Camellins A—C, Three New Triterpenoids from the Roots of Camellia sinensis

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Camellia sinensis is an evergreen shrub plant of the Theaceae family. Their leaves and leaf buds are used to produce tea. Since the tea was discovered for the first time in China in 2737 BC, it is at present, only next to water, the most popular beverage, and is currently grown and cultivated in at least 30 countries around the world. Phytochemical and pharmacological studies of tea have provided convincing evidence that the polyphenolic antioxidants present in tea are capable of affording chemoprevention in cancer. Root of Camellia sinensis is a common traditional Chinese medicine, which has been used to cure rheumatic and hypertensive cardiopathy, coronary heart disease and arrhythmia. Previous phytochemical and pharmacological studies of tea have provided convincing evidence that the polyphenolic antioxidants present in tea are capable of affording chemoprevention in cancer. Root of C. sinensis is a common traditional Chinese medicine, which has been used to cure rheumatic and hypertensive cardiopathy, coronary heart disease and arrhythmia. Previous phytochemical and pharmacological studies of roots have led to the identification of some sugars, phenolic components, steroids, and triterpene saponins. With the aim of searching new natural compounds with interesting biological activities, we carried out phytochemical investigations on the roots of this plant collected in China. Three new triterpenoids, camellins A—C (1—3), together with 15 known compounds have been isolated. Here, we report the structure elucidation of these new triterpenoids on the basis of their spectroscopic data and the bioassay of their cytotoxicity against five kinds of the human tumor cell lines, including HL-60, SMMC-7721, A-549, PANC-1 and SK-BR-3.

Results and Discussion

Phytochemical studies on the 70% aqueous acetone extract of the roots of C. sinensis led to the isolation of three new triterpenoids, camellins A—C (1—3), and 15 known compounds including one triterpenoid, ursolic acid, two steroids, chondrillasterol and α-spinasterone, six phenolic compounds, lariciresinol, pinoresinol, 4-O-methylcedrusin, (+)-balanophonin, α-hydroxypropyguaiacol, (E)-ferulaldehyde, and six other constituents, 5-megastigmene-3,9-diol, 4,5-dihydrobromlumen A, 6R,9R-9-hydroxy-4-megastigmene-3-one, blumenol B, glycerol 1-hexadecanoate, and hexadecanoic acid. The known compounds were determined by comparing their mass spectra and NMR data with those of literatures. The new compounds were characterized on the basis of comprehensive spectroscopic analysis.

Camellisin A (1) was obtained as colorless needle crystals from MeOH. The high-resolution-electrospray mass spectrometry (HR-ESI-MS) exhibited a pseudo-molecular ion peak [M−H]− at m/z 517.3166 (Calcd. 517.3165) corresponding to the molecular formula C30H46O7, indicating eight degrees of unsaturation. The 1H- and 13C-NMR spectra displayed 30 carbon resonances comprising seven methyl groups, 12 methylenes, eight methines, three oxygenated at C-3, nine olefinic carbons, and five quaternary carbons (Table 1). This information, coupled with the molecular formula indicated that compound 1 was a triterpenoid with five rings and four hydroxyls.

The 1H−1H shift correlation spectroscopy (COSY) spectrum revealed five groups of correlations including H-1/H-2/H-3, H-5/H-6/H-7, H-9/H-11, H-15/H-16, H-18/H-19/H-29/H-20 (H-30)/H-21 (Fig. 1). The heteronuclear multiple bond correlation (HMBC) spectrum displayed distinct correlations from five singlet methyl groups: from H-24 (δH 1.40) to C-3, C-4, C-5; from H-25 (δH 1.10) to C-1, C-5, C-9, C-10; from H-26 (δH 1.09) to C-7, C-8, C-9, C-14; from H-27 (δH 1.32) to C-13, C-14, C-15; and from H-28 (δH 1.03) to C-16, C-17, C-18, and C-22 (Fig. 1). The COSY correlations above and the key HMBC cross peaks built up the five rings framework as shown and assigned three of the four hydroxyls to be located at C-3 (δC 78.9), C-11 (δC 70.6), and C-21 (δC 77.7), respectively. The remained hydroxyl could only construct an enol system with the olefinic bond of C-12 and C-13, which can be deduced by the HMBC correlations from H-9 and H-18 to C-12, H-27 and H-18 to C-13, respectively (Fig. 1). The carbonic acid was

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assigned at C-23 from the distinct HMBC correlations from H-3, H-5, and H$_3$-24 to signal at $\delta_C$ 180.9 (Fig. 1). And the carbonyl carbon ($\delta_C$ 216.7) was confirmed to be C-22, since the HMBC correlations existed in both H-21 and H$_3$-28 with $\delta_C$ 216.7 (Fig. 1). Thus, the planar structure of compound 1 was established.

