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A New Triterpenoid from *Amoora dasyclada*

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Abstract: Five compounds were isolated from the EtOH extraction of the stem of *Amoora dasyclada* (How et T. Chen) C. Y. Wu (Meliaceae). On the basis of spectroscopic methods, their structures were elucidated as 24, 25-epoxy-tirucall-7-ene-3, 23-dione (**1**), 24, 25, 26, 27-tetranortirucall-7-ene-3-oxo-23 (21)-lactone (**2**), taraxerone (**3**), taraxerol (**4**) and β-sitosterol (**5**). Among them, compound **1** was a new triterpenoid, compounds **3**−**5** were firstly obtained from this plant; compound **2**, an tetranortriterpenoid, was firstly isolated from natural sources, and its NMR data were assigned for the first time. Moreover, the Δ^7-bond and the Me-14 in compound **2** were never changed, which has never been found in other tetranortriterpenoids. And the biosynthetic pathway of tetranortriterpenoid was further discussed.

Key words: *Amoora dasyclada*; Meliaceae; tetranortriterpenoid; 24, 25-epoxy-tirucall-7-ene-3, 23-dione; 24, 25, 26, 27-tetranortirucall-7-ene-3-oxo-23(21)-lactone

The genus *Amoora*, comprising about 25−30 species, is mainly distributed in India and the Malay Peninsula. And six species are found in Yunnan Province of China, one of which is *Amoora dasyclada* (How et T. Chen) C. Y. Wu (Yunnan Institute of Botany, 1977). Up to now, few studies on its chemical constituents have been reported (Daulatabad and Jamkhandi, 1997; Aboutabl, 2000; Luo *et al.*, 2000a; 2000b; 2001), and tetranortriterpenoids or protolimonoids that were considered as chemotaxonomic markers of the family Meliaceae have not been isolated from this genus. However, a new protolimonoid (**1**) and a tetranortriterpenoid (**2**), together with three known compounds (**3**−**5**), were obtained from the stems of *A. dasyclada*. In this paper, we describe the isolation and structural elucidation of these compounds, and discuss the biosynthetic pathway of tetranortriterpenoid.

1 Results and Discussion

The ethanolic extract of the stems from *A. dasyclada* was partitioned between H_2O and $CHCl_3$, and the $CHCl_3$-soluble fraction was subjected to repeated silica gel CC to yield five compounds: 24, 25-epoxy-tirucall-7-ene-3, 23-dione (**1**), 24, 25, 26, 27-tetranortirucall-7-ene-3-oxo-23 (21)-lactone (**2**), taraxerone (**3**), taraxerol (**4**) and β-sitosterol (**5**). The known compounds **3** and **4** were identified by comparison of their spectroscopic data with those reported in the literature (Sakurai *et al.*, 1987), and compound **5** was identified by co-TLC with an authentic sample. The structures of compounds **1** and **2** were established by using spectroscopic method.

Compound **1** was assigned the molecular formula of $C_{30}H_{46}O_3$ by HREIMS. The ^{1}H- and ^{13}C-NMR spectra of compound **1** showed signals for seven tertiary methyls, eight methylenes, four methines (δ_C 52.6 (C-5), 48.7 (C-9), 53.2 (C-17), 32.9 (C-20)), four quaternary carbons (δ_C 48.1 (C-4), 35.3 (C-10), 43.9 (C-13), 51.6 (C-14)), a double bond (δ_C 118.4 (C-7), 145.9 (C-8)), two ketonyl carbons (δ_C 217.1 (C-3), 207.2 (C-23)), and an epoxide group (δ_C 65.9 (C-24), 61.3 (C-25)). These data are similar to those of 24, 25-epoxy-3β, 23-dihydroxy-7-tirucallene (**6**) (Luo *et al.*, 2000a; 2000b), which indicated compound **1** possessing a tirucallane or euphane skeleton. Comparing the 1D-NMR data of compound **1** with compound **6**, compound **1** contained two carbonyl groups instead of two hydroxyl groups. These structural features were confirmed by HMQC and HMBC (Fig.1). In the HMBC spectrum of compound **1**, cross signals between C-3 with H-1, H-2, H-28, H-29, and C-23 with H-20, H-22, H-24, H-26 were observed. It was presumed that the C-20 configuration belongs to the tirucallane rather than the euphane series, since tirucallane derivatives occur widely in the Meliaceae while euphanes are restricted to Melia species (Purushothaman *et al.*, 1985). And the optical rotation of compound **1**, $[\alpha]_D^{22}$ −56.6° (*c* 0.52, $CHCl_3$), was similar to that of compound **6**, $[\alpha]_D^{22}$ − 47° (*c* 0.075,

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$CHCl_3$) (Gray *et al.*, 1988), which indicated it to be the tirucallane rather than euphane series (Itoh *et al.*, 1976; Sherman *et al.*, 1980). Thus, compound **1** was determined to be 24, 25-epoxy-tirucall-7-ene-3, 23-dione.

