Oxylipins from *Dracontium loretense*

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Four novel oxylipins (**1**−**4**) were isolated from the *n*-butanol extract of the corms of *Dracontium loretense*. Their structures were assigned by 1D and 2D NMR analyses and electrospray ionization multistage ion trap mass spectrometry (ESI-ITMS^n) data. Relative configurations were assigned on the basis of combined analysis of homonuclear and heteronuclear $^{2,3}J$ couplings, along with ROE data. Oxylipin **2** exhibited an immunostimulatory effect on human PBMC proliferation.

With over 3300 species and 105 genera of herbs and vines, Araceae is one of the dominant tropical families. Many species are used as traditional remedies or food. *Dracontium loretense* Engl., belonging to the Araceae family (subfamily Lasioideae), is a plant widely distributed in the Peruvian Amazon, where it is known as "jergón sacha". The infusion obtained from the corms of *D. loretense* has been traditionally used in Peruvian folk medicine to enhance immune function. In particular, together with the extract or infusion from *Uncaria tomentosa*, it is used by AIDS patients to reinforce the immune system.[1−3] Jergón sacha is also well known in current Peruvian herbal medicine and can be found in many natural pharmacies and stores, where it is provided as dried powder, tincture, alcoholic, and aqueous-alcoholic preparations. No phytochemical investigation has been carried out on *D. loretense* to date.

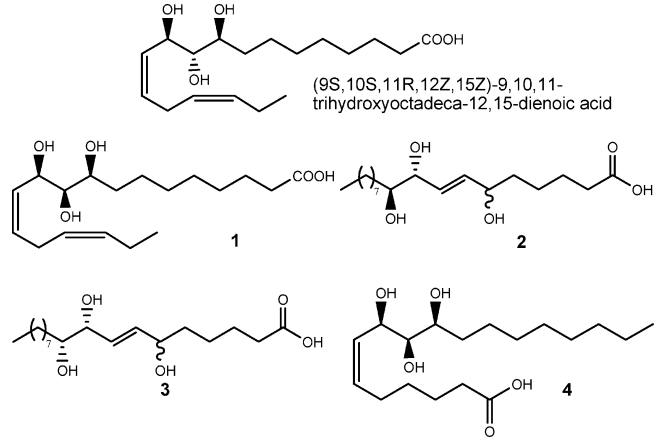
In our continuing search for bioactive secondary metabolites from Amazon plants, the *n*-butanol extract of *D. loretense* corms has been studied. This investigation led to the isolation of four novel oxylipins (**1**−**4**), namely, (9*S**,10*R**,11*R**,12*Z*,15*Z*)-9,10,11-trihydroxyoctadeca-12,15-dienoic acid (**1**), (9*R**,10*S**,7*E*)-6,9,10-trihydroxyoctadec-7-enoic acid (**2**), (9*R**,10*R**,7*E*)-6,9,10-trihydroxyoctadec-7-enoic acid (**3**), and (8*R**,9*R**,10*S**,6*Z*)-trihydroxyoctadec-6-enoic acid (**4**). The structures were elucidated by extensive spectroscopic methods, including 1D (^{1}H, ^{13}C, TOCSY) and 2D NMR experiments (DQF-COSY, HMBC, HSQC, and ROESY) as well as electrospray ionization multistage ion trap mass spectrometry (ESI-ITMS^n) analysis. The relative configurational assignment was performed by a PFG-HETLOC experiment and by applying the *J*-based method developed by Murata.[4]

On the basis of biological activities reported for *D. loretense*, the immunostimulatory effect of isolated compounds was tested on human peripheral blood mononuclear cells (PBMCs), in a proliferation assay by ^{3}H-thymidine incorporation.

Results and Discussion

The corms of *D. loretense* were extracted with petroleum ether, $CHCl_3$, and MeOH. The dried MeOH extract was partitioned between H_2O and *n*-BuOH, and then the *n*-butanol extract fractionated over Sephadex LH-20. The fractions obtained were chromatographed by reversed-phase HPLC to yield four novel oxylipins (**1**−**4**).

