Proctoriones A−C: 2-Acylcyclohexane-1,3-dione Derivatives from *Peperomia proctorii*

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The structures of three new compounds isolated from *Peperomia proctorii*, named proctoriones A−C, have been established by spectroscopic and chemical methods as 2,3-dihydro-5,8-dihydroxy-2-pentadecyl-4*H*-benzopyran-4-one (**1**) and enolic forms of 4-hydroxy-2-octadecanoylcyclohexane-1,3-dione (**2**) and 4-hydroxy-2-octadec-(11*Z*)-enoylcyclohexane-1,3-dione (**3**).

Recent studies have shown that the phytochemistry of *Peperomia*[1] (Piperaceae) differs significantly from that of the closely related and more well-studied genus *Piper*.[2] Many *Peperomia* metabolites, especially those of mixed acetate and mevalonate biogenesis, possess interesting biological activity.[1] We have examined extracts of *Peperomia proctorii* Yuncker, one of the 13 *Peperomia* species endemic to Jamaica.[3] *P. proctorii* is a small glabrous succulent herb of infrequent occurrence that grows in restricted and vulnerable habitats on rock ledges in woodlands.[3]

Chromatography of the hexane extract of whole plants of *P. proctorii* resulted in the isolation of proctoriones A, B, and C (**1**−**3**), in order of increasing polarity.

Proctorione A (**1**) was obtained as a yellow solid, mp 100−103 °C, and HREIMS provided a molecular formula of $C_{24}H_{38}O_4$, requiring six degrees of unsaturation. The IR bands at 3481, 3232, 1640, 1622, and 1584 cm^{-1} indicated the presence of hydroxyl, hydrogen-bonded carbonyl, and aromatic groups. The ^1H NMR spectrum of **1** (Table 1) contained a singlet at δ 10.94 ascribable to a chelated hydroxyl group, a pair of *ortho*-coupled one-proton aromatic doublets at δ 7.08 and 6.43, a fairly complex one-proton multiplet at δ 4.49 due to an oxymethine group, and signals for two deshielded methylene groups at δ 2.75 (2H) and 1.90 and 1.73. In addition, there were a large methylene envelope (20H) centered at δ 1.4, a distorted methyl triplet at δ 0.87, and a broad peak at δ 4.93 for an unchelated hydroxyl group. Associations in the $^1H-^1H$ COSY spectrum indicated vicinal coupling between the oxymethine proton and both deshielded methylene groups, with the methylene pair at higher field (δ 1.90, 1.73) also coupled to protons in the methylene envelope; this established the side-chain and C-3 as branching from the oxygen-substituted C-2.

The ^{13}C NMR spectrum of **1** (Table 1) showed the expected carbonyl signal at δ 197.65 and peaks for six aromatic carbons; all the remaining peaks were due to sp^3 centers. The most deshielded of the sp^3 carbons occurred at δ 78.9, 42.6, and 34.7, and all the other carbons resonated upfield of δ 32. On the basis of an HMBC correlation, the carbonyl group was located at position C-4, vicinal to the deshielded C-3 methylene group. Positions C-1 through C-4 were then fused to the aromatic ring, fulfilling the requisite hydrogen deficit. A hydroxyl group, chelated to the carbonyl, was placed at C-5. Strong three-bond and weak two-bond HMBC cross-peaks in the aromatic region enabled placement of the second hydroxyl group at C-8. Key connectivities were C-5→OH-5,H-6, H-7 and C-8a→OH-8,H-7; these ruled out the alternative isomer in which the second nonchelated hydroxyl group is located at C-6.

Table 1. NMR Data for Proctorione A (**1**)

position	δ_C	δ_H (mult; $J_{H,H}$ in Hz)	HMBC[a]
2	78.9	4.49 (ddd; 9.4, 5.6, 3.8)	3, 1′
3	42.6	2.78 (dd; 17.0, 11.0)	1′
		2.72 (dd; 17.0, 3.9)	
4	197.7		3
4a	107.9		
5	154.5		OH-5, 6, 7,
6	108.3	6.43 (d; 8.6)	OH-5
7	124.1	7.08 (d; 8.6)	OH-8
8	136.7		7
8a	146.9		7, OH-8
1′	34.7	1.9 (m; 5.7)	3
		1.74 (dddd;14.0, 5.7)	
2′	31.9	1.26 (m)	
3′−12′	29.4−29.7	1.20−1.64 (m)	
13′	24.9	1.50 (m)	
14′	22.9	1.20−1.64 (m)	15′
15′	14.1	0.87 (t; 5.0)	
OH-5		10.94 (s)	
OH-8		4.93 (s)	

[a] Protons correlating with carbon shift.

