Cytotoxic Polyacetylenes from the Twigs of *Ochanostachys amentacea*

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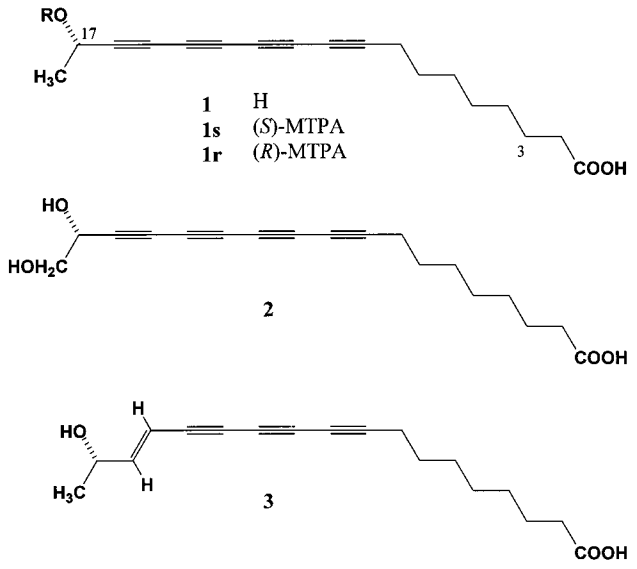
Bioassay-guided investigation of the twigs of *Ochanostachys amentacea* using LNCaP (hormone-dependent human prostate cancer) cells as a monitor led to the isolation of three alkynes, the known (*S*)-17-hydroxy-9,11,13,15-octadecatetraynoic acid (minquartynoic acid, **1**) and two novel analogues, (*S*)-17,18-dihydroxy-9,11,13,15-octadecatetraynoic acid (**2**) and (*S*)-17-hydroxy-15*E*-octadecen-9,11,13-triynoic acid (**3**). Compounds **1**−**3** were tested against a panel of human tumor cell lines and found to be significantly cytotoxic.

Ochanostachys amentacea Mast. (Olacaceae) is a tree indigenous to western Malaysia and parts of Indonesia. Only one species occurs in this genus, and the fruits are edible.[1] There is no information in the literature on the biological activity or constituents of *O. amentacea*. A decoction of the leaves of this species is used as an antipyretic in Indonesian traditional medicine.[2]

As a part of our ongoing program for the discovery of anticancer agents from plants, a chloroform-soluble extract of the twigs of *O. amentacea* was found to exhibit significant cytotoxic activity when evaluated against a panel of human cancer cell lines. Bioassay-guided phytochemical investigation of this extract, using a hormone-dependent human prostate cancer (LNCaP) cell line as a monitor, led to the isolation of (*S*)-17-hydroxy-9,11,13,15-octadecatetraynoic acid (minquartynoic acid, **1**), together with two novel polyacetylene analogues, (*S*)-17,18-dihydroxy-9,11,-13,15-octadecatetraynoic acid (18-hydroxyminquartynoic acid, **2**) and (*S*)-17-hydroxy-15*E*-octadecen-9,11,13-triynoic acid (*E*-15,16-dihydrominquartynoic acid, **3**), as active principles. The structures of compounds **2** and **3** were elucidated using 1D- and 2D-NMR spectroscopic methods, and compounds **1**−**3** were evaluated against a human cancer cell line panel.

Compound **1** was identified as minquartynoic acid, previously isolated from the stem bark of *Minquartia guianensis* Aubl.[3] and *Coula edulis* Baill.[4] (Olacaceae), on the basis of physical and spectral data comparison with literature values.[3] However, the ^{1}H NMR assignments for H-2, H-3, H-7, and H-8 obtained in CD_3OD were revised in the present investigation, based on HMQC and HMBC experiments conducted on **1**. The stereochemistry of the hydroxy group attached to C-17 of **1** was not established when this compound was isolated initially.[3,4] In the present investigation, the absolute stereochemistry of **1** was determined directly on the natural product by Mosher ester methodology.[5−7] On treatment of **1** with (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, the (*S*)-ester (**1s**) and (*R*)-ester (**1r**) derivatives at C-17 were obtained. Analysis of the $\Delta\delta_{H(S-R)}$ values showed a negative chemical shift difference at H_3-18 [$\Delta\delta_{H(S-R)}$ −0.064]. Thus, the absolute configuration at C-17 was determined as *S*. Consequently, compound **1** (minquartynoic acid) was established as (*S*)-17-hydroxy-9,11,13,15-octadecatetraynoic acid.[8]



