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Chemical Constituents of *Ficus drupacea* Leaves and Their α-Glucosidase Inhibitory Activities

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α-Glucosidase is a carbohydrate-hydrolyzing enzyme for digestion of polysaccharide and oligosaccharide to monosaccharides.[1] Inhibition of the enzyme decreases blood glucose levels by delaying the digestion of the poly- and oligosaccharides to absorbable monosaccharides in the small intestine.[2] Several α-glucosidase inhibitors (AGIs) are available, such as acarbose (Glucobay®) from *Actinoplane* sp.,[3] voglibose (Basen®) from *Streptomyces hygroscopicus* var. *limoneus*[4] and miglitol (Glyset®) from *S. roseochromogenus*.[5] Acarbose, when taken as directed, has been shown to effectively reduce the intestinal absorption of sugars in humans.[6] However, it is necessary to take this medication three times daily with meals and often leads to non-compliance along with a decrease in the efficacy of the drug. The main drawback of acarbose is its side-effects of abdominal distention, flatulence, meteorism, and diarrhea.[7] Therefore, AGIs from natural sources, such as plants, are more potent and may have fewer side-effects and thus may be useful tool for identifying new sources of treatment for elevated blood glucose evident in diabetes.[8]

Ficus (Moraceae) constitutes one of the largest genera of flowering plants including deciduous trees, hemi-epiphyte shrubs, and climbers occurring in tropical and subtropical regions of both hemispheres. In Vietnam, the botanists estimate that there are about 100-120 *Ficus* species.[9] *Ficus drupacea* Thund. is a giant strangler about 40 m tall with aerial roots. The leaves of *F. drupacea* are often used to treat malaria, paragonimiasis, nasosinusitis, sinusitis, and anasarca.[9] However, its chemical constituents have not been investigated thoroughly. In our screening project for α-glucosidase inhibition from natural sources, we found *F. drupacea* to possess α-glucosidase inhibitory effect with 39% at a concentration of 100 μg/mL. As part of our continuing research to elucidate the biological activities of this plant, we report the isolation, structural elucidation, and evaluation of α-glucosiase inhibitory activity of one new megastigmane, 4'-dihydrophaseate sodium, one new benzenediol glucoside, 1,4-di-*O*-β-glucopyranosyl-2-(1,1-dimethylpropenyl)benzene, along with nine known compounds from the leaves of *F. drupacea*.

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{15}H_{21}O_5Na$ by HR-ESI-MS at *m/z* 327.1197 (Calcd. for $C_{15}H_{21}O_5Na_2$, 327.1179) and 339.0985 (Calcd. for $C_{15}H_{21}O_5NaCl$, 339.0981). The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of 4'-dihydrophaseic acid[10] except for a carboxylic group replaced by carboxylate sodium group in C-1 (see Supporting information). The presence of carboxylate sodium group at C-2 in **1** was confirmed by HR-ESI-MS and by comparing ^{13}C-NMR chemical shifts of C-1 (174.9), C-2 (127.1), and C-3 (142.1) in **1** and 4'-dihydrophaseic acid[10] [δ_C values for C-1 (170.6), C-2 (120.3), and C-3 (151.0)]. In addition, a coupling constant between proton H-4 and H-5, $J_{4\text{-}5}$ = 16.0 Hz, and ROESY correlations between H-6 (δ_H 1.98) and H-2 (δ_H 5.84) and H-5 (δ_H 6.27) were observed (see Figure 3). This suggested that the *Z* and *E* configurations were at C-2/C-3 and C-4/C-5, respectively. Chemical shift of C-3' (δ_C 45.9), C-4' (δ_C 66.1), and C-5' (δ_C 44.5) in **1** confirmed the stereochemistry of the hydroxyl group at C-4' to be β configuration, based on a comparison to the corresponding data of the cucubalol[11] [δ_C values for C-3' (45.1), C-4' (65.9), and C-5' (44.9)] and 4'-dihydrophaseic acid[10] [δ_C values for C-3' (46.0), C-4' (66.1), and C-5' (44.5)] and on ROESY correlations between H_a-4' (δ_H 4.10) and H_e-3' (δ_H 1.84); H_a-4' (δ_H 4.10) and H_e-5' (δ_H 2.02) (see Figure 3). Consequently, compound **1** was elucidated to be 4'-dihydrophaseate sodium.

