New Onoceranoid Triterpene Constituents from *Lansium domesticum*

Tadamitsu Tanaka,[†] Masami Ishibashi,*[,†] Haruhiro Fujimoto,[†] Emi Okuyama,[†] Takashi Koyano,[‡] Thaworn Kowithayakorn,[§] Masahiko Hayashi,[⊥] and Kanki Komiyama[⊥]

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan, Temko Corporation, 4-27-4 Honcho, Nakano, Tokyo 164-0012, Japan, Department of Horticulture, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand, and The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

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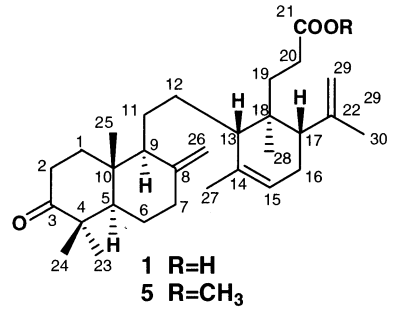
Three new natural onoceranoid triterpenes, lansionic acid (**1**), 3β-hydroxyonocera-8(26),14-dien-21-one (**2**), and 21α-hydroxyonocera-8(26),14-dien-3-one (**3**), were isolated from the fruit peel of *Lansium domesticum* together with two known triterpenoids (**4** and **5**), and their structures were elucidated from spectral data. These triterpenoids exhibited mild toxicity against brine shrimp (*Artemia salina*).

Lancium domesticum Corr. (Meliaceae) is a popular fruit in southern Asia, and the peel of this fruit, traditionally, is said to be toxic to animals. Previous studies revealed that this plant contained several types of triterpenoids.[1−4] During our search for bioactive natural products from tropical plants, we investigated the chemical constituents of the peel of *L. domesticum* collected in Thailand. Here we describe isolation and structure elucidation of three new naturally occurring triterpenoids (**1**−**3**) together with two known triterpenoids (**4** and **5**).

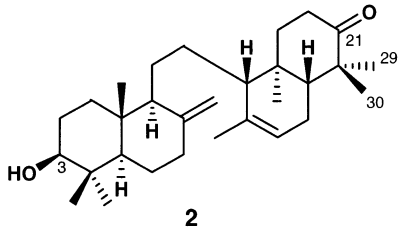
The peel of *L. domesticum*, collected in Thailand, was extracted with MeOH, and the extract showed toxicity against *Artemia salina*.[5] The MeOH extract was subjected to solvent partitioning and repeated chromatographies to give three triterpenoids (**1**−**3**), together with known compounds, lansic acid (**4**)[1,3] and methyl ester (**5**).[3] Compounds **1**−**3** have been isolated here as natural products, but **2** and **3** were previously reported as synthetic intermediates for chemical synthesis of lansic acid (**4**) from α,γ-onoceradienedione.[6,7]

Lansionic acid (**1**) was shown to have the molecular formula $C_{30}H_{46}O_3$ by HRFABMS. The IR spectrum of **1** showed a broad absorption band at 3400−2800 cm^{-1} and a strong absorption at 1710 cm^{-1}, indicating the presence of carboxyl and ketone groups. The ^{1}H NMR spectrum of **1** showed signals due to six tertiary methyls, and its ^{13}C NMR spectrum aided by HMQC experiments revealed the presence of a trisubstituted olefin (δ_C 135.8 and 121.8), two exomethylenes (δ_C 147.5, 147.6, 107.6, and 114.0), a ketone (δ_C 217.2), and a carboxyl group (δ_C 178.0). Since five out of eight unsaturation degrees were thus accounted for, **1** was inferred to have three rings. The HMBC spectrum of **1** showed correlations consistent with the 21,22-secoonocerane skeleton, and the spectral features of **1** were similar to those of methyl lansionate (**5**),[3,7] concurrently isolated here, and lansiolic acid,[3,6] which possesses a hydroxyl group on C-3 and was previously isolated from the same plant. Treatment of **1** with trimethylsilyldiazomethane afforded the methyl ester (**5**). Thus, lansionic acid (**1**) was concluded to be 3-dehydrolansiolic acid.

