MALLOAPELTIC ACID, A NEW BENZOPYRAN DERIVATIVE FROM *Mallotus apelta*

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*A new benzopyran derivative, malloapeltic acid (**1**), along with seven known compounds, was isolated from the roots of* Mallotus apelta*. The structure of the new compound **1** was elucidated by spectroscopic data analysis. The known compounds **2–8** were identified by comparison of their spectroscopic data with reported values in the literature. Compound **1** showed strong anti-HIV activity* in vitro*.*

Keywords: benzopyran, malloapeltic acid, Euphorbiaceae, *Mallotus apelta*.

Mallotus apelta (Lour.) Muell.-Arg. (Euphorbiaceae) is widely distributed in the east and south of China. The roots of *Mallotus apelta* have been used in traditional chinese medicine (TCM) for the treatment of chronic hepatitis and enteritis [1]. Pharmacological studies showed that their ethanolic extract exhibited strong inhibitory effects on murine retroviral reverse transcriptase and human deoxyribonucleic (DNA) polymerase *in vitro* [2]. Recently, in an extensive screen for effective anti-HIV natural products, extract of the roots of *Mallotus apelta* also showed significant activity [2]. However, only four triterpenoids and a pyridine-type alkaloid have been described as its constitutions [1, 3]. In our present research, a new benzopyran derivative, 2-(3′,4′,5′-trimethoxyphenyl)-3,7-dihydroxy-6-(*Z*)-carboxyvinyl-8-methyl-2,3-dihydro-1-benzopyran (**1**, 7 mg), along with seven known compounds, including erythrodiol-3-acetate (**2**, 43 mg) [4], acetyl aleuritolic acid (**3**, 39 mg) [5], *β*-sitosterol (**4**, 48 mg) [6], 5-hydroxy-7-methoxychromone (**5**, 12 mg) [7], scopoletin (**6**, 21 mg) [8], 4,5,4′-trimethyl ellagic acid (**7**, 10 mg) [9], and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**8**, 23 mg) [10], was isolated from the ethanolic extract of the roots of *Mallotus apelta*. Compound **1** was identified by spectroscopic data and named malloapeltic acid, which showed strong anti-HIV activity *in vitro*.

Malloapeltic acid (**1**) was obtained as pale yellow needles, mp 282°C. Its molecular formula was determined to be $C_{22}H_{24}O_8$ by means of analyzing 1D, 2D NMR spectroscopic data, and was also verified by EI-MS data (found *m/z*: 416.0 $[M]^+$; calcd 416.0). The UV spectrum showed absorption of the conjugated system (331 nm). The IR spectrum indicated the presence of OH (3418 cm^{-1}), =C-H (3081 cm^{-1}), C-H (2938 cm^{-1}), OCH_3 (2845 cm^{-1}), C=O (1710 cm^{-1}), benzene ring (1645, 1510 cm^{-1}), -CH_2- (1447 cm^{-1}), -CH=CH- (1417 cm^{-1}), and C-O (1139 cm^{-1}). The ^{13}C NMR spectrum displayed 22 carbon resonances comprising two sp^3 methines, four sp^3 methyls, one sp^3 methylene, five sp^2 methines, one carboxyl, and nine quaternary carbons. Furthermore, the ^1H and ^{13}C NMR spectral data (Table 1) revealed the presence of three methoxyls, two benzene rings, a double bond, and the partial structure –CHOR-CHOR-CH_2, The complete structure of **1** was determined by analyzing the 2D NMR data including the HMQC and HMBC spectra in $(CD_3)_2CO$. The HMQC spectrum allowed us to connect the protons and carbons as shown in Table 1. In the HMBC spectrum, H-6′ and H-3 showed cross-peaks with C-2, H-2 with C-4, C-9, H-11 with C-5, C-6, C-7, H-8-CH_3 with C-7, H-5 with C-10, H-12 with C-13, and H-2′ with C-1′. There are cross-peaks between H-3′-OCH_3, H-4′-OCH_3, H-5′-OCH_3, and C-3′, C-4′, C-5′. Based on the above data, compound **1** was determined to be 2-(3′,4′,5′-trimethoxyphenyl)-3,7-dihydroxy-6-(*Z*)-carboxyvinyl-8-methyl-2,3-dihydro-1-benzopyran, which was named melloapeltic acid (**1**).

