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12β-Acetyloxyperforatin, a New Limonoid from *Harrisonia Perforata*

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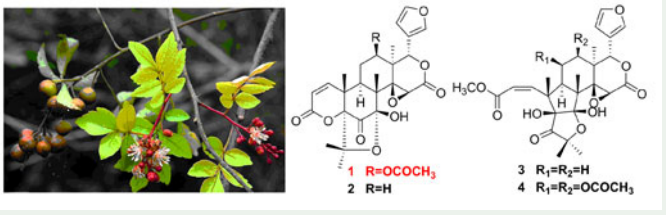
ABSTRACT

A new A, D-seco limonoid, named 12-acetyloxyperforatin (**1**), along with three known ones, were isolated from the leaves of *Harrisonia perforata*. Their structures were elucidated on the basis of spectroscopic analysis, including extensive NMR techniques and computational modelling. These compounds showed no inhibitory activity against the 11β-HSD1 enzyme.



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1. Introduction

Limonoids with diverse structures and significant bioactivities are found mainly within the Meliaceae, Rutaceae, Cneoraceae, and the Harrisonia genera of Simaroubaceae (Fang X. et al. 2011; Tan and Luo 2011). *Harrisonia perforata* is the only species of this genus grown in China, and its root and leaves have been applied in Chinese folk medicine for the treatment of wound healing and malaria(Wang M et al. 1983; Huong D-T-T et al. 2000). Previous chemical investigations of its leaves and branches led to isolation of quassinoids (Kamiuchi et al. 1996), chromones (Wang M et al. 1983; Wang M et al. 1984; Wei et al. 1985; Tanaka et al. 1995; Tuntiwachwuttikul et al. 2006), polyketides (Yin et al. 2009), and limonoids of tremendous structural diversity (Kamiuchi et al. 1996; Khuong-Huu et al. 2000, 2001; Rugutt et al. 2001). Recently, unprecedented

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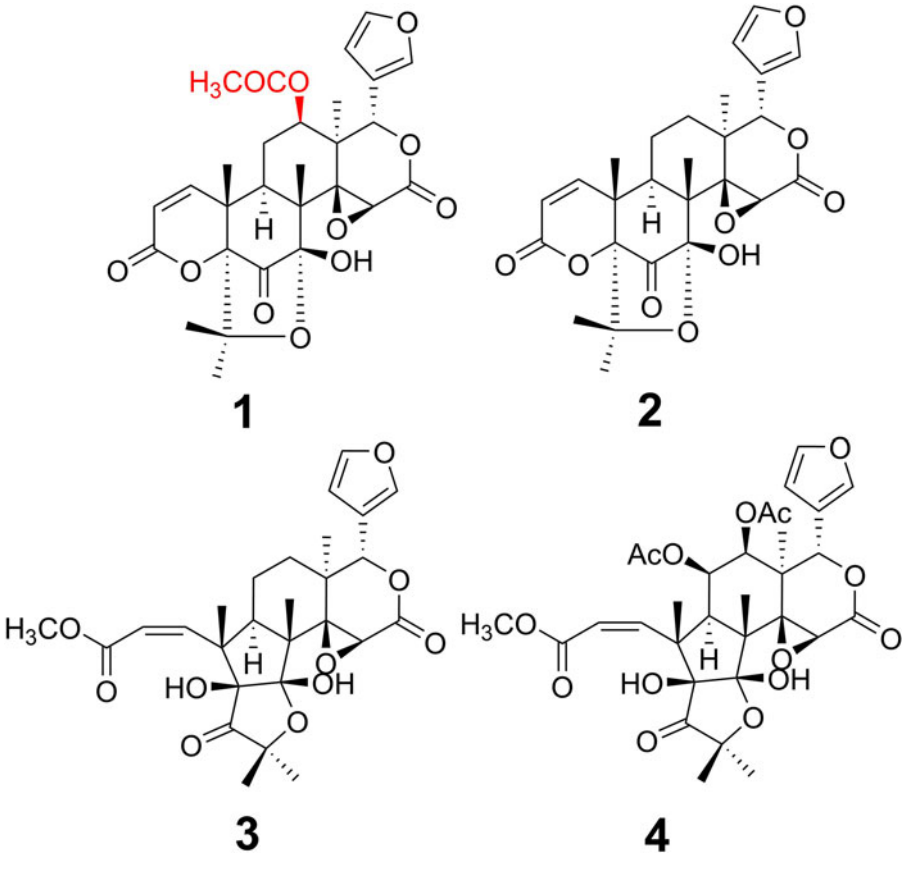

