Diterpene Esters and Phenolic Compounds from *Sapium insigne* (Royle) Benth. ex Hook. fil.

Hari Prasad Devkota,[a] Purusotam Basnet,[b,1)] and Shoji Yahara*,[a]

[a] *Graduate School of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan: and* [b] *The School of Pharmaceutical and Biomedical Sciences, Pokhara University; P.O. Box 427, Lekhnath, Kaski, Nepal.*
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From the leaves of *Sapium insigne* (Royle) Benth. ex Hook. fil., two new phorbol derivatives, such as 16-hydroxyphorbol-16-acetate (4) and 4β-deoxy-16-hydroxyphorbol-16-acetate (5) along with twelve known phenolic compounds such as 3-*O*-caffeoyl quinic acid (1), 1-*O*-galloyl-β-D-glucose (2), 1,3-di-*O*-galloyl-β-D-glucose (3), rutin (6), 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (7), quercetin (8), guaijaverin (9), nicotiflorin (10), scopolin (11), methyl gallate (12), corilagin (13) and 1,3,6-tri-*O*-galloyl-β-D-glucose (14) were isolated. All of these compounds have been isolated for the first time from this plant.

Key words *Sapium insigne*; Euphorbiaceae; phorbol derivative

Sapium insigne (Royle) Benth. ex Hook. fil. (Euphorbiaceae) is a deciduous tree (about 10 m high), distributed throughout Nepal, India, Sri Lanka, China and Southeast Asia. Traditionally, in Nepal, juice of the bark is applied on wounds to dispel worms or germs and, therefore, accelerate wound healing. The milky latex is mixed with water and given in indigestion. Bark and leaf are widely used as fish poisons.[2)] Previous phytochemical studies have reported the phorbol esters,[3)] flavone derivative,[4)] and 3-*O*-acetylcycloart-23-en-25-ol[5)] from this plant. The present report deals with the isolation and structure elucidation of two new phorbol derivatives, such as 16-hydroxyphorbol-16-acetate (**4**) and 4β-deoxy-16-hydroxyphorbol-16-acetate (**5**) along with twelve known phenolic compounds from the leaves of *Sapium insigne*.

The fresh leaves of *Sapium insigne* were collected from Lumle, Nepal. The shed dried powdered leaves were extracted with MeOH and 40% MeOH successively. The MeOH extract was suspended in water and then partitioned successively with hexane and EtOAc to give hexane, EtOAc and water soluble fractions. The water soluble fraction was then subjected to repeated column chromatography on MCI gel CHP20P, Sephadex LH20, octadecyl silica (ODS) and silica gel to obtain 16-hydroxyphorbol-16-acetate (**4**), 4β-deoxy-16-hydroxyphorbol-16-acetate (**5**) along with, 3-*O*-caffeoyl quinic acid (**1**),[6)] 1-*O*-galloyl-β-D-glucose (**2**),[7)] 1,3-di-*O*-galloyl-β-D-glucose (**3**),[8)] rutin (**6**),[9)] 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**7**),[10)] quercetin (**8**)[11)] and guaijaverin (**9**).[12)] The 40% methanol extract was subjected to MCI gel CHP20P, Sephadex LH20, ODS and silica gel column chromatography to afford compounds nicotiflorin (**10**),[9)] rutin (**6**),[9)] scopolin (**11**),[13)] methyl gallate (**12**),[14)] 1,3,4,6-tetra-*O*-galloyl-β-D-glucose (**7**),[10)] corilagin (**13**)[15)] and 1,3,6-tri-*O*-galloyl-β-D-glucose (**14**).[16)] All of the known compounds

