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# *ent*-Kaurene Glycosides from *Ageratina cylindrica*

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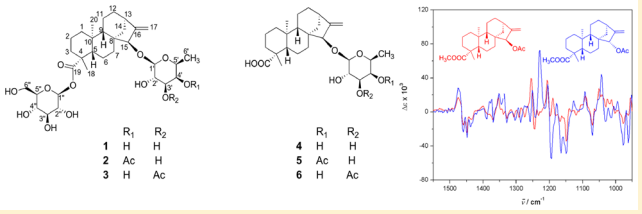
Supporting Information

**ABSTRACT:** The aqueous extract of the leaves of *Ageratina cylindrica* afforded six new *ent*-kaurenoic acid glycosides together with the known diterpenoid paniculoside V, the flavonoid astragalin, chlorogenic acid, and L-*chiro*-inositol. The structures were elucidated mainly by NMR and MS methods, and the absolute configuration was established by vibrational circular dichroism spectroscopy. The new compounds showed moderate antiprotozoal activity against *Entamoeba histolytica* and *Giardia lamblia* trophozoites.




As a continuation of our search for antiparasitic compounds,[1] the chemical investigation of the aqueous extract of *Ageratina cylindrica* (McVaugh) R. M. King & H. Rob (Asteraceae) yielded six new *ent*-kaurenoic acid glycosides. This type of compound is predominantly found in species belonging to several families, but best represented in the Asteraceae, although they are rarely found in the genera *Eupatorium* and *Ageratina*. Only three kaurenes have been isolated from *Ageratina vacciniaefolia*.[2] Kaurene glycosides and related compounds have attracted significant attention due to their therapeutic potential and economic value.[3] In this regard, steviol glycosides, isolated from the leaves of *Stevia rebaudiana*, are natural sugar substitutes sweeter than sucrose, in addition to their pharmacological activities as hypoglycemic, antitumor and anticancer, antihypertensive, and antidiarrheal agents.[4]

*Entamoeba histolytica* and *Giardia lamblia* are protozoa associated with parasitic infections for which metronidazole has proven to be effective. However, due to its adverse side effects, efforts for improving the therapy for giardiasis and amoebic dysentery are needed. In this sense, vegetal extracts have been used for many years in the treatment and prevention of several diseases, including parasitic infections,[5] since medicinal plants could be a source of new drugs with high activity and low toxicity. Herein, the isolation, structural characterization, and antiprotozoal activity of six new *ent*-kaurenoic glycosides from *A. cylindrica* are discussed.

## RESULTS AND DISCUSSION

Repeated chromatography of the aqueous extracts of the leaves of *A. cylindrica* on Sephadex LH-20 and octadecyl-functionalized silica gel yielded six new compounds (**1**−**6**) together with the known *ent*-kaurene paniculoside V (**7**),[6] astragalin,[7] chlorogenic acid,[8] and L-*chiro*-inositol.[9] The compounds were fully characterized by their physical and spectroscopic properties, including $^{1}$H and $^{13}$C NMR data (Tables 1 and 2). The resonances were assigned using COSY, HSQC, ROESY, and HMBC correlations.

Compound **1** gave the molecular formula $C_{32}H_{50}O_{12}$, as determined by HRESIMS and $^{13}$C NMR data. The IR spectrum showed absorption bands for OH and COOR groups at 3370 and 1732 cm$^{-1}$, respectively. The $^{1}$H and $^{13}$C NMR spectra of **1** were similar to those of **7**. The $^{1}$H NMR spectrum (Table 1) showed the presence of two methyl singlets at $\delta$ 1.28 and 1.30 and two olefinic singlets at $\delta$ 5.08 and 5.88 of an exocyclic methylene group, which showed cross-peaks with the methylene carbon signal at $\delta$ 106.5 in the HSQC experiment. The above data suggested an *ent*-kaurenoic acid derivative. Two doublets at $\delta$ 4.80 ($J$ = 8.0 Hz) and $\delta$ 6.29 ($J$ = 8.0 Hz), coupled with the carbon resonances at $\delta$ 107.3 and 96.2, respectively, were assigned to anomeric hydrogens, suggesting the presence of two sugar units. Their $\beta$-orientation follows from the

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Table 1. NMR Data ($^1$H 400 MHz, $^{13}$C 100 MHz, Pyridine-$d_5$) of 1 and 2

