H_{eq}−C(2)); 6.05 (*t*, $J = 2.9$, H−C(4)); 2.03 (*ddd*, $J = 2.9$, 12.6, 15.4, H_{ax}−C(5)); 1.88 (*ddd*, $J = 2.9$, 3.7, 15.1, H_{eq}−C(5)); 2.73 (br. *ddd*, $J = 2.2$, 3.7, 12.6, H−C(6)); 5.36 (*sept.*, $J = 1.3$, H−C(10)); 1.90 (*s*, 3 H−C(12)); 6.99 (*d*, $J = 2.2$, H−C(3′)); 5.49 (br. *s*, H_a−C(7′)); 5.24 (br. *s*, H_b−C(7′)); 1.82 (*s*, 3 H−C(11′)); 2.04 (*s*, AcO−C(4)); 2.16 (*s*, AcO−C(3′)). ^{1}H-NMR Experiment with [Yb(hfbc)$_3$]: to a soln. of **5** in $CDCl_3$ (0.6 ml) was added 0.042M [Yb(hfbc)$_3$] in $CDCl_3$ in 5-μl portions; at 0.2 mol-equiv. of [Yb(hfbc)$_3$], the following downfield shifts were observed[2]): 0.9 ppm for H−C(1), 0.6 ppm for H−C(4), 0.4 ppm for H_{ax}−C(5), and 0.3 ppm for both 2 H−C(2) and H−C(6); no doubling or broadening of either these or other

signals could be noticed. ^{13}C-NMR[2]): 67.92 (*d*, C(1)); 28.51 (*t*, C(2)); 116.27 (*s*, C(3)); 65.46 (*d*, C(4)); 33.04 (*t*, C(5)); 42.78 (*d*, C(6)); 132.40 (*s*, C(7)); 90.80 (*s*, C(8)); 89.19 (*s*, C(9)); 104.76 (*d*, C(10)); 149.74 (*s*, C(11)); 24.92 (*q*, C(12)); 132.12 (*d*, C(3′)); 121.16 (*t*, C(7′)); 21.22 (*q*, C(11′)); 170.02 (*s*, Me*C*OO−C(4)); 167.71 (*s*, *Me*COO−C(3′)); 20.70 (*q*, *Me*COO−C(4)); 21.31 (*q*, *Me*COO−C(3′)). EI-MS: 332 (2, $M^{+\cdot}$), 290 (1, [M − CH_2CO]$^{+\cdot}$), 272 (1.5, [M − CH_3COOH]$^{+\cdot}$), 230 (7.5, [M − CH_2CO − CH_3COOH]$^{+\cdot}$), 212 (9), 201 (7), 183 (10), 169 (7), 43 (100).

4. *Cyclizations of (+)-(*R*)-Citronellal* (**8**). 4.1. *With $ZnBr_2$*. To a soln. of **8** (30.0 mg, 0.19 mmol) in CH_2Cl_2 (4 ml), $ZnBr_2$ (1 equiv.) was added at r.t. and stirring was continued for 30 min until all **8** had disappeared (TLC monitoring). Precipitated $ZnBr_2$ was filtered off and the eluate evaporated: **9**/**10** 9:1, as established by integration of ^{1}H-NMR signals [10].

4.2. *With $EtAlCl_2$*. The reagents were mixed as described in *4.1.*, except for using $EtAlCl_2$ as *Lewis* acid. After a few minutes, complete conversion of **8** to **9**/**10** 7:3 was observed.

