Chinese Chemical Letters Vol. 13, No. 12, pp 1181 – 1184, 2002 1181
http://www.imm.ac.cn/journal/ccl.html

Two New Triterpene Esters from *Daphniphyllum oldhami*

Zhi Yu SHAO, Da Yuan ZHU, Yue Wei GUO*

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031

Abstract: Two new triterpene esters, 2-*O*-caffeoylalphitolic acid (**1**) and methyl 2-*O*-caffeoyl-alphitolate (**2**), together with two known triterpenes were isolated from the shrub *Daphniphyllum oldhami*. The structures of new compounds were elucidated on the basis of detailed spectroscopic analysis and compared with related compound.

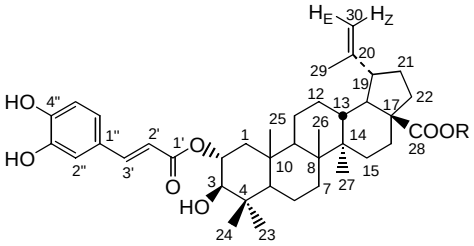
Keywords: *Daphniphyllum oldhami*, triterpenoid, 2-*O*-caffeoylalphitolic acid, methyl 2-*O*-caffeoyl -alphitolate.

The shrub *Daphniphyllum oldhami* is widely distributed in southern China, which has been used in folk medicine for the healing of wounds and as an antiinflammatory remedy[1]. A previous phytochemical investigation performed on the genus *Daphniphylllum* resulted in the isolation of alkaloids of the daphniphyline group[2] and antioxidant flavonoid[3]. No any phytochemical investigation had been done on the species *D. oldhami*. On our search for plant-derived medicinal agents, the stems of *D. oldhami* collected from Guangdong province were chemically studied.

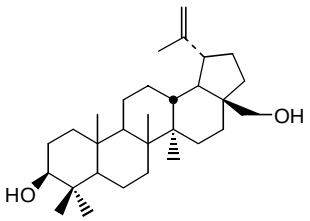
Two new triterpene esters, 2-*O*-caffeoylalphitolic acid (**1**) and methyl 2-*O*-caffeoyl-alphitolate (**2**), together with two known triterpenes, betulin (**3**)[4] and 28-hydroxyl--lup-20(30)-ene-3-one (**4**)[5], were isolated from the EtOAc-soluble fraction of an EtOH extract of the plant. This paper deals with the isolation and the structural elucidation of new triterpene esters.

The EtOH extract of the powdered stems of *D. oldhami* was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was repeatedly chromatographed over sillca gel and Sephadex LH-20 columns to afford the new compounds **1** and **2**, as well as known compounds **3** and **4**, respectively.

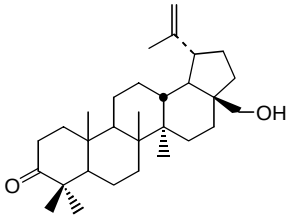
*E-mail:ywguo@mail.shcnc.ac.cn



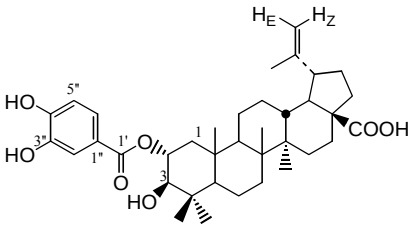
1 R=H
2 R=CH_3



3



4



5

Compound **1**[6] was isolated as a white amorphous powder, mp 234-236 ^{0}C, $[\alpha]^{20}_D$ + 3.6 (*c* 0.63, MeOH). Its negative ESIMS spectrum displays a quasimolecular ion $[M-H]^-$ at *m/z* = 633 corresponding to a molecular formula of $C_{39}H_{54}O_7$ for the compound, which was further confirmed by high resolution ESIMS spectrum (657.3776 $[M+Na]^+$, calcd. 657.3767). Analysis of ^{1}H NMR spectrum of **1** revealed that **1** contained a caffeoyl group, reflected in the ^{1}H NMR spectrum by an AX spin system for H-2′(δ 6.24, d, 1H, *J* = 16.1 Hz) and H-3′ (δ 7.51, d, 1H, *J* = 16.1 Hz), and an ABX spin system for H-2″ (δ 7.12, d, 1H, *J* = 2.2 Hz), H-5″ (δ 6.84, d, 1H, *J* = 8.1 Hz) and H-6″ (δ 6.96, dd, 1H, *J* = 8.1, 2.2 Hz). The triterpene moiety containing an isopropylidene group is very clear [IR 1640 and 890 cm^{-1}; ^{1}H NMR δ 4.67 (d, 1H, *J* = 2.1Hz, H_E-30), 4.54 (d, 1H, *J* = 2.1Hz, H_Z-30) and 1.65 (s, 3H, H_3-29)][4]. In addition, the ^{1}H NMR spectrum of **1** showed five additional methyl signals (δ 1.02, 0.99, 0.97, 0.94, and 0.84, each 3H, s), and two methine protons (δ 5.03, ddd, *J* =10.0, 10.0, 4.5 Hz, and δ 3.24, d, *J* =10.0 Hz,) which were assigned to H-2$_\beta$ and H-3$_\alpha$, respectively, on the basis of ^{1}H-^{1}H COSY spectrum. These data suggested **1** to be a derivative of alphitolic acid[7]. An overall comparison ^{13}C NMR data (**Table 1**) between the model compound **5,** 2-*O*-protocatechuoyl alphitolic acid, previously isolated from the root of *Zizyphus jujuba* var. *spinosa*[7], and compound **1** revealed that except for the difference in signals for the aromatic carboxylic acid moiety, the ^{13}C NMR data of **1** is almost the same to **5**. Hence, the structure of compound **1** was determined as 2-*O*-caffeoylalphitolic acid.

