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# Biovalorization of Friedelane Triterpenes Derived from Cork Processing Industry Byproducts

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Here, we describe the synthesis, bioactivity screening, and structure−activity relationships of various synthetic triterpenoids prepared from the cork processing byproducts friedelin (**1**) and 3-hydroxyfriedel-3-en-2-one (**2**) via oxidative procedures. The synthesis of compounds 2α-trimethylsiloxyfriedelan-3-one (**17**), friedelin-2,3-lactone (**18**), friedelin-3-oxime (**19**), and friedelin-3,4-lactam (**20**) is also described. We have studied the insecticidal and phytotoxic potential of these compounds, their selective cytotoxic effects on insect and mammalian cells, and their antiparasitic effects. Structural modifications of the A-ring of friedelin (**1**) improved its insecticidal activity with derivatives **5**, 2,3-secofriedelan-2-al-3-oic acid (**6**), its acetylated derivative **6a**, 3*β*- and 3α-hydroxyfriedelane (**9** and **10**), 3α-hydroxyfriedel-2-one (**11**), 4*β*-hydroxyfriedel-3-one (**16**), the acetylated **10a**, 3,4-secofriedelan-4-oxo-3-oic-acid (**14**), lactone **18**, and the oxime **19** being stronger insecticides than the parent compound. Methyl-3-nor-2,4-secofriedelan-4-oxo-2-oic acid (**12**) and its acetylated derivative **12a** also showed insecticidal activity in contrast to their inactive parent compound **2**. The postingestive effects and cytotoxicity of these compounds suggest a multifaceted insecticidal mode of action. These structural modifications did not result in better phytotoxic agents than the parent compounds except for lactam **20** and yielded several moderately active antiparasite derivatives (seco acids **6**, **12**, **14**, and 4*β* -hydroxyfriedel-3-one **16**) with cytotoxic effects on mammalian cells.

**KEYWORDS:** ***Quercus suber*; cork smoker wash solids; triterpene friedelanes; *Spodoptera littoralis*; *Lactuca sativa*; *Trypanosoma cruzi*; *Leishmania infantum*; antifeedant; phytotoxic; antiparasitic**

## INTRODUCTION

Biobased products derived from nature contribute to sustainable industrial growth in sectors such as health care, agrifood, forest products, chemicals, plastics, energy, and the environment. The cork industry comprises one of the world's most important biobased, nontimber forest products. Natural cork is a renewable resource harvested from the living bark of the cork oak, *Quercus suber* L. (Fagaceae), with minimal environmental impact. This tree is native to the Mediterranean forest with Portugal and Spain being among the main producers worldwide. The *Quercus* forest provides immeasurable ecological value to the fragile ecosystems of its arid native habitat (*1*, *2*).

The cork processing industry generates large amounts of cork powder and cork smoker wash solids (ca. 200 tons/year of cork smoker wash solids in Portugal), a black wax obtained in the process of making corkboard from ground cork under pressure by treatment with superheated steam. This black wax has no practical application and represents an environmental problem (Comissão de Coordenação e Desenvolvimento Regional de Lisboa e Vale do Tejo" Portuguese Environmental Department).

This residue is a convenient source of friedelane triterpenes friedelin (**1**) (8%, w/w) and 3-hydroxyfriedel-3-en-2-one (**2**) (5.4%, w/w). Small amounts of *β*-sitosterol (1%), campesterol (0.3%), *β*-amyrin and sitost-4-en-3-one (0.3%), and waxes have also been isolated from this residue (*3*). Triterpenoids are abundant in the plant kingdom, and pharmacological (antiinflammatory, antibacterial, antifungal, antiviral, cytotoxic, and antiparasitic) and pesticidal (insecticidal, phytotoxic) effects have been reported (*4*).

As part of our ongoing assessment of cork-derived biobased products, we previously prepared compounds **3**−**16** by structurally modifying the A-ring of triterpenes **1** and **2** (**Scheme 1**), including seco acid derivatives with antitumoral activity (*3*, *5*).

In this work, we have obtained a mixture of compounds **5**, **6**, **11**, and the new derivative **17** from compound **3** via oxidative

