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THE ALKALOIDS OF ANODENDRON AFFINE DRUCE

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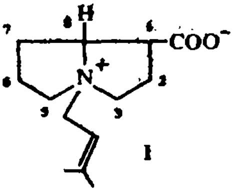
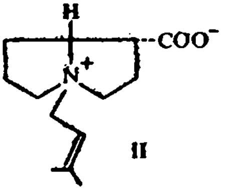
Abstract—Two new zwitterionic alkaloids, anodendrine (I) and alloanodendrine (II), were isolated from *Anodendron affine* Druce. Characterization by chemical and physical methods and chemical synthesis have shown the former to be N-isopropenyl-laburninic acid and the latter to be N-isopropenyl (+)-isoretronecanolic acid.

SEVERAL indole alkaloids occur in the plant family apocyanaceae. In *Anodendron affine* Druce, we found water soluble alkaloids, which could not be taken up in ether even if the aqueous solution was made basic. We wish to report the isolation, structures and syntheses of two new pyrrolizidine alkaloids, anodendrine (I) and alloanodendrine (II).[1] In addition, a quarternary amine, choline (III), was isolated from the above plant.



I



II

Paper chromatography of the alkaloidal components, extracted from the leaves and stems of *Anodendron affine*, showed two spots (A and B). The fraction corresponding to the part A was isolated as a precipitate of the reineckate and purified by paper chromatography to give only one alkaloid, choline (III). From the second fraction B, anodendrine was crystallized as a picrate, m.p. 123–124° and molecular formula $C_{13}H_{21}O_2N$–$C_6H_3O_7N_3$, on the basis of elemental analysis and ε value of its UV spectrum. The mother liquor, free from picric acid, was concentrated, and treated with *p*-bromophenacyl bromide to give an ester, m.p. 82–84°, $C_{13}H_{21}O_2N$–$C_8H_6OBr_2.4/5CHCl_3$. We named this ester, alloanodendrine *p*-bromophenacyl ester bromide, as its IR spectrum was different from that of corresponding ester derived from anodendrine (Fig. 1).

Anodendrine (I), an oily substance shows a CO band (1606 cm^{-1} in chloroform) and a double bond absorption (1665 cm^{-1}) and exhibits UV end absorption. The IR spectrum of anodendrine (I) shows a strong absorption band at 1606 cm^{-1}, but as this band appears at 1712 cm^{-1} in the picrate, I probably has a carboxylate group. Further, anodendrine (I) must be a quarternary amine, since it shows only one *pKa'* value at 3·7, which is attributable to the carboxyl group. Accordingly, anodendrine (I) is a zwitterionic alkaloid with a quarternary N and a carbonyl group. Catalytic