Compound **3** was obtained as a white amorphous powder, mp 182-185 °C and its molecular formula, $C_{23}H_{34}O_{12}$, was determined on the HR-ESI-MS at *m/z* 501.1972 $[M-H]^-$ (calcd. for $C_{23}H_{33}O_{12}$, 501.1978). The ^{1}H-NMR of **3** (in CD_3OD) (Table 1) showed the following signals: two

Table 1. NMR data for compound **3**

Pos.	δ_C[a,b]	δ_H[a,c] (mult., *J* in Hz)	HMBC (H→C)
Aglycone			
1	152.5	-	
2	138.6	-	
3	117.9	7.10 (d, 2.5)	4, 1, 2, 7
4	153.5	-	
5	116.1	6.97 (dd, 2.5, 8.5)	1, 3, 4
6	116.1	7.09 (d, 8.5)	1, 2, 4
7	41.8	-	
8	149.8	6.31 (dd, 10.5, 17.5)	2, 7, 10, 11
9	110.5	4.97 (d, 10.5) 5.01 (d, 17.5)	7, 8
10	28.1	1.50 (s)	5, 7, 8
11	27.7	1.50 (s)	5, 7, 8
1-*O*-Glc			
1'	101.6	4.95 (d, 7.5)	1, 5'
2'	75.0	3.53 (t, 7.0)	
3'	77.9	3.43*	
4'	71.3	3.43 (m)	
5'	78.4	3.51	
6'	62.6	3.74 (dd, 6.0, 12.0) 3.91 (dd, 2.5, 12.0)	4'
4-*O*-Glc			
1"	103.2	4.80 (d, 7.5)	4, 5"
2"	74.8	3.47*	
3"	77.9	3.43*	
4"	71.3	3.43 (m)	
5"	78.0	3.49*	
6"	62.5	3.74 (dd, 6.0, 12.0) 3.91 (dd, 2.5, 12.0)	4"

[a]Measured in CD_3OD. [b]125 MHz. [c]500 MHz. *Overlapped signals. Assignments were done by HMQC and HMBC experiments

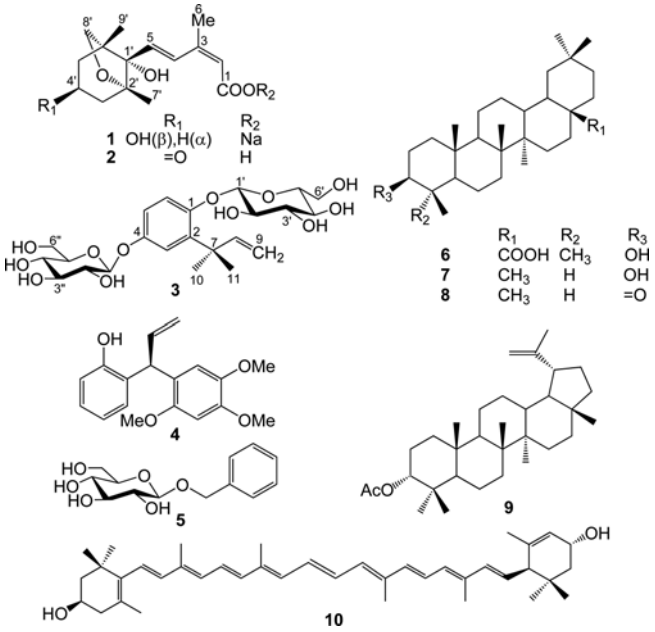

Figure 1. Structures of isolated compounds **1-10** from the leaves of *F. drupacea*.

