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CALAMISTRINS H AND I, TWO LINEAR ANNONACEOUS ACETOGENINS FROM THE ROOTS OF *UVARIA CALAMISTRATA* HANCE

Guang-Xiong Zhou,[1*] Yanjun Zhang,[2] Ruo-Yun Chen,[2] and De-Quan Yu[2]

[1] College of Pharmacy, Jinan University, Guangzhou 510632, China; [2] Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China

Abstract – Two linear Annonaceous acetogenins, calamistrins H and I (**1** and **2**) were isolated from an ethanolic extract of the roots of *Uvaria calamistrata* Hance (Annonaceae) with silica gel column and preparative HPLC. Compounds **1** and **2** belong to a class of acetogenins without tetrahydrofuran (THF) ring on the chain. The structures of **1** and **2** were determined by spectroscopic analysis (NMR and MS) and chemical derivatives.

INTRODUCTION

Annonaceous plant *Uvaria calamistrata* Hance distributed on Hainan island in China.[1] The ethanolic extract of roots from this plant showed significant *in vitro* cytotoxicity against human tumor cell lines. We had previously isolated several mono-tetrahydrofuran (THF) ring type and bis-THF ring Annonaceous acetogenins from the active fractions.[2-4] In further investigation of acetogenin constituents in the plant, two linear Annonaceous acetogenins have been isolated and identified by chemical and spectroscopic methods. However, both **1** and **2** did not show any cytotoxicity on several human cancer cell lines at concentration of 100 μg/mL in an *in vitro* MTT test. This paper reported their isolation and elucidation.

RESULTS AND DISCUSSION

Compound **1** was obtained as amorphous solid. The test of compound **1** with Kedde reagent showed a positive pink color, which indicated the presence of an α,β-unsaturated γ-lactone ring in the structure.[2,5] HRFABMS gave out a peak ($[M+H]^+$) at *m/z* 611.5264, suggesting its molecular formulae as $C_{37}H_{70}O_6$. FABMS showed ion peaks at *m/z* 611 $[M+H]^+$, 633 $[M+Na]^+$, 649 $[M+K]^+$. The FABMS also showed

fragment peaks at *m/z* 593 $[MH\text{-}H_2O]^+$, 575 $[MH\text{-}2H_2O]^+$, 557 $[MH\text{-}3H_2O]^+$, and 539 $[MH\text{-}4H_2O]^+$, supporting the successive lose of four H_2O molecules. Four proton signals at δ 6.98, 4.99, 2.26, and 1.42 as well as six carbon signals at δ173.3 (C-1), 151.1 (C-35), 134.3 (C-2), 77.3 (C-36), 25.2 (C-3), and 18.9 (C-37) revealed the *γ*-methyl *α,β*-unsaturated *γ*-lactone moiety was present in **1**.[2] All other data in ^1H and ^{13}C NMR spectra supported that it was a linar acetogenin without THF ring.[2]

Compound **1** was acetylated by the treatment of sample with Ac_2O in pyridine and yield **1a**. Four acetyl groups in the proton NMR of **1a** confirmed the existence of four hydroxyl groups in the structure of **1**. Further, two pairs of vicinal diols were confirmed by the observation of two formaldehyde acetals in the NMR of **1b** after formaldehyde acetal derivative (**1b**) of **1** was prepared. The TMS derivative experiment of **1** was employed to determine the positions of hydroxyl groups as usually used for determination of hydroxyl groups in acetogenin compounds.[2] The EIMS fragmentation pattern (see Figure 1) of **1** and its TMS derivative (**1c**) revealed the location of two pairs of diols at C-15/16, and 21/22. The same chemical shift of four ketal protons suggested that both adjacent diols have *threo* relative configuration, because A

