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PRENYLATED PROTOCATECHUIC ACID DERIVATIVES WITH ANTI-OXIDANT ACTIVITY FROM *PIPER HETEROPHYLLUM*

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ABSTRACT

Two new protocatechuic acid derivatives, arieianoic acid (**1**) and arieianol (**2**), were isolated from the aerial parts of *Piper heterophyllum* Ruíz & Pavon and their structures were determined by spectroscopic techniques. From **1**, the semi-synthetic derivatives **3**–**8** were prepared. The antioxidant activity of the natural and semi-synthetic derivatives were evaluated using the DPPH assay.

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INTRODUCTION

In the course of our search for bioactive compounds from the Bolivian flora, we noted that few investigations of Bolivian Piperaceae species have been reported, although several of them are used in the traditional medicine. An example is *Piper heterophyllum* Ruíz & Pavon, a medicinal plant, from which the leaves are used in the Tacana community to treat kidney pain and fever[1], but never investigated scientifically. The results reported here consequently constitute the first examination of the secondary metabolites of the inflorescences of this plant.
From the ethanol extract of the inflorescences of *Piper heterophyllum*, two new prenylated protocatechuic acid derivatives were isolated and characterized. Due to their structural resemblance with the ant–repellent arieianal (**3**), previously isolated from *P. arieianum*,[2] they were named arieianoic acid (**1**) and arieianol (**2**). Protocatechuic acid (3,4-dihydroxybenzoic acid),[3,4] reported from varios higher plants like *Hibiscus sabdariffa*,[3] and *Zanthoxylum piperitum*[4] is a well known natural antioxidant and posses protective effects against oxidative damage in rats,[5] is an inhibitor of blood platelet aggregation,[6] inhibits LDL (low-density lipoprotein) oxidation,[7] and is a dietary chemopreventive agent (inhibits development of neoplasms in animal models).[8] Prenylated protocatechuic acid derivatives are rare, and so far only the ant–repellent activity is known for the related compound arieianal,[2] motivating the assay of the antioxidant properties of arieianoic acid (**1**), arieianol (**2**). As arieianoic acid (**1**) is the major component in the ethanol extract, (8.3% w/w of dried material) from which it precipitates when dichloromethane is added, it was possible to isolate **1** on a preparative scale. **1** was subsequently used as a starting material for the preparation of semi-synthetic derivatives, which also were assayed.

RESULTS, DISCUSSION

Arieianoic acid (**1**) was obtained as a white amorphous solid, mp. 124-125°C, from dichloromethane. The FABMS indicated a molecular formula of $C_{27}H_{36}O_6$ from the ion peak at *m/z* 479.2406 $[M+Na]^+$ (calcd. 479.2408), showing an unsaturation index of 10 degrees. The ^{1}H NMR spectrum displayed signals for 6 methylene groups, 4 methyl groups, 4 olefinic hydrogens and 2 aromatic hydrogens. The ^{13}C NMR spectrum displayed 27 signals, with chemical shifts indicating two conjugated carboxylic acids, 14 aromatic/olefinic carbons and 11 sp^3-hybridized carbons. These spectral data suggested a prenylated tetra-substituted aromatic ring in which one of the methyl units of the prenylated chain was oxidized to a carboxylic acid. The observation of two downfield aromatic carbon signals at 142.9 and 147.8 ppm suggested a diphenol, i.e. a catechol (Table 1). A HMBC spectrum permitted the complete assignment of **2**. A HMBC correlation between the olefinic hydrogen (H-10') to the carboxylic signal at δ 171.6 (C-18') and to the carbon signal at 133.2 (C-11') established C-10', C-11', and C-18’ as a conjugated system. Correlations between the

