Dimeric Aporphine Alkaloids of *Phoenicanthus obliqua* from Sri Lanka

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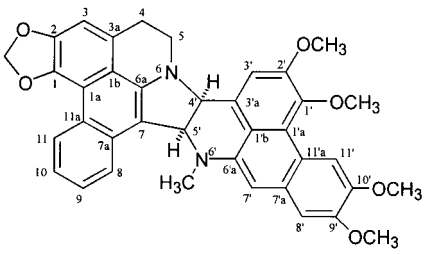
A new dimeric aporphine alkaloid, phoenicanthusine (**1**), and six known alkaloids were isolated from the stem bark of *Phoenicanthus obliqua*. The structure of **1** was elucidated by 1D (^{1}H, ^{13}C) and 2D (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) NMR and HRMS studies. Phoenicanthusine represents the first example of a N-6−C-4′ and C-7−C-5′ linked dimeric aporphine alkaloid.

In an ongoing effort to uncover bioactive and/or novel natural products from Sri Lankan Annonaceae[1] we have investigated the constituents of the stem bark of *Phoenicanthus obliqua* Hook.f. & Thoms. (Annonaceae). The genus *Phoenicanthus* contains only two species, *P. obliqua* and *P. coriacea*, both of which are endemic to Sri Lanka.[2] These have not been subjects of previous chemical investigations. We report herein the isolation and structure elucidation of a new dimeric aporphine alkaloid, phoenicanthusine (**1**), with hitherto unprecedented linkages between the two monomers, together with three known dimeric aporphines [7,7′-bis(dehydro-*O*-methylisopiline) (**2**), 7-dehydronuciferyl-7′-dehydro-*O*-methylisopiline (**3**), urabaine (**4**)], glaucine, *N*-methylsecoglaucine, and pseudocolumbamine.

The dried stem bark of *P. obliqua* was successively extracted with hexane and methyl ethyl ketone. TLC analysis indicated these extracts to be similar. Therefore, these were combined and subjected to separation and purification by repeated MPLC (medium-pressure liquid chromatography) over silica gel to obtain phoenicanthusine (**1**), 7,7′-bis(dehydro-*O*-methylisopiline) (**2**),[3] 7-dehydronuciferyl-7′-dehydro-*O*-methylisopiline (**3**),[3] urabaine (**4**),[4] and glaucine.[5] Repeated silica gel MPLC of the methanolic extract of the fresh stem bark of *P. obliqua* afforded *N*-methylsecoglaucine[5] and pseudocolumbamine.[6] The structures of all known alkaloids were established by comparison of their physical and spectroscopic data with those reported previously.

The new alkaloid phoenicanthusine (**1**) exhibited UV maxima characteristic of an aporphine chromophore[3] and its molecular formula ($C_{38}H_{32}N_2O_6$) indicated it to be a dimeric aporphine. The ^{1}H NMR spectrum of **1** in the aromatic region had a signal at δ 8.92 (d, J = 8.0 Hz), which formed part of a four-spin system [δ 8.05 (d, J = 8.0 Hz), 7.60 (t, J = 8.0 Hz), and 7.35 (t, J = 8.0 Hz)] and five singlets at δ 9.03, 7.34, 7.04, 6.66, and 6.60. It also exhibited protons of a methylenedioxy group [δ 6.15 (d, J = 1.5 Hz) and 6.10 (d, J = 1.5 Hz)] and five 3H singlets at δ 3.97, 3.93, 3.74, 3.55, and 3.44. The singlet at δ 3.44 was assigned to an *N*-CH_3 group based on HMBC and HMQC spectra (see below). Additional signals were present in the ^{1}H NMR spectrum of **1** at δ 3.53 (1H, m), 3.23 (1H, m), 2.94 (1H, m), and 2.90 (1H, m), and in comparison with other dimeric aporphine alkaloids[3] these were assigned to the $ArCH_2-CH_2-N$ spin system of an aporphine moiety. Absence of signals due to the second $ArCH_2-CH_2-N$ system suggested that it has participated in the dimerization process. The remaining ^{1}H NMR signals consisted of two ^{1}H doublets (J = 6.8 Hz) at δ 4.89 and 4.85 due to a CH−CH spin system.



