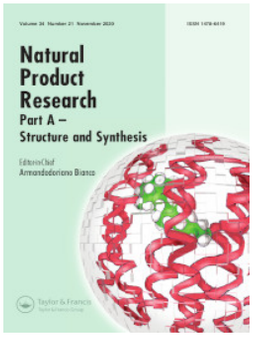

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A new *ent*-abietane lactone from *Glycosmis pentaphylla*

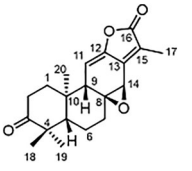
Suwadee Chokchaisiri[a], Nuttapon Apiratikul[b] and Thitima Rukachaisirikul[a]

[a]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand; [b]Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand

ABSTRACT

A new *ent*-abietane lactone, 3-oxojolkinolide A (**1**), together with 16 known compounds, helioscopinolide E (**2**), helioscopinolide A (**3**), 3-methyl-9*H*-carbazole (**4**), carbalexin (**5**), carbalexin B (**6**), glycaborinine (**7**), arborinine (**8**), 1*H*-indole-3-carbaldehyde (**9**), glycoamide A (**10**), glycoamide B (**11**), 2-(*N*-methyl-2-phenylacetamido)benzoic acid (**12**), 2-(methylamine)-methylbenzoate (**13**), fraxidin (**14**), scopoletin (**15**), (-)-syringaresinol (**16**) and ferulic acid (**17**) were isolated from *Glycosmis pentaphylla*. The structures of these compounds were elucidated using spectroscopic techniques such as NMR and MS. Among them, compounds **1**–**3**, **9** and **12**-**17** were isolated from the genus *Glycosmis* for the first time.

Glycosmis pentaphylla



3-oxojolkinolide A (**1**)

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Glycosmis pentaphylla; Rutaceae; *ent*-abietane lactones; alkaloids; coumarins; lignan; cytotoxicity

1. Introduction

The genus *Glycosmis* (Rutaceae) comprises about 40 species of glabrous shrub, distributed in warm and temperate regions of the world including 14 species in Thailand (Smitinand 2011). Previous reports indicated that the genus is a rich source of various types of alkaloids, sulphur-containing amides, and flavonoids (Cuong et al. 1999; Hofer et al. 2000; Ito et al. 2000, 2004; Lukaseder et al. 2009).

Glycosmis pentaphylla, known in Thai as Khey Tay, is a small shrub which grows to a height of 5 meters and it has been used as a folk medicine in the treatment of cough, worms, jaundice, fever, inflammation, rheumatism, anaemia and vermifuge. This plant was also found to possess several pharmacological activities, such as

CONTACT Thitima Rukachaisirikul thitima@ru.ac.th
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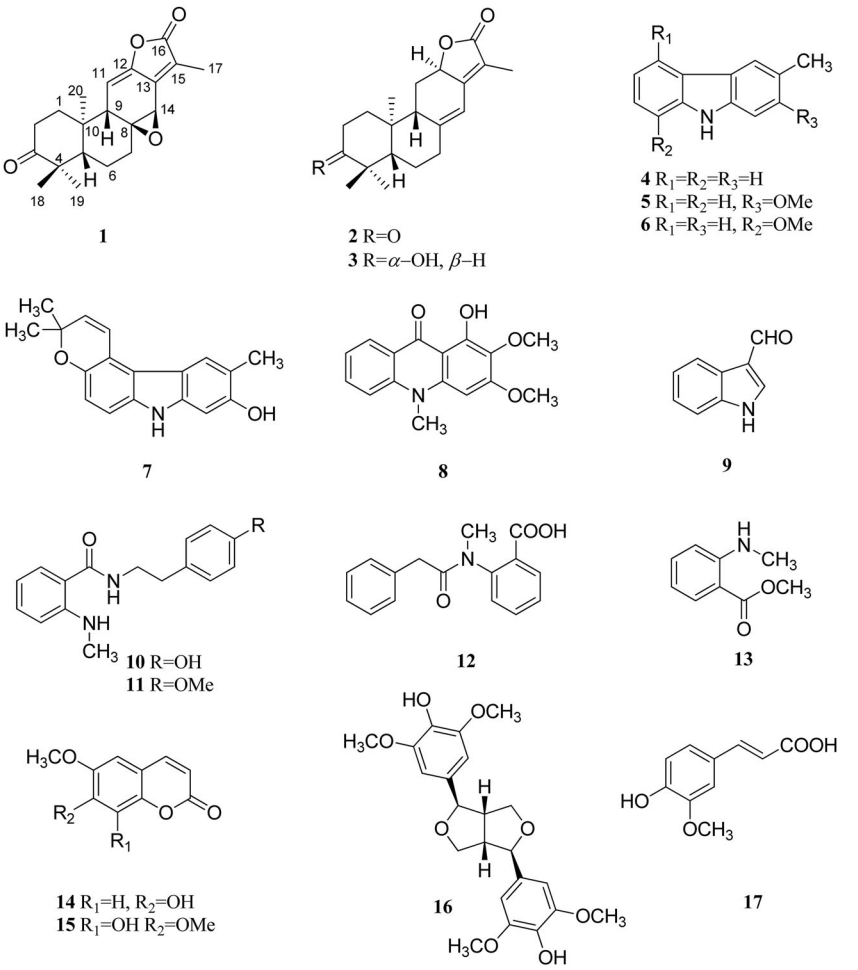