The ^{1}H NMR spectrum of compound **2** was similar to that of **1**, except for the presence of signals consistent with diastereotopic protons of a hydroxymethyl group at δ_H 3.67 (1H, dd, J = 11.2, 6.2 Hz) and δ_H 3.60 (1H, dd, J = 11.2, 5.3 Hz), instead of the resonance for a C-18 methyl group at δ 1.40. In the ^{13}C NMR spectrum of **2**, when compared with that of **1**, a hydroxymethylene carbon at δ_C 66.7 was observed, in place of a methyl signal of C-18 (δ_C 21.4). Although EIMS, CIMS, and FABMS did not afford any molecular ion for **2**, the negative-ion ESMS of **2** showed a deprotonated molecular ion at m/z 299.1 $[M - H]^-$, while the positive-ion ESMS analysis of **2** showed a sodium adduct ion at m/z 323.1 $[M + Na]^+$. The elemental formula of **2** was determined as $C_{18}H_{20}O_4$ from MALDI-FTMS and DEPT ^{13}C NMR spectral data. The stereochemistry at C-17 was inferred as *S*, since **2** exhibited the same optical sign as **1**. The structure of **2** was characterized therefore as (*S*)-17,18-dihydroxy-9,11,13,15-octadecatetraynoic acid (18-hydroxyminquartynoic acid).

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Table 1. Cytotoxic Activity of Compounds **1**−**3**[a,b]

compd	BC1	Lu1	Col2	KB	KB-V$^+$	KB-V$^-$	LNCaP	SW626	SKNSH	M109
1	3.5	4.1	5.5	3.7	2.8	4.3	1.6	4.1	1.4	3.7
2	>20	14.6	>20	2.6	11.9	13.4	9.2	19.4	6.7	10.1
3	>20	>20	9.9	1.1	>20	>20	0.30	0.36	3.7	5.4

[a] Results are expressed as ED_{50} values (μg/mL). [b] Key to cell lines used: BC1 = human breast cancer; Lu1 = human lung cancer; Col2 = human colon cancer; KB = human oral epidermoid carcinoma; KB-V$^+$ = multidrug-resistant KB assessed in the presence of vinblastine (1 μg/mL); KB-V$^-$ = multidrug-resistant KB assessed in the absence of vinblastine; LNCaP = hormone-dependent human prostate cancer; SW626 = human ovarian cancer; SKNSH = human neuroblastoma cancer; M109 = mouse lung cancer.

