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Chemical constituents of *Allophylus longipes*

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[ABSTRACT] AIM: To investigate the chemical constituents of *Allophylus longipes.* **METHODS:** Compounds were isolated and purified by various chromatographic techniques and their structures were elucidated by physicochemical characteristics and spectral data. **RESULTS:** Twenty-five compounds were isolated and identified as cycloart-24-en-3*β*, 26-diol (**1**), 3-oxotrirucalla-7, 24-dien-21-oic acid (**2**), zizyberenalic acid (**3**), colubrinic acid (**4**), *ent*-4(15)-eudesmene-1*β*, 6*a*-diol (**5**), 4(15)- eudesmene-1*β*, 8*a*-diol (**6**), 4(15)-eudesmene-1*β*, 5*a*-diol (**7**), methyl asterrate (**8**), betulin (**9**), betulinic aldehyde (**10**), betulinic acid (**11**), 3*β*-hydroxy-5*a*, 8*a*-epidioxyergosta-6, 22-dien (**12**), 3-oxo-19*a*-hydroxyurs-12-en-28-oic acid (**13**), ursolic acid (**14**), scopoletin (**15**), fraxidin (**16**), cleomiscosin A (**17**), 4-hydroxy-3-methoxybenzaldehyde (**18**), 4-hydroxy-3-methoxycinnamaldehyde (**19**), 2',6'-dihydroxy-4'-methoxyacetophenone (**20**), *p*-(aminoalkyl)-benzoic acid (**21**), 4-hydroxy-3-methoxybenzoic acid (**22**), 1-*O*-*p*-coumaroylglucose (**23**), *β*-sitosterol (**24**), and poriferast-5-ene-3*β*, 4*β*-diol (**25**). **CONCLUSION:** All the compounds were isolated from *Allophylus longipes* for the first time.

[KEY WORDS] *Allophylus longipes*; Chemical constituents

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1 Introduction

The genus *Allophylus* comprises about 255 species, 6 of which are distributed in China including 3 in Yunnan Province[1]. *A. longipes* has been historically used as a folk remedy in 'Dai' ethnopharmacy for the treatment of cold and inflammation, but its chemical constituents have not been investigated up to now. In the present phytochemical study on this species, twenty-five compounds were isolated and identified as cycloart-24-ene-3*β*, 26-diol (**1**)[2], 3-oxotrirucalla-7, 24-dien-21 -oic acid (**2**)[3], zizyberenalic acid (**3**)[4], colubrinic acid (**4**)[5], *ent*-4(15)-eudesmene-1*β*, 6*a*-diol (**5**)[6], 4(15)-eudesmene-1*β*, 8*a*-diol (**6**)[7], 4(15)-eudesmene-1*β*, 5*a*-diol (**7**)[8], methyl asterrate (**8**)[9], betulin (**9**)[10], betulinic aldehyde (**10**)[10], betulinic acid (**11**)[11], 3*β*-hydroxy-5*a*, 8*a*-epidioxyergosta-6, 22-dien (**12**)[12], 3-oxo-19*a*-hydroxyurs-12-en-28-oic acid (**13**)[13], ursolic acid (**14**)[14], scopoletin (**15**)[15], fraxidin (**16**)[16], cleomiscosin A (**17**)[17], 4-hydroxy-3-methoxybenzaldehyde (**18**)[18], 4-hydroxy-3- methoxycinnamaldehyde (**19**)[19], 2', 6'- dihydroxy-4'-methoxyacetophenone (**20**)[20], *p*-(aminoalkyl)-benzoic acid (**21**)[21], 4-hydroxy-3-methoxybenzoic acid (**22**)[22], 1-*O*-*p*-coumaroylglucose (**23**)[23], *β*-sitosterol (**24**), and poriferast-5-ene-3*β*, 4*β*-diol (**25**).

