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Campylopin from *Delphinium campylocentrum*, the first hetidane C_{20}-diterpene, suggests a new alkaloid biogenetic pathway

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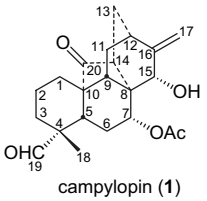
Abstract—A novel C_{20}-diterpene, campylopin (**1**), was isolated from the whole herb of *Delphinium campylocentrum.* The elucidation of its structure was accomplished through extensive spectroscopic methods. Compound **1** represents the first hetidane-type diterpene skeleton, which may imply a new biosynthetic pathway from the atisane or hetidane-type diterpene to the hetidine-type diterpenoid alkaloid.

1. Introduction

Delphinium plants (also known as Larkspur) are a large species within the genus Ranunculaceae, largely distributed throughout the northern hemisphere region, such as Asia, Europe, and North America, while a few occur in equatorial Africa. It is estimated that about 350 species of *Delphinium* exist in the world, and 173 species (150 endemic) are found in China.[1]

Immense phytochemical investigations of species of *Delphinium* have been carried out, and the C_{18}-, C_{19}-diterpenoid alkaloids, and C_{20}-diterpene as well as other biological and pharmacological components were found in these species. Representative alkaloids, methyllycaconitine (MLA) and its analogues, are the selective antagonists of the neuronal α7 nAChR receptors, which are potential targets for drug design in the treatment of Alzheimer's disease.[2]

In this study, *Delphinium campylocentrum* Maxim. was examined. Campylopin (**1**) was isolated and has a novel C_{20}-diterpene skeleton, which may imply a new biogenetic pathway in the formation of hetidine-type C_{20}-diterpenoid alkaloids.



campylopin (**1**)

Keywords: Campylopin; *Delphinium campylocentrum*; C_{20}-diterpene; Hetidane; Alkaloid biogenetic pathway.
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2. Results and discussion

Campylopin (**1**) was obtained as a colorless amorphous solid and showed $[\alpha]^{20}_{436\ nm}-18.0$ (*c* 0.2, $CHCl_3$).[3] Its molecular formula $C_{22}H_{28}O_5$ was determined by HR-ESIMS 395.1826 [M+Na] (calcd 395.1829) and indicated nine degrees of unsaturation. Each of the 22 carbon atoms displayed a corresponding signal in the ^{13}C NMR spectrum. With the aid of an HSQC experiment, 27 proton signals were assigned to each of the non-quaternary carbon atoms present in the structure. The remaining one hydrogen signal (δ 2.92, 1H, br s, disappeared when D_2O was added) was assigned to a hydroxyl group proton attached to the methine carbon atom at δ 65.7, based on the correlation between its signal and the proton signal (δ 4.26, 1H, br s) of that tertiary carbon atom in the ^1H–^1H COSY spectrum. Interpretation of the ^{13}C NMR spectrum (and DEPT experiments), as well as the ^1H NMR spectrum, revealed a ketone group, an acetate group, an aldehyde group, and an exocyclic double bond. Since each of these functional groups accounted for one degree of unsaturation, the five degrees left suggested a pentacyclic skeleton for this compound.

The planar pentacyclic skeleton was constructed through 2D-NMR experiments (Fig. 1). Four structural moieties, **I** [H_2-1–H_2-2–H_2-3], **II** [H-5–H_2-6–H-7], **III** [H-9–H_2-11–H-12–H_2-13–H-14], and **IV** [H-15–H_2-17] were established using the ^1H–^1H COSY spectrum, and were further connected sequentially involving all the quaternary carbon atoms based on the linkages implicated by the HMBC experiment. The combination of the structural moieties **I** and **II** via a quaternary carbon atom C-5 was deduced from the HMBC correlations H_3-18/C-3, H_3-18/C-5, H_3-18/C-19, and from the correlation H-19/C-4. Another series of HMBC correlation, H-7/C-8, H-7/C-9, and couplings H_2-1/C-20, H_2-11/C-10, H_2-13/C-8, as well as H-9/C-14, allowed

