Note

Novel Monoterpene Lactones from *Cinnamomum inunctum*

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Myanmar is located in the westernmost part of Southeast Asia. It is 1.8 times larger than Japan and stretches from south to north. While northern Myanmar has a temperate climate, the middle and southern parts are tropical. Myanmar is surrounded by several other countries, and in the border regions with Thailand and Laos (the so-called “GOLDEN TRIANGLE”), opium poppy is cultivated illegally. Due to the influence of tropical monsoons and its subtropical climate, Myanmar abounds in plant resources. However, to date, there have been inadequate investigations of these resources.

*Cinnamomum inunctum* MEISN. (Lauraceae) (Local name: Karawe dea) grows in Myanmar’s tropical region. Its dried fruits (Local name: Karawe dea) are available in herbal medicine markets and are used for the treatment of inflammation, diarrhea, asthma, fever, headache, malaria and menstrual disorders.1 The dried fruits measure about 5 mm in diameter and have a wrinkled surface like a walnut. The fruits are sometimes confused with those of *Piper cubeba* times confused with those of *Cinnamomum cubeba* because of their physical resemblance, and they also have similar efficacies as medicines.

In general, the main constituents of *Cinnamomum* species fruits are essential oils. However, there have been few reports on the constituents of *C. inunctum.*2

In this paper, we describe the chemical constituents of the fruit of *C. inunctum.*

Experimental

Plant Material Dried fruits of *C. inunctum* were purchased at an herbal medicine market in Yangon, Myanmar in 2002, and identified by Dr. Satake (Ochanomizu University). Sample specimens (Sample specimen No. MCI-01) are deposited at the Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation (NIBIO).

General Experimental Procedures Optical rotations were determined with a JASCO P-1200 automatic polarimeter. 1H- and 13C-NMR spectra were measured with a JEOL Alpha 500 spectrometer (500 MHz). IR spectra were recorded on a JASCO FTIR-5300 spectrometer. HPLC was run on a Shimadzu LC-10ADVP system. LC/MS was measured on an AB Sciex QSTAR XL (ESI-TOFMS), a Thermo Fischer Scientific LTQ-Orbitrap XL (ESI-Orbitrap MS) and a JEOl JMS-HX-110 (FAB-MS, EI-MS). GC-MS was run on a Shimadzu GCMS-QP5050A system.

Extraction and Isolation Dried fruits of *C. inunctum* (30.7 g) were extracted with methanol (MeOH) to give an extract (11.2 g). The extract was chromatographed on silica gel using chloroform (CHCl3)-MeOH as an eluent to give 20 fractions (Fr. 1–20). Fractions 2–5 (eluted with 5% MeOH–CHCl3) were combined and rechromatographed repeatedly on silica gel (solvent system; 1st: n-hexane–ethyl acetate (EtOAc), 2nd: CHCl3–MeOH), and then purified by HPLC using an ODS column (CAPCELPK C18 SGI20A 5µm (10 mm×250 mm), solvent system; 50–95% MeOH–water (gradient condition)) to afford 3-hydroxy-4,4-dimethyl-4-butyrolactone (5).3 (10.5 mg), 1 (9.9 mg) and 2 (7.1 mg). Fractions 6–9 (eluted with 20% MeOH–CHCl3) were combined and subjected to Sephadex LH-20 column chromatography using 90% MeOH–water as an eluent and then purified by HPLC using an ODS column (CAPCELPK C18 SGI20A 5 µm, Shimseido Co., Ltd. (10 mm×250 mm), solvent system: 50–95% MeOH–water (gradient condition)) to obtain compounds 3 (7.1 mg) and 4 (10.5 mg).

Compound 1 [α]D +2.1° (c=0.06, MeOH), IR νmax (CHCl3) cm⁻¹: 3676, 3577, 3024, 2980, 1782, 1231. Electron ionization (EI)-MS m/z: 203 [M–CH3]⁺, 185 [M–CH3–H2O], 159 [M–(CH3)2C(OH)]⁺, 142, 129 [M–(CH3)2C(OH)CH(OH)]⁺, 115 [M–(CH3)2C(OH)CH(OH)CH3]⁺, 113, 96, 71, 59 [(CH3)2C(OH)]⁺ (base peak). Electrospray ionization (ESI)-Orbitrap MS z: 217.1073 [M–H]⁻ (Caled for C10H17O5: 217.1076).

