JOURNAL OF NATURAL PRODUCTS

Article

Cite This: J. Nat. Prod. XXXX, XXX, XXX−XXX

pubs.acs.org/jnp

Preparation and Phytotoxicity Evaluation of 11,13-Dehydro *seco*-Guaianolides

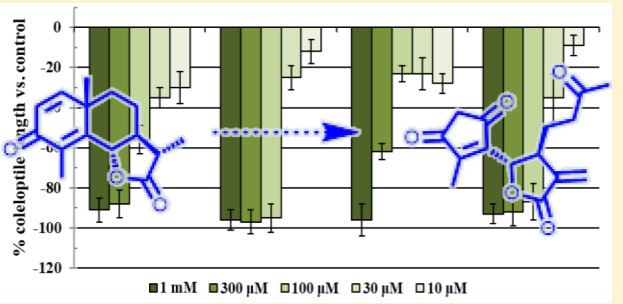
Nuria Chinchilla,[†] Alejandro Santana,[†] Rosa M. Varela,[†] Frank R. Fronczek,[‡] José M. G. Molinillo,[†] and Francisco A. Macías*[,†]

[†]Allelopathy Group, Department of Organic Chemistry, School of Sciences, Institute of Biomolecules (INBIO), University of Cadiz, C/República Saharaui, s/n, 11510 Puerto Real (Cádiz), Spain

[‡]Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, United States

Supporting Information

ABSTRACT: 11,13-Dehydro *seco*-guaianolides, a particular type of sesquiterpene lactones, were synthesized from the commercially available α-santonin (**11**) using a facile strategy involving a high-yielding photochemical reaction. Natural products **10** and **17** from *Artemisia gorgonum* were synthesized in good yields. Specifically, compound **10** was obtained in five steps with an overall yield of 17%. The sesquiterpene lactones were tested in the etiolated wheat coleoptile bioassay, and the most active compounds were assayed on standard target species. Guaianolide **13** showed the highest phytotoxic activities when compared with the known herbicide Logran.



The biological activities of plant-derived extracts are attributed to the presence of secondary metabolites. An important group of these metabolites are the sesquiterpene lactones (SLs), which are widely distributed in plants, mainly in the Asteraceae family. More than 8000 structures have been described with a regular skeleton, and *seco*-germacranolide and *seco*-guaianolides have also been reported.[1,2]

Sesquiterpene lactones exhibit a wide spectrum of biological activities, and these include anti-inflammatory and antimicrobial activities, cytotoxicity against tumor cell lines, and potential allelopathic activity.[3−5] It has been proposed that some of these activities of SLs are mediated by a Michael-type addition reaction on the electrophilic α,β-unsaturated carbonyl groups (α-methylene-γ-lactone or α,β-unsaturated cyclopentenone) with thiol-containing enzymes and proteins. This interaction adversely affects cellular function, and the extent of the effect depends on the conformation, geometry, lipophilicity, chemical environment, and accessibility of the sesquiterpene molecule to the target sites.[6,7]

The iso-*seco*-tanapartholide family of natural products isolated from plants of the genera *Artemisia* and *Achillea* were described in the literature as members of the *seco*-guaianolide family, and these provide interesting activity as inhibitors of the NF-κB signaling pathway. This pathway plays a key role in inflammation, immunology, and cancer.[8] The *seco*-guaianolides are also known as allelopathic agents, and in many cases they show high levels of activity (Figure 1).[8−18]

In 2008, *seco*-guaianolide **6** was isolated from the endemic Cape Verdean *Artemisia gorgonum* used in local folk medicine as a treatment for fever. This compound showed good antimalarial activity.[9] Previously we described the total synthesis of this compound along with its phytotoxicity evaluation.[19] High activity levels were observed in comparison with the commercial herbicide Logran, which was used as the internal standard. This interesting activity may be related to the presence of a cyclopentenedione ring in the structure of **6**.

As a continuation of this study into the outstanding results of *seco*-guaianolide **6**, our group was motivated to exploit the allelopathic characteristics to identify natural products with similar structures for weed and pest control. *seco*-Guaianolide **10** was obtained by oxidation of **7**, which was isolated from *Tanacetum macrophillum* L., a plant that is widespread in southeastern Europe.[17] This plant grows in shadowy and humid mountain valleys up to an altitude of 1500 m. This compound is similar to **6** but contains an additional Michael acceptor at the α-methylene-γ-lactone moiety. Herein, we report an alternative synthesis of *seco*-guaianolide **10** and some derivatives with the aim of assessing their phytotoxic activity for the production of new natural product-based herbicides (Scheme 1).

RESULTS AND DISCUSSION

There are only a few synthetic strategies to gain access to the guaianolide skeletons.[20,21] The easiest approach relies on the structurally advanced natural product α-santonin, which has a 6−6−5 fused ring system (eudesmanolide). This compound undergoes a photochemical rearrangement to produce a 5−7−5 ring system (guaianolide). *seco*-Guaianolides are obtained by oxidative cleavage of the guaiane derivative.

