molecules

MDPI

Article

Chemical Constituents from *Flueggea virosa* and the Structural Revision of Dehydrochebulic Acid Trimethyl Ester

Chih-Hua Chao [1,2,*], Ying-Ju Lin [3,4], Ju-Chien Cheng [5], Hui-Chi Huang [6], Yung-Ju Yeh [5], Tian-Shung Wu [7,8], Syh-Yuan Hwang [9] and Yang-Chang Wu [1,2,10,11,*]

1 School of Pharmacy, China Medical University, Taichung 40402, Taiwan
2 Chinese Medicine Research and Development Center, China Medical University Hospital, Taichung 40447, Taiwan
3 School of Chinese Medicine, China Medical University, Taichung 40402, Taiwan; yjlin@mail.cmu.edu.tw
4 Genetic Center, Department of Medical Research, China Medical University Hospital, Taichung 40447, Taiwan
5 Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung 40402, Taiwan; jccheng@mail.cmu.edu.tw (J.-C.C.); star740101@gmail.com (Y.-J.Y.)
6 Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung 40402, Taiwan; hchuang@mail.cmu.edu.tw
7 Department of Pharmacy, National Cheng Kung University, Tainan 70101, Taiwan; tswu@mail.ncku.edu.tw
8 Department of Pharmacy and Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung 90741, Taiwan
9 Endemic Species Research Institute, Council of Agriculture, Nantou 55244, Taiwan; son757780@yahoo.com.tw
10 Center for Molecular Medicine, China Medical University Hospital, Taichung 40447, Taiwan
11 Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 80708, Taiwan
* Correspondence: chaochihhua@hotmail.com (C.-H.C.); yachwu@mail.cmu.edu.tw (Y.-C.W.); Tel.: +886-4-2205-3366 (ext. 5157) (C.-H.C.)

Academic Editor: Derek J. McPhee
Received: 9 August 2016; Accepted: 12 September 2016; Published: 16 September 2016

Abstract: In an attempt to study the chemical constituents from the twigs and leaves of *Flueggea virosa*, a new terpenoid, 9(10→20)-*abeo*-*ent*-podocarpane, 3β,10α-dihydroxy-12-methoxy-13-methyl-9(10→20)-*abeo*-*ent*-podocarpa-6,8,11,13-tetraene (**1**), as well as five known compounds were characterized. Their structures were elucidated on the basis of spectroscopic analysis. In addition, the structure of dehydrochebulic acid trimethyl ester was revised as (2*S*,3*R*)-4*E*-dehydrochebulic acid trimethyl ester based on a single-crystal X-ray diffraction study. The in vitro anti-hepatitis C virus (anti-HCV) activity and cytotoxicity against Huh7.5 cells for the isolated compounds were evaluated.

Keywords: *Flueggea virosa*; dehydrochebulic acid trimethyl ester; *ent*-podocarpane; anti-HCV; HCVcc

1. Introduction

Flueggea virosa (Roxb. ex Wild) Baill, belonging to the Euphorbiaceae family, is a common medicinal plant in Africa and China [1,2]. In previous reports, *Securinega* alkaloids have been widely regarded as a representative group of the genus *Flueggea* [3], formerly classified as *Securinega* genus. However, our prior studies have demonstrated that the roots of *F. virosa* contained a series of non-alkaloids, including *ent*-podocarpanes [4,5], 9(10→20)-*abeo*-*ent*-podocarpanes [6], 3,4-*seco*-*ent*-podocarpanes [7], and 3,4-*seco*-30-*nor*-friedelanes [6], some of which were endowed with anti-HCV activities and weak toxicities. Among them, 4-hydroxy-12-methoxy-13-methyl-3,4-*seco*-*ent*-podocarpa-6,8,11,13-tetraen-3-oic acid was found to be a brand new type anti-HCV

agent [7]. The non-alkaloids in the other parts of this plant still received less attention. Thus, the isolation of active constituents from the twigs and leaves of *F. virosa* was carried out, which resulted in the isolation of a new 9(10→20)-*abeo-ent*-podocarpane, namely 3β,10α-dihydroxy-12-methoxy-13-methyl-9(10→20)-*abeo-ent*-podocarpa-6,8,11,13-tetraene (**1**), alone with five known compounds. In addition, the structural revision of dehydrochebulic acid trimethyl ester (Chart 1, original **2**) was also reported in this study.

