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Bioactive alkenylphenols from *Piper obliquum*

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Abstract—Various parts of *Piper obliquum* Ruíz & Pavon yielded the new alkenylphenols obliquol A (**1**) and obliquol B (**2**), the new 4-chromanone **3** together with the known compounds **4** and **5**. A synthesis of obliquol B (**2**) was developed in order to confirm its structure and to provide sufficient amounts for biological testing. Compounds **1** and **2** have antibacterial activity comparable to that of ampicillin, **2** in addition possesses potent anti-NF-κB activity by targeting early events in the TNFα-induced NF-κB inflammatory pathway, which may explain the effects reported for the traditional use of the plant.

1. Introduction

Piper species are known as 'thuda' by the Tacana community in Bolivia. The Tacana recognize different classes of thuda by the smell, color, shape, and place of collection. Several thudas have been used for many medicinal purposes, for example as antiseptics, to treat skin diseases, as painkillers, and against fever,[1] although it is difficult to determine exactly which species that were used traditionally due to the number of *Piper* species that grow in the area. Here we wish to report a chemical and biological investigation of *Piper obliquum*, from which two new alkenylphenols and a new 4-chromanone derivative were obtained together with known metabolites. Motivated by the traditional use of thuda, the isolated metabolites were assayed for inhibition of the NF-κB pathway. The transcription factor NF-κB plays a key role for the inducible expression of genes mediating proinflammatory effects, and is thus an important target for the development of anti-inflammatory drugs.[2,3] NF-κB is an inducible transcription factor made up of homo- and heterodimers that interact with a family of inhibitory IκB proteins, of which IκBα is the best characterized. In most cell types, these proteins sequester NF-κB in the cytoplasm by masking its nuclear localization sequence. Stimulation of cells with a variety of physiological or pathogenic stimuli leads to phosphorylation, ubiquitination, and the subsequent degradation of IκBα proteins.[4] The degradation of IκB results in the translocation of NF-κB from the cytoplasm to the nucleus, and IκBα kinases (IKKs)-dependent phosphorylation of IκBα is a key step involved in the activation of NF-κB complexes.[3] NF-κB is implicated in the regulation of several cytokines, chemokines, adhesion molecules, acute phase proteins, and inducible effector enzymes that participate in inflammatory diseases such as rheumatoid arthritis, asthma, and inflammatory bowel disease.[2,5,6] In addition, the metabolites were assayed for antibacterial activity.

2. Chemistry

An ethanol extract of the inflorescences of *P. obliquum* yielded the two new alkenylphenols obliquol A (**1**), and obliquol B (**2**) (see Fig. 1 for structures, names pro-

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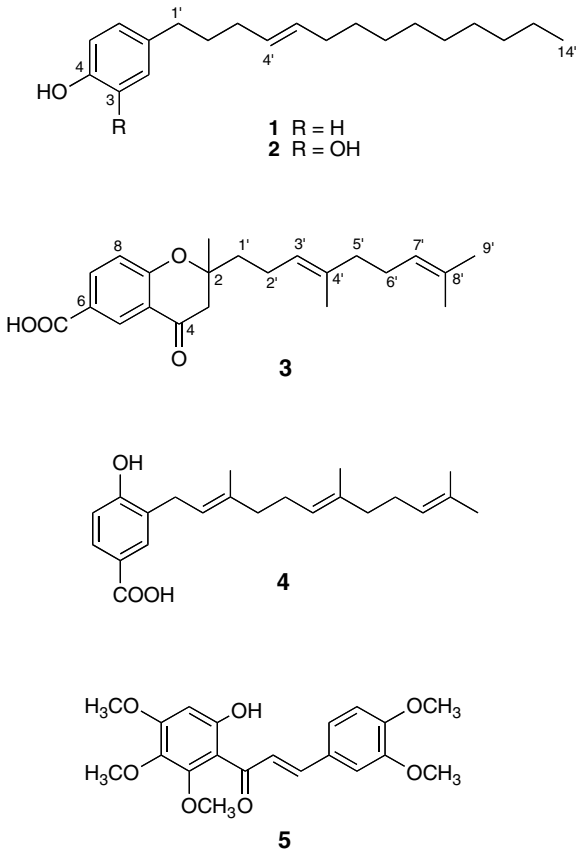


Figure 1. Secondary metabolites isolated from the inflorescences (**1** and **2**) and the leaves (**3**, **4**, and **5**) of *Piper obliquum*.

posed by us), while an ethanol extract of the leaves gave the new 4-chromanone (**3**) together with the known 3-farnesyl-4-hydroxybenzoic acid[7–12] (**4**) and 2′-hydroxy-3,4,4′,6′-tetramethoxychalcone[13–15] (**5**). Despite the fact that the genus *Piper* has been intensively studied, alkenylphenols have rarely been isolated. In fact, only the occurrence of a C_{16} alkenylphenol in *Piper hispidum*[16] and the C_8 and C_{10} alkenylphenols gibbilimbols A–D from *Piper gibbilimbum*[17] have been reported.

