PERGAMON

Phytochemistry 61 (2002) 461–464

PHYTOCHEMISTRY

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Iridoid and phenolic diglycosides from *Canthium berberidifolium*

Tripetch Kanchanapoom[a], Ryoji Kasai[b], Kazuo Yamasaki[b,*]

[a]*Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand*
[b]*Institute of Pharmaceutical Sciences, Faculty of Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan*

Received 7 January 2002; received in revised form 10 April 2002

Dedicated to Professor Vichiara Jirawongse on the occasion of his 84th birthday.

Abstract

An iridoid diglycoside, 6-*O*-β-D-apiofuranosyl-mussaenosidic acid, and four phenolic diglycosides, canthosides A–D, were isolated from the aerial part of *Canthium berberidifolium*, along with seven known compounds. Structural elucidations were based on analyses of spectroscopic data. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Canthium berberidifolium*; Rubiaceae; Iridoid diglycoside; Phenolic diglycosides; Canthosides A–D; Hydroquinone diglycoside

1. Introduction

Canthium berberidifolium Geddes (Rubiaceae, Thai name: Ngiang-Pla-Duk) is a shrub, distributed in Southeast Asia. It is used in Thai traditional medicine for antifever purposes, as well as an anti-inflammatory agent. In continuing studies on Thai Medicinal plants, the constituents of this plant was investigated, following plant collection in the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. To our knowledge, no phytochemical investigation has previously been carried out in this species. The present paper deals with the isolation and structural determination of 12 compounds (**1**–**12**), isolated from the aerial part of this plant, including a new iridoid diglycoside (**2**), four new phenolic diglycosides (**7**, **9**–**11**) and the known iridoid glucoside (**1**), the five phenolic glycosides (**3**–**6**, **8**), and the coumarin diglycoside (**12**), respectively.

2. Results and discussion

From the methanolic extract of the aerial part of *C. berberidifolium*, 12 compounds (**1**–**12**) were isolated. Seven were identified as known compounds: geniposidic acid (**1**) (Guarnaccia et al., 1972; Toda et al., 1985), 4-*O*-β-D-glucopyranosylacetophenone (picein, **3**) (Ushiyama and Furuya, 1989), 4-hydroxyacetophenone 4-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (**4**) (Wang et al., 1998), benzyl β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (icariside F_2, **5**) (Miyase et al., 1988), phenethyl β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside (icariside D_1**6**) (Miyase et al., 1987), koaburaside (**8**) (Ogawa et al., 1973; Gewali et al., 1988) and hymexelsin (**12**) (Rao et al., 1988) by comparison of physical data with literature values and from spectroscopic evidence.

The molecular formula of compound **2** was determined as $C_{21}H_{31}O_{14}$ by HR-FAB MS. The ^1H and ^{13}C NMR spectral data revealed the presence of a β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit, compared to **4**, in addition to a cyclopentanopyran ring, corresponding to an iridoid skeleton. The chemical shifts of compound **2** were very similar with those of mussaenosidic acid (Damtoft et al., 1984) except for additional signals of an apiofuranosyl unit. The carbon signal of C-6′ was shifted downfield by 7.2 ppm. Thus, compound **2** was elucidated as 6′-*O*-β-D-apiofuranosyl-mussaenosidic acid.

Compound **7** has the molecular formula $C_{19}H_{26}O_{12}$, as deduced from HR-FAB MS. The ^1H and ^{13}C NMR spectral data indicated the presence of a 1,2-disubstituted aromatic ring, with one carbomethoxyl group in addition to β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit. The chemical shifts of compound **7** coincided with those reported data for methyl 2-*O*-β-D-glucopyranosyl benzoate (Ushiyama and Furuya, 1989). However, additional signals of the apiofuranosyl unit were observed from the spectra of compound **7**. Thus, compound **7** was methyl 2-

* Corresponding author. Tel.: 82-257-5285; fax: 82-257-5289.
E-mail address: kazuoy@hiroshima-u.ac.jp (K. Yamasaki).

