*Note*

# Diterpenoids and Flavonoids from *Andrographis paniculata*

Tran Thi Hong Hanh,[a] Nguyen Thi Thuy My,[b] Pham Thi Cham,[a] Tran Hong Quang,*[,a] Nguyen Xuan Cuong,[a] Tran Thu Huong,[b] Nguyen Hoai Nam,[a] and Chau Van Minh*[,a]

[a] *Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST); Hanoi 122102, Vietnam: and* [b] *School of Chemical Engineering, Hanoi University of Science and Technology; Hanoi 113143, Vietnam.*
Received August 7, 2019; accepted October 16, 2019

**Chemical investigation of the aerial parts of *Andrographis paniculata* resulted in isolation of nine compounds, including a new *ent*-labdane diterpenoid, andrographic acid methyl ester (1), a new chalcone glucoside, pashanone glucoside (5), and seven known metabolites, andrograpanin (2), andrographolide (3), andropanolide (4), andrographidine A (6), andrographidine F (7), 6-*epi*-8-*O*-acetyl-harpagide (8), and curvifloruside F (9). Their chemical structures were elucidated based on comprehensive analyses of the spectroscopic data, including NMR and MS. Among the isolated compounds, andropanolide exerted cytotoxicity toward LNCaP, HepG2, KB, MCF7, and SK-Mel2 carcinoma cells, with $IC_{50}$ values ranging from 31.8 to 45.9 $\mu$M. In addition, andropanolide significantly inhibited the overproduction of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages, with an $IC_{50}$ value of 13.4 $\mu$M.**

**Key words** *Andrographis paniculata*; Acanthaceae; diterpenoid; flavonoid; cytotoxicity; nitric oxide overproduction

## Introduction

The herbaceous plant *Andrographis paniculata* (Burm. f.) Nees (Acanthaceae) is the well-known medicinal plant that is widely found in Asian countries, such as India, China, Malaysia, Indonesia, Philippines, and Vietnam. It has long been used in the Vietnamese traditional medicine to treat fever, flu, cough, sore throat, pneumonia, tonsillitis, urethritis, vaginitis, cervical ulcers, inflammatory bowel disease, hypertension, *etc*.[1)] As reported by the pharmacological investigations, *A. paniculata* possesses anti-microbial, anti-inflammatory, hypotensive, antihyperglycemic, antioxidant, anti-atherosclerotic, anti-malarial, anti-human immunodeficiency virus (HIV), antiplatelet aggregation, hepatoprotective, and anti-cancer activities.[2)] Previous chemical studies have indicated that *ent*-labdane diterpenoids and flavonoids are the major chemical constituents and these compounds were considered to be responsible for the pharmacological effects of this medicinal plant.[2,3)] In the present study, we report isolation and structural elucidation of a new *ent*-labdane diterpenoid (**1**), a new chalcone glucoside (**5**), and five known compounds from the methanol extract of the aerial parts of *A. paniculata* growing in Vietnam. In addition, cytotoxic and nitric oxide (NO) inhibitory effects of the isolated compounds were also evaluated.

## Results and Discussion

The aqueous part obtained from the methanol extract of *A. paniculata* aerial parts was subjected to combined chromatographic separations to afford nine compounds (**1**–**9**) (Fig. 1). Their chemical structures were unambiguously deduced by detailed analyses of the spectroscopic evidence as well as comparison with the data reported in the literature.

