Tetrahedron. Vol 24, pp. 5691 to 5700. Pergamon Press 1968. Printed in Great Britain

THE STRUCTURES OF DAPHNIPHYLLINE AND CODAPHNIPHYLLINE

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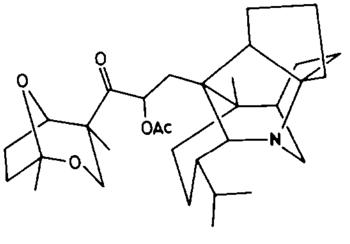
(*Received in Japan* 26 *March* 1968; *Received in the UK for publication* 16 *April* 1968)

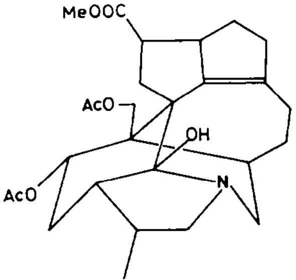
Abstract—Isolation and structures of two alkaloids, daphniphylline and codaphniphylline, are presented. The structure of the former, established by X-ray analysis, is in full agreement with its chemical and spectral data. The structure of the latter was deduced by comparison of the NMR, IR and mass spectra of both compounds. Finally, chemical transformation of daphniphylline to codaphniphylline was carried out.

AN ALKALOID of *Daphniphyllum macropodum* Miquel was first isolated in 1909[1] as a white amorphous powder, m.p. 75–84°, $C_{27}H_{41}O_4N$, and named daphnimacrine.*

We have examined the alkaloidal components of the same plant and isolated seven new alkaloids which were structurally divided into two groups.† The first group comprises daphniphylline, codaphniphylline and neodaphniphylline.[2,3] In addition to the presence of five Me groups, all have characteristic peaks at *m/e* 286 and 272 in their mass spectra. The second group comprises yuzurimine, yuzurimine-A, yuzurimine-B and neoyuzurimine.[4] These have a secondary Me group, a tertiary Me group (or a tertiary acetoxy- or hydroxymethyl group) and a carbomethoxyl group, and there are no typical fragmentation peaks in their mass spectra. The chemical and spectral properties of the major alkaloids, daphniphylline and yuzurimine, indicated that it would be difficult to determine the structures by chemical methods alone, but X-ray diffraction studies indicated the unusual structures I and II for these compounds respectively.[5,6]



I



II

In the present paper,‡ the structures of daphniphylline and codaphniphylline will be discussed.§

* Daphnimacrine described in the Ref 1 seems to be a mixture of more than two alkaloids.

† The relative amounts of each alkaloid varied with the season of collection as well as parts of the plant.

‡ Chemical studies of the other alkaloids will be reported soon.

§ We are interested in the structure of an alkaloid A, which was determined by T. Nakano *et al.* However, from a biogenetic point of view it seems difficult to believe its structure. Structures of the other alkaloids were also reported by the same authors on the basis of our results.[9]

Physical and chemical data of daphniphylline, m.p. 238–240° (as hydrochloride, in a sealed tube), $C_{32}H_{49}O_5N \cdot HCl$, are in full agreement with the structure I; the NMR spectrum of daphniphylline is shown in Fig. 1, and its remarkable signals as well as their assignments are also described. Characteristic peaks at *m/e* 272 and 286 in the mass spectrum can be attributable to the fragment ions III and IV (or V), respectively, as shown below.

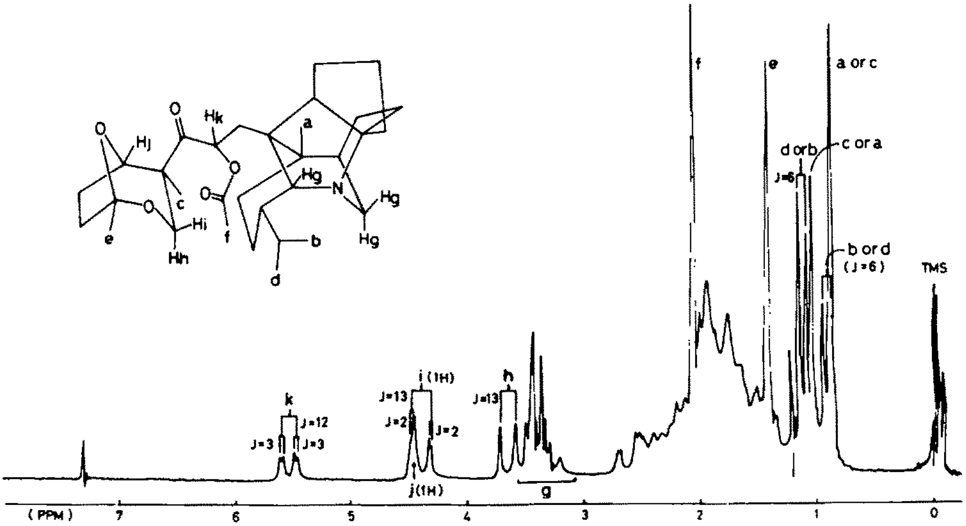
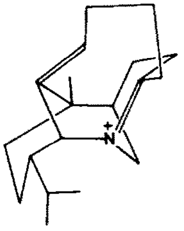
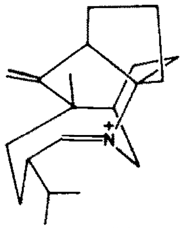


