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LIMONOIDS FROM FRUITS OF *MELIA DUBIA* CAV. IN VIET NAM

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Abstract. Although the genus *Melia* of the Meliaceae family contains about five poorly defined species in the old-world tropics, almost every part of the plants of this genus are used as traditional herbal medicines, such as being anthelmintics, for the treatment of leprosy, eczema, asthma, malaria, fevers, and venereal diseases, as well as cholelithiasis, acariasis, and pain. The genus *Melia* is well-known as a rich and valuable source of bioactive limonoids. The genus *Melia* has 3 species (*M. azedarach*, *M. dubia*, and *M. toosendan*) in Viet Nam. *Melia dubia* Cav. has been shown to be a source of interesting tetranortriterpenoids. In addition, it is well known for its traditional medicinal properties and other biological activities such as insect or larval growth inhibition and as an antifeedant. Therefore, *Melia dubia* has been used to anti-insect and larval in agriculture in recent days. A phytochemical study from fruits of *Melia dubia* Cav. collected in Quy Hop, Nghe An province led to the isolation of three secondary metabolites, including 21*α*-O-methylmelianodiol (**1**), 21*β*-*O*-methylmelianodiol (**2**) and (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21*β*-methoxytirucalla-7,25-dien-3-one (**3**). The chemical structures of these compounds were determined based on 1D-, 2D- NMR, UV, IR, and MS analytical results and compared with reported data. All three compounds (**1**–**3**) were isolated from this plant for the first time.

Keywords: Melia dubia, limonoid, 21*α*-O-methylmelianodiol, 21*β*-O-methylmelianodiol, (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21*β*-methoxytirucalla-7,25-dien-3-one.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Although the genus *Melia* of the Meliaceae family contains about five poorly defined species in the old world tropics, almost every part of the plants of this genus are used as traditional herbal medicines, such as being anthelmintics, for the treatment of leprosy, eczema, asthma, malaria, fevers, and venereal diseases, as well as cholelithiasis, acariasis, and pain [1, 2]. The genus *Melia* is well-known as a rich and valuable source of bioactive limonoids [3]. The genus *Melia* has 3 species (*M. azedarach*, *M. dubia*, and *M. toosendan*) in Viet Nam [4, 5]. Therein, *M. dubia* Cav. is listed as a synonym of *Melia azedarach* L., it is not clear they are the same species or different. Therefore, *M. dubia* and *M. azedarach* were analyzed by DNA barcoding. The results based on Bayesian analysis of the concatenated dataset strongly support the view that *M. dubia* be considered a distinct species; not a synonym of *M. azedarach* [6].

Melia dubia is well known as a high tree in the Ghat forests of India and is generally termed the giant neem [7, 8]. The tree enjoys a broad geographical distribution (e.g., in Viet Nam, Indonesia and Philippines) and is well known for its traditional medicinal properties and other biological activities such as insect or larval growth inhibition and as an antifeedant [8]. *M. dubia* has been shown to be a source of interesting tetranortriterpenoids [9, 10]. However, to our knowledge, there are no documents on phytochemical research of fruits of *M. dubia* in Viet Nam. In this study, three triterpenoids such as 21α-*O*-methylmelianodiol (**1**), 21β-*O*-methylmelianodiol (**2**) and (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21β-methoxytirucalla-7,25-dien-3-one (**3**) have been isolated from fruits of *M. dubia*.

2. MATERIALS AND METHODS

2.1. General

Melting points were measured using Yanagimoto MP-S3 apparatus without corrections. Optical rotations were determined using a JASCO DIP-370 polarimeter. The UV spectra were obtained on an Agilent UV-3210 spectrophotometer and IR spectra were recorded on a Bruker FTIR-8501 spectrophotometer. ^{1}H- and ^{13}C-NMR, DEPT, COSY, NOESY, HSQC, and HMBC spectra were recorded on the Bruker AV-III 500 NMR spectrometer, with tetramethylsilane (TMS) as the internal standard and chemical shifts were reported in δ values (ppm). The electrospray ionization mass spectra (ESI-MS) were measured using an Agilent 1200 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230 - 400 mesh, E. Merck). The preparative HPLC was conducted on an Agilent 218 Purification System. Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F 254 plates (Merck) and the compounds were visualized by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110 °C for 10 min.

2.2. Plant material

The fruits of *Melia dubia* Cav. were collected in Quy Hop, Nghe An province, Viet Nam, in July 2018 and identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST. A voucher specimen (TDT-20180718) was deposited at the Herbarium of the School of Chemistry, Biology and Environment, Vinh University, Viet Nam.