Proctorione B (**2**), a colorless solid, mp 45−48 °C, gave a molecular ion in the HREIMS corresponding to a formula of $C_{24}H_{42}O_4$. Infrared absorptions at 3426, 1659, 1557, and 1445 cm^{-1} provided evidence for hydroxyl, chelated carbonyl, and vinyl functionalities. The ^1H NMR spectrum of **2** (Table 2) was initially determined using a normal spectral width (12 ppm). In this window the peaks at lowest field were a well-defined multiplet at δ 4.08 due to an oxymethine proton and a broad signal at δ 4.04 for an unchelated hydroxyl group. There were also three deshielded methylene pairs (δ 3.16−1.80) and signals for a long saturated side chain consisting of a methylene envelope (30H) centered at δ 1.6 and a distorted methyl triplet at δ 0.88. Analysis of the $^1H-^1H$ COSY spectrum led to the formulation of two linear protonated sequences. The oxymethine and two of the deshielded methylene groups formed one

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Table 2. NMR Data for Proctoriones B (**2**) and C (**3**)

	2		**3**		
position	δ_C	δ_H (mult; $J_{H,H}$ in Hz)	δ_C	δ_H (mult; $J_{H,H}$ in Hz)	HMBC[a] **2**, **3**
1	197.9		197.9		OH-1[b], 5[b], 6[b]
2	110.3		110.2		OH-1[b], 6[b]
3	195.5		193.5		OH-4[b], 4[b], 5-ax[c]
4	71.6	4.08 (ddd; 15.0, 7.5, 2.0)	71.6	4.08 (ddd; 15.0, 7.5, 2.0)	OH-4[b], 5-ax, 6
5	27.1	1.80 (ddt; 15.0, 13.2, 8.4) 2.40 (m)	27.1	1.81 (ddt; 15.0, 13.2, 8.4) 2.40 (m)	OH-4[b], 4[b], 6[b]
6	31.3	2.80 (m)	31.8	2.76 (m)	OH-1[b], 5-ax[b]
1′	206.1		206.1		2′, 3′
2′	40.3	2.96 (ddd; 13.0, 7.8, 5.3) 3.06 (ddd; 13.0, 7.8, 5.3)	40.2	2.96 (ddd; 13.0, 7.8, 5.3) 3.09 (ddd;13.0, 7.8, 5.3)	3′
3′	24.5	1.62 (m)	24.5	1.62 (m)	2′
4′	29.3				2′
5′	29.5		29.1−29.7	1.20−1.42 (m)	11′[c], 12′[c]
6′−9′					
10′			27.2	2.01 (m)	11′[c], 12′[c]
11′			129.9	5.35 (d; 10.5)[d]	10′[c], 13′[c]
12′	29.4−29.7	1.20−1.42 (m)	129.8	5.35 (d; 10.5)[d]	10′[c], 13′[c]
13′			27.2	2.01 (m)	11′[c], 12′[c]
14′			29.1−29.7	1.20−1.42 (m)	
15′					
16′			31.6		14′[c], 15′[c], 18′[c]
17′	22.7	1.28 (m)	22.5	1.48 (q; 7.5)	18′
18′	14.1	0.88 (t; 7.0)	14.1	0.88 (t; 7.5)	
OH-1		18.28 (s)		18.26 (s)	
OH-4		4.04 (br s)		4.01 (br s)	

[a] Protons correlating with carbon shift. [b] **2** only. [c] **3** only. [d] From ^{13}C satellites.

sequence, i.e., H-4−H_2-5−H_2-6. The *J* values in the multiplets for this fragment indicated that it was part of a six-membered ring with the oxymethine H-4 being ψ-axial and the hydroxyl group ψ-equatorial. In the other sequence, the third deshielded methylene pair (δ 2.96, 3.06) was coupled into the methylene envelope via a CH_2 group at δ_H 1.62, giving the H_2-2′−H_2-3′−H_2-4′ through H_3-18′ side chain.