High resolution MS experiments suggested that the elemental composition of obliquol A (**1**) is $C_{20}H_{32}O$, and this was confirmed by 1D NMR data. HMBC correlations between 1′-H_2 and C-1, C-2, and C-6 established a *para* substituted phenol. The position of the double bond in the chain was determined by the COSY correlations between the benzylic methylene (1′-H_2) at δ 2.54 with 2′-H_2 at δ 1.63 and further on to 3′-H_2 which, in turn, correlate to the olefinic hydrogens (4′-H/5′-H), confirming that the double bond is between C-4′ and C-5′. The *E* stereochemistry was assigned by comparison of the chemical shifts of C-3′ and C-6′ at δ 31.9 and δ 32.7, respectively, for a *Z* configuration chemical shifts between δ 26 and 27 would be expected.[18] The NMR data of obliquol B (**2**) are very similar to those of **1**, although it contains one additional oxygen according to the MS data. An analysis of the HMBC spectrum of **2** provided sufficient information to position it, as an hydroxyl group, on C-3. For the 4-chromanone **3**, the elemental composition was by HRESIMS experiments suggested to be $C_{22}H_{28}O_4$. COSY and HMBC correlations established the presence of a disubstituted phenolic moiety, substituted with an acyl group in position 2 and a carboxylic functionality in position 4. In addition, a C_{15} isoprenoid chain ending with the acyl carbonyl could be deduced. C-2 is oxygenated, as evidenced by its chemical shift and the fact that 2-CH_3 only give HMBC correlations to C-2, C-3, and C-1′. As the unsaturation index (**9**) of **3** required an additional ring, this has to be between C-2 and C-8a. Pertinent COSY and HMBC correlations for **3** are shown in Figure 2.

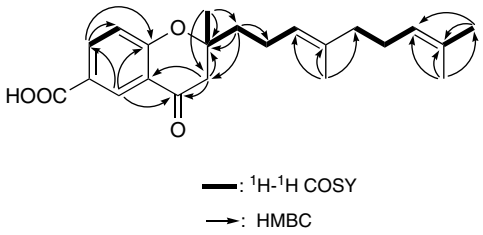


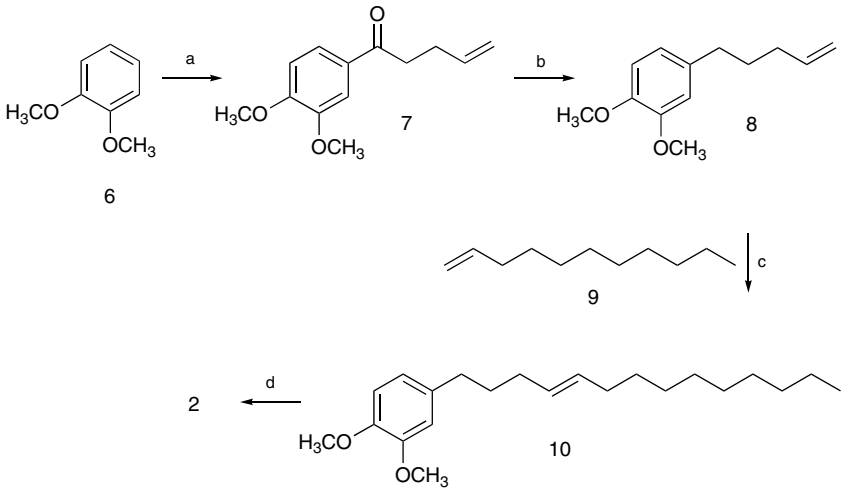
Figure 2. Pertinent COSY and HMBC correlations observed with chromanone **3**.