| | 1 | | | 2 | |
|---|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$, type | HMBC | $\delta_H$ ($J$ in Hz) | $\delta_C$ |
| 1a | 1.82, d (13.2) | 41.2, $CH_2$ | 2, 3, 9, 10, 20 | 1.75, m | 41.0, $CH_2$ |
| 1b | 0.83, td (13.2, 3.6) | | 2, 3, 10 | 0.75, td (13.2, 3.6) | |
| 2a | 2.23, m | 20.0, $CH_2$ | 5,[a] 9,[a] 20[a] | 2.18, m | 20.0, $CH_2$ |
| 2b | 1.40, m | | 1, 3, 9[a] | 1.38, m | |
| 3a | 2.39, m | 38.8, $CH_2$ | 2, 18, 19 | 2.35, dd (13.6, 2.8) | 38.9, $CH_2$ |
| 3b | 1.00, m | | 2, 4, 5, 19 | 0.85, td (13.6, 4.4) | |
| 4 | | 44.6, C | | | 44.5, C |
| 5 | 1.25, dd (12.0, 1.6) | 57.1, CH | 6, 10, 18, 19, 20 | 1.12, dd (12.0, 1.2) | 56.8, CH |
| 6a | 2.48, d (13.6) | 22.5, $CH_2$ | 4, 7, 8, 10 | 2.48, m | 22.4, $CH_2$ |
| 6b | 2.13, dd (13.6, 1.6) | | 7 | 2.10, m | |
| 7a | 2.35, m | 39.0, $CH_2$ | 20,[a] 11,[a] 6 | 2.33, m | 37.0, $CH_2$ |
| 7b | 1.46, m | | 6, 5 | 1.47, m | |
| 8 | | 46.8, C | | | 46.7, C |
| 9 | 1.90, m | 46.7, CH | 8, 10, 12, 14 | 1.83, m | 46.7, CH |
| 10 | | 40.1, C | | | 40.1, C |
| 11a | 1.60, m | 18.9, $CH_2$ | 8, 10, 12 | 1.91, m | 18.8, $CH_2$ |
| 11b | 1.42, m | | 10 | 1.83, m | |
| 12a | 1.61, m | 34.4, $CH_2$ | 11 | 1.60, m | 34.3, $CH_2$ |
| 12b | 1.46, m | | 11, 14, 16, 17[a] | 1.44, m | |
| 13 | 2.58, br s | 41.1, CH | 8, 11, 12, 15, 16 | 2.58, br s | 41.1, CH |
| 14a | 2.25, m | 37.2, $CH_2$ | 9, 12, 13 | 2.21, m | 38.6, $CH_2$ |
| 14b | 1.02, m | | 9, 12, 13, 15, 16 | 1.03, dd (12.0, 4.4) | |
| 15 | 4.11, t (2.0) | 91.1, CH | 9, 16, 17 | 4.10, t (2.4) | 90.1, CH |
| 16 | | 157.3, C | | | 157.2, C |
| 17a | 5.88, br s | 106.5, $CH_2$ | 12,[a] 13, 15, 16 | 5.91, br s | 106.4, $CH_2$ |
| 17b | 5.08, br s | | 12,[a] 13, 15, 16 | 5.09, dd (1.2) | |
| 18 | 1.28, s | 29.1, $CH_3$ | 3, 4, 5, 19 | 1.23, s | 28.9, $CH_3$ |
| 19 | | 177.4, C | | | 177.4, C |
| 20 | 1.30, s | 16.6, $CH_3$ | 1, 5, 9, 10 | 1.28, s | 16.5, $CH_3$ |
| 1′ | 4.80, d (8.0) | 107.3, CH | 15 | 4.87, d (8.0) | 107.0, CH |
| 2′ | 4.41, t (8.8) | 72.9, CH | 1′ | 4.36, dd (9.2) | 73.0, CH |
| 3′ | 4.12, dd (9.2, 3.6) | 76.0, CH | 1′, 5′ | 4.28, dd (9.6, 4.0) | 73.6, CH |
| 4′ | 4.06, dd (3.6, 0.8) | 73.1, CH | 3′ | 5.64, dd (3.6, 1.2) | 74.6, CH |
| 5′ | 3.79, qd (6.4,0.8) | 71.8, CH | 1′, 2′,[a] 6′ | 3.90, qd (6.4, 0.8) | 70.1, CH |
| 6′ | 1.59, d (6.4) | 17.8, $CH_3$ | 4′, 5′ | 1.37, d (6.4) | 17.4, $CH_3$ |
| 1″ | 6.29, d (8.0) | 96.2, CH | 3″, 5″, 19 | 6.28, d (8.0) | 96.2, CH |
| 2″ | 4.23, t (8.8) | 79.6, CH | 1″, 5″,[a] 6″[a] | 4.25, t (8.8) | 79.6, CH |
| 3″ | 4.28, t (8.8) | 74.5, CH | 2″, 4″ | 4.28, t (8.8) | 74.5, CH |
| 4″ | 4.36, t (9.2) | 71.5, CH | 2″, 3″, 6″ | 4.37, dd (9.6, 9.2) | 71.5, CH |
| 5″ | 4.04, ddd (9.2, 4.4, 2.4) | 79.8, CH | 1″, 2″, 3″, 4″[a] | 4.04, ddd (9.6, 4.4, 2.4) | 79.8, CH |
| 6″a | 4.48, dd (12.0, 2.4) | 62.6, $CH_2$ | 1″,[a] 5″ | 4.47, dd (12.0, 2.4) | 62.5, $CH_2$ |
| 6″b | 4.42, dd (12.0, 4.0) | | 5″ | 4.40, dd (12.0, 4.4) | |
| $OCO\underline{C}H_3$ | | | | 1.80, s | 21.1, $CH_3$ |
| $O\underline{C}OCH_3$ | | | | | 171.4, C |

[a] $^4J$ interaction.

magnitude (8 Hz) of the coupling constants. An additional methyl doublet at $\delta$ 1.59 ($J$ = 6.4, 3H) suggested one of the sugar residues to be a methyl pentose moiety.

The location of the sugar moieties was determined by the HMBC correlations between the carbonyl group at $\delta$ 177.4 (C-19) and the anomeric hydrogen at $\delta$ 6.29, indicating that one sugar moiety must be located at C-19 as an ester, while the other one must be located at C-15, since its anomeric doublet at $\delta$ 4.80 showed long-range coupling with the carbon signal at $\delta$ 91.1 ($\delta_H$ 4.11) assigned to C-15. Identification of the spin system of the individual monosaccharide moieties and complete assignment of the hydrogen resonances were achieved through COSY and selective 1D-TOCSY experiments. The NMR correlations showed two spin systems, from anomeric H-1″ [$\delta_H$ 6.29 ($\delta_C$ 96.2)] to $H_2$-6″ [$\delta_H$ 4.42 and 4.48 ($\delta_C$ 62.6)] and from anomeric H-1′ [$\delta_H$ 4.80 ($\delta_C$ 107.3)] to $H_3$-6′ [$\delta_H$ 1.59 ($\delta_C$ 17.8)] (Table 1). Carbon chemical shifts and H−H coupling constants permitted identification of the sugar moieties as $\beta$-glucopyranosyl and $\beta$-fucopyranosyl residues, respectively.

The $^{13}$C NMR data (Table 1) showed 32 resonances due to three methyl, 10 methylene, and 14 methine groups, four quaternary carbon atoms, and one carbonyl carbon evidenced by DEPT experiments. Eighteen signals at low frequencies, together with a carbonyl ($\delta$ 177.4) and an olefinic methylene ($\delta$ 106.5), are assignable to the kaurene skeleton, while 10 methines and one methylene in the $\delta$ 62.6−107.3 range,


