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THE ALKALOIDS FROM THE FRUITS OF THE PLANT *DAPHNIPHYLLACEAE*

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Abstract—Six alkaloids have been isolated from the fruits of the plant *Daphniphyllaceae.* Two of them are new alkaloids, namely methyl homodaphniphyllate (1) and daphnilactone-B (2). The structure of the former was deduced by chemical transformation from daphniphylline (3). The structure of daphnilactone-B was estimated by the exhaustive spectral analysis as well as by chemical evidences, and finally determined by an X-ray crystallographic analysis of the free base.

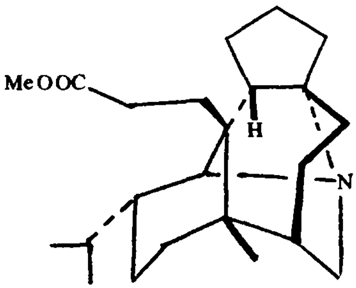
A great variety of related alkaloids have been isolated from the plant *Daphniphyllum macropodum* Miquel (Yuzuriha in Japanese), whose structures are divided into four types of N-heterocyclic carbon skeleton represented by Daphniphylline (3),[1] yuzurimine (4),[2] secodaphniphylline (5),[3] and daphnilactone-A (6).[5] In connection with the structural relationship between secodaphniphylline (5), one of the C_{30}-alkaloids, and methyl homosecodaphniphyllate (7)[3] included in a group of the C_{22}-alkaloids, further great efforts have been made to search for the C_{22}-alkaloid (1) corresponding to codaphniphylline (8),[1] and led to the isolation of the expected alkaloid, methyl homodaphniphyllate (1), as well as a new lactone (2), named "Daphnilactone-B". In particular, the latter has a new type of N-heterocyclic carbon skeleton. Accordingly, the daphniphyllum alkaloids should be structurally divided into five groups. In the present paper, we wish to describe the isolation and structures of these two new alkaloids, namely methyl homodaphniphyllate (1) and daphnilactone-B (2).[5]

Isolation. In November, the fruits of the plant *Daphniphyllum teijsmanni* Zollinger (Hime-yuzuriha) were collected at Irako Cape in Aichi-ken and pulverized with a mixer, and then immersed in a lot of MeOH for 2 weeks. According to essentially the same procedure as reported earlier,[3] an oily material containing alkaloidal components were obtained, and then chromatographed on basic alumina. Successive elution with n-hexane, n-hexane-benzene (1 : 1), benzene, benzene–$CHCl_3$ (1 : 1) and $CHCl_3$ gave six alkaloids containing daphnilactone-B (II) as a major product, as shown in the Table. Furthermore, alkaloidal components of the fruits of the other kinds of the plant were examined according to the similar procedure, and the similar results were also obtained in the cases of the plants *Daphniphyllum macropodum* Miquel (Yuzuriha) and *Daphniphyllum humile* Maxim (Ezo-yuzuriha).

Of six daphniphyllum alkaloids so far obtained, methyl homodaphniphyllate (1) and daphnilactone-B (2) are new alkaloids. The other bases (3, 4, 7 and 9) were identified by comparing their physical data with that of each authentic sample.

The structure of methyl homodaphniphyllate (1). As mentioned earlier, our considerable efforts to isolate this alkaloid from the bark and leaves of the plant *Daphniphyllum macropodum* Miquel have been made, and failed. However, this expected alkaloid (1) could be obtained from the fruits of the plants *Daphniphyllum macropodum* Miquel and *Daphniphyllum teijsmanni* Zollinger, but not from the bark and leaves of the same plants. The alkaloid thus obtained was completely identical with methyl homodaphniphyllate (1) which had been already derived from daphniphylline (3) in several steps, as follows.[7]

