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CHEMICAL CONSTITUENTS FROM THE AERIAL PART OF *CLINACANTHUS NUTANS* (BURM. F.) LINDAU

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Summary

Chemical Constituents from the Aerial Part of *Clinacanthus nutans* (Burm. f.) Lindau

Investigation of the aerial part of *Clinacanthus nutans* afforded two triterpenoids, two sulfur-containing compounds and a flavonoid. Their structures were identified by spectroscopic methods (MS and NMR) and comparison with published spectroscopic data as lupeol (**1**), betulin (**2**), clinamide E (**3**), *cis*-entadamide C (**4**), and vitexin (**5**). Compound **4** was first isolated from this species.

Keywords: *Clinacanthus nutans, Triterpenoid, Sulfur-containing compound, Flavonoid.*

1. Introduction

Clinacanthus nutans (Burm. f.) Lindau (Acanthaceae), a widely used medicinal plant, is mainly distributed in tropical Asia and Southeast Asian countries. This plant has been known for the treatment of herpes infection, cancer, diabetes, inflammation, and various skin problems [1]. *C. nutans* has been reported to have various phenolic compounds, terpenoids, and some other bioactive compounds, such as benzenoids, cerebrosides, glycoglycerolipids, glycosylglycerides, fatty acids, chlorophyll derivatives, phytosterols, and sulfur-containing glucosides that contribute to its diverse bioactivities [2]. Recently, in Vietnam, there are two publications on the chemical composition of *C. nutans*; some compounds were isolated from *C. nutans* such as fiedelin, morolic acid acetate, betulinic acid, (3β-hydroxyolean-12-en-28-oic acid [3], methyl tetracosanoate, candenatenin B, calycosin [4]. This study investigated the constituents of the aerial part of C. *nutans* of Vietnam and resulted in the isolation and determination of the chemical structures of five compounds.

2. Materials and methods

2.1. Plant material

The aerial part of *Clinacanthus nutans* (Burm. f.) Lindau were collected in Hung Yen province in October 2020 and were taxonomically identified by MSc. Phan Van Truong (Department of Medicinal Material Resources, NIMM). A voucher specimen (DV-13032021) was deposited at the National Institute of Medicinal Materials.

2.2. General experimental procedures

NMR spectra were recorded at 294 K on Bruker AM 500 and 600 FT-NMR spectrometers (Karlsruhe, Germany) with TMS as the internal standard, *J* in Hz. MS data were analyzed with an LC-MS/MS (Shimadzu, Japan). *Silica gel* (Merck, 0.040 - 0.063 mm particle size), RP-C_{18} (Merck, 30 - 50 µm particle size) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ (Merck, 0.25 mm) and RP-$18F_{254}$ (Merck, 0.25 mm) plates. Spots were detected under UV 254 and 366 nm or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating.

2.3. Extraction and isolation

The dry aerial part of *C. nutans* (5.0 kg) was extracted with 80% aqueous ethanol by reflux for 3 hours. The extraction was repeated three times. The extracts were combined and evaporated at 50°C under low pressure to obtain a residue (199.5 g). The residue was suspended in water and successively extracted with *n*-hexane and ethyl acetate. Solvents were then evaporated *in vacuo* to obtain the corresponding *n*-hexane (MCH, 30.6 g) and ethyl acetate (MCE, 42.1 g) fractions.

The *n*-hexane fraction (30.5 g) was chromatographed over *silica gel* using *n*-hexane - acetone (100:1 to 0:1) solvent gradient into 8 fractions (H1-H8). Fr. H2 gave compound **1** (150 mg) after recrystallization from acetone. Fr. H4 was subjected to *silica gel* CC eluted with *n*-hexane - acetone (100:1 to 0:1) to give compound **2** (20 mg).

The ethyl acetate fraction (40.5 g) was

chromatographed over *silica gel* using dichloromethane - methanol (DCM-MeOH) (100:1 to 0:1) solvent gradient into 15 fractions (E1-E15). Fraction E8 (4.0 g) was chromatographed on *silica gel* CC eluting with DCM-MeOH (30:1 - 2:3, v/v) to yield compounds **3** (10 mg) and **4** (8 mg). Fraction E12 (2.18 g) was separated by RP-C_{18} gel CC employing MeOH-water (1:3, 2:3, 1:1, v/v) to yield compound **5** (15 mg).