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Table 2. NMR Data ($^1$H 400 MHz, $^{13}$C 100 MHz, Pyridine-$d_5$) of 3−5

| | 3 | | 4 | | 5[a] | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ |
| 1a | 1.80, s | 41.2, $CH_2$ | 1.81, s | 41.4, $CH_2$ | 1.84, m | 41.2, $CH_2$ |
| 1b | 0.83, td (12.0, 4.0) | | 0.87, td (13.6, 4.0) | | 0.81, td (13.5, 4.0) | |
| 2a | 2.21, s | 20.0, $CH_2$ | 2.25, m | 20.3, $CH_2$ | 2.24, m | 20.3, $CH_2$ |
| 2b | 1.39, m | | 1.48, m | | 1.45, m | |
| 3a | 2.31, m | 39.0, $CH_2$ | 2.38, td (13.2, 4.0) | 39.0, $CH_2$ | 2.42, m | 38.9, $CH_2$ |
| 3b | 1.45, m | | 1.01, m | | 0.89, td (13.0, 4.0) | |
| 4 | | 44.5, C | | 44.3, C | | 44.2, C |
| 5 | 1.87, m | 46.8, CH | 1.23, m | 56.6, CH | 1.1,1 dd (12.0, 2.0) | 56.4, CH |
| 6a | 2.45, m | 22.4, $CH_2$ | 2.21, m | 22.8, $CH_2$ | 2.42, d (12.0) | 22.8, $CH_2$ |
| 6b | 2.11, m | | 2.14, m | | 2.14, m | |
| 7a | 2.22, m | 37.0, $CH_2$ | 2.05, d (12.0) | 37.3, $CH_2$ | 1.05, dd (11.5, 4.5) | 37.2, $CH_2$ |
| 7b | 1.01, m | | 1.05, m | | 2.04, d (11.5) | |
| 8 | | 46.8, C | | 46.8, C | | 46.7, C |
| 9 | 1.22, m | 57.2, CH | 1.21, m | 46.7, CH | 1.85, d (7.5) | 46.7, CH |
| 10 | | 40.1, C | | 40.1, C | | 40.1, C |
| 11a | 1.88, m | 18.8, $CH_2$ | 1.92, m | 18.8, $CH_2$ | 1.91, m | 18.8, $CH_2$ |
| 11b | 1.43, m | | 1.44, m | | 1.42, m | |
| 12a | 1.56, m | 34.3, $CH_2$ | 1.58, m | 34.4, $CH_2$ | 1.58, m | 34.4, $CH_2$ |
| 12b | 1.43, m | | 1.48, m | | 1.46, m | |
| 13 | 2.57, br s | 41.1, CH | 2.61 s | 41.1, CH | 2.62, s | 41.1, CH |
| 14a | 2.37, m | 38.8, $CH_2$ | 2.46, d (12.8) | 38.9, $CH_2$ | 2.05, d (11.5) | 37.2, $CH_2$ |
| 14b | 1.01 m | | 1.00, dd (13.2, 4.0) | | 1.06, dd (11.5, 4.5) | |
| 15 | 4.10, t (2.4) | 91.3, CH | 4.13, m | 91.0, CH | 4.11, t (2.5) | 90.9, CH |
| 16 | | 157.0, C | | 157.2, C | | 157.1, C |
| 17a | 5.84, s | 106.6, $CH_2$ | 5.92, s | 106.4, $CH_2$ | 5.94, s | 106.4, $CH_2$ |
| 17b | 5.07, dd (1.2, 1.2) | | 5.11, t (1.2) | | 5.12, s | |
| 18 | 1.27, s | 29.0, $CH_3$ | 1.33, s | 29.7, $CH_3$ | 1.28, s | 29.7, $CH_3$ |
| 19 | | 177.4, C | | 180.7, C | | 180.8, C |
| 20 | 1.30, s | 16.5, $CH_3$ | 1.21, s | 16.7, $CH_3$ | 1.19, s | 16.6, $CH_3$ |
| 1′ | 4.87, d (8.0) | 107.1, CH | 4.85, d (8.0) | 107.3, CH | 4.91, d (8.0) | 107.1, CH |
| 2′ | 4.62, dd (10.0, 8.0) | 69.7, CH | 4.44, dd (9.2, 8.0) | 72.9, CH | 4.39, dd (9.5, 8.0) | 73.0, CH |
| 3′ | 5.42, dd (10.0, 3.2) | 78.5, CH | 4.14, dd (9.2, 3.2) | 76.0, CH | 4.30, dd (9.5, 3.5) | 73.6, CH |
| 4′ | 4.25, dd (4.0, 0.8) | 70.5, CH | 4.09, dd (3.2, 0.8) | 73.1, CH | 5.66, d (3.5) | 74.6, CH |
| 5′ | 3.82, td (8.0, 0.8) | 71.5, CH | 3.85, td (8.0, 0.8) | 71.8, CH | 3.96, q (6.5) | 70.1, CH |
| 6′ | 1.54, d (8.0) | 17.6, $CH_3$ | 1.64, d (8.0) | 17.8, $CH_3$ | 1.42, d (6.5) | 17.4, $CH_3$ |
| 1″ | 6.28 d (8.0) | 96.2, CH | | | | |
| 2″ | 4.23 t (8.0) | 79.6, CH | | | | |
| 3″ | 4.28 t (8.8) | 74.5, CH | | | | |
| 4″ | 4.35 t (8.8) | 71.5, CH | | | | |
| 5″ | 4.04 ddd (9.2, 4.4, 2.4) | 79.8, CH | | | | |
| 6″a | 4.47 dd (12.0, 2.8) | 62.5, $CH_2$ | | | | |
| 6″b | 4.39 dd (12.0, 4.4) | | | | | |
| $OCO\underline{C}H_3$ | 1.95 | 21.4, $CH_3$ | | | 1.82, s | 21.1, $CH_3$ |
| $O\underline{C}OCH_3$ | | 171.2, C | | | | 171.0, C |

[a]NMR was recorded at 500 MHz for $^1$H and 125 MHz for $^{13}$C.

together with a methyl signal at $\delta$ 17.8, are due to the sugar moieties.

In the case of *ent*-kaurenoic acid derivatives the C-15 configuration has been assigned by NOE effects between H-15/H-9 or H-15/H-14 for the $\alpha$- and $\beta$-epimers, respectively,[10] although these interactions were not observed in **1**−**6**. Analysis of the multiplicity of the H-15 signal has also been used to assign the configuration at C-15. Nevertheless, inspection of the $^1$H NMR data of related molecules reveals that the multiplicities for H-15 have been reported either as a broad singlet or as triplets or a doublet of doublets, thus causing conflicting assignments of the configuration at C-15.[11] Consequently, it was essential to unequivocally assign the C-15 absolute configuration (AC) of the new compounds and at the same time to verify that they are *ent*-kaurenes.