When treated with *p*-TsCl in pyridine at room temperature overnight, daphnialcohol (10) which was produced from 3 in three steps[1] afforded a tosylate (**11**, m.p. 107–107·5°) in 53% yield. Further treatment of **11** with KCN in aqueous EtOH (under reflux, 100 h) gave a mixture of two compounds, a nitril (**12**, m.p. 155–156°; ν_{max} (KBr) 2280 cm^{-1}) and an amide (**13**, m.p. 280–281°; ν_{max} (KBr) 3420, 3220 and 1670 cm^{-1}) in 35 and 21% yields, respectively. Accordingly, the above mixture was directly hydrolyzed with 6N. HCl (under reflux 6 h), and then further treated with 20% methanolic HCl to give methyl homodaphniphyllate as hydrochloride (**1**, m.p. 233–234°; $C_{23}H_{37}O_2N \cdot HCl$; ν_{max} (KBr) 1735



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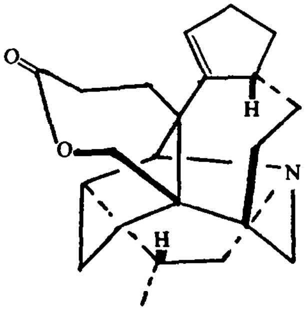

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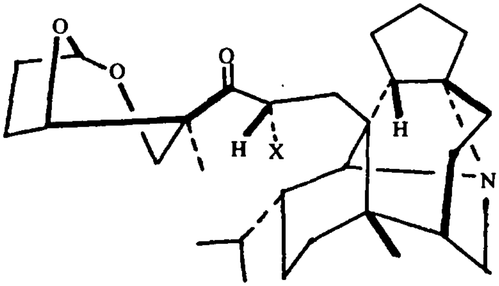
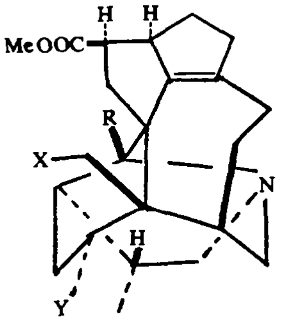
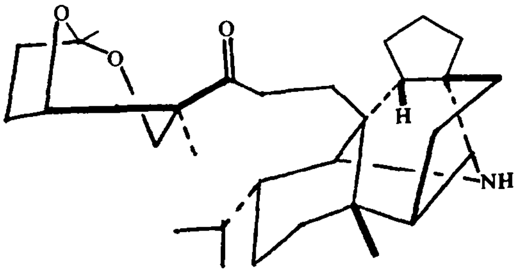


3: X = OAc
8: X = H



4: R = OH, X = OAc, Y = OAc
9: R = H, X = OH, Y = H



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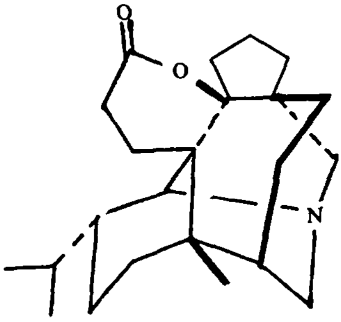

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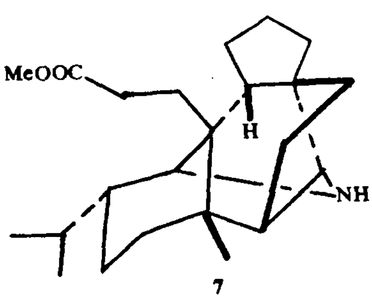

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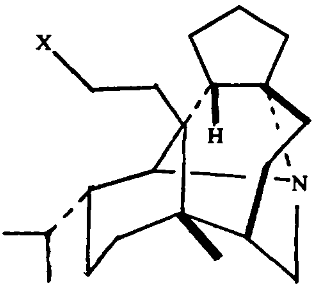
Fig 1.