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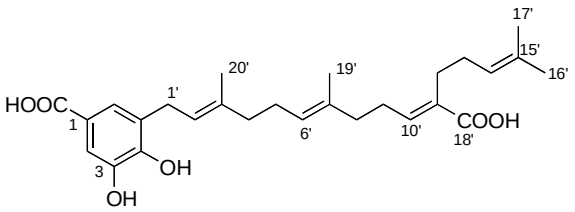
hydrogen at δ 7.49 (H-6) with the carboxylic group at 171.6 ppm and the benzylic carbon δ 39 (C-1'), indicated that the hydrogen was positioned between these two groups. The remaining aromatic hydrogen at δ 7.45 (H-2) also showed a correlation with the carboxylic acid group at δ 170.2. Consequently a protocatechuic acid ring structure was confirmed. The carbon shifts of the vinylic methyl groups at C-3' and C-7' suggested an *E* configuration by comparison to the shifts reported by Green *et al.*[2] A NOESY correlation between the C-9' methylene hydrogens at δ 2.22 with C-10' olefinic hydrogen could not be clearly seen, due to the close chemical shift at δ 2.24 for the C-12' methylene hydrogens, but comparison of the chemical shift for the C-9' and C-12' methylenes with the linear triterpene *Z*, *E*, *E*-cupanosin A[9] allowed us to assigned the *E* configuration for the C-10', C11' olefin. Then, compound **1** was assigned as [3,4-dihydroxy-5-(*E*,*E*,*E*-11'-carboxyl-3',7',15'-trimethylhexadeca-2',6',10',14'tetraenyl).



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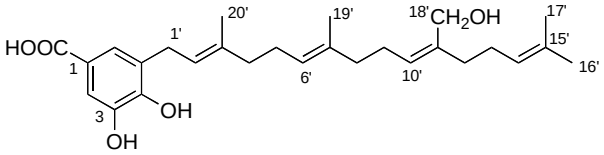

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Figure 1.

Arieianol (**2**) was obtained as a light brown wax. The FABMS of arieianol revealed an ion peak at *m/z* 465.2622 $[M+Na]^+$ (calcd. 465.2627) given the molecular formula $C_{27}H_{38}O_5$ requiring 9 degrees of unsaturation. The ^1H NMR and ^{13}C spectrum is very similar to that of arieianoic acid, the presence of an additional signal in the ^1H spectrum at 4.17 ppm and at 60.3 ppm in the ^{13}C spectrum clearly indicate a methylene bonded to an hydroxyl group. HMBC and HMQC spectra were used to make the complete assignment of the structure **2**. Correlation of the C-10' olefinic hydrogen with the carbon signal at δ 60.3, established the C-18' as the vinylic alcohol. The geometries of both C-2'and C-3', C-6' and C-7' double bonds were shown to be *E* by comparison of the NMR data with those of arieianal and arieianoic acid. Furthermore the NOESY spectrum showed correlation of H-9' with H-18', but not with H-10', revealing a *Z* configuration, supported also by comparison chemical shifts of the ^{13}C NMR spectrum with those of the prenylated chain of the benzoquinone derivative chabrolobenzoquinone E.[10] Consequently compound **2** was assigned unambiguously as [3,4-dihydroxy-5-(*E*,*E*,*Z*-18'-hydroxy-3',7',15'-trimethylhexadeca-2',6',10',14',tetraenyl) benzoic acid. The *E* configuration was assigned to each of the synthetic derivatives assigned mainly by the observation of the NOESY correlation between H-10' and the methyl of the carboxylate for the methylated compounds (**3**-**8**), H-10' and the methylene alcohol for the tetraol (**6**), supported also by comparison with the synthetic 3,4-dihydroxy-5-((2*E*,6*E*,10*E*)-11(hydroxymethyl)-3,7-15-trimethylhexadeca-2,6,6,10,14-tetraenyl) benzoic acid),[11] H-10' and the aldehydic hydrogen for the dialdehyde (**7**) and by comparison of the NMR data of arieianoic acid for the chromane (**8**). Preparation of the tetraol and dialdehyde derivatives started by preparing the diester of arieianoic acid (**1**) using MeI and K_2CO_3. Tetra– (**3**) and trimethylated (**4**) derivatives were also prepared using an excess of the potassium carbonate and MeI. The catechol moiety was protected as the corresponding diisopropylmethyl ether, which is not cleaved by DIBAL-H when performing the reduction step. These reactions were done in one pot. The protecting groups were removed using a solution of 1% HCl in ethanol, and after purification the tetraol derivative (**6**) was obtained. For the synthesis of the dialdehyde (**7**), the protected dialcohol

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was oxidized using MnO_2 to afford the protected dialdehyde, which was then deprotected in the same way as for the tetraol.