There were HMBC cross-peaks from three lowfield carbons to the methylene, methine, and hydroxyl protons of the first sequence. The C-3→H-4,OH-4,H_2-5 correlations suggested that this carbon (δ 195.5) was vicinal to the hydroxymethine group. The C-1→H_2-6,H_2-5 correlations placed C-1 (δ 197.9) vicinal to C-6, and the six-membered ring was completed by C-2, (δ 110.3), which showed a cross-peak to H_2-6. The chemical shifts of C-1, C-2, and C-3 (δ 197.9, 110.3, and 195.5) corresponded closely to those observed for an enolized β-triketone system.[4] The remaining carbonyl signal (δ 206.1) showed two- and three-bond correlations to protons in the side chain to provide the acyl group, which was placed at C-2. This completed the enolized β-triketone and the structure of proctorione B (**2**) except for the C-1 enolic proton; this was observed at δ 18.28 when the ^1H NMR spectrum was acquired in a spectral window of 20 ppm. HMBC cross-peaks between this signal and those for C-1, C-2, and C-6 established that in **2** the C-1 carbonyl is enolized and ruled out the alternative C-3 enol.

Proctorione C (**3**), an oil, had a molecular formula of $C_{24}H_{40}O_4$. Similarities in the spectral data to that of proctorione B (**2**) indicated that **3** was also a 2-acylcyclohexane-1,3-dione derivative. From the molecular formula and NMR signals for two vinyl methine groups (Table 2), it was deduced that **3** was a side chain dehydro derivative of **2**.

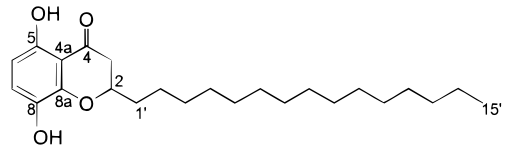
The vinyl protons in the side chain of **3** are degenerate, and the ^1H NMR signal (δ 5.35, 2H) gave no coupling or stereochemical information. The *Z* stereochemistry was deduced by analysis of the ^{13}C satellites, which gave $^3J_{H,H} = 10.5$ Hz, with a chemical shift difference between the vinyl protons of 0.004 ppm (2 Hz at 500 MHz). Additionally, the carbons determined by COSY and HMBC to be allylic to the double bond both appeared at δ 27.2, corresponding to known chemical shifts (δ 27.2)[5] of carbons allylic to cis double bonds and differing significantly from carbon shifts of positions allylic to trans double bonds (δ 32.5).[5]

The double bond was tentatively placed at C-11′ by comparison of the ^{13}C NMR shifts of the vinyl carbons with reported data for model compounds. Gunstone and co-workers have correlated chemical shifts of vinyl carbons in monoene C_{18}-*cis*-alkenoic acids with double-bond position and configuration.[6] The chemical shifts of the vinyl carbons of proctorione C (**3**; δ 129.9, 129.8) corresponded more closely to those of octadec-(11*Z*)-enoic acid (δ 129.89, 129.86) than to octadec-(9*Z*)-enoic acid (δ 129.78, 130.02), with the latter being almost identical to the values reported for octadec-(9*Z*)-enoylcyclohexane-1,3-dione (δ 130.0, 130.0).[9c,10a]

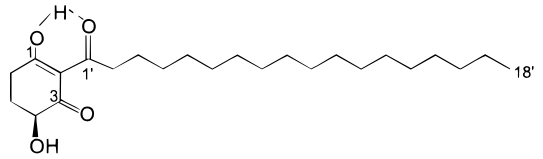
Confirmation of the C-11′ location of the double bond of **3** was obtained by methylthiomethylation−MS.[7] LREIMS of the product obtained by treatment of the acetate derivative (**4**) with dimethyl disulfide produced major ions at *m*/*z* 383 (20%) and *m*/*z* 145 (54%), corresponding to cleavage of a C-11′−C-12′ dithiomethyl adduct (**5**).

