

Two New Tirucallane Triterpenes with Six-Membered Hemiacetal from *Amoora dasyclada*

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Two novel tirucallane derivatives, 3 α ,21 β ,25-triol-tirucalla-21,24-epoxy-23-one (**1**) and 21 β ,25-diol-tirucalla-21,24-epoxy-3,23-dione (**2**), were isolated from *Amoora dasyclada*. Their structures were identified by spectroscopic and chemical means.

Key words: *Amoora dasyclada*, Tirucallane, Hemiacetal

Introduction

Usually, the side chains of tirucallane derivatives are long chains or cyclize to form five-membered rings [1–4]. Some five-membered ring hemiacetal side chains at C-17 were also reported successively [5–7]. However a tirucallane with a six-membered ring hemiacetal side chain at C-17 has not been published until now. In continuation of our phytochemical screening of *Amoora* species (Meliaceae), we report here the isolation and structure elucidation of two analogous tirucallane-type triterpenoids 3 α ,21 β ,25-triol-tirucalla-21,24-epoxy-23-one (**1**) and 21 β ,25-diol-tirucalla-21,24-epoxy-3,23-dione (**2**) from the twigs of *Amoora dasyclada* (a bush mainly growing in Yunnan, Hainan, and Guangdong Province of China).

Results and Discussion

Compound **1**, colorless crystals, had a molecular formula of C₃₀H₄₈O₅, as established by HR-ESIMS (found 511.3387, calcd. 511.3399 for C₃₀H₄₈O₅Na). Its IR spectrum revealed absorption bands for hydroxyl groups (3508, 3449 and 3313 cm⁻¹), a carbonyl group (1696 cm⁻¹) and a double bond (1640 cm⁻¹). The ¹³C NMR spectra showed the resonances for all thirty carbon atoms in the molecular. The DEPT spectra revealed the presence of seven methyl, eight methylene, and seven methine groups, three of which were oxygenated, six quaternary carbons. The three downfield carbons at δ = 211.2 (C-23), 118.3 (C-7),

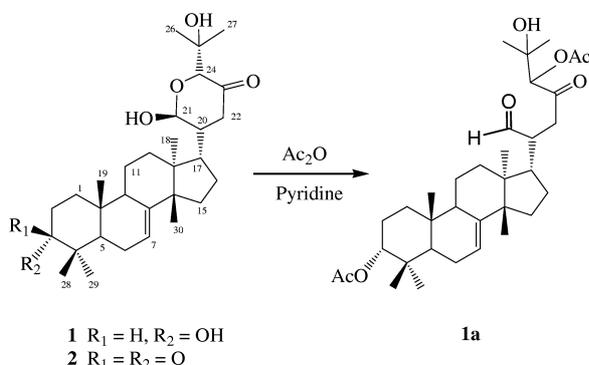
145.3 (C-8) indicated the presence of a carbonyl group and a trisubstituted double bond. These data suggested that **1** belongs to a tirucallane-euphane system with a double bond between C-7 and C-8 and a hydroxy group in 3-position [1, 4]. The chemical shifts typical of the double bond δ = 118.3 (C-7) and 145.3 (C-8) revealed the tirucallane skeleton [8]. The oxymethine proton at δ = 3.32 was attributed to C-3 and the broad singlet suggested that its configuration was eq- β H.

In the HMBC spectrum the cross signal between the hemiacetal proton [δ = 5.25 (H-21)] and the oxymethine carbon [δ = 78.4 (C-24)] indicated that there was an ether bridge across the hemiacetal and oxymethine. In the HMBC spectrum, two methyl group protons H-26 [δ = 1.14 (s)] and H-27 [δ = 1.10 (s)] also showed cross peaks to the oxymethine carbon (C-24), which suggested that the 2-hydroxyisopropyl group (C-25, C-26, C-27) was connected to the oxymethine. In addition, in the ¹H NMR spectrum, the doublets of doublets at δ = 2.35 (dd, J = 16.6, 12.7 Hz) and 2.23 (dd, J = 16.6, 5.0 Hz) indicating a methylene attached with a carbonyl group [δ = 211.2 (C-23)]. The cross signal between oxymethine proton [δ = 3.96 (H-24)] and C-23 in the HMBC spectrum indicated that C-24 was connected with this carbonyl group. Thus the side chain was determined as 21,25-diol-21,24-epoxy-23-one. Acetylation of **1** gave the derivative **1a**, which showed the presence of two acetate groups and an aldehyde group, and a downfield shift for C-24 to 90.3 was

Table 1. ^{13}C NMR data of **1**^a, **2**^b, **1a**^a (125 MHz).

