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Facile Preparation of Bioactive *seco*-Guaianolides and Guaianolides from *Artemisia gorgonum* and Evaluation of Their Phytotoxicity

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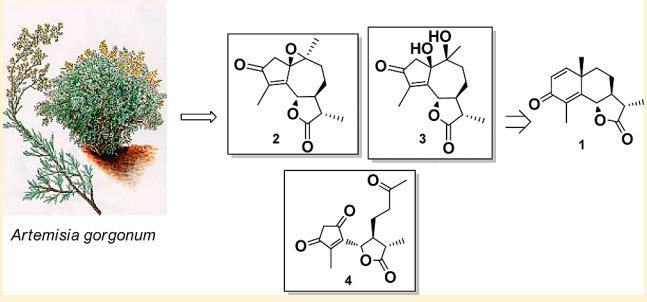
Supporting Information

ABSTRACT: Commercially available santonin was used to synthesize seven sesquiterpene lactones using a facile strategy that involved a high-yielding photochemical reaction. Three natural products from *Artemisia gorgonum* were synthesized in good yields, and in the case of two compounds, absolute configurations were determined from X-ray quality crystals. The structures previously reported for these compounds were revised. Sesquiterpene lactones were tested using the etiolated wheat coleoptile bioassay, and the most active compounds were assayed in standard target species. *seco*-Guaianolide (**4**) showed higher phytotoxic activities than the known herbicide Logran. This high activity could be due to the presence of a cyclopentenedione ring. These results suggest that compound **4** should be involved in defense of *A. gorgorum*, displaying a wide range of activities that allow proposing them as new leads for development of a natural herbicide model with a *seco*-guaianolide skeleton.



The intensive use of agrochemicals with identical modes of action has led to the development of more evolved biotypes with adaptations to the most common herbicides through several mechanisms, e.g., detoxification, changes in the active center, etc.[1] In the case of weeds, resistance (cross and multiple) has been reported to almost all commercial herbicides, including glyphosate.[2] The biotypes that have more than one mechanism of resistance to one or several different herbicides that act on a wide range of molecular targets are the most problematic weeds. This phenomenon is known as multiple resistance.[3] The control of these biotypes is extremely difficult, as the number of possible alternative herbicides diminishes. New pesticides, including natural-based systems, are needed to combat this multiple resistance.[4]

Natural products provide a wider diversity of structures, stereostructures, skeletons, and functionalizations than synthetic compounds.[5] Allelochemicals are natural herbicides that are synthesized by plants when in competition with other plants for soil and resources.[6] These compounds exert their effect on numerous—as yet unknown—molecular targets. One obvious application of allelopathy is the use of allelopathic traits to develop methods for weed and pest control. Sesquiterpene lactones have been reported as allelopathic agents with high levels of bioactivity, and these compounds are particularly abundant in plants of the family Compositae.[5,7] The sunflower is particularly noteworthy because of the economic importance of this crop and the abundance and variety of compounds of interest.[8] A number of sesquiterpene lactones, such as the guaianolide dehydrozaluzanine C,[9] have significant phytotoxic activity.

The endemic Cape Verdean *Artemisia gorgonum* is used in local folk medicine as a treatment for fever. Recently, sesquiterpene lactones **2**, **3**, and **4** have been isolated from this plant, and their good antimalarial activity has been demonstrated.[10] Compound **3** was previously isolated from *Artemisia adamsii*, but the structure was initially proposed as **2**.[11] These compounds were all isolated in small amounts, and their synthesis is therefore required for further biological studies.

Herein we report the synthesis of these sesquiterpene lactones and an assessment of their phytotoxic activity and potential for production as new natural product-based herbicides. The key step in the proposed retrosynthetic reaction sequence (Scheme 1) is a high-yielding photochemical transformation of the readily available α-santonin **1**. The structures of the natural products and their isomers **2**, **3**, **7**, and **8** have been revised. We also report the evaluation of the phytotoxic effects of these sesquiterpene lactones on several crops.

RESULTS AND DISCUSSION

Synthesis. Sesquiterpene lactones **2**−**8** were prepared by the reactions outlined in Scheme 2. The synthetic strategy

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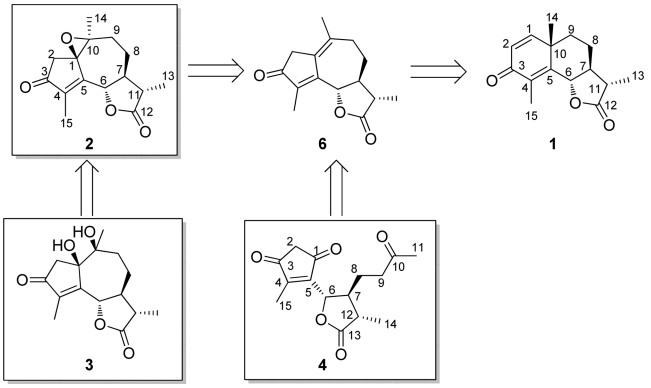
Scheme 1. Retrosynthetic Analysis of the *seco*-Guaianolides and Guaianolides 2, 3, and 4



involves the transformation of α-santonin, a commercial sesquiterpene lactone with the eudesmanolide structure, into the guaianolide isophotosantonin (**5**).