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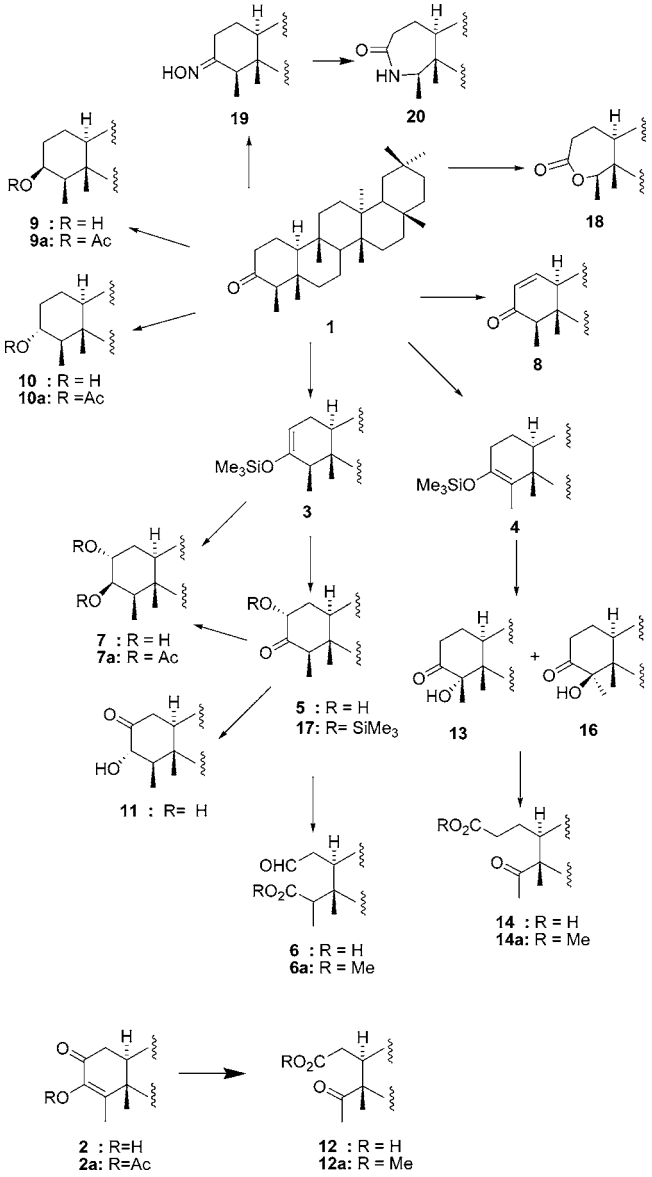
**Scheme 1.** Friedelane Derivatives




procedure. We have also synthesized derivatives **18**−**20**. We have studied the biopesticide potential (insecticidal and phytotoxic) of these compounds on the insect pest *Spodoptera littoralis* and *Lactuca sativa* seeds. We have also tested their selective cytotoxic effects on insect Sf9 cells derived from *S. frugiperda* pupal ovarian tissue and mammalian chinese hamster ovary (CHO) cells. Additionally, we studied their antiparasitic effects against *Trypanososma cruzi* and *Leishmania infantum*, which are associated with diseases responsible for high morbidity and mortality in developing countries. In both cases, the etiologic agent is a flagellated protozoa and the chemotherapies used are not effective in all phases of the infection.

## MATERIAL AND METHODS

**General Experimental Procedures.** Melting points were determined using a Reichert microscopic Thermovar model and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1725X spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AMX 300 MHz spectrometer using $CDCl_3$ solutions with $SiMe_4$ as an internal standard. Multiplicities of $^{13}$C signals were determined by distortionless enhancement of polarization transfer experiments. All NMR experiments were carried out at constant temperature (298 K). Electron impact mass spectra (EIMS) were recorded on a Kratos model MS-25 RF mass spectrometer at 70 eV. Thin-layer chromatography was carried out using precoated silica gel plates 60 F254, and detection was achieved by spraying with methanolic DNP and heating to 150 °C. Column chromatography was performed using silica gel 60 (0.040−0.063 Merck, 9385). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was from Sigma-Aldrich. Cell and parasite viability were measured in a microplate reader SLT Lab Instruments (Austria).

**Material.** Cork smoker wash solids were a gift from Corticeira Amorim Algarve (Portugal).

**Extraction and Isolation.** Friedelin (**1**) and 3-hydroxyfriedel-3-en-2-one (**2**) were extracted from powdered cork smoker wash solids (*3*).

**Synthesis.** Details on the synthesis of compounds **3**−**16** and their physical and spectral data have been previously reported (*3*, *5*). Here, we scaled-up the synthetic method to prepare compound **5**. The synthesis of compounds **17**−**20** is also described along with their spectroscopic data.

**Synthesis of 2α-Hydroxyfriedel-3-one (5).** A solution of $NaHCO_3$ (0.5 M, 70 mL) at 0 °C was added to 3-trimethylsiloxyfriedel-2-ene (**3**) (932 mg) dissolved in $CH_2Cl_2$ (117 mL), and the mixture was vigorously stirred at room temperature. Then, *m*-CPBA (776,7 mg) was added at 0 °C and the mixture was stirred at room temperature for 1 h. The organic phase was extracted and washed with a saturated solution of sodium sulfite, $NaHCO_3$ (0.5 M), and $H_2O$, and the residue (1.20 g) was purified by column chromatography (silica gel with a $CH_2Cl_2$:MeOH gradient) to obtain a mixture of 2α-hydroxyfriedel-3-one (**5**) (28.3 mg; 2.7%), 2,3-secofriedelan-2-al-3-oic acid (**6**) (133.4 mg; 12.4%), 3α-hydroxy-friedel-2-one (**11**) (548 mg; 53.2%), 2α-trimethylsiloxyfriedelan-3-one (**17**) (41.7 mg; 3.5%), and friedelin (**1**) (55.8 mg; 5.6%). Compound **5** isomerized in the presence of silica gel to **11**. Compound **17** was obtained for the first time in trace amounts.