2.3. Extraction and isolation

The dried fruits powder of *Melia dubia* (4.5 kg) was extracted with methanol at ambient temperature for five times, and total methanolic extract was evaporated under reduced pressure to give the crude methanolic extract (854.0 g). After that, the methanolic extract was suspended in water and partitioned successively with *n*-hexane, ethyl acetate and *n*-butanol to afford *n*-hexane (MDH-68.0 g), ethyl acetate (MDE-272.0 g), *n*-butanol extracts (MDB-133.0 g) and a water-soluble fraction (80.0 g), respectively. The ethyl acetate extract (272.0 g) was applied to silica gel column chromatography and was eluted by a mixture of chloroform/methanol with gradient 100:0, 50:1, 30:1, 20:1, 10:1, 5:1, 2:1, 1:1 to afford ten fractions (Frs. F1-F10). Fraction F2 (16.5 g) was subjected to silica gel column chromatography (150.0 g, 80 × 2 cm) eluting with a mixture of *n*-hexane/acetone 15:1 to obtain six fractions (Frs. F2.1-F2.6). Fraction F2.1 (2.5 g) was subjected to silica gel column chromatography (300 g, 80 × 3 cm) eluting with a mixture of *n*-hexane/acetone (7:1) to give compound **3** (21.0 mg). Fraction F2.2 (0.2 g) was separated by preparative HPLC ($MeOH/H_2O$, 80:40 to 90:10, 10 min, 16 ml min^{-1}) to afford compound **1** (15.0 mg) and **2** (12.0 mg).

Compound 1: 21α-*O*-Methylmelianodiol (**1**) White powder; $[\alpha]_D^{18}$ = -98.4 (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242 nm; IR (KBr) cm^{-1}: 3505, 2953, 1707, 1468, 1386, 1099, 1037; ^{1}H-NMR (500 MHz) and ^{13}CNMR (125 MHz), see Table 1; ESI-MS (positive ion mode) m/z: 503.4 $[M+H]^+$.

Compound 2: 21β-*O*-Methylmelianodiol (**2**) White powder; $[\alpha]_D^{18}$ = -12.9 (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 242 nm; IR (KBr) cm^{-1}: 3444, 2952, 1707, 1467, 1385, 1093; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz), see Table 1; ESI-MS (positive ion mode) m/z: 503.4 $[M+H]^+$.

Compound 3: (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21β-methoxytirucalla-7,25-dien-3-one (**3**). White needles (MeOH). m.p. 148 – 150°C. $[\alpha]_D^{18}$ = -12.4 (c = 0.1, $CDCl_3$). IR (KBr) cm^{-1}: 3447 (OH), 2950, 1714 (C=O), 1453, 1385, 1365, 1092, 986. ^{1}H- and ^{13}C-NMR: see Tables 1. ESI-MS m/z: 507.3 $[M+Na]^+$.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. A molecular formula of **1** ($C_{31}H_{50}O_5$) was assigned on the basis of its ESI-MS, ^{13}C-NMR and DEPT spectral data. The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra of **1** (Table 1) displayed characteristic signals for seven methyl groups at $\delta_{C/H}$ 23.1/C-18, 0.90/H_3-18; 13.1/C-19, 1.07/H_3-19; 20.8/C-26, 1.20/H_3-26; 22.6/C-27, 1.26/H_3-27; 22.0/C-28, 1.15/H_3-28; 25.1/C-29, 1.05/H_3-29 and 27.8/C-30, 1.08/H_3-30, a methoxy group at $\delta_{C/H}$ 55.6/3.37, three oxygenated methine groups at $\delta_{C/H}$ 110.2/C-21, 4.79/H-21; 76.6/C-23, 4.19/H-23; 76.9/C-24, 3.24/H-24, an olefinic bond at $\delta_{C/H}$ 119.3/C-7, 5.37/H-7; 147.1/C-8, and several overlapping protons for other aliphatic methines and methylenes. Besides, based on the ^{13}C-NMR chemical shifts, it was apparent that a saturated carbonyl ketone at δ_C 219.2/C-3 and an oxygenated tertiary carbon at δ_C 78.7/C-25 were present in the molecule of **1**. All of the above-mentioned NMR observations suggested that compound **1** is a triterpene possessing one methoxy group. The locations of seven methyl groups were assigned at C-4, C-10, C-13, C-14 and C-25 on the basis of the HMBC correlations seen in Fig. 1. Also, the presence of a tetrahydrofuran ring in the side chain was assigned based on the observed correlations in its 2D NMR (^{1}H–^{1}H COSY, HMQC and HMBC) spectra.