Obliquol B (**2**) was isolated in quite small amounts and in order to facilitate the biological testing we decided to synthesize it. Natural alkenylphenols have been synthesized previously,[19,20] but we were interested in making use of the efficient metathesis reaction. Veratrole (**6**) underwent a Friedel–Crafts acylation with 4-pentenoyl chloride to provide the coupled product **7** (Scheme 1), and a Clemensen reduction afford 4-pent-4-enyl-1,2-dimethoxybenzene (**8**). A cross-metathesis reaction between the two terminal alkenes **8** and 1-undecen gave **10** in good yields. The subsequent demethylation of the catechol ring gave obliquol B (**2**) as the only product, in all ways identical to the natural product.

3. Results and discussion

The isolated compounds were tested in a NF-κB-dependent luciferase gene reporter assay, where the amount of the luciferase gene product reflects the extent of NF-κB transcriptional activation. The HIV-1 promoter is highly responsive to TNFα-induced NF-κB pathway and by using the 5.1 stably transfected cell line it could be shown that obliquol B (**2**), in a concentration-dependent manner, inhibited TNFα-induced HIV-1-LTR transactivation (Fig. 3).

Interestingly, obliquol A (**1**) did not show a significant anti-NF-κB activity, demonstrating that the 1,2-dihydroquinone moiety is critical for the biological activity of obliquol B in the NF-κB pathway. Compounds **3**, **4**, and **5** were inactive in this biological assay. The IC_{50} inhibitory activity for obliquol B (**2**) was calculated



Scheme 1. Reagents and conditions: (a) 1-pentenoyl-chloride, $AlCl_3$, CH_2Cl_2, 0 °C (74%); (b) Zn, $HgCl_2$, 50% aq HCl (85%); (c) $CH_2{=}CH(CH_2)_8CH_3$ (**9**), Grubbs catalyst, 2nd generation, CH_2Cl_2, reflux (74%); (d) TMSI, 20% mol 2,6-di-*tert*-butyl-4-pyridine, $CDCl_3$ (87%) (the reaction was carried out in a NMR tube with a sealed cap).

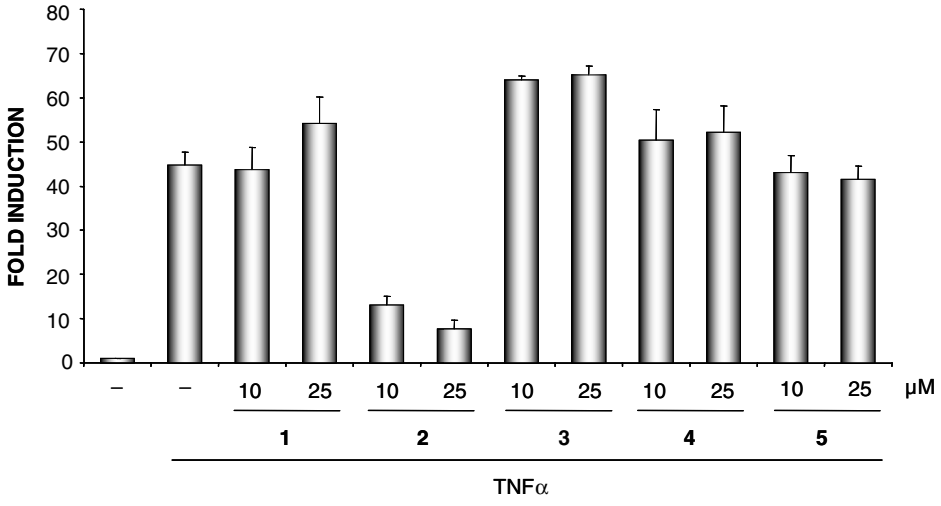


Figure 3. Effects of *Piper obliquum* compounds on NF-κB activation. The 5.1 cells were pre-treated with increasing concentrations of the isolated compounds for 15 min and then treated with TNFα for 6 h, after which the luciferase activities were measured in the cell lysates and expressed as a fold induction.

to be close to 5 μM. We also determined that **2** does not affect the luciferase activity induced by doxycycline in the HeLa-Tet-On-Luc cells, ruling out the possibility that the NF-κB inhibitory activity of **2** is mediated by either non-specific inhibition of the luciferase activity or cytotoxicity (Fig. 4A). It has been shown that natural compounds isolated from medicinal plants with anti-inflammatory activities, such as *Salix alba* and *Tanacetum parthenium*, have potent anti-NF-κB activity by acting a different levels of the NF-κB pathway.[6] Therefore the biochemical pathways affected by obliquol B (**2**) was investigated by immunoanalysis of proteins involved in the NF-κB pathway. In 5.1 cells we detected that TNFα induced a rapid phosphorylation and degradation of the cytoplasmic IκBα protein, and this was prevented by preincubation of the cells with obliquol B (10 μM) prior to TNFα stimulation (Fig. 4B).

