Two New Alkaloids from *Artabotrys uncinatus*

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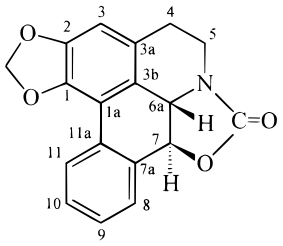
Received March 3, 1999

A novel oxazoloaporphine, artabonatine A (**1**), and a new 7-hydroxyaporphine, artabonatine B (**2**), have been isolated and characterized from the fresh unripe fruits of *Artabotrys uncinatus*, along with five known compounds. Structure elucidation of **1** and **2** was based on UV, IR, NMR, and MS analyses.

Artabotrys uncinatus (Lam) Merr. (Annonaceae) is widely distributed throughout southern Taiwan. As a traditional folk medicine, its roots and fruits are used for treatment of malaria and scrofula.[1] Previous investigations have shown the plant to contain sesquiterpenes[2−4] and alkaloids[5] possessing antimalarial and anticancer activities, respectively. As part of a continuing search for bioactive compounds of Formosan annonaceous plants, two new aporphinoid alkaloids, artabonatine A (**1**) and artabonatine B (**2**), were obtained by systematic extraction and isolation from the fresh unripe fruits of *A. uncinatus*. Five known alkaloids, liriodenine,[5,6] anonaine,[7] norushinsunine,[7] asimilobine,[7] and stepharine,[8] were also obtained. All of these compounds, except for liriodenine, are found for the first time in this plant.



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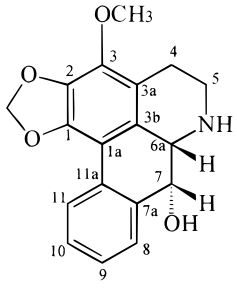
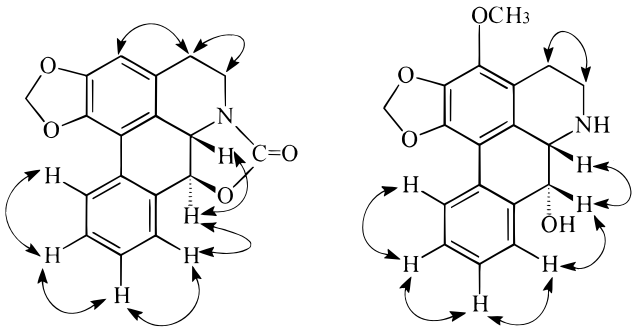


2

Artabonatine A (**1**) was obtained as a white amorphous powder from $CHCl_3$, positive to Dragendorff's test. HREIMS revealed a $[M]^+$ ion at *m/z* 307.0847, corresponding to the molecular formula $C_{18}H_{13}O_4N$. The EIMS revealed fragments at *m/z* 279 $[M - 28]^+$ and 263 $[M - CO_2]^+$, which suggested the existence of an ester group. The UV spectrum of **1** showed intense absorption bands at λ 204, 275, and 325 nm, which were typical of an aporphine skeleton.[9] The IR spectrum of **1** exhibited absorption bands at ν_{max} 1745, 1067, and 978 cm^{-1}, indicating carbonyl and methylenedioxy groups, respectively.[10] The ^1H NMR spectrum of **1** contained a signal at δ 8.18 for H-11 and a multiplet at δ 7.55−7.49 for H-8 and H-10, a signal at δ 7.37 for H-9, and a singlet at δ 6.73 for H-3 in the aromatic region, in addition to two singlets due to methylenedioxy protons at δ 6.04 and 6.14, accounting for seven protons.[11] The ^1H NMR pattern was consistent with substitution where the methylenedioxy group was placed at the 1,2-position of an aporphine skeleton.[9] Two significant downfield signals at δ 4.72 for H-6a and δ 5.61 for H-7 indicated that an electron-withdrawing group was bonded to the nitrogen and C-7.[10−14] The coupling constant between H-6a and H-7 (J = 7.9 Hz) indicated a trans relationship between H-6a and H-7.[10−14] The ^1H NMR spectrum displayed signals at δ 3.73 for H-5b, δ 2.90 for H-5a, δ 2.84 for H-4b, and δ 2.81 for H-4a.



1 **2**

Figure 1. The NOESY Correlations of **1** and **2**.

Various 2D NMR spectra gave further support for the structure of **1**. Complete assignments, and the relative configuration of aliphatic and aromatic protons of **1** were established by $^1H-^1H$ COSY and $^1H-^1H$ NOESY experiments. Significant correlations between H-3, H-4, and H-5, as well as H-6a, H-7, H-8, H-9, H-10, and H-11, were observed in the $^1H-^1H$ NOESY spectrum (Figure 1). Contrasting with the *N*-carbonyl carbon at δ 161.7[15] in other aporphines, the signal at δ 161.8 in compound **1** was assigned to an *N*-carbonyl carbon. By comparison with literature data, the downfield-shifted resonance of H-6a and H-7 in the ^1H NMR suggested an ester bridge between N-6 and C-7.[10−14] Thus, **1** (artabonatine A) is a novel alkaloid and is the second example of an oxazoloaporphine.

