*ORIGINAL ARTICLE*

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*Rec. Nat. Prod.* 11:4 (2017) 356-361

records of natural products

# A New Triterpene from *Buddleja lindleyana* with Neuroprotective Effect

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*(Received December 19, 2016; Revised March 11, 2017; Accepted March 24, 2017)*

**Abstract:** In the phytochemical investigation of *Buddleja lindleyana*, a new 3-acetyl substituted triterpene, 13, 28-epoxy-23-hydroxy-3*β*-acetoxy-olean-11-ene (**1**), together with four same skeleton type known compounds (**2-5**) were isolated. The structure of **1** was elucidated by means of extensive spectroscopic analysis. Their neuroprotective effect against 1-methyl-4 -phenylpyridinium ion-induced ($MPP^+$-induced) neurotoxicity in SH-SY5Y cells were evaluated. The structure activity relationship of compounds **1**-**5** has been discussed preliminarily.

**Keywords:** *Buddleja lindleyana*; oleanolic triterpene; neurotoxicity effect. 

## 1. Introduction

The *Buddleja lindleyana* Fortune (Buddlejaceae, "zui-yu-cao" in Chinese) is a perennial bushes tree, mainly distributed in Province Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Yunnan and Zhejiang on the mountains beside trails, scrub by streams or forest edges [1]. Its flowers, leaves and roots have been used as a traditional Chinese medicine, applied for the treatment of rheumatism, cough, blood stasis, and so on [2]. Phytochemical studies on *Buddleja* species have led to the isolation of kinds of triterpenoids [3, 4], flavonoids [5], iridoid glycosides [6, 7] and phenylethanoids [8]. In the present study, we report the isolation and structure eluciation of a new triterpenoid, 13, 28-epoxy-23-hydroxy-3*β*-acetoxy-olean-11-ene (**1**), along with four known compounds. The compounds' activity against $MPP^+$-induced neurotoxicity in SH-SY5Y cells has also been evaluated.

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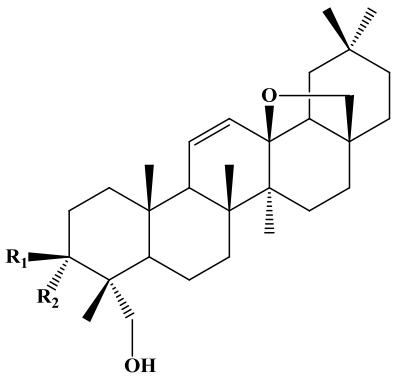
The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/15/2017 EISSN:1307-6167




| | | |
|---|---|---|
| 1 | $R_1$= O-COCH3 | $R_2$=H |
| 2 | $R_1$+$R_2$= O | |
| 3 | $R_1$= OH | $R_2$=H |
| 4 | $R_1$= O-fuc | $R_2$=H |
| 5 | $R_1$= O-fuc-(3←1)-glc | $R_2$=H |

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

## 2. Experimental

### *2.1. Material*

The *B. lindleyana* were collected in November 2013 from Wanfo Mountain of Shucheng County, Anhui Province of China, and authenticated by Prof. Shou-Jin Liu, Anhui University of Traditional Chinese Medicine. A voucher specimen (AHTCM-201311) has been deposited at the herbarium of AHTCM.

Melting points were determined on an XT digital melting point apparatus with microscope and have not been corrected (Shanghai Eastern Co., Ltd., Shanghai, China). Optical rotations were measured on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. NMR spectra were recorded on Bruker DRX-500 instruments with TMS as the internal standard (Bruker, Bremerhaven, Germany). The chemical shifts were given in δ (ppm) scale with reference to the solvent signal. HR-EI-MS spectra were recorded on Waters AutoSpec Premier P776 mass spectrometer (Waters Co., Milford, MA, USA). Silica gel (200–300 mesh; Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences Co., Ltd., Sweden) were used for column chromatography (CC). Preparative HPLC was performed on Waters Auto Purification 2545-2489 system equipped with a Shimadzu ODS-18, 9.4 mm × 250 mm column. Fractions monitored by TLC, and spots were detected by spraying with 10% ethanol-$H_2SO_4$ reagent followed by heating. IR, HR-EI-MS, $^1$H-NMR, $^{13}$C-NMR and 2D NMR spectra of compound **1** are available as supporting information file.