This result indicates that **2** targets the IKK complex, and we are currently investigating this possibility. The use of plant extracts in the healing of inflammatory diseases is millenia old and continues to this day. Alkenylphenols such as gibbilimbols show potent antibacterial properties toward *Staphylococcus epidermis* and *B. cereus*, and also display cytotoxic properties toward KB nasopharyngal carcinoma cells.[17] Compounds **1**, **2**, **3**, and **5** were evaluated for antibacterial activity (the antibacterial properties of compound **4** have been reported previously),[21] and the minimum inhibitory concentration (MIC) with *Escherichia coli* and *S. epidermis* was determined by the broth dilution method[22] (see Table 3). It is obvious that both **1** and **2** possess potent antibacterial activity, comparable to that of ampicillin which was used as positive control. Compound **5** is less potent while **3** is only weakly active.

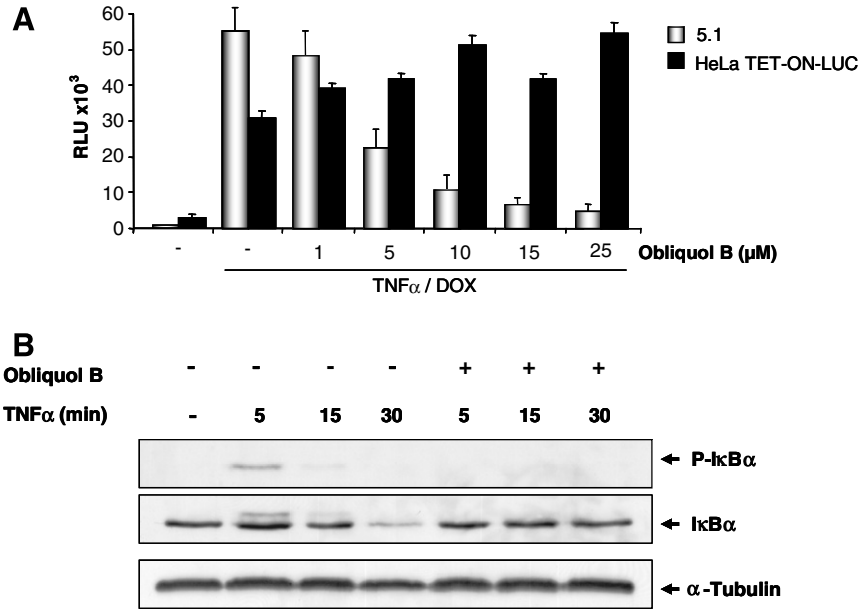


Figure 4. (A) Determination of the IC_{50} inhibitory anti-NF-κB activity. Both the 5.1 and the HeLa-Tet-On-Luc were pre-incubated increasing concentrations of obliquol B (**2**) for 15 min and then stimulated with TNFα (2 ng/mL) or Doxycyclin (DOX; 1 μg/mL) for 6 h, after which the luciferase activities (R.L.U.; relative light units) were measured in the cell lysates. (B) Effects of obliquol B (**2**) on the NF-κB activation pathway 5.1 cells were incubated with the compounds for 15 min and then treated with TNFα for the indicated times and the then tested for IκBα phosphorylation and degradation, and α-tubulin expression by Western blots.

4. Conclusion

A large number of compounds isolated from plants are currently known as NF-κB modulators,[6] and our results have set the biochemical principles that explain the anti-inflammatory activity of *P. obliquum*, a plant used in traditional medicine. In addition, obliquol B (**2**) should be expected to have also an antioxidative activity, although this was not tested. However, it should be noted that **2** is a catechol and that similar compounds have been shown to possess allergenic properties.

5. Experimental

5.1. General experimental procedure

The NMR spectra were recorded in a Bruker DRX300 spectrometer at 300 MHz (^{1}H) and at 75 MHz (^{13}C) and a Bruker DRX400 spectrometer (at 400/100 MHz). The chemical shifts (δ) are reported in parts per million relative to chloroform (7.27 and 77.0 ppm for $CDCl_3$). HRESIMS spectra (H_3PO_4 for calibration and as internal standard) were recorded with a Micromass Q-tof Micro spectrometer and FABMS spectra were recorded with a JEOL SX 102 spectrometer. Organic extracts were dried over $MgSO_4$. All chromatography was performed on 60 Å 35–70 μm Matrex silica gel (Grace Amicon). Preparative TLC were performed on 1 mm plates (20 × 20 cm) (Aldrich). TLC analyses were made on Silica Gel 60 F_{254} (Merck) plates and visualized with*p*-anisaldehyde and heating.

