Peyssonenynes A and B, Novel Enediyne Oxylipins with DNA Methyl Transferase Inhibitory Activity from the Red Marine Alga *Peyssonnelia caulifera*

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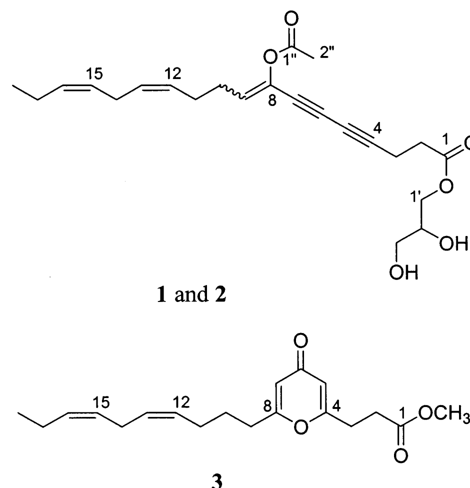
Two novel ω3 fatty acids, obtained as monoacyl glycerol derivatives, were isolated as DNA methyl transferase inhibitors following bioassay-guided fractionation of the Fijian red marine alga *Peyssonnelia caulifera*. Both active metabolites, peyssonenynes A (**1**) and B (**2**), possess an unusual enediyne motif, whereas an inactive co-metabolite, peyssopyrone (**3**), contains an unusual γ-pyrone functionality. The molecular structures of all three compounds were determined by NMR spectroscopy in combination with UV, IR, and MS data analysis. The instability of the enediyne monoacyl glycerol derivatives prevented their complete stereochemical assignments.

Recognition of the importance of DNA methyltransferases (e.g., DNMT-1) as well as other enzymes involved in regulating the mammalian cell cycle has led to a broad search for natural product modulators of these key systems.[1] DNMT-1 causes methylation of the cytosine phosphodiester linked guanine dinucleotide (CpG) by catalyzing the transfer of a methyl group from *S*-adenosylmethionine to the 5′ position on cytosine residues residing at CpG sites. In many cancers, promoters of tumor suppressor genes are silenced by hypermethylation at CpG sites, and thus, the inhibition of DNMT-1 could potentially reverse tumor growth. Thus, a homogeneous scintillation proximity assay was developed to screen for small molecule inhibitors of DNMT-1. It is hoped that new molecular scaffolds showing activity on these pathways may hold promise both as new cancer chemotherapeutics and as new "tool compounds" for study of the enzymes, their reaction mechanisms, and interaction with other cellular pathways.[2]

Screening of the organic extracts of marine algae and cyanobacteria for mechanism-based anticancer agents has been quite productive and has led to the discovery of new chemotypes showing antiproliferative properties.[3] In this regard, we found that the organic extract of a South Pacific red alga, *Peyssonnelia caulifera*, possessed strong inhibitory activity to the DNMT1 isoform; hence, it was subjected to a rigorous bioassay-guided isolation scheme, which resulted in two novel enediyne inhibitors, peyssonenynes A (**1**) and B (**2**). Biosynthetically, these new DNMT1 inhibitors appear to derive from an ω3 polyunsaturated fatty acid and, thus, represent novel oxylipin metabolites.

Structurally diverse oxylipins have been isolated from a large number of red marine algae and result from a widespread lipoxygenase metabolism of predominantly C_{18} and C_{20} ω3 fatty acids in these algae.[4] Marine oxylipins are remarkable for the diversity of oxygenated, halogenated, and unsaturated functional groups they possess and also for the range of carbocycles they produce, which vary in ring size and position in the lipid chain.[5] For example, the red algal metabolite hybridalactone is a macrolactone derived from eicosapentaenoic acid (EPA) and is functionalized with epoxycyclopentyl and cyclopropyl rings.[6] Another red algal derivative of EPA, agardhilactone, incorporates a δ-pyrone in combination with an epoxycyclopentyl moiety.[7] However, among this diversity of functional groups found in red algal oxylipins, acetylenic moieties are considerably less common; the only other report of lipid acetylenes produced by a red alga is from *Liagora farinosa*.[8] Similarly, secondary metabolite glycerides are relatively rare and have been previously reported as red algal natural products only from *Liagora farinosa*,[8] *Peyssonnelia caulifera*,[9] and *Gracilariopsis lemaneiformis*.[10] Here, we report the isolation from *P. caulifera* of two enediyne ω3 monoacyl glycerides, peyssonenynes A (**1**) and B (**2**), together with a pyrone-containing ω3 fatty acid methyl ester, peyssopyrone (**3**). Previously reported metabolites from *P. caulifera* include two anti-HIV sesquiterpene hydroquinones,[11] a cytotoxic *homo*-monoterpene lactone,[12] a fatty acid ester, and a glycosidic diacyl glyceride.[9]



