New Alkaloids from *Daphniphyllum longeracemosum* ROSENTH.

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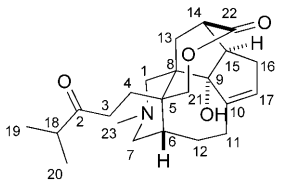
Two new pentacyclic alkaloids, daphnilongertone (**1**) and 11-hydroxydaphnigracine (**2**), together with the known congener dehydrodaphnigracine (**3**), were isolated from the fruits of *Daphniphyllum longeracemosum.* Their structures were elucidated spectroscopically. Compound **3** was isolated for the first time as a *natural* product.

Introduction. – Plants of the genus *Daphniphyllum* (Daphniphyllaceae) comprise *ca*. 30 species, of which ten grow in China [1]. They are distinguished for metabolizing a group of highly complex and diversified polycyclic alkaloids that have been challenging subjects in natural-product, synthetic, and biologically oriented chemistry. So far, more than 100 alkaloids have been isolated from the genus *Daphniphyllum* [2–10].

Daphniphyllum longeracemosum ROSENTH. is an evergreen tree distributed in Yunnan Province, China. In our search for structurally unique and biogenetically interesting *Daphniphyllum* constituents, we herein report the isolation and characterization of two new alkaloids from the fruits of *D. longeracemosum* [11]: daphnilongertone (**1**) and 11-hydroxydaphnigracine (**2**). These compounds were obtained together with the known alkaloid dehydrodaphnigracine (**3**), which had been synthesized before from daphnigracine [12].



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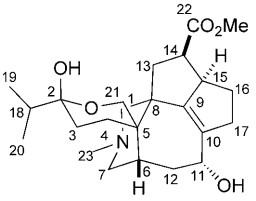

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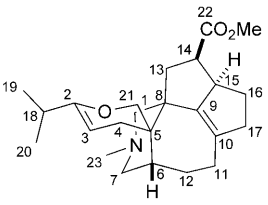

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Results and Discussion. – Daphnilongertone (**1**) was isolated as an optically active, colorless solid. Its molecular formula was determined as $C_{23}H_{33}NO_4$ (*m*/*z* 388.2499 ([*M*+H]$^+$; calc. 388.2487)) by positive-ion HR-ESI-MS, implying eight degrees of unsaturation. The IR spectrum suggested the presence of OH (3441) and C=O (1715, 1725

cm^{-1}) functions. The 1D- and 2D-NMR spectra (*Table 1*) of **1** displayed 23 carbon signals, including two C=O, one trisubstituted C=C, four CH, nine CH_2, and three Me groups, together with three quaternary C-atoms. Among them, Me(23) (δ(C) 46.5; δ(H) 2.24) and both CH_2(1) and CH_2(7) (δ(C) 58.2, 59.6; δ(H) 2.80/2.20 and 2.61/2.55, resp.) were linked to an N-atom[1]). The chemical shifts of CH_2(21) (δ(C) 70.6; δ(H) 4.98/3.69) and the quaternary C(9) at δ(C) 99.5 suggested linkage to O-atoms [9]. Since the C=O groups and the single C=C bond accounted for three out of eight degrees of unsaturation, **1** was inferred to possess five rings. Extensive analysis of the ^{1}H- and ^{13}C-NMR , ^{1}H,^{1}H-COSY, TOCSY, HSQC, and HMBC data (*Table 1*) allowed us to assign **1** an yuzurine-type alkaloidal framework [2][12].

Table 1. *^{1}H-, ^{13}C-, and HMBC-NMR Data for* **1**. At 500 and 125 MHz, resp., in CD_3OD; δ in ppm, *J* in Hz.

