The Daphniphyllum Alkaloids with A New Nitrogen Heterocyclic Skeleton

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The isolation and structures of six new alkaloids [yuzurine, daphnigracine, daphnigraciline, oxodaphnigracine, oxodaphnigraciline, and epioxodaphnigraciline] are described. The structure of yuzurine, which has been elucidated by means of an X-ray crystallographic analysis of the corresponding methiodide, is in full agreement with its spectral data. The structures of the other five alkaloids, all of which were isolated from the plant *Daphniphyllum gracile* Gage collected in New Guinea, were deduced on the basis of an exhaustive comparison of their spectral data coupled with chemical evidences. Finally, a plausible biogenesis of these alkaloids with a new type of nitrogen heterocyclic skeleton is discussed.

The plant *Daphniphyllum macropodium* Miquel ("yuzuriha" in Japanese) contains a great variety of related alkaloids, which can be regarded as a triterpene alkaloid. From a structural point of view, they are divided into five types of nitrogen heterocyclic skeleton represented by daphniphyldine, secodaphniphylline, daphnilactone A, daphnilactone B, and yuzurimine.[1] However, all of them possess in common the 2-azabicyclo[3.3.1]nonane system [A], a part of which constitutes a part of the bicyclo[5.3.0]decane system. Thus, these daphniphyllum alkaloids are reasonably related to one another by bond formation or fission on the basis of our biosynthetic study indicating that they are biosynthesized from six molecules of mevalonic acid *via* a squalene-like intermediate.[2] In particular, the isolation of daphnilactones A and B strongly suggests that such compounds as [B] and [C] must be key intermediates between two main groups represented by daphniphylline and yuzurimine.[3] In connection with the above biogenetic consideration, our considerable efforts have been made to search for the biogenetically important intermediates. In the present paper, we wish to describe the novel structures of six new daphniphyllum alkaloids that have no 2-azabicyclo[3.3.1]nonane system and differ from the other structurally known alkaloids.

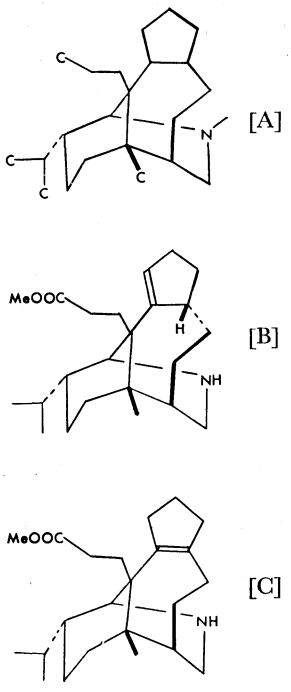

Fig. 1.

The Structure of Yuzurine. Yuzurine (**1**) has been isolated from the bark and leaves of the plant *Daphniphyllum macropodum* M. as one of the minor components, and cited as the alkaloid A_2.[4] This alkaloid is a colorless viscous liquid with a molecular formula [$C_{24}H_{37}O_4N$ (*m/e* 403 (M^+))] and characterized as the corresponding methiodide (**7**); mp 229—230 °C; $C_{25}H_{40}O_4NI$ [*m/e* 403 (M^+—MeI)]. Although this base can be regarded as one of the C_{22}-alkaloids from its molecular formula, the spectral data of **1** and **7** indicate that the carbon skeleton of yuzurine seems to be considerably different from those of the other daphniphyllum alkaloids, whose structures have been already established.

Firstly, yuzurine (**1**) has one ethyl group [δ 0.85 (3H, t, J=7.4 Hz)]. Furthermore, the NMR spectrum of the free base has two methyl singlets at δ 2.17 and 3.21 due to each one of NMe and OMe groups in addition to the presence of a methoxycarbonyl group (ν_{max} 1740 cm^{-1} and δ 3.64) and a $-CH_2-O-$ grouping [δ 3.93 (2H, s)]. In the case of the corresponding methiodide (**7**), the methyl singlet at δ 2.17 in **1** was shifted to lower magnetic field and two singlets were observed at δ 3.57 and 3.63 assignable to two methyl groups attached to the newly formed quaternary nitrogen atom. Secondly, three doublets with a geminal coupling constant (J=12—13.5 Hz) were newly observed at δ 3.35, 4.06, and 4.10 in the NMR spectrum of **7**. In addition, the NMR signal corresponding to the geminal doublet at δ 4.06, is present at δ 3.67—3.85. From these data, the methiodide (**7**) must have a partial structure $(-CH_2-\overset{\overset{Me}{|}}{\overset{+}{N}}-CH_2-)$. Finally, the complex structure of yuzurine (**1**) was elucidated by means of an X-ray crystallographic analysis of the methiodide (**7**),[5] which was subjected to Hofmann degradation to give the

