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Chemical constituents of Thai propolis

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ABSTRACT

Phytochemical investigation on the constituents of Thai propolis led the isolation of a new phenylallylflavanone, (7″*S*)-8-[1-(4′-hydroxy-3′-methoxyphenyl)prop-2-en-1-yl]-(2*S*)-pinocembrin (**1**) and (*E*)-cinnamyl-(*E*)-cinnamylidenate (**2**) from methanolic extract of Thai propolis. Their structures were determined on the basis of extensive NMR spectroscopic analysis. In addition to this, 19 compounds (**3**–**21**) belonging to flavonoids and phenolic esters were isolated and identified.

1. Introduction

Propolis is a sticky hive substances collected by bees from various plant sources. It is used to seal holes in the beehive, smooth out the internal walls and to protect the hive entrance against intruders. It is also an important chemical weapon of honey bees against pathogen microorganisms. It has been used as a folk medicine since ancient times in many parts of the world [1]. Propolis is known to possess versatile biological activities such as antibacterial, antifungal, immunostimulating, antitumor, antiinflammatory, antioxidant etc. [2,3]. Recently, propolis has been a subject of intense research for its activity against human cancers and a number of compounds possessing the anticancer activity have been reported from propolis [4–7]. Previous study in Brazilian red propolis showed interesting preferential cytotoxicity against PANC-1 human pancreatic cancer cells. 7-Hydroxy-6-methoxyflavanone and mucronulatol isolated from Brazilian red propolis has been shown to possess potent cyctotoxicity against the panel of six cancer cell lines [8]. These evidences suggested the beneficial effect of propolis and its constituents in human health. Therefore, there has been increasing interest on the study of constituents of propolis in different parts of the world. The uses of propolis products are gaining interest among the peoples in Thailand and are consumed as health supplements and alternative medicines. However, only a little is known about the chemical constituents from Thailand until now. Therefore, we carried out the extensive phytochemical investigation and isolated and identified 21 compounds (Fig. 1) including two new ones. We herein report these constituents and the structure new compounds by spectroscopic methods.

2. Experimental

2.1. General

Optical rotations were measured on a JASCO DIP-140 digital polarimeter. CD measurements were carried out on a JASCO J-805 spectropolarimeter. IR spectra were measured with a Shimadzu IR-408 spectrophotometer in $CHCl_3$ solutions. NMR

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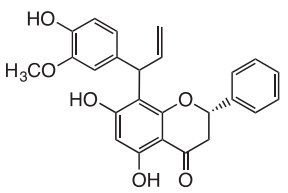
spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values. HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer and glycerol was used as matrix. Column chromatography was performed with normal-phase silica gel (Silica Gel 60N, Spherical, neutral, 40–50 μm, Kanto Chemical Co., Inc.). Analytical and preparative TLC was carried out on precoated silica gel $60F_{254}$ and RP-$18F_{254}$ plates (Merck, 0.25 or 0.50 mm thickness).

2.2. Biological material

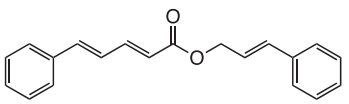
Propolis was collected from a hive of *Apis mellifera* from Chiangmai province, Thailand in August, 2006. A voucher specimen (SWU 0212) was deposited at the Faculty of Pharmacy, Srinakharinwirot University, Nakhon nayok province, Thailand.

