

Short Note

(S)-2-Methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one

Yasmiwar Susilawati ^{1,2}, Ricky Nugraha ¹, Ahmad Muhtadi ², Supriyatna Soetardjo ² and Unang Supratman ^{1,*}

¹ Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jalan Raya Bandung-Sumedang Km 21, Jatinangor 45363, Sumedang, Indonesia;

E-Mails: yasmiwar_usie@yahoo.co.id (Y.S.); nugraky@gmail.com (R.N.)

² Faculty of Pharmacy, Padjadjaran University, Jalan Raya Bandung-Sumedang Km 21, Jatinangor 45363, Sumedang, Indonesia; E-Mails: muhtadi_utad@yahoo.com (A.M.);

supriyatna_soetadjo@yahoo.com (S.S.)

* Author to whom correspondence should be addressed; E-Mail: u_supratman@unpad.ac.id; Tel./Fax: +62-22-7794391.

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Abstract: A novel chromene, (S)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one (**1**), was isolated from the leaves of *Peperomia pellucida* (Piperaceae). The chemical structure of **1** was determined by spectroscopic methods and comparison with those related compounds previously reported.

Keywords: chromone; *Peperomia pellucida*; Piperaceae

Introduction

The genus *Peperomia* is the second largest in the Piperaceae family and comprises more than 600 species widely distributed in Indonesia [1]. Previous phytochemical studies on the genus *Peperomia* have revealed the presence of a variety of compounds with interesting biological activities, including flavonoids [2–4], benzopyran derivatives [5–7], secolignans [8–11], terpenes, arylpropanoids, phenolic compounds [12–15] and essential oils [16]. Species of *Peperomia* have found application in folk medicine for the treatment of asthma and gastric ulcers, inflammation, and exhibit analgesic and

antibacterial activities [17]. As part of our studies on the Indonesian *Peperomia* species, we have performed a phytochemical examination of leaves of *P. pellucida*. The plant, known as “sasaladaan” in Indonesia is a perennial herb that typically grows in wet rock crevices, and can be found from the northeast to the southeast of Indonesia [1]. The plant is used in Indonesian folk medicine for the treatment of fever, contused wound and skin diseases [18]. Although secondary metabolites of other *Peperomia* species have been investigated previously, the chemical composition of *P. pellucida* which grows in Indonesia is yet to be reported. The isolation and structure elucidation of the isolated compounds are described herein.

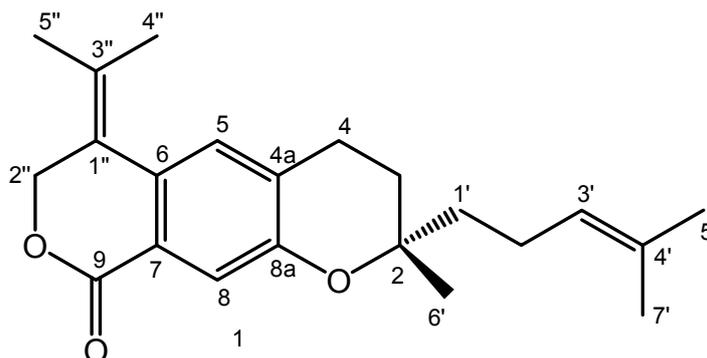


Figure 1. (S)-2-Methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one (**1**).

Results and Discussion

(S)-2-Methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-g]chromen-9(2H)-one (**1**) (Figure 1), was obtained as an orange amorphous powder, $[\alpha]_D^{20} +25.1^\circ$ (*c*, 0.2, MeOH), completely dissolved in acetone and showed green-light fluorescence. Its molecular formula $C_{22}H_{28}O_3$, was established from a combination analysis of the HR-ESI-TOFMS (m/z 341.2072 $[M+H]^+$, calcd. for $C_{22}H_{28}O_3$ 340.2038) and NMR data (Table 1), thus requiring nine degrees of unsaturation. The IR spectrum showed the presence of ester lactone (1700 cm^{-1}), *gem*-dimethyl 1385 and 1360 cm^{-1} , aromatic (1590 and 1480 cm^{-1}) and ether (1078 cm^{-1}) moieties, while the UV spectrum showed three absorption maxima at 328, 280 and 256 nm ($\log \epsilon$ 4.40, 3.76 and 3.20), indicating the aromatic character of **1**. The $^1\text{H-NMR}$ spectrum of **1** revealed two sets of singlet proton at δ_H 7.68 (1H, s) and 7.70 (1H, s), which implied the presence of a 2,3,5,6-tetrasubstituted benzene ring, a singlet at δ_H 2.08, corresponding to a methyl group attached to an oxygen bearing carbon. The $^1\text{H-NMR}$ spectrum also showed signs of an olefinic proton at δ_H 4.10 (1H, m), of eight coupled aliphatic protons at δ_H 1.34 (2H, m) and 1.64 (2H, m), 1.26 (2H, m) and 1.27 (2H, m), of oxygenated methylene protons at δ_H 4.13 (2H, br.s), besides four methyl groups at δ_H 0.87 (6H, s), 1.26 (3H, s) and 1.27 (3H, s). The $^{13}\text{C-NMR}$ spectrum showed 22 carbon resonances, which were classified by their chemical shifts and the HMQC spectrum as one carbonyl lactone, five methyls, five sp^3 methylenes, three sp^2 methines, seven sp^2 quaternary carbons and one sp^3 quaternary carbons. These functionalities accounted for six out of the total nine degrees of unsaturation. The remaining three degrees of unsaturation were consistent to chromene with one additional lactone ring. A comparison of the NMR data of **1** with those of lhotzchromene [19], revealed that the structures of the compounds are closely related, the main

