Full Papers

New Acetylenes Isolated from the Bark of *Heisteria acuminata*

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Five new linear acetylenic compounds, namely, pentadeca-6,8,10-triynoic acid (**1**), octadeca-8,10,12-triynoic acid (**2**), *trans*-pentadec-10-en-6,8-diynoic acid (**3**), *cis*-hexadec-11-en-7,9-diynoic acid (**4**), and *cis*-octadec-12-en-7,9-diynoic acid (**5**), were isolated from the bark of *Heisteria acuminata* by bioassay-guided fractionation, using cyclooxygenase (COX) and 5-lipoxygenase (5-LO) assays as models for antiinflammatory activity. The structures of compounds **1**−**5** were established by NMR, MS, IR, and Raman spectroscopy. These isolated compounds were found to be potent inhibitors of COX. Compounds **4** and **5** were the most potent inhibitors of 5-LO, whereas the other compounds only showed a weak inhibition at the same concentration.

The bark of *Heisteria acuminata* (Humb. & Bonpl.) Engl. (Olacaceae) is used in Ecuadorian traditional medicine to treat toothache, rheumatism, wounds, and swellings (Ghia, F. Personal communication, 1992).[1] Other *Heisteria* species are used by South American Indians for the treatment of rheumatism, abscesses, headache, throat infections, swellings, nose bleeds, and pain in the joints and muscles.[1−6] In the Congo, *H. parvifolia* Sm. and *H. zimmereri* Engl. are used for the treatment of eye disorders, migraine, asthma, gynecological problems, pains, and convulsions.[7] Two varieties of *H. acuminata* (Santo Domingo and Oriente) have been investigated for antiinflammatory activity in the in vivo rat-paw edema model, and one of the extracts showed a moderate inhibition of carrageenan-induced edema.[8] It has also been proven to have an antiphlogistic effect after external application in patients with rheumatism and arthritis.[9] In the literature, *H. latifolia* Standl. has been reported to be psychoactive.[10]

From various *Heisteria* species scopolamine, triterpene quinone methides (pristimerin, netzahualcoyen, tingenone, and 22-hydroxytingenone), ourateacatechin, ourateaproanthocyanidin A, (−)-epiafzelechin-(4β→8)-(−)-epiafzelechin-(4β→8)-4′-*O*-methyl-(−)-epigallocatechin, and fatty acids have been isolated.[10−14] The triterpene quinone methides were proven to be strong inhibitors of 5-lipoxygenase (5-LO) and lymphocyte transformation, whereas ourateacatechin and the proanthocyanidins exerted inhibitory effects on cyclooxygenase (COX).[14]

In an initial screening of medicinal plants from Ecuador, the effects of crude plant extracts on the biosynthesis of inflammatory mediators were investigated, and a petroleum ether extract from the bark of *H. acuminata* inhibited COX by 75% at 50 μg/mL and 5-LO by 32% at 390 μg/mL in vitro. The aim of this study was to isolate and characterize the principles responsible for the observed antiinflammatory activity in vitro.

Results and Discussion

Bioassay-guided fractionation using a combination of liquid−liquid extraction followed by chromatography on Si gel and liquid partition chromatography on Sephadex LH-20[15] provided an active fraction containing mainly acetylenic compounds (COX inhibition in vitro: 88% at 250 μg/mL and 79% at 50 μg/mL, respectively). After fractionation on a Lobar RP-18 column, compound **2** (7 mg) was isolated, whereas the other compounds needed further purification using semipreparative reversed-phase HPLC. Thus, acetylenes **1** (3 mg), **3** (7 mg), **4** (7 mg), and **5** (7 mg) were also obtained.

HREIMS indicated for **1** an elemental composition of $C_{15}H_{18}O_2$ (found *m/z* [M^+] 230.13298, calcd 230.13068), indicating the presence of a carboxylic acid function. ^{1}H and ^{13}C NMR spectra of this compound taken in $CDCl_3$ and CD_3OD solutions could be interpreted in terms of two independent aliphatic spin systems separated by three conjugated triple bonds, with three pairs of singlet lines observed between 60 and 80 ppm in the ^{13}C NMR spectrum (Table 2). For **1,** all ^{1}H−^{1}H correlations could be deduced from the COSY spectrum. Knowing the ^{1}H NMR connectivities, we could assign the protonated carbons by a gradient-enhanced HMQC method. However, because of the dilute solution, we could not assign the lines of the conjugated triple bonds. Based on the literature[16−20] we suggest a parabolic dependence of these chemical shifts along the chain (Tables 1 and 2).

