Polyacetylene Carboxylic Acids from *Mitrephora celebica*

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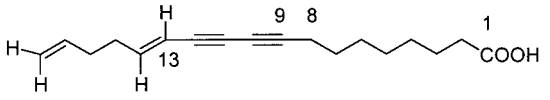
A new polyacetylene carboxylic acid, 13(*E*),17-octadecadiene-9,11-diynoic acid (13,14-dihydrooropheic acid, **1**), and the known 17-octadecene-9,11,13-triynoic acid (oropheic acid, **2**) were isolated from the stem bark of *Mitrephora celebica* through bioassay-guided fractionation. Both compounds demonstrated significant activity against methicillin-resistant *Staphylococcus aureus* and *Mycobacterium smegmatis*.

Mitrephora celebica Scheff (Annonaceae) is a plant native to the Indonesian archipelago, specifically North Sulawesi. There are only two other species in this genus,[1,2] and no reports have appeared in the literature on the phytochemistry or biological activity of the plant. In the course of our investigation of over 1000 organic plant extracts from the Natural Product Repository of the National Cancer Institute for antimicrobial activity, a CH_2Cl_2-soluble extract of the stem bark of *M. celebica* was found to exhibit significant activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium smegmatis* (the latter is a model of the pathogenic mycobacteria). Bioassay-guided investigation of the extract, using a 96-well-plate microdilution assay,[3] led to the isolation of 13(*E*),17-octadecadiene-9,11-diynoic acid (13,14-dihydrooropheic acid, **1**), a new polyacetylene carboxylic acid, and the known 17-octadecene-9,11,13-triynoic acid (oropheic acid, **2**).[4] The structure of compound **1** was completely elucidated by spectral means. Both compounds **1** and **2** were found to be significantly active against MRSA and *M. smegmatis*.



1

2

The molecular formula of 13,14-dihydrooropheic acid, $C_{18}H_{24}O_2$, was derived from HRMS, DCIMS, and NMR data. The CH_4 DCIMS of **1** showed $[M + H]^+$ at *m*/*z* 273, and the NH_3 DCIMS displayed an ammonium ion adduct $[M + NH_4]^+$ at *m*/*z* 290. The molecular formula indicated the presence of seven degrees of unsaturation. The ND_3 DCIMS of **1** showed $[M + ND_4]^+$ at *m*/*z* 295, indicating the presence of one exchangeable hydrogen. The ^{13}C GASPE and HMQC NMR spectra contained 18 signals, including a carbonyl at δ_C 177.2, nine methylene carbons, four olefinic carbons, and four additional quaternary carbons. The ^1H NMR spectrum of compound **1** revealed the presence of two signals at δ_H 5.03 (1H, dd, J = 17.0, 1.8 Hz) and δ_H 4.99 (1H, dd, J = 10.4, 1.8 Hz), indicating the presence of a terminal olefin, and a signal at δ_H 5.78 (1H, ddt, J = 17.0, 10.4, 6.5 Hz) corresponding to the adjacent olefinic proton. Two other signals at δ_H 6.28 (1H, dt, J = 15.9, 6.8 Hz) and δ_H 5.51 (1H, dm, J = 15.9 Hz) implied the presence of another double bond positioned between a quaternary carbon and an aliphatic CH_2 group. Due to the magnitude of the coupling constant, 15.9 Hz, this double bond was assigned as trans. The COSY data clearly indicated that these two double bonds were connected to one another through two methylene groups, $CH_2{=}CH{-}CH_2{-}CH_2{-}CH{=}CH{-}$, and the HMBC data confirmed that proposal. Considering the opposite end of the molecule, the COSY and HMBC NMR data further indicated that the C-1 carboxylic acid resonance at δ_C 177.2 was flanked by a seven-carbon straight chain consisting of aliphatic methylene groups, $-(CH_2)_7COOH$. Collectively these two fragments of the molecule account for $C_{14}H_{24}O_2$, leaving only four quaternary carbon atoms between δ_C 83.6 and δ_C 65.2 to be assigned in the structure of **1**. The H-8 triplet at δ_H 2.31 and its neighbors shared HMBC correlations of diminishing intensities with C-9 at δ_C 83.6, C-10 at δ_C 65.2, C-11 at δ_C 73.1, and C-12 at δ_C 73.8, respectively. These assignments were confirmed from the opposite end of the four-carbon alkyne chain via HMBC correlations of diminishing intensities between the H-13 doublet of multiplets at δ_H 5.51, its neighbors, and the four alkyne carbons, in the reverse order.