Table 1. *^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, in 10% CD_3OD in $CDCl_3$ for **1** and $CDCl_3$ for **2**, with the $CDCl_3$ signal (7.27 and 77.0 ppm) as reference.*

	^{1}H (δ; multiplicity; *J* [Hz])		**^{13}C** (δ)	
Position	**1**	**2**	**1**	**2**
1	-	-	122.2	120.4
2	7.34 (d, *J*=2.0)	7.50 (s)	115.2	114.8
3	-	-	145.5	143.0
4	-	-	149.5	148.0
5	-	-	129.2	127.8
6	7.30 (d, *J*=2.0)	7.49 (s)	124.3	124.6
1'	3.31 (d, *J*=7.5)	3.37 (d, *J*=7.1)	29.1	28.4
2'	5.32 (br t, J_1=6.3, J_2=7.3)	5.09 (br t, J_1=6.3, J_2=7.3)	123.8	121.7
3'	-	-	137.1	137.0
4'	2.05 m	2.14/2.07 (m)	149.5	39.3
5'	2.13 (dd, J_1=14.3, J_2=7.0)	2.14/2.07 (m)	27.6	26.4
6'	5.16 (br t, J_1=5.0, J_2=7.0)	5.32 (br t, J_1=5.0, J_2=7.0)	126.3	124.1
7'	-	-	135.3	134.8
8'	2.05 (m)	2.14/2.07 (m)	39.7	39.7
9'	2.24 (m)	1.96 (br t, J_1=8.0, J_2=7.1)	28.6	26.0
10'	6.73 (t, *J*=7.4)	5.32 (br t, *J*=7.3)	144.4	129.7
11'	-	-	133.2	137.0
12'	2.24 (m)	2.14/2.07 (m)	27.9	35.0
13'	2.05 (m)	2.14/2.07 (m)	28.8	27.1
14'	5.0 (br t, *J*=7.4)	5. 09 (br t, *J*=7.3)	125.0	124.1
15'	-	-	133.1	131.8
16'	1.63 (s)	1.56 (s)	26.0	25.8
17'	1.56 (s)	1.66 (s)	17.8	16.1
18'	-	4.17 (s)	171.6	60.3
19'	1.59 (s)	1.57 (s)	16.3	16.0
20'	1.71 (s)	1.72 (s)	16.4	17.7
COOH	-	-	170.6	171.7

The chromane derivative (**8**) was unexpectedly obtained in an attempt to protect the catechol as an acetonide derivative, by refluxing the arieianoic acid in acetone with a catalytic amount of *p*-toluensulfonic acid (*p*-TsOH) (Figure 2).