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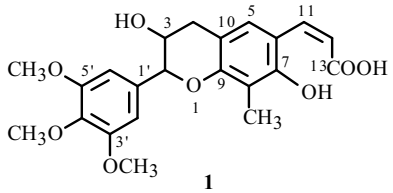
TABLE 1. ^{1}H and ^{13}C NMR Data of **1**[a], J/Hz

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2	5.08 (1H, d, J = 8.4)	77.764	11	7.91 (1H, d, J = 9.6)	145.183
3	4.30 (1H, ddd, J_1 = 8.4, J_2 = 3.6, J_3 = 2.4)	79.414	12	6.28 (1H, d, J = 9.6)	114.238
4	3.58 (1H, dd, J_1 = 13.6, J_2 = 3.6)	61.286	13		160.946
	3.89 (1H, dd, J_1 = 13.6, J_2 = 2.4)		1′		127.191
5	6.86 (1H, s)	101.653	2′, 6′	6.87 (2H, d)	106.357 (2C)
6		112.402	4′		146.833
7		139.562	3′, 5′		148.788 (2C)
8		112.192	8-CH_3	1.29 (3H, s)	29.896
9		133.086	4′-OCH_3	3.86 (3H, s)	56.289
10		138.524	3′-OCH_3, 5′-OCH_3	3.87 (6H, s)	56.472 (2C)

[a]Data were recorded in $(CD_3)_2CO$ on an Inova-600 MHz spectrometer (^{1}H NMR, ^{13}C NMR, HMQC and HMBC); chemical shifts are shown in δ (ppm) with TMS as internal standard.



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The anti-HIV activity *in vitro* of melloapeltic acid (**1**) was assayed on MT-4 cell (a kind of $CD4^+$ lymphocytes). The MIC (minimal inhibitory concentration) was determined for 1.6 μg/mL. The median toxic concentration (TC_{50}) of compound **1** was 120.0 μg/mL, and it inhibited uninfected MT-4 cell growth with an IC_{50} value of 3.2 μg/mL and a calculated therapeutic index (TI) value of 37.5. In general, TI > 5.0 is considered to denote significant activity; compound **1** showed strong anti-HIV activity *in vitro* with a TI value over 5.

EXPERIMENTAL

Roots of *Mallotus apelta* were purchased from Guangdong Province of China in June, 2006. The dried and powdered roots of *Mallotus apelta* (15 kg) were extracted with 95% ethanol (60000 mL × 3, 2 h) at room temperature, and the combined extracts were evaporated under reduced pressure to afford 660 g of extract. A portion of the extract (500 g) was suspended in water and further partitioned with petroleum ether (1500 mL × 3) and ethyl acetate (1500 mL × 3), successively. The petroleum ether extract (60 g) was subjected to silica gel column chromatography and eluted with increasing concentrations of ethyl acetate in petroleum ether. Each fraction (80 mL) was monitored by TLC; fractions with similar TLC patterns were combined to yield three major fractions (F_1–F_3). Fraction F_2 was rechromatographed on a silica gel column eluted with petroleum ether–ethyl acetate (12:1–7:1), followed by recrystallization, to yield pure **2**, **3**. Fraction F_3 was rechromatographed on a silica gel column eluted with petroleum ether–ethyl acetate (7:1–4:1), followed by recrystallization, to yield pure **4**, **5**. The ethyl acetate extract (180 g) was subjected to silica gel column chromatography, eluted with increasing concentrations of MeOH in $CHCl_3$. Each fraction (80 mL) was monitored by TLC; fractions with similar TLC patterns were combined to yield four major fractions (F'_1-F'_4). Fraction F'_2 was rechromatographed on a silica gel column eluted with MeOH–$CHCl_3$ (16:1–9:1), followed by recrystallization, to yield pure **1**, **6**. Fraction F'_3 was rechromatographed on a silica gel column eluted with MeOH–$CHCl_3$ (12:1–7:1), followed by recrystallization, to yield pure **7**. Fraction F'_4 was rechromatographed on a silica gel column eluted with MeOH–$CHCl_3$ (9:1–6:1), followed by recrystallization, to afford pure **8**.

Malloapeltic Acid (1). Pale yellow needles; mp 282°C; UV (acetone, λ_{max}, nm): 331; IR (KBr, ν_{max}, cm^{-1}): 3418, 3081, 2938, 2845, 1710, 1645,1510, 1447, 1417, 1139; ^{1}H and ^{13}C NMR data, see Table 1; FAB-MS *m/z:* 417.0 $[M+H]^+$, 387.0 $[M+H–CH_2O]^+$; EI-MS *m/z:* (relative intensity): 416 $[M]^+$ (65), 398 $[M–HO_2]^+$ (25), 386 $[M–CH_2O]^+$ (44), 368 $[M–CH_2O–HO_2]^+$ (23), 356 (7), 249 (31), 210 (100), 180 (60), 167 (67), 137 (57) (calcd for $C_{22}H_{24}O_8$, 416).

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