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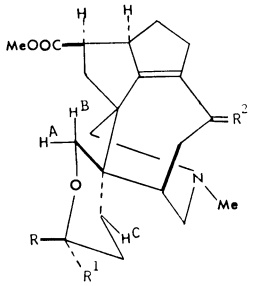
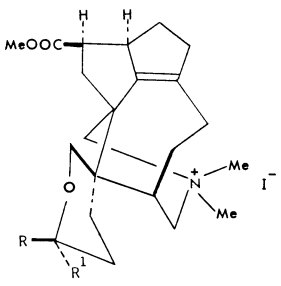
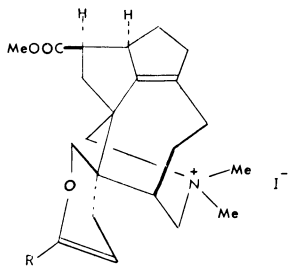


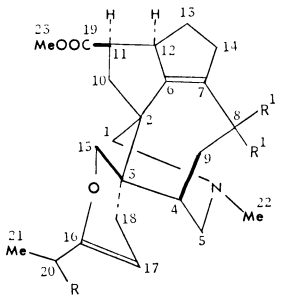
1 R=OMe, R^1=Et, R^2=H_2
2 R=OH, R^1=*i*-Pr, R^2=H_2
3 R=OH, R^1=Et, R^2=H_2
4 R=OH, R^1=*i*-Pr, R^2=O
5 R=OH, R^1=Et, R^2=O
6 R=Et, R^1=OH, R^2=O
12 R=Et, R^1=OMe, R^2=H_2



7 R=OMe, R^1=Et
8 R=OH, R^1=*i*-Pr
9 R=OH, R^1=Et
13 R=Et, R^1=OMe



10 R=*i*-Pr
11 R=Et



14 R=Me, R^1=H_2
15 R=H, R^1=H_2
16 R=H, R^1·R^1=O

Fig. 2.

original alkaloid (**1**). Further study on this quaternary base[6] has revealed that yuzurine (**1**) has the same absolute configuration as those of daphmacrine[7] and methyl homosecodaphniphyllate.[8] The structure of yuzurine so far obtained is in full agreement with its spectral and chemical data.

As described earlier, many number of alkaloids have been isolated from the plant *Daphniphyllaceae* growing in Japan.[1,3] Recently, an extensive survey was made of the occurrence of alkaloids in New Guinea plants,[9] and according to general procedures (see Experimental) the crude alkaloidal components were obtained from the plant *Daphniphyllum gracile* Gage by one of us (J. A. L). Interestingly, an analytical TLC of the crude oil so far obtained did not show any spots corresponding to daphniphylline and yuzurimine, both of which had been already isolated from the Japanese species as a main product.[10] This crude oil was further separated carefully by repeated preparetive TLC [Kieselgel PF_{254}; hexane–Et_2O–Et_2NH (10 : 10 : 1)] to give five new alkaloids, namely daphnigracine (**2**), daphnigraciline (**3**), oxodaphnigracine (**4**), oxodaphnigraciline (**5**) and in epioxodaphnigraciline (**6**) in 0.0046, 0.023, 0.0041, 0.016, and 0.019% overall yields, respectively.[11]

The Structures of Daphnigracine and Daphnigraciline. Daphnigracine is a colorless viscous liquid [**2**, $C_{24}H_{37}O_4N$; *m/e* 403 (M^+) and 385 (M^+-18)]. This alkaloid has each one of hydroxyl and methoxycarbonyl

Table 1. NMR spectra of daphnigracine (**2**) and daphnigraciline (**3**)[a]

2	**3**
0.93(6H, d, J=7.0 Hz)	0.94(3H, t, J=7.0 Hz)
2.15(3H, s)	2.19(3H, s)
3.62(3H, s)	3.62(3H, s)
3.89(1H, dd, J=12.5, 2 Hz)	3.90(1H, dd, J=12.5, 2 Hz)
4.32(1H, d, J=12.5 Hz)	4.35(1H, d, J=12.5 Hz)

a) In $CDCl_3$.