Figure 1. Structures of compounds **1**–**17** from *G. pentaphylla*.

hepatoprotective, anti-inflammatory, anti-tumor, antibacterial, antioxidant, anti-viral, anti-ulcer, chemo protective and antiseptic activities (Sreejith et al. 2012). Previous phytochemical studies on this plant have resulted in the isolation of various alkaloids (Jash et al. 1992; Ito et al. 1999a; Yang et al. 2012; Sripisut et al. 2012; Chen et al. 2015; Zhang et al. 2016, Kumar et al. 2018), hydroquinone diglycoside acyl esters (Wang et al. 2006a) and isoflavone diglycosides (Wang et al. 2006b). In the course of our continuing search for new bioactive compounds from *G. pentaphylla*, we further investigated the chemical constituents of its leaves and twigs, which led to the isolation and identification of one new *ent*-abietane lactone, 3-oxojolkinolide A (**1**), together with 16 known compounds, helioscopinolide E (**2**) (Borghi et al. 1991), helioscopinolide A (**3**) (Borghi et al. 1991), 3-methyl-9*H*-carbazole (**4**) (Yan et al. 2017), carbalexin (**5**) (Jash et al. 1992), carbalexin B (**6**) (Pacher et al. 2001), glycaborinine (**7**) (Chakravarty et al. 1999), arborinine (**8**) (Rahmani et al. 2010), 1*H*-indole-3-carbaldehyde (**9**) (Cordero-Pérez et al. 2014), glycoamide A (**10**) (Ito et al. 1999b), glycoamide B (**11**) (Ito et al. 1999b), 2-(*N*-methyl-2-phenylacetamido) benzoic acid (**12**) (Biabani et al.

Table 1. Cytotoxic activities of some isolated compounds from *G. pentaphylla*.

	Cytotoxicity (IC_{50}, μM)		
Compound	HeLa	HT 29	MCF-7
5	31.84	33.74	inactive
7	8.85	14.75	29.87

1998), 2-(methylamine)-methylbenzoate (**13**) (Correa et al. 2016), fraxidin (**14**) (Rumzhum et al. 2012), scopoletin (**15**) (Mofiz Uddin Khan and Sagar Hossain 2015), (-)-syringaresinol (**16**) (Ragasa et al. 2015) and ferulic acid (**17**) (Sajjadi et al. 2012) (Figure 1). The structure of the new compound was elucidated by spectroscopic techniques whereas those of the known compounds were identified by comparisons of spectroscopic data with those of reported compounds. Compounds **6**, **10** and **11** were firstly found from *G. pentaphylla* while compounds **1–3**, **9** and **12–17** were isolated from the genus *Glycosmis* for the first time. This paper deals with the isolation and structural elucidation of a new compound as well as the cytotoxicity evaluation of some isolates.

