Cytotoxic Cardenolides from the Leaves of *Calotropis gigantea*

Chonticha SEEKA and Somyote SUTTHIVAIYAKIT*

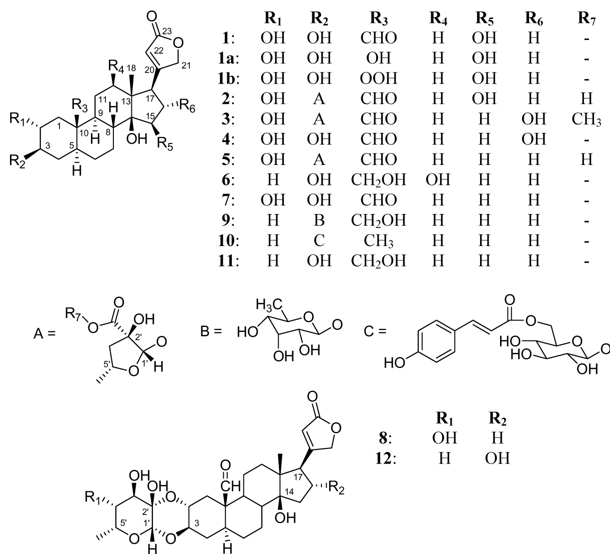
Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University; Ramkhamhaeng Road, Bangkapi, Bangkok 10240, Thailand.
Received November 14, 2009; accepted February 2, 2010; published online February 18, 2010

Two 15β-hydroxycardenolides (1,2) and a 16α-hydroxycalactinic acid methyl ester (3) along with eleven known compounds including 16α-hydroxycalotropagenin, coroglaucigenin, 16α-hydroxycalotropin, calactinic acid, calotoxin, 6′-*O*-(*E*-4-hydroxycinnamoyl)desglucouzarin, 12β-hydroxycoroglaucigenin, frugoside, calotropagenin, 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid and *R*-(−)-mevalonolactone were isolated from the polar fraction of the CH_2Cl_2 extract, and *n*-BuOH extract of the leaves of this plant. The isolated compounds were evaluated for their inhibitory activities against a panel of cell lines.

Key words *Calotropis gigantea*; 15β-hydroxycardenolide; 16α-hydroxycalactinic acid methyl ester

In continuation to our recent study on the bioactive compounds from *Calotropis gigantea*,[1] we herein report the isolation of two 15β-hydroxycardenolides (**1**, **2**), 16α-hydroxycardenolide (**3**) along with eleven known compounds including 16α-hydroxycalotropagenin (**4**),[2] calactinic acid (**5**),[3—5] 12β-hydroxycoroglaucigenin (**6**),[6] calotropagenin (**7**),[7] calotoxin (**8**),[7] coroglaucigenin (**11**),[6] 16α-hydroxycalotropin (**12**),[2] frugoside (**9**),[8] 6′-*O*-(*E*-4-hydroxycinnamoyl)desglucouzarin (**10**),[9] 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid,[10] and *R*-(−)-mevalonolactone,[11,12] from the polar fraction of the CH_2Cl_2 extract, and *n*-BuOH extract of the leaves of this plant. Structural identification was established by spectroscopic methods.

