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CHEMOTAXONOMICAL ALKALOID STUDIES STRUCTURES OF LIPARIS ALKALOIDS

K. NISHIKAWA, M. MIYAMURA and Y. HIRATA

Chemical Institute, Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan

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Abstract—The isolation and structures of the Liparis alkaloids, (*Orchidaeae*) nervosine (I), kuramerine (IX), kumokirine (XIII), malaxin (XV) and auriculine (XX) obtained from *L. nervosa* Lindl., *L. Krameri* Franch et. Sav., *L. Kumokiri* F. Maekawa, *L. bicallosa* Schltr. or *L. hachijoensis* Nakai and *L. auriculata* Blume, respectively, have been studied from the point of chemotaxonomy.

INTRODUCTION

THE plants of Liparis groups are perennial herbs belonging to the *Orchidaceae*. In 1936, Eguchi *et al.* reported[1] the presence and isolation of an alkaloid with a bitter taste from *L. Krameri* Franchi et. Sav. in a fresh state.

The paper prompted us to reinvestigate the plants of Liparidinae in the following respects: (1) Have the other plants of Liparis species an alkaloidal bitter substance? and what are the structures? (2) If all these plants contain an alkaloid, what is the relation between the structures and species of the plants? On a basis of mlrphorogy, it is very difficult to classify some plants of this group, therefore, in addition to the structural elucidation, we also examined the possibility of a chemotaxonomical classification. As some plants of this group contain new types of related alkaloids, the isolation and the constitutions are reported in this paper.

Nervosine

A main basic compound, nervosine,[2] was isolated as a picrate from the water soluble parts of the methanolic extracts of fresh *L. nervosa* Lindl.*

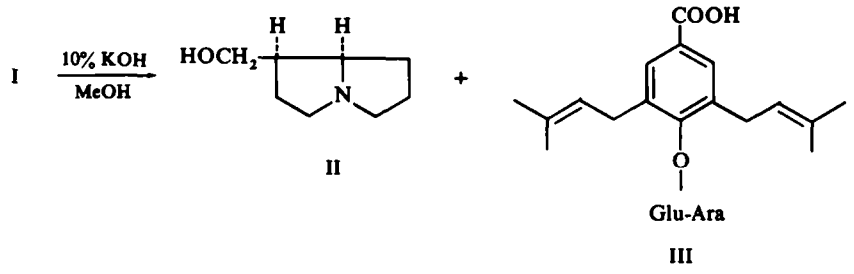
The molecular formula of the picrate is $C_{36}H_{53}O_{12}N.H_2O$–$C_6H_3O_7N_3$, m.p. 130–131° corresponding also to its neutrallization equivalent and nervosine (I) which was obtained from the picrate, as a colorless amorphous solid soluble in acidified water, MeOH and EtOH, and insoluble in benzene, $CHCl_3$ and Et_2O. A positive Dragendorff reaction and p*Ká* value (10·0 in 66% MeOH) of I indicate that one nitrogen is tertiary.

The IR (Fig. 5) and UV (Fig. 2) spectral findings indicate that nervosine (I) contains polyhydroxyl groups (ν_{max}^{KBr} (cm^{-1}) 3400 (broad), 1120–980 (complicated bands)) and an ester function of a benzoic acid derivative (ν_{max}^{KBr} (cm^{-1}) 1715, 1605; λ_{max}^{MeOH} (ε) 248, 280, 290 mμ (14,000, 2300, 2000)).

Alkaline hydrolysis of nervosine (I) afforded two degradation products.

One basic (II) $C_8H_{15}ON$ and the other acidic (III) $C_{28}H_{40}O_{12}.H_2O$.

* Japanese name is Kokuran.



The NMR spectrum of II in pyridine shows the signals at 1·90–2·50 (7H, multiplet), 2·65–4·10 (4H, distributed m), 3·95 (2H, d, J = 7 c/s) and 4·54 ppm (1H, m). These data suggest the presence of —CH_2OH, two —N—CH_2—, and —N—CH— groups in II. Treatment of II with *p*-nitrobenzoyl chloride in pyridine gave a mono-benzoate, the NMR spectrum of which had a signal of a broad doublet at 4·48 ppm (2H, J = 7 c/s) instead of the doublet at 3·95 ppm in II. This finding concluded that II exactly contains a secondary hydroxymethyl group, which is also supported by the fact that the fragment peaks at *m/e* 124, 110 were found in the mass spectrum of II (Fig. 1). A characteristic peak at *m/e* 83 was attributed to the fragment ion IIa or IIb.