The reaction occurs through a photochemical process that has been known since 1957[12] to provide an interesting source of functionalized guaianolide sesquiterpene lactones. The reaction was initially carried out in an acidic aqueous medium following the procedure recently published by Barbosa et al. to give the guaianolide isophotosantonin (**5**) in 32% yield.[13] The photochemical reaction was optimized through an extensive study of the reaction conditions, and the yield was increased to 75%. These results have no literature precedent. The main results are summarized in Table 1. The yields were quantified by ^{1}H NMR spectroscopy on the crude reaction mixture with 1,3,5-trimethoxybenzene used as internal standard. This simple, rapid, and easy to implement approach was carried out according to the methodology developed by different groups.[14]

Photochemical reactions are renowned for the large amounts of byproducts that are often obtained, a characteristic that decreases the yields of target products. The use of filter solutions can improve results, and, for example, our group has

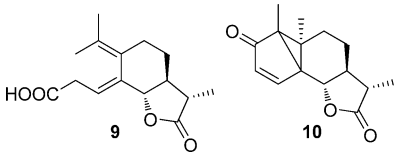
Table 1. Summary of the Optimization Study of the Photochemical Reaction to Obtain Isophotosantonin (5)

no.	time (h)	acid/water ratio (mL/mL)	atmosphere	filter sol.	temperature (°C)	yield (%)
1	7	40/40	Ar	no	rt	30
2	5	20/60	Ar	no	rt	17
3	5	10/50	Ar	no	rt	33
4	3	20/60	Ar	no	reflux	0[a]
5	5	10/50	Air	no	rt	11
6	5	10/70	Ar	yes	rt	24
7	12	20/80	Ar	yes	10	74
8	7	15/65	Ar	Yes	~2	75
9	7	48/60		no	reflux	17[b]
10	27	80/80	N_2	no	rt	32[c]

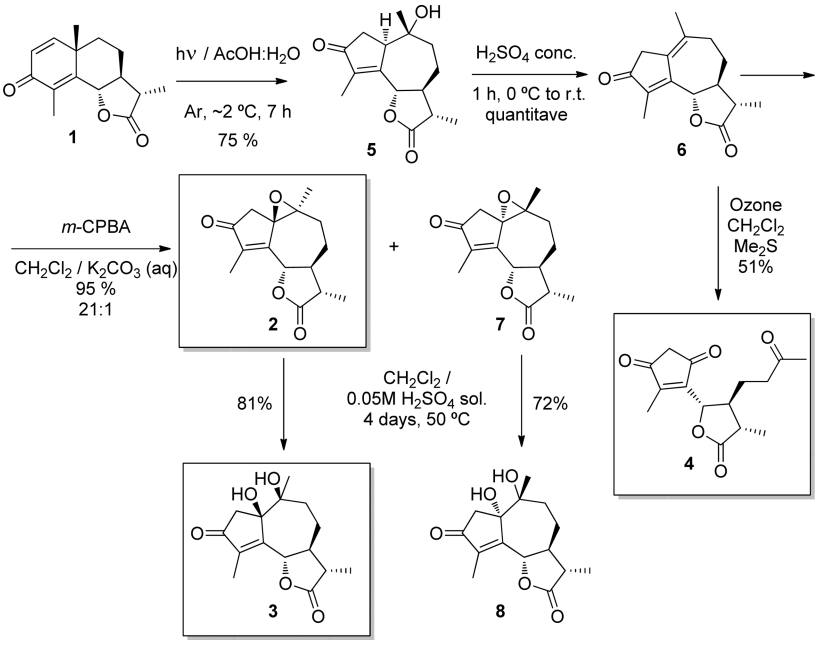
[a]Mainly isophotosantonic acid (**9**) was obtained. Isophotosantonin (**5**) was not observed. [b]Conditions described by Barton.[12] [c]Conditions described by Barbosa.[23]

reported the use of a solution of Ni(II) and Co(II) to restrict radiation below 3000 Å,[15] thus providing cleaner reactions without the formation of photoreduction products and with fewer byproducts. In this study cleaner reactions and better yields were obtained when the filter solution was used (entries 6–8). It is important to note that under these conditions only three products were observed: the starting material α-santonin (**1**), isophotosantonic acid (**9**), and isophotosantonin (**5**). Lumisantonin (**10**) was not observed in any case.