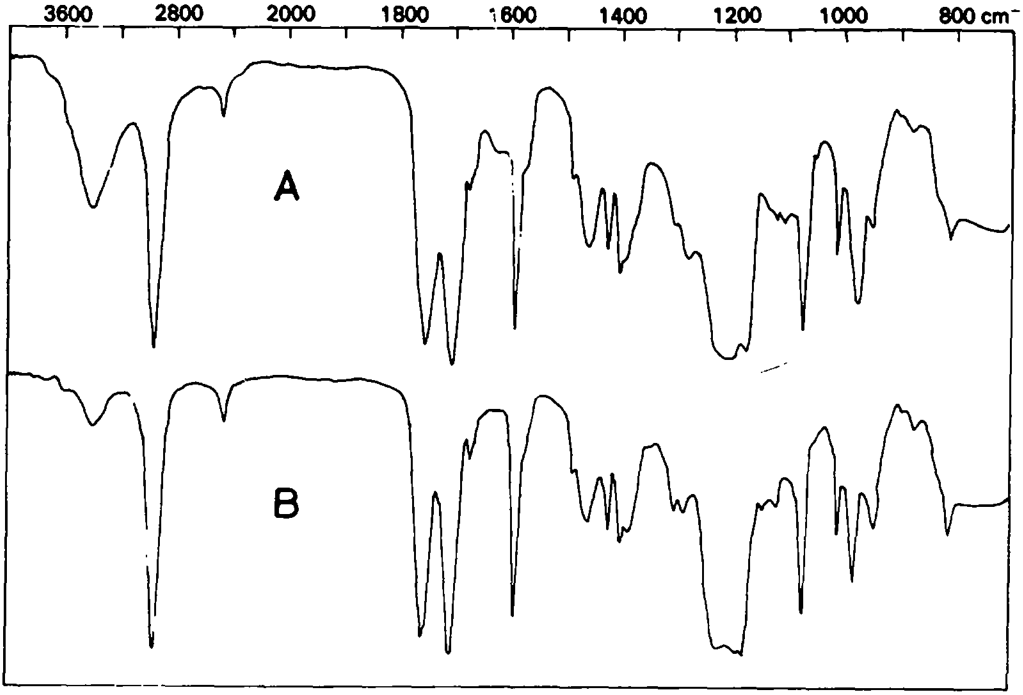
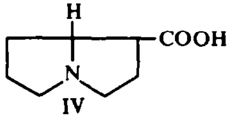
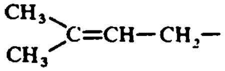


FIG 1. IR spectra of anodendrine p-bromophenacyl ester bromide (A) and corresponding ester derived from alloanodendrine (B)

hydrogenolysis of I afforded laburninic acid[2] (IV). Laburninic acid (IV) contains eight C atoms less than anodendrine (I) by C_5-unit.



IV



$$\begin{matrix}CH_3 \\ CH_3\end{matrix}\!\!>C{=}CH{-}CH_2{-}$$

V

The mass spectrum of anodendrine (I) could not be taken because of its low vaporization pressure. However, the mass spectrum (Fig. 2) of the methyl ester shows the highest mass *m/e* peak at 169, corresponding to the molecular peak of laburninic acid methyl ester. The base peak was observed at *m/e* 67 and the peak at *m/e* 68 (C_5H_8) may be attributable to the isopentenyl group.

The NMR spectrum (Fig. 3) of anodendrine shows signals at 1·86 and 1·90 ppm for two methyls attached to a double bond, 3·9–4·5 for two allylic protons, 1·7–2·2 (6H), 2·93 (m, 1H), 3·3–3·9 (4H), and 4·55 (m, 1H) arising from laburninic acid (IV), and 5·43 (broad t, 1H). Irradiation of two Me signals at 1·86 and 1·90 ppm converts the broad olefinic signal at 5·43 ppm (broad t, $J = 7{\cdot}5$, 1H) to a sharp triplet. On irradiation of the olefinic signal at 5·43 ppm, the complex signal at 3·9–4·5 ppm (2H) is changed to two pairs of doublets. These results indicate the presence of the isopentenyl group (V), which can be attached to the N atom of laburninic acid (IV). Therefore, the structure I must be given to anodendrine.

The second alkaloid, alloanodendrine obtained from the *p*-bromophenacyl ester bromide on hydrolysis, was an oily substance. The physical data are very similar to that of anodendrine (I) except for the optical rotation value. Particularly, the very close