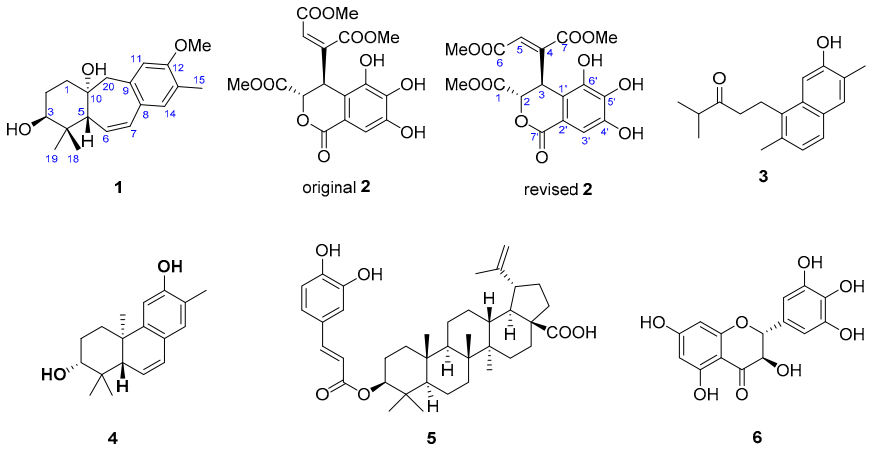

Chart 1. Structures of compounds **1**–**6**.

2. Results and Discussion

The MeOH extract from the twigs and leaves of *F. virosa was* concentrated and the alkaloids were removed by partition with acidic water. The resulting nonalkaloid layer was separated repeatedly by column chromatography to afford a new 9(10→20)-*abeo-ent*-podocarpane (**1**) and five known compounds, which were identified as 4*E*-dehydrochebulic acid trimethyl ester (**2**) [8,9], 12-hydroxy-20(10→5)-*abeo*-4,5-*seco*-podocarpa-5(10),6,8,11,13-pentaen-3-one (**3**) [10], 3β,12-dihydroxy-13-methylpodocarpa-6,8,11,13-tetraene (**4**) [10], betulinic acid 3β-calfeate (**5**) [11], and (+) ampelosin E (**6**) [12].

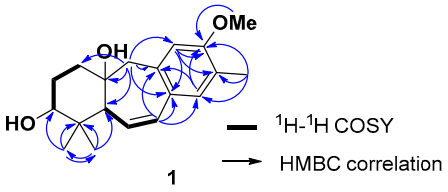

Figure 1. Selected ^{1}H−^{1}H COSY (▬) and HMBC (→) correlations of **1**.

Compound **1** was obtained as a colorless oil and a molecular formula of $C_{19}H_{26}O_3$ was deduced for this compound based on the molecular ion peak $[M - H]^-$ at m/z 301.1805 in the (−)-HR-APCI-MS (calcd for $C_{19}H_{25}O_3$, 301.1809). Inspection of the overall ^{1}H- and ^{13}C-NMR data revealed that it is a member of 9(10→20)-*abeo-ent*-podocarpanes [6], with slight chemical shift differences for resonances of substituent patterns. The 12-methoxy group was readily assigned from its proton chemical shift at δ_H 3.82 (s), while the 6,7-double bond was deduced according to the proton chemical shifts at δ_H 6.58 (1H, dd, J = 12.1, 2.6 Hz) and 5.78 (1H, dd, J = 12.1, 4.4 Hz) (Table 1) [6]. The above assignment was confirmed by the interpretation of ^{1}H–^{1}H COSY and HMBC correlations (Figure 1). The 3β-OH was determined based on the chemical shift and coupling patterns of H-3 [δ_H 3.51 (1H, t, J = 2.8 Hz)]. The relative configuration of **1** was further confirmed by the analysis of NOE correlations (Figure 2). The NOE correlations of H-5/H-20a, H-5/H-1a, and H-5/H_3-18 confirmed that **1**

possessed the same relative configurations (10α-OH, 5β-H) as those of 9(10→20)-*abeo*-*ent*-podocarpanes isolated previously from the roots of *F. virosa* [6] Accordingly, the structure of **1** was determined as 3β,10α-dihydroxy-12-methoxy-13-methyl-9(10→20)-*abeo*-*ent*-podocarpa-6,8,11,13-tetraene.

