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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 6259–6262

Daphnilongerine, an unprecedented fused pentacyclic ring system alkaloid from *Daphniphyllum longeracemosum* Rosenth.

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Received 22 February 2006; revised 22 June 2006; accepted 26 June 2006

Abstract—An unusual yuzurine-type alkaloid daphnilongerine (**1**), with an unprecedented fused pentacyclic skeleton in addition to seven known ones, daphnigracine (**2**), daphnezomine R, daphnigraciline, yuzurine, longistylumphyllines A, daphnilongeranin C, and calycinine A, was isolated from the fruits of *Daphniphyllum longeracemosum*. The structure and relative stereochemistry of **1** were determined by spectroscopic analysis.

Daphniphyllum alkaloids are a group of highly complex and diversified polycyclic alkaloids, which have been the challenging subjects of natural products, biogenetic pathways, and synthetic programs.[1] Despite more than 100 *Daphniphyllum* alkaloids having been isolated from the genus *Daphniphyllum* by Kobayshi, Jossang, Yue, Bodo, and their co-workers,[1–5] there is still a great interest for the discovery of unique ring system alkaloids from a biogenetic point of view.[6,7]

Daphniphyllum longeracemosum Rosenth. is an evergreen tree distributed in the Yunnan Province of China. Yue and co-workers have reported four new *Daphniphyllum* alkaloids daphnilongeranins A–D, along with four known ones, which were isolated from the leaves and stems of this plant.[4a] Our investigation has shown that the major alkaloids of the fruits of this plant, however, are of the yuzurine type.[1] Herein we reported an unprecedented pentacyclic fused ring system alkaloid daphnilongerine (**1**), together with seven known alkaloids, daphnigracine (**2**),[8] daphnezomine R,[2f] daphnigraciline,[8b] yuzurine,[8b] longistylumphyllines A,[4c] daphnilongeranin C,[4a] and calycinine A,[9] which was isolated from the fruits of *D. longeracemosum* Rosenth.

The fruits of *D. longeracemosum* were extracted with 95% EtOH. The crude extract was adjusted with 10% HCl to pH ~ 2. The acidic mixture was defatted with petroleum ether (PE), and then extracted with $CHCl_3$. The aqueous phase was basified to pH ~ 10 with saturated Na_2CO_3 and extracted with $CHCl_3$ to obtain crude alkaloids (8.0 g). The crude alkaloids were subjected to a silica gel column ($CHCl_3$/MeOH, 1:0 → 0:1, v/v) to give four fractions (1–4). Fraction 2 was chromatographed over a silica gel column (PE/EtOAc/Et_2NH, 10:1:0.25 to 1:1:0.25) followed by silica gel (PE/acetone, 8:2) to yield daphnilongerine (**1**, 0.0004%).

Daphnilongerine (**1**) was isolated as an optically active colorless solid, $[\alpha]_D^{21.5}$ +65.0 (*c* 0.98, CH_3OH). The ESIMS showed the molecular ion $[M+H]^+$ at *m*/*z* 402 and the HRESIMS established the molecular formula $C_{24}H_{36}NO_4$ (*m*/*z* 402.2641, $[M+H]^+$; calcd: 402.2644). Thus, the structure of **1** possessed eight degrees of unsaturation. The IR absorption spectrum suggested the presence of hydroxyl (3443 cm^{-1}) and two carbonyls (1735 cm^{-1} and 1705 cm^{-1}). The 1D and 2D NMR spectra (CD_3OD, Table 1) displayed 24 carbon signals consisting of two carbonyls, two sp^2 quaternary carbons assignable to one tetrasubstituted double bond, two sp^3 quaternary carbons, six sp^3 methines (two methines at δ_C 42.3 were observed by HSQC and HMBC), eight sp^3 methylene, and four methyl groups. Among them, one methylene (δ_C 60.0; δ_H 2.62 and 2.08), one methine (δ_C 71.9; δ_H 2.87–2.86), and one methyl (δ_C 43.1; δ_H

Keywords: *Daphniphyllum longeracemosum*; *Daphniphyllum* alkaloid; Daphnilongerine.
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doi:10.1016/j.tetlet.2006.06.144

