# Three New Eudesmanolactones (= Eudesmanolides) from *Camchaya loloana*

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Three new eudesmanolactones (=eudesmanolides), loloanolide A (**1**), loloanolide B (**2**), and 1-*O*-acetylloloanolide B (**3**), together with five known compounds, were isolated from the $CHCl_3$ extract of the aerial parts of *Camchaya loloana*. Their structures were elucidated on the basis of spectroscopic analysis. This type of eudesmanolactones bearing a 2-(hydroxymethyl)acryloyloxy group at C(8) was discovered for the first time. Compounds **1**–**3** exhibited cytotoxicity against the HepG2 cell line, with $GI_{50}$ values of 22.9, 18.1, and 12.8 nmol/ml, respectively.

**Introduction.** – The large family Asteraceae contains 25000 to 30000 species belonging to *ca.* 1000 genera. Many species have been used as sources for medicines, edible oils, vegetables, pesticides, *etc.* [1]. The extensive chemical and biological investigations showed that this family is a rich source of sesquiterpenoids, especially sesquiterpene lactones [2–4]. Many compounds from this family possess biological activities including antitumor [5], cytotoxic [6], antimicrobial [7], and anti-inflammatory effects [8]. The genus *Camchaya* with four species is distributed in the Indo-China Peninsula. *C. loloana* Kerr is the only Chinese representative of this genus [9]. Previous studies of *C. calcarea* have resulted in the isolation of antiplasmodial and antimycobacterial germacrolide sesquiterpenes [10]. However, there has been no other report on chemical compositions of *C. loloana* subsequently. The present study reports the presence of eudesmanolactones (=eudesmanolides) in *C. loloana*, a rare kind of constituent in the genus *Camchaya*. On the basis of spectral data, the new compounds were determined to be (3$\beta$,6$\alpha$,8$\alpha$)-3-(acetyloxy)-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-1,11(13)-dieno-12,6-lactone[1]) (=loloanolide A; **1**), (1$\beta$,6$\alpha$,8$\alpha$)-1-hydroxy-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-2,4(14),11(13)-trieno-12,6-lactone[1]) (=loloanolide B; **2**), and (1$\beta$,6$\alpha$,8$\alpha$)-1-acetyloxy)-8-{[2-(hydroxymethyl)acryloyl]oxy}-eudesma-2,4(14),11(13)-trieno-12,6-lactone[1]) (=1-*O*-acetylloloanolide B; **3**) (*Fig. 1*). The known compounds were characterized as $\beta$-sitosterol, $\beta$-daucosterol [11], lupenol, betulin [12], and caffeic acid [13]. To the best of our knowledge, compounds **1**–**3** are the first examples of eudesmanolactones with a 2-(hydroxymethyl)acryloyloxy group at C(8).

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

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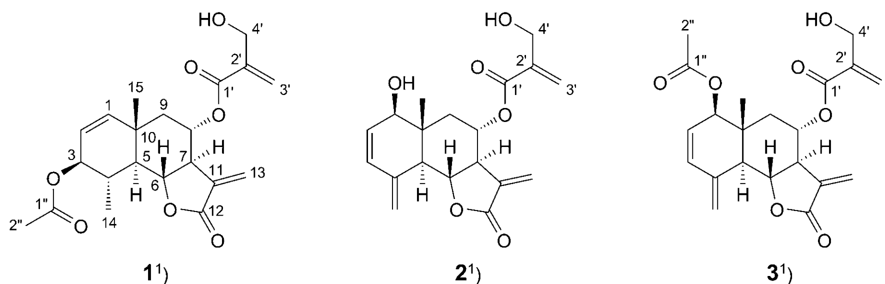