Scheme 3. Structures of Isophotosantonic Acid (9) and Lumisantonin (10)[13]



Scheme 2. Synthetic Route to *seco*-Guaianolides and Guaianolides 2, 3, and 4



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Reaction temperature was found to be an important parameter. Lower temperatures led to higher yields of isophotosantonin (**5**) (entries 7, 8), whereas high reaction temperatures generated only isophotosantonic acid (**9**) (entry 4). The reaction atmosphere also played a key role because the use of argon led to fewer byproducts and better yields (entries 3, 5). Finally, the acid/water ratio has some influence on the results, with higher ratios giving the best yields.

The photochemical study revealed that isophotosantonin can be obtained in high yields by judicious choice of conditions and with significantly lower reaction times than previously reported (entry 8). An X-ray quality crystal of isophotosantonin (**5**) (as the monohydrate) was obtained for the first time (see Supporting Information, Figure S17). The configuration at C-10 was confirmed to be *R*, with the OH group in an α-orientation.

The diene **6** was obtained quantitatively by acid-mediated elimination of water from **5**. The procedure, adapted from Westwood et al., was previously used to eliminate an acetyl group at C-10 from the acetylated isophotosantonin.[16,17] Products from the elimination at C-14 or C-9 were not observed.

Subsequent epoxidation of **6** by treatment with *m*-CPBA in a two-phase system [CH_2Cl_2/$KHCO_3$(aq)] led to a 21:1 mixture of natural compound **2** and the isomer **7**, with an overall yield of 95%. Cleavage of the oxirane ring or rearrangements of acid-sensitive species observed in other previously reported epoxidations were avoided by using this method.[18] The spectroscopic data for compound **2** are identical to those described for the natural product isolated in 2008[10] and prepared in 2009.[16]

Both previously described epoxides were reported with a different stereochemistry. This discrepancy was initially resolved by carrying out NMR NOESY studies on both isomers, with key correlations obtained for H-6/H-11/H-8β and H_3-14/H-2α/H-2β/H-9α/H-9β for the natural product and H-6/H-11/H-8β and H_3-14/H-2α for the isomer of the natural product. The expected key correlation H-6/H-14 was not observed, although this evidence is inconclusive. An X-ray quality crystal of the 1:5 EtOAc solvate of epoxide **2** was obtained by slow solvent evaporation (Figure 1). The β-

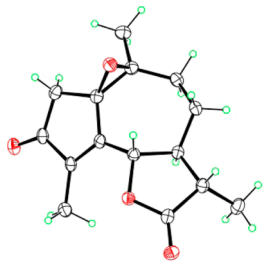


Figure 1. Crystal structure of compound **2** (ORTEP diagram).

orientation of the oxirane moiety in **2** was unequivocally established. The structure described[10] for the natural compound should therefore be corrected as indicated. The ^{1}H NMR spectrum of compound **7** showed slight differences compared to compound **2**. The spectroscopic data for both compounds are shown in Table 2. The most significant results are the chemical shifts for H-6 and H-7, which clearly indicate that **7** is the corresponding isomer of **2**, with the opposite configuration for the oxirane ring (i.e., the α-orientation).

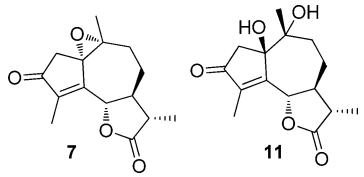
Table 2. ^{1}H NMR[a] and ^{13}C NMR[b] Spectroscopic Data (400 MHz, $CDCl_3$) for 1,10-Dioxa-isophotosantonin (2) and 1,10-Dioxa-isophotosantonin (7)

	2		**7**	
position	δ_H (ppm) (*J* in Hz)	δ_C (ppm)	δ_H (ppm) (*J* in Hz)	δ_C (ppm)
1		68.3		68.6
2a	2.72, d (18.8)	40.8	2.67, d (19.2)	39.3
2b	2.57, d (18.8)		2.44, d (19.2)	
3		203.1		203.3
4		141.4		146.1
5		159.1		157.0
6	4.82, dd (10.4; 1.6)	82.2	4.97, dd (11.0)	77.5
7	1.61, m	50.2	2.86, m	43.4
8a	1.84, m	24.5	1.45, m	23.6
8b	1.59, m		1.45, m	
9a	2.36, dt (4.4; 2.8)	33.5	2.25, dt (11.2; 4.0)	28.8
9b	2.01, dt (4.9; 3.6)		2.06, dt (11.2; 4.0)	
10		66.0		66.3
11	2.30, dq (14.0; 2.6)	41.4	2.17, dq (12.4; 2.3)	42.5
12		177.3		177.5
13	1.26, d (7.2)	12.9	1.22, d (7.2)	12.8
14	1.41, s	25.7	1.41, s	24.8
15	2.00, d (2.0)	9.3	2.01, d (1.6)	10.0

[a]400 MHz, $CDCl_3$ signal of residual $CHCl_3$ centered at 7.25 ppm. [b]100 MHz, signals of $CDCl_3$ centered at 77.0 ppm.