Table 2. NMR spectra of the methiodides (**8** and **9**)[a]

8	**9**
0.95(3H, d, J=7.0 Hz)	0.97(3H, t, J=7.0 Hz)
0.96(3H, d, J=7.0 Hz)	
3.45(3H, s)	3.44(3H, s)
3.50(3H, s)	3.48(3H, s)
3.68(3H, s)	3.70(3H, s)
3.00—3.90(5—6H, complex)	3.00—3.90(5—6H, complex)
4.42(1H, d, J=12.5 Hz)	4.43(1H, d, J=12.5 Hz)

a) In $CDCl_3$-CD_3OD.

groups [ν_{max} 3450 cm^{-1}; ν_{max} 1730 cm^{-1} and δ 3.62 (3H, s)], and can be characterized as the corresponding methiodide [**8**, mp (dec) 198—199 °C; $C_{25}H_{40}O_4NI$]. Daphnigraciline [**3**, mp 76—78 °C; $C_{23}H_{35}O_4N$; *m/e* 389 (M^+) and 371 (M^+-18)] has a hydroxyl group (ν_{max} 3500 cm^{-1}) and a methoxycarbonyl group [ν_{max} 1735 cm^{-1} and δ 3.62 (3H, s)]. Similarly, treatment of **3** with MeI in acetone afforded the corresponding methiodide [**9**, mp (dec) 163—165 °C; $C_{24}H_{38}O_4NI$], in an almost quantitative yield. As shown in Tables 1 and 2, the spectral data, particularly NMR spectra, of both alkaloids are quite similar to each other except for the following points: the NMR signal due to an isopropyl group is observed at δ 0.93 in **2**, while daphnigraciline (**3**) has a methyl triplet at δ 0.94. Furthermore, when treated with Ac_2O–AcOH (1 : 1), both of the methiodides (**8** and **9**) were readily converted into the corresponding dehydration products (**10** and **11**) in high yields [**10**; mp (dec) 243—245 °C; $C_{25}H_{38}O_3NI$; *m/e* 385 (M^+—MeI); ν_{max} 1675 cm^{-1}; δ 4.44 (1H, m). **11**; mp (dec) 235—237 °C; $C_{24}H_{36}O_3NI$; *m/e* 371 (M^+—MeI); ν_{max} 1680 cm^{-1}; δ 4.39 (1H, m)]. From these data, clearly, daphnigracine and daphnigraciline must have the same carbon skeleton except for the different alkyl groups. Finally, the structures of these alkaloids were elucidated by conversion of daphnigraciline (**3**) into yuzurine (**1**), as follows.

When treated with MeOH containing one drop of AcOH (room temp, overnight), daphnigraciline (**3**) was converted into yuzurine (**1**), in a high yield, in addition to a small amount of an epimer (**12**) which was characterized as the corresponding methiodide [**13**: mp (dec) 144—146 °C; $C_{25}H_{40}O_4NI$; *m/e* 403 (M^+—MeI); ν_{max} 1730 cm^{-1} and no OH absorption band; δ 3.11 (3H, s)]. On Hofmann degradation, **13** was easily re-

converted into the starting base (**12**).

Finally, although the configuration of the hydroxyl group in a acetal moiety is not determined chemically, the NMR spectrum of **2** (or **3**) indicates that this hydroxyl group must be in an axial configuration, as discussed below. In the NMR spectrum of daphnigracine (**2**), as expected, a sharp doublet (δ 4.32) due to the geminal proton (H^A), which is in a 1,3-diaxial relationship to the hydroxyl group, is observed in lower magnetic field than the NMR signal at δ 3.89 assignable to the equatorial geminal proton (H^B) which can couple with a proton (H^C) along the 'W' path.