	1	2	1a		1	2	1a
1	31.1	38.0	32.0	18	22.5	22.0	23.8
2	25.6	34.5	22.9	19	12.8	12.0	12.9
3	76.0	218.2	78.3	20	43.6	43.1	49.1
4	37.2	48.6	36.6	21	91.3	91.9	204.3
5	44.4	51.9	45.6	22	40.2	39.8	41.5
6	23.8	23.7	23.7	23	211.2	210.8	207.3
7	118.3	117.7	118.6	24	78.4	78.3	90.3
8	145.3	144.8	145.1	25	72.1	71.6	71.8
9	48.4	47.4	48.5	26	25.2	25.0	26.6
10	34.6	34.3	34.8	27	24.6	24.3	25.4
11	17.7	17.5	17.7	28	27.2	23.7	27.4
12	32.0	31.4	33.0	29	21.6	20.8	21.4
13	43.4	43.1	43.6	30	27.6	26.7	27.1
14	51.0	50.5	50.9	CH ₃ COO			21.4
15	33.6	33.2	33.8				20.6
16	26.2	25.7	27.7	CH ₃ COO			170.7
17	47.6	47.3	49.2				170.6

^a Measured in CDCl₃; ^b measured in CDCl₃ and CD₃OD.

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

also observed. These indicated the cleavage of the side chain by the acetylation.

The methylene proton at $\delta = 2.35$ (H-22a) and 2.23 (H-22b) showed a large coupling constant ($J = 12.7$ Hz) and a small coupling constant ($J = 5.0$ Hz) with H-20, which revealed axial orientations for H-20 and H-22a. The hemiacetal proton at $\delta = 5.25$ (H-21) showed a small coupling constant ($J = 2.2$ Hz) with H-20 indicating H-21 was in equatorial position. In the ROESY spectrum the cross peak between $\delta = 2.35$ (H-22a) and $\delta = 3.96$ (H-24) indicated their *cis*-relationship. The NOE interactions between $\delta = 0.77$ (s, 3H, Me-18) with 2.01 (H-20) and 5.25 (H-21) revealed an H-20 α (C-20S) stereochemistry indicating a tirucallane triterpene [8, 9]. So **1** was determined as 3 α ,21 β ,25-triol-tirucalla-21,24-epoxy-23-one.

Compound **2**, colorless crystals, had a molecular formula of C₃₀H₄₆O₅, as established by HR-ESIMS

Table 2. ^1H NMR data of **1**^a, **2**^b, **1a**^a (500 MHz).

	1	2	1a
1 β	1.36 (m)	1.70 (ddd 13.1, 5.3, 3.0)	1.41 (m)
1 α	1.25 (m)	1.15 (dd 12.7, 5.0)	1.32 (m)
2 β	1.79 (m)	2.46 (td 14.4, 5.5)	1.85 (m)
2 α	1.35 (m)	1.89 (dt 14.4, 3.4)	1.64 (m)
3	3.32 (br s)		4.66 (br s)
5	1.65 (dd 12.2, 5.0)	1.41 (dd 14.8, 6.9)	1.71 (dd 14.8, 6.9)
6	2.0, 1.84 (m)	1.78, 1.56 (m)	2.02, 1.92 (m)
7	5.15 (br d 3.0)	5.0 (br d 3.3)	5.25 (br d 3.0)
9	2.22 (m)	1.99 (m)	2.27 (m)
11	1.45–1.50 (m)	1.23–1.28 (m)	1.59, 1.43 (m)
12	1.93, 1.39 (m)	1.76, 1.23 (m)	1.59, 1.51 (m)
15	1.38 (m)	1.21–1.18 (m)	1.51–1.40 (m)
16	1.58, 1.23 (m)	1.52, 1.20 (m)	1.92 (m); 1.30 (m)
17	1.78 (m)	1.54–1.58 (m)	1.82 (m)
18	0.77 (s)	0.56 (s)	1.03 (s)
19	0.66 (s)	0.70 (s)	0.74 (s)
20	2.01 (m)	1.83 (m)	3.01 (br t 9.4)
21	5.25 (d 2.2)	5.03 (d 2.3)	9.90 (s)
22 β	2.35 (dd 16.6, 12.7)	2.07 (dd 16.1, 12.7)	3.15 (dd 14.2, 12.9)
22 α	2.23 (dd 16.6, 5.0)	2.01 (dd 16.1, 5.6)	2.52 (dd 14.2, 4.9)
24	3.96 (s)	3.72 (s)	4.78 (s)
26	1.14 (s)	0.92 (s)	1.23 (s)
27	1.10 (s)	0.88 (s)	1.23 (s)
28	0.79 (s)	0.71 (s)	0.81 (s)
29	0.80 (s)	0.80 (s)	0.93 (s)
30	0.88 (s)	0.70 (s)	0.93 (s)
CH ₃ COO			2.20, 2.07 (s)

^a Measured in CDCl₃; ^b measured in CDCl₃ and CD₃OD.