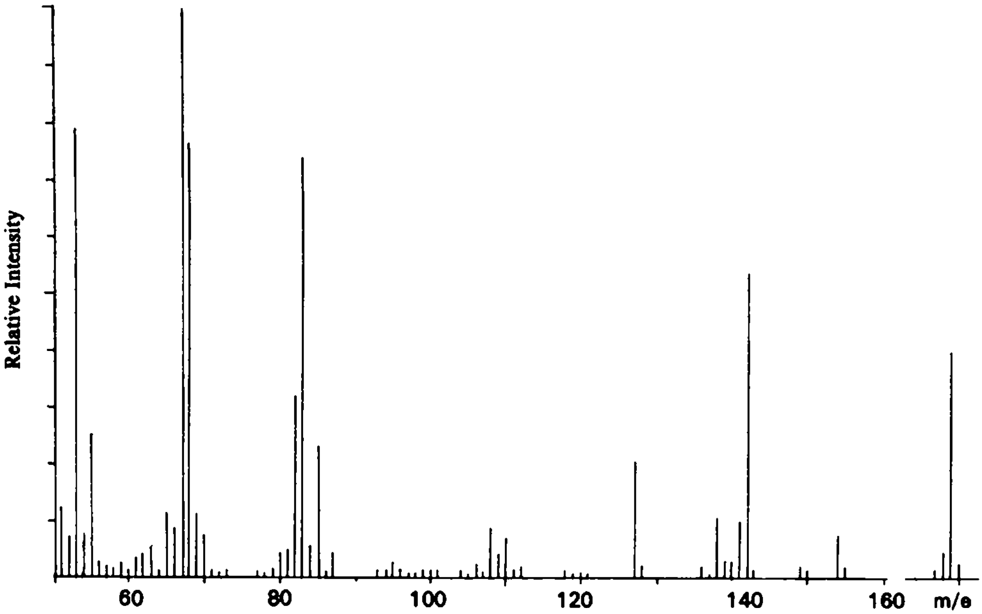
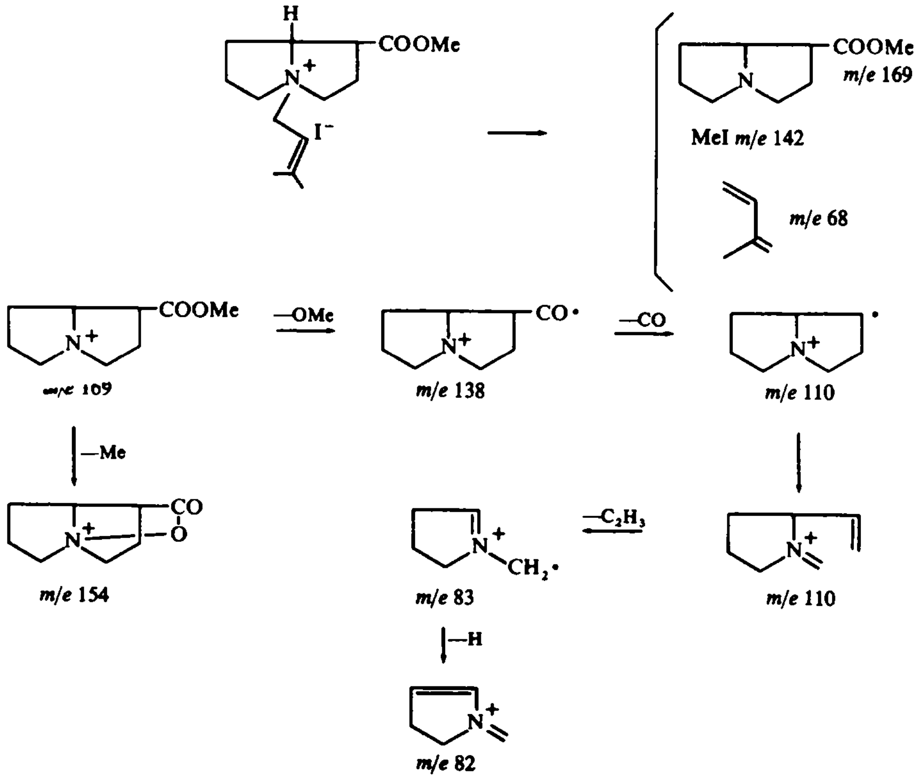


