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Glánvillic Acids A and B and Methyl Capucinoate A, New Metabolites Isolated from the Caribbean Sponges *Plakortis halichondrioides* and *Plakinastrella onkodes*

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Glánvillic acids A (**2**) and B (**3**) and the cytotoxic cyclic peroxides methyl capucinoate A (**4**) and **5** were isolated from the Dominican marine sponges *Plakortis halichondrioides* and *Plakinastrella onkodes*, respectively. The structures have been elucidated by spectroscopic analysis of **4** and **5** and of methyl glánvillates A (**6**) and B (**7**).

Marine sponges continue to attract attention as a rich source of structurally novel cytotoxic secondary metablites that are potential lead compounds for the development of new anticancer drugs.[1] As part of an ongoing search for new bioactive metabolites from tropical sponges,[2] it was found that crude extracts of the Dominican sponges *Plakortis halichondrioides* (Wilson, 1902) and *Plakinastrella onkodes* (Uliczka, 1929) (order Homosclerophorida, family Plakinidae) exhibited in vitro cytotoxicity. Fractionation of the *P. halichondrioides* extract led to the isolation of the known cytotoxic cyclic peroxide **1**[3] and the new inactive metabolites glánvillic acids A (**2**) and B (**3**), while fractionation of the *P. onkodes* extract led to the isolation of the new cytotoxic cyclic peroxide methyl capucinoate A (**4**) and the previously reported, but incompletely characterized, aromatic peroxide **5**.[4] The structures of the new metabolites **2**, **3**, and **4** and the complete characterization of **5** are described below.

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Results and Discussion

Plakortis halichondrioides (Wilson, 1902) was harvested by hand using scuba in Prince Rupert Bay, southwest of Glánvillia, Dominica, in July 1997. Freshly collected sponge was frozen on site and transported frozen to Vancouver. The sponge was repeatedly extracted with MeOH. The MeOH extracts were combined, concentrated in vacuo, and then partitioned between H_2O and EtOAc. Bioassay-guided fractionation of the EtOAc soluble materials via Sephadex LH-20 and flash silica gel and reversed-phase column chromatographies followed by reversed-phase HPLC gave a sample of pure peroxide **1** and a fraction that contained a ~1:2 mixture of glánvillic acids A (**2**) and B (**3**). The mixture of **2** and **3**, obtained as a pale yellow optically active oil, resisted all attempts at further fractionation. In the positive-ion HRFABMS, the mixture of **2** and **3** gave $[M + H]^+$ ions at *m/z* 307.2272 and 295.2273, consistent with the molecular formulas $C_{19}H_{30}O_3$ and $C_{18}H_{30}O_3$ for A and B, respectively. The mixture of glánvillic acids streaked on normal phase silica gel TLC. One resonance at δ 139.9 (C-3) in the ^{13}C NMR spectrum of the mixture was very broad, and not all the carbons required by the molecular formulas could be accounted for. These observations sug-

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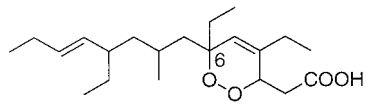

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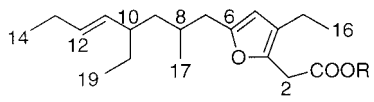

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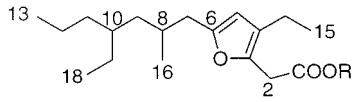

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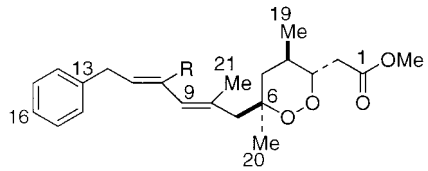

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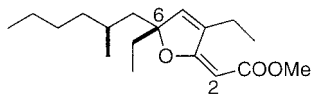

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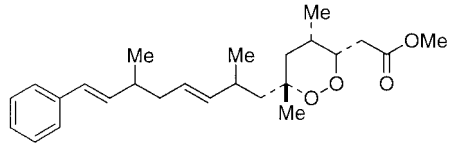

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gested that the compounds contained a carboxylic acid functionality. The mixture was reacted with diazomethane to give the methyl esters **6** and **7**, which were routinely separated by normal phase HPLC.