The relative stereochemistry of 1 was ascertained by the nuclear Overhauser effects (NOEs) and X-ray diffraction. From the biosynthetic point of view, H-5 and CH$_3$-27 were $\alpha$-orientations, while H-18, CH$_3$-25, CH$_3$-26, and CH$_3$-28 were $\beta$-orientations. Thus, the rotating frame Overhauser enhancement (ROE) correlations of H-5 with CH$_3$-23 and of H-11 with CH$_3$-25 indicated both CH$_3$-23 and HO-11 were $\alpha$-directed. Correlations of both H-18 and CH$_3$-28 with CH$_3$-29 suggested that CH$_3$-29 was $\beta$-orientation, while H-19 with CH$_3$-30 and H-21 indicated the $\alpha$-orientations of both CH$_3$-30 and H-21 (Fig. 2). The X-ray diffraction analysis of compound 1 further confirmed its relative stereochemistry (Fig. 3). The circular dichroism (CD) spectrum of 1 showed the relatively strong $\pi\rightarrow\pi^*$ transition around 208 nm for the olefin group are positive, while the weak $n\rightarrow\pi^*$ transition

![Image 1](image1.png)

![Image 2](image2.png)

![Image 3](image3.png)
around 285 nm for the cyclohexanone group is negative. Analysis of the Cotton effect according to the olefin octant rule\(^2\) and the cyclohexanone octant rule\(^2\) indicated the rings B/C is trans while the D/E system is cis in 1. Thus, the absolute configuration of 1 was established as shown. Therefore, compound 1 was elucidated to be an ursane triterpenoid analog, 3β, 11α, 12, 21β-tetrahydroxy-22-oxo-urs-12-en-24-oic acid, named camellisin A.

Camellisin B (2) was isolated as colorless needle crystals in MeOH. Its molecular formula, C\(_{30}H\_{44}O\_6\), was deduced from the positive HR-ESI-MS ion peak [M+Na]\(^+\) m/z 541.3148 (Calcd 541.3141). The \(^1\)H-NMR spectrum displayed seven quaternary methyl groups at \(δ_{HH}\) 0.96, 1.04, 1.13, 1.57, 1.66, 1.77, 1.78, and a hydroxyl singlet signal at \(δ_{HH}\) 7.08 (Table 1). The \(^{13}\)C-NMR data revealed 30 carbon signals including seven methyls, eight methylenes, five methines (two oxygenated), seven \(sp^3\) quaternary carbon (one oxygenated), two carbonyl groups, and one carboxyl carbon. The 1D-NMR information, together with the eight degrees of unsaturation, indicated that compound 3 was a five ring triterpenoid with three hydroxyls. Detailed comparison of its 1D-NMR data with those of compounds 1 and 2 showed that they possessed the same rings A and B. This can be further confirmed by the \(^1\)H–\(^1\)H COSY correlations of H-1/H-2/H-3 and H-5/H-6/H-7, along with the key HMBC cross peaks of H-124 with C-3 and C-5, H-25 with C-1, C-5, C-9, C-10, and H-26 with C-7, C-8, C-9, and C-14 (Fig. 1). The hydroxyl at \(δ_{HH}\) 7.08 (s) was assigned to be located at C-13 by the obvious HMBC correlation of \(δ_{HH}\) 7.08 with C-13. The third hydroxyl group was placed at C-11, as H-9 showed obvious COSY correlation with a doublet proton at \(δ_{HH}\) 5.15 (J = 12.1 Hz, H-11) (Fig. 1). The distinct HMBC correlations of H-11 with \(δ_{HH}\) 212.8 (s) assigned C-12 to be a carbonyl group (Fig. 1). The 6/6 membered rings D and E were established by \(^1\)H–\(^1\)H COSY correlations of H-15/H-16 and H-18/H-19, along with three groups of HMBC correlations from H-27 to C-13, C-14, C-15, from H-28 to C-16, C-17, C-18, and from H-29 to C-19, C-20, C-21, C-30 (Fig. 1). The other carbonyl group was located at C-22, which can be deduced from the key HMBC correlations of \(δ_{HH}\) 217.5 (s) with both H-28 and H-21 (Fig. 1).

The relative stereochemistry of 3 was established by the ROE correlations. As an oleanane triterpenoid, H-5, H-9, and CH\(_{3}\)–27 were biogenetically α-orientations, while CH\(_{2}\)–25, CH\(_{2}\)–26, and CH\(_{3}\)–28 were biogenetically β-orientations, just as those of compound 1.\(^2\)\(^9\) Thus, CH\(_{2}\)–24 showed ROE correlation with H-2β, H-6β, and CH\(_{2}\)–26, indicated the β-orientation of CH\(_{2}\)–24 and the S* configuration of C-4. H-11, presenting correlation with both CH\(_{2}\)–25 and CH\(_{2}\)–26, and HO-13, exhibiting correlations with H-11 and H-18, suggested that all of them were β-orientations (Fig. 4). Thus, compound 3 was elucidated to be a new oleanane triterpenoid, 3β, 11α, 3β-trihydroxy-12,22-dioxo-olean-23-oic acid.