The positive HRMALDITOFMS spectrum of **1** showed an [M + Na]$^+$ ion peak at *m*/*z* 351.2155, supporting the molecular formula



$C_{18}H_{32}O_5$ (calcd for $C_{18}H_{32}O_5Na$, 351.2147). Analysis of NMR data (Table 1) indicated a structural homology to (9*S*,10*S*,11*R*,12*Z*,15*Z*)-9,10,11-trihydroxyoctadeca-12,15-dienoic acid, a trihydroxyoxylipin whose configurational assignment has been recently clarified.[5]

The signal of the methyl group at δ 1.00 was downfield shifted, indicating thereby a proximity to the double bond of the chain, and a detailed analysis revealed the presence of an ω-3 acyl chain. The signals at δ 2.95−2.90 were ascribable to methylene protons located between two double bonds. Although the coupling constants of the olefinic protons were not clearly detectable, it was evident that the *J*-values for the two double bonds were in the 11 Hz region, characteristic of *Z* geometry. Moreover, the ^{1}H NMR spectrum displayed signals corresponding to the protons of three secondary carbinol functions (δ 4.53, 3.78, and 3.31), whose positions, confirmed by HMBC correlations, were identical to those of the known trihydroxyoxylipin.[5] Negative full and tandem mass spectrometric experiments allowed us to define the length of this fatty acid chain as a C-18 chain and to confirm the assigned double bonds and secondary alcoholic group positions, according to literature data.[6,7] In particular, upon collision-induced dissociation of the deprotonated oxylipin **1**, characteristic product ions at *m*/*z* 171, 201, 143, 199, and 231, unambiguously indicating the hydroxy group positions at C-9, -10, and -11, respectively, were formed.[6−8] Product ions resulting from formal loss of three molecules of hydrogen (H_2) were observed starting from ions at *m*/*z* 327, 309 and 291, respectively. Although it does not indicate the position of the hydroxy groups, this latter MS/MS pattern represents an important fragmentation route of deprotonated vicinal hydroxylated fatty acids in low-energy CID, especially since this loss is not observed for nonhydroxylated fatty acids under the same experimental conditions.[6] According to this trend, the introduction of a third vicinal hydroxy group in the alkyl chain, such as in compound **1**, makes

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**4** (Methanol-d_4)

	1		**2**		**3**		**4**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	178.0		179.4		179.4		178.0	
2	34.9	2.29 (t, 7.5)	36.3	2.27 (t, 7.3)	37.3	2.24 (t, 7.3)	35.0	2.29 (t, 7.3)
3	26.1	1.62 (m)	26.4	1.63 (m)	26.7	1.63 (m)	25.8	1.63 (quintet, 7.3)
4	30.4	1.37 (m)	30.5	1.37 (m)	30.3	1.37 (m)	30.3	1.43, 1.38 (m)
5	30.4	1.37 (m)	38.0	1.56, 1.50 (m)	38.2	1.57, 1.51 (m)	28.5	2.19, 2.14 (m)
6	30.4	1.37 (m)	73.1	4.07 (q, 6.5)	73.1	4.07 (q, 6.5)	135.1	5.62 (dt, 10.9, 7.3)
7	26.2	1.37 (m)	136.9	5.70 (dd, 15.3, 6.5)	136.7	5.72 (dd, 15.3, 6.5)	131.0	5.50 (ddt, 10.9, 8.9, 1.6)
8	33.8	1.57, 1.53 (m)	130.8	5.75 (dd, 15.3, 6.5)	131.1	5.69 (dd, 15.3, 6.5)	68.9	4.50 (dd, 8.9, 6.5)
9	71.4	3.78 (m)	76.5	3.95 (dd, 6.5, 4.4)	76.0	3.91 (t, 6.5)	76.8	3.30 (dd, 6.5, 3.2)
10	76.6	3.31 (dd, 6.5, 2.8)	75.5	3.52 (m)	75.6	3.43 (m)	71.5	3.76 (m)
11	68.7	4.53 (dd, 8.3, 6.9)	33.4	1.54, 1.39 (m)	33.6	1.55, 1.37 (m)	34.7	1.55 (m)
12	131.0	5.52 (m)	26.6	1.36 (m)	26.4	1.36 (m)	26.7	1.52, 1.38 (m)
13	132.7	5.57 (m)	30.1	1.37 (m)	30.4	1.37 (m)	30.3	1.38 (m)
14	28.0	2.95, 2.90 (m)	30.1	1.37 (m)	30.4	1.37 (m)	30.1	1.38 (m)
15	127.9	5.36 (m)	30.1	1.37 (m)	30.4	1.37 (m)	30.1	1.38 (m)
16	133.1	5.43 (m)	33.3	1.39 (m)	32.9	1.34 (m)	32.9	1.36 (m)
17	21.0	2.12 (quintet, 7.3)	23.9	1.36 (m)	23.4	1.36 (m)	23.7	1.36 (m)
18	14.0	1.00 (t, 7.7)	14.5	0.94 (t, 6.7)	14.1	0.94 (t, 6.7)	14.3	0.94 (t, 7.3)