Comparison of the calculated and experimental vibrational circular dichroism (VCD) spectra is a sensitive technique for determining the AC of a single stereogenic center in the presence of several centers and has allowed the determination of the AC of a significant number of natural products.[12] VCD of natural product ester derivatives has advantages over the analysis of free alcohols or acids, which often show intermolecular solute−solute associations that confuse comparison of experimental and density functional theory (DFT)-calculated spectra,[13] and therefore **8** was selected as a truncated model compound.



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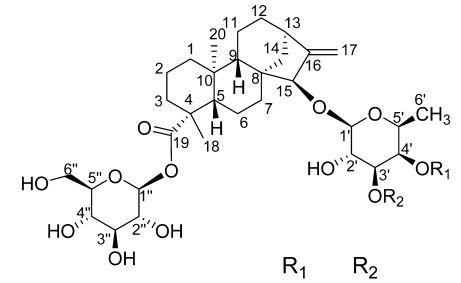
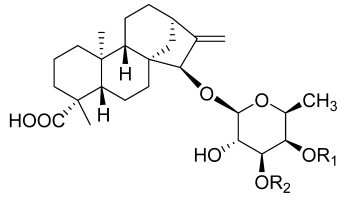
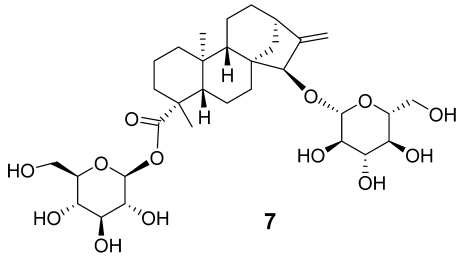
**Chart 1**



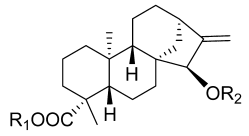
| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | H | H |
| **2** | Ac | H |
| **3** | H | Ac |



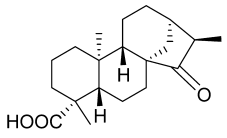
| | $R_1$ | $R_2$ |
|---|---|---|
| **4** | H | H |
| **5** | Ac | H |
| **6** | H | Ac |



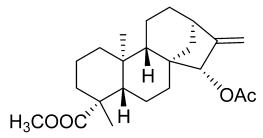
**7**



| | $R_1$ | $R_2$ |
|---|---|---|
| **8** | Me | Ac |
| **9** | H | H |



**10**



**11**

**Table 3. Relative Energies and Conformational Populations of 8 and 11**

| conformer | $\Delta E_{MMFF94}$ [a] | $\%_{MMFF94}$ [b] | $\Delta E_{SP}$ [c] | $\%_{SP}$ [d] | $\Delta E_{OPT}$ [e] | $\%_{OPT}$ [d] | $\Delta G_{B3PW91}$ [f] | $\%_{B3PW91}$ [d] |
|---|---|---|---|---|---|---|---|---|
| **8a** | 0.00 | 51.8 | 0.42 | 33.0 | 0.34 | 36.0 | 0.70 | 23.5 |
| **8b** | 0.04 | 48.2 | 0.00 | 67.0 | 0.00 | 64.0 | 0.00 | 76.5 |
| **11a** | 0.00 | 56.0 | 0.24 | 39.8 | 0.30 | 37.4 | 0.00 | 52.5 |
| **11b** | 0.14 | 44.0 | 0.00 | 60.2 | 0.00 | 62.6 | 0.06 | 47.5 |

[a]Molecular mechanics energy relative to 73.03 and 72.74 kcal/mol for **8** and **11**, respectively. [b]Molecular mechanics population in %. [c]Single-point relative energy; the lowest energy values for **8** and **11** were −751 396.8 and −751 396.5 kcal/mol, respectively. [d]Conformational population. [e]Energy of the optimized structures; data are relative to −751 439.7 kcal/mol for **8** and −751 439.1 kcal/mol for **11**. [f]Free energy relative to −751 135.1 kcal/mol for **8** and −751 133.9 kcal/mol for **11**.

Acid hydrolysis of **1** with aqueous 5% HCl did not afford target compound **9**. Instead, the keto acid **10** was obtained, in addition to D-glucose and L-fucose, which were identified as their trimethylsilyl derivatives by GC and the positive (+35.4) and negative (−33.2) specific rotations of their acetate derivatives, respectively. It is known that *ent*-kaurenes with a 15$\beta$-hydroxy group undergo a Wagner−Meerwein rearrangement when heated with mineral acid to afford (16*R*)-*ent*-kauran-15-one (**10**), while the epimeric 15$\alpha$-hydroxy group is stable under these reaction conditions.[14] This result suggested the $\beta$-orientation of the fucosyloxy moiety at C-15 in **1**.

With a view to obtaining **9** and to prevent the proton-catalyzed Wagner−Meerwein rearrangement of **1** to **10**, the oxidation/elimination sequence[15] was selected. Alkaline hydrolysis of **1** gave fucosyl derivative **4**, which when treated with $NaIO_4$ and KOH gave desacetylxylopic acid (**9**),[16] which without purification was subjected to acetylation followed by esterification with diazomethane to give methyl xylopate (**8**).

The absolute configuration of **8** and of its C-15 epimer **11**, prepared from an authentic sample of grandifloric acid, was obtained by comparing the experimental and calculated IR and VCD spectra. A molecular mechanics conformational search using the MMFF94 force field provided 12 minimum energy conformers for each molecule in a 10 kcal/mol energy window. Two conformers of each epimer with energy in the 0.2 kcal/mol gap (Table 3) accounted for 99.99% of the conformational populations of **8** and **11**. These four conformations were submitted to single-point calculations with the B3PW91 hybrid functional and the DGDZVP basis set. Since conformers **8a** and **8b**, as well as **11a** and **11b**, remained within similar energy ranges, they were optimized at the same level of theory (Table 3). The dipole and rotational strengths, calculated at the B3PW91/DGDZVP level, were converted into molar absorptivity of each conformer using Lorentzian functions with a bandwidth of 6 $cm^{-1}$. Finally, the Boltzmann-weighted factor for each conformer obtained with the Gibbs free energy was



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used to generate the calculated IR and VCD spectra. The solvent-subtracted and calculated IR and VCD spectra for **8** and **11** are shown in Figures 1 and 2, respectively. Table 4 shows

