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Triterpenoids and Phenolics from the Leaves of *Ficus hirta* Vahl. in Tuyen Quang Province, Vietnam

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Abstract

Two usane-type triterpenoids as *α*-amyrin acetate (**1**), 3*β*-acetoxy-11*α*-hydroxy-12-ursenes (**2**) and two phenolics as *p*-coumaric acid (**3**), isovitexin (**4**) were isolated from the leaves of *Ficus hirta* collected in Yen Son district, Tuyen Quang province, Vietnam. Their structures were determined from analysis of MS and 1D-, 2D-NMR spectra data and by comparison with data reported in the literatures.

Keywords

Ficus hirta, triterpenoids, phenolics, *α*-amyrin acetate, 3*β*-acetoxy-11*α*-hydroxy-12-ursene, *p*-coumaric acid, isovitexin

1. Introduction

The genus Ficus (Moraceae) comprising about 1,000 species grows mainly in tropical and subtropical [1,2]. Where it is traditionally used as a medicinal plant for nephritis, hepatitis, mastitis, bruises, injuries, rheumatism, cough and to promote milk secretion during childbirth, treatment of constipation, postpartum hypogalactia, tumors, and cancer [2,3,4]. Certain studies regarding the chemical composition and harmacological activities of *F. hirta* Vahl. reported that benzene derivatives, phenolics, and glycosides of flavonoid are majorly present of the roots [5,6,7,8] and fruits of *F. hirta* [9]. Dao Duc Thien et al. have separated and analyzed the chemical components in the extract of *F. hirta*. The result has identified a new 5-*O*-[*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranosyl] bergaptol (**1**), 9 known compounds [10,11] from the Roots of *F. hirta* and 04 triterpenoid with a new 3*β*-hydroxy-11-oxo-olean-12-enyl-3-stearate [12] from leaves of *F. hirta*. This paper reports the isolation and structure identification of two triterpenoids, *α*-amyrin acetate (**1**), 3*β*-acetoxy-11*α*-hydroxy-12-ursenes (**2**) and two phenolics, *p*-coumaric acid (**3**), isovitexin (**4**) from leaves of *F. hirta* collected in Yen Son district, Tuyen Quang province, Vietnam. Their structures were determined from analysis of MS and 1D-, 2D-NMR spectra data and by

comparison with data reported in the literatures.

2. Experimental

2.1. Plant materials

The leaves of *F. hirta* were collected in Yen Son district, Tuyen Quang province, Vietnam in December, 2019. Identified at the site by Dr. Nguyen The Hai, Faculty of medicine and pharmacy, Tan Trao University. A voucher specimen (FH-12/2019) is deposited in Center for Pharmacy Practice Accreditation, Tan Trao University.

2.2. General experimental procedures

Thin layer chromatog-raphy (TLC) was performed on silica gel $60F_{254}$ aluminum plates (0.25 mm, Merck, Darmstadt, Germany). TLC spots were viewed at 254, 302 and 366 nm and visualized by spraying with vanilin-10% H_2SO_4 solution and heating for 5 minutes. Column chromatography was carried out on silica gel 60 (0.040 – 0.063 mm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan). HR-ESI-MS was obtained on a Agilent introduces 1,100 Series LC/MSD Trap SL. NMR spectra were taken on a Bruker Avance III 500 spectrometer (Bruker, Fällanden, Switzerland) at Institute of Chemistry, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried leaves of *F. hirta* (4 kg) were ground and extracted three times with methanol-water (95:5, v/v) at room temperature. After the combined extracts, were evaporated under reduced pressure at 45 ^oC, the residue was suspended in H_2O and then partitioned in turn between *n*-hexane, ethyl acetate and *n*-butanol, successively. The organic solvents were evaporated to yield the corresponding extracts of 40 g, 50 g, and 100 g, respectively.

The *n*-hexane extract (40 g) was subjected to silica gel column chromatography with a gradient mixture of *n*-hexane: EtOAc (from 50:1 to 1:1, v/v) to give 7 fractions (A-G). Fraction B was repeatedly chromatographed on silica gel column (*n*-hexane: EtOAc, 15:1, v/v) to afford compound **1** (0,3 g). Fraction F was chromatographed on silica gel column (*n*-hexane: EtOAc, 9:1, v/v) and then on Sephadex LH-20 column (*n*-hexane : CH_2Cl_2 : MeOH = 1:1:2) to give compound **2** (4 mg).

