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New Mono- and Dimeric Members of the Secalonic Acid Family: Blennolides A–G Isolated from the Fungus *Blennoria* sp.**

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Abstract: Blennolides A–G (**2**–**8**), seven unusual chromanones, were isolated together with secalonic acid B (**1**) from *Blennoria* sp., an endophytic fungus from *Carpobrotus edulis*. This is the first reported isolation of the blennolides **2** and **3** (hemisecalonic acids B and E), the existence of which as the monomeric units of the dimeric secalonic acids had long been postulated. A compound of the proposed structure **4** (β-diversonolic ester) will need to be revised, as its reported data do not fit those of the established structure of blennolide C (**4**). Other monomers, the blennolides D–F (**5**–**7**) seem to be derived from blennolides A (**2**) and B (**3**) by rearrangement of the hydroaromatic ring. The heterodimer **8**, composed of the monomeric blennolide A (**2**) and the rearranged 11-dehydroxy derivative of blennolide E (**6**), extends the ergochrome family with an ergoxanthin type of skeleton. The structures of the new compounds were elucidated by detailed spectroscopic analysis and further confirmed by an X-ray diffraction study of a single crystal of **2**. The absolute configurations were determined by TDDFT calculations of CD spectra, including the solid-state CD/TDDFT approach. Preliminary studies showed strong antifungal and antibacterial activities of these compounds against *Microbotryum violaceum* and *Bacillus megaterium*, respectively. They were also active against the alga *Chlorella fusca* and the bacterium *Escherichia coli*.

Keywords: absolute stereochemistry · antifungal agents · blennolides A–G · natural products · total synthesis

Introduction

The ergochromes (synonyms ergoflavin, ergochrysin, secalonic acids) are an important group of biologically highly active mycotoxins, produced by a variety of microorganisms.[1] These fungal metabolites, named ergoflavins, were first isolated in pure form from *Claviceps purpurea* (ergot) in 1958,[2] although the initial investigations of ergot compounds can be traced back to 130 years ago.[1] Great epidemics, particularly in medieval Europe, were caused by toxic ergot alkaloids and mycotoxins such as the ergochromes, due to contamination of flour by *C. purpurea*. At present, at least twenty-two members of the ergochrome family have been isolated and structurally identified.[3] They are dimers of six different monoxanthones (hemisecalonic acids A–F), and ergochrome diversity is attributable to different homo- and heterodimers of these six monomeric units.[1] The secalonic acids usually contain a 2,2′-linkage,[1,3d] while another class of dimers, the eumitrins,[3e,f] and isoergochrysin,[3g] are coupled through the 4,2′ positions. The eumitrins have recently been identified as new inhibitors for nitric oxide formation.[4] More recently, another 4,4′-cou-

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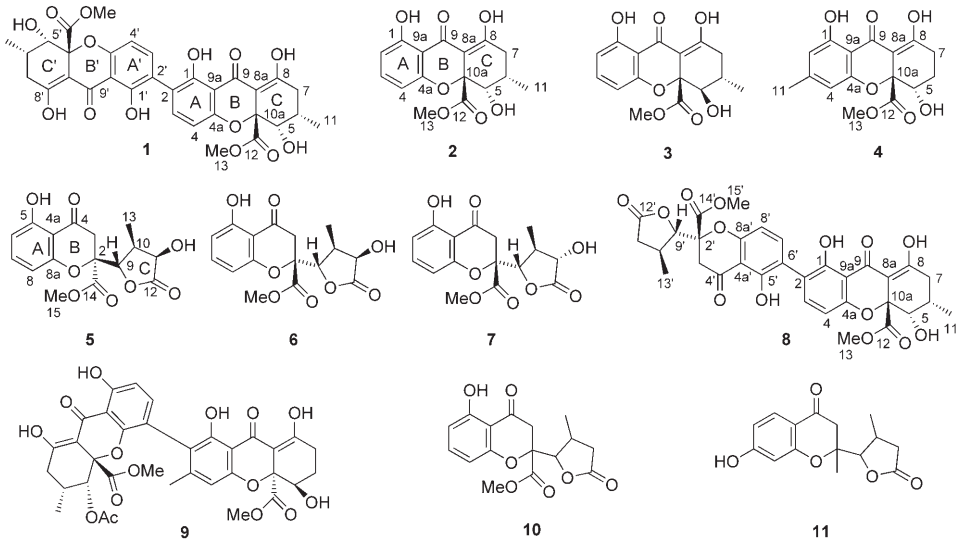
pling dimer, phomoxanthon A, was isolated from *Phomopsis* sp.[3a,b] Surprisingly, however, in spite of more than 130 years of investigation,[1] none of the six hypothetical hemisecalonic acids A–F had previously been isolated from natural sources.

During our ongoing screening for biologically active secondary metabolites from fungi,[5] we investigated *Blennoria* sp. (internal strain no. 7064), isolated as an endophytic fungus from the succulent *Carpobrotus edulis*, growing on Gomera, in the Canary Islands. The fungus was cultivated on biomalt agar medium. The crude ethyl acetate extract of the culture showed pronounced antifungal activity against *Microbotryum violaceum* and moderate algicidal activity against *Chlorella fusca*. Fractionation of the crude ethyl acetate extract led to the isolation and structural determination of the known secalonic acid B (**1**), together with a series of new monomer derivatives and a mixed dimer, which we named blennolides A–G (**2**–**8**). The stereoisomeric compounds **2** and **3** are in fact the long sought-after monomeric units of the dimeric secalonic acids, namely hemisecalonic acids B and E. Their rearrangement products **5**–**7** are structurally unique new natural products, in each of which a highly substituted γ-lactone moiety is linked to a dihydrobenzopyranone. The isomeric monomer **4** shows a different carbon skeleton (Me on C-3 instead of C-6) and can be correlated with diversonol, isolated by Turner from *Penicillium diversum* and synthesized by Bräse.[6b,c] In blennolide G (**8**), the usual ergochrome monomer **2** is linked to the deoxy analogue of rearranged monomer **6**, extending the secalonic acid family with a novel heterodimer. Here we report on the isolation, structural elucidation (including relative and absolute configurations), and bioactivities of these compounds.



Results and Discussion

The fungus *Blennoria* sp. was cultivated on biomalt agar medium for four weeks, and was then extracted with ethyl acetate. The crude extract was fractionated on silica gel, followed by Sephadex LH-20 column chromatography, yielding a crude mixture of secalonic acid B (**1**) and blennolides A–G (**2**–**8**), which were purified by preparative TLC.

The structure of secalonic acid B (**1**), the major metabolite of the title fungus, was determined by detailed spectroscopic analysis and comparison with reported data.[3i,7] In addition, extensive analysis of ^{1}H and ^{13}C NMR spectra led to a complete assignment of all signals; the ^{13}C and 2D NMR data of the compound had not been previously reported. The absolute configurations of 2,2′-secalonic acids such as secalonic acid B (**1**) had been determined by their n–π* CD bands around 330 nm, which were correlated with the configurations of the C-10a and C-10a′ stereogenic centers.[8] A positive n–π* CD band (332 nm, $\Delta\varepsilon = +13.5$) indicated C-10a*R*, C-10a′*R* configuration and also allowed the assignment of the other stereogenic centers on the basis of the relative stereochemistry. Thus, the absolute configuration of (+)-(5*S*,6*S*,10a*R*,5′*S*,6′*S*,10a′*R*)-secalonic acid B (**1**) was assigned in accordance with the reported data.[7a] In contrast, the CD spectra of 4,4′-ergochromes such as phomoxanthone A, which has a hindered rotation about the biaryl axis, are characteristic of their axial—rather than the central—chirality.[3a]

Blennolide A (**2**) was obtained as optically active, light yellow crystals. The molecular formula $C_{16}H_{16}O_7$, indicating nine double bond equivalents, was established by HREIMS. The IR spectrum of **2** showed the presence of hydroxy groups (3593 cm^{-1}), a carbonyl functionality (1742 cm^{-1}), and a typical 1,2,3-trisubstituted aromatic system (3029, 1621, 1586, 801, 716 cm^{-1}). These observations were in agreement with the observation of signals in the ^{13}C NMR and DEPT spectra (Table 1) for one secondary oxygenated carbon ($\delta_C = 71.3$ ppm, d), one enolic group ($\delta_C = 179.8$ ppm, s; 100.0 ppm, s), the ester carbonyl atom ($\delta_C = 171.1$ ppm, s) and the conjugated ketone carbonyl atom ($\delta_C = 187.5$ ppm, s), and six aromatic atoms ($\delta_C = 162.1$, s; 157.7, s; 137.6, d; 111.2, d; 107.9, d; 107.2 ppm, s) accounting for seven double bond equivalents. The remaining double bond equivalents were due to the presence of two more rings in the molecule.

A comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** revealed a great similarity, except that a sp^2 quaternary carbon ($\delta_C = 118.7$ ppm) in **1** was replaced by an aromatic methine ($\delta_C = 111.2$ ppm, $\delta_H = 6.55$ ppm) in **2**. This suggested that **2** could be a monomer of **1**. The relative stereochemistry of **2**, deduced from the NOESY and NOEDIFF experiments, proved to be the same as that of the monomeric units in **1**.

Table 1. NMR data[a,b] for blennolides A (**2**) and B (**3**).

No.	Blennolide A (**2**)		Blennolide B (**3**)	
	δ_H, m, *J* in Hz	δ_C, m[c]	δ_H, m, *J* in Hz	δ_C, m[c]
1		162.1, s		162.1, s
2	6.55, dd, 8.4, 0.7	111.2, d	6.53 dd, 8.3, 0.8	110.7, d
3	7.32, t, 8.3	137.6, d	7.36, t, 8.3	138.0, d
4	6.49, dd, 8.1, 0.7	107.9, d	6.55, dd, 8.2, 0.8	107.9, d
4a		157.7, s		158.8, s
5	4.11, s	71.3, d	3.92, dd, 11.2, 2.6	77.0, d
6	2.10, m	28.5, d	2.41, m	29.3, d
7α	2.52, dd, 18.9, 11.2	32.6, t	2.30, dd, 19.1, 10.6	36.3, t
7β	2.39, dd, 18.9, 6.1		2.74, dd, 19.1, 6.2	
8		179.8, s		177.5, s
8a		100.0, s		101.7, s
9		187.5, s		187.1, s
9a		107.2, s		107.2, s
10a		84.7, s		84.7, s
11	1.17, d, 6.8	17.5, q	1.17, d, 6.5	18.0, q
12		171.1, s		170.3, s
13	3.68, s	53.4, q	3.69, s	53.1, q
1-OH	11.33, s		11.22, s	
5-OH	2.54, s			
8-OH	14.00, s		13.80, s	

[a] Bruker Avance 500 NMR spectrometer; ^{1}H and ^{13}C chemical shifts with reference to $CHCl_3$ ($\delta_H = 7.26$ ppm) and $CDCl_3$ ($\delta_C = 77.0$ ppm), respectively. [b] Assignments made by 2D NMR (COSY, NOESY, HMQC and HMBC) experiments. [c] By DEPT sequence.

The structure and the relative stereochemistry of **2** were further confirmed by a single-crystal X-ray analysis (Figure 1), the atomic coordinates from which were then

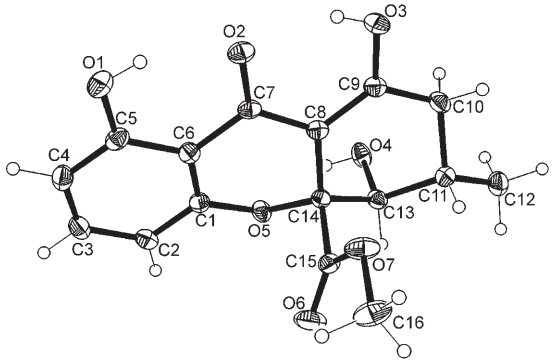


Figure 1. Molecular structure of **2** in the crystal (ORTEP drawing showing 50% ellipsoids).

used to determine the absolute configuration of **2** by the novel solid-state CD/TDDFT approach.[9] By this method, the single-crystal structure is directly used as input for TDDFT[10] CD calculations,[11] and the resultant computed CD spectrum is compared with the solid-state CD measured as a KCl disc. The solution and solid-state CDs of **2** were nearly identical, indicating that the solid-state structure of the rigid molecule is also dominant in solution. In addition to the positive low-energy n–π* CD band at 328 nm (331 nm in dichloromethane), a more intense negative one was observed at 220 nm (Figure 2).

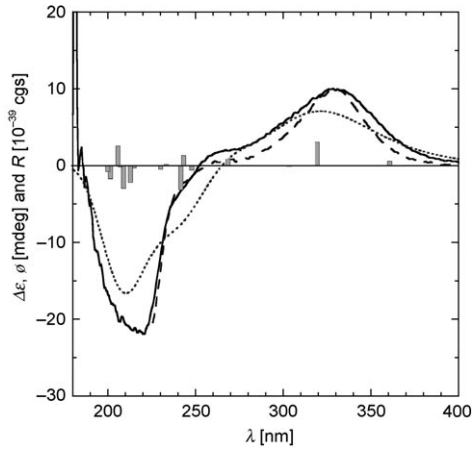


Figure 2. Experimentally measured CD spectra of blennolide A (5*S*,6*S*,10a*R*)-(**2**) in dichloromethane solution (——) and in the solid state as a KCl disc (-----), compared with the calculated TDB3LYP/TZVP CD spectrum (·····). Vertical bars are computed rotational strengths *R* (▪, in 10^{-39} cgs).

The TDB3LYP/TZVP-computed CD spectrum of (5*S*,6*S*,10a*R*)-**2**, with the X-ray structure as input geometry, reproduced well both the signs and the shape of the measured solid-state CD spectrum (Figure 2). The positive band between 260–400 nm is due to three transitions (n–π* and π–π* types), all of which are allied to positive computed rotational strengths. The absolute configuration of the three stereogenic centers of **2** can thus be assigned as 5*S*, 6*S*, and 10a*R*, which was also corroborated by the reported absolute configurations of the monomers of secalonic acid B (**1**) and the positive n–π* CD transition around 330 nm.[7a,8] The positive values of optical rotation for both compounds—$[\alpha]_D^{20} = +133.7$ for **1** (literature value[3i,7] +196) and +181.8 for **2**—also support the above conclusion.

Blennolide B (**3**) was isolated as an optically active, light yellow gum. Its molecular formula of $C_{16}H_{16}O_7$, established by HREIMS, was the same as that of **2**. The IR and UV spectra of **3** were nearly identical to those of **2**, and the ^{1}H and ^{13}C NMR spectra of **3** also resembled those of **2**, suggesting the same polycyclic skeleton. However, a difference was observed in the NMR resonances of the ring C atoms, mainly from C-5 to C-8 (Table 1). In particular, the singlet of H-5 in **2** ($\delta_H = 4.11$ ppm, s) was replaced by a doublet of doublets in **3** ($\delta_H = 3.92$ ppm, dd, $J = 11.2$, 2.6 Hz), indicating a pseudoaxial orientation of the α-orientated H-5. Obviously, the upfield shift of C-7 in **2** ($\delta_C = 32.6$ ppm), with respect to the corresponding shift value in **3** ($\delta_C = 36.3$ ppm), was due to a γ-*gauche* effect of α-OH at C-5.[12] The observation of a NOE effect between H-5 and H-7α confirmed the related α configuration. Blennolide B (**3**) was thus assigned as the C-5 epimer of **2**, and is the monomeric unit of secalonic acid D. In view of the determination of the absolute configuration of blennolide A (**2**) described above, the chirality of the monomeric blennolide B (**3**) can be assigned as 5*R*, 6*S*, and 10a*R*; this information can also be extended to the dimeric secalonic acid D.[8]

Blennolide C (**4**), an optically active, white powder, has the same molecular formula as **2**, as deduced from HREIMS. The ^{13}C NMR shifts of **4** were closely related to those of **2**, except that two methine signals ($\delta_C = 137.6$, d; 28.5 ppm, d) in **2** were replaced in **4** by a quaternary carbon ($\delta_C = 149.9$ ppm, s) and a methylene ($\delta_C = 23.1$ ppm, t). In the ^1H NMR spectrum of **4**, two aromatic protons showed no ^3J coupling ($\delta_H = 6.38$, s; 6.35 ppm, d, $J = 0.4$ Hz). The methyl group, however, was markedly downfield-shifted to $\delta_H = 2.29$ ppm (d, $J = 0.4$ Hz) with respect to that in **2** ($\delta_H = 1.17$ ppm, d, $J = 6.8$ Hz). This evidence, in conjunction with the modified coupling pattern for the aromatic protons, suggested that the methyl group in structure **4** was attached at C-3 instead of at C-6 as in **2**. The HMBC correlations from H_3-11 to C-2, C-3, and C-4, as well as the proton connectivity of H-5/H_2-6/H_2-7, deduced from the ^1H–^1H COSY spectrum, confirmed the above conclusion. Moreover, the NOE enhancement between 5-OH and H-7α showed that these groups both have the α-configuration.