	δ(H)	δ(C)	HMBC (H → C)
CH_2(1)	2.80 (*m*, H_α),	58.2	C(5), C(7), C(8), C(13)
	2.20 (*m*, H_β)		C(9)
C(2)	–	217.0	–
CH_2(3)	2.50 (*m*),	35.6	C(2), C(4)
	2.46 (*m*)		C(2), C(4)
CH_2(4)	2.16 (*m*),	24.4	C(6)
	1.95 (*m*)		C(6), C(8)
C(5)	–	55.7	–
H–C(6)	1.81 (*m*)	37.0	C(8)
CH_2(7)	2.61 (*m*, H_α),	59.6	C(1), C(6), C(8), C(12), C(21)
	2.55 (*m*, H_β)		
C(8)	–	42.0	–
C(9)	–	99.5	–
C(10)	–	150.2	–
CH_2(11)	2.91 (*m*, H_α),	26.7	
	2.28 (*m*, H_β)		C(9)
CH_2(12)	1.81 (*m*, H_α),	34.8	–
	1.55 (*m*, H_β)		
CH_2(13)	2.30 (*m*, H_β),	37.2	C(8), C(9), C(14), C(15), C(22)
	1.71 (*dd*, *J* = 14.1, 6.7, H_α)		C(1), C(8), C(14), C(15)
H–C(14)	3.38 (*dd*, *J* = 10.3, 6.7)	51.7	C(9), C(13), C(15), C(22)
H–C(15)	2.98 (*m*)	55.8	C(9), C(14), C(16), C(22)
CH_2(16)	2.77 (*m*, H_α),	32.8	C(10), C(17)
	2.04 (br. *d*, *J* = 19.0, H_β)		C(10), C(14), C(15), C(17)
H–C(17)	5.44 (*s*)	128.2	C(9), C(10), C(11), C(15), C(16)
H–C(18)	2.68 (*m*)	42.2	C(2), C(19), C(20)
Me(19)	1.07 (*d*, *J* = 6.9)	18.7	C(2), C(18), C(20)
Me(20)	1.07 (*d*, *J* = 6.9)	18.7	C(2), C(18), C(19)
CH_2(21)	4.98 (br. *d*, *J* = 11.5, H_α),	70.6	C(4), C(5), C(22)
	3.69 (br. *d*, *J* = 11.5, H_β)		C(4), C(5), C(8), C(22)
C(22)	–	179.2	–
MeN	2.24 (*s*)	46.5	C(1), C(7)

[1]) Arbitrary atom numbering. For systematic names, see the *Exper. Part*.

Analysis of the 2D-NMR spectra of **1** (*Table 1*) established the fragments **a**–**d** (*Fig. 1,a*). The C(2)=O group at δ(C) 217.0 connected fragments **a** and **b**, on the basis of its HMBC correlations with CH_2(3), H–C(18), Me(19), and Me(20). HMBC correlations between Me(23) and both C(1) and C(7), H_α–C(7) and C(1), and H_α–C(1) and C(7) suggested that C(1), C(7), and C(23) were all linked to the same N-atom. Atoms C(21), C(4), C(8), and C(6) were attached to C(5), based on HMBC correlations between CH_2(21) and both C(4) and C(5), H_β–C(21) and C(8), as well as CH_2(4) and both C(6) and C(8). The linkage of the two quaternary C(8) and C(9) atoms was determined by HMBC correlations between CH_2(13) and C(8), H_β–C(13) and C(9), and H_β–C(1) and C(9). The bond between C(9) and C(15) was evident from the HMBC correlations between C(9) and both H–C(14) and H–C(15). Further, C(9) was linked to C(10), on the basis of the HMBC correlation between H–C(17) and both C(9) and C(10). The connectivity between fragments **c** and **d** *via* C(10) was inferred from HMBC correlations between H–C(17) and C(11), and between CH_2(16) and C(10). A HMBC correlation between CH_2(21) and C(22) indicated the presence of a seven-membered lactone. Thus, the planar structure of **1** could be derived as shown.

