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Xylomaticin and Gonionenin, Cytotoxic Annonaceous Acetogenins from the Seeds of *Annona cherimolia*

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Abstract – Further bioactivity-directed fractionation of the ethanol extract of the seeds of *Annona cherimolia* has led to the isolation of two mono-tetrahydrofuran acetogenins, xylomaticin (**1**) and gonionenin (**2**). The structures of these compounds were characterized on the basis of chemical and spectroscopic data. Compounds **1** and **2** have a relative stereochemistry relationship of *threo*/*trans*/*threo* across the mono-tetrahydrofuran ring with its two flanking hydroxyls. Compounds **1** and **2** are known, but are first isolated from this plant. In brine shrimp lethality test (BST), **1** and **2** exhibited cytotoxic activity.
Keywords – Annonaceous acetogenins, *Annona cherimolia*, xylomaticin, gonionenin, cytotoxic activity

Introduction

Annona cherimolia Mill. is a tropical tree native to South America (Peru), and is cultivated world-wide for its edible fruits (Fies, 1959). The plant has been used in traditional medicine as an insecticide and a parasiticide (Barriga, 1974). The brine shrimp lethality test (BST) (Meyer, *et al*., 1982; McLaughlin, 1991) detected bioactivity and was used to direct the fractionation. In a previous paper, we reported the isolation, identification, and cytotoxic activities of seven new compounds such as annocherin, (2,4)-*cis*- and *trans*-annocherinones (Woo, *et al*., 1999a), annomolin, annocherimolin (Kim, *et al*., 2001), the epimer mixtures of annomolon A and 34-epi-annomolin, and annomolon B and 34-epi-annomolon B (Son, *et al*., 2003), and five known compounds, *cis*-annonacin, (2,4)-*cis*- and *trans*-isoannonacins (Woo, *et al*., 1999b), corrosolin and compound-2 (Kim, *et al*., 1999) from the seeds. In this paper, we describe the isolation and structural determination of mono-tetrahydrofuran (THF) acetogenins (**1-2**).

Experimental

Instruments and reagents – Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a JASCO FT/IR-300E spectrophotometer. UV spectra were obtained on a Shimadzu UV-1601PC spectrophotometer. NMR spectroscopy was taken on a Bruker DMX-250 spectrometer in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on a Quattro II spectrometer. For TLC, silicagel 60 F_{254} (EM 5717) glass plates (0.25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in MeOH, followed by heating. HPLC was performed on a Waters 600 apparatus equipped with a Waters 486 UV detector at 225 nm using the Autochrowin software system (Young Su Scientific Co., Seoul, Korea). For preparation of tetra-TMSi derivative, *N,O-bis*-(trimethylsilyl) acetamide (BSA) and pyridine in silylation grade were purchased from the Aldrich Company.

Plant material – The seeds of *Annona cherimolia* were obtained in 1996 from fruits grown commercially in plantations in southern California and purchased from Hurov Botanicals and Seeds located in Chula Vista, California, USA. A voucher specimen is preserved at the Department of Pharmacy, Catholic University of Daegu, Korea.

Extraction and isolation – The seeds of *A. cherimolia* (8 kg) were extracted with 95% ethanol (4 times). The EtOH extract (F001, BST LC_{50} 1.33×10^{-1} μg/mL, 700 g) was partitioned between H_2O (F002, BST LC_{50} 2.79×10^{2} μg/mL, 300 g) and CH_2Cl_2 (F003, BST LC_{50} 1.19×10^{-2} μg/mL, 400 g), and F003 was partitioned between 10%

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H_2O in MeOH (F005, BST LC_{50} 1.13×10^{-2} μg/mL, 250 g) and *n*-hexane (F006, BST LC_{50} 1.78 μg/mL, 150 g). F005 (250 g) was subjected to open column chromatography over silica gel (2.8 kg) eluted with a gradient of hexane/$CHCl_3$/MeOH. Fractions were collected and pooled according to their similar TLC patterns. The BST active pool F-12 (130 g) was further resolved on another silica gel (1.5 kg) open column, eluted with a gradient of hexane/$CHCl_3$/MeOH. Fractions were collected into 13 pools (A to M) on the basis of TLC patterns. Further purification of the most bioactive BST fraction (H) was carried out by HPLC eluted with 85% acetonitrile in water to afford **1** and **2**.