Table 2. NMR data[a,b] for blennolides D–F (**5**–**7**).

No.	Blennolide D (**5**)		Blennolide E (**6**)		Blennolide F (**7**)	
	δ_H, m, J in Hz	δ_C, m[c]	δ_H, m, J in Hz	δ_C, m[c]	δ_H, m, J in Hz	δ_C, m[c]
2		84.4, s		84.2, s		83.6, s
3α	3.07, d, 17.3	40.3, t	3.17, d, 17.0	39.9, t	3.25, d, 16.7	39.8, t
3β	3.45, d, 17.3		3.02, d, 17.0		3.11, d, 16.7	
4		194.5, s		193.4, s		194.0, s
4a		107.3, s		107.6, s		107.6, s
5		161.9, s		162.0, s		161.9, s
6	6.56, dd, 8.4, 0.9	110.8, d	6.57, dd, 8.3, 0.6	110.8, d	6.57, dd, 8.3, 0.9	110.8, d
7	7.41, t, 8.3	138.8, d	7.42, t, 8.3	139.2, d	7.42, t, 8.3	139.0, d
8	6.48, dd, 8.3, 0.9	107.3, d	6.52, dd, 8.2, 0.6	107.5, d	6.54, dd, 8.3, 0.9	107.7, d
8a		159.0, s		159.0, s		158.8, s
9	4.41, d, 1.9	85.1, d	4.46, d, 1.8	86.4, d	4.45, d, 9.0	83.3, d
10	2.94, m	34.7, d	3.01, m	34.7, d	2.62, m	39.2, d
11	4.89, d, 8.4	68.6, d	4.80, d, 8.4	68.4, d	4.12, d, 9.7	74.2, d
12		176.2, s		176.0, s		174.2, s
13	1.16, d, 7.3	13.4, q	1.25, d, 7.3	13.6, q	1.37, d, 6.8	16.5, q
14		168.9, s		168.6, s		168.4, s
15	3.76, s	53.7, q	3.73, s	53.7, q	3.73, s	53.6, q
5-OH	11.42, s		11.40, s		11.43, s	

[a] Bruker Avance 500 NMR spectrometer; ^1H and ^{13}C chemical shifts with reference to $CHCl_3$ ($\delta_H = 7.26$ ppm) and $CDCl_3$ ($\delta_C = 77.0$ ppm), respectively. [b] Assignments made by 2D NMR (COSY, NOESY, HMQC and HMBC) experiments. [c] By DEPT sequence.

A compound believed to have the structure assigned to blennolide C (**4**) had previously been isolated from *Penicillium diversum* and named β-diversonolic ester.[13] However, a careful comparison of the relevant NMR data revealed a marked difference between the two data sets, so the structure of β-diversonolic ester will need to be revised. In contrast, in recent reports the correct structure, with data that match those for blennolide C (**4**), was suggested as a monomeric part of the dimeric neosartorin (**9**), a eumitrin analogue isolated from the fungus *Neosartorya fischeri*.[3c] In addition, its C-5 epimer was isolated from *Penicillium* sp. as the monomer of the homodimers rugulotrosins A and B with 2,2′- and 4,2′-coupling.[14]

Blennolide D (**5**) was isolated as an optically active, colorless oil with the molecular formula of $C_{16}H_{16}O_8$, as deduced from HREIMS. The IR and UV spectra of **5** were reminiscent of those of blennolides A (**2**) and B (**3**), showing functional absorption bands for hydroxyl groups, carbonyl groups, and a typical 1,2,3-trisubstituted phenyl group. The presence of a chelated proton, resonating at $\delta_H = 11.42$ ppm in the ^1H NMR spectrum, indicated the unchanged rings A and B. This was also confirmed by comparison of the ^{13}C NMR spectra with those of **2**, with similar signals related to ring A. In contrast, the signals related to ring C were completely different (Table 2). Analysis of the ^1H–^1H COSY spectrum readily allowed us to establish the proton spin system of H-9/H-10/(H_3-13)/H-11, which is cyclized to give a β-methyl-γ-lactone moiety, as deduced from significant HMBC correlations of both H-9 and H-11 to C-12. Diagnostic HMBC correlations of H_2-3 with C-2, C-4, C-4a, C-9, and C-14 led to connections being established between the chromone moiety and the ester group and the γ-lactone moiety to give the planar structure of **5**.

The same planar structure was also found for blennolide E (**6**), with the same molecular formula as **5**, as determined by HREIMS. Interestingly, the ^{13}C NMR shift values of **6** were almost identical to those of **5** (Table 2), with some differences being observed for several proton signals in the ^1H NMR spectra. Moreover, NOESY experiments suggested the same relative stereochemistry of the two γ-lactone moieties in **5** and **6**, through the observation of NOE effects between H-9 and H_3-13 and between H-10 and H-11, as well as by the absence of NOE enhancement between H-11 and H-9 and H_3-13. The relative configuration of the chiral centers on ring C could then be assigned either as 9*S**,10*S*,11*R* or 9*R**,10*R*,11*S*. Clearly, **5** and **6** cannot be enantiomers, as they were separated through non-enantioselective methods, have different chemicophysical data, and non-opposite optical rotations. This leaves us with two possibilities: either **5** and **6** are epimers at C-2, retaining the same configuration of ring C, or the configuration at C-2 is the same but that of ring C is opposite. The different spatial arrangements of **5** and **6** are further demonstrated by the NOE network between H-3 (α and β), H-9, and H-10 (see Figure 4 below). For **5** there is no H-10/H-3 NOE, and that for H-9/H-3α is much larger than that for H-9/H-3β; for **6** there is also a distinct H-10/H-3α NOE.

The overall relative and absolute stereochemistry of blennolides D and E (**5**, **6**) was ultimately established by means of a combination of spectroscopic (NOESY, heteronuclear

$^3J_{C,H}$ couplings, CD) and computational (molecular mechanics conformational searches, DFT geometry optimizations, TDDFT excited-state calculations) techniques. CD spectra of blennolides D and E recorded in acetonitrile and dichloromethane solutions showed a quasi-mirror image pattern (Figure 3). In the case of blennolide E (**6**), the CD con-

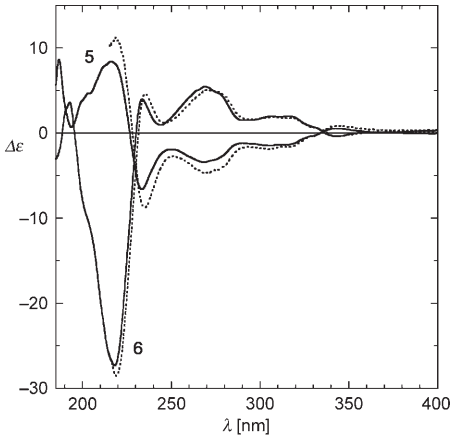


Figure 3. CD spectra of blennolides D (**5**) and E (**6**) in acetonitrile (——) and dichloromethane (•••••). Sample concentrations ≈7 mM, cell length 0.01 cm.