**2α-Trimethylsiloxyfriedelan-3-one (17).** Amorphous brown solid. $^1$H NMR ($CDCl_3$): 0.10 (9H, *s*, $OSiMe_3$), 0.66 (3H, *s*, Me-24), 0.83 (3H, *s*, Me-25), 0.84 (3H, *d*, $J$ = 6,4 Hz, Me-23), 0.95 (3H, *s*, Me-30), 1.00 (6H, *s*, Me-26 and Me-29), 1.07 (3H, *s*, Me-27), 1.17 (3H, *s*, Me-28), 1.90 (1H, *m*, H-10), 2.87 (1H, *q*, $J$ = 6.4 Hz, H-4), 3.96 (1H, *t*, $J$ = 3.0 Hz, H-2). $^{13}$C NMR ($CDCl_3$): 0 ($O SiMe_3$), 32.16 (C-1), 74.89 (C-2), 212.73 (C-3), 52.45 (C-4), 43.03 (C-5), 40.93 (C-6), 18.21 (C-7), 52.90 (C-8), 36.72 (C-9), 51.91 (C-10), 35.28 (C-11), 30.35 (C-12), 39.66 (C-13), 38.28 (C-14), 32.74 (C-15), 35.98 (C-16), 29.93 (C-17), 42.76 (C-18), 35.28 (C-19), 28.10 (C-20), 32.29 (C-21), 39.17 (C-22), 6.28 (C-23), 14.00 (C-24), 18.05 (C-25), 18.54 (C-26), 20.07 (C-27), 32.05 (C-28), 31.79 (C-29), 34.92 (C-30). EIMS *m*/*z* (%): 514 (52) $[M]^+$, 499 (43) $[M - CH_3]^+$, 424 (4) $[M - SiMe_3OH]^+$, 409 (5) $[M - (SiMe_3O + O)]^+$, 341 (5) $[C_{25}H_{41}]^+$, 273 (22) $[C_{19}H_{29}O]^+$, 205 (40) $[C_{15}H_{25}]^+$, 191 (20) $[C_{15}H_{25}-CH_2]^+$, 159 (98), 143 (100), 137 (31), 125 (38), 123 (58) $[C_9H_{15}]^+$, 109 (73), 95 (91), 81 (71), 69 (69), 73 (89) $[Me_3Si]^+$, 55 (77) $[C_3H_3O]^+$, 43 (45).

**Synthesis of Friedelin-2,3-lactone (18).** Friedelin (**1**, 65.8 mg) was dissolved in $CHCl_3$ (1.3 mL), and then, *m*-CPBA (98 mg) was added. The mixture was stirred continuously at 60 °C for 17 h. A $NaHCO_3$ saturated solution was added to the ice-cooled mixture, the pH was adjusted to 7, and the organic phase was extracted with $CHCl_3$. The residue was purified by preparative liquid chromatography [silica gel, $CH_2Cl_2$:$(CH_3)_2CO$, 98:2] to obtain friedelin-2,3-lactone (48 mg, 71% yield).

**Friedelin-2,3-lactone (18).** White crystals; mp 288−290 °C [lit. (*8*) 247−248 °C]. IV $\upsilon_{max}$ (KBr) ($cm^{-1}$): 2944, 1734 (C═O), 1073 (C−O), 752. $^1$H NMR ($CDCl_3$): 0.83 (3H, *s*, Me-25), 0.89 (3H, *s*, Me-24), 0.95 (3H, *s*, Me-26), 0.99 (6H, *s*, Me-27 and Me-30), 1.00 (3H, *s*, Me-29), 1.17 (3H, *s*, Me-28), 1.20 (3H, *d*, $J$ = 6,3 Hz, Me-23), 1.94 (1H, *m*, H-$1_{ax}$), 2.52 (1H, *td*, $J$ = 1.5, 13.0, 13.0 Hz, H-$2_{ax}$), 2.63 (1H, *ddd*, $J$ = 1.5, 7.0, 13.0 Hz, H-$2_{eq}$), 4.22 (1H, *q*, $J$ = 6,3 Hz, H-4). $^{13}$C NMR ($CDCl_3$): 18.55 (C-1), 34.35 (C-2), 175.64 (C═O), 84.91(C-4), 40.76 (C-5), 38.44 (C-6), 18.03 (C-7), 52.72 (C-8), 38.18 (C-9), 63.94 (C-10), 35.29 (C-11), 30.59 (C-12), 39.33 (C-13), 38.37 (C-14), 32.35 (C-15), 35.40 (C-16), 29.98 (C-17), 42.71 (C-18), 35.95 (C-19), 28.16 (C-20), 32.73 (C-21), 39.22 (C-22), 13.45 (C-23), 16.22 (C-24), 17.90 (C-25), 18.59 (C-26), 20.20 (C-27), 32.05 (C-28), 31.75 (C-29), 35.03 (C-30). EIMS *m*/*z*: 442 (9) $[M]^+$, 427 (3) $[M - CH_3]^+$, 398 (17) $[M - CO_2]^+$, 383 (6) $[M - (CO_2 + CH_3)]^+$, 274 (10), 245 (15), 218 (24),

205 (44), 191 (23), 179 (16), 163 (20), 149 (23), 135 (27), 123 (53) 109 (67), 69 (100), 95 (95).