Artabonatine B (**2**) was isolated as a yellow amorphous powder from MeOH, positive to Dragendorff's test. HREIMS revealed a $[M]^+$ ion at *m/z* 311.1157, corresponding to molecular formula $C_{18}H_{17}O_4N$. The UV spectrum of **2** contained absorption bands typical of the aprophine skeleton.[9] The IR spectrum of **2** exhibited absorption bands, indicating hydroxyl and methylenedioxy groups, respectively.[13] The ^1H NMR spectrum of **2** presented a signal at δ 8.11 for H-11, a multiplet at δ 7.42−7.37 for H-8 and H-10, and a signal at δ 7.27 for H-9, in the aromatic region. A singlet at δ 4.05 (3H, s) was assigned to 3-OMe, and signals at δ 4.66 and 4.10 were assigned to H-7 and H-6a.[7] The coupling constant between H-6a (1H, d, J = 3.2 Hz)

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10.1021/np990085l CCC: $18.00
Published on Web 07/31/1999

and H-7 (1H, d, J = 3.2 Hz) proved a cis relationship between them.[7,13] Two singlets (δ 5.98 and 6.11) were indicative of methylenedioxy protons. The ^{1}H NMR spectrum also contained signals at δ 3.66 (H-5b), 3.50 (H-5a), 2.79 (H-4b), and 3.12 (H-4a). The relative configuration of **2** was established by a ^{1}H−^{1}H NOESY experiment (Figure 1). Significant correlations between H-4 and H-5, H-6a, H-7, H-8, H-9, H-10, and H-11, were observed in the NOESY spectrum. In the ^{13}C NMR spectrum of **2**, 12 aromatic carbon atoms between δ 147.3 and 106.8, a methylenedioxy carbon atom at δ 102.4, one methoxy carbon atom at δ 60.0, two signals for methylene carbons at δ 43.1 and 23.2, and two signals for methine carbons at δ 58.1 and 70.5, were consistent with structure **2**.

The only known oxazoloaporphine alkaloid, oxazoloaporphine—a synthetic,[10] possessed a cis configuration between H-6a and H-7 (J = 3.5).[12] A versatile intermediate, oxazoloaporphine was used to prepare the 7-hydroxyaporphines via a series of selective reductions.[10,12] Compound **1** is the first oxazoloaporphine from natural sources possessing a trans configuration between H-6a and H-7. We propose **1** as a likely precursor of 7-hydroxyaporphines such as norushinsunine[7] and **2** that have also been isolated from this plant.

Five known alkaloids, liriodenine, anonaine, norushinsunine, asimilobine, and stepharine, were isolated and characterized by comparing their physical and spectral data (UV, IR, ^{1}H and ^{13}C NMR) with those in the literatures.[5−8]

Experimental Section

General Experimental Procedures. The UV spectra were obtained on a Hitachi 200−20 spectrophotometer; IR spectra were measured on a Hitachi 260−30 spectrophotometer. ^{1}H NMR (400 and 200 MHz, using $CDCl_3$ and CD_3OD as solvents for measurement), COSY, and NOESY spectra were obtained on a Varian NMR spectrometer (Unity Plus). LREIMS were collected on a JEOL JMS−SX/SX 102A mass spectrometer or Quattro GC−MS spectrometer having a direct inlet system. HREIMS were measured on a JEOL JMS−HX 110 mass spectrometer. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography, precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.20 mm) were used for analytical TLC, and precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0,50 mm) were used for preparative TLC. The spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and then heating on a hot plate.

Plant Material. Fresh unripe fruits of *A. uncinatus* were collected from Pingtung, Taiwan, in September 1997. A voucher specimen was characterized by Dr. Hsin-Fu Yen and deposited in the Graduate Institute of Natural Products, Kaohsiung Medical College, Kaohsiung, Taiwan.

