Chinese Chemical Letters Vol. 17, No. 1, pp 38-40, 2006
http://www.imm.ac.cn/journal/ccl.html

A New Daphnane Diterpene from *Daphne tangutica*

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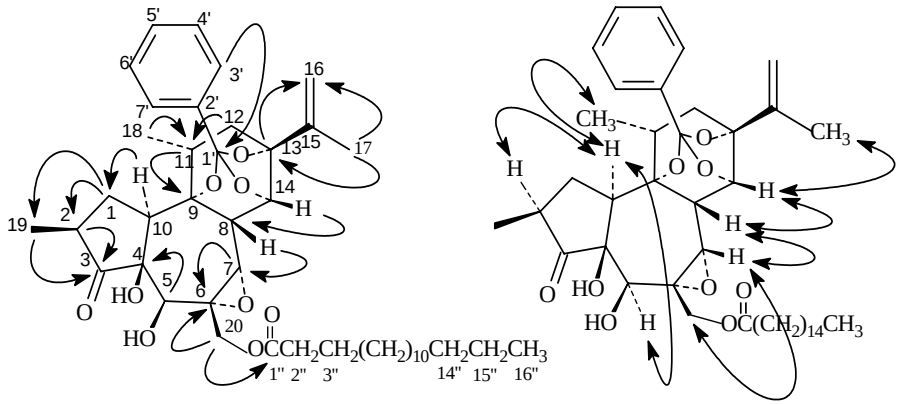
Abstract: A new daphnane diterpene was isolated from the root barks of *Daphne tangutica* Maxim. Its structure was elucidated as 1, 2α-dihydro-20-palimoyldaphnetoxin by the spectroscopic evidence including 2D-NMR.

Keywords: *Daphne tangutica*, daphnane diterpene, 1, 2α-dihydro-20-palimoyldaphnetoxin.

Daphne tangutica Maxim., a shrub of Thymelaeaceae family, meanly grows in northwest area of China at altitude of 1500-4000 m. The root barks of this plant are utilized as a traditional Tibetan medicine for releasing pain, curing rheumatism and as an abortifacient[1]. According to previous research, daphnane diterpenes were found only in the families of Euphorbiaceae and Thymelaeaceae, and were considered to be the major toxic and active constituents of Thymelaeaceae plants [2-6]. In this paper, the structural elucidation of a new daphnane diterpene isolated from the root barks of *D. tangutica* was described.

Figure 1 The key HMBC (left) and NOESY (right) correlations of **1**



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The fresh root barks (15 kg) of *D. tangutica* collected from Huzhubei mountain in Qinghai province of China were extracted three times (3×7 days) with 30 L 80% EtOH at room temperature. The concentrated syrup was suspended in H_2O and then extracted successively with petroleum ether, EtOAc and BuOH. The EtOAc extract was separated by silica gel column and then purified on preparative TLC to give **1**.

Compound **1** was obtained as a colorless glassy resin. $[\alpha]_D^{25}$ +57 (C 0.1, MeOH). It gave quasi-molecular ion peaks at *m/z* 723 ($[M+H]^+$) and 745 ($[M+Na]^+$) in positive ESI-MS, indicating its molecular weight of 722. The molecular formula was determined as $C_{43}H_{62}O_9$ by HR-ESI-MS ($[M+H]^+$ *m/z* 723.4449; calcd. 723.4467). The presence of an orthobenzoyl group was suggested based on the ^1H signals at δ 7.36 (m, 2H), 7.38 (m, 1H) and 7.76 (m, 2H), and ^{13}C signals at δ117.6 (s), 126.1 (d), 127.8 (d), 129.3 (d) and 136.0 (s). A palmitoyl group could be recognized from the ^1H signals at δ 0.87 (t, 3H, *J* = 7.0 Hz), 2.36 (t, 2H, *J* = 6.8 Hz), 1.57 (m, 2H) and 1.24-1.29 (m, 24H), and ^{13}C signals at δ 14.1 (q), 22.7 (t), 24.9 (t), 29.1-29.7 (t), 31.9 (t), 34.1 (t) and an ester carbonyl signal at δ 173.7. The daughter ions at *m/z* 640 ($[M+Na–C_6H_5CO]^+$), 624 ($[M+Na–C_6H_5CO_2]^+$), 623 ($[M+Na-C_6H_5COOH]^+$), 607 ($[M+Na–C_6H_5CO_3H]^+$) and 489 $[M+Na–C_{15}H_{31}COOH]^+$ were obtained from the cleavage of the parent ion of *m/z* 754 ($[M+Na]^+$) in tandem mass spectroscopy. These dada gave excellent support for the existence of *ortho*benzoyl and palmitoyl groups. In addition, 20 carbon signals in ^{13}C NMR belonged to one keto group, eight oxygenated C-atoms (one primary, three secondary and four tertiary), and carbons of one terminal olefinic, four methines, two methylenes and three methyls. Combining these data with a quaternary C-atom (δ 117.6, s) of orthobenzoyl group, linking to three oxygen atoms, **1** was suggested to have a daphnane diterpene skeleton of resiniferonol-9, 13, 14-orthobenzoate type.