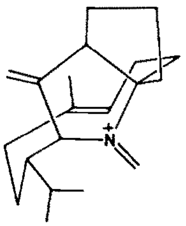
Fig. 1 The NMR spectrum of daphniphylline



m/e 272 III

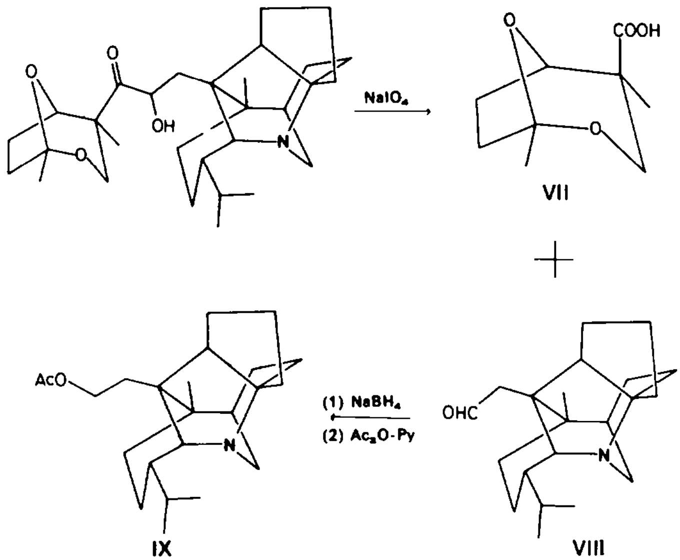


m/e 286 IV



V

The structure I of daphniphylline consists of three main parts (an amine, an α-acetoxy–ketone and a ketal). Hydrolysis of I with 0·6N NaOH in aqueous methanol gave desacetyl daphniphylline (VI), which was oxidized with sodium metaperiodate in aqueous methanol to afford an acid (VII), m.p. 122–123°, *m/e* 186 (M^+), ν_{max} 1700 cm^{-1}, $C_9H_{14}O_4$, and an unstable aldehyde (VIII), which was directly used for the next experiment. Sodium borohydride reduction of VIII in ethanol followed by acetylation with acetic anhydride in pyridine afforded daphnialcohol acetate (IX), m.p. 268–270° (as hydrochloride, in a sealed tube), *m/e* 359 (M^+), ν_{max} 1741 cm^{-1}, no OH group, $C_{23}H_{37}O_2N$. The structures VII and IX are confirmed by their NMR spectra (Figs. 2 and 3). The above chemical experiments are fully consistent with the structure I.



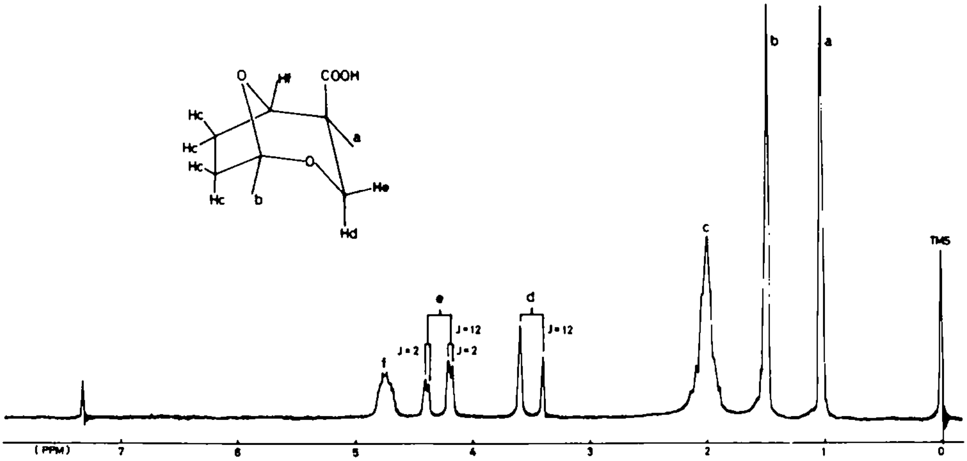


Fig. 2 The NMR spectrum of VII

The structure of codaphniphylline, m.p. 266–267° (as hydrochloride, in a sealed tube), $C_{30}H_{47}O_3N \cdot HCl$, can be deduced by comparison of its spectral data (NMR, IR and mass spectra) with those of daphniphylline (I). The multiplet at 2·90 ppm (2H), which can be assigned to two methylene protons adjacent to the carbonyl group, is observed only in the NMR spectrum of codaphniphylline. On the other hand, daphniphylline has the singlet at 2·05 ppm (3H) and the quartet at 5·52 ppm (1H), which can be assigned to a proton attached to the C atom bearing an acetoxyl group, in its NMR spectrum. The remaining signals are similar in both compounds (Figs. 1 and 4). Furthermore, the IR spectrum of codaphniphylline indicates the presence of a carbonyl group and no ester group, whereas daphniphylline has an α-acetoxyketone as a partial structure. The mass spectrum of codaphniphylline also gives