The *n*-butanol extract (100 g) was applied on a silica gel column eluded with (EtOAc:MeOH:H_2O = 8:2:0.2 → 3.5:2:0.2) to yield 9 fractions. Fraction 1 was chromatographed on silica gel column (*n*-hexane: EtOAc = 2:1) to give compound **3** (2 mg). Fraction 7 was chromatographed on silica gel column (CH_2Cl_2: MeOH= 8.5:1.5) and then on Sephadex LH-20 column (MeOH:H_2O = 2:1) to give compound **4** (3 mg).

***α*-Amyrin acetate (1):** White solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 5.13 (1H, t, *J* = 3.5 Hz, H-12), 4.51 (1H, m, H-3), 2.04 (3H, s, $\underline{CH_3}CO$), 1.07 (3H, s, H-27), 1.01 (3H, s, H-26), 0.98 (3H, s, H-25), 0.92 (3H, d, *J* = 6.0 Hz, H-30), 0.88 (3H, s, H-23), 0.87 (3H, s, H-24), 0.80 (3H, s, H-28), 0.79 (3H, d, *J* = 4.0 Hz, H-29). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 170.96 ($CH_3\underline{CO}$), 139.64 (C-13), 124.34 (C-12), 80.96 (C-3), 59.09 (C-18), 55.29 (C-5), 47.67 (C-9), 42.09 (C-14), 41.55 (C-22), 40.05 (C-8), 39.67 (C-19), 39.62 (C-20), 38.49 (C-1), 37.72 (C-4), 36.81 (C-10), 33.76 (C-17), 32.89 (C-7), 31.26 (C-21), 28.75 (C-23), 28.11 (C-28), 28.08 (C-15), 26.62 (C-16), 23.62 (C-2), 23.38 (C-11), 23.24 (C-27), 21.39 (C-30), 21.29 ($\underline{CH_3}CO$), 18.26 (C-6), 17.50 (C-29), 16.88 (C-26), 16.74 (C-24), 15.73 (C-25).

3*β*-Acetoxy-11*α*-hydroxy-12-ursene (2): White solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 5.19 (1H, d, *J* = 3.5 Hz, H-12), 4.52 (1H, dd, *J* = 10.0, 6.5 Hz, H-3), 4.24 (1H, dd, *J* = 9.0, 4.0 Hz, H-11), 2.04 (3H, s, $\underline{CH_3}CO$), 1.16 (3H, s, H-27), 1.12 (3H, s, H-24), 1.06 (3H, s, H-26), 0.93 (3H, s, H-30), 0.89 (6H, s, H-23&H-25), 0.86 (3H, d, *J* = 5.5 Hz, H-29), 0.80 (3H, s, H-28). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 170.93 ($CH_3\underline{CO}$), 142.90 (C-13), 128.75 (C-12), 80.70 (C-3), 68.36 (C-11), 58.16 (C-18), 55.77 (C-9), 55.43 (C-5), 43.30 (C-8), 42.15 (C-14), 41.31 (C-22), 40.51 (C-1), 39.42 (C-20), 39.30 (C-19), 37.99 (C-4), 33.64 (C-7), 33.61 (C-17), 31.09 (C-21), 28.67 (C-28), 28.22 (C-22), 27.94 (C-16), 26.69 (C-15), 23.77 (C-2), 23.12 (C-27), 21.32 (C-30), 21.30 ($\underline{CH_3}CO$), 18.25 (C-6), 17.99 (C-26), 17.57 (C-29), 16.77 (C-24&C-25).

***p*-Coumaric acid (3):** White solid. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 7.66 (1H, d, *J* = 16.0 Hz, H-7), 7.46 (2H, d, *J* = 8.5 Hz, H-2&H-6), 6.82 (2H, d, *J* = 8.5 Hz, H-3&H-5, 6.40 (1H, d, *J* = 16.0 Hz, H-8). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 168.72 (C-9), 161.16 (C-4), 146.56 (C-7), 131.10 (C-2&C-6), 127.32 (C-1), 116.80 (C-3&C-5), 115.39 (C-8).