In addition, the UV spectrum of compound **1** was typical of polyacetylenes with absorption maxima at 208, 215, 227, 239, 253, 267, and 282 nm.[5,6] The IR spectrum confirmed the presence of a carboxylic acid group (3439, 1693 cm^{-1}). Therefore, the structure of compound **1** was determined as 13(*E*),17-octadecadiene-9,11-diynoic acid (13,14-dihydrooropheic acid).

Based on its spectroscopic properties, compound **2** was identified as oropheic acid, which was previously isolated from the leaves of *Orophea enneandra* Bl. (Annonaceae). This compound has not been reported from any other species. The UV and DCIMS data of compound **2** were in agreement with literature values.[4] The NMR data were comparable to those previously reported taking into account solvent differences.[4]

Both compounds **1** and **2** were somewhat unstable, and there was some decomposition into blue compounds, which were insoluble in MeOH. Acetylenic acids are known to be unstable even at room temperature. They tend to undergo

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a spontaneous oxidation and polymerization to viscous oils with distinctive odors.[7]

The antimicrobial testing on compounds **1** and **2** was performed using a 96-well-plate microdilution method.[3] The method was developed to screen large numbers of extracts in a timely manner and to overcome the solubility problem of organic extracts tested in water-based media. The minimum inhibitory concentrations (MICs) of 13,14-dihydrooropheic acid (**1**) and oropheic acid (**2**) against MRSA were determined to be 25 and 12.5 μg/mL, respectively. Vancomycin was used as a positive control (MIC of 0.8 μg/mL).[8] The MIC values of **1** and **2** were both 12.5 μg/mL against *M. smegmatis*, and the MIC of isoniazid, the positive control, was 0.8−1.6 μg/mL.[3]

Polyacetylenes are not commonly found in the Annonaceae. To our knowledge, 13,14-dihydrooropheic acid (**1**) has not been found in the plant or animal kingdom before, and there is only one literature report on the previous isolation of oropheic acid (**2**) from a plant source.[4] Since the phytochemistry of the *Mitrephora* genus has not been intensively investigated, the compounds **1** and **2** might be of chemotaxonomic value.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-265 spectrophotometer in MeOH at 0.01 mg/mL concentration. FT-IR spectra were obtained using a Perkin-Elmer Spectrum 1000 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ with a secondary reference standard (^{1}H, 7.27 ppm, and ^{13}C, 77.0 ppm). The number of attached protons for each carbon was determined from a spectral editing GASPE experiment. ^{1}H, ^{13}C, COSY, HMQC, and HMBC were recorded using standard Bruker pulse sequences. The HRMS were recorded on a Finnigan FT/MS Newstar T70 instrument and DCIMS on a Finnigan 4610 Quadrupole instrument. HPLC was performed on a Dionex P580 system equipped with a Dionex UVD340S photodiode array detector.

Plant Material. *M. celebica* was collected in North Sulawesi, Indonesia, under NCI contract with the University of Illinois at Chicago by Dr. John Burley of the Harvard University Arnold Arboretum and Turkin et al. at the Bogor Herbarium (Indonesia) in March 1990. The plant was identified by Dr. Burley in November 1990. A voucher specimen (Q66O-3746) has been deposited at the Smithsonian Museum of Natural History Botany Department.

