Two New C-Glucosyl Benzoic Acids and Flavonoids from Mallotus nanus and Their Antioxidant Activity

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Two new 2-C,β-D-glucopyranosyl benzoic acid derivatives named mallonanosides A (1) and B (2) were isolated from the methanolic extract of the leaves of Mallotus nanus along with five known flavonoids, kaempferin (3), juglanin (4), quercitrin (5), myricitrin (6), and rhoifolin (7). Their structures were established on the basis of spectral and chemical evidence. Their antioxidant activities were shown to depend on the number of hydroxyl groups, and the location and species of sugar moiety.

Key words: Mallotus nanus, C-glucosyl benzoic acid, Mallonanoside A, Mallonanoside B, Oxygen radical absorbance capacity

INTRODUCTION

Mallotus nanus Airy Shaw (Ba bet lun) belongs to Euphorbiaceae family, which is widely distributed from South Asia to South-East Asia. In Vietnam, this plant is found only in Kontum and Gialai provinces (Ho, 2003; Hoi et al., 2005). No studies on the chemical and bioactivities of this plant have yet been done. In the course of a phytochemical and biological study on Mallotus genus, we report herein the isolation, structural elucidation, and antioxidant activity of two new benzoic acid derivatives, mallonanosides A (1) and B (2), and five known flavonoids from the methanolic extract of the leaves of M. nanus.

The antioxidant activity of two benzoic acids and five flavonoids were measured using the oxygen radical absorbance capacity (ORAC) which is an acknowledged method for antioxidant assessment. The ORAC assay involves the completion of free radicals activities as a mean of quantitation, and combines both the extent of inhibition and the length of inhibition time of free radical action by antioxidants into a single quantity. The ORAC assay provides important information regarding the antioxidant capacity of various biological samples from pure compounds such as phenolic acids and flavonoids to complex matrices such as tea, fruits, vegetables, and animal tissues (Prior and Cao, 1999).

MATERIALS AND METHODS

General methods

Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. All NMR spectra (1H, 13C, DEPT, HSQC, HMBC, COSY and ROESY) were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for 1H and 125 MHz for 13C), and chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. The ESI-MS was obtained on an AGILENT 1200 SERIES LC-MSD Trap spectrometer. HR-ESI-MS was recorded on a JEOL JMS-DX 303 mass spectrometer. Column chro-
matography (CC) was performed on silica gel 230-400 mesh (0.040-0.063 mm, Merck) or YMC RP-18 resins (30-50 µm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F 254 (Merck 1.05715) or RP18 F 254s (Merck) plates. Spots were visualized by spraying 10% H2SO4 aqueous and heating for 5 min.

Plant materials
The leaves of *M. nanus* were collected in Kontum, Vietnam in May 2006 and identified by Prof. Nguyen Nghia Thin, College of Natural Sciences, Hanoi National University. An authentic sample (No MA23) was deposited at the Herbarium of Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and isolation
The leaves of *M. nanus* (1.8 kg) were powdered and extracted with methanol (MeOH, 3 L) three times using a Sonicator (Ultrasonic 2010, 950W) at temperature of 50°C in 60 min. The extract was concentrated under low pressure to obtain a MeOH extract (150.0 g), which was suspended in water and successively partitioned with n-hexane and ethyl acetate (EtOAc) to obtain n-hexane (35.0 g) and EtOAc (65.0 g) extracts, successively. The aqueous layer was passed through a Dianion HP-20 column using a gradient concentration of MeOH in H2O (0:100; 30:70; 70:30; 100:0, v/v, 2 L) as an eluent to give four fractions designated W0 (removed), W1 (15.0 g), W2 (19.0 g), and W3 (11.0 g). The EtOAc fraction (65.0 g) was chromatographed on a silica gel column and eluted with CHCl3/MeOH gradient (10:1 - 1:1, v/v) to obtain three sub-fractions designated E3A (26.5 g), E3B (13.4 g), and E3C (24.0 g). The E3A sub-fraction (26.5 g) was chromatographed on an YMC RP-18 column and eluted with MeOH/H2O (4:1, v/v) to yield 3 (20.5 mg) and 4 (9.0 mg) as yellow amorphous powders. The E3B sub-fraction (13.4 g) was chromatographed on an YMC RP-18 column and eluted with MeOH/H2O (5:2 v/v) to give 5 (15.0 mg) and 6 (12.0 mg) as yellow amorphous powders. The E3C sub-fraction (24.0 g) was chromatographed on a silica gel column and eluted with CHCl3/MeOH/H2O (3:1:0.1, v/v/v) yielding 1 (120.0 mg) as a white amorphous powder. The W1 fraction was chromatographed on a silica gel column and eluted with CHCl3/MeOH/H2O (2:1:0.2, v/v/v) to obtain 7 (35.0 mg) as a yellow amorphous powder. Compound 2 (100.0 mg) was recrystallized in MeOH from the W2 fraction.

**Mallonanoside A (1)**
White amorphous powder; mp 196-197°C; [α]D25 +55° (c 0.50, CHCl3); IR (KBr) cm−1: 3440 (OH), 2954 (CH), 1725 (C=O), 1620 and 1580 (C=C); positive ESI-MS m/z 347 [M + H]+, negative ESI-MS m/z 327 [M-H2O -H]−; HR-ESI-MS m/z 351.0710 [M-H 2O+Na]+ (Calcd for C14H16O9Na 351.0692); 1H NMR (500 MHz, CD3OD) and 13C NMR: See Table I.

**Mallonanoside B (2)**
White crystals; mp 201-202°C; [α]D25 +57° (c 0.50, CHCl3); IR (KBr) cm−1: 3450 (OH), 2953 (CH), 1723 (C=O), 1625 and 1580 (C=C); positive ESI-MS m/z 315 [(M-H2O) + H] +, negative ESI-MS m/z 313 [M-H2O-H]+; HR-ESI-MS m/z 337.0550 [M-H2O+Na]+ (Calcd for C13H14O9Na 337.0536); 1H NMR (500 MHz, DMSO-d6) and 13C NMR (125 MHz, CD3OD): See Table I.

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![Fig. 1. Structures of isolated compounds (1-7)](image-url)