1 and **2**

3

Results and Discussion

The organic extract of *P. caulifera* collected near Yanuca Island, Fiji (1997), was crudely fractionated by normal-phase vacuum-liquid chromatography (NPVLC), and the fractions were submitted for bioactivity testing. Two relatively polar fractions (100% EtOAc and 5% MeOH/EtOAc) were active as DNA methyltransferase inhibitors (4.0 and

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Table 1. ^{13}C and ^{1}H NMR Data for Peyssonenyne A (**1**, $CDCl_3$), Peyssonenyne B (**2**, $CDCl_3$), and Peyssopyrone (**3**, C_6D_6) at 150 and 600 MHz, respectively

	peyssonenyne A (**1**)		peyssonenyne B (**2**)		peyssopyrone (**3**)	
atom no.	δ_C	δ_H (mult., *J*/Hz)	δ_C ^{a}	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)
1	171.8 C		171.8 C		171.7 C	
2	33.0 CH_2	2.61 (ddd, 14.4, 2.0, 1.0) 2.63 (ddd, 14.4, 9.1, 1.4)	32.8 CH_2	2.62 (m)	31.0 CH_2	2.05 (t, 8.6)
3	15.8 CH_2	2.65 (ddd, 14.4, 2.0, 1.4) 2.66 (ddd, 14.4, 9.1, 1.0)	15.6 CH_2	2.66 (m)	28.9 CH_2	2.24 (t, 8.6)
4	83.5 C		83.3 C		166.3 C	
5	65.81 C		65.7 C		114.08 CH	5.93 (s)
6	74.1 C		73.6 C		178.9 C	
7	69.8 C		70.0 C		114.13 CH	5.96 (s)
8	129.5 C		128.9 C		168.0 C	
9	131.1 CH	5.78 (t, 9.4)	131.1 CH	5.78 (m)	33.1 CH_2	1.89 (t, 7.7)
10	26.2 CH_2	2.13 (d, 10.5)	26.2 CH_2	2.13 (m)	26.91 CH_2	1.29 (m)
11	26.6 CH_2	2.13 (d, 10.5)	26.2 CH_2	2.13 (m)	26.86 CH_2	1.84 (br q, 7.3)
12	129.9 CH	5.40 (m)	130.5 CH	5.40 (m)	129.1 CH	5.22 (br q, 7.3)
13	128.2 CH	5.31 (m)	128.0 CH	5.31 (m)	129.9 CH	5.43 (m)
14	25.8 CH_2	2.75 (br t, 7.1)	b	2.75 (t, 7.4)	26.3 CH_2	2.74 (br t, 6.8)
15	127.1 CH	5.29 (m)	127.2 CH	5.29 (m)	127.7 CH	5.37 (m)
16	132.3 CH	5.38 (m)	132.2 CH	5.38 (m)	132.6 CH	5.43 (m)
17	20.8 CH_2	2.06 (br p, 7.6)	20.5 CH_2	2.06 (m)	21.3 CH_2	2.02 (br p, 7.6)
18	14.5 CH_3	0.97 (t, 7.6)	14.1 CH_3	0.97 (t, 7.4)	14.8 CH_3	0.93 (t, 7.6)
1′	65.83 CH_2	4.19 (dd, 14.4, 7.5) 4.24 (dd, 14.4, 5.8)	*b*	4.19 (m)		
2′	70.3 CH	3.96 (br s)	*b*	3.96 (m)		
3′	63.5 CH_2	3.63 (dd, 14.1, 7.6) 3.72 (dd, 14.1, 4.5)	*b*	3.63 (m) 3.72 (m)		
1″	168.4 C		168.1 C			
2″	20.8 CH_3	2.17 (s)	*b*	2.17 (s)		
OCH_3					51.7 CH_3	3.26 (s)

^{a} These ^{13}C data were obtained from a 2D HMBC experiment. ^{b} Shifts not obtainable from $CDCl_3$ HMBC data.