Compound **1** was obtained as a colorless gum. Its high resolution-electrospray ionization (HR-ESI)-MS exhibited a quasi-molecular ion $[M+Na]^+$ at *m*/*z* 401.1931 (calcd for $C_{21}H_{30}NaO_6^+$, 401.1935), corresponding with the molecular formula of $C_{21}H_{30}O_6$ (seven indexes of hydrogen deficiency). The $^1$H-NMR spectrum contained olefinic proton signals characteristic of three double bonds, including an exocyclic methylene [$\delta_H$ 4.57 and 4.75 (each d, $J$ = 1.5 Hz, $H_a$-17 and $H_b$-17)], a *trans*-disubstituted olefin [$\delta_H$ 6.31 (1H, dd, $J$ = 10.0, 16.0 Hz, H-11) and 6.11 (1H, d, $J$ = 16.0 Hz, H-12)], and a trisubstituted double bond [$\delta_H$ 5.47 (1H, s, H-14)] (Table 1). The $^1$H-NMR spectrum additionally exhibited signals for one oxymethine group at $\delta_H$ 3.41 (H-3), one oxymethylene group [$\delta_H$ 4.14 and 3.37 (each d, $J$ = 11.0 Hz, $H_a$-19 and $H_b$-19), two methyl groups at $\delta_H$ 1.24 (3H, s, $H_3$-18) and 0.83 (3H, s, $H_3$-20), and one methoxy group at $\delta_H$ 3.70 (3H, s). Analysis of $^{13}$C-NMR and heteronuclear single quantum coherence (HSQC) spectra pointed out the presence of eight $sp^2$ carbons [including two carbonyl carbons at $\delta_C$ 168.4 (C-15) and 175.2 (C-16) and six olefinic carbons] and 13 $sp^3$ carbons of which one oxymethine at $\delta_C$ 81.3 (C-3), one oxymethylene at $\delta_C$ 65.0 (C-19), two methines, two methylenes, and three methyls were recognized (Table 1). This spectroscopic evidence suggested that **1** possesses the *ent*-labdane diterpenoid skeleton which has been demonstrated as one of the major chemical types of *A. paniculata*.[2,3)] Comparative analysis of the $^1$H- and $^{13}$C-NMR spectroscopic data of **1** with those of the reported *ent*-labdane derivative, andrographic acid revealed that both structures are closely related, except that **1** has an additional methoxy group [$\delta_H$ 3.70 (s)/$\delta_C$ 51.7] at C-15 position.[4)] This feature was confirmed by an heteronuclear multiple bond connectivity (HMBC) correlation from $\delta_H$ 3.70 to $\delta_C$ 168.4 (C-15) (Fig. 2) observed in the HMBC spectrum of **1**. The relative configuration of **1** was deduced by analysis of the nuclear Overhauser effect spectroscopy (NOESY) spectrum in comparison with that of andrographic acid[4)] (Fig. 2). In the NOESY spectrum, nuclear Overhauser effect (NOE) correlations between H-3 and H-5, between H-3 and $H_3$-18, between H-5 and H-9, and between H-9 and H-12 were observed, suggesting that H-3, H-5, H-9, and $H_3$-18 are $\beta$-oriented. On the opposite spatial side of the structure, NOE

* To whom correspondence should be addressed. e-mail: quangtranhong@imbc.vast.vn; cvminh@vast.vn