2. Results and discussion

Compound **1** was obtained as white amorphous powder with optical rotation $[\alpha]_D^{30}$ $+147.2^{\circ}$ (*c* 0.10, $CHCl_3$). Its molecular formula was determined as $C_{20}H_{24}O_4$ by HRESIMS (*m/z* 351.1560 $[M+Na]^+$, calcd for $C_{20}H_{24}O_4Na$ 351.1566), indicating nine degrees of unsaturation. The UV spectrum suggested the presence of a diene carbonyl group from the absorption maximum at λ 283 nm. The IR spectrum indicated the presence of an α,β-unsaturated γ-lactone (1766 cm^{-1}), a keto carbonyl group (1704 cm^{-1}), and a conjugated double bond (1663 cm^{-1}). The ^1H NMR spectrum displayed the signals of four tertiary methyl groups (δ_H 0.93, 1.06, 1.14, and 2.06, each 3H, s), an oxygenated methine group (δ_H 3.77, 1H, s), and a trisubstituted olefinic moiety (δ_H 5.42, 1H, d, $J = 5.2$ Hz). A combination of ^{13}C NMR, DEPT and HSQC experiments indicated the presence of 20 carbon signals assigned to four tertiary methyls, four methylenes, four methines and eight quaternary carbons. Careful analysis of the ^1H and ^{13}C NMR data suggested the structure of **1** share the similar *ent*-abietane lactone skeleton with the known compound, 3α-hydroxyjolkinolide A (Wang et al. 2004), except the absence of the C-3 hydroxy (δ_H 3.38, δ_C 78.2) group in 3α-hydroxyjolkinolide A and the presence of the C-3 keto (δ_C 214.9) group in **1**. Additionally, the ^{13}C NMR data of **1** showed the signals for C-2 and C-4 deshielded from δ_C 27.0 and 39.2 in 3α-hydroxyjolkinolide A to δ_C 33.9 and 47.9 in **1**, which may be due to the anisotropic effect from the keto group. The keto group located at C-3 was confirmed by the HMBC correlations from H-2, H_3-18 and H_3-19 to C-3. The HMBC correlations between the vinylic methyl proton (CH_3-17) and C-13, C-15 and C-16 revealed the presence of an α,β-unsaturated γ-lactone moiety. The location of a $\Delta^{11,12}$ double bond was corroborated by the HMBC correlations from H-11 to C-8, C-9, C-12 and C-13. Futhermore, the HMBC correlations from H-14 to C-7, C-8, C-12 and C-13 determined the epoxy group at C-8 and C-14 (Figure S11). The correlations in the ROESY spectrum between Me-19/Me-20, H-5/H-9 and H-5/Me-18 confirmed the α-orientation of Me-19, Me-20 and β-orientation of H-5,

H-9 and Me-18 whereas the correlations between H-14/Me-20 indicated the β-orientation of 8,14-epoxy ring (Figure S12). The stereochemistry at C-5, C-9 and C-10 of **1** was inferred by analogy with that of the co-occurring helioscopinolide E (**2**) and helioscopinolide A (**3**) of which the relative configuration was confirmed by X-ray crystallographic analysis (Shizuri et al. 1983). In addition, compounds **1**-**3** should have the same biosynthetic pathway as that of the *ent*-abietane diterpenoids from the roots of *Euphorbia fischeriana* (Zhang et al. 2017). Therefore, the stereostructure of **1** was unambiguously established as identical to that of 3α-hydroxyjolkinolide A and jolkinolide A (Wang et al. 2004). Additional evidence to support the stereostructure of **1** came from the similarity of their ^{13}C NMR data (Table S1) and optical rotation [3α-hydroxyjolkinolide A, $[\alpha]_D^{23}$ +115.2° (*c* 0.19, $CHCl_3$), (Wang et al. 2004) and jolkinolide A, $[\alpha]_D^{25}$ +128° (c 0.02), (Lal et al. 1990)]. On the basis of these data, the structure of compound **1** was established as 3-oxojolkinolide A.

