Synthesis of Pertyolides A, B, and C: A Synthetic Procedure to C$_{17}$-Sesquiterpenoids and a Study of Their Phytotoxic Activity

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ABSTRACT: C$_{17}$-sesquiterpenoids are a group of natural products that have been recently discovered. These compounds have the peculiarity of lacking the α,β-methylene butyrolactone system, which is known to be quite relevant for many of the biological activities reported for sesquiterpene lactones. Unfortunately, the biological interest of C$_{17}$-sesquiterpenoids has not been studied in-depth, mainly due to the poor isolation yields in which they can be obtained from natural sources. Therefore, in order to allow a deeper study of these novel molecules, we have worked out a synthetic pathway that provides C$_{17}$-sesquiterpenoids in enough quantities from easily accessible sesquiterpene lactones to enable a more thorough investigation of their bioactivities. With this synthesis method, we have successfully synthesized, for the first time, three natural C$_{17}$-sesquiterpenoids, pertyolides A, B, and C, with good overall yields. Furthermore, we have also evaluated their phytotoxicity against etiolated wheat coleoptiles and corroborated that pertyolides B and C present strong phytotoxic activity.
unsaturated system conjugated to the lactone ring of C17-sesquiterpenoids has been modified and that a C2 unit has been appended to it, generally, with polar functionalizations, such as a keto or hydroxy group. This fact may suggest that these molecules perform their bioactivity through a mechanism of action not related to the αβ-methylene-butyrolactone system or that the modified fragment improves the physicochemical properties of the molecule, like, for example, the aqueous solubility. SLs are known to exhibit poor aqueous solubility, which may hinder their bioactivity because of their inability to reach their site of action. The modifications that take place in their αβ-unsaturated system may just serve to optimize their physicochemical properties or their spatial arrangement, which would result in an increase of their activities.22

Even though C17-sesquiterpenoids have been successfully isolated from natural sources, not many studies have been found regarding their biological interest. Up to the present, only a handful of studies regarding their anti-inflammatory and antitumor activities have been conducted.18–21 No data regarding their phytotoxic activity or their mechanism of action have been reported, mainly due to the poor yields that have been obtained until now through isolation.

In order to further study the biological properties of C17-sesquiterpenoids, we have synthesized three C17-sesquiterpenoids and evaluated their phytotoxic activity. The synthesis of the C17-sesquiterpenoids comprises two key steps: a photochemical addition of acetaldehyde to the αβ-unsaturated double bond of the lactone ring in order to add the C2 chain and a hydroxylation in the α position of the lactone group. By applying this synthesizing procedure, we have succeeded to synthesize for the first time pertolides A (1), B (2), and C (3) using isoalantolactone (4), alantolactone (5), and dehydrocostuslactone (6) as starting materials, respectively. We have also evaluated, for the first time, the phytotoxic activities of these molecules against etiolated wheat coleoptiles.

### RESULTS AND DISCUSSION

Isoalantolactone (4) and alantolactone (5) were isolated by column chromatography from the methanolic extract of dried Inula helenium roots.23 From 160 g of methanolic extract, 1.5 g of 4 (0.9% yield) and 4.5 g of 5 (2.8% yield) were obtained as colorless crystalline solids. Compound 6 was obtained from a Saussurea lappa root extract following the procedure described in the literature.24 From 50 g of S. lappa root extract, 2.3 g of 6 (5.0% yield) were obtained as a colorless crystalline solid. After successfully isolating the starting materials, we applied the synthesizing routes shown in Schemes 1 and 2 to obtain pertolides A, B, and C. Compounds 1 and 2 were synthesized in four steps. The photochemical addition of acetaldehyde to the starting sesquiterpene lactones yields the 1,4-dicarbonyl derivatives 7 (68% yield) and 8 (54% yield) with a C17 skeleton following the procedure described in the literature.25–27 (For NMR data, see Table S3, Supporting Information).

When the photochemical addition was carried out with alantolactone, the epoxide byproduct 9 (Figure 1) was also generated along with the desired 1,4-dicarbonyl derivative 8. It is unclear how the photoaddition would lead to the formation of the epoxide from the endocyclic double bond. The authors believe that two parallel reactions take place: the desired photochemical addition of acetaldehyde at position C-13 and also an undesired epoxidation which may be due to an interaction between the singlet oxygen generated and the already mentioned double bond. Different trials were conducted modifying the reaction time to optimize the yield of the desired product, and the best results were obtained with 2 h of reaction time. NOESY experiments were carried out to determine the relative configuration of the C-11 center of derivatives 7 and 8 (Figure 1), as well as the orientation of the epoxide group of 9. The C3 chain was determined to be β-oriented due to the positive NOE effects between H-8 and H-7 and H-11. On the other hand, the epoxy group was determined to be β-oriented due to the positive NOE correlations between H-6 and H-4, H-7 and H-13. The absolute configuration of the molecules has been established from the known absolute configuration of the precursors.