The Structures of Oxodaphnigracine, Oxodaphnigraciline and Epioxodaphnigraciline. The IR, UV, and NMR spectra of oxodaphnigracine (**4**: mp 116—117 °C; $C_{24}H_{35}O_5N$) and oxodaphnigraciline (**5**: mp 107—109 °C; $C_{23}H_{33}O_5N$) indicate that these two alkaloids must be quite similar to each other except for the alkyl group [δ 0.88 (6H, d, J=7.0 Hz) in **4**; δ 0.94 (3H, t, J=7.0 Hz) in **5**], as seen in the cases of daphnigracine (**2**) and daphnigraciline (**3**). In the UV spectra of **4** and **5**, particularly, the absorption maximum is observed at 253 nm, indicating the presence of an α,β-unsaturated CO group which must be included in the seven-membered ring.[12]

Epioxodaphnigraciline (**6**: mp 102—104 °C; $C_{23}H_{33}O_5N$) has the same molecular formula as that of oxodaphnigraciline (**5**), and their spectral data are also quite similar to each other. However, a remarkable difference is seen in the following points: the NMR spectrum of the former has the sharp doublet (δ 3.88) due to the geminal proton (H^A) at slightly higher magnetic field than that of **5** (δ 4.03). Therefore, the structure of epioxodaphnigraciline must be represented by **6**. Furthermore, these results are supported by the ^{13}C NMR spectra of the dehydration products (**14**, **15**, and **16**), which have been obtained on dehydration of the corresponding alkaloids **2**, **3**, and **5** (or **6**) with Ac_2O–AcOH (1 : 1).

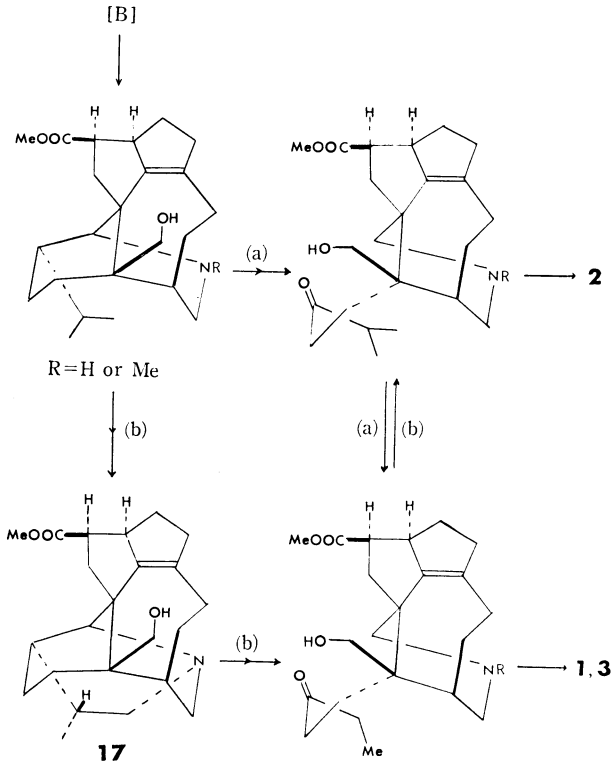
As demonstrated in the previous paper,[13] the methyl, methylene, methine and quaternary carbon signals in the ^{13}C NMR spectra of these dehydro compounds are easily differentiated by measurements of their partially relaxed FT ^{13}C NMR spectra coupled with off-resonance experiments, and the results are summarized in Table 3. In the spectrum of **14**, nine methylene and four methine signals are observed, while ten methylene and three methine signals are found in that of **15**. In the case of **16**, nine methylene and three methine signals are found in addition to one new CO signal (δ 208.9). Furthermore, the olefinic signals at δ 138.6 and 183.3 strongly suggest that this CO group must conjugate with the tetra-substituted double bond (C_6–C_7) in **16**. In the cases of **15** and **16**, the ^{13}C NMR signals of the NMe group [δ 26.6 in **15**; δ 26.5 in **16**] appear at extraordinary high magnetic field. This may be due to complicated steric compression. The other signals of these three dehydration products show the one-to-one correspondence to one another.