**Synthesis of Friedelin-3-oxime (19).** Friedelin (**1**, 80 mg) and hydroxylamine hydrochloride (59 mg) were dissolved in $C_5H_5N$ (1 mL) and absolute $C_2H_5OH$ (1 mL), and the mixture was refluxed for 3 h and 30 m. The solvents were evaporated, a solution of HCl (10%) was added, and the organic phase was extracted with EtOAc and washed with a solution of $NaHCO_3$. The residue was purified by column chromatography (silica gel, $CH_2Cl_2$) to obtain friedelin-3-oxime (70 mg, 85% yield).

**Friedelin-3-oxime (19).** White crystals; mp 280−282 °C [lit. (*9*) 289−292 °C]. IV $\upsilon_{max}$ (KBr) ($cm^{-1}$): 3293 (OH), 2928, 1686 (C═N), 1458 (C−O), 945 (N−O); $^1H$ NMR ($CDCl_3$): 0.74 (3H, *s*, Me-24), 0.84 (3H, *s*, Me-25), 0.94 (3H, *d*, $J = 6.9$ Hz, Me-23), 1.00 (3H, *s*, Me-30), 1.03 (6H, *s*, Me-27 and Me-29), 1.17 (3H, *s*, Me-28), 1.21 (3H, *s*, Me-26), 1.77 (1H, *m*, H-2eq), 2.02 (1H, *q*, $J = 6.9$ Hz, H-4), 3.43 (1H, *ddd*, $J = 2.4$, 4.5, 13.0 Hz, H-$2_{ax}$). $^{13}C$ NMR ($CDCl_3$): 20.58 (C-1), 36.03 (C-2), 162.39 (C═N), 50.96 (C-4), 41.15 (C-5) 39.67 (C-6), 18.35 (C-7), 53.10 (C-8), 37.29 (C-9), 60.00 (C-10), 35.34 (C-11), 30.54 (C-12), 40.30 (C-13), 38.30 (C-14), 32.40 (C-15), 35.57 (C-16), 30.02 (C-17), 42.79 (C-18), 35.90 (C-19), 28.18 (C-20), 32.79 (C-21), 39.27 (C-22), 8.37 (C-23), 14.21 (C-24), 17.97 (C-25), 18.67 (C-26), 20.24 (C-27), 32.09 (C-28), 31.79 (C-29), 35.04 (C-30). EIMS *m*/*z*: 441 (11) $[M]^+$, 426 (37) $[M - CH_3]^+$, 410 (48) $[M - NOH]^+$, 272 (11), 220 (10), 218 (18), 205 (19), 179 (16), 149 (20), 137 (28), 125 (21), 109 (58), 95 (81), 81 (77), 69 (97), 55 (27).

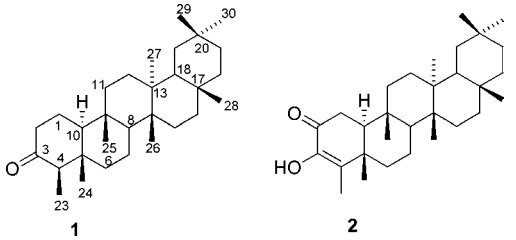
**Synthesis of Friedelin-3,4-lactam (20).** Friedelin-3-oxime (**19**, 60 mg) and *p*-toluenesulfonyl chloride (19.6 mg) were dissolved in $C_5H_5N$ (1.14 mL), and the mixture was refluxed for 5 h. The reaction was cooled and diluted with $H_2O$, and the product was extracted with $CHCl_3$. The $CHCl_3$ phase was washed with HCl (10%), NaCl (saturated solution), and $H_2O$ to afford pure friedelin-3,4-lactam (59.9 mg, 99.8% yield).