Methyl glánvillate A (**6**) was obtained as a clear optically active oil that gave a $[M + H]^+$ ion in the HRFABMS at *m/z* 321.2428, appropriate for a molecular formula of $C_{20}H_{32}O_3$, consistent with glánvillic acid A (**2**) having undergone monomethylation. The ^1H, ^{13}C, DEPT, and HMQC NMR data obtained for **6** identified five methyl, six methylene, five methine, and four quaternary carbons. A series of downfield ^{13}C NMR resonances were assigned to a disubstituted olefin (δ 133.3 (CH), 132.1 (CH)), a trisubstituted furan (δ 154.2 (C), 140.2 (C), 124.0 (C), 107.6 (CH)), and an ester carbonyl (δ 170.4). These funtionalities accounted for all five sites of unsaturation demanded by the molecular formula. The observation that a sharp methyl singlet at δ 3.67 in the ^1H NMR spectrum correlated to a carbon resonance at δ 52.0 in the HMQC experiment confirmed the presence of a methyl ester.

COSY, HMQC, and HMBC data routinely established the spin systems including H-8 and Me-17 through H-10, H-11 through Me-14, and those of the H-15/Me-16 and H-18/Me-19 ethyl branches. Both the OMe resonance (δ 3.67) and a methylene resonance at δ 3.55 (s, H-2) showed HMBC correlations to the carbonyl at δ 170.4, establishing the connectivity between C-1 and C-2. In the COSY experiment two weak allylic correlations were observed between the isolated methylene singlet resonating at δ 3.55 (H-2) and a broad singlet at δ 5.82 (H-5) and a quartet at δ 2.31 (H-15). In the HMBC experiment the H-2 (δ 3.55), H-5 (δ 5.82), H-15 (δ 2.31), and Me-16 (δ 1.10) resonances were all observed to correlate with the furan C-4 resonance at δ 124.0, and both the H-2 (δ 3.55) and H-5 (δ 5.82) resonances correlated with the C-3 resonance at δ 140.2. In addition, HMBC correlations were observed between both H-5 (δ 5.82) and the allylic methylene resonances at δ 2.45 and δ 2.34 (H-7/7′) and C-6 at δ 154.2, and between H-7/7′ (δ 2.45 and 2.34) and H-15 (δ 2.31) and the carbon resonance at δ 107.6 (C-5). These observations unambiguously established the presence of a 2,3,5-trisubstituted furan, the nature of the substituents, and the connectivity from C-1 through C-7 inclusive.

Further correlations observed in the HMBC data of methyl glánvillate A (**6**) extended the chain to complete the structural assignment. HMBC correlations between the H-7 methylene resonances (δ 2.45 and 2.34) and C-8 (δ 30.0), C-9 (δ 42.4), and C-17 (δ 19.2), and between Me-17 (δ 0.82) and C-7 (δ 36.4), established the connectivity between C-7 and C-8 and extended the chain to C-9. Since the H-18 methylene resonance at δ 1.14 and the Me-19 resonance at δ 0.80 both correlated with the C-10 resonance at δ 42.2, the C-18/Me-19 ethyl branch was placed at C-10. Finally, HMBC correlations observed between the two olefinic resonances at δ 5.36 (H-12) and δ 4.98 (H-11) and C-10 (δ 42.2), and between one of the H-9 methylene resonances at δ 1.06 and both the H-18 methylene resonances (δ 1.30 and 1.14) and C-11 (δ 133.3), established the connectivity between C-9 and C-10 and between C-10 and C-11. The magnitude of the coupling between the olefinic protons H-11 and H-12 ($J = 15.3$ Hz) and difference NOE experiments established the *E* configuration for the $\Delta^{11,12}$ olefin in **6**.