5.2. Plant material

Piper obliquum Ruíz & Pavon, was collected in the region of Valle del Sacta in Cochabamba, Bolivia in November 2003 a voucher specimen (RB-1111) was authenticated by Rosario Barco, botanist at Martin Cardenas National Herbarium, where it is deposited.

5.3. Extraction and isolation

The dried inflorescences of *P. obliquum* (200 g) were extracted with ethanol (95%) at room temperature for 3 days, and after the extract was concentrated under reduced pressure, 5 g of crude extract was obtained. This extract was subjected to silica gel column chromatography, eluting with a petroleum ether/EtOAc gradient (0%, 25%, 50%, and 75% EtOAc). Four fractions were obtained, fractions 3 and 4 were further purified by flash column chromatography using petroleum ether/EtOAc (3:2), to afford obliquol A (1) (300 mg) and obliquol B (2), which was difficult to isolate as a pure compound so further purifications were done by preparative thin layer chromatography using petroleum ether/EtOAc (3:2). Running the plate two times afforded 6 mg of obliquol B (2). The dried leaves of *P. obliquum* (300 g) were extracted with ethanol at room temperature for 3 days. After evaporation of the solvent using a rotavaporator 8 g of crude extract was obtained. The ethanolic extract was fractionated by flash column chromatography using petroleum ether/EtOAc gradient (0%, 25%, 50%, 75%, and 100% EtOAc) to afford 5 fractions. From fractions 4 and 5, 20 mg of the known 2′-hydroxy-3,4,4′,6-tetramethoxychalcone (**5**) were isolated as orange needles.

Fractions 4 and 5 were re-purified by flash column chromatography using petroleum ether/EtOAc (2:3) from which a mixture of two compounds was obtained. Further purification by preparative thin layer chromatography using CH_2Cl_2/MeOH (9:1) as eluent afforded 50 mg of a new 4-chromanone derivative (**3**) and 100 mg of the known 3-farnesyl-*p*-hydroxybenzoic acid (**4**).

5.4. Antibacterial activities

The antibacterial properties of compounds **1**, **2**, **3**, and **5** were evaluated against *E. coli* and *S. epidermis* (isolates characterized and stored at the division of Applied Microbiology, Lund University). *E. coli* were grown in LB-broth medium, *S. epidermis* were grown in a buffered peptone water medium at 37 °C. The minimal inhibitory concentration of the compounds previously dissolved in DMSO (dimethyl sulfoxide) was estimated by the broth dilution method, using an inoculum of 10^6–10^7 cells/mL. The antibacterial properties were determined by measuring the optical density at 620 nm (OD_{620}) read after 24 h incubation at 37 °C. All the experiments were performed in duplicate.

5.5. Cell lines and reagents

The 5.1 cell line is a Jurkat derived clone stably transfected with a plasmid containing the luciferase gene driven by the HIV-1-LTR promoter and was maintained in exponential growth in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% heat inactivated FCS, 2 mM L-glutamine, 1 mM Hepes and antibiotics.[23] The HeLa-Tet-On-Luc cell line was previously described.[24] Briefly, this cell line contains two plasmids: the pTET-ON codifies constitutively for rtTA protein that in response to doxycycline, gets active, and binds to the pTRE2hyg-Luc starting the synthesis of luciferase gene. The anti-IκBα mAb 10B was a gift from R.T. Hay (St. Andrews, Scotland), the mAb anti-tubulin was purchased from Sigma Co. (St. Louis, MO, USA), and anti-phospho-IκBα was from New England Biolabs (Hitchin, UK).

5.6. Luciferase assays

To determine NF-κB-dependent transcription of the HIV-1-LTR-luc, 5.1 cells were preincubated for 30 min with the compounds tested as indicated, followed by stimulation with TNFα (2 ng/mL) for 6 h. Then, the cells were lysed in 25 mM Tris–phosphate, pH 7.8, 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, and 7% glycerol. Luciferase activity was measured using an Autolumat LB 9510 (EG&G Berthold, USA) following the instructions of the luciferase assay kit (Promega, Madison, WI, USA) and protein concentration was measured by the Bradford method. The background obtained with the lysis buffer was subtracted from each experimental value, the relative light units (RLU)/mg of protein was calculated and the specific transactivation expressed as the percentage of transcriptional activity compared to TNF-α alone (fold induction) or by showing the absolute RLU numbers. All the experiments were repeated at least four times.