Table. Alkaloidal components of the fruits

Alkaloid	M.p. (°C)	Molecular formula	Yield (%)
Methyl homodaphniphyllate (1)	233–234 (B·HCl)	$C_{23}H_{37}O_2N$	0·00006
Daphnilactone-B (2)	92–94	$C_{22}H_{31}O_2N$	0·033
Daphniphylline (3)	238–240 (B·HCl)	$C_{32}H_{49}O_5N$	0·00031
Yuzurimine (4)	150–152	$C_{27}H_{37}O_7N$	0·0006
Methyl homoseco-daphniphyllate (7)	102·5–103	$C_{23}H_{37}O_2N$	0·00013
Yuzurimine-B (9)[6]	282–284 (B·HCl)	$C_{23}H_{33}O_3N$	0·0006

cm^{-1}) in 53% yield. This methyl ester was completely identical with the alkaloid which was isolated from the fruits of the plant *Daphniphyllaceae* (mixed m.p. and IR spectrum).



10: X = OH
11: X = OTs
12: X = CN
13: X = CONH$_2$

Fig 2.

Generally, the fundamental carbon skeleton of the daphniphyllum alkaloids consists of 30 or 22 carbon atoms. Furthermore, the C_{30}-alkaloids have been known to be biosynthesized from six molecules of mevalonic acid *via* a squalene-like intermediate.[8] Accordingly, methyl homodaphniphyllate (1) must be produced by oxidative cleavage of 8 carbon atoms from codaphniphylline (8)[1] or its precursor.

The structure of daphnilactone-B. Daphnilactone-B (2, m.p. 92–94°), which has a molecular formula ($C_{22}H_{31}O_2N$; *m/e* 341 (M^+); ν_{max} ($CHCl_3$) 1726 cm^{-1} and no NH band), is regarded as one of the C_{22}-alkaloids. As shown in the NMR spectrum of this alkaloid, a methyl doublet is observed at δ 1·00 (3H, d, J = 6·0 Hz). Furthermore, a broad singlet at δ 5·67 (1H, Wh = 5·5 Hz) arising from an olefinic proton indicates the presence of a tri-substituted double bond. In fact, catalytic hydrogenation of 2 over PtO_2 afforded a dihydro-compound (14, *m/e* 343 (M^+)), which had no NMR signal due to an olefinic proton. The presence of a lactone ring A is confirmed by the spectral data of daphnilactone-B (ν_{max} ($CHCl_3$) 1726 cm^{-1}; AB-quartet at δ 3·63 and 4·73 (each 1 H, d, J_{AB} = 13 Hz)) coupled with the chemical evidences, as follows.

Action of sodium methoxide in MeOH on daphnilactone-B (room temp, overnight) converted it into the corresponding methyl ester (15), which was easily reconverted into the original lactone when

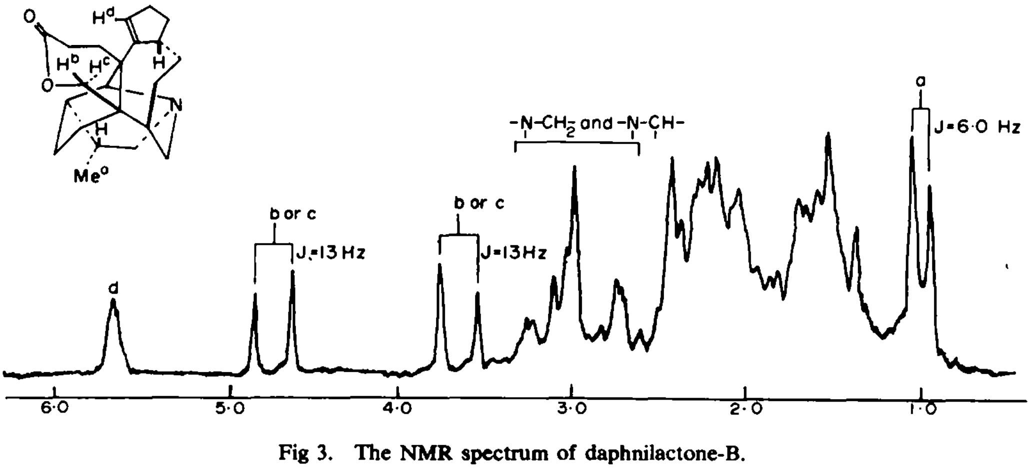
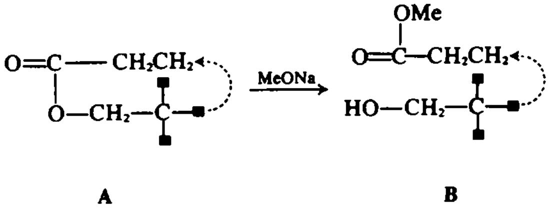