Some isolated compounds **4**, **5**, **7–11**, **13** and **15–17** were evaluated for their cytotoxicity against three human cancer cell lines HeLa (human cervical cancer cells), HT29 (human colon cancer cells) and MFC-7 (human breast cancer cells). Compounds **5** and **7** showed medium to weak cytotoxicity (Table 1) while the other compounds were inactive.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO-1020 polarimeter. UV spectra were measured on an UV-1800 Shimadsu UV spectrophotometer. ATR-FTIR spectra were obtained using a Perkin Elmer FT-IR spectrum 400 spectrometer. 1D and 2D NMR spectra were recorded on a Bruker ASCEND 400 NMR spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. ES-MS and HR-ESI-MS were recorded on a Finnigan LC-Q mass spectrometer and a micrOTOP-II mass spectrometer, respectively. Column chromatography (CC) was carried out using SaliCycle SiliaFlash silica gel 60 (<60–200 μm) and Amersham Biosciences Sephadex LH-20. For TLC, Merck precoated silica gel 60 F254 plates were used. Spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

3.2. Plant material

The leaves and twigs of *G. pentaphylla* were collected from Khao Hin Sorn royal development study center, Chachoengsao, in August 2016. A voucher specimen (Thitima Rukachaisirikul, No. 011) was deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

3.3. Extraction and isolation

The air-dried, powdered leaves of *G. pentaphylla* (9 kg) were extracted successively with n-hexane and MeOH at room temperature. The hexane and MeOH extracts

were filtered and concentrated to dryness under reduced pressure. The hexane extract (517.6 g) was subjected to CC using gradient solvent system of hexane, hexane-EtOAc and EtOAc to give 6 fractions (H1–H6). Fr. H5 (80.2 g) was purified by CC (30% EtOAc in hexane) to give 5 subfractions (H5.1–H5.5). Subfr. H5.2 (14.7 g) was subjected to CC (10% EtOAc in hexane) to furnish **10** (30.6 mg). Subfr. H5.3 (1.69 g) was further purified by CC twice in succession (20% EtOAc in hexane and 40% hexane in CH_2Cl_2) to afford **11** (24.8 mg), whereas subfr. H5.5 (5.57 g) was rechromatographed by CC (25% EtOAc in hexane) to furnish **8** (12.0 mg). The MeOH extract (961.0 g) was subjected to CC using gradient solvent system of hexane, hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH to give 5 fractions (M1–M5). Fr. M3 (32.1 g) was rechromatographed by CC twice in succession (1% MeOH in CH_2Cl_2) to afford **12** (18.5 mg).

The air-dried, powdered twigs of *G. pentaphylla* (5.8 kg) were extracted successively with n-hexane, EtOAc and MeOH at room temperature. The hexane, EtOAc and MeOH extracts were filtered and concentrated to dryness under reduced pressure. The hexane extract (15.7 g) was subjected to CC using gradient solvent system of hexane, hexane-EtOAc and EtOAc to give 8 fractions (H1-H8). Fr. H2 (3.25 g) was purified by CC twice in succession (1% EtOAc in hexane and hexane) to afford **13** (11.1 mg). The EtOAc extract (52.8 g) was subjected to CC using gradient solvent system of hexane, hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH to give 8 fractions (E1-E8). Fr. E3 (2.60 g) was fractionated on Sephadex LH-20 (MeOH) and further purified by CC (5% EtOAc in hexane) to yield **4** (4.1 mg) and **5** (3.1 mg). Fr. E5 (5.34 g) was separated on Sephadex LH-20 (20% CH_2Cl_2 in MeOH) and further purified by CC (0.5% MeOH in CH_2Cl_2) to afford **6** (3.5 mg) and **7** (3.7 mg). The MeOH extract (417.0 g) was subjected to CC using gradient solvent system of hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH to give 9 fractions (M1–M9). Fr. M5 (6.11 g) was separated by CC twice in succession (20% EtOAc in hexane) to afford **2** (9.1 mg). Fr. M6 (7.71 g) was rechromatographed by CC (30% EtOAc in hexane) to obtain 6 fractions (M6.1-M6.6). Subfr. M6.1 gave **1** (1.0 mg), whereas subfr. M6.3 (0.55 g) was rechromatographed by CC (0.5% MeOH in CH_2Cl_2) to obtain **3** (13.1 mg) and **9** (10.0 mg). Subfr. M6.4 (0.19 g) was separated by CC (0.5% MeOH in CH_2Cl_2) to give **14** (3.8 mg), **15** (17.3 mg), and **17** (8.8 mg). Fr. M7 (12.8 g) was further purified by CC twice in succession (0.5% MeOH in CH_2Cl_2) to give **16** (9.4 mg).