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (400 and 100 MHz, resp.; $CDCl_3$) of **1**.

	1		
	δ_H (*J* in Hz)	**δ_C (Mult.)**	**HMBC**
1	2.05 td (14.1, 4.4) 1.61 m	33.0 (CH_2)	2, 3, 20
2	2.27 m 1.62 m	25.5 (CH_2)	1
3	3.51 t (2.8)	75.6 (CH)	1, 5
4		38.2 (C)	
5	2.45 dd (4.4, 2.6)	49.6 (CH)	4, 6, 7, 10, 18, 19
6	5.78 dd (12.1, 4.4)	128.0 (CH)	4, 5, 8, 10
7	6.58 dd (12.1, 2.6)	131.3 (CH)	5, 9, 14
8		128.4 (C)	
9		135.2 (C)	
10		76.4 (C)	
11	6.56 s	112.2 (CH)	8, 12, 13, 20
12		156.9 (C)	
13		124.4 (C)	
14	6.95 s	132.4 (CH)	7, 9, 12
15	2.17 s	15.6 (CH_3)	12, 13, 14
18	1.00 s	27.2 (CH_3)	3, 4, 5, 19
19	1.09 s	22.4 (CH_3)	3, 4, 5, 18
20	2.96 d (14.2) 2.68 d (14.2)	51.6 (CH_2)	1, 5, 8, 9, 10, 11
12-OMe	3.82 s	55.3 (CH_3)	12

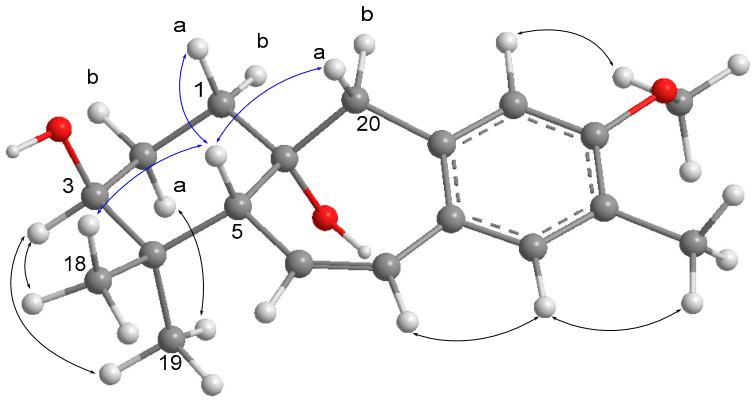

Figure 2. Selected NOE correlations of **1**.

The structure of dehydrochebulic acid trimethyl ester (original **2**, Chart 1) with a *cis*-methylbutenedioate scaffold was reported from *Phyllanthus urinaria* by Yao et al. in 1993 [9]. However, the authors proposed the wrong geometry for the methylbutenedioate moiety because of failure to compare the NMR data for this moiety with those reported in the literature. The ^{1}H- and ^{13}C-NMR data of **2** measured in acetone-d_6 and pyridine-d_6 were in good agreement with those reported in the literature [8,9]. In the selective 1D NOESY experiment (Figure 3), irradiation of olefinic H-5 did not show enhancements with other signals, suggesting that the 4,5-double bond in **2** has a *trans* geometry. A single-crystal X-ray analysis was performed on **2** (Figure 4), which led the absolute configurations to be defined as 2*S*,3*R* according to the value of Flack parameter 0.09 (14),

and confirmed the geometry of a 4*E*-double bond. Accordingly, the structure of **2** was revised as (2*S*,3*R*)-4*E*-dehydrochebulic acid trimethyl ester (Chart 1, revised **2**).

