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# New Cytotoxic Benzopyrans from the Leaves of *Mallotus apelta*

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Two new benzopyrans 6-[1'-oxo-3'(*R*)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran (**1**) and 6-[1'-oxo-3'(*R*)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran (**2**) were isolated from the leaves of *Mallotus apelta* Muell.-Arg., (Euphorbiaceae). Their chemical structures were elucidated by spectroscopic analyses, especially by 1D-, 2D-NMR and MS spectra. Compound **1** was found to have strong cytotoxic effect against two human cancer cell lines as human hepatocellular carcinoma (Hep-2, $IC_{50}$: 0.49 μg/mL) and rhabdosarcoma (RD, $IC_{50}$: 0.54 μg/mL), while compound **2** showed moderate activity against Hep-2 cell line ($IC_{50}$, 4.22 μg/mL) by *in vitro* assay.

**Key words:** Euphorbiaceae, *Mallotus apelta*, Benzopyran, Cytotoxic activity

## INTRODUCTION

*Mallotus apelta* Muell.-Arg., (Euphorbiaceae), is distributed widely around Vietnam and south of China. Its leaves have been used as traditional Vietnamese medicine for treatment of chronic hepatitis, white blood, and enteritis (Chi, 1997; Loi, 2001). Previous investigations of the *M. apelta* have isolated triterpenoids (Shan and Feng, 1985), diterpenoids (Cheng *et al.*, 1999a, 1999b), alkaloid (Cheng *et al.*, 1998) and coumarino-lignoids (Cheng and Chen, 2000) from the root, triterpenoids (Kiem *et al.*, 2004) and benzopyran derivatives (An *et al.*, 2001, 2003) from the leaves of this plant. Only a few biological effects have been reported, e.q., inhibitory effects of reverse transcriptase and various DNA polymerase (Ono *et al.*, 1989) and antioxidant effect (Zhao *et al.*, 2002). As a part of our ongoing research program on bioactive compounds from Vietnamese medicinal plants, we herein report the isolation and structural determination of two new benzopyrans from the leaves of *M. apelta* and their cytotoxic effects against two cancer cell lines Hep-2 (human hepatocellular carcinoma) and RD (rhabdosarcoma) in an *in vitro* assay system.

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## MATERIALS AND METHODS

### General experimental procedures

The IR spectra were obtained on a Hitachi 270-30 type spectrometer using KBr discs. The optical rotations were determined on a JASCO DIP-1000 KUY polarimeter. The Electron Spray Ionization mass (ESI) spectrum was obtained using an AGILENT 1100 LC-MSD trap spectrometer. The HR-FAB-MS spectrum was obtained using a JEOL JMS-DX 300 spectrometer. The $^1$H-NMR (500 MHz) and $^{13}$C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as the internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or YMC * GEL ODS-A.

### Plant material

The leaves of *M. apelta* were collected at Tam Dao Mountain, Vinh Phuc province on December 2002, and identified by Prof. Vu Van Chuyen, Hanoi University of Pharmacy. A voucher specimen (INPC 2847) was deposited at the herbarium of the Institute of Natural Product Chemistry, Vietnamese Academy of Science and Technology.

### Extraction and isolation

The dried and powdered leaves of *M. apelta* (4.5 kg) were extracted with methanol at room temperature to give

methanol extract (200 g), which was suspended in water and then partitioned with chloroform (v/v: 1/1) to give chloroform extract (50 g). The chloroform extract was then chromatographed on a silica gel column (Φ 70 × L 500 mm) eluted with hexane-acetone as eluent containing increasing concentrations of acetone [hexane (2 L), hexane-acetone (100:1; 2 L), hexane-acetone (10:1; 2 L), hexane-acetone (2:1; 2 L), and acetone 100%] to give MA1 (5.4 g), MA2 (13.0 g), MA3 (15.0 g), MA4 (6.6 g), and MA5 (10.0 g) fractions, respectively. The MA3 fraction (15.0 g) was then chromatographed on a silica gel (Φ 30 × L 700 mm) eluted with hexane-acetone (50:1; 2 L) as eluent to give MA3A (4.6 g), MA3B (6.3 g), and MA3C (4.1 g) fractions. The MA3B fraction (6.3 g) was then rechromatographed on a YMC RP-18 column (Φ 25 × L 500 mm) eluted with methanol-water (10:1; 1.5 L) as eluent to give **1** (9.0 mg) and **2** (20.0 mg) as colourless oils.

### 6-[1'-Oxo-3'(*R*)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran (1)

Colourless oil; $[\alpha]_D^{25}$: -3.5° ($CHCl_3$, *c* 0.5); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 1750 (C=O), 1642 (C=C); positive ESI *m/z*: 307 $[M+H]^+$; HR-FAB-MS *m/z*: 307.1542 $[M+H]^+$ (Calcd. for $C_{17}H_{23}O_5$: 307.1546); The $^{13}$C-NMR (125 MHz) and $^1$H-NMR (500 MHz): see Table I.