FIG 2. Mass spectrum of anodendrine methyl ester iodide



SCHEME 1

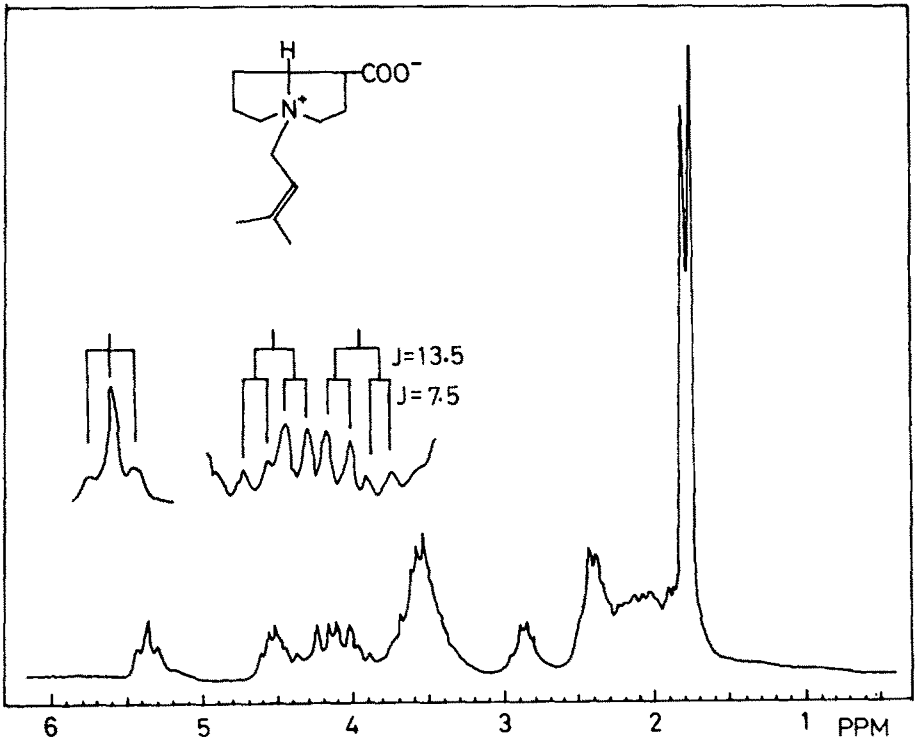
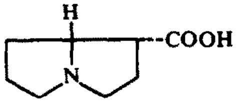


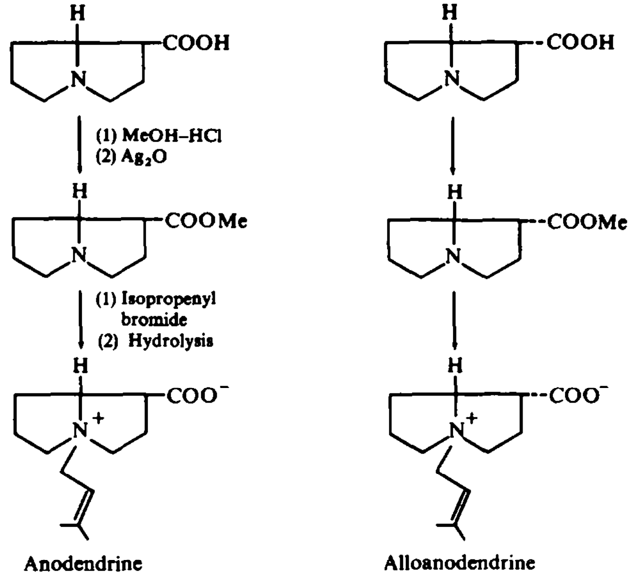
FIG 3. NMR spectrum of anodendrine in $CDCl_3$

similarity of its NMR spectrum to that of anodendrine suggests that alloanodendrine (II) must be an epimer of anodendrine (I) at C_1 or C_8-position. Hydrogenolysis of alloanodendrine gave (+)-isoretronecanolic acid (VI).[3] The result of hydrogenolysis coupled with the physical data indicate that alloanodendrine has the structure II.