Received: March 30, 2019

DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

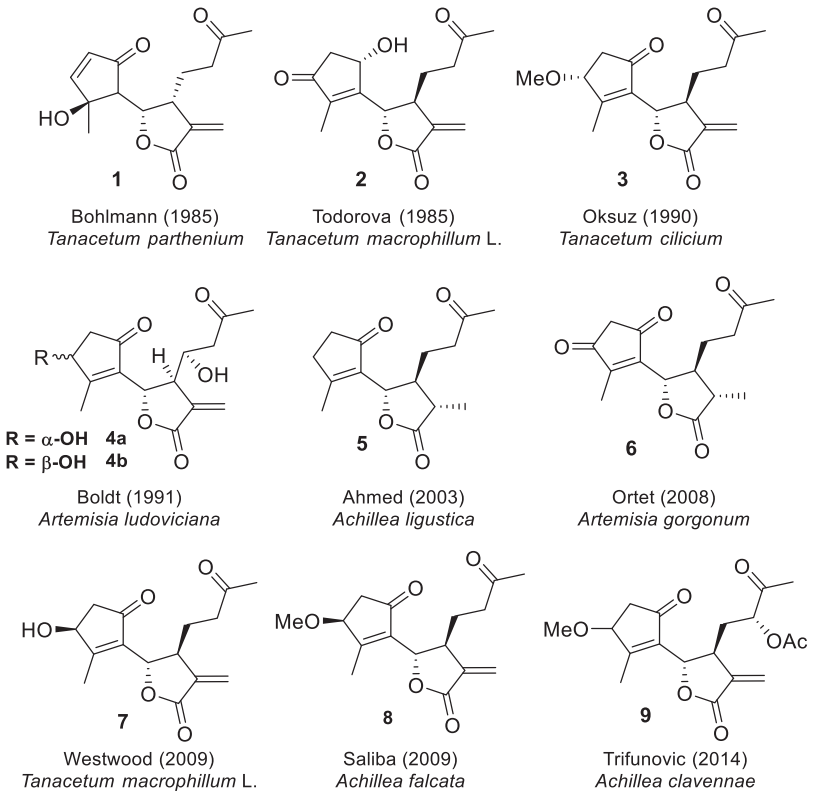
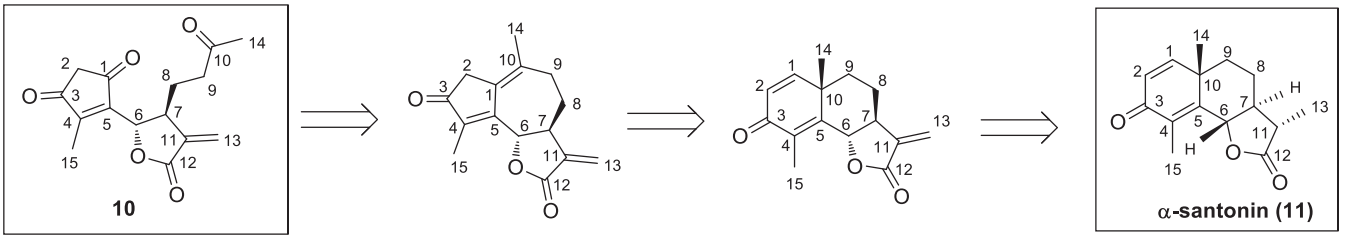
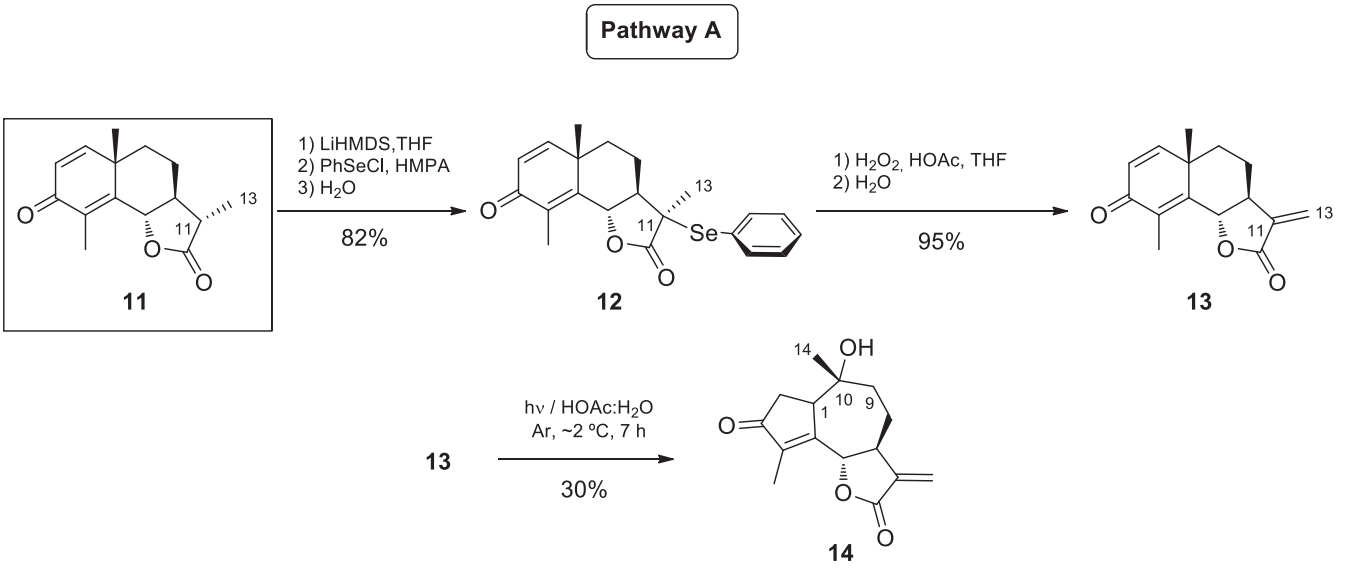


Figure 1. Structures of isolated *seco*-guaianolides.

Scheme 1. Retrosynthetic Analysis of the 1,10-*seco*-Guaianolides



Scheme 2. Proposed Synthetic Route to *seco*-Guaianolide 10, Pathway A



DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

The first approach for this synthesis is shown in Scheme 1, where the procedure is similar to that used in the preparation of **6**.[19] The synthetic strategy involves the transformation of α-santonin (**11**), a commercial sesquiterpene lactone with a eudesmanolide structure, into a guaianolide structure, followed by dehydrogenation and ozonolysis.

The first proposed methodology (pathway A) is shown in Scheme 2. Initially, synthesis of phenylseleno derivative **12** and subsequent selenium oxidation and spontaneous, regioselective *syn* elimination afforded **13** with a $\Delta^{11(13)}$ double bond by a commonly used procedure.[8,22] Conversion to intermediates **12** and **13** gave excellent yields of 82% and 95%, respectively. Compound **12** was obtained as a colorless crystalline solid suitable for X-ray diffraction analysis, which confirmed the β-orientation of the phenylselenide moiety (Figure 2).

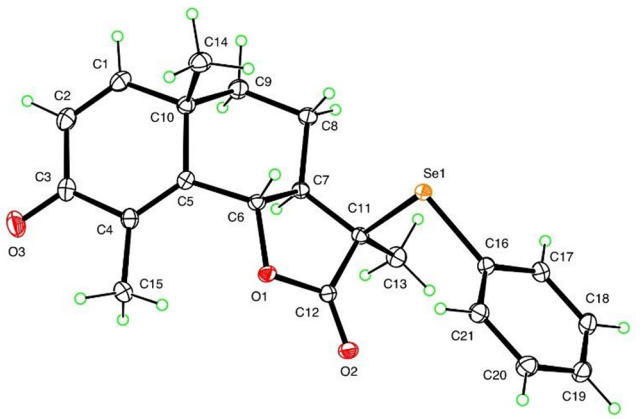


Figure 2. ORTEP diagram of phenylselenylsantonin (**12**).

Compound **13** has been also reported by Barbosa et al.,[22] and its spectroscopic data are identical, except the assignation of H_β-8 and H_β-9. Barbosa et al. report these signals as a multiplet (2H) at δ 1.56−1.63 ppm, instead a dddd signal (1H) at δ 2.19 ppm, corresponding to H_β-8, and a dt signal (1H) at 1.57 ppm assigned to H_β-9 (see Supporting Information, p S10).