Compound **1**: White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 3.18 (1H, dd, J = 5.0, 11.5 Hz, H-3), 2.37 (1H, dt, J = 6.0, 11.5 Hz, H-19), 0.94 (3H, s, H-23), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 1.03 (3H, s, H-26), 0.97 (3H, s, H-27), 0.79 (3H, s, H-28), 4.68 (1H, d, J = 2.0 Hz, H-29a), 4.56 (1H, d, J = 2.0 Hz, H-29b), 1.68 (3H, s, H-30); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 38.7 (C-1), 27.4 (C-2), 79.0 (C-3), 38.9 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.9 (C-8), 50.5 (C-9), 37.2 (C-10), 21.0 (C-11), 25.2 (C-12), 38.1 (C-13), 42.9 (C-14), 27.5 (C-15), 35.6 (C-16), 43.0 (C-17), 48.3 (C-18), 48.0 (C-19), 151.0 (C-20), 29.9 (C-21), 40.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.6 (C-27), 18.0 (C-28), 109.3 (C-29), 19.3 (C-30).

Compound **2**: White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 3.19 (1H, dd, J = 4.8, 11.4 Hz, H-3), 0.76 (3H, s, H-24), 0.83 (3H, s, H-25), 0.98 (3H, s, H-26), 1.02 (3H, s, H-27), 3.33 (3H, d, J = 10.8 Hz, H-28a), 3.79 (3H, d, J = 10.8 Hz, H-28b), 4.58 (1H, d, H-29a), 4,68 (1H, d, J = 2.4 Hz, H-29b), 1.68 (3H, s, H-30); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 38.7 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.9 (C-8), 50.4 (C-9), 37.3 (C-10), 20.9 (C-11), 25.2 (C-12), 37.1 (C-13), 42.7 (C-14), 27.1 (C-15), 29.2 (C-16), 47.8 (C-17), 47.8 (C-18), 48.7 (C-19), 150.5 (C-20), 29.8 (C-21), 34.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.8 (C-27), 60.6 (C-28), 109.7 (C-29), 19.1 (C-30).

Compound **3**: White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 6.75 (1H, d, J = 14.4 Hz, H-2), 7.60 (1H, d, J = 14.4 Hz, H-3), 2.73 (3H, s, H-4), 3.56 (2H, m, H-5), 3.80 (2H, t, J = 5.4 Hz, H-6); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 163.3 (C-1), 128.1 (C-2), 146.9 (C-3), 39.8 (C-4), 42.7 (C-5), 61.9 (C-6).

Compound **4**: White powder; ESI-MS: 199.5. $[M+Na]^+$; ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 6.50 (1H, d, J = 10.2 Hz, H-2), 6.74 (1H, d, J = 10.2 Hz, H-3), 2.88 (3H, s, H-4), 3.37 (2H, m, H-5), 3.65 (2H, t, J = 6.0 Hz, H-6); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 165.7 (C-1), 129.0 (C-2), 153.6 (C-3), 41.4 (C-4), 43.2 (C-5), 61.3 (C-6).

Compound **5**: Yellow amorphous powder; ESI-MS: 433.1 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H: 6.77 (1H, s, H-3), 6.27 (1H, s, H-6), 8.02 (2H, d, J = 9.0 Hz, H-2', 6'), 6.89 (2H, d, J = 8.5 Hz, H-3', 5'), 4.69 (1H, d, J = 9.5 Hz, H-1"), 3.84 (1H, t, J = 9.5 Hz, H-2"), 3.28 (1H, s, H-3"), 3.23 (1H, s, H-5"), 3.52 (1H, dd, J = 6.0; 11.5 Hz, H-6"a), 3.77 (1H, dd, J = 5.5; 11.0 Hz, H-6"b); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 163.9 (C-2), 102.4 (C-3), 182.1 (C-4), 160.4 (C-5), 98.1 (C-6), 162.6 (C-7), 104.6 (C-8), 156.0 (C-9), 104.0 (C-10), 121.6 (C-1'), 128.9 (C-2'), 115.8 (C-3'), 161.1 (C-4'), 115.8 (C-5'), 128.9 (C-6'), 73.4 (C-1"), 70.8 (C-2"), 78.65 (C-3"), 70.6 (C-4"), 81.8 (C-5"), 61.3 (C-6").

3. Results and discussion

Compound **1** was obtained as a white powder. The ^{1}H NMR spectrum showed 7 angular methyl protons at δ_H 0.76 (s), 0.79 (s), 0.83(s), 0.94 (s), 0.97 (s), 1.03 (s) and 1.68 (s) corresponding to C-24, C-28, C-25, C-23, C-27, C-26, and C-30. The proton NMR showed H-3 as a doublet of doublet at δ_H 3.2 and two olefinic protons of an exocyclic double bond at δ_H 4.56 and 4.68. The ^{13}C-NMR and DEPT spectrum indicated 30 carbon signals [seven methyl, eleven methylene, six methine and six quaternary carbons] for the lupane skeleton which includes a hydroxylmethine carbon at C-3 at δ_C 79.0. The olefinic carbons of the exocyclic double bond C20-C29 appeared at δ_C 151.0 and 109.3. Thus, compound **1** was assigned as **lupeol** (Fig. 1) [5],[6].