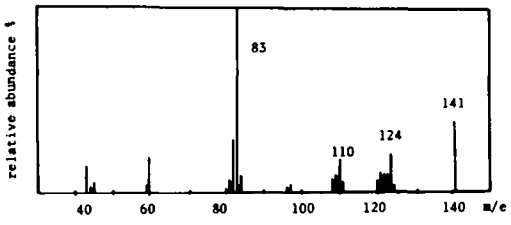
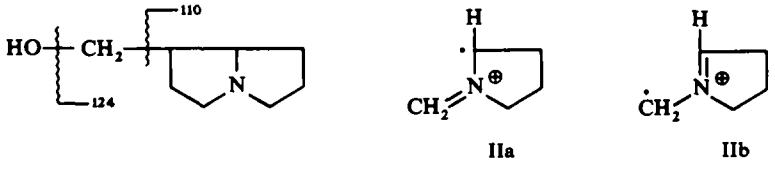


FIG. 1 The mass spectrum of II.



From these results, the structure of II is considered to be 1-hydroxymethylpyrrolizidine. The m.p. of the picrate (193–194°, corrected) and $[\alpha]_D^{20}$ value (+73° in MeOH) of II are in agreement with those of linelofidine.[3] In fact, the oxidation product of II with CrO_3 in dil H_2SO_4 is also identical with the corresponding amino acid, $C_8H_{13}O_2N$.[4]

The IR spectrum of the acidic compound, nervosinic acid (III), has strong bands of OH groups at about 3400 (broad) and 1120–980 (complicated absorptions) cm^{-1} and the UV spectrum of III (Fig. 2) in neutral MeOH is very similar to that of nervosine indicating the presence of the same chromophore as I. But, the hypsochromic shift in alkaline MeOH (8 mμ) suggests the presence of a new carboxyl function in

III in conjugation with the chromophore. When hydrogenated on PtO_2 in EtOH, III absorbed quantitatively 2 moles of hydrogen to give hydronervosinic acid (IV) $C_{28}H_{44}O_{12}.H_2O$, the UV spectrum of which was almost identical with that of III. This fact indicates that III contains two isolated double bonds.

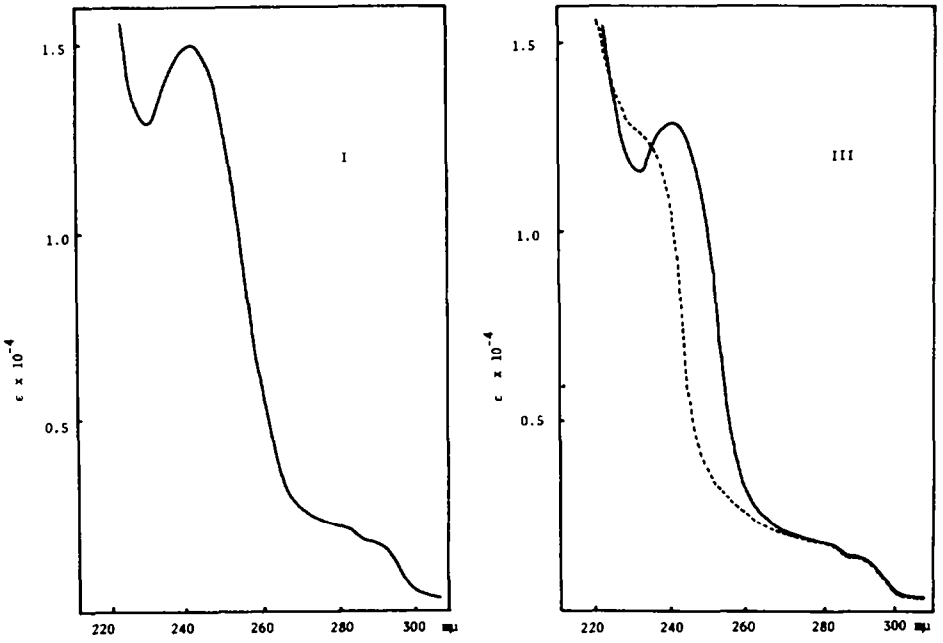
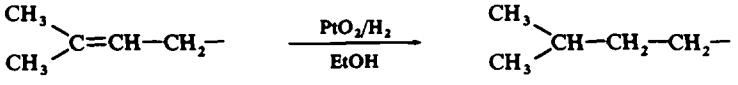


FIG. 2 The UV spectra of I and III. ——— MeOH, - - - - - alkaline MeOH.