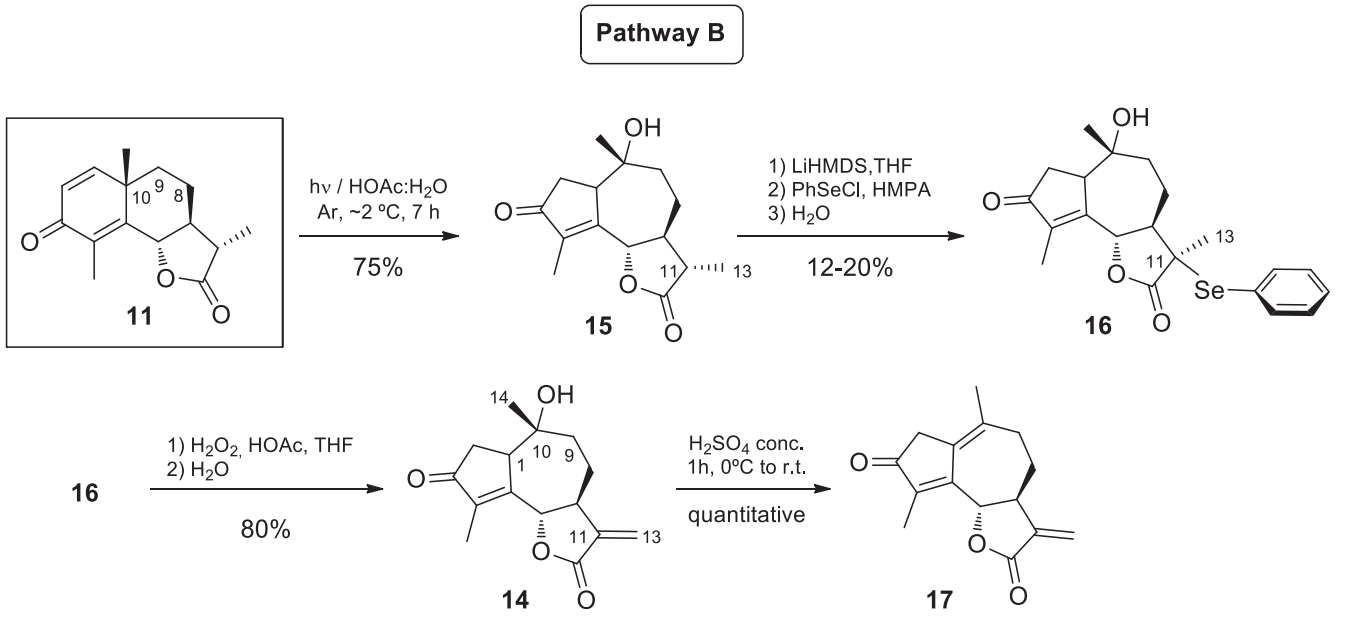
Parishin A (**14**)[23,24] was obtained by a photochemical reaction that has been known since 1957, i.e., one of the first photoreactions reported, to provide an interesting source of functionalized guaianolide sesquiterpene lactones.[25,26] This reaction was optimized in a previous project in which an extensive study of the reaction conditions permitted the synthesis of guaianolide **15** in 75% yield using α-santonin (**11**). However, when **13** was subjected to these reaction conditions, the yield decreased considerably to 30%, which is similar to that reported by Barbosa et al.[27]

With the aim of achieving better results, an alternative route (pathway B) was devised in which the isophotosantonin (**15**) (75% yield) was prepared before modifying the C-11−C-13 bond, as shown in Scheme 3. However, in this new strategy the yields of **16** were poor due to competition with the other α-carbon at C-2 of **15**. Compound **16** is described here for the first time. The absolute configuration of C-10 was assigned by comparison of the spectroscopic data collected in the literature for compound **14**.[23] However, the overall yields of SL **14** were relatively similar, i.e., 23% for pathway A and 12% for pathway B.

Dehydration of compound **14** using H_2SO_4 gave 1,10-dehydroparisin A (**17**),[8] which is described here for the first time. Compound **10** was not obtained when compound **17** was subjected to ozonolysis under the conditions reported for the preparation of **6**.[19] The reductive ozonolysis reaction was also tried using dry pyridine as the catalyst, but similar results were obtained.[28,29]

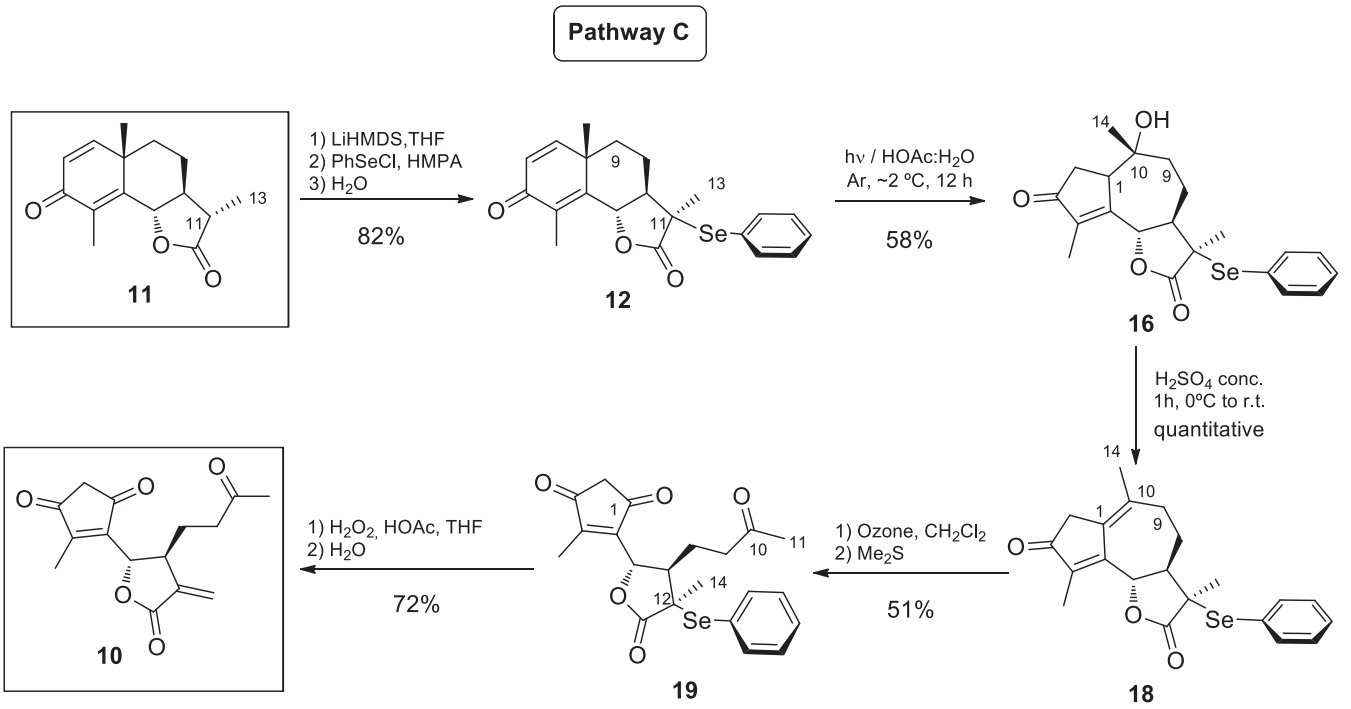
Given the low yields obtained in the second step, a third alternative (pathway C) was proposed in which the phenylselenium group was retained in the structure until the ozonolysis reaction, as shown in Scheme 4. The aim of this change was to avoid any reaction of the $\Delta^{11(13)}$ double bond. Photochemical reaction of **12** under similar conditions gave the target product in 58% yield. Although the double bond does not seem to participate significantly in this reaction, the absence of this unsaturation is crucial to obtain high yields, as