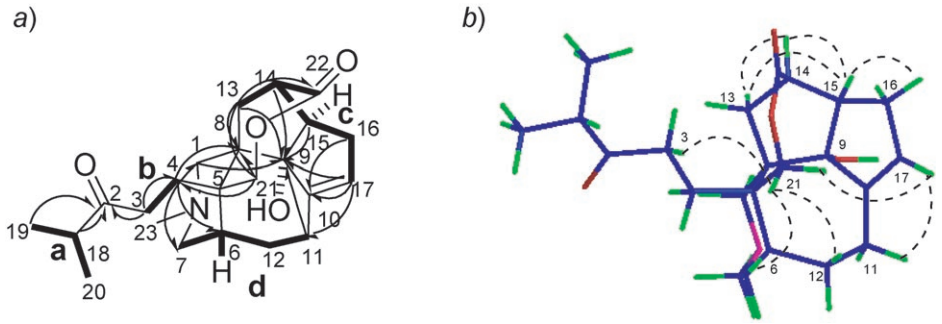

Fig. 1. a) *^{1}H,^{1}H-COSY and TOCSY* (▬), *as well as key HMBC* (H → C) *correlations for* **1**; b) *key ROESY* (- - -) *correlations for* **1**

The relative configuration of **1** was deduced from the ROESY spectrum, in combination with a computer-generated 3D model (*Fig. 1,b*). The ROESY correlations between H_α–C(13) and H–C(14), between H–C(14) and H–C(15), and between H–C(15) and H_α–C(16) indicated that H–C(14) and H–C(15) were α-oriented, and that the lactone C=O group was β-configured. Since the 9-OH function had to be on the opposite side of the seven-membered lactone ring, it was α-oriented. From these data, the structure of daphnilongertone (**1**) was identified as (4*S**,4a*R**,8*R**,8a*R**,11a*R**,11b*R**)-1,2,3,4,4a,5,8,8a,9,11a-decahydro-11a-hydroxy-2-methyl-4a-(4-methyl-3-oxopentyl)-7*H*-4,11-ethano-8,11b-methanocyclopenta[5,6]oxocino[4,3-*c*]pyridin-7-one.

11-Hydroxydaphnigracine (**2**) was isolated as an optically active, colorless solid. Positive-ion HR-ESI-MS established the molecular formula $C_{24}H_{37}NO_5$ (*m/z* 420.2755 ($[M+H]^+$, calc. 420.2749)), in accord with seven degrees of unsaturation. The IR spectrum indicated the presence of OH (3424) and C=O (1738 cm^{-1}) functions. The ^{13}C-NMR spectrum of **2** (*Table 2*) showed 24 signals, including one C=O group, a

Table 2. *^{1}H-, ^{13}C-, and HMBC-NMR Data for* **2**. At 400 and 100 MHz, resp., in CD_3OD; δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC H → C
CH_2(1)	2.32 (*d*, J = 11.7, H_α),	59.9	C(5), C(7), C(8), C(9), C(13), C(23)
	2.30 (*d*, J = 11.7, H_β)		C(5), C(7), C(8), C(9), C(13), C(23)
C(2)	–	98.9	–
CH_2(3)	1.69 (*m*, H_α),	25.0	C(4), C(2)
	1.41 (*m*, H_β)		C(4), C(5)
CH_2(4)	1.97 (*m*, H_α),	23.2	C(2), C(5)
	1.69 (*m*, H_β)		C(2), C(3), C(6)
C(5)	–	36.6	–
H–C(6)	2.39 (*m*)	34.0	C(23)
CH_2(7)	2.66 (*d*, J = 12.9, H_β),	55.6	C(1), C(5), C(12)
	2.57 (*dd*, J = 12.9, 3.5, H_α)		C(1), C(12), C(23)
C(8)	–	47.7	–
C(9)	–	150.3	–
C(10)	–	136.6	–
H–C(11)	3.98 (*t*, J = 3.1)	68.4	C(6), C(9), C(10), C(17)
CH_2(12)	2.39 (*m*, H_β),	35.5	C(6)
	1.86 (*m*, H_α)		C(6), C(5), C(11)
CH_2(13)	2.82 (*dd*, J = 15.0, 2.5, H_β),	40.9	C(1), C(5), C(8), C(14), C(15), C(22)
	1.76 (*m*, H_α)		C(1), C(5), C(8), C(9), C(14), C(22)
H–C(14)	2.95 (*ddd*, J = 9.7, 7.4, 2.5)	43.2	C(8), C(9), C(13), C(22)
H–C(15)	3.48 (*m*)	56.4	C(9), C(10)
CH_2(16)	1.86 (*m*, H_β),	28.4	C(9), C(10), C(15), C(17)
	1.39 (*m*, H_α)		C(14), C(15), C(17)
CH_2(17)	2.88 (*m*, H_β),	41.1	C(9), C(10), C(16)
	2.45 (*dd*, J = 15.0, 8.2, H_α)		C(9), C(10), C(15), C(16)
H–C(18)	1.72 (*m*)	39.9	C(2), C(3), C(19), C(20)
Me(19)	0.92 (*d*, J = 6.9)	17.5	C(2), C(18), C(20)
Me(20)	0.92 (*d*, J = 6.9)	17.0	C(2), C(18), C(19)
CH_2(21)	4.32 (*d*, J = 12.6, H_β),	63.8	C(2), C(4), C(6), C(8)
	3.57 (*d*, J = 12.6, H_α)		C(2), C(4), C(5), C(6)
C(22)	–	176.5	–
MeN	2.25 (*s*)	45.4	C(1), C(6), C(7)
MeO	3.61 (*s*)	51.5	C(22)