The epoxide cleavage reactions of **2** and **7** gave the unexpected *syn*-diols **3** and **8**, respectively, in good yields of 81% and 72%, respectively. Usually this reaction affords the *trans*-1,2-diols.[10] The synthesis was carried out following a two-phase acid hydrolysis with dilute H_2SO_4 and DCM. The spectroscopic data for the products are identical to those previously reported by the group of Bohlmann (1985)[11] and Ortet (2008)[10] for natural products whose structures were assigned as **7** and **11** (Scheme 4).

Scheme 4. Structures of Natural Products Proposed by Bohlmann and Ortet



An X-ray quality crystal of *syn*-diol **3** was obtained under similar conditions to epoxide **2** (Figure 2), and the structure was corrected to **3**, a *syn*-diol with the hydroxy groups in a β-

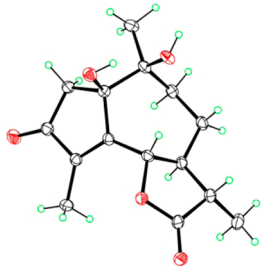


Figure 2. Crystal structure of compound **3** (ORTEP diagram).

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orientation. This finding suggests that the structure of the natural compound must also be revised. In 2009 Westwood and co-workers synthesized the *syn*-diols **3** and **8** using a different approach to obtain 87% yield of a 3:1 mixture described as inseparable diastereomers. Only the *syn*-diol **3** has been characterized with identical ^{1}H NMR and ^{13}C NMR spectra to those obtained in this work. The spectroscopic data for both *syn*-diols are shown in Table 3. The most significant differences

Table 3. ^{1}H NMR[a] and ^{13}C NMR[b] Spectroscopic Data (400 MHz, $CDCl_3$) for 1,10-Diol-isophotosantonin (3) and 1,10-Diol-isophotosantonin (8)

	3		8	
position	δ_H (ppm) (*J* in Hz)	δ_C (ppm)	δ_H (ppm) (*J* in Hz)	δ_C (ppm)
1		80.4		82.9
2a	2.52, d (18.0)	46.6	2.89, d (12.0)	48.4
2b	2.43, d (18.4)		2.35, d (12.0)	
3		205.4		206.5
4		137.3		141.3
5		165.2		163.0
6	5.25, dd, (10.4; 1.6)	78.0	4.97, dd (11.0)	82.4
7	1.76, m	52.6	2.66, m	46.2
8a	1.84, m	23.2	1.66, m	25.0
8b	1.78, m		1.60, m	
9a	2.10, dt, (4.4;2.8)	34.3	2.80, dt, (11.2;4.0)	37.3
9b	1.46, dt (4.9; 3.6)		2.33, dt (11.2; 4.0)	
10		74.0		75.8
11	2.37, dq (14.0;2.6)	42.7	2.25, dq (12.4;2.3)	41.6
12		177.8		178.3
13	1.22, d (7.2)	12.8	1.23, d (7.2)	12.3
14	1.24, s	26.2	1.40, s	29.0
15	1.80, s	8.4	1.86, s	9.6

[a]400 MHz, $CDCl_3$ signal of residual $CHCl_3$ centered at 7.25 ppm. [b]100 MHz, signals of $CDCl_3$ centered at 77.0 ppm.

are the chemical shifts of the signals for H-6, H-7, H-9a, and H-9b. These results indicate that **8** is the corresponding isomer of **3**, with the opposite orientation for the hydroxy groups (i.e., α-orientation). This is the first time that **3** and **8** have been prepared, isolated, and unequivocally characterized separately.

The next challenge was to synthesize natural product **4**. Diene **6** was converted into *seco*-guaianolide (**4**) in good yield by oxidation with ozone in a cold solution using dry DCM and Me_2S. The ^{1}H and ^{13}C NMR spectra of the product are identical to those reported for the natural product.[10]

In summary, the natural products **2**, **3**, and **4** were obtained in 3 or 4 steps with overall yields of 68%, 55%, and 38%, respectively.