### *2.2. Extraction and isolation*

Air-dried and powdered *B. lindleyana* (4.6 kg) were percolated with 95% ethanol (45 L) and 50% ethanol (30 L) at room temperature. The combined extracts were concentrated in vacuo yielding a crude residue (473g), which was suspended in $H_2O$ and then partitioned successively with petroleum ether, ethyl acetate and n-butanol. The n-butanol extract (180 g) was passed through a macroporous absorbent resin (AB-8) column and washed with $H_2O$, and different concentrations (30%, 50%, 70% , 95%) of ethanol, to give five fractions 1-5. The 50% ethanol portion (Fraction 3, 39.2 g) was subjected to silica gel (100-200 mesh, 400g) CC, and eluted with a gradient of $CHCl_3$-MeOH (v/v = 98:2, 93:7, 85:15, 70:30, 50:50), to afford subfractions3-a-3-e. Fraction 3-b (11.2 g) was chromatographed over repeated silica gel CC and eluted with $CHCl_3$-MeOH gradient systems and Sephadex LH-20, yielding compound **4** (33 mg). Fraction 3-c (8.6 g) was extensively purified by chromatography through

columns of silica gel, Sephadex LH-20, and preparative HPLC to yield compounds **5** (25 mg). The 70% ethanol portion (Fraction 4, 84.3 g) was chromatographed on a silica gel (100-200 mesh, 800g) CC, and eluted with a $CHCl_3$-MeOH gradient system (95: 5, 90:10, 85:15, 80: 20) to give subfractions 4-a-4-d. Fractions 4-a (6.7 g) was applied to crystallize in $CHCl_3$-MeOH (1: 1), followed by chromatographed on silica gel CC repeatedly, to yield compound **2** (38 mg)**, 3** (23 mg). The 95% ethanol portion (Fraction 5, 15.3 g) was separated by silica gel (100-200 mesh, 200g) CC with $CHCl_3$-MeOH (25:1), and followed by preparative HPLC to yield compound **1** (14mg).

2.3. *Spectroscopic data*

*13,28-epoxy-23-hydroxy-3β-acetoxy-olean-11-ene (**1**):* mp: 236-238ºC. $[\alpha]_D$: + 51.5 (*c* 0.01, $CH_3OH$). IR (KBr): 3486, 2919, 2857, 1714, 1470, 1383, 1259, 1033 $cm^{-1}$. HR-EI-MS: m/z 498.3715 $[M]^+$ (calcd. 498.3709 $[M]^+$ for $C_{32}H_{50}O_4$). $^1$H-NMR: (pyridine-$d_5$, 500 MHz) $\boldsymbol{\delta_H}$: 5.96 (1H, d, *J* = 10.0 Hz, H-12) , 5.58 (1H, dd, *J* = 10.0 Hz, 3.0 Hz, H-11), 4.49 (1H, d, *J* = 11.0 Hz, H-23a), 4.29 (1H, d, *J* = 11.0 Hz, H-23b), 3.94 (1H, dd, *J* = 10.5 Hz, 5.5 Hz, H-3),3.72 (1H, d, *J* = 6.5 Hz, H-28a), 3.33 (1H, d, *J* = 6.5 Hz, H-28b), 2.06 (3H, s, $CH_3$-32), 1.32(3H, s, $CH_3$-26), 0.97(3H, s, $CH_3$-24), 0.96(3H, s, $CH_3$-25), 0.96(3H, s, $CH_3$-27), 0.92(3H, s, $CH_3$-29), 0.81 (3H, s, $CH_3$-30). $^{13}$C-NMR: (pyridine-$d_5$, 125 MHz) $\boldsymbol{\delta_C}$: 38.5 (C-1), 27.5 (C-2), 71.3 (C-3), 44.1 (C-4), 48.3 (C-5), 18.1 (C-6), 31.0 (C-7), 42.0 (C-8), 53.8 (C-9), 37.4 (C-10), 131.8 (C-11), 131.9 (C-12), 84.9 (C-13), 44.1 (C-14), 25.6 (C-15), 25.8 (C-16), 41.7 (C-17), 51.5 (C-18), 38.5 (C-19), 31.7 (C-20), 35.0 (C-21), 31.6 (C-22), 66.8 (C-23), 12.3 (C-24), 18.6 (C-25), 19.6 (C-26), 19.8 (C-27), 77.1 (C-28), 33.6 (C-29), 23.6 (C-30), $CH_3CO$-: 170.9 (C-31), 20.8 (C-32).

*2.4. Cell viability assay*

Human neuroblastoma SH-SY5Y cells were obtained from the Chinese Academy of Sciences (Shanghai, China), were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO), supplemented with 10% (v/v) heat-inactivated fetal calf serum and 100 units/ml penicillin/streptomycin. Cells were incubated in a humid environment with 5% $CO_2$ at 37°C. All experiments were carried out 24–48 h after cells were seeded.