differences are the absence of *cis*-olefine, carboxyl group and one of the aromatic proton and the presence of a lactone ring in the side chain and two additional methyl groups. The gross structure of **1** was deduced from the ^1H - ^1H COSY and HMBC spectra (Figure 2). The aromatic proton at δ_{H} 7.68 was correlated to C-4a (δ_{C} 127.1), C-6 (δ_{C} 129.2) and C-7 (δ_{C} 129.8), whereas another aromatic at δ_{H} 7.70 was correlated to C-8a (δ_{C} 152.1), C-7 (δ_{C} 129.8) and C-4a (δ_{C} 127.1) indicating a tetrasubstituted aromatic ring and the location of the aromatic protons in C-5 and C-8, respectively. The presence of a lactone ring is confirmed from correlation from oxygenated methylene protons at δ_{H} 4.13 to C-8 (δ_{C} 167.0), C-6 (δ_{C} 129.2) and C-1 (δ_{C} 131.7) and aromatic proton at C-8 (δ_{H} 7.70) to carbonyl lactone at C-9 (δ_{C} 167.0), demonstrating that the lactone ring should be attached at C-6 and C-7 formed by cyclization of carboxylic acid and hydroxyl from the side chain. The methyl proton at δ_{H} 0.87 and 1.26 were correlated to C-3'' (δ_{C} 126.2) and C-1'' (δ_{C} 131.7), suggesting that one of an isoprenyl moiety was attached at C-1'' of the lactone ring. The methylene protons at δ_{H} 1.23 (H-3) and 1.34 (H-4) are mutually coupled and correlated to C-4a (δ_{C} 127.1), C-8a (δ_{C} 152.1) and C-2 (δ_{C} 56.5), suggested that pyran ring was located at C-2, C-3, C-4, C-4a, and C-8a. The correlations of methyl group at δ_{H} 2.08 with C-2 (δ_{C} 56.5), C-1' (δ_{C} 29.8) and C-3 (δ_{C} 29.8) observed for the one of the methyl group confirmed its position to an oxygenated carbon at C-2. The methylene protons at δ_{H} 1.27 (H-1') and 1.42 (H-2') are mutually coupled and correlated to C-2 (δ_{C} 56.5) and C-3' (δ_{C} 114.5), whereas olefinic proton at δ_{H} 4.10 (H-3') was correlated to C-2' (δ_{C} 22.4) and sp^2 quaternary carbon at C-4' (δ_{C} 131.7), suggesting that the 4-methyl-3-pentenyl units were attached to C-2 of the of a pyrane ring. From the results of a NOESY experiment, it was evident that Me-5'-H-3' and Me-5''-H-2'' had regiochemical relationship, therefore Me-5'-H-3' and Me-5''-H-2'' has *Z*-configuration, respectively. The relative stereochemistry of C-2 was determined by NOESY experiments, pairs of NOESY correlations, H-1'/H-2' and Me-6'/H-3, suggested that the Me-6' was in β -oriented. The relative stereochemistry of C-2 is also confirmed according the structural similarity and optical rotation, $[\alpha]_{\text{D}}^{20} +25.1^\circ$ to blandachromene I [19] and lhotzchromene [20], therefore the *S*-configuration at C-2 was suggested. Based on physicochemical properties and spectral data along with biogenetic point of view occurrence of chromone constituents in *Piperomia* genus, consequently, the structure of the new chromene was determined to be (*S*)-2-methyl-2-(4-methylpent-3-enyl)-6-(propan-2-ylidene)-3,4,6,7-tetrahydropyrano[4,3-*g*]chromen-9(2*H*)-one, and was named peperochromen A.