Methyl glánvillate B (**7**) was obtained as a clear optically active oil that gave a $[M + H]^+$ ion in the HRFABMS at *m/z* 309.2422 that was appropriate for a molecular formula of $C_{19}H_{32}O_3$, differing from the molecular formula of methyl glánvillate A (**6**) simply by the loss of a carbon. Comparison of the NMR data (see Table 1) obtained for **7** with that of **6** indicated that the two compounds were closely related. The $\Delta^{11,12}$ in **2** and **6** was missing and the chain length, after C-10, was reduced by one carbon. Structure **7** was assigned to methyl glánvillic acid B and structure **3** to glánvillic acid B.

Plakinastrella onkodes (Uliczka, 1929) was harvested by hand using scuba at Capucin, on the northwestern tip of Dominica. Freshly collected sponge was frozen on site and transported frozen to Vancouver. The sponge was repeatedly extracted with MeOH, and the MeOH extracts were combined, concentrated in vacuo, and then partitioned between H_2O and EtOAc. Bioassay-guided fractionation of the EtOAc soluble materials via Sephadex LH-20 and flash silica gel column chromatographies followed by reversed-phase HPLC gave pure samples of methyl capucinoate A (**4**) and **5**.

Methyl capucinoate A (**4**) was obtained as a clear optically active oil that failed to give an $[M + H]^+$ ion in the HRFABMS. However, two strong ions were observed at *m/z* 187.09729 and 171.11805 that were appropriate for fragments with molecular formulas of $C_9H_{15}O_4$ and $C_{13}H_{15}$, respectively. Although only 20 carbon resonances with 28 attached hydrogen atoms were observed in the $^1H/^{13}C$/HMQC NMR data obtained for **4** (4 × CH_3, 4 × CH_2, 8 ×

Table 1. 500 MHz NMR Data for Methyl Glánvillates A (**6**) and B (**7**) Recorded in $CDCl_3$[a]

	δ ^{1}H		δ ^{13}C	
atom	**6**	**7**	**6**	**7**
1			170.4	170.4
2	3.55 s, 2H	3.55 s, 2H	32.2	32.2
3			140.2	140.3
4			124.0	124.1
5	5.82 bs, 1H	5.83 bs, 1H	107.6	107.6
6			154.2	154.2
7	2.45 dd (14.9, 6.4), 1H 2.34 dd (14.9, 7.6), 1H	2.49 dd (14.8, 5.6) 2.32, 1H	36.4	35.9
8	1.77 m, 1H	1.81 m, 1H	30.0	30.1
9	1.23 m, 1H 1.06 m, 1H	1.14−1.18 1.03 m, 1H	42.4	41.1
10	1.87 m, 1H	1.30	42.2	36.1
11	4.98 ddt (15.3, 9.1, 1.4), 1H	1.19−1.24	133.3	19.5
12	5.36 dt (15.3, 6.4), 1H	1.14−1.18	132.1	35.5
13	1.98 m, 2H	0.85 t (6.2), 3H	25.6	14.5
14	0.95 t (7.5), 3H	2.31 q (7.7), 2H	14.2	18.1
15	2.31 q (7.6), 2H	1.10 t (7.7), 3H	18.1	14.8
16	1.10 t (7.6), 3H	0.84 d (6.4), 3H	14.8	20.1
17	0.82 d (6.7), 3H	1.27 1.19−1.24	19.2	26.5
18	1.30 m, 1H 1.14 m, 1H	0.81 t (7.2), 3H	28.9	10.8
19	0.80 t (7.4), 3H		11.7	
OCH_3	3.67 s, 3H	3.67 s, 3H	52.0	52.0

[a] Assignments based on DEPT, HMQC, HMBC, and COSY data.