**Friedelin-3,4-lactame (20).** Amorphous yellowish solid; mp 266−268 °C. IV $\upsilon_{max}$ (KBr) ($cm^{-1}$): 2928, 2867, 1672 (C═O), 1458 (N−H), 1362 (C−N), 734 (N−H). $^1H$ NMR ($CDCl_3$): 0.81 (3H, *s*, Me-24), 0.82 (3H, *s*, Me-25), 0.95 (3H, *s*,Me-30), 0.98 (3H, *s*, Me-27), 0.99 (3H, *s*, Me-29), 1.00 (3H, *s*, Me-26), 1.04 (3H, *d*, $J = 6.9$ Hz, Me-23), 1.17 (3H, *s*, Me-28), 1.88 (1H, *m*, H-$1_{ax}$), 2.40 (2H, *m*, H-$2_{ax}$ a nd H-$2_{eq}$), 3.27 (1H, *quint.*, $J = 6.4$ Hz, H-4), 5.30 (1H, *brs*, N−H). $^{13}C$ NMR ($CDCl_3$): 18.24 (C-1), 36.39 (C-2), 177.01 (C-3), 58.91 (C-4), 39.14 (C-5), 39.14 (C-6), 18.33 (C-7), 52.63 (C-8), 38.30 (C-9), 65.09 (C-10), 35.16 (C-11), 30.57 (C-12), 39.72 (C-13), 38.37 (C-14), 32.27 (C-15), 35.37 (C-16), 29.87 (C-17), 42.60 (C-18), 35.89 (C-19), 28.03 (C-20), 32.64 (C-21), 39.12 (C-22), 15.82 (C-23), 13.75 (C-24), 17.81 (C-25), 18.45 (C-26), 20.07 (C-27), 31.91 (C-28), 31.63 (C-29), 34.89 (C-30). EIMS *m*/z (%) 441 (9) $[M]^+$, 426 (3) $[M - CH_3]^+$, 398 (2) $[M - (CH_3 + CO)]^+$, 273 (2) $[C_{19}H_{31}N]^+$, 205 (8) $[C_{15}H_{25}]^+$, 191 (5) $[205 - CH_2]^+$, 179 (2), 163 (4), 137 (7), 125 (3), 123 (11) $[C_9H_{15}]^+$, 109 (18), 95 (22), 81 (23), 69 (21), 55 $[C_3H_3O]^+$ (27), 42 (100).

**Insect Bioassays.** *S. littoralis* and *Leptinotarsa decemlineata* were reared on artificial diet (*6*) and potato foliage, respectively, and maintained at 22 ± 1 °C, >70% relative humidity, with a photoperiod of 16:8 h (L:D) in a growth chamber.

*Choice Feeding Assays*. These experiments were conducted with newly emerged sixth-instar *S. littoralis* larvae and adult *L. decemlineata*. The percent feeding inhibition (%FI) was calculated as previously described (*6*). Compounds with an FI > 70% were tested in a dose−response experiment to calculate their relative potency ($EC_{50}$ values, the effective dose for 50% feeding reduction), which was determined from linear regression analysis (% FI on log dose).

*Oral Cannulation*. This experiment was performed with preweighed newly emerged *S. littoralis* L6 larvae. Each experiment consisted of 20 larvae orally dosed with 40 μg of the test compound (*6*). An analysis of covariance (ANCOVA1) on biomass gains with initial biomass as the covariate (covariate $p > 0.05$) showed that initial insect weights were similar among all treatments. A second analysis (ANCOVA2) was performed on biomass gains with food consumption as the covariate to test for postingestive effects (*6*).




1 2

**Figure 1.** Natural fridelane triterpenes isolated from cork smoker wash solids.

**Phytotoxicity Tests.** These experiments were conducted with *L. sativa* var. Carrascoy seeds placed on paper disks (Whatman no. 1, 2.0 $cm^2$) treated with 50 μg/$cm^2$ of the test compound or solvent for the control. The disks were placed in lidded clear plastic boxes (2 × 2 $cm^2$) lined with 4 g of sterilized sand soil humidified with 200 μL of deionized water and then placed in a plant growth chamber (25 ± 1 °C, >70% relative humidity, with a photoperiod of 16:8 h L:D) for 6 days. A total of 100 seeds were used (20 seeds/box, five boxes). The germination was monitored daily, and the radicle length was measured at the end of the experiment (20 digitalized radicles randomly selected for each experiment) with the application Scion Image for Windows release Alpha 4.0.3.2 (www.scioncorp.com). An analysis of variance (ANOVA) was performed on germination and radicle length data.

**Cytotoxicity.** Sf9 cells derived from *S. frugiperda* pupal ovarian tissue (European Collection of Cell Cultures, ECCC) and mammalian CHO cells (a gift from Dr. Pajares, I. C. Biomédicas, CSIC) were grown as previously described (*7*). Cell viability was analyzed by an adaptation of the MTT colorimetric assay method (*7*). The active compounds were tested in a dose−response experiment to calculate their relative potency ($EC_{50}$ values, the effective dose to give 50% cell viability), which was determined from linear regression analysis (% cell viability on log dose).

**Trypanocidal Activity.** This activity was assayed on epimastigote forms of *T. cruzi*, Y strain, as described (*7*). The active compounds were tested in a dose−response experiment to calculate their relative potency ($EC_{50}$ values, the effective dose to give 50% parasite growth reduction), which was determined from linear regression analysis (% cell viability on log dose).

**Leishmanicidal Activity.** The leishmanicidal activity was assayed on promastigote forms of *L. infantum*, PB75 strain, cultured at 28 °C in RPMI medium supplemented with fetal calf serum. Parasites in logarithmic growth phase were distributed in 96-well flat bottom plates. The compounds to be tested were dissolved in DMSO and added to the cultures at several concentrations (100, 50, 25, 10, 5, and 1 μg/mL; except for **1**, assayed at 100, 10, and 1 μg/mL) for 72 h. Amphotericin B was used as a reference drug, and parasite viability was analyzed by a modified MTT colorimetric assay (*7*). The activity was calculated as described for *T. cruzi*.