sists of a small negative band at 340 nm, followed by three moderate positive bands in the 230–330 nm region, and a stronger negative band centered at 220 nm; for blennolide D (**5**) the sequence of bands is similar but their signs are inverted. The CD of compounds **5** and **6** is mainly allied to the transitions of the chromanone chromophore perturbed by various chiral elements, the dominant one being the chiral ring B. In fact, with respect to the aromatic chromophore, ring B belongs to the so-called second chiral sphere, the helicity of which provides the leading contribution to the observed CD, similarly to the related situation of the tetralins.[15] Higher-order spheres—to which, for example, the remote chiral centers on ring C belong—are expected to influence the CD only to a lesser extent. In particular, the first three bands in the CD spectra of **5**–**6** may be assigned, from right to left, to the n–π*, the ^1L_b-type π–π*, and the ^1L_a-type (or K-) π–π* transitions[16] of the chromanone chromophore. In the case of **5**, the n–π* transition at 345 nm is positive, while the two π–π* transitions below 330 nm are negative; the opposite is true for **6**. The signs of the ^1L_a and n–π* bands of chromanones are known to correlate with the helicity of ring B (expressed for example, in terms of $\omega_{8a\text{-}1\text{-}2\text{-}3}$ torsion).[17–19] Therefore, it may be inferred that blennolides D and E differ in their configurations at C-2, because these determine the helicity adopted by ring B. Consequently, a 2*S**,9*S*,10*S*,11*R* relative configuration is assumed for blennolide D (**5**), and 2*R**,9*S*,10*S*,11*R* for blennolide E (**6**).

Molecular mechanics conformational searches (with MMFF force field) revealed the presence, in both compounds **5** and **6**, of three main degrees of conformational freedom: 1) the conformation of ring B, with the substituents at C-2 assuming either an axial or an equatorial position, 2) the rotamerism around the C-2/C-14 bond, and 3) the rotamerism around the C-2/C-9 bond. In both cases, MMFF predicts the more stable conformations (within 1.5 kcal mol^{-1}) to be those with the COOMe group occupying the axial, and ring C the equatorial position at C-2. The situation is confirmed by DFT (B3LYP/6-31G(d)) optimization: here the energy difference between conformers with equatorial (more stable) and axial C ring at C-2 is even larger (2.3 kcal mol^{-1} for **6**, >5 kcal mol^{-1} for **5**). Calculation results are also in line with heteronuclear $^3J_{C,H}$ couplings (Table 3), measured by means of *J*-modulated HMBC ex-

Table 3. Relevant $^3J_{C,H}$ couplings[a,b] for blennolides D, E, and G (**5**, **6**, **8**).

C and H[b]	**5**[c]	**6**[d]	C and H[e]	**8**[c]
C-9/H-3α	4.2	4.2	C-9′/H-3α′	4–5
C-9/H-3β	4.2	3.6	C-9′/H-3β′	nd
C-14/H-3α	5.0	10.0	C-14′/H-3α′	>10
C-14/H-3β	10.0	≤ 3.5	C-14′/H-3β′	4.5

[a] Varian INOVA 600 NMR spectrometer; *J* values in Hz, measured with *J*-modulated HMBC experiments. [b] See Table 2 and structure for numbering. [c] In $CDCl_3$. [d] In CD_3CN. [e] See Table 4 and structure for numbering.

periments.[20] In particular, for both compounds, C-9 has two *gauche*-type couplings[21] (J = 3.5–4.2 Hz) with both H-3α and H-3β, while C-14 has a *gauche* coupling (J = 3.5–5.0 Hz) with H-3α in **5** and with H-3β in **6**, and an *anti* coupling ($J \geq$ 10.0 Hz) with H-3β in **5** and with H-3α in **6**. These findings also establish that H-3α is equatorial and H-3β axial in **5**, and, vice-versa, that H-3α is axial and H-3β equatorial in **6** (Figure 4).

As for the three possible rotamers around the C-2/C–9 bond, that with H-9 *anti* to O-1 is predicted to be strongly preferred over the others in the cases both of compound **5** (DFT energy difference >3 kcal mol^{-1}) and of compound **6** (>1.7 kcal mol^{-1}). Finally, the COOMe group tends to assume a position with either C–OMe or C=O eclipsed by O-1, the first possibility being favored over the second by 0.24 kcal mol^{-1} (DFT energy, compound **5**) or 0.53 kcal mol^{-1} (compound **6**). Overall, the conformational situation around C-2 seems to be the result of a complicated balance of steric and electronic factors; the absolute DFT energy minima for **5** and **6** are shown in Figure 4. The consistency with observed diagnostic NOEs between H-3α and β with H-9 and H-10 (indicated by arrows) definitely confirmed the relative configuration assumed above for compounds **5** and **6**.

The absolute configurations of blennolides D and E were determined by comparing experimentally measured CD spectra with those calculated[11] by the TDDFT method.[10] In contrast to the above situation with blennolide B, DFT-optimized structures were employed as input, due to the lack of solid-state structures, and the Boltzmann-weighted averages for two minima for each compound were considered.[22] In Figure 5, the experimentally measured CD is plotted along with the CD calculated for both the absolute energy minimum and the weighted average for

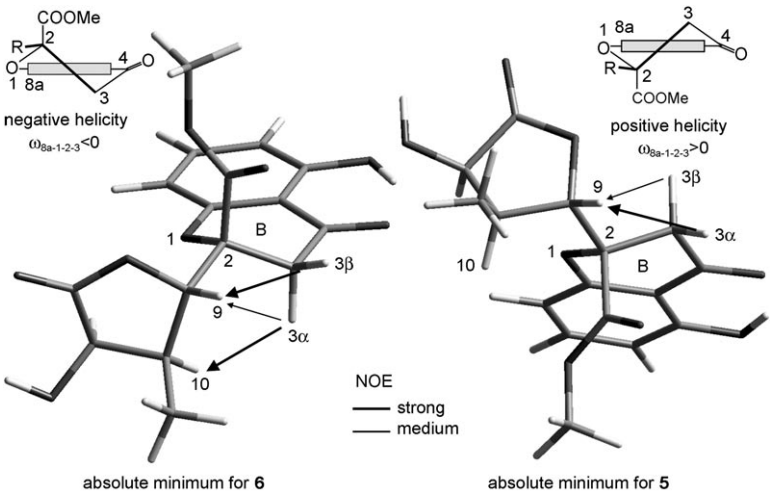


Figure 4. Lowest-energy B3LYP/6-31G(d) structures of **5** (right) and **6** (left). Diagnostic NOEs are indicated by arrows, with thicknesses proportional to their relative strengths. The sign of the chirality assumed by ring B is also shown (R = γ-lactone ring C).

(2*S*,9*S*,10*S*,11*R*)-**5** and (2*R*,9*S*,10*S*,11*R*)-**6**; the observed agreement is sufficient for the absolute configuration assignment. The two major CD bands between 250–330 nm are associated with the two π–π* (^1L_a and ^1L_b) transitions of the chromanone chromophore; they are negative for the 2*S* configuration as in **5**, and positive for the 2*R* as in **6**. These configurations in turn correspond to positive and negative helicity, respectively, assumed by ring B (positive or negative $\omega_{8a\text{-}1\text{-}2\text{-}3}$ torsion) as shown in Figure 4. The positive sign for the ^1L_b band is thus correlated to a negative helicity of ring B, in keeping with the known trend.[18,19] It is instead regrettable to observe that the n–π* transition is wrongly predicted by TDDFT in terms of both position (calculated at 310 nm) and rotational strength. The stronger band at high energy (below 230 nm) is due to the superposition of several transitions also involving the two ester chromophores.

In conclusion, the absolute stereochemistry of blennolides D and E is established as (−)-(2*S*,9*S*,10*S*,11*R*)-**5** and (+)-(2*R*,9*S*,10*S*,11*R*)-**6**. Apparently, blennolide E (**6**) is biogenetically derived from blennolide A (**2**) by a cleavage of the enolic double bond, followed by an esterification of C-8 with 5-OH, with the original configurations of all chiral atoms remaining intact, while for blennolide D (**5**) the stereochemistry at C-2 is inverted.