Scheme 3. Synthetic Route to *seco*-Guaianolide 10, Pathway B



DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 4. Synthetic Route to *seco*-Guaianolide 10, Pathway C



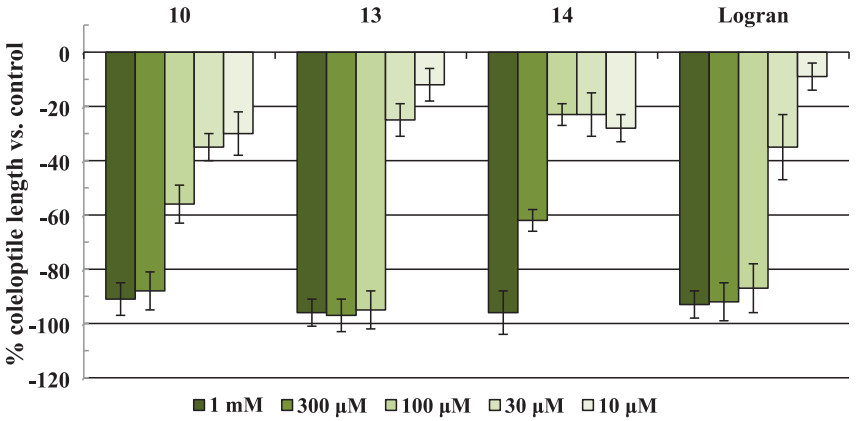


Figure 3. Results of the wheat coleoptile bioassay.

can be seen for compounds **11** and **12** vs **13**. SL **18** was obtained in quantitative yield by following the same procedure, i.e., keeping the phenylselenium group intact under extreme acidic conditions. The ozonolysis reaction was carried out using dimethyl sulfide as reductant to give **19** (58% yield), described here for the first time. Finally, *seco*-guaianolide **10** was obtained by oxidative elimination of the phenylselenium group using 30% H_2O_2 to give the target product in 72% yield.[17]

Thus, the target compound **10** was obtained in five steps with an overall yield of 17%. The importance of the sequence used for the process is worth noting. The α-methylene-γ-lactone moiety seems to interfere with the photochemical rearrangement, and it is reactive under the ozonolysis conditions. As a consequence, this moiety was formed in the last step.[30]

Bioassay Data and Discussion. Compounds **10**, **13**, and **14** were tested in the etiolated wheat coleoptile bioassay.[31] This bioassay is easy and fast (24 h) and is highly sensitive to a wide range of biological activities, including phytotoxic and antimicrobial properties. All of the above compounds showed high levels of bioactivity [**13**, −96% 1 mM, −97% 300 μM, −95% 100 μM; **14**, −96% 1 mM, −62% 300 μM; **10**, −91% 1 mM, −88% 300 μM, −560% 100 μM, −35% 30 μM]. The most active metabolite was **13**, which showed significant inhibition of elongation [100% at 100 μM ($P < 0.01$)]. The results are shown in Figure 3.

The bioactivity of these compounds was also evaluated in a phytotoxicity bioassay using seeds of the standard target species (STS)[32] *Lepidium sativum* (cress), *Allium cepa* (onion), *Lactuca sativa* (lettuce), and *Lycopersicum esculentum* (tomato) and Logran, a commercial herbicide, as the internal standard (IS). The concentrations tested in the phytotoxicity assay were identical to those in the wheat coleoptile assay. The results are shown in Figure 4. The most affected parameters were root and shoot length for all of the species tested. *L. esculentum* (tomato) was affected the most. In general, guaianolide **13** showed the best activity profiles for all parameters and all of

DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

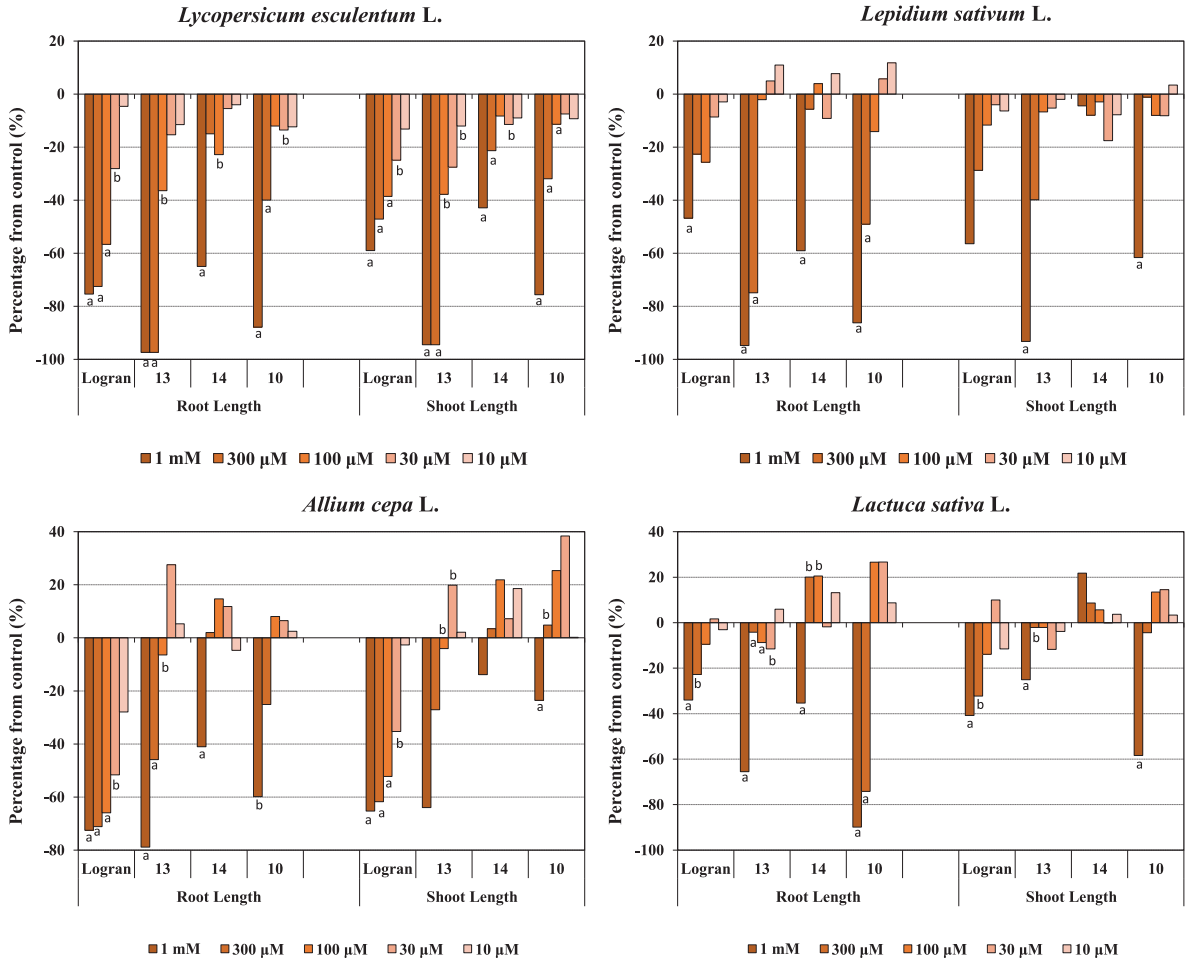


Figure 4. Effects of compounds **10**, **13**, **14**, and Logran on STS. Statistical treatment of the data was carried out by Welch's test. Values are expressed as percentage difference from control. The absence of a letter indicates $P > 0.05$, (a) denotes values significantly different from control at $P < 0.01$, and (b) denotes values significantly different at $0.01 < P < 0.05$.