Compound **1** was obtained as a solid, mp 226—228 °C, and the HR-electrospray ionization (ESI)-MS spectrum indicated molecular formula of $C_{23}H_{32}O_7$. FT-IR spectrum indicated absorption maxima for a hydroxyl (3401 cm^{-1}) and α,β-unsaturated-γ-lactone (1738, 1623 cm^{-1}) functional groups. ^{13}C-NMR spectrum exhibited the presence of twenty three carbon signals comprising one methyl, eight methylene, nine methine including three oxymethine and one formyl, and five quaternary carbons. ^{1}H- and ^{13}C-NMR spectra showed characteristic signals of an α,β-unsaturated-γ-lactone moiety commonly found in cardenolide (δ_H 6.13 assignable to H-22, δ_H 5.30, 5.03 to H_2-21 and δ_C 175.8, 74.3, 118.3, 175.2 to C-20, C-21, C-22, C-23, respectively). The methyl proton signal at δ_H 0.83 (assigned for CH_3-18) showed heteronuclear multiple bond coherence (HMBC) correlations with ^{13}C-NMR signals at δ_C 49.4 (C-17) and 81.9 (C-14). The two hydroxyl groups at C-2, and C-3 were evident from HMBC correlations between H-1 (at δ_H 2.97, 1.34) and C-2, C-3, C-5, C-10 and C-19 (at δ_C 73.3, 76.1, 43.5, 53.4, 209.5, respectively), in addition to ^{1}H–^{1}H correlation spectroscopy (COSY) cross-peaks between H-2 (δ_H 4.08)/H-1 and H-3 (δ_H 3.90). Vicinal coupling constants, $J_{1a,2}$ of 11.5 Hz, $J_{2,3}$ of 8.9 Hz and $J_{3,4a}$ of 11.3 Hz, were used as evidences for the assignment of orientations of 2-OH and 3-OH groups as α- and β-, respectively. Placement of the third hydroxyl group at C-15 was revealed from ^{1}H–^{1}H COSY cross-peaks between H_2-16 (δ_H 2.75, 1.97)/H-15 (δ_H 4.75) and H-17(δ_H 2.69), as well as HMBC correlations between H-17/C-15 (δ_C 72.6), C-16 (δ_C 37.9) and C-21 (δ_C 74.3). The OH-15 was proposed to have β-orientation based on the nuclear Overhauser effect spectroscopy (NOESY) spectrum which revealed cross-peaks between H-15/H-7 and H-17. The $J_{15,16}$ value of 8.0 Hz is also consistent to those values reported in the 15β-hydroxycardenolide analogs.[6,13)]



	R1	R2	R3	R4	R5	R6	R7
1:	OH	OH	CHO	H	OH	H	-
1a:	OH	OH	OH	H	OH	H	-
1b:	OH	OH	OOH	H	OH	H	-
2:	OH	A	CHO	H	OH	H	H
3:	OH	A	CHO	H	H	OH	CH_3
4:	OH	OH	CHO	H	H	OH	-
5:	OH	A	CHO	H	H	H	H
6:	H	OH	CH_2OH	OH	H	H	-
7:	OH	OH	CHO	H	H	H	-
9:	H	B	CH_2OH	H	H	H	-
10:	H	C	CH_3	H	H	H	-
11:	H	OH	CH_2OH	H	H	H	-

	R1	R2
8:	OH	H
12:	H	OH

Compound **1** was thus proposed as 2α,15β-dihydroxy-19-oxo-uzarigenin. Full assignment of ^{1}H- and ^{13}C-NMR data was as shown in Tables 1 and 2. In this study, compound **1** was found to be very unstable and further transformed to 19-nor-2α,10,15β-trihydroxyuzarigenin (**1a**), and 19-nor-10-hydroperoxy-2α,15β-dihydroxyuzarigenin (**1b**) upon standing at room temperature for 2 d with or without solvent in a well capped vial. These types of transformations were previously documented,[14,15)] but no ^{1}H- and ^{13}C-NMR spectroscopic evidences of the 10-hydroperoxide derivative were reported. In this study we include full assignment of the ^{1}H- and ^{13}C-

Fig. 1. Selected HMBC Correlations of **1**

* To whom correspondence should be addressed. e-mail: s_somyote@ru.ac.th
© 2010 Pharmaceutical Society of Japan

Table 1. ^{1}H-NMR Spectroscopic Data of Compounds **1**—**3** with *J* Values (in Hz) in Parentheses

