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广东冬青中的三萜及三萜皂甙成分

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摘要: 从广东冬青(*Ilex kwangtungensis*)的叶中分离得到四个三萜皂甙和三个三萜成分, 通过光谱解析及化学方法, 三个三萜成分分别鉴定为齐墩果酸(1)、熊果酸(2)和常春藤皂甙元(3); 四个三萜皂甙成分分别鉴定为齐墩果酸3-O-β-D-吡喃葡萄糖基-(1→3)-α-L-吡喃阿拉伯糖甙(4)、齐墩果酸3-O-β-D-吡喃葡萄糖基-(1→2)-α-L-吡喃阿拉伯糖甙(5)、齐墩果酸3-O-β-D-吡喃葡萄糖基-(1→2)-β-D-吡喃葡萄糖甙(6)和齐墩果酸3-O-β-D-吡喃葡萄糖基-(1→2)-β-D-吡喃半乳糖甙(7). 本文系首次报道广东冬青的化学成分.

关键词: 冬青属; 广东冬青; 三萜; 三萜皂甙
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TRITERPENOID SAPONINS AND TRITERPENES FROM *ILEX KWANGTUNGENSIS*

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Abstract: Four triterpenoid saponins along with three triterpenes, oleanolic acid (1), ursolic acid (2) and hederagenin (3) were isolated from the leaves of *Ilex kwangtungensis*. By spectroscopic and chemical methods, the structures of the four saponins were identified as 3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-oleanolic acid (4), 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopy-ranosyl-oleanolic acid (5), 3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-oleanolic acid (6) and 3-O-β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid (7). This paper is the first report of the chemical constituents from this plant.

Key words: *Ilex; Ilex kwangtungensis;* Triterpene; Triterpenoid saponin

Ilex kwangtungensis Merr., an evergreen tree endemic to South China[1], has no medicinal or health-promoting use. Another species of the same genus with the same distribution, *I. kudingcha* C. J. Tseng, is a medicinal plant, whose leaves are used as a popular health-promoting tea in South China[2, 3]. Because of the close similarity in appearance, specifically in leaves between two plants, *I. kwangtungensis* is easily to be

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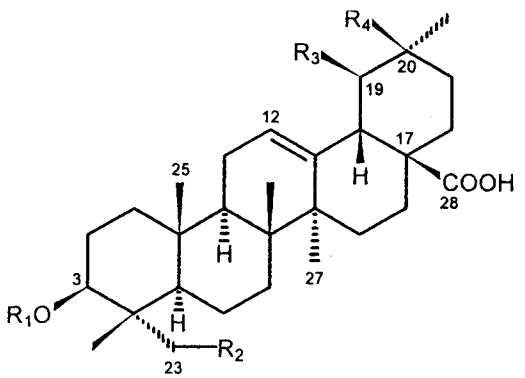
confused with *I. kudingcha* when collecting the plant in the wild. The chemical constituents of *I. kwangtungensis* were previously unknown, while triterpenes and triterpenoid saponins of *I. kudingcha* have been reported[4-8]. In oder to understand the chemical difference between the two plants and find bioactive natural compounds, we made a chemical investigation of *I. kwangtungensis*. This paper describes the isolation and identification of four triterpenoid saponins along with three triterpenes from the leaves of this species.

1 Results and discussion

The EtOH percolate of the fresh leaves was fractionated with C_6H_6, EtOAc and *n*-BuOH. The EtOAc-soluble fraction was subjected to chromatography on a silica gel column, followed by repeated low-pressure chromatography on a reverse-phase (ODS) column. Four triterpenoid saponins were isolated along with three triterpenes, oleanolic acid (1), ursolic acid (2) and hederagenin (3). By spectroscopic and chemical means, the structures of these saponins were identified as 3-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-oleanolic acid (4), 3-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl-oleanolic acid (5), 3-O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-oleanolic acid (6) and 3-O-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-oleanolic acid (7).