After obtaining 7 and 8, the new carbonyl group at position 16 must be masked to enable the later regioselective hydroxylation at position 11. The procedures described in our previous work were applied to mask the keto group.24 In the first instance, the keto group was treated with 2-ethyl-2-methyl-1,3-dioxolane (MED) as a protective agent, to obtain the corresponding cyclic ketal. However, after several attempts using different Lewis acids (p-TsOH, aluminum trichloride (AlCl3) and boron trifluoride-etherate (BF3·C2H5O), as well as different temperature levels and reaction times, the target cyclic ketal was never obtained. A number of trials were also conducted by applying a Dean–Stark trap procedure, where ethylene glycol was used as the protective agent, but in most cases the starting material remained unchanged or degradation products were observed.

In order to mask the carbonyl group and obtain the desired acyclic ketals (10 and 11), a different strategy was devised that consisted in the protection of the keto group by means of trimethyl orthoformate (TMOF) in the presence of anhydrous MeOH and catalytic amounts of p-TsOH.28 As a result, the acyclic ketal derivatives 10 and 11 were obtained at 58% and 65% yields, respectively (refer to Table S4 for NMR data).

Once the ketal derivatives (10 and 11) had been obtained, hydroxylation at the α position of the lactone group was carried out by peroxidation followed by a reduction step in one pot according to the procedure described in the literature.29 The treatment of the derivative 10 yielded a mixture of the desired hydroxylated ketal 12 (63%) and 1 (22%), while the derivative 11 yielded 62% of 13 and 18% of 2. The attempts to purify the hydroxylated ketals 12 and 13 were conducted by means of column chromatography and HPLC. However, they could not be successfully separated and instead, 9:1 mixtures with their respective pertolide as minor constituent was obtained.

It is well-known that ketals are labile in acidic media and can be easily deprotected through hydrolysis or transketalization. Some reports suggest that acyclic acetals can be alternatively deprotected in aqueous solution without the presence of an acid.30 Accordingly, during the workup of the reaction, the
crude is treated with Sörensen buffer (an aqueous solution of Na₂HPO₄ and KH₂PO₄, pH 7.2). The authors believe that this step is responsible for the partial hydrolysis of the derivatives 12 and 13.

The complete deprotection of the ketals 12 and 13 was performed by transketalization in an acetone:H₂O 95:5 mixture with catalytic amounts of p-TsOH according to the procedure described in the literature. This procedure produced pertyolide A (1) and B (2) both as crystalline solids in 89% and 95% yields, respectively. The NMR and the spectroscopy data of the synthesized 1 and 2 match those reported in the bibliography (see Table S1 for more detailed NMR data and Tables S6 and S7 for a comparison with the NMR data reported for the isolated 1 and 2).

Pertyolide C (3) was synthesized through six steps as shown in Scheme 2. According to the procedure described in our previous work, 14 was synthesized from 6 in three steps. Then, an allylic oxidation step using selenium dioxide and tert-butyl hydroperoxide (TBHP) yielded the dihydroxylated ketal 15 in 59%. The compound 15 was then deprotected via transketalization under the same conditions used for pertyolides A and B, and a 93% yield of the compound 16 was obtained (see Table S5 for the NMR data of the compounds 15 and 16).

The last step of the synthetic pathway consists of the esterification of the hydroxy group at C-3 using isovaleric acid with complete Walden inversion of the alcohol stereocenter. This can be done in one pot through the Mitsunobu reaction. The treatment of the compound 16 under the conditions described in the literature, i.e., with isovaleric acid, disopropylazodicarboxylate (DIAD), and triphenylphosphine (PPh₃), produced pertyolide C (3) as a colorless oil at 43% yield. The relative configuration of the C-3 center was determined by the positive NOE correlations between H-3 and H-1 and H-5, which proved that the isovalerate group introduced is β-oriented. The absolute configuration of the molecule has been established from the known absolute configuration of the precursors. The NMR data and the spectroscopy data of the synthesized pertyolide C match those reported in the bibliography (see Table S2 for more detailed NMR data and Table S8 for a comparison with the NMR data reported for isolated 3).