Compounds **2** and **3** had the same molecular formula ($C_{30}H_{48}O_2$) as determined by their HRFABMS data. The



1 R=H
5 R=CH$_3$



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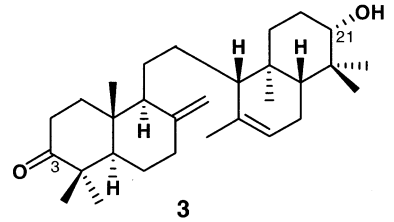

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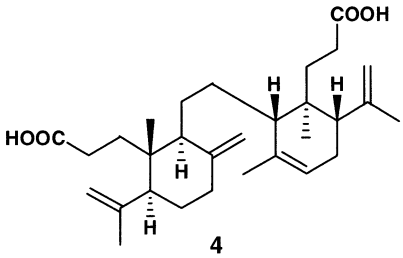

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^{1}H NMR spectrum of **2** showed signals due to seven tertiary methyls, and its ^{13}C NMR and HMQC spectra revealed the presence of a trisubstituted olefin (δ_C 135.8 and 121.7), an exomethylene (δ_C 148.1 and 106.9), a ketone (δ_C 217.3), and an oxymethine group (δ_C 79.5). The IR spectrum of **2** suggested the presence of hydroxyl and ketone groups. The HMBC spectrum of **2** showed the ketone group on C-21 and the hydroxy group on C-3. Double bonds at $\Delta^{8,26}$ and $\Delta^{14,15}$ were also indicated from its HMBC correlations. The

* To whom correspondence should be addressed. Tel and Fax: +81-43-290-2913. E-mail: mish@p.chiba-u.ac.jp.
† Chiba University.
‡ Temko Corporation.
§ Khon Kaen University.
⊥ The Kitasato Institute.

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hydroxy group on C-3 was suggested to be β-equatorial from the NOE correlations observed for H-3/H_3-23, H_3-23/H-5, and H_3-24/H_3-25. Compound **3** had spectral data very similar to those of **2**; the differences between **2** and **3** were the positions of the ketone and hydroxy groups. The HMBC spectrum of **3** showed that the ketone group was on C-3 and the hydroxy group on C-21. The hydroxy group on C-21 was shown to be α-equatorial from the NOE correlations observed for H-21/H_3-30, H_3-30/H-17, and H_3-29/H_3-28. Thus, compounds **2** and **3** were identified as 3β-hydroxyonocera-8(26),14-dien-21-one (**2**) and 21α-hydroxyonocera-8(26),14-dien-3-one (**3**), both of which were previously prepared from α,γ-onoceradienedione by $NaBH_4$ reduction.[6,8] The NMR data of **2** and **3** are also reported here for the first time.

Triterpenes **1**−**5** all showed moderate toxicity against *Artemia salina* (brine shrimp) at a concentration of 100 μg/mL. Trypsin inhibitory activities of these onoceranoid triterpenes (**1**−**5**) were also examined, but all were inactive.[9]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO J-20 instrument. IR spectra were measured on KBr disks in a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on JEOL JNM GSX-A400, A500, and ecp600 spectrometers. High-resolution fast atom bombardment (HRFAB) mass spectra were acquired on a JMS HX-110 mass spectrometer.

Plant Materials. Fruit peels of *Lansium domesticum* were collected in Khon Kaen, Thailand, in September 2000. A voucher specimen is maintained at the Department of Horticulture, Faculty of Agriculture, Khon Kaen University.