the seeds evaluated. In the case of *L. esculentum*, compound **13** showed moderate to high levels of inhibition, even at low concentrations, with IC_{50} values of 125 μM for the root and 109 μM for the shoot. In the case of *L. sativum*, the most affected parameter was root length. Compounds **10** and **13** showed high levels of inhibition, with better IC_{50} values (370 and 244 μM, respectively) than those of the internal standard Logran (1521 μM). Furthermore, compound **13** showed a high activity on shoot length for the two highest concentrations tested. In the bioassay on onion, only compound **13** showed moderate activity on the two evaluated parameters, while in the bioassay on lettuce, only moderate inhibition on the root was observed for the first two concentrations tested.

In conclusion, the synthesis of *seco*-11,13-dehydroguaianolide **10** is described for the first time, and this compound was obtained in an overall yield of 17%. The synthesis of 1,10-dehydroparisin A (**17**) is also described. Compound **13** was the most active in the coleoptile bioassay, and this showed higher activity than the commercial herbicide. However, compound **10** was more active at the lowest concentration of 30 μM. Phytotoxicity bioassays using the STS showed that guaianolide **13** and *seco*-guaianolide **10** have high levels of inhibition when compared to Logran.

EXPERIMENTAL SECTION

General Experimental Procedures. All melting points are uncorrected, and $[\alpha]_D$ values were measured using a PerkinElmer polarimeter (model 241) set on the sodium D line. Infrared spectra were recorded on a PerkinElmer FT-IR Spectrum 1000 Mattson 5020 system. HRMS data were acquired using VG 1250 or VG Autospec instruments at 70 eV. ^{1}H NMR, ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C gHSQC, and ^{1}H−^{13}C g-HMBC NMR spectra were obtained at 500 and 125 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 7.25) and ^{13}C signals of $CDCl_3$ (77.00 ppm). 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), 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 spectroscopic grade or were distilled from glass prior to use. α-Santonin was obtained from Sigma-Aldrich.

General Procedure for the Preparation of Phenylseleno Derivatives (GP1). To a solution of SL (8.28 mmol) in dry tetrahydrofuran (THF) at −78 °C was added a 1.0 M THF solution of lithium bis(trimethylsilyl)amide (LiHMDS) (25 mmol) dropwise, and the mixture was stirred at this temperature for 1 h. A solution of

DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

phenylselenium chloride (10.93 mmol) in THF and hexamethylphosphoramide (HMPA) (11.18 mmol) was slowly added at −78 °C. The mixture was stirred at the same temperature for 2 h and was then allowed to warm up to room temperature. The mixture was stirred for a further 3 h. The reaction was quenched by the addition of 0.1 M HCl (15 mL), and the product was extracted with EtOAc. The organic phases were combined, washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by CC (SiO_2, EtOAc/*n*-hexane, 3:7).

General Procedure for the Preparation of α-Methylene-γ-lactone Derivatives (GP2). To a solution of phenylseleno lactones (0.60 mmol) in THF at 0 °C was added successively glacial HOAc and 30% H_2O_2. The mixture was stirred for 2 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (10 mL). The product was extracted with Et_2O, and the combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CC) (SiO_2, EtOAc/*n*-hexane, 4:6).

General Procedure for the Photochemical Reaction (GP3). Based on a reported methodology,[19] SL (0.49 mmol) was dissolved in glacial HOAc and H_2O. The mixture was stirred in a modified Hanovia reactor cooled with ice/water with an aqueous solution of Ni(II) and Co(II) as the 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 argon for 5 min and irradiated with a 125 W mercury lamp at 5 °C for 12 h. The mixture was concentrated under reduced pressure after the addition of cyclohexane. The product was purified by CC (SiO_2, EtOAc/*n*-hexane, 7:3).

General Procedure for the Dehydration of Compound 14 (GP4). The corresponding SL (0.75 mmol) was added portionwise over 10 min to H_2SO_4 (10 mL) at 0 °C, 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. The product was extracted with CH_2Cl_2 (3 × 60 mL). The combined organic layers were washed with a 5% NaOH solution (20 mL), water, and brine (20 mL) and dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure.

General Procedure for the Ozonolysis Reaction (GP5). Ozone was introduced to a solution of the corresponding SL (0.41 mmol) in dry CH_2Cl_2 at −75 °C, and the mixture was stirred until the solution became blue, with the addition of ozone stopped after 15−20 min. Dimethyl sulfide was carefully added (2 mL) dropwise. The reaction was continued for 2 days, and the organic phase was washed with saturated aqueous NaCl. The dried compounds were purified by column chromatography using silica gel.

General X-ray Experimental Data. Crystal structures of **12** was determined using data collected with Cu Kα radiation (λ = 1.541 78 Å) at low temperature on a Bruker Kappa Apex-II-DUO diffractometer. Absolute configuration of the structure was determined by refinement of the Flack parameter.[33]

Crystal data for **12**: $C_{21}H_{22}O_3Se$, M_r = 401.35, orthorhombic space group $P2_12_12_1$, a = 7.7213(3) Å, b = 10.2396(4) Å, c = 22.5926(8) Å, V = 1786.24(12) Å^3, Z = 4, ρ_{calcd} = 1.492 mg cm^{-3}, μ = 2.12 mm^{-10}, T = 90 K, 9258 independent reflections collected with θ = 2.1−37.5°. Refinement on F^2: $R[F^2 > 2\sigma(F^2)]$ = 0.019, $wR(F^2)$ = 0.043 (all data) for 9258 reflections and 229 refined parameters, Flack x = 0.014(3) for 4025 Friedel pairs, CCDC 1907207.

General Procedure for Bioassay. *Wheat Coleoptile Bioassay.* The bioassay test involved the analysis of the elongation of the cereals' apical zones (wheat in this case) in the presence of liquid media containing the potentially bioactive agents. For the sample preparation, the compounds were first dissolved in dimethyl sulfoxide (DMSO) (0.1%, v/v) and diluted in phosphate−citrate buffer at pH 5.6 (2% sucrose) to obtain the desire concentrations (1000, 300, 100, 30, and 10 μM). Parallel controls were also carried out. The buffer described above was used as a negative control, and the commercial herbicide Logran (59.4% terbutrine and 0.6% triasulfuron) was used as an internal reference.[32] Three test tubes were used per dilution, with five coleoptiles and 2 mL of solution, and they were placed in the dark at 25 °C and 6 rpm in a roller-tube apparatus for 24 h. The coleoptile elongations are expressed as percentage differences from the control. Welch's test was used for the statistical analysis. Stimulation was represented as positives values, and inhibition was represented as negatives.