Extraction and Purification. The dried bark (328.0 g) was extracted with MeOH−CH_2Cl_2 (1:1) to yield 23.24 g of a crude extract. A portion of the crude extract (1.03 g) was fractionated by flash chromatography on diol resin and eluted with hexane (2.20 mg), CH_2Cl_2 (593.0 mg), ethyl acetate (129.2 mg), acetone (154.1 mg), and MeOH (110.1 mg). A portion of the biologically active CH_2Cl_2 fraction (202.6 mg) was further fractionated by Sephadex LH-20 column chromatography and eluted with MeOH−CH_2Cl_2 (1:1), affording fractions A−F. The active fraction C (27.8 mg) was separated by gradient HPLC on a YMC C_{18} column (5 μm, 1 × 25 cm) using CH_3CN−0.1% TFA in H_2O (80:20 → 95:5 over 15 min) as mobile phase, yielding compounds **1** (2.6 mg) and **2** (1.6 mg).

13(*E*),17-Octadecadiene-9,11-diynoic acid (13,14-dihydrooropheic acid, 1): unstable white amorphous powder; UV (MeOH) λ_{max} (log ϵ) 208 (4.48), 215 (4.60), 227 (3.43), 239 (3.72), 253 (4.04), 267 (4.21), 282 (4.10) nm; IR (NaCl) ν_{max} 3439, 2920, 2848, 1693, 1643, 1462, 1275, 956, 908, 726 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.50 (1H, b, COOH), 6.28 (1H, dt, J = 15.9, 6.8 Hz, H-14), 5.78 (1H, ddt, J = 17.0, 10.4, 6.5 Hz, H-17), 5.51 (1H, dm, J = 15.9 Hz, H-13), 5.03 (1H, dd, J = 17.0, 1.8 Hz, H-18 trans), 4.99 (1H, dd, J = 10.4, 1.8 Hz, H-18 cis), 2.37 (2H, t, J = 7.2 Hz, H-2), 2.31 (2H, t, J = 7.0 Hz, H-8), 2.22 (2H, m, H-15), 2.16 (2H, m, H-16), 1.65 (2H, m, H-3), 1.53 (2H, m, H-7), 1.40 (2H, m, H-6), 1.34 (4H, m, H-4, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.2 (C-1), 147.0 (C-14), 137.3 (C-17), 115.4 (C-18), 109.2 (C-13), 83.6 (C-9), 73.8 (C-12), 73.1 (C-11), 65.2 (C-10), 33.7 (C-2), 32.6 (C-16), 32.5 (C-15), 28.9 (C-4), 28.7 (C-5), 28.6 (C-6), 28.2 (C-7), 24.6 (C-3), 19.5 (C-8); DCIMS (CH_4, positive ion mode) m/z 313 [M + $C_3H_5^+$] (3), 301 [M + $C_2H_5^+$] (6), 273 [M + H^+] (64), 255 (25), 231 (56), 213 (36), 199 (15), 185 (28), 171 (34), 159 (34), 145 (99), 131 (60), 115 (100), 107 (30), 91 (54), 81 (48); DCIMS (NH_3, positive ion mode) m/z 290 [M + NH_4^+] (100), 273 [M+H^+] (3); DCIMS (ND_3, positive ion mode) m/z 295 [M + ND_4^+] (100); HRMS m/z 272.1776, calcd for $C_{18}H_{24}O_2$ 272.1781.

17-Octadecene-9,11,13-triynoic acid (oropheic acid, 2): unstable white amorphous powder; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data were comparable to the literature values taking into account solvent differences;[4] DCIMS (CH_4, positive ion mode) m/z 299 [M + $C_2H_5^+$] (19), 271 [M + H^+] (60), 253 (74), 225 (22), 211 (41), 197 (21), 183 (41), 169 (61), 155 (37), 143 (71), 128 (81), 115 (100), 105 (26), 91 (100), 79 (29); DCIMS (NH_3, positive ion mode) m/z 288 [M + NH_4^+] (100), 271 [M + H^+] (2); DCIMS (ND_3, positive ion mode) m/z 293 [M + ND_4^+] (100).

Bioassay Evaluation. Compounds **1** and **2** were evaluated for antimicrobial activity against MRSA and *M. smegmatis*, according to an established protocol for screening natural products for activity against bacteria and fungi.[3]

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