Blennolide F (**7**) was isolated as an optically active, colorless oil. The HREIMS displayed the same molecular formula as blennolides D and E (**5**, **6**). IR, UV, and NMR spectra suggested that **7** was an additional analogue of **5** and **6**. ^{13}C NMR shift values in rings A and B of **7** were parallel to those of **5** and **6**. A difference was only apparent in the signals of ring C, originating from the different orientation of H-11. In contrast to the cases of **5** and **6**, diagnostic cross peaks between H_3-13 and H-9 and H-11 were observed in the NOESY spectrum of **7**, indicating that all these protons were oriented on the same side of the five-membered ring. These observations suggested that **7** was a C-11 epimer of **5** or **6**. A definite assignment was again made possible by CD and NMR spectroscopy and molecular modeling. The CD spectrum of blennolide F (**7**, Figure 6), was clearly reminiscent of that of **6** and might analogously be taken as a confir-

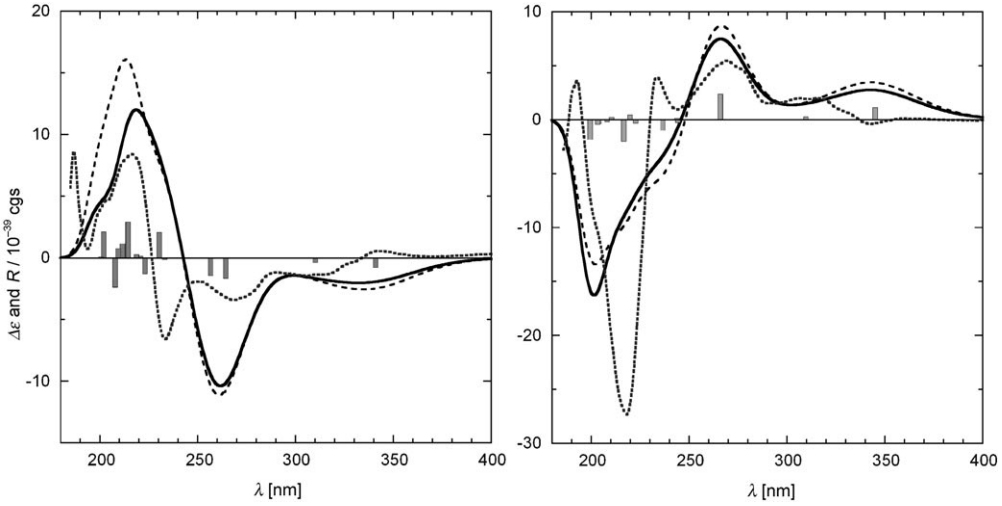


Figure 5. Experimentally measured CD spectra of blennolides D (**5**, left) and E (**6**, right) in acetonitrile (•••••) compared with TDB3LYP/TZVP calculated CD spectra for the lowest-energy DFT structure (-----) and Boltzmann weighted averages (——). Vertical bars are rotational strengths *R* (▮, in 10^{-39} cgs) calculated for the absolute minima.

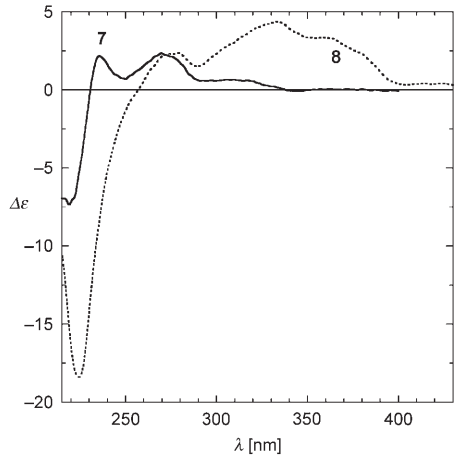


Figure 6. CD spectra of blennolides F (**7**, ——) and G (**8**, ·····) in dichloromethane.

mation of the negative helicity assumed by ring B. H-3α/β-H-9/H-10 NOE patterns and MMFF conformational search results were also similar to those of compound **6**, showing distinct NOE effects between H-3α and H-10 and between H-3β and H-9, together with a very weak NOE effect between H-3α and H-9. In addition, the similar shift values of H-3 in **7** ($\delta_{H\text{-}3\alpha}=3.25$ ppm, $\delta_{H\text{-}3\beta}=3.11$ ppm) and **6** ($\delta_{H\text{-}3\alpha}=3.17$ ppm, $\delta_{H\text{-}3\beta}=3.02$ ppm) with respect to those in **5** ($\delta_{H\text{-}3\alpha}=3.07$ ppm, $\delta_{H\text{-}3\beta}=3.45$ ppm) supported the above conclusion. The structure of **7** was thus determined as a C-11 epimer of **6**, and may be assigned as (+)-(2*R*,9*S*,10*S*,11*S*)-**7**.

Two structures related to blennolides D–F—compounds **10** and **11**—with unreported NMR data have been prepared by different groups.[23] Blennolides D–F (**5**–**7**) represent the first examples of such a skeleton from natural sources.

Blennolide G (**8**) was an optically active, light yellow gum with a molecular formula of $C_{32}H_{30}O_{14}$ as deduced from HREIMS. The IR and UV spectra of **8** indicated a chromanone derivative. A careful comparison of the ^{1}H and ^{13}C NMR spectra of **8** (Table 4) with those of **1**–**7** immediately revealed **8** to be an asymmetric dimer of blennolide A (**2**) and an analogue of the rearranged blennolides D–F (**5**–**7**). Subtraction of the signals of the blennolide A (**2**) subunit confirmed the similarity of the remaining NMR data with those of blennolide E (**6**), though differing in the γ-lactone moiety. The replacement of a secondary alcohol group ($\delta_H=$ 4.80 ppm, d, $J=8.4$ Hz; $\delta_C=68.4$ ppm, d) in **6** by a methylene group ($\delta_H=2.91$ ppm, dd, $J=17.5$, 9.4 Hz and 2.23 ppm, dd, $J=17.5$, 4.3 Hz; $\delta_C=36.1$ ppm, d) in **8** showed that the planar structure of the second monomer coincided with 11-dehydroxy-blennolide E. This conclusion was further confirmed by the proton sequence from H-9′ to H_2-11′, as established by a ^{1}H–^{1}H COSY experiment, and the observation in the HMBC spectrum of long-range correlations of H-9′ with C-12′ and C-13′, and of H-3′ with C-2′ and C-9′. The monomers are connected by a C-2–C-6′ linkage, as shown (Figure 7) by the diagnostic HMBC correlations both of H-3 with C-6′ and of H-7′ with C-2. Moreover, the significant NOE effects in the NOESY spectrum of H-9′ with H-11′β and H_3-13′ indicated that all these protons are oriented on the same side of the five-membered ring.

The CD spectrum of blennolide G (**8**; Figure 6) may in principle be the result of a combination of the central chirality of each of the monomers **2** and **6**, plus the axial chirality due to the C-2/C-6′ linkage, as in atropisomeric biaryls. The existence of true atropisomerism in **8** is not attainable because, as in the case of the parent 2,2′-dihydroxybiphenyl (2,2′-biphenol), the steric hindrance exerted by the substituents *ortho* to the biaryl junction (two OH and two H) is in-

Table 4. NMR data[a,b] for blennolide G (**8**).