The phytotoxic activities of the new compounds were evaluated against etiolated wheat coleoptiles following the procedure developed by our research group. The usage of etiolated wheat coleoptiles is a common practice in the field of allelopathy in order to evaluate phytotoxic activity. This is a rapid and sensitive procedure that can be applied to a wide range of bioactive substances, such as plant growth regulators, herbicides, or mycotoxins.

The recorded data on the activity profiles of each molecule (Figure 2) were fitted to a sigmoidal dose-response curve to determine their IC₅₀ values (Table 1) and establish a
The FTIR spectra were obtained using a PerkinElmer Spectrum 2000 FTIR spectrophotometer. The absorption bands of the ketone system only caused a slight drop in the phytotoxic activity of the compounds. Another slight drop of the activity by both compounds 15 and 16 was observed when the second hydroxy group was introduced at position C-3. However, pertyolide C (3) experienced a large improvement of its activity after the hydroxy group at position C-3 had been modified by adding the isovalerate group. In fact, pertyolide C (3) exhibited the strongest inhibitory profile of all the molecules and at every concentration level within the range tested (1000 μM to 10 μM) with an outstanding IC50 value of 12 μM, which is lower than that of dehydrocostuslactone (6) (170 μM)24 or the commercial herbicide Logran (38 μM).

According to the results obtained from the bioassays with etiolated wheat coleoptiles, some of the target C17-sesquiterpenoids and intermediates synthesized present similar or even superior activity to the original sesquiterpene lactone containing the α-methylene-butyrolactone system. No clear trend could be observed to support that the elimination of the said moiety has a negative impact on the phytotoxic activity of the molecules. This led us to think that the phytotoxic activity of C17-sesquiterpenoids may take place at a different site of action in the molecule and that they may have a specific mechanism of action other than the Michael addition reactions that are commonly associated with the activity of sesquiterpene lactones.

In conclusion, we have applied a previously designed general synthetic pathway that has allowed the synthesis, for the first time, of three natural C17-sesquiterpenoids, pertyolides A (1), B (2), and C (3) with 29%, 26%, and 4% overall yields through two key steps: the photochemical addition of acetaldehyde to the α-methylene-butyrolactone system of the sesquiterpene lactone and a hydroxylation at the α position of the lactone group. Furthermore, the phytotoxic activities of the target molecules, as well as their intermediates, were evaluated against etiolated wheat coleoptiles to determine their profile of activity and their IC50 values. Pertyolide C (3) presented the highest inhibitory activity with an outstanding IC50 value of 12 μM. The data obtained from the bioassays suggest that the α-methylene-butyrolactone moiety is not essential for these types of molecules to present phytotoxic properties.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** The melting points were determined by means of a Kofler hot bench. The optical rotations were measured on a Jasco P-2000 polarimeter using CHCl3 as solvent. The FTIR spectra were obtained using a PerkinElmer Spectrum TWO IR spectrophotometer. The major absorptions in the IR spectra are given as wavenumbers (ω) in cm−1. 1H NMR and 13C NMR spectra were recorded on Agilent spectrometers at 400/100 and 500/125 MHz using CDCl3 (Magnisolv, Merck) or CD2Cl2 (Magnisolv, Merck) as internal reference. The solvents residual peaks were set to δ 7.26 ppm for 1H NMR and δ 77.0 ppm for 13C NMR in the case of...
CDCl3 and δ 7.16 ppm for 1H NMR and δ 128.1 ppm for 13C NMR in the case of C6D6. The exact masses were measured on a UPLC-QTOF-ESI (Waters Synapt G2) high-resolution mass spectrometer (HRTOFSIMS). The reactions were monitored by thin layer chromatography using Merck Kieselgel 60 F254 normal phase plates. The resulting products were purified by column chromatography using silica gel Gelran Si 60 (0.063–0.200 mm) or by HPLC using Merck-Hitachi D-2500 with refractive index detector and a Merck LiChrospher 60 (10 μm, 250 × 10 mm) column. The reagents for the synthetic procedures were supplied by either Sigma-Aldrich Co., Merck, Alfa Aesar, or Acros Organics. The solvents used for purification were supplied by VWR International. Logran Extra 60 WG (Syngenta Agro, S. A.) was used as positive control in the etiolated wheat coleoptile bioassay.