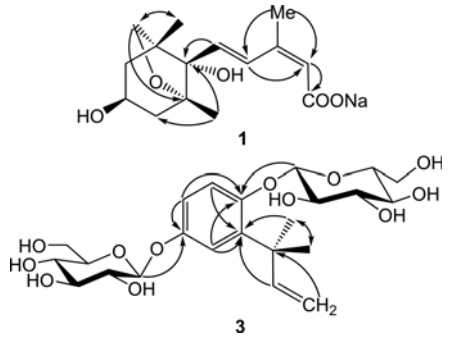

Figure 2. Important HMBC correlations of compounds **1** and **3**.

tertiary methyl groups at δ_H 1.50 (s); three olefinic protons at δ_H 4.97 (d, *J* = 10.5 Hz), 5.01 (d, *J* = 17.5 Hz), and 6.31 (dd, *J* = 10.5, 17.5 Hz); two anomeric protons at δ_H 4.80 (d, *J* = 7.5 Hz) and 4.95 (d, *J* = 7.5 Hz); and one 1,2,4-trisubstituted aromatic ring with ABX coupling patterns [δ_H 6.97 (dd, *J* = 2.5, 8.5 hz), 7.09 (d, *J* = 8.5 Hz), and 7.10 (d, *J* = 2.5 Hz)]. The ^{13}C-NMR and DEPT spectra (Table 1) revealed 23 carbon signals, of which, 6 were assigned to a benzene ring, 5 belonged to branched-chain, and 12 contributed to two sugar moieties. The ^{1}H- and ^{13}C-NMR data of **3** were similar to those of 1,3-di-*O*-β-glucopyranosyl-4-(1,1-dimethyl-propenyl)benzene except for an position on the benzene ring.[12] The HMBC correlations between tertiary methyl protons H-10, H-11 (δ_H 1.50) and C-7 (δ_C 41.8) and C-8 (δ_C 149.8); between H-9 (δ_H 4.97 and 5.01) and C-7 (δ_C 41.8) and C-8 (δ_C 149.8) suggested that branched-chain was 1,1-dimethylpropenyl (see Figure 2). This branched-chain was located at C-2 of the benzene ring by HMBC correlations between protons H-3 (δ_H 7.10), H-6 (δ_H 7.09), and H-10/H-11 (δ_H 1.50) and C-2 (δ_C 138.6). Furthermore, in the HMBC spectrum, a proton signal at δ_H 6.97 (H-5, dd, *J* = 2.5, 8.5 Hz) correlated with carbons C-1 (δ_C 152.5), C-3 (δ_C 117.9), and C-4 (δ_C 153.5); a proton signal at δ_H 7.10 (H-3, d, *J* = 2.5 Hz) correlated with carbons C-1 (δ_C 152.5), C-2 (δ_C 138.6), C-5 (δ_C 116.1), and C-7 (δ_C 41.8); and two anomeric protons at δ_H 4.80 (H-1") and 4.95 (H-1') correlated with C-4 (δ_C

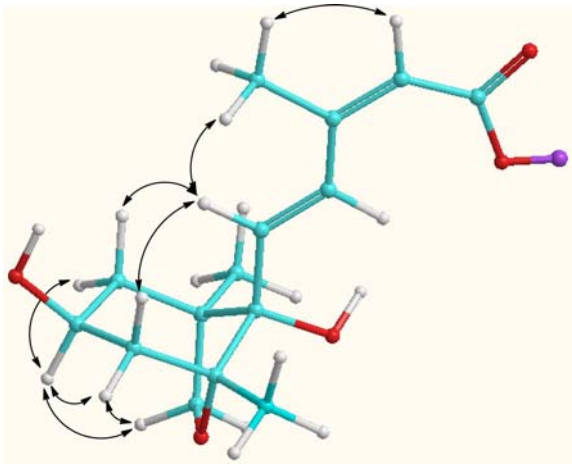

Figure 3. ROESY correlations of compound **1**.