## RESULTS AND DISCUSSION

The triterpene derivatives studied here were obtained through simple chemical modifications of natural triterpenes **1** and **2** (**Figure 1**). These reactions are suitable for possible large-scale applications (**Scheme 1**). The conversions focused mainly on the modification of the carbonyl function at C-3 of friedelin **1**, via controlled silyl enol ethers **3** and **4**, which were modified to hydroxyl ketones (**5** and **16**) to obtain seco acids **6** and **14**, respectively. Seco acid **12** was prepared by oxidation of **2** in the presence of a phase transfer catalyst and potassium permanganate (**Scheme 1**), a convenient method for the preparation of the seco acids.

Oxidation of **3** with *m*-chloroperbenzoic acid under buffered pH conditions produced quantitatively **5** (*3*). However, the scale-up of this reaction based on the procedure described above and the subsequent purification with silica gel gave a mixture of **5**

**Table 1.** Consumption ($\Delta I$) and Biomass Gain ($\Delta B$) of Orally Injected *S. littoralis* L6 Larvae, Expressed as Percent of the Control and Cytotoxic Effects on *Spodoptera frugiperda* Sf9 and Mammalian CHO Cells[a]

| | | | | $EC_{50}$ ($\mu$g/mL) | |
|---|---|---|---|---|---|
| compd | $\Delta B$ | $\Delta I$ | pANCOVA2[b] | Sf9 | CHO |
| **1** | 73* | 77* | >0.05 | >100 | >100 |
| **2** | 97 | 105 | | ~100 | >100 |
| **2a** | 102 | 115 | | 23.10 (17.77, 30.02) | >100 |
| **5** | 35** | 80 | 0.0001 | 47.6 (24.31, 93.25) | 41.92 (26.52, 66.27) |
| **6** | 51* | 89 | 0.007 | 7.26 (12.63, 23.60) | 9.32 (7.08, 12.27) |
| **6a** | 34** | 73* | 0.008 | >100 | >100 |
| **7** | | | | 6.99 (4.62, 10.58) | 27.70 (20.89, 36.72) |
| **7a** | | | | 73.46 (56.36, 95.76) | 52.35 (38.63, 70.94) |
| **8** | 79 | 93 | | >100 | >100 |
| **9** | 53* | 73 | >0.05 | >100 | >100 |
| **9a** | | | | 79.56 (41.87, 151.21) | >100 |
| **10** | 36* | 62* | >0.05 | >100 | >100 |
| **10a** | 58* | 74* | >0.05 | >100 | >100 |
| **11** | 57* | 71* | >0.05 | 8.32 (6.25, 11.07) | 50> $EC_{50}$ >25 |
| **12** | 65* | 77* | >0.05 | 20.23 (14.28, 28.65) | 34.33 (29.41, 40.07) |
| **12a** | 75* | 79* | >0.05 | >100 | >100 |
| **14** | 61* | 73* | >0.05 | 23.84 (18.78, 30.27) | 20.11 (17.98, 22.50) |
| **14a** | | | | 33.40 (28.17, 39.61) | 19.88 (8.30, 47.57) |
| **16** | 53* | 65* | >0.05 | >100 | >100 |
| **17** | 77 | 65* | | >100 | >100 |
| **18** | 44* | 46* | >0.05 | >100 | ~100 |
| **19** | 47* | 49* | >0.05 | 62.55 (27.05, 144.62) | ~100 |
| **20** | 73* | 77* | >0.05 | ~100 | >100 |

[a] *$p < 0.05$ and **$p < 0.0005$, ANCOVA1 (initial larval weight as covariate). [b] $p$ Level, ANCOVA2 ($\Delta I$ as covariate).

(2.7%), **11** (53.2%), seco acid **6** (12.4%), and the new reaction product **17** (3.5%). The purification of the mixture on silica gel gave the hydroxy ketone **11** by epimerization of **5**, which was formed via an epoxide intermediate.

The selective reduction of the carbonyl function of hydroxyl ketones **5** and **1** by a mild treatment with $NaBH_4$ produced alcohols **7** and **9**. Additionally, the reduction of **1** with sodium produced alcohol **10**. Alcohols **7**, **9**, and **10** were subjected to acetylation (with acetic anhydride and pyridine). The $\alpha,\beta$-unsaturated carbonyl **8** derivative was prepared directly from **1** by phenylselenenyl chloride (PhSeCl) oxidation in 93% yield, by contrast DDQ oxidation via sylil enol ether **3** procedure (76% yield) (*3*).

The preparation of the 3,4-lactone (**18**) and 3-oxime of friedelin (**19**) was carried out as a modification of a previously reported protocol (*8*, *9*). Subsequent reaction of **19** with *p*-toluenesulfonyl chloride yielded the 3,4-lactam **20** (99.8%). These compounds were identified by their spectroscopic data (see Experimental Procedures). The NMR data of oxime **20** showed signals associated with a single product.