Position	**1** (C_5D_5N)	**1a** (C_5D_5N)	**1b** (C_5D_5N)	**2** (C_5D_5N)	**3** (C_5D_5N)[19]	**3** ($CDCl_3$+ MeOH-d_4, 30 : 1)
1	2.97 dd (12.8, 4.8), 1.34	2.80 dd (13.1, 4.8), 1.53 dd (12.7, 11.8)	3.43 dd (13.4, 4.6), 1.49	2.90, 1.25	2.88 dd (12.8, 4.9), 1.15	2.55 dd (13.2, 5.0), 0.91 t (12.4)
2	4.08 ddd (11.5, 8.9, 4.8)	4.53 ddd (11.5, 9.0, 4.9)	4.56 ddd (11.3, 9.3, 4.7)	3.99	3.87	3.38 ddd (11.4, 8.7, 5.1)[i]
3	3.90 ddd (11.3, 8.9, 4.8)	3.97 ddd (11.3, 9.1, 4.8)	3.95 ddd (11.3, 9.1, 4.7)	3.89	3.74	3.30 ddd (11.0, 8.8, 5.1)
4	1.95, 1.62 ddd (12.8, 11.3, 11.3)	2.20 dd (12.1, 11.9), 1.90	2.10 dt (12.2, 11.8), 1.88	1.99, 1.31		1.52[f], 1.14
5	1.91	1.40	1.56	1.88		1.28
6	2.00, 1.49	1.47	1.68, 1.34	1.53		1.78, 1.52[f]
7	2.40, 1.90	2.31, 1.80	2.33, 1.82	2.42, 1.89		2.10
8	2.48	2.28	2.41	2.48		1.41 dt (12.1, 2.9)
9	1.43	1.39[a]	1.45	1.27		1.22
11	1.70, 1.21	1.72	2.48, 1.90[c]	1.67, 1.26		1.70, 1.19
12	1.63, 1.30	1.39[a]	1.35	1.32		1.57
14	OH-14 5.30	—	—	—	OH-14 5.92	—
15	4.75 t (8.0)	4.77 t (7.7)	4.71 t (7.3)	4.74 t (7.5)	2.59, 2.46	1.87, 1.50
16	2.75, 1.97	2.67[b], 1.94	2.67,[d] 1.92[c]	2.68,[e] 1.90	5.11	4.43[g]
17	2.69	2.66[b]	2.66[d]	2.68[e]	3.06 d (4.0)	2.51 d (4.3)
18	0.83 s	0.98 s	1.04 s	0.92 s	0.95 s	0.71 s
19	10.20 s	—	—	10.10 s	10.1 s	9.88 s
21	5.30 dd (18.3, 1.7), 5.03 dd (18.3, 1.7)	5.33 d (18.6), 5.01 dd (18.2, 1.5)	5.30[h], 4.96 d (18.2)	5.29 d (18.4), 5.04 d (18.0)	5.23 d (18.6), 5.03 d (18.6)	4.85 d (17.3), 4.72 d (18.1)
22	6.13 s	6.10 s	6.06 s	6.14 s	6.28 s	5.88 s
1′				5.65[j]	5.20 s	4.84 s
4′				1.88	2.67 dd (13.8, 9.9), 2.52	2.23 dd (13.1, 10.1), 2.04 dd (13.3, 5.7)
5′				4.82	4.83	4.43[g]
6′				1.53 d (5.7)	1.47 d (6.2)	1.32 d (6.2)
OMe					3.78 s	3.76 s

a—f) Overlapped signals. *h—j*) Partially obscured by solvent signal.

NMR spectroscopic data of these two transformed products. The ^{13}C-NMR resonance of C-10 with hydroperoxyl group of **1b** was notably found at less shielded position (δ_C 82.9) than that of the corresponding C-10 with a hydroxyl group (δ_C 72.8) of **1a**.[16]

Compound **2** was isolated as colorless solid with a molecular formula of $C_{29}H_{40}O_{11}$ based on the HR-ESI-MS spectrum. The characteristic IR absorption maxima, ^{1}H- and ^{13}C-NMR shifts of the α,β-unsaturated-γ-lactone moiety were observed as of **1**. The dideoxyfuranosyl moiety was detected from signals of a dioxygenated methine group at δ_H 5.65 (s) and δ_C 107.6, in addition to a doublet signal at δ_H 1.53 (d, J=5.7 Hz) of H-6′ in the ^{1}H-NMR spectrum as observed in calactinic methyl ester recently isolated from *Asclepias curassavica*.[14] and also from this plant.[1] The ^{1}H–^{1}H COSY and HMBC spectra also revealed the presence of an OH-15 group. The 15-oxymethine proton was detected as a triplet at δ_H 4.74 with J value of 7.5 Hz indicating OH-15 as β-oriented.[6,13] The ^{13}C-NMR signals of C-15, C-16 and C-17 (at δ_C 76.7, 38.5, 49.4, respectively) were also consistent to those of **1**. Compound **2** was thus elucidated as 15β-hydroxycalactinic acid. ^{1}H- and ^{13}C-NMR data of **2** are shown in Tables 1 and 2.