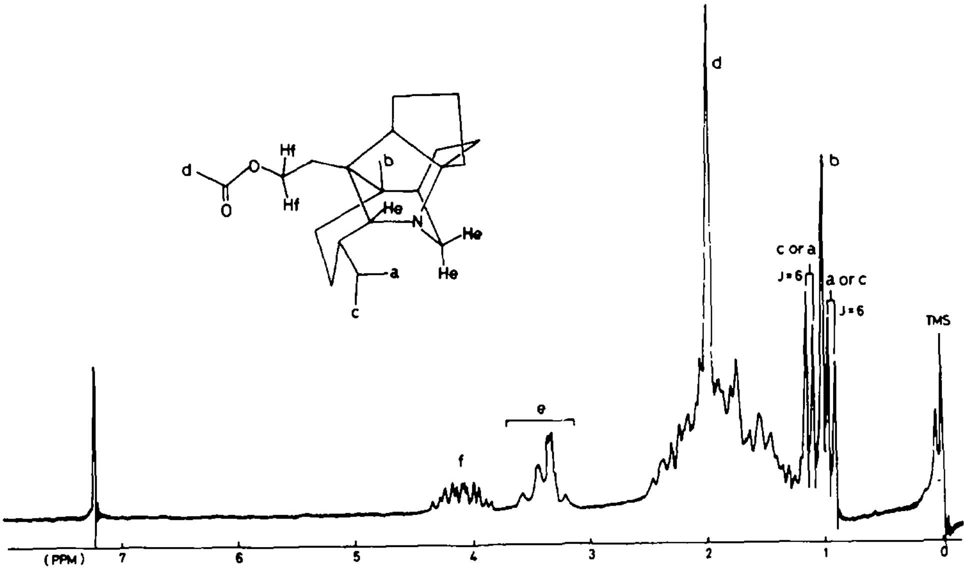


FIG. 3 The NMR spectrum of IX

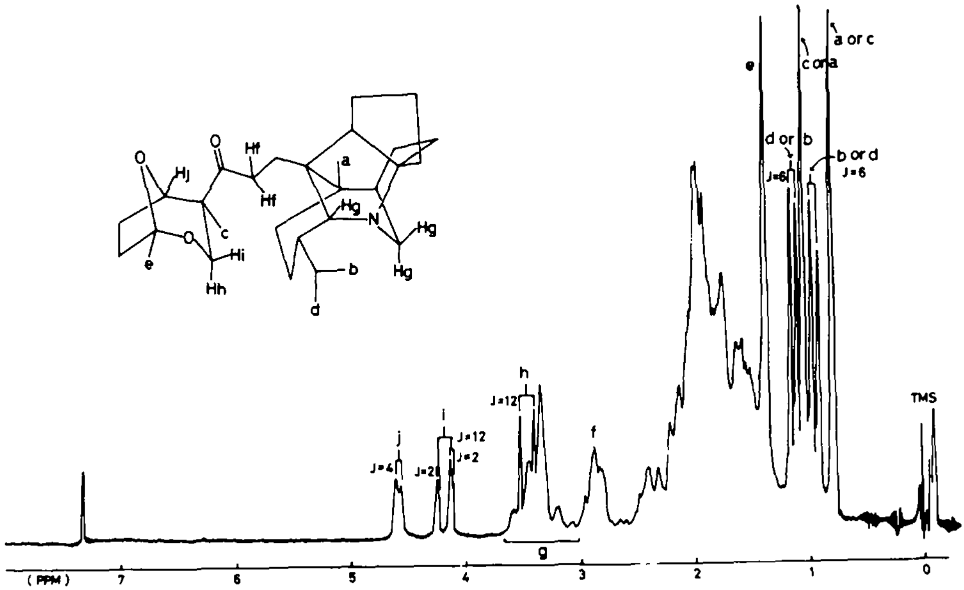
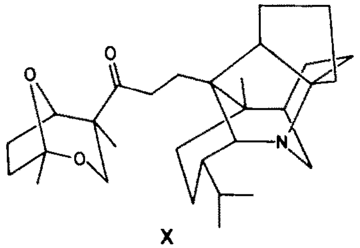


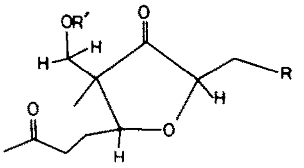
FIG. 4 The NMR spectrum of codaphniphylline

characteristic peaks at *m/e* 272 and 286, suggesting that it has the same carbon skeleton around the N atom as that of daphniphylline. Therefore, the structure X should be assigned to codaphniphylline. In fact, codaphniphylline which has no α-acetoxy-ketone as a partial structure is very stable to mineral acids.[7] On the other hand, when treated with 6N HCl at 80° for 45 min, daphniphylline as well as desacetyl

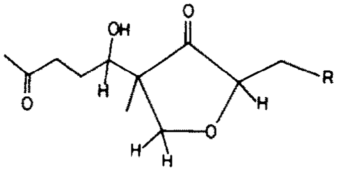


X

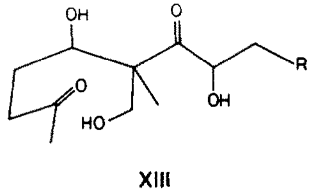
daphniphylline was converted into white powder, desacetyl isodaphniphylline (*m/e* 485 (M^+), 455, 286 and 272 (a pair of characteristic peaks); ν_{max} 3400 (OH), 1753 (5-membered ring ketone) and 1714 cm^{-1}; no acetoxyl band) in quantitative yield. In the NMR spectrum of desacetyl-isodaphniphylline, in addition to four Me signals in the 0·90–1·15 ppm region, a sharp singlet (acetyl group) at 2·14 ppm was observed instead of a Me singlet at 1·41 ppm in daphniphylline (I). Furthermore, there are an AB-quartet (2H, J = 11 c/s) centered at 3·49 ppm and multiplets (2H) at the 4·00–4·30 ppm region. On the basis of the above spectral data, desacetyl-isodaphniphylline should have the structure XI or XII, which was formed through an intermediate XIII. Desacetyl isodaphniphylline hydrochloride was acetylated with acetic