Fig 3. The NMR spectrum of daphnilactone-B.

allowed to stand at room temperature. The presence of a partial structure B in 15 is based on its exhaustive spectral analysis, as described below. The IR and NMR spectra indicate the presence of a carbomethoxyl group (ν_{max} (CHCl$_3$) 1730 cm^{-1} and δ (CDCl$_3$) 3·62) as well as of a hydroxy-methyl group (ν_{max} (CHCl$_3$) 3600 and 3400 cm^{-1}; AB-quartet at δ 3·60 and 4·22 (each 1 H, d, J_{AB} = 9·5 Hz)). In particular, the mass spectrum of this methyl ester has a pair of the remarkable peaks at *m/e* 300 and 286 in addition to the molecular ion peak at *m/e* 373, suggesting the presence of -CH$_2$CH$_2$COOMe grouping in [B]. Furthermore, only one Me doublet at δ 1·04 is observed in its NMR spectrum, which is similar to those of the yuzurimine group of alkaloids.



On the basis of the above results coupled with co-occurrence of methyl homodaphniphyllate (1) and yuzurimine-B (9), the structure (2) of daphnilactone-B can be derived from a common intermediate (16) except for a biogenetically expected position of the tri-substituted double bond (δ 5·67 (1H, br.s, Wh = 5·5 Hz)).[3] The stereostructure (2) of daphnilactone-B was finally determined by an X-ray crystallographic analysis of the free base.[9]

Recent studies on the biosynthesis of daphnilactone-B (2) have demonstrated that this alkaloid must be derived from methyl homosecodaphniphyllate (7), as shown below.[10]

EXPERIMENTAL

All m.ps were uncorrected. The IR spectra were recorded on a Hitachi Grating Infrared Spectrophotometer EPI-G3 or a JASCO Model IR-S. The NMR spectra were obtained on a Varian Associates AH-100 (100 Mc) or a Nihondenshi JNM-C 60 H (60 Mc). Chemical shifts for all NMR spectra are given in ppm from TMS as internal standard using CDCl$_3$ as solvent, unless otherwise stated. Only prominent peaks

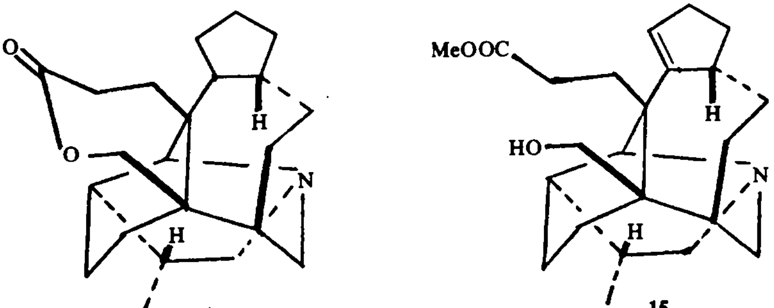


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Fig 4.