the (OH)C−H-9, (OH)C−H-10 and (OH)C−H-11 bonds, and other bonds adjacent to them, sufficiently weak to undergo charge-remote homolytic cleavage.

Further NMR studies devoted to the relative configurational assignment of compound **1** were performed by using the PFG-HETLOC experiment, from which heteronuclear $^{2,3}J_{CH}$ coupling constants can be accurately extracted, and by applying the *J*-based method developed by Murata.[4] For the segment C-10−C-11, in the PFG-HETLOC the large heteronuclear coupling of $^{2}J_{H10-C11}$ −2.2 and $^{2}J_{H11-C10}$ −3.9 Hz suggested an *anti* configuration between H-10 and H-11, while an ROE effect between H-9 and H-12 was decisive and consistent with their *gauche* orientation. To establish the configuration of the C-9−C-10 segment, small values of $^{2}J_{CH}$ and of $^{3}J_{H-H}$ coupling constants allowed us to identify unequivocally the arrangement depicted in Table 2. Finally, ROE correlations between H-9 and H-10, H-10 and H-8, and H-9 and H-11 were fully consistent with this result.

Thus a 9*S**, 10*R**, 11*R** relative configuration was assessed, and the compound defined as the new (9*S**,10*R**,11*R**,12*Z*,15*Z*)-9,10,11-trihydroxyoctadeca-12,15-dienoic acid (**1**).

Compound **4** exhibited an $[M + Na]^+$ ion peak at *m*/*z* 353.2317 in the positive HRMALDITOFMS spectrum, corresponding to the molecular formula $C_{18}H_{34}O_5$. For compound **4** the signal of the terminal methyl group at δ 0.94 rather than at δ 1.00, as observed for **1** and for its diastereoisomer, suggested that more methylene groups separated the methyl protons from the unsaturated sites. As for compound **1**, along with the protons of three secondary alcoholic functions (δ 4.50, 3.76, and 3.30), the ^{1}H NMR spectrum displayed signals corresponding to two olefinic protons (δ 5.62 and 5.50), several methylene protons including the allylic protons at δ 2.19−2.14, and the protons at δ 2.29 and 1.63 located $\alpha-\beta$ to the carboxyl group, respectively. The *Z* configuration was assigned on the basis of the coupling constants exhibited by the olefinic protons and on the basis of the high-field chemical shifts of the allylic methylene carbons in the ^{13}C NMR spectrum.[9] The location of the hydroxy groups at C-8, C-9, and C-10 as well as the double-bond positions were established by HMBC correlations (see Supporting Information). As for compound **1**, negative ESIMS and ESIMS/MS profiles confirmed these allocations and the presence of a C-18 fatty acid, according to previous literature data.[6,7]

Concerning the relative configuration the C-8−C-9 segment, the observed 6.5 Hz value of $^{3}J_{8,9}$, along with the two large couplings of $^{2}J_{H8-C9}$ −3.3 and $^{2}J_{H9-C8}$ −2.7 Hz, suggested an *anti* orientation between H-8 and H-9. Furthermore, an ROE correlation between H-7 and H-10 supported a relative 8*R*, 9*R* configuration. For the C-9−C-10 fragment, a small $^{3}J_{H9-H10}$ of 3.2 Hz indicated a *gauche* relation between H-9 and H-10, while the $^{2}J_{H-C}$ coupling constant of −1.4 Hz between H-10 and C-9 and the $^{3}J_{H-C}$ coupling constant of 1.9 Hz between H-10 and C-8 and between H-9 and C-11 permitted definition of a 9*R**, 10*S** relative configuration (Table 2). Compound **4**, (8*R**,9*R**,10*S**,6*Z*)-trihydroxyoctadec-6-enoic acid, is new and possesses the same relative configuration as **1**.

