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Three novel 3,4-*seco*-podocarpane trinorditerpenoids from *Aleurites moluccana*

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ABSTRACT

Three novel 3,4-*seco*-podocarpane-type trinorditerpenoids, moluccanic acid (**1**), moluccanic acid methyl ester (**2**), and 6,7-dehydromoluccanic acid (**3**), were isolated from the twigs and leaves of *Aleurites moluccana*. Their structures were elucidated by spectroscopic methods including 2D NMR analysis. The cytotoxicity of compounds **1**–**3** was evaluated.

Aleurites moluccana (L.) Willd (Euphorbiaceae), an energy and ornamental plant, is distributed in the tropic regions of Asia and Australia. Its oil of seed kernels can be converted into biological diesel fusel.[1] Our previous phytochemical investigation of *A. moluccana* proved that it was a source of bioactive diterpenoids.[2] As part of a program aimed at searching bioactive substances from this species, continuing study on the *A. moluccana* led to the isolation of three novel 3,4-*seco*-podocarpane trinorditerpenoids, moluccanic acid (**1**), moluccanic acid methyl ester (**2**), and 6,7-dehydromoluccanic acid (**3**).[3] To the best of our knowledge, this is the first report of a *seco*-podocarpane trinorditerpenoid. All compounds (**1**–**3**) were tested for the cytotoxic activities.

Moluccanic acid (**1**)[4] was obtained as a colorless and optically active gum. Its HRESIMS displayed a quasi-molecular ion peak at *m*/*z* 297.1479 $[M+Na]^+$, consistent with a molecular formula of $C_{17}H_{22}O_3$, requiring seven degrees of unsaturation. While the UV spectrum in MeOH showed two absorption peaks at 202 (4.2), 283 (4.0) nm, the IR spectrum KBr clearly suggested the hydroxyl group at 3430 cm^{-1}, aromatic ring at 2960, 1629 1506, 1446 cm^{-1}, and carbonyl functionality at 1683 cm^{-1}. The ^1H NMR spectrum of 1 (Table 1) indicated the presence of two olefinic protons [δ_H 4.72 (1H, br s) and 4.95 (1H, br s)], three aromatic protons[δ_H 6.54 (1H, dd, J = 2.4, 8.3 Hz), 6.71 (1H, d, J = 2.4 Hz), and 6.85 (1H, d, J = 8.3 Hz)], an olefinic methyl [δ_H 1.79 (3H, s)], and one additional methyl singlet [δ_H 1.19 (3H, s)]. The ^{13}C NMR spectrum, in combination with DEPT experiments (Table 2), showed the presence of 17 carbons: two olefinic carbons at δ_C 148.2 (C-4) and 114.7 (C-18), one carboxylic carbon at δ_C 178.1 (C-3), and six aromatic carbons between δ_C 113.7 and 156.5, as well as two methyls, four methylenes, one methine, and one quaternary carbon. Since the carbonyl group, the double bond and phenyl group account for six of the seven degrees of unsaturation, compound **1** must be bicyclic. The ^1H and ^{13}C NMR data were typically a podocarpane trinorditerpenoid and similar to those of 3,4-*seco*-ring A

Table 1
^1H NMR data of compounds **1**–**3**

No.	**1**[a,c]	**2**[a,d]	**3**[b,c]
1a	2.19 (m)	2.13 (m)	2.08 (m)
1b	2.05 (m)	1.99 (m)	1.55 (overlap)
2a	2.20 (m)	2.26 (m)	2.18 (m)
2b	1.88 (m)	1.96 (m)	1.81 (overlap)
5	2.43 (dd, 2.8, 11.7)	2.40 (dd, 3.5, 11.5)	2.74 (d, 6.1)
6α	1.87 (m)	1.90 (m)	5.53 (dd, 6.1, 9.6)
6β	1.75 (m)	1.81 (m)	
7α	2.70 (m)	2.73 (m)	6.38 (d, 9.6)
7β	2.68 (m)	2.71 (m)	
11	6.71 (d, 2.4)	6.76 (d, 2.5)	6.62 (d, 2.3)
13	6.54 (dd, 2.4, 8.3)	6.62 (dd, 2.5, 8.2)	6.53 (dd, 2.3, 8.1)
14	6.85 (d, 8.3)	6.91 (d, 8.2)	6.84 (d, 8.1)
18a	4.95 (br s)	4.96 (br s)	4.74 (d, 2.2)
18b	4.72 (br s)	4.71 (br s)	4.58 (d, 2.2)
19	1.79 (s)	1.79 (s)	1.22 (s)
20	1.19 (s)	1.21 (s)	1.18 (s)
OMe		3.62 (s)	

[a] 400 MHz.
[b] 500 MHz.
[c] Measured in CD_3OD.
[d] Measured in $CDCl_3$.