Fig. 1. *Isolated new compounds* **1**–**3** *from* C. loloana

**Results and Discussion.** – Loloanolide A (**1**) was obtained as a colorless gum. The HR-ESI-MS of **1** showed the quasi-molecular-ion peak at *m/z* 413.1569 ($[M+Na]^+$), corresponding to the molecular formula $C_{21}H_{26}O_7$ with nine degrees of unsaturation. The IR spectrum of **1** exhibited absorption bands assigned to OH (3514 $cm^{-1}$), $\alpha,\beta$-unsaturated ester (1731 $cm^{-1}$), and $\gamma$-lactone C=O groups (1747 $cm^{-1}$). The $^1$H- and $^{13}$C-NMR spectra (*Table*) showed signals typical of an eudesmanolactone-like skeleton [4]. The $^{13}$C-NMR spectrum revealed the presence of 21 C-atoms, which were determined as three Me, two sp$^3$ $CH_2$ (one O-bearing at $\delta$(C) 62.0), six sp$^3$ CH (three O-bearing at $\delta$(C) 70.7, 77.7, and 71.5), and three C=O groups ($\delta$(C) 170.7, 169.8, and 165.0), besides three C=C bonds and a quaternary C-atom (*Table*), attributed on the basis of chemical shifts, DEPT, and HSQC spectra. The $^1$H-NMR spectrum of **1** showed one Me *s* ($\delta$(H) 1.06), one Me *d* ($\delta$(H)1.16 (*d*, $J=6.3$ Hz)), two terminal olefinic H-atoms ($\delta$(H) 5.80 and 6.32 (each *d*, $J=2.0$ Hz)), and two olefinic H-atoms ($\delta$(H) 5.66 and 5.69 (each *d*, $J=9.6$ Hz)). The HSQC and HMBC spectra led to the identification of two partial structures: CH(5)–CH(6)–CH(7)–CH(8)–$CH_2$(9)–C(10)–Me(15) and CH(7)–C(11)–$CH_2$(13) (*Fig. 2*). Moreover, the Me(14)–CH(4)–CH(5)–C(10)–CH(1)–CH(2)–CH(3)–CH(4) structure was readily deduced by HMBC cross-peaks. Connection of these fragments established the framework of an eudesmanolactone. The $^1$H-NMR signals at $\delta$(H) 5.88 (br. *s*, 1 H), 6.22 (br. *s*, 1 H), and 4.29 (*s*, 2 H), as well as the $^{13}$C-NMR signals at $\delta$(C) 165.0 (C(1′)), 139.0 (C(2′)), 126.6 (C(3′)), and 62.0 (C(4′)), were undoubtedly assigned to a 2-(hydroxymethyl)acryloyloxy moiety, which was located at C(8) in view of the HMBC H–C(8)/C(1′). In addition, $^1$H-NMR signals at $\delta$(H) 2.03 (*s*, 3 H) and the $^{13}$C-NMR signals at $\delta$(C) 170.7 (C(1″)) were attributed to an AcO group which was attached to C(3) in view of the HMBC H–C(3)/C(1″). The lactone ring should be formed between C(6) and C(12) as suggested by the HMBC H–C(6)/C(12). The relative configurations of the various groups were assigned on the basis of NOESY experiments (*Fig. 3*) and $^1$H,$^1$H-coupling-constant analysis. In the NOESY plot, Me(15) correlated with H–C(4) ($\delta$(H) 2.08–2.11), H–C(6) ($\delta$(H) 4.59), and H–C(8) ($\delta$(H) 5.44) suggesting that Me(15), H–C(4), H–C(6), and H–C(8) were oriented to the same $\beta$ side. The NOESY correlation of the H–C(7) signal ($\delta$(H) 3.54–3.56) with H–C(5) ($\delta$(H) 2.10) and the coupling constant $J(7,8)=8.4$ Hz) indicated that H–C(7) and H–C(5) were $\alpha$-oriented. H–C(3) was also $\alpha$-oriented based on the NOESY correlation H–C(3)/H–C(5).