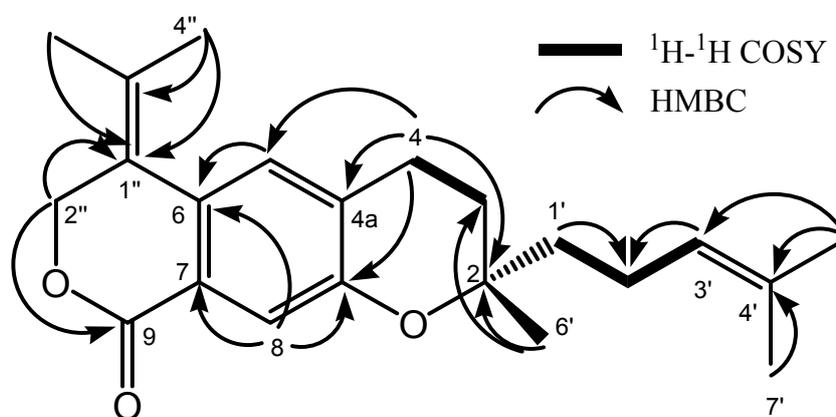


Figure 2. Selected HMBC and COSY correlations for **1**.

Table 1. NMR data (500 MHz for ^1H and 125 MHz for ^{13}C , in CDCl_3) for **1**.

Position	^{13}C -NMR	^1H -NMR
	δ_{C} (mult.)	δ_{H} (integral, mult., J Hz)
2	56.5 (s)	-
3	29.8 (t)	1.23 (1H, m) 1.28 (1H, m)
4	22.4 (t)	1.34 (1H, m) 1.64 (1H, m)
4a	127.1 (s)	-
5	128.7 (d)	7.68 (1H, s)
6	129.2 (s)	-
7	129.8 (s)	-
8	131.6 (d)	7.70 (1H, s)
8a	152.1 (s)	-
9	167.0 (s)	-
1'	29.8 (t)	1.26 (1H, m) 1.27 (1H, m)
2'	31.0 (t)	1.27 (1H, m) 1.42 (1H, m)
3'	114.5 (d)	4.10 (1H, m)
4'	131.7 (s)	
5'	10.8 (q)	0.87 (3H, s)
6'	39.5 (q)	2.08 (3H, s)
7'	13.9 (q)	1.27 (3H, s)
1''	131.7 (s)	-
2''	67.4 (t)	4.13 (2H, br.s)
3''	126.2 (s)	-
4''	13.9 (q)	1.26 (3H, s)
5''	10.8 (q)	0.87 (3H, s)

Experimental Section

General

Optical rotations were recorded on an ATAGO AP-300 automatic polarimeter. The UV-Visible spectra were obtained on Shimadzu series 1800 spectrophotometer (Kyoto, Japan). The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR (Waltham, MA, USA) in KBr. Mass spectra were obtained with a Water, Qtof HR-MS XEV^otm mass spectrometer. (Waters, Milford, MA, USA). ^1H - and ^{13}C -NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as internal standard (Tokyo, Japan). Chromatographic separations were carried out on silica gel 60 (Merck, Darmstadt, Germany). PTLC glass plates were precoated with silica gel GF₂₅₄ (Merck, 0.25 mm). TLC plates were precoated with silica gel GF₂₅₄ (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating.

Plant Material

The leaves of *P. pellucida* were collected in Sumedang, West Java Province, Indonesia in August 2012. The plant was identified by the staff of the Laboratory of Plant Taxonomy, Department of Biology, Padjadjaran University and a voucher specimen was deposited at the herbarium.

Extraction and Isolation

Dried ground leaves (4.5 kg) of *P. pellucida* were successively extracted in Soxhlet apparatus with *n*-hexane, EtOAc, *n*-BuOH and EtOH. Evaporation resulted in the crude extracts of *n*-hexane (117 g), EtOAc (125 g), *n*-BuOH (86 g), and MeOH (104 g), respectively. A portion of the EtOAc extract (120 g) was subjected to vacuum liquid chromatography using gradient elution of *n*-hexane/EtOAc/MeOH to afford 12 fractions (A01-A12). Fraction A01 (3.3 g) was subjected to silica gel column chromatography using mixtures of CH₂Cl₂/EtOAc (7:3) as eluting solvents to afford 10 fractions (B01-B10). Fraction B02 and B03 were combined (500 mg) and was subjected to silica gel column chromatography using mixtures of *n*-hexane/EtOAc (3:2) as eluting solvents to afford 10 fractions (D01-D10). Fraction D04 (300 mg) was preparative TLC on silica gel GF₂₅₄, eluted with *n*-hexane/EtOAc (3:2) and CH₂Cl₂/EtOAc (9.5:0.5), to give **1** (11.5 mg).

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Author Contributions

Unang Supratman designed the whole experiment and contributed to the manuscript. Yasmiwar Susilawati, Ricky Nugraha researched data and wrote the manuscript. Ahmad Muhtadi and Supriyatna Soetardjo analyzed the NMR and LCMS/MS spectra. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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