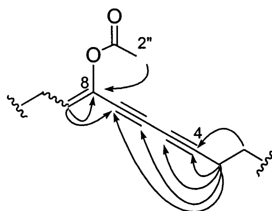

Figure 1. Key HMBC correlations for the enediyne partial structure of peyssonenyne A (**1**).

2.5 μg/mL, respectively). Solid-phase extraction (SPE) and reversed-phase HPLC of the 100% EtOAc VLC fraction yielded peyssonenyne A (**1**) as the major component. HRFABMS data for **1** gave a molecular formula of $C_{23}H_{30}O_6$ with an inherent nine degrees of unsaturation. After careful processing using Gaussian broadening of a truncated FID (free induction decay), the ^{13}C NMR spectrum for **1** revealed 23 resonances, interpreted from multiplicity-edited HSQC data as seven quaternary, seven methine, seven methylene, and two methyl carbons. In the ^{1}H NMR spectrum of **1**, its unsaturated fatty acid nature was immediately evident from five olefinic proton resonances (δ 5.31−5.79) accompanied by six deshielded methylene resonances (δ 1.80−2.80). Also apparent in the ^{1}H NMR spectrum were an acetate methyl singlet (δ 2.17), a pattern of midfield signals characteristic of a monosubstituted glycerol moiety (see Table 1, H_2-1′, H-2′, H_2-3′), and a shielded methyl triplet (δ 0.98).

Five of the nine degrees of unsaturation were accounted for by two carbonyl carbon and six olefinic carbon resonances in the ^{13}C NMR spectrum. The remaining four degrees of unsaturation were attributed to the presence of two acetylenic bonds, suggested by four midfield, low-intensity ^{13}C resonances (δ 83.5, 74.1, 69.8, and 65.8), none of which displayed an HSQC correlation. An acetylated enediyne partial structure (Figure 1) was delineated by two-, three-, four-, and five-bond HMBC correlations evident from a standard HMBC experiment optimized for 8 Hz (evolution delay d6 = 63 ms) and run at 400 MHz. The diyne segment was established through correlations from H_2-3 (δ 2.65) to all four acetylenic carbons (C-4 to C-7) and from H_2-2 (δ 2.62) to acetylenic carbon C-4 (δ 83.5). The contiguity of the C-8/C-9 double bond to the diyne arrangement was shown by correlations from H-9 (δ 5.78) to C-7 (δ 69.8) and C-8 (δ 129.5). Additionally, the acetate methyl protons (H-2″, δ 2.17) correlated only to C-8.

The unsaturated tail of the fatty acid chain (C-10 to C-18) could be assembled from COSY90 data and by chemical shift comparison with literature values (Table 1). HMBC correlations from H-9 to C-10/C-11 (δ 26.2/26.6) linked the enediyne moiety to this latter section. The *cis* geometry of the C-12 and C-15 olefins was evident from the shielded ^{13}C NMR shifts of the flanking and bis-allylic methylenes (δ 26.6, C-11; 20.8, C-17; 25.8, C-14).[13] HMBC correlations from H_2-2 and H_2-3 to the carboxylate carbon C-1 completed the C_{18} fatty acid backbone. Finally, an HMBC correlation from H_2-1′ (δ 4.22) to carboxylate C-1 linked the lipid residue with the isolated glycerol spin system, thus completing the enediyne monoacyl glycerol structure for **1**. Unfortunately, degradation of the compound during the acquisition of NMR data in $CDCl_3$ precluded determination of its C-8 double bond geometry or C-2′ glycerol methine stereocenter.

