New Constituents from Stems of *Artabotrys uncinatus*

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Two new compounds, 4,5-dioxoartacinatine (1) and 24-methylenelanosta-7(11)-diene-3-one (2), together with thirty known compounds were isolated and characterized from the stems of *Artabotrys uncinatus*. Structures of the new compounds were determined by spectral analysis.

Key words *Artabotrys uncinatus*; 4,5-dioxoartacinatine; biological assay; 24-methylenelanosta-7(11)-diene-3-one; antioxidant activity

There are more than 100 species of the genus *Artabotrys* throughout tropical Africa and East Asia. 1,2 *Artabotrys uncinatus* (Lam.) Merr. (Annonaceae) is widely distributed throughout southern Taiwan, and the roots and fruits are used for the treatment of malaria and scrofula. 2,3 Previous literature has shown this genus to contain alkaloids, triterpenoids, lignans, flavonoids, and steroids. 4-6 Among them, yingzhaosu analogues showed notable antimalarial activities in vitro; 7-9 alkaloids showed cytotoxic and antithrombotic activities. 5,10 In this study, we investigated the stem parts of *A. uncinatus*, and two new compounds, 4,5-dioxoartacinatine (1) and 24-methylene lanosta-7,9(11)-diene-3-one (2), along with thirty known compounds: cloven-2,9α-di-ol (3), 7 caryolane-1,9β-diol (4), 7 1-methoxy-9-caryolanol (5), 8 spathulenol (6), 9 (-)-ent-4β-hydroxy-10α-methoxymaradendrene (7), 10 4β-hydroxy-10α-methoxymaradendrene (8), 10 β-caryophyllene-8R,9R-oxide (9), 7 artabotryside A (10),11 artabotryside B (11),11 apigenin (12),12 luteolin (13),13 5-hydroxy-7,4′-dimethoxyflavone (14),14 (+)-catechin (15),15 liriodenine (16),16 atherospermidine (17),16 artacinatine (18),15 (-)-asimilobine (19),2 2N-β-p-coumaryltyramine (21),18 (+)-syringaresinol (22),19 (2R,3R)-3-hydroxy-2-methylbutyrolactone (23),20 tetrahydrofururan-4-methylidine-3-ol (24),21 phytol (25),22 24-methylenelanosta-7,9(11)-dien-3β-ol (26),21 (24R)-stigmaster-5-en-3β,7β-α-diol (27),21 (22E,24S)-stigmaster-5,22-dien-3β,7β-α-diol (28),21 β-sitosterol (29) and stigmasterol (30), 21β-sitosteryl-3-O-β-D-glucoside (31), and stigmasteryl-3-O-β-D-glucoside (32) were isolated. Compounds 13, 16, 17, and 22 were evaluated for their cytotoxicity against several cancer cell lines. Compounds 10, 11, 12, 13, and 15 were tested for their antioxidant activity.