3-Oxojolkinolide A (**1**): White amorphous powder, $[\alpha]_D^{30}$ +147.2° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} 283 nm; IR (ATR) ν_{max} 2948, 2867, 1766, 1704, 1663, 1607, 1458, 1433, 1386, 1216, 1135, 1118, 1067, 1016, 879, 850, 756, 743 cm^{-1}, ^1H NMR ($CDCl_3$, 400 MHz): δ 0.93 (3H, s, H_3-20ax), 1.06 (3H, s, H_3-19ax), 1.14 (3H, s, H_3-18eq), 1.63 (1H, m, H-5ax)[a], 1.65 (1H, m, H-7eq)[a], 1.66 (1H, m, H-6eq)[a], 1.73 (1H, m, H-1ax), 1.75 (1H, m, H-6ax), 2.02 (1H, ddd, *J* = 13.2, 6.0, 3.2 Hz, H-1eq), 2.06 (3H, s, H_3-17), 2.13 (1H, m, H-7ax), 2.34 (1H, ddd, *J* = 15.6, 4.6, 3.2 Hz, H-2eq), 2.62 (1H, ddd, *J* = 15.4, 14.2, 6.0 Hz, H-2ax), 2.68 (1H, d, *J* = 5.6 Hz, H-9ax), 3.77 (1H, s, H-14), 5.42 (1H, d, *J* = 5.2 Hz, H-11), [a] overlapping signal; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 8.7 (C-17), 14.7 (C-20), 21.4 (C-6), 22.1 (C-19), 25.5 (C-18), 33.6 (C-7), 33.9 (C-2), 37.8 (C-1), 40.6 (C-10), 47.9 (C-4), 50.7 (C-9), 53.8 (C-5), 54.2 (C-14), 60.7 (C-8), 102.7 (C-11), 125.9 (C-15), 144.4 (C-13), 147.8 (C-12),

170.3 (C-16), 214.9 (C-3); HR-ESI-MS *m/z* 351.1560 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_4$ Na 351.1566).

3.4. Cytotoxicity assay

Human colon adenocarcinoma (HT29) and cervical epithelial adenocarcinoma (HeLa) cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 mg/mL) and l-glutamine (4 mM) at 37 °C, 5% CO_2. Human breast adenocarcinoma (MCF-7) was also grown as mentioned above except that the medium contained 1% of insulin. For the cytotoxicity assay, the cells were seeded up to 1×104 cells/well in a 96-well plate, to give 50–70% confluence to be used on the next day. Monolayers of cancer cells were treated with various concentrations of pure compound in DMSO for 48 h. Cell viability was determined by MTT assay. Cells were incubated with 0.5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution and incubated at 37 °C under a humidified 5% CO_2 incubator for 4 h. Then, the culture medium was removed and 100 μL of DMSO was added before the measurement of absorbance at 550 nm by Tecan U.S., Durham, NC, USA. Half maximal inhibitory concentration (IC_{50}) values were determined by regression analysis (Radchatawedchakoon et al. 2015)

4. Conclusion

In conclusion, a new *ent*-abietane lactone, 3-oxojolkinolide A (**1**), and 16 known compounds, were isolated from the leaves and twigs of *G. pentaphylla*. Three compounds were firstly found from this plant while ten compounds were isolated from this genus for the first time. All the isolated compounds showed no significant cytotoxic activities against cancer cell lines HeLa, HT29 and MFC-7.

Disclosure statement

No potential conflict of interest was reported by the authors.

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