CH, 4 × C), the COSY, HMQC, and HMBC NMR data clearly identified a phenyl moiety (δ ^{1}H (^{13}C) 7.18, m (126.0 (CH)); 7.28 m (128.4 (2 × CH)); 7.18 m (128.5 (2 × CH)); (140.5 (C)), indicating that the molecular formula was $C_{22}H_{30}O_4$. A series of downfield ^{13}C NMR resonances were routinely assigned to two olefins (δ 132.6 (C), 129.2 (CH), 127.6 (CH), 131.5 (CH)) and an ester carbonyl (δ 171.0). The observation that a sharp methyl singlet at δ 3.68 in the ^{1}H NMR spectrum was correlated to a carbon resonance at δ 51.9 in the HMQC experiment established the presence of a methyl ester. Two carbinol carbons were observed at δ 83.4 (CH) and 82.2 (C). Additional upfield ^{1}H and ^{13}C NMR resonances could be assigned to an allylic methyl (δ 1.77 s (18.6)), to an aliphatic methyl singlet (δ 1.30 (21.1)) and methyl doublet (δ 0.88 J = 6.6 Hz (17.1)), and to an aliphatic methylene (δ 1.39 t, J = 12.8 Hz and δ 1.55 dd, J = 13.3, 4.5 Hz (41.9)) and methine (δ 1.87 m (30.6)). The remaining NMR resonances were assigned to a doubly allylic methylene (δ 3.42 d, J = 7.1 Hz (39.3)), an allylic methylene (δ 2.19 bd, J = 13.8 Hz and δ 2.11 d, J = 13.8 Hz) correlating in the HMQC with an unusually deshielded carbon resonance at δ 50.9, and an aliphatic methylene adjacent to an ester carbonyl (δ 2.62 dd, J = 15.7, 3.5 Hz and δ 2.35 dd, J = 15.7, 8.8 Hz (36.1)).

COSY and HMQC data routinely established the spin system from Me-21 through H-12, and the constitution of this fragment was confirmed by the HMBC data. The observation of HMBC correlations between the H-14/18 phenyl (δ 7.18) resonance and the C-12 (δ 39.3) resonance, and both the H-11 (δ 5.73) and H-12 (δ 3.42) resonances and the C-13 (δ 140.5) resonance (and also H-12 and C-14/18), required attachment of the phenyl moiety at C-12. HMBC correlations observed between both the Me-21 and H-9 resonances (δ 1.77 and 5.79, respectively) and C-7 (δ 50.9) and between the allylic H-7 methylene protons (δ 2.19 and 2.11) and C-8 (δ 132.6) and C-9 (δ 129.2) established the connectivity between C-8 and C-7. In addition, the Me-20 singlet at δ 1.30 and the methylene resonance of H-5 (δ 1.39) also correlated with C-7 (δ 50.9), and along with the methylene resonances of H-7 (δ 2.19 and 2.11) these four proton resonances each correlated with the carbinol resonance at δ 82.2 (C-6). These results unambiguously established the connectivity from C-5 through C-18. Further, COSY, HMQC, and HMBC correlations readily established the connectivity through C-2. Finally, the observation that the H-3 methine resonance at δ 4.07, the methylene resonances of H-2 (δ 2.62 and 2.35), and the OMe singlet at δ 3.68 all showed HMBC correlations to the carbonyl at δ 171.0 confirmed that the carbonyl of the methyl ester was situated at C-1.

The methyl ester, phenyl residue, and two alkene functional groups identified in methyl capucinoate A (**4**) accounted for seven of the eight sites of unsaturation demanded by the molecular formula. Therefore, it was apparent that methyl capucinoate A (**4**) also contained a ring. With the structural constraints established above, a ring could only be accommodated by introducing a peroxide via the two carbinol oxygens at C-3 and C-6. This led to the structure **4** for the new polyketide-derived cyclic peroxide methyl capucinoate A.