No.	δ_H, m, J in Hz	δ_C, m[c]	No.	δ_H, m, J in Hz	δ_C, m[c]
1		159.4, s	2′		84.2, s
2		118.3, s	3′α	3.05, d, 17.0	39.7, t
3	7.43, d, 8.5	139.6, d	3′β	3.21, d, 17.0	
4	6.57, d, 8.5	107.6, d	4′		194.1, s
4a		157.3, s	4a′		107.5, s
5	4.13, s	71.3, d	5′		159.2, s
6	2.12, m	28.5, d	6′		118.1, s
7α	2.40, dd, 19.0, 6.2	32.6, t	7′	7.53, d, 8.5	141.3, s
7β	2.53, dd, 19.0, 11.3		8′	6.62, d, 8.5	107.3, d
8		179.9, s	8a′		158.6, s
8a		99.9, s	9′	4.45, d, 3.9	87.6, d
9		187.6, s	10′	2.85, m	30.0, d
9a		107.0, s	11′α	2.91, dd, 17.5, 9.4	36.1, t
10a		84.8, s	11′β	2.23, dd, 17.5, 4.3	
11	1.18, d, 6.9	17.5, q	12′		175.0, s
12		171.2, s	13′	1.29, d, 6.8	20.8, q
13	3.72, s	53.5, q	14′		168.8, s
1-OH	11.84, s		15′	3.76, s	53.6, q
5-OH	2.52, s		5′-OH	11.87, s	
8-OH	13.94, s				

[a] Bruker Avance 500 NMR spectrometer; ^{1}H and ^{13}C chemical shifts with reference to $CHCl_3$ ($\delta_H=$ 7.26 ppm) and $CDCl_3$ ($\delta_C=77.0$ ppm), respectively. [b] Assignments made by 2D NMR (COSY, NOESY, HMQC and HMBC) experiments. [c] By DEPT sequence.

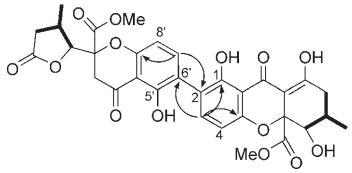


Figure 7. ^{1}H–^{1}H COSY (bold bonds) and selected HMBC correlations (curved arrows) of **8**.

sufficient.[24] The two axial chiral isomers M and P will therefore interconvert rapidly at room temperature. On the other hand, for compound **8**, M and P forms are not enantiomers but diastereomers, with different energies and non-mirror image CD spectra, and therefore capable of providing sizable contributions to the average CD spectrum.[25] As a matter of fact, the CD spectrum of **8** (Figure 6) does not amount to the sum of the CD spectra of **2** (Figure 2) and **6** (Figure 3), and is therefore not easy to interpret. For example, the moderate positive signals above 350 nm, in association with a shoulder on the strong 330 nm absorption band, have no correspondence in the constituent spectra.

MMFF conformational searches for **8** led to several low-energy minima due to the many degrees of conformational freedom. The two lowest-energy structures with opposite axial chirality, optimized with AM1, are shown in Figure 8. They differed by 0.41 kcal mol^{-1} with MMFF and <0.1 kcal with AM1. The interconversion barrier was estimated to be 13.3 kcal mol^{-1} by MMFF and 8.6 kcal mol^{-1} by AM1, in keeping with recent DFT calculations on 2,2′-biphenol.[26] These results did not give confidence for the use of TDDFT calculations for an independent configurational assignment of **8**, in view of the large molecular size combined with the pronounced conformational flexibility.

However, we were able to base the absolute configuration of blennolide **8** on that of its monomeric constituents: the secalonic moiety was assigned the same configuration as blennolide A (**2**), which is preserved through the series, while the configuration of the 11-dehydroxyblennolide moiety was ascertained on the basis of DFT geometry optimizations and NMR experiments. The $^3J_{C,H}$ couplings between H-3′α/β and C-9′/C-14′ (see Table 3) indicated the high-field H-3′ proton ($\delta_H = 3.05$ ppm) to be axial (indicated as H-3α′) and the low-field H-3β′ proton ($\delta_H = 3.21$ ppm) to be equatorial. The NOESY spectrum showed appreciable NOEs between H-9′ and both H-3α′ and H-3β′, in the order 3β′/9 > 3α′/9, as well as a moderate H-3α′/H-10′ NOE. Thus, except for a reverse chemical shift order of protons H-3′, the diagnostic NMR data for **8** strongly resembled those for **6**, and the same relative configuration of the blennolide moiety might therefore be inferred. This was also supported by MMFF conformational searches followed by DFT geometry optimizations run on the blennolide half of compound **8**, which again were in keeping with similar results on blennolide E (**6**). As the only difference, a C-2′/C-14′ rotamer with H-9′ *gauche* to O-1 becomes quite populated with respect to the H-9′/O-1 *anti*. This has no large effect on the expected NOEs, but may help to explain the chemical shift discrepancy with respect to blennolide E (**6**). Once the relative configuration had been established, and since the absolute configuration of the γ-lactone moiety is preserved through the whole series, we assign the absolute structure of blennolide G as (+)-(5*S*,6*S*,10a*R*,2′*R*,9′*S*,10′*S*)-**8**.

Blennolide G (**8**) is structurally correlated to ergoxanthin,[3f–i] the only secalonic acid member containing a rearranged monomeric unit. Ergoxanthin was isolated from a Portuguese ergot drug by Mayo and co-workers, with its two monomeric subunits as planar structures being assigned separately.[3h,i] Later, Whalley et al. showed that the monomers were connected by a C-2,C-2′ linkage (C-2,C-6′, if using our numbering system, see above), but again no stereochemistry was assigned to ergoxanthin.[3f,g]

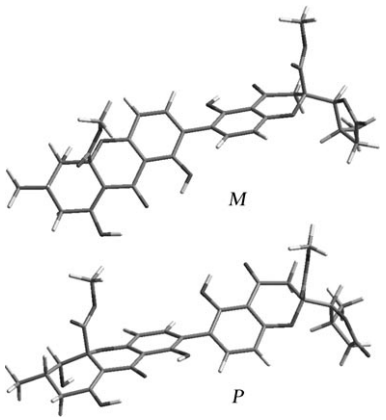


Figure 8. Lowest-energy AM1 structures of **8** with opposite axial chirality.

Obviously, blennolide G (**8**) is biogenetically correlated to blennolide A (**2**) and secalonic acid B (**1**). A cleavage of the enolic double bond, followed by an esterification of C-8 with 5-OH on one monomer of secalonic acid B (**1**) should give dimer **8**, extending the secalonic acid family formed by C-2, C-6′ coupling of hetero monomers.

Bioactivity: The isolated compounds **1**, **2**, **3**, **5**, and **6** were tested in an agar diffusion assay for their antibacterial, antifungal and algicidal properties (Table 5). Whereas compound **2** inhibited all four test organisms, compound **1** was the most inhibitory. All the metabolites were antialgal against *Chlorella fusca* and antifungal against *Microbotryum violaceum*. Compounds **1**–**3** also inhibited the Gram-positive bacterium *Bacillus megaterium*, and compounds **2** and **3** also inhibited the Gram-negative bacterium *Escherichia coli.*

Table 5. Agar diffusion assays for antibacterial, antifungal, and antialgal activities.[a]

Compound	*Escherichia coli*	*Bacillus megaterium*	*Microbotryum violaceum*	*Chlorella fusca*
1	0	15	13	gi 5
2	gi 7	gi 8	gi 9	gi 5
3	gi 8	gi 8	gi 8	gi 9
5	0	0	gi 7	gi 6
6	0	0	gi 8	gi 7

[a] Radii of the zones of inhibition are given in mm (gi = growth inhibition); that is, some growth within the zone of inhibition. Otherwise, the inhibition zone was clear.

Experimental Section

General experimental procedures: Commercial silica gel (Merck, 0.040–0.063 mm) was used for column chromatography. Precoated silica gel plates (Merck, G60 F-254 or G50 UV-254) were used for analytical and preparative thin-layer chromatography (TLC), respectively. Spots were

detected on TLC under UV or by heating after spraying with 0.5 mL of anisaldehyde in HOAc (50 mL) and H_2SO_4 (1 mL). TLC R_f values are reported. The NMR spectra were recorded at 293 K on a Bruker Avance 500 (11.7 T) and a Varian INOVA 600 (14.1 T) spectrometer. Chemical shifts are reported in parts per million (δ), with use of the residual $CHCl_3$ signal ($\delta_H = 7.26$ ppm) as an internal standard for ^1H NMR and $CDCl_3$ ($\delta_{C=} 77.0$ ppm) for ^{13}C NMR; coupling constants (J) in Hz. ^1H and ^{13}C NMR assignments were supported by ^1H–^1H COSY, HMQC, HMBC, and NOESY experiments. $^3J_{C,H}$ couplings were measured by means of pulsed field gradient HMBC spectra, recorded by varying the J-refocusing time (τ) between 0.04–0.16 s (10 ms interval), corresponding to $J = 1/(2\tau) = 3.1$–12.5 Hz. $^3J_{C,H}$ values were estimated with least-squares sinusoidal fits of the experimentally determined cross-peak intensities as a function of J.[20] The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublets of doublets, m = multiplicity. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter at the sodium D-line. Infrared spectra were recorded on a Nicolet-510P spectrophotometer; peaks are reported in cm^{-1}. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. UV absorption spectra were recorded on a UV-2101PC spectrophotometer; peak wavelengths are reported in nm. CD spectra were recorded on a J-810 spectropolarimeter. For the solid-state CD protocol, see ref. [9] The mass spectra and high-resolution mass spectra were performed on a MAT 8200 mass spectrometer, resolution 7000. An isopropyl alcohol solution of sodium iodide (2 mg per mL) was used as a reference compound.

