Asian Journal of Chemistry; Vol. 25, No. 17 (2013), 9457-9459

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15024

Triterpenoides from the Leaves of *Ilex pubescens* and their Potential Xanthine Oxidase Inhibitory Activity

ZHONG-LIU ZHOU[1,*], ZONG-CAI FENG[2] and JING-MIN XIA[1]

[1]Chemistry Science and Technology School, Zhanjiang Normal University, Zhanjiang, P.R. China
[2]Development Center for New Materials Engineering & Technology in Universities of Guangdong, Zhanjiang Normal University, Zhanjiang, P.R. China

*Corresponding author: Tel./Fax: +86 759 3183176; E-mail: zlzhou@hotmail.com

(*Received*: 26 December 2012; *Accepted*: 1 October 2013) AJC-14204

A new triterpene saponin, ilexsaponin B (**1**), along with three known triterpenoides, **2a**, **3β**, **19a**, 23-tetrahydroxy oleanolic acid (**2**), ilexgenin A (**3**) and ilexolide A (**4**) were isolated from the leaves of *Ilex pubescens*. Their chemical structures were elucidated on the basis of UV, IR, MS, NMR spectroscopic analyses coupled with chemical degradation. In addition, the xanthine oxidase inhibitory activity of the isolated compounds was reported. Compound **1** exhibited moderate xanthine oxidase inhibitory activity in the test with an IC_{50} value of 26.01 μmol/L.

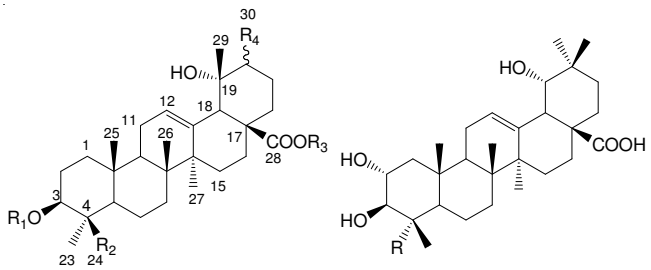
Key Words: *Ilex pubescens*, Triterpenoide, Ilexsaponin B, Xanthine oxidase.

INTRODUCTION

Ilex pubescens is an evergreen bush distributed widely in Southern China and is used as a traditional Chinese medicine for the treatment of cardiovascular diseases and inflammatory diseases[1-5]. The leading function of xanthine oxidase (XOD) is to catalyze the oxidation of xanthine to uric acid. The overaccumulation of uric acid can lead to hyperuricemia, which can be linked to gout because of the deposition of uric acid in the joints leading to painful inflammation. Therefore, the xanthine oxidase inhibitor that blocks the synthesis of uric acid in the body should be one of the approaches for the treatment of hyperuricemia[5]. Our studies indicated that the 70 % aqueous ethanol extract of the leaves of *Ilex pubescens* showed considerable xanthine oxidase inhibitory activity. To further investigate the constituents and screen the bioactive compouds from its leaves, a phytochemical study was performed that resulted in the isolation of four triterpenoides (Fig. 1). In the present study, we describe the structural elucidation of a novel compound (**1**), together with the xanthine oxidase inhibitory activity test performed for all isolated compounds (**1-4**).

EXPERIMENTAL

UV spectra were recorded on a Hewlett-Packard HP-845 UV-visible spectrophotometer. Specific rotation measurements were recorded on a Perkin-Elmer 242 MC polarimeter. IR spectra were recorded on a Nicolet 470 spectrometer and MS on a Varian MAT-212 mass spectrometer and a Micromass Q-TOF mass spectrometer, respectively. NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. Column chromatography separations were carried out on D101 macroporous resin (Chemical Factory of Nankai University, Tianjin, P.R. China), silica gel (200-300 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, P.R. China), ODS (50 mesh, AA12S50, YMC) and Sephadex LH-20 (Pharmacia). All other chemicals used were of biochemical reagent grade.



