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\(\text{Ac} = \) \(\text{2-Mebu} = \) \(\text{Tig} = \) \(\text{Tig-5-OH} = \) \(\text{OH} = \)
Three new inositol derivatives from *Chisocheton paniculatus*

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ABSTRACT

Three new inositol derivatives (1–3) were isolated from the leaves of *Chisocheton paniculatus* collected in Vietnam, along with four known compounds, including a limonoid (4), two triterpenoids (5–6), and a tocopherol (7). The structures of the new compounds were elucidated by 1D- and 2D-NMR and HRESIMS analyses. Compound 4 showed the highest cytotoxicities against the human lung cancer A549 and cervical cancer HeLa cell lines, with IC\(_{50}\) values of 7.3 and 8.8 µM, respectively, among the isolated compounds. Compounds 5 and 7 displayed moderate to weak cytotoxicities against the A549, HeLa, and human stomach cancer GSU cell lines, with IC\(_{50}\) values ranging from 17.7 to 68.0 µM.

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1. Introduction

The *Chisocheton* genus consists of 53 species and is regarded as the second largest genus in the Meliaceae family. This genus is distributed in the tropical and subtropical regions of Asia. The extracts and isolated compounds from the *Chisocheton* genus have gained a great amount of attention from scientists over the last decade because of their bioactivities, including their anticancer, anti-inflammatory, antibacterial, antifungal, antimalarial, and anti-obesity activities. Previous phytochemical studies of 11 species of the *Chisocheton* genus have led to the isolation and characterization of numerous limonoids, protolimonoids, and triterpenes, together with steroids, sesquiterpenes, anthraquinones, spermidine alkaloids, coumarines, and phenolics. In a previous study, we reported that the *C. paniculatus* fruit contains a new limonoid, 6α,7α-diacetoxy-3-oxo-24,25,26,27-tetranorapotirucall-1,14,20(22)-trien-21,23-lactam, and two known 5α,8α-epidioxysterols with NO production inhibitory activities against LPS stimulated BV2 cells. In the course of our search for naturally occurring cytotoxic compounds from Vietnamese medicinal plants, we isolated three new inositol derivatives (1–3), together with four known compounds (4–7), from the leaves of *C. paniculatus* collected in Vietnam (Fig. 1). Herein, we report the structural elucidations of the new compounds (1–3) and cytotoxicities of the isolated compounds.
COSY spectrum, as well as the lack of the anomeric signal in the $^{13}$C NMR spectrum, indicated the presence of an inositol moiety in 1. This assignment is also supported by the coupling patterns of six oxygenated methine protons in 1, where three small $J$ values ($J_{H_3-1b/H_4} = 3.7$ Hz, $J_{H_4-1b/H_5} = 3.8$ Hz, $J_{H_4-2b/H_3} = 3.3$ Hz) and three large $J$ values ($J_{H_4-1b/b'/C-1} = 10.5$ Hz, $J_{H_4-4b/C-5} = 10.0$ Hz, $J_{H_5-3b/a/C-3} = 10.0$ Hz) were observed in a manner similar to those of the reported chiro-inositol derivatives. Furthermore, the ROESY correlations of H-3 ($\delta_H 5.16$) to H-5 ($\delta_H 5.47$) and of H-4 ($\delta_H 5.71$) to H-6 ($\delta_H 4.30$) were observed (Fig. 3). Thus, chiro-form was assigned for inositol moiety of 1.

The presence of two tigloyloxy moieties was deduced by the HMBC correlations of H$_{-1}$-4a ($\delta_H 1.92$)/H$_{-1}$-5a ($\delta_H 1.75$) to C-2a ($\delta_C 129.4$)/C-3a ($\delta_C 139.8$), of H$_{-1}$-5a to C-1a, of H$_{-1}$-4c ($\delta_H 1.88$)/H$_{-1}$-5c ($\delta_H 1.75$) to C-2c ($\delta_C 129.1$)/C-3c ($\delta_C 140.0$), and of H$_{-1}$-5c to C-1c (Fig. 2). Meanwhile, the HMBC correlations between H$_{-1}$-4d ($\delta_H 1.86$)/H$_{-1}$-5d ($\delta_H 4.22$) to C-2d ($\delta_C 133.2$)/C-3d ($\delta_C 143.6$) and of H$_{-1}$-5d to C-1d ($\delta_C 167.7$), as well as the ROESY correlation between H$_{-1}$-4d and H$_{-1}$-5d (Fig. 3), suggested the presence of (E)-2-hydroxymethylbut-2-enoyloxy moieties (trivial name: 5-hydroxytigloxyloxy) in the molecule. In the same manner, the presence of another 5-hydroxytigloxyloxy moiety in 1 was determined from the 2D NMR spectra analyses (Figs. 2 and 3). The HMBC correlations from H-1 ($\delta_H 5.31$) to C-1a, from H-3 ($\delta_H 5.16$) to C-1c, from H-4 ($\delta_H 5.71$) to C-1d, and from H-5 ($\delta_H 5.47$) to C-1e allowed us to locate the tigloxyloxy moieties at C-1 and C-3 and the 5-hydroxytigloxyloxy moieties at C-4 and C-5, respectively. Consequently, compound 1 was elucidated to be chiro-inositol-4,5-di-5-hydroxytiglate-1,3-di-tiglate.