From a biogenetic point of view, these new alkaloids may be produced from the plausible intermediate [B] according to a pathway (a) or (b), as shown in Scheme

TABLE 3. ^{13}C CHEMICAL SHIFTS OF **14**, **15**, AND **16**

Functional group	**14**	**15**	**16**	Assignment
CH_3–	20.4 (2 Me)	11.6	11.4	C-21
	32.1	26.6	26.5	C-22
	51.0	51.0	51.5	C-23
–CH_2–	27.2	27.2	21.1	C-9, 13, 14, 20
	27.2	27.2	26.3	
	28.1	27.4	27.7	
		28.0	38.2[a]	
	39.8[a]	39.9[a]	38.6[a]	C-18
	42.6[a]	42.6[a]		C-8
	46.6[a]	46.6[a]	46.1	C-10
	56.5	56.4	56.1	C-5
	62.1	62.2	62.9	C-1
	69.4	69.6	69.1	C-15
–CH–	34.2	34.3	34.4	C-4
	42.6	42.6	42.6	C-12
	54.9	54.9	45.0	C-11
	27.4			C-20
–C–	36.6	36.6	37.1	C-3
	46.5	46.4	50.3	C-2
>C=C<	90.3	92.0	91.8	C-17
	133.6	133.5	138.6	C-7
	159.5	156.0	156.1	C-16
	145.8	145.9	183.3	C-6
>C=O	175.4	175.5	173.7	C-19
			208.9	C-8

a) Assignment of chemical shifts for close-lying peaks may be reversed.



Scheme 1.

1. Of two possibilities, the isolation of daphnigracine (**2**) with an isopropyl group from the plant suggests that yuzurine (**1**) and daphnigraciline (**3**) both are not necessarily derived from yuzurimine B (**17**), but may be produced by oxidative demethylation of daphnigracine (**2**) or its precursor Biosynthetic experiments are further required to solve these problems.

Experimental

All the mps are uncorrected. IR spectra were recorded on a JASCO Model IR-S spectrophotometer. UV spectra were obtained on a Perkin Elmer 202 spectrophotometer, using MeOH as the solvent. NMR spectra were recorded on a Varian Associate AH-100 or A-60, or JEOL JNM-PS 100 NMR spectrometer, using $CDCl_3$ as the solvent, unless otherwise stated. The chemical shifts are given in ppm relative to the internal TMS, and only prominent signals are cited (d, doublet; m, multiplet; q, quartet; s, singlet; t, triplet). Mass spectra were taken on a Hitachi RMU-6C mass spectrometer, operating with an ionization energy of 70 eV.

Column chromatography was carried out on basic alumina (Nakarai Chemical Co., Ltd., *ca.* 300 mesh) or on silicic acid (Mallinckrodt, 100 mesh). Thin layer chromatography was performed on Kieselgel PF_{254} using hexane–Et_2O–Et_2NH (10 : 10 : 1) or Et_2O–Et_2NH (40 : 3), unless otherwise stated.

*Physical Properties of Yuzurine (**1**).* Isolation of this alkaloid was reported in the reference 4. Yuzurine is a colorless viscous liquid (**1**); ν_{max} (film) 1740 cm^{-1}; δ 0.85(3H, t, J=7.4 Hz), 2.17(3H, s), 3.21(3H, s), 3.64(3H, s), and 3.93 (2H, s); *m/e* 403 (M^+ for $C_{24}H_{37}O_4N$), 388, 372, 360, and 344.

*Formation of Yuzurine Methiodide (**7**).* A solution of yuzurine (40 mg) and MeI (1 ml) in acetone (2 ml) was allowed to stand at room temperature overnight, and then concentrated under reduced pressure to give pale yellow crystals (45 mg). Recrystallization from benzene–MeOH afforded pale yellow needles, which were subjected to an X-ray crystallographic analysis.[5] Its physical data are as follows: mp (dec) 229—230 °C; ν_{max} (KBr) 2700 and 1740 cm^{-1}; δ 0.87 (3H, t, J=7.0 Hz), 3.20(3H, s), 3.35(1H, br d, J=13.5 Hz,) 3.57(3H, s), 3.64(3H, s), 3.67(3H, s), 3.67—3.85(1H, superimposed on Me signals), 3.85(2H, br s), 4.06 (1H, d, J=12 Hz), and 4.10(1H, br d, J=13.5 Hz); *m/e* 403 (M^+−MeI) (Found: C, 54.51; H, 7.44; N, 2.51%. Calcd for $C_{25}H_{40}O_4NI$: C, 55.04; H, 7.39; N, 2.57%).