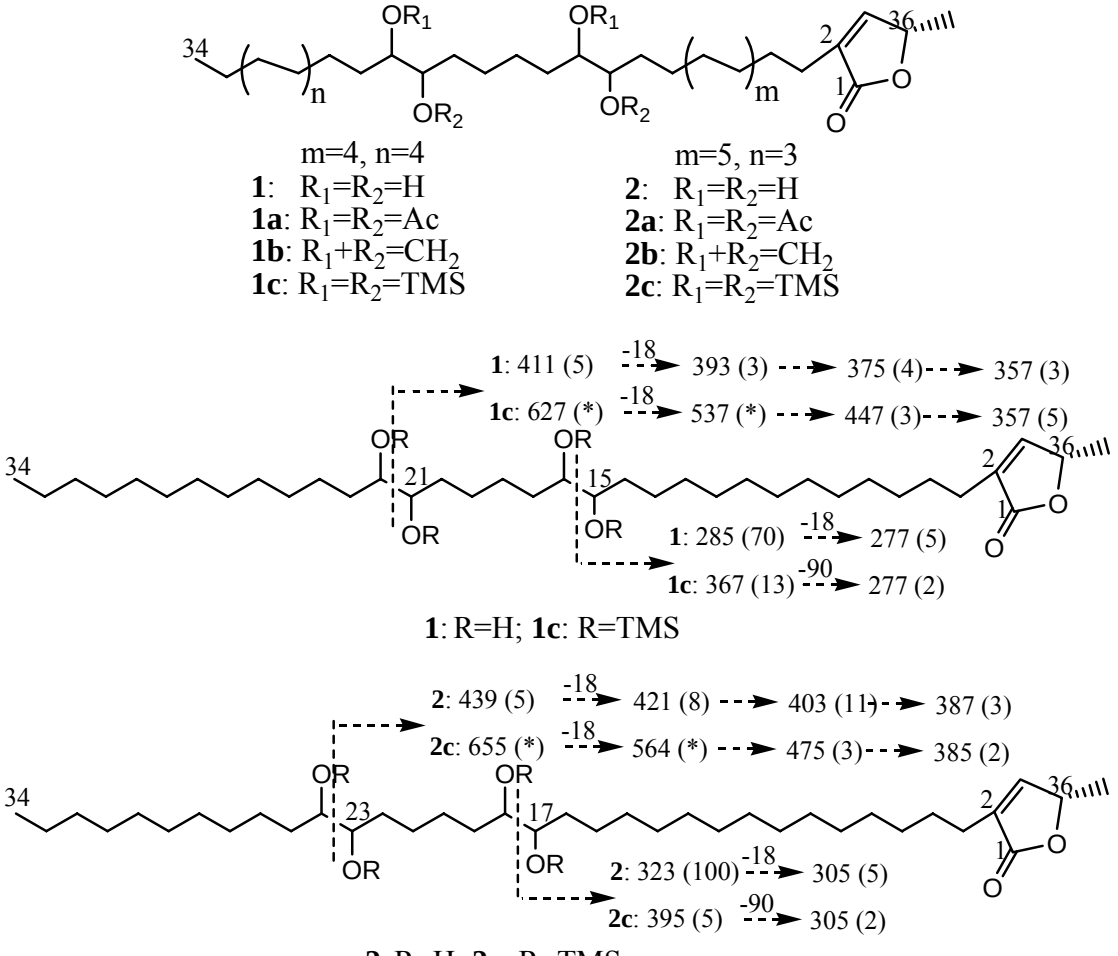

Figure 1. Diagnostic fragments from EIMS of **1**/**1c** and **2**/**2c**

series of acetogenins with threo vicinal diols has the ^{1}H NMR signals at δ about 3.43 and ^{13}C NMR signals at δ about 74.0 for the carbinols, and methylene protons of two formaldehyde acetals from **1b** appear together as a four-proton signlet.[6] The absolute configuration of C-36 was assumed to be *S* due to all natural Annonaceous acetogenins with an *S* configuration at C-36.[7] The structure of **1** was determined as (5*S*)-5-methyl-3-(13,14,19,20-tetrahydroxydotriacontyl) furan-2(5*H*)-one (in IUPAC nomenclature), or 15,16,21,22-tetrahydroxyacetogenin. It is a new linear Annonaceous acetogenins, named as calamistrin H. Compound **2** was obtained as amorphous solid. The positive test of **2** with Kedde reagent indicated the presence of an α,β-unsaturated γ-lactone ring in the structures. **2** has the same molecular formula $C_{37}H_{70}O_6$ as that of compound **1**, as a identical $[M+H]^+$ ion (*m/z* 611.5252) was observed in HRFABMS. It also had the very similar IR, ^{1}H and ^{13}C NMR spectra to those of **1**, which indicated that **2** had the same structure skeleton as that of **1**. As described in **1**, the acetyl derivative (**2a**), formaldehyde acetal derivatives (**2b**) and TMS derivatives (**2c**) were prepared in the similar procedure for the preparation of **1a**-**1c** to determine the number and positions of hydroxyl groups. After carefully analysis of EIMS fragmentation pattern (see Figure 1) of the derivatives (**2a** and **2c**) as well as **2**, It turned out that the only difference between **2** and **1** was the difference of substituted positions of hydroxyl groups. The fragment ions at *m/z* 323.3, 429.2 in the EIMS of **2c** suggested that two pairs of adjacent diols at C-17/18, and C-23/24, respectively in the structure of **2**. The relative configuration of two diols and the absolute configuration of C-36 were respectively assumed to *threo* and *S* due to the same consideration as those in **1**. Therefore, the structure of **2** was deduced as (5*S*)-5-methyl-3-(13,14,21,22-tetrahydroxydotriacontyl) furan-2(5*H*)-one (in IUPAC nomenclature), or 17,18,23,24-tetrahydroxyacetogenin. **2** also were a new liner acetogenin, designed as calamistrin I.