Table. *$^1$H- and $^{13}$C-NMR Data* ($CDCl_3$; 600 and 150 MHz, resp.) *of Compounds* **1**–**3**[a]). $\delta$ in ppm, *J* in Hz.

| | **1**[a]) | | **2**[a]) | | **3**[a]) | |
|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) | $\delta$(H) | $\delta$(C) |
| H–C(1) | 5.66 (*d*, *J* = 9.6) | 141.8 | 3.53 (*d*, *J* = 5.5) | 71.8 | 4.76 (*d*, *J* = 5.5) | 73.2 |
| H–C(2) | 5.69 (*d*, *J* = 9.6) | 121.8 | 5.82 (*dd*, *J* = 9.3, 5.5) | 125.9 | 5.79 (*dd*, *J* = 9.6, 5.5) | 122.2 |
| H–C(3) | 5.15 (*t*, *J* = 3.1) | 70.7 | 6.25 (*d*, *J* = 9.3) | 133.0 | 6.31 (*d*, *J* = 9.6) | 134.4 |
| H–C(4) or C(4) | 2.08–2.11 (*m*) | 42.0 | – | 134.7 | – | 134.8 |
| H–C(5) | 2.10 (*d*, *J* = 9.6) | 32.9 | 2.69 (*d*, *J* = 9.4) | 41.0 | 2.75 (*d*, *J* = 9.4) | 41.7 |
| H–C(6) | 4.59 (*dd*, *J* = 9.6, 7.3) | 77.7 | 4.94 (*t*, *J* = 8.1) | 76.7 | 4.92 (*dd*, *J* = 9.4, 7.6) | 76.7 |
| H–C(7) | 3.54–3.56 (*m*) | 41.6 | 3.60–3.64 (*m*) | 42.5 | 3.60–3.64 (*m*) | 42.3 |
| H–C(8) | 5.44 (*ddd*, *J* = 8.4, 6.0, 4.0) | 71.5 | 5.45–5.48 (*m*) | 70.7 | 5.41 (*ddd*, *J* = 9.0, 7.7, 4.2) | 70.6 |
| $CH_2$(9) | 1.84 (*dd*, *J* = 14.8, 4.0, $H_\alpha$), 1.97 (*dd*, *J* = 14.8, 6.0, $H_\beta$) | 40.5 | 1.56 (*dd*, *J* = 14.0, 4.2) 2.60 (*dd*, *J* = 14.0, 8.1) | 34.1 | 1.60 (*dd*, *J* = 14.0, 4.2) 2.21 (*dd*, *J* = 14.0, 7.7) | 34.2 |
| C(10) | – | 34.8 | – | 37.3 | – | 35.9 |
| C(11) | – | 135.4 | – | 140.6 | – | 140.3 |
| C(12) | – | 169.8 | – | 169.6 | – | 169.5 |
| $CH_2$(13) | 5.80 (*d*, *J* = 2.0), 6.32 (*d*, *J* = 2.0) | 123.6 | 5.92 (*d*, *J* = 2.6), 6.36 (*d*, *J* = 2.6) | 123.8 | 5.91 (*d*, *J* = 2.7), 6.37 (*d*, *J* = 2.7) | 123.7 |
| Me(14) or $CH_2$(14) | 1.16 (*d*, *J* = 6.3) | 15.1 | 5.44, 5.23 (2 br. *s*) | 116.9 | 5.49, 5.28 (2 br. *s*) | 117.8 |
| Me(15) | 1.06 (*s*) | 22.3 | 0.86 (*s*) | 20.1 | 0.94 (*s*) | 20.3 |
| C(1′) | – | 165.0 | – | 165.2 | – | 165.1 |
| C(2′) | – | 139.0 | – | 139.1 | – | 139.0 |
| $CH_2$(3′) | 5.88, 6.22 (2 br. *s*) | 126.6 | 5.86, 6.23 (2 br. *s*) | 127.1 | 5.92, 6.26 (2 br. *s*) | 126.7 |
| $CH_2$(4′) | 4.29 (*s*) | 62.0 | 4.26–4.34 (*m*) | 62.3 | 4.32 (*s*) | 62.1 |
| C(1″) | – | 170.7 | – | – | – | 170.3 |
| Me(2″) | 2.03 (*s*) | 20.7 | – | – | 1.98 (*s*) | 20.9 |

[a]) Assignments based on HSQC and HMBC experiments.