The neuroprotective effect of compound **1-5** against $MPP^+$-induced neurotoxicity in SH-SY5Y cells has been evaluated by MTT assay [9]. The SH-SY5Y cells were inoculated in a 96-well microplates ($2\times 10^4$ /well in 100 μL medium) for 24h.Dissolve dried sample with 100% (v/v) DMSO to 1 mg/ml. Then dilute the dissolved compound to 0.1 $mg\cdot ml^{-1}$ with serum free medium. Finally, make a ten fold serial dilution from 0.1 $\mu g\cdot ml^{-1}$ to 100 $\mu g\cdot ml^{-1}$ in serum free medium for each compound. The complete blank control group without SH-SY5Y cells added serum free medium. The blank-control well added nothing except the medium. $MPP^+$ at the concentration of $1mmol\cdot L^{-1}$ was added to the model group well. The medical group well were pretreated with different concentrations (0.1, 1, 10 and 100 $\mu g\cdot mL^{-1}$) of compounds **1-5** for 1h prior to exposure to $MPP^+$ ($1mmol\cdot L^{-1}$). Incubate the plate at 37℃ in a humidified incubator with 5% $CO_2$ for 24h. After the treatment, the MTT (20μL, 5 mg·mL-1) was added to each well and the plates were incubated for another 4 h. The cells were lysed with 150 μL DMSO after removing the medium, and the plates were shaken vigorously to ensure complete solubilization. The optical density (OD) of the lysate was measured at 570 nm by a Spectra Max M5 microplate reader.

The cell viability was expressed as a percentage relative to the untreated control. Cell viability (%) = (OD medical group − OD complete blank control group )/(OD blank control group − OD complete blank control group)×100%.

## 3. Results and Discussion

### *3.1. Structure elucidation*

The new compound **1** was obtained as a white amorphous powder. The molecular formula was determined to be $C_{32}H_{50}O_4$ by HR-EI-MS, $[M]^+$ at *m/z* 498.3715 (calcd for 498.3709), indicating eight degrees of unsaturation. In the $^1$H-NMR spectrum of **1**, signals were observed for six tertiary methyl singlets at $\delta_H$ 1.32, 0.97, 0.96, 0.96, 0.92, 0.81 (each 3H, s), and two olefinic protons at $\delta_H$ 5.96 (1H, d, $J$ = 10.0 Hz) and 5.58 (1H, dd, $J$ = 10.0 Hz, 3.0 Hz). Their carbon resonances were assigned unambiguously by HSQC experiments at $\delta_C$ 19.6 (C-26), 12.3 (C-24), 18.6 (C-25), 19.8 (C-27), 33.6 (C-29), 23.6 (C-30), 131.9 (C-12) and 131.8 (C-11), respectively. Two AB systems of methylene protons at $\delta_H$ 4.49 (1H, d, $J$ = 11.0 Hz), 4.29 (1H, d, $J$ = 11.0 Hz), and $\delta_H$ 3.72 (1H, d, $J$ = 6.5 Hz), 3.33 (1H, d, $J$ = 6.5 Hz) were also observed. The $^{13}$C-NMR and DEPT spectra displayed the presence of 32 carbon signals, including a disubstituted double bond ($\delta_C$ 131.9, 131.8), an oxygenated methine ($\delta_C$ 71.3), two hydroxylmethyls ($\delta_C$ 66.8, 77.1), one oxygenated quaternary carbon ($\delta_C$ 84.9), a carboxyl carbon ($\delta_C$ 170.9), seven methyls, nine methylenes, three methines and six quaternary carbons. On the basis of above datas, compound **1** was concluded to be a triterpene based on the oleanane skeleton. And these signals showed many similarities to those of 13, 28-epoxy-3*β*, 23-dihydroxy-11-oleanene [10, 11] except a carboxyl carbon at $\delta_C$ 170.9. In addition, the $^1$H-NMR at $\delta_H$ 2.06 (3H, s) was demonstrated to be a methyl protons signal of acetyl group.

In HMBC spectrum, the proton signals at $\delta_H$ 3.94 (H, m) corresponding to $\delta_C$ 71.3 in its HSQC spectrum, showed cross peak to $\delta_C$ 170.9 in the HMBC spectrum. And the chemistry shift of $\delta_C$ 71.3 was attributed to C-3 by comparison with the literature [10, 11]. Moreover, It could be observed that the signal at $\delta_H$ 3.94 (H, m, H-3) of compound **1** showed significant downfield shift by comparing with the $\delta_H$ 3.51 (H, m, H-3) [11,12] in compound **3** (esterification effect). All of these evidences suggested that acetyl substitution took place at C-3. Thus, the structure of compound **1** was elucidated as 13, 28-epoxy-23-hydroxy-3*β*-acetoxy-olean-11-ene.