Table 1. ^{1}H and ^{13}C NMR, HMBC, and ROESY correlations of **1** in CD_3OD

	δ_H[a], multi, *J* (Hz)	δ_C[b]	HMBC H → C	ROESY H → H
1α	2.62 (br d, 11.7)	60.0 (CH_2)	5, 7, 8, 9	15, 1β
β	2.08 (br d, 11.7)		7, 8, 9, 13, 24	3, 1α, 13α
2		218.1 (C)		
3	3.25 (dd, 9.2, 5.4)	44.8 (CH)	2, 4, 6, 7	18, 24, 1β, 4α, 19, 20
4α	1.51 (dd, 9.2, 13.4)	34.1 (CH_2)	2, 5, 6, 7, 8	21α, 3, 13β, 4β
β	2.21–2.15 (m)		2, 3, 5, 21	21α, 21β, 4α
5		51.6 (C)		
6	2.04–2.00 (m)	42.3 (CH)	5, 7, 8, 11	21β, 7, 12β
7	2.87–2.86 (m)	71.9 (CH)	1, 2, 4, 5, 24	24, 6
8		47.6 (C)		
9		145.6 (C)		
10		138.9 (C)		
11α	2.24–2.21 (m)	26.4 (CH_2)	9, 10	
β	1.93–1.89 (m)		17	12α
12α	2.29–2.27 (m)	25.9 (CH_2)	5, 10	11β, 12β
β	1.74–1.71 (m)		5, 6, 11	12α, 6
13α	1.65 (dd, 14.0, 8.7)	39.8 (CH_2)	1, 8, 9, 14, 15, 22	14, 13β, 1β
β	2.45 (dd, 14.0, 6.9)		1, 5, 8, 14, 22	21α, 13α, 4α
14	2.97–2.91 (m)	42.3 (CH)	9, 15, 22	15, 13α
15	3.53–3.50 (m)	53.7 (CH)		14, 1α, 16α
16α	1.95–1.92 (m)	29.3 (CH_2)	9, 10, 15	15, 17α, 16β
β	1.32–1.28 (m)		14, 15	17β, 16α
17α	2.59–2.54 (m)	41.6 (CH_2)		17β, 16α
β	2.35–2.31 (m)		9, 10, 15, 16	17α, 16β
18	2.86–2.80 (m)	40.9 (CH)	2, 19, 20	3, 19, 20
19	1.09 (d, 7.0)	19.4 (CH_3)	2, 18, 20	18
20	1.06 (d, 7.0)	18.8 (CH_3)	2, 18, 19	18
21α	3.85 (br d, 11.5)	65.7 (CH_2)	4, 5, 6, 8	21β, 13β, 4β, 4α
β	3.59 (br d, 11.5)		4, 5, 6, 8	21α, 4β, 6
22		177.5 (C)		
23	3.62 (3H, s)	51.8 (CH_3)	22	
24	2.27 (3H, s)	43.1 (CH_3)	1, 7	3, 7

[a] Measured at 400 MHz.
[b] Measured at 100 MHz.

2.27) were ascribed to those linking to a nitrogen. The chemical shift of the methylene at δ_C 65.7 suggested its linking to an oxygen. Since the carbonyl groups and the only double bond accounted for 3 out of 8 unsaturations, **1** was inferred to possess five rings.

By an extensive comparison of ^{1}H and ^{13}C NMR data of **1** with daphnigracine,[8] **1** was suggested to possess a partial structure (Fig. 1, part A) of daphnigracine. However, the presence of an unprecedented fused pentacyclic ring system for **1** has not been observed from other *Daphniphyllum* alkaloids. So, 2D NMR experiments were required to determine the whole structure of **1**.

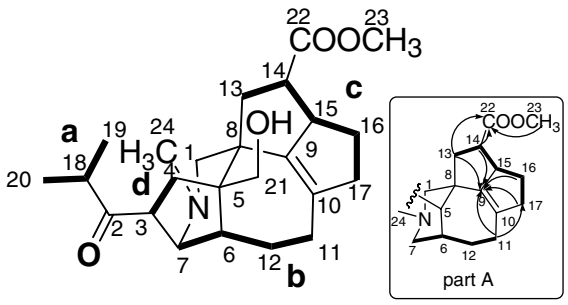


Figure 1. ^{1}H–^{1}H COSY, TOCSY, and part A of **1**.

Analysis of the ^{1}H–^{1}H COSY, TOCSY, HSQC, and HMBC spectra established four fragments, **a** (C-18 to C-19 and C-20), **b** (C-12 to C-6 and C-11,C-6 to C-7), **c** (C-14 to C-13 and C-15, C-15 to C-14 and C-16, C-16 to C-15 and C-17), and **d** (C-3 and C-4) drawn with bold bonds (Fig. 1). In part A, C-9 attaching to C-15 could be determined by the HMBC correlations of H-14/C-9, H-16α/C-9. The HMBC correlations of H-11α/C-10, C-9, H-11β/C-17 and H-17β/C-10, C-9 suggested that C-10 connected fragments **c** and **b**. The correlations between C-22 (δ_C 177.5) with the methoxyl (δ_C 51.8, δ_H 3.62) and H-14 (δ_H 2.97–2.91), indicated the only ester group was located at C-14 (δ_C 42.3). H-6 (δ_H 2.04–2.00) displayed correlations with C-7, C-5 and C-11 (δ_C 26.4) in HMBC. The connectivity between C-1 and C-13 through C-8 was provided by HMBC correlations of H_2-1/C-8, H-1β/C-13, H_2-13/C-1. The linkage of two quaternary carbons C-8 and C-9 could be determined by the HMBC correlations between H-13α and C-9, H_2-1 and C-9. A ketone carbonyl (δ_C 218.1) connected fragments **a** and **d** on the basis of its HMBC correlations with H-18 (δ_H 2.86–2.80), H_3-19 (δ_H 1.09), H_3-20 (δ_H 1.06), H-3 (δ_H 3.25) and

