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A NEW IRIDOID GLYCOSIDE FROM *SANTISUKIA PAGETII*

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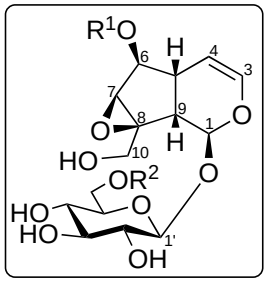
Abstract – A new iridoid glycoside, 6-*O*-[(2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoyl]-catalpol (kanchanikoside, **1**) was isolated from the leaves and twigs of *Santisukia pagetii*. In addition to the new compound, the 17 known glycosides were isolated: nemoroside, 6''(*Z*)-nemoroside, ambiguuside, specioside, verminoside, 6-*trans*-feruloylcatalpol, 6,6'-di-*O*-caffeoylcatalpol, amphicoside, 6-*O*-veratroylcatalpol, citrusin B, (7*R*,8*S*)-balanophonin 4-*O*-β-D-glucopyranoside, (7*R*,8*S*)-dehydrodiconiferyl-*O*-β-D-glucopyranoside, martynoside, benzyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside, benzyl *O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, icariside D_1 and (6*S*,9*R*)-roseoside. Their structures were determined based on the spectroscopic evidence including 1D and 2D NMR, and HR-ESI-TOF-MS experiments.

Santisukia pagetii (Thai name: Kan-Cha-Ni-Ka) belongs to the family Bignoniaceae, tribe Tecomeae. This plant is an evergreen tree that grows up to 20 meters high and is found in Thailand's southwestern. Continuing systematic studies on plants from tribe Tecomeae of this family,[1] the secondary metabolites of this species were investigated. In previous phytochemical investigation, terpenoids, iridoid glycosides, flavonoids and phenolic compounds with anti-HIV-1 activities were reported.[2] This present paper deals with the isolation and structure identification of a new iridoid (**1**), and 17 known compounds, including nine iridoid glucosides (**2-10**, Figure 1), three neolignan glycosides (**11-13**), a phenylethanoid glycoside (**14**), simple aromatic glycosides (**15-17**), and a megastigmane glycoside (**18**) from the water soluble fraction of the MeOH extract of the leaves and twigs of this plant.

The methanolic extract of the leaves and twigs of *S. pagetii* was partitioned between Et_2O and H_2O. The aqueous soluble fraction was separated by combination of chromatographic methods to afford a new iridoid (**1**) together with 17 known compounds. The known compounds were identified as nemoroside (**2**), 6"(*Z*)-nemoroside (**3**), ambiguuside (**4**),[3] specioside (**5**),[4] verminoside (**6**),[5] 6-*trans*-feruloylcatalpol (**7**),[6] 6,6'-di-*O*-caffeoylcatalpol (**8**),[7] amphicoside (**9**),[8] 6-*O*-veratroylcatalpol (**10**),[9] citrusin B (**11**),[10] (7*R*,8*S*)-balanophonin 4-*O*-β-D-glucopyranoside (**12**),[11] (7*R*,8*S*)-dehydrodiconiferyl *O*-β-D-glucopyranoside (**13**),[12] martynoside (**14**),[13] benzyl *O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**15**),[14] benzyl *O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (icariside F_2**, 16**),[15] icariside D_1 (**17**),[16] and (6*S*,9*R*)-roseoside (**18**)[17] by comparison of physical data with literature values and from spectroscopic evidence.