Hofmann Degradation of Yuzurine Methiodide. To a solution of yuzurine methiodide (80 mg) in MeOH–H_2O [(4 : 1) 5 ml] was added excess amounts of Ag_2O (*ca.* 200 mg). The reaction mixture was stirred at room temperature for 1 h, and then filtered. The filtrate was concentrated under reduced pressure to leave a crystalline residue, which was heated *in vacuo* at *ca.* 200 °C for 10 min, and then cooled. The resulting oily residue was purified by preparative TLC, using hexane–Et_2O–Et_2NH (10 : 10 : 1) to afford a colorless oil of yuzurine (47.2 mg) (analytical TLC and IR spectrum).

*Isolation of Daphnigracine (**2**), Daphnigraciline (**3**), Oxodaphnigracine (**4**), Oxodaphnigraciline (**5**), and Epioxodaphnigraciline (**6**).* The leaves of the plant *Daphniphyllum gracile* Gage growing in New Guinea were collected late in January. According to a usual procedure,[9] the air-dried leaves were milled and extracted by continuous percolation with MeOH at 40 °C. The methanolic extracts were concentrated under reduced pressure so that the temperature of the solution did not exceed 40 °C. The concentrate was diluted with water, and then acidified by addition of 1M H_2SO_4. The solution was filtered and made basic by addition of ammonia, and then extracted repeatedly with chloroform. Evaporation of the combined chloroform extracts gave the crude alkaloids as a dark brown residue. This residue was dissolved in chloroform (apart from a small amount of largely nonalkaloidal gum) and the alkaloids were extracted from the solution by repeatedly shaking with successive lots of 1M H_2SO_4. The crude alkaloids as a dark brown oil were recovered from the aqueous acidic solution by basification with ammonia followed by extraction with chloroform. Yield of the crude alkaloids from the air-dried leaves is 0.15% in weight.

The dark brown oil (556 mg) was further separated carefully by repeated preparative TLC using hexane–Et_2O–Et_2NH (10 : 10 : 1) to give five new alkaloids in the following order from the less polar to polar fractions: daphnigracine (**2**) (17.2 mg), daphnigraciline (**3**) (84.3 mg), oxodaphnigracine (**4**) (15 mg), oxodaphnigraciline (**5**) (61.4 mg) and epioxodaphnigraciline (**6**) (70.8 mg). Their physical data are shown below.

*Daphnigracine (**2**)* as a colorless viscous liquid: ν_{max} (film) 3450 and 1730 cm^{-1}: *m/e* 403 (M^+) and 385 (Found: *m/e* 403.27186. Calcd for $C_{24}H_{37}O_4N$: *m/e* 403.27224).

*Daphnigraciline (**3**):* Mp 76—78 °C (from hexane–Et_2O); ν_{max} (Nujol) 3500 and 1735 cm^{-1}; *m/e* 389 (M^+) and 371 [Found: C, 70.70; H, 9.25; N, 3.23% (*m/e* 389.25396). Calcd for $C_{23}H_{35}O_4N$: C, 70.92; H, 9.06; N, 3.60% (*m/e* 389.25659)].

*Oxodaphnigracine (**4**):* Mp 116—117 °C (from hexane–Et_2O); ν_{max} (Nujol) 3400 br, 1730, 1685, and 1650 cm^{-1}; λ_{max} 253 nm (ε, 7500); δ 0.88(6H, d, J=7.0 Hz), 2.10(3H, s), 3.60(1H, dd, J=11, 2 Hz), 3.69(3H, s), and 4.03(1H, d, J=11 Hz); *m/e* 417 (M^+) and 399 (Found: *m/e* 417.25334. Calcd for $C_{24}H_{35}O_5N$: *m/e* 417.25151).

*Oxodaphnigraciline (**5**):* Mp 107—109 °C (from hexane–Et_2O); ν_{max} (Nujol) 3380 br, 1730, 1685, and 1648 cm^{-1}; λ_{max} 253 nm (ε, 8060); δ 0.94(3H, t, J=7.0 Hz), 2.12(3H, s), 3.58(1H, dd, J=11.5, 3 Hz), 3.70(3H, s), and 4.03(1H, d, J=11.5 Hz); *m/e* 403 (M^+) and 385 (Found: *m/e* 403.23692. Calcd for $C_{23}H_{33}O_5N$: *m/e* 403.23585).