5. *Alkaline Hydrolysis of* **3** *and* **5**. 5.1. To a soln. of **3** (2.3 mg, 0.007 mmol) in MeOH (2 ml), solid K_2CO_3 was added. Complete conversion of **3** to volvatellin (**4a**) was observed (TLC monitoring) within a few minutes. The mixture was passed through a strong anion-exchanger (*Merck LiChrolut SAX* (500 mg), H_2O (elimination of salts), then MeOH): **4a** (2.1 mg, 90%). HPLC (hexane/AcOEt 96:4, flow 1 ml min^{-1}; λ 230 nm) gave *(4*R,5S*)-5-hydroxy-4-(5-methyl-1-methylenehex-4-en-2-ynyl)cyclohex-1-ene-1-carbaldehyde* (**4a**; t_R 9.2) of high purity for polarimetric measurements. $[\alpha]_D^{20} = +7$, $[\alpha]_{577}^{20} = +43$ ($c = 0.1$, $CHCl_3$) ([3]: $[\alpha]_D = -88.3$ ($c = 0.04$, Et_2O)). ^{1}H-NMR and low-resolution MS: in accordance with [3]. HR-MS: 230.1302 ± 0.002 ($C_{15}H_{18}O_2^+$; calc. 230.1307).

5.2. On addition of solid K_2CO_3 to a soln. of **3** or **5** (2 mg) in CD_3OD (0.6 ml) in a NMR tube, clean and complete formation of **4a** or the diastereoisomeric aldehyde **7**, respectively, was observed. ^{1}H-NMR (CD_3OD; only the most relevant spectral differences are reported[2]): 3.90 (*dt*, $J = 5.5$, 9.1, H−C(1) (**4a**)); 4.40 (*dt*, $J = 3.6$, 2.6, H−C(1) (**7**)); 6.88 (*m*, H−C(4) (**4a**)); 6.97 (*m*, H−C(4) (**7**)); 2.47 (*dt*, $J = 5.8$, 9.5, H−C(6) (**4a**)); 2.39 (*dt*, $J = 2.0$, 3.8, H−C(6) (**7**)); 5.35, 5.38 (2 br. *s*, 2 H−C(7′) (**4a**)); 5.37, 5.45 (2 br. *s*, 2 H−C(7′) (**7**)).

6. *Acetylation of* **4a**. Aldehyde **4a** (1.8 mg, 0.008 mmol) was treated with excess Ac_2O in dry pyridine (0.5 ml) under stirring at r.t. overnight. Then a sat. aq. $CuSO_4$ soln. (1 ml) and AcOEt (1 ml) were added, and the mixture was passed through a *Whatman* phase-separation filter. The org. phase was evaporated and subjected to prep. TLC (hexane/AcOEt 7:3): pure **4b** (2.0, 94%). $[\alpha]_D^{20} = +15$, $[\alpha]_{577} = +54$ ($c = 0.1$, $CHCl_3$) ([3]: $[\alpha]_D = -57.2$ ($c = 0.08$, $CHCl_3$). ^{1}H-NMR: in accordance with [3].

7. *Synthesis of the MTPA Esters* **6a** *and* **6b**. Compound **3** (5 mg; 0.015 mmol) was treated with (−)-(*R*)-MTPA-Cl (3 mol-equiv.) and 4-(dimethylamino)pyridine (1.0 mg) in dry pyridine (0.5 ml) under stirring for 24 h at r.t. The mixture was then quenched with sat. aq. $CuSO_4$ soln. (1 ml), followed by Et_2O (4 ml), and passed through a *Whatman* phase-separation filter. The org. phase was evaporated and subjected to prep. TLC (hexane/AcOEt 7:3): **6a** (7.3 mg, 88%). By a similar procedure, the same amounts of **3** and (+)-(*S*)-MTPA-Cl gave **6b** (7.4 mg, 88%). NMR and TLC examination of the crude reaction mixtures showed that both **6a** and **6b** were obtained as single diastereoisomers.