### 6-[1'-Oxo-3'(*R*)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran (2)

Colourless oil; $[\alpha]_D^{25}$: -3.0° ($CHCl_3$, *c* 0.5); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1750 (C=O), 1640 (C=C), 1040 (C-O-C); positive ESI *m/z*: 321 $[M+H]^+$; Elemental Anal: $C_{18}H_{24}O_5$ (Calcd., C, 67.48; H, 7.55; O, 24,97. Found: C, 67.42; H, 7.55; O, 24,86); The $^{13}$C-NMR (125 MHz) and $^1$H-NMR (500 MHz): see Table I.

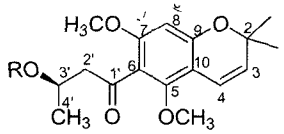
**Table I.** $^1$H- and $^{13}$C-NMR data for **1** and **2**

| C | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$[a,b] | $\delta_H$[a,c] (*J*. Hz) | $\delta_C$[a,b] | $\delta_H$[a,c] (*J*. Hz) |
| 2 | 76.9 s | - | 76.8 s | - |
| 3 | 127.9 d | 5.53 d (10.5) | 127.8 d | 5.52 d (10.5) |
| 4 | 116.3 d | 6.46 d (10.5) | 116.5 d | 6.46 d (10.5) |
| 5 | 154.2 s | - | 154.3 s | - |
| 6 | 117.8 s | - | 118.2 s | - |
| 7 | 157.5 s | - | 157.7 s | - |
| 8 | 96.3 d | 6.19 (s) | 96.1 d | 5.99 (s) |
| 9 | 156.3 s | - | 156.0 s | - |
| 10 | 108.1 s | - | 108.1 s | - |
| 2-$Me_2$ | 27.9 q | 1.56 (s) | 27.9 q | 1.42 (s) |
| 1' | 205.1 s | - | 202.2 s | - |
| 2'a<br>2'b | 53.2 t | 3.00dd (16.5. 6.5)<br>2.85 dd (16.5. 6.5) | 51.7 t | 3.11dd (16.5. 6.5)<br>2.80 dd (16.5. 6.5) |
| 3' | 64.5 d | 4.32 (m) | 73.2 d | 3.87 (m) |
| 4' | 22.4 q | 1.22 d (6.5) | 19.6 q | 1.20 d (6.5) |
| 3'-$OCH_3$ | | | 56.2 q | 3.30 s |
| 5-$OCH_3$ | 63.8 q | 3.75 s | 63.3 q | 3.70 s |
| 7-$OCH_3$ | 55.9 q | 3.76 s | 55.8 q | 3.72 s |

[a]Measured in $CDCl_3$, [b]125 MHz, [c]500 MHz, Chemical shift (δ) in ppm

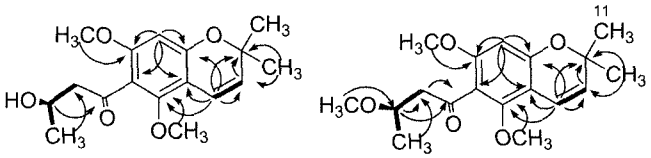
## RESULTS AND DISCUSSION

The methanol extract obtained from the leaves of *M. apelta* led to the isolation of two new benzopyrans (**1** and **2**). Compound **1** was obtained as colourless oil. The molecular formula $C_{17}H_{22}O_5$ of **1** was deduced from the HR FAB-MS spectrum (Found *m/z*: 307.1542 $[M+H]^+$; Calcd. for $C_{17}H_{23}O_5$: 307.1546). The IR spectrum of **1** showed the presence of OH, C=O, and C=C groups at 3420, 1750, and 1642 $cm^{-1}$, respectively. The $^1$H-NMR of **1** showed two doublets at δ 5.53 and 6.46 and one singlet at δ 6.19 of the three olefinic protons; two doublet of doublet of the methylene protons at δ 2.85 and 3.00; three methyl protons at δ 1.56 (6H) and 1.22; one methine carbinol proton at δ 4.32, and two singlets of the methoxyl groups at δ 3.75 and 3.76. The heteronuclear multiple quantum coherence (HMQC) spectrum of **1** led to connect the protons and carbons as shown in Table I. In the $^1$H-$^1$H chemical shift correlation spectroscopy ($^1$H-$^1$H COSY), H-3 proton at δ 5.53 correlated with H-4 at δ 6.46, while the signal of a proton attached to carbon bearing an oxygen function at δ 4.32 showed correlation with a doublet signal of the methyl group at δ 1.22 and with doublet of doublet signals of the methylene group at δ 2.85 and 3.00. This elucidated the partial structure $CH_3$-CH(OH)-$CH_2$-CO-. The $^{13}$C-NMR spectrum of **1** indicated the presence of 17 carbons including five olefinic quaternary carbons (δ 108.1, 117.8, 154.2, 156.3, and 157.5), three olefinic methine carbons (δ 96.3, 116.3, and 127.9), quaternary carbon bearing to oxygen atom (δ 76.9), a *gem*-dimethyl groups at δ 27.9. All substances [δ 157.5, 156.3, 154.2, 127.9, 117.8, 116.3, 108.1, 96.3, 76.9, and 2 × 27.9] showed the typical NMR signals of 2,2-dimethyl-2*H*-1-benzopyrans (Kamperdick *et al.*, 1997). Comparison the NMR data of **1** with those of 6-(1-methoxyethyl)-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran (Kamperdick *et al.*, 1997) suggested the C-5 and C-7 positions of two methoxyl groups, and the 1-oxo-3-hydroxy-butyl group connected to C-6 of the benzene ring. The suggested structure of **1** was shown in Fig. 1, and confirmed by detailed analyses of the heteronuclear multiple bonds correlation spectrum (HMBC) (Fig. 2). Cross peaks were observed between the methoxyl proton at δ 3.75 and carbon C-5 (δ 154.2), between the other methoxyl proton at δ 3.76 and carbon C-7 (δ 157.5), and between H-2' proton at δ 2.85/3.00 and