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Table 1. ^{1}H-NMR Data of Compounds **1**−**5** Determined at 500 MHz and Room Temperature in CD_3OD[a,b]

position	**1**	**2**	**3**	**4**	**5**
1	2.27 t 7.5				
2	1.68 (2H) quint 7.5	2.25 (2H) t 7.4	2.31 (2H) t 7.5	2.29[c] (2H) t 7.3	2.28 (2H) t 7.4
3	1.56 (2H) quint 7.5	1.58 (2H) quint 7.3	1.70 (2H) quint 7.3	1.63 (2H) quint 7.6	1.61 (2H) quint 7.3
4	2.23 (2H) t 7.4	1.32 (2H) quint	1.57 (2H) quint	1.55[d] (2H) quint	1.35 (2H) quint
5		1.40 (2H) quint	2.35 (2H) td 7.1[e]	1.55[d] (2H) quint	1.42 (2H) quint
6		2.29 (2H) t 7.3		2.35 (2H) td 7.0; 0.7[f]	1.31 (2H) quint
7					2.31 (2H) td 7.4; 0.6[f]
8					
9					
10			5.51 dt 15.8; 1.4[g]		
11	2.29 (2H) t 6.6		6.25 dt 15.8; 7.2	5.48 dt 10.7; 1.1[g]	
12	1.50 (2H) quint 7.6		2.13 (2H) qd 7.2; 1.4[g]	6.07 dt 10.8; 7.5	5.42 dt 10.9; 1.2[g]
13	1.40 (2H) hex 7.6	2.28 (2H) t 7.1	1.14 (2H) quint	2.29[c] (2H) qd 7.5; 1.1[g]	6.07 td 10.9; 7.6
14	0.91 (3H) t 7.3	1.50 (2H) quint	1.33 (2H) hex	1.39 (2H) quint	2.34 (2H) qd 6.9; 1.2[g]
15		1.49 (2H) quint	0.91 (3H) t 7.2	1.36 (2H) hex	1.41 (2H) quint
16		1.40 (2H) quint		0.93 (3H) t 7.2	1.39 (2H) quint
17		1.41 (2H) hex			1.36 (2H) hex
18	ca. 4.9	0.90 (3H) t 7.5			0.93 (3H) t 7.2
OH		no[h]	no	no	ca. 3.8

[a] After the multiplicities coupling constants are reported (Hz). [b] ^{1}H−^{13}C connectivities were determined by g HMQC method. [c,d] Assignments may be interchanged. [e] $^{4}J_{HH}$ present but not distinguishable. [f] $^{7}J_{HH}$. [g] $^{4}J_{HH}$. [h] no: not observed.

Table 2. ^{13}C-NMR Data of Compounds **1**−**5** Determined at 125 MHz and Room Temperature in $CDCl_3$

position	**1**	**2**	**3**	**4**	**5**
1	no[a]	no	178.0 s	no	no
2	no	no	33.8 t	34.0 t	no
3	23.8 t	no	23.8 t	24.3 t	24.8 t
4	27.4 t	28.7 t	27.6 t	28.4 t	29.0 t
5	19.1 t	28.8 t	19.3 t	28.0 t	28.8 t
6	78.3[b] s	28.1 t	65.8 s	19.5 t	28.7 t
7	60.2[c] s	19.2[b] t	72.7[b] s	65.5 s	19.7 s
8	60.6[c] s	79.2[c] s	74.3[b] s	72.3[b] s	65.3 s
9	65.6[c] s	60.4[d] s	82.6 s	78.2[b] s	72.2[b] s
10	66.2[c] s	60.5[d] s	108.5 d	84.3 s	78.2[b] s
11	79.5[b] s	65.8[d] s	148.4 d	108.1 d	84.7 d
12	19.1 t	65.9[d] s	33.3 t	147.8 d	108.2 d
13	30.1 t	79.4[c] d	30.7 t	31.3 t	147.9 t
14	21.9 t	19.4[b] d	22.1 t	30.4 t	31.0 t
15	13.5 q	29.8 t	13.8 q	22.3 t	28.3 t
16		30.2 t		13.9 q	30.5 t
17		22.0 t			22.3 t
18		13.6 q			14.0 t

[a] no: not observed. [b,c,d] Assignments may be interchanged.