*(α*S*)-α-Methoxy-α-(trifluoromethyl)benzeneacetic Acid (1*S*,2*R*,4*S*,5*Z*)-4-(Acetyloxy)-5-[(acetyloxy)methylene]-2-(5-methyl-1-methylenehex-4-en-2-ynyl)cyclohexyl Ester* (**6a**). ^{1}H-NMR[2]): 5.10 (*td*, $J = 10.7$, 4.8, H−C(1)); 2.25 (*ddd*, $J = 2.3$, 10.7, 13.1, H_{ax}−C(2)); 2.60 (*dd*, $J = 4.8$, 13.1, H_{eq}−C(2)); 5.92 (*t*, $J = 2.8$, H−C(4)); 1.85 (*ddd*, $J = 2.8$, 12.8, 14.8, H_{ax}−C(5)); 2.05 (*ddd*, $J = 2.8$, 3.8, 14.8, H_{eq}−C(5)); 2.85 (*ddd*, $J = 3.8$, 10.7, 12.8, H−C(6)); 5.37 (*sept.*, $J = 1.3$, H−C(10)); 1.86 (*s*, 3 H−C(12)); 7.06 (*d*, $J = 2.0$, H−C(3′)); 5.33, 5.30 (2*d*, $J = 1.8$, 2 H−C(7′)); 1.81 (*s*, 3 $J = 11'$)); 2.04 (*s*, AcO−C(4)); 2.17 (*s*, AcO−C(3′)); 7.50 (*m*, 2 arom. H); 7.38 (*m*, 3 arom. H); 3.54 (*q*, ^5J(H,F) = 1.1, MeO). ^{13}C-NMR[2]): 75.68 (*d*, C(1)); 30.56 (*t*, C(2)); 116.91 (*s*, C(3)); 64.52 (*d*, C(4)); 35.11 (*t*, C(5)); 44.49 (*d*, C(6)); 132.64 (*s*, C(7)); 90.30 (*s*, C(8)); 88.09 (*s*, C(9)); 104.74 (*d*, C(10)); 149.80 (*s*, C(11)); 24.95 (*q*, C(12)); 132.67 (*d*, C(3′)); 122.65 (*t*, C(7′)); 21.24 (*q*, C(11′)); 169.86 (*s*, Me*C*OO−C(4)); 167.67 (*s*, Me*C*OO−C(3′)); 20.66 (*q*, *Me*COO−C(4)); 21.24 (*q*, *Me*COO−C(3′)); 165.79 (*s*, RO*C*O−C(1)); 84.60 (*q*, ^2J(C,F) = 28, *C*CO−C(1)); 123.12 (*q*, ^1J(C,F) = 288, CF_3); 55.72 (*s*, MeO); 127.30 (*d*, 2 arom. C); 128.28 (*d*, 2 arom. C); 129.52 (*d*, arom. C); 129.65 (*s*, arom. C). EI-MS: 548 (8, $M^{+\cdot}$), 506 (3), 488 (3), 229 (7), 213 (15), 212 (40), 77 (12), 43 (100).

*(α*R*)-α-Methoxy-α-(trifluoromethyl)benzeneacetic Acid (1*S*,2*R*,4*S*,5*Z*)-4-(Acetyloxy)-5-[(acetyloxy)methylene]-2-(5-methyl-1-methylenehex-4-en-2-ynyl)cyclohexyl Ester* (**6b**). ^{1}H-NMR[2]): 5.13 (*td*, $J = 10.7$, 4.8, H−C(1)); 2.45 (*ddd*, $J = 2.2$, 11.2, 13.1, H_{ax}−C(2)); 2.63 (*dd*, $J = 4.8$, 13.1, H_{eq}−C(2)); 5.93 (*t*, $J = 2.8$, H−C(4)); 1.85 (*ddd*, $J = 2.8$, 12.6, 15.0, H_{ax}−C(5)); 2.04 (*ddd*, $J = 2.8$, 3.9, 15.0, H_{eq}−C(5)); 2.82 (*ddd*, $J = 3.9$, 10.7, 12.6, H−C(6)); 5.38 (*sept.*, $J = 1.3$, H−C(10)); 1.89 (*s*, 3 H−C(12)); 7.07 (*d*, $J = 2.1$, H−C(3′)); 5.08, 5.07 (2*d*, $J = 1.7$, 2 H−C(7′)); 1.82 (*s*, 3 H−C(11′)); 2.07 (*s*, AcO−C(4)); 2.17 (*s*, AcO−C(3′)); 7.50, 7.38 (2*m*,