Compound 1 was isolated as yellow needles, positive to Dragendorff’s test. Its molecular formula was determined as C_{19}H_{17}O_{6}N on the basis of its HR-EI-MS spectrum (m/z 355.1058 [M]+, Calcd 355.1055). The UV spectrum showed absorption at λ_{max} 230, 252, and 282 nm. The IR spectrum showed absorption bands at 1664, 1734, and 3421 indicating carbonyl and hydroxyl groups, respectively. The 1H-NMR spectra revealed signals for two methoxy groups (δ_{H} 4.08, 4.14), two aromatic protons at the 8,9-positions of the D-ring dehydroaporphine moiety (Fig. 3).5 The 13C-NMR spectrum exhibited the presence of three methyl, two methylene, three methine, and eleven quaternary carbons. In comparison with the literature data, 25 the di-ketone groups in 4,5-dioxoaporphines usually resonate at δ_{C} 178 and 157 ppm, respectively. Compound 1 showed the signals at δ_{C} 175.0 and 152.4 ppm, which are coincident with the assignments of 4,5-di-ketone groups. H-3 appeared at δ_{H} 8.25 indicating the existence of a carbonyl group at the peri-position. The HMBC spectra gave further support for the structure determination of 1, the correlations between 1-OCH_{3} and C-1, and 2-OCH_{3} and C-2 confirmed the methoxy groups at C-1 and C-2. The correlations between H-3 and C-2/C-11c/C-4, and between N-CH_{3} and C-9/C-11d/C-17 confirmed the unusual dehydroaporphine moiety. 5,25 The proton signals at δ_{H} 2.18, 2.73, 3.15, and 3.29 were ascribed to methylene protons at the 8,9-positions of the D-ring dehydroaporphine moiety (Fig. 3).5 The 13C-NMR spectrum exhibited the presence of three methyl, two methylene, three methine, and eleven quaternary carbons. In comparison with the literature data, 25 the di-ketone groups in 4,5-dioxoaporphines usually resonate at δ_{C} 178 and 157 ppm, respectively. Compound 1 showed the signals at δ_{C} 175.0 and 152.4 ppm, which are coincident with the assignments of 4,5-di-ketone groups. H-3 appeared at δ_{H} 8.25 indicating the existence of a carbonyl group at the peri-position. The HMBC spectra gave further support for the structure determination of 1, the correlations between 1-OCH_{3} and C-1, and 2-OCH_{3} and C-2 confirmed the methoxy groups at C-1 and C-2. The correlations between H-3 and C-2/C-11c/C-4, and between N-CH_{3} and C-5/C-6a indicated the ketone groups at C-3 and C-5. The significant NOESY correlation between H-7 and H-8 together with the aforementioned assignments also proved the carbonyl group was located at C-11. The above evidence and comparison with the spectral data reported for artacinatine (18), cepharonidine-A, and aristolodione indicated the structure of 1 was 4,5-dioxoartacinatine. 25 In our previous study, artacinatine (18) isolated from this plant with the same D-ring moiety had been evidenced by X-ray crystalline analysis. Compound 18 possesses a 10α-hydroxyl function. Com-
Compound 2 was obtained as a colorless solid. The UV spectrum showed characteristic absorptions at $\lambda_{\text{max}}$ 242, 235 nm. The IR spectrum of 2 contained absorption for the carbonyl group at 1707 cm$^{-1}$. The EI-MS spectrum showed a molecular ion peak at $m/z$ 436 and HR-EI-MS spectrum gave $m/z$ 436.3708 for the [M]$^+$ ion (Calcd 436.3705) corresponding to the molecular formula $C_{31}H_{48}O$. The $^1$H-NMR spectrum of 2 indicated three secondary methyl groups (d$_H$ 0.92, 1.02, 1.03), five tertiary methyl groups (d$_H$ 0.59, 0.89, 1.09, 1.13, 1.20), two olefinic protons (d$_H$ 5.39, 5.50), and geminal protons for one terminal double bond (d$_H$ 4.66, 4.72). The $^{13}$C-NMR spectrum of 2 showed signals due to a 7,9(11)-conjugated diene at d$_C$ 119.8, 142.9, 144.5, and 117.3, eight methyls at d$_C$ 25.4, 25.3, 22.5, 22.0, 22.0, 21.9, 18.5, and 15.7, and a ketone at d$_C$ 216.9. Further evidence from COSY, HMQC, and HMBE spectra also confirmed the planar structure of 2. The HMBC cross peaks between H-2 and C-3, and CH$_3$-28 and C-3 indicated the carbonyl group was located at C-3, the COSY correlations from H-15 to H-23 and HMBC cross peaks between CH$_3$-21 and C-17/C-20/C-22, CH$_2$-31 and C-23/C-24/C-25, and CH$_3$-26 and C-24/C-25/C-27 revealed the side chain was situated at C-17. The stereochemistry of 2 was deduced by NOESY experiments, the NOE correlation between H-5 and H-28 indicated that H-5 was assigned to be in a $\alpha$ orientation. According to the aforementioned evidence, analyses of 1D and 2D NMR spectra and comparison with data of 24-methylenelanista-7,9(11)-dien-3$\beta$-ol (26) (Fig. 3) confirmed that the structure of 2 was 24-methylenelanosta-7,9(11)-dien-3-one.

According to the previous literature, caryolane-1,9$\beta$-diol
(4), liriophenone (16), atherospermidine (17), (+)-syringaresinol (22), and 24-methylenelanoasta-7,9(11)-dien-3β-ol (26) had significant cytotoxicity, anti-HIV activity, and anti-inflammatory activity.26,27

In biological assay, atherospermidine (17) and (+)-syringaresinol (22) show significant inhibition against several cancer cell lines,29 including Hep G2 (human hepato cellular carcinoma) cell line with IC₅₀ values of 0.97 and 0.35 μg/mL, respectively. Flavonoids were reported to possess antioxidant and free radical scavenging activities.29 Compounds 10, 11, 12, 13, and 15 were tested for their antioxidant activity.30 Among them, arbutabyside A (10), luteolin (13), and (−)-catechin (15) were found to be powerful scavengers of DPPH free radicals with IC₅₀ values of 14.09, 15.32, and 5.55 μg/mL, respectively. Arbutabyside A (10) showed a significant effect (IC₅₀ 10.19 μg/mL) on scavenging hydroxyl radical and luteolin (13) showed good SOD-like activity (IC₅₀ 24.52 μg/mL).

Experimental

Melting points were measured on a Yanagimoto micro-melting point apparatus and were uncorrected. The UV spectra were recorded on a Jasco V-530 UV/Vis spectrophotometer. The IR spectra were recorded on a Mattson Genesis II spectrophotometer. 1H-NMR (400 MHz) and 13C-NMR (100 MHz) spectra were recorded with Varian NMR spectrometers. HR-ESI-MS were collected on a Bruker DALTONICS Apex II mass spectrometer. HR-EI-MS were collected on a Finnigan POLARISQ mass spectrometer. HR-EI-MS: Caled for C₁₇H₂₉NO₄/H₂O (M+H)+ 355.1055, found 355.1058.

References