1 R_1 = H, R_2 = COOGlc, R_3 = H, R_4 = α-CH_3
3 R_1 = H, R_2 = COOH, R_3 = H, R_4 = α-CH_3
4 R_1 = Xyl, R_2 = CH_3, R_3 = Glc, R_4 = β-CH_3

2 R = CH_2OH

Fig. 1. Compounds (**1-4**) isolated from the leaves of *Ilex pubescens*

The leaves of *Ilex pubescens* were collected in Baise, Guangxi Province of China in October 2010 and a voucher specimen (No. 2010007) was kept at the Chemistry Science and Technology School, Zhanjiang Normal University.

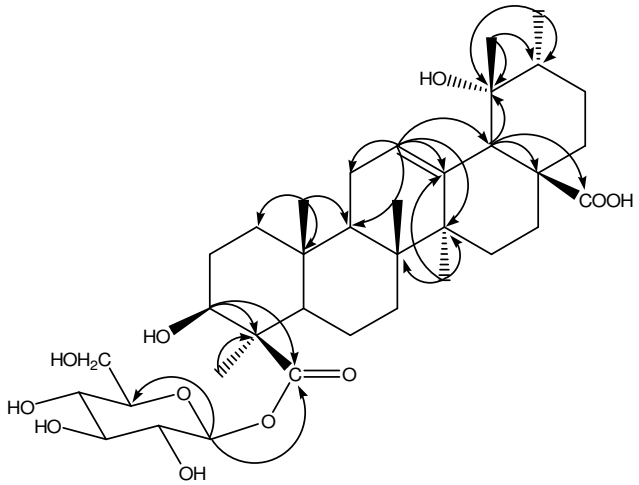


Fig. 2. Structure of **1** and its important H-C correlations in the HMBC spectrum

Extraction and isolation: The leaves of *Ilex pubescens* (6 kg) were refluxed with 70 % aq. EtOH (3 L × 75 L). The extract was condensed in a rotary evaporator to obtain 284 g precipitate which was subjected to chromatographic separation over D101 macroporous resin eluted with H_2O, 60 % EtOH-H_2O and acetone by turns to give three fractions with the yields 28, 97 and 45 g, respectively. The 60 % EtOH-H_2O fraction (97 g) was further fractionated by silica gel column chromatography (CC) (200-300 mesh) using a mobile phase composed of $CHCl_3$/MeOH/H_2O (100:1:0-10:10:4) and finally with MeOH alone to collect three additional fractions (F.1-Fr.3). Fr.1 (20 g) was subjected to repeated CC (SiO_2; $CHCl_3$/MeOH/H_2O 9:1:0.1) to afford 2 (13 mg) and 3 (22 mg). Fr. 2 (31 g) was then subjected to CC over silica gel (10-40 μm) using $CHCl_3$/MeOH/H_2O (9:1:0.1, 8:2:0.2, 7:3:0.5 and 6:4:1) and ODS C_{18} CC using MeOH/H_2O (1:1-9:1) repeatedly to afford 1 (17 mg) and 4 (26 mg).

Acid hydrolysis of ilexsaponin B: A solution (1.5 mg) of 1 in 1 M HCl (1 mL) was heated at 100 ºC for 2 h under an N_2 atmosphere. After cooling, the solution was removed by blowing with N_2. The residue was dissolved in the solution of 1-(trimethylsilyl) imidazole in pyridine, stirred at 60 ºC for 5 min. After removal of the solvent with a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1:1 v/v). The CH_2Cl_2 fraction was analyzed by GC using a L-chirasil-Val column (0.32 mm × 25 m). The temperature of the injector and detector were 200 ºC. A temperature gradient system was used for the oven, starting at 100 ºC for 1 min and increasing up to 180 ºC at a rate of 5 ºC/min. The peaks of the hydrolysates of **1** were confirmed by comparison of retention time of authentic sample D-glucose treated with 1-(trimethylsilyl) imidazole[6,7].