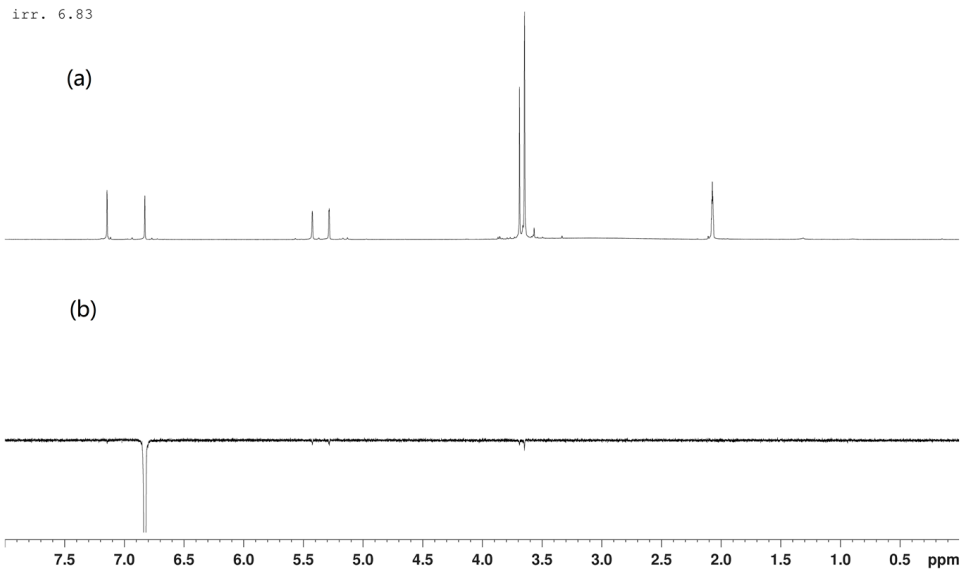

Figure 3. (**a**) ^{1}H-NMR spectrum of **2** measured in acetone-d_6 and (**b**) selective 1D NOESY spectrum of **2**: selective irradiation of H-5 (δ_H = 6.83 ppm) using *selnogp.3* Bruker program (parameter set: ns 128, p12 = 80 ms, and d8 = 400 ms).

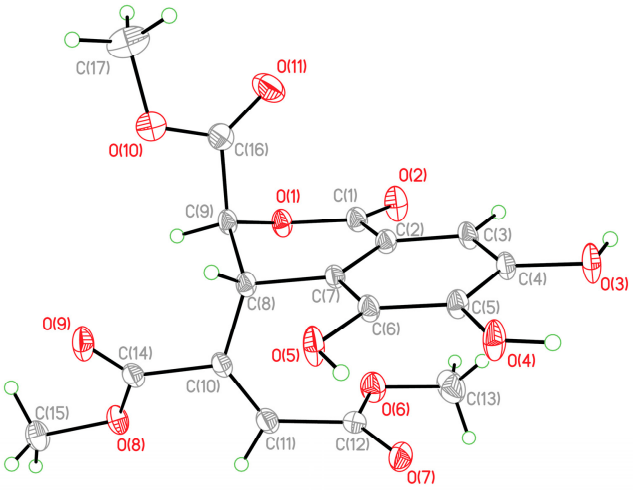

Figure 4. X-ray ORTEP drawing of **2**.

In order to continue our exploration for anti-HCV agents from a natural source, the isolated compounds were subjected to anti-HCV and cytotoxic evaluation. As shown in Table 2, the anti-HCV activity of compounds **1**–**6** was assayed using the cell-based HCV cell culture (HCVcc) infection system, while the toxicity toward human hepatoma Huh7.5 cells was also measured using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt (MTS) assay. In addition, the therapeutic indices (TI = IC_{50}/EC_{50}) were also calculated to estimate their potency as anti-HCV agents. The result showed that compounds **3**–**5** possessed better potencies than honokiol (TI = 2.1) [13], a natural anti-HCV agent from *Magnolia officinalis* [14], with TI values of 6.8, 9.2, and 2.9, respectively.

Table 2. Anti-HCV activities (EC_{50}) and cytotoxicity (IC_{50}) of **1**–**6**.

Compound	EC_{50} (μM) [a]	IC_{50} (μM) [b]	(TI) [c]
1	27.4 ± 1.4	>100	- [d]
2	98.4 ± 2.1	>100	- [d]
3	12.8 ± 3.1	87.1 ± 5.1	6.8
4	7.7 ± 2.7	70.5 ± 4.2	9.2
5	20.8 ± 2.0	60.7 ± 2.0	2.9
6	66.7 ± 1.8	>100	- [d]
honokiol	22.4 ± 0.5	48.8 ± 0.8	2.1

[a] EC_{50}: concentration that inhibits HCVcc infection by 50%. [b] IC_{50}: concentration that inhibits cell growth by 50%. [c] Therapeutic index (TI) = IC_{50}/EC_{50}. [d] not calculated.