2 Apparatus and Reagents

NMR spectra were run on Bruker DRX-500 and AV-400 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Autospec-3000 spectrometer or an API QSTAR Pulsar 1 spectrometer. Column chromatography was performed on silica gel (48−75 μm, Qingdao Marine Chemical Ltd., Qingdao, China), Rp-18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF_{254}, Qingdao

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Marine Chemical Co., Ltd., Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

3 Plant Material

A. longipes was collected in Xishuangbanna, Yunnan Province, China, and identified by Mr. CUI Jing-Yun, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. Cui20081124) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

4 Extraction and Isolation

The air-dried and powdered stems of *A. longipes* (10 kg) were extracted with methanol at room temperature for three times (2 d × 3) and concentrated under reduced pressure to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (107 g) was subjected to silica gel (48−75 μm, 2 kg) column chromatography (CC), eluted with a $CHCl_3$-Me_2CO (1 : 0,70 : 1, 50 : 1, 30 : 1, 20 : 1, 10 : 1, 6 : 1, 3 : 1, 2 : 1, 1 : 2) to yield 6 fractions Fr. 1−6. Fraction 2 (15 g) was chromatographed on silica gel (petroleum ether-Me_2CO, 30 : 1-1 : 2) to give five subfractions 2.1-2.5. Subfraction 2.2 (3 g) was chromatographed on silica gel (petroleum ether- Me_2CO, 30 : 1−1 : 2) to give **1** (8 mg) and **4** (5 mg) while subfraction 2.3 (4 g) was subjected to silica gel (petroleum ether- EtOAc, 20:1−1:2) to yield **2** (5 mg) and **5** (8 mg). Subfraction 2.4 (5 g) was subjected to an Rp-18 CC (MeOH-H_2O, 50%−95%) to give **19** (6 mg) and **20** (7 mg). Fraction 3 (9 g) was chromatographed on silica gel ($CDCl_3$- Me_2CO, 25 : 1−1 : 2) to give **10** (1 012 mg), **25** (622 mg) and a mixture, which was chromatographed on silica gel (petroleum ether- EtOAc, 15 : 1−1 : 2) to yield **21** (7 mg), **24** (6 mg). Fraction 4 (12 g) was subjected to an Rp-18 CC (MeOH-H_2O, 40%−95%) to give **26** (27 mg) and a mixture, which was chromatographed on silica gel (petroleum ether - Me_2CO, 25 : 1−1 : 2) to yield **13** (23 mg), **16** (31 mg) and **3** (71 mg), respectively. Fraction 5 (16 g) was subjected to an Rp-18 CC (MeOH-H_2O, 30%−95%) to give **18** (88 mg) and 5 subfractions 5.1-5.5. Subfraction 5.4 (5 g) was chromatographed on silica gel (petroleum ether-EtOAc, 20 : 1−1 : 2) to yield **11** (27 mg) and **12** (6 mg). **14** (11 mg), **22** (14 mg) and **27** (3 mg) were obtained by repeated silica gel CC, Sephadex LH-20 CC (MeOH) and further recrystallized from subfraction 5.3 (4 g). Fraction 6 (20 g) was subjected to an Rp-18 column (MeOH-H_2O, 20%−95%) to give 6 subfractions 6.1-6.6. Subfractions 6.3 (4 g) was chromatographed on silica gel (petroleum ether-Me_2CO, 16 : 1−1 : 2) to yield **6** (8 mg), **8** (24 mg) and **23** (6 mg), respectively. Subfractions 6.4 (3 g) was chromatographed on silica gel (petroleum ether-EtOAc, 18 : 1-1 : 2) to give **7** (10 mg) and **9** (40 mg). **15** (8 mg) and **17** (17 mg) were obtained by repeated silica gel CC and further recrystallized from subfraction 6.5 (2 g).