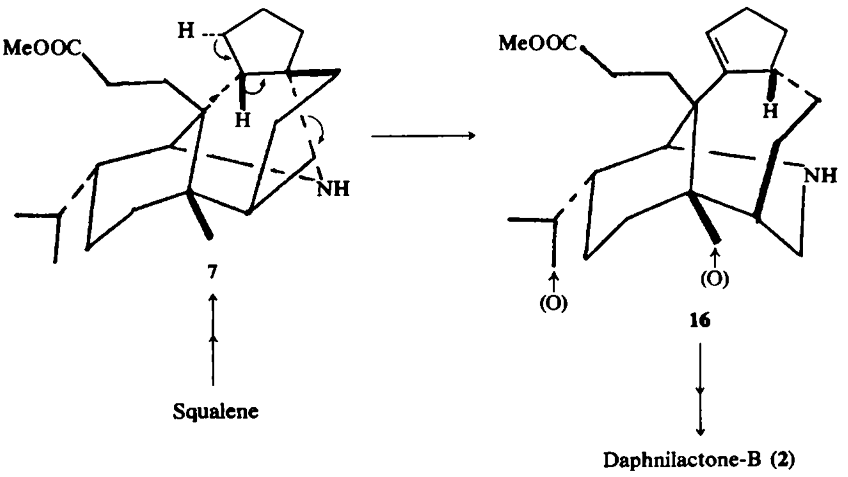
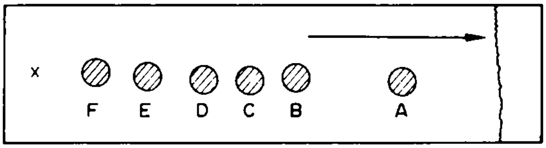


Fig 5.

are cited (d, doublet; m, multiplet; q, quartet; s, singlet; t, triplet). The mass spectra were recorded on a Hitachi RMU-6C mass spectrometer 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 silica gel GF_{254} (E. Merck, A. G., Germany) using n-hexane–ether–diethyl amine (20 : 20 : 3) or ether–diethyl amine (40 : 3) as solvent system.

Isolation. Wet fruits of the plant *Daphniphyllum teijsmanni* Zollinger (10 Kg)* were pulverized with a mixer and immersed in MeOH (10l) at room temp for 2 weeks, and then filtered. The methanolic soln was concentrated under reduced pressure to about 1l, diluted with equal volume of water, and then filtered. The filtrates were acidified with conc HCl to pH 2, and then washed with a lot of ether (10l) to remove chlorophyll and acidic and neutral substances. The aqueous soln was made basic with 4N NaOH gradually (pH 9–13), and then extracted with a lot of ether (20l). The ethereal layer was then extracted with 0·1 N HCl (1l) for purification. This aqueous soln was made basic again with 4N NaOH gradually (pH 9–13) and extracted with a lot of ether (20l). The ethereal extracts were washed with water, and then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave an oil (12g), which showed several spots on a TLC plate using n-hexane–ether–diethyl amine (20 : 20 : 3) as solvent system, as described below. The oil so far obtained was chromatographed on alumina (60 g) and eluted, in the following order, with n-hexane, n-hexane–benzene (1 : 1), benzene, benzene–$CHCl_3$ (1 : 1) and $CHCl_3$ to give five fractions.



TLC of the alkaloids on silicic acid. A: Methyl homosecodaphniphyllate (7); B: Methyl homodaphniphyllate (1); C: Daphniphylline (3); D: Daphnilactone-B (2); E: Yuzurimine (4); F: Yuzurimine-B (9).

The first three fractions (total weight, *ca* 5·3 g) were combined and crystallized from n-hexane–benzene to give pure daphnilactone-B **2** (4·8 g). The mother liquor was rechromatographed on alumina (1 g) using n-hexane and n-hexane–benzene (1 : 1) to give three alkaloids in the following order: Compounds **7** (0·02 g), **1** (0·01 g) and **3** (0·05 g).

On the other hand, the remaining two fractions (total weight, *ca* 2 g) were also combined, and rechromatographed on alumina (4g) using benzene and benzene–$CHCl_3$ (1 : 1) to give three alkaloids in the following order: **2** (0·5 g), **4** (0·1 g) and **9** (0·1 g). The physical data of two new alkaloids thus obtained are cited below.

Methyl homodaphniphyllate (**1**): m.p. 233–234° (as hydrochloride, in a sealed tube, from MeOH–ether); ν_{max} (KBr) 2750–2400 br., 1735, 1472, 1445, 1380 and 1177 cm^{-1}; δ 0·93 (3H, d, J = 6·0 Hz), 1·04 (3H, s), 1·12 (3H, d, J = 6·0 Hz), 3·1–3·5 (3H, br. complex) and 3·63 (3H, s); *m/e* 359 (M^+–HCl), 344, 328, 317, 286 and 272 (Found: C, 69·60; H, 9·81; N, 3·62. $C_{23}H_{37}O_2N \cdot HCl$ requires: C, 69·76: H, 9·67; N, 3·54%).