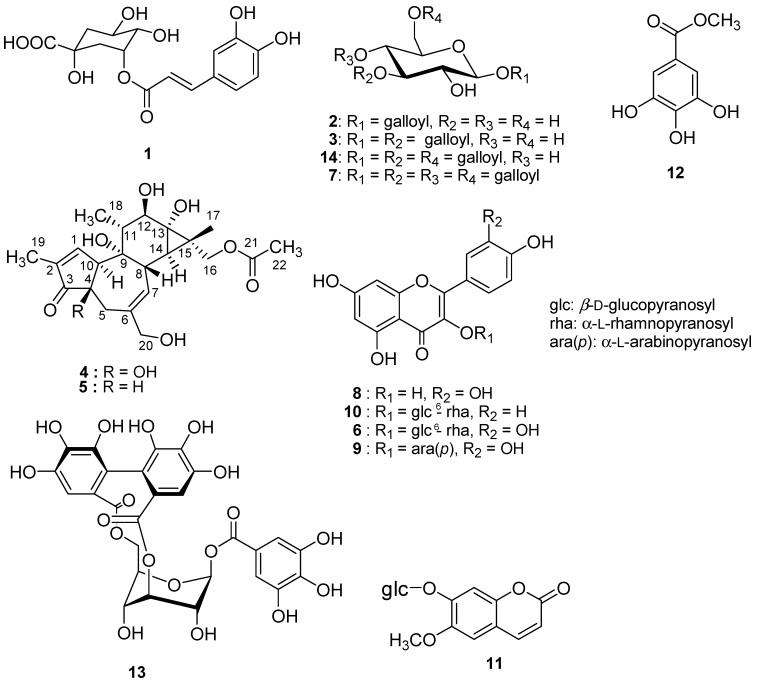

Fig. 1. Structures of Isolated Compounds

* To whom correspondence should be addressed. e-mail: yaharas1@gpo.kumamoto-u.ac.jp

Table 1. NMR Spectroscopic Data for **4** and **5**

Position	**4**			**5**		
	δ_C, mult.	δ_H (J in Hz)	HMBC[a)]	δ_C, mult.	δ_H (J in Hz)	HMBC[a)]
1	160.8, CH	7.66, br s	3, 4, 10	161.8, CH	7.65, br s	2, 3, 4, 10
2	133.9, qC			137.5, qC		
3	210.3, qC			212.5, qC		
4	74.1, qC			45.7, CH	2.60, m	3, 5
5	38.1, CH_2	2.45, d (18.9)	3, 6, 7, 20	30.5, CH_2	2.14, dd (18.1, 9.2)	3, 6, 7, 20
		2.53, d (18.9)	4, 6		2.75, dd (18.1, 10.0)	6, 7, 10
6	141.6, qC			143.4, qC		
7	129.6, CH	5.59, br s	8, 20	128.6, CH	5.46, br s	5, 14, 20
8	38.9, CH	3.34, br s	7, 14	42.9, CH	2.32, br s	5, 6, 7, 14
9	79.2, qC			79.2, qC		
10	58.1, CH	3.09, br s		58.1, CH	3.15, br s	
11	45.1, CH	1.93, m	12	45.0, CH	1.40, m	12, 18
12	80.9, CH	4.03, d (11.0)	15, 18	81.9, CH	4.02, d (9.8)	11, 13, 18
13	62.6, qC			63.2, qC		
14	34.8, CH	1.01, d (5.5)	7, 12, 16	35.2, CH	0.99, d (5.5)	13, 6
15	30.1, qC			30.7, qC		
16	71.4, CH_2	4.15, d (11.0)	14, 17, 21	71.9, CH_2	4.13, d (10.9)	14, 15, 17, 21
		4.26, d (11.0)	12, 17, 21		4.19, d (10.9)	13, 15, 17, 21
17	12.8, CH_3	1.20, s	12, 14, 15, 16	13.1, CH_3	1.15, s	13, 14, 15, 16
18	15.2, CH_3	1.09, d (6.7)	9, 11, 12	16.1, CH_3	1.05, d (6.7)	9, 11, 12
19	10.2, CH_3	1.78, s	1, 2, 3	10.2, CH_3	1.70, d (2.5)	1, 2, 3
20	67.7, CH_2	3.93, d (12.8)	6, 7	67.9, CH_2	3.92, s	5, 6, 7
		3.97, d (12.8)	6, 7			
21	173.2, qC			173.6, qC		
22	21.1, CH_3	2.10, s	21	20.9, CH_3	2.04, s	21

a) HMBC correlations are from proton(s) stated to the indicated carbon.

were identified by using physical data and spectroscopic data including optical rotation, 1 dimensional (1D) and 2D NMR data and with comparison to literature data. All these 14 compounds are isolated and reported for the first time from this plant.