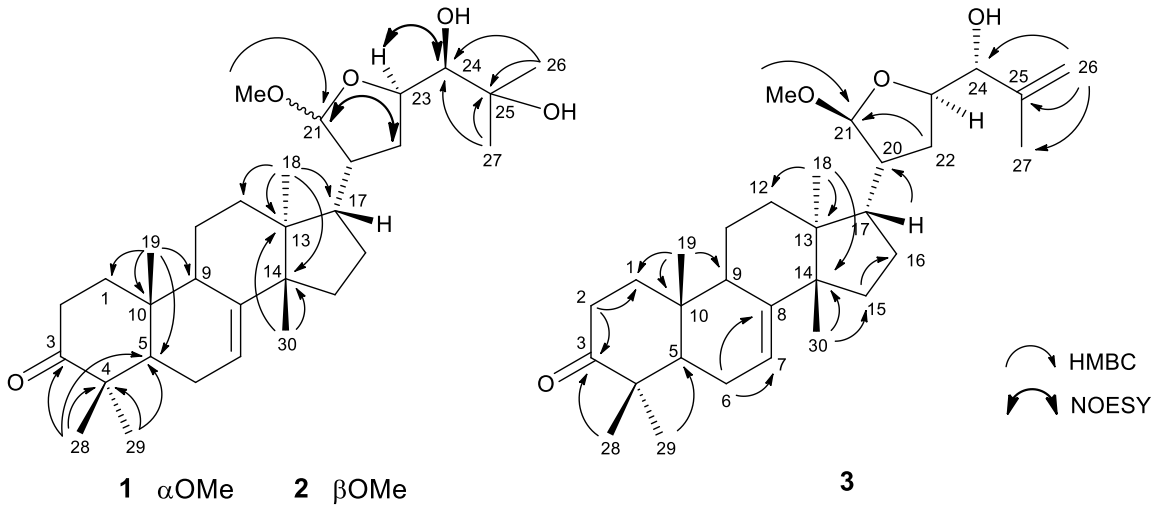

Figure 1. Chemical structures and NOESY, HMBC correlations of **1-3**.

Table 1. The NMR data of compounds **1-2** in CD_3OD, compounds **3** in $CDCl_3$, 500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR.

Position	**Compound 1**		**Compound 2**		**Compound 3**	
	δC	***δ*** H (***J*** in Hz)	***δ***C	***δ*** H (*J* in Hz)	***δ*C**	***δ*** H (*J* in Hz)
1	39.6	1.47 *m* 2.05 *m*	39.6	1.48 *m* 2.05 *m*	38.5	1.98-2.01 *m*
2	36.3	2.23 *m* 2.89 *dt* (14.5, 5.5)	36.3	2.22 *m* 2.89 *dt* (14.5, 5.5)	34.7	1.28-1.35 *m*
3	219.2	-	219.2	-	216.8	-
4	48.7	-	48.7	-	47.9	-
5	53.9	1.77 *m*	54.0	1.78 *m*	52.4	1.70-1.76 *m*
6	25.4	2.14 *m*	25.4	2.13 *m*	24.4	2.03-2.01 *m*
7	119.3	5.37 br d (3.5)	119.3	5.37 br d (3.0)	118.2	5.31 d (3.0)
8	147.0	-	147.0	-	145.6	-
9	49.8	2.39 *m*	49.8	2.39 *m*	48.3	2.31-2.36 *m*
10	36.9	-	35.9	-	35.1	-
11	18.9	1.68 *m*	18.9	1.67 *m*	17.8	1.53-1.57 *m*
12	32.9	1.54 *m* 1.86 *m*	32.5	1.53 *m* 1.94 *m*	31.7	1.66-1.70 *m*
13	44.9	-	44.8	-	43.7	-
14	52.2	-	52.0	-	50.5	-
15	35.9	2.86 *m* 2.23 *m*	35.4	2.84 *m* 2.20 *m*	34.9	1.47-1.55 *m*
16	28.4	1.36 *m* 1.97 *m*	28.3	1.39*m* 1.94 *m*	27.5	1.03 *s*
17	51.8	1.84 *m*	48.0	1.98 *m*	45.1	2.19-2.23 *m*