5.7. Western blots

5.1 cells (1×10^6 cells/mL) were stimulated with TNFα (2 ng/mL) in the presence or absence of the compounds for the indicated period of time. Cells were then washed with PBS and proteins extracted in 50 μl of lysis buffer (20 mM Hepes, pH 8.0, 10 mM KCl, 0.15 mM EGTA, 0.15 mM EDTA, 0.5 mM Na_3VO_4, 5 mM NaFl, 1 mM DTT, leupeptin 1 μg/mL, pepstatin 0.5 μg/mL, aprotinin 0.5 μg/mL, and 1 mM PMSF) containing 0.5% NP-40. Protein concentration was determined by the Bradford assay (Bio-Rad, Richmond, CA, USA) and 30 μg of proteins were boiled in Laemmli buffer and electrophoresed in 10% SDS/polyacrylamide gels. Separated proteins were transferred to nitrocellulose membranes (0.5 A at 100 V; 4 °C) for 1 h. Blots were blocked in TBS solution containing 0.1% Tween 20 and 5% non-fat dry milk overnight at 4 °C, and immunodetection of specific proteins was carried out with primary antibodies using an ECL system (Amersham, UK).

5.8. Obliquol A (1)

Yellowish oil; see Table 1 for ^1H and ^{13}C NMR data; HRESIMS *m/z* $M+H^+$ observed 289.2518, Calcd for $C_{20}H_{33}O$ 289.2531.

5.9. Obliquol B (2)

Yellowish oil; see Table 1 for ^1H and ^{13}C NMR data; HRESIMS *m/z* $M+Na^+$ observed 327.2281, Calcd for $C_{20}H_{32}NaO_2$ 327.2300.

5.10. 2-Methyl-2,2[(3′*E*)-4′,8′-dimethylnona-3′,7′-dienyl]-2,4-dihydro-2H-1-benzopyran-6-carboxylic acid-4-one (3)

Colorless oil; $[\alpha]_D^{22}$ +8.5° (*c* 4, chloroform); see Table 2 for ^1H and ^{13}C NMR data; HRESIMS *m/z* $M+H^+$ observed 357.2076, Calcd for $C_{22}H_{29}O_4$ 357.2066.

5.11. 4-(1-Oxo-4-pentenyl)veratrole (7)

Veratrole (1 g, 7.2 mmol) was dissolved in dry CH_2Cl_2 (15 mL). The mixture was cooled to 0 °C and $AlCl_3$ (2 g, 13 mmol) were added in portions over 10 min with vigorous stirring. After 5 min, 4-pentenoyl chloride (859 mg, 7.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise via addition funnel. The resulting brown solution was stirred at 0 °C for 3 h, then poured onto conc. hydrochloric acid (20 mL) and ice (20 g). The organic layer was separated, the aqueous layer was extracted with ether (2× 15 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was fractionated by chromatography on silica gel (petroleum ether/EtOAc, 4:1) to give 1.2 g (74%) of **7** as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 2.34 (2H, d, J = 7.7 Hz, 3′-H), 2.89 (2H, dd, J_1 = 7.6 Hz, J_2 = 2.5 Hz, 2′-H), 3.79 (6H, s, OCH_3), 4.87 (1H, d, J = 10.2 Hz, 5′-H), 4.96 (1H, d, J = 17.1, 5′-H), 5.76 (1H, m, 4′-H), 6.74 (1H, dd, J = 8.3 Hz, J = 2.5 Hz, 5-H), 7.4 (1H, d, J = 2.3 Hz, 3-H), 7.45 (1H, d, J = 8.3 Hz, 6-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.0 (C-3′), 36.8 (C-2′), 55.4 (OCH_3), 55.6 (OCH_3), 109.6 (C-6), 114.8

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for obliquol A (**1**) and obliquol B (**2**) ($CDCl_3$, δ 7.27 and 77.0)