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Table 2
^{13}C NMR data of compounds **1**–**3**

Position	**1**[a,c]	**2**[a,d]	**3**[b,c]
1	36.1 t	34.6 t	37.5 t
2	30.4 t	29.4 t	30.8 t
3	178.1 s	174.8 s	178.0 s
4	148.2 s	146.6 s	146.9 s
5	48.4 d	47.1 d	56.1 d
6	26.1 t	24.7 t	126.7 d
7	30.5 t	29.5 t	128.2 d
8	129.2 s	129.1 s	126.7 s
9	145.4 s	144.5 s	144.2 s
10	41.9 s	41.0 s	39.3 s
11	113.7 d	112.9 d	113.6 d
12	156.5 s	154.0 s	158.3 s
13	114.3 d	113.3 d	113.7 d
14	130.9 d	130.1 d	129.1 d
18	114.7 t	114.3 t	114.2 t
19	23.3 q	22.8 q	18.9 q
20	28.3 q	27.7 q	20.9 q
Ome		51.6 q	

[a] 100 MHz.
[b] 125 MHz.
[c] Measured in CD_3OD.
[d] Measured in $CDCl_3$.

diterpenoids 3,4-*seco*-sonderianol,[5] formosanic acid,[6] and *seco*-hinokiol.[7]

Careful analysis of the 2D NMR data (HMQC, HMBC, and COSY) helped to establish the structure of **1**, as shown (Fig. 1). The HMBC cross-peaks from H-5 (δ_H 2.43) and Me-19 (δ_H 1.79) to both C-4 (δ_C148.2) and C-18 (δ_C 114.7) indicated the position of the terminal double bond at C-4(18). The propionic acid group (C-1–C-3) was attached at C-10 by the observed ^{1}H–^{1}H COSY correlation of H-1 with H-2 and HMBC correlations (Me-20/C-10; H-1/C-3, C-10, C-20; H-2/C-1, C-3, C-10). The hydroxyl group was placed at C-12 based on the ^{1}H–^{1}H COSY correlation of H-13 with H-14 and HMBC correlations (H-11/C-8, C-10, C-12, C-13; H-14/C-7, C-9, C-12, C-13). With regard to the podocarpane derivatives that coexist in *A. molucca*,[2] the β relative configuration of H-5, and the α orientation of Me-20 could be proposed. This was further supported by the negative optical rotation ($[\alpha]_D^{16}$ −68. 94) of **1**, which is very similar to that of (5β,10α)-12-hydroxy-13-methylpodocarpa-8,11,13-trien-3-one.[2] Thus, the structure of **1** was elucidated as (5β,10α)-12-hydroxy-3,4-*seco*-podocarpa-4(18),8,11,13-tetraen-3-oic acid, named moluccanic acid.

Moluccanic acid methyl ester (**2**)[8] was isolated as a colorless gum, and was assigned the molecular formula $C_{18}H_{24}O_3$, as deduced from the positive HRESIMS molecular ion peak (*m*/*z* 311.1626 [M+Na]$^+$). The ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 2) were quite similar to those of **1**. The major difference was the presence of only one carbomethoxy signal at δ_H 3.62 in ^{1}H NMR spectrum and at δ_C 174.8 and 51.6 in ^{13}C NMR spectrum. The correlation of the methoxyl (δ_H 3.62) with the carboxyl group (C-3, δ_C 174.8) in the HMBC spectrum of **2** established the esterification position at C-3. The above evidence established the structure of **2** as (5β,10α)-12-hydroxy-3,4-*seco*-podocarpa-4(18),8,11,13-tetraen-3-oic acid A 3-methyl ester, named moluccanic acid methyl ester.

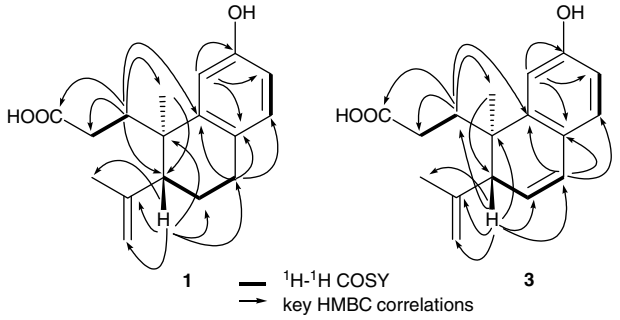

Figure 1. Structure units from ^{1}H–^{1}H COSY NMR spectra and selected HMBC correlations for **1** and **2**.