Bioactivity. The natural products were tested for growth of *Plasmodium falciparum*, and they displayed weak activity. The cytotoxic activity was subsequently evaluated on the growth of the VERO cell line, and the compounds showed weak activity in all cases. (Complete bioassay data are provided in the Supporting Information, Tables S1 and S2.) Following our procedure for the preselection of compounds to be tested for phytotoxicity, we initially assessed the sesquiterpene lactones **1**–**8** using the etiolated coleoptile bioassay carried out with the following concentrations: 1 mM, 500 μM, 100 μM, 50 μM, 10 μM. Bioassay data are shown in Figure 3, where negative values signify inhibition, positive values denote activation, and zero represents the control. All compounds, with the exception of

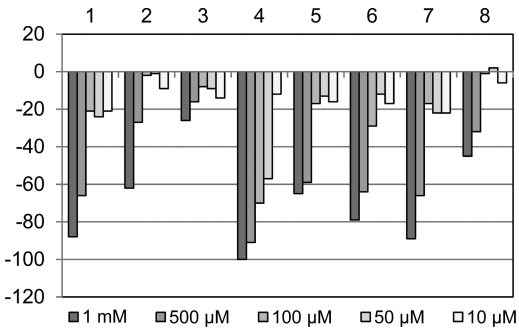

Figure 3. Etiolated coleoptile bioassay.

diols **3** and **8**, displayed high levels of phytotoxic activity [**1**, −88% 1 mM, −66% 500 μM; **2**, −62% 1 mM; **5**, −65% 1 mM, −59% 500 μM; **6**, −79% 1 mM, −64% 500 μM; **7**, −89% 1 mM, −66% 500 μM]. In particular, *seco*-guaianolide (**4**) gave a remarkable activity even when tested at the lowest concentration [50 μM], and this is clearly the most active compound in the series [**4**, −100% 1 mM, −91% 500 μM, −70% 100 μM, −57% 50 μM].

All the sesquiterpene lactones except diols **3** and **8** were evaluated in the phytotoxicity bioassay using the standard target species *Lepidium sativum* (cress), *Allium cepa* (onion), *Lactuca sativa* (lettuce), and *Lycopersicum esculentum* (tomato) and the commercial herbicide Logran as internal standard.[19] The concentrations tested in the phytotoxicity assay were identical to those in the previous bioassay. Once again, *seco*-guaianolide (**4**) gave the highest phytotoxic activity [*L. sativum* (root, −85% 1 mM, −82% 500 μM; shoot, −71% 1 mM, −58% 500 μM), *A. cepa* (root, −84% 1 mM, −80% 500 μM, −74% 100 μM; shoot, −86% 1 mM, −82% 500 μM), *L. sativa* (root, −91% 1 mM, −88% 500 μM, −66% μM; shoot, −77% 1 mM, −45% 500 μM), and *L. esculentum* (germination, −89% 1 mM, −70% 500 μM; root, −90% 1 mM, −87% 500 μM; shoot, −78% 1 mM, −73% 500 μM)]. The results for compound **4** and for Logran, for each plant seed in germination and root and shoot lengths, are shown in Figures 4–7. In general, **4** showed

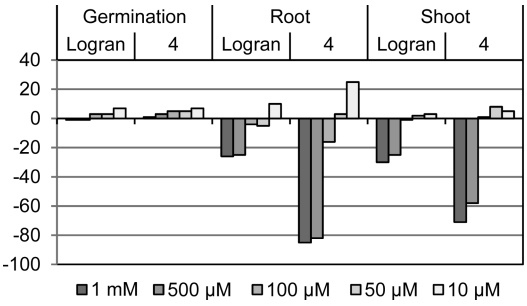

Figure 4. Cress bioassay compared to Logran.

inhibition levels higher than or similar to Logran, particularly at higher concentrations [1 mM and 500 μM]. This activity is more significant on cress and onion, where the activity is greater than that shown by the commercial herbicide.

The natural product *seco*-guaianolide (**4**) has a cyclopentenedione ring, which is uncommon in natural products. Such compounds are rare, but they have shown significant biological activities. One such example is spiromamakone A, which was isolated from the extract of a cultured non-sporulating fungal endophyte derived from the New Zealand

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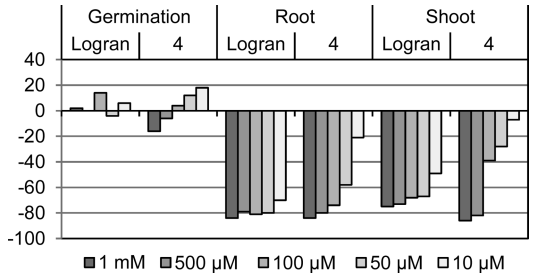

Figure 5. Onion bioassay compared to Logran.

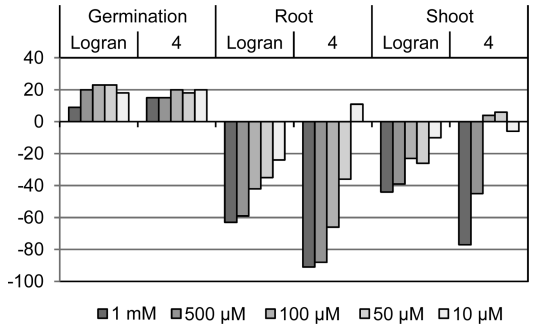

Figure 6. Lettuce bioassay compared to Logran.