The ^1H NMR spectra of compounds 4－7 showed proton signals for 7 tertiary methyl groups at δ 0.80－1.29, a broad singlet for an olefinic proton at δ 5.45 or 5.46, and a double doublet (J＝11.2 and 4.0 Hz) for a proton of an oxygenated methine carbon at δ 3.20 to 3.33. By the combined analysis of their ^{13}C NMR and DEPT data (Table 2), all these saponins were indicated as oleanolic acid glycosides which were confirmed by their acid hydrolysis to give the same aglycone, oleanolic acid. The ^{13}C NMR spectra of all these saponins indicated the presence of two anomeric carbon signals: δ 107.5 and 106.3 in 4, δ 104.9 and 106.3 in 5, δ 105.0 and 105.8 in 6, and δ 105.1 and 105.9 in 7. In addition, the downfield shift of C-3 to about δ 88.7 and the carbon signal for a free carboxylic group at δ 180.5 or 180.4 evidently indicated the presence of 3-O-glycosidic linkage and the absence of 28-O-glycosidic linkage.



	R_1	R_2	R_3	R_4
1	H	H	H	Me
2	H	H	Me	H
3	H	OH	H	Me
4	Ara^{3}-Glc	H	H	Me
5	Ara^{2}-Glc	H	H	Me
6	Glc^{2}-Glc	H	H	Me
7	Gal^{2}-Glc	H	H	Me

The acid hydrolysis of 4 yielded glucose and arabinose besides oleanolic acid. The proton and carbon signals of the sugar chain could be assigned by the careful examination of ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY. The ^{13}C NMR signals for the terminal sugar were coincident with β-D-glucopyranoside[9]. The C-3 signal of the arabinose unit was downfield to δ 84.1, indicating that the terminal sugar in 4 was linked to the 3-hydroxyl of the arabinose unit[9]. The glycosidic and interglycosidic linkages were supported by the NOESY experiment in which the strong cross peaks were observed between the signal at δ 4.71 for the anomeric proton of arabinose moiety and the signal at δ 3.33 for the proton at C-3, and between the anomeric proton of glucose moiety (δ 5.36) and the proton H-3 of arabinose moiety (δ 4.22). The α-linkage of arabinose moiety was suggested by the J value of the anomeric proton (J=8.0 Hz)[10]. From all these results, 4 was determinded as 3-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-oleanolic acid. all spectral data of 4 were identical with those in reference [11], and the structure was confirmed.

The acid hydrolysis of 5 also yielded glucose and arabinose. The ^{1}H and ^{13}C NMR spectra of 5 were very similar to those of 4, except that the carbon signals for arabinose unit were changed. The significant changes were that the anomeric carbon signal and the C-3 signal of arabinose unit were upfield to δ 104.9 and 73.5, respectively, while the resonance corresponding to C-2 was downfield to δ 81.0. This indicated that the terminal glucose in 5 was linked to the 2-hydroxyl of the arabinose unit[9]. Thus, compound 5 was concluded to be 3-O-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl-oleanolic acid[12].

Compound 6, on acid hydrolysis, yield only glucose besides oleanolic acid, indicating that both terminal and inner sugars were glucose. In the ^{13}C NMR spectrum of 6, the signal assignable to C-2 of one of the two glucose moieties was downfield to δ 83.2, showing that the interglycosidic linkage in 6 was 1 → 2[9]. The linkages of both glucose units were suggested as β orientation by the J values of the anomeric protons (J=8.0 Hz). Therefore, compound 6 was determined as 3-O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-oleanolic acid[13].

Compound 7 yielded glucose and galactose besides oleanolic acid on acid hydrolysis. The ^{13}C NMR signals for the terminal sugar in 7 were identical with those in 6. The carbon signal assignable to C-2 of galactose unit was downfield to δ 82.0, indicating that the terminal glucose in 7 was linked to the 2-hydroxyl of galactose unit[9]. The β-linkage for galactose moiety was suggested by the J value of the anomeric proton (J=8.0 Hz). Compound 7 was thus elucidated as 3-O-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-oleanolic acid[14].

