5-Oxonoraporphines from *Mitrephora* cf. *maingayi*

Nancy H. S. Lee, Yuan-Jian Xu, and S. H. Goh*

Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260

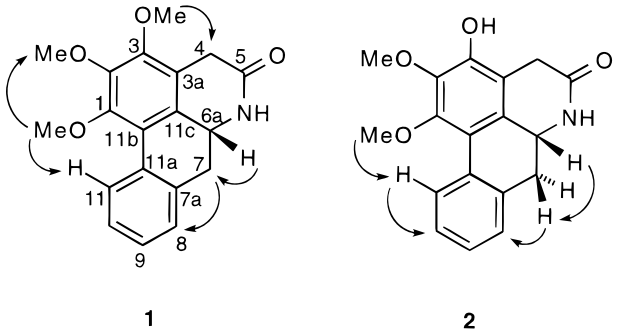
Received December 11, 1998

Two new 5-oxonoraporphines, **1** and **2**, together with three known compounds, ouregidione, 3-methoxycepharadione B, and isoelemicin, have been isolated from the bark of *Mitrephora* cf. *maingayi*. Structures of **1** and **2** were determined to be 1,2,3-trimethoxy-5-oxonoraporphine and 1,2-dimethoxy-3-hydroxy-5-oxonoraporphine on the basis of NMR and MS studies.

In continuation of our studies on Malaysian plants with cytotoxic principles, we have investigated *Mitrephora* cf. *maingayi* (Annonaceae). It is well-known that plants of this family provide a ready source of diverse natural products,[1] and a number of plants have also been used in folk medicine for various purposes[2,3] as well as for insecticides. Bioactive acetogenins isolated from plants of the Annonaceae family have attracted considerable attention,[4] while alkaloids, also widely distributed in plants within this family, remain of interest as many are pharmacologically important.[5] The Borneo plant *M. maingayi* had been shown to have cytotoxic and larvicidal activities[6] from preliminary screening of crude extracts, and we now report the isolation of two rare 5-oxonoraporphine alkaloids.

Two new oxonoraporphines, **1** and **2**, were isolated from the bark of *Mitrephora* cf. *maingayi*. The HREIMS of **1** showed $[M]^+$ at *m/z* 325.1320, suggesting a molecular formula $C_{19}H_{19}NO_4$. The fragment ion at *m/z* 282 in the LREIMS indicated the loss of HNCO via a *retro* Diels−Alder fragmentation, which is characteristic of aporphines,[7,8] and revealed **1** as a lactam. The ^{1}H NMR of **1** showed the presence of three methoxyl groups at δ 3.96, 3.95, 3.77 and a typical aromatic proton, H-11, of the aporphine nucleus at δ 8.32. Thus, **1** was deduced to be a mono-oxonoraporphine alkaloid. Although oxygenation of aporphines is usually at position 7, **1** was found to have the relatively rare 5-oxonoraporphine structure. This was evident from the spectral data showing the presence of five nonaromatic protons and a lactam group. Further HMQC, HMBC, and NOE difference data provided confirmation of the 5-oxonoraporphine skeleton. Assignments for the 1-, 2-, and 3-methoxyl groups were determined from HMBC and NOE difference spectra (Figure 1). The lowest field aromatic proton, H-11, appeared as a broad doublet (J = 7.8 Hz); H-10 was observed as a doublet of triplets, with *ortho*-coupling (J = 7.8 Hz) to H-9 and H-11 and *meta*-coupling (J = 1.9 Hz) to H-8; H-8 and H-9 overlapped as a multiplet. These data and the carbon chemical shifts showed that ring D was not substituted by an oxygenated group. The ^3J HMBC correlation for H-4 and C-3 (δ 149.6) confirmed the position of a 3-methoxyl group. The other two methoxyl substitutions were in accord with the general observation that positions 1 and 2 of an aporphine are usually oxygenated.[9,10] Trimethoxylation of ring A was also found in dioxoaporphines isolated from this plant.