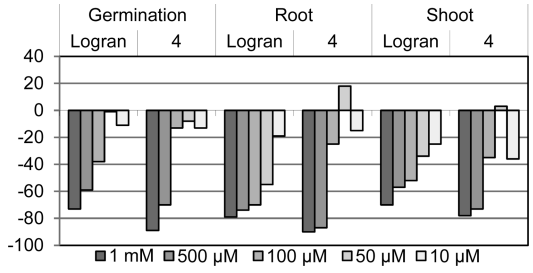

Figure 7. Tomato bioassay compared to Logran.

native tree *Knightia excelsa* (rewarewa). This represents a new structural entity and is a potent cytotoxic and antimicrobial agent.[20] Another example is found in the chemical constituents of the endophytic fungus *Preussia* sp., which led to the isolation of new spirobisnaphthalene analogues such as spiropreussione A. This compound showed cytotoxicity toward A2780 and BEL-7404 cells with IC_{50} values of 2.4 and 3.0 μM, respectively.[21]

CONCLUSIONS

The synthesis of natural products **2**, **3**, and **4** was achieved in good overall yields, and the structures of guaianolides **2** and **3** were established by X-ray crystallography, with the structures previously reported by Bohlmann and Ortet revised. All compounds showed good phytotoxic activity, especially the *seco*-guaianolide (**4**), which showed remarkable activity that was higher than the internal standard Logran. This interesting activity could be related to the presence of a cyclopentenedione ring in the structure of the natural product. We will continue to elucidate the structural requirements for activity in these compounds based on intensive structure−activity studies.

EXPERIMENTAL SECTION

General Experimental Procedures. All melting points are uncorrected, and optical rotations were measured using a Perkin-Elmer polarimeter (model 241) set on the sodium D line. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrum 1000 Mattson 5020 system. HR-MS results were determined using VG 1250 or VG Autospect instruments at 70 eV. ^{1}H NMR, ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C gHSQC, and ^{1}H−^{13}C gHMBC NMR spectra were obtained at 399.954 and 100.565 MHz for ^{13}C NMR on a Varian INOVA-400 spectrometer using $CDCl_3$ as solvent. Chemical shifts are given in parts per million with respect to residual ^{1}H signals of $CDCl_3$ (δ_H 7.25) and ^{13}C signals of $CDCl_3$ (δ_C 77.00). Column chromatography was performed on silica gel (35−75 mesh), and TLC analysis was carried out using aluminum precoated silica gel plates. Synthetic products were purified by preparative HPLC using a Lichrosorb silica 60 semipreparative column (Lichrospher SiO_2, Merck, 7 and 10 μm, 150 × 10 nm) and analytical columns Lichrosorb silica 60 (LiChrospher SiO_2, Merck, 7 and 10 μm, 250 × 10 mm) and Phenomex Luna [Phenomex Luna Silica (2), 10 μm, 100A] in conjunction with a Hitachi Lachrom D-7000 PLC system with a Hitachi L-7490 RI detector and Hitachi L-7420 UV detector. All solvents were spectroscopy grade or were distilled from glass prior to use. α-Santonin was obtained from Sigma-Aldrich.

General Photochemical Reactions: NMR Yield Calculation Procedure. Photochemical reaction crude mixtures were analyzed by ^{1}H NMR spectroscopy using the internal standard 1,3,5-trimethoxybenzene to calculate the molecular weight of a given compound by eq 1, where the subscript (u) means unknown in reference to the different products present in the crude mixture and the subscript (i) means internal standard. The (m) represents the moles, (P) the number of protons per molecule, and (A) the area of the peaks or cluster of peaks. The basis for the quantitative use of ^{1}H NMR is derived from the direct proportional relationship between the area of the proton resonance signal and the number of protons in the observed resonance. This relationship has been proposed as a method for the determination of the molecular weight of unknown compounds by different groups.[14] Additionally the molecular weight permits the calculation of yield, which is compared with the yield obtained by traditional purification of products.

$$m_u = \frac{A_u P_i m_i}{A_i P_u} \quad (1)$$

General X-ray Experimental Data. Crystal structures of **5** monohydrate, **2**·1/5 EtOAc, and **3** were determined using data collected with Cu Kα radiation (λ = 1.54178 Å) at low temperature on a Bruker Kappa Apex-II diffractometer. Absolute configurations of all structures were determined by refinement of the Flack parameter.[22]