The relative stereochemistry of the substituents on the six-membered cyclic peroxide in methyl capucinoate A (**4**) was established by examining the proton coupling constants and the results of a series of difference NOE experiments. Difference NOEs were observed between H-3 and H-5β (δ 1.39) and between Me-20 and H-4, indicating the axial orientation of H-3, H-4, H-5β, and Me-20. This was supported by the magnitude of the H-3/H-4 (J = 9.5 Hz) and H-4/H-5β (J = 12.8 Hz) coupling constants and the observation of W coupling between H-5β (δ 1.39) and Me-20 (δ 1.30) in the COSY, which can only occur if a diaxial arrangement exists between Me-20 and H-5β. Difference NOE experiments established the E configurations for the $\Delta^{8,9}$ and $\Delta^{10,11}$ olefins in **4**.

Compound **5** was obtained as a clear optically active oil. Comparison of the NMR and MS data (see Table 2 and Experimental Section) obtained for **5** with that of **4** indicated that the two compounds were very closely related. Diene **5** differed from methyl capucinoate A (**4**) simply by the addition of CH_2. The olefinic H-10 in **4** was replaced by an additional allylic methyl (Me-22: δ 1.80 s (17.2)) in **5**, leading to the assigned structure. Difference NOE

Table 2. 500 MHz NMR Data for Methyl Capucinoate A (**4**) and **5** Recorded in $CDCl_3$[a]

	δ ^{1}H		δ ^{13}C	
atom	**4**	**5**	**4**	**5**
1			171.0	171.1
2	2.62 dd (15,7, 3.5), 1H 2.35 dd (15.7, 8.8), 1H	2.62 dd (15.7, 3.4), 1H 2.34 dd (15.7, 8.9), 1H	36.1	36.1
3	4.07 ddd (9.5, 8.8, 3.5), 1H	4.07 td (9.5, 3.4), 1H	83.4	83.4
4	1.87 m, 1H	1.87 m, 1H	30.6	30.5
5	1.55 dd (13.3, 4.5), 1H 1.39 t (12.8), 1H	1.56 dd (13.3, 4.4), 1H 1.39 t (12.8), 1H	41.9	42.1
6			82.2	82.2
7	2.19 bd (13.8), 1H 2.11 d (13.8), 1H	2.18 bd (13.8), 1H 2.09 d (13.8), 1H	50.9	51.5
8			132.6	131.5
9	5.79 bd (10.8), 1H	5.65 bs, 1H	129.2	133.4
10	6.30 ddt (15.0, 10.8, 1.4), 1H		127.6	133.5
11	5.73 dt (15.0, 7.1), 1H	5.43 bt (7.4), 1H	131.5	127.9
12	3.42 d (7.1), 2H	3.43 d (7.4), 2H	39.3	34.5
13			140.5	141.3
14, 18	7.18 m, 2H	7.18 m, 2H	128.5[b]	128.4
15, 17	7.28 m, 2H	7.27 m, 2H	128.4[b]	128.4
16	7.18 m, 1H	7.18 m, 1H	126.0	125.8
19	0.88 d (6.6), 3H	0.87 d (6.6), 3H	17.1	17.2
20	1.30 s, 3H	1.31 s, 3H	21.1	21.0
21	1.77 s, 3H	1.77 s, 3H	18.6	20.1
22		1.80 s, 3H		17.2
OCH_3	3.68 s, 3H	3.68 s, 3H	51.9	51.9