Position	^{1}H (δ, multiplicity, J [Hz])		^{13}C	
	1	**2**	**1**	**2**
1	—	—	153.3 (s)	143.3 (s)
2	7.06 (d, J = 8.1)	6.73 (d, J = 1.8)	129.5 (d)	141.3 (s)
3	6.76 (d, J = 8.1)	—	115.2 (d)	120.7 (d)
4	—	—	134.9 (s)	135.8 (s)
5	6.76 (d, J = 8.1)	6.79 (d, J = 8)	115.2 (d)	115.1 (d)
6	7.06 (d, J = 8.1)	6.62 (dd, J_1 = 8, J_2 = 1.8)	129.5 (d)	115.5 (d)
1′	2.54 (t, J = 7.5)	2.50 (t, J = 7.5)	34.4 (t)	34.6 (t)
2′	1.63 (m)	1.64 (m)	31.6 (t)	31.4 (t)
3′	1.99 (m)	2.01 (m)	31.9 (t)	32.0 (t)
4′	5.41 (m)	5.40 (m)	129.8 (d)	129.7 (d)
5′	5.41 (m)	5.40 (m)	131.0 (d)	130.9 (d)
6′	1.99 (m)	2.01 (m)	32.7 (t)	32.6 (t)
7′	1.29 (m)	1.29 (m)	29.4 (t)	29.3 (t)
8′	1.29 (m)	1.29 (m)	29.2 (t)	29.2 (t)
9′	1.29 (m)	1.29 (m)	29.6 (t)	29.5 (t)
10′	1.29 (m)	1.29 (m)	29.7 (t)	29.6 (t)
11′	1.29 (m)	1.29 (m)	29.7 (t)	29.6 (t)
12′	1.29 (m)	1.29 (m)	31.9 (t)	31.9 (t)
13′	1.29 (m)	1.29 (m)	22.7 (t)	22.7 (t)
14′	0.89 (t, J = 7)	0.90 (t, J = 6.7)	14.2 (q)	14.1 (q)

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for 2-methyl 2-2 [(3′*E*)-4′,8′-dimethylnona-3′,7′-dienyl]-2,4-dihydro-2H-1-benzopyran-6-carboxylic acid-4-one (**3**) ($CDCl_3$, δ 7.27 and 77.0)

Position	^{1}H δ, multiplicity, J (Hz)	^{13}C δ
2	—	82.4 (s)
3	2.85 (d, J = 16.4); 2.72 (1H, d, J = 16.4)	47.2 (d)
4	—	191.4 (s)
4a	—	119.9 (s)
5	8.64 (d, J = 2.2)	129.9 (d)
6	—	122.0 (s)
7	8.19 (dd, J_1 = 8.7, J_2 = 2.2)	137.2 (s)
8	7.01 (d, J = 8.7)	118.8 (d)
8a	—	163.7 (s)
1′	1.84 (m); 1.73 (m)	39.3 (t)
2′	2.13 (m)	22.1 (t)
3′	5.08 (t, J = 6.6)	122.8 (d)
4′	—	136.2 (s)
5′	1.96 (m)	39.6 (t)
6′	2.04 (m)	26.6 (t)
7′	5.06 (t, J = 6.6)	124.1 (d)
8′	—	131.4 (s)
9′	1.66 (s)	25.6 (q)
4′-CH_3	1.57 (br s)	15.9 (q)
8′-CH_3	1.57 (br s)	17.6 (q)
2-CH_3	1.45 (s)	24.0 (q)
6-COOH	—	170.9 (s)

Table 3. MIC's values of compounds **1**, **2**, **3**, and **5**

Compound	MIC (μg/mL)	
	E. coli	*S. epidermis*
Ampicillin	5	1
1	5	2.5
2	5	5
3	100	50
5	10	10

(C-3), 122.2 (C-4), 129.7 (C-4′), 137.1 (C-5′), 148.6 (C-2), 152.8 (C-1), 197.5 (C-1′). HRESIMS *m/z* M+Na^+ observed 243.1002, calculated for $C_{13}H_{16}NaO_3$ 243.0997.