Compound **2** was purified as a white powder. The ^{1}H NMR spectrum showed 6 methyl groups at δ_H 0.76 (s), 0.83 (s), 0.98 (s), 1.02 (s) and 1.68 (s) and doublets for geminal protons at δ_H 4.68 and 4.58, along with a vinylic methyl group at δ_H 1.68, suggesting that **2** was a lupeol-type triterpene. Another pair of doublets at δ_H 3.79 and 3.33, instead of the seventh methyl singlet around δ_H 0.8 as in **1** confirmed the presence of a second hydroxyl group at C-28. The ^{13}C NMR spectrum revealed thirty carbon atoms and further established **2** to be a lupeol-type triterpene. Particularly, the characteristic pair of sp^2 carbons comprising the double bond of lupeol was observed at δ_C 150.5 and 109.7. Signals of the oxygenated carbons C-3 and C-28 were observed at δ_C 79.0 and 60.6, respectively. Consequently, compound **2** was determined to be 20(29)-lupene-3,28-diol, commonly known as **betulin** (Fig. 1). Experimental NMR data were

compared and perfectly matched to those reported in the literature [7].

Compound **3** was isolated as a white powder. Its molecular formula was suggested as $C_6H_{11}NO_3S$ based on the positive-ion peak at *m/z* 178 $[M+H]^+$ and NMR spectra. The ^{1}H-NMR spectrum of **3** contained signals corresponding to five protons involved in two doublets a *trans*-disubstituted olefin at δ_H 6.75 (d, J = 14.4 Hz) and 7.60 (d, J = 14.4 Hz), one isolated methyl group (δ_H 2.73), and two methylene groups at δ_H 3.56 (m) and 3.80 (t, J = 5.4) connected to each other. The ^{13}C-NMR spectrum showed six carbon signals, consisting of one methylsulfinyl group (δ_C 39.8), one oxymethylene group (δ_C 61.9), one methylene group (δ_C 42.7), two methine groups (δ_C 128.1 and 146.9), and one amide carbonyl group (δ_C 163.3). The ^{1}H- and ^{13}C NMR spectra of compound **3** consisted with the corresponding data of the published clinamide E [8]. Thus compound **3** was defined as **clinamide E** (*trans-N*-(2-hydroxyethyl)-3-methylsulfinylpropenamide) (Fig. 1). This compound showed antioxidant activity on DPPH and ferric reducing antioxidant potential assay [8].

Compound **4** was isolated as a white powder. The ^{1}H NMR spectrum of **4** showed nine proton signals. The two doublets at δ_H 6.50 (d, J = 10.2 Hz) and 6.74 (d, J = 10.2 Hz) were attributed to olefinic protons in *cis* relationship. The signals appearing as a triplet and a multiplet at δ_H 3.65 (t, J = 6.0 Hz) and 3.37 (m) corresponded to the methylene protons. The sharp singlet at δ_H 2.88 revealed the presence of a methylsulfinyl group. The carbons of **4** were assigned by ^{13}C NMR experiments: one methyl at δ_C 40.5, two methylenes at δ_C 43.2 (C-5) and 61.3 (C-6), two methines at δ_C 129.0 (C-2) and 153.6 (C-3), and one carbonyl carbon at δ_C 165.4 (C=O). By referencing the published spectral data in the literature [9], the structure of **4** was finally determined to be *cis-N*-(2-hydroxyethyl)-3-methylsulfinylpropenamide (***cis*-entadamide C**) (Fig. 1). Compound **4** was isolated from *C. siamensis* [9] and this is the first time compound **4** has been isolated from *C. nutans*.