Comparison of the NMR and MS data with reported values led to the identification of the structures of the known compounds **2-5** as 13, 28-epoxy-23-hydroxy-11-oleanene-3-one (**2**) [11], 13, 28-epoxy-3*β*, 23-dihydroxy-11-oleanene (**3**) [11], Mimengoside I (**4**) [4], Mulleinsaponin I (**5**) [13].

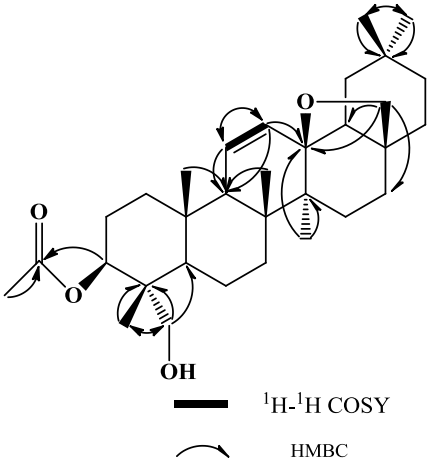



**Figure 2.** Selective $^1$H-$^1$H COSY, HMBC of compound 1

### *3.2 Neuroprotective effect*

We evaluated the protective effects of compounds **1-5** against $MPP^+$-induced loss of cell viability in dopaminergic neuroblastoma SH-SY5Y cells by MTT assay [9]. The results showed that compounds **1-5** demonstrated preventive effects on $MPP^+$-induced SH-SY5Y cells death compared with model group. And compounds **4, 5** showed a stronger activity than compound **1, 2, 3** by

inhibiting cell apoptosis at low concentrations (Fig. 3). However, the structures of the five compounds were found to be similar, except for the substituents located at C-3, demonstrating that the glycoside activity of the same structure type was stronger than that of aglycones in this experiment. Moreover, the activity of compound **5** (diglycoside) was stronger than that of compound **4** (monoglycoside) at each concentration, suggesting that the number of 3-substituted sugar chains has an influence on compound's activity. On the basis of experimental data, It can be revealed that the substituents of C-3 play an essential role in the neuroprotective activity of compounds **1-5**.

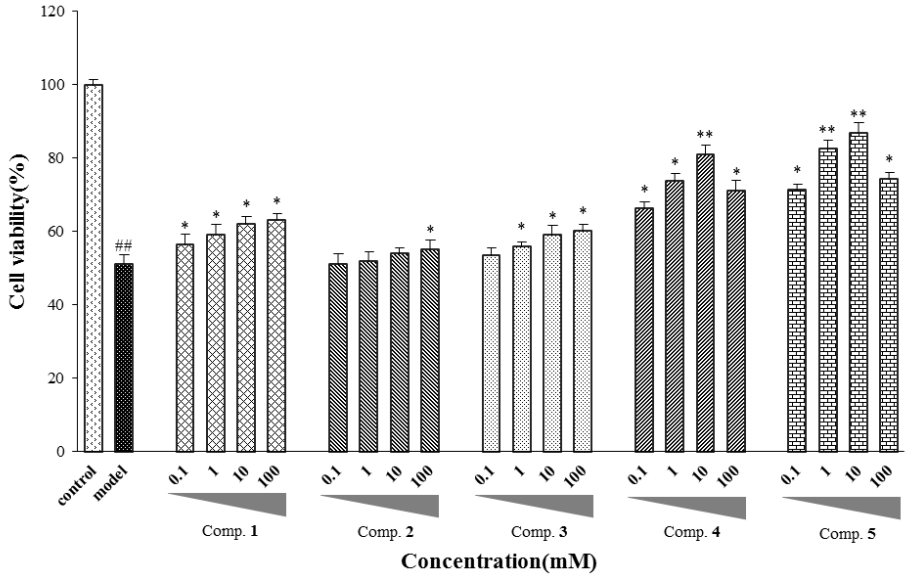



**Figure 3.** Effect of compounds against $MPP^+$-induced neuronal cell death in SH-SY5Y cells. The values were presented as the mean ± SE (n = 6, $^{\#}P < 0.05$, $^{\#\#}P<0.01$, compared with control group. *P < 0.05, **P<0.01, compared with the model group)

## Acknowledgments

This research was financially supported by National Natural Science Foundation of China (81373841), and the Natural Science Foundation of Anhui University of Chinese Medicine (No. 2015zr008), republic of China. We also thank the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for all spectra tests.

## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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