Extraction and Isolation. Fresh unripe fruits of *A. uncinatus*. (11.6 kg) were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated under reduced pressure to yield a dark-brown syrup (372.4 g). The syrup was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ solution was extracted with 3% HCl to give a $CHCl_3$ solution (Part A) (276 g) and an acidic aqueous layer. The latter was basified with NH_4OH and extracted with $CHCl_3$ (Part B) (3.8 g). Part B gave a positive alkaloid test with Dragendorff's reagent. The crude alkaloid portion (Part B) was chromatographied over Si gel and eluted with increasing polarities of $CHCl_3$−MeOH mixtures to obtain 14 fractions. Liriodenine[5,6] (10 mg) ($CHCl_3$−MeOH 10:1 R_f = 0.65) was eluted from fraction 3 (0.6 g), and anonaine[7] (5 mg) (*n*-hexane−EtOAc 1:10 R_f = 0.32) was obtained from fraction 6 (0.8 g) on elution with *n*-hexane−EtOAc 3:2. Fraction 5 (0.4 g), eluted with EtOAc−Me_2CO 11:1, was further separated and purified by Si gel column chromatography and preparative TLC to afford the stepharine[8] (4 mg) (EtOAc−Me_2CO 4:1 R_f = 0.66). Norushinsunine[7] (17 mg) (EtOAc−Me_2CO 1:2 R_f = 0.44) was isolated from the column using EtOAc−Me_2CO 8:1 as the solvent system in the fraction 9 (0.8 g). Fraction 10 (0.2 g), eluted with EtOAc−Me_2CO 7:1, was further separated and purified by Si gel column chromatography and preparative TLC to obtain artabonatine A (**1**) (2 mg) ($CHCl_3$−MeOH 10:3 R_f = 0.65). Fraction 12 (0.3 g), eluted with EtOAc−Me_2CO 8:4, was further separated by Si gel column chromatography and preparative TLC to afford artabonatine B (**2**) (4 mg) ($CHCl_3$−MeOH 10:3.5 R_f = 0.55). Fraction 13 (0.2 g) was fractionated on Si gel, eluted with EtOAc−Me_2CO 2:1, to afford asimilobine[7] (7 mg) (EtOAc−Me_2CO 16:9 R_f = 0.42).

Artabonatine A (**1**): obtained as white amorphous powder; $[\alpha]^{24}_D$ −102.7°(*c* 0.4, $CHCl_3$); UV (EtOH)λ_{max} (log ϵ) 205 (4.11), 275 (3.94) and 325 (3.76) nm; IR (neat) ν_{max} 1745, 1067, and 978 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, dd, J = 8.2, 1.2 Hz, H-11), 7.55−7.49 (2H, m, H-8 and H-10), 7.37 (1H, td, J = 8.1, 1.2 Hz H-9), 6.73(1H, s, H-3), 6.14 and 6.04 (each 1H, d, J = 1.6 Hz, OCH_2O), 4.72 (1H, d, J = 7.9 Hz, H-6a), 5.61 (1H, d, J = 7.9 Hz, H-7), 3.73 (1H, ddd, J = 12.5, 4.6, 1.5 Hz, H-5b), δ 2.90 (1H, ddd, J = 12.5, 12.5, 2.7 Hz, H-5a), δ 2.84 (1H, ddd, J = 16.0, 2.7, 1.5 Hz, H-4b), δ 2.81 (1H, ddd, J = 16.0, 12.5, 4.6 Hz, H-4a); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.8 (s, N*C*OO), 101.1 (t, OCH_2O), 52.6 (d, C-6a), 72.8 (d, C-7); EIMS (70 eV) *m*/*z*: 307 [M]$^+$ (100), 279 (10), 263 (54), 262 (81); HREIMS *m*/*z* [M]$^+$ 307.0847 (calcd for $C_{18}H_{13}O_4N$, 307.0845).

Artabonatine B (**2**): isolated as yellow amorphous powder. $[\alpha]^{24}_D$ −121.5°(*c* 0.8, $CHCl_3$); UV (EtOH)λ_{max} (log ϵ) 214 (4.25), 256 (4.18), 295 (3.86), and 325 (3.56) nm; IR (neat) ν_{max} 3455, 1072 and 920 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.11 (1H, dd, J = 8.0, 1.2 Hz, H-11), 7.42−7.37 (2H, m, H-8 and H-10), 7.27 (1H, td, J = 8.0, 1.2 Hz, H-9), 4.05 (3H, s, C3−OCH_3), 6.11 and 5.98 (each 1H, d, J = 1.6 Hz, OCH_2O), 4.10 (1H, d, J = 3.2 Hz, H-6a), 4.66 (1H, d, J = 3.2 Hz, H-7), 3.66 (1H, ddd, J = 12.5, 4.5, 1.3 Hz, H-5b), 3.50 (1H, ddd, J = 12.5, 12.5, 2.6 Hz, H-5a), 2.79 (1H, ddd, J = 16.0, 2.6, 1.3 Hz, H-4b), 3.12 (1H, ddd, J = 16.0, 12.5, 4.5 Hz, H-4a); ^{13}C NMR (CD_3OD, 100 MHz) δ 60.0 (s, C3−OCH_3), 102.4 (t, OCH_2O), 58.1 (d, C-6a), 70.5 (d, C-7); EIMS (70 eV) *m*/*z* 311 [M]$^+$(100), 310 (86), 293 (24), 280 (23), 206 (44); HREIMS *m*/*z* [M]$^+$ 311.1157 (calcd for $C_{18}H_{17}O_4N$, 311.1158).

Acknowledgment. This investigation was supported by a grant from the National Science Council of the Republic of China.

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NP990085L