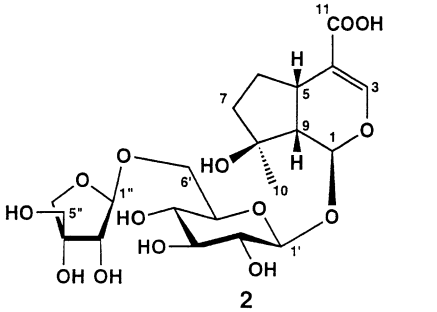
0031-9422/02/$ - see front matter
PII: S0031-9422(02)00140-1

hydroxybenzoate 2-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside, named canthoside A.

The molecular formula of compound **9** was determined as $C_{19}H_{28}O_{13}$ by HR-FAB MS. The ^1H and ^{13}C NMR spectral data revealed the presence of a tetrasubstituted symmetrical aromatic ring, two equivalent methoxyl groups together with a β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit. The chemical shifts of the core portion were in agreement with those of koaburaside (**8**) (Geweli et al., 1988). The position of the two equivalent methoxyl groups were located at C-3 and C-5 by a NOE difference experiment, in which irradiation of the anomeric signal of the glucosyl unit at δ 4.73 caused an NOE enhancement at H-2,6 (δ 6.47). Therefore, compound **9** was determined as 3,5-dimethoxy-4-hydroxyphenol 1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside, named canthoside B.

Compound **10** has the molecular formula $C_{18}H_{26}O_{12}$, as deduced from HR-FAB MS analysis. The ^1H and ^{13}C NMR spectra indicated the presence of 1,2,4-trisubstituted aromatic ring, with one methoxyl group as well as a β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl unit. The spectroscopic data of compound **10** suggested the same skeleton as compound **9**, but lacking a methoxyl group. The complete assignment was established by the NOE difference experiments. Irradiation of the anomeric signal at δ 4.70 of glucosyl unit resulted in the intensity of the signals at δ 6.75 (*d*, $J=2.7$ Hz) and δ 6.58 (*dd*, $J=8.5$, 2.7 Hz) being enhanced, and irradiation of the methoxyl signal at δ 3.82 caused an enhancement at δ 6.75. From this evidence, compound **10** was identified as 3-methoxy-4-hydroxyphenol 1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside, named canthoside C.

Compound **11** has the molecular formula $C_{18}H_{26}O_{12}$, based on HR-FAB MS spectrometry. The ^1H and ^{13}C NMR and mass spectral data indicated that compound **11** is an isomer of compound **10**, suggesting a different location of the methoxyl group. Its structure was sup-



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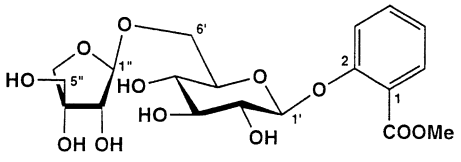

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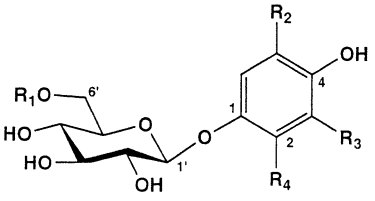

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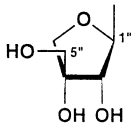
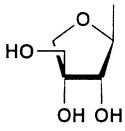
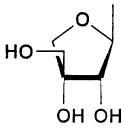
9		OMe	OMe	H
10		H	OMe	H
11		H	H	OMe

Table 1
NMR spectral data of compound **2** (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR, CD_3OD)