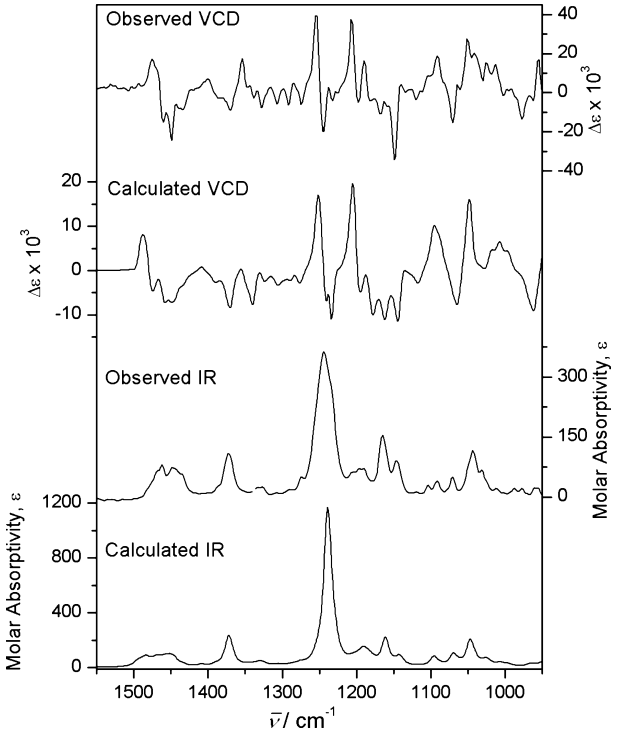


**Figure 1.** Comparison of the experimental and calculated, at the B3PW91/DGDZVP level, IR and VCD spectra of **8**.

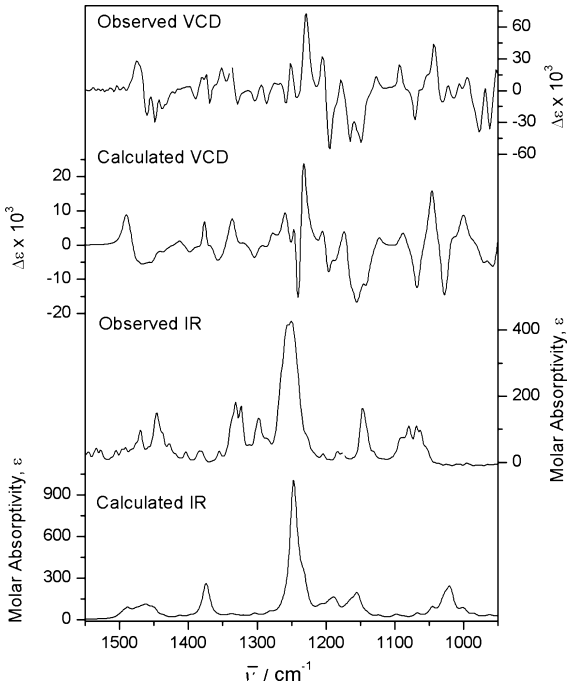


**Figure 2.** Comparison of the experimental and calculated, at the B3PW91/DGDZVP level, IR and VCD spectra of **11**.

the data obtained using the CompareVOA software.[17] For compound **8** the IR and VCD spectra similarities, $S_{IR}$ and $S_E$,[18] are 95.1 and 86.1, respectively, with a confidence level of 100%, while for compound **11** the values are 97.3 and 83.9 with the

**Table 4. Confidence Level Data for the IR and VCD Spectra of 8 and 11 and Cross-Comparisons**

| compound | anH[a] | $S_{IR}$[b] | $S_E$[c] | $S_{-E}$[d] | ESI[e] | $C$[f] (%) |
|---|---|---|---|---|---|---|
| **8** | 0.972 | 95.1 | 86.1 | 17.7 | 68.8 | 100 |
| **11** | 0.974 | 97.3 | 83.9 | 18.5 | 65.5 | 100 |
| **8**E vs **11**C[g] | 0.969 | 94.2 | 67.0 | 24.8 | 42.2 | 82 |
| **11**E vs **8**C[h] | 0.976 | 93.1 | 67.7 | 24.9 | 42.8 | 85 |

[a]Anharmonicity factor. [b]IR spectral similarity. [c]VCD spectral similarity for the correct enantiomer. [d]VCD spectral similarity for the incorrect enantiomer. [e]Enantiomer similarity index, calculated as $S_E - S_{-E}$. [f]Confidence level for the stereochemical assignments. [g]Experimental **8** vs calculated **11** spectra comparison. [h]Experimental **11** vs calculated **8** spectra comparison.

same confidence level. As also shown in Table 4, cross-comparisons of the calculated and experimental spectra of **8** and **11** and vice versa show high values of spectra similarity of the IR and VCD spectra, but the enantiomer similarity index (ESI) and the confidence level are lower than those of the correct configuration, thus eliminating any doubt of the assignment of the 15*R* absolute configuration of **8**. It is important to note that the VCD spectra of **8** and **11** did not exhibit VCD exciton coupling[19] of the carbonyl group signals.

The configuration at C-15 in compound **8** was also supported by the NOE effect between H-15 at $\delta$ 5.15 t (2.5) and H-14 at $\delta$ 4.04 d (12.0). Thus, the structure of **1** was defined as *ent*-15$\beta$-($\beta$-L-fucosyloxy)kaur-16-en-19-oic acid $\beta$-D-glucopyranosyl ester.

Compound **2** displayed an $[M + Na]^+$ ion at $m/z$ 691.3306, as determined by HRESIMS, which supports the molecular formula $C_{34}H_{52}O_{13}$. The $^1$H and $^{13}$C NMR spectra (Table 1) of **2** displayed features similar to those of **1**, except for the presence of a sharp methyl singlet at $\delta$ 1.81 in the $^1$H NMR spectrum and two extra carbon resonances ($\delta$ 171.4 and 21.1) in the $^{13}$C NMR spectrum, associated with the presence of an acetate group. The $^1$H NMR chemical shifts of the sugar moieties of **2** were comparable with those of **1**, except for the deshielding of H-4′ at $\delta$ 5.64 (dd, 3.6, 1.2 Hz) indicative of the esterification at C-4′. The HMBC correlations between the signal at $\delta$ 5.64 (H-4′) and the methyl protons at $\delta$ 1.81 with the carbon resonance at $\delta$ 171.4 revealed the presence of an acetate group at C-4′. Consequently the structure of **2** was defined as *ent*-15$\beta$-(4-acetoxy-$\beta$-L-fucosyloxy)kaur-16-en-19-oic acid $\beta$-D-glucopyranosyl ester.