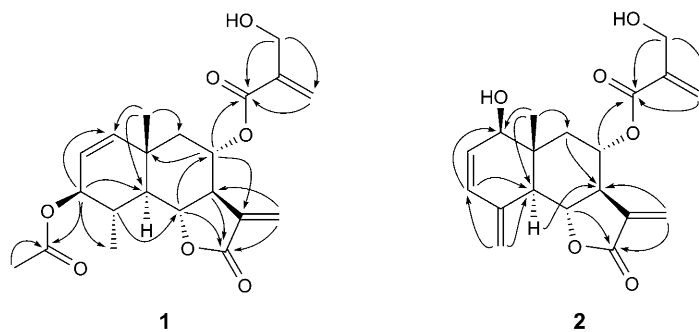


Fig. 2. *Selected HMBCs* (H → C) *of* **1** *and* **2**

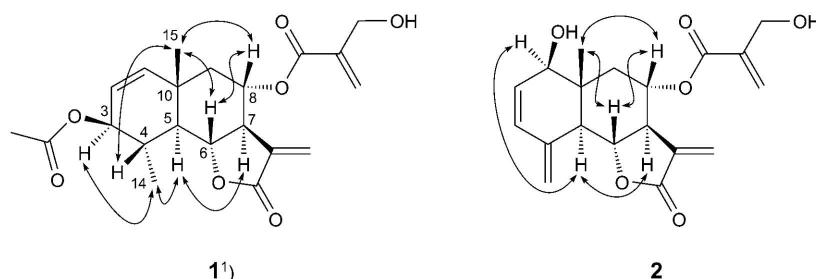


Fig. 3. *Selected NOESY correlations* (H ↔ H) *of* **1** *and* **2**

Therefore, loloanolide A (**1**) could be characterized as (3$\beta$,6$\alpha$,8$\alpha$)-3-(acetyloxy)-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-1,11(13)-dieno-12,6-lactone[1]).

Loloanolide B (**2**) was isolated as a colorless gum. Its molecular formula $C_{19}H_{22}O_6$ was deduced from the quasi-molecular-ion peak at *m/z* 369.1298 ($[M+Na]^+$) in the HR-ESI-MS. The IR spectrum showed maxima at 3433 $cm^{-1}$ (OH), 1760 $cm^{-1}$ ($\gamma$-lactone), 1715 $cm^{-1}$ ($\alpha$,$\beta$-unsaturated ester), 1633 $cm^{-1}$ (C=C bond), and 1160 $cm^{-1}$ ($\tilde{\nu}_{C-O}$ of ester). Interpretation of the $^1$H- and $^{13}$C-NMR (DEPT) and HSQC revealed that compound **2** possessed one Me, two sp$^3$ $CH_2$ (an O-bearing at $\delta$(C) 62.3), five sp$^3$ CH (three O-bearing at $\delta$(C) 71.8, 76.7, and 70.7), and two C=O groups ($\delta$(C) 169.6 and 165.2), besides four C=C bonds (*Table*). Careful study of the NMR spectra suggested that compound **2** was also an eudesmanolactone containing a 2-(hydroxymethyl)acryloyloxy group at C(8). Compared to **1**, one Me and one Ac group were absent, and one more C=C bond was present in **2**. The presence of conjugated C=C bonds ($\delta$(H) 5.82 (*dd*, $J$ = 9.3, 5.5 Hz, 1 H), 6.25 (*d*, $J$ = 9.3 Hz, 1 H), 5.44 (br. *s*, 1 H), and 5.23 (br. *s*, 1 H)) was confirmed by the HMBCs $CH_2$(14)/C(3) and C(4), and H–C(3)/C(2) and C(4) (*Fig. 2*). In addition, the HMBCs Me(15)/C(1) and H–C(2)/C(1) established the presence of a $\beta$-OH group at C(1), which was confirmed by the key NOESY correlation H–C(1)/H–C(5). The relative configurations of other groups were also assigned on the basis of NOESY experiments (*Fig. 3*). Thus, the structure of loloanolide B (**2**) was determined as (1$\beta$,6$\alpha$,8$\alpha$)-1-hydroxy-8-{[2-(hydroxymethyl)-acryloyl]oxy}eudesma-2,4(14),11(13)-trieno-12,6-lactone[1]).