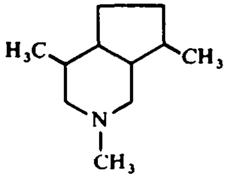
Finally, the structures I and II for anodendrine and alloanodendrine have been confirmed by the syntheses (Scheme 2). Laburninic acid (IV) as the starting material for the synthesis of I, was esterified to the methyl ester, which reacted with isopentenyl bromide. Hydrolysis of the product afforded an amino acid, the picrate of which is identical with natural anodendrine picrate. Alloanodendrine (II) was synthesized from (+)-isoretronecanolic acid (VI) in a procedure similar to that of I. The synthetic sample was identical with natural alloanodendrine.

The discovery of anodendrine (I) and alloanodendrine (II) adds another class of alkaloids to the variety already elaborated by the family apocyanaceae. From this

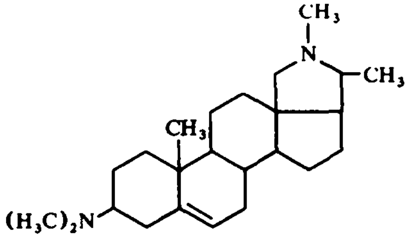

Syntheses of Anodendrine and Alloanodendrine

SCHEME 2

family many indole alkaloids and two other types[4] (monoterpene alkaloids: skytanthine, and steroidal alkaloids: conessine) have been reported. These new zwitterionic alkaloids belonging to pyrrolizidine group, which usually consists of necine (amino alcohol) and necic acid (carboxylic acid), have an amino acid and isopentenyl group attached to the N atom forming a quarternary amine. From a biogenetic point of view, these alkaloids may be formed from laburninic acid (IV) (or (+)-isoretronecanolic acid (VI)) and isoprene unit.



skytanthine



conessine

EXPERIMENTAL

All m.ps are uncorrected. The UV spectra were measured in MeOH soln with a Perkin–Elmer 202 Spectrophotometer. The IR spectra were recorded with a Nihon–Bunko IR-S Spectrophotometer and with a Nihon–Bunko DS-402G Spectrophotometer. The NMR spectra were recorded with JOEL Spectrometers (C-60H and JNM-4H-100). Chemical shifts for all NMR spectra are given in ppm relative to internal TMS in $CDCl_3$ and pyridine, and to sodium 3-(trimethylsilyl) propanesulfonate in D_2O; s, singlet; d, doublet; t, triplet; m, multiplet; coupling constants are given in c/s. Optical rotation were taken on a

JASCO ORD/UV-5 Spectropolarimeter. The mass spectra were recorded with a Hitachi RMU-6D mass spectrometer equipped with a heating system and operating with an ionization energy of 70 eV. Column chromatography were performed on Mallinckrodt silicic acid (100 mesh Mallinckrodt U.S.A.). Paper chromatography was carried out on Toyo-roshi filter paper No. 51 (Toyo-roshi, Japan) using ethyl acetate—ethanol—water (5:3:2) as a developing solvent. Visualization was affected with Dragendorff reagent. Ion-exchange resin chromatography was performed on Amberlite IR-4B and IRC-50 (Organo, Japan).