Compound **2**[8]**,** a white amorphous powder, mp 173-175°C, $[\alpha]^{20}_D$ + 10.2 (*c* 0.32, MeOH), has the molecular formular $C_{40}H_{56}O_7$ as indicated by HREIMS spectrum (648.4023 $[M]^+$, calcd. 648.4026). The ^{1}H NMR spectrum of **2** is very similar to that of **1**, except for an additional methyl singlet (δ 3.64, s, 3H). This fact suggests that compound **2** is a methylated derivative of **1**. Detailed analysis of the HMQC and

HMBC data of **2** ambiguously indicated that the methyl was located on the 28-carboxylic group. An overall comparison ^{13}C NMR data (**Table 1**) between compounds **2** and **1** also confirmed this conclusion. Consequently, **2** is methyl 2-*O*-caffeoylalphitolate.

The immune activities of compounds **1**, **2** were tested, both of them showed no significant bioactivity. Other biological assays of compounds **1**, **2** are currently being investigated.

Table 1 ^{13}C NMR data of compounds **1, 2** and **5** [100 MHz, $(CD_3)_2CO$, δ ppm][a]

Position	**1**	**2**	**5**[7]	Position	**1**	**2**	**5**[7]
1	44.5	44.6	44.8	21	30.9	30.9	31.1
2	73.2	73.3	73.8	22	37.2	37.0	37.4
3	79.8	79.8	80.0	23	28.6	28.6	28.8
4	40.1	40.1	40.4	24	17.2	17.2	17.4
5	55.7	55.7	55.9	25	16.8	16.8	17.0
6	18.6	18.7	18.9	26	16.1	16.0	16.3
7	34.6	34.6	34.8	27	14.6	14.7	14.8
8	41.1	41.2	41.3	28	177.5	176.5	178.2
9	50.8	50.8	51.0	29	19.1	19.1	19.2
10	38.8	38.9	38.7	30	109.6	109.7	109.8
11	21.4	21.4	21.6	1′	167.4	167.4	167.1
12	25.8	25.8	26.0	2′	115.7	115.8	
13	38.5	38.7	39.0	3′	145.1	145.1	
14	42.8	42.8	43.0	1″	127.0	127.1	122.9
15	30.1	30.1	30.8	2″	114.4	114.5	117.1
16	32.4	32.3	32.6	3″	146.0	146.0	145.4
17	56.3	56.9	56.6	4″	148.4	148.4	150.6
18	49.5	49.8	49.7	5″	115.9	115.9	115.4
19	47.5	47.6	47.7	6″	122.0	122.0	123.1
20	151.2	151.0	151.4	28-OCH_3		51.2	

[a] Assignments aided by 2D NMR experiments.

Acknowledgments

We are thankful to Associate Professor Bing-Hui Chen from South China Institute of Botany, Chinese Academy of Sciences for taxonomic identification. Thanks are also due to Prof. Jie-Ping Zuo for biological tests. This work was financially supported by the National Natural Science Foundation for Outstanding Youth (30125044). This work was also partly funded by the "foundation for scholars come back from abroad" provided by Ministry of Education, Ministry of Personnel, and Chinese academy of Sciences.