Table 2. α-Glucosidase inhibitory activity of compounds **1-10** at 100 μM concentration

Compound	Inhibition (%)
1	NI
2	NI
3	9.8 ± 0.4
4	9.9 ± 0.1
5	8.6 ± 0.4
6	49.9 ± 0.1*
7	NI
8	20.1 ± 0.1*
9	16.1± 0.1*
10	NI
Acarbose[a]	82.5 ± 2.9

[a]Acarbose (10 μM) was used as positive control. Data presented is the mean ± SD of samples run in triplicate. *P < 0.05 compared with acarbose, NI: No inhibition.

153.5) and C-1 (δ_C 152.5), respectively, confirming that two sugar moieties were at C-1 and C-4 of the benzene ring. The presence of D-glucose was confirmed by acid hydrolysis of **3** (identified as TMS derivative, see Experimental). Consequently, the structure of compound **3** was determined to be 1,4-di-*O*-β-D-glucopyranosyl-2-(1,1-dimethylpropenyl)benzene.

The known compounds were identified as phaseic acid **2**,[13] 5-*O*-methyllatifolin **4**,[14] benzyl-*O*-β-D-glucopyranoside (**5**),[15] oleanolic acid **6**,[16] epifriedelanol **7**,[17] friedelin **8**,[17] epilupeol acetate **9**,[18] and xanthophyll **10**[19] (see Figure 1). Their structures were established on the basis of spectral and chemical evidence, which were in good agreement with those reported in the literature. These compounds were isolated from *F. drupacea* for the first time.

Compounds **1-10** were then evaluated for α-glucosidase inhibitory activity at a concentration of 100 μM. Acarbose was used as a positive control, with inhibition of 82.5% at a concentration of 10 μM. As shown in Table 2, oleanolic acid **6** showed the strongest inhibitory activity with inhibition percent of 49.9% at a concentration of 100 μM, followed by compounds **8** and **9**. All other compounds showed weak or no activity. In addition, oleanolic acid was found to simulate secretion of insulin in pancreatic β-cells.[20] Our results demonstrate that *Ficus drupacea* is a possible source of oleanolic acid, which may be useful for the treatment of diabetes mellitus.

Experimental

Plant Material. The leaves of *F. drupacea* were collected in Tam Dao, Vinh Phuc province, Vietnam in June, 2010, and identified by Dr. Ninh Khac Ban, Institute of Marine Biochemistry, VAST, Vietnam. A voucher specimen (FD1006) was deposited at the Herbarium of Institute of Marine Biochemistry.

4'-Dihydrophaseate Sodium (1): A white amorphous powder, mp 213-216 °C, $[\alpha]_D^{25}$: −22 (c = 0.5, MeOH), positive ESI-MS: *m/z* 305 $[M+H]^+$, 327 $[M+Na]^+$, negative ESI-MS: *m/z* 281 $[M-Na]^-$, $C_{15}H_{21}O_5Na$, HR-ESI-MS found *m/z* 327.1197 $[M+Na]^+$ (Calcd. $C_{15}H_{21}O_5Na_2$, 327.1179), 339.0985 $[M+Cl]^-$ (Calcd. $C_{15}H_{21}O_5NaCl$, 339.0981), ^{1}H- and ^{13}C-NMR: see Supporting information.

1,4-Di-*O*-β-D-glucopyranosyl-2-(1,1-dimethylpropenyl)-benzene (3): A white amorphous powder, mp 182-185 °C, $[\alpha]_D^{25}$: +45 (c = 0.5, MeOH), negative ESI-MS: *m/z* 501 $[M-H]^-$, $C_{23}H_{34}O_{12}$, HR-ESI-MS found *m/z* 501.1972 $[M-H]^-$ (Calcd $C_{23}H_{33}O_{12}$ for 501.1978), ^{1}H- and ^{13}C-NMR: see Table 1.

Supporting Information. General procedures, extraction, isolation, hydrolysis procedure, α-glucosidase assays, NMR, and MS spectra of **1** and **3** are available as Supporting information.