Compound **3** was obtained as colorless solid with molecular formula $C_{30}H_{42}O_{11}$ based on HR-ESI-MS spectrum. Most of the ^{1}H- and ^{13}C-NMR chemical shifts are very similar to those of compound **2** (Tables 1, 2), with additional NMR resonances at δ_H 3.76 (s, 3H) and δ_C 52.4 (CH_3) indicating compound **3** to be a calactinic acid methyl ester analog. The oxymethine proton at δ_H 4.43 which showed cross-peak with H-17 at δ_H 2.51 (d, J=4.3 Hz) in the ^{1}H–^{1}H COSY spectrum help disclose the presence of 16-hydroxyl group. The relative configuration at C-16, although could not be obtained from the NOESY spectrum, was deduced from the $J_{16,17}$ value of 4.3 Hz, which is close to the values reported in 16α-hydroxycalotropagenin[2] (also isolated in this study), and in 16α-acetoxycalactin,[17] thus indicated an α-oriented 16-OH group. Compound **3** could therefore be concluded as being 16α-hydroxycalactinic acid methyl ester.

Some of the isolates were evaluated for their cytotoxic activities against small-cell lung (NCI-H187), oral epidermal carcinoma (KB) and breast cancer (MCF 7) cell lines. The results are as shown in Table 3. Compounds **8** and **9** showed most potent inhibitory activity against all cell lines.

Experimental

General Experimental Procedures Optical rotations were recorded on a Jasco DIP 1020 polarimeter. IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. HR-ESI-MS spectra were recorded on a Bruker Daltonics microTOF instrument. ^{1}H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

Plant Material The leaves of *Calotropis gigantea* Linn. (Asclepiadaceae) were collected from the Suwinthawong road area, Minburi District, Bangkok in June, 2002. Botanical identification was achieved through comparison with a voucher specimen No. 11 SN 227321, BK 15951 kept in the herbarium collection of the Sirindhorn Museum (Bangkok Herbarium), Botanical section, Botany and weed section, Department of Agriculture, Ministry of Agriculture and Cooperatives. Voucher specimen SSCG/2002 is

Table 2. ^{13}C-NMR Spectroscopic Data of Compounds **1**—**3**

Position	**1** (C_5D_5N)	**1a** (C_5D_5N)	**1b** (C_5D_5N)	**2** (C_5D_5N)	**3** (C_5D_5N)	**3** ($CDCl_3$+MeOH-d_4 30 : 1)
1	40.6	44.2	39.3	39.9	39.7	37.8
2	73.3	73.3	73.3	70.9	72.0	70.3
3	76.1	76.3	76.1	81.9	85.1	85.1
4	38.5	36.4	37.2	33.8	34.8	34.2
5	43.5	44.3	44.6	43.2	43.9	42.5
6	28.6	28.7	28.4	28.5	29.1	27.5
7	27.2	26.7	27.0	27.2	29.1	27.2
8	42.8	41.1	41.6	42.8	43.8	42.0
9	48.4	49.5	48.8	48.5	49.8	48.1
10	53.4	72.8	82.9	52.9	52.7	51.9
11	22.6	21.3	23.0	22.5	21.5	22.0
12	38.2	38.7	39.2	38.0	40.5	40.1
13	49.1	49.1	49.3	49.1	49.5	48.7
14	81.9	81.9	82.2	82.0	84.7	84.3
15	72.6	73.2	73.2	76.7	42.2	40.6
16	37.9	38.0	38.1	38.5	76.9	76.2
17	49.4	48.3	49.6	49.4	63.1	60.5
18	16.9	17.0	17.1	17.0	17.3	15.5
19	209.5	—	—	209.6	209.9	207.8
20	175.8	175.7	175.8	176.1	174.0	173.4
21	74.3	74.0	74.1	74.5	74.8	74.3
22	118.3	118.1	118.1	118.4	119.2	117.6
23	175.2	174.8	174.8	175.5	174.6	173.0
1′				107.6	109.2	108.6
2′				nd	85.6	84.2
3′				174.0	171.9	171.6
4′				43.0	41.5	39.9
5′				72.7	77.9	76.5
6′				23.1	22.0	21.9
OMe					53.2	52.4

deposited at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University.