Compound **3**, as in the case of **2**, showed an $[M + Na]^+$ ion at $m/z$ 691.3306, which together with the $^{13}$C NMR data indicates the molecular formula $C_{34}H_{52}O_{13}$. The $^1$H and $^{13}$C NMR spectra (Table 1) showed resonances similar to those of **2**, except for the chemical shifts of H-3′ and H-4′. While in **3** the H-3′ signal was deshielded to $\delta$ 5.42 (dd, 10.0, 3.2 Hz), H-4′ was shielded as compared with compound **2**, indicating the presence of the acetate group at C-3′ in **3**, and therefore **3** is a regioisomer of **2** possessing the *ent*-15$\beta$-(3-acetoxy-$\beta$-L-fucosyloxy)kaur-16-en-19-oic acid $\beta$-D-glucopyranosyl ester structure.

The HRESIMS data of **4** showed an $[M + Na]^+$ ion at $m/z$ 487.2667 (calculated for $C_{26}H_{40}O_7$ + Na, 487.2672), which supported the molecular formula $C_{26}H_{40}O_7$. The $^{13}$C NMR spectrum of **4** showed 26 carbon signals, 20 of them due to the aglycone moiety and six due to a sugar unit. The $^1$H and $^{13}$C NMR data (Table 2) of **4** were similar to those of **1**, but lacked the glucose moiety resonances. Therefore, the structure of **4**


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was established as *ent*-15$\beta$-($\beta$-L-fucosyloxy)kaur-16-en-19-oic acid.

A minor amount of a mixture of **5** and **6** (4.5 mg) was isolated. Their $^1$H and $^{13}$C NMR data (Table 2 for **5** and Experimental Section for **6**) were similar to those of **2** and **3**, respectively, except for the absence of the glucose moiety resonances at C-19. Although **5** and **6** were isolated as natural products, they could not be fully identified. Thus, enzymatic hydrolysis of a mixture of **1**–**3** using $\beta$-glucosidase from almonds gave a sufficient quantity of **5** for its complete characterization, as shown in the Experimental Section and in Table 2.

The antiprotozoal activity of **1**–**4** was tested on *Entamoeba histolytica* and *Giardia lamblia* trophozoites (Table 5). In general, all compound showed moderate activity against both protozoa with $IC_{50}$ values ranging from 43.3 to 73.5 $\mu$M for *E. histolytica* and from 41.9 to 98.5 $\mu$M for *G. lamblia*.

**Table 5. In Vitro Antiprotozoal Activity of Compounds 1–4**

| compound | $IC_{50}$ $\mu$M (CI)[a] | |
|---|---|---|
| | *Entamoeba histolytica* | *Giardia lamblia* |
| **1** | 43.3 (43.6–43.1) | 41.9 (42.2–41.5) |
| **2** | 49.5 (50.1–49.0) | 69.5 (69.9–69.2) |
| **3** | 52.7 (52.9–52.3) | 48.9 (49.3–48.7) |
| **4** | 73.5 (73.9–72.8) | 98.5(98.7–97.8) |
| emetine[c] | 2.18 (2.2–2.14) | 0.83 (0.87–0.82) |
| metronidazole[c] | 0.23 (0.58–0.17) | 1.22 (1.57–0.81) |

[a]Results are expressed as mean ($n$ = 6), CI = 95% confidence intervals; correlation coefficient >0.9500 and $p < 0.05$. [c]Positive controls.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Melting points were measured on a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained on a Bruker Tensor 27 spectrometer. VCD data were acquired on a BioTools dualPEM ChiralIR FT-VCD spectrophotometer (Jupiter, FL, USA). The 1D and 2D NMR experiments were performed on a Bruker Advance III spectrometer at 400 MHz for $^1$H and 100 MHz for $^{13}$C and on a Varian Unity Inova 500 MHz for $^1$H and 125 MHz for $^{13}$C. Chemical shifts were referenced to TMS, and $J$ values are given in Hz. The HRESIMS data were recorded on a Waters Synap G2S HDMS Q-TOF mass spectrometer. The DART-MS data were obtained on a Jeol AccuTOF JMS-T100LC mass spectrometer. Preparative TLC was carried out on precoated Macherey Nagel Sil G/UV$_{254}$ plates of 1.0 mm thickness. Silica gel 230–400 mesh (Macherey-Nagel), Sephadex LH-20 (Pharmacia Biotech), and octadecyl-functionalized silica gel (Sigma-Aldrich) were used for column chromatography.

**Plant Material.** *A. cylindrica* was collected from Tenancingo, State of Mexico, Mexico, in February 2012. The plant material was identified by Jose Luis Villaseñor, and a voucher specimen (MEXU-1333 472) was deposited at the National Herbarium (MEXU) of the Instituto de Biologia, UNAM, México City, Mexico.

**Extraction and Isolation.** The dried and powdered leaves of *A. cylindrica* (100 g) were added to boiling $H_2O$ (1 L × 3) and filtered immediately. The infusion was filtered, and after cooling to room temperature it was lyophilized to yield 33 g of residue. This material was fractionated over a Sephadex LH-20 column, using MeOH as eluent, to give nine fractions (A–I). Fraction D (950 mg) was subjected to flash column chromatography on octadecyl-functionalized silica gel, with an isocratic mobile phase of MeOH/$H_2O$ (1:3, v/v) with a 5.0 mL min$^{-1}$ flow rate, to obtain 50 fractions (25 mL each), which were combined into 15 major fractions (D1–D15) by TLC evaluation. D1 (35 mg) was subjected to silica gel CC eluting with EtOAc/MeOH/$H_2O$ (200:16:7), affording **7** (25 mg). Pure **1** (30 mg) was obtained from fraction D3, **2** (100 mg), **3** (25 mg), and **4** (15 mg) were obtained from fractions D8, D11, and D13, respectively, D15 (4.7 mg) gave a mixture of **5** and **6**, L-*chiro*-inositol[9] (150 mg) crystallized from fraction F, fraction G (30 mg) was subjected to silica gel TLC eluting with *n*-BuOH/2-propanol/formic acid/$H_2O$ (48:17:18:17) to give chlorogenic acid[8] (15 mg), and astragalin[7] (25 mg) was isolated from fraction I (67 mg) in the same way.