Compound **1** was obtained in an amorphous powder form. The molecular formula was determined as $C_{25}H_{38}O_{12}$ by the high-resolution electrospray ionization time-of-flight mass spectrometric (HR-ESI-TOF-MS) analysis. The ^1H and ^{13}C NMR spectroscopic data indicated that compound **1** is an iridoid glycoside ester of a monoterpenic acid, closely related to compounds **2-5**. The chemical shift at δ_C 95.0 was characteristic of an acetal group of C-l. The methine signals at δ_C 142.4 and 102.9 were assigned to a disubstituted olefinic group at C-3 and C-4, respectively. The chemical shifts of two methine carbons at δ_C 60.2 and 66.8 belonged to an epoxyl group at C-7 and C-8 of the cyclopentanopyran ring. Compound **1** has the same core structure as compounds **2-10**, and could be identified to be catapol. This compound displayed two mass units larger than compounds **2** and **3**, and the signals of the double bond at C-6" and C-7" were not observed in the spectra, suggesting these positions were hydrogenated. The assignment of the structure was supported by 2D-NMR experiments. In the HMBC spectrum, the correlations were observed from i) H-6 (δ_H 4.96) to C-1" (δ_C 169.4), ii) H-3" (δ_H 6.85) to C-1" (δ_C 169.4), C-2" (δ_C 128.3), C-4" (δ_C 27.2), C-5" (δ_C 36.9) and C-10" (δ_C 12.4), iii) H-6" (δ_H 1.64) to C-4" (δ_C 27.2), C-5" (δ_C 36.9), C-7" (δ_C 40.6), C-8" (δ_C 61.3) and C-9" (δ_C 19.7), as illustrated in Figure 2. Thus, the monoterpenyl group was concluded to be 8-hydroxy-2,6-dimethyl-2-octenoyl moiety. The absolute configuration at C-6" was determined to be *R* by alkaline hydrolysis of this compound to provide compound **1a**, which was identified to be (2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoic acid by comparison of NMR spectroscopic data and optical rotation ($[\alpha]_D^{24}$ +7.8, MeOH) with literature values ($[\alpha]_D^{20}$ +7.9).[18] Consequently, the structure of compound **1** was elucidated to be 6-*O*-[(2*E*,6*R*)-8-hydroxy-2,6-dimethyl-2-octenoyl]-catalpol, namely, kanchanikoside.