Daphnilactone-B (**2**). m.p. 92–94° (from n-hexane–benzene); ν_{max} ($CHCl_3$) 1726 cm^{-1}, and no absorption band in the region of OH and NH; *m/e* 341 (M^+) and no strong peak in higher region than *m/e* 150 except for the molecular ion peak (Found: C, 77·71; H, 8·96; N, 3·92. $C_{22}H_{31}O_2N$ requires: C, 77·37; H, 9·15; N, 4·10%).

The similar results were also obtained in the cases of the fruits of the plants *Daphniphyllum macropodum* Miquel† and *Daphniphyllum humile* Maxim.‡

Reaction of daphnialcohol (**10**) *with* p-*toluensulfonyl chloride.* p-Toluenesulfonyl chloride (230 mg) was added to a soln of daphnialcohol (223 mg) in dry pyridine (10 ml) at 0°, and then the resulting soln was allowed to stand at room temp overnight with stirring. The mixture was poured into water, and made basic with Na_2CO_3 aq (pH > 10). The aqueous soln was extracted with $CHCl_3$. The extracts were successively washed with water and sat NaCl aq, and then dried over Na_2SO_4. The solvent was removed under reduced pressure to give an oil, which was chromatographed on alumina (2g) and eluted with EtOAc to afford a colourless oil which was crystallized on addition of ether. Recrystallization from iso-propyl ether gave white crystals of **11** (176 mg); m.p. 107–107·5°; ν_{max} (KBr) 1600, 1502, 1364, 1188, 1178, 938 and 814 cm^{-1}; δ 0·73 (3H, s), 0·87 (3H, d, J = 6·0 Hz), 0·97 (3H, d, J = 6·0 Hz), 2·46 (3H, s), 2·6–3·4 (3H, br. complex), 3·9–4·6 (2H, m), 7·37 (2H, d, J = 8·0 Hz), 7·82 (2H, d, J = 8·0 Hz); *m/e* 471 (M^+), 428, 388, 316, 306, 286, 274 and 272 (Found: C, 71·40; H, 8·91; N, 2·98. $C_{28}H_{41}O_3NS$ requires: C, 71·40; H, 8·77; N, 2·97%).

Reaction of **11** *with KCN.* KCN (1·5 g) was added to a soln of **11** (212 mg) in EtOH–water (1 : 1, 10 ml), and then the resulting soln was heated under reflux for 100 h. The soln was poured into a lot of water, and then extracted with a lot of $CHCl_3$. The extracts were successively washed with water and sat NaCl aq, and then dried over Na_2SO_4. Removal of the solvent under reduced pressure gave an oil which was crystallized on addition of EtOAc and collected by filtration. Recrystallization from acetone gave plates of **13** (32 mg); m.p. 280–281° (in a sealed tube); ν_{max} (KBr) 3420, 3220, 1670, 1473 and 1455 cm^{-1}; δ 0·85 (3H, d, J = 6·0 Hz), 0·87 (3H, s), 0·95 (3H, d, J = 6·0 Hz), 2·6–2·8 (2H, m), 2·8–3·3 (3H, br. complex) and 5·0–5·9 (2H, br.); *m/e* 344 (M^+), 329, 302, 286 and 272 (Found: C, 76·48; H, 11·05; N, 8·10. $C_{22}H_{36}ON_2$ requires: C, 76·69; H, 10·53; N, 8·13%).

On the other hand, the filtrates were concentrated under reduced pressure to leave an oil, which was chromatographed on alumina (3 g) and eluted with benzene to give a colourless oil. Crystallization from n-hexane afforded white crystals of **12** (51 mg); m.p. 155–156° (from n-hexane); ν_{max} (KBr) 2280, 1474, 1445, 1385, 1167 and 939 cm^{-1}; *m/e* 326 (M^+), 311, 286, 283 and 272 (Found: C, 80·44; H, 10·77; N, 8·49. $C_{22}H_{34}N_2$ requires: C, 80·92; H, 10·50; N, 8·58%).