3. Experimental Section

3.1. General Experimental Procedures

Melting point and optical rotation data were recorded on a Yanako MP-500P micro melting point apparatus (Yanaco, Kyoto, Japan) and a JASCO P2000 digital polarimeter (JASCO Corporation, Tokyo, Japan), respectively. IR spectra were recorded as a thin film on a KBr plate or using a conventional KBr pellet on a Shimadzu IR Prestige21 FT-IR spectrometer (Shimadzu, Milan, Italy). UV data were obtained using a Shimadzu UV-1700 UV/Vis spectrometer (Shimadzu). NMR spectra (400 MHz) were recorded using a Bruker Avance 400 spectrometer equipped with a 5-mm Dual *z*-gradient probe $^{1}H/^{13}C$ (Bruker, Rheinstetten, Germany). HR-APCI-MS and HR-ESI-MS were measured with an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific Corp., Waltham, MA, USA). Column chromatography (CC) was performed on silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) and RP-18 gel (230–400 mesh, SiliaBond C18, SiliCycle, Quebec City, QC, Canada). Silica gel plates (Kieselgel 60 F254, 0.25 mm, Merck) were used for thin-layer chromatography (TLC) analysis, and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating.

3.2. Plant Material

Twigs and leaves of *F. virosa* were collected in North Pingtung, Taiwan, in August 2013. Botanical identification was performed by Dr. S.-Y. Hwang. A voucher specimen (chao-002) was deposited in the School of Pharmacy, China Medical University.

3.3. Extraction and Isolation

The twigs and leaves of *F. virosa* (35 kg) were crushed and extracted exhaustively with MeOH (5 × 40 L). The organic extract was concentrated to an aqueous suspension and was further partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract was washed with 3% aqueous tartaric acid three times to remove alkaloids. The resulting nonalkaloid extract (420 g) was fractionated by open column chromatography on silica gel using hexane/EtOAc and EtOAc/MeOH mixtures of increasing polarity to yield 14 fractions. Fraction 9, eluted with EtOAc/MeOH (9:1), was repeatedly separated by RP-18 column chromatography with gradient elution (MeOH/H_2O, 5% to 40%) to yield **2** (200.2 mg) and **6** (12.1 mg). Fraction 7 was fractionated by silica gel column chromatography using gradient elution (hexane/EtOAc, 100:0 to 53:47) to afford 20 subfractions (7A to 7T). Compounds **1** (100.2 mg), **4** (266.1 mg), **3** (19.1 mg), and **5** (80.0 mg) were obtained from fraction 7L by repeated column chromatography over silica gel with (hexane/EtOAc, 100:0 to 60:40).

3β,10α-Dihydroxy-12-methoxy-13-methyl-9(10→20)-abeo-ent-podocarpa-6,8,11,13-tetraene (**1**): pale yellow oil; $[\alpha]_D^{25}$ +139 (*c* 1.65, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.23), 261 (3.94) nm; IR (neat) υ_{max} 3444, 3003, 2953, 2931, 2870, 1610, 1568, 1504, 1446, 1435, 1384, 1336, 1315, 1257, 1217, 1114, 1097, 1045, 1020,

and 981 cm^{-1}; for ^{1}H- and ^{13}C-NMR data in $CDCl_3$, see Table 2; (−)-APCI-MS *m/z* 301 $[M - H]^-$; (−)-HR-APCI-MS *m/z* 301.1805 $[M - H]^-$ (calcd for $C_{19}H_{25}O_3$, 301.1809).

(2S,3R)-4E-Dehydrochebulic acid trimethyl ester (**2**): colorless crystal; mp 216–218 °C (MeOH), $[\alpha]_D^{25}$ +15 (*c* 28.45, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.47), 287 (3.97) nm; IR (KBr) υ_{max} 3394, 3257, 1761, 1752, 1730, 1708, 1649 1622, 1608, 1537, 1494, 1431, 1390, 1369, 1352, 1309, 1278, 1244, 1209, 1180, 1114, 1066, and 1006 cm^{-1}.