Isovitexin (4): Yellow solid. ^{1}H-NMR (500 MHz, CD_3OD): δ_H 7.76 (1H, d, J = 8.0 Hz, H-2′&H-6′), 6.90 (1H, d, J = 8.0 Hz, H-3′&H-5′), 6.51 (1H, s, H-3), 6.43 (1H, s, H-8), 4.92 (1H, d, J = 10.0 Hz, H-1″), 4.22-4.18 (1H, m, H-2″), 3.92 (1H, dd, J = 12.0, 2.0 Hz, H-6a″), 3.79 (1H, dd, J = 12.0, 5.5 Hz, H-6b″), 3.53 (1H, m, H-4″), 3.51 (1H, m, H-3″), 3.48-3.44 (1H, m, H-5″). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 183.88 (C-4), 166.02 (C-2), 164.81 (C-7), 162.69 (C-4′), 161.91 (C-5), 158.58 (C-9), 129.35 (C-2′&C-6′), 122.99 (C-1′), 117.00 (C-3′&C-5′), 109.09 (C-6), 105.15 (C-10), 103.78 (C-3), 95.29 (C-8), 82.54 (C-5″), 80.12 (C-3″), 75.30 (C-1″), 72.60 (C-2″), 71.74 (C-4″), 62.77 (C-6″).

3. Discussion

The structure of **1** was determined to be $C_{32}H_{52}O_2$ with the aid of spectral comparison of NMR and ESI-MS ion at m/z 469.6 $[M + H]^+$. The ^{13}C-NMR and heteronuclear single-quantum correlation (HSQC) spectra displayed 32 carbon atoms inclusive: a carbonyl (δ_C 170.96), nine methylene, seven methine, nine methyl, six quaternary. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** displayed signals characteristic for a triterpene of the ursan-12-ene skeleton, eght methyl carbon signals inclusive: six tertiary methyl groups [δ_H 1.07 (3H, s, H-27), 1.01 (3H, s, H-26), 0.98 (3H, s, H-25), 0.88 (3H, s, H-23), 0.87 (3H, s, H-24), 0.80 (3H, s, H-28)] and two methyl groups [δ_H 0.93 (3H, d, J = 6.0 Hz, H-30), 0.79 (3H, d, J = 4.0 Hz, H-29)], a proton of (>C=C-) group [δ_H 5.13 (1H, t, J = 3.5 Hz, H-12)]. The ^{1}H-, ^{13}C-NMR spectrum of **1** exhibited signals characteristic of a acetoxy group, a oxymethine group [δ_H 4.52-4.49 (1H, m); δ_C 80.96], a acetyl group [δ_H 2.04 (3H, s, $\underline{CH_3}CO$), δ_C 170.96 ($CH_3\underline{CO}$), 21.29 ($\underline{CH_3}CO$)]. The exact acetoxy group were located at C-3 additional HMBC correlations between the protons H-23 (δ_H 0.88), H-24 (δ_H 0.87) and carbon C-3 (δ_C 80.96); H-3 (δ_H 4.51) and carbons C-23 (δ_C 28.75), C-24 (δ_C 16.74). From the above spectral data and with published data [13], the structure of **1** was determined to be α-amyrin acetate.

Combining all of the above ^{1}H-, ^{13}C-NMR, HSQC spectra data and ESI-MS ion at m/z 485.6 $[M + H]^+$ the structure of **2** was determined to be $C_{32}H_{52}O_2$. Combining all of the MS, NMR spectral data showed that compounds **2** and **1** indicated they had the same skeleton and **2** connect an -OH group, the presence of an –OH group was indicated by the observed resonance at δ_H 4.24 (1H, dd, J = 9.0, 4.0 Hz) and δ_C 68.36. The HMBC correlations between the proton oxymethine H-11 (δ_H 4.24) and C-9 (δ_C 55.77), C-12 (δ_C 128.75), determined the hydroxy group to be at C-5. The hydroxyl group is bonded to an α-carbon atom was indicated by correlations between the proton H-9 and H-11, J = 9.0 Hz. Comparison with ^{13}C-NMR spectroscopic data of 3β-acetoxy-11α-hydroxy-12-ursene reported in the literature [14] for same compounds. Combining all of the above information, the structure of **2** was determined to be 3β-acetoxy-11α-hydroxy-12-ursene.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** displayed signals characteristic for four aromatic protons of system is AA'BB' at δ_H 6.82 (2H, d, J = 8,5 Hz), 7.46 (2H, d, J = 8,5 Hz) and two carbons of the trans-olefin have a very large chemical shift difference at δ_H 7.66 (1H, d, J = 16.0 Hz), 6.34 (1H, d, J = 16,0 Hz). The 13C-NMR spectra displayed nine carbon atoms inclusive: a carbonyl at δ_C 168.72, six methine carbon and two quaternary composed of a quaternary carbon atom bonded to an oxygen atom at δ_C 146.6. From the above spectral data and with published data [15], the structure of **3** was determined to be *p*-coumaric acid.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of compound **4** displayed signals characteristic of a flavonoid for four aromatic protons of system is AA'BB' at δ_H 7.76 (1H, d, J = 8.0 Hz, H-2′&H-6′), 6.90 (1H, d, J = 8.0 Hz, H-3′&H-5′) and singles at δ_H 6.51 (1H, s, H-3), 6.43 (1H, s, H-8). The signals characteristic of a glucose unit inclusive: a oxymethylene at [δ_H 3.92 (1H, dd, J = 12.0, 2.0 Hz, H-6a″), 3.79 (1H, dd, J = 12.0, 5.5 Hz, H-6b″), δ_C 62.77] and five oxymethine at [δ_H 4.22-3.44, δ_C 82.54-71.74]. The HSQC spectrum the correlations between proton anomeric at (δ_H 4.92) and a quaternary carbon at (δ_C 75.30), show that C-linkage/glycosidic bond. Direct carbon-proton coupling constants J = 10.0 Hz are β-D-glucopyranose configuration. The ^{1}H-, ^{13}C-NMR spectra compound **4** displayed signals 15 carbon atoms of a flavonoid inclusive: nine quaternary carbon, six methine and six carbon atoms of a glucose unit at δ_C 82.54-62.77 ppm. The HMBC correlations between the proton H-1″ (δ_H 4.92) and carbon atoms at C-5 (δ_C 161.91), C-6 (δ_C 109.09) và C-7 (δ_C 164.81) show that C_6-glycosidic bond. From the above spectral data and with published data [16], the structure of **4** was determined to be isovitexin.