Figure 1. Structures of compounds **1–4**

quassinoids and limonoid derivatives with notable biological properties have been discovered and evaluated by our group (Yan et al. 2011; Fang Xin et al. 2015; Lv et al. 2016; Yan et al. 2016). As part of our continuous effort to search for bioactive natural products (Chen et al. 2009; Yan et al. 2010; Wang S et al. 2013; Li et al. 2016; Yuan et al. 2017), one new A, D-seco limonoid, 12β-Acetyloxyperforatin (**1**), with rare 6/6/6/6/5 ring system, as well as three known ones, perforatin (**2**), harrisonin (**3**) and haperforine A (**4**), were isolated from the leaves of the title plant (Figure 1). Herein, the isolation, structural elucidation, and inhibitory effects of **1**-**4** on 11β-HSD1 are described.

2. Results and Discussion

2.1. Chemistry

12-acetyloxyperforatin (**1**) was isolated as colorless crystals (MeOH). The HRESIMS data of this compound exhibited a sodium adduct ion $[M + Na]^+$ at *m/z* 565.1696 (calcd for 565.1685) corresponding to a molecular formula of $C_{28}H_{30}O_{11}$, corresponding to 14 degrees of unsaturation. The IR absorption bands at 3433, 1796, 1744 and 1628 cm^{-1} indicated the presence of hydroxyl, carbonyls and double bonds. The ^{13}C NMR and DEPT spectra of **1** (Table S1) showed 28 well-resolved resonances, including sp^3

carbons (7 quaternary carbons, 4 methines, 1 methylene, and 6 tertiary methyls), and sp^2 carbons (5 methines and 5 quaternary carbons). Among them, the carbon resonances at δ_C 201.6, δ_C 169.9, and δ_C 167.0 were assigned to one ketonic carbonyl and two ester carbonyls, respectively. Two sp^3 quaternary carbons (δ_C 88.7, δ_C 80.6) and two methine carbons (δ_C 74.5, δ_C 71.2) were ascribed to those bearing an oxygen atom, while the sp^3 quaternary carbon at δ_C 99.3 was attributed to a hemiketal carbon bearing two oxygen atoms. Analysis of the ^1H and ^{13}C NMR spectroscopic data further indicated the presence of one trisubstituted epoxy moiety (δ_H 4.69 (s), δ_C 56.1, C-15; δ_C 68.5, C-14), one α,β-unsaturated ester moiety (δ_H 6.89 (d, 10.0), δ_C 149.5, C-1; 5.91 (d, 10.0), δ_C 119.2, C-2; δ_C 159.8, C-3), and one characteristic β-substituted furyl group [δ_H 7.42 (brs), δ_C 119.4, C-20; δ_H 7.36 (brs), δ_C 141.4, C-21; δ_H 6.14 (brs), δ_C 108.9, C-22; and δ_H 7.42 (brs), δ_C 143.8, C-23]. The epoxy moiety, three double bonds, and four carbonyls accounted for eight of the 14 of unsaturation, and the remaining six of unsaturation indicated that compound **1** was hexacyclic. The above-mentioned spectroscopic features and comparison to known compounds implied that **1** might has a partial structure, including the A, B, E and furan rings, similar to compound **2** (Byrne et al. 1991) .

The planar structure of **1** was confirmed by the HMBC NMR spectrum. HMBC correlations (Figure S1) of Me-18/C-12, C-13, C-14 and C-17, Me-30/C-7, C-8, C-9, and C-14, and H-15/C-13, C-14, and C-16 showed a six-membered ring C connected to ring D at C-13 and C-14. Cross-peaks between H-12 and the acetyl carbonyl signal (δ_C 169.9) in the HMBC spectrum were used to place the acetoxy group at C-12. Therefore, the planar structure of compound **1** was established as shown.