tetrasubstituted C=C bond, three additional quaternary C-atoms, five CH, nine CH_2, and four Me groups. Thereby, CH_2(1) (δ(C) 59.9; δ(H) 2.32/2.30), CH_2(7) (δ(C) 55.6; δ(H) 2.66/2.57), and Me(23) (δ(C) 45.4; δ(H) 2.25) were connected to the N-atom. Since the C=O and tetrasubstituted C=C moieties accounted for two out of seven degrees of unsaturation, compound **2** also contained five rings.

The NMR spectroscopic data of **2** (*Table 2*) indicated a structure similar to that of daphnigracine [12][13], except for the presence of one more OH group. One CH (δ(C) 68.4; δ(H) 3.98) was linked to an O-atom. On the basis of HMBC correlations between H–C(11) (δ(H) 3.98) to C(6), C(9), C(10), and C(17), the OH group was located at C(11) (*Fig. 2*, *a*).

The relative configuration of **2** was deduced by a ROESY experiment, as illustrated in a computer-generated 3D drawing (*Fig. 2*, *b*). The ROESY correlations between H_α–C(13) and H–C(14), and between H–C(15) and H_α–C(16) indicated that H–

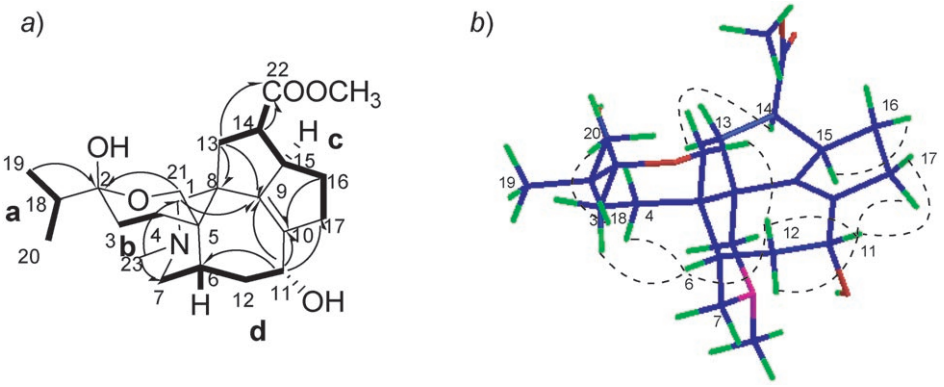

Fig. 2. a) *^{1}H,^{1}H-COSY* (▬) *and key HMBC* (H → C) *correlations for* **2**; b) *key ROESY* (- - -) *correlations for* **1**

C(14) and H–C(15) were both α-oriented. The correlation between H–C(6) and H_β–C(12) suggested β-configuration for H–C(6). Also, the H-atom at C(11) was β-oriented, on the basis of the corresponding NMR signal at δ(H) 3.98 (*t*, $J = 3.1$ Hz), which was coupling with H_α–C(12) and H_β–C(12) due to similar dihedral angles (*Fig. 2*, *b*). Thus, the relative configuration of the 11-OH group was α.