Phytotoxicity Bioassays. Several STS were proposed, including the monocotyledon onion (*Allium cepa* L.) and dicots tomato (*Lycopersicon esculentum* Will.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.), which were assayed.[32,34] Test compounds were assayed at 1000, 333, 100, 33, and 10 μM. In the same way as for the coleoptile bioassay, the herbicide Logran was used as positive control, and buffered nutritive aqueous solution with DMSO (5 μL DMSO solution/mL buffer) without any test compound was used as negative control. Moreover, germination rate, root length, and shoot length were recorded using a Fitomed system for evaluation of the data and statistical analysis for this study.[35]

*4-Methyl-5-[(2S,3S)-4-methylene-5-oxo-3-(3-oxobutyl)-tetrahydrofuran-2-yl]cyclopent-4-ene-1,3-dione (**10**).* According to GP2, phenylseleno *seco*-guaianolide **19** (17 mg, 0.038 mmol) in THF (5 mL) was treated with glacial HOAc (0.1 mL) and 30% H_2O_2 (0.5 mL) to give **10** (140 mg, 0.57 mmol) as a colorless oil in 72% yield: $[\alpha]^{20}_D$ −3 (*c* 0.5, $CHCl_3$); IR ν_{max} (thin film) 3443, 2920, 2851, 1768, 1735, 1697, 1681, 1659, 1633, 1455, 1378, 1316, 1220, 1093, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.39 (d, J = 2.3 Hz, H-13), 5.72 (d, J = 2.3 Hz, H′-13), 5.23 (1H, d, J = 6.7 Hz, H-6), 3.07 (1H, ddt, J = 6.7, 4.0, 2.4 Hz, H-7), 2.91 (2H, s, H-2), 2.70 (1H, dt, J = 18.0, 7.4 Hz, H-9), 2.57 (1H, dt, J = 18.1, 6.7 Hz, H′-9), 2.18 (3H, s, H-14), 2.08 (3H, s, H-15), 1.97 (2H, dt, J = 7.1, 6.7 Hz, H-8). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 207.2 (C-10), 199.0 (C-3), 198.6 (C-1), 169.3 (C-12), 157.3 (C-5), 153.5 (C-4), 137.1 (C-11), 124.2 (C-13), 76.0 (C-6), 43.7 (C-7), 40.9 (C-2), 39.2 (C-9), 30.0 (C-14), 28.1 (C-8), 9.3 (C-15); HRESIMS m/z 299.0869 $[M + Na]^+$ (calcd for $C_{15}H_{16}O_5Na$, 299.0895).

*(3S,3aR,5aS,9bS)-3,5a,9-Trimethyl-3-(phenylselenyl)-3a,4,5,5a-tetrahydronaphtho[1,2-b]-furan-2,8(3H,9bH)-dione (**12**).* According to GP1, α-santonin (**11**) (2.04 g, 8.28 mmol) in dry THF (40 mL) was treated with a 1.0 M THF solution of LiHMDS (25 mL, 25 mmol), PhSeCl (2.09 g, 10.93 mmol) in THF (5 mL), and HMPA (2.00 g, 11.18 mmol) to give the target product (2.85 g, 7.10 mmol) as pale yellow crystals in 85% yield. The ^{1}H NMR spectrum was identical to that reported by Barbosa and co-workers.[22]

*(3aS,5aS,9bS)-5a,9-Dimethyl-3-methylene-3a,4,5,5a-tetrahydronaphtho[1,2-b]-furan-2,8(3H,9bH)-dione (**13**).* According to GP2, phenylselenosantonin **12** (242 mg, 0.60 mmol) in THF (5 mL) was treated with glacial HOAc (0.1 mL) and 30% H_2O_2 (0.5 mL) to give **13** (140 mg, 0.57 mmol) as a yellow solid in 95% yield: $[\alpha]^{20}_D$ −68.0 (*c* 10, CH_3Cl); IR ν_{max} (thin film) 3421, 2937, 2873, 1778, 1705, 1663, 1636, 1615, 1458, 1379, 1305, 1257, 1109, 1041, 987, 906, 835, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.68 (d, J = 10.0 Hz, H-1), 6.23 (d, J = 10.2 Hz, H-2), 6.21 (d, J = 3.2 Hz, H-13), 5.54 (d, J = 3.2 Hz, H′-13), 4.74 (1H, dC, J = 11.6, 1.4 Hz, H-6), 2.68 (1H, tc, J = 11.7, 3.6 Hz, H-7), 2.18 (1H, dddd, J = 13.2, 4.6, 3.6, 2.2 Hz, H_β-8), 2.13 (3H, d, J = 10.0 Hz, H-15), 1.91 (1H, ddd, J = 13.4, 3.6, 2.2 Hz, H_α-9), 1.76 (1H, tdd, J = 13.2, 11.9, 3.6 Hz, H_α-8), 1.57 (1H, td, J = 13.2, 4.6 Hz H_β-9), 1.29 (3H, s, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.8 (C-3), 167.0 (C-12), 154.7 (C-1), 137.4 (C-11), 128.6 (C-4), 1275.7 (C-2), 119.5(C-13), 81.3 (C-6), 50.1 (C-7), 34.5 (C-9), 30.8 (C-15), 25.0 (C-14), 21.46 (C-8); HRESIMS m/z 299.0969 $[M + Na]^+$ (calcd for $C_{15}H_{16}O_3Na$, 267.0997).

*(3S,3aR,6R,9bS)-6-Hydroxy-3,6,9-trimethyl-3-(phenylselenyl)-3a,4,5,6,6a,7-hexahydroazuleno[4,5-b]furan-2,8(3H,9bH)-dione (**16**).* According to GP1 (pathway B), isophotosantonin (**15**) (225 mg, 0.85 mmol) in dry THF (20 mL) was treated with a 2.0 M THF solution of lithium diisopropylamide rather than LiHMDS (0.5 mL, 1 mmol) and PhSeCl (195 mg, 1.02 mmol) in THF (3 mL) to give the target product as pale yellow oil (107 mg, 12% yield): $[\alpha]^{20}_D$ +13 (*c* 1, $CHCl_3$); IR ν_{max} (thin film) cm^{-1} 3423, 2927, 2856, 1773, 1702, 1640, 1438, 1289, 1228, 1139, 1102, 998, 752, 691; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.61 (2H, ddd, J = 6.6, 3.2, 1.6 Hz, H-17, H-

DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

21), 7.39−7.43 (1H, m, H-19), 7.29−7.34 (2H, m, H-18, H-20), 5.09 (1H, d, J = 11.0 Hz, H-6), 3.14−3.19 (1H, m, H-1), 2.58 (1H, dd, J = 19.9, 2.43 Hz, H_{α}-2), 2.46 (1H, dd, J = 20.1, 5.3 Hz, H_{β}-2), 2.27−2.34 (1H, m, H-7), 2.03−2.11 (2H, m, H_{β}-8, H-9), 1.82 (1H, s, H-15), 1.73−1.81 (1H, m, H′-9), 1.56−1.64 (1H, m, H-8α), 1.54 (1H, s, H-13), 0.89 (1H, s, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 207.9 (C-3), 174.7 (C-12), 161.6 (C-5), 143.3 (C-4), 138.2 (C-17, C-21), 130.0 (C-19), 129.1 (C-18, C-20), 123.8 (C-16), 79.1 (C-6), 74.1 (C-10), 52.1 (C-7), 50.4 (C-11), 50.3 (C-1), 45.0 (C-9), 37.1 (C-2), 23.5 (C-8), 21.8 (C-13), 21.0 (C-14), 9.3 (C-15). It was not possible to assign the orientation of H-9. HRESIMS m/z 443.0715 $[M + Na]^+$ (calcd for $C_{21}H_{24}NaO_4Se$, 443.0738). According to GP3 (pathway C), phenylselenosantonin **12** (200 mg, 0.50 mmol) was treated with glacial HOAc (60 mL) and H_2O (30 mL) to give the target product as a pale yellow solid (85.7 mg, 0.20 mmol) in 58% yield.