Culture, extraction, and isolation: The endophytic fungus *Blennoria* sp., internal strain No. 7064, was isolated after surface sterilization from *Carpobrotus edulis*, from El Cedro, Gomera, and was cultivated on biomalt solid agar media (5% *w/v*, 12 L) at room temperature for 28 d.[27] The culture media were then extracted with ethyl acetate to afford a residue (35 g) after removal of the solvent under reduced pressure. The extract was subjected to column chromatography (CC) on silica gel, with elution with a gradient of petroleum ether in ethyl acetate (90:10, 50:50, 0:100), to give a mixture of the metabolites (430 mg). The mixture was recrystallized from CH_2Cl_2/MeOH 1:1 and was then filtered to yield **1** (280 mg). The filtrate was split by CC on Sephadex LH-20 (CH_2Cl_2/MeOH 5:1) into two subfractions, which were purified by preparative TLC (CH_2Cl_2/isopropanol 30:1). The first subfraction gave the two dimers **1** (32 mg) and **8** (2.9 mg), while the second subfraction yielded all the monomers: namely **2** (16.2 mg), **3** (1.8 mg), **4** (0.7 mg), **5** (3.6 mg), **6** (21.7 mg), and **7** (1.1 mg).

Secalonic acid B (1): Yellow crystals (CH_2Cl_2/CH_3OH 4:1); $R_f = 0.42$ (CH_2Cl_2/isopropanol 96:4); m.p. 231–232 °C; $[\alpha]_D^{20} = +133.7$ ($c = 0.38$ in $CHCl_3$); CD (CH_2Cl_2, $c = 2.0\times10^{-4}$): λ ($\Delta\varepsilon$) = 374 sh (2.9), 332 (13.5), 293 sh (2.6), 226 nm (-35.5 $M^{-1}cm^{-1}$); ^1H NMR (500 MHz, $CDCl_3$): δ = 13.96 (s, 2H; OH-8, OH-8′), 11.85 (s, 2H; OH-1, OH-1′), 7.42 (d, J = 8.5 Hz, 2H; H-3, H-3′), 6.57 (d, J = 8.5 Hz, 2H; H-4, H-4′), 4.12 (d, J = 1.4 Hz, 2H; H-5, H-5′), 3.72 (s, 6H; H_3-13, H-13′), 2.57 (s, 2H; OH-5, OH-5′), 2.52 (dd, J = 19.0, 11.3 Hz, 2H; H-7α, H-7′α), 2.41 (dd, J = 19.0, 6.1 Hz, 2H; H-7β, H-7′β), 2.12 (m, 2H; H-6, H-6′), 1.18 ppm (d, J = 6.8 Hz, 6H; H_3-11, H_3-11′); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 187.7 (s; C-9, C-9′), 179.8 (s; C-8, C-8′), 171.2 (s; C-12, C-12′), 159.4 (s; C-1, C-1′), 157.2 (s; C-4a, C-4a′), 139.7 (d; C-3, C-3′), 118.7 (s; C-2, C-2′), 107.5 (d; C-4, C-4′), 107.0 (s; C-9a, C-9a′), 99.9 (s; C-8a, C-8a′), 84.8 (s; C-10a, C-10a′), 71.4 (d; C-5, C-5′), 53.4 (q, C-13, C-13′), 32.6 (t, C-7, C-7′), 28.5 (t, C-6, C-6′), 17.5 ppm (q, C-11, C-11′); IR ($CHCl_3$): $\tilde{\nu}$ = 3582, 3014, 1747, 1611, 15891, 1214, 1058, 796, 726 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 338 (32840), 260 (13136), 228 nm (21086 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{32}H_{30}O_{14}$: 638.16356; found: 638.16376 $[M]^+$.

Blennolide A (2): Light yellow crystal (acetone/*n*-hexane 1:4) $R_f = 0.44$ (CH_2Cl_2/isopropanol 96:4); m.p. 159–160 °C; $[\alpha]_D^{20} = +181.8$ ($c = 1.62$ in $CHCl_3$); CD (CH_2Cl_2, $c = 1.6\times10^{-4}$): λ ($\Delta\varepsilon$) = 364 sh (1.77), 331 (9.9), 266 sh (0.74), 245 sh (-1.8), 224 nm (-21.8 $M^{-1}cm^{-1}$); CD (KCl): λ (φ) = 366 sh (5.6), 328 (17.3), 267 sh (3.5), 243 (-5.0), 220 nm (-37.8 mdeg); ^1H and ^{13}C NMR spectroscopic data see Table 1; IR ($CHCl_3$): $\tilde{\nu}$ = 3593, 3029, 1742, 1621, 1586, 1234, 801, 716 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 329 (13422), 278 (4327), 229 nm (9747 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{16}H_{16}O_7$: 320.08960; found: 320.08965 $[M]^+$.

Blennolide B (3): Light yellow gum; $R_f = 0.44$ (CH_2Cl_2/isopropanol 96:4); $[\alpha]_D^{25} = +96.7$ ($c = 0.18$ in CH_2Cl_2); ^1H and ^{13}C NMR spectroscopic data see Table 1; IR ($CHCl_3$): $\tilde{\nu}$ = 3602, 3024, 1742, 1621, 1586, 1209, 796, 722 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 330 (10629), 278 (4517), 229 nm (10220 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{16}H_{16}O_7$: 320.08960; found: 320.08974 $[M]^+$.

Blennolide C (4): White powder; $R_f = 0.44$ (CH_2Cl_2/isopropanol 96:4); $[\alpha]_D^{25} = +181.7$ ($c = 0.06$ in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ = 14.03 (s; OH-8), 11.27 (s; OH-1), 6.38 (s, 1H; H-2), 6.35 (d, J = 0.4 Hz, 1H; H-4), 4.31 (brs, 1H; H-5), 3.70 (s, 3H; H_3-13), 2.82 (ddd, J = 19.2, 11.7, 7.2 Hz, 1H; H-7α), 2.64 (d, J = 1.1 Hz; 5-OH), 2.38 (dd, J = 19.2, 6.2, 1H; H-7β), 2.29 (d, J = 0.4, 3H; H_3-11), 2.14 (m, 1H; H-6α), 1.95 ppm (m, 1H; H-6β); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 187.0 (s; C-9), 179.1 (s; C-8), 171.2 (s; C-12), 161.9 (s; C-1), 157.6 (s; C-4a), 149.9 (s; C-3), 111.7 (d; C-2), 108.7 (d; C-4), 104.9 (s; C-9a), 100.1 (s; C-8a), 83.9 (s; C-10a), 67.0 (d; C-5), 53.4 (q; C-13), 24.3 (t; C-7), 23.1 (t; C-6), 22.5 ppm (q, C-11); IR ($CHCl_3$): $\tilde{\nu}$ = 3598, 3029, 1742, 1626, 1465, 1214, 796, 712 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 332 (16329), 280 (4825), 229 nm (10763 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{16}H_{16}O_7$: 320.08960; found: 320.08970 $[M]^+$.