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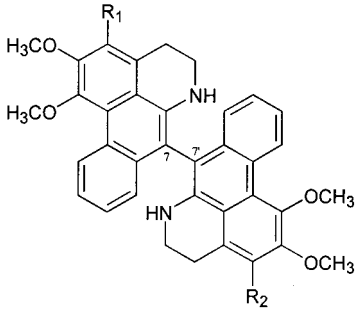

2 $R_1 = R_2 = OCH_3$
3 $R_1 = OCH_3$, $R_2 = H$
4 $R_1 = R_2 = H$

The ^{13}C NMR spectrum of **1**, when analyzed with the help of its DEPT spectrum, showed the presence of five methyl, three methylene, 11 methine, and 19 quaternary carbons. From these and the above ^{1}H NMR data it was apparent that **1** is not a 7,7′-bisaporphine alkaloid (e.g., **2**−**4**) and that in one aporphine moiety of this dimeric alkaloid C-7 is free and that C-4/C-4′ and C-5/C-5′ have participated in the dimerization process. The unsaturation number (24) of phoenicanthusine together with the absence of an absorption band due to a free *N*H in its IR spectrum further confirmed the presence of an additional ring

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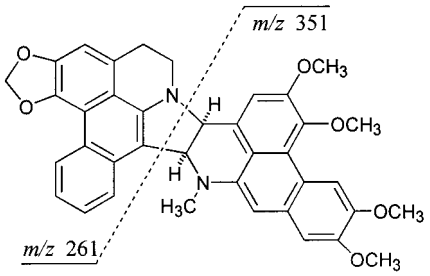

Figure 1. Mass spectral fragmentation of **1**.

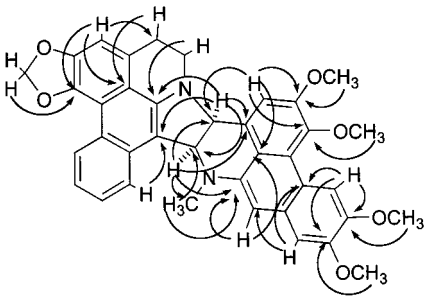

Figure 2. Selected HMBC correlations for **1**.