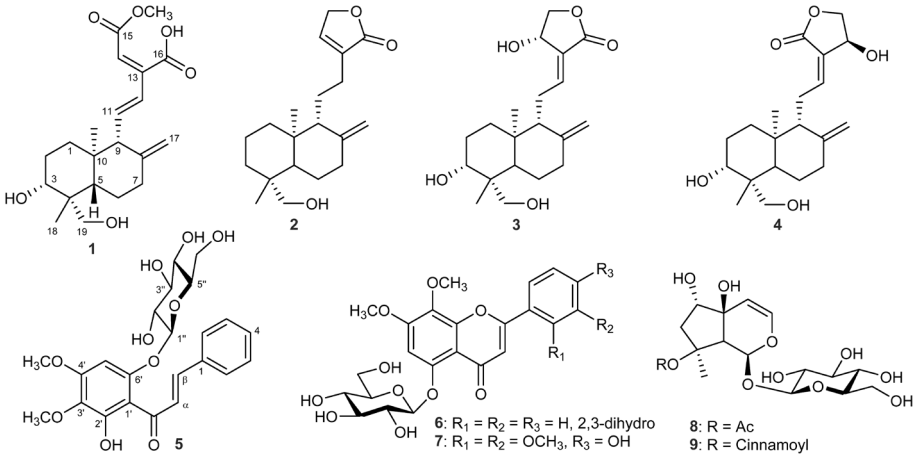


Fig. 1. Chemical Structures of Compounds **1**–**9**

Table 1. $^{1}$H- and $^{13}$C-NMR Data for Compounds **1** and **5**

| Position | **1** | | Position | **5** | |
|---|---|---|---|---|---|
| | $\delta_C$ [a,b] | $\delta_H$ [a,c,d] mult. (*J* in Hz) | | $\delta_C$ [a,b] | $\delta_H$ [a,c,d] mult. (*J* in Hz) |
| 1 | 39.4 | 1.19 td (5.0, 13.5) | 1 | 136.8 | — |
| | | 1.60 dt (3.5, 13.5) | | | |
| 2 | 28.9 | 1.72 | 2 | 129.9 | 7.42 dd (2.0, 9.0) |
| 3 | 81.3 | 3.41 | 3 | 130.0 | 7.76 t (9.0) |
| 4 | 43.8 | — | 4 | 131.3 | 7.41 t (9.0) |
| 5 | 55.9 | 1.25 dd (2.5, 13.0) | 5 | 130.0 | 7.76 t (9.0) |
| 6 | 24.4 | 1.40 | 6 | 129.9 | 7.42 dd (2.0, 9.0) |
| | | 1.82 | | | |
| 7 | 37.8 | 2.08 td (5.0, 13.0) | *α* | 129.1 | 8.12 d (15.5) |
| | | 2.46 | | | |
| 8 | 149.7 | — | *β* | 144.3 | 7.74 d (15.5) |
| 9 | 62.4 | 2.43 | C=O | 195.3 | — |
| 10 | 39.9 | — | 1′ | 109.3 | — |
| 11 | 140.6 | 6.31 dd (10.0, 16.0) | 2′ | 158.0 | — |
| 12 | 132.3 | 6.11 d (16.0) | 3′ | 132.8 | — |
| 13 | 157.8 | — | 4′ | 159.5 | — |
| 14 | 113.0 | 5.47 s | 5′ | 92.8 | 6.58 s |
| 15 | 168.4 | — | 6′ | 157.2 | — |
| 16 | 175.2 | — | 3′-$OCH_3$ | 61.0 | 3.79 s |
| 17 | 109.6 | 4.57 d (1.5) | 4′-$OCH_3$ | 56.7 | 3.96 s |
| | | 4.75 d (1.5) | | | |
| 18 | 23.3 | 1.24 s | 1″ | 102.8 | 5.19 d (7.0) |
| 19 | 65.0 | 4.14 d (11.0) | 2″ | 75.1 | 3.55 dd (7.0, 9.0) |
| | | 3.37 d (11.0) | | | |
| 20 | 16.3 | 0.83 s | 3″ | 78.5 | 3.54 dd (9.0, 9.5) |
| 15-$OCH_3$ | 51.7 | 3.70 s | 4″ | 71.6 | 3.38 dd (9.0, 9.5) |
| | | | 5″ | 78.9 | 3.54 |
| | | | 6″ | 62.7 | 3.70 dd (6.5, 12.0) |
| | | | | | 3.94 dd (2.0, 12.0) |

*a*) Recorded in CD3OD, *b*) 125 MHz, *c*) 500 MHz, *d*) overlapped signals are shown without multiplicity.

interactions observed between $H_2$-19 and $H_3$-20 and between $H_3$-20 and H-11 enabled to identify *α*-orientation for $H_2$-19 and $H_3$-20. The *Z* geometry of the 13,14-double bond was assigned by an NOE correlation between H-12 and H-14. Thus, the structure of **1** was established as shown in Fig. 1, named andrographic acid methyl ester. It is noted that the natural occurrence of **1** was confirmed by its presence in both of MeOH and EtOH extracts, as deduced by HPLC analysis (Figs. S15 and S16, Supplementary material).

Compound **5** was obtained as a yellow, amorphous powder. Its molecular formula was determined to be $C_{23}H_{26}O_{10}$ by a sodium adduct ion $[M+Na]^+$ at *m/z* 485.1414 (calcd for $C_{23}H_{26}O_{10}Na^+$, 485.1418) found in the HR-ESI-MS, along with analysis of the $^{1}$H- and $^{13}$C-NMR spectroscopic data. The $^{1}$H-NMR spectrum showed signals for a *trans*-configured double bond [$\delta_H$ 8.12 and 7.74 (each d, $J=15.5$ Hz, H-*α* and H-*β*],

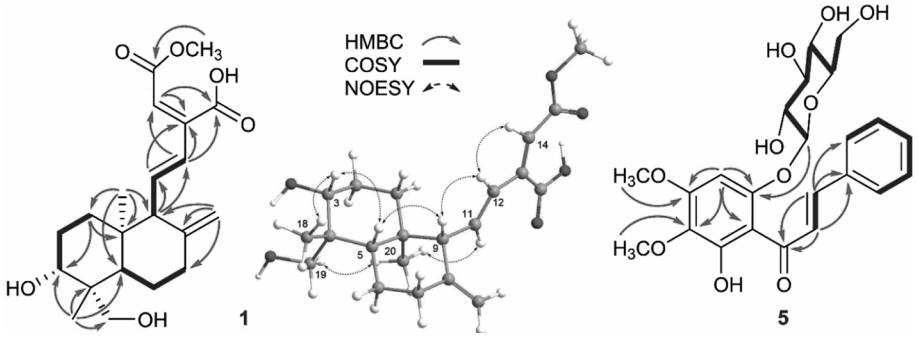


Fig. 2. Key HMBC and COSY Correlations of **1** and **5** and NOESY Interactions of **1**