6,7-Dehydromoluccanic acid (**3**)[9] was shown to have molecular formula $C_{17}H_{20}O_3$ by HRESIMS (*m*/*z* 295.1322 ([M+Na]$^+$), corresponding to 8° of unsaturation in the molecule. Step-by-step comparison of the spectral features of **3** with those of **1** revealed that the other signals of **3** were similar to those of **1** except for the substructure between C-6 and C-7. Observation of the presence of a double bond (δ_C 126.7d, and 128.2d) and the disappearance of two methylenes in the ^{13}C NMR spectrum of **3** (Table 2) showed that a double bond exists between C-6 and C-7, which was confirmed by the mass difference of *m*/*z* = 2 and the ^{1}H–^{1}H COSY, and HMBC spectrum. The ^{1}H–^{1}H COSY correlations of H-6 with H-5 and H-7, and HMBC correlations between H-5 and C-1,C-4, C-6, C-7, C-10, C-18, Me-20, H-6 and C-4, C-5, C-8, C-9, C-10, and H-7 and C-5, C-8, C-9, C-14 were observed. Therefore, **3** was determined as (5β,10α)-12-hydroxy-3,4-*seco*-podocarpa-4(18),6,8,11,13-pentaen -3-oic acid, named 6,7-dehydromoluccanic acid.

Compounds **1**–**3** were tested for cytotoxic activities in vitro against Raji (Burkitt's lymphoma) and HepG2 (hepatocellular carcinoma) human cell lines using the method described in the literature,[10] with DDP as positive control (IC_{50} = 0.63, and 0.77 μg/ml, respectively). Compounds **1**–**3** showed weak activities against Raji cell line, with IC_{50} values of 33.71, 35.15, and 13.95 μg/ml, respectively. Compound **2** exhibited moderate cytotoxic activity against HepG2 cell line with IC_{50} value of 9.31 μg/ml, while compounds **1** and **3** were inactive in the tested systems (IC_{50} >100 μg/ml).

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3. The air-dried twigs and leaves of *A. moluccana* (6.0 kg) were extracted with 70% Me_2CO (3 × 15 L) at room temperature for three weeks and filtered. The filtrate was then concentrated, and the gummy remainder (318 g) was subjected to column chromatography over silica gel (3 kg, 200–300 mesh) eluting with $CHCl_3$–Me_2CO (1:0–0:1) to afford fractions A–E, of which faction D was separated by column chromatography over Rp-18 eluting with MeOH–H_2O (5:5–7:3) to afford fractions D_1–D_4. Fractions D_1–D_4 were combined and chromatographed on silica gel eluting with petroleum–EtOAc (2:1–1:1) and Sephadex LH-20 eluting with MeOH or Me_2CO to give **1** (15 mg), **2** (7 mg), and **3** (18 mg).
4. Moluccanic acid (**1**): colorless gum; $[\alpha]_D^{16}$ −68. 94 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.2), 283 (4.0) nm; IR (KBr) ν_{max} 3430, 2960, 2925, 1683, 1629, 1506, 1446, 1283, 1204, 1033 cm^{-1}; EIMS: *m*/*z* 274 [M]$^+$ (43), 231 (30), 213 (81), 201 (80), 173 (54), 171 (55), 159 (100), 145 (72), 131 (27), 115 (20), 107 (10), 91 (11); positive HRESIMS [M+Na]$^+$ *m*/*z* 297.1479 (calcd for $C_{17}H_{22}O_3Na$, 297.1466); ^{1}H and ^{13}C NMR, see Tables 1 and 2.
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8. Moluccanic acid methyl ester (**2**): Colorless gum; $[\alpha]_D^{13}$ −40.20 (*c* 0.40, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 207 (2.75), 283 (3.0) nm; IR (KBr) ν_{max} 3412, 3020, 2931, 1738, 1713, 1612, 1583, 1498, 1294, 1203, 1019, 895, 870 cm^{-1}; EIMS:*m*/*z* 288 [M]$^+$ (21), 245 (12), 213 (50), 201 (61), 173 (43), 159 (100), 145 (71), 131 (27); positive HRESIMS [M+Na]$^+$ *m*/*z* 311.1626 (calcd for $C_{18}H_{24}O_3Na$, 311.1623); ^{1}H and ^{13}C NMR, Tables 1 and 2.
9. 6,7-Dehydromoluccanic acid (**3**): Colorless gum; $[\alpha]_D^{16}$ +292.46 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.3), 280 (3.7) nm; IR (KBr) ν_{max} 3440, 2966, 2925, 1687, 1629, 1509, 1444, 1290, 1205, 1034, 895, 833 cm^{-1}; EIMS:*m*/*z* 272 [M]$^+$ (25), 200 (15), 199 (100), 171 (21), 158 (12), 157 (15), 128 (5); positive HRESIMS [M+Na]$^+$ *m*/*z* 295.1322 (calcd for $C_{17}H_{20}O_3Na$, 295.1310); ^{1}H and ^{13}C NMR, Tables 1 and 2.
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