5.12. 4-(4-Pentenyl)veratrole (8)

A solution of **7** (900 mg, 4 mmol) in ethanol (10 mL) was added to freshly prepared amalgamated zinc (2 g) in concd HCl (10 mL) and water (10 mL), and the mixture was stirred vigorously under nitrogen for 16 h. After the extraction of the mixture with ether, the extract was washed with water, dried ($MgSO_4$), and the solvent was evaporated to leave a yellow oil of 4-(4-pentenyl)veratrole (**8**) (700 mg, 85%). NMR δ_H (300 MHz, $CDCl_3$): 1.66 (2H, pt, J_1 = 8.1, J_2 = 7.0 Hz, 2′-H), 2.05 (2H, q, J = 7.2 Hz, 3′-H), 2.53 (2H, t, J = 8.1 Hz, 1′-H), 3.78 (3H,s, OMe), 3.81 (3H, s, OMe), 4.94 (1H, d, J = 10.2 Hz, 5′-H), 4.99 (1H, d, J = 17.7, 5′-H), 5.79 (1H, m, 4′-H), 6.67 (2H, br s, 5-H and 3-H), 6.73 (1H, d, J = 8.4 Hz, 6-H). NMR (75 MHz, $CDCl_3$) δ 30.4 (C-2′), 32.9 (C-3′), 34.5 (C-1′), 55.3 (OCH_3), 55.4 (OCH_3), 110.7 (C-6), 111.3 (C-3), 114.3 (C-4), 119.8 (C-5), 134.6 (C-5′), 138.1 (C-4′), 146.7 (C-1), 148.3 (C-2). HRESIMS *m/z* M+Na^+ observed 229.1204, calculated for $C_{13}H_{18}NaO_2$ 229.1204.

5.13. 4-(4-Tetradecenyl)veratrole (10)

4-(4′-Pentenyl)veratrole (**8**) (125 mg, 0.61 mmol) and 1-undecene (260 μL, 1.2 mmol) were simultaneously added via a syringe to a stirring solution of Grubb's catalyst (53 mg, 0.06 mmol, 10% mol) in dry CH_2Cl_2 (15 mL). The flask was fitted with a condenser and refluxed under N_2 for 12 h. The reaction mixture was then reduced in volume and purified directly on a silica gel column eluting with 9.5:0.5 petroleum ether/EtOAc. 4-(4-Tetradecenyl)veratrole (**10**) was obtained as a light

yellow oil (153 mg, 74% yield, 4:1 *E*/*Z* as determined by the relative intensity of alkene ^{13}C peaks at 129.6 and 129.1 ppm). NMR δ_H (300 MHz, $CDCl_3$): 0.87 (3H, t, $J = 6.8$ Hz, 14′-H), 1.26 (14H, br s, 7′H to 13′-H), 1.65 (2H, q, $J = 7.6$ Hz, 2′-H), 1.99 (4H, m, 3′-H and 6′-H), 2.54 (2H, t, $J = 7.5$ Hz, 1′-H), 3.83 (3H, s, OMe), 3.85 (3H, s, OMe), 5.4 (m, 4′H and 5′H), 6.69 (2H, br s, 3-H and 5-H), 6.76 (1H, d, $J = 8.6$ Hz, 6-H). NMR δ_c (75 MHz, $CDCl_3$): 13.9 (C-14′), 22.5 (C-13′), 29.0 (C-8′), 29.2 (C-7′), 29.4 (C-9′), 29.5 (C-10′ and C-11′), 31.4 (C-2′), 31.8 (C-12′), 31.9 (C-6′), 32.5 (C-3′), 34.8 (C-1′), 55.6 (–OMe), 55.7 (–OMe), 111.0 (C-6), 111.6 (C-3), 120.0 (5), 129.6 (C-4′), 130.8 (C-5′), 135.2 (C-4), 146.9 (C-1), 148.6 (C-2). HRESIMS *m*/*z* $M+K^+$ observed 371.2364, calculated for $C_{22}H_{36}KO_2$ 371.2352.

5.14. Obliquol B (2)

To 30 mg (0.1 mmol) of 4-(4-tetradecenyl)veratrole in 0.5 mL of $CDCl_3$ in an NMR tube with a tightly sealed cap, 3.7 mg (0.02 mmol, 20%) of 2,6-di-*tert*-butyl-4-methylpyridine was added. To this solution, 25 μL of freshly distilled trimethylsilyl iodine (TMSI) was added. The solution was kept at room temperature, the reaction was monitored by NMR periodically. After 24 h the signals for the OCH_3 protons in the NMR spectrum had disappeared and a signal at 2.15 ppm indicated the presence of CH_3I. The brown solution was poured into 5 mL MeOH and the volatile components were removed at reduced pressure. The residue was purified by column chromatography using petroleum ether/EtOAc (4:1) as eluent, to afford obliquol B (**2**) as a yellowish oil (24 mg, 87%), identical in all respects with the natural product.

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References and notes

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