*(3aS,9bS)-6,9-Dimethyl-3-methylene-3,3a,4,5-tetrahydroazuleno[4,5-b]furan-2,8(7H,9bH)-dione (**17**).* According to GP4 (pathway B), isophotosantonin derivate **14** (100 mg, 0.38 mmol) was treated with concentrated sulfuric acid (10 mL) to give conjugated diene **17** (93 mg, 0.38 mmol) as a colorless oil in quantitative yield: $[\alpha]^{20}_D$ −0.3 (c 0.5, CH_3Cl); IR ν_{max} (thin film) 3267, 3193, 2973, 2932, 1770, 1747, 1703, 1699, 1633, 1446, 1381, 1315, 1221, 1090, 753 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.28 (1H, d, J = 3.5 Hz, H-13b), 5.57 (1H, d, J = 3.5 Hz, H-13a), 5.30 (1H, d, J = 10.5 Hz, H-6), 3.01−3.09 (1H, m, H-7), 2.94 (2H, s, H-2), 2.58−2.67 (1H, m, H-9), 2.35−2.44 (1H, m, H-8), 2.24−2.30 (1H, m, H′-9), 2.03 (1H, s, H-15), 1.89 (1H, s, H-14), 1.82−1.88 (1H, m, H′-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 208.0 (C-3), 168.9 (C-12), 161.0 (C-5), 138.9 (C-11), 138.5 (C-4), 132.7 (C-10), 130.1 (C-1), 121.1 (C-13), 79.5 (C-6), 43.8 (C-7), 40.0 (C-2), 31.3 (C-9), 26.2 (C-8), 24.4 (C-14), 9.4 (C-15); HRESIMS m/z 267.0985 $[M + Na]^+$(calcd for $C_{15}H_{16}NaO_3$, 267.0997).

*(3S,3aR,9bS)-3,6,9-Trimethyl-3-(phenylselenyl)-3,3a,4,5-tetrahydroazuleno[4,5-b]furan-2,8(7H,9bH)-dione (**18**).* According to GP4 (pathway C), phenylselenolactone **16** (60 mg, 0.14 mmol) was treated with concentrated sulfuric acid (10 mL) to give the target product as a colorless oil in quantitative yield: $[\alpha]^{20}_D$ −0.7 (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (2H, dd, J = 8.1, 1.3 Hz, H-17, H-21), 7.40−7.45 (1H, m, H-19), 7.31−7.36 (2H, m, H-18, H-20), 5.35 (1H, d, J = 11.0 Hz, H-6), 2.94 (2H, s, H-2), 2.53−2.61 (1H, m, H-9), 2.33−2.38 (1H, m, H-7), 2.25−2.33 (1H, m, H′-9), 2.07−2.11 (2H, m, H-8, H′-8), 2.00 (3H, s, H-15), 1.87 (3H, s, H-14), 1.54 (3H, s, H-13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 204.1 (C-3), 174.7 (C-12), 159.5 (C-5), 140.0 (C-4), 138.2 (C-17, C-21), 133.5 (C-10), 130.0 (C-19), 129.2 (C-18, C-20), 128.9 (C-1), 124.1 (C-16), 79.1 (C-6), 51.6 (C-11), 51.5 (C-7), 40.4 (C-2), 33.6 (C-9), 24.4 (C-14), 23.8 (C-8), 21.9 (C-13), 9.9 (C-15). It was not possible to assign the orientation of H-9 and H-8. HRESIMS m/z 425.0645 $[M + Na]^+$ (calcd for $C_{21}H_{22}NaO_3Se$, 425.0632).

*4-Methyl-5-[(2S,3R,4S)-4-methyl-5-oxo-3-(3-oxobutyl)-4-(phenylselenyl)tetrahydrofuran-2-yl]cyclopent-4-ene-1,3-dione (**19**).* According to GP5, phenylseleno diene-isophotosantonin **18** (100 mg, 0.41 mmol) was treated with ozone at −75 °C and dimethyl sulfide to give the *seco*-guaianolide **19** as a colorless oil (51%, 53.0 mg): $[\alpha]^{20}_D$ +26 (c 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 (2H, dd, J = 7.6, 1.3 Hz, H-17, H-21), 7.44 (1H, tt, J = 7.6, 1.3 Hz, H-19), 7.34 (2H, dd, J = 7.6, 7.6 Hz, H-18, H-20), 4.99 (1H, d, J = 10.2 Hz, H-6), 2.94 (2H, d, J = 2.6 Hz, H-2), 2.55−2.67 (1H, m, H-7), 2.55−2.67 (1H, m, H-9), 2.49 (1H, dt, J = 18.0, 6.4 Hz H′-9), 2.11 (3H, s, H-11), 2.01−2.09 (1H, m, H-8), 2.01 (3H, s, H-15), 1.79−1.83 (1H, m, H′-8); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.6 (C-10), 199.1 (C-3), 198.2 (C-1), 175.0 (C-13), 159.7 (C-5), 151.4 (C-4), 138.3 (C-17, C-21), 130.1 (C-19), 129.3 (C-18, C-20), 124.1 (C-16), 74.0 (C-6), 51.1 (C-12), 50.8 (C-7), 41.3 (C-2), 40.7 (C-9), 31.6 (C-11), 23.7 (C-14), 22.2 (C-8), 9.5 (C-15).). It was not possible to assign the orientation of H-9 and H-8. HRESIMS m/z 457.0548 $[M + Na]^+$ (calcd for $C_{21}H_{22}NaO_5Se$, 457.0530).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00285.

NMR spectra of sesquiterpene lactones **10**, **15**, and **17**−**19**, crystal structures of **12**, and additional bioassay data (PDF)

X-ray crystallographic data (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +34.956012770. Fax: +34.956 016288. E-mail: famacias@uca.es.

ORCID

Nuria Chinchilla: 0000-0002-6659-373X
Rosa M. Varela: 0000-0003-3616-9134
Frank R. Fronczek: 0000-0001-5544-2779
José M. G. Molinillo: 0000-0002-7844-9401
Francisco A. Macías: 0000-0001-8862-2864

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Ministerio de Ciencia y Competitividad, Spain (Project No. AGL2017-88083-R).