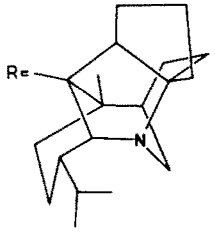
XI: R' = H
XIV: R' = Ac



XII



XIII



anhydride in pyridine to afford an isodaphniphylline hydrochloride, m.p. 197–198·5°, ν_{max} 2600 br., 1756, 1744, 1709 and 1231 cm^{-1}; no OH band, $C_{32}H_{49}O_5N$ HCl, in 73% yield. Isodaphniphylline hydrochloride (XIV) was also obtained by treatment of desacetyl-isodaphniphylline hydrochloride with only acetic acid at 95° for 21 hr in 66% yield. In the NMR spectrum of isodaphniphylline, in addition to the presence of an acetoxyl signal at 2·00 ppm, an AB-quartet at 3·49 ppm in desacetyl isodaphniphylline was shifted to an AB-quartet at 3·99 ppm (2H, J = 11 c/s). From the above result, isodaphniphylline should have the structure XIV.

Finally, the chemical transformation from daphniphylline to codaphniphylline was carried out, as described below.[8] At first, we tried to remove the acetoxyl group of daphniphylline with active zinc powder in glacial acetic acid under various conditions

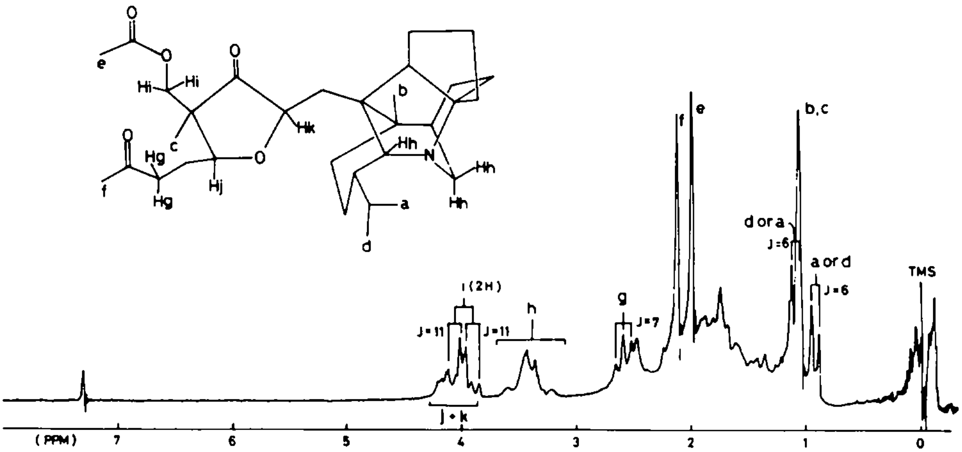
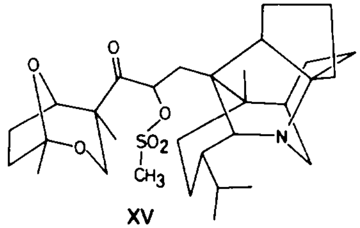


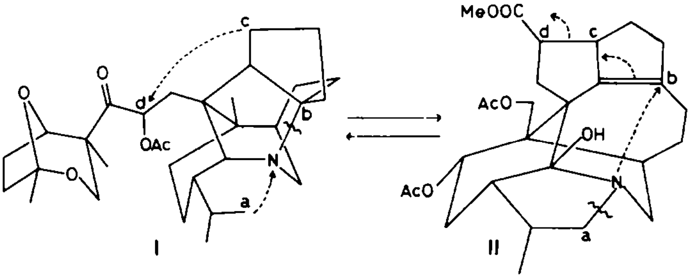
Fig. 5 The NMR spectrum of isodaphniphylline

without success. Therefore, to make a derivative of daphniphylline, which has a better leaving group than an acetoxyl group at the α-position of the ketone, desacetyldaphniphylline (VI) was heated under reflux with *p*-toluenesulfonyl chloride in pyridine for 6 hr, but the starting material was recovered.* However, when treated with methanesulfonyl chloride in pyridine at 65° for 7 hr, VI gave a methanesulfonate (XV), m.p. 162–164°, $C_{31}H_{49}O_6NS$, in 61% yield. Reduction of the methanesulfonate (XV) as hydrochloride with active zinc powder in methanol gave desacetoxydaphniphylline (X) in 78% yield. This compound was identical with codaphniphylline in m.p., IR and mass spectra.



XV

The carbon skeleton of daphniphylline (or codaphniphylline) can be transformed into that of yuzurimine (II) by the following formal procedures; bond formation from C_a to N and C_c to C_d, bond fission between N and C_b. From a biogenetic point of view, these natural products are regarded as a new type of alkaloid, the main carbon skeleton of which consists of four isoprene units and an acetate.



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* The molecule of *p*-toluenesulfonyl chloride seems to be too bulky to react with the hindered OH group of VI, as compared with methanesulfonyl chloride.