Compound **2** possessed the molecular formula $C_{26}H_{38}O_3$ as determined by EIMS and the 1D-NMR spectra. The ^1H- and ^{13}C-NMR spectra of compound **2** were similar to those of compound **1**, except for the side chain. In the 1D-NMR spectrum of compound **2**, resonances for three methyls, an epoxide group and a ketonyl carbon in the side chain were disappeared. However, it showed signals for an ester group (δ_C 176.9 (C-23), 72.3 (C-21)), a methylene (δ_C 34.7 (C-22)) and a methine (δ_C 39.0 (C-20)). These facts may be rightly interpreted as that the side chain is cyclized with the loss of four carbons to form a lactone. The above inference was confirmed by the following HMBC correlations (Fig.1): H-22 with C-17, C-20, C-21 and C-23; H-21with C-17, C-20, C-22 and C-23; H-20 with C-17, C-21 and C-23; H-17 with C-12, C-13, C-15, C-16, C-18, C-20 and C-21. Therefore, compound **2** was assigned as 24, 25, 26, 27-tetranortirucall-7-ene-3-oxo-23 (21)-lactone. Although compound **2** had previously been synthesized by oxidation in the process of ascertaining several tirucallane derivatives' structures (Breen *et al.*, 1966; Chan *et al.*, 1970; Kumar *et al.*, 1991), compound **2** is not an artefact formed from compound **1** during isolation, which was shown by the failure of synthesizing compound **2** by subjecting compound **1** to the isolation condition.

Tetranortriterpenoids were thought to arise from Δ^7-tirucallol or Δ^7-euphol. According to Champagne *et al.* (1992), in the biosynthetic pathway of tetranortriterpenoid, the Δ^7-bond is epoxidized to a 7-epoxide, which is then opened inducing a Wagner-Meerwein shift of Me-14 to C-8, formation of OH-7, subsequently the side chain is cyclized with the loss of four carbons. However, it is interesting that the Δ^7-bond and the Me-14 in compound **2** have never been changed, which has never been found in tetranortriterpenoids from natural sources previously. The above inference indicated that the 7-epoxide and shift of Me-14 to C-8 may not occur from a precursor to compound **2**. And it was supported by the reactions in the references (Breen *et al.*, 1966; Chan *et al.*, 1970; Kumar *et al.*, 1991).

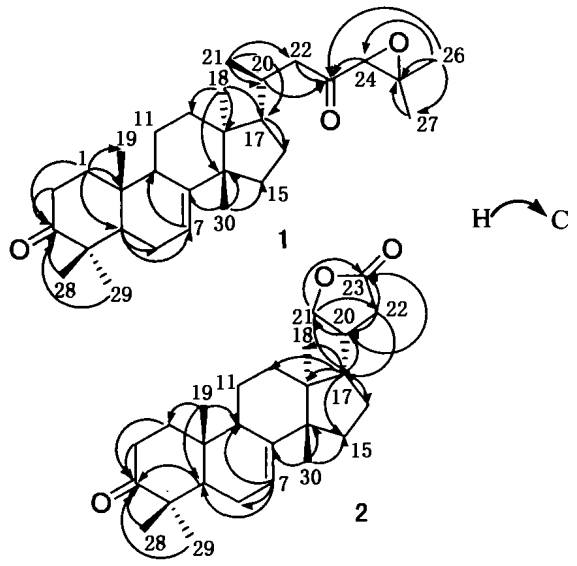

Fig.1. The structures and key HMBC correlations of compounds **1** and **2**.

2 Experimental

2.1 General experimental procedures

All melting points were measured on an XRC-1 micromelting apparatus and uncorrected. Optical rotations were measured with a Horbia SEAP-300 spectropolarimeter. IR spectrum was obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. UV spectrum was taken on a Shimadzu double-beam 210A spectrophotometer. MS spectrum was obtained with a VG Auto Spec-3000 spectrometer, at 70 eV for EI. 1D- and 2D-NMR spectra were recorded on a Bruker AM-400 and a DRX-500 MHz spectrometer with TMS as internal standard. Silica gel (200–300 mesh) for CC and GF254 for analytical TLC were from the Qindao Marine Chemical Factory, China.