Because of an unfavorable signal:noise ratio, the carboxylic carbon and the carbon in the α position to the carboxylic function were not observed directly in the ^{13}C NMR spectrum of **1**; however, when comparing the chemical shifts of the carbon in the β position to the suggested carboxylic function, and the ^{1}H NMR shifts for protons in the α and β positions to those published for various polyacetylenes, the presence of a carboxylic acid function was corroborated. [Reported values: δ_c C_α 34 ppm and C_β 24 ppm,[17, 21−23] δ_H H_α 2.37 t (6.9) and H_β 1.55 br t (7.49)].[24]

The ^{1}H NMR spectra of **1** recorded in $CDCl_3$ solution showed a clear pattern with two different, flexible aliphatic spin systems, one giving rise to broader peaks than the other. Resonances at the carboxylic acid end of the molecule were broader than those at the other end, both in the ^{13}C NMR and in the ^{1}H NMR spectrum. The broadened line increased regularly when going toward the carboxylic acid terminus. These findings can be interpreted by an association process in $CHCl_3$ solution, thus making the inner part of the chain (containing the carboxylic acid functions) move slower than the outer (pure aliphatic, nonassociating) portions. Similar phenomena have been observed in the associa-

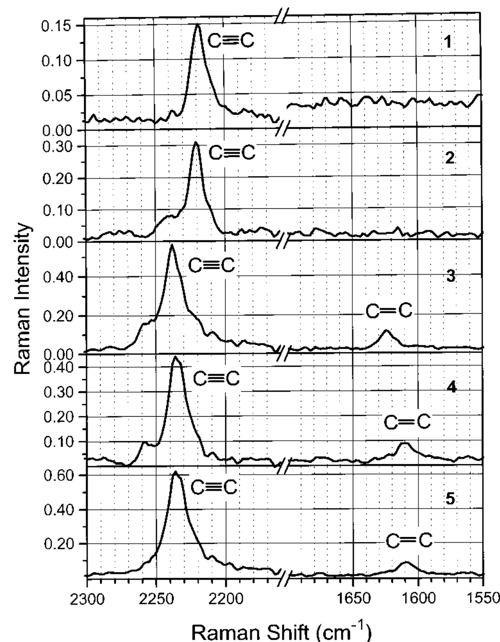


Figure 1. Raman shifts ($\nu_{C\equiv C}$ and $\nu_{C=C}$ regions) of **1**−**5** in CD_3-OD.

tion of phytol[25] and in a complex formed from benzylated sugars with cyclodextrin.[26] In both cases relaxation gradients[27] can be used to describe the mobility of carbon atoms in different locations of the same molecule. The dimerization may cause even stronger broadening of the $-CH_2COOH$ carbon signals. These could not be observed in the ^{13}C NMR spectrum of **1** because of the limited signal:noise ratio (at chemical shifts about 35 and 178 ppm).

In the solid-state IR spectrum of **1**, the existence of the terminal carboxylic acid function could be deduced from an absorption around 1700 cm^{-1} assigned to the carboxylic function forming a carboxyl dimer that is also indicated by a broad band between 2400 and 3700 cm^{-1} and the out-of-plane vibration band around 942 cm^{-1}. The presence of triple bonds in the molecule of **1** was also seen as an absorption band at 2216 cm^{-1}. The solid-state Raman spectrum of **1** (Figure 1) provided additional proof for triple bonds showing a characteristic

Raman shift at 2218 cm^{-1}. The UV spectrum recorded on-line during isolation had a λ_{max} at 210 nm and also contained very weak additional absorption maxima between 250 and 310 nm, which corresponded to maxima reported in the literature as being characteristic for polyacetylenes containing three conjugated triple bonds, that is, maxima at 210, 255, 269, and 286 nm.[28] Thus, the structure of **1** could be elucidated as pentadeca-6,8,10-triynoic acid.