References and Notes

1. D. E. Gray, W. N. Leung, *Asian J. Med.*, **1971**, *7*, 409.
2. H. R. Arthur, R. P. K. Chan, S. N. Loo, *Phytochemistry*, **1965**, *4*, 627.
3. E. J. C. Gamez, L. Luyengi, S. K. Lee, L. F. Zhu, B. N. Zhou, H. H. S. Fong, J. M. Pezzuto, A. D. Kinghorn, *J. Nat. Prod.*, **1998**, *61*, 706.
4. W. F. Tino, L. C. Blair, A. Ali, W. F. Reynolds, S. McLean, *J. Nat. Prod*., **1992**, *55*, 395.
5. C. M. Hasan, J. A. Armstrong, A. I. Gray, *Biochem. Syst. Ecol.*, **1993**, *21* (5), 625.
6. Spectral data of compound **1**: UV (MeOH), λ_{max} (log ε) 327 (4.23) nm; IR (KBr) ν 3432, 2946, 1736, 1689, 1640 and 890 cm^{-1}; ^1H NMR (CD_3COCD_3, δ ppm): 0.94, 2.04 (m, 2H, H_2-1), 5.03 (ddd, 1H, *J*=10.0, 10.0, 4.5 Hz, H-2_β), 3.24 (d, 1H, *J*=10.0Hz, H-3_α), 0.89 (m, 1H, H-5), 1.44, 1.62 (m, 2H, H_2-6), 1.41 (m, 2H, H_2-7), 1.40 (m, 1H, H-9), 1.25, 1.37 (m, 2H, H_2-11), 1.04, 1.68 (m, 2H, H_2-12), 2.30 (m, 1H, H-13), 1.11, 1.50 (m, 2H, H_2-15), 1.40, 2.19 (m, 2H, H_2-16), 1.60 (m, 1H, H-18), 2.97 (m, 1H, H-19), 1.33, 1.84 (m, 2H, H_2-21), 1.45, 1.85 (m, 2H, H_2-22), 1.02 (s, 3H, H_3-23), 0.97 (s, 3H, H_3-24), 0.84 (s, 3H, H_3-25), 0.94 (s, 3H, H_3-26), 0.99 (s, 3H, H_3-27), 1.65 (s, 3H, H_3-29), 4.54 (d, 1H, *J*=2.1Hz, H_E-30), 4.67 (d, 1H, *J*=2.1Hz, H_Z-30), 6.24 (d, 1H, *J*=16.1Hz, H-2′), 7.51 (d, 1H, *J*=16.1Hz, H-3′), 7.12 (d, 1H, *J*=2.2Hz, H-2″), 6.84 (d, 1H, *J*=8.1Hz, H-5″), 6.96 (dd, 1H, *J*=8.1, 2.2Hz, H-6″); ^{13}C NMR data see **Table 1**.
7. S. S. Lee, B. F. Lin, K. C. Lin, *Phytochemistry*, **1996**, *43*, 847.
8. Spectral data of compound **2**: UV (MeOH), λ_{max} (log ε) 327 (4.19) nm; IR (KBr) ν 3432, 2946, 1736, 1697, 1633 and 890 cm^{-1}; ^1H NMR (CD_3COCD_3, δ ppm): 0.94, 2.04 (m, 2H, H_2-1), 5.03(ddd, 1H, *J*=10.0, 10.0, 4.5 Hz, H-2_β), 3.24 (d, 1H, *J*=10.0Hz, H-3_α), 0.89 (m, 1H, H-5), 1.44, 1.62 (m, 2H, H_2-6), 1.41 (m, 2H, H_2-7), 1.40 (m, 1H, H-9), 1.25, 1.37 (m, 2H, H_2-11), 1.04, 1.68 (m, 2H, H_2-12), 2.30 (m, 1H, H-13) 1.11, 1.50 (m, 2H, H_2-15), 1.40, 2.19 (m, 2H, H_2-16), 1.60 (m, 1H, H-18), 2.97 (m, 1H, H-19), 1.33, 1.84 (m, 2H, H_2-21), 1.45, 1.85 (m, 2H, H_2-22), 1.02 (s, 3H, H_3-23), 0.98 (s, 3H, H_3-24), 0.84 (s, 3H, H_3-25), 0.92 (s, 3H, H_3-26), 1.00 (s, 3H, H_3-27), 1.65 (s, 3H, H_3-29), 4.55 (d, 1H, *J*=2.1Hz, H_E-30), 4.69 (d, 1H, *J*=2.1Hz, H_Z-30); 3.64 (s, 3H, OCH_3), 6.23 (d, 1H, *J*=16.1Hz, H-2′), 7.51 (d, 1H, *J*=16.1Hz, H-3′), 7.12 (d, 1H, *J*=2.2Hz, H-2″), 6.84 (d, 1H, *J*=8.1Hz, H-5″), 6.96 (dd, 1H, *J*=8.1, 2.2Hz, H-6″); ^{13}C NMR data see **Table 1**.

Received 25 March, 2002