Compound **2** was isolated as straw-colored needles, mp 160−162 °C. The IR spectrum of **2** showed the presence of a lactam group and a hydroxyl group. The HREIMS of **2** showed $[M]^+$ at *m/z* 311.1178, suggesting a molecular formula $C_{18}H_{17}NO_4$. Like compound **1**, the fragment ion at *m/z* 268 due to a *retro*-Diels−Alder type fragmentation was observed in the EIMS of **2**, indicating that it is a structurally analogous lactam. The ^{1}H NMR of **2** showed the signals of two methoxyl groups, one hydroxyl group, and a low-field aromatic proton at δ 8.25, typical of a deshielded H-11 of an aporphine. Thus, **2** was also deduced to be a mono-oxonoraporphine alkaloid, similar to **1**, but having one hydroxyl group unmethylated. Similar to compound **1**, H-11 appeared as a broad doublet, and H-9 showed *ortho* couplings (J = 7.5 Hz) with H-8 and H-10 and a *meta* coupling (J = 1.3 Hz) with H-11, indicating that ring D was not substituted. The 1,2-dimethoxy- and 3-hydroxy-substitution patterns were deduced from ^3J HMBC correlations observed for H-4/C-3, O−H/C-3a, OMe-1/C-1, and OMe-2/C-2. Noteworthy is the large geminal coupling (20 Hz) of the H-4 protons for both 5-oxonoraporphines **1** and **2**.



1 **2**

Figure 1. Selected NOE-difference correlations for **1** and **2**.

Only one natural 5-oxonoraporphine alkaloid, fuseine, has been previously isolated from *Fusea longifolia* (Annonaceae).[11] Other 5-oxonoraporphines described in the literature were derived by synthetic means.[12,13]

Two dioxoaporphines, ouregidione and 3-methoxycepharadione B, and an aromatic hydrocarbon, *trans*-isoelemicin, were also isolated. In previous studies, ouregidione exhibited larvicidal activity (LC_{50} 10−25 mg/mL) against mosquito (*Aedes aegypti*) larvae[6] and cytotoxic activity.[14] Ouregidione is likely to be responsible for the bioactivity found in the plant because the 5-oxoaporphines **1** and **2** showed no larvicidal or cytotoxic activity.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were recorded on Büchi 535 or Bausch and Lamb hot-stage instruments. UV spectra were recorded on a Hewlett−Packard 8452A diode-array spectrophotometer, and IR spectra

* To whom correspondence should be addressed. Tel.: +65-8743511. Fax: +65-7791691. E-mail: chmgsh@leonis.nus.edu.sg.

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were recorded on a Bio-Rad FT−IR spectrometer. EIMS were run on a Micromass VG7035F mass spectrometer at 70 eV. NMR spectra were recorded using Brucker AMX 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using $CDCl_3$ or Me_2CO-d_6 solutions with TMS as an internal standard. The NMR (^{1}H and ^{13}C) assignments were made by HMQC, HMBC, and NOE difference experiments. Liquid chromatography was performed on Si gel 60 (particle size 0.04−0.063 mm) and Sephadex LH-20. TLC was performed on precoated Si gel plates (Merck Si gel $60F_{254}$).

Plant Material. The specimen of *Mitrephora* cf. *maingayi* Hook f. (Annonaceae) was collected in Sabah, Malaysia. A voucher specimen (SAN135246) has been deposited in the Herbarium of the Forest Research Centre, Sepilok, Sandakan, Sabah.

Extraction and Isolation. The dry, powdered bark (750 g) was exhaustively extracted with MeOH (4 L × 5). Evaporation in vacuo reduced the extract to a residue (45 g) that was suspended in 90% MeOH in 10% H_2O and then successively reextracted with hexane, $CHCl_3$, and *n*-BuOH, respectively. After evaporation in vacuo, further separations of hexane (5.5 g) and $CHCl_3$ (3.5 g) fractions were carried out by chromatography on Si gel eluting with solvent mixtures of increasing polarity (*n*-hexane−Me_2CO) from 20:1 to pure Me_2CO and then on Sephadex LH-20 with $CHCl_3$−MeOH (1:1). Compound **1** (15 mg, 0.0020%), from the hexane fraction, was purified by preparative TLC using Si gel and $CHCl_3$−MeOH (50:1). Compound **2** (10 mg, 0.0013%), from the hexane fraction, was similarly purified by TLC using Si gel and *n*-hexane−Me_2CO (2:1). Alkaloids ouregidione (7 mg, 0.00093%) and 3-methoxycepharadione B (4 mg, 0.00053%) and *trans*-isoelemicin (20 mg, 0.0027%) were also isolated from the $CHCl_3$ and hexane fractions, respectively, using Si gel preparative TLC.