(calcd for C₃₀H₄₆O₅Na 509.3242, found 509.3229), which revealed that the molecular formula of **2** comprised two hydrogens less than that of **1**. Comparing the ^1H and ^{13}C NMR spectra of it with those of **1** indicated that instead of three oxymethine groups and one carbonyl group in **1**, two oxymethine groups and two carbonyl groups were presented in **2**, except the one at C-23, another carbonyl group were attributed to C-3 because chemical shift values of C-4 and C-5 shifted downfield significantly in the ^{13}C NMR spectrum of **2**. The assignment was further confirmed by cross peaks between two methyl group protons $\delta = 0.71$ (s, 3H, Me-28) and 0.80 (s, 3H, Me-29) to $\delta = 218.2$ (C-3) in the HMBC spectrum. Therefore **2** was determined to be 21 β ,25-diol-tirucalla-21,24-epoxy-3, 23-dione.

Experimental Section

General

All melting points were measured on an XRC-1 apparatus and uncorrected. Optical rotations were measured with a Horiba SEAP-300 polarimeter. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. 1D and 2D-NMR spectra were recorded on Bruker DRX-

500 spectrometers with TMS as internal standard, δ in ppm, J in Hz. MS data were recorded on a VG Autospec-3000 spectrometer.

Plant material

The twigs of *A. dasyclada* were collected at Xishuangbanna County of Yunnan Province, P.R. China, in January 2002. The plant was identified by Professor Jing-Yun Cui, Xishuangbanna Botanical Garden, Academia Sinica, the Chinese Academy of Sciences.

Extraction and isolation

The crashed and air-dried twigs (10.0 kg) were extracted with 25 l × 4 of 95% EtOH/H₂O at r.m. The extract was evaporated *in vacuo* to give a black-brown gum, which was suspended in H₂O, and extracted with CHCl₃. The CHCl₃ extract (210 g) were subjected to CC silica gel and eluted with petroleum ether-EtOAc (from 1:0 to 1:1). Nine fractions were obtained by monitoring with TLC (F1-F9). F8 were repeatedly chromatographed over silica gel and then purified on reverse phase chromatography (RP-18), eluted with CH₃OH-H₂O (from 1:1 to 100% CH₃OH) to afford **1** (87 mg) and **2** (31 mg).

3 α ,21 β ,25-triol-tirucalla-21,24-epoxy-23-one (1). Colorless crystals. – M. p. 160–162 °C. – $[\alpha]_D^{17}$ – 4.31 (CHCl₃, c 0.116). – ¹H and ¹³C NMR spectral data see Table 1 and 2, respectively. – IR (KBr): ν = 3508, 3449, 3313,

2966, 2923, 2870, 2850, 1696, 1640, 1447, 1393, 1385, 1357, 1279, 1237, 1174, 1140, 1114, 1088, 1070, 1030, 1009, 972 cm⁻¹. – MS (EI, 70 eV): m/z (%) = 430 (1), 415 (1), 397 (3), 379 (3), 213 (4), 197 (5), 187 (13), 175 (10), 187 (13), 161 (13), 133 (13), 119 (22), 105 (21), 97 (45), 81 (29), 69 (25), 58 (100). – MS (FAB⁺): m/z (%) = 489 [M+1]⁺ (11), 488 (32), 471 (51), 451 (46), 430 (22), 413 (100), 395 (12), 381 (32), 315 (9), 297 (55), 135 (77), 83 (45), 59 (63). – MS (HRESI): m/z = 511.3387 (C₃₀H₄₈O₅Na. calcd. 511.3399).

21 β ,25-diol-tirucalla-21,24-epoxy-3,23-dione (2), Colorless crystals. – M. p. 147–149 °C. – $[\alpha]_D^{17}$ – 15.38 (CHCl₃, c 0.195). – ¹H and ¹³C NMR spectral data see Table 1 and 2, respectively. – IR (KBr): ν = 3441, 2951, 1707, 1634, 1465, 1386, 1082, 1032 cm⁻¹. – MS (EI, 70 eV): m/z (%) = 428 (11), 413 (20), 395 (48), 377 (8), 321 (6), 312 (8), 299 (39), 281 (11), 271 (20), 245 (18), 213 (19), 199 (17), 187 (26), 173 (27), 159 (32), 145 (45) 133 (57), 119 (67), 105 (76), 97 (100), 81 (72), 69 (80), 55 (95). – MS (FAB⁺): m/z (%) = 487 [M+1]⁺ (100), 469 (67), 428 (37), 338 (26), 111 (88). – MS (HRESI): m/z = 509.3229 (C₃₀H₄₆O₅Na. calcd. 509.3242).

Acetylation of compound **1**. **1** (30 mg) was dissolved in pyridine (10 ml) and acetic anhydride (10 ml). The reaction mixture was left to stand for 48 h at room temperature. After the usual work-up, the product was purified by TLC [solv., CHCl₃-EtOAc (9:1)] to give the acetate **1a** (13 mg). – ¹H and ¹³C NMR spectral data see Table 1 and 2. – MS (FAB⁻): m/z = 571 [M-1]⁺ (92).

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