H_2-4 (δ_H 2.21–2.15 and 1.51). HMBC correlations of H-3/C-7 (δ_C 71.9), H-3/C-6 (δ_C 42.3), H-7/C-4 (δ_C 34.1), and H-4α/C-7 indicated the connectivity of C-3 to C-7. HMBC correlations of H_3-24 to C-1 (δ_C 60.0) and C-7, H-7 to C-1 and C-24, H-1 to C-24 and C-7 suggested that C-1, C-7, and C-24 were all linked to the same nitrogen atom. C-21 (δ_C 65.7), C-4, C-8 (δ_C 47.6) and C-6 were attached to the C-5 (δ_C 51.6) on the grounds of HMBC correlations of H_2-21/C-4, C-6, C-8, C-5, H_2-4/C-5, H-6/C-8. The above data could be explained by the presence of a new carbon ring connecting C-3–C-4–C-5–C-6–C-7 in **1** in contrast to other yuzurine-type alkaloids. Thus, the gross planar structure of daphnilongerine was assigned as shown in Figures 1 and 2.

The relative stereochemistry of **1** was deduced by the ROESY spectrum as shown in the computer-generated three-dimensional drawing (Fig. 3). The ROESY correlations of H-13α/H-14, H-14/H-15, H-15/H-16α, and H-16α/H-17α indicated that H-14, H-15 were α-oriented. H-6 and H-7 were assigned β-configuration on the basis of the correlation of H-6/H-12 β, H-6/21β, H-6/H-7. The correlation of H-3/H-4α determined H-3 was α-oriented.

The configuration of daphnilongerine (**1**) may also be deduced by the inspection of the proposed biosynthesis. From a biogenetic point of view, it could be admitted

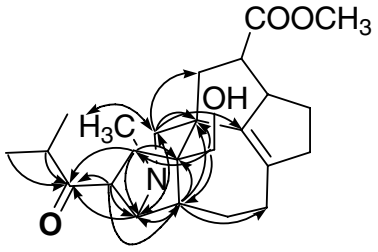


Figure 2. Key HMBC correlations of **1** (H⌒C).

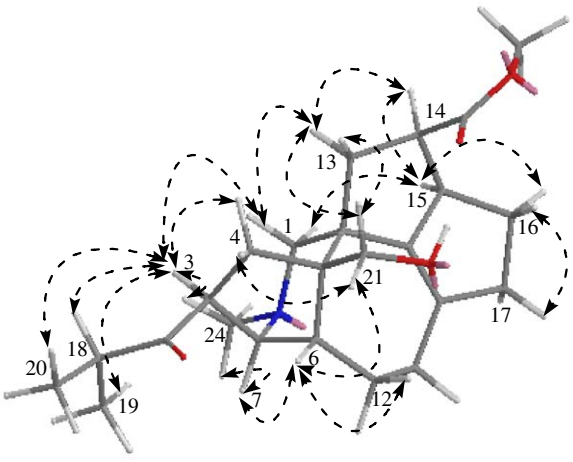
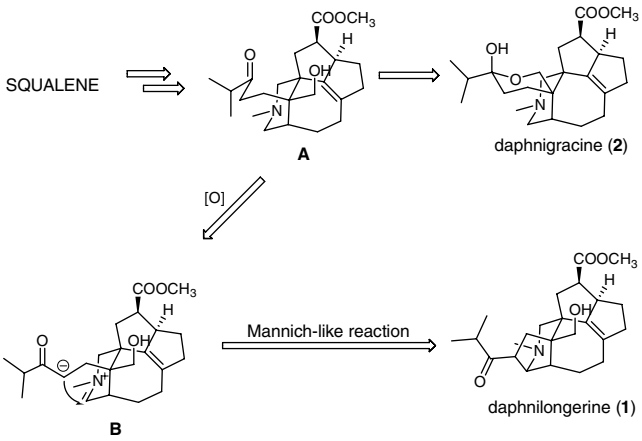


Figure 3. Key ROESY correlations and conformation generated from computer modeling (Chem. Draw. 9.0 3D).



Scheme 1. Biogenetic pathway proposed for daphnilongerine (**1**).

that yuzurine-type alkaloids represented by daphnilongerine (**1**) and daphnigracine (**2**)[8] may be produced from an intermediate A derived from squalene.[8b] The several-step oxidative reaction may convert A to imine B, the latter underwent plausible Mannich reaction to give daphnilongerine (**1**). On the other hand, the condensation reaction of A yields hemiketal product daphnigracine (**2**) (Scheme 1).

A cytotoxicity assay showed that compound **1** was not active against the human glioblastoma (U251), human lung cancer (A549), the acute myelogenous leukemia (HL60), human liver carcinoma (BEL-7402), murine leukemia (P388), and murine melanoma (B16) cell lines.

Acknowledgements

We are grateful to Professor Xun Gong, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for the collection and identification of the plant material.

Supplementary data

1D and 2D NMR; HRESIMS; and IR. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.06.144.

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