Table 1 ^1H and ^{13}C NMR data of **1** ($CDCl_3$, δ ppm, J_{Hz})

No.	δ_C	δ_H	No.	δ_C	δ_H
1	33.3 t	2.36 m, 1.64 m	18	20.9 q	1.32 d (7.0)
2	42.9 d	2.28 m	19	12.7 q	1.11 d (6.8)
3	220.1 s		20	66.3 t	4.78 d (12), 3.71 d (12)
4	75.0 s		orthobenzoyl		
5	69.3 d	4.09 s	1'	117.6 s	
6	59.4 s		2'	136.0 s	
7	64.0 d	3.40 s	3', 7'	127.8 d	7.36 m
8	36.5 d	2.96 d (2.6)	4', 6'	126.1 d	7.76 m
9	80.1 s		5'	129.3 d	7.38 m
10	44.4 d	3.07 m	palmitoyl		
11	35.3 d	2.51 m	1"	173.7 s	
12	36.0 t	2.22 m, 1.78 m	2"	34.1 t	2.36 t (6.8)
13	84.3 s		3"	24.9 t	1.57 m
14	82.6 d	4.51 d (2.6)	4"-13"	29.1-29.7 t	1.24-1.29 m
15	146.3 s		14"	31.9 t	
16	111.4 t	5.06 brs, 4.96 brs	15"	22.7 t	
17	19.1 q	1.83 s	16"	14.1 q	0.87 t (7.0)

Comparison of the NMR data of **1** with those of daphnetoxin (5β-hydroxy-6α, 7α-epoxyresiniferonol-9, 13, 14-orthobenzoate)[7,8] showed that **1** did not possess an olefinic proton (H-1) at δ 7.66 nor a vinylic methyl (H-19) at δ 1.81 in ^{1}H NMR. A doublet of three protons at 1.11 with coupling value 6.8 Hz was assigned to H-19 (methyl at C-2). The signal for C-3 of **1** shifted downfield by 10.6 ppm in ^{13}C NMR comparing with daphnetoxin, for the absence of conjugate effect between the carbonyl and the double bond. All these showed that **1** represents a 1, 2-dihydro derivative of daphne diterpene. The NOE effect between H-2 and H-10 (**Figure 1**) indicated that the methyl (C-19) at C-2 was β oriented. According to the previous report[2-7], the long-chain fatty acid group is often locating at C-20, but seldom at C-4 and C-5. The location of the palmitoyl group at C-20 in **1** could be deduced from the cross peak between H-20 (δ 3.71, 4.78) and the carboxyl carbon (δ 173.7, s) of palmitoyl group in HMBC (**Figure 1**). This esterification led to the downfield shifts of a proton of H-20 from δ 3.90 to δ 4.78 and C-20 from δ 64.2 to δ 66.3. Therefore, the structure of **1** was elucidated as 1, 2α-dihydro-20-palimoyl-daphnetoxin.

Acknowledgments

We are grateful to the staff of the analytical group of Chengdu Institute of Biology, Chinese Academy of Sciences for measuring the MS and NMR spectra and Prof. Pan Jing-Tang of the Northwest Plateau Institute of Biology, Chinese Academy of Sciences, who identified the plant.

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Received 18 April, 2005