18	23.1	0.90 *s*	23.6	0.90 *s*	22.6	0.86 *s*
19	13.1	1.07 *s*	13.1	1.07 *s*	12.8	1.11 *s*
20	48.5	2.15 *m*	46.5	2.08 *m*	48.6	1.99-2.06 *m*
21	110.2	4.79 *br s*	106.0	4.75 *br s*	108.7	4.72 *d* (3.5)
22	35.0	1.61 *m* 1.96 *m*	33.9	1.75 *m* 1.96 *m*	33.8	1.85-1.94 *m*
23	76.9	4.19 *br s*	79.6	4.33 *br s*	78.7	4.16- 4.18 *m*
24	76.6	3.24 *br s*	78.3	3.32 *br s*	78.3	3.86 *d* (4.0)
25	78.7	-	78.5	-	144.5	-
26	26.8	1.20 *s*	26.7	1.19 *s*	113.3	5.01 *s*
27	26.6	1.26 *s*	26.4	1.24 *s*	18.2	1.76 *s*
28	22.0	1.15 *s*	22.0	1.15 *s*	24.5	1.04 *s*
29	25.1	1.05 *s*	25.1	1.05 *s*	21.6	1.11 *s*
30	27.8	1.08 *s*	28.0	1.07 *s*	27.3	1.01 *s*
21-OCH_3	55.6	3.37 *s*	55.1	3.36 *s*	55.5	3.35 *s*

The side chain possessing tetrahydrofuran ring in compound **1** was found to be similar to that of holstinone A [11], the 21-methoxy group analogue of melianodiol [12]. Furthermore, the NOESY correlations between OMe-21 and H-23 (δ_H 3.37/4.19), OMe-21/H-23 (δ_H 3.37/4.79) also showed the relative configuration of the methoxy group at C-21 in the α-orientation with respect to the tetrahydrofuran ring. Based on ESI-MS, ^{1}H-, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC spectral data and comparison with the reported spectral data of 21α-O-methylmelianodiol [13], compound **1** had been identified as 21α-*O*-methylmelianodiol.

Compound **2** was isolated as a white powder. The ESI-MS of **2** determined the molecular formula $C_{31}H_{50}O_5$, which is the same as that of **1**. Both the ^{1}H- and ^{13}C-NMR spectroscopic data of compound **2** (Table 1) were comparable to those of **1**, suggesting it was also a triterpene possessing a methoxy group. The gross structure of compound **2** was assigned in the same way as that of compound **1** based on the observed correlations in its 2D NMR (^{1}H-^{1}H COSY, HSQC and HMBC) spectra. The same correlations as that of **1** were observed in the HMBC spectrum of **2** (Fig. 1). However, the signals for C-17 and C-21 were relatively upfield at δ_C 48.0 and 106.0, while the signal for C-23 was downfield at δ_C 79.6, suggesting a γ-gauche effect of the oxygenated substituent on C-21β [14]. Additionally, in contrast to compound **1**, the NOESY correlation between OMe-21 and H-23 (δ_H 3.37/4.19) was not observed in its NOESY experiment. Therefore, the relative configuration of the methoxy group at C-21 in compound **2** was assigned as a 21β. Based on the above spectral evidence and comparison of these data with those in the literatures [13], the structure of **2** was elucidated as 21β-*O*-methylmelianodiol.

Compound **3** was attributed to molecular formula $C_{31}H_{48}O_4$ by ESI-MS giving *m/z* 507.3 ($[M+Na]^+$). The optical rotation of **3** is the same with those reported of compound in the literatures [15]. The ^{1}H-NMR and ^{13}C-NMR signals of **3** typical for the tirucallane triterpenoids showed six methyl groups at $\delta_{C/H}$ 22.6/C-18, 0.86/H_3-18; 12.8/C-19, 1.01/H_3-19; 18.2/C-27, 0.85/H_3-27; 24.5/C-28, 1.04/H_3-28; 21.6/C-29, 1.11/H_3-29; 27.3/C-30, 1.01/H_3-30, a methoxy group at $\delta_{C/H}$ 55.5/3.35/ OCH_3, nine methylenes at $\delta_{C/H}$ 38.5/C-1, 1.98-2.01/H_2-1; 34.7/C-2, 1.28-1.35/H_2-25; 24.4/C-6, 2.03-2.10/H_2-6; 17.8/C-11, 1.53-1.57/H_2-11; 31.7/ C-12, 1.66-1.70/H_2-12; 34.9/C-15, 1.47-1.55/H_2-15; 27.5/C-16, 1.03/H_2-16; 33.8/C-22, 1.85-1.94/H_2-22 and a terminal olefinic ethylene at $\delta_{C/H}$ 113.3/ C-26, 4.91-5.01/H_2-26; eight methine groups at $\delta_{C/H}$ 52.4/C-5,