From the above data, compound **2** was identified as methyl (4*S**,6*R**,6′*R**,8a*R**,9-*R**,10a*R**,11*S**)-2,3,4,5,5′,6,6′,7,8,8a,9,10-dodecahydro-6,6′-dihydroxy-2-methyl-6′-(1-methylethyl)-1*H*,4′*H*-spiro[4,10a-methanopentaleno[1,6-*cd*]azonine-11,3′-pyran]-9-carboxylate.

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.

Experimental Part

General. Column chromatography (CC) was performed on silica gel, 200–300 or 300–400 mesh (*Qingdao Haiyang Chemical Industry Co., Ltd*). Optical rotation: *JASCO DIP-300* spectropolarimeter. IR Spectra: *Perkin-Elmer-577* spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm rel. to Me_4Si, *J* in Hz. MS: *VG Auto Spec3000* spectrometer; in *m*/*z*.

Plant Material. Fruits of *Daphniphyllum longeracemosum* ROSENTH. were collected from Malipo County, Yunnan Province, P. R. China, in November 2004, and were identified by Prof. *Xun Gong*. A voucher specimen (No. 2004202) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, P. R. China.

Extraction and Isolation. The air-dried fruits (5 kg) of *D. longeracemosum* were extracted with 95% EtOH. The crude extract was adjusted with 10% aq. HCl to pH 2. The acidic mixture was defatted with petroleum ether (PE), and then extracted with $CHCl_3$. The aq. phase was brought to pH 10 by addition of sat. aq. Na_2CO_3 soln., and then extracted with $CHCl_3$ to obtain crude alkaloids (8.0 g). The crude alkaloids were subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0 → 0:1): fractions *Fr. 1–4*. *Fr. 2* was repeatedly chromatographed (SiO_2; 1. PE/AcOEt/Et_2NH 50:1:0.2 → 10:1:0.2; 2. PE/Me_2CO 8:2; 3. $CHCl_3$/MeOH 50:1) to yield **1** (5 mg) and **2** (2 mg). *Fr. 1* was also chromatographed (SiO_2; 1. PE/Me_2CO 10:1; 2. PE/Et_2NH 10:0.2) to yield **3** (8 mg).

Daphnilongertone (= *(4S*,4aR*,8R*,8aR*,11aR*,11bR*)-1,2,3,4,4a,5,8,8a,9,11a-Decahydro-11a-hydroxy-2-methyl-4a-(4-methyl-3-oxopentyl)-7H-4,11-ethano-8,11b-methanocyclopenta[5,6]oxocino[4,3-c]-pyridin-7-one*; **1**). Colorless solid. $[\alpha]_D^{21.1} = -18.1$ ($c = 0.82$, MeOH). IR (KBr): 3441, 2927, 2854, 1725, 1715, 1466, 1455, 1298, 1029, 1018. ^{1}H-, ^{13}C-, and 2D-NMR: see *Table 1*. ESI-MS (pos.): 388 ($[M+H]^+$). HR-ESI-MS (pos.): 388.2499 ($[M+H]^+$; $C_{23}H_{34}NO_4^+$; calc. 388.2487).

11-Hydroxydaphnigracine (= *(4S*,6R*,6′R*,8aR*,9R*,10aR*,11S*)-2,3,4,5,5′,6,6′,7,8,8a,9,10-Dodecahydro-6,6′-dihydroxy-2-methyl-6′-(1-methylethyl)-1H,4′H-spiro[4,10a-methanopentaleno[1,6-cd]azonine-11,3′-pyran]-9-carboxylate*; **2**). Colorless solid. $[\alpha]_D^{21.4} = -13.7$ ($c = 0.81$, MeOH). IR (KBr): 3424, 2926, 2853, 1738, 1454, 1167, 1077, 1034. ^{1}H-, ^{13}C-, and 2D-NMR: see *Table 2*. ESI-MS (pos.): 420 ($[M+H]^+$). HR-ESI-MS (pos.): 420.2755 ($[M+H]^+$, $C_{24}H_{38}NO_5^+$; calc. 420.2749).

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