compared with 7,7′-bisaporphines and that one of the *N* atoms of an aporphine moiety has participated in the formation of this additional ring. The presence of two significant fragments at *m*/*z* 351 and 261 (see Figure 1) further supported this contention. The alternate structure in which *N*-6 is linked to C-5′ and C-7 to C-4′ was ruled out on the basis of the observed close proximity of the ^{1}H NMR chemical shifts of the protons at C-4′ and C-5′ (δ 4.85 and 4.89, respectively) and long-range correlations observed in the HMBC spectrum. The proton at δ 4.85 had HMBC correlations with three quaternary aromatic carbons (δ 115.8, 129.5, and 143.3) and an aliphatic methine carbon at δ 41.0 (C-5′), whereas the proton at δ 4.89 showed HMBC correlations with two quaternary aromatic carbons (δ 129.5 and 137.8), an aliphatic methine carbon at δ 42.3 (C-4′), and one of the methyl carbons at δ 41.0. In the HMQC spectrum the methyl group at δ 41.0 showed a correlation with the 3H singlet at δ 3.44. The presence of HMBC correlations between this signal and the quaternary aromatic carbon signal at δ 137.8 (C-6′a) and the aliphatic methine carbon signal at δ 41.0 (C-5′) suggested that this 3H singlet at δ 3.44 was due to an *N*-CH_3 group found sandwiched between C-5′ and a quaternary aromatic carbon (C-6′a) of an aporphine moiety. The significant low-field shift of the *N*-CH_3 signal in **1** compared with other dimeric aporphine alkaloids (commonly found at δ 2.10−2.55)[7] may be due to the anisotropic effect caused by the aromatic ring system of the 1,2-methylenedioxyaporphine moiety of **1**. The remaining four 3H singlets at δ 3.97, 3.93, 3.74, and 3.55 were assigned to protons of methoxy groups at C-9′, C-10′, C-1′, and C-2′ based on HMBC correlations. All ^{1}H and ^{13}C NMR signals for **1** were assigned by careful analysis of its ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. Some key HMBC correlations are shown in Figure 2. Coupling constants (6.8 Hz) together with the analysis of the NOESY spectrum of **1** confirmed the *cis* orientation of the protons at C-4′ and C-5′.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot stage apparatus equipped with a microscope and are uncorrected. UV spectra were recorded in EtOH with a Shimadzu UV 160 A spectrometer, IR spectra were recorded on a KBr disk on a Shimadzu IR 408 spectrometer, and ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX 400 instrument at 395.75 and 99.45 MHz, respectively. For HMBC experiments the long-range *J* value of 8.0 Hz was used. MS were recorded on a JEOL JMS D-300 spectrometer. Column chromatography (CC and MPLC) employed silica gel 60 (Merck, 70−230 mesh). Chemical shifts are reported in ppm (δ) downfield from internal TMS.

Plant Material. Stem bark of *P. obliqua* was collected at Labugama forest reserve in the Western province of Sri Lanka and identified by Dr. D. A. S. Wijesundara of Royal Botanic Garden, Peradeniya. A voucher specimen is deposited at the Medical Research Institute, Sri Lanka.

Extraction and Isolation. Dried and powdered stem bark (3 kg) of *P. obliqua* was extracted at room temperature (28−30 °C) with hexane followed by methyl ethyl ketone (MEK). Concentration under reduced pressure afforded the hexane (14 g) and the MEK extracts (21 g). Both extracts were combined and chromatographed over a silica gel column (600 g) made up in hexane and eluted with hexane containing increasing amounts of EtOAc, followed by EtOAc containing increasing amounts of MeOH. Early fractions eluted with 40% EtOAc in hexane were combined and purified by recrystallization to give **2** (650 mg). Middle fraction eluted with 40% EtOAc in hexane yielded **3** (1400 mg). The late fraction eluted with the same solvent was subjected to MPLC over silica gel (10 g) using 20% EtOAc in hexane as the mobile phase to give **4** (30 mg). Fractions eluted with 60% EtOAc in hexane from the first column was purified by recrystallization to give **1** (10 mg). The fraction eluted with 20% MeOH in EtOAc was further purified by MPLC over silica gel (10 g) using 50% EtOAc in Et_2O as the mobile phase to give glaucine (900 mg).

Fresh stem bark (400 g) of *P. obliqua* was powdered and extracted with cold MeOH. Evaporation afforded the MeOH extract (8 g), a part (7.5 g) of which was chromatographed over a column of silica gel (300 g) made up in EtOAc and eluted with EtOAc containing increasing amounts of MeOH and finally with MeOH. Early fractions eluted with 20% MeOH in EtOAc after recrystallization gave *N*-methylsecoglaucine[5] (90 mg). Late fractions eluted with the same solvent mixture were combined and further purified by recrystallization to give pseudocolumbamine[6] (20 mg).