Peyssonenyne B (**2**) was isolated by HPLC of the 100% EtOAc NPVLC fraction as a minor component (0.4 mg). Considering the instability of **1** in $CDCl_3$, NMR data for **2** were acquired in C_6D_6. By ^{1}H NMR, compound **2** appeared to contain many of the same structural elements as compound **1**. In fact, analysis of its 2D NMR data (HSQC, HMBC, COSY90) led to the same bond connectivity as peyssonenyne A (Table 1). Thus, the likely relationship between **1** and **2** was that they were geometrical isomers about the C-8 double bond; however, we were hampered in these further investigations by insufficient intact mate-

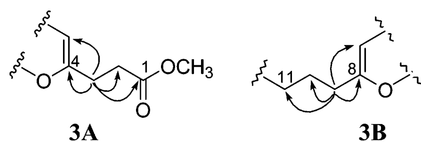

Figure 2. Partial structures for peyssopyrone (**3**) showing key HMBC correlations.

rial. In an effort to isolate more of these enediyne monoacyl glycerides for stereochemical analysis, the extracts of five other collections of *P. caulifera* (one from Madagascar and four from Papua New Guinea) were evaluated by a combination of VLC and subsequent reversed-phase HPLC profiling (see Supporting Information). None of these was found to contain any trace of the enediyne metabolites. Therefore, to directly compare the two compounds, ^{1}H NMR and HMBC spectra were acquired in $CDCl_3$ for peyssonenyne B (**2**, 80 μg/20 μL $\approx$ 10 μM) using a Microcoil NMR probe. While peyssonenynes A and B possessed nearly identical ^{1}H NMR spectra in this solvent, significant differences were observed between the C-6 (**1**, δ 74.1; **2**, 73.6) and C-8 (**1**, δ 129.5; **2**, 128.9) ^{13}C NMR signals for each compound (Table 1).

A paucity of protons in the enediyne moiety precluded the use of NOE data to investigate the C-8 geometries of **1** and **2**. Preliminary molecular modeling studies suggested that the 8*Z* isomer would possess an extended molecular conformation in contrast to the more folded 8*E* isomer; the latter was predicted to show long-range NOE interactions. Unfortunately, the 2D ROESY data we obtained at this point in the study (80 μg of compound **2**, microcoil probe) were of insufficient quality to assign this structural feature.

Peyssopyrone (**3**) was obtained by careful HPLC of fractions containing peyssonenynes **1** and **2**. While the ^{1}H NMR spectrum for compound **3** showed olefinic proton signals characteristic of an ω3 fatty acid, it did not contain midfield multiplets indicative of the monosubstituted glycerol moiety present in **1** and **2**. Rather, a methoxy methyl singlet at δ 3.26 suggested that **3** was a fatty acid methyl ester. HRCIMS data provided a molecular formula of $C_{19}H_{26}O_4$ for **3**, in agreement with the 19 dispersed resonances present in its ^{13}C NMR spectrum. Four of these ^{13}C resonances possessed chemical shifts indicative of oxygenation and/or unsaturation (δ 166.2, 168.0, 171.6, and 178.9). The two more deshielded resonances were assigned to the terminal ester group (δ_C 171.6; IR $\nu_{C=O}$ 1740 cm^{-1}) and a cross-conjugated ketone (δ_C 178.9; IR $\nu_{C=O}$ 1664 cm^{-1}), while the two more shielded ^{13}C shifts were assigned to olefinic carbons with pendant oxygen atoms. Determination of the nature of this unusual functional group was simplified by assignment of the remainder of peyssopyrone (**3**). A C-10 to C-18 fragment containing ω3 and ω6 *cis* double bonds was readily recognized in compound **3** from its COSY90 spectrum and by ^{13}C chemical shift comparison with NMR data for peyssonenynes A and B. Two further segments of the fatty acid chain were delineated by two- and three-bond HMBC correlations from H_2-3 (δ 2.24) to C-1, C-2, C-4, and C-5 (Figure 2, **3A**) and from H_2-9 (δ 1.89) to C-7, C-8, C-10, and C-11 (Figure 2, **3B**). However, the connectivity of these two segments was initially obscured by near overlap of the ^{13}C resonances for C-5 (δ 114.08) and C-7 (δ 114.13) as well as for their respective ^{1}H signals (δ 5.93 and 5.96). In fact, it was unclear from HSQC data whether the H-5 and H-7 ^{1}H NMR signals were a pair of closely spaced singlets or a pair of superimposed mutually coupled doublets (J = 9.3 Hz) arising from vicinal protons. Connection was further complicated by the absence of HMBC correlations to the C-6 carbonyl (δ 178.9). However, close examination of the HMBC spectrum finally revealed a "skewed" cross-peak representing two correlations: one from the broadened H-5 singlet resonance to C-4 and a second from the broadened H-7 singlet to C-8. In concert with molecular formula considerations (i.e., only "CO" was not assigned), these correlations required the placement of a ketone carbonyl at C-6 to complete a γ-pyrone ring structure in peyssopyrone (**3**).