Extraction and Isolation. The air-dried fruit peels (125 g) were extracted with MeOH (700 mL × 2). The MeOH extract (27.1 g) was partitioned between hexane (100 mL × 2) and 10% aqueous MeOH (100 mL), and the aqueous phase was further extracted with EtOAc (100 mL × 3) and *n*-BuOH (100 mL × 2) to give four fractions (hexane phase, 8.7 g; EtOAc phase, 6.1 g; n-BuOH phase, 2.1 g; aqueous phase, 8.6 g). A part of the EtOAc-soluble fraction (5.7 g) was subjected to silica gel column chromatography (column A; 4.0 × 23 cm) eluted with 0−100% EtOAc/hexane. The fraction eluted with EtOAc/hexane (2:1) contained **4** (566 mg). The fraction eluted with EtOAc/hexane (1:2) was further separated by gel filtration with Sephadex LH-20 (2.2 × 40 cm) eluted with EtOAc, followed by separation by MPLC on ODS (Ultrapack ODS-S-50A, 10 × 300 mm, Yamazen; eluent, 90% CH_3CN; flow rate, 7 mL/min) to yield **1** (113.5 mg, t_R 22 min), **2** (14.3 mg, t_R 30 min), and **5** (3.7 mg, t_R 39 min). Another fraction from column A eluted with EtOAc/hexane (1:2) was further separated by repeated chromatographies on Sephadex LH-20 (2.2 × 50 cm, eluted with MeOH), on a silica gel column [1.5 × 50 cm, eluted with EtOAc/hexane (3:1→1:0)], and finally purified with HPLC on ODS (Develosil ODS HG-5, 10 × 250 mm; eluent, 95% MeOH) to give **3** (2.5 mg, t_R 24 min). The spectral data of **4** and **5** were identical with those published in the literature.[3]

Lansionic acid (1): colorless amorphous solid; $[\alpha]^{25}_D$ +34° (*c* 2.7, MeOH); IR (KBr) ν_{max} 3400−2800, 1710, 1640, 1460, 1380, and 890 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.36 (1H, br s; H-15), 4.89 and 4.61 (each 1H, s; H_2-26), 4.81 and 4.77 (each 1H, s; H_2-29), 2.61 and 2.38 (each 1H, m; H_2-2), 2.43 and 2.27 (each 1H, m; H_2-20), 2.43 (2H, m; H_2-7), 2.20 (H, m; H_2-17), 2.19 and 1.86 (each 1H, m; H_2-16), 2.02 and 1.57 (each 1H, m; H_2-1), 1.82 (1H, m; H-13), 1.76 (3H, s; H_3-30), 1.72 (1H, m; H_3-27), 1.69 and 1.48 (each 1H, m; H_2-6), 1.68 (2H, m; H_2-19), 1.67 and 1.39 (each 1H, m; H_2-11), 1.66 (1H, m; H-5), 1.61 (1H, m; H-9), 1.21 (2H, m; H_2-12), 1.07 (3H, s; H_3-23), 1.00 (3H, s; H_3-24), 0.84 (3H, s; H_3-25), and 0.81 (3H, s; H_3-28); ^{13}C NMR (Table 1); FABMS *m*/*z* 455 $(M + H)^+$; HRFABMS *m*/*z* 455.3492 [calcd for $C_{30}H_{47}O_3$, (M + H) 455.3525].

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**3** in $CDCl_3$

position	**1** δ_C	**2** δ_C	**3** δ_C
1	37.6	37.2	37.8
2	34.7	27.9	34.7
3	217.2	79.5	217.2
4	47.8	39.1	47.8
5	55.1	54.7	55.2
6	25.1	24.8	25.7
7	37.8	38.0	37.9
8	147.5	148.1	147.4
9	57.5	56.6	56.6
10	39.3	39.2	38.7
11	26.3	25.5	23.5
12	27.2	23.9	25.1
13	48.3	54.3	55.3
14	135.8	135.8	135.2
15	121.8	121.7	122.1
16	29.4	24.0	25.8
17	49.2	51.5	49.6
18	38.7	36.4	36.5
19	32.6	38.1	37.2
20	28.6	34.7	27.4
21	178.0	217.3	79.2
22	147.6	47.5	39.2
23	25.9	28.2	26.0
24	21.7	15.4	21.6
25	14.1	14.6	14.2
26	107.6	106.9	107.6
27	22.9	22.2	22.3
28	16.3	13.3	13.6
29	114.0	22.1	15.1
30	22.8	24.9	17.9