Assessment of these derivatives involved their screening for insecticidal, phytotoxic, and antiparasitic effects. None of the triterpene derivatives had antifeedant effects on *S. littoralis* or *L. decemlineata* (data not shown). However, most of the compounds tested (84%) had negative postingestive effects on *S. littoralis* larvae (**Table 1**). A covariance analysis (ANCOVA1) of food consumption ($\Delta I$) and biomass gains ($\Delta B$) with initial larval weight (BI) as the covariate (covariate $p > 0.05$) was performed to test for significant effects of the test compounds on these variables. An additional ANOVA analysis and covariate adjustment on $\Delta B$ with $\Delta I$ as the covariate (ANCOVA2) was performed for those compounds that significantly reduced $\Delta B$ to understand their postingestive mode of action (antifeedant and/or toxic) (*6*).

The following compounds reduced biomass gains ($\Delta B$) and food consumption ($\Delta I$) of orally injected *S. littoralis* larvae, ranking as follows: **6a**, **10** > **18**, **19** > **9**, **10a**, **11**, **16** > **12**, **14** > **1**, **12a**, **17**, and **20** (**Table 1**). Treatment effects on $\Delta B$ disappeared with covariance adjustment except for **6a**. Therefore, these compounds acted as postingestive antifeedants with varying potencies, while **6a** had additional toxic action. Compounds **5** and **6** reduced $\Delta B$ but not $\Delta I$. Treatment effects of these compounds on $\Delta B$ did not disappear with covariance adjustment, indicating that they acted as strong postingestive toxins without antifeedant effects.

A lower number of derivatives had cytotoxic effects to insect-derived Sf9 and mammalian CHO cells (45 and 40%, respectively). Compound **6** had the strongest cytotoxic effect followed by **7**, **11**, **12**, **14**, **14a**, **5**, and **7a**, while **2a**, **9a**, and **19** showed selective and moderate-low cytotoxic effects to Sf9 (**Table 1**). Similarly, compound **6** has been reported as a potent inhibitor of human lymphocyte and cancer cell proliferation, followed by **5**, **7**, **12**, and **14** (*3*, *5*). Other friedelane, oleanane, lupane, and ursane triterpenes have also been reported as tumoral cell growth inhibitors (*10*−*12*), but this is the first report on this class of compounds acting on insect-derived cells.

The lack of short-term feeding behavior modulating the effects of these terpenes on *S. littoralis* indicates that the noncytotoxic insect growth regulators (**6a**, **10**, **18**, **19**, **9**, **16**, **1**, **12a**, and **17**) could be digestive toxins, while the cytotoxic ones (**5**, **6**, **11**, **12**, and **14**) could act unspecifically on cell membranes in addition to being digestive toxins. Therefore, the growth regulation effects observed here could be the result of a multifaceted biological action. Triterpenes such as oleanolic and ursolic acid have fluidity-modulating effects on liposomal membranes (*13*). Asiatic acid reduced the consumption and growth of the rice grasshopper *Oxya fuscovita* and inhibited the digestive enzymes amylase and invertase (*14*). Angiotensin blockers induced ecdisteroid secretion in the lepidopteran *Lymantria dispar* (*15*), and triterpene acids including oleanolic and $\beta$-glycyrrhetinic blocked agiotensin II receptors (*16*), suggesting that these triterpenes could interfere with the insect's ecdysteriod levels.

Compounds **1**, **6** > **18** > **17**, **19**, **20** > **9**, **10a**, **11**, and **16** inhibited *L. sativa* germination during the first 24−48 h of the experiment (**Table 2**). All of the compounds tested inhibited *L. sativa* radicule elongation, *N*-derivative **19** being the most active, followed by **1**, **10**, **18**, **12**, **16**, and **17**. Therefore, modification of the A-ring of parent terpenes **1** and **2** did not result in improved phytotoxic effects except for the oxime **19**. The lack of molecular selectivity observed suggests a nonspecific phytotoxic effect for these compounds on *L. sativa*. Previous reports have shown that **1** affected the radicule growth of *Echinochloa crusgalli* (*17*). The phytotoxic effects of these type of molecules have been attributed to their membrane-modifying properties (*13*, *18*).

The triterpene derivatives tested did not show relevant antiparasite effects. Seco acids **6** and **12** were moderately active against both parasite species ($EC_{50}$ values of 37.6 and 44.3 $\mu$g/mL, respectively, against *L. infantum* and 46.3 and 50.4 $\mu$g/mL against *T. cruzi*). Compound **16** and its seco acid derivative **14** had selective antiparasite affects against *L. infantum* ($EC_{50}$ of 19.2 $\mu$g/mL) and *T. cruzi* ($EC_{50}$ of 49.3 $\mu$g/mL). There are previous reports on the leishmanicidal and trypanocidal effects of some pentacyclic triterpenes with **1** being inactive (*19*). Furthermore, the presence of a carboxyl moiety in these types of compounds was found to be important for their antileishmanial activity (*20*).