Compound 2 [α]D –3.0° (c=0.57, MeOH), UV λmax (MeOH) nm: 208, IR νmax (CHCl3) cm⁻¹: 3575, 3027, 2988, 1748, 1644, 1305. EI-MS m/z: 185 [M–CH3]⁺, 156, 141 [M–(CH3)2C(OH)]⁺, 125, 111 [M–(CH3)2C(OH)CH(OH)]⁺, 98 [M–(CH3)2C(OH)CH(OH)CH3]⁺, 71 [M–(CH3)2C(OH)]⁻ (base peak), 59 [(CH3)2C(OH)]⁻. High resolution
(HR)-FAB-MS m/z: 201.1116 [M+H]+ (Calcd for C_{10}H_{18}O_{5}: 201.1127).

**Compound 3**

[α]D = +2.4° (c=0.49, MeOH). IR ν<sub>max</sub> (CHCl<sub>3</sub>) cm<sup>−1</sup>: 1764, 1657. FAB-MS (positive mode) m/z: 397 [2M+H]<sup>+</sup>, 199 [M+H]<sup>+</sup>, 181 [M–H<sub>2</sub>O+H]<sup>+</sup>. HR-FAB-MS m/z: 199.0975 [M+H]<sup>+</sup> (Calcd for C_{10}H_{18}O_{5}: 199.0970). HR-ESI-Orbitrap MS m/z: 197.0812 [M−H]<sup>−</sup> (Calcd for C_{10}H_{18}O_{5}: 197.0819).

**Compound 4**

[α]D = −0.3° (c=0.43, MeOH). IR ν<sub>max</sub> (CHCl<sub>3</sub>) cm<sup>−1</sup>: 1763, 1659. FAB-MS (positive mode) m/z: 397 [2M+H]<sup>+</sup>, 199 [M+H]<sup>+</sup>, 181 [M–H<sub>2</sub>O+H]<sup>+</sup>. HR-FAB-MS m/z: 199.0954 [M+H]<sup>+</sup> (Calcd for C_{10}H_{18}O_{5}: 199.0970).

**Benzoylation of 3 and 4**

Compounds 3 (1.5 mg) and 4 (1.5 mg) were separately dissolved in pyridine (2 mL) and benzoyl chloride (2 mg) was added to each solution. The respective mixtures were warmed for 5 min, and then stirred for 2 h at room temperature. The solvent was then removed from the resulting mixture by a vacuum pump for 1 d. The residues were purified by HPLC (column: Mightysil Si60 from KANTO Chemicals), solvent system: n-hexane–ethyl acetate, gradient condition, flow rate: 2.0 mL/min. The results by MS chromatography: 3: retention time (t<sub>R</sub>) 8.6 min, 9.7 min. MS: m/z 217.1073 [M+H]<sup>+</sup> (Calcd 217.1076). In the IR spectrum, the presence of hydroxyl and γ-lactone moieties were suggested from the absorption at 3577 cm<sup>−1</sup> and 1782 cm<sup>−1</sup>, respectively. 1H- and 13C-NMR spectra, together with 1H-detected heteronuclear multiple quantum coherence (HMQC) experiment, showed three methyl (δ<sub>H</sub> 24.3 (δ<sub>C</sub> 68, 43)).

**Results and Discussion**

From the methanolic extract of *C. inunctum*, four new compounds (1–4) were isolated together with a known compound, 3-hydroxy-4,4-dimethyl-4-butyrolactone. 3

Compounds 1 was obtained as a colorless oil with a sweetish odor, and the molecular formula was determined as C_{10}H_{18}O_{5} based on the HR-ESI-Orbitrap MS (m/z 217.1073 [M+H]+ (Calcd 217.1076)). In the IR spectrum, the presence of hydroxyl and γ-lactone moieties were suggested from the absorption at 3577 cm<sup>−1</sup> and 1782 cm<sup>−1</sup>, respectively. 1H- and 13C-NMR spectra, together with 1H-detected heteronuclear multiple quantum coherence (HMQC) experiment, showed three methyl (δ<sub>H</sub> 24.3 (δ<sub>C</sub> 68, 43)), two methylene (δ<sub>H</sub> 2.12 (δ<sub>C</sub> 32.2 [δ<sub>C</sub> 13.9, 10.7, 4.4 Hz) and 2.19 (δ<sub>C</sub> 13.9, 10.7, 4.4 Hz) and 2.19 (δ<sub>C</sub> 13.9, 10.7, 4.4 Hz)), and two methine (δ<sub>H</sub> 8.3 Hz) groups. In the 13C-NMR spectrum, in addition to the above hydrogen bearing carbons, three oxygen bearing (δ<sub>C</sub> 69.9, 85.7, 88.8) and an ester carbonyl (δ<sub>C</sub> 175.3) carbons were observed. Based on the molecular formula and the observed carbon signals, 1 was considered to be a monoterpenoid. The observed wavelength of the C–O stretching of the carboxyl group (1782 cm<sup>−1</sup>) in-

| Compd. | H-3′ | H-7′ | H-8 |
|--------|------|------|-----|
| 1      | 2.66 d | 2.84 d | 2.12 ddd |
| 2      | 5.82 q | 5.16 brd | 4.88 ddd |
| 3      | 5.81 q | —     | 2.30 dd |
| 4      | 5.88 q | —     | 2.00 dd |
| 3b     | 5.85 d | —     | 2.47 dd |
| 4b     | 5.89 d | —     | 2.32 dd |