5 arom. H); 3.51 (*q*, ^5J(H,F) = 1.1, MeO). ^{13}C-NMR[2]): 75.27 (*d*, C(1)); 31.02 (*t*, C(2)); 116.94 (*s*, C(3)); 64.56 (*d*, C(4)); 34.96 (*t*, C(5)); 44.67 (*d*, C(6)); 132.19 (*s*, C(7)); 89.94 (*s*, C(8)); 88.21 (*s*, C(9)); 104.90 (*d*, C(10)); 149.52 (*s*, C(11)); 24.94 (*q*, C(12)); 132.63 (*d*, C(3′)); 122.87 (*t*, C(7′)); 21.25 or 21.19 (*q*, C(11′)); 169.84 (*s*, Me*C*OO–C(4)); 167.64 (*s*, Me*C*OO–C(3′)); 20.66 (*q*, *Me*COO–C(4)); 21.19 or 21.25 (*q*, *Me*COO–C(3′)); 165.52 (*s*, RO*C*O–C(1)); 84.59 (*q*, ^2J(C,F) = 28, *C*CO–C(1)); 123.20 (*q*, ^1J(C,F) = 288, CF_3); 55.44 (*s*, MeO); 127.44 (*d*, 2 arom. C); 128.21 (*d*, 2 arom. C); 129.44 (*d*, arom. C); 130.57 (*s*, arom. C).

REFERENCES

[1] V. J. Paul, 'Ecological Roles of Marine Natural Products', Cornell University Press, Ithaca, N.Y., 1992.

[2] M. J. Garson, *Nat. Prod. Rep.* **1989**, *6*, 143; M. T. Davies-Coleman, M. J. Garson, *Nat. Prod. Rep.* **1998**, *15*, 477.

[3] A. Fontana, M. L. Ciavatta, E. Mollo, C. G. Naik, S. Wahidulla, L. D'Souza, G. Cimino, *J. Nat. Prod*. **1999**, *62*, 931.

[4] a) I. Mancini, G. Guella, A. Defant, M. L. Candenas, C. P. Armesto, D. Depentori, F. Pietra, *Helv. Chim. Acta* **1998**, *81*, 1681; b) A. Guerriero, D. Depentori, M. D'Ambrosio, F. Pietra, *Helv. Chim. Acta* **1995**, *78*, 1759.

[5] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc*. **1991**, *113*, 4092.

[6] V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno, L. Mayol, *Tetrahedron Lett*. **1978**, 3593.

[7] a) W. Oppolzer, V. Snieckus, *Angew. Chem*. **1978**, *90*, 506; b) B. B. Snider, *Acc. Chem. Res.* **1980**, *13*, 426; c) R. J. Loncharich, K. N. Houk, *J. Am. Chem. Soc*. **1987**, *109*, 6947; d) B. B. Snider, M. Karras, R. T. Price, D. J. Rodini, *J. Org. Chem.* **1982**, *47*, 4538.

[8] R. W. Hoffmann, *Chem. Rev*. **1989**, *89*, 1841.

[9] G. Guella, F. Marchetti, F. Pietra, *Helv. Chim. Acta* **1997**, *80*, 684.

[10] Y. Nakatani, K. Kawashima, *Synthesis* **1978**, 147.

[11] Y.-R. Naves, P. Ochsner, *Helv. Chim. Acta* **1964**, *47*, 5; Y. Nakatani, K. Kawashima, *Synthesis* **1995**, *78*, 1755; G. D. Yadav, J. J. Nair, *Chem. Commun.* **1998**, 2369.

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