The ^{1}H NMR spectrum of compound **3** was similar to that of **1**, except for the appearance of two olefinic protons at δ_H 6.40 (1H, dd, J = 15.9, 5.2 Hz) and δ_H 5.76 (1H, dd, J = 15.9, 1.4 Hz). In the ^{13}C NMR spectrum of **3**, 18 signals were observed, including a carbonyl at δ_C 178.2, seven methylene carbons (δ_C 35.3, 30.1, 29.9, 29.8, 29.2, 26.2, and 20.0), a hydroxymethine carbon at δ_C 68.4, and a methyl carbon at δ_C 23.0. Also, two olefinic carbons (δ_C 154.1 and δ_C 107.4) and six quaternary carbons (δ_C 83.5, 75.2, 75.0, 67.3, 66.2, and 60.1) were observed, in place of eight quaternary carbons for **1**. The HMBC spectrum showed cross-peaks between H_3-18 and C-16 and C-17, between H-16 and C-15, C-17, and C-18, and between H-15 and C-13, C-14, C-16, and C-17. These interactions gave evidence for the linkage adjacent to the methyl group, which included a hydroxy methine group and a double bond. Further correlations in the HMBC spectrum between H-8 and C-7, C-9, and C-10, between H-3 and C-1, C-2, and C-4, and between H-2 and C-1 and C-3 provided information on the functionality of the remainder of **3**. Due to the magnitude of the coupling constant between H-15 and H-16 (J = 15.9 Hz), the double bond was assigned as *trans*. The negative-ion ESMS analysis of **3** showed a deprotonated molecular ion at m/z 285.1 [M − H]$^-$, with a sodium adduct ion peak at m/z 309.1 [M + Na]$^+$ by positive-ion ESMS. The elemental formula of **3** was determined as $C_{18}H_{22}O_3$ from MALDI-FTMS analysis and DEPT ^{13}C NMR spectral data. Like **2**, the stereochemistry at C-17 in **3** was assigned as *S* by comparison of the optical rotation with **1**. Therefore, the structure of compound **3** was determined as (*S*)-17-hydroxy-15*E*-octadecen-9,11,13-triynoic acid (*E*-15,16-dihydrominquartynoic acid).

As summarized in Table 1, compounds **1**−**3** were evaluated against a panel of human tumor cell lines. Minquartynoic acid (**1**) showed broad but weak cytotoxicity against all of the cell lines in which it was tested. Previously, this compound was reported to exhibit strong cytotoxicity against the P-388 (murine lymphocytic leukemia) cell line, with an ED_{50} of 0.18 μg/mL.[3] In contrast to **1**, 18-hydroxyminquarytynoic acid (**2**) demonstrated significant cytotoxicity against only the KB cell line in the panel (human oral epidermoid carcinoma; ED_{50} 2.6 μg/mL). Accordingly, the presence of the C-18 methyl group in **1** appears to augment cytotoxic activity. However, the most potent activity among the three *O. amentacea* isolates obtained in this investigation was shown by **3**, against the KB, LNCaP (human hormone-dependent prostate cancer), and SW626 (human ovarian) cell lines (Table 1). Some selectivity was expressed by this compound, with, for example, the BC1 and Lu1 cell lines (human breast and lung cancer, respectively) not being susceptible to **3**. Therefore, replacement of the C-15/C-16 alkyne group of **1** by a *trans*-oriented double bond in **3** was found to have a significant effect on the resultant cytotoxic potency and selectivity.

Including the present report, minquartynoic acid (**1**) has now been found in three species of the family Olacaceae.[3,4,8] Accordingly, since **1** has not been found elsewhere in the plant kingdom to date, this compound may have some chemotaxonomic value, as previously suggested by Fort et al.[4]

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. UV spectra were measured on a Beckman DU-7 spectrometer. IR spectra were taken on a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR data (including DEPT, HMQC, HMBC, and ^{1}H−^{1}H COSY spectra) were measured on a Bruker DRX-500 instrument operating at 500.1 and 125.7 MHz, respectively. Compounds were analyzed in $CDCl_3$, with tetramethylsilane (TMS) as internal standard. ^{13}C NMR multiplicity was determined using DEPT experiments. EIMS were recorded on a Finnigan MAT-90 mass spectrometer. Electrospray mass spectra (ESMS) were acquired using a Hewlett-Packard 5989 mass spectrometer. Matrix-assisted laser desorption/ionization (MALDI) FT-ICR/MS experiments were performed on an IonSpec FTMS mass spectrometer (IonSpec Co., Irvine, CA). An aliquot (50 mM) of each sample was mixed with 2,5-dihydroxybenzoic acid in methanol at a 1:1 ratio. A portion (0.5 μL) of the sample−matrix mixture solution was deposited into a MALDI sample plate and inserted into the ionization chamber of the instrument. Samples were ionized with a focused 337 nm nitrogen laser (Laser Science, Inc., Franklin, MA). The generated ions were then differentiated according to their m/z values in an ion cyclotron resonance mass spectrometer.