*Epioxodaphnigraciline (**6**):* Mp 102—104 °C (from hexane–Et_2O); ν_{max} (Nujol) 3400 br, 1735, 1685, and 1650 cm^{-1}; λ_{max} 252 nm (ε, 7000); δ 0.92 (3H, t, J=7.5 Hz), 2.15(3H, s), 3.61(4H, s)*, and 3.88(1H, d, J=12 Hz); *m/e* 403 (M^+) and 385 (Found: *m/e* 403.23532. Calcd for $C_{23}H_{33}O_5N$: *m/e* 403.23585).

*Formation of Daphnigraciline Methiodide (**9**).* According to the similar procedure as that of yuzurine (**1**), a solution of daphnigraciline (20 mg) in acetone (5 ml) was treated with MeI (1 ml) at room temperature overnight to give pale yellow crystals in quantitative yield:; mp (dec) 163—165 °C (from acetone–Et_2O); ν_{max} (Nujol) 3300 and 1710 cm^{-1}; *m/e* 371 (M^+−MeI−H_2O) (Found: C, 54.17; H, 7.36; N, 2.45%. Calcd for $C_{24}H_{38}O_4NI$: C, 54.23; H, 7.21; N, 2.64%).

*Formation of Daphnigracine Methiodide (**8**).* Under the same conditions as that of daphnigraciline (**3**), daphnigracine methiodide was also produced in an almost quantitative yield; mp (dec) 198—199 °C (from acetone–Et_2O); ν_{max} (Nujol) 3450 br, 3320 and 1710 cm^{-1}; *m/e* 385 (M^+−MeI−H_2O) (Found: C, 53.57; H, 7.15; N, 2.46%. Calcd for $C_{25}H_{40}O_4NI \cdot H_2O$: C, 53.28; H, 7.15; N, 2.64%).

*Dehydration of Daphnigraciline Methiodide (**9**).* A solution of **9** (15 mg) in Ac_2O–AcOH [(1 : 1) 2 ml] was allowed to stand at room temperature overnight, and then concentrated under reduced pressure at 100 °C to leave a crystalline

* One of the geminal protons (H^B) is included.

solid (13 mg). Recrystallization from acetone–Et_2O gave pale yellow needles (**11**): mp (dec) 235—237 °C; ν_{max} (Nujol) 1730 and 1680 cm^{-1}; δ 1.00(3H, t, J=7.2 Hz), 3.29 (1H, d, J=13.5 Hz), 3.54(3H, s), 3.61(3H, s), 3.62(3H, s), 3.73—3.92(2H, br), 4.02(1H, d, J=12 Hz), 4.18(1H, d, J=13.5 Hz), 4.38(1H, d, J=12 Hz), and 4.39(1H, m); *m/e* 371 (M^+—MeI), 356, 343, 340, 328, 314, 312, and 300 (Found: C, 55.99; H, 7.13; N, 2.31%. Calcd for $C_{24}H_{36}O_3NI$: C, 56.14; H, 7.07; N, 2.72%).

Dehydration of Daphnigracine Methiodide (8). Under the similar conditions as that of **9**, daphnigracine methiodide (20 mg) was treated with Ac_2O–AcOH [(1 : 1) 3 ml] to give a crystalline solid (*ca.* 20 mg), which was recrystallized from acetone–Et_2O to give pale yellow needles (**10**): mp (dec) 243—245 °C; ν_{max} (Nujol) 1730 and 1675 cm^{-1}; δ 1.01(6H, d, J=7.0 Hz), 3.30(1H, d, J=13.5 Hz), 3.57(3H, s), 3.63(3H, s), 3.65(3H, s), 3.7—3.90(2H, br), 4.02(1H, d, J=12 Hz), 4.21(1H, d, J=13.5 Hz), 4.44(1H, d, J=12 Hz), and 4.44 (1H, m); *m/e* 385 (M^+—MeI), 370, 357, 354, 342, 326, 314, and 300 (Found: C, 57.45; H, 6.83; N, 2.38%. Calcd for $C_{25}H_{38}O_3NI$: C, 56.92; H, 7.26; N, 2.66%).