For compound **2**, HREIMS indicated an elemental composition of $C_{18}H_{24}O_2$ (found *m*/*z* [M^+] 272.18421, calcd 272.17763). In the IR spectrum of **2** only one band was seen in the triple-bond region at 2217 cm^{-1}, and the Raman spectrum (Figure 1) also showed the characteristic Raman shift for triple bonds at 2220 cm^{-1}. The UV spectrum recorded on-line was almost superimposable on that of **1**, suggesting a conjugated arrangement of three triple bonds.

Because the NMR spectra of compound **2** showed very similar structural features to **1** with regard to the triple bonds and relating to **5** in the aliphatic regions, we could assign the structure of **2** as octadeca-8,10,12-triynoic acid by analogy to compounds **1** and **5** and by comparison with literature.[18] The NMR data for **2** are compiled in Tables 1 and 2.

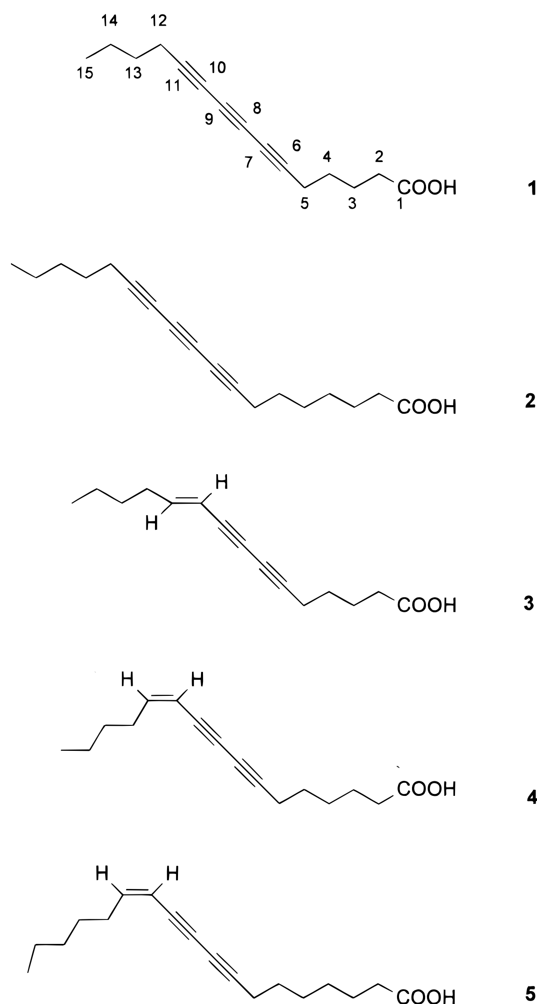
The on-line recorded UV spectra suggested compounds **3**−**5** were diacetylenes containing conjugated arrangements of two triple bonds and a double bond, that is, 210, 240, 254, 268, and 280 nm (values reported in the literature: λ_{max} 210, 238, 251, 264, and 280 nm).

The IR spectra of all three compounds exhibited absorption bands characteristic of a carboxylic acid function, as previously mentioned for **1**, as well as absorption bands of triple bonds between 2200 and 2270 cm^{-1}. In **3**−**5** these bands were shifted to 2233−2234 cm^{-1}, and in **3** and **4** they were also accompanied by an additional, weaker band at 2251−2252 cm^{-1}. Raman spectra in CD_3OD (Figure 1) provided additional proof for the presence of triple bonds in all three compounds (stretching frequencies observed: **3** at 2238 cm^{-1}, **4** at 2236 cm^{-1}, and **5** at 2236 cm^{-1}). For compounds **3**−**5** well-defined Raman shifts at 1624, 1609, and 1609 cm^{-1} ($\nu_{C=C}$), respectively, supported the occurrence of conjugated arrangements of triple and double bonds.