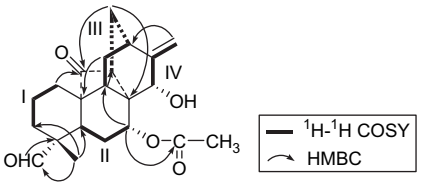


Figure 1. Key 2D-NMR correlations for **1**.

the moieties **I**, **II**, and **III** to be constructed. The correlations H_2-17/C-12 and H_2-13/C-16 inferred the linkage between C-12 and C-16. C-15, at the other end of moiety **IV**, was readily connected to the quaternary carbon atom C-8. In addition, the ketone group (δ 224.6) was assigned to C-20 in the cyclopentaxane ring based on its low chemical shift and the H_2-1/C-20 and H_2-13/C-20 HMBC correlations. The OAc group was placed at C-7 from the H-7/C-22 correlation observed in the HMBC spectrum. Thus, the planar structure of **1** was confirmed.

The relative stereochemistry of **1** was determined from the NOESY spectrum (Fig. 2). Cross-peaks between H-5/H-7 and H-7/H-15 indicated that H-7 and H-15 shared a β-orientation with H-5 and that the 7-OAc and 15-OH groups were both α-oriented. H-9 was β-oriented based on the NOESY correlation of H-9/H-15. Although the relative stereochemistry of H-12 was ambiguous at first glance, the D and E rings were assumed to be below the plane, requiring H-12β, since the NOESY correlations of H-9/H-13 or H-14/H-15 were not found to support the alternative configuration for those rings. The 18-methyl group and the 19-aldehyde group were assigned to be β- and α-oriented, respectively, due to their correlations with H-6β and H-6α. The possible three-dimensional presentation of **1** was carried out by spatial structure calculations.[4]

Interpretation of the absolute stereochemistry of **1** was completed from the CD spectrum.[5] The mild negative Cotton effect curve (λ_{max} 309 nm, θ −3950) corresponded to the C-20 ketone group's n–π* absorption. It should be noted that most

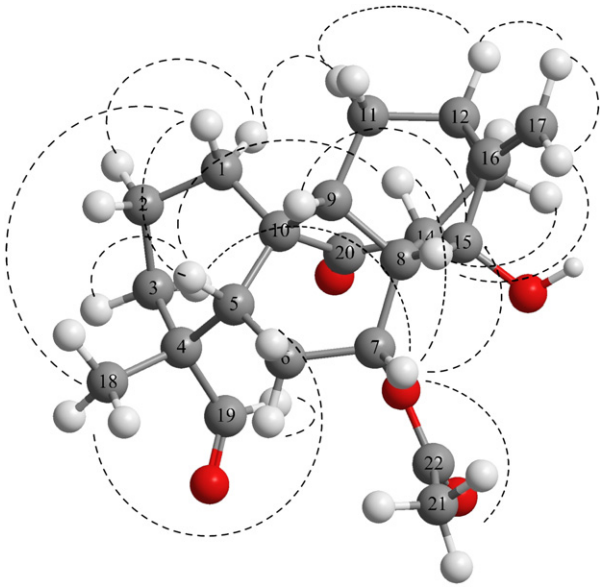


Figure 2. Selected NOESY correlations.

of the carbon atoms placed in the octant were located in the four 'back' quadrants, yet C-2 and C-19 were located in the front quadrants, having the 'opposite' sign compared to the ones at the back. From these findings, campylopin was established to be *ent*-7β-acetoxy-15β-hydroxy-16-hetiden-19-al-20-one. The structure of **1** was thus elucidated, and the compound was named as campylopin.

Plausible biogenetic pathways for campylopin (**1**) were considered (Scheme 1). Compound **1** might be derived from the hetidine-type alkaloids, which coexist with **1** in *D. campylocentrum*.[6,7] An alternative biogenetic pathway for **1** through sequential oxidation of an atisane-type diterpene is considered quite plausible. It was inferred that the hetidine-type diterpenoid alkaloids are not formed only via the atisine-type alkaloids as previously thought (Scheme 2).[8]

In conclusion, campylopin (**1**) is the first naturally occurring hetidane-type diterpene and has important significance for the biosynthesis of diterpenoid alkaloids.