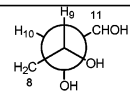
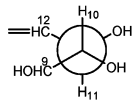
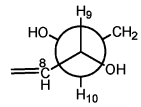
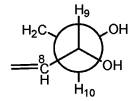
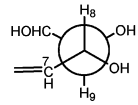
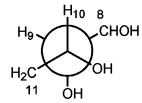
By NMR and MS analyses, the same basic structure was established for compounds **2** and **3**. The *E* geometry of the double bond was assigned on the basis of the large (15.3 Hz) coupling constant, while COSY and HMBC experiments allowed us to establish the position of the three protons of secondary alcoholic functions with respect to the double bond.

From the positive HRMALDITOFMS for both compounds the molecular formula $C_{18}H_{34}O_5$ was assigned, and the interpretation of ESIMS and ESIMS/MS spectra recorded by flow injection in the negative ion mode confirmed the NMR results and permitted identification of a C-18 fatty acid chain. In particular, the ESIMS/MS spectra showed fragmentation patterns in agreement with proposed mechanisms.[6−8] Noteworthy, both compounds produced two abundant fragment ions at *m*/*z* 229 and 211, originating from a different fragmentation mechanism, likely corresponding to loss of a pent-4-enoic acid and subsequent loss of water from the $[M - H]^-$ anion, respectively (see Supporting Information). The high abundance of the observed product ions could be explained considering that the presence of a double bond at C-7−C-8 contributed to enhance the stability of the formed anions by increasing the number of possible resonance structures.

^{1}H and ^{13}C NMR data of compound **3** (Table 1) were almost superimposable on those of compound **2**. Some minor differences were observed for the chemical shifts of H-9 (δ 3.90 in compound **3** vs 3.95 in compound **2**) and H-10 (δ 3.43 in compound **3** vs 3.52 in compound **2**). These differences could be due to configurational isomerism. A 6,9,10-trihydroxy-7(*Z*)-octadec-7-enoic acid, sanleng acid, has been found in a common herb, *Sparganium stoloniferum*, and partly identified.[10] The large value of $^{3}J_{H-H}$ and the pattern of $^{2,3}J_{C-H}$ couplings ruled out all the 3D arrangements with H-9 and H-10 in a *gauche* relationship, leaving only two H-9−H-10 *anti* conformers with opposite relative configurations. Corroboration of the configurational assignment was obtained by analysis of the ROE cross-peaks. In particular, the presence of a cross-peak between H-8 and H-11 observed in the ROESY spectrum of compound **3** and the absence in the spectrum of compound **2** were consistent with the configurational hypothesis. Thus, a 9*R**, 10*R** relative configuration was established for the segment C-9−C-10 of compound **3**, and a 9*R**, 10*S** relative configuration for compound **2**.

In order to assess the potential immunostimulatory effect of the *n*-butanol extract, Sephadex fractions 16−18, and isolated com-

Table 2. Dominant Rotamers of Compounds **1**, **2**, **3**, and **4** Side Chain C_2-Fragments along with Their Relative Configurations