EXPERIMENTAL

All m.ps were uncorrected. Optical rotations were determined in $CHCl_3$ with a JASCO ORD/UV-5 spectropolarimeter. IR spectra were recorded in KBr with a JASCO IR-S spectrometer. UV spectra were measured in EtOH with a Cary Model 15 instrument. NMR spectra were obtained on a Varian Associates AH-100 spectrometer. Chemical shifts for all NMR spectra are given in ppm from an internal TMS standard with $CDCl_3$ as solvent, Abbreviations: d, doublet; m, multiplet; q, quartet; s, singlet. Mass spectra were recorded with a Hitachi RMU-6D mass spectrometer with an ionization energy of 70 eV. Samples were introduced into the source by the direct inlet system. Column chromatography was performed on neutral alumina (E. Merk) and silicic acid (Mallinckrodt).

Extraction of yuzurimine, daphniphylline and codaphniphylline. Wet, chopped bark of *Daphniphyllum macropodum* Miquel (15 Kg)* was immersed in MeOH (60 l.) at room temp for 2 weeks, and then filtered off. The methanolic soln (Dragendorff test +) was concentrated under reduced press to about 5 l., diluted with equal volume of water, and then filtered. The filtrate (Dragendorff test +) was acidified with conc HCl to lower than pH 2, and then washed with a lot of ether (5 l.) 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 (20 l.). The ethereal layer (Dragendorff test +) was extracted with 0·1N HCl (1 l.) for purification. This aqueous soln was made basic again with 4N NaOH gradually (pH 9–13) and extracted with ether (15 l.). The ethereal extracts were washed with water (1 l.), dried over Na_2SO_4, and then concentrated under reduced press to give an oil (ca. 30 g), which showed several spots on a TLC plate, as described in Fig. 6. The oil was chromatographed on silicic acid (200 g) and eluted with $CHCl_3$ and $CHCl_3$–MeOH to give 3 fractions. *Fraction* 1. Elution with $CHCl_3$ (1 l.) gave an oil (ca. 10 g) which was a mixture of hydrocarbons. *Fraction* 2. Elution with $CHCl_3$–MeOH (100:1, 1·5 l.) gave an oil (ca. 10 g), part of which was solved in the mixed solvent of $CHCl_3$ and ether saturated with HCl gas. The mixed solvent was removed under reduced press to afford a colourless oil which was crystallized from $CHCl_3$–EtOAc to give white crystals (2·6 g). Recrystallization from $CHCl_3$–ether gave yuzurimine hydrochloride, m.p. 247·5–249° (in a sealed tube); ν_{max} 3440, 2940, 1740 and 1247 cm^{-1}; NMR spectrum: 1·11 (3H, d, $J = 7$ c/s), 1·98 (6H, s), 3·53 (3H, s), 3·20–4·00 (4H, br), 4·00–4·60 (1H, br), 4·32 (2H, q, $J = 11$ c/s), 5·36 (1H, q, $J = 7, 12$ c/s) and 6·66 (1H, s, disappeared on addition of D_2O) ppm; *m/e* 487 (1, $M^+ - HCl$), 469 (44), 410 (45), 334 (13), 85 (65) and 83 (100). The mass spectrum of the hydrochloride is superimposed on that of the corresponding hydrobromide, as described below. According to the above mentioned procedure, crystallization of the remaining oil from $CHCl_3$–ether saturated with HBr gas afforded white crystals (30 mg). Recrystallization from MeOH gave colourless plates of yuzurimine hydrobromide, m.p. 251–253° (in a sealed tube); $[\alpha]_D$ +7·9 (*c*, 1·01); ν_{max} 3440, 2940, 1733 and 1246 cm^{-1}; $\lambda_{210\,m\mu}$ 7400 (end absorption). (Found: C, 57·23; H, 6·76; N, 2·87. $C_{27}H_{37}O_7N$ HBr requires: C, 57·04; H, 6·74; N, 2·46%). This hydrobromide was used for X-ray analysis. *Fraction* 3. Elution with $CHCl_3$–MeOH (20:1, 1·5 l.) gave an oil (ca. 10 g), which was rechromatographed on alumina (300 g) and eluted with benzene-diethylamine (100:0·45) to afford an oil (5 g), which was solved in $CHCl_3$–ether saturated with HCl gas. The solvent was removed under reduced press to give an oil, which was crystallized from $CHCl_3$–ether to give white crystals (1 g). Recrystallization from $CHCl_3$–ether afforded colourless needles, m.p. 238–240° (in a sealed tube), $[\alpha]_D$ +43·7° (*c*, 2·18); ν_{max} 2500 br, 1742, 1714, 1239, 1052, 898 and 826 cm^{-1}; $\lambda_{210\,m\mu}$ 527 (end absorption); NMR spectrum: 0·87 (3H, s), 0·93 (3H, d, $J = 6$ c/s), 1·04 (3H, s), 1·11 (3H, d, $J = 6$ c/s), 1·41 (3H, s), 2·05 (3H, s), 3·1–3·6 (3H, br), 3·65 (1H, d, $J = 13$ c/s), 4·38 (1H, q, $J = 13, 2$ c/s), 4·44 (1H, m) and 5·52 (1H, q, $J = 12, 3$ c/s) ppm; *m/e* 527

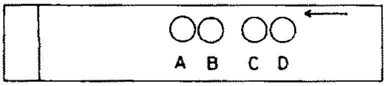

FIG. 6 Thin layer chromatography of the alkaloids on silicic acid (Kiesel gel G) in ether–n-hexane–diethylamine (20:15:3·5). A: daphniphylline and codaphniphylline: B: neoyuzurimine and yuzurimine-A; C: yuzurimine and neodaphniphylline; D: yuzurimine-B.