The MS recorded on-line during analytical HPLC exhibited *m*/*z* [M^+] 232 for **3** and *m*/*z* [M^+] 246 for **4**. From HREIMS the elemental composition of $C_{18}H_{26}O_2$ was deduced for compound **5** (found *m*/*z* [M^+] 274.18756, calcd 274.18756). The collected spectroscopic and spectrometric data thus indicated that compounds **3**−**5** were acetylene homologues.

^1H and ^{13}C NMR spectroscopy confirmed that compounds **3**−**5** consisted of two aliphatic spin systems separated by a conjugated arrangement of two triple bonds and a double bond. Coupling constants of olefinic protons indicated the conformation of **3** to be trans (15.8 Hz) and cis in diacetylenes **4** and **5** (10.8 and 10.9 Hz, respectively).

In the ^{13}C NMR spectrum of **3** the two triple bonds resulted in four resonances between 65 and 84 ppm, and the double bond at 108.5 and 148.4 ppm (Table 2). The carboxylic carbon resonance (expected around 178 ppm) could only be seen in the ^{13}C NMR spectrum of **3**; however, when comparing the chemical shifts of protons



and carbons in the α and β positions to the carboxylic acid function, the presence of a carboxylic function in all compounds **1**−**5** could be corroborated.

Although all $^1H-^1H$ NMR correlations for **1** could be deduced from the COSY spectrum, this was not possible for **3**−**5** due to the overlapping in the spectra. Therefore, a selective 1D-TOCSY[29] experiment was carried in order to select only those methylene groups that form a coupled spin system with the methyl group. This methyl group was irradiated (at 86 dB, 100 ms duration) with a spin-lock enabling magnetization transfer as far as the double bond. Thus, these compounds were identified as *trans*-pentadec-10-en-6,8-diynoic acid (**3**), *cis*-hexadec-11-en-7,9-diynoic acid (**4**), and *cis*-octadec-12-en-7,9-diynoic acid (**5**).

A diacetylene structurally related to **5**, octadec-13-en-9,11-diynoic acid, has been isolated previously from *Ongokea gore* (Hua) Pierre and from *Ximenia americana* L., two members of the Olacaceae.[30] The information obtained from the 1D-TOCSY NMR spectrum showed that **5** is structurally different from this acetylene. We have found no reports on isolation of compounds structurally related to **1** and **2** from the Olacaceae. A polyacetylenic acid containing four conjugated triple bonds has been reported from *Minquartia guianensis* Aubl. (Olacaceae).[24] Most natural fatty acids have cis configuration and, because acetylenes are biosynthesized from fatty acids by further unsaturation, the trans configuration of **3** is unusual. Hence, one can speculate

whether it is a natural compound or an artifact of isolation and/or purification procedures. An isomerization of a cis double bond as well as a partial reduction of the 10,11-triple bond of **1**, would result in an artifact identical to **1**. The on-line UV spectrum as well as the retention time of purified **3** are, however, identical to those observed before and during fractionation.

During isolation, despite the applied protecting procedures, valuable material was lost as a consequence of breakdown due to the highly unstable nature of compounds **1**−**5**. When isolated as pure compounds, **1** and **2** were very unstable, easily deteriorating after exposure to air or light, forming insoluble blue compounds. This type of reaction has been reported in the literature as occurring for other highly unsaturated polyacetylenes.[28,31]

The isolated compounds were found to be potent inhibitors of COX (inhibition at 13 μM: **1** 55%, **2** 45%, **3** 56%, **4** 51%, and **5** 56%, inhibition at 6.5 μM: **1** 36%, **2** 33%, **3** 44%, **4** 36%, and **5** 40%). Compounds **4** and **5** were the most potent inhibitors of 5-LO, with 45% and 55% inhibition at 5 μM, whereas the other compounds showed only weak inhibition at the same concentration (**1** 18%, **2** 21%, and **3** 14%). Polyacetylenes have previously been reported in the literature to be potent inhibitors of arachidonic acid metabolism.[32−36] Therefore it may be inferred that compounds **1**−**5** are, at least in part, responsible for the antiinflammatory activity of folklore preparations of *H. acuminata* bark.