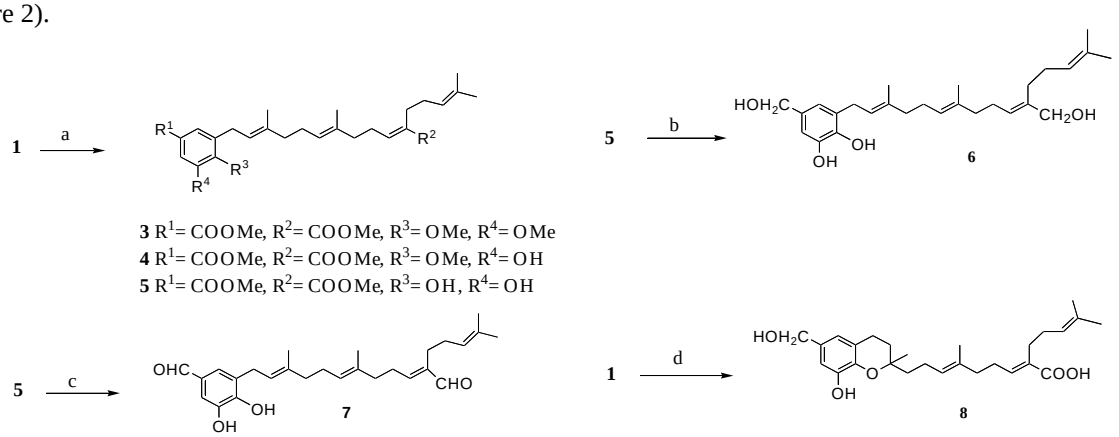

Figure 2. *Reagents and conditions: a) i) K_2CO_3, DMF, 70°C ii) MeI (tetramethylated (62%) and trimethylated (15%), using and excess of MeI, and dimethylated (75%); b) i) **5**, CH_2Cl_2, 0°C, diisopropylmethylsilylchloride, DIPEA, ii)DIBAL (85%); iii) 1% HCl in ethanol (63%); c) i) **5**, CH_2Cl_2, diisopropylmethylsilylchloride, DIPEA; ii) DIBAL (85%); iii) MnO_2, 24 hrs (21%); iv) 1% HCl in ethanol (63%); d) p-TsOH, acetone, reflux (73%).*

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Antioxidant properties

The antioxidant properties of the natural products and the semi-synthetic derivatives were determined adding aliquots of 30% of the methanolic solution containing each compound to 3 mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min. IC_{50} values denote the concentration of a sample required to scavenge 50% of DPPH free radicals.

The tetramethylated (3), trimethylated (4) and chromane (8) derivatives did not show scavenger activity.

The IC_{50} values for the active compounds 1, 2, 5, 6 and 7 are summarized in Table 2.

***Table 2**. Antioxidant activities for compounds*

COMPOUND	DPPH IC_{50} (μM) ± SD
protocatechuic acid	14.30 ± 0.06
arieianoic acid (**1**)	3.00 ± 0.07
arieianol (**2**)	4.20 ± 0.18
dimethylated (**5**)	3.12 ± 0.02
tetraol (**6**)	4.80 ± 0.02
dialdehyde (**7**)	2.85 ± 0.01

The natural products, arieianoic acid (**1**), arieianol (**2**) and the semi-synthetic diester (**5**), tetraol (**6**) and dialdehyde (**7**), showed a scavenger activity better to that of protocatechuic acid used as a reference. Some conclusions can be drawn from the comparison of the structures, like the cathecol ring is the most important feature for the compounds to show antioxidant activity, confirmed by the loss of activity of the tetramethylated (**3**), trimethylated (**4**) and chromane (**8**), in which no cathecol system is present. The prenylated chain bonded to the ring seems to increase the scavenger activity, when compared to the reference. A small increase in the potency is observed, when the following groups are present in both the aromatic ring and the prenylated chain: dialdehyde (**7**)> dicarboxylic acid (**1**) > diester (**5**) > tetraol (**6**). The importance of both groups for the antioxidant activity of this class of compounds, can be noticed also when comparing the IC_{50} values of the tetraol (**6**) derivative and the natural compound arieianol (**2**), in which even though a carboxylic acid is present in the aromaric ring, the scavenger activity of this compound is lower compared with that of arieianoic acid (**1**), which presents two carboxylic groups.

EXPERIMENTAL SECTION

General

The melting point was recorded with a Reichter microscope. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded in CD_3OD and $CDCl_3$ using a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in parts per million relative to solvent 7.27 and 77.00 ppm for $CDCl_3$, while the coupling constants (*J*) are given in Hertz. The Mass spectra were recorded with a Jeol SX 102 spectrometer at 70 eV.

Column chromatography was run on Matrex silica gel 60, while TLC was on Silica gel GF_{254} pre-coated plates with detection accomplished by spraying with an ethanolic solution of *p*-anisaldehyde and H_2SO_4 followed by heating at 100°C, or by visualizing with an UV lamp at 254 nm.

Plant material

The inflorescences of *Piper heterophyllum* Ruíz & Pavon were collected from Valle del Sacta, a region belonging to Carrasco National Park in Bolivia, in February 2002. Authentication was done by Rosario Barco, botanist at Martin Cardenas National Herbarium in Cochabamba, where voucher specimens were deposited, and compared with the voucher GB-1538 deposited in 1997 at National Herbarium of Bolivia in La Paz.

Extraction and isolation

The inflorescences of *P. heterophyllum* (300 g) were macerated with ethanol 95% during 5 days. Removal of the solvent from the filtrate under vacuum in a rotatory evaporator provided an organic extract (58 g), which was subjected to column chromatography on silica gel, using mixtures of petroleum ether, dichloromethane and methanol as eluents to afford four main fractions. From the polar fraction, arieianoic acid (**1**) precipitated as a white solid (25

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g) from dichloromethane. Further purifications of the dichloromethane residue by CC on silica gel eluted with a mixture of Petroleum ether-EtOAc (2:3) afforded arieianol (**2**, 338 mg).