Pure peyssonenynes A (**1**) and B (**2**) showed comparable activity (16 and 9 μM, respectively) in a DNA methyltransferase (DNMT-1) enzyme inhibition assay, while peyssopyrone (**3**) was inactive. As such, the peyssonenynes represent fundamentally new chemotypes to show inhibitory activity to this key enzyme target. However, a related enediyne motif has previously been reported in the antifungal metabolite siphonodiol, from the marine sponge *Siphonochalina truncata*.[14] Similar sulfonated derivatives, the callyspongins, were subsequently isolated from the marine sponge *Callyspongia*.[15] The latter metabolites show antifertilization activity against starfish gametes. Fischerellin A is a fresh/brackish water cyanobacterial metabolite (*Fischerella muscicola*) that contains two heterocyclic ring systems as well as an enediyne moiety. This metabolite showed potent inhibitory activity to photosystem-II.[16] The terrestrial plant natural product dendroarboreol B also possesses this enediyne motif.[17] However, none of these previously reported enediyne metabolites possesses an oxygenated "ene" component as found in the peyssonenynes. To our knowledge, this is the first reported naturally occurring enol acetate-containing enediyne.

It is intriguing to speculate on the potential biosynthetic relationship of the co-occurring peyssonenynes (e.g., **1**, **2**) and peyssopyrone (**3**). It is conceivable that the acetate functionality is cleaved, resulting in a diynone intermediate. This could then add two molecules of water at the β and δ positions and undergo tautomerization of the one at C-6 to produce a symmetrical cross-conjugated ketone. Condensation of the two ketonic centers with loss of water would yield peyssopyrone (**3**).

Experimental Section

General Experimental Procedures. UV data were acquired on a Beckman DU640B spectrophotometer, while IR data were obtained on a Nicolet 510 FTIR spectrophotometer. NMR spectra were recorded on Bruker Avance DPX 300, Bruker Avance DPX 400, and Bruker Avance DRX 600 spectrometers. Mass spectra were recorded on a Kratos MS50TC mass spectrometer. HPLC separations were performed using Waters 515 HPLC pumps, a Rheodyne 7725i injector, and a Waters 996 photodiode array detector.

Collection, Extraction, and Isolation Procedures. *Peyssonnelia caulifera* was collected by hand using scuba (6−8 ft) in 1997 at Yanuca Island, Fiji. The alga was stored at −20 °C in 70% EtOH until workup. A voucher specimen is available from W.H.G. (collection number VYI-5FEB97-05). The alga (365 g dry wt) was extracted three times with CH_2Cl_2−MeOH (2:1) to give a crude organic extract (2.17 g). A portion of the extract (2.03 g) was fractionated on silica gel by NPVLC to give nine fractions using a stepwise gradient of hexanes−EtOAc and EtOAc−MeOH. Fraction 7 (100% EtOAc) was further chromatographed on a Waters C_{18} solid-phase extraction cartridge (RP-18 SPE, 500 mg) using 7:3 MeOH−H_2O, 100% MeOH, and CH_2Cl_2. The fraction eluting in 7:3 MeOH−H_2O (2.9 mg) was subjected to RPHPLC (Varian microsorb-mv, 5 μm, 250 × 4.6 mm; 3:1 MeOH−H_2O, 0.7 mL/min) to yield, in succession, peyssopyrone (**3**, 0.7 mg, 0.03% of extract), peyssonenyne B (**2**, 0.4 mg, 0.02% of extract), and peyssonenyne A (**1**, 1.3 mg, 0.06% of extract). RPHPLC of VLC fraction 8 (3:1 EtOAc−MeOH) using the same conditions as before yielded more of peyssopyrone (**3**, 1.0 mg).

Biological Evaluation. The DNMT-1 assay was carried out as a homogeneous scintillation proximity assay (SPA) in the following manner. Using a 50 mM Tris pH 8.0 buffer in a 96-well Isoplate (Wallac, 1450-51), preparations of partially purified DNMT-1 (0.25 mg/mL) were combined with test compounds, biotinylated double-stranded polynucleotide synthetic substrate (poly(dI-dC)·poly(dI-dC) at 1 nM), and the methyl donor *S*-adenosyl-L-[*methyl*-^{3}H]methionine (^{3}H-SAM at 10 μM) and mixed for 3.5 h at 37 °C. Following addition of streptavidin-coated SPA beads, bead-associated methylated substrate was quantitated in a microplate scintillation counter.