The residue of polar fractions obtained from chromatography of the acetone extract of *P. proctorii* was acetylated. Further chromatography of the crude product afforded the diacetate of (*E*)-*N*-feruoyltyramine, identified from its spectral data and comparison of these and physical constants with literature values.[8]

Proctoriones A−C (**1**−**3**) are likely of acetogenin and fatty acid biogenetic origin. Proctoriones B and C (**2**, **3**) are closely related to the series of kairomonal 2-acylcyclohexane-1,3-diones identified in the mandibular gland secretion of larvae of the Mediterranean flour moth *Ephestia kuehniella*.[9] In these kairomones, the C_{16} and C_{18} side chains are all unsaturated at one or more positions, with the Δ^9 isomer of proctorione C (**3**) being one member of the C_{18}



1



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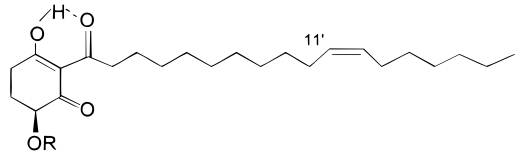
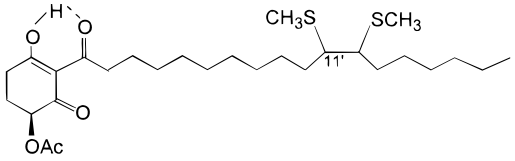


3 R = H

4 R = Ac



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group. The occurrence of these lepidopterous kairomones in plants is not unprecedented, as similar structures have been reported from *Virola* species (Myrsinaceae).[10] The accumulation of such large quantities in *P. proctorii* (0.5% of dried plant material) is intriguing, but at present the ecological significance remains a matter for speculation.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. IR spectra were recorded on a FT−IR SPECTRUM 1000 spectrometer with KBr pellets for solids or NaCl disks for liquids. UV spectra were recorded in EtOH on a Perkin-Elmer UV/VIS/NIR Lambda 19 spectrometer. NMR spectra were obtained on Varian GEMINI-200 and UNITY-500 spectrometers with $CDCl_3$ as solvent and TMS as internal standard. EIMS were obtained at 70 eV on VG 70-250S mass spectrometer. Adsorption column chromatography was performed with Si gel 60 (230−400 mesh). TLC analysis was performed with Whatman precoated Si gel 60 F_{254} plates. Spots were visualized under UV and by spraying with 4% phosphomolybdic acid in 5% H_2SO_4 followed by heating.

Plant Material. Entire plants of *P. proctorii* were collected at Mango Tree Hill, Trelawny and Gourie Forest, Manchester, Jamaica, in September 1996. A voucher specimen (no. 34251) is deposited in the Herbarium, Department of Life Sciences, University of the West Indies, Mona, Jamaica.

Extraction and Isolation. The dried, ground plant material (83 g) was percolated with hexane at room temperature, and the extract taken to dryness under reduced pressure. The hexane extract (9 g) was chromatographed over Si gel and eluted with Me_2CO−hexane mixtures. The residue from the 5% Me_2CO−hexane fraction (3.3 g) was rechromatographed, eluting with EtOAc−hexane mixtures. Proctorione A (**1**) (14 mg) crystallized from the 5% EtOAc−hexane fractions. The residue from evaporation of the 10% EtOAc−hexane fractions was triturated with MeOH leaving a MeOH-insoluble white solid consisting of proctorione B (**2**) (20 mg). Proctorione C (**3**) (408 mg) was obtained as an oil after evaporation of the 15% EtOAc−hexane fraction.

The Me_2CO extract (2 g) of the marc after hexane extraction was chromatographed, eluting with Me_2CO−hexane mixtures. The residue (497 mg) from the 70% Me_2CO−hexane fractions was stirred overnight with Ac_2O−pyridine. Workup provided a gum (250 mg) that was chromatographed twice, again eluting with Me_2CO−hexane. Fractions eluted with 40% Me_2CO−hexane provided (*E*)-*N*-feruolyltyramine diacetate (40 mg), identified by comparison of physical and spectral data to literature values.[8]

Proctorione A (1): yellow needles (EtOAc−hexane); mp 100−103°; $[\alpha]_D$ −75.0° (*c* 0.01, EtOH); UV λ_{max} (log ϵ) 242 (5.03), 276 (4.49) nm, +KOH (log ϵ) 262 (5.09), 292 (4.99) nm; IR ν_{max} 3481, 3232, 1640, 1622, 1584 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 390 $[M]^+$ (71), 179 (74), 153 (100), 124 (6); HREIMS *m*/*z* 390.2772 (calcd for $C_{28}H_{38}O_4$) 390.2770.