EXPERIMENTAL

3.1 General

Melting points were determined on a micromelting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AM 500 spectrometer with TMS as internal standard. EIMS were obtained on a ZAB-2F mass spectrometer. HRESIMS spectra were carried using a Micromass Q-TOF mass spectrometer. FABMS were obtained on a Zabspec E mass spectrometer. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarmeter. Preparative HPLC was performed using an ODS column (19 mm × 300 mm, 10 μm, XTerra Prep. Rp18, Detector: RID). Silica gel (200-300 mesh) was purchased from Qingdao Factory of Marine Chemical Industry, Qingdao, China. All the solvents are R grade and made in China.

3.2 Plant Material

Plant material was collected from Jian-Feng Mountain on Hainan Island in July 1996 and identified as *Uvaria calamistrata*. A voucher specimen (Annonaceae No. 46) was deposited in the Institute of Material Medica, Chinese Academy of Medical Science, Beijing.

3.3 Extraction and isolation

Procedures employed for the extraction and primary isolation were previously described.[2] Briefly, the dried and pulverized roots (10 kg) of *U. calamistrata* were exhaustively extracted with 30 L (×3) 95% EtOH under reflux. The extract was concentrated in vacuum to yield a 1.9 kg residue (F001), which was partitioned between H_2O and $CHCl_3$ (1:1), giving 575 g of $CHCl_3$ soluble extract (F002). The $CHCl_3$ extract was partitioned between 90% aqueous MeOH and petroleum ether (1:1) to supply 350 g gum-like solid, which was chromatographed on silica gel column (16-200 mesh, 3.5 kg) eluting with gradient petroleum ether-acetone solvent to result in several fractions with positive Kedde test. The fraction 33 (560 mg) was re-chromatographed on silica gel column with $CHCl_3$-MeOH (10:1-2:1) as eluting solvent to yield an amorphous solid (70.5 mg). The solid (30.0 mg) was further separated by preparative reverse phase HPLC eluting with 87% aqueous MeOH to obtain **1** (10.7 mg) and **2** (8.9 mg).