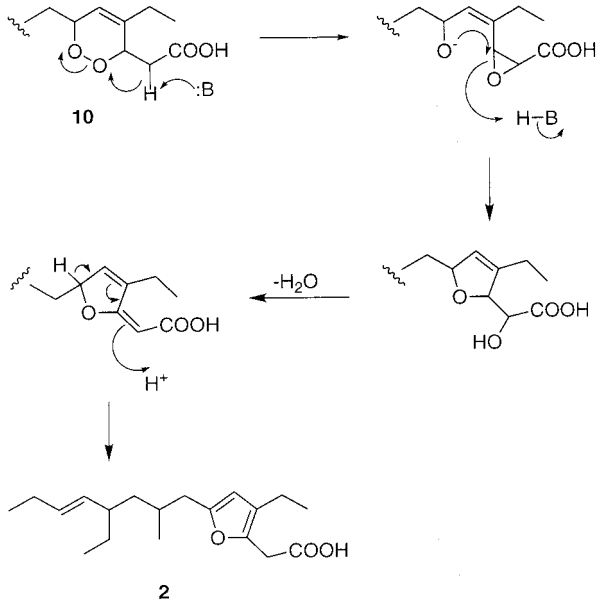
[a] Assignments based on HMQC, HMBC, COSY, and NOE data. [b] Assignments within a column are interchangeable.

experiments established the *E* configuration for the $\Delta^{10,11}$ olefin. Since *P. onkodes* was extracted in MeOH, the methyl esters **4** and **5** may be isolation artifacts.

The cyclic peroxides **1** (P388: IC_{50} = 55 ng/mL), **4** (B16F1: IC_{50} = 12 μg/mL), and **5** (B16F1: IC_{50} = 12 μg/mL) all showed in vitro cytotoxicity, but none of them showed in vivo activity against murine leukemia P388. Neither of the furans **2** or **3** showed sinificant in vitro cytotoxicity against P388 (IC_{50}'s > 20 μg/mL). Previous chemical studies of sponges in the family Plakinadae have resulted in the isolation of numerous cytotoxic, antimicrobial, and ichthyotoxic furan and cyclic peroxides containing polyketide-derived metabolites.[5,6] Several of the cyclic peroxides activate cardiac SR Ca^{2+} pumping ATPase,[7] and recently, a number of both classes of these compounds have been shown to be active against the protozoan *Leishmania mexicana*.[8] Glánvillic acids A (**2**) and B (**3**) appear to be the first examples of *Plakotis* metabolites without the normal C-6 alkyl substitution found for example in compound **8** isolated from the Palauan *Plakortis* aff. *angulospiculatus*.[8] As a consequence, compounds **2** and **3** are apparently the first polyketides in this family to have a C-3 to C-6 aromatic furan. Glánvillic acids A (**2**) and B (**3**) may be derived from a cyclic peroxide precursor such as **10** by the mechanism outlined in Scheme 1.[8,9]

Compound **5**, although previously reported from a *Plakortis* sp., appears to have never been fully characterized.[4] Peroxides **4** and **5** are related to the antifungal metabolite plakinic acid B (**9**) isolated from two unidentified Caribbean sponges.[10] However, the relative stereochemistry of the substituents on the peroxide ring differs, as does the length of the acyclic portion and the positioning of the methyl substituents along the polyketide backbone.

Scheme 1. Proposed Mechanism for the Conversion of Peroxide **10** to Furan **2**



Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500 spectrometer. ^{1}H chemical shifts are referenced to the residual $CDCl_3$ signal (δ 7.24 ppm), and ^{13}C chemical shifts are referenced to the $CDCl_3$ solvent peak (δ 77.0 ppm). Low- and high-resolution FABMS were recorded on a Kratos Concept II HQ mass spectrometer with xenon as the bombarding gas and a thioglycerol or 3-NBA sample matrix. Optical rotations were measured using a Jasco J-710 spectrophotometer.

Merck type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography. Waters 2 g Sep-Pak silica and C_{18} cartridges were used for normal and reversed-phase flash chromatography. Reversed-phase HPLC purifications were performed on a Waters 600E System Controller liquid chromatograph attached to a Waters 996 photodiode array detector. All solvents used for HPLC were Fisher HPLC grade.