Arieianoic acid (**1**)
Amorphous white solid, mp 124-125°C. ^{1}H NMR (10% CD_3OD in $CDCl_3$, 500 MHz) and ^{13}C (10% CD_3OD in $CDCl_3$, 125 MHz) see Table 1; FABMS *m/z* 479.2406 calcd for $C_{27}H_{36}O_6$ + Na, 479.2408.

Arieianol (**2**)
Light brown wax. NMR data^{1}H NMR($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 125 MHz) see Table 1; FABMS *m/z* 465.2622 calcd for $C_{27}H_{38}O_5$ + Na, 465.2627.

[3,4-Dimethoxy-5-(E,E,E-11'-methyl-carboxylate-3',7',15'-trimethylhexadeca-2',6',10',14',-tetraenyl)methyl benzoate], (**3**)
To a solution of arieianoic (500 mg, 1.1 mmol) in DMF (10 mL), K_2CO_3 (393 mg; 2.6 mmol, 4 eq) and MeI (1.4 g; 4.4 mmol) were added and the mixture was heated at 70°C for 5 hours. After cooling the mixture was diluted with water (20 mL) and extracted with 20 mL of ether 4 times. The organic phases were combined and dried over $MgSO_4$. After evaporation of the solvent, the mixture was purified by column chromatography with petroleum ether and ethyl acetate (4:1) as eluent to afford 350 mg (62%) of the tetramethylated (**3**) and 80 mg (15%) of trimethylated (**4**). **^{1}H–NMR** (400 MHz, $CDCl_3$) δ 7.4 (d, *J* = 1.7, 1H), 7.3 (d, *J* = 1.7, 1H), 6.6 (t, *J* = 7.3, 1H), 5.20 (q, 2H), 5.07 (q, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.65 (s, 3H), 3.30 (d, *J* = 7.2), 2.19 (m, 4H), 2.02 (m, 6H), 1.67 (s, 3H), 1.60 (s, 3H), 1.54 (s, 3H), 1.52 (s, 3H) .**^{13}C-NMR** (100 MHz, $CDCl_3$) δ 168.2, 166.8, 152.3, 151.0, 142.7, 136.1, 135.4, 133.9, 132.0, 131.6, 125.3, 124.9, 123.7, 122.4, 111.2, 60.4, 55.7, 51.9, 51.5, 39.6, 38.5, 28.4, 27.6, 27.2, 26.9, 26.4, 25.6, 17.5, 17.4, 16.1, 15,9. HRESIMS *m/z* 535.2972 calcd for $C_{31}H_{44}O_6$ + Na, 535.3036.

[3-Methoxy-4-hydroxy-5-(E,E,E-11'-methyl-carboxylate-3',7',15'-trimethylhexadeca-2',6',10',14',-tetraenyl)methyl benzoate], (**4**)
Isolated from the same reaction mixture of compound **3**, as a yellow oil (80 mg, 15%). ^{1}H–NMR (500 MHz, $CDCl_3$) δ 7.4 (d, *J* = 1.7, 1H), 7.3 (d, *J* = 1.7, 1H), 6.6 (t, *J* = 7.3, 1H), 5.20 (q, 2H), 5.07 (q, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.69 (s, 3H), 3.30 (d, *J* = 7.2), 2.19 (m, 4H), 2.02 (m, 6H), 1.67 (s, 3H), 1.60 (s, 3H), 1.54 (s, 3H), 1.52 (s, 3H) .^{13}C-NMR (125 MHz, $CDCl_3$) **δ** 168.4, 166.7, 149.1, 148.8, 142.7, 136.1, 135.4, 133.9, 132.0, 131.6, 125.3, 124.9, 123.7, 122.4, 111.2, 61.1, 52.0, 51.5, 39.6, 38.5, 28.4, 27.6, 27.2, 26.9, 26.4, 25.6, 17.5, 17.4, 16.1, 15,9. HRESIMS [M+Na] *m/z* 521.2859 calcd for $C_{30}H_{42}O_6$ + Na, 521.2879.