3.4. Crystallographic Data of **2**

A colorless crystal of **2** with sizes of 0.62 × 0.50 × 0.47 mm^3 was obtained at room temperature by slow evaporation in MeOH solution. Diffraction intensity data were acquired with a CCD area detector with graphite-monochromated CuKα radiation (λ = 1.54178 Å). Crystal data for **2**: $C_{17}H_{16}O_{11}$, M = 396.30, orthorhombic, a = 7.1567(2) Å, b = 9.7147(3) Å, c = 25.1934(7) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 1751.58(9) $Å^3$, T = 150(2) K, space group $P2_12_12_1$, Z = 4, μ(CuKα) = 1.115 mm^{-1}, 6642 reflections collected, 3372 independent reflections (R_{int} = 0.0199). The final R_1 values were 0.0298 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0787 ($I > 2\sigma(I)$). The final R_1 values were 0.0315 (all data). The final $wR(F^2)$ values were 0.0800 (all data). The goodness of fit on F^2 was 1.028. Flack parameter = 0.09(14). Crystallographic data for this compound have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 1484756) (Supplementary Materials). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.5. Infection Inhibition Assay

The JC1-Luc2A HCV reporter viral particles were prepared as described previously [15,16]. In brief, in vitro-transcribed genomic JC1-Luc2A RNA was delivered into Huh7.5.1 cells by electroporation. The virus-containing supernatant was collected for 4 days, clarified by low-speed centrifugation, passed through a filter with a pore size of 0.45 μm, and quantitated by fluorescent focus assay. The MTS assay was used to determine the cell viability, while HCV infection inhibition assay was performed according to the published protocol in [6,15]. Huh7.5.1 cells were seeded into the 96-well culture plates at a cell density of 1 × 10^4 cells/well for 24 h followed by incubation with HCV reporter virus (with 0.1 MOI) for an additional 4 h. After removing the virus containing supernatant, the cells were washed 3 times with PBS and then replaced with medium containing indicated compound for 72 h. The luciferase activity assay was performed by the collected cell lysates. Honokiol, a known natural product with anti-HCV activity, was used as an inhibition positive control.

4. Conclusions

A new 9(10→20)-*abeo*-*ent*-podocarpane (**1**) and five known compounds were isolated from the twigs and leaves of *F. virosa*. The structures of the isolates were identified on the basis of NMR, MS, and IR spectroscopic data. The structure 4*E*-dehydrochebulic acid trimethyl ester was revised and its absolute configuration was determined for the first time. Comparing the present study with our prior investigation [4–7], it was found that the diversity in chemical constitutions of the roots were quite different from those of the twigs and leaves of *F. virosa*. Furthermore, 3β,12-dihydroxy-13-methylpodocarpa-6,8,11,13-tetraene (**4**) was found to be abundant in the twigs and leaves; however, the 12-*O*-methyl analogue of **4** was isolated as the main constituent in the roots [4]. Although the 9(10→20)-*abeo*-*ent*-podocarpane skeleton had been reported from the roots of this plant [6], it was still rare in a natural source. The present anti-HCV evaluation suggested that the *ent*-podocarpane derivatives might be the active ingredients in this plant.

Supplementary Materials: Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/21/9/1239/s1.

Acknowledgments: The authors are grateful for financial support from the Ministry of Science and Technology (MOST102-2113-M-039-001, MOST103-2320-B-039-004-MY3, and MOST104-2911-I-002-302). This study was also supported in part from the grant of Chinese Medicine Research Center, China Medical University (the Ministry of Education, the Aim for the Top University Plan).