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* The bark was collected at Sanage, Japan in June. Isolation and chemical studies of the other minor alkaloids except for yuzurimine, daphniphylline and codaphniphylline will be reported later, because attempted chemical correlation among them has not yet been successful.

(33, M$^+$), 512 (18), 484 (7), 443 (10), 442 (34), 287 (22), 286 (100), 272 (38) and 230 (10), (Found: C, 68·14; H, 8·81; N, 2·29; AcO, 7·12. $C_{32}H_{49}O_5N$ HCl requires: C, 68·12; H, 8·93; N, 2·48; AcO, 7·63%).

In order to transform the hydrochloride into the hydrobromide, a small amount of water saturated with $NaHCO_3$ was poured into an aqueous soln of daphniphylline hydrochloride (100 mg) with precipitate of white solid. After the ppt (43 mg) was washed with water, it was crystallized from benzene–n-hexane saturated with HBr gas. Recrystallization from the mixed solvent of benzene and n-hexane (5:1) gave monoclinic crystals, m.p. 245–246°; ν_{max}^{Nujol} 2500 br, 1734, 1707, 1230, 1064 and 1053 cm^{-1}. The mass spectrum could be superimposed on that of the corresponding hydrochloride. This hydrobromide was used for X-ray analysis.

The remaining oil of the fraction was rechromatographed on alumina (300 g) and eluted with benzene–diethylamine (100:0·5). After a small amount of oil was eluted with 1·8 l. of the solvent, the following elution with 0·6 l. of the same solvent gave an oil (2 g), from which daphniphylline (0·94 g) was obtained as hydrochloride, according to the usual manner. Further elution with benzene–diethylamine (100:0·5, 0·2 l.) gave an oil (0·3 g), which was solved in $CHCl_3$–ether saturated with HCl gas. The solvent was removed under reduced press to afford an oil, which was crystallized from $CHCl_3$–ether and then recrystallized from the same mixed solvent to give white crystals of codaphniphylline hydrochloride (0·13 g), m.p. 266–267° (in a sealed tube); $[\alpha]_D$ +4·2° (c, 2·40); ν_{max} 2500 br, 1707, 1388, 1321, 1182, 1142, 1095 and 1053 cm^{-1}; $\lambda_{210\,m\mu}$ 170 (end absorption); NMR spectrum: 0·79 (3H, s), 0·94 (3H, d, J = 6 c/s), 1·04 (3H, s), 1·13 (3H, d, J = 6 c/s), 1·38 (3H, s), 2·90 (2H, m), 3·1–3·7 (3H, br), 3·47 (1H, d, J = 12 c/s), 4·19 (1H, q, J = 12, 2 c/s) and 4·60 (1H, broad d, J = 4 c/s) ppm; *m/e* 469 (100, M), 454 (50), 386 (20), 292 (20), 286 (56), 272 (67), 259 (23), 258 (36), and 230 (26). (Found: C, 70·75; H, 9·41; N, 2·82. $C_{30}H_{47}O_3N\cdot HCl$ requires: C, 71·21; H, 9·56; N, 2·77%).

Desacetyl daphniphylline (VI). To a soln of daphniphylline hydrochloride (23 mg) in MeOH (1 ml) was added 2N-50% methanolic NaOH aq (1 ml). The reaction soln was allowed to stand at room temp overnight, diluted with a lot of water, and then extracted with $CHCl_3$. The extract was washed with a lot of water and dried over Na_2SO_4. The solvent was removed under reduced press to afford an oil, which was dissolved in 0·1N HCl (2 ml). To this acidic soln several drops of 2N NaOH were added to afford a white ppt, which was washed with water and dried under reduced press to give an amorphous powder of desacetyl-daphniphylline (14 mg), ν_{max}^{film} 3400, 1705, 1390, 1322, 1179, 1145, 1059 and 830 cm^{-1}; NMR spectrum: 0·85 (3H, s), 0·87 (3H, d, J = 6 c/s), 0·97 (3H, d, J = 6 c/s), 1·03 (3H, s), 1·40 (3H, s), 2·9–3·4 (3H, br), 3·56 (1H, d, J = 13 c/s), 4·27 (1H, q, J = 13, 2 c/s), 4·76 (1H, m), and 4·90 (1H, q, J = 8, 3 c/s) ppm; *m/e* 485 (31, M$^+$), 470 (9), 443 (5), 287 (25), 286 (100), 273 (12), 272 (46), 245 (17), and 230 (7). Attempted crystallization from various kinds of solvent was not successful, but acetylation of this amorphous powder with acetic anhydride in pyridine afforded daphniphylline in quantitative yield (TLC, IR and mass spectra).

Sodium periodate oxidation of desacetyl daphniphylline. To a soln of desacetyl–daphniphylline, which was produced from daphniphylline hydrochloride (233 mg), in 50% aqueous MeOH (3 ml), sodium periodate (197 mg) in 50% aqueous methanol (4 ml) was added with stirring. The resulting soln was allowed to stand at room temp overnight, and then diluted with water. After addition of 0·5N NaOH (0·5 ml), the reaction product was extracted with a lot of chloroform. The extract was washed with a small amount of water and dried over Na_2SO_4. The solvent was removed under reduced press to give an unstable aldehyde (Tollens test +), which was directly used for the next experiment. On the other hand, the basic soln was acidified with 2N HCl, and then extracted with a lot of $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated under reduced press to afford white crystals (55·3 mg). Recrystallization from n-hexane–benzene gave colourless plates of VII, m.p. 122–123°; ν_{max} 1700, 1393, 1140, 1068, 944 and 811 cm^{-1}; NMR spectrum: 1·03 (3H, s), 1·50 (3H, s), 2·03 (4H, m), 3·63 (1H, d, J = 12 c/s), 4·30 (1H, q, J = 12, 2 c/s), and 4·77 (1H, m) ppm; *m/e* 186 (18, M$^+$), 156 (10), 138 (24), 126 (32) 124 (45), 113 (45), 101 (91), 95 (82) and 72 (100). (Found: C, 58·01; H, 7·47. $C_9H_{14}O_4$ requires: C, 58·05; H, 7·58%).