Compound **5** was obtained as a yellow amorphous powder. The molecular formula of **4** was found to be $C_{21}H_{20}O_{10}$ by NMR data and an ESI-MS positive-ion peak at *m/z* 433.2 $[M+H]^+$. The ^{1}H-NMR of compound **5** (in DMSO-d_6) showed the following signals: four aromatic protons of an AA′BB′ coupling system in B ring at δ_H 6.89 (2H, d, J = 8.5 Hz) and 8.02 (2H, d, J = 9.0 Hz); two singlet protons at δ_H 6.27 (1H, s) and 6.77 (1H, s) assigned to a flavone aglycone; one anomeric proton at δ_H 4.69 (1H, d, J = 9.5 Hz), assigned to a sugar unit. The ^{13}C-NMR and HSQC spectra revealed signals of 21 carbons, including one carbonyl at δ_C 182.1, eight non-protonated carbons at δ_C 104.6, 104.0, 121.6, 156.0, 160.4, 161.1, 162.6 and 163.9, eleven methine carbons at δ_C 70.6, 70.8, 73.4, 78.6, 81.8, 98.1, 102.4, 115.8 × 2, 128.9 × 2, and one methylene carbon at δ_C 61.3. The ^{1}H and ^{13}CNMR data of **5** were similar to those of vitexin [10]. The HMBC cross peaks from H-3 (δ_H 6.77) to C-1′ (δ_C 121.6)/C-2 (δ_C 163.9)/C-4 (δ_C 182.1)/C-10 (δ_C 104.0); from H-6 (δ_H 6.27) to C-5 (δ_C 160.4)/C-7 (δ_C 162.6)/C-8 (δ_C 104.6)/C-10 (δ_C 104.0) confirmed that two singlet protons were at C-3 and C-6. The large coupling constant $J_{H\text{-}1''/H\text{-}2''}$ = 9.5 Hz and ^{13}C-NMR chemical shifts of sugar moiety (δ_C 73.4, 70.8, 78.6, 70.6, 81.8, and 61.3) were very typical of a *C*-glucopyranosyl moiety [11]. In addition, this sugar was attached to C-8 confirming by the HMBC correlation between glc H-1″ (δ_H 4.69) and C-8 (δ_C 104.6). The HMBC correlations between H-2′ (δ_H 8.02)/H-3′ (δ_H 6.89) and C-4′ (δ_C 161.1) suggested that the hydroxyl group was at C-4′ of flavone. Consequently, the structure of **5** was determined to be **vitexin** (Fig. 1) [10].

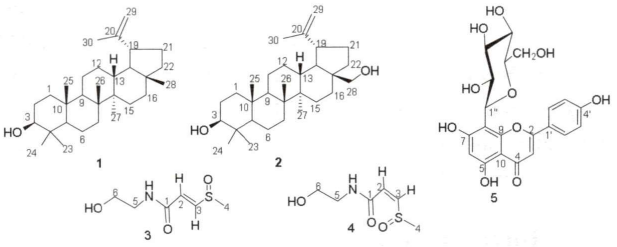

Fig. 1. Chemical structures of compounds **1-5**

4. Conclusion

Five compounds (**1-5**) were isolated from *C. nutans* collected in Hung Yen, Vietnam. Their chemical structures were identified as lupeol (**1**), betulin (**2**), clinamide E (**3**), *cis*-entadamide C (**4**) and vitexin (**5**) based on MS, 1D and 2D-NMR spectroscopic analysis, and comparison with the published literatures. Compounds **4** were isolated for the first time from this plant.

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IRIDOID AND PHENOLIC COMPOUNDS FROM THE RHIZOMES OF *STACHYS SIEBOLDII*

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Summary

Iridoid and Phenolic Compounds from the Rhizomes of *Stachys sieboldii*

The rhizomes of *Stachys sieboldii* in Vietnam were investigated on the chemical constituents. Seven compounds were isolated and determined by NMR and MS analysis as palmitic acid, oleanolic acid, acteoside, isoacteoside, 8-*O*-acetyl harpagide, chrysoeriol 7-*O*-(2"-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside, and *trans*-cinnamic acid.

Keywords: *Stachys sieboldii, Iridoid, Oleanolic acid, Phenethyl glycoside, Flavone glycoside.*

1. Introduction

Stachys sieboldii Miq. (Lamiaceae) commonly called as Chinese artichoke is a perennial herbaceous plant originating from China, Japan, and France [1],[2]. Its rhizomes are used in Chinese traditional medicine for the treatment of inflammation, cold, pneumonia, and heart disease [3],[4]. Components isolated from *S. sieboldii* are iridoid and phenylethanoid glycosides. Acteoside is recognized in *S. sieboldii* as the component responsible for improving experimental autoimmune encephalomyelitis [5]. Harpagide and 8-*O*-acetyl harpagide are the principal iridoids in *S. sieboldii* with antibacterial, antiinflammatory, and antiviral activities [6]. However, the study on the chemical and biological activities of *S. sieboldii* rhizomes has not been reported in Vietnam. In this study, the chemical constituents of the *S. sieboldii* rhizomes of Vietnam were investigated. Seven compounds including palmitic acid, oleanolic acid, acteoside, isoacteoside, 8-*O*-acetyl harpagide, chrysoeriol 7-(2"-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside, *trans*-cinnamic acid were isolated and structurally determined.

2. Material and methods

2.1. Plant material

The rhizomes of *Stachys sieboldii* Miq. were collected by Mr. Pham Ngoc Khanh in Sa Pa province, Vietnam in March 2021. A voucher specimen of plant (NIMM-0019206) was conducted by Miss Nguyen Quynh Nga (Department of Medicinal Plant Resources - National Institute of Medicinal Materials (NIMM)).