Extraction of anodendrine and alloanodendrine (part B). The leaves and stems (2·0 Kg) of *Anodendron affine* Druce collected in May–June in Kii and Atsumi peninsula (Japan) were extracted with MeOH (8 l.) at room temp for a week. The soln was concentrated to about 300 ml, which was filtered through supercell. The filtrate was washed with ether several times to remove chlorophyll, and then concentrated *in vacuo* to about 100 ml. A gumlike material, insoluble in EtOH–MeOH (1:1), was separated, and discarded after having been found to give a negative test with Dragendorff reagent. On removal of the solvent, the brown residue was chromatographed on Amberlite IRC-50 (H-form, 100 ml) using water as eluent. The crude alkaloids were absorbed on a column packed with active charcoal (10 g) (Wako, Japan, activated for chromatography) and then with MeOH (1 l.). The removal of the solvent afforded a colourless oily material (400 mg). To a soln of the oily substance in water (0·5 ml) was added a soln of picric acid (170 mg) in water (10 ml). The mixture was allowed to stand overnight to give a yellow solid, which was separated by decantation. Recrystallization from EtOAc gave yellow needles of anodendrine picrate, 250 mg, m.p. 123–124°; IR bands at 3420, 1712, 1672, 1615, 1565, 1550 cm^{-1} (KBr); (Found: C, 50·20; H, 5·27; N, 12·22. $C_{13}H_{21}O_2N.C_6H_3O_7N_3$ requires: C, 50·44; H, 5·35; N, 12·39%).

A soln of anodendrine picrate (200 mg) in MeOH–H_2O (1:4) (20 ml) was passed through IR-4B (OH-form) and successively treated with active charcoal (Darco G-60, 0·3 g) to exclude picric acid. On removal of the solvent, a colourless oily residue of anodendrine (95 mg) remained, R_f 0·37 on paper chromatography; IR bands at 1606, 1665 cm^{-1} ($CHCl_3$); UV, end absorption, ε (212 mμ) = 645 (MeOH); $[\alpha]_D^{25°}$ = 9·5° (c = 4·0, EtOH); *pKa′* = 3·7 (50% MeOH); NMR signals at 1·86 (s, 3H), 1·90 (s, 3H), 1·7–2·2 (6H), 2·93 (m, 1H), 3·3–3·9 (4H), 4·55 (m, 1H), 5·43 (broad t, J = 7·5, 1H) in $CDCl_3$ (Fig 3).

Separation of anodendrine (I) *and alloanodendrine* (II). To a mixture (2·3 g) of I and II in $CHCl_3$ (ca. 30 ml) was added *p*-bromophenacyl bromide (2·7 g), and the resulting soln was refluxed for 6 hr. The soln was then concentrated under reduced press to give a pale yellow oil, which was chromatographed over silicic acid (60 g). Early fraction (total amount, 800 ml) eluted by $CHCl_3$–MeOH (96:4) gave a crystalline solid (0·97 g), recrystallization of which from $CHCl_3$–benzene (2:7) yielded *p*-bromophenacyl ester bromide, plates, m.p. 82–84°, IR bands at 1749, 1705, 1670, 1590, 1456, 1425, 1404, 1076, 1013, 983 cm^{-1} ($CHCl_3$); NMR signals at 1·87 (broad s, 6H), 1·9–2·9 (6H), 3·2–5·0 (8H), 5·43 (broad d, 3H), 7·72 (A_2B_2 type, 4H); (Found: C, 43·81; H, 4·66; N, 2·40. $C_{13}H_{21}O_2N.C_8H_6OBr_2$. 4/5$CHCl_3$ requires: C, 43·87; H, 4·70; N, 2·35%).

To a methanolic soln of IV (200 mg/3 ml) was added dropwise 0·2 N $Ba(OH)_2$ soln (6 ml) at room temp with stirring. The mixture was kept overnight, and then neutralized with 0·2 N H_2SO_4 with cooling. Precipitated $BaSO_4$ was filtered off and 0·2 N H_2SO_4 was further added to the filtrate for complete precipitation of $BaSO_4$. Additional $BaSO_4$ was filtered off. The filtrate was washed with ether several times, and then neutralized with Amberlite IR-4B (OH-form). The soln was put on a column packed with active charcoal (1·5 g) and washed with water and then eluted with MeOH (50 ml). Removal of the solvent afforded a colorless oily product (70 mg), IR bands at 1597, 1665 cm^{-1}; UV, end absorption, ε (212 mμ) = 640 (MeOH); $[\alpha]_D^{25°}$ = 18° (c = 2·9, EtOH); *pKa′* = 3·2 (water).