*ent-15β-(β-L-Fucosyloxy)kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**1**):* white powder; mp 145–150 °C; $[\alpha]^{25}_D$ −31.6 ($c$ 0.01, MeOH); IR (KBr) $\nu_{max}$ 3370, 2924, 2856, 1732, 1655, 1061 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; HRESIMS $m/z$ [M + Na]$^+$ 649.3222 (calculated for $C_{32}H_{50}O_{12}$ + Na, 649.3200).

*ent-15β-(4-Acetoxy-β-L-fucosyloxy)kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**2**):* white powder; mp 150–152 °C; $[\alpha]^{25}_D$ −46.6 ($c$ 0.01, MeOH); IR (KBr) $\nu_{max}$ 3373, 2925, 2857, 1724, 1655, 1602, 1240, 1070 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; HRESIMS $m/z$ [M + Na]$^+$ 691.3316 (calculated for $C_{34}H_{52}O_{13}$ + Na, 691.3306).

*ent-15β-(3-Acetoxy-β-L-fucosyloxy)kaur-16-en-19-oic acid β-D-glucopyranosyl ester (**3**):* white powder; mp 145–148 °C; $[\alpha]^{25}_D$ −33.0 ($c$ 0.01, MeOH); IR (KBr) $\nu_{max}$ 3691, 3597, 2929, 1856, 1734, 1602, 1022 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 2; HRESIMS $m/z$ [M + Na]$^+$ 691.3316 (calculated for $C_{34}H_{52}O_{13}$ + Na, 691.3306).

*ent-15β-(β-L-Fucosyloxy)kaur-16-en-19-oic acid (**4**):* white powder; mp 142–146 °C; $[\alpha]^{25}_D$ −36.7 ($c$ 0.01, MeOH); IR (KBr) $\nu_{max}$ 3368, 1731, 1706, 1655, 1231 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; HRESIMS $m/z$ [M + Na]$^+$ 487.2667 (calculated for $C_{26}H_{40}O_7$ + Na, 487.2672).

*ent-15β-(4-Acetoxy-β-L-fucosyloxy)kaur-16-en-19-oic acid (**5**):* white powder; mp 149–152 °C; $[\alpha]^{25}_D$ −28.0 ($c$ 0.01, MeOH); IR (KBr) $\nu_{max}$ 3596, 1735, 1693, 1449, 1370, 1242 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; DART-MS $m/z$ [M + H]$^+$ 507.

*ent-15β-(3-Acetoxy-β-L-fucosyloxy)kaur-16-en-19-oic acid (**6**):* $^1$H NMR partial data extracted from a **5** and **6** mixture (pyridine-$d_5$, 500 MHz) $\delta$ 2.61 (1H, s, H-13), 5.88 (1H, s, H-17a), 5.11 (1H, s, H-17b), 1.32 (3H, s, $CH_3$-18), 1.21 (3H, s, $CH_3$-20), 1.96 (3H, s, $OCOCH_3$), 4.92 (1H, d, $J$ = 7.6 Hz, H-1′), 4.65 (1H, dd, $J$ = 10.0, 7.6 Hz, H-2′), 5.46 (1H, dd, $J$ = 10.0, 3.2 Hz, H-3′), 4.28 (1H, d, $J$ = 4.0 Hz, H-4′), 3.88 (1H, qd, $J$ = 6.4, 0.8 Hz, H-5′), 1.58 (3H, d, $J$ = 6.4 Hz, $CH_3$-6′); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 41.1 ($CH_2$, C-13), 106.6 ($CH_2$, C-17), 29.7 ($CH_3$, C-18), 180.7 (C, C19), 16.6 ($CH_3$, C-20), 171.2 (C, O$\underline{C}$OCH$_3$), 21.4 ($CH_3$, OCO$\underline{C}$H$_3$), 107.1 (CH, C-1′), 72.8 (CH, C-2′), 78.6 (C, C-3′), 70.5 (CH, C-4′), 71.6 (CH, C-5′), 17.6 ($CH_3$, C-6′).

*Paniculoside V (**7**):* white powder; mp 168–172 °C (reported[6] 173–175 °C); $^{13}$C NMR data were essentially the same as reported;[6] $^1$H NMR (pyridine-$d_5$, 400 MHz) $\delta$ 1.82 (1H, d, $J$ = 12.4, H-1a), 0.83 (1H, td, $J$ = 12.4, 3.2, H-1b), 2.19 (1H, m, H-2a), 1.38 (1H, m, H-2b), 2.34 d (1H, d, $J$ = 12.8, H-3a), 0.95 (1H, dd, $J$ = 13.6, 4.4, H-3b), 1.30 (1H, m, H-5), 2.43 (1H, m, H-6a), 2.08 (1H, m, H-6b), 2.44 (1H, m, H-7a), 1.48 (1H, m, H-7b), 1.92 (1H, m, H-9), 1.90 (1H, m, H-11a), 1.43 (1H, m, H-11b), 1.62 (1H, m, H12a), 1.46 (1H, m, H-12b), 2.57 (1H, br s, H-13), 2.22 (1H, m, H-14a), 1.00 (1H, m, H-14b), 4.11 (1H, t, $J$ = 2.4, H-15), 5.89 (1H, br s, H-17a), 5.09 (1H, dd, $J$ = 1.6, 0.8, H-17b), 1.24 (3H, s, $CH_3$-18), 1.29 (3H, s, $CH_3$-20), 5.04 (1H, d, $J$ = 7.6, H-1′), 4.12 (1H, t, $J$ = 8.8, H-2′), 4.27 (1H, m ov, $J$ = 9.2, H-3′), 4.25 (1H, m ov, H-4′), 3.92 (1H, ddd, $J$ = 8.8, 4.8, 2.8, H-5′), 4.54 (1H, dd, $J$ = 11.2, 2.8, H-6′a), 4.42 (1H, dd, $J$ = 12.0, 4.0, H-6′b), 6.27 (1H, d, $J$ = 8.0, H-1″), 4.21 (1H, t, $J$ = 8.8, H-2″), 4.28 (1H, t, $J$ = 8.4, H-3″), 4.34 (1H, t, $J$ = 9.2, H-4″), 4.03 (1H, ddd, $J$ = 9.2, 4.4, 2.4, H-5″), 4.46 (1H, dd, J = 12.0, 2.4, H-6″a), 4.38 (1H, dd, $J$ = 12.0, 4.8, H-6″b).