**1** R = H
**2** R = $CH_3$

Fig. 1. Structures of compounds **1** and **2**



HMBC
$^1$H-$^1$H COSY

Fig. 2. Selected $^1$H-$^{13}$C long-range correlations in the HMBC spectra and H$^1$-H$^1$ correlations in the $^1$H-$^1$H COSY spectra of **1** and **2**

carbons C-6 (δ 117.8)/C-1' (205.1) in the HMBC spectrum indicating that two methoxyl groups connected to C-5 and C-7, and the carbonyl carbon connected to C-6. Comparing the $^1$H- and $^{13}$C-NMR data of the partial structure $CH_3$-CH(OH)-$CH_2$-CO- of **1** with those of 1-(2-hydroxy-4,6-dimethoxyphenyl)-3(*S*)-hydroxybutane-1-one (Hori *et al.*, 1990) showed the quite difference of the chemical shifts of proton H-2' and H-3' and methyl carbon chemical shifts at C-4'. That difference suggested the stereochemistry at C-3' was (*R*). Accordingly, the structure of **1** was determined to be 6-[1'-oxo-3'(*R*)-hydroxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran.

The NMR spectra of **2** were very similar to those of **1**, except for the more appearance of the methoxyl signals in the NMR spectra of **2** ($\delta_H$ 3.30 and $\delta_C$ 56.20). This evidence regarded **2** as a methoxyl derivative of **1**. The molecular formula $C_{18}H_{24}O_5$ of **2** was confirmed from the quasi-molecular ion peaks at *m/z* 321 $[M+H]^+$ in the positive electron spray ionization mass spectrum (ESI) as well as from the elemental analysis. In addition, the lack of the hydroxy group of **2** confirmed by the absence of OH stretching in the IR spectrum. The 2,2-dimethyl-2*H*-1-benzopyran skeleton was identical to that of **1**, and 6-(1-methoxyethyl)-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran (Kamperdick *et al.*, 1997). The stereochemistry at C-3' of **2** was suggested to be (*R*) by comparing the $^1$H- and $^{13}$C-NMR data of the partial structure $CH_3$-CH($OCH_3$)-$CH_2$-CO of **2** with those of **1** and 1-(2-hydroxy-4,6-dimethoxyphenyl)-3(*S*)-hydroxybutane-1-one (Hori *et al.*, 1990). All the chemical shift assignment (Table I) established by evaluation of the $^1$H-$^{13}$C correlations from the HMQC spectrum, and of the long-range correlations from the $^1$H-$^1$H COSY and HMBC spectra (Fig. 2) to yield the new structure **2** as 6-[1'-oxo-3'(*R*)-methoxy-butyl]-5,7-dimethoxy-2,2-dimethyl-2*H*-1-benzopyran.

### Cytotoxicity

The cytotoxic activities of compounds **1** and **2** were assayed on Hep-2 (human hepatocellular carcinoma) and RD (rhabdosarcoma) cells by SRB method (Lee *et al.*, 2003; Likhitwitayawuid *et al.*, 1993). As a result, **1** was found to be strongly cytotoxic to both cancer cell lines Hep-2 and RD with the 50% inhibition concentration ($IC_{50}$) of 0.49 μg/mL and 0.54 μg/mL, respectively, while compound **2** showed moderate cytotoxic activity to Hep-2 cell line with the $IC_{50}$ value of 4.22 μg/mL.

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