Compound 2 was isolated as a pale yellow oil. The HRESIMS analysis exhibited a quasi-molecular ion peak at $m/z$ 523.1792 [M+Na$^+$], corresponding to the molecular formula C$_{23}$H$_{32}$O$_{12}$. The $^1$H and $^{13}$C NMR spectroscopic data (Table S1) were similar to those of 1. The main difference was the presence of an acetoxyl group [$\delta_C 171.2$ (C-1b), 20.7 (C-2b); $\delta_H 2.13$ (H$_{-1}$-2b)] in 2, instead of the tigloxyloxy group in 1. The acetoxyl group was attached at C-2, based on the HMBC correlation from H-2 ($\delta_H 5.43$) to C-1b. This assignment was supported by the significant upfield shift of H-1 ($\delta_H 4.00$)/C-1 ($\delta_C 71.0$) and the downfield shift of H-2 ($\delta_H 5.43$)/C-2 ($\delta_C 71.9$) of 2, as compared to those of 1. The remaining tigloxyloxy group and the two 5-hydroxytigloxyloxy groups in 2 were located at the same positions as 1, based on the downfield chemical shifts of H-3 ($\delta_H 5.47$), H-4 ($\delta_H 5.55$), and H-5 ($\delta_H 5.52$) and their HMBC correlations to...
the carbonyl carbons at δc 168.2, 167.7, and 168.1, respectively (Fig. 2). Thus, compound 2 was assigned as chiro-inositol-2-acetate-4,5-di-5-hydroxytiglate-3-tiglate.

Compound 3 was isolated as a pale yellow oil, and its molecular formula was determined to be C34H43O12 from the NMR and HRESIMS data. The NMR data of compound 3 (Table S1) revealed a close structural resemblance to 2, except for the replacement of the tigloyloxy moiety at C-3 in 2 by a saturated ester group in 3. The cross peaks of H-3(δh 0.78)/H-5c(δh 1.03) to C-2c(δc 42.3)/C-3c(δc 27.6) and of H-5c to C-1c(δc 176.9) in the HMBC spectrum, and the linear spin system [(C-4c(δh 1.6)/C-3c(δh 2.4)/C-2c(δh 1.8)/C-5c(δh 2.1)] in the COSY spectrum (Fig. 2), supported the presence of a 2-methylbutanoyloxy moiety in 3. The position of this moiety at C-3 was verified by the HMBC correlation from H-3 to C-1c.

Hence, the structure of 3 was identified as chiro-inositol-2-acetate-4,5-di-5-hydroxytiglate-3-2-methylbutyrate. To the best of our knowledge, this is the first report for the isolation of inositol derivatives with 5-hydroxytigloyl group.

The other isolated compounds were identified as 6α,7α-diaceoctoxy-23-hydroxy-3-oxo-26,27-tetranorapotirucall-1,4,20(22)-tien-21,23-olide (4), (24E)-3-oxo-dammara-20,24-dien-26-ol (5), viasmiaefolic acid (6), and δ-tocopherol (7), (Fig. 1), based on their spectroscopic data including 1D and 2D NMR data and comparisons with those reported in the literature.

Natural products have played a very important role as established cancer chemotherapeutic agents, and we have so far searched naturally occurring cytotoxic compounds against human cancer cell lines. Inositol is a natural constituent possessing moderate anticancer activity. Besides, the importance of phosphate moiety in the structure of inositol phosphate to improve cytotoxicity was reported.11-13 Thus, we tried to evaluate the structure-activity relationship of inositol derivatives with 5-hydroxytigloyl group against cancer cell lines. Inositol is a natural constituent possessing anticancer agents from Vietnamese natural sources,14-21 the isolated compounds 1–7 were assessed for their cytotoxic activities against three human cancer cell lines (A549, lung cancer; HeLa, cervical cancer; and GSU, stomach cancer). The results of the cytotoxic assays are summarized in Table 1. Among the isolated compounds, compound 4 exhibited the highest cytotoxicities against the A549 and HeLa cell lines, with IC50 values of 7.3 and 8.8 µM, respectively, at levels comparable to those of the positive control, 5-FU. This compound also showed moderate cytotoxicity against the GSU cell line, with an IC50 value of 31.7 µM. In addition, 5 and 7 displayed moderate to weak inhibitions against the three tested cancer cell lines, with IC50 values ranging from 17.7 to 68.0 µM. In contrast, 6 did not show any cytotoxicities against the tested cell lines. Furthermore, no activities were detected for inositol derivatives 1–3, suggesting that the presence of 5-hydroxytigloyl group in the structure is not important for the cytotoxicity.