3. Experimental

3.1. General

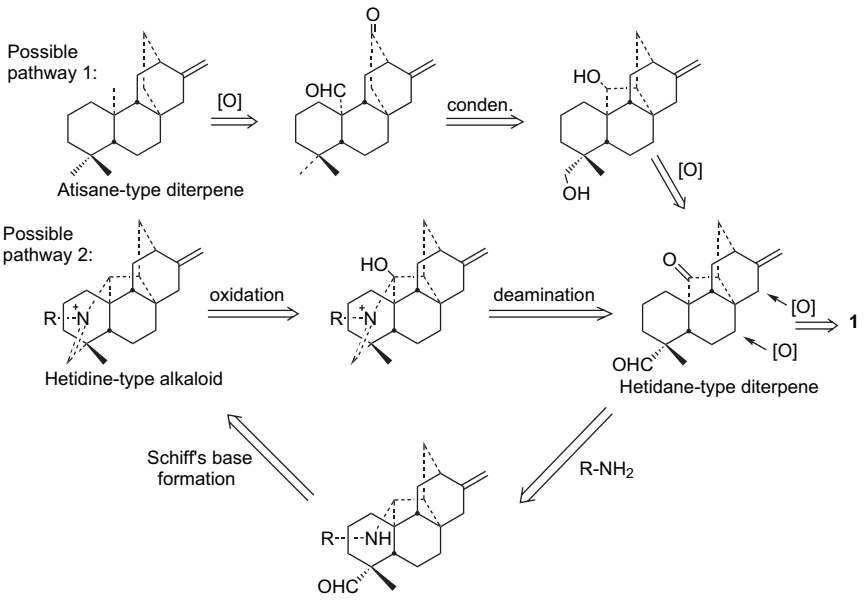
Optical rotation was determined on a Perkin–Elmer 341 polarimeter. 1D- and 2D-NMR spectra were recorded by a Bruker AV-600 spectrometer with TMS as internal standard. HR-ESIMS and ESIMS were analyzed on a VGAutoSpec 3000 spectrometer and a Finnigan TSQ 7000 mass spectrometer, respectively. The CD spectrum was recorded on a JASCO J-500C CD spectrometer. Silica gels G and H (Qingdao Haiyang Chemical Co. Ltd., China) were used for TLC and column chromatography, respectively.

3.2. Isolation and structure identification

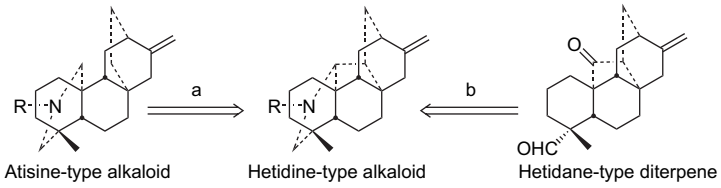
3.2.1. Plant material. *D. campylocentrum* Maxim. was collected in July 2002, in Aba autonomous prefecture in Sichuan province of China, by De Zhou, Food and Drug Administration of Aba, Sichuan. The plant was identified by Professor Qing'er Yang of the Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 00-9-14-B3) has been deposited in Sichuan Institute of Traditional Chinese Medicine, Chengdu, China.

3.2.2. Extraction and isolation. Air-dried and powdered whole herbs (2.6 kg) were percolated with 0.1 mol HCl (40 L).[9] The acidic aqueous solution was basified with concd ammonia (8 mol) to pH 10 and then extracted with ethyl acetate (15 L×3). Removal of the solvent at reduced pressure afforded a dark brown residue (17.3 g), which was chromatographed with silica gel H (200 g) and eluted with petroleum ether–acetone (3:1) to furnish 10 fractions (A–J). Fraction A (1.5 g) was subjected to silica gel H column chromatography and was eluted with cyclohexane–acetone (6:1) to yield fractions A-1 to A-4. Fraction A-4 (100 mg) was chromatographed repeatedly on a silica gel H column (cyclohexane–acetone, 5:1) to give campylopin **1** (5 mg).