Animal Material. A specimen of *Plakortis halichondrioides* (Wilson, 1902) was collected by hand using scuba at a depth of 15−20 m in Prince Rupert Bay, southwest of Glánvillia, and specimens of *Plakinastrella onkodes* (Uliczka, 1929) were collected at a depth of 5−10 m at Capucin, on the northwestern tip of Dominica in July 1997. Freshly collected sponges were frozen on site and transported to Vancouver over dry ice. Voucher samples have been deposited at the Zoological Museum of Amsterdam (ZMA POR. 13487 and ZMA POR. 13488, respectively).

Extraction of *P. halichondrioides* and Isolation of Glánvillic Acids A (2) and B (3). A sample of sponge (150 g) was cut into small pieces, immersed in MeOH, and subsequently extracted repeatedly with MeOH (3 × 150 mL) at room temperature. The combined methanolic extracts were concentrated in vacuo, and the resultant cytotoxic brown gum (3.1 g) (P_{388}: IC_{50} = 3.14 μg/mL) was then partitioned between EtOAc (4 × 15 mL) and H_2O (50 mL). The combined EtOAc extract was evaporated to dryness, to give 581.4 mg of brown oil (P_{388}: IC_{50} = 0.30 μg/mL), which was chromatographed on a Sephadex LH-20 column with MeOH to give a fraction (210.7 mg) exhibiting cytotoxic activity. This material was further fractionated using Si gel flash chromatography, employing a step gradient from 1:19 EtOAc/hexane to EtOAc and on to MeOH. A 97.7 mg fraction, eluting with 1:3−1:1 EtOAc/hexane, elicited cytotoxic activity (P_{388}: IC_{50} = 120 ng/mL) and was further chromatographed on Sephadex LH-20 in 20:5:2 EtOAc/MeOH/H_2O to give a cytotoxic fraction (74.5 mg) (P_{388}: IC_{50} = 98.1 ng/mL). The cytotoxic fraction was next purified using reversed-phase flash chromatography employing a step gradient from H_2O to MeOH. A 56.3 mg fraction, eluting with 4:1 MeOH/H_2O, exhibited cytotoxic activity against P_{388} with

an IC_{50} = 89.2 ng/mL. An inactive mixture of glánvillic acids A (**2**) and B (**3**) (13.3 mg) (P_{388}: IC_{50} > 10 μg/mL), isolated as a sharp peak, and a pure sample of the known cytotoxic cyclic peroxide **1** (15.5 mg) (P_{388}: IC_{50} = 54.6 ng/mL) were obtained from this mixture via C_{18} reversed-phase HPLC using a Whatman Magnum-9 Partisil 10 ODS-3 column, with 4:1 MeCN/(0.05% TFA/H_2O) as eluent. The glánvillic acids A (**2**) and B (**3**) were obtained as an inseparable mixture.

Glánvillic acids A (2) and B (3): pale clear oil; positive ion HRFABMS $[M + H]^+$ *m/z* 307.2272 (calcd for $C_{19}H_{31}O_3$, 307.2274) and 295.2273 (calcd for $C_{18}H_{31}O_3$, 295.2274) for A (**2**) and B (**3**), respectively.

Preparation of the Methyl Glánvillates A (6) and B (7). Methylation of glánvillic acids A (**2**) and B (**3**) was accomplished using a MNNG-diazomethane kit. MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) (238 mg, 1.78 mmol) was placed in the inside tube through its screw cap opening with 0.75 mL of water. Along with a stir bar **2** and **3** (4.1 mg, 13.9−13.3 μmol) were placed in the outside tube dissolved in 3 mL of EtOEt. The two parts were assembled with a butyl O-ring and held with a pinch-type clamp. The lower part was immersed in a dry ice/acetone bath, and approximately 3 mL of 5 N NaOH (enough to use all the MNNG) was injected dropwise through the silicone rubber septum. The solution was left stirring for 1 h and then allowed to warm to room temperature (over 45 min). The procedure was repeated, and the volume of the resulting solution was reduced in vacuo and chromatographed on a Whatman Magnum-9 Partisal 10 column using 85:15 hexane/CH_2Cl_2 as eluent to yield 1.3 mg (4.1 μmol) of methyl ester **6** and 2.1 mg (6.8 μmol) of **7**.