The relatively large number of bioactive derivatives allowed us to draw some structure−activity relationships for these

**Table 2.** Effects of the Test Compounds on Germination and Radicle Length of *L. sativa*

| | germination (% control) | | | | | | |
|---|---|---|---|---|---|---|---|
| compd | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h | radicle length (% control)[a] |
| **1** | 31* | 76* | 100 | 100 | 100 | 100 | 37.5 |
| **2** | 97 | 96 | 100 | 100 | 100 | 100 | 49.3 |
| **2×bb** | 94 | 91 | 98 | 100 | 100 | 100 | 65.0 |
| **5** | 81 | 97 | 100 | 100 | 100 | 100 | 57.8 |
| **6** | 34* | 93 | 94 | 94 | 94 | 94 | 51.2 |
| **6a** | 97 | 93 | 99 | 99 | 99 | 99 | 79.6 |
| **7** | 89 | 100 | 100 | 100 | 100 | 100 | 56.5 |
| **7a** | 89 | 100 | 100 | 100 | 100 | 100 | 68.0 |
| **8** | 94 | 100 | 100 | 100 | 100 | 100 | 52.1 |
| **9** | 78* | 95 | 99 | 100 | 100 | 100 | 52.2 |
| **9a** | 100 | 100 | 100 | 100 | 100 | 100 | 67.7 |
| **10** | 96 | 100 | 100 | 100 | 100 | 100 | 35.6 |
| **10a** | 70* | 94 | 100 | 100 | 100 | 100 | 51.5 |
| **11** | 77* | 100 | 100 | 100 | 100 | 100 | 55.6 |
| **12** | 81 | 98 | 100 | 100 | 100 | 100 | 42.9 |
| **12a** | 97 | 100 | 100 | 100 | 100 | 100 | 51.0 |
| **14** | 99 | 100 | 100 | 100 | 100 | 100 | 72.0 |
| **14a** | 94 | 100 | 100 | 100 | 100 | 100 | - |
| **16** | 72* | 99 | 99 | 99 | 99 | 99 | 40.07 |
| **17** | 67* | 100 | 100 | 100 | 100 | 100 | 44.26 |
| **18** | 59* | 87* | 98 | 100 | 100 | 100 | 38.43 |
| **19** | 66* | 86* | 96 | 97 | 97 | 97 | 25.82 |
| **20** | 68* | 100 | 100 | 100 | 100 | 100 | 56.5 |

[a] $p < 0.05$ in all cases. *$p < 0.005$, LSD test.

triterpenes. The presence of a hydroxyl group at C-2 (**5**) or a lactone/hydroxyl at C-4 (**18**, **16**) gave rise to a strong insecticidal effect with respect to **1**. The presence of a bulky substituent at C-2 (**17**) and the substitution of the C-4 lactone of **18** by a lactam (**20**) lowered the insecticidal activity and increased its phytotoxic effect. Substitution of the ketone at C-3 by hydroxyl (**10**, **11**, and **9**), acetate (**10a**), or oxime (**19**) group also resulted in elevated activity. The stereochemistry of the C-3 substituent also played a role, with the α-hydroxyl (**10**) resulting in stronger insecticidal and lower phytotoxic effects than the β-hydroxyl (**9**). Similarly, recent studies have shown that lower polarity of the C-3 functional group of oleanane triterpene acids reduced their postingestive insect toxicity (*21*).

The A-ring resulted in inactive compounds (**2**, **2a**, and **8**). Among the open A-ring terpenes, seco acid **6** showed the strongest insecticidal effect, followed by **12** and **14**. The methylated derivatives **6a** and **12a** were stronger insecticides than their parent compounds lacking cytotoxic or antiparasite effects. Similarly, diacetate **7a** was less cytotoxic than the parent alcohol **7**, suggesting a polarity-dependent mode of action of these compounds on the cell/parasite. The phytotoxic (radicule elongation) and insecticidal effects had a similar structure−activity pattern with the acetylated derivatives being less active.

In summary, structural modifications of the A-ring of the cork processing byproduct **1** improved its insecticidal activity with the C-2 hydroxyl derivative **5**, the aldehyde seco acid **6**, its acetylated derivative **6a**, alcohols **9**−**11** and **16**, the acetylated **10a**, seco acid **14**, lactone **18**, and the oxime **19** being stronger insecticides than the parent compound. Additionally, seco acid **12** and its acetylated derivative **12a** were also insecticides in contrast to their inactive parent compound **2**, another cork processing byproduct. The larval postingestive effects and insect cell cytotoxicity of these compounds suggest a multifaceted mode of action. These structural modifications did not result in better phytotoxic agents than the parent compounds **1** and **2**, except for lactam **20**, and yielded several moderately active antiparasite compounds (seco acids **6**, **12**, **14**, and **16**) with cytotoxic effects on mammalian CHO cells. Therefore, the cork processing byproduct friedelin is a convenient model for the design of insect growth regulators with phytotoxic effects.

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