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References

1. Kakavanos, R.; Hopwood, J. J.; Lang, D.; Meikle, P. J.; Brooks, D. A. *FEBS Lett.* **2006**, *580*, 4365.
2. McCulloch, D. K.; Kurtz, A. B.; Tattersall, R. B. *Diabetes Care* **1983**, *6*, 483.
3. Shinoda, Y.; Inoue, I.; Nakano, T.; Seo, M.; Sassa, M.; Goto, S.; Awata, T.; Komoda, T.; Katayama, S. *Metabolism* **2006**, *55*, 935.
4. Chen, X.; Zheng, Y.; Shen, Y. *Curr. Med. Chem.* **2006**, *13*, 109.
5. Lee, A.; Patrick, P.; Wishart, J.; Horowitz, M.; Morley, J. E. *Diabetes Obes. Metab.* **2002**, *4*, 329.
6. Jenkins, D. J.; Taylor, R. H.; Goff, D. V.; Fielden, H.; Misiewicz, J. J.; Sarson, D. L.; Bloom, S. R.; Alberti, K. G. *Diabetes* **1981**, *30*, 951.
7. Bischoff, H.; Puls, W.; Krause, H. P.; Schutt, H.; Thomas, G. *Diabetes Res. Clin. Pract.* **1981**, *1*, 53.
8. Kwon, Y. I.; Vattem, D. A.; Shetty, K. *Asia Pac. J. Clin. Nutr.* **2006**, *15*, 107.
9. Bich, D. H.; Chung, D. Q.; Chuong, B. X.; Dong, N. T.; Dam, D. T.; Hien, P. V.; Lo, V. N.; Mai, P. D.; Man, P. K.; Nhu, D. T.; Tap, N.; Toan, T. Hanoi Science and Technology Publishing House: Hanoi, 2004; Vol. 1, p 716.
10. Zhang, Z.; Zhang, W.; Ji, Y.-P.; Zhao, Y.; Wang, C.-G.; Hu, J.-F. *Phytochemistry* **2010**, *71*, 693.
11. Cheng, Y. X.; Zhou, J.; Deng, S. M.; Tan, N. H. *Planta Med.* **2002**, *68*, 91.
12. Izumikawa, M.; Khan, S. T.; Komaki, H.; Nagai, A.; Inaba, S.; Takagi, M.; Shin-Ya, K. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 2138.
13. Hirai, N.; Kondo, S.; Ohigashi, H. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2408.
14. Sekine, N.; Ashitani, T.; Murayama, T.; Shibutani, S.; Hattori, S.; Takahashi, K. *J. Agric. Food Chem.* **2009**, *57*, 5707.
15. Ky, P. T.; Anh, P. T.; Kiem, P. V.; Minh, C. V.; Nhiem, N. X.; Tai, B. H.; Ngan, N. T. T.; Quang, T. H.; Thao, N. P.; Kim, Y. H. *Bull. Korean Chem. Soc.* **2012**, *43*, 3763.

16. Seebacher, W.; Simic, N.; Weis, R.; Saf, R.; Kunert, O. *Magn. Reson. Chem.* **2003**, *41*, 636.
17. Kiem, P. V.; Minh, C. V.; Huong, H. T.; Nam, N. H.; Lee, J. J.; Kim, Y. H. *Arch. Pharm. Res.* **2004**, *27*, 1109.
18. Lee, J. H.; Lee, K. R. *Kor. J. Pharmacogn.* **2005**, *36*, 145.
19. Barayal, M.; Molnal, P.; Szabolcs, J.; Radics, L.; Kajtar-Peredy, M. *Tetrahedron* **1981**, *37*, 203.
20. Teodoro, T.; Zhang, L.; Alexander, T.; Yue, J.; Vranic, M.; Volchuk, A. *FEBS Lett.* **2008**, *582*, 1375.
21. Li, H. L.; Song, F. R.; Xing, J. P.; Tsao, R.; Liu, Z. Q.; Liu, S. Y. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1496.