### Table 1. Cytotoxic activities of 1–7 against three human cancer cell lines

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</table>

*Positive control

### 3. Experimental

#### 3.1. General experimental procedures

Infrared spectra were recorded with KBr pellets on a Jasco FT/IR-460 Plus spectrophotometer (Hachioji, Tokyo, Japan). UV–Vis spectroscopy was recorded on a NanoDrop™ 2000C spectrophotometer (Thermo Fisher Scientific, Wallata, MA, USA). Optical rotations were measured on a Jasco P-2100 polarimeter (Hachioji, Tokyo, Japan). NMR spectra were recorded using a Bruker Avance 500 spectrometer (500 MHz for 1H NMR, 125 MHz for 13C NMR) (Bruker, MA, USA), with TMS as an internal reference. High-resolution electron spray ionization mass spectrometry data were acquired with an Agilent 6530 Accurate-Mass spectrometer (Agilent, CA, USA). Chromatography was performed with silica gel (60 N, spherical, neutral, 40-50 µm, Kanto Chemical Co., Inc., Tokyo, Japan), RP-18 (Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan), and Sephadex LH-20 (Dowex® 50WX2-100, Sigma–Aldrich, USA). Analytical TLC was performed on pre-coated silica gel 60F254 and RP-18 F254 plates (0.25 or 0.50 mm thickness, Merck KgaA, Darmstadt, Germany). Preparative HPLC was conducted on an Agilent 1260 Infinity II system (Agilent, CA, USA), using a Zorbax SB-C18 column (5µm particle size, 9.4 x 250 mm) and a DAD detector. The 96-well plates were purchased from TPP (TTP Techno Plastic Products AG, Switzerland).

#### 3.2. Plant material

The C. paniculatus leaves were collected from Quang Tri province, Vietnam, in August 2018 and identified by Dr. Vu Tien Chinh, Vietnam National Museum of Nature, VAST, Vietnam. A voucher specimen (CP-02) was deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

#### 3.3. Extraction and isolation

The dried leaves of C. paniculatus (4.5 kg) were extracted with MeOH (3 times, 10.0 L each) at room temperature to yield 357 g of a dark solid extract. The extract was then suspended in water (2.0 L) and successively partitioned with n-hexane and ethyl acetate (3 times, 5.0 L each) to obtain the n-hexane (H, 127 g), ethyl acetate (E, 105 g), and water (W, 98 g)-soluble portions, after the removal of the solvents in vacuo. The H extract was chromatographed on a silica gel column, eluted with an n-hexane–acetone gradient system (100:0, 95:5, 90:10, 50:10, 10:10, 0:10 v/v, each 1.0 L) to obtain 4 fractions (H1–H4). Fraction H2 (42 g) was fractionated on a silica gel column, eluted with n-hexane–acetone (20:1, v/v) to obtain 13 fractions (H2.1–H2.13). Fraction H2.4 (3.1 g) was applied to a Sephadex LH-20 column, eluted with CHCl3–MeOH (1:1, v/v) to give 6 fractions (H2.4.1–H2.4.6). Fraction H2.4.3 (380 mg) was then partitioned on a Sephadex LH-20 column, eluted with CHCl3–MeOH (1:1, v/v) to give 3 fractions (H2.4.3.1–H2.4.3.3). Fraction H2.4.3.3 (100 mg) was purified by preparative reversed phase HPLC, using MeOH–water (96:4, flow rate 2 mL/min) as the eluent, to afford 7 (63.0 mg). Fraction H2.4.4 (250 mg) was then partitioned on a Sephadex LH-20 column, eluted with MeOH to furnish 6 (12.1 mg). Fraction H2.6 (33.3 g) was then partitioned on a Sephadex LH-20 column, eluted with CHCl3–MeOH (1:1, v/v) to give 3 fractions (H2.6.1–H2.6.3). Fraction H2.6.2 (0.98 g) was purified by preparative reversed phase HPLC, using MeOH–water (92.5:7.5, flow rate 2 mL/min) as the eluent, to afford 5 (7.5 mg).
The E extract (105 g) was loaded onto an open silica gel column, which was eluted with CH$_2$Cl$_2$–MeOH (10:1, v/v) to obtain 8 fractions (E1–E8). Fraction E4 (10.2 g) was partitioned on an RP-18 column eluted with MeOH–water (1:1, v/v) to obtain 4 fractions (E4-1–E4-4). Fraction E4-1 (2.6 g) was applied on an RP-18 column eluted with acetone–MeOH–water (3:1:1, v/v/v) to obtain 6 fractions (E4-1.1–E4-1.6). Fraction E4-1.4 (400 mg) was then partitioned on a Sephadex LH-20 column eluted with CH$_2$Cl$_2$–MeOH (10:1, v/v), to give 3 fractions (E4-1.4-1–E4-1.4-3). Fraction E4-1.4-3 (150 mg) was purified by preparative reversed phase HPLC, using MeOH–TFA in water 0.05% (50:50, flow rate 3.53 mL/min) as eluent, to afford 1 (4.8 mg), 2 (5.7 mg), and 3 (3.1 mg). Fraction E4-3 (1.9 g) was chromatographed on an RP-18 column eluted with MeOH–acetonitrile–water (7:4:1, v/v/v), followed by fractionation on a Sephadex LH-20 column eluted with MeOH, to obtain 4 (5.5 mg).