Extraction of choline (III) *(part A). Anodendron affine* Druce (4·0 Kg) was extracted with MeOH, and the methanolic soln was concentrated *in vacuo* to about 300 ml. The remaining soln was washed with ether several times. To the aqueous soln, a satd ammonium reineckate soln was added at 60°, and then the mixture was cooled in an icebox. The ppt was collected and dissolved in acetone. The acetone soln of the reineckate was filtered, and a warm satd soln of Ag_2SO_4 was added to the filtrate, and then the resulting ppt was filtered off. The filtrate was evaporated under reduced press to give a pale yellow solid, paper chromatography of which showed only one spot. Crystallization of the corresponding picrate from EtOH afforded yellow crystals (14 mg), m.p. 145–246°, which were identical with the authentic sample of choline picrate (mixed m.p., IR and NMR spectra).

Hydrogenolysis of anodendrine (I). A soln of I (150 mg) in MeOH (10 ml) was hydrogenated over 10% Pd–C (50 mg) for 4 hr at room temp. After removal of the catalyst, the solvent was evaporated *in vacuo* to give a crystalline residue, which was crystallized from EtOAc–EtOH to give IV (91 mg), m.p. 210–212°

(dec); $[\alpha]_D^{25°} = 44.2°$ ($c = 1.6$, water); mass, 155 (M^+); IR bands at 3400, 1740, 1440, 1388, 1220, 1200 cm^{-1} (KBr). To an aqueous soln of IV was added a soln of picric acid to give crude crystals, which were recrystallized from EtOH to afford yellow needles (183 mg), m.p. 175–176°, IR bands at 3420, 1756, 1724, 1636, 1564, 1323, 1277, 1143, 1083, 915 cm^{-1} (KBr); $pKa' = 3.95 \cdot 10.55$ (50% MeOH); (Found: C, 43·61; H, 4·03; N, 14·44. $C_{14}H_{16}O_9N_4$ requires: C, 43·75; H, 4·20; N, 14·58%). IV was identical with laburninic acid ($[\alpha]_D$ value, IR and mass spectra). Furthermore, the picrate of IV was identical with the authentic sample of laburninic acid picrate (mixed m.p., IR and mass spectra).

Hydrogenolysis of alloanodendrine (II). A soln of II (100 mg) in MeOH (10 ml) was hydrogenated over 10% Pd–C (80 mg) for 3 hr at room temp. After removal of the catalyst, the solvent was evaporated under reduced press leaving a crystalline residue, which was recrystallized from EtOAc–EtOH to give VI (55 mg), m.p. 223–225° (dec); $[\alpha]_D^{25°} = 72°$ ($c = 2.0$, EtOH); mass, 155 (M^+). To the aqueous soln of I was added aqueous soln saturated with picric acid to give crude crystals, which was recrystallized from EtOH to afford yellow needles (129 mg), m.p. 219–220° (dec); IR bands at 3230, 1749, 1710, 1635, 1562, 1503, 1320, 1200, 1078, 918 cm^{-1} (KBr); $pKa' = 3.95, 10.55$ (50% MeOH); (Found: C, 43·74; H, 3·94; N, 14·49. $C_{14}H_{16}O_9N_4$ requires: C, 43·75; H, 4·20; N, 14·58%). VI was identified as (+)-isoretronecanolic acid (mixed m.p. and IR spectra).

Anodendrine p-bromophenacyl ester bromide. A mixture of I (100 mg) and *p*-bromophenacyl bromide (125 mg) in $CHCl_3$ (10 ml) was refluxed for 5 hr. Removal of the solvent under reduced press, gave a pale yellow oily product which was chromatographed on silicic acid (5 g) using $CHCl_3$–MeOH (95:5) as eluent to give an oil. Paper chromatography as well as TLC of this oily substance showed one spot; IR bands at 1745, 1702, 1667, 1589, 1453, 1421, 1400, 1076, 1011, 971, 943 cm^{-1} ($CHCl_3$); NMR signals at 1·86 (broad s, 6H), 5·46 (broad d, 3H), 7·72 (A_2B_2 type, 4H) in $CDCl_3$.