Blennolide D (5): Colorless oil; $R_f = 0.43$ (CH_2Cl_2/isopropanol 96:4); $[\alpha]_D^{25} = -18.1$ ($c = 0.31$ in CH_2Cl_2); CD (CH_2Cl_2, $c = 7.0\times10^{-3}$): λ ($\Delta\varepsilon$) = 345 (+0.83), 317 sh (-1.56), 306 (-1.94), 276 sh (-4.37), 270 (-4.72), 235 (-8.68), 219 (+11.1 $M^{-1}cm^{-1}$); ^1H and ^{13}C NMR spectroscopic data see Table 2; IR ($CHCl_3$): $\tilde{\nu}$ = 3597, 3019, 1802, 1742, 1616, 1586, 1224, 796, 721 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 350 (2809), 271 (38537), 229 nm (6883 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{16}H_{16}O_8$: 336.08452; found: 336.08461 $[M]^+$.

Blennolide E (6): Colorless oil; $R_f = 0.43$ (CH_2Cl_2/isopropanol 96:4); $[\alpha]_D^{20} = +69.0$ ($c = 2.17$ in $CHCl_3$); CD (CH_2Cl_2, $c = 6.6\times10^{-3}$): λ ($\Delta\varepsilon$) = 345 (-0.1), 317 sh (+0.6), 307 (+0.6), 276 sh (+2.1), 270 (+2.3), 235 (+2.2), 219 (-7.3 $M^{-1}cm^{-1}$); ^1H and ^{13}C NMR spectroscopic data in $CHCl_3$, see Table 2; ^1H NMR (600 MHz, CD_3CN): δ = 11.43 (s, 1H; OH-5), 7.46 (t, ^3J(H,H) = 8.5 Hz, 1H; H-7), 6.57 (d, ^3J(H,H) = 8.5 Hz, 1H; H-6), 6.52 (d, ^3J(H,H) = 8.5 Hz, 1H; H-8), 4.74 (d, ^3J(H,H) = 8.3 Hz, 1H; H-11), 4.48 (d, ^3J(H,H) = 2.2 Hz, 1H; H-9), 3.67 (s, 3H; H_3-15), 3.25 (d, ^2J(H,H) = 17.0 Hz, 1H; H-3α), 3.06 (d, ^2J(H,H) = 17.0 Hz, 1H; H-3β), 2.97 (dq, ^3J(H,H) = 7.6 and 2.0 Hz, 1H; H-10), 1.13 ppm (d, J = 7.3 Hz, 3H; H_3-13); IR ($CHCl_3$): $\tilde{\nu}$ = 3578, 3024, 1797, 1742, 1656, 1576, 1224, 786, 711 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 353 (3686), 272 (10294), 229 nm (8314 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{16}H_{16}O_8$: 336.08452; found: 336.08456 $[M]^+$.

Blennolide F (7): Colorless oil; $R_f = 0.43$ (CH_2Cl_2/isopropanol 96:4); $[\alpha]_D^{25} = +12.7$ ($c = 0.11$ in CH_2Cl_2); CD (CH_2Cl_2, $c \approx 7\times10^{-3}$): λ ($\Delta\varepsilon$) = 345 (-0.41), 318 sh (+1.74), 307 (+1.94), 278 sh (+4.58), 271 (+5.01), 235 (+4.51), 219 (-29.4 $M^{-1}cm^{-1}$); ^1H and ^{13}C NMR spectroscopic data, see Table 2; IR ($CHCl_3$): $\tilde{\nu}$ = 3682, 3053, 1795, 1742, 1657, 1271, 896, 737 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 339 (2157), 271 (5187), 229 nm (6041 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{16}H_{16}O_8$: 336.08452; found: 336.08453 $[M]^+$.

Blennolide G (8): light yellow gum; $R_f = 0.42$ (CH_2Cl_2/isopropanol 96:4); $[\alpha]_D^{25} = +81.1$ ($c = 0.29$, $CHCl_3$); CD (CH_2Cl_2, $c = 6.2\times10^{-3}$): λ ($\Delta\varepsilon$) = 379 sh (+2.37) 361 (+3.35), 333 (+4.37), 279 (+2.33), 274 sh (+2.30), 224 (-19.4 $M^{-1}cm^{-1}$); ^1H and ^{13}C NMR spectroscopic data, see Table 4; IR ($CHCl_3$): $\tilde{\nu}$ = 3599, 3018, 1782, 1738, 1606, 1528, 1225, 790, 668 cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 380 (sh, 6700), 335 (13801), 267 nm (14041 $mol^{-1}m^3cm^{-1}$); HRMS (EI): m/z: calcd for $C_{32}H_{30}O_{14}$: 638.16356; found: 638.16360 $[M]^+$.

X-ray crystallographic studies of blennolide A (2): Light yellow block crystals of **2** were obtained by recrystallization from acetone/*n*-hexane (1:4). $C_{16}H_{16}O_7$ (Mr = 320.29), monoclinic, space group $P2_1$ with a = 8.9805(11) Å, b = 7.5036(10) Å, c = 11.1525(14) Å, β = 104.497 (3)°, V = 727.60(16) Å^3, Z = 2, D_{calcd} = 1.462 g cm^{-3}, λ = 0.71073 Å. Intensity data were measured on a Bruker-AXS SMART APEX CCD diffractometer. A total of 6234 reflections were collected to a maximum 2Θ value of 55.7° at 120(2) K. Data reduction and semiempirical absorption correc-

tion were from equivalents with the Bruker package.[28] The structure was solved by direct methods and refined by full-matrix, least-squares procedures. The title compound crystallizes in the non-centrosymmetric space group $P2_1$; however, in the absence of significant anomalous scattering effects, the Flack parameter is essentially meaningless. Accordingly, Friedel pairs were merged. All non-hydrogen atoms were given anisotropic thermal parameters; hydrogen atoms were located from difference Fourier maps and refined at idealized positions riding on their parent atoms. The refinement converged at $R1(I > 2\sigma(I)) = 0.036$, $wR2$ (all data) $= 0.092$ for 1861 independent reflections and 214 variables.

CCDC-668574 (**2**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Agar diffusion test for biological activity: Compounds **1**–**3**, **5**, and **6** were dissolved in acetone at a concentration of 1 mg mL^{-1}. A sample of the solution (50 μL, 0.05 μg) was pipetted onto a sterile filter disc (Schleicher & Schuell, 9 mm), which was placed on an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism.[29] The test organisms were the Gram-negative bacterium *Escherichia coli*, the Gram-positive bacterium *Bacillus megaterium* (both grown on NB medium), the fungus *Microbotryum violaceum*, and the alga *Chlorella fusca* (both grown on MPY medium).[29] These microorganisms were chosen because a) they are non-pathogenic, and b) they had in the past proved to be accurate initial test organisms for antibacterial, antifungal, and antialgal/herbicidal activities. Commencing at the outer edge of the filter disc, the radius of zone of inhibition was measured in mm.

Computational section: MMFF (Molecular Merck force field) and AM1 (Austin model 1) calculations were executed with Spartan′06 (Wavefunction, Inc, Irvine CA). DFT and TDDFT calculations were executed with Gaussian′03W, Revision D.01 (Gaussian, Inc., Pittsburgh PA).

Conformational searches were run with MMFF, with standard parameters and convergence criteria. The minima thus found for compounds **5** and **6**, and for the blennolide monomer of **8**, were optimized with DFT at the B3LYP/6-31G(d) level. The input geometries of **2** for TDDFT calculations were obtained from the solid-state structure upon re-optimization, by use of the DFT method at the B3LYP/6-31G(d) level, of the H-atoms' positions.

TDDFT calculations on compounds **2**, **5**, and **6** were executed with the hybrid functional B3LYP with TZVP basis set.[30] All computed transitions responsible for the CD bands above 190 nm had energies below the estimated ionization potentials, and involved virtual orbitals with negative eigenvalues.[31] CD spectra were generated by use of the rotational strengths computed with dipole-length gauge formulation, to which a Gaussian band-shape was applied with 5800 (**2**) or 4200 cm^{-1} (**5**, **6**) half-height width, corresponding to 60 and 48 nm, respectively, at 340 nm. Rotational strengths computed for all transitions with dipole-velocity gauge formulation differed from dipole-length values by less than 5% for all compounds.

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