The relative configuration of **1** was constructed from the analysis of ROESY correlations and the energy minimized molecular models using density functional theory (DFT) at 3-21G* basis set level (Figure S1). As shown in the ROE data ($CDCl_3$), the correlations of H-9/H-11a, H-9/Me-18, H-9/Me-28 indicated that H-9, H-11a, and Me-18, and Me-28 were cofacial, arbitrarily assigned as the α-oriented. The ROE correlations of H-11b/Me-19, H-11b/Me-30, and Me-30/7-OH suggested that H-11b, Me-19, Me-30 and 7-OH were α-oriented. Moreover, 12-OAc and H-17 took α-orientation due to the ROE correlations of Me-18/H-12 and Me-18/H-22. Although ROESY correlations of 7-OH/H-15 were unambiguous, it is not sufficient to determine the orientation of C-14/C-15 epoxy ring (Zhang et al. 2009; Ma et al. 2018). Therefore, molecular modeling was performed for the two possible structures of **1**, corresponding to the β (**A**) or α (**B**) orientation of the C-14/C-15 epoxy as shown in Figure S2. Two optimized structures were obtained, in which the calculated distance of the proton pairs near the epoxide oxygen of **B** was fully consistent with the corresponding ROESY data. Therefore, the orientation of the C-14/C-15 epoxy ring was determined to be β.

The structures of three known compounds isolated from *Harrisonia perforata* were identified as Perforatin (**2**) (Byrne et al. 1991), Harrisonin (**3**) (Kubo et al. 1976) and Haperforine A (**4**) (Khuong-Huu et al. 2000). Their structures were established using NMR spectral method and their spectral data were compared to previous literature values.

2.2. Biological activity

The inhibitory activity of compounds **1**–**4** on murine and human 11β-HSD1 were evaluated using the scintillation proximity assay (SPA) (Yu et al. 2012). In intact CHOP cells transfected with murine *HSD11B1*, compounds **1–4** showed no inhibitory effects (an $IC_{50} > 40$ μM).

3. Experimental

3.1. General experiment procedures

^{1}H and ^{13}C NMR, Bruker AM-400 spectrometer; 2D NMR, Bruker DRX-500 instrument; ESIMS and HRESIMS, VG ZABHS and Auto Spec-3000 spectrometers, respectively; Optical rotations, Perkin-Elmer model 241 polarimeter; IR, Bio-Rad FTS - 135 spectrometer; Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Waters X-Bridge Prep Shield RP18 (10 × 150 mm) column. CC was performed on silica gel (90 - 150 μm; Qingdao Marine Chemical Inc. Qingdao, China). Precoated silica gel GF_{254} and HF_{254} plates (Qingdao Marine Chemical Inc. Qingdao, China) were used for thin-layer chromatography (TLC).

3.2. Plant material

Leaves of *H. perforata* were collected from the Hainan province of China in July 2014, and were authenticated by Dr. Hao-Fu Dai of the Chinese Academy of Tropical Agricultural. A voucher specimen (accession number KIB-20140702) was deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and Isolation

The air-dried leaves of *H. perforata* (5.0 kg) were powdered and extracted exhaustively by maceration with MeOH at room temperature. The extract solution was concentrated under diminished pressure to afford 110 g of dried extract. The extracts were then suspended in H_2O, with one latter extracted successively with petroleum ether, and 5L EtOAc at 60 °C. The EtOAc extract was next subjected to silica gel CC and eluted with petroleum ether/Me_2CO (from 1:0 to 0:1) to give 10 fractions (A1–A10). Fraction A3 (petroleum ether/Me_2CO 5:1, 5 g) was further fractionated via MCI gel CC and eluted with a MeOH/H_2O gradient from 5:5 to 9:1 to obtain five fractions (B1–B5). Fraction B2 (1.5 g) was first subjected to Sephadex LH-20 CC to afford **1** (50 mg) and **2** (300 mg), and further separated by semi-preparative HPLC on a Waters X-Bridge Prep Shield RP18 column (MeOH/H2O, 20:80 → 100:0, v/v, 3mL/min) afford to 3 (135 mg) and 4 (80 mg). The purities of compounds **1–4** were 95%, as determined by TLC and HPLC.

3.3.1. 12β-Acetyloxyperforatin (1)

Colorless crystals (MeOH); mp: 242–244 °C; $C_{28}H_{30}O_{11}$; $[\alpha]_D^{26} = -109.2°$ (c = 0.06, MeOH); IR: ν_{max}(KBr) cm^{-1}: 3433, 1796, 1744, 1710, 1628, 1231, 1027 cm^{-1}; ^{1}H NMR

(400 MHz, $CDCl_3$) and ^{13}C NMR data for detail see table 1; ESIMS: *m/z* 543 $[M + H]^+$, 565 $[M + Na]^+$; HRESIMS: *m/z* 565.1696 $[M + Na]^+$, (calcd for 565.1685).