No.	C	H[a]
1	95.6	5.34 (1H, *d*, $J=4.9$ Hz)
3	152.1	7.40 (1H, *brs*)
4	113.5	
5	32.5	3.16 (1H, *m*)
6	30.8	1.46 (1H, *m*) 2.29 (1H, *m*)
7	40.5	1.71 (2H, *brt*, $J=7.6$ Hz)
8	80.4	
9	52.3	2.18 (1H, *dd*, $J=8.8$, 4.9 Hz)
10	24.8	1.34 (3H, *s*)
11	170.8	
Glc-1′	99.9	4.66 (1H, *d*, $J=7.8$ Hz)
2′	74.7	3.19 (1H, *dd*, $J=9.0$, 7.8 Hz)
3′	78.0	3.36 (1H, *dd*, $J=9.0$, 8.8 Hz)
4′	71.7	3.26 (1H, *dd*, $J=9.5$, 8.8 Hz)
5′	77.3	3.45 (1H, *m*)
6′	68.7	3.61 (1H, *dd*, $J=11.2$, 6.3 Hz)
		3.98 (1H, *dd*, $J=11.2$, 2.2 Hz)
Api-1″	111.0	5.01 (1H, *d*, $J=2.4$ Hz)
2″	78.0	3.88 (1H, *d*, $J=2.4$ Hz)
3″	80.8	
4″	75.0	3.75 (1H, *d*, $J=9.8$ Hz)
		3.95 (1H, *d*, $J=9.8$ Hz)
5″	65.6	3.56 (2H, *s*)

[a] ^1H NMR chemical shifts assigned on the basis of a HSQC experiment.

Table 2
^{1}H NMR spectral data of compounds **7**, **9**–**11** (400 MHz, CD3OD)[a]

H	7	9	10	11
2		6.47 (1H, *s*)	6.75 (1H, *d*, *J*=2.7 Hz)	
3	7.38 (1H, *dd*, *J*=8.6, 1.0 Hz)			6.46 (1H, *d*, *J*=2.7 Hz)
4	7.56 (1H, *ddd*, *J*=8.6, 7.6, 1.7 Hz)			
5	7.12 (1H, *ddd*, *J*=7.8, 7.6, 1.0 Hz)		6.70 (1H, *d*, *J*=8.5 Hz)	6.99 (1H, *d*, *J*=8.5, 2.7 Hz)
6	7.76 (1H, *dd*, *J*=7.8, 1.7 Hz)	6.47 (1H, *s*)	6.58 (1H, *dd*, *J*=8.5, 2.7 Hz)	6.31 (1H, *d*, *J*=8.5 Hz)
1-COOMe	3.88 (3H, *s*)			
2-OMe				3.80 (3H, *s*)
3-OMe		3.81 (3H, *s*)	3.82 (3H, *s*)	
5-OMe		3.81 (3H, *s*)		
Glc-1′	4.84 (1H, *d*, *J*=7.6 Hz)	4.73 (1H, *d*, *J*=7.3 Hz)	4.70 (1H, *d*, *J*=7.3 Hz)	4.65 (1H, *d*, *J*=7.8 Hz)
2′	3.36 (1H, *dd*, *J*=9.0, 7.8 Hz)	3.33 (1H, *d*, *J*=9.0, 7.3 Hz)	3.35 (1H, *dd*, *J*=9.0, 7.3 Hz)	3.34 (1H, *dd*, *J*=9.0, 7.8 Hz)
3′	3.51 (1H, *dd*, *J*=9.0, 8.3 Hz)	3.53 (1H)[b]	3.53 (1H, *dd*, *J*=9.0, 7.8 Hz)	3.56 (1H, *dd*, *J*=8.6, 7.8 Hz)
4′	3.46 (1H, *dd*, *J*=9.0, 8.3 Hz)	3.43 (1H, *dd*, *J*=8.8, 8.6 Hz)	3.39 (1H, *dd*, *J*=9.0, 7.8 Hz)	3.42 (1H, *dd*, *J*=9.0, 8.6 Hz)
5′	3.59 (1H, *m*)	3.55 (1H, *m*)	3.52 (1H, *m*)	3.46 (1H, *m*)
6′	3.64 (1H, *dd*, *J*=11.0, 6.6 Hz)	3.61 (1H, *dd*, *J*=10.3, 4.3 Hz)	3.60 (1H, *dd*, *J*=10.7, 6.4 Hz)	3.61 (1H, *dd*, *J*=11.0, 5.9 Hz)
	4.03 (1H, *dd*, *J*=11.0, 1.5 Hz)	4.02 (1H, *brd*, *J*=10.3 Hz)	4.00 (1H, *brd*, *J*=10.7 Hz)	4.00 (1H, *dd*, *J*=11.0, 2.0 Hz)
Api-1	4.99 (1H, *d*, *J*=2.5 Hz)	4.97 (1H, *d*, *J*=2.2 Hz)	4.97 (1H, *d*, *J*=2.2 Hz)	4.97 (1H, *d*, *J*=2.2 Hz)
2	3.91 (1H, *d*, *J*=2.5 Hz)	3.88 (1H, *d*, *J*=2.2 Hz)	3.89 (1H, *d*, *J*=2.2 Hz)	3.87 (1H, *d*, *J*=2.2 Hz)
4	3.75 (1H, *d*, *J*=9.8 Hz)	3.74 (1H, *d*, *J*=9.8 Hz)	3.74 (1H, *d*, *J*=9.5 Hz)	3.73 (1H, *d*, *J*=9.8 Hz)
	3.97 (1H, *d*, *J*=9.8 Hz)	3.94 (1H, *d*, *J*=9.8 Hz)	3.95 (1H, *d*, *J*=9.5 Hz)	3.92 (1H, *d*, *J*=9.8 Hz)
5	3.58 (2H, *s*)	3.55 (2H, *s*)	3.57 (2H, *s*)	3.56 (2H, *s*)