■ REFERENCES

(1) Fraga, B. M. *Nat. Prod. Rep.* **2005**, *22*, 465−486.
(2) Schmidt, T. J.; Khalid, S. A.; Romanha, A. J.; Alves, T. M.; Biavatti, M. W.; Brun, R.; Da Costa, F. B.; de Castro, S. L.; Ferreira, V. F.; de Lacerda, M. V. G.; Lago, J. H. G.; Leon, L. L.; Lopes, N. P.; Amorim, R. C. D.; Niehues, M.; Ogungbe, I. V.; Pohlit, A. M.; Scotti, M. T.; Setzer, W. N.; Soeiro, M. D. C.; Steindel, M.; Tempone, A. G. *Curr. Med. Chem.* **2012**, *19*, 2128−2175.
(3) Theodori, R.; Karioti, A.; Rančić, A.; Skaltsa, H. *J. Nat. Prod.* **2006**, *69*, 662−664.
(4) dos Santos, F. A.; Da Costa, F. B. *Crit. Rev. Plant Sci.* **2016**, *35*, 18−37.
(5) Konstantinopoulou, M.; Karioti, A.; Skaltsas, S.; Skaltsa, H. *J. Nat. Prod.* **2003**, *66*, 699−702.
(6) Schmidt, T. J. *Curr. Org. Chem.* **1999**, *3*, 577−608.
(7) de Oliveira, R. B.; de Paula, D. A. C.; Rocha, B. A.; Franco, J. J.; Gobbo-Neto, L.; Uyemura, S. A.; dos Santos, W. F.; Da Costa, F. B. *J. Ethnopharmacol.* **2011**, *133*, 434−441.
(8) Makiyi, E. F.; Frade, R. F.; Lebl, T.; Jaffray, E. G.; Cobb, S. E.; Harvey, A. L.; Slawin, A. M.; Hay, R. T.; Westwood, N. J. *Eur. J. Org. Chem.* **2009**, *2009*, 5711−5715.
(9) Ortet, R.; Prado, S.; Mouray, E.; Thomas, O. P. *Phytochemistry* **2008**, *69*, 2961−2965.
(10) Kawazoe, K.; Tsubouchi, Y.; Abdullah, N.; Takaishi, Y.; Shibata, H.; Higuti, T.; Hori, H.; Ogawa, M. *J. Nat. Prod.* **2003**, *66*, 538−539.
(11) Trifunovic, S.; Isakovic, A. M.; Isakovic, A.; Vuckovic, I.; Mandic, B.; Novakovic, M.; Vajs, V.; Milosavljevic, S.; Trajkovic, V. *Planta Med.* **2014**, *80*, 297−305.
(12) Saikali, M.; Ghantous, A.; Halawi, R.; Talhouk, S.; Saliba, N.; Darwiche, N. *BMC Complementary Altern. Med.* **2012**, *12*, 89.
(13) Ahmed, A. A.; Gati, T.; Hussein, T. A.; Ali, A. T.; Tzakou, O. A.; Couladis, M. A.; Mabry, T. J.; Toth, G. *Tetrahedron* **2003**, *59*, 3729−3735.
(14) Jakupovic, J.; Tan, R. X.; Bohlmann, F.; Boldt, P. E.; Jia, Z. J. *Phytochemistry* **1991**, *30*, 1573−1577.
(15) Oksuz, S. *Phytochemistry* **1990**, *29*, 887−90.

DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX

(16) Bohlmann, F.; Hartono, L.; Jakupovic, J.; Huneck, S. *Phytochemistry* **1985**, *24*, 1003−7.
(17) Todorova, M.; Ognyanov, I. *Planta Med.* **1985**, *51*, 174−175.
(18) Ghantous, A.; Nasser, N.; Saab, I.; Darwiche, N.; Saliba, N. A. *Eur. J. Med. Chem.* **2009**, *44*, 3794−7.
(19) Macias, F. A.; Santana, A.; Yamahata, A.; Varela, R. M.; Fronczek, F. R.; Molinillo, J. M. G. *J. Nat. Prod.* **2012**, *75*, 1967−1973.
(20) Santana, A.; Molinillo, J. M. G.; Macias, F. A. *Eur. J. Org. Chem.* **2015**, *2015*, 2093−2110.
(21) Schall, A.; Reiser, O. *Eur. J. Org. Chem.* **2008**, *2008*, 2353−2364.
(22) Arantes, F. F.; Barbosa, L. C.; Alvarenga, E. S.; Demuner, A. J.; Bezerra, D. P.; Ferreira, J. R.; Costa-Lotufo, L. V.; Pessoa, C.; Moraes, M. O. *Eur. J. Med. Chem.* **2009**, *44*, 3739−45.
(23) Zhang, S.; Wang, J.; Xue, H.; Deng, Q.; Xing, F.; Ando, M. *J. Nat. Prod.* **2002**, *65*, 1927−1929.
(24) Zdero, C.; Bohlmann, F.; King, R. M.; Robinson, H. *Phytochemistry* **1987**, *26*, 1207−1209.
(25) Barton, D. H. R.; De Mayo, P.; Shafiq, M. *J. Chem. Soc.* **1957**, 929−35.
(26) Ulubelen, A.; Goeren, N.; Jiang, T.-Y.; Scott, L.; Tianasoa-Ramomojy, M.; Snyder, J. K. *Magn. Reson. Chem.* **1995**, *33*, 900−4.
(27) Arantes, F. F. P.; Barbosa, L. C. A.; Maltha, C. R. A.; Demuner, A. J.; Marçal da Costa, P.; Ferreira, J. R. O.; Costa-Lotufo, L. V.; Moraes, M. O.; Pessoa, C. *Eur. J. Med. Chem.* **2010**, *45*, 6045−6051.
(28) Willand-Charnley, R.; Fisher, T. J.; Johnson, B. M.; Dussault, P. H. *Org. Lett.* **2012**, *14*, 2242−2245.
(29) Molander, G. A.; Cooper, D. J. *J. Org. Chem.* **2007**, *72*, 3558−3560.
(30) Fisher, T. J.; Dussault, P. H. *Tetrahedron* **2017**, *73*, 4233−4258.
(31) Hancock, C. R.; Barlow, H. W. B.; Lacey, H. J. *J. Exp. Bot.* **1964**, *15*, 166−176.
(32) Macias, F. A.; Castellano, D.; Molinillo, J. M. G. *J. Agric. Food Chem.* **2000**, *48*, 2512−2521.
(33) Flack, H. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *A39*, 876−881.
(34) Rial, C.; Varela, R. M.; Molinillo, J. M. G.; Bautista, E.; Hernandez, A. O.; Macias, F. A. *Phytochem. Lett.* **2016**, *18*, 68−76.
(35) Castellano, D.; Macías, F. A.; Castellano, M.; Cambronero, R. M. *FITOMED (automated system for measurement of variable lengths)*; Spain Patent No. P9901565; 2001.

DOI: 10.1021/acs.jnatprod.9b00285
J. Nat. Prod. XXXX, XXX, XXX−XXX