5 Identification

Compound 1 White amorphous powder (petroleum ether-EtOAc). ESI-MS *m/z* 443 $[M + H]^+$, $C_{30}H_{50}O_2$. ^1H NMR ($CDCl_3$, 500 MHz) δ : 5.29 (1H, m, H-24), 4.13 (2H, s, H-26), 3.28 (1H, dd, *J* = 4.4, 8.5 Hz, H-3), 1.79 (3H, s, H-27), 0.33 (1H, d, *J* = 4.2 Hz, H-19), 0.55 (1H, d, *J* = 4.2 Hz, H-19), 3.28 (1H, dd, *J* = 4.4, 8.5 Hz, H-3), 0.81, 0.87, 0.88, 0.95, 0.96, 1.78 (each 3H × 6). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 133.8 (s, C-25), 129.2 (d, C-24), 78.8 (d, C-3), 61.6 (t, C-26), 52.2 (d, C-17), 48.1 (s, C-10), 47.9 (d, C-8), 45.2 (s, C-13), 40.3 (s, C-4), 36.6 (t, C-22), 35.8 (d, C-20), 35.5 (t, C-12), 32.8 (t, C-15), 31.9 (t, C-1), 30.3 (t, C-2), 29.8 (t, C-19), 28.1 (t, C-7), 26.4 (t, C-16), 25.9 (t, C-23), 24.5 (t, C-11), 21.1 (t, C-6), 25.4 (q, C-29), 21.3 (q, C-27),19.3 (q, C-28), 18.1 (q, C-21), 18.0 (q, C-18), 13.9 (q, C-30). It was characterized as cycloart-24-en-3*β*, 26-diol by comparison of the spectral data with the literature[2].

Compound 2 White amorphous powder (petroleum ether-EtOAc). EI-MS *m/z* 454, $C_{30}H_{46}O_3$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 5.18(1H, m, H-7), 4.96(1H, m, H-24), 0.75, 0.87, 0.88, 0.91, 0.99, 1.35, 1.54 (each 3H × 7). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 178.9 (s, C-21), 145.4 (s, C-8), 131.8 (s, C-25), 123.4 (d, C-24), 117.8 (d, C-7), 52.2 (d, C-5), 50.1 (s, C-14), 49.4 (d, C-17), 48.6 (d, C-9), 48.4 (s, C-4), 47.4 (d, C-20), 42.1 (s, C-13), 38.2 (t, C-1), 34.8 (s, C-10), 34.7 (t, C-2), 33.2 (t, C-15), 33.2 (t, C-22), 29.8 (t, C-12), 27.0 (t, C-16), 26.9 (q, C-30), 25.7 (t, C-23), 25.3 (q, C-27), 24.1 (t, C-6), 21.4 (q, C-18), 21.3 (q, C-29), 17.4 (q, C-26), 17.3 (t, C-11), 12.4 (q, C-19). It was characterized as 3-oxotrirucalla-7, 24-dien-21-oic acid by comparison of the physical and spectral data with the literature[3].

Compound 3 White amorphous powder (petroleum ether-EtOAc). EI-MS *m/z* 452, $C_{30}H_{44}O_3$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 9.70 (1H, s, H-2), 6.57 (1H, s, H-3), 4.76 (1H,s, H-30), 4.63 (1H, s, H-30), 1.77 (3H, s, H-29), 1.01 (3H, s, H-25), 1.00 (3H, s, H-26), 0.99 (3H, s, H-27), 0.94 (3H, s, H-24). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 191.4 (d, C-2), 182.3 (s, C-28), 163.3 (d, C-3), 157.3 (s, C-29), 150.1 (s, C-20), 109.8 (t, C-29), 63.0 (d, C-5), 56.2 (s, C-17), 52.2 (d, C-18), 49.4 (d, C-9), 46.9 (d, C-19), 42.5 (s, C-8), 38.2 (t, C-13), 37.1 (t, C-22), 35.1 (t, C-7), 32.3 (t, C-21), 30.6 (t, C-15), 25.1 (t, C-12), 24.1 (t, C-11), 28.1 (q, C-23), 19.3 (q, C-29), 18.9 (q, C-25), 17.6 (q, C-26), 16.8 (t, C-6), 14.7 (q, C-27). It was characterized as zizyberenalic acid by comparison of the physical and spectral data with the literature[4].