Experimental Section

General Experimental Procedures. IR spectra were recorded with a Nicolet Magna 750 FT-IR spectrometer as KBr micro pellets (2-mm diameter) in the 4000−400 cm^{-1} range. The resolution was 4 cm^{-1}. Raman spectra were recorded using a Nicolet System 950 FT-Raman spectrometer equipped with a Nd:YAG laser. Spectra were measured in the 4000−100 cm^{-1} range under 4 cm^{-1} resolution. The Raman spectrum of compound **1** was taken in the solid state (with the KBr micro pellet used for the IR spectrum). Other Raman spectra (compounds **2**−**5**) were measured in CD_3OD solution. The Raman spectrum of the solvent (CD_3OD) was subtracted from the spectra of the solutions. In addition, the Raman spectra of **2**−**5** were also examined in $CHCl_3$.

All homonuclear and heteronuclear, 1D and 2D NMR spectra were recorded on a Bruker DRX-500 spectrometer at room temperature using standard pulse programs of the Bruker library. Chemical shifts are given in parts per million referred to TMS (i). ^1H and gradient-enhanced ^1H−^1H correlation (gCOSY) NMR[29] spectra were taken both in $CDCl_3$ and CD_3OD. Selective 1D TOCSY experiments were performed in CD_3OD solution. ^{13}C NMR spectra, including APT and gradient-enhanced HMQC (gHMQC) data, were measured in $CDCl_3$. Impurities at 1.28 ppm (hexane) and 2.01 ppm (Me_2CO) were present in all compounds. HREIMS spectra were recorded on an AEI MS-902 double-focusing, direct-inlet MS spectrometer (EI, 70 eV).

The experimental setup used for LC/MS was as follows: HPLC configuration, 600MS (Waters) solvent delivery system, on-line UV HP 1050 photodiode array detector (Hewlett-Packard) and 590MS (Waters) pump for postcolumn addition of buffer. MS detection was carried out on a Finnigan MAT TSQ 700 triple-stage quadrupole instrument equipped with a thermospray 2 (Finnigan MAT) interface. Spectra (350−500 amu) were recorded every 2 s. HPLC conditions for LC/MS: column, NovaPak (Waters) C_{18} 4 μm 300 × 3.9 mm i.d.

Gradients systems used (1 mL/min): A $MeCN−H_2O$ (0.05% TFA) [65:35→80:20 (15 min) →100:0 (10 min) →100:0 (10 min)]; B $MeCN−H_2O$ (0.05% TFA) [50:50→90:10 (60 min) →100:0 (10 min)]. The LC−UV traces were recorded at 210 and 254 nm. The UV spectra were recorded from 200 to 500 nm. To obtain satisfactory thermospray (TSP) ionization, ammonium acetate buffer (0.5 M, 0.2 mL/min) was added postcolumn. The TSP MS was operated in the positive-ion mode with the filament off. The optimal TSP conditions were: source 280 °C, vaporizer 95 °C, aerosol 306 °C, repeller 50 V. UV spectra for all compounds were recorded on-line during analytical HPLC and LC/MS.

Analytical TLC was carried out on precoated Si gel (Kieselgel 60 F_{254}), with hexane−EtOAc−MeOH (10:10:1) as mobile phase. Detection was carried out with UV light at 254 nm and by spraying with 1% vanillin in H_2SO_4 acid followed by heating.

Accelerating gradient chromatography (AGC)[37] was performed on Si 60 (70−230 mesh) using Separo AB AGC equipment (Baeckström Separo AB, Lidingö, Sweden), column diameter 2.5 cm, eluted at 25 mL/min. Fractions (12 mL) were collected and analyzed by TLC.

Reversed-phase liquid−liquid partition chromatography was performed on Sephadex LH-20 (Pharmacia, Uppsala, Sweden), 15 × 2.5 cm, flow rate 0.5 mL/min. The Sephadex LH-20 was pre-swollen in the upper phase of $CHCl_3−MeOH−H_2O$ (200:100:75), and the column was slurry packed and washed with 1 volume of lower phase.[15] The sample was dissolved in a mixture of the two phases and applied to the column. The column was then eluted with two volumes of lower phase followed by two volumes of upper phase. Fractions (5 mL each) were collected, and separation was monitored by TLC.