Author Contributions: Chih-Hua Chao and Yang-Chang Wu conceived and designed the experiments; Ying-Ju Lin, Yung-Ju Yeh, and Ju-Chien Cheng performed the biological assay; Hui-Chi Huang and Tian-Shung Wu analyzed the data; Syh-Yuan Hwang contributed to the collection and species identification.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kamatenesi-Mugisha, M.; Oryem-Origa, H. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. *Afr. Health Sci.* **2005**, *5*, 40–49. [PubMed]
2. Chen, S.-K.; Chen, B.-Y.; Li, H. *Flora of China*; Science Press: Beijing, China, 1997; Volume 44, pp. 68–71.
3. Wu, Z.-L.; Zhao, B.-X.; Huang, X.-J.; Tang, G.-Y.; Shi, L.; Jiang, R.-W.; Liu, X.; Wang, Y.; Ye, W.-C. Suffrutines A and B: a pair of *Z*/*E* isomeric indolizidine alkaloids from the roots of *Flueggea suffruticosa*. *Angew. Chem. Int. Ed.* **2014**, *53*, 5796–5799. [CrossRef] [PubMed]
4. Chao, C.-H.; Cheng, J.-C.; Shen, D.-Y.; Wu, T.-S. Anti-hepatitis C virus dinorditerpenes from the roots of *Flueggea virosa*. *J. Nat. Prod.* **2014**, *77*, 22–28. [CrossRef] [PubMed]
5. Chao, C.-H.; Cheng, J.-C.; Hwang, T.-L.; Shen, D.-Y.; Wu, T.-S. Trinorditerpenes from the roots of *Flueggea virosa*. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 447–449. [CrossRef] [PubMed]
6. Chao, C.-H.; Cheng, J.-C.; Shen, D.-Y.; Huang, H.-C.; Wu, Y.-C.; Wu, T.-S. Terpenoids from *Flueggea virosa* and their anti-hepatitis C virus activity. *Phytochemistry* **2016**, *128*, 60–70. [CrossRef] [PubMed]
7. Chao, C.-H.; Cheng, J.-C.; Shen, D.-Y.; Huang, H.-C.; Wu, Y.-C.; Wu, T.-S. 13-Methyl-3,4-*seco*-*ent*-podocarpanes, rare C18-diterpenoids from the roots of *Flueggea virosa*. *RSC Adv.* **2016**, *6*, 34708–34714. [CrossRef]
8. Camacho-Luis, A.; Gayosso-De-Lucio, J.A.; Torres-Valencia, J.M.; Munoz-Sanchez, J.L.; Alarcon-Hernandez, E.; Lopez, R.; Barron, B.L. Antioxidant Constituents of *Geranium bellum* Rose. *J. Mex. Chem. Soc.* **2008**, *52*, 103–107.
9. Yao, Q.-Q.; Zuo, C.-X. Chemical studied on the constituents of *Phyllanthus urinaria* L. *Acta Pharm. Sin.* **1993**, *28*, 829–835. [CrossRef] [PubMed]
10. Yuan, W.; Lu, Z.; Liu, Y.; Meng, C.; Cheng, K.-D.; Zhu, P. Three new podocarpane-type diterpenoids from callus of *Securinega suffruticosa*. *Chem. Pharm. Bull.* **2005**, *53*, 1610–1612. [CrossRef] [PubMed]
11. Pan, H.; Lundgren, L.N.; Andersson, R. Triterpene caffeates from bark of *Betula pubescens*. *Phytochemsitry* **1994**, *37*, 795–799. [CrossRef]
12. Yang, J.-H.; Lu, H.; Huang, R.; Wang, Y.S. Flavonoids from leaves and twigs of *Stachyurus himalaicus* var. *himalaicus*. *Chem. Nat. Compd.* **2011**, *47*, 112–113. [CrossRef]
13. Lan, K.-H.; Wang, Y.-W.; Lee, W.-P.; Lan, K.-L.; Tseng, S.-H.; Hung, L.-R.; Yen, S.-H.; Lin, H.-C.; Lee, S.-D. Multiple effects of Honokiol on the life cycle of hepatitis C virus. *Liver Int.* **2012**, *32*, 989–997. [CrossRef] [PubMed]
14. Shih, H.-C.; Hwang, T.-L.; Chen, H.-C.; Kuo, P.-C.; Lee, E.-J.; Lee, K.-H.; Wu, T.-S. Honokiol dimers and magnolol derivatives with new carbon skeletons from the roots of *Magnolia officinalis* and their inhibitory effects on superoxide anion generation and elastase release. *PLoS ONE* **2013**, *8*, e59502. [CrossRef] [PubMed]
15. Huang, J.-T.; Tseng, C.-P.; Liao, M.-H.; Lu, S.-C.; Yeh, W.-Z.; Sakamoto, N.; Chen, C.-M.; Cheng, J.-C. Hepatitis C virus replication is modulated by the interaction of nonstructural protein NS5B and fatty acid synthase. *J. Virol.* **2013**, *87*, 4994–5004. [CrossRef] [PubMed]
16. Blight, K.J.; McKeating, J.A.; Rice, C.M. Highly permissive cell lines for subgenomic and genomic hepatitis C virus RNA replication. *J. Virol.* **2002**, *76*, 13001–13014. [CrossRef] [PubMed]

Sample Availability: Samples of the compounds **1** and **2** are available from the authors.