***Assignments may be interchanged.
dicated the presence of a five-membered lactone. From these data together with the two dimensional (2D)-NMR correlations shown in Fig. 1, the structure of 1 was concluded as shown in Fig. 1. The EI-MS fragmentation patterns of 1 also supported this structure (Fig. 2). The stereochemical relationship between the methyl group (H-3/3') and H-4 was determined to be syn by the correlation in the nuclear Overhauser effect spectroscopy (NOESY) spectrum (Fig. 1). However, the stereochemistry at C-6 could not be determined. This compound has not been reported previously.

Compound 2 was assigned a formula of C_{10}H_{16}O_{4} based on the HR-ESI-FAB-MS (m/z 201.1116 [M+H]^+, Calcd 201.1127). The ^1H- and ^13C-NMR spectra were similar to those of 1, except for the γ-lactone part. In the ^13C-NMR spectrum of 2, signals of the oxygenated tertiary carbon (δ_C 85.7, C-3) and the isolated methylene carbon (δ_C 42.1, C-2) in 1 were replaced by two olefinic signals (δ_C 169.8, 116.5), suggesting a β-elimination of the 3-hydroxy group of 1 to form an α,β-unsaturated γ-lactone. This was supported by the ^1H-NMR of 2: the isolated methylene signals (H-2) in 1 were replaced by an olefinic proton signal [δ_H 5.82 (q, J=1.5 Hz)], which showed allyl coupling with the H-3/3' methyl group [δ_C 2.09 (dd, J=1.5, 0.7 Hz)]. The presence of a 4-methyl-2(5H)-furanone moiety was also deduced based on a comparison of the ^13C-NMR chemical shifts of 2 with those of 3-methyl-7-oxo-2-octen-4-olide. Finally, the structure was established by 2D-NMR techniques (double quantum filtered (DQF) correlation spectroscopy (COSY), HMQC, heteronuclear multiple bond connectivity (HMBC) and NOESY), as shown in Fig. 1. The EI-MS fragmentation patterns of 2 supported this structure (Fig. 2). This compound has also not been reported previously.

Compound 3 was assigned a formula of C_{10}H_{14}O_{4} based on the HR-ESI-Orbitrap MS (m/z 197.0812 [M−H]^−, Calcd 197.0819). The IR spectrum revealed the presence of a conjugated carbonyl group (1764 cm\(^{-1}\)) and an olefinic bond (1657 cm\(^{-1}\)). Its NMR spectra resembled to those of 2, except for the signals around C-4. As the molecular formula was two hydrogens less from 2, an additional ring structure was deduced for 3. The ^13C-NMR showed an acetal carbon signal (δ_C 113.2), and HMBC correlations (Fig. 3) were observed between this acetal carbon and H-3', 5 and 6, indicating the
acetal carbon to be C-4. Based on the above data, compound 3 was estimated to have a spiroacetal in its structure. This part structure was supported by comparison of the 13C-NMR spectra with those of the spiroacetal moieties of α- and β-lavantenolide.5,6) Figure 3 shows the correlations observed in 2D-NMR. To clarify the stereochemistry of 3, a benzoate (3b) was prepared and subjected to an NOE experiment. As shown in Fig. 4, the key relay correlation observed in the NOE spectrum (between H-3/uni2032 and benzoyloxy proton) indicated that the benzoyloxy group on C-6 resides in the same side with the allyl methyl group on C-3.

Compound 4 was assigned a formula of C_{10}H_{14}O_{4} based on the HR-FAB-MS (m/z 199.0954 [M+H]^+) Calcd 199.0970). The NMR data were similar to those of 3 except for the coupling constants between H-5 and H-6. Therefore, 4 was considered to be a stereoisomer of 3 at C-6. An NOE experiment with its benzoate 4b supported anti relationship between the allyl methyl (C-3) and the benzoate group, as shown in Fig. 4.

Since GC-MS analysis of 3 and 4 using a chiral capillary column showed two peaks with completely the same MS spectra in each sample, 3 and 4 were determined to be racemates. In this study, we isolated four new monoterpenoids together with a known compound. These structures showed unique skeletons. Compounds 3 and 4 contain spirolactone moieties, which can be formed by an oxidation of C-4 of 2 followed by intramolecular cyclization.

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Conflict of Interest The authors declare no conflict of interest.

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