Crystal data for **5** monohydrate: $C_{15}H_{20}O_4{\cdot}H_2O$, M_r = 282.33, orthorhombic space group $P2_12_12_1$, a = 7.6345(10) Å, b = 13.862(2) Å, c = 13.927(2) Å, V = 1473.9(4) Å^3, Z = 4, ρ_{calcd} = 1.272 g cm^{-3}, μ = 0.78 mm^{-1}, T = 90 K, 9639 data collected with θ < 68.7°, R = 0.025 (I > 2σ(I)), R_w = 0.066 (all data) for 2624 unique data and 194 refined parameters, Flack x = 0.01(13) for 1076 Friedel pairs, CCDC 884581. Crystal data for **2**·EtOAc solvate: $C_{15}H_{18}O_4{\cdot}1/5C_4H_8O_2$, M_r = 279.91, orthorhombic space group $P2_12_12_1$, a = 7.3918(2) Å, b = 29.7040(10) Å, c = 32.1937(10) Å, V = 7068.6(4) Å^3, Z = 20, ρ_{calcd} = 1.315 g cm^{-3}, μ = 0.78 mm^{-1}, T = 90 K, 57 945 data collected with θ < 68.4°, R = 0.029 (I > 2σ(I)), R_w = 0.077 (all data) for 12 751 unique data and 929 refined parameters, Flack x = 0.00(7) for 5549 Friedel pairs, CCDC 884582. Crystal data for **3**: $C_{15}H_{20}O_5$, M_r = 280.31, orthorhombic space group $P2_12_12_1$, a = 9.0581(4) Å, b = 9.5429(4) Å, c = 15.5537(7) Å, V = 1344.47(10) Å^3, Z_e = 4, ρ_{calcd} = 1.385 g cm^{-3}, μ = 0.86 mm^{-1}, T = 90 K, 12 926 data collected with θ < 69.5°, R = 0.027 (I > 2σ(I)), R_w = 0.072 (all data) for 2467 unique data and 191 refined parameters, Flack x = 0.10(14) for 1024 Friedel pairs, CCDC 884583. Supplementary data in CIF format can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

General Methodology. *Isophotosantonin (**5**).* Based on a modification of the method described by Barbosa and co-workers[23] and Barton and co-workers,[12] α-santonin (100 mg, 0.41 mmol) was dissolved in HOAc (15 mL) and H_2O (65 mL) and stirred in a modified Hanovia reactor cooled with ice−water with a Ni(II) and

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Co(II) aqueous solution as a filter (the filter solution contained 46 g of $NiSO_4 \cdot 6H_2O$ and 14 g of $CoSO_4 \cdot 7H_2O$ per 100 mL of water). The reaction mixture was degassed by a flow of Ar for 5 min and was then irradiated with a 125 W mercury lamp at 0 °C for 7 h. The mixture was concentrated under reduced pressure after addition of cyclohexane. The product was purified by column chromatography (EtOAc/*n*-hexane = 70:30). Isophotosantonin (**5**) was obtained as a white solid (81 mg, 75% yield): mp 145−146 °C; $[\alpha]^{20}_D$ +105.0 (*c* 1.12, CH_3Cl); IR (thin film) ν_{max} 3466, 2973, 2932, 2878, 1775, 1701, 1641, 1456, 1340, 1312, 1232, 1177, 1154, 1134, 1099, 991,752 cm^{-1}; ^1H NMR data are identical to those reported by Barbosa et al.[13] X-ray quality crystals of pure isophotosantonin (**5**) were obtained by slow evaporation of an EtOAc solution (data submitted to CCDC (CCDC 884581)).

*3-Oxo-11β(S)-H-4,11(13)-Guaiadien-6α(S),12-olide (**6**).* To concentrated H_2SO_4 (10 mL) at 0 °C was added isophotosantonin (**5**) (200 mg, 0.75 mmol) portionwise over 10 min, and the mixture was stirred for a further 10 min. The ice bath was removed, and the mixture was allowed to warm to room temperature and stirred for 50 min. The resulting brown solution was poured into ice−water and was allowed to warm to room temperature before extracting with DCM (3 × 60 mL). The combined organic layers were washed with 5% NaOH solution (20 mL) and H_2O and brine (20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the diene **6** (185 mg, 0.75 mmol) as a white solid in quantitative yield: mp 87−90 °C; ^1H NMR data are identical to those reported by Westwood et al.[16]

*1β(R),10β(S)-Epoxy-3-oxo-11β(S)H-4,11(13)-guaien-6α(S),12-olide (**2**) and 1α(S),10α(R)-Epoxy-3-oxo-11β(S)H-4,11(13)-guaien-6α(S),12-olide (**7**).* To a solution of diene **6** (200 mg, 0.81 mmol) in DCM (14 mL) was added m-CPBA (280 mg, 1.62 mmol) and saturated aqueous $KHCO_3$ (14 mL). The mixture was stirred for 3 h at room temperature. The reaction was quenched with NaOH (5 mL, 0.5 M). DCM was added, the layers were separated, and the aqueous phase was extracted with DCM. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated, and the residue was purified by HPLC (EtOAc/*n*-hexane = 55:45, flow 12 mL/min) to give two fractions. The major fraction was the naturally occurring epoxide **2**, and the other contained the isomer **7**. Natural epoxide **2** was obtained as a white solid (193 mg, yield 91%). The fraction corresponding to its isomer was purified by HPLC using an analytical column (EtOAc/*n*-hexane = 55:45, flow 3 mL/min). The isomer and the natural compound were separated in a 21:1 ratio of **2**:**7** with an overall yield 95%. The spectroscopic data for the two compounds are shown in Table 2. Epoxide **2**: mp 125−127 °C (reported: mp 134−136 °C[16]); $[\alpha]^{20}_D$ −59.0 (*c* 1, CH_3Cl); IR (thin film) ν_{max} 3499, 2971, 2930, 2878, 1786, 1714, 1644, 1413, 1379, 1313, 1297, 1232, 1167, 1079, 1042, 1011, 957, 804, 752, 633, 603 cm^{-1}; X-ray quality crystals of **2** were obtained by slow evaporation of an EtOAc solution of pure epoxide, and the structure of the natural compound was confirmed (data submitted to CCDC (CCDC 884582)).