Compound 4 White amorphous powder (petroleum ether-EtOAc). ESI-MS *m/z* 471 $[M + H]^+$, $C_{30}H_{46}O_4$. ^1H NMR ($CDCl_3$, 500 MHz) δ: 9.72 (1H, s, H-2), 4.13 (1H, d, *J* = 8.5 Hz, H-3), 5.21 (1H,s, H-30), 4.98 (1H, s, H-30). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 206.5 (d, C-2), 179.8 (s, C-28), 150.3 (s, C-20), 109.4 (t, C-29), 80.5 (d, C-1), 72.5 (d, C-3), 62.1 (d, C-5), 17 (s, C-17), 49.7 (d, C-18), 48.9 (d, C-9), 47.8

(s, C-4), 46.8 (d, C-19), 42.5 (t, C-8), 37.9 (d, C-13), 36.9 (t, C-22), 33.9 (t, C-7), 32.1 (t, C-16), 30.3 (t, C-15), 29.5 (t, C-21), 25.0 (q, C-25), 24.5 (t, C-11), 18.9 (q, C-30), 17.9 (t, C-6),16.3 (q, C-24), 14.5 (q, C-26), 14.4 (q, C-27). It was characterized as colubrinic acid by comparison of the physical and spectral data with the literature[5].

Compound 5 Colorless oil (petroleum ether- Me_2CO). EI-MS *m/z* 238, $C_{15}H_{26}O_2$. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.02 (1H, s, H-15), 4.74 (1H, s, H-15), 3.70 (1H, t, *J* = 9.8 Hz, H-1), 3.42 (1H, br, s, H-6), 0.70 (3H, s, H-14), 0.86 (3H, d, *J* = 7.0 Hz, H-12), 0.94 (3H, d, *J* = 7.0 Hz, H-13). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 146.2 (s, C-4), 107.8 (t, C-15), 78.9 (d, C-1), 66.9 (d, C-6), 55.8 (d, C-5), 49.3 (d, C-7), 41.6 (s, C-10), 36.2 (t, C-9), 35.1 (t, C-3), 31.9 (t, C-2), 25.9 (d, C-11), 21.1 (q, C-12), 18.1 (t, C-8), 16.1 (q, C-13), 11.6 (q, C-14). It was characterized as ent-4(15)-eudesmene-1*β*, 6*a*-diol by comparison of the physical and spectral data with the literature[6].

Compound 6 Colorless oil (petroleum ether-Me_2CO). EI-MS *m/z* 238, $C_{15}H_{26}O_2$. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.94 (1H, s, H-15), 4.80 (1H, s, H-15), 3.58 (1H, dd, *J* = 4.8, 11.4 Hz, H-8), 3.58 (1H, dd, *J* = 4.8, 11.4 Hz, H-8), 3.22 (1H, br, s, H-1), 0.99 (3H, d, *J* = 7.0 Hz, H-13), 0.90 (3H, d, *J* = 7.0 Hz, H-12), 0.66 (3H, s, H-14). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 150.4 (s, C-4), 109.1 (t, C-15), 84.2 (d, C-8), 80.4 (d, C-1), 57.9 (d, C-5), 50.9 (s, C-10), 43.5 (d, C-5), 40.8 (d, C-7), 38.7 (t, C-9), 36.3 (t, C-3), 33.3 (t, C-2), 32.8 (d, C-11), 27.4 (t, C-6), 22.0 (q, C-13), 16.1 (q, C-12), 13.7 (q, C-14). It was characterized as 4(15)-eudesmene-1*β*, 8*a*-diol by comparison of the physical and spectral data with the literature[7].

Compound 7 Colorless oil (petroleum ether- Me_2CO). EI-MS *m/z* 238, $C_{15}H_{26}O_2$. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.85 (1H, s, H-15), 4.75 (1H, s, H-15), 4.05 (1H, dd, *J* = 4.9, 11.6 Hz, H-1), 0.76 (3H, s, H-14). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 150.6 (s, C-4), 108.6 (t, C-15), 76.1 (s, C-5), 73.1 (s, C-1), 42.2 (s, C-10), 38.2 (d, C-7), 34.3 (t, C-6), 32.8 (d, C-11), 30.5 (t, C-2), 29.9 (t, C-9), 29.7 (t, C-3), 23.6 (t, C-8), 19.9 (q, C-13), 19.7 (q, C-12), 12.7 (q, C-14). It was characterized as 4(15)-eudesmene-1*β*, 5*a*-diol by comparison of the physical and spectral data with the literature[8].