Calamistrin H (**1**) was obtained as a colorless solid, mp 71-72 °C, $[\alpha]_D^{14}$ +7.5 (c 0.06, MeOH); IR ν_{max} (KBr): 3356, 2916, 1849, 1753, 1466, 1070, 723 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ7.35 (1H, s, H-35), 5.06 (1H, s, H-36), 3.43 (4H, brs, 4×OH), 3.19 (4H, s, 4×carbinols), 2.14 (2H, s, H-3), 1.50-1.10 (m), 1.44 (3H, d, *J*=6.5 Hz, H-37), 0.84 (3H, t, *J*=6.6 Hz, H-34); ^{13}C NMR (125 MHz, DMSO-d_6) δ173.3, 151.1, 134.3 ,77.25, 73.1, 40.0-39.0, 32.3, 31.2, 22.4, 22.0, 18.9, 13.9; HRFABMS m/z 611.5264 (calcd. for $C_{37}H_{70}O_6$, 610.5282); FABMS m/z (rel. Int.) 649 $[M+K]^+$ (70), 633 $[M+Na]^+$ (100), 611 $[M+H]^+$ (15), 593 ($[MH]^+$- H_2O) (10), 574 ($[MH]^+$-$2H_2O$) (20), 557 ($[MH]^+$-$3H_2O$) (30), 539 ($[MH]^+$-$4H_2O$) (15); EIMS, see Figure 1.

Acetyl derivative (**1a**) A small amount (5 mg) of **1** was treated with Ac_2O-pyridine at rt for 12 h. The reacted solution was subjected to preparative TLC developed with $CHCl_3$-MeOH (9:1) and yielded the purified tetraacetyl derivative (**1a**, 3.5 mg). ^1H NMR (300 MHz, $CDCl_3$) δ6.99 (1H, s, H-35), 2.30 (2H, t, *J*=7.1 Hz, H-3), 2.08 (12H, brs, 4×OAc), 1.50-1.10 (m), 1.40 (3H, d, *J*=6.6 Hz, H-37), 0.88 (3H, t, *J*=6.5 Hz, H-34).

Formaldehyde acetal derivative (**1b**) 5-mg of **1**, 20 mg of paraformaldehyde, and a few crystals of *p*-toluenesulfonic acid were mixed well in 1 mL of $CHCl_3$, and stirred at rt for 24 h. The reaction solution was afforded for preparative TLC to yield **1b**. ^1H NMR (300 MHz, $CDCl_3$) δ7.01 (1H, s, H-35), 4.99 (5H, brs, H-36, 2×-OCH_2O-), 3.56 (4H, brs, 4×carbinols, H-15, 16, 21 and 22), 2.30 (2H, t, *J*=7.1 Hz, H-3), 1.50-1.10 (m), 1.44 (3H, d, *J*=6.5 Hz, H-37), 0.84 (3H, s, H-34).

Preparation of TMS derivative (**1c**) **1** (0.5 mg) were treated with 150 μL of mixture of *N*,*O*-bis-(trimethylsilyl)acetamide and pyridine (1:1) and heated at 70 °C for 30 min to yield TMS derivative (**1c**). The solution was used to obtain EI mass spectra directly. EIMS data, see Figure 1.

Calamistrin I (**2)** was obtained as a colorless solid, mp 73-74 °C, $[\alpha]_D^{14}$ +5.5 (c 0.05, MeOH); IR (KBr), ^{1}H NMR (500 MHz, DMSO-d_6) ^{13}C NMR (125 MHz, DMSO-d_6), and FABMS data of **2** were same as those of **1**; EIMS data for **2**, see Figure 1. The acetyl derivative (**2a**), formaldehyde acetal derivatives (**2b**) and TMS derivatives (**2c**) were prepared in the similar procedure with the preparation of **1a**-**1c**. Both **2a** and **2b** have similar ^{1}H NMR spectra data as those of **1a** and **1b**.

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