Proctorione B (2): white solid (MeOH), mp 45−48°; $[\alpha]_D$ +53.6° (*c* 0.01, EtOH); UV λ_{max} (log ϵ) 232 (4.94), 272 (5.07) nm, +KOH (log ϵ) 268 (5.29) nm; IR ν_{max} 3426 1659, 1557, 1445 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 394 $[M]^+$ (71), 376 (13), 358 (8), 330 (6), 183 (100), 170 (40), 155 (17), 137 (84); HREIMS *m*/*z* 394.3074 (calcd for $C_{24}H_{42}O_4$ 394.3084).

Proctorione C (3): oil, $[\alpha]_D$ −50° (*c* 0.01, EtOH); UV λ_{max} (log ϵ) 234 (4.40), 270 (4.65) nm, +KOH (log ϵ) 268 (4.78) nm; IR ν_{max} 3435, 1669, 1654, 1634 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; EIMS *m*/*z* 392 $[M]^+$ (83), 183 (68), 155 (56), 127 (100); HREIMS *m*/*z* 392.2911 (calcd for $C_{24}H_{40}O_4$ 392.2927).

Proctorione C Acetate (4). The residue (1.30 g) obtained after evaporation of the MeOH triturate from the isolation of **2** was stirred with Ac_2O and pyridine for 12 h. The oily product (1.0 g) obtained on workup was chromatographed on a Si gel column in an Me_2CO−hexane solvent system to afford proctorione C acetate (**4**) (71 mg) as a white solid: mp 55−58 °C; IR ν_{max} 1736, 1660, 1535 cm^{-1}; ^1H NMR (500 MHz) δ 5.35 (2H, m H-11′, H-12′), 5.32 (1H, dd, J = 13.4, 5.2 Hz, H-4), 3.05, 2.94 (each 1H, ddd, J = 13.4, 7.6, 5.0 Hz, H-2′), 2.88−2.83 (3H, m, H-5, H-6), 2.22 (3H, s, $OCOCH_3$), 2.13 (1H, m, H-5), 2.01 (4H, m, H-10′, H-13′), 1.62 (2H, m, H-3′), 1.22−1.38 20H, m, H-4′−H-9′, H-14′−H-17′), 0.88 (3H, t, J = 7.0 Hz, H-18′); ^{13}C NMR (125 MHz) δ 206.5 (C-1′), 196.6 (C-1), 189.5 (C-3), 170.2 ($OCOCH_3$), 129.9 (C-11′), 129.9 (C-12′), 111.4 (C-2), 72.6 (C-4), 40.5 (C-2′), 31.2 (C-6′), 29.8, 29.7, 29.5, 29.4, 29.3, 29.0, 27.2 (C-4′−C10′, C-13′−C16′), 24.5 (C-3′), 24.3 (C-5), 22.7 (C-17′), 20.9 ($OCOCH_3$), 14.1 (C-18′); EIMS *m*/*z* 434 $[M]^+$ (7), 392 (36), 183 (26), 165 (100); HREIMS *m*/*z* 434.3022 (calcd for $C_{26}H_{42}O_5$ 434.3032).

Methylthiomethylation of 4. Proctorione C acetate (**4**) (0.22 mg) in hexane (500 μL) was treated with dimethyl disulfide (250 μL) and I_2 (2.6 mg in 150 μL Et_2O), and the reaction mixture was kept overnight at 40−45 °C. The mixture was allowed to cool to room temperature, diluted with hexane (500 μL), and washed with 0.1 M $Na_2S_2O_3$ (2 × 200 μL). The organic phase was removed, the aqueous solution washed with hexane (3 × 200 μL), and the combined organic extracts dried (Na_2SO_4), filtered, and evaporated in a stream of argon to produce an amorphous residue: EIMS *m*/*z* 408 (30), 383 (20), 272 (29), 257 (19), 229 (36), 190 (17), 180 (94), 173 (57), 163 (31), 145 (54), 105 (48), 91 (100), 81 (55), 55 (54).

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Supporting Information Available: Structure and NMR data for (*E*)-*N*-feruloyltyramine diacetate (**6**). This material is available free of charge via the internet at http://pubs.acs.org.

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