Extraction and Isolation The fresh leaves of *C. gigantea* (24.4 kg) were ground and extracted successively with hexane, CH_2Cl_2 and MeOH to obtain hexane (125 g), CH_2Cl_2 (76 g) and MeOH (385 g) extracts. The MeOH extract was further partitioned with water and *n*-BuOH to obtain *n*-BuOH (67.9 g) and aqueous (317 g) extracts after solvent evaporation. The CH_2Cl_2 extract was subjected to gradient column chromatography (silica gel, hexane–CH_2Cl_2 95 : 5 to CH_2Cl_2–MeOH 1 : 9) to yield ten fractions. The nonpolar and moderately polar fractions were studied and their constituents reported.[1] Polar fraction P1 (9.44 g) was subjected to column chromatography (silica gel, CH_2Cl_2–MeOH 98 : 2 to 40 : 60) to obtain five fractions. Fraction 3 was purified using reversed-phase column chromatography (C_{18}, H_2O–MeOH 98 : 2 to 0 : 100) to give six subfractions (3.1—3.6), subfraction 3.2 gave compound **7** (120.9 mg) and subfraction 3.4 gave **8** (45.1 mg). Fraction 4 after purification (C_{18}, H_2O–MeOH 90 : 10 to 0 : 100) gave five subfractions (4.1—4.5). Subfraction 4.1 (200.2 mg) was subjected to reversed-phase column chromatography (C_{18}, H_2O–MeOH 85 : 15 to 0 : 100) and gave five subfractions (4.1.1—4.1.5). Subfraction 4.1.2 was purified (reversed-phase C_{18}, H_2O–MeOH 85 : 15 to 0 : 100) and gave **12** (6.1 mg), subfraction 4.1.3 gave **1** (58.7 mg). Subfraction 4.2 (237.6 g) was purified using reversed-phase column chromatography (C_{18}, H_2O–MeOH 80 : 20 to 0 : 100) then Sephadex LH-20 eluted with MeOH to give **3** (7.7 mg). Subfraction 4.3 (265.3 mg) after column chromatography (silica gel, EtOAc–MeOH 99 : 1 to 80 : 20) gave **11** (28.7 mg). Subfraction 4.4 after purification (Sephadex LH-20, MeOH), then reversed-phase column chromatography (C_{18}, H_2O–MeOH 80 : 20 to 0 : 100) gave three subfractions (4.4.1—4.4.3). Subfraction 4.4.2 gave 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid (22.9 mg). Subfraction 4.4.3 after column chromatography (silica gel, CH_2Cl_2–MeOH 99 : 1 to 98 : 2), then reversed-phased column chromatography (C_{18}, H_2O–MeOH 85 : 15 to 0 : 100) gave **10** (7.3 mg). Fraction 5 (1.97 g) was subjected to Sephadex LH-20 column chromatography eluted with MeOH to give 3 subfractions (5.1—5.3) and subfraction 5.2 (1.16 g) was further purified (C_{18}, H_2O–MeOH 60 : 40 to 0 : 100) to give 4 subfractions (5.2.1—5.2.4). Subfraction 5.2.1 (134.1 mg) yielded **4** (20.1 mg) after reversed-phase column chromatography (C_{18}, H_2O–MeOH 95 : 5 to 0 : 100). Subfraction 5.2.3 (186.5 mg) after silica gel column chromatography (EtOAc–MeOH 100 : 0 to 98 : 2) gave **9** (29.2 mg). Polar fraction P2 (6.4 g) was fractionated by column chromatography (C_{18}, H_2O–MeOH 50 : 50 to 10 : 90) to give four subfractions (P2.1—P2.4). Subfraction P2.3 (78.2 mg) after purification (Sephadex LH-20, H_2O–MeOH 40 : 60) gave **2** (15.9 mg), subfraction 10.4 yielded pure **5** (80.4 mg).