2 Experimental

General　Mps: uncorr. IR: KBr discs. NMR: 400 MHz (^{1}H) or 100 MHz (^{13}C), chemical

shifts as δ values (ppm) relative to TMS, C_5D_5N as solvent. FAB-MS: positive ion mode with *m*-nitrobenzyl alcohol as a matrix. HPTLC: silica gel 60 F_{254} and RP-18 F254 plates (Merk), using the following solvent systems: (a) $CHCl_3$-MeOH (9:1 or 8:2) for triterpenes and sapogenins, (b) $CHCl_3$-MeOH-H_2O (70:30:5) and MeOH-H_2O (6:4) for saponins, and (c) EtOAc-MeOH-HOAc-H_2O (60:15:15:10) for sugars; spray reagent 10% H_2SO_4 in EtOH followed by heating. CC: silica gel 60 (100−200 mesh). Low-pressure CC: ODS.

Plant material The leaves of *I. kwangtungensis* were collected in Botanical Garden of South China Institute of Botany, the Chinese Academy of Sciences, in Spring 1995, and identified by Dr. T Chen. A voucher specimen has been deposited at the Herbarium of South China Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation Fresh leaves (5.4 kg) of *I. kwangtungensis* were extracted by percolation with 90% EtOH three times at room temperature. The EtOH extracts were concentrated to syrup under reduced pressure. This syrup was suspended in H_2O and the aqueous suspension was extracted 3 times with benzene. The aqueous layer was then extrated 6 times with ethyl acetate (EtOAc). The combined EtOAc extract after concentration *in vacuo*, yielded a light-brown mass (130 g). This mass was then subjected to a silica gel CC, eluted with $CHCl_3$-MeOH mixtures of increasing polarity [(95:5) to (70:30)], yielding six fractions (I−VI). Fraction Ⅱ, on further chromatography over a silica gel column using $CHCl_3$-MeOH (9:1) as the solvent, afforded 1 (530 mg, 0.0098%), 2 (110 mg, 0.002%) and 3 (40 mg, 0.00074%). Fraction Ⅳ was further chromatographed on a low-pressure reversed phase (ODS) column eluting with MeOH-H_2O (35:65), to give 4 (150 mg, 0.0028%) and 5 (130 mg, 0.0024%). Fraction V was further applied to a low-pressure reversed phase (ODS) column eluting with MeOH-H_2O (30:70), yielding 6 (60 mg, 0.0011%) and 7 (30 mg, 0.00056%).

Oleanolic acid (1), ursolic acid (2) and hederagenin (3) Identified by mp, co-TLC and comparison of their ^{13}C NMR data (Table 1) with those in literatures [15,16].

Table 1 ^{13}C NMR chemical shifts of 1−3 in C_5D_5N

Carbon	1	2	3	Carbon	1	2	3	Carbon	1	2	3
1	38.6t	38.4t	38.6t	11	23.7t	23.4t	23.6t	21	34.2t	30.7t	34.0t
2	27.7t	27.2t	27.6t	12	122.7d	125.3d	122.6d	22	33.1t	36.7t	33.0t
3	78.4d	78.4d	73.3d	13	145.0s	138.9s	144.9s	23	28.2q	28.9q	67.7q
4	39.7s	38.8s	42.7s	14	42.2s	41.9s	42.2s	24	16.8q	15.5q	13.1q
5	55.7d	55.6d	48.4d	15	28.2t	27.9t	28.2t	25	15.4q	15.6q	16.4q
6	18.4t	18.1t	18.7t	16	23.7t	24.5t	23.6t	26	17.0q	16.4q	17.3q
7	33.1t	32.9t	33.0t	17	46.6s	47.7s	46.2s	27	26.1q	23.6q	25.9q
8	39.4s	39.5s	39.5s	18	42.2d	53.2d	41.7d	28	180.5s	179.6s	180.1s
9	47.8d	47.7d	48.0d	19	46.4t	39.0d	46.3t	29	33.2q	23.4q	32.9q
10	36.9s	36.9s	37.2s	20	30.9s	39.0d	30.7s	30	23.7q	21.2q	23.4q