fragment	segment	$^3J_{HH}$ (Hz)	$^{2,3}J_{HC}$ (Hz)	ROE [a]
		1		
C-9-C-10		2.8	$^2J_{H_9\text{-}C_{10}}$ small	H-8-H-10 (m)
			$^2J_{H_{10}\text{-}C_9}$ small	H-9-H-11 (m)
C-10-C-11		6.9	$^2J_{H_{10}\text{-}C_{11}}$ -2.2	H-9-H-12 (m)
			$^3J_{H_{10}\text{-}C_{12}}$ 2.5	
			$^2J_{H_{11}\text{-}C_{10}}$ -3.9	
		2		
C-9-C-10		6.5	$^3J_{H_9\text{-}C_{11}}$ small	-
			$^2J_{H_9\text{-}C_{10}}$ -3.1	
			$^2J_{H_{10}\text{-}C_9}$ -2.5	
		3		
C-9-C-10		6.5	$^3J_{H_9\text{-}C_{11}}$ small	H-8-H-11 (m)
			$^2J_{H_9\text{-}C_{10}}$ -2.9	
			$^2J_{H_{10}\text{-}C_9}$ -1.9	
		4		
C-8-C-9		6.6	$^3J_{H_9\text{-}C_7}$ 2.3	H-10-H-7 (m)
			$^2J_{H_9\text{-}C_8}$ -2.7	
			$^2J_{H_8\text{-}C_9}$ -3.3	
C-9-C-10		3.2	$^3J_{H_9\text{-}C_{11}}$ 1.9	
			$^3J_{H_{10}\text{-}C_8}$ 1.9	
			$^2J_{H_{10}\text{-}C_9}$ -1.4	

[a] ROESY contacts are classified as strong (s), medium (m), and weak (w) effects.

pounds (**1**−**4**), human PBMCs were used to perform a proliferation assay by ^{3}H-thymidine incorporation. An efficient and significant cell activation was obtained by Sephadex fractions 16−18 and the *n*-butanol extract at 10 μg/mL and by compound **2** at 10 μM. At the highest concentration used (100 μg/mL for the Sephadex fractions 16−18 and the *n*-butanol extract and 100 μM for compounds **1**−**4**), all the samples seemed to exert a toxic effect on these cells, since a reduced cell proliferation was observed compared with the control, nontreated PBMCs. To evaluate the response of these cells to mitogens, control OKT3-activated PBMCs (counts per minute (cpm) = 82 644, not shown) were used in all experiments. An independent experiment repeated in triplicate is shown in Figure 1, and the statistical significance is reported in the graphs (*p < 0.05 in the Student's t test). Compound **2** was the only compound exhibiting activity on cell proliferation. It is noteworthy that compound **2** differs from compound **3** only by the configuration at C-10, thus emphasizing the crucial role of this carbon for the activity. On the other hand, the *n*-butanol extract and the Sephadex fractions 16−18, preliminarly analyzed by ESI-ITMS^n, were shown to also contain ceramides and cerebrosides, which could also be responsible for the activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 1000 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-600 spectrometer equipped with a Bruker 5 mm TCI cryoprobe; chemical shifts were referenced to the residual solvent signal (methanol-d_4, δ_H = 3.34, δ_C = 49.0). The ^{1}H, gCOSY, ROESY, gHSQC, and gHMBC NMR experiments were run under standard conditions at 300 K. The ROESY spectra were executed with a mixing time of 400 ms. For **1**, **3**, and **4**, $^{2,3}J_{C-H}$ values were obtained from phase-sensitive PFG-HETLOC spectra with 16 scans/t_1, acquiring 4K points in ω_2, and with a t_{1max} value of 38.0 ms, while for **2** 96 scans/t_1 were acquired with 4K points in ω_2 and with a t_{1max} of 28.8 ms.[11] A spin lock pulse of 50 ms was used. Exact masses were measured by a Voyager DE mass spectrometer equipped with a 337 nm laser and delay extraction and operated in positive ion reflector mode. Samples were analyzed by MALDITOF mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from angiotensin III at 931.5154 Da and α-cyano-4-hydroxycinnamic acid at 190.0504 Da as internal standard. ESIMS analysis in negative ion mode was performed using a ThermoFinnigan LCQ Deca ion-trap mass spectrometer, and the mass spectra were acquired and processed using the software provided by