Table 3. Cytotoxic Activities of the Isolated Compounds

Compound	KB[a)]	MCF 7[a)]	NCI-H187[a)]
1	Inactive[b)]	Inactive[b)]	Inactive[b)]
2	Inactive	Inactive	Inactive
3	0.94 (1.63)	46.61 (80.60)	5.74 (9.93)
4	Inactive	Inactive	Inactive
5	17.15 (31.28)	Inactive	Inactive
6	0.68 (1.67)	34.35 (84.55)	6.24 (15.36)
7	2.56 (6.33)	42.87 (106.06)	19.42 (48.04)
8	0.002 (0.003)	3.26 (5.95)	0.002 (0.003)
9	0.02 (0.03)	1.96 (3.65)	0.11 (0.20)
10	11.81 (17.30)	Inactive	39.56 (57.97)
Ellipticine[c)]	0.45 (1.82)	—	0.68 (2.78)
Doxorubicin[c)]	0.25 (0.46)	0.57 (1.05)	0.04 (0.06)

a) Values indicated are IC_{50} values in μg/ml, whereas in parentheses in μM. *b)* Inactive at 50 μg/ml. *c)* Positive control substance.

Fractionation of the *n*-BuOH extract (67.9 g) using silica gel column chromatography (CH_2Cl_2–MeOH 100 : 0 to 0 : 100) gave six fractions. Fraction 2 was fractionated by column chromatography (silica gel, hexane–EtOAc 80 : 20 to EtOAc–MeOH 80 : 20) and gave three subfractions (2.1—2.3). Subfraction 2.2 (48.5 g) was purified using column chromatography (silica gel, CH_2Cl_2–MeOH 98 : 2) to give *R*-(−)-mevalonolactone (13.1 mg). Fraction 3 (1.53 g) was subjected to reversed-phase column chromatography (C_{18}, H_2O–MeOH 80 : 20 to 0 : 100), then Sephadex LH-20 eluted with MeOH and yielded **7** (14.7 mg). Fraction 5 (1.57 g) was purified (silica gel, CH_2Cl_2–MeOH 98 : 2 to 50 : 50), then reversed-phase column chromatography (C_{18}, H_2O–MeOH 95 : 5 to 0 : 100) to give an additional quantity of **4** (15.5 mg) and **6** (5.9 mg). Column chromatography of the more polar fractions led to the isolation of polymeric material which is insoluble in most of the NMR solvents. The decomposition mixture of **1** (45 mg) was purified by column chromatography (reversed-phase C_{18}, H_2O–MeOH 95 : 5) to obtain pure **1a** (10.6 mg), **1b** (4.1 mg) and **1** (10.0 mg).

2α,15β-Dihydroxy-19-oxo-uzarigenin (**1**): Colorless solid, mp 226—228 °C; $[\alpha]_D$ +12.00° (c=0.075, MeOH, 29 °C). HR-ESI-MS *m/z*: $[M+Na]^+$ 443.2040 (Calcd for $C_{23}H_{32}O_7Na$: 443.2037). IR ν_{max} (KBr) cm^{-1}: 3401, 2923, 2853, 1738, 1623, 1456, 1383, 1166, 1019. ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N), see Tables 1 and 2.

19-Nor-2α,10,15β-trihydroxyuzarigenin (**1a**): Colorless solid, mp 258—260 °C; $[\alpha]_D$ +5.59° (c=0.56, MeOH, 28.4 °C). HR-ESI-MS *m/z*: $[M+Na]^+$ 431.2053 (Calcd for $C_{22}H_{32}O_7Na$, 431.2037). IR ν_{max} (KBr) cm^{-1}: 3423, 2924, 2854, 1745, 1615, 1459, 1383, 1163, 1053, 1018. ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N), see Tables 1 and 2.

19-Nor-10-hydroperoxy-2α,15β-dihydroxyuzarigenin (**1b**): Sticky oil; $[\alpha]_D$ −3.71° (c=0.205, MeOH, 28.5 °C). HR-ESI-MS *m/z*: $[M+Na]^+$ 447.1987 (Calcd for $C_{22}H_{32}O_8Na$, 447.1986). IR ν_{max} (KBr) cm^{-1}: 3437, 2923, 2853, 1733, 1619, 1459, 1379, 1170, 1024. ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N), see Tables 1 and 2.