Sodium borohydride reduction of the aldehyde. Sodium borohydride (100 mg) in EtOH (15 ml) was added to a soln of the above aldehyde in EtOH (10 ml). The resulting soln was allowed to stand at room temp overnight with stirring, and then the reaction soln was concentrated to about 3 ml, diluted with a lot of water, and then extracted with $CHCl_3$. The extract was washed with a small amount of water and dried over Na_2SO_4. The solvent was removed under reduced press to give an oil, which was dissolved in 5 ml of $CHCl_3$–diethylamine (100:0·4) and chromatographed on silicic acid (10 g). After a small amount of oil was eluted with 200 ml of the mixed solvent, further elution gave an oil, which was solved in $CHCl_3$–ether saturated with HCl gas. The solvent was removed under reduced press to give an oil, which was crystallized from $CHCl_3$–ether and recrystallized from $CHCl_3$–ether to afford colourless needles of daphnialcohol hydrochloride (76 mg), m.p. 239–241·5° (in a sealed tube); ν_{max} 3400, 2600 br, 1476, 1455, 1030 and 927 cm^{-1};

NMR spectrum: 0·91 (3H, d, $J = 6$ c/s), 0·97 (3H, s), 1·06 (3H, d, $J = 6$ c/s), and 3·10–3·90 (5H br) ppm; m/e 317 (100, M^+), 302 (74), 286 (42), 275 (27), 274 (24), 273 (16), 272 (62), 234 (30) and 231 (36). (Found: m/e 317·2734. $C_{21}H_{35}ON$ requires: m/e 317·2718).

Acetylation of daphnialcohol hydrochloride. 0·5 ml of Ac_2O was added to a soln of daphnialcohol hydrochloride (112 mg) in pyridine (1 ml). The resulting soln was allowed to stand at room temp overnight, and then the solvent and excess amounts of Ac_2O were removed under reduced press to give an oil, which was crystallized from n-hexane–benzene and recrystallized from $CHCl_3$–ether to afford colourless needles of daphnialcohol acetate hydrochloride (116 mg), m.p. 268–270° (in a sealed tube); ν_{max} 2500 br, 1741, 1368, 1231, 1065, 1036 and 1026 cm^{-1}; NMR spectrum: 0·91 (3H, d, $J = 6$ c/s), 1·00 (3H, s), 1·10 (3H, d, $J = 6$ c/s), 1·99 (3H, s), 3·10–3·70 (3H, br) and 3·80–4·40 (2H, m) ppm; m/e 359 (100, M^+), 344 (77), 317 (26), 316 (28), 286 (30), 274 (42), 272 (78), 258 (39) and 230 (28). (Found: C, 69·70; H, 9·52; N, 3·34. $C_{23}H_{37}O_2N \cdot HCl$ requires: C, 69·76; H, 9·42; N, 3·54%).

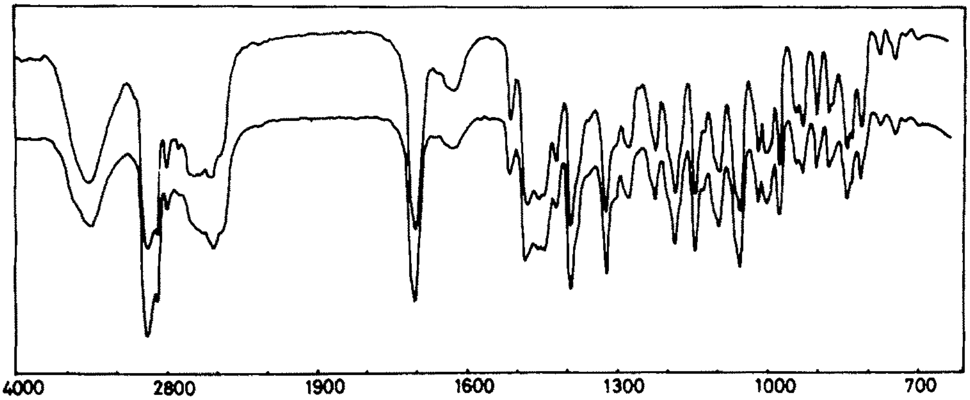
Acid isomerization of daphniphylline hydrochloride. A soln of daphniphylline hydrochloride (92 mg) in 3 ml of 6N HCl was warmed at 80° for 45 min, and then the reaction soln was cooled and diluted with a small amount of water. The reaction product was extracted with $CHCl_3$ and dried over Na_2SO_4. The solvent was evaporated under reduced press to give an oil, which was dissolved in 10 ml of n-hexane–ether–diethylamine (100:100:3) and chromatographed on silicic acid (5 g). Elution with EtOAc (50 ml) gave an oil which was dissolved in 0·1N HCl. 1N NaOH was added gradually to this soln to afford a white ppt, (50 mg), which was washed with a lot of water; ν_{max} 3400 br, 1753, 1714, 1363, 1165, 1064, 1005 and 932 cm^{-1}; NMR spectrum: 0·87 (3H, d, $J = 6$ c/s), 0·97 (9H, broad s), 2·14 (3H, s), 3·00–3·40 (3H, br), 3·37 (1H, d, $J = 11$ c/s), 3·61 (1H, d, $J = 11$ c/s) and 4·00–4·30 (2H, m) ppm; m/e 485 (11, M^+), 470 (2), 455 (24), 440 (5), 286 (100), 272 (21), 245 (16) and 230 (9). Attempted crystalization of the amorphous desacetylisodaphniphylline from various solvents was not successful but we did obtain an acetyl derivative as white crystals, as shown below.