[3,4-Dihydroxy-5-(E,E,E-11'-methyl-carboylate-3',7',15'-trimethylhexadeca-2',6',10',14',-tetraenyl)methyl benzoate], (**5**)
3g (6.5 mmol) of arieianoic acid (**1**) were dissolved in 20 mL of DMF, followed by addition of 0.8 mL (13 mmol) of MeI and 830 mg (6.5 mmol, 2 eq.) of K_2CO_3. The mixture was heated at 65°C for 2 hours. The work up was done as described above. The product was purified by column chromatography using petroleum ether and ethyl acetate (3:2) as eluent to afford 2.5 g (75%) of the **5** as a yellow oil. ^{1}H –NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* =1.9 Hz, 1H), 7.44 (d, *J* = 1.9 Hz, 1H), 6.75 (t, *J* = 7.3 Hz, 1H), 5.33 (t, J_1 = 7.3, J_2 = 6.3 Hz, 1H), 5.12 (dt, J_1 = 7.3, J_2 = 6.6 Hz, 2H), 3.38 (d, *J* = 7.2, 2H), 2.30 (dd, J_1 = 7.3, J_2 = 8.2 Hz, 2H), 2.25 (t, *J* = 7.7 Hz, 2H), 2.07 (m, 8H), 1.74 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.57 (s, 3H), 3.86 (s, 3H), 3.74 (s, 3H).^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.2, 167.9, 147.5, 143.4, 137.3, 134.2, 132.4, 131.7, 127.7, 125.1, 123.9, 123.7, 121.8, 121.4, 114.5, 52.2, 51.9, 39.7, 38.6, 28.6, 27.8, 27.4, 27.1, 26.5, 25.8, 17.7, 16.3, 16.1. HRESIMS *m/z* 507.2702 calcd for $C_{29}H_{40}O_6$ + Na, 507.2723.

[3,4-Dihydroxy-5-(E,E,E-18'-hydroxy-3',7',15'-trimethylhexadeca-2',6',10',14',-tetraenyl)benzyl alcohol, (**6**)
To a solution of 3 (1.1 g, 2.2 mmol) in dichloromethane, isopropyldimethylsilylchloride (IPDMS) (7 mL, 4.4 mmol) and diisopropyl ethyl amine (0.8 mL, 4.4 mmol) were added dropwise at room temperature and the mixture was stirred for 30 minutes and then cooled to –78°C. To this mixture a solution of DIBAL-H (12 mL, 4.4 mmol) was added and stirred for 2 hours. The work up was done adding 30 mL of MeOH, a transparent gel was formed which was filtered and washed 3 times with MeOH. After concentration the yellow oil obtained was filtered over silica gel with petroleum ether and ethyl acetate (100 mL, 60:40). After evaporation of the solvent, 1.19 g (85%) of the

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protected diol was obtained. 500 mg of the protected diol was dissolved in 10 ml of 1% solution of conc. HCl in EtOH and the mixture was stirred for 5 hours, and then 10 mL of a saturated solution of $NaHCO_3$ was added. The mixture was extracted 3 times with 15 mL of dichloromethane. The organic phase was combined and dried over $MgSO_4$, and after evaporation of the solvent, the tetraol (**6**) was purified by column chromatography (silica gel, petroleum ether: EtOAc, 3:2) to obtain 219 mg (63%) of pure tetraol (**6**) ^{1}H–NMR (400 MHz, $CDCl_3$) δ: 6.71 (d, *J* = 1.6 Hz, 1H), 6.63 (d, *J* = 1.6 Hz, 1H), 5.39 (t, *J* = 7 Hz, 1H), 5.34 (t, *J* = 7 Hz, 1H), 5.13 (br t, 2H), 4.4 (s, 2H), 4.0 (s, 2H) 3.34 (d, *J* = 7.2 Hz, 2H), 2.09 (m, 12H), 1.74 (s, 3H), 1.69 (s, 3H), 1.61(s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 143.9, 142.3, 138.3, 136.6, 134.8, 131.9, 129.3, 127.7, 127.6, 124.5, 124.2, 122.4, 121.2, 113.1, 67.1, 65.5, 39.8, 39.7, 28.7, 28.2, 27.1, 26.4, 26.2, 25.8, 17.8, 16.2, 16.1. HRESIMS *m/z* 451.2858 calcd for $C_{27}H_{40}O_4$ + Na, 451.2824.