1,2,3-Trimethoxy-5-oxonoraporphine (1): obtained as yellow needles ($CHCl_3$); mp 185−187 °C; $[\alpha]^{25}_D$ −31.6° (*c* 0.037, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 228 (4.10), 274 (4.04), 316 (3.26) nm; IR (KBr) ν_{max} 3227, 2928, 2855, 1688, 1416, 1354, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.32 (1H, br d, J = 7.8 Hz, H-11), 7.33 (1H, dt, J = 1.9, 7.8, Hz, H-10), 7.23−7.27 (2H, m, H-8 and H-9), 6.56 (1H, br s, N−H), 4.57 (1H, m, H-6a), 3.96 (3H, s, MeO-2), 3.95 (3H, s, MeO-3), 3.82 (1H, br d, J = 20.6 Hz, H-4b), 3.77 (3H, s, MeO-1), 3.39 (1H, dd, J = 20.6, 3.6 Hz, H-4a), 2.93 (2H, d, J = 9.0 Hz, H-7); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2 (s, C-5), 151.1 (s, C-1), 149.6 (s C-3), 146.3 (s, C-2), 133.4 (s, C-7a), 131.8 (s, C-11a), 128.0 (d, C-8), 127.8 (d, C-11), 127.5 (d, C-10), 127.4 (d, C-9), 127.0 (s, C-11c), 121.8 (s, C-11b), 117.4 (s, C-3a), 60.9 (q, MeO-2 and 3), 60.7 (q, MeO-1), 51.6 (d, C-6a), 37.6 (t, C-7), 30.0 (t, C-4); EIMS *m*/*z* 325 $[M]^+$ (100), 324 (86), 310 (60), 294 (92), 282 (17); HREIMS *m*/*z* 325.1320 (calcd for $C_{19}H_{19}NO_4$, 325.1314).

1,2-Dimethoxy-3-hydroxy-5-oxonoraporphine (2): obtained as light straw needles ($CHCl_3$); mp160−162 °C; $[\alpha]^{25}_D$ −52.0° (*c* 0.070, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 228 (4.08), 282 (4.08), 320 (2.85) nm; IR (KBr) ν_{max} 3305, 3227, 2928, 2855, 1665 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) δ 8.53 (1H, br s, O−H), 8.25 (1H, br d, J = 7.5 Hz, H-11), 7.34 (1H, br s, N−H), 7.28−7.33 (2H, m, H-8 and H-10), 7.17 (1H, dt, J = 7.5, 1.3 Hz, H-9), 4.52 (1H, m, H-6a), 3.91 (3H, s, MeO-2), 3.79 (3H, s, MeO-1), 3.60 (1H, br d, J = 20.5 Hz, H-4b), 3.26 (1H, dd, J = 20.5, 3.2 Hz, H-4a), 3.13 (1H, dd, J = 13.9, 4.8 Hz, H-7a), 2.77 (1H, dd, J = 13.9, 13.9 Hz, H-7b); ^{13}C NMR ($CDCl_3$, 125 MHz) d 170.2 (s, C-5), 149.6 (s, C-1), 145.6 (s C-3), 139.6 (s, C-2), 133.2 (s, C-7a), 131.9 (s, C-11a), 128.2 (s, C-11c), 128.0 (d, C-8), 127.33 (d, C-10), 127.28 (d, C-11), 126.9 (d, C-9), 118.1 (s, C-11b), 112.6 (s, C-3a), 61.1 (q, MeO-1), 60.3 (q, MeO-2), 51.7 (d, C-6a), 37.6 (t, C-7), 29.5 (t, C-4); EIMS *m*/*z* 311 $[M]^+$ (100), 310 (41), 296 (24), 280 (50), 268 (5); HREIMS *m*/*z* 311.1178 (calcd for $C_{18}H_{17}NO_4$, 311.1158).

Ouregidione: orange needles ($CHCl_3$); mp >260 °C (lit. 262−264 °C);[13] EIMS, ^{1}H and ^{13}C NMR were identical to earlier reported data.[14]

3-Methoxycepharadione B: yellow needles ($CHCl_3$); mp 198−201 °C (lit. 198−201 °C); EIMS, ^{1}H and ^{13}C NMR were identical to earlier reported data.[15]

***trans*-Isoelemicin or 1,2,3-trimethoxy-5-(1-propenyl)-benzene:** light brown oil; ^{1}H NMR and EIMS spectra were identical to earlier reported data;[16] ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.2, 136.5, 133.7, 130.8, 125.2, 102.9, 63.2, 55.9, and 18.2.

Cytotoxicity. Determination of ED_{50} of the extracts or purified compounds were carried out as described previously.[17] Compounds **1** and **2** were bioassayed against the P-388 cell line and considered inactive at >30 mg/mL.

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