Plant Material. The twigs of *O. amentacea* (local name "Kayu kacang") were collected in a tropical rainforest at Kintap, South Kalimantan, Indonesia, in August 1994. A voucher specimen (2182166) representing this collection has been deposited at the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The dried twigs of *O. amentacea* (750 g) were extracted three times with MeOH at room temperature. The resultant extracts were combined, concentrated under a vacuum, dissolved in MeOH (500 mL), and washed with hexane (3 × 300 mL). The lower layer was concentrated to dryness under reduced pressure and partitioned between 5% MeOH (300 mL) and $CHCl_3$ (3 × 300 mL). The $CHCl_3$-soluble extract [24 g, ED_{50} 5.7 μg/mL against the LNCaP (hormone-dependent human prostate cancer) cell line] was subjected to Si gel column chromatography and eluted with gradient mixtures of hexane−Me_2CO−MeOH (8:1:0.1→2:1:0.1, 100 mL per fraction) to give seven fractions. Fractions 4 and 6 were active when tested against the LNCaP cell line (ED_{50} 1.6 and 6.2 μg/mL, respectively). Additional chromatographic separation of active fraction 4 over Si gel with hexane−Me_2CO−MeOH (5:1:0.1) yielded compound **1** (20 mg). Further chromatography of fraction 6 over Si gel with hexane−Me_2CO−MeOH (4:1:0.1) afforded a mixture of **2** and **3**. Compounds **2** (8 mg) and **3** (1 mg) were purified by HPLC with an ODS-AQ column (250 × 20 mm; YMC, Inc., Wilmington, NC) using CH_3CN−H_2O (7:3) as eluant at a flow rate of 8 mL/min.

***(S)*-17-Hydroxy-9,11,13,15-octadecatetraynoic acid (minquartynoic acid, 1):** needles (MeOH); mp 97 °C (lit.[3] 95 °C); $[\alpha]_D$ −37.6° (*c* 0.1, MeOH) [lit.[3] −29° (*c* 0.1 MeOH)], and UV, IR, ^{13}C NMR, EIMS data consistent with literature values;[3] ^{1}H NMR (CD_3OD, 500 MHz) δ 4.52 (1H, q, J = 6.7 Hz, H-17),

2.36 (2H, t, J = 7.0 Hz, H_2-8), 2.28 (2H, t, J = 7.4 Hz, H_2-2), 1.60 (2H, m, H_2-3), 1.54 (2H, m, H_2-7), 1.30−1.38 (6H, m, overlapped, H_2-4, 5, 6), 1.40 (3H, d, J = 6.7 Hz, H_3-18).

(*S*)-17,18-Dihydroxy-9,11,13,15-octadecatetraynoic acid (18-hydroxyminquartynoic acid, 2): needles (MeOH); mp 130−135 °C (dec); $[\alpha]_D$ −39.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (4.54), 218 (4.92), 229 (5.09), 238 (5.10) nm; IR (NaCl) ν_{max} 3227, 2335, 2224, 1714, 1649, 1457, 1385, 1087, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.38 (1H, dd, J = 6.2, 5.5 Hz, H-17), 3.67 (1H, dd, J = 11.2, 6.2 Hz, H-18), 3.60 (1H, dd, J = 11.2, 5.3 Hz, H-18), 2.37 (2H, t, J = 7.0 Hz, H_2-8), 2.28 (2H, t, J = 7.4 Hz, H_2-2), 1.61 (2H, m, H_2-3), 1.54 (2H, m, H_2-7), 1.44−1.32 (6H, m, overlapped, H_2-4, 5, 6); ^{13}C NMR ($CDCl_3$, 75.6 Hz) δ 177.9 (s, C-1), 83.0 (s, C-9), 79.2 (s, C-16), 70.0 (s, C-10), 66.7 (t, C-18), 65.9 (s, C-15), 64.6 (d, C-17), 64.0 (s, C-11), 63.7 (s, C-14), 60.9 (s, C-12), 60.3 (s, C-13), 35.1 (t, C-2), 30.1 (t, C-4), 29.8 (t, C-6), 29.8 (t, C-5), 29.0 (t, C-7), 26.1 (t, C-3), 19.9 (t, C-8); EIMS m/z M^+ missing, 253 (14), 167 (21), 149 (85), 71 (100); negative-ion ESMS m/z $[M - H]^-$ 299.1; positive-ion ESMS m/z 323.1 $[M + Na]^+$; HR-MALDI-FTMS m/z 323.1252 $[M + Na]^+$ (calcd for $C_{18}H_{20}O_4Na$ 323.1254).