**Extraction and Isolation of the Starting Materials (4, 5, and 6).** Isolated lactones (4) and alantolactone (5) were isolated from commercial *Inula helenium* roots purchased from “Centro dietetico Viquez-Herbolario Cádiz” (Cádiz, Spain). Dry roots (1.0 kg) were extracted with MeOH for 3 days. The methanolic extract was filtered under vacuum and the solvent evaporated under reduced pressure. The extract obtained (160 g) was redissolved in MeOH and further purified by column chromatography using a hexane:EtOAc 95:5 mixture; 1.5 g of isoalantolactone (0.9% yield) and 4.5 g of alantolactone (2.8% yield) were obtained as colorless crystalline solids.

**Dehydrolactone (6) was isolated from a *Saussurea lappa* root oil extract purchased from Pierre Chauvet S.A. The extract (50 g) was dissolved in CH2Cl2 and purified by column chromatography using a hexane:EtOAc 95:5 mixture; 2.3 g of dehydrolactone (5.0% yield) was obtained as a colorless crystalline solid.

**Synthesis of the 1,4-Dicarboxyl Derivatives (7 and 8).** The sesquiterpene lactones 4 and 5 (100 mg each) were each dissolved in 100 mL of previously distilled acetaldehyde and introduced in a modified Hanovia reactor fitted with a Pyrex jacket. A 125 W medium pressure Hg lamp (Radium from Radium) was set into the reactor at a distance of approximately 10.0 cm from the reaction mixture. An aqueous solution of NiSO4·6H2O (46 g) and CoSO4·7H2O (14 g) in 100 mL of H2O was used as filter solution to restrict the formation of undesired byproducts. The reaction was stirred for 1 or 2 h (1 h for 4 and 2 h for 5) at room temperature (rt) and the solvent was evaporated under reduced pressure. During this process small amounts of cyclohexane were added to eliminate the acetic acid generated as a reaction byproduct. The crude product was purified by column chromatography using a mixture of hexane:EtOAc 9:1 to 6:4 and either the corresponding 1,4-dicarboxyl derivative 7 at 68% yield (8 mg, 2.3% 10−3 mol/mol) or a mixture of the compound 8 at 54% yield (64.2 mg, 2.32 × 10−3 mol/mol) as well as a 19% yield of the compound 9 (23.9 mg, 8.17 × 10−3 mol/mol) were obtained.

(3S,3aR,4aS,8aR,9aR)-8a-Methyl-5-methylene-3-(2-oxopropyl)-octahydro-2H-oxireno[2,3-b]furan-2(3H)-one (8). Toluene (1 mL) and a solution of 48% HBF4 in CH3OH (10 μL) were added. The reaction was stirred for 1 h and then neutralized using 0.1 mL of trimethylamine. Saturated aqueous Na2CO3 solution (20 mL) was added to the crude and then it was extracted thrice using EtOAc. The organic layers were combined, dried by means of Na2SO4 and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography using a mixture of hexane:EtOAc 9:1 to 6:4 and the corresponding ketal derivative 10 at 58% yield (67.7 mg, 2.10 × 10−3 mol/mol) or the compound 11 at 65% yield (75.8 mg, 2.35 × 10−3 mol/mol) was obtained.

(3S,3aR,4aS,8aR,9aR)-3-(2,2-Dimethylpropoxy)-8a-methyl-5-methyleneoctahydro-2H-oxireno[2,3-b]furan-2(3H)-one (10). Toluene (1 mL) and 4 mL of 48% HBF4 in CH3OH were added. The reaction was stirred for 2 h and then neutralized using 0.1 mL of trimethylamine. Saturated aqueous Na2CO3 solution (20 mL) was added to the crude and then it was extracted thrice using EtOAc. The organic layers were combined, dried by means of Na2SO4 and the solvent evaporated under reduced pressure. The crude product was purified by column chromatography using a mixture of hexane:EtOAc 9:1 to 6:4 and the corresponding ketal derivative 12 at 81% yield (710 mg, 2.08 × 10−2 mol/mol) was obtained.
Synthesis of the Hydroxylated Ketal Derivatives (12, and 13).
Each of the ketal derivatives (12 and 13) (82.5 mg, 2.82 × 10⁻² mmol) and 93% yield of the dihydroxylated 1,4-dicarbonyl derivative 16 (81.3 mg, 2.65 × 10⁻¹ mmol) were obtained.

Pertyolide B (2) (Crystalline solid, δ(CDCl₃) 0.100, MeOH); HREFMS calculated for C₂₀H₂₂O₆Na: m/z 373.1635 [M + H]+ (calcd for C₂₀H₂₂O₆Na: m/z 373.1627).