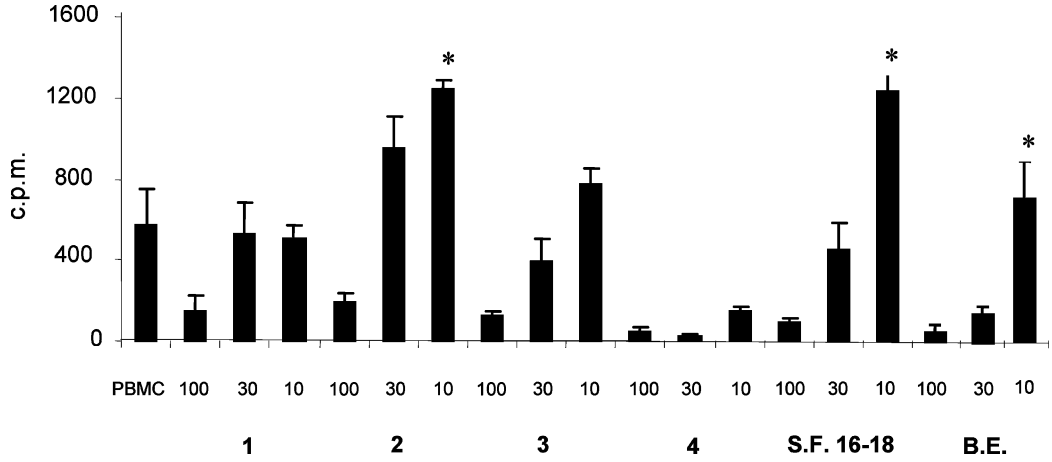


Figure 1. Proliferation assay by ^{3}H-thymidine incorporation of the *n*-butanol extract (B.E.), Sephadex fractions 16−18 (S.F. 16−18), and compounds **1**−**4**. PBMCs (10^5 cells/well) were treated with the samples at μM concentrations for compounds **1**−**4** and at μg/mL concentrations for B.E. and S.F. 16−18. Cells were cultured in triplicate for 48 h. Proliferation was measured after 18 h of ^{3}H-thymidine incorporation (1 μCi). The counts per minutes (cpm) of all the compounds are shown in the histograms with ±SD. The statistical significance is indicated (*$p < 0.05$ calculated with respect to the nontreated cells).

the manufacturer. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow rate was 5 μL/min. The capillary voltage was set at −4.0 V, the spray voltage at 5 kV, and the tube lens offset at 20 V. The capillary temperature was 280 °C. Data were acquired in MS^1 and MS^n scanning modes. Column chromatography was performed over Sephadex LH-20 (Pharmacia). HPLC separations were carried out on a Waters System, equipped with a refractive index detector, a μ-Bondapak C_{18} column (300 × 7.6 mm i.d., 10 μm), and a Rheodyne injector. TLC was performed on silica gel F254 (Merck) plates.

Reagents and Materials. All solvents for chromatographic separation were of analytical grade from Carlo Erba (Rodano, Italy). HPLC grade MeOH was purchased from J.T. Baker (Baker Mallinckrodt, Phillipsburg, NJ). HPLC grade water (18 mΩ) was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA).

Plant Material. *D. loretense* corms were collected in Iquitos, Peru, in May 2005. The plant material was identified by the biologist Elsa Rengifo (Instituto de Investigaciones de la Amazonía Peruana (IIAP), Iquitos, Perú). A voucher specimen (no. 8662) has been deposited in the Herbarium of the IIAP.

Extraction and Isolation. Dried and powdered corms (800 g) of *D. loretense* were extracted for a week, three times, at room temperature using solvents of increasing polarity, namely, petroleum ether 1.5 L, $CHCl_3$ 1.5 L, and MeOH 1.5 L. The solutions were evaporated to dryness in vacuo to give 0.4, 1.3, and 57.6 g of crude extract, respectively. The MeOH extract was partitioned between H_2O and *n*-BuOH (1:1), yielding 6.3 g of *n*-butanol extract. Part of this extract (3 g) was fractionated on a Sephadex LH-20 column (100 × 5 cm) using MeOH as the mobile phase. Seventy fractions (8 mL each) were obtained. Each fraction was isocratically analyzed by HPLC using MeOH/H_2O mixtures as eluents (flow rate of 2 mL/min).