3.4. 11β-HSD enzyme activity assay

The inhibitory activities of the compounds on human or mouse 11β-HSD1 and 11β-HSD2 were determined using the scintillation proximity assay (SPA). The full-length cDNAs of human or murine11β-HSD1 and 11β-HSD2 were isolated from the cDNA libraries provided by NIH Mammalian Gene Collection. The cDNAs were cloned into pcDNA3 expression vectors. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in 5 the presence of 700 μg/ml of G418. The microsomal fraction overexpressing 11β-HSD1 or 11β-HSD2 was prepared from the HEK-293 cells, which were stably transfected with 11β-HSD1 or 11β-HSD2. The fraction was then used as the enzyme source for SPA. Microsomes containing human or mouse 11β-HSD1 were incubated with NADPH and [3H] cortisone. The product, [3H] cortisol, was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. The 11β-HSD2 screening was performed by incubating 11β-HSD2 microsomes with [3H] cortisol and NAD^+ and monitoring substrate disappearance. All tests were done twice with glycyrrhizinic acid as a positive control. IC_{50} (X + SD, n = 2) values were calculated by using Prism Version 4 (GraphPad Software, SanDiego, CA).

3.5. Computation Methodology

The Gaussian 03 package was used in the computations of two isomers of **1** with different configuraton of epoxy ring. Hartree-Fork (HF) theory was applied to search for the low energy conformations at the 3-21G* level. The obtained geometries were further optimized at the B3LYP/6-31G* level of theory in the gas phase.

4. Conclusion

In conclusion, a new A, D-seco limonoid, named 12-acetyloxyperforatin (**1**), along with three known ones, were isolated from the leaves of *Harrisonia perforata*. Their structures were elucidated on the basis of spectroscopic analysis, including extensive NMR techniques and computational modelling.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Byrne LT, Mai VT, Nguyen MP, Sargent MV, Skelton BW, White AH. 1991. Perforatin: a novel tetranortriterpenoid from *Harrisonia perforata*. Aust J Chem. 44(1):165–169.

Chen J, Yan XH, Dong JH, Sang P, Fang X, Di YT, Zhang ZK, Hao XJ. 2009. Tobacco Mosaic Virus (TMV) Inhibitors from *Picrasma quassioides* Benn. J Agric Food Chem. 57(15):6590–6595.

Fang X, Di YT, Hao XJ. 2011. The Advances in the Limonoid Chemistry of the Meliaceae Family. Current Organic Chemistry. 15(9):1363–1391.

Fang X, Di YT, Zhang Y, Xu ZP, Lu Y, Chen QQ, Zheng QT, Hao XJ. 2015. Unprecedented quassinoids with promising biological activity from *Harrisonia perforata*. Angew. Chem. Int. Ed. 54(19):5592–5595.

Kamiuchi K, Mitsunaga K, Koike K, Ouyang Y, Ohmoto T, Nikaido T. 1996. Quassinoids and limonoids from *Harrisonia perforata*. Heterocycles. 43(3):653–664.

Qui KH, Angèle C, Claude R, Hanh NN, Kinh NV and Françoise KH. 2000. New Rearranged Limonoids from *Harrisonia perforata*. J Nat Prod. 63(7):1015–1018.

Qui KH, Angèle C, Claude R, Hanh NN, Kinh NV and Françoise KH. 2001. New Rearranged Limonoids from *Harrisonia perforata*. J Nat Prod. 64(5):634–637.

Kubo I, Tanis SP, Lee YW, Miura I, Nakanishi K, Chapya A. 1976. The structure of harrisonin. Heterocycles. 5(1):485–498.

Li Y, Xu M, Ding X, Yan C, Song Z, Chen L, Huang X, Wang X, Jian Y, Tang G et al. 2016. Protein kinase C controls lysosome biogenesis independently of mTORC1. Nature Cell Biology. 18:1065.

Lv C, Yan XH, Tu Q, Di YT, Yuan CM, Fang X, Ben-David Y, Xia L, Gong JX, Shen YM, Yang Z and Hao XJ. 2016. Isolation and Asymmetric Total Synthesis of Perforanoid A. Angew Chem. 128:7665–7669.