Low-pressure column chromatography was carried out on Lobar RP-18, size B (E. Merck, Darmstadt, Germany), isocratic solvent systems; $CH_3CN−MeOH−H_2O$ (30:20:4), (400 mL), followed by 70% CH_3CN in H_2O (400 mL), and finally 75% CH_3CN in H_2O (400 mL) at a flow rate of 1.0 mL/min. Fractions (12 mL) were collected, and separation was monitored by TLC.

Analytical HPLC was performed on LiChrospher RP-18 in a LiChroCART 125 × 4 mm, 5 μ (E. Merck), using a linear gradient elution (50−80% CH_3CN, 30 min) of $H_2O−CH_3CN$ containing 1% 0.1 N H_3PO_4; flow rate 1.0 mL/min (Hewlett−Packard, HPLC model 1090, with photodiode array detection at 200, 210, 254 nm).

Semipreparative HPLC was performed with a Licrosorb RP-18 column, 250 × 10 mm, 7 μm (E. Merck), using various linear gradients of $H_2O−CH_3CN$ containing 0.01% HCOOH, flow rate 3 mL/min, UV detection at 210 nm.

Due to the unstable nature of the isolated compounds[28, 38] when solvents were evaporated under reduced pressure, the flasks were covered with aluminum foil, and N_2 was slowly introduced instead of air

in order to protect the compounds from exposure to air and light. After isolation, the compounds were stored in MeOH or $CHCl_3$ solution in darkness at maximum 4 °C.

Bioassays. Cyclooxygenase Assay. The test was performed in microtiter scale as a modification of a previously described method[39, 40] with COX isolated from sheep seminal vesicle microsomes according to the method of Nutgeren and Hazlehof.[41] The CO inhibition was calculated on the basis of the reduced PGE_2-concentration against the control. The results are means of a minimum of three experimental values.

5-Lipoxygenase Assay. This assay was been performed according to the method of Kuhl[40] using isolated porcine leukocytes as previously described by Wagner et al.[42] Quantification of 5-HETE was performed via peak area determination. The results are means of a minimum of four experimental values.

Plant Material. Bark of *H. acuminata* was collected and identified by Dr. F. Ghia in 1992, at the Reserva Biologica, Jatun Sancha, Provincia del Napo, Ecuador. A voucher specimen (F. G. 480) is deposited in the Herbario Economico, Escuela Politecnica Nacional, Quito, Ecuador.

Extraction and Isolation. The bark of *H. acuminata* (500 g) was milled and extracted with petroleum ether (1.6 L) in a Soxhlet apparatus for 24 h. The crude petroleum ether extract (13 g) was extracted twice using a three-phase liquid−liquid system; $CHCl_3-CH_3CN-$hexane$-H_2O$ (1:3.4:2:1). The solvents were evaporated, and the $CHCl_3-CH_3CN$ fraction (1.2 g) was further partitioned between hexane and CH_3CN (3 × 100 mL). The CH_3CN fraction (784 mg) was further fractionated using AGC:[37] the extract was dissolved in MeOH, mixed with 2.3 g of silica and the solvent evaporated. The dried silica/sample mixture was applied to a Separo column and Si gel was added on top (column length, 2 cm). The column was eluted with a nine-step accelerating gradient of EtOAc (containing 25% MeOH) in hexane (0, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25, 50, and 100% EtOAc + 25% MeOH, 50-mL portions). Fractions were collected, monitored by TLC, and then combined. Combined fractions were analyzed by analytical HPLC (diode array). Fraction 4 (490 mg), containing mainly acetylenic compounds, exhibited inhibition of COX, and 5-LO was further fractionated using Sephadex partition column chromatography on Sephadex LH 20.[15] A fraction enriched for acetylenes (170 mg) was collected and further purified on a Lobar RP-18 column. Thus, 7 mg (1.4×10^{-3}% dry wt) of polyacetylene **1** was obtained.

Final purification of acetylenes **3**, **4** (7 mg; 1.4×10^{-3}% each), and **5** (7 mg; 1.4×10^{-3}%) from Lobar column fraction 3 was obtained by means of semipreparative HPLC (65−75% CH_3CN in H_2O, 30 min, linear gradient). From Lobar column fraction 4, polyacetylene **2** was obtained (2.5 mg; 6×10^{-4} %) (67−72% CH_3CN in H_2O, 30 min, linear gradient).