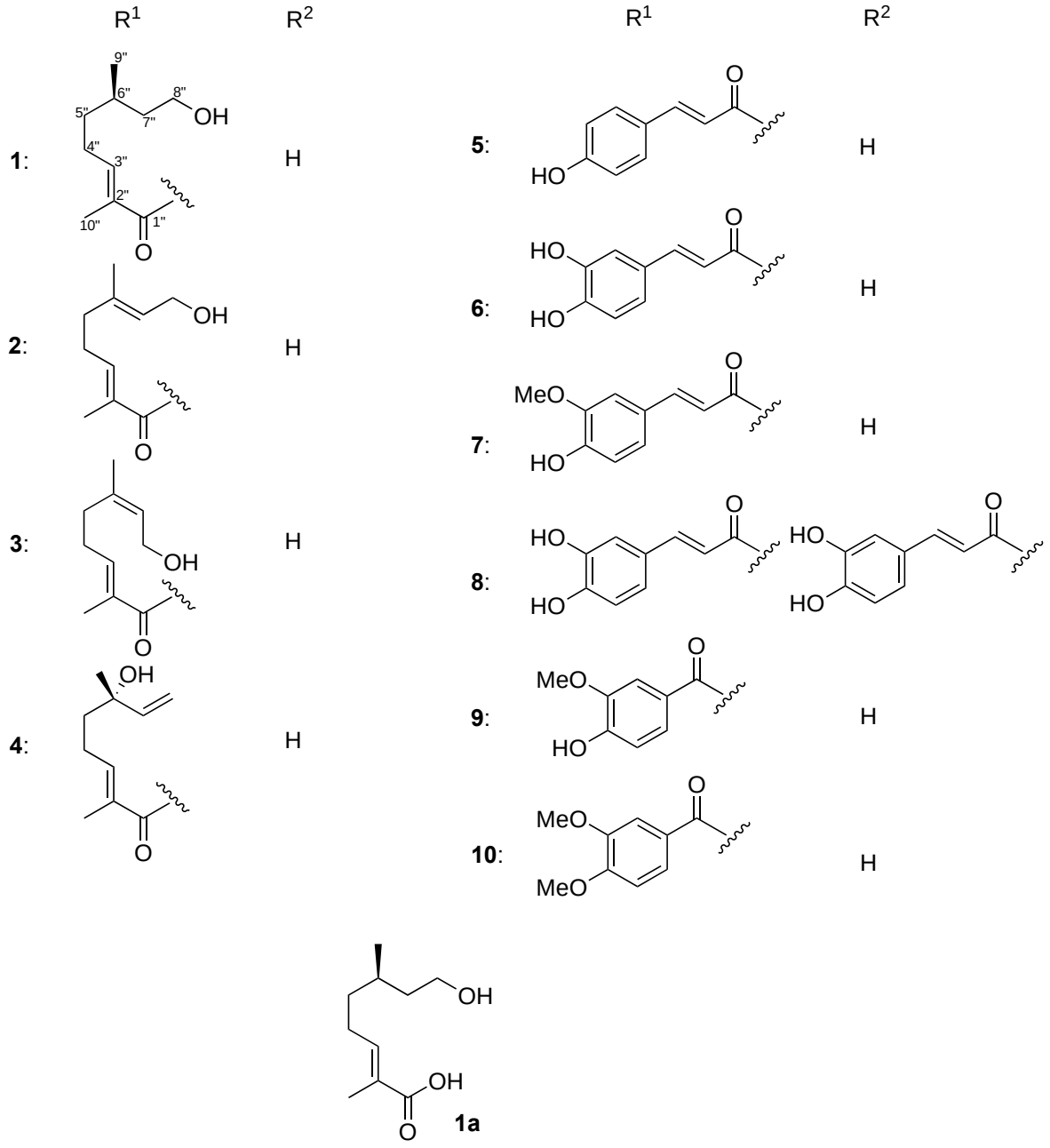


Figure 1. Structures of compounds **1-10** and **1a**

Table 1. NMR spectroscopic data of compounds **1** and **1a**

(400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, in CD_3OD)

Position	**1**		**1a**	
	δ_H	δ_C	δ_H	δ_C
1	5.15 (1H, d, *J* = 9.1 Hz)	95.0		
3	6.36 (1H, dd, *J* = 5.9, 1.4 Hz)	142.4		
4	4.94 (1H, dd, *J* = 5.9, 4.4 Hz)	102.9		
5	2.56 (1H, m)	36.6		
6	4.96 (1H, brd, *J* = 8.5 Hz)	81.6		
7	3.68 (1H, s)	60.2		
8		66.8		
9	2.61 (1H, dd, *J* = 9.1, 7.7 Hz)	43.1		
10	4.16 (1H, d, *J* = 13.1 Hz)	60.9		
	3.82 (1H, d, *J* = 13.1 Hz)			
1'	4.78 (1H, d, *J* = 8.0 Hz)	99.7		
2'	3.25 (1H, dd, *J* = 8.8, 8.0 Hz)	74.8		
3'	3.40 (1H, dd, *J* = 9.1, 8.8 Hz)	78.6		
4'	3.27 (1H, dd, *J* = 9.1, 8.0 Hz)	71.8		
5'	3.32 (1H, m)	77.7		
6'	3.92 (1H, dd, *J* = 11.9, 1.8 Hz)	62.9		
	3.65 (1H, dd, *J* = 11.9, 5.8 Hz)			
1"		169.4		171.7
2"		128.3		128.8
3"	6.85 (1H, td, *J* = 7.4, 1.1 Hz)	144.9	6.78 (1H, td, *J* = 7.5, 1.1 Hz)	144.0
4"	2.26 (2H, m)	27.2	2.22 (2H, m)	27.2
5"	1.50 (1H, m)	36.9	1.47 (1H, m)	37.0
	1.31 (1H, m)		1.27 (1H, m)	
6"	1.64 (1H, m)	30.5	1.61 (1H, m)	30.5
7"	1.60 (1H, m)	40.6	1.59 (1H, m)	40.6
	1.38 (1H, m)		1.37 (1H, m)	
8"	3.60 (2H, m)	61.3	3.60 (2H, m)	60.9
9"	0.95 (3H, d, *J* = 6.5 Hz)	19.7	0.94 (3H, d, *J* = 6.4 Hz)	19.7
10"	1.87 (3H, s)	12.4	1.81 (3H, s)	12.4

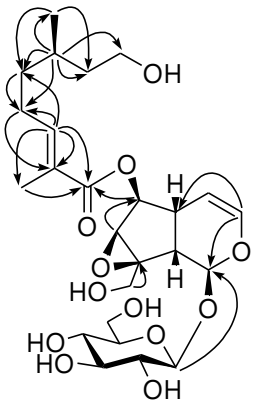


Figure 2. Significant HMBC correlations of compound **1**

Plants from tribe Tecomeae of the family Bignoniaceae are well known to contain iridoid glycosides, phenylethanoid glycosides, and lignan glycosides.[1] Iridoid glycosides, especially those lacking carboxylic acid functionality at C-4 (**1-10**) were expected to isolate from this species. There are no significant differences in the chemical patterns among tribe Tecomeae of Thai Bignoniaceous plants studied to date. However, it provides further confirmation of the typical profile of secondary metabolites found in this family.