Xylomaticin (1) – white amorphous powder, mp 67.4 - 69.6 °C; $[\alpha]_D^{21}$ +16.0° (*c* 0.07, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ε): 216 (4.0); IR (KBr) ν_{max} cm^{-1}: 3421 (OH), 1736 (C = O α,β-unsaturated γ-lactone); ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 7.0, H_3-34), 1.43 (3H, d, *J* = 6.8 Hz, H_3-37), 1.67 (2H, m, H-17a, 18a), 1.97 (2H, m, H-17b, 18b), 2.41 (1H, m, H-3a), 2.50 (1H, m, 3b), 3.41 (2H, m, H-15, 20), 3.60 (1H, m, H-10), 3.80 (3H, m, H-4, 16, 19), 5.06 (1H, qq, *J* = 7.0, 1.5 Hz, H-36), 7.18 (1H, q, *J* = 1.5 Hz, H-35)

Tetra-TMSi derivative (1a) of xylomaticin – Mass (EI) *m/z*: Fig. 1

Gonionenin (2) – white amorphous powder, mp 80.0 - 81.0 °C, $[\alpha]_D^{25}$ +10.3° (*c* 0.07, CH_2Cl_2); UV (MeOH) λ_{max} nm (log ε): 209 (4.2); IR (KBr) ν_{max} cm^{-1}: 3448 (OH), 1737 (C = O α,β-unsaturated γ-lactone); ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 7.0 Hz, H_3-34), 1.44 (3H, d, *J* = 6.8 Hz, H_3-37), 1.70 (2H, m, H-15a, 16a), 2.02 (2H, m, H-15b, 16b), 2.43 (1H, m, H-3a), 2.50 (1H, m, 3b), 3.45 (2H, m, H-13, 18), 3.63 (1H, m, H-10), 3.83 (3H, m, H-4, 14, 17), 5.06 (1H, qq, *J* = 7.0, 1.5 Hz, H-36), 5.37 (2H, m, H-21, 22), 7.19 (1H, q, *J* = 1.5 Hz, H-35)

Tetra-TMSi derivative (2a) of gonionenin – Mass (EI) *m/z*: Fig. 2

Preparation of TMSi-derivatives – Approximately 10 μg of **1** and **2** were separately treated with 0.2 μL of pyridine and 2 μL of *N,O-bis*-(trimethylsilyl)acetamide (BSA) for 5 hrs to give **1a** and **2a**.

Bioassay – The extract, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp lethality test (BST) (Meyer, *et al*., 1982; McLaughlin, 1991).

Results and Discussion

Compound **1** was isolated as a white amorphous powder. The prominent absorption peak at 3567 cm^{-1} in the IR spectrum and the four successive losses (*m/z* 822, 732, 642, 552) of TMSiOH (*m/z* 90) from the molecular ion (*m/z* 912) in the EIMS of **1a** indicated the presence of four hydroxyl groups in the molecule. In addition, a prominent IR carbonyl absorption at 1737 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone. The EIMS and NMR spectral characteristics of **1** showed that it belongs to the familiar class of bioactive mono-THF acetogenins. The structure of a terminal α,β-unsaturated γ-lactone ring and a hydroxy group at C-4 was readily recognized as a similar fragment found in annonacin (Alkofahi, *et al*., 1988). Mass fragmentation analysis of the tetra-TMSi derivative (**1a**) demonstrated that the four OH groups were located at C-4, C-10, C-15 and C-20, as shown in Fig. 1. The presence of a mono-THF ring, with two OH groups adjacent to the ring was suggested by ^{1}H-NMR resonance at δ 3.41 (H-15, H-20) and 3.80 (H-16, H-19) for **1** (Alkofahi, *et al*., 1988). These NMR data also indicated that the relative stereochemistries of the centers at C-15/16 and C-19/20 were both *threo* (Laprevote, *et al*., 1992). Compound **1** showed proton signals at *ä* 1.97 and 1.67, corresponding to H-17 and H-18, which are

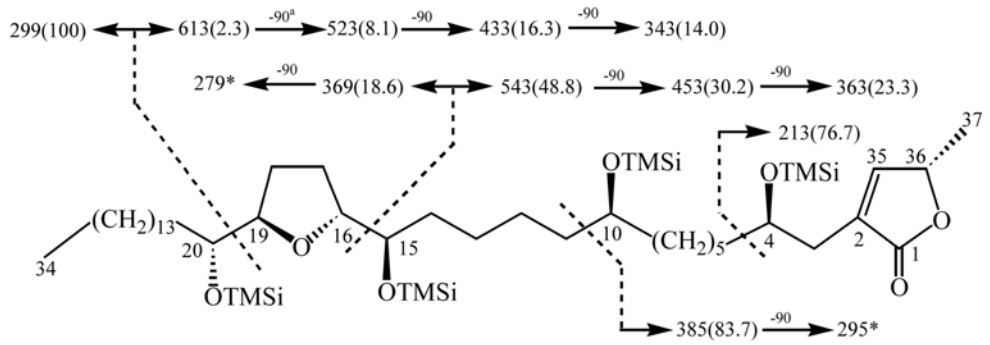

Fig. 1. Diagnostic EIMS fragmentations of tetra-TMSi (**1a**) derivative.
[a]: TMSiOH (Intensities are indicated in parentheses) *Ions not observed.