Laburninic acid methyl ester. To a methanolic soln of IV was added a methanolic soln saturated with HCl gas. The mixture was kept at room temp overnight, the resulting soln was neutralized with IR-4B (OH-form) and evaporated under reduced press to give an oil, which was chromatographed on silicic acid using $CHCl_3$–MeOH (95:5) as eluent to afford a pure oil. Paper chromatography as well as TLC of this oily substance showed one spot. Its mass spectrum shows peaks at 169 (M^+) (13), 154 (7·1), 142 (53), 141 (9·7), 138 (10), 127 (20), 110 (68), 108 (6·8), 85 (22), 83 (74), 82 (31), 68 (76), 67 (100), 55 (25), 53 (79).

Synthesis of anodendrine (I). To a methanolic soln of IV was added a methanolic soln (0·2 ml) saturated with HCl gas, and then the mixture was kept at room temp overnight with stirring. The reaction soln was concentrated to about 0·5 ml, and diluted with water (3 ml). The resulting soln was neutralized with IR-4B (OH-form), and then concentrated under reduced press to give an oil. To the oily product in MeOH (5 ml) was added anhyd Ag_2O (50 mg). The resulting soln was allowed to stand at room temp overnight with stirring. AgCl and Ag_2O were filtered off, and the filtrate was evaporated under reduced press to give an oily product. A mixture soln of the oily material and isopentenyl bromide (100 mg) in $CHCl_3$–benzene (1:1) (10 ml) was warmed at 60° overnight with stirring, and then the solvent was removed under reduced press to give an oil, which was dissolved in MeOH (2 ml) and 1N NaOH soln (2 ml). The mixed soln was kept at room temperature for 6 hr with stirring. The resulting soln was washed with ether (30 ml) and neutralized with IRC-50 (H-form), and then concentrated to about 0·5 ml. The product showed only one spot on paper chromatography. To a resulting soln was added an aqueous saturated with picric acid to give needles. Recrystallization from water gave anodendrine picrate (35 mg), m.p. 123–124°. This synthetic sample was found to be completely identical with natural anodendrine picrate (mixed m.p., IR and NMR spectra).

Synthesis of alloanodendrine (II). II was synthesized from (+)-isoretronecanolic acid (VI), according to the similar procedure as that of I.

To a methanolic soln (10 ml) of VI (52 mg) was added a methanolic soln (1 ml) saturated with HCl gas. The resulting soln was neutralized with IR-4B (OH-form), and then concentrated. To a methanolic soln of the product was added Ag_2O (100 mg) with stirring, and the AgCl and Ag_2O were filtered off and then the filtrate was concentrated to give an oil. A mixed soln of the oily product and isopentenyl bromide (120 mg) in $CHCl_3$–benzene (2:1) (10 ml) was kept at 50° for a day, and then the solvent was removed *in vacuo* to give an oil. A soln of the oil in MeOH (2 ml) and 0·4 N NaOH soln was kept at room temp for 6 hr with stirring. The mixture was neutralized with IRC-50 (H-form) and then the solvent was removed under reduced press to give an oil, which showed only one spot on paper chromatography and was identical with II by comparison with NMR spectra. A soln of the oily substance (60 mg) and *p*-bromophenacyl bromide (75 mg) in $CHCl_3$ (5 ml) was refluxed for 5 hr, and then the solvent was removed under reduced press to give an oil, which was chromatographed on silicic acid (2 g) using $CHCl_3$–MeOH (96:4) as eluent to afford plates, 26 mg, m.p. 82–84°, which was completely identical with *p*-bromophenacyl ester bromide of II (mixed m.p. and IR spectra).

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