EXPERIMENTAL

General experimental procedures. NMR spectra were recorded in CD_3OD using Bruker Ascend^{TM}-400 spectrometer (Bruker Biospin AG, Fällanden, Switzerland). MS data were obtained from the Bruker Micro TOF-LC mass spectrometer (Bruker Daltonik, Bremen, Germany). Optical rotations were measured using a Jasco P-1020 digital polarimeter (Japan). Diaion HP-20 (Mitsubishi Chemical Industries Co., Ltd., Japan), SiliaFlash® P60 (230-400 mesh, SiliCycle Inc., Canada), and RP-18 (50 μm, YMC Co, Ltd., Japan) were used to create a column chromatography. Semi-preparative HPLC (Jasco PU-980 intelligent HPLC pump, Japan) was carried out using a ODS column (column 20 mm i.d. x 250 mm length, ODS-AQ, YMC Co., Ltd., Japan) and a Jasco UV-970 detector (Japan) at 220 nm and flow rates were 6 mL/min. TLC spraying reagent was 10% H_2SO_4 in H_2O-EtOH (1:1, v/v).

Plant material. The leaves and twigs of *Santisukia pagetii* (Craib) Brummitt were collected from Kanchanaburi Province, Thailand, in November 2018. Plant specimen was identified by one of the research team (TK). Voucher specimens (TK-PSKKU-0088) are on files in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation. The air dried leaves and twigs of *S. pagetii* (7.5 kg) were extracted three times with MeOH, and concentrated to dryness. The brown-greenish residue (494.7 g) was suspended in H_2O and partitioned with Et_2O. The water soluble part (391.9 g) was subjected to a Diaion HP-20 column, and eluted with H_2O, and MeOH, successively. The fraction eluted with MeOH (117.9 g) was applied to a silica gel column and washed with solvent systems of EtOAc-MeOH (9:1, 11.0 L), EtOAc-MeOH-H_2O (40:10:1, 8.0 L), EtOAc-MeOH-H_2O (70:30:3, 8.0 L) and EtOAc-MeOH-H_2O (6:4:1, 7.0 L), respectively to produce six fractions (A to F), monitored by TLC. Fraction B (11.8 g from 58.3 g) was applied to a RP-18 column using a gradient solvent system, H_2O-MeOH (90:10 $\rightarrow$ 20:80, v/v) to provide 12 sub-fractions. Sub-fraction B-3 was purified by semi-preparative HPLC-ODS using solvent system H_2O-MeCN (90:10, v/v) to provide compounds **16** (45.2 mg), **17** (43.2 mg) and **18** (34.2 mg). Sub-fraction B-5 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (80:20, v/v) to afford compounds **6** (493.3 mg), **9** (74.1 mg) and **12** (9.3 mg). Sub-fraction B-8 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (80:20, v/v) to obtain compounds **8** (11.7 mg) and **14** (8.8

mg). Sub-fraction B-11 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (75:25, v/v) to give compound **1** (37.1 mg). Fraction C (12.0 g from 38.6 g) was separated on a RP-18 column using solvent system, H_2O-MeOH (90:10 → 20:80, v/v) to provide 14 sub-fractions. Sub-fraction C-3 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (90:10, v/v) to yield compound **15** (52.0 mg). Sub-fraction C-4 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (88:12, v/v) to afford compounds **11** (27.9 mg). Sub-fraction C-5 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (80:20, v/v) to obtain compound **13** (59.3 mg). Sub-fraction C-9 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (75:25, v/v) to obtain compounds **5** (513.7 mg), **7** (71.4 mg) and **10** (88.1 mg). Sub-fraction C-12 was purified by semi-preparative HPLC-ODS with H_2O-MeCN (75:25, v/v) to provide compounds **2** (18.4 mg), **3** (12.3 mg) and **4** (219.2 mg).

Kanchanikoside (1): Amorphous powder, $[\alpha]_D^{25}$ −101.7 (MeOH, *c* 0.15); ^1H and ^{13}C NMR (CD_3OD): Table 1; HR-ESI-TOF-MS, *m/z*: 565.2057 $[M+Cl]^-$ (calcd for $C_{25}H_{38}ClO_{12}$, 565.2057).

Alkaline hydrolysis of kanchanikoside (1): Compound **1** (20.0 mg) was hydrolyzed using 0.5 N NaOH (5.0 mL) at 45 °C for 1 h. The reaction mixture was acidified by addition of 2 N HCl, and extracted with EtOAc (20 mL x 3). The combined EtOAc part was washed with H_2O, dried with Na_2SO_4, and evaporated to provide compound **1a** (7.0 mg).

(2*E*,6*R*)-8-Hydroxy-2,6-dimethyl-2-octenoic acid (1a): Oil, $[\alpha]_D^{24}$ +7.8 (MeOH, *c* 0.42); ^1H and ^{13}C NMR (CD_3OD): Table 1; HR-ESI-TOF-MS, *m/z*: 185.1179 $[M-H]^-$ (calcd for $C_{10}H_{17}O_3$, 185.1183).

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