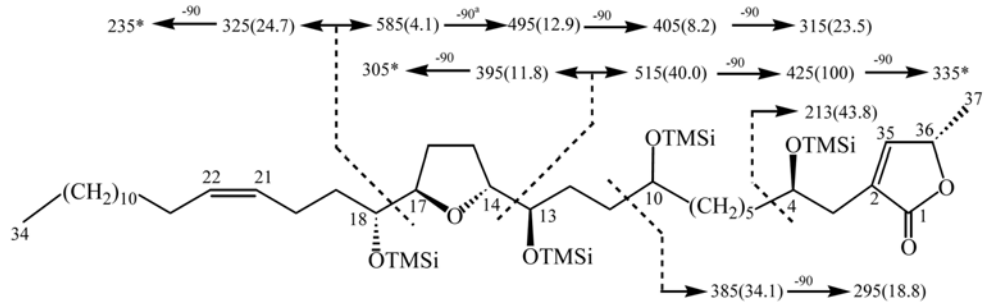

Fig. 2. Diagnostic EIMS fragmentations of tetra-TMSi (**2a**) derivative.
[a]: TMSiOH (Intensities are indicated in parentheses) *Ions not observed.

typical methylene proton signals for a *trans*-THF ring (Gu, *et al*., 1994). Thus, the relative configuration for these four chiral centers are *threo/trans/threo* for **1**. From the above data, we concluded that the structure of xylomaticin (Colman-Saizarbitoria, *et al*., 1994) is as illustrated in **1**.

Compound **2** was obtained as a white amorphous powder. The presence of four OH moieties was suggested by successive losses (*m/z*, 820, 730, 640, 550) of four TMSiOH molecules (*m/z*, 90) in the formation of a tetra-TMSi derivative (**2a**). The presence of an α,β-unsaturated γ-lactone moiety with a hydroxyl group at position C-4 was confirmed by the following characteristic features: a strong IR absorption peak at 1745 cm^{-1} and six proton resonances at δ 7.19 (H-35), 5.06 (H-36), 3.83 (H-4), 2.43 (H-3a), 2.50 (H-3b) and 1.44 (H-37) (Ratnayake, *et al*., 1993). The presence of a mono-THF ring in **2**, with two OH groups at the adjacent carbons of the ring, was deduced by the ^{1}H-NMR resonances at δ 3.83 (H-14 and H-17) and 3.45 (H-13 and H-18), which are characteristic for mono-THF acetogenins having two OH groups adjacent to the ring (Rupprecht, *et al*., 1990; Fang, *et al*., 1993). From the abundant ion signals at *m/z* 315 and 515, both of which contain the unsaturated lactone ring, it was obvious that the THF ring is located between C-14 and C-17 in the molecule of **2a**. The signals at δ 3.60 and 3.63 in the ^{1}H-NMR spectra of **1** and **2** are characteristic of a hydroxyl group in an alkyl chain. The position of the OH group was determined by the fragment at *m/z* 385, which indicated that this hydroxyl was at C-10 (Woo, *et al*., 1999b). On the basis of the spectral data described above, the four OH groups in **2** were assigned at C-4, C-10, C-13 and C-18 positions. The relative stereochemistry of C-13/C-14 and C-17/C-18 was defined as *threo* by comparing the ^{1}H NMR signals of H-13, H-18 (δ 3.45) and H-14, H-17 (δ 3.83) in **2**, with those of model compounds of known relative configuration between C-13/C-14 and C-17/C-18 (Harmange, *et al*., 1992; Fujimoto, *et al*., 1994). The presence of an isolated double bond in the molecule was determined by the proton signals at *d* 5.37. So, this compound was identified as gonionenin (Gu, *et al*., 1994). Both compounds **1** and **2** showed potent bioactivities with BST LC_{50} (μg/mL) values of 3.00×10^{-4} and 6.00×10^{-1}, respectively.

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