Compounds 1–3 were tested for cytotoxicity against HL-60 (human myeloid leukemia cell line), SMMC-7721 (human hepatocarcinoma cell line), A-549 (lung cancer cell line), PANC-1 (human pancreatic carcinoma) and SK-BR-3 (breast cancer cell line) cell lines. All compounds were inactive with \(IC_{50}\) values greater than 40 μM, while the positive control cisplatin showed \(IC_{50}\) values of 1.7, 19.4, 29.7, 38.0, and

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\text{Thus, the oxygenated methine C-21 in 1 was changed into a methylene in 2, which can be confirmed from the} \quad ^1H-^1H \quad \text{COSY correlations of H-18/H-19/H-20/H-21 (Fig. 1). ROE} \quad \text{experiment of compound 2 showed correlations of CH}_2\text{-23 with H-3 and H-5, which indicated the R* configuration of C-4. The other chiral centers had the same stereochemistry as that of 1. Thus, compound 2 was established to be another ursane triterpenoid, 3β,12-dihydroxy-11,22-dioxo-urs-12-en-24-oic acid.} \]
General Procedure

Extraction and Isolation

Plant Material

Extraction and Isolation

E. Preparative HPLC (MeOH–H2O) to give compound camellisin A (6), 56 mg), 5-megastigmene-3,9-diol (27 mg), and CC (200—300 mesh) with CHCl3/acetone (20:1) to afford 3 fractions.

Fig. 4. Key ROE Correlations of 3

Camellisins A (2) Katiyar S. K., Mukhtar H., 3) 1223–336–033; or deposit@ccdc.cam.ac.uk.

Camellisin C (3): Colorless needle crystals in MeOH; mp 189—190 °C; [a]D 14.23 (c=0.15, CH3CN); UV max (MeOH) nm (log ε): 202 (3.88), 287 (4.20), 374 (2.70), IR (KBr) νmax cm⁻¹: 3426, 2974, 2933, 2874, 1694, 1665, 1637, 1460, 1388, 1379, 1363, 1307, 1284, 1251, 1116, 1040; 1H- and 13C-NMR: listed in Table 1; ESI-MS: m/z (%): [M+Na]+ 500 [M+Na]+ 100 [M+Cl]− 100, 471 (20), 317 (56), 303 (62), 233 (80); HR-ESI-MS (pos.): m/z 523.3037 (5+Na)+, C30H44O6Na, Calcd 523.3036.

Camellisin B (2): Colorless needle crystals in MeOH; mp 199.0—200.8 °C; [a]D 6.235 (c=0.10, CH3CN); UV max (MeOH) nm (log ε): 197 (3.40), 206 (3.67), 352 (0.67); IR (KBr) νmax cm⁻¹: 3430, 3004, 2977, 2955, 1738, 1709, 1673, 1637, 1475, 1461, 1401, 1349, 1361, 1236, 1275, 1229, 1196, 1089, 1063, 1029, 1002, 985; 1H- and 13C-NMR: listed in Table 1; ESI-MS (neg.): m/z 541.3148 (5+Na)+, C16H12O8Na, Calcd 541.3141.

Cytotoxic Assay

The following human tumor cell lines were used: HL-60 (human myeloid leukemia cell line), SMCC-771 (human hepatocarcinoma cell line), A-549 (human lung cancer cell line), Panc-1 (human pancreatic carcinoma) and SK-BR-3 (breast cancer cell line). All the cells were cultured in RPMI-1640 or Dulbecco’s modified Eagle’s medium (DMEM) (HyClone, Logan, UT, U.S.A.), supplemented with 10% fetal bovine serum (HyClone, U.S.A.) at 37 °C in a humidified atmosphere with 5% CO2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, U.S.A.). Briefly, 100 μl adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 24 h before drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1×10⁵ cells/ml in 100 μl medium each. Each culture plate was exposed to the tested compound at various concentrations in triplicates for 48 h, with cis-platin (Sigma, U.S.A.) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μl 20% sodium dodecyl sulfate (SDS)-50% N,N-dimethylformamide (DMF) after removal of 100 μl medium. The optical density of the lysate was measured at 595 nm in a 96-well microplate reader (Bio-Rad 680, U.S.A.). The IC50 value of each compound was calculated by the Reed and Muench’s method.

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References