Assay of the xanthine oxidase inhibitory activity: In order to test the xanthine oxidase inhibitory activity of 1-4, the xanthine oxidase activities with xanthine as the substrate were measured with spectrophotometer at λ_{max} 295 nm using the method reported previously[5]. Allopurinol was used as positive control with IC_{50} = 1.61 μmol/L.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. Its molecular formula $C_{36}H_{56}O_{11}$ was deduced from the ^{13}C NMR data and the positive ion HR-ESI-MS, which showed a molecular peak at *m/z* 687.3973 $[M + Na]^+$. Positive results of both Liebermann-Burchard and Molisch reactions indicated that **1** could be a triterpenoid saponin. The ^1H NMR spectrum showed that the aglycon of **1** contained five tertiary methyl groups (δ_H 1.16, 1.23, 1.47, 1.63 and 1.75, s, 3H each) and a methyl doublet at δ_H 1.09 (3H, d, *J* = 7.5 Hz), as well as an olefinic proton at δ_H 5.61 (1H, br t). Six methyl carbons (δ_C 12.1, 15.4, 16.3, 17.3, 24.1 and 26.9), two olefinic carbons (δ_C 127.7, 139.6) and two carbonyl carbons (δ_C 176.9, 180.3) were exhibited in the ^{13}C NMR spectrum (Table-1), respectively. ^1H and ^{13}C NMR spectra of **1** indicated that the structure of the aglycone moiety corresponded to that of ilexgenin A1[8].

TABLE-1
^1H NMR AND ^{13}C NMR SPECTROSCOPIC DATA FOR COMPOUND **1** IN C_5D_5N[a]

Carbon No.	δ_H	δ_C	Carbon No.	δ_H	δ_C
1	1.22 (m), 1.53 (m)	40.6 (t)	20	1.32 (m)	41.8 (d)
2	1.58 (m), 2.03 (m)	28.5 (t)	21	1.61 (m), 1.73 (m)	26.6 (t)
3	3.67 (dd, 4.1, 11.9)	75.1 (d)	22	–	37.4 (t)
4	–	54.8 (s)	23	1.63 (s)	12.1 (q)
5	1.83 (m)	51.8 (d)	24	–	176.9 (s)
6	1.48 (m), 1.67 (m)	20.6 (t)	25	1.23 (s)	15.4 (q)
7	1.24 (m), 1.35 (m)	33.6 (t)	26	1.16 (s)	17.3 (q)
8	–	40.2 (s)	27	1.75 (s)	24.1 (q)
9	1.58 (m)	46.9 (d)	28	–	180.3 (s)
10	–	37.9 (s)	29	1.47 (s)	26.9 (q)
11	2.01-2.17 (m)	24.4 (t)	30	1.09 (d, 7.1)	16.3 (q)
12	5.61 (br t, 3.4)	127.6 (d)	Glc	–	–
13	–	138.6 (s)	1'	6.48 (d, 8.0)	96.1 (d)
14	–	42.2 (s)	2'	4.16 (m)	74.1 (d)
15	1.62 (m), 1.73 (m)	28.9 (t)	3'	4.24 (m)	78.8 (d)
16	2.33-2.38 (m)	26.0 (t)	4'	4.31 (m)	71.2 (d)
17	–	47.1 (s)	5'	3.99 (m)	79.1 (d)
18	2.98 (s)	53.5 (d)	6'a	4.37 (dd, 4.5, 12.0)	62.2 (t)
19	–	71.9 (s)	6'b	4.41 (dd, 2.0, 12.0)	–

[a]Recorded in DMSO at 500 MHz (^1H NMR) or 125 MHz (^{13}C NMR spectra). Chemical shifts and coupling constants are given in ppm and Hz, respectively.

Therefore, the aglycone of **1** was believed to be 3β,19α-dihydroxyurs-23-oxo-12-en-24,28-dioic acid.