2.2 Plant materials

The stems of *Amoora dasyclada* (How et T. Chen) C. Y. Wu were collected from Xishuangbanna, Yunnan Province, China, in 2002, and identified by Prof. CUI Jing-Yun, Xishuangbanna Botanical Garden, The Chinese Academy of Sciences. A voucher specimen was deposited in the herbarium of the Department of Taxonomy, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, China.

2.3 Extraction and isolation

The air-dried stems (10 kg) of *A. dasyclada* were extracted with EtOH four times at room temperature, and the solvent was evaporated *in vacuo*. The residue was suspended in H_2O and then extracted with $CHCl_3$ three times. The $CHCl_3$ layer was concentrated *in vacuo* to give 258 g of residue. Two hundred and ten grams of it was chromatographed over silica gel. The column was eluted with petroleum ether-EtOAc (from petroleum ether to petroleum-EtOAc 1:1). According to differences in composition monitored by TLC (GF_{254}), 14 fractions were obtained. Crystal from fraction 2 (13.0 g) was washed intensively with petrol-acetone (10:1) to afford compound **3** (620 mg). Crystal from fraction 4 (5.0 g) was washed intensively with

acetone to afford compound **4** (360 mg). Fraction 6 (4.18 g) was chromatographed on silica gel column eluted with petrol-Me_2CO (23:2) to give four subfractions (A–D). Crystals from fraction B (910 mg) and C (927 mg) were washed intensively with petrol-acetone (5:1) to afford compound **1** (44 mg) and **5** (300 mg), respectively. Ten grams of fraction 7 (27.2 g) was subjected to CC on silica gel with petrol-EtOAc (24:4). Ten subfractions (E–N) were collected. Crystals from fraction I (298 mg) and J (1.730 g) were washed intensively with petrol-acetone (5:1) to afford compounds **1** (90 mg) and **5** (900 mg). Fraction M (2.2 g) was subjected to CC on silica gel with petrol-EtOAc (9:1) to give three subfractions (a–c). Fraction b (1.2 g) was chromatographed over silica gel eluted with $CHCl_3$ to get four subfractions (Ⅰ–Ⅳ). Crystal from fraction Ⅰ (164 mg) was washed intensively with petrol-acetone (5:1) to afford compound **2** (35 mg).

2.4 Identification

24, 25-Epoxy-tirucall-7-ene-3, 23-dione (1) $C_{30}H_{46}O_3$, colorless needles, mp 150–151 ℃, $[\alpha]_D^{22.4}$ –56.62° ($CHCl_3$, *c*

Table 1 ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of compounds **1** and **2**

H	1	2	C	1	2
1	1.95 (1H, m)	1.92 (1H, m)	1	38.8 t	38.3 t
	1.44 (1H, m)	1.40 (1H, m)			
2	2.72 (1H, td, 5.5, 14.5)	2.70 (1H, td, 5.5, 14.5)	2	35.2 t	34.4 t
	2.19 (1H, m)	2.19 (1H, m)			
			3	217.1 s	216.5 s
			4	48.1 s	47.7 s
5	1.68 (1H, m)	1.67 (1H, m)	5	52.6 d	52.2 d
6	2.05 (2H, m)	2.06 (2H, m)	6	24.6 t	24.2 t
7	5.27 (1H, d, 3.0)	5.28 (1H, dd, 3.2, 6.4)	7	118.4 d	118.5 d
			8	145.9 s	144.6 s
9	2.25 (1H, m)	2.21 (1H, m)	9	48.7 d	48.0 d
			10	35.3 s	34.9 s
11	1.53 (2H, m)	1.55 (2H, m)	11	18.5 t	17.5 t
12	1.75 (1H, m)	1.67 (1H, m)	12	33.8 t	31.6 t
	1.61 (1H, m)	1.43 (1H, m)			
			13	43.9 s	43.6 s
			14	51.6 s	50.5 s
15	1.47 (2H, m)	1.52 (2H, m)	15	34.2 t	34.0 t
16	1.91 (1H, m)	1.90 (1H, m)	16	28.6 t	27.2 t
	1.30 (1H, m)	1.30 (1H, m)			
17	1.48 (1H, m)	1.71 (1H, m)	17	53.2 d	50.8 d
18	0.82 (3H, s)	0.78 (3H, s)	18	22.3 q	22.5 q
19	0.97 (3H, s)	0.95 (3H, s)	19	13.1 q	12.6 q
20	2.01 (1H, m)	2.15 (1H, m)	20	32.9 d	39.0 d
21	0.87 (3H, d, 6.3)	4.33 (1H, t, 8.2)	21	19.8 q	72.3 t
		3.87 (1H, t, 9.1)			
22	2.51 (1H, dd, 2.5, 15.8)	2.50 (2H, m)	22	48.3 t	34.7 t
	2.25 (1H, m)				
			23	207.2 s	176.9 s
24	3.31 (1H, s)	–	24	65.9 d	–
			25	61.3 s	–
26	1.39 (3H, s)	–	26	25.1 q	–
27	1.23 (3H, s)	–	27	18.8 q	–
28	1.01 (3H, s)	0.99 (3H, s)	28	24.8 q	24.4 q
29	1.08 (3H, s)	1.06 (3H, s)	29	21.9 q	21.4 q
30	0.98 (3H, s)	0.97 (3H, s)	30	27.7 q	27.1 q