Pentadeca-6,8,10-triynoic acid (1): white amorphous solid; UV λ_{max} (CH_3CN-H_2O, approximately 7:3) (log $\epsilon \times 10^{-3}$) 210, 255 (<0.2), 270 (<0.2), 285 (<0.2) 307 (<0.2) nm; IR solid state $\nu_{C=O}$ 1700 cm^{-1}, ν_{OH} 2400−3700 cm^{-1}, out of plane OH 942 cm^{-1}, $\nu_{C\equiv C}$ 2216 cm^{-1}; Raman spectrum, solid state $\nu_{C\equiv C}$ 2218 cm^{-1}; ^{1}H NMR data see Table 1; ^{13}C NMR data see Table 2; EIMS *m/z* 230 (59), 187 (12), 171 (13), 128 (100), 91 (51), 41 (32); HREIMS *m/z* [M$^+$] 230.13298.

Octadeca-8,10,12-triynoic acid (2): white amorphous solid; UV λ_{max} (CH_3CN-H_2O, approximately 7:3) 210 nm; IR solid state $\nu_{C=O}$ 1709 cm^{-1}, ν_{OH} 2500−3700 cm^{-1}, out of plane OH 941 cm^{-1}, $\nu_{C\equiv C}$ 2217 cm^{-1}; Raman spectrum, solution $\nu_{C\equiv C}$ 2220 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 272 (26), 230 (10), 129 (100), 41 (79); HREIMS *m/z* [M$^+$] 272.18421.

***trans*-Pentadec-10-en-6,8-diynoic acid (3):** white amorphous solid; UV λ_{max} (CH_3CN-H_2O, approximately 7:3) (log $\epsilon \times 10^{-3}$) 210, 240 (1.0), 254 (1.8), 268 (2.4), 280 (2.0) nm; IR, solid state $\nu_{C=O}$ 1706 cm^{-1}, ν_{OH} 2400−3700 cm^{-1}, out of plane OH 955 cm^{-1}, $\nu_{C\equiv C}$ 2234 and 2252 cm^{-1}; Raman spectrum, solution $\nu_{C\equiv C}$ 2238 cm^{-1}, $\nu_{C=C}$ 1624 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 232 (52), 165 (31), 129 (100), 91 (71), 41 (35).

***cis*-Hexadec-11-en-7,9-diynoic acid (4):** white amorphous solid; UV λ_{max} (CH_3CN-H_2O, approximately 7:3) (log $\epsilon \times 10^{-3}$) 210, 240 (0.4), 254 (0.8), 268 (1.3), 280 (1.0) nm; IR solid state $\nu_{C=O}$ 1710 cm^{-1}, ν_{OH} 2300−3700 cm^{-1}, out of plane OH 937 cm^{-1}, $\nu_{C\equiv C}$ 2233 and 2251 cm^{-1}; Raman spectrum, solution $\nu_{C\equiv C}$ 2236 cm^{-1}, $\nu_{C=C}$ 1609 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 246 (5), 146 (30), 129 (56), 117 (100), 91 (71), 41 (34).

***cis*-Octadec-12-en-7,9-diynoic acid (5):** white amorphous solid; UV λ_{max} (CH_3CN-H_2O, approximately 7:3) (log $\epsilon \times 10^{-3}$) 210, 240 (0.55), 254 (1.1), 268 (1.6), 280 (1.3) nm; IR, solid state $\nu_{C=O}$ 1710 cm^{-1}, ν_{OH} 2300−3700 cm^{-1}, out of plane OH 926 cm^{-1}, $\nu_{C\equiv C}$ 2233 cm^{-1}; Raman spectrum, solution $\nu_{C\equiv C}$ 2236 cm^{-1}, $\nu_{C=C}$ 1609 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* 274 (7), 245 (4), 187 (24), 164 (24), 146 (83), 117 (100), 91 (74), 41 (37); HREIMS *m/z* [M$^+$] 274.18756.

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