[a] Assignments confirmed by HSQC and difference NOE experiments.
[b] Signal unclear due to overlapping.

ported by difference NOE experiments, in which irradiation of the anomeric signal of glucosyl unit gave rise to a NOE enhancement only at δ 6.31. Upon irradiation of the methoxy signal at δ 3.80, an NOE enhancement was observed for the signal at δ 6.46. Consequently, compound **11** was elucidated as 2-methoxy-4-hydroxyphenol 1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside, named canthoside D.

It should be noted that the isolated glycosides from this plant (**2**, **4**–**7**, **9**–**12**) all have β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranosyl units for their sugar moieties.

Table 3
^{13}C NMR spectral data of compounds **7**, **9**–**11** (400 MHz, CD_3OD)

C	7	9	10	11
1	122.4	152.2	152.7	154.9
2	158.7	96.9	103.7	152.0
3	119.2	149.3	149.2	101.9
4	135.2	132.1	143.0	141.0
5	123.7	149.3	116.1	107.7
6	132.1	96.9	110.1	120.6
1-COOMe	168.5			
1-COOMe	52.8			
2-OMe				56.6
3-OMe		56.9	56.5	
5-OMe		56.9		
Glc-1′	104.1	103.7	104.0	104.3
2′	75.0	74.9	74.9	75.0
3′	78.1	77.9	78.0	78.0
4′	71.5	71.5	71.6	71.6
5′	77.3	76.8	76.9	77.0
6′	68.8	68.7	68.7	68.6
Api-1″	111.1	110.7	110.9	110.9
2″	77.6	77.8	77.9	77.8
3″	80.5	80.5	80.5	80.5
4″	75.0	74.9	74.9	75.2
5″	65.5	65.4	65.5	65.7

3. Experimental

3.1. General

NMR, MS, HPLC and CC were performed as described (Kanchanapoom et al., 2001). The solvent systems were: (I) EtOAc–EtOH–H_2O (4:1:0.1), (II) EtOAc–EtOH–H_2O (7:3:0.3), (III) EtOAc–EtOH–H_2O (6:4:1), (IV) 10–50% MeOH, (V) 8% MeCN, (VI) 15% MeCN, (VII) 20–70% MeOH and (VIII) 10% MeCN. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