0.521). UV λ_{max}^{MeOH} nm (log ε): 204 (3.62); IR ν_{max}^{KBr} cm^{-1}: 2 963, 2 875, 1 707, 1 454, 1 386, 1 242, 1 158, 1 111, 1 062, 999, 968, 962, 836,765; ^{1}H- and ^{13}C-NMR spectral data see Table 1; EIMS *m/z* (rel. int.): 454 $[M]^+$ (94), 439 (92), 421 (10), 397 (6), 381 (58), 367 (69), 349 (17), 340 (68), 325 (100), 311 (17), 297 (17), 283 (11), 271 (32), 258 (5), 243 (23), 229 (10), 215 (9), 201 (13), 187 (17), 173 (14), 159 (13), 141 (15), 125 (21), 113 (42), 96 (16), 83 (22), 72 (10), 59 (6); HREIMS *m/z* $[M]^+$ 454.345 8 (calcd. for $C_{30}H_{46}O_3$, 454.344 7; error: -2.4×10^{-6}).

24, 25, 26, 27-Tetranortirucall-7-ene-3-oxo-23(21)-lactone (2) $C_{26}H_{38}O_3$, colorless needles. Mp 189–190 ℃ (mp 194 ℃ (Breen *et al.*, 1966); mp 188–189 ℃ (Chan *et al.*, 1970; Kumar *et al.*, 1991)). $[\alpha]_D^{17.7}$ –73.53° ($CHCl_3$; *c* 0.102) ($[\alpha]_D$ –73° (*c* 1.1) (Breen *et al.*, 1966); $[^a]_D$ –61.5° (*c* 0.4) (Kumar *et al.*, 1991)). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 239 (2.64); IR ν_{max}^{KBr} cm^{-1}: 2 952, 1 784, 1 708, 1 472, 1 457, 1 386, 1 368, 1 178, 1 037, 1 020, 993; ^{1}H- and ^{13}C-NMR spectral data see Table 1; EIMS *m/z* (rel. int.): 398 $[M]^+$ (28), 383 (100), 365 (7), 341 (2), 325 (1), 297 (3), 271 (2), 260 (5), 245 (7), 219 (4), 203 (3), 185 (5), 178 (5), 159 (7), 145 (8), 133 (12), 119 (14), 105 (14), 95 (13), 81 (12), 67 (4), 55 (8).

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粗枝崖摩中一个新的三萜

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摘要： 从粗枝崖摩(*Amoora dasyclada* (How et T. Chen) C. Y. Wu)中分离到5个化合物。通过波谱方法鉴定为：24, 25-epoxy-tirucall-7-ene-3, 23-dione (**1**)，24, 25, 26, 27-tetranortirucall-7-ene-3-oxo-23(21)-lactone (**2**)，taraxerone (**3**)，taraxerol (**4**) and β-sitosterol (**5**)。其中化合物**1**为一个新的三萜，**3~5**为首次从该植物中分离得到。化合物**2**是首次从天然植物中分离得到的一个四降三萜，对它的碳谱和氢谱数据进行了全归属。此外化合物**2**在碳7位上的双键和14位上的甲基并未发生变化，以前文献中没有报道过与此类似的四降三萜，据此进一步讨论了四降三萜的生物合成路径。

关键词： 粗枝崖摩；楝科；四降三萜；24, 25-epoxy-tirucall-7-ene-3, 23-dione；24, 25, 26, 27-tetranortirucall-7-ene-3-oxo-23(21)-lactone

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