Phoenicanthusine (1): pale yellow crystals; mp 261−263 °C; UV λ_{max} EtOH (log ϵ) 214 (4.24), 264 (4.60), and 328 (3.28) nm; IR ν_{max} 3440, 2985, 1611, 1507, 1460, 1408, 1330, 1286, 1241, 1130, and 1049 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.03 (1H, s, H-11′), 8.92 (1H, d, J = 8.0 Hz, H-11), 8.05 (1H, d, J = 8.0 Hz, H-8), 7.60 (1H, t, J = 8.0 Hz, H-9), 7.35 (1H, t, J = 8.0 Hz, H-10), 7.34 (1H, s, H-3′), 7.04 (1H, s, H-8′), 6.66 (1H, s, H-3), 6.60 (1H, s, H-7′), 6.15 and 6.10 (each 1H, d, J = 1.5, OCH_2O), 4.89 (1H, d, J = 6.8 Hz, H-5′), 4.85 (1H, d, J = 6.8 Hz, H-4′), 3.97 (3H, s, 9′-OCH_3), 3.93 (3H, s, 10′-OCH_3), 3.74 (3H, s, 1′-OCH_3), 3.55 (3H, s, 2′-OCH_3), 3.53 and 2.94 (1H each, m, H-5), 3.44 (3H, s, NCH_3), 3.23 and 2.90 (1H each, m, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.8 (s, C-2′), 149.1 (s, C-9′), 146.0 (s, C-10′), 145.8 (s, C-2), 144.6 (s, C-1′), 143.3 (s, C-6a), 141.7 (s, C-1), 137.8 (s, C-6′a), 131.4 (s, C-7a), 130.1 (s, C-7′a), 129.5 (s, C-3′a), 128.2 (d, C-11), 127.3 (d, C-9), 126.3 (s, C-3a), 124.7 (s, C-1b), 124.7 (s, C-11a), 124.6 (s, C-1′a), 122.2 (d, C-8), 122.2 (d, C-10), 118.5 (s, C-1′b), 118.1 (s, C-11′a), 116.4 (s, C-1a), 115.8 (s, C-7), 111.1 (d, C-3′), 109.3 (d, C-11′), 107.6 (d, C-3), 106.5 (d, C-8′), 101.7 (d, C-7′), 101.0 (t, OCH_2O), 59.9 (q, 1′-OCH_3), 55.9 (q, 2′-OCH_3), 55.7 (q, 10′-OCH_3), 55.6 (q, 9′-OCH_3), 47.1 (t, C-5), 42.3 (d, C-4′), 41.0 (d, C-5′), 41.0 (q, NCH_3), 29.4 (t, C-4); EIMS *m*/*z* 612 [M]$^+$ (68), 597 (100), 351 (26), 336 (57), 261 (26); HREIMS *m*/*z* 612.6839 (calcd for $C_{38}H_{32}O_6N_2$ *m*/*z* 612.6819).

7,7′-Bis(dehydro-*O*-methylisopiline) (2): off-white crystals; mp 276−278 °C; EIMS *m*/*z* 616 [M]$^+$; UV, IR, ^{1}H NMR, and ^{13}C NMR data were consistent with literature values.[3]

7-Dehydronornuciferyl-7′-dehydro-*O*-methylisopiline (3): light yellow crystals; mp 256−258 °C; EIMS *m*/*z* 586 [M]$^+$; UV, ^{1}H NMR, and ^{13}C NMR values were consistent with literature data.[3]

Urabaine (4): off-white crystals; mp 316−318 °C; EIMS *m*/*z* 556 $[M]^+$; UV, IR, ^{1}H NMR, and ^{13}C NMR data were consistent with literature values.[4]

Glaucine: colorless crystals; mp 119−120 °C; EIMS *m*/*z* 355 $[M]^+$; UV, IR, and ^{1}H NMR data were consistent with literature values.[5]

***N*-Methylsecoglaucine:** colorless crystals; mp 244−245 °C; EIMS *m*/*z* 369 $[M]^+$; UV, IR, and ^{1}H NMR data were consistent with literature values.[5]

Pseudocolumbamine: off-white crystals; mp 250−252 °C; EIMS *m*/*z* 338 $[M]^+$; UV, IR, and ^{1}H NMR data were consistent with literature values.[6]

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