Canthium berberidifoium Geddes was collected in May, 2001 from the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The identity of the plant was confirmed by Professor Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU-0026) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

3.3. Extraction and isolation

The dried aerial part (1.4 kg) of *C. berberidifolium* was extracted with hot MeOH (8 l×5, refluxed for 24 h). After removal of the solvent in vacuo, the resulting residue (66.4 g) was suspended in H_2O (600 ml) and defatted with Et_2O (1.8 l). The aqueous layer was applied to a column of highly porous copolymer resin of styrene and divinylbenzene and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (15.2 g) was subjected to silica gel CC solvent (systems I, II and III, respectively) affording five fractions (monitored by TLC). Fraction 2 (2.3 g) was applied to a column of RP-18 (solvent system IV) to provide nine fractions. Fraction 2-2 was purified by prep. HPLC-ODS (solvent system V) to give compound **3** (5 mg). Fractions 2-3 and 2-4 were combined, then purified by prep. HPLC-ODS (solvent system VI) to afford compounds **6** (2 mg) and **7** (2 mg). Fraction 3 (5.9 g) was subjected to a RP-18 column (solvent system VII) to provide 12 fractions. Fraction 3-3 was purified by prep. HPLC-ODS solvent (system VIII) to give compounds **1** (62 mg), **2** (15 mg) and **4** (234 mg). Fraction 3-4 was similarly purified by prep. HPLC-ODS (system VIII) to afford compounds **5** (22 mg) and **12** (57 mg). Fraction 4 (4.6 g) was further separated by using a column of RP-18 (system VII), then followed by prep. HPLC-ODS (system XIV) to give compounds **8** (4 mg), **9** (86 mg), **10** (47 mg) and **11** (28 mg).

3.4. 6-O-β-D-Apiofuranosyl-mussaenosidic acid (2)

Amorphous powder, $[\alpha_D]$ −97.1° (MeOH, *c* 1.02); for ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD3OD) spectra: Table 1; negative HR-FAB-MS, *m*/*z*: 507.1722 ($C_{21}H_{31}O_{14}$ requires 507.1713).

3.5. Canthoside A (7)

Amorphous powder, $[\alpha_D]$ −61.1° (MeOH, *c* 0.18); for ^{1}H NMR and (CD_3OD). ^{13}C NMR (CD3OD): see Tables 2 and 3; negative HR-FAB-MS, *m*/*z*: 445.1340 ($C_{19}H_{25}O_{12}$ requires 445.1346).

3.6. Canthoside B (9)

Amorphous powder, $[\alpha_D]$ −72.4° (MeOH, *c* 3.13); for ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) spectra: Tables 2 and 3; negative HR-FAB-MS, *m*/*z*: 463.1465 ($C_{19}H_{27}O_{13}$ requires 463.1451).

3.7. Canthoside C (10)

Amorphous powder, $[\alpha_D]$ −84.1° (MeOH, *c* 3.18); for ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) spectra: Tables 2 and 3; negative HR-FAB-MS, *m*/*z*: 433.1355 ($C_{18}H_{25}O_{12}$ requires 433.1709).

3.8. Canthoside D (11)

Amorphous powder, $[\alpha_D]$ −73.7° (MeOH, *c* 1.53); for ^{1}H NMR (CD_3OD): and ^{13}C NMR (CD3OD) spectra: Tables 2 and 3; negative HR-FAB-MS, *m*/*z*: 433.1349 ($C_{18}H_{25}O_{12}$ requires 433.1345).

Acknowledgements

The authors thank the Research Center for Molecular Medicine, Hiroshima University for the use of its NMR facilities. Also, we are grateful to Mr. Bunlert Khamya of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, for help in obtaining the plant material.

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