HREFMS m/z 373.1627 (calcd for C₂₀H₂₂O₆Na: 373.1635).
3H, d, 10.5, 9.6, 5.0 Hz, H-7), 1.78 (1H, m, H-8), 1.76 (1H, m, H-2b), 0.97 (3H, d, J = 6.6 Hz, H-4a, b, c; H-5a, b, c). 13C NMR (CDCl3, 125 MHz) δ 209.9 (C, C-16), 175.8 (C, C-12), 154.1 (C, C-4), 148.7 (C, C-10), 113.4 (CH2, C-15), 112.8 (CH2, C-14), 83.9 (CH, C-6), 76.3 (C, C-11), 74.3 (CH, C-3), 51.7 (CH, C-7), 49.5 (CH, C-5), 44.4 (CH2, C-13), 44.1 (CH, C-1), 39.5 (CH2, C-2), 36.2 (CH2, C-9), 32.1 (CH2, C-17), 25.5 (CH2, C-8); HRESIMS m/z 329.1371 [M + Na]+ (calcd for C16H16O4Na, 329.1365).

Synthesis of Pertyolide C (3). Triphenylphosphine (92 mg, 3.09 × 10−4 mol) was dissolved in 2 mL of anhydrous THF in a 25 mL flask under a nitrogen atmosphere. Isolaveric acid (39 μL, 3.59 × 10−4 mol) was added into the mixture and then it was cooled to 0 °C using an ice/water bath. Compound 16 (100 mg, 3.26 × 10−4 mol) dissolved in 1 mL of anhydrous THF (under a nitrogen atmosphere) was added to the mixture. Then, 69 μL (3.59 × 10−4 mol) of diisopropylazodicarboxylate were also added and the mixture was allowed to react for 90 min at rt. When the reaction was completed, the solvent was evaporated under reduced pressure and the crude was redissolved in EtOAc and filtered through silica to eliminate the triphenylphosphine oxide generated. The crude product was purified by column chromatography with a mixture of hexane:EtOAc 9:1 to give the title compound as amorphous solid. 1H NMR (CDCl3, 125 MHz) δ 5.55 (1H, ddd, J = 13.9, 7.9, 7.9 Hz, H-1b), 4.91 (1H, s, H-14b), 4.36 (1H, dd, J = 8.9, 1.7 Hz, H-2a), 2.80 (1H, ddd, J = 9.6, 9.6 Hz, H-6), 2.87 (1H, ddd, J = 13.0, 9.8, 5.3 Hz, H-9), 2.63 (1H, d, J = 16.8 Hz, H-13a), 2.51 (1H, ddd, J = 8.9, 1.7 Hz, H-17a, b, c), 2.31 (3H, s, H-17a, b, c), 2.23 (2H, brdd, J = 7.2, 1.7 Hz, H-2a, b), 2.12 (1H, brsepd, J = 6.6, 0.8 Hz, H-3), 1.99 (1H, ddd, J = 13.0, 9.8, 5.3 Hz, H-9b), 1.96 (1H, ddd, J = 10.5, 9.5, 5.0 Hz, H-7), 1.78 (1H, m, H-8), 1.76 (1H, m, H-2b), 0.97 (3H, d, J = 6.6 Hz, H-4a, b, c; H-5a, b, c). 13C NMR (CDCl3, 125 MHz) δ 209.9 (C, C-16), 175.8 (C, C-12), 172.9 (C, C-1), 148.2 (C, C-10), 148.0 (C, C-4), 114.2 (CH2, C-15), 113.8 (CH2, C-14), 82.9 (CH, C-6), 76.2 (C, C-11), 74.3 (CH, C-3), 51.7 (CH, C-7), 50.1 (CH, C-5), 44.3 (CH2, C-13), 44.2 (CH, C-1), 43.6 (CH2, C-2′), 36.2 (CH2, C-2′), 34.5 (CH, C-9), 32.0 (CH2, C-17), 25.7 (CH, C-3′), 23.0 (CH2, C-8), 22.40 (CHV, C-4′), 22.37 (CHV, C-5′); HRESIMS m/z 413.1938 [M + Na]+ (calcd for C21H20O4Na, 413.1940).

Coleoptile Bioassay. Wheat seeds (Triticum aestivum L. cv. Burgos) were sown in water-moistened 15 cm diameter Petri dishes and grown in the dark by means of a tube roller device. The shoots had 2.0 mm of root. The latter had 2.0 mm of coleoptile. The coleoptile bioassay was conducted in DMSO and diluted in a phosphate/citrate buffer. The results were expressed as percentage di- 

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Notes
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