[3,4-Dihydroxy-5-(E,E,E-18'-aldehyde-3',7',15'-trimethylhexadeca-2',6',10',14',-tetraenyl)benzyl aldehyde, (**7**)

To 500 mg of the protected tetraol (**6**) 5g of freshly prepared MnO_2,[12] was added and the mixture stirred for 24 hours. The mixture was extracted with dichloromethane and filtered over Celite. After evaporation of the solvent the residue was purified by column chromatography (silica gel, petroleum ether: ethyl acetate, 4:1) to afford 100 mg (21%) of the protected dialdehyde. To this was added 10 ml of 1% solution of conc. HCl in ethanol. The mixture was stirred for 30 minutes, and then a 5% solution of $NaHCO_3$ was added. The mixture was extracted 3 times with 15 ml of ether, the organic phase was dried over $MgSO_4$, and after evaporation of the solvent, the residue was purified by column chromatography (silica gel, petroleum ether: ethyl acetate 4:1) to afford 50 mg (69%) of the dialdehyde (**7**) ^{1}H–NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 9.3 (s, 1H), 7.35 (d, *J* = 1.6 Hz, 1H), 7.26 (d, *J* = 1.6 Hz, 1H), 6.45 (t, *J* = 7.2 Hz, 1H), 5.34 (t, *J* = 6.2 Hz, 1H), 5.16 (t, *J* = 5.6 Hz, 1H), 5.08 (t, *J* = 7.2 Hz, 1H), 3.42 (d, *J* = 7.2 Hz, 2H), 2.44(q, *J* = 7.3, 2H), 2.26 (t, J=7.6 Hz, 2H), 2.04 (dd, J=7.3 Hz, *J* = 7.6 Hz, 2H), 2.15 (m, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 196.1, 192.1, 156.3, 149.3, 144.3, 143.4, 138.0, 133.9, 132.7, 129.3, 128.2, 126.7, 125.5, 123.7, 121.5, 112.6, 39.8, 38.5, 28.6, 27.6, 27.2, 26.5, 25.9, 24.5, 17.9, 16.4, 16.2. HRESIMS *m/z* 447.2502 calcd for $C_{27}H_{36}O_4$ + Na, 447.2511.

2-[(E,E,E-18'-Carboxyl--4',12'-dimethyl-3',7',11'-tridecatrienyl)]-3,4-dihydro-2-methyl-1-benzopyran-8-hydroxy-6-carboxylic acid (**8**)

To a solution of (400 mg, 0.9 mmol) in acetone (20 mL), *p*-TsOH (5 mg, 0.02 mmol) were added and the mixture refluxed for 6 hrs. After cooling, ether was added (30 mL) and the mixture was washed with water (3 times , 20 mL). After evaporation of the organic phase, the mixture was subjected to flash column chromatography using CH_2Cl_2 : MeOH (9:1) to afford 300 mg (73%) of the chromane derivative. ^{1}H –NMR (300 Mhz, 10% CD_3OD in $CDCl_3$) δ 7.49 (br s, 2H), 6.9 (ps t J_1 = 7.2 Hz and J_2 = 7.7 Hz, 1H), 5.16 (br t, 2H), 2.81 (m, 2H), 2.33 (m, 2H), 2.12 (m, 4H), 1,87 (m, 2H), 1.69 (s, 3H), 1.60 (br s, 6H), 1.36 (d, *J* = 5.9 Hz, 3H). ^{13}C-NMR (75 MHz, 10% CD_3OD in $CDCl_3$) δ 173.9, 172.6, 146.0, 145.3, 145.0, 132.5, 131.5, 136.9, 134.5, 124.8, 123.7, 121.0, 120.6, 113.9, 78.9, 39.8, 38.5, 30.9, 27.8, 27.4, 26.8, 25.8, 24.2, 22.3, 21.8, 17.8, 16.0. HRESIMS *m/z* 479.2380 calcd for $C_{27}H_{36}O_6$ + Na, 479.2410.

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