Fractions 16−18 (1 g), eluted with MeOH/H_2O (3:2), yielded four new compounds structurally related to oxylipin: compound **1** (2.4 mg t_R = 20 min), compound **2** (1.0 mg, t_R = 22 min), compound **3** (1.1 mg, t_R = 24 min), and compound **4** (3.3 mg, t_R = 29 min).

Compound 1: white, amorphous powder; $[\alpha]_D^{25}$ −14.6 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS *m/z* 327.1 $[M - H]^-$; ESIMS/MS (collision energy 32%) *m/z* 327.1 (5), 324.9 (0.1), 309.0 (100), 307.3 (0.2), 291.1 (23), 289.4 (0.1), 273.1 (7), 269.1 (0.2), 252.9 (0.1), 247.1 (0.3), 239.3 (0.1), 231.0 (2), 227.1 (0.1), 213.1 (7), 211.1 (0.2), 201.1 (21), 199.1 (0.3), 185.1 (0.2), 183.1 (0.1), 171.1 (30), 165.1 (0.2), 155.0 (0.1), 153.1 (0.1), 142.9 (0.1), 137.0 (2); HRMALDITOFMS $[M + Na]^+$ *m/z* 351.2155 (calcd for $C_{18}H_{32}O_5Na$, 351.2147).

Compound 2: white, amorphous powder; $[\alpha]_D^{25}$ −10.7 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS *m/z* 329.2 $[M - H]^-$; ESIMS/MS (collision energy 38%) *m/z* 329.2 (5), 327.1 (0.7), 311.2 (71), 309.2 (18), 293.2 (29), 291.1 (6), 285.2 (0.6), 275.2 (0.3), 265.2 (0.7), 257.3 (0.1), 249.2 (0.7), 232.9 (0.1), 229.2 (100), 227.2 (1.5), 225.1 (0.2), 215.2 (0.1), 213.2 (3.6), 211.2 (47), 201.3 (6), 199.3 (1.5), 193.2 (2.3), 185.2 (2), 173.1 (0.8), 171.2 (61), 169.1 (0.4), 157.1 (5), 155.2 (1.7), 153.1 (1), 141.3 (0.3), 139.2 (3.4), 129.1 (0.2), 127.1 (2), 125.1 (2); HRMALDITOFMS $[M + Na]^+$ *m/z* 353.2315 (calcd for $C_{18}H_{34}O_5Na$, 353.2304).

Compound 3: white, amorphous powder; $[\alpha]_D^{25}$ −32.6 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS *m/z* 329.2 $[M - H]^-$; ESIMS/MS (collision energy 35%) *m/z* 329.2 (65), 327.1 (11), 311.3 (81), 309.1 (27), 293.3 (18), 291.1 (0.5), 275.2 (0.1), 265.1 (0.1), 257.3 (0.1), 249.1 (1), 232.9 (0.1), 229.1 (100), 227.1 (3), 215.0 (0.1), 213.0 (1.4), 211.3 (29), 201.3 (3.5), 199.3 (1), 185.3 (1), 173.1 (0.2), 171.3 (66), 169.1 (0.1), 157.2 (8), 155.2 (3), 153.1 (0.1), 141.1 (1.7), 139.1 (3.5), 129.1 (0.7), 127.1 (5.5), 125.3 (3.5); HRMALDITOFMS $[M + Na]^+$ *m/z* 353.2313 (calcd for $C_{18}H_{34}O_5Na$, 353.2304).

Compound 4: white, amorphous powder; $[\alpha]_D^{25}$ −17.4 (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; ESIMS *m/z* 329.2 $[M - H]^-$; ESIMS/MS (collision energy 35%) *m/z* 329.2 (6), 327.2 (0.1), 311.1 (100), 309.2 (1.6), 293.1 (24), 291.1 (0.5), 275.3 (6), 265.3 (0.2), 257.4 (0.1), 247.1 (0.2), 231.2 (0.5), 229.1 (1.7), 227.1 (1.7), 215.3 (0.2), 213.2 (2), 211.3 (7.5), 209.1 (0.3), 201.3 (28), 199.3 (5), 197.2 (1), 185.3 (0.5), 183.1 (0.5), 181.0 (1), 171.3 (19), 169.1 (1.4), 165.3 (0.3), 157.3 (0.1), 155.3 (0.6), 139.2 (0.8), 128.8 (0.7), 127.1 (0.2), 125.3 (0.3), 120.9 (0.2); HRMALDITOFMS $[M + Na]^+$ *m/z* 353.2317 (calcd for $C_{18}H_{34}O_5Na$, 353.2304).