15β-Hydroxycalactinic Acid (**2**): Colorless solid, mp 290—295 °C; $[\alpha]_D$ −48.21° (c=0.10, MeOH, 32 °C). HR-ESI-MS *m/z*: $[M+Na]^+$ 587.2463 (Calcd for $C_{29}H_{40}O_{11}Na$, 587.2457). IR ν_{max} (KBr) cm^{-1}: 3429, 2922, 2853, 1709, 1632, 1384, 1157, 1046, 1017. ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N), see Tables 1 and 2.

16α-Hydroxycalactinic Acid Methyl Ester (**3**): Colorless solid, mp 230—232 °C; $[\alpha]_D$ −31.50° (c=0.14, MeOH, 32 °C); IR ν_{max} (KBr) cm^{-1}: 3430, 2933, 1741, 1626, 1437, 1384, 1262, 1115, 1074, 1048, 1021. HR-ESI-MS *m/z*: $[M+Na]^+$ 601.2625 (Calcd for $C_{30}H_{42}O_{11}Na$, 601.2613). ^{1}H-NMR (400 MHz, C_5D_5N and $CDCl_3$+MeOH-d_4 30 : 1) and ^{13}C-NMR (100 MHz, C_5D_5N and $CDCl_3$+MeOH-d_4 30 : 1), see Tables 1 and 2.

Calactinic Acid (**5**): Colorless solid, mp 196—198 °C; $[\alpha]_D$ −40.48° (c=0.25, MeOH, 32 °C); HR-ESI-MS *m/z*: $[M+Na]^+$ 571.2523 (Calcd for $C_{29}H_{40}O_{10}Na$, 571.2519). IR ν_{max} (KBr) cm^{-1}: 3429, 2920, 1738, 1615,

1438, 1383, 1156, 1109, 1017. ^{1}H-NMR (400 MHz, C_5D_5N) δ_H: 10.00 (1H, s, C-19), 6.15 (1H, s, H-22), 5.74 (1H, s, H-1′), 5.25 (1H, d, J=18.1 Hz, H-21). 5.01 (1H, d, J=18.0 Hz, H-21), 4.88 (1H, dq, J=9.7, 5.9 Hz, H-5′), 3.91 (1H, ddd, J=11.2, 8.6, 4.9 Hz, H-2), 3.89 (1H, ddd, J=10.9, 8.6, 4.7 Hz, H-3), 2.91 (1H, dd, J=12.8, 4.8 Hz, H-1), 2.87 (1H, dd, J=12.9, 5.8 Hz, H-4′), 2.74 (1H, dd, J=9.0, 4.6 Hz, H-17), 2.58 (1H, dd, J=13.0, 9.8 Hz, H-4′), 2.44 (1H, dt, J=10.2, 2.4 Hz, H-6), 2.07 (1H, m, H-16), 2.01 (1H, m, H-16), 1.96 (1H, m, H-15), 1.83 (1H, m, H-15), 1.80 (1H, m, H-8), 1.72 (1H, m, H-11), 1.63 (1H, m, H-7), 1.50 (3H, d, J=6.2 Hz, H-6′), 1.43 (2H, m, H-4), 1.32 (2H, m, H-6, H-12), 1.29 (1H, m, H-7), 1.25 (1H, m, H-5), 1.23 (3H, m, H-1, H-9, H-11), 1.19 (1H, m, H-12), 0.89 (3H, s, H-18), and ^{13}C-NMR (100 MHz, C_5D_5N) δ_C: 208.6 (CH, C-19), 176.1 (C, C-20), 174.8 (C, C-3′), 174.5 (C, C-23), 117.9 (CH, C-22), 108.7 (CH, C-1′), 85.8 (C, C-2′), 84.3 (CH, C-3), 84.2 (C, C-14), 76.9 (CH, C-5′), 73.9 (CH_2, C-21), 71.0 (CH, C-2), 52.5 (C, C-10), 51.3 (CH, C-17), 49.9 (C, C-13), 42.9 (CH, C-5), 48.7 (CH, C-9), 42.7 (CH, C-8), 41.8 (CH_2, C-4′), 39.7 (CH_2, C-1), 39.3 (CH_2, C-12), 34.7 (CH_2, C-4), 32.5 (CH_2, C-15), 28.1 (CH_2, C-11), 27.9 (CH_2, C-6), 27.3 (CH_2, C-16), 22.4 (CH_2, C-7), 22.9 (CH_3, C-6′), 16.0 (CH_3, C-18).