Methyl glánvillate A (6): clear oil; $[\alpha]^{25}_D$ −23.8° (*c* 0.21, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 233 (ϵ 5297) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRFABMS $[M + H]^+$ *m/z* 321.2428 (calcd for $C_{20}H_{33}O_3$, 321.2431).

Methyl glánvillate B (7): clear oil; $[\alpha]^{25}_D$ −23.1° (*c* 0.13, CH_2Cl_2); UV (CH_2Cl2) λ_{max} 230 (ϵ 5815) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRFABMS $[M + H]^+$ *m/z* 309.2422 (calcd for $C_{19}H_{33}O_3$, 309.2431).

Extraction of *P. onkodes* and Isolation of Methyl Capucinoate A (4) and 5. A sample of *P. onkodes* (150 g) was cut into small pieces and immersed in and subsequently extracted repeatedly with MeOH (3 × 150 mL) at room temperature. The combined methanolic extracts were concentrated in vacuo, and the resultant cytotoxic brown gum (1.1 g) (B16F1: IC_{50} = 38 μg/mL) was then partitioned between EtOAc (4 × 40 mL) and H_2O (100 mL). The combined EtOAc extract was evaporated to dryness to give 159.7 mg of brown oil, which was chromatographed on a Sephadex LH-20 column with MeOH to give a fraction (82.8 mg) exhibiting cytotoxic activity (B16F1: IC_{50} = 5.0 μg/mL). This material was further fractionated using Si gel flash chromatography, employing a step gradient from 1:19 EtOAc/hexane to EtOAc and on to MeOH. A 42.2 mg fraction, eluting with 1:19 EtOAc/hexane, elicited mild cytotoxic activity against B16F1 (IC_{50} = 14 μg/mL). Pure methyl capucinoate A (**4**) (15.3 mg) and **5** (11.2 mg) were obtained from this mixture via C_{18} reversed-phase HPLC using a Whatman Magnum-9 Partisil 10 ODS-3 column, with 4:1 MeCN/H_2O as eluent.

Methyl capucinoate A (4): clear oil; $[\alpha]^{25}_D$ −44.8° (*c* 0.67, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 227 (ϵ 5330) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRFABMS $[M - 2H + H]^+$ *m/z* 357.20652 (calcd for $C_{22}H_{29}O_4$, 357.20667), $[M - C_{13}H_{15}]^+$ 187.09729 (calcd for $C_9H_{15}O_4$, 187.09705), $[M - C_9H_{15}O_4]^+$ 171.11805 (calcd for $C_{13}H_{15}$, 171.11745), $[187 - C_3H_6O_2]^+$ 113.06005 (calcd for $C_6H_9O_2$, 113.06027), 91.05470 (calcd for C_7H_7, 91.05481).

5: clear oil; $[\alpha]^{25}_D$ −48.7° (*c* 0.37, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 229 (ϵ 3827) nm; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; positive ion HRFABMS $[M + H]^+$ *m/z* $[M - 2H + H]^+$ *m/z* 371.22208 (calcd for $C_{23}H_{31}O_4$, 371.22233), $[M - C_{14}H_{16}]^+$ 187.09737 (calcd for $C_9H_{15}O_4$, 187.09705), $[M - C_9H_{15}O_4]^+$ 185.13273 (calcd for $C_{14}H_{16}$, 185.13311), $[187 - C_3H_6O_2]^+$ 113.05976 (calcd for $C_6H_9O_2$, 113.06027), 91.05458 (calcd for C_7H_7, 91.05481).

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