Compound **4** was obtained as amorphous powder, $[\alpha]_D^{29}$ +70.0° (c=0.44, MeOH). The HR-FAB-MS of **4** showed the molecular ion $[M+Na]^+$ at m/z: 445.1864, supporting the formula $C_{22}H_{30}O_8$ (Calcd for $C_{22}H_{30}O_8Na$, 445.1838). The ^{1}H- and ^{13}C-NMR spectroscopic data of compound **4** (Table 1) showed typical signals of phorbol derivative.[17,18)] The ^{1}H-NMR displayed signals for four methyl groups (δ_H 1.09, d, C_{18}-H_3; 1.20, s, C_{17}-H_3; 1.78, s, C_{19}-H_3 and 2.10, s, C_{22}-H_3) and two olefinic protons (δ_H 7.66, br s, C_1-H; and 5.59, br s, C_7-H). The doublet at δ_H 1.01 with the coupling constant J=5.5 Hz was assigned for the C_{14}-H. One of the germinal methyl groups (C-16) on the cyclopropane ring in phorbol was replaced by an hydroxymethyl group (δ_H 4.15, d, J=11.0 Hz, C_{16}-Ha and δ_H 4.26, d, J=11.0 Hz, C_{16}-Hb) which was also supported by the literature data.[3,19)] An oxymethylene groups corresponding to AB system (δ_H 3.93, d, J=12.8 Hz, C_{20}-Ha and δ_H 3.97, d, J=12.8 Hz, C_{20}-Hb) was also detected. All of these assignments were made on the basis of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) correlations (Table 1). In the ^{13}C-NMR spectrum, δ_C 210.3 and δ_C 173.2 were assigned for a ketone (C-3) and ester (C-21) carbonyl groups, respectively. Similarly, δ_C 173.2 (C-21) and δ_C 21.1 (C-22) were assigned for the acetate group. The HMBC data suggested that the acetate group could be assigned to C-16 on the basis of the correlations from C_{16}-Ha (δ_H 4.15) and C_{16}-Hb (δ_H 4.26) to the ester carbonyl carbon (δ_C 173.2). In the differential NOE spectra, the irradiation of C_{14}-H signal caused the enhancement of the C_{16}-H_2 signal and the irradiation of C_{18}-H_3 signal caused the enhancement of the C_{12}-H and C_1-H. Similarly, the irradiation of C_8-H signal caused the enhancement of the C_{11}-H and C_{17}-H_3. These data indicated that C_8-H and C_{11}-H were in β-axial configuration and C_{17}-H_3 in β configuration whereas C_{12}-H and C_{14}-H were found to be in α-axial and α-equatorial configuration respectively. The β-axial configuration of the hydroxyl group at C-4 was also supported by the positive optical rotation.[19)] On the basis of these observations, compound **4** was assigned to be 16-hydroxyphorbol-16-acetate.

Compound **5** was obtained as amorphous powder, $[\alpha]_D^{29}$ +41.2° (c=0.99, MeOH). The HR-FAB-MS of **5** showed the molecular ion $[M+Na]^+$ at m/z: 429.1909, supporting the formula $C_{22}H_{30}O_7$ (Calcd for $C_{22}H_{30}O_7Na$, 429.1889). The ^{1}H- and ^{13}C-NMR spectra of **5** were similar to that of **4** except a multiplet signals observed at δ_H 2.60 and signal for C-4 was observed at δ_C 45.7 instead of 74.1 (with the low field shielding of Δ25.7 ppm indicated that it did not bear a hydroxyl group[20)] which was also supported by the observation of HMBC correlation of C_4-H to C-3 and C-5 and appearance of the C_5-Ha (δ_H 2.14, dd, J=18.1, 9.2 Hz) and C_5-Hb (δ_H 2.53, dd, J=18.1, 10.0 Hz) as double doublets instead of doublets in the spectrum of **4**. The given coupling constants for the C_5-Ha and C_5-Hb have revealed that the C_4-H is in the β configuration[21)] which has been reported to be double doublets of 15.6, 5.0 Hz and 15.6, 2.6 Hz, respectively in the case of the α-isomer. In the differential NOE spectra, the irradiation of C_4-H signal caused the enhancement of the C_8-H and C_{11}-H signals, which also supported that C_4-H is in β-axial

configuration. All other NOE observations were similar to that of compound **4**. Similarly, these data also supported the β-axial configuration of the hydroxyl group at C-4 in compound **4**. On the basis of these observations, compound **5** was assigned to be 4β-deoxy-16-hydroxyphorbol-16-acetate.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^{1}H-, ^{13}C- and 2D-NMR spectra were measured on a JEOL α-500 spectrometer. Chemical shifts are given in ppm with reference to TMS. Mass spectra were recorded on JOEL JMS 700 MStation mass spectrometer. Column chromatography was carried out with silica gel 60 (0.040—0.063 mm, Merck), MCI gel CHP20P (75—150 μm, Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH20 (Pharmacia Biotech) and Chromatorex ODS (30—50 μm, Fuji Silysia Chemical Co., Ltd.). TLC was performed on a precoated silica gel 60 F_{254} (0.2 mm, aluminum sheet, Merck).