Acid hydrolysis of **1** yielded 3β,19α-dihydroxyurs-23-oxo-12-en-24, 28-dioic acid and the released monosaccharide unit identified as D-glucose was detected by GC-MS with standard sugar. The D-glucose residue of **1** was deduced from the presence of an anomeric proton signal at δ_H 6.48 (1H, d, J = 8.0 Hz) in the ^{1}H NMR spectrum and an anomeric carbon signal at δ_C 96.1 in the ^{13}C NMR spectra. In the HMBC spectrum, correlations between H1' of the D-glucose residue and C24 of the aglycone, H3 and C24 and H23 and C24 indicated the D-glucose moiety was concluded to be attached to the C24 carboxyl group on the aglycon of **1**. The relative stereochemistry of D-glucose was determined as β-D-glucopyranose based on the characteristic $J_{H1',H2'}$ coupling constant (J = 8.0 Hz) and ^{13}C NMR data. Accordingly, compound **1** was elucidated as 3β,19α-dihydroxyurs-12-en-24,28-dioic aicd 24-O-β-D-glucopyranosyl ester, named ilexsaponin B.

The structures of the other isolated components 2a, 3β,19a,23-tetrahydroxy oleanolic acid (**2**)[9], ilexgenin A (3)[8], and ilexolide A (4)[10] were determined by comparison to the ^{1}H and ^{13}C NMR spectral data in the literatures. To the best of our knowledge, the known compound, 2a,3β,19a,23-tetrahydroxy oleanolic acid (**2**) was the first reported from the *Ilex pubescens*. Compounds **1-4** were examined for their inhibitory activities on xanthine oxidase[5]. Allopurinol was used as positive control with IC_{50} = 1.61 μmol/L. All compounds **1-4** showed moderate xanthine oxidase inhibitory activity with IC_{50} values of 26.01, 73.19, 90.83 and 67.14 μmol/L, respectively.

Conclusion

A new triterpene saponin, ilexsaponin B (**1**), was isolated from the leaves of *Ilex pubescens*, along with three known triterpenoides. Compound **1** showed considerable activity with IC_{50} value of 26 μM. And the others compounds showed inhibitory effect with higher IC_{50}.

ACKNOWLEDGEMENTS

This study was supported by Foundation for Distinguished Young Talents in Higher Education of Guangdong, China (2012LYM-0095) and Key Science and Technology Program of Zhanjiang City (2012C3102017).

REFERENCES

1. Jiangsu New Medical College, Directory of Chinese Materia Medica (Zhong Yao Da Ci Dian), Shanghai Scientific and Technological Press, Shanghai, China, p. 441 (1977).
2. F. Feng, M.X. Zhu, N. Xie, W.Y. Liu, D.J. Chen and Q.D. You, *J. Asian Nat. Prod. Res*., **10**, 71 (2008).
3. C.X. Zhang, C.Z. Lin, T.Q. Xiong, C.C. Zhu, J.Y. Yang and Z.X. Zhao, *Fitoterapia*, **81**, 788 (2010).
4. J.R. Wang, H. Zhou, Z.H. Jiang and L. Liu, *Chem. Biodivers*., **5**, 1369 (2008).
5. L.P. Lin, W. Qu and J.Y. Liang, *Chin. Chem. Lett*., **22**, 697 (2011).
6. E. Innocent, J.J. Magadula, C. Kihampa and M. Heydenreich, *Nat. Prod. Commun*., **5**, 903 (2010).
7. W.W. Fu, J.N. Fu, W.M. Zhang, L.X. Sun, Y.H. Pei and P. Liu, *Molecules*, **16**, 4371 (2011).
8. K. Hidaka, M. Ito, Y. Matsuda, H. Kohda, K. Yamasaki and J. Yamahara, *Phytochemistry*, **26**, 2023 (1987).
9. X.H. Zhou, R. Kasai, K. Ohtani, O. Tanaka, R.L. Nie, C.R. Yang, J. Zhou and K. Yamasaki, *Phytochemistry*, **31**, 3642 (1992).
10. C.X. Zhang, C.Z. Lin, J.Y. Yang, T.Q. Xiong, J.W. Deng, X.L. Wei, Z. X. Zhao and C.C. Zhu, *Acta Sci. Nat. Univ. Sunyatseni*, **50**, 70 (2011).