Bioassays Cytotoxicity assays were performed using the colorimetric method of Skehan and co-workers.[18]

Acknowledgment We are grateful to the Thailand Research Fund and Ramkhamhaeng University for financial support. CS acknowledges Center for Innovation in Chemistry, Postgraduate Education and Research Program in Chemistry (PERCH-CIC) for a scholarship. Thanks are also due to Mr. N. Chimnoi, Chulabhorn Research Institute and Ms. S. Chaichana, Mahidol University, for HR-MS measurements, the Bioassay Laboratory of the National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, for biological activity assays, and Ms. T. Lhinhatrakool for her valuable technical assistance.

References and Notes

1) Lhinhatrakool T., Sutthivaiyakit S., *J. Nat. Prod.*, **69**, 1249—1251 (2006).
2) Abe F., Mori Y., Yamauchi T., *Chem. Pharm. Bull.*, **40**, 2917—2920 (1992).
3) Brueschweiter F., Stoecklin W., Reichstein T., *Helv. Chim. Acta*, **52**, 2086—2105 (1969).
4) Crout D. H. G., Curtis R. F., Hassall C. H., Jones T. L., *Tetrahedron Lett.*, **1963**, 63—67 (1963).
5) Crout D. H. G., Curtis R. F., Hassell C., *J. Chem. Soc.*, **1963**, 1866—1875 (1963).
6) Abe F., Mori Y., Yamauchi T., *Chem. Pharm. Bull.*, **39**, 2709—2711 (1991).
7) Cheung H. T. A., Nelson C. J., *J. Chem. Soc. Perkin Trans. 1*, **1989**, 1563—1570 (1989).
8) Elgamal M. H. A., Hanna A. G., Morsy N. A. M., Duddeck H., Simon A., Gati T., Toth G., *J. Mol. Struct.*, **477**, 201—208 (1999).
9) Martin R. A., Lynch S. P., Schmitz F. J., Pordesimo E. O., Toth S., Horton R. Y., *Phytochemistry*, **30**, 3935—3939 (1991).
10) Oueslati M. H., Jannet H. B., Mighri Z., Chriaa J., Abreu P. M., *J. Nat. Prod.*, **69**, 1366—1369 (2006).
11) Tschesche R., Struckmeyer K., Wulff G., *Chem. Ber.*, **104**, 3567—3572 (1971).
12) Kishida M., Yamauchi N., Sawada K., Ohashi Y., Eguchi T., Kakinuma K., *J. Chem. Soc. Perkin Trans. 1*, **1997**, 891—895 (1997).
13) El-Askary H., Holzl J., Hilal S., El-Kashoury E., *Phytochemistry*, **34**, 1399—1402 (1993).
14) Roy M. C., Chang F.-R., Huang H.-C., Chiang M. Y.-N., Wu Y.-C., *J. Nat. Prod.*, **68**, 1494—1499 (2005).
15) The transformation in **1** was likely to occur through nucleophilic attack of an active oxygen species, such as peroxide radical, at the bridge-head formyl group. The addition product after rearrangement (as in Baeyer Villiger oxidation), and further hydrolysis, may give rise to **1a** and **1b**. See: M. Hudlický, "Oxidations in Organic Chemistry," ACS Monograph 186, American Chemical Society, Washington DC, 1990, pp. 180—181.
16) In previous report, ^{13}C-NMR chemical shifts of the quaternary carbons baring OOH groups were also found to resonate at less shielded positions than those with OH groups. See: Lee C.-K., Fang J.-M., Cheng Y.-S., *Phytochemistry*, **39**, 391—394 (1995).
17) Warashina T., Noro T., *Phytochemistry*, **37**, 801—806 (1994).
18) Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., *J. Natl. Cancer Inst.*, **82**, 1107—1112 (1990).
19) Some of the ^{1}H-NMR assignments of compound **3** (measured in C_5H_5N) was not possible because of the HMQC and HMBC correlations were not so clear in some regions, partly due to scarcity of pure compound since most of the compound was used for the bioactivity evaluation.