Peyssonenyne A (1): colorless oil; UV (MeOH) λ_{max} (log ϵ) 208 (3.25) 215 (3.36), 240 (2.83), 253 (2.81) 267 (2.87), 282 (2.78) nm; ^{1}H and ^{13}C NMR data (CDCl$_3$), see Table 1; LRFABMS *m*/*z* (%) 403 (18), 391 (79), 341 (18), 307 (36), 289 (21), 136 (92), 107 (28), 89 (31); HRFABMS (nba) obsd [M + H]$^+$ *m*/*z* 403.2131 (calcd for $C_{23}H_{31}O_6$, 403.2121).

Peyssonenyne B (2): colorless oil; UV (MeOH) λ_{max} (log ϵ) 215 (2.28), 239 (2.01), 253 (2.04), 267 (2.08), 283 (2.05) nm; ^{1}H and ^{13}C NMR data (CDCl$_3$), see Table 1; ^{1}H NMR (C_6D_6, 600 MHz) δ 5.62 (1H, t, J = 6.9 Hz, H-9), 5.42 (2H, m, H-13, H-16), 5.37 (1H, m, H-15), 5.23 (1H, m, H-12), 3.91 (2H, d, J = 5.8 Hz, H$_2$-1′), 3.52 (1H, br s, H-2′), 3.29 (1H, m, H-3′), 3.23 (1H, m, H-3′), 2.71 (2H, t, J = 6.6 Hz, H$_2$-14), 2.07 (2H, m, H$_2$-3), 2.02 (1H, m, H-17), 1.99 (2H, m, H$_2$-10), 1.92 (2H, m, H$_2$-11), 1.90 (2H, m, H$_2$-2), 1.55 (3H, s, H$_3$-20), 0.92 (3H, t, J = 7.5 Hz, H$_3$-18); ^{13}C NMR (C_6D_6, 150 MHz) δ 170.9 (C, C-1), 167.2 (C, C-19), 130.6 (CH, C-9), 130.4 (CH, C-16), 129.9 (2CH, C-8, C-13), 128.1 (CH, C-12), 126.8 (CH, C-15), 83.8 (C, C-4), 74.4 (C, C-7), 70.5 (C, C-7), 69.9 (CH, C-2′), 65.7 (C, C-5), 65.3 (CH$_2$, C-1′), 63.2 (CH$_2$, C-3′), 32.4 (CH$_2$, C-2), 26.4 (CH$_2$, C-11), 26.2 (CH$_2$, C-10), 25.4 (CH$_2$, C-14), 21.3 (CH$_2$, C-17), 19.5 (CH$_3$, C-20), 15.4 (CH$_2$, C-3), 15.2 (CH$_3$, C-18); HRFABMS (nba) obsd [M + H]$^+$ *m*/*z* 403.2131 (calcd for $C_{23}H_{31}O_6$, 403.2121).

Peyssopyrone (3): colorless oil; UV (MeOH) λ_{max} (log ϵ) 212 (3.18), 246 (3.19) nm; IR ν_{max} (film) 2925, 1740, 1664, 1621, 1393 cm^{-1}; ^{1}H and ^{13}C NMR data (C_6D_6), see Table 1; LRFABMS *m*/*z* (%) 319 (100), 235 (2), 209 (5), 149 (4), 136 (6), 109 (5), 89 (6); HRCIMS obsd [M + H]$^+$ *m*/*z* 319.1910 (calcd for $C_{19}H_{27}O_4$, 319.1909).

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Supporting Information Available: 1D and 2D NMR data in CDCl$_3$ for peyssonenyne A (**1**) and in C_6D_6 for peyssonenyne B (**2**) and peyssopyrone (**3**); ^{1}H NMR and HMBC microcoil spectra for **2** in CDCl$_3$; HPLC trace profiles of other *P. caulifera* extracts from Papua New Guinea and Madagascar collections. This material is available free of charge via the Internet at http://pubs.acs.org.

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