Compound **3** was a colorless gum. The molecular formula $C_{21}H_{24}O_7$ was established from the quasi-molecular-ion peak at *m/z* 411.1427 ($[M+Na]^+$) in the HR-ESI-MS. The UV, IR, $^1$H- and $^{13}$C-NMR data were very similar to those of **2**, except for the presence of one more Ac group ($\delta$(H) 1.98 (*s*, Me(2″)); $\delta$(C) 20.9 (C(2″)) and 170.3 (C(1″))). The 1$\beta$-AcO group was deduced from the HMBC H–C(1) ($\delta$(H) 4.76 (*d*, $J$ = 5.5 Hz))/C(1″) and the NOESY correlation H–C(5)/H–C(1). The remaining structure and relative configuration of **3** were the same as those of **2**. Thus, the structure of **3** was elucidated as (1$\beta$,6$\alpha$,8$\alpha$)-1-(acetyloxy)-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-2,4(14),11(13)-trieno-12,6-lactone[1]) (=1-*O*-acetylloloanolide B).

Compounds **1**, **2**, and **3** showed cytotoxicity against the HepG2 cell line, with $GI_{50}$ values of 22.9, 18.1, and 12.8 nmol/ml, respectively.

## Experimental Part

*General. TLC: Merck* 0.25 mm thick precoated silica gel ($SiO_2$) *60 F254* plates. HPLC: *Perkin-Elmer-600* prep. HPLC instrument, with a *Kromasil-100-10-C$_{18}$* column (250 × 20 mm). Column chromatography (CC): $SiO_2$ (200–300 mesh). Optical rotations: *Perkin-Elmer-341* automatic polarimeter. UV Spectra: *Perkin-Elmer Lambda-35* spectrometer; $\lambda_{max}$ (log $\varepsilon$) in nm. IR Spectra: *Perkin-Elmer* FT-IR spectrometer; KBr disk; in KBr pellets in $cm^{-1}$. 1D- and 2D-NMR Spectra: *Bruker-Advance-600* spectrometer; at 295 K; $\delta$ in ppm rel. to $Me_4Si$, $J$ in Hz. MS: *Bruker-Daltonics-Bio-TOF-Q* mass spectrometer; in *m/z*.

*Plant Material*. The aerial parts of *Camchaya loloana* were collected from Xishuangbanna, Yunnan Province, China, in September 1998. The plant sample was identified by Prof. *Jingyun Cui* at Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences (CAS). A voucher specimen (Z-005) was deposited with the Herbarium of the Chengdu Institute of Biology, CAS.

*Extraction and Isolation*. The 95% EtOH extract (80 g) of air-dried aerial parts of *C. loloana* (2.1 kg) was suspended in $H_2O$ and extracted successively with petroleum ether, $CHCl_3$, and BuOH. The $CHCl_3$ fraction (18 g) was separated by CC ($SiO_2$ (600 g; 150 mm × 330 mm), petroleum ether/acetone 5:1, 4:1, 3:1, 2:1, 1:1, 1:2 (each 3000 ml)): *Fractions A–D*. The separation of *Fr. A* (2.5 g) by CC ($SiO_2$ (80 g; 40 mm × 250 mm), petroleum ether/AcOEt 10:1) afforded $\beta$-sitosterol (0.8 g) and lupeol (0.4 g). Betulin (0.2 g) and caffeic acid (0.72 g) were obtained, resp., by crystallizing *Fr. B* from petroleum ether/acetone 1:1 and *Fr. C* from acetone. The residue of the mother liquid (1.1 g) of the betulin crystallization was applied to CC ($SiO_2$ (50 g; 30 mm × 200 mm), $CHCl_3$/acetone 4:1): **1** (14 mg). Separation of the residue of the mother liquid (65 mg) of caffeic acid by semi-prep. HPLC (MeOH/$H_2O$ 55:45) yielded **2** (7 mg) and **3** (15 mg). *Fr. D* (4.0 g) was subjected to CC ($SiO_2$ (150 g; 50 mm × 30 mm), $CHCl_3$/MeOH 5:1): $\beta$-daucosterol (2.7 g).