Conversion of Daphnigraciline Methiodide (9) into Yuzurine Methiodide (7). Daphnigraciline methiodide (10 mg) was dissolved in MeOH (2 ml) containing one drop of AcOH. The resulting solution was allowed to stand at room temperature overnight, and then concentrated under reduced pressure on a boiling water-bath to give a pale brown solid (*ca.* 10 mg), which was crystallized from MeOH–benzene to afford pale yellow crystals of yuzurine methiodide (**7**) (mp and IR spectrum).

Conversion of the Dehydration Product (11) into Yuzurine Methiodide (7). According to the same procedure as that of daphnigraciline methiodide (**9**), **11** (10 mg) was treated with MeOH (2 ml) containing one drop of AcOH to give a crystalline solid quantitatively, which was recrystallized from MeOH–benzene to give yuzurine methiodide (**7**) (mp and IR spectrum).

Conversion of Daphnigraciline (3) into Yuzurine (1) and Epiyuzurine (12). A solution of **3** (100 mg) in MeOH (5 ml) containing AcOH (*ca.* 0.01 ml) was stirred at room temperature for 4 h, and then concentrated under reduced pressure at 100 °C to leave a slightly brown residue, which was separated by preparative TLC [hexane–Et_2O–Et_2NH (20 : 20 : 3)] to give yuzurine (75 mg) (TLC and IR spectrum) and a colorless viscous liquid of epiyuzurine (*ca.* 7 mg) which was characterized as the corresponding methiodide (**13**), mp (dec) 144—146 °C (from acetone–Et_2O); ν_{max} (Nujol) 1730 cm^{-1}; δ 0.85(3H, t, J=7.0 Hz), 3.11(3H, s), 3.42(2H, br s), 3.55(3H, s), 3.65(3H, s), 3.66 (3H, s), 3.34—3.72(1H, superimposed on Me signals), and 3.84—4.22(3H, complex); *m/e* 403 (M^+—MeI), 388, 371, 343, 340, 328, 314, 312, and 300 (Found: C, 54.59; H, 7.15; N, 2.20%. Calcd for $C_{25}H_{40}O_4NI$: C, 55.04; H, 7.39; N, 2.57%).

Dehydration of Daphnigracine (2). A solution of **2** (20 mg) in Ac_2O–AcOH [(1 : 1) 2 ml] was allowed to stand at room temperature overnight, and then concentrated to dryness under reduced pressure at 100 °C. The resulting pale brown oil was purified by preparative TLC, using hexane–Et_2O–Et_2NH (10 : 10 : 1) to afford a colorless viscous liquid (15 mg) [**14**: ν_{max} (film) 1735 and 1675 cm^{-1} (no OH absorption band) (Found: *m/e* 385.25956. Calcd for $C_{24}H_{35}O_3N$: *m/e* 385.26168) which was characterized as the corresponding methiodide (**10**).

Dehydration of Daphnigraciline (3). According to the same procedure as that of daphnigracine (**2**), treatment of **3** (20 mg) with Ac_2O–AcOH [(1 : 1) 2 ml] afforded the corresponding dehydration product (12 mg) as a colorless viscous liquid [**15**: ν_{max} (film) 1740 and 1678 cm^{-1} (no OH absorption band) (Found: *m/e* 371.24465. Calcd for $C_{23}H_{33}O_3N$: *m/e* 371.24603)]. This product was also characterized as the corresponding methiodide (**11**).

Dehydration of Oxodaphnigraciline (5). According to the same conditions as that of daphnigracine (**2**), **5** (22 mg) was treated with Ac_2O–AcOH [(1 : 1) 2 ml] to give a brown oil, which was purified by preparative TLC using Et_2O–Et_2NH (40 : 3) to afford an almost colorless viscous liquid (10 mg) [**16**: ν_{max} (film) 1735, 1690 br, and 1665 cm^{-1} (no OH absorption band); δ 1.00(3H, t, J=7.5 Hz), 2.16(3H, s), 3.55 (1H, d, J=11.5 Hz), 3.64(3H, s), 4.11(1H, dd, J=11.5, 2 Hz), and 4.36(1H, m) (Found: *m/e* 385.22619. Calcd for $C_{23}H_{31}O_4N$: *m/e* 385.2253)].

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