Plant Material Fresh leaves of *Sapium insigne* were collected in August, 2007 from Lumle (1600 m), Kaski District, Nepal and shade dried for one month. The specimen was identified by Mr. Kuber Jung Malla, Scientic Officer, Department of Plant Resources, Thapathali, Kathmandu, Nepal. The voucher specimen (No. 432) has been deposited at The School of Pharmaceutical and Biomedical Sciences, Pokhara University, Nepal.

Extraction and Isolation The dried ground powder of leaves of *Sapium insigne* (3.0 kg) was extracted first with MeOH (12 l) and then with 40% MeOH (10 l) and extracts were evaporated separately under reduced pressure to give MeOH extract (215 g) and 40% MeOH extract (322 g) respectively. The MeOH extract (74 g) was suspended in water and then partitioned successively with hexane and EtOAc to give hexane (38 g), EtOAc (14 g) and water (22 g) soluble fractions. The water soluble fraction (22 g) was then subjected to MCI gel CHP20P column and eluted with water, 40% MeOH, 70% MeOH and 100% MeOH to give 17 fractions. Fraction 6 was then subjected to ODS column (10% MeOH) to obtain **1** (18 mg) and **2** (17 mg). Similarly, fraction 7 was subjected to ODS column (20% MeOH) to obtain compound **3** (14 mg). Fraction 10 was then subjected to ODS column (15% MeOH) and then to silica gel column ($CHCl_3$: MeOH : H_2O=9 : 2 : 0.1) to obtain compound **4** (4.6 mg). Fraction 11 was subjected to ODS column (30% MeOH) to obtain compound **5** (86 mg), **6** (43 mg), **7** (107 mg) and **8** (3 mg). Fraction 15 was subjected to Sephadex LH20 column (MeOH) to obtain compound **9** (79 mg). Then, the 40% MeOH extract (302 g) was then subjected to MCI gel CHP20P column and eluted successively with water, 40% MeOH, 70% MeOH and 100% MeOH to give nine fractions. The water eluted fraction 2 was again subjected to MCI gel CHP20P column and eluted with water, 40% MeOH, 70% MeOH and 100% MeOH to give nine subfractions. The subfraction 8 was then applied on ODS column and eluted with 45% MeOH to obtain compound **10** (93 mg). Subfraction 6 was applied to ODS column and eluted with 45% MeOH to obtain compound **8** (236 mg) and **7** (661 mg). Similarly, subfraction 4 was subjected to ODS column and eluted with 20% and 30% MeOH. The 20% MeOH eluted fraction was then applied on Sephadex LH20 column and then silica gel column ($CHCl_3$: MeOH : H_2O=8 : 2 : 0.1) to obtain compound **11** (6 mg) and **12** (42 mg). The 30% MeOH eluted fraction was subjected to Sephadex LH20 column (MeOH) and then ODS column (28% MeOH) to obtain compound **13** (113 mg) and **14** (145 mg).

16-Hydroxyphorbol-16-acetate (**4**): A white amorphous powder. $[\alpha]_D^{29}$ +70.0° (c=0.44, MeOH). ^{1}H-NMR (CD_3OD : $CDCl_3$=1 : 1, 500 MHz) and ^{13}C-NMR (CD_3OD : $CDCl_3$=1 : 1, 125 MHz), Table 1; HR-FAB-MS *m/z*: 445.1864 $[M+Na]^+$ (Calcd for $C_{22}H_{30}O_8Na$, 445.1838).

4β-Deoxy-16-hydroxyphorbol-16-acetate (**5**): A white amorphous powder. $[\alpha]_D^{29}$ +41.2° (c=0.99, MeOH). ^{1}H-NMR (CD_3OD : $CDCl_3$=1 : 1, 500 MHz) and ^{13}C-NMR (CD_3OD : $CDCl_3$=1 : 1, 125 MHz), Table 1; HR-FAB-MS *m/z*: 429.1909 $[M+Na]^+$ (Calcd for $C_{22}H_{30}O_7Na$, 429.1889).

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