*Methyl xylopate (**8**):* A solution of **4** (17 mg) in $H_2O$ (2.0 mL) was treated with 23 mg of $NaIO_4$ and stirred at rt for 18 h. Then, 20 mg of KOH was added and the reaction mixture refluxed for 1 h. After addition of $H_2O$ (2.0 mL) the pH was adjusted to 4–5 with HOAc, and the resulting mixture was extracted with EtOAc (3 × 10 mL). The EtOAc fraction was washed with $H_2O$ (25 mL), dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure to yield **9**, which without purification was subjected to esterification with diazomethane, followed by acetylation with pyridine and $Ac_2O$ to give **8** (1 mg),


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which was identified by NMR spectroscopic comparison with literature data.[20]

**Alkaline Hydrolysis.** A solution of **1** (30 mg) in 10% KOH/EtOH (5 mL) was heated at 70 °C for 4 h. After acidification with HOAc, the solvent was removed and the residue was partitioned between *n*-BuOH and $H_2O$ (10 mL × 3). The *n*-BuOH layer was concentrated in vacuo to yield a white powder, which was identified as **4** (18 mg) by NMR spectroscopic comparison.

**Acid Hydrolysis.** To a mixture of **2**–**4** (35 mg) in MeOH (5 mL) was added 10% HCl (10 mL), and the mixture was refluxed for 8 h, followed by extraction with $CH_2Cl_2$ (3 × 20 mL) to give an aqueous fraction containing sugars and a $CH_2Cl_2$ fraction containing **10**, which was characterized[23] by $^1$H and $^{13}$C NMR data. The aqueous phase was neutralized with 1 N KOH, extracted with *n*-BuOH (20 mL), and washed with $H_2O$ (3 × 10 mL), and after removal of the solvent under reduced pressure, the sugars were converted into their silylated derivatives by treatment with bis(trimethylsilyl)trifluoracetamide and then analyzed by GC-MS, as described.[24]

A solution of **4** (60 mg) in MeOH was subjected to acid hydrolysis as described above. The aqueous phase was neutralized with $Na_2CO_3$ and extracted with *n*-BuOH (20 mL × 3). After removal of the solvent under reduced pressure L-fucose was acetylated without isolation and purified by TLC using hexanes/EtOAc (2:1), affording 3 mg of the corresponding acetate, which was identified by $^1$H NMR and the specific rotation $[\alpha]^{25}_D$ −33.2 (*c* 0.0028, $CHCl_3$).

**Enzymatic Hydrolysis.** To a solution of a mixture of **1**–**3** (60 mg) in 20 mL of 0.1 M NaOAc buffer (pH 5.0) was added β-glucosidase from almonds (12 000 μL, Sigma-Aldrich 49290). The mixture was stirred at 50 °C for 96 h, followed by extraction with EtOAc (3 × 20 mL), to give an organic fraction containing **4**–**6**, which were identified by TLC using reference samples. The aqueous phase was extracted with *n*-BuOH (3 × 20 mL) and treated as described above to give 5 mg of D-glucose peracetate identified by $^1$H NMR and specific rotation $[\alpha]^{25}_D$ +35.4 (*c* 0.01, $CHCl_3$).

*15-Acetoxykaurenoic Acid Methyl Ester (**11**):* Grandifloric acid, isolated from *Montanoa gigas*, showed spectroscopic data identical to those published.[21] Grandifloric acid (15 mg) was treated, using the esterification and acetylation procedure for **8**, to obtain **11** (12 mg). NMR characterization agreed with the literature.[22]

*Astragalin:* yellow powder; mp 175–177 °C (reported 177–178 °C); NMR data were essentially the same as reported.[7]

*Chlorogenic acid:* white powder; mp 203–205 °C (reported 208 °C); NMR data were the same as reported.[8]

*L-chiro-Inositol:* colorless crystals; mp 237–241 °C (reported 240–243 °C); $[\alpha]^{25}_D$ −50.0 (*c* 0.0101, $H_2O$) (reported $[\alpha]^{20}_D$ −70 (*c* 0.55, $H_2O$)); NMR data were essentially the same as reported.[9]

**VCD Analysis.** Samples of 7.0 mg of **8** and 9.4 mg of **10** were dissolved in 150 μL of 100% atom-D $CDCl_3$ and placed in a $BaF_2$ cell with a path length of 0.1 mm. Data were acquired during 6 h with 4 $cm^{-1}$ resolution for samples and solvent. The six 1 h blocks were averaged, and the final spectra were obtained by subtracting the solvent spectrum. The samples' stability was monitored by $^1$H NMR immediately before and after the VCD measurement.

**Computational Methods.** Monte Carlo conformational searches of the starting structures for compounds **8** and **11** were carried out by molecular mechanics using the MMFF94 force field included in the ComputeVOA software (Biotools). Starting from the 12 conformers obtained in the 10 kcal/mol window for each molecule, an energy cutoff of 0.2 kcal/mol was done to cover 99.99% of the conformational population. The conformers in Table 3 were submitted to a single-point energy calculation at the B3PW91/DGDZVP level of theory. The geometry of each of the conformers **8a**, **8b**, **11a**, and **11b** was optimized at the same level of theory employing the Gaussian 09W program.[25] The optimized structures of the two conformers for **8** and **11** resulted in no imaginary frequencies in the vibrational analysis and relative free energy in the interval of 0.7 kcal/mol for **8** and 0.06 kcal/mol for **11**. These four conformers were considered for the calculation of dipole moments and rotational strengths. The Boltzmann-weighted calculated IR and VCD spectra were obtained considering Lorentzian bands with half-widths of 6 $cm^{-1}$. Calculated and experimental spectra were compared using the CompareVOA program (Biotools). All Gaussian calculations were carried out using a server node with 16 processors at 2.60 GHz and 16 Gb of RAM.

**Antiprotozoal Assays.** The antiprotozoal activity of isolated compounds against *Entamoeba histolytica* HM1-IMSS and *Giardia lamblia* IMSS:1090:1 strains was determined using the reported method.[26] The resulting data are given in Table 5.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00488.

$^1$H, $^{13}$C, DEPT 135, HSQC, HMBC, COSY, NMR, ESIMS, and IR spectra for compounds **1** and **4**; $^1$H and $^{13}$C NMR spectra for compounds **2**, **3**, and **5** and a mixture of **5** and **6** (PDF)

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### Notes

The authors declare no competing financial interest.

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