Biological Assays. Isolation of Human Peripheral Blood Mononuclear Cells (PBMCs). PBMCs derived from buffy coats of healthy volunteers were isolated by density gradient centrifugation over Lymphoprep. The cells were washed three times with PBS and resuspended in culture medium RPMI, 10% heat-inactivated fetal calf serum (FCS). All assays were performed in RPMI 1640 supplemented with penicillin/streptomycin, 2 mM L-glutamine, and 10% heat-inactivated FCS.[12,13]

Proliferation Assays on Human PBMCs. Isolated PBMCs (10^5 cells/well) were cultured in triplicate in round-bottomed 96-well plates in a final volume of 200 μL of RPMI 10% FCS. Cells were cultured in the presence of extracts, dissolved in MeOH to achieve final concentrations of 100, 30, and 10 μM for compounds **1**−**4** and 100, 30, and 10 μg/mL for B.E. and S.F. 16−18. The vehicle was used as control in all experiments. Cells were incubated 48 h at 37 °C, pulsed with 1 μCi of ^{3}H-thymidine, and incubated an additional 18 h before the cultures were processed for scintillation counting. After the incubation, cultures were harvested onto fiberglass filters to capture the labeled DNA. After drying, the scintillation fluid was added to the filters, which were sealed and counted in a scintillation counter to detect radioactivity measured as ^{3}H-thymidine incorporation. The counts per minute obtained provided the counting results for cell proliferation.

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Supporting Information Available: Spectroscopic data for **1**, **2**, **3**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Brack Egg, A. *Diccionario Enciclopedico de Plantas utiles del Perú*; Centro de Estudios Regionales Andinos Bartolome de las Casas: Cuzco, Peru, 1999; p 187.
(2) Miyake, M.; Sasaki, K.; Ide, K.; Matsukura, Y.; Shijima, K.; Fujiwara, D. *J. Immunol.* **2006**, *176*, 5797–5804.
(3) Lovera, A.; Bonilla, C.; Hidalgo, J. *Rev. Perú Med. Exp. Salud Publica* **2006**, *23*, 177–181.
(4) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
(5) de Marino, S.; Borbone, N.; Gala, F.; Zollo, F.; Fico, G.; Paliotti, R.; Iorizzi, M. *J. Agric. Food Chem.* **2006**, *54*, 7508–7516.
(6) Moe, M. K.; Strom, M. B.; Jensen, E.; Claeys, M. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 1731–1740.
(7) Moe, M. K.; Anderssen, T.; Strom, M. B.; Jensen, E. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 46–59.
(8) Moe, M. K.; Anderssen, T.; Strom, M. B.; Jensen, E. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2121–2130.
(9) Gunstone, F. D.; Pollard, M. R.; Scrimgeour, C. M.; Vedanayagam, H. S. *Chem. Phys. Lipids* **1977**, *18*, 115–129.
(10) Zhang, W.; Xiao, K.; Yang, G.; Chen, H. *Zhongcaoyao* **1995**, *26*, 125–126.
(11) Uhrín, D.; Batta, G.; Hruby, V. J.; Barlow, P. N.; Kövér, K. E. *J. Magn. Reson.* **1998**, *130*, 155–161.
(12) Malfitano, A. M.; Laezza, C.; Pisanti, S.; Gazzerro, P.; Bifulco, M. *Br. J. Pharmacol.* **2008**, *153*, 1003–1010.
(13) Malfitano, A. M.; Cahill, R.; Mitchell, P.; Frankel, G.; Dougan, G.; Bifulco, M.; Lombardi, G.; Lechler, R. I.; Bamford, K. B. *Helicobacter* **2006**, *11*, 21–30.

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