1.70-1.76/H-5; 118.2/C-7, 5.31/H-7; 48.3/C-9, 2.31-2.36/H-9; 45.1/C-17, 2.19-2.23/H-17; 48.6/C-20, 1.99-2.06/H-20; 108.7/C-21, 4.72/H-21; 33.8/C-22, 1.85-1.94/H-22; 78.7/C-23, 4.16-4.18/H-23; 78.3/C-24, 3.86/H-24, six quaternary carbons at $\delta_{C/H}$ 47.9/C-4; 35.1/C-10; 43.7/C-13; 50.5/C-14; 145.6/C-8; 145.6/C-25 and a carbonyl ketone group at δ_C 216.8/C-3. The main HMBC correlations of **3** were showed in Fig. 1. The configuration at C-24 for **3** was determined by comparison of the NMR spectral data with those reported of (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21*β*-methoxytirucalla-7,25-dien-3-one in the literatures [15]. Based on the spectral evidence the structure of **3** was elucidated as (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21*β*-methoxytirucalla-7,25-dien-3-one [15].

4. CONCLUSIONS

From the methanolic extract of *Melia dubia* fruits collected in Nghe An province, three compounds were isolated and structurally elucidated as 21*α*-*O*-methylmelianodiol (**1**), 21*β*-*O*-methylmelianodiol (**2**), and (21*S*,23*R*,24*R*)-21,23-epoxy-24-hydroxy-21*β*-methoxytirucalla-7,25-dien-3-one (**3**). All three compounds (**1**–**3**) were isolated for the first time from this plant.

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CRediT authorship contribution statement. This study was conceived and designed by T.D.T. T.D.T contributed reagents/materials/analysis tools. The experiments were conducted by T.T.H, N.N.T, N.T.N.T. N.T.T., N.Q.T. and V.D.H. analyzed the data. The manuscript was drafted by N.N.T, N.Q.T., and T.D.T. T.D.T. finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Styles B. T., Pennington T. D. - A generic monograph of the Meliaceae, Blumea **22** (1975) 419.
2. Kirtikar K. R., Basu B. D. - Indian Medicinal Plants, Allahabad **1** (1935) 542.
3. Zhao L., Huo C. H., Shen L. R., Yang Y., Zhang Q., Shi Q. W. - Chemical constituents of plants from the genus *Melia*, Chem. Biodivers. **7** (2010) 839.
4. Ban N. T. - Checklist of Plant Species of Vietnam, Agriculture Publishing House **2**, 2003. (in Vietnamese)
5. Ho P. H. - An Illustrated Flora of Vietnam, Young Publisher, Ho Chi Minh, Vol. 2, 1999, pp. 82. (in Vietnamese)
6. Sivaraj I., Nithaniyal S., Bhooma V., Senthilkumar U., Parani M. - Species delimitation of *Melia dubia* Cav. from *Melia azedarach* L. complex based on DNA barcoding, Botany **96** (2018) 329.
7. Indrayan A. K., Priya. - Physical properties of the fixed oil isolated from the seeds of *Melia composita*, Indian Drugs **36** (1999) 410.
8. Koul O., Jain M. P., Sharma V. K. - Growth inhibitory and antifeedant activity of extracts from *Melia dubia* to *Spodoptera litura* and *Helicoverpa armigera* larvae, Indian J. Exp. Biol. **38** (2000) 63.

9. Purushothaman K. K., Duraiswamy K., Connolly J. D. - Tetranortriterpenoids from *Melia dubia*, Phytochemistry **23** (1984) 135.
10. Silva D. L. B., Stoecklin W., Geissman T.A. - The isolation of salannin from *Melia dubia*, Phytochemistry **8** (1969) 1817.
11. Mulholland D. A., Monkhe T. V., Taylor D. A. H., Rajab M.S. - Triterpenoids from *Turraea holstii*, Phytochemistry **52** (1999) 123.
12. Puripattanavong J., Weber S., Brecht V., Frahm A. W. - Phytochemical investigation of *Aglaia andamanica*, Planta. Med. **66** (2000) 740.
13. Xu G. H., Kim J. A., Kim S. Y., Ryu J. C., Kim Y. S. - Terpenoids and coumarins isolated from the fruits of *Poncirus trifoliata*, Chem. Pharm. Bull. **56** (2008) 839.
14. Nakanishi T., Inada A., Nishi M., Miki T., Hino R., Fujiwara T. - The structure of a new natural apotirucallane-type triterpene and the stereochemistry of the related terpenes. X-ray and ^{13}C-NMR spectral analyses, Chem. Lett. **9** (1986) 69.
15. Zhou F., Ma X. H., Li Z. J., Li W., Zheng W. M., Wang Z. B., Zeng X. M., Sun K. H., Zhang Y. H. - Four new tirucallane triterpenoids from the fruits of *Melia azedarach* and their cytotoxic activities, Chem. Biodivers. **13** (2016) 1738.