**chiro-Inositol-4,5-di-5-hydroxytiglate-1,3-di-tiglate (1):** Pale yellow oil; [c]$_D$$^2$ +52.5 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 209 (4.09), 215 (4.27), 219 (4.31), 225 (4.29), 228 (4.32); IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3436, 2951, 1716, 1648, 1267, 1137; $^1$H NMR (500 MHz, CD$_3$OD): see Table S1; HRESIMS $m/z$: 563.2108 [M+Na]$^+$ (calcd. for C$_9$H$_9$O$_7$Na$_2$, 563.2104).

**chiro-Inositol-2-acetate-4,5-di-5-hydroxytiglate-3-tiglate (2):** Pale yellow oil; [c]$_D$$^2$ +45.9 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 209 (3.38), 215 (3.56), 219 (3.59), 225 (3.59), 228 (3.61); IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3420, 2921, 2851, 2359, 1716, 1652; $^1$H NMR (500 MHz, CD$_3$OD) and $^{13}$C NMR (125 MHz, CD$_3$OD): see Table S1; HRESIMS $m/z$: 523.1792 [M+Na]$^+$ (calcd. for C$_{10}$H$_{11}$O$_7$Na$_2$, 523.1791).

**chiro-Inositol-2-acetate-4,5-di-5-hydroxytiglate-3,2'-methylbutyrate (3):** Pale yellow oil; [c]$_D$$^2$ +49.9 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 209 (4.06), 215 (4.16), 219 (4.15), 225 (4.07); IR (KBr) $\nu_{max}$ (cm$^{-1}$): 3434, 2926, 2347, 1685, 1638, 1385, 1205, 1139; $^1$H NMR (500 MHz, CD$_3$OD) and $^{13}$C NMR (125 MHz, CD$_3$OD): see Table S1; HRESIMS $m/z$: 525.1953 [M+Na]$^+$ (calcd. for C$_{12}$H$_{14}$O$_7$Na, 525.1948).

### 3.4. Cytotoxicity assay

**α-Minimum essential medium with d-(-)-glucose, L-glutamine, phenol red, and sodium pyruvate (α-MEM, Nacalai Tesque)** was used for the HeLa and A549 cell cultures, while RPMI 1640 (Nacalai Tesque) with d-(-)-glucose, L-glutamine, and phenol red was used for the GSU cell culture. All media were supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% antibiotic antimycotic solution (Sigma). Briefly, each cell line was seeded in 96-well plates (2 × 10$^3$ cells per well) and incubated in the respective medium at 37 °C, under a 5% CO$_2$ and 95% air atmosphere, for 24 h. After the cells were washed with PBS (Nissui Pharmaceuticals), the concentrations of the serial dilutions of the tested samples were added to the wells. 5-Fluorouracil was used as the positive control. After a 72 h incubation, 10 μL of MTT working solution (5 mg/mL in phosphate buffer solution) was added to each well, the plate was incubated for 3 hours at 37°C in a CO$_2$ incubator, and then the medium was aspirated. The formazan crystals thus formed were solubilized with 100 μL of DMSO per well for 30 min at 37°C in a CO$_2$ incubator, and the absorbance at 540 nm was measured. The cell viability was calculated from the mean values of data from three wells by using the following equation, and the cytotoxicity was expressed as the IC$_{50}$ (50% inhibitory concentration) value.
Highlights

- Three new inositol derivatives were isolated from *Chisocheton paniculatus* leaves
- The structures of the new compounds were elucidated by NMR and HRESIMS analyses
- The new inositol derivatives did not show any