Formation of methyl homodaphniphyllate (**1**). The mixture (**12** and **13**), which was obtained from the tosylate (**11**) (132 mg) according to the above mentioned procedure, was directly dissolved in 6N HCl (25 ml) without separation of **12** and **13**. The acidic soln was heated under reflux for 6 h, and then concentrated under reduced pressure to leave an oily residue, which was dissolved in 20% anhyd methanolic HCl and allowed to stand at room temp overnight with stirring. The soln was concentrated again under reduced pressure to give an oil, which was dissolved in $CHCl_3$. The $CHCl_3$ soln was successively washed with dil Na_2CO_3 aq and water, and then dried over Na_2SO_4. The solvent was removed under reduced pressure to give an oily substance, which was

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*Collected at Irako Cape in Aichi-ken (in November).

†Collected in the suburbs of Gifu-shi (in November).

‡Collected at Shiratori in Gifu-ken (in November).

chromatographed on alumina (2 g) and eluted with benzene to give a colourless oil. This oil was dissolved in ether saturated with HCl gas. Crystallization from $CHCl_3$-ether afforded fine crystals of **2** as hydrochloride (58 mg), whose physical data were completely identical with those of natural methyl ester isolated from the fruits of the plant *Daphniphyllaceae.*

Catalytic hydrogenation of daphnilactone-B (**2**). Catalytic hydrogenation of **2** (50 mg) in EtOAc (1 ml) was carried out over PtO_2 (50 mg) at room temp overnight. After filtration of the catalyst, the solvent was removed under reduced pressure to give a white solid, which showed two spots on analytical TLC plate, corresponding to a reduction product and the original lactone (**2**). The former was easily separated by preparative TLC (silica gel PF_{254}, Merck) using n-hexane–ether–diethyl amine (20:20:3) as solvent system to give a white powder of **14** (20 mg), which was not crystallized but showed one spot on analytical TLC plate. The structure of this compound was confirmed by the following physical data: ν_{max} ($CHCl_3$) 1730 cm^{-1}; δ 1·06 (3H, d, J = 6·0 Hz), 3·71 (1H, d, J_{AB} = 13·0 Hz) and 5·02 (1H, d, J_{AB} = 13·0 Hz); *m/e* 343 (M^+ for $C_{22}H_{33}O_2N$).

Reaction of daphnilactone-B (**2**) *with sodium methoxide* To a solution of **2** (110 mg) in anhyd MeOH (1 ml) was added 0·16 N NaOMe in MeOH (3 ml), and then stirred under N_2 at room temp overnight. The mixture was then diluted with cold water (3 ml), and then extracted with benzene. The extracts were washed with water, and then dried over $MgSO_4$. The solvent was removed under reduced pressure to give a colourless oil, which showed two spots on analytical TLC plate. The upper spot corresponds to the starting material, and the lower one to a methyl ester (**15**). These two compounds were separated by preparative TLC (silica gel PF_{254} Merck) using n-hexane–ether–diethyl amine (20:20:3) as solvent system. Elution with EtOAc–MeOH (4:1) gave an oily substance of **15** (20 mg), whose mass, IR and NMR spectra were immediately measured as shown below, since this oil was gradually recyclized to the original **2** at room temp: ν_{max} ($CHCl_3$) 3600, 3400 and 1730 cm^{-1}; δ 1·04 (3H, d, J = 6·0 Hz), 3·00 (1H, br.s, OH), 3·60 (1H, d, J_{AB} = 9·5 Hz), 3·62 (3H, s), 4·22 (1H, d, J_{AB} = 9·5 Hz) and 5·64 (1H, br.m) *m/e* 373 (M^+ for $C_{23}H_{35}O_3N$), 358 (M^+–Me), 342 (M^+–CH_2OH), 314, 300 and 286.

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