Formation of isodaphniphylline hydrochloride (XIV). 1·5 ml of Ac_2O was added to a soln of desacetyl isodaphniphylline, which was obtained by hydrolysis of daphniphylline hydrochloride (190 mg) with 6N HCl, in dry pyridine (3 ml). The resulting soln was allowed to stand at room temp overnight, and then the solvent and excess amounts Ac_2O were removed under reduced press to give an oil, which was crystallized from acetone–ether saturated with HCl gas and recrystallized from $CHCl_3$–ether to give a 73% yield of colourless needles of isodaphniphylline hydrochloride (138 mg), m.p. 197–198·5°; ν_{max} 2600 br, 1756, 1744, 1709, 1377, 1359, 1231, 1063 and 1045 cm^{-1}; NMR spectrum: 0·93 (3H, d, $J = 6$ c/s), 1·07 (6H, s), 1·10 (3H, d, $J = 6$ c/s), 2·00 (3H, s), 2·13 (3H, s), 3·10–3·60 (3H, br), 3·90 (1H, d, $J = 11$ c/s), 4·07 (1H, d, $J = 11$ c/s), and 3·80–4·30 (2H, m) ppm; m/e 527 (14, M^+), 512 (3), 485 (2), 426 (4), 350 (3), 286 (100), 272 (23), 245 (18) and 230 (13). (Found: C, 68·36; H, 8·88; N, 2·59. $C_{32}H_{49}O_5N \cdot HCl$ requires: C, 68·12; H, 8·93; N, 2·48%).

Acetylation of desacetyl isodaphniphylline hydrochloride with acetic acid. A soln of desacetyl-isodaphniphylline hydrochloride (92 mg) in 10 ml of glacial AcOH was warmed at 95° for 21 hr, and then concentrated under reduced press to give an oil, which was crystallized from $CHCl_3$–ether to afford an acetate (61 mg), which was identified as isodaphniphylline hydrochloride by m.p., IR and mass spectra.

Reaction of desacetyl daphniphylline with methanesulfonyl chloride. 0·5 ml of methanesulfonyl chloride was added to a soln of desacetyl-daphniphylline, which was obtained from daphniphylline hydrochloride (134 mg), in dry pyridine (5 ml). The resulting soln was warmed at 65° for 7 hr. After cooling, the reaction soln was diluted with a lot of water, washed with ether, and then extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated under reduced press to afford an oil, which was dissolved in 10 ml benzene–diethylamine (100:0·5) and chromatographed on 1 g of alumina. Elution with 50 ml of the same solvent gave white solid which was recrystallized from acetone–water to afford colourless plates of daphniphylline methanesulfonate (68 mg), m.p. 162–164°; ν_{max} 1720, 1388, 1355, 1328, 1173, 1144, 1052, 950, 938, 922, 885, 825 and 795 cm^{-1}; NMR spectrum: 0·86 (3H, d, $J = 6$ c/s), 0·92 (3H, s), 0·98 (3H, d, $J = 6$ c/s), 1·12 (3H, s), 1·46 (3H, s), 2·14 (3H, s), 3·69 (1H, d, $J = 13$ c/s), 4·3 (1H, q, $J = 13$, 1·6 c/s), 4·68 (1H, m) and 5·81 (1H, q, $J = 13$, 2 c/s) ppm; m/e 563 (43, M^+), 548 (12), 468 (12), 467 (23), 442 (44), 286 (100), 272 (32), 244 (8) and 230 (8). (Found: C, 66·25; H, 9·00; N, 2·30. $C_{31}H_{49}O_6NS$ requires: C, 66·02; H, 8·78; N, 2·48%).

Reduction of the methanesulfonate (XV) *with active zinc powder.* A soln of the methanesulfonate hydrochloride (103 mg) in 20 ml MeOH was refluxed with 5 g of active Zn powder for 15 hr with stirring, and then cooled. After the Zn powder was removed by filtration, the solvent was removed under reduced press to give an oil which was dissolved in 10 ml benzene–diethylamine (100:0·5) and chromatographed on alumina (4 g). Elution with 50 ml of the above solvent afforded an oil, which was crystallized from chloroform–ether saturated with HCl and recrystallized from $CHCl_3$–ether to give 72 mg of desacetoxydaphniphylline hydrochloride in 78% yield. This compound was identified as codaphniphylline by m.p., IR and mass spectra. IR spectra of both compounds are shown below.



Acknowledgements—The authors are grateful to Takeda Chemical Industries, Ltd. for measurement of NMR spectra. They are also indebted to the National Institutes of Health, U.S.A., which supported this work through Grant RG-7969 and GM-7969.

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