Table 2 ^{13}C NMR chemical shifts of 4–7 in C_5D_5N

Carbon	4	5	6	7	Carbon	4	5	6	7
1	38.6t	38.6t	38.6t	38.6t	30	23.7q	23.7q	23.7q	23.7q
2	26.4t	26.5t	26.6t	26.6t	Inner Ara				
3	88.6d	88.9d	88.8d	88.7d	1	107.5d	104.9d		
4	39.7s	39.7s	39.4s	39.4s	2	71.8d	81.0d		
5	55.7d	55.7d	55.7d	55.7d	3	84.1d	73.5d		
6	18.4t	18.4t	18.4t	18.4t	4	69.3d	68.3d		
7	33.1t	33.1t	33.1t	33.1t	5	67.0t	64.9t		
8	39.4s	39.4s	39.7s	39.7s	Inner Glc				
9	47.8d	47.8d	47.8d	47.8d	1			105.0d	
10	36.9s	36.9s	36.9s	36.9s	2			83.2d	
11	23.7t	23.7t	23.7t	23.7t	3			78.5d*	
12	122.7d	122.7d	122.7d	122.7d	4			71.6d	
13	145.0s	145.0s	144.9s	144.9s	5			78.2d*	
14	42.2s	42.2s	42.2s	42.2s	6			62.8t	
15	28.2t	28.2t	28.2t	28.2t	Inner Gal				
16	23.7t	23.7t	23.7t	23.7t	1				105.1d
17	46.6s	46.6s	46.6s	46.6s	2				82.0d
18	42.2d	42.2d	42.1d	42.1d	3				75.4d
19	46.4t	46.4t	46.4t	46.4t	4				69.9d
20	30.9s	30.9s	30.9s	30.9s	5				76.3d
21	34.2t	34.2t	34.2t	34.2t	6				62.3t
22	33.1t	33.1t	33.1t	33.1t	Terminal Glc				
23	28.2q	28.2q	28.3q	28.3q	1	106.3d	106.3d	105.8d	105.9d
24	16.8q	16.8q	17.0q	17.0q	2	75.7d	76.4d	77.0d	77.0d
25	15.4q	15.4q	15.6q	15.6q	3	78.7d	78.2d	78.0d*	78.0d
26	17.0q	17.0q	17.4q	17.4q	4	71.5d	71.5d	71.6d	71.6d
27	26.1q	26.1q	26.1q	26.1q	5	78.1d	78.1d	78.0d*	78.0d
28	180.5s	180.5s	180.4s	180.4s	6	62.5t	62.5t	62.6t	62.8t
29	33.2q	33.2q	33.2q	33.2q					

* Assignments may be interchanged.

3-O-β-D-Glucopyranosyl-(1→3)-α-L-arabinopyranosyl-oleanolic acid (4) $C_{41}H_{66}O_{12}$, M750; white amorphous powder, mp 249–254 ℃; $[\alpha]_D^{28}$+42.5°(c 1.0, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3411, 1690, 1631, 1170; FABMS m/z (%): 751 $[M+1]^+$ (100); ^1H NMR (δ): 0.81, 0.96, 0.99, 1.26 and 1.29 (each 3H, s, *tert.* Me×5), 0.95 (6H, s, *tert.* Me×2), 3.33 (1H, dd, J=11.2, 4.0 Hz, H-3α), 4.73 (1H, d, J=8.0 Hz, Ara H-1), 5.36 (1H, d, J=8.0 Hz, Glc H-1), 5.45 (1H, br s, H-12), ^{13}C NMR: see Table 2.

3-O-β-D-Glucopyranosyl-(1→2)-α-L-arabinopyranosyl-oleanolic acid (5) $C_{41}H_{66}O_{12}$, M750; white amorphous powder, mp 261–264 ℃; $[\alpha]_D^{28}$+40.8°(c 0.8, MeOH); IR $\nu_{max}^{KBr}$$cm^{-1}$: 3411, 1693, 1635, 1170; FABMS m/z (%): 751 $[M+1]^+$ (100); ^1H NMR (δ): 0.81, 0.96, 0.99, 1.26 and 1.29 (each 3H, s, *tert.* Me×5), 0.95 (6H, s, *tert.* Me×2), 3.33 (1H, dd, J=11.2, 4.0 Hz, H-3α), 4.73 (1H, d, J=8.0 Hz, Ara H-1), 5.36 (1H, d, J=8.0 Hz, Glc

H-1), 5.45 (1H, br s, H-12); ^{13}C NMR: see Table 2.