(*S*)-17-Hydroxy-15*E*-octadecen-9,11,13-triynoic acid (*E*-15,16-dihydrominquartynoic acid, 3): amorphous powder; $[\alpha]_D$ −12.8° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.58), 212 (4.62), 231 (4.80), 243 (4.92), 258 (3.81), 273 (3.92), 290 (4.12), 309 (4.21), 330 (4.07) nm; IR (NaCl) ν_{max} 3403, 2210, 1722, 1651, 1550, 1357, 1277, 1147, 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.40 (1H, dd, J = 15.9, 5.2 Hz, H-16), 5.76 (1H, dd, J = 15.9, 1.4 Hz, H-15), 4.30 (1H, ddq, J = 6.6, 5.2, 1.4 Hz, H-17), 2.35 (2H, t, J = 7.0 Hz, H_2-8), 2.27 (2H, t, J = 7.4 Hz, H_2-2), 1.60 (2H, m, H_2-3), 1.55 (2H, m, H_2-7), 1.44−1.31 (6H, m, overlapped, H_2-4, 5, 6), 1.22 (3H, d, J = 6.6 Hz, H_3-18); ^{13}C NMR ($CDCl_3$, 75.6 Hz) δ 178.2 (s, C-1), 154.1 (d, C-16), 107.4 (d, C-15), 83.5 (s, C-9), 75.2 (s, C-14 or C-15), 75.0 (s, C-15 or C-14), 68.4 (d, C-17), 67.3 (s, C-11), 66.2 (s, C-10), 60.1 (s, C-12), 35.3 (t, C-2), 30.1 (t, C-4), 29.9 (t, C-6), 29.8 (t, C-5), 29.2 (t, C-7), 26.2 (t, C-3), 23.0 (q, C-18), 20.0 (t, C-8); EIMS m/z M^+ missing, 279 (11), 167 (31), 149 (100), 71 (32); negative-ion ESMS m/z $[M - H]^-$ 285.1; positive-ion ESMS m/z 309.1 $[M + Na]^+$; HR-MALDI-FTMS m/z 309.1457 $[M + Na]^+$ (calcd for $C_{18}H_{22}O_3Na$ 309.1461).

Preparation of Mono-(*S*)- and Mono-(*R*)-MTPA Ester Derivatives of 1. To a solution of **1** (0.8 mg in 0.5 mL of $CHCl_3$ in a NMR tube) were sequentially added pyridine-d_5 (100 μL), 4-(dimethylamino)pyridine (0.5 mg), and (*R*)-(−)α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (8.0 μL). The mixture was heated at 50 °C for 4 h under a N_2 atmosphere to give the *S*-Mosher ester (**1s**). Treatment of **1** (0.8 mg) with (*S*)-(+)α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as described above yielded the *R*-Mosher ester (**1r**). ^1H NMR: δ 1.500 (*S*-MTPA ester derivative H_3-18), δ 1.564 (*R*-MTPA ester derivative H_3-18).

Bioassay Evaluation. Compounds **1**−**3** were evaluated for cytotoxicity against a panel of human cancer cell lines, according to established protocols.[9] Compounds demonstrating ED_{50} values of >5 μg/mL are regarded as inactive.

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