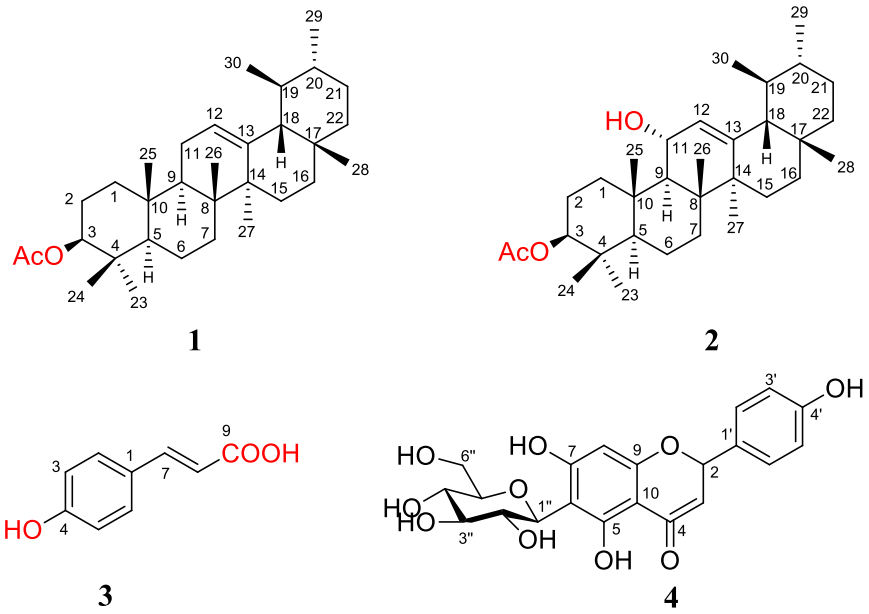

Figure 1. Chemical structures of compounds 1–4 isolated from the leaves of *F. hirta.*

Compounds **2** and **4** were discovered in leaves of *F. hirta* for the fist time collected in Yen Son district, Tuyen Quang province, Vietnam. Compounds **1** widely distributed in genus Ficus inclusive: *Ficus sur*, *Ficus exasperate*, *Ficus bengalensis* and roots of *F. hirta.* Compound **1** has anti-inflammatory activities [17], lowering the glucose level in the blood [18]. Compound **3** was discovered in roots of *F. hirta* in China [8]. Compound **4** was discovered in leaves of *Ficus deltoidea* [19].

4. Conclusion

From *n*-hexane and *n*-butanol extracts leaves of *Ficus hirta* collected in Yen Son district, Tuyen Quang province, Vietnam, four compounds were isolated and chemical structurally elucidated, namely pinoresinolv α-amyrin acetate (**1**), 3β-acetoxy-11α-hydroxy-12-ursene (**2**), *p*-coumaric acid (**3**) và isovitexin (**4**). Among them compound **4** was identified for the first time in this plant. Their chemical structures were confirmed by spectroscopic methods including MS, 1D-, 2D NMR and compared with reported data.

5. Consent For Publication

Not applicable.

6. Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

7. Acknowledgement

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