2.3. Extraction and isolation

Thai propolis (1 kg) was extracted with MeOH under sonication (2 L, 90 minutes × 3) at room temperature, and the solvent was evaporated under reduced pressure to give a MeOH extract (517 g). A part of MeOH extract (140 g) was chromatographed on silica gel with a MeOH–CH_2Cl_2 gradient system to give 16 fractions (fr.1: 100% CH_2Cl_2 eluate, 0.39 g; fr.2: 100% CH_2Cl_2 eluate, 0.7 g; fr.3: 0–1% MeOH–CH_2Cl_2 eluate, 0.8 g; fr.4: 1% MeOH–CH_2Cl_2 eluate 5.1 g; fr.5: 1–1.5% MeOH–CH_2Cl_2 eluate, 3.0 g; fr.6: 1.5–2% MeOH–CH_2Cl_2 eluate, 7.1 g; fr.7: 2% MeOH–CH_2Cl_2 eluate, 5.2 g; fr.8: 2% MeOH–CH_2Cl_2 eluate, 9.5 g; fr.9: 2–5% MeOH–CH_2Cl_2 eluate, 1.4 g; fr.10: 2–5% MeOH–CH_2Cl_2 eluate, 2.7 g; fr.11: 5–10% MeOH–CH_2Cl_2 eluate, 6.0 g; fr.12: 10–15% MeOH–CH_2Cl_2 eluate, 7.7 g; fr.13: 15–20% MeOH–CH_2Cl_2 eluate, 13.9 g; fr.14: 20–30% MeOH–CH_2Cl_2 eluate, 5.4 g; fr.15: 30–40% MeOH–CH_2Cl_2 eluate, 28.9 g; fr.16: 40–60% MeOH–CH_2Cl_2 eluate, 35 g). Fraction 2 (0.7 g) was left overnight, which gave the crystals of **12** (95 mg). The mother liquor (600 mg) was subjected to normal-phase preparative TLC (pTLC) with 5% EtOAc–hexane, followed by reversed-phase pTLC with acetone–water (5:1), to give **2** (2.4 mg) and **4** (36 mg), respectively. A part of fraction 3 (400 mg) was purified with reversed-phase pTLC with CH_3CN–CH_3COCH_3–H_2O (3:3:2), followed by normal-phase pTLC with 15% EtOAC–hexane to give **3** (12 mg). Fraction 5 (3.0 g) was left overnight, which gave the crystals of **19** (314 mg). The mother liquor (2.4 g) was rechromatographed on reversed-phase silica gel with CH_3CN–CH_3COCH_3–H2O (1:1:1) to afford six subfractions (fr.5-1, 42 mg; fr.5-2, 55 mg; fr.5-3, 1.6 g; fr.5-4, 420 mg; fr.5-5, 134 mg; fr.5-6, 38 mg). Subfraction 5-4 was further purified by reversed-phase pTLC with MeOH–H_2O (4:1) to give **5** (224 mg). Subfraction 5-3 was subjected to normal-phase pTLC with EtOAc–hexane (15:85), followed by MeOH–$CHCl_3$ (1:99), to give **6** (75 mg), **14** (90 mg) and mixture (1.2 g). This mixture was further purified by reversed-phase pTLC with CH_3CN–MeOH–H_2O (1:1:1), to give **1** (9.5 mg), **3** (157 mg), **8** (54 mg), **9** (212 mg), **11** (198 mg), respectively. Fraction 6 (7.1 g) was left overnight, which gave the crystals of **13** (584 mg). The mother liquor (4.2 g) was subjected to reversed-phase pTLC with MeOH–H_2O (3:1) to give **17** (38 mg). Fraction 8 (9.5 g) was left overnight, which gave the crystals of **20** (732 mg). The mother liquor (8.5 g) was rechromatographed in reversed-phase silica gel with CH_3CN–MeOH–H_2O (1:1:1) to obtain 8 subfractions (fr.8-1, 1.4 g; fr.8-2, 1.2 g; fr.8-3, 773 mg; fr.8-4, 1.8 g; fr.8-5, 532 mg; fr.8-6, 880 mg; fr.8-7, 103 mg; fr.8-8, 1.5 g). Purification of subfractions 8-2 and 8-5 by normal-



1



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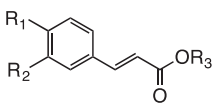
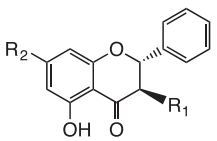
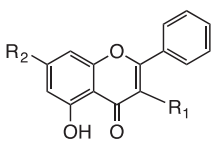


	R_1	R_2	R_3
3	OCH_3	H	CH_2CH=CHPh
4	H	H	CH_2CH=CHPh
5	OH	OCH_3	CH_2CH=CHPh
6	OH	H	CH_2CH=CHPh
7	OH	OH	CH_2CH_2Ph
8	OH	OCH_3	CH_2CH_2Ph
9	OH	OCH_3	CH_2Ph
10	OH	H	CH_2Ph
11	OCH_3	OH	CH_2Ph



	R_1	R_2
12	H	OCH_3
13	H	OH
14	$OCOCH_3$	OCH_3
15	$OCOCH_3$	OH
16	$OCOCH(CH_3)_2$	OH
17	$OCOCH(CH_3)CH_2CH_3$	OH
18	$OCOCH_2CH_3$	OH



	R_1	R_2
19	H	OCH_3
20	H	OH
21	OH	OCH_3

Fig. 1. Structure of compounds isolated from Thai propolis.