Compound 8 White amorphous powder (petroleum ether-Me_2CO). EI-MS *m/z* 362, $C_{18}H_{18}O_8$. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 11.33, 11.80 (each s, OH), 6.91 (1H, d, *J* = 2.7 Hz, H-3), 6.82 (1H, d, *J* = 2.7 Hz, H-5), 6.37 (1H, m, H-6'), 5.84 (1H, m, H-4'), 3.93 (3H, s, H-9'), 3.74 (3H, s, H-7), 3.63 (3H, s, H-9), 2.16 (3H, s, H-7'). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 136.0 (s, C-1), 126.9 (s, C-2), 108.7 (d, C-3), 156.0 (s, C-4), 105.5 (d, C-5), 154.7 (s, C-6), 56.5 (q, C-7), 166.0 (s, C-8), 52.2 (q, C-9), 164.3 (s, C-1'), 102.0 (s, C-2'), 160.9 (s, C-3'), 106.6 (d, C-4'), 146.5 (s, C-5'), 111.1 (d, C-6'), 21.9 (q, C-7'), 172.2 (s, C-8'), 52.6 (q, C-9'). It was characterized as methyl asterrate by comparison of the physical and spectral data with the literature[9].

Compounds **9**−**23** isolated from this plant were identified as betulin[10], betulinic aldehyde[10], betulinic acid[11], 3*β*-hydroxy-5*a*, 8*a*-epidioxyergosta-6, 22-dien[12], 3-oxo-19*a*-hydroxyurs-12-en-28-oic acid[13], ursolic acid[14], scopoletin[15], fraxidin[16], cleomiscosin A[17], 4-hydroxy-3-methoxybenzaldehyde[18], 4-hydroxy-3-methoxycinnamaldehyde[19], 2', 6'-dihydroxy-4'-methoxyaceto-phenone[20], *p*-(aminoalkyl)-benzoic acid[21], 4-hydroxy-3-methoxybenzoic acid[22] and 1-*O*-*p*-coumaroylglucose[23], respectively, by comparison of the spectral data with those reported in the literatures. Compounds **24-25** were identified as *β*-sitosterol, poriferast-5-ene-3*β*, 4*β*-diol by comparing with the standard compounds. All the twenty-five compounds were isolated from *Allophylus longipes* for the first time.

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长柄异木患的化学成分

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【摘　要】 **目的**: 研究长柄异木患(*Allophylu logipess*)茎中的化学成分。**方法：**对长柄异木患茎甲醇提取物的乙酸乙酯部分进行色谱分离，根据光谱数据和理化性质确定各化合物的结构。**结果：**分离得到 25 个化合物，分别鉴定为：ycloart-24-ene-3*β*, 26-diol (**1**), 3-oxotrirucalla-7,24-dien-21-oic acid (**2**), zizyberenalic acid (**3**), 蛇藤酸 (**4**), *ent*-4(15)-eudesmene-1*β*, 6*a* -diol (**5**), 4(15)-eudesmene-1*β*, 8*a* -diol (**6**), 4(15)-eudesmene-1*β*, 5*a*-diol (**7**), 甲基埃斯特瑞 (**8**), 白桦脂醇 (**9**), 白桦脂醛(**10**),白桦脂酸(**11**), 3*β*-hydroxy-5*a*, 8*a*-epidioxyergosta-6, 22-dien (**12**), 3-oxo-19*a*-hydroxyurs-12-en-28-oic acid (**13**), 熊果酸 (**14**), 东莨菪内酯 (**15**), 楮皮啶(**16**),黄花菜木脂素 A (**17**), 香草醛 (**18**), 松柏醛(**19**), 2', 6'-dihydroxy-4'-methoxyacetophenone (**20**), *p*-(aminoalkyl)-benzoic acid (**21**), 香草酸 (**22**), 1-*O*-*p*-coumaroylglucose (**23**), *β*-谷甾醇 (**24**), poriferast-5-ene-3*β*, 4*β*-diol (**25**)。**结论：**所有化合物均为首次从长柄异木患(*Allophylu logipess*)中分离得到。

【关键词】 长柄异木患；化学成分

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