3.2.3. Campylopin (1). Colorless amorphous solid; $[\alpha]^{20}_{436\ nm}$ −18.0 (*c* 0.2, $CHCl_3$). ESIMS *m*/*z* 395.3 (100%);



Scheme 1. Two biogenetic pathways for **1**.



Scheme 2. An alternative biogenetic pathway for hetidine-type alkaloid proposed by the discovery of campylopin (**1**). (a) Previous biogenetic hypothesis; (b) new biogenetic hypothesis.

Table 1. ^{1}H and ^{13}C NMR data, ^{1}H–^{1}H COSY, HMBC, and NOESY correlations of **1**

	δ_C (mult)	δ_H, [mult, *J* (Hz)]	^{1}H–^{1}H COSY	HMBC	NOESY
1	28.9 (t)	α 2.16 (m)	1β		1β,2α,11α,13β
		β 1.15 (td, 13.5, 4.5)	1α,2α,2β	2,10,20	1α,2α,5,18
2	19.1 (t)	β 2.26 (dt, 14.1, 3.6)	1β,2α,3β		2α,3α,19
		α 1.67 (dt, 14.1, 3.6)	1β,2β,3β		1α,1β,2β,3β
3	35.3 (t)	α 2.02 (br s)	3β		2β,3β,18,19
		β 1.25 (dd, 5.9, 3.1)	2α,2β,3α		2α,3α,5,18
4	48.2 (s)				
5	49.1 (d)	1.52 (dd, 14.3, 3.1)	6α		1β,3,7,14,18
6	27.3 (t)	β 2.36 (dt, 9.5, 2.9)	6α,7	5	6α,7,18
		α 1.47 (dt, 11.2, 2.4)	5,6β,7		6α,19
7	71.4 (d)	5.42 (dd, 10.6, 6.8)	6α,6β	8,9,22	5,6β,14,15,21
8	47.5 (s)				
9	47.3 (d)	2.37 (dd, 10.5, 3.6)	11α	14	15
10	52.3 (s)				
11	27.5 (t)	α 1.83 (t, 2.5)	9,12	10	1α
		β 1.80 (dt, 9.7, 2.5)			
12	34.5 (d)	2.33 (d, 3.4)	11α,13α		11α,13α,17e
13	32.2 (t)	β 1.87 (d, 14.1)	13α		1α,13α,15
		α 1.75 (dd, 14.1, 4.5)	12,13β,14	8,12,16,20	13β,12
14	45.0 (d)	2.00 (dd, 9.4, 3.1)	13α		5,7
15	65.7 (d)	4.26 (br s)	17z, 17e		7,9,13β,17z,21
16	153.4 (s)				
17	108.0 (t)	z 5.08 (br s)	15	12,16	15,17e
		e 5.00 (br s)	15	12	12,17z
18	24.0 (q)	1.00 (s)		3,5,19	1β,3α,3β,5,6β,19
19	205.5 (d)	9.80 (s)		4	2β,3α,6α,18
20	224.6 (s)				
21	21.1 (q)	2.11 (s)		22	7,15
22	171.7 (s)				

HR-ESIMS *m/z* 395.1826 [M+Na] (calcd 395.1829) $C_{22}H_{28}O_5Na$; ^1H-, ^{13}C-, and 2D-NMR data see Table 1; IR ($CHCl_3$) ν_{max} 3440, 2933, 2987, 1730, 1657, 1461, 1376, 1240, 948 cm^{-1}.

Acknowledgements

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3. The rotation at the sodium D line was too small to give meaningful value.
4. The 3D presentation of **1** was generated by the Cambridgesoft™ Chem3D Ultra 2006. In such processing, MM2 energy minimization had been applied.
5. Compound **1** could not be cultured to form crystals for X-ray single crystal diffraction due to the paucity of the material. Its absolute configuration was deduced by 2D-NMR and CD experiments.
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9. The use of HCl in the processing raised the question of whether **1** was of genuine natural occurrence. However, based on a re-examination of the extraction using methanol rather than acidic condition, we believe that **1** does coexist with other components also isolated from this plant.