3β-Hydroxyonocera-8(26),14-dien-21-one (2): colorless amorphous solid; $[\alpha]^{25}_D$ −19° (*c* 1.1, MeOH); IR (KBr) ν_{max} 3450, 1710, 1450, and 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.40 (1H, br s; H-15), 4.85 and 4.54 (each 1H, s; H_2-26), 3.26 (1H, dd, J = 11.8 and 4.3 Hz; H-3), 2.76 and 2.26 (each 1H, m; H_2-20), 2.46 and 1.46 (each 1H, m; H_2-19), 2.12 and 1.80 (2H, m; H_2-12), 2.07 and 1.97 (each 1H, m; H_2-7), 1.96 and 1.43 (each 1H, m; H_2-16), 1.81 and 1.17 (each 1H, m; H_2-1), 1.76 and 1.62 (each 1H, m; H_2-2), 1.71 (1H, m; H_3-27), 1.65 (H, m; H_2-17), 1.64 and 1.42 (each 1H, m; H_2-6), 1.64 (1H, m; H-13), 1.59 (1H, m; H-9), 1.28 (2H, m; H_2-11), 1.13 (1H, m; H-5), 1.08 (3H, s; H_3-29), 1.04 (3H, s; H_3-30), 0.99 (3H, s; H_3-23), 0.93 (3H, s; H_3-28), 0.76 (3H, s; H_3-24), and 0.67 (3H, s; H_3-25); ^{13}C NMR (Table 1); FABMS *m*/*z* 441 $(M + H)^+$; HRFABMS *m*/*z* 441.3719 [calcd for $C_{30}H_{49}O_2$, (M + H) 441.3733].

21α-Hydroxyonocera-8(26),14-dien-3-one (3): colorless amorphous solid; $[\alpha]^{25}_D$ −7.5° (*c* 0.11, MeOH); IR (KBr) ν_{max} 3450, 1710, 1460, and 1390 cm^{-1}; ^1H NMR ($CDCl_3$) δ_H 5.37 (1H, br s; H-15), 4.89 and 4.60 (each 1H, s; H_2-26), 3.23 (1H, dd, J = 11.0 and 5.0 Hz; H-21), 2.41 (2H, m; H_2-7), 2.37 (2H, m; H_2-2), 2.01 and 1.93 (each 1H, m; H_2-1), 1.95 and 1.92 (2H, m; H_2-11), 1.76 and 1.06 (each 1H, m; H_2-19), 1.69 and 1.51 (2H, m; H_2-12), 1.68 (1H, m; H_3-27), 1.64 (1H, m; H-9), 1.60 (2H, m; H_2-20), 1.57 (1H, m; H-13), 1.57 (1H, m; H-5), 1.49 (2H, m; H_2-16), 1.35 (2H, m; H_2-6), 1.16 (H, m; H_2-17), 1.08 (3H, s; H_3-23), 1.01 (3H, s; H_3-24), 0.95 (3H, s; H_3-30), 0.83 (3H, s; H_3-25), 0.82 (3H, s; H_3-29), and 0.69 (3H, s; H_3-28); ^{13}C NMR (Table 1); FABMS *m*/*z* 441 $(M + H)^+$; HRFABMS *m*/*z* 441.3745 [calcd for $C_{30}H_{49}O_2$, (M + H) 441.3733].

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(6) Nishizawa, M.; Nishide, H.; Kuriyama, K.; Hayashi, Y. *Chem. Pharm. Bull*. **1986**, *34*, 4443−4446. No spectral data of compounds **2** and **3** were described in this literature.
(7) Compound **5** (methyl lansionate) was also first isolated here from extracts of natural source, but it had been prepared from lansiosides A−C through methanolysis, and the spectral data of **5** were previously described in the literature.[3]
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