Ma YL, Tang XH, Yuan WJ, Ding X, Di YT, Hao XJ. 2018. Abietane Diterpernoids from the Roots of *Euphorbia ebracteolata*. Natur Prod & Bioprosp.

Rugutt JK, Rugutt KJ, Berner DK. 2001. Limonoids from Nigerian *Harrisonia* abyssinica and their stimulatory activity against *Striga hermonthica* Seeds. J Nat Prod. 64(11):1434–1438.

Tan QG, Luo XD. 2011. Meliaceous Limonoids: Chemistry and Biological Activities. Chemical Reviews. 111(11):7437–7522.

Tanaka T, Koike K, Mitsunaga K, Narita K, Takano S, Kamioka A, Sase E, Ouyang Y, Ohmoto T. 1995. Chromones from *Harrisonia perforata*. Phytochemistry. 40(6):1787–1790.

Tuntiwachwuttikul P, Phansa P, Pootaeng-on Y, Taylor WC. 2006. Chromones from the branches of *Harrisonia perforata*. Chem Pharm Bull. 54(1):44–47.

Wang MX, Zhang MS, Liu WZ, Zhu YL. 1984. Isolation and structural determination of perforatic acid from the traditional Chinese medicine *Harrisonia perforata*. Acta Pharmaceutica Sinica. 19(10):760–763.

Wang MX, Zhang MS, Zhu YL. 1983. Studies on the chemical constituents of a Chinese folk medicine *Harrisonia perforate*. Acta Pharmaceutica Sinica. 18(2):113–118.

Huong DTT, Mai VT, Nguyen BN, Thierry S, Mary P & Marie-Thérèse M. 2000. Foritin, a new Limonoid from *Harrisonia Perforata*. Nat Prod Lett. 14(3):191–195.

Wang S, Yin J, Chen D, Nie F, Song X, Fei C, Miao H, Jing C, Ma W, Wang L et al. 2013. Small-molecule modulation of Wnt signaling via modulating the Axin-LRP5/6 interaction. Nature chemical biology. 9(9):579–585.

Wei XC, Pan ZH, Gu XC, Wang MX, Zhu YL. 1985. Chemical constituents of the Chinese medicine *Harrisonia perforate* IV Molecular and crystal structure on perforate V. Chinese J Struc Chem. 4(4):281–287.

Yan XH, Cao P, Fang X, Zhang Y, Bin W, Di YT, Hao XJ, Yan XH, Yi P, Yang SY et al. 2016. 16-nor Limonoids from *Harrisonia perforata* as promising selective 11β-HSD1 inhibitors. Sci Rep. 6:36927.

Yan XH, Chen J, Di YT, Fang X, Dong JH, Sang P, Wang YH, He HP, Zhang ZK, Hao XJ. 2010. Anti-Tobacco Mosaic Virus (TMV) Quassinoids from *Brucea javanica* (L.) Merr. J Agric Food Chem. 58(3):1572–1577.

Yan XH, Di YT, Fang X, Yang SY, He HP, Li SL, Lu Y, Hao XJ. 2011. Chemical constituents from fruits of *Harrisonia perforata*. Phytochemistry. 72(6):508–513.

Yin S, Chen X, Su ZS, Yang SP, Fan CQ, Ding J, Yue JM. 2009. Harrisotones A-E, five novel prenylated polyketides with a rare spirocyclic skeleton from *Harrisonia perforata*. Tetrahedron. 65(6):1147–1152.

Yu J, Liu H, Xia G, Liu L, Xu Z, Chen Q, Ma C, Sun X, Xu J, Li H et al. 2012. Discovery of 2-Alkyl-1-arylsulfonylprolinamides as 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitors. ACS Med Chem Lett. 3(10):793–798.

Yuan WJ, Ding X, Wang Z, Yang BJ, Li XN, Zhang Y, Chen DZ, Li SL, Chen Q, Di YT et al. 2017. Two novel diterpenoid heterodimers, Bisebracteolasins A and B, from *Euphorbia ebracteolata* Hayata, and the cancer chemotherapeutic potential of Bisebracteolasin A. Sci Rep. 7(1):14507.

Zhang Z, Di YT, Wang YH, Zhang Z, Mu SZ, Fang X, Zhang Y, Tan Cj, Zhang Q, Yan XH et al. 2009. Gelegamines A–E: five new oxindole alkaloids from *Gelsemium elegans*. Tetrahedron. 65(23):4551–4556.