an unsubstituted phenyl ring [$\delta_H$ 7.42 (2H, dd, $J = 2.0$, 9.0 Hz, H-2 and H-6), 7.76 (2H, t, $J = 9.0$ Hz, H-3 and H-5), and 7.41 (1H, t, $J = 9.0$ Hz, H-4)], a singlet characteristic of a pentasubstituted aromatic ring at $\delta_H$ 6.58 (1H, H-5′), and two methoxy groups at $\delta_H$ 3.79 (3H, s, 3′-$OCH_3$) and 3.96 (3H, s, 4′-$OCH_3$). A signal of an anomeric proton at $\delta_H$ 5.19 (d, $J = 7.0$ Hz, H-1″) observed in the $^1$H-NMR spectrum was indicative of the presence of a sugar unit which was further determined to be glucose by the typical carbon signals at $\delta_C$ 102.8 (C-1″), 75.1 (C-2″), 78.5 (C-3″), 71.6 (C-4″), 78.9 (C-5″), and 62.7 (C-6″) observed in the $^{13}$C-NMR spectrum.[4] The relative large coupling value ($J = 7.0$ Hz) of the anomeric proton was characteristic of the $\beta$ configuration of the glucose. Analysis of the $^{13}$C-NMR and HSQC spectra showed that, in addition to six typical carbon signals of a the glucose, 17 signals of the aglycone, including a carbonyl carbon at $\delta_C$ 195.3 (C=O), one double bond, one phenyl ring, one 1,2,3,4,6-pentasubstituted aromatic ring, and two methoxy groups are recognized, suggesting that **5** possesses the chalcone carbon skeleton.[5] Comparison of the $^1$H- and $^{13}$C-NMR data of **5** with those of the reported chalcone, pashanone revealed that the structures of both compounds are relatively similar, except for the additional occurrence of the glucose moiety in **5**.[5] This was supported by analysis of the HMBC spectrum (Fig. 2). The anomeric proton of the glucose at $\delta_H$ 5.19 (H-1″) was shown to have an HMBC correlation with a non-protonated carbon signal at $\delta_C$ 157.2 (C-6′), indicating that the glucose is located at C-6′ position. This was supported by an HMBC correlation from H-5′ to C-6′ as well as an NOE correlation observed between H-1″ and H-5′. Besides, HMBC cross-peaks from $\delta_H$ 3.79 to $\delta_C$ 132.8 (C-3′) and from $\delta_H$ 3.96 to $\delta_C$ 159.5 (C-4′) along with an NOE interaction between H-5′ and $\delta_H$ 3.96 (4′-$OCH_3$) enabled to confirm the location of the two methoxy groups at C-3′ and C-4′ positions, respectively. On the basis of the spectroscopic evidence, compound **5** was identified as pashanone glucoside.

By using the same spectroscopic methods and comparing with the reported data, the known metabolites were identified as: andrograpanin (**2**),[6] andrographolide (**3**),[7] andropanolide (**4**),[8] andrographidine A (**6**),[4] andrographidine F (**7**),[9] 6-*epi*-8-*O*-acetyl-harpagide (**8**),[10] and curvifloruside F (**9**).[11] The previous pharmacological studies have shown that andrograpanin (**2**) exerts anti-inflammatory activity through down-regulating the p38 mitogen-activated protein kinase (MAPKs) signaling pathways[12] and selective enhancement of chemokine SDF-1$\alpha$-induced leukocyte chemotaxis[13] and an anti-proliferative effect toward HL-60 cells[14]; andrographolide (**3**) possesses various pharmacological effects such as anti-inflammatory and anti-cancer activities[2,3]; andropanolide (**4**) shows a beneficial effect in the treatment of progressive forms of multiple sclerosis[15]; while andrographidine A (**6**) exhibits an anti-proliferative effect against HL-60 cells.[16] Therefore, except **2** and **3**, all of the isolates were evaluated for their cytotoxicity toward five human cancer cell lines (including LNCaP, HepG2, KB, MCF7, and SK-Mel2) using sulforhodamine B (SRB) assay[17,18] and their NO inhibitory effects (at the noncytotoxic concentrations) in lipopolysaccharide (LPS)-stimulated RAW264.7 cells using Griess assay.[19] As the result, among the compounds tested, andropanolide (**4**) was shown to exert cytotoxic effect toward all five human cancer cell lines, including LNCaP, HepG2, KB, MCF7, and SK-Mel2 cells, with $IC_{50}$ values of 31.8, 43.5, 37.9, 45.9, and 42.1 $\mu$M, respectively. In addition, **4** also significantly inhibited the overproduction of NO in LPS-stimulated RAW264.7 macrophages, with an $IC_{50}$ value of 13.4 $\mu$M.