3-O-β-D-Glucopyranosyl-(1→2)-β-D-glucopyranosyl-oleanolic acid (6) $C_{42}H_{68}O_{13}$; M780; white amorphous powder, mp 259−263 ℃; $[\alpha]_D^{28}$+ 31.4° (c 0.6, MeOH); IR $\nu_{max}^{KBr}cm^{-1}$: 3450, 1692, 1631, 1160; FABMS m/z (%): 781 $[M+1]^+$(100); ^1H NMR (δ): 0.80, 0.93, 0.97, 0.98 and 1.08 (each 3H, s, *tert.* Me×5), 1.27 (6H, s, *tert.* Me×2), 3.28 (1H, dd, J=11.2, 2.8 Hz, H-3α), 4.91 (1H, d, J=8.0 Hz, inner Glc H-1), 5.25 (1H, d, J=8.0 Hz, terminal Glc H-1), 5.46 (1H, br s, H-12); ^{13}C NMR: see Table 2.

3-O-β-D-Glucopyranosyl-(1→2)-β-D-galactopyranosyl-oleanolic acid (7) $C_{42}H_{68}O_{13}$, M780; white amorphous powder, mp 265−283 ℃; $[\alpha]_D^{28}$+27.3° (c 0.5, MeOH); IR $\nu_{max}^{KBr}cm^{-1}$: 3448, 1691, 1634, 1160; FABMS m/z (%): 781 $[M+1]^+$(100); ^1H NMR (δ): 0.80, 0.93, 0.97, 0.98 and 1.08 (each 3H, s, *tert.* Me×5), 1.27 (6H, s, *tert.* Me×2), 3.28 (1H, dd, J=11.2, 2.8 Hz, H-3α), 4.86 (1H, d, J=8.0 Hz, Gal H-1), 5.25 (1H, d, J=8.0 Hz, Glc H-1), 5.46 (1H, br s, H-12); ^{13}C NMR: see Table 2.

Acid hydrolysis of 4−7 A solution of each saponin (5−10 mg) in 2 ml of ethanolic HCl (10%) was refluxed for 4 hours. The reaction mixture was diluted with 3 ml of water and evaporated to remove ethanol. The aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ layer, after evaporation, afforded oleanolic acid which was identified by the direct comparison on TLC with authentic sample. The aqueous layer was neutralized with 10% NaOH and concentrated under reduced pressure. The residue was compared with standard sugars on TLC and shown to consist of Ara and Glc in 4 and 5, Glc in 6, and Gal and Glc in 7.

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昆明山梅花及其变种的指定模式标本

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摘要: 昆明山梅花 *Philadelphus kunmingensis* S. M. Hwang 及其变种小叶山梅花 var. *parvifolius* S. M. Hwang 已发表于中国科学院华南植物研究所集刊 7:5－7. 1991. 有拉丁文的描述及标本引证，但未指定模式标本，为使其名称符合植物命名法规，现补充指定模式标本。

关键词: 昆明山梅花; 模式指定

中图分类号: Q949.751.2　　**文献标识码**: A　　**文章编号**: 1005－3395(2000)03－0263－01

INDICATION OF THE TYPE FOR *PHILADELPHUS KUNMINGENSIS* AND ITS VARIETY

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Abstract: The type specimens of *Philadelphus kunmingensis* S. M. Hwang and var. *parvifolius* S. M. Hwang was indicated.

Key words: *Philadelphus kunmingensis*; Indication of type

Philadelphus kunmingensis S. M. Hwang in Acta Bot. Austro. Sin. 7: 5. f. 1. 1991. (Type was not indicated). Yunnan: Kunming alt. 2 100 m. June 1964 刘慎谔(T. N. Liou) 16385 (holotype SCBI).

Philadelphus kunmingensis var. *parvifolius* S. M. Hwang in Acta Bot. Austro. Sin. 7: 6－7. 1991. (Type was not indicated). Yunnan: Kunming alt. 2100 m. June 1964. 刘慎谔(T. N. Liou) 16382 (holotype SCBI).