*1β(R),10β(S)-Dihydroxy-3-oxo-11β(S)H-4,11(13)-guaien-6α(S),12-olide (**3**) and 1α(S),10α(R)-Dihydroxy-3-oxo-11β(S)H-4,11(13)-guaien-6α(S),12-olide (**8**).* To a solution of epoxide **2** or **7** (100 mg, 0.38 mmol) in DCM (20 mL) was added a solution of H_2SO_4 (20 mL, 0.05 M). The mixture was heated under reflux and stirred for 4 d at 50 °C. The reaction was quenched with saturated aqueous K_2CO_3 (~pH 9). H_2O (20 mL) and DCM (20 mL) were added. The product was extracted with DCM (4 × 20 mL), and the combined organic layers were washed with brine. Compound **3** was obtained in 81% yield (87 mg), and compound **8** in 72% yield (72 mg). The spectroscopic data for both compounds are shown in Table 3. Product **3**: mp 125−127 °C; $[\alpha]^{20}_D$ −82.1 (*c* 1, CH_3Cl); IR (thin film) ν_{max} 3448, 2974, 2934, 2876, 1777, 1710, 1654, 1457, 1379, 1300, 1237, 1167, 1141, 1086, 1060, 1026, 999, 879, 754 cm^{-1}; X-ray quality crystals of **3** were obtained by slow evaporation of an EtOAc solution of pure epoxide, and the structure of the natural compound was confirmed (data submitted to CCDC (CCDC 884583)). Diol **8**: $[\alpha]^{20}_D$ +71.8 (*c* 0.05, CH_3Cl); IR (thin film) ν_{max} 3442, 2926, 1762, 1700, 1458, 1376, 1316, 1261, 1235, 1175, 1014, 986, 919, 801, 733 cm^{-1}.

*4-Methyl-5-[(2S,3S,4S)-4-methyl-5-oxo-3-(3-oxobutyl)-tetrahydrofuran-2-yl]-cyclopent-4-ene-1,3-dione (**4**).* Ozone was introduced to a solution of diene **6** (100 mg, 0.41 mmol) in dry DCM (30 mL) at −75 °C, and the mixture was stirred until the solution became blue, at which point the addition of ozone was stopped. Me_2S was carefully added (2 mL) dropwise. The reaction mixture was stirred for 2 d, and the solvent was washed with saturated aqueous NaCl. The dried compounds were separated by CC using silica gel (EtOAc/*n*-hexane = 40:60, 50:50). *seco*-Guaianolide (**4**) was obtained as a white solid in 51% yield (53 mg): mp 125−127 °C; $[\alpha]^{20}_D$ −82.1 (*c* 1, CH_3Cl); IR (thin film) ν_{max} 3448, 2974, 2934, 2876, 1777, 1710, 1654, 1457, 1379, 1300, 1237, 1167, 1141, 1086, 1060, 1026, 999, 879, 754 cm^{-1}; ^1H and ^{13}C NMR data are identical to those reported by Ortet et al.[10]

Coleoptiles Bioassay. Wheat seeds (*Triticum aestivum* L. cv. Duro) were sown in 15 cm Petri dishes moistened with 10 mL of distilled H_2O and were grown in the dark at 22 ± 1 °C for 3 days. The roots and caryopses were removed from the shoots. Roots were placed in a Van der Wijt guillotine, and the apical 2 mm were cut off and discarded. The next 4 mm portion of each coleoptile was cut and used for the bioassay. All manipulations were performed under a green safelight. Fractions and extracts were predissolved in DMSO, and this led to the final bioassay concentration with a maximum of 0.1% DMSO, with dilution using phosphate-citrate buffer (2 mL) containing 2% sucrose at pH 5.6. Parallel controls were performed with the same DMSO concentration.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of sesquiterpene lactones **2**−**8**, crystal structures of **2**, **3**, and **5**, and additional bioassay data are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

In memory of Horace G. Cutler.

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