**Acknowledgments** This work was financially supported by Vietnam Academy of Science and Technology (VAST) under Grant number KHCBHH.02/18-20.

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Material** The online version of this article contains supplementary materials.

## References

1) Chi V. V., "Dictionary of medicinal plants in Vietnam," Vol. 2, Vietnam Publisher of Medicine, 2012, p. 1224.
2) Dai Y., Chen S.-R., Chai L., Zhao J., Wang Y., Wang Y., *Crit. Rev. Food Sci. Nutr.*, **59** (Suppl.), S17–S29 (2019).
3) Chao W. W., Lin B. F., *Chin. Med.*, **5**, 17 (2010).
4) Li W., Xu X., Zhang H., Ma C., Fong H., van Breemen R., Fitzloff J., *Chem. Pharm. Bull.*, **55**, 455–458 (2007).
5) Patra A., Mitra A. K., Bhattacharyya A., Adityachaudhury N., *Org. Magn. Reson.*, **18**, 241–242 (1982).
6) Fujita T., Fujitani R., Takeda Y., Takaishi Y., Yamada T., Kido M., Miura I., *Chem. Pharm. Bull.*, **32**, 2117–2125 (1984).
7) Matsuda T., Kuroyanagi M., Sugiyama S., Umehara K., Ueno A., Nishi K., *Chem. Pharm. Bull.*, **42**, 1216–1225 (1994).
8) Pramanick S., Banerjee S., Achari B., Das B., Sen A. K. Sr., Mukhopadhyay S., Neuman A., Prangé T., *J. Nat. Prod.*, **69**, 403–405 (2006).
9) Kuroyanagi M., Sato M., Ueno A., Nishi K., *Chem. Pharm. Bull.*, **35**, 4429–4435 (1987).
10) Morvai M., Nagy T., Kocsis Á., Szabó L. F., Podányi B., *Magn.*

*Reson. Chem.*, **38**, 343–359 (2000).
11) Lai G. F., Wang X. Y., Wang Y. F., Cao J. X., Luo S. D., Ju P., *Helv. Chim. Acta*, **92**, 470–480 (2009).
12) Liu J., Wang Z.-T., Ge B.-X., *Int. Immunopharmacol.*, **8**, 951–958 (2008).
13) Ji L.-L., Wang Z., Dong F., Zhang W.-B., Wang Z.-T., *J. Cell. Biochem.*, **95**, 970–978 (2005).
14) Chen L., Zhu H., Wang R., Zhou K., Jing Y., Qiu F., *J. Nat. Prod.*, **71**, 852–855 (2008).
15) Hancke J. O. *PCT Int. Appl.*, WO 2017214346 A1 20171214 (2017).
16) Chen L.-X., He H., Xia G.-Y., Zhou K.-L., Qiu F., *Nat. Prod. Res.*, **28**, 138–143 (2014).
17) Monks A., Scudiero D., Skehan P., Shoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise P., Vaigro-Wolff A., Gray-Goodrich M., Campbell H., Mayo J., Boyd M., *J. Natl. Cancer Inst.*, **83**, 757–766 (1991).
18) Ngoc N. T., Hanh T. T. H., Thanh N. V., Cuong N. X., Nam N. H., Thung D. C., Kiem P. V., Minh C. V., *Nat. Prod. Res.*, 2019, in press.
19) Van Q. T. T., Vien L. T., Hanh T. T. H., Huong P. T. T., Cuong N. T., Thao N. P., Thuan N. H., Dang N. H., Thanh N. V., Cuong N. X., Nam N. H., Kiem P. V., Minh C. V., *Nat. Prod. Res.*, 2019, in press.