*Loloanolide A* (*=(3β,6α,8α)-3-(Acetyloxy)-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-1,11(13)-dieno-12,6-lactone* = rel-*(3a*R*,4*S*,5a*S*,8*S*,9*S*,9a*S*,9b*R*)-8-(Acetyloxy)-2,3,3a,4,5,5a,8,9,9a,9b-decahydro-5a,9-dimethyl-3-methylene-2-oxonaphto[1,2-*b*]furan-4-yl 2-(Hydroxymethyl)prop-2-enoate*; **1**): Colorless gum. $[\alpha]_D^{25} = -9.0$ ($c = 0.10$, MeOH). UV (MeOH): 269 (3.34), 206 (4.14). IR: 3514, 2925, 1747, 1731, 1644, 1378, 1337, 1239, 1165, 1130, 1059, 1016, 986, 968, 919. $^1$H- and $^{13}$C-NMR: *Table*. HR-ESI-MS (pos.): 413.1569 ($[M + Na]^+$; calc. 413.1571).

*Loloanolide B* (*=(1β,6α,8α)-1-Hydroxy-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-2,4(14),11(13)-trieno-12,6-lactone* = rel-*(3a*R*,4*S*,5a*R*,9a*S*,9b*R*)-2,3,3a,4,5,5a,6,9,9a,9b-Decahydro-6-hydroxy-5a-methyl-3,9-bis(methylene)-2-oxonaphtho[1,2-*b*]furan-4-yl 2-(Hydroxymethyl)prop-2-enoate*; **2**): Colorless gum. $[\alpha]_D^{25} = +141.0$ ($c = 0.10$, MeOH). UV (MeOH): 266 (3.15), 213 (4.07). IR: 3433, 2924, 2855, 1760, 1715, 1633, 1385, 1322, 1271, 1160, 1109, 1031, 817. $^1$H- and $^{13}$C-NMR: *Table*. HR-ESI-MS (pos.): 369.1298 ($[M + Na]^+$; calc. 369.1309).

*1-*O*-Acetylloloanolide B* (*=(1β,6α,8α)-1-(Acetyloxy)-8-{[2-(hydroxymethyl)acryloyl]oxy}eudesma-2,4(14),11(13)-trieno-12,6-lactone* = rel-*(3a*R*,4*S*,5a*R*,9a*S*,9b*R*)-6-(Acetyloxy)-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-5a-methyl-3,9-bis(methylene)-2-oxonaphtho[1,2-*b*]furan-4-yl 2-(Hydroxymethyl)prop-2-enoate*; **3**): Colorless gum. $[\alpha]_D^{25} = +46.0$ ($c = 0.12$, MeOH). UV (MeOH): 268 (3.22), 218 (4.35). IR: 3442, 2928, 1764, 1725, 1636, 1372, 1240, 1157, 1054, 1018, 990, 948, 817. $^1$H- and $^{13}$C-NMR: *Table*. HR-ESI-MS (pos.): 411.1427 ($[M + Na]^+$; calc. 411.1414).

*Cytotoxicity Assays.* Hepatoma cell line HepG2 was obtained from the *American Type Culture Collection* (*ATCC*) and cultured according to the supplier's instruction. The cells were seeded into 96-well plates, incubated for 16 h at 37°, and treated with compounds **1**, **2**, and **3** at different concentrations for 48 h. Taxol was used as pos. control. The cytotoxic activity was examined by means of a colorimetric chemosentivity assay with SRB (sulforodhamine B). The $GI_{50}$ value (the drug concentration required to inhibit the cell growth by 50%) was used as a parameter for cytotoxicity.

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