Comparison of the NMR spectra of III and IV showed a doublet at 0·97 ppm (12H, $J = 5$ c/s) in IV instead of two singlets at 1·66 and 1·73 ppm (both 6H) in III. Furthermore, a doublet at 3·92 ppm (4H, $J = 7$ c/s) and a broad triplet at 5·56 ppm (2H, $J = 7$ c/s) in III disappeared in IV, while a broad multiplet at 1·71 ppm (6H) and a broad triplet at 3·17 ppm (4H, $J = 7$ c/s) appeared in IV. These spectral properties are interpreted as follows:



IIIa IVa

When treated with 2N HCl, IV gave D-glucose, L-arabinose and hydronervogenic acid (V) $C_{17}H_{26}O_3$, characteristic bands of which were observed at 3500 (—OH), 3200–2400 and 1673 (conjugated —COOH), 1605 (benzene ring), 1390 and 1375 cm^{-1} (*gem*-dimethyl group) in the IR spectrum.

The UV spectrum of V has a max at 258 mμ ($\varepsilon = 11{,}500$) in neutral MeOH, 291 mμ ($\varepsilon = 17{,}000$) in alkaline MeOH, which suggests that V has a *p*-hydroxybenzoic acid chromophore. The p*K'a* values, 6·3 and 11·8 (in 66% MeOH), are attributable to the carboxyl and phenolic OH groups, respectively.

The latter may be newly formed by the cleavage of phenolic glycoside bond in IV. The nature of other hydrogens of V were clearly disclosed from the NMR spectrum (Fig. 3): the signals at 0·97 (12H, d, J = 5·1 c/s), 1·2–1·8 (6H, m) and 2·66 ppm (4H, tr, J = 7·5 c/s) are interpreted by assuming the presence of two side chains as being IVa and the other signal at 7·79 ppm (2H, s) is attributable to the isolated and symmetrical two benzene protons.

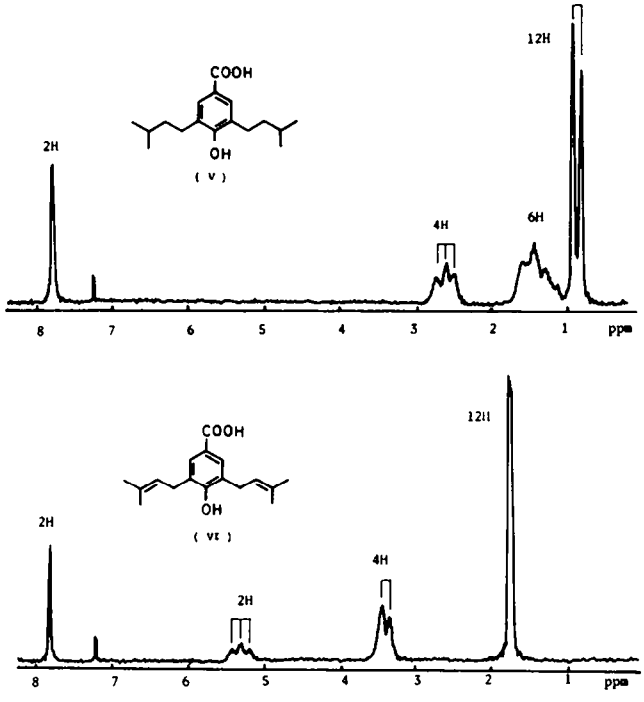


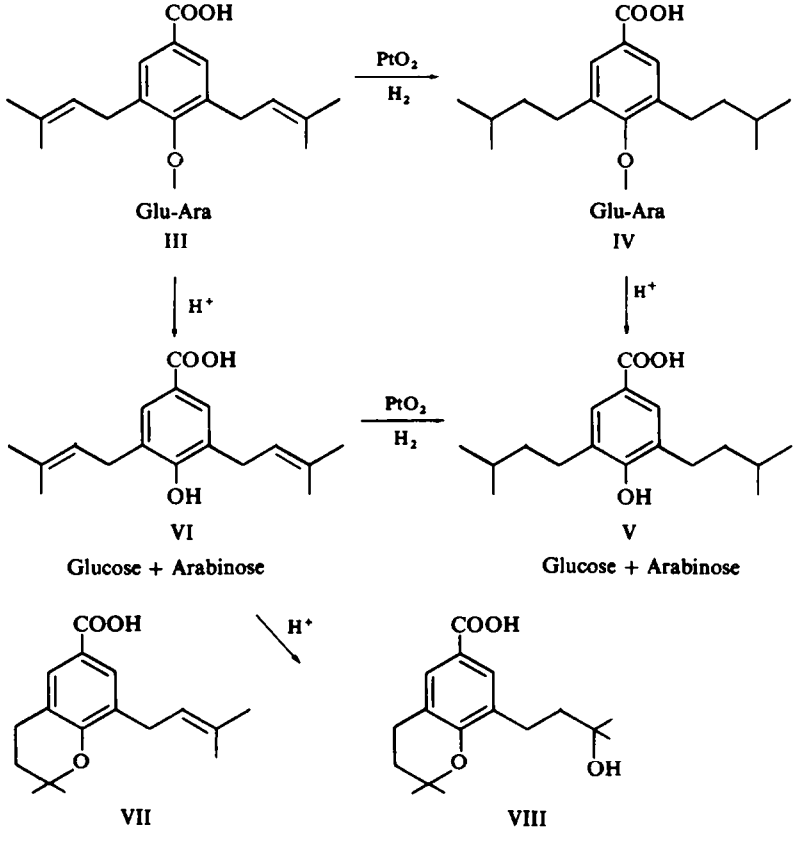
FIG. 3 The NMR spectra of V and VI (in $CDCl_3$).

On the other hand, acid hydrolysis of nervosinic acid (III) under mild condition gave nervogenic acid (VI), $C_{17}H_{22}O_3$, which also gave V by catalytic hydrogenation. All the signals in the NMR spectrum of VI (Fig. 3) are distinctly accounted for by the two groups of IIIa and two benzene protons. From biogenetic consideration* together with the findings described above, the structures of hydronervogenic acid and nervogenic acid can be represented by V and VI, respectively. In fact, these presumed structures were confirmed by the following experiment: acid treatment of VI gave two 2,2-dimethyl-6-carboxychroman derivatives, (VII) $C_{17}H_{22}O_3$ and its hydration product, (VIII) $C_{17}H_{24}O_4$, the latter of which was partly obtained by the prolonged treatment of VII under similar conditions.

* From the above NMR spectral findings, the position of isoamyl or 2-isopentenyl substituent is not decided. But in naturally occurring phenolic compounds, 2-isopentenyl groups are all located at *ortho* or *para* position to the phenolic OH function, excluding the O-isopentenylated compounds. Biogenetically, nervogenic acid is considered to be formed by the enzymatic reaction of *p*-hydroxybenzoic acid with two moles of γ,γ-dimethylallyl pyrophosphate to introduce the two 2-isopentenyl groups at both *ortho* positions.

In the NMR spectrum of VII, two triplets at 1·83 (2H, J = 7 c/s) and 2·84 ppm (2H, J = 7 c/s) and a sharp singlet at 1·37 ppm (6H) indicate the presence of the chroman ring in VII. Accordingly, the structure of nervogenic acid (VI) has been established as 3,5-di-(2-isopentenyl)-4-hydroxybenzoic acid and therefore, the structure of hydronervogenic acid as V. The relation of these derivatives are shown in scheme I.

Scheme I. The relation of nervosine derivatives.



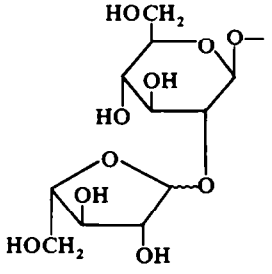
Although the structure of the aglycone part in nervosinic acid is represented as VI, the constitution of the sugar moiety remains unknown. From the molecular formula of III, it is clear that nervosinic acid (III) must contain one mole of D-glucose and one mole of L-arabinose. The negative Fehling and Tollens tests of III indicate that the C_1 positions of glucose and arabinose are involved in formation of glycosidic linkage.

When III was treated with 0·1N HCl in dioxan at 82–85° for 1·5 hr, the disaccharide (nervosiose), as well as glucose and arabinose, was obtained which surely gave glucose and arabinose on acid hydrolysis. On the other hand, only arabinose was detected, when the disaccharide was reduced by $NaBH_4$ and hydrolysed by treatment with dil HCl.

These results show that in the disaccharide, the aldehyde group of glucose is reduced and therefore, the C_1 position of glucose must be in glycosidic linkage with the phenolic OH group of the aglycone (VI).

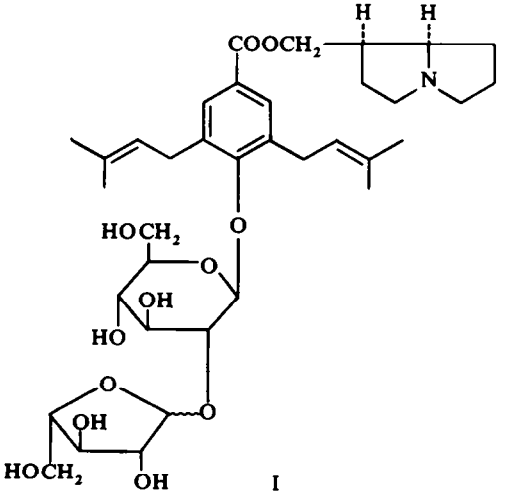
On titration of III with periodic acid in phosphate buffer (pH 6·1), two moles of periodate was consumed. The first mole of periodic acid was taken rapidly but the second mole was very slowly.

This experiment concludes that the glucose is present as a pyranoside and the arabinose as a furanoside, and the C_1 position of arabinose should be combined with 2 or 4 position of the glucose. The possibility of 1–4 bonding between arabinose and glucose was excluded from the following result: i.e., the fully methylated nervosinic acid was decomposed by refluxing with 1N methanolic hydrogen chloride for 12 hr to give a mixture of methylated monosaccharide. The peaks corresponding to α and β methyl-2,3,6-tri-O-methyl-glucospyranoside were not observed, but the peaks corresponding to α and β methyl-3,4,6-tri-O-methyl-glucopyranoside were detected by the comparison with the authentic samples on VPC of the mixture. From the above discussion, the structure of the sugar moiety of nervosinic acid, nervosiose, can be described as IIIb.



IIIb

At least, the conformation of the glycosidic bond of the glucose may be deduced to be a β-linkage from the NMR spectral finding, the coupling constant between the C_1 and C_2 protons having $J = 6$ c/s.



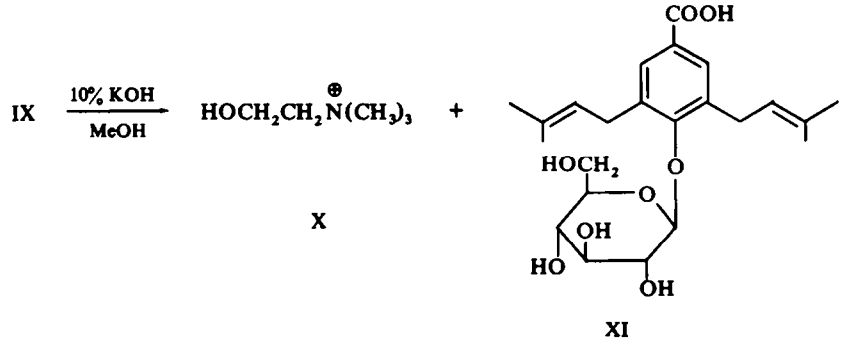
I

Here, the total structure of nervosine have been represented as I, constructed with lindelofidine (II), nervogenic acid (VI) and the disaccharide (IIIb).

Kuramerine

Kuramerine (IX)[5] was isolated from *L. Krameri* Franch et. Sav.* as a main alkaloidal component, $C_{28}H_{44}O_8N$–$C_6H_2O_7N_3$ m.p. 105–107° (as picrate), which is a quarternary basic substance (p*Ka'* over 12) and has some similarity to nervosine (I) in the spectral properties. For examples, the IR spectrum is characterized by bands at 3400 (broad) and 1120–980 (complicated bands) polyhydroxyl groups), 1715 (conjugated ester carbonyl) and 1605 (benzene ring) cm^{-1}, and in the UV spectrum, IX shows the closely similar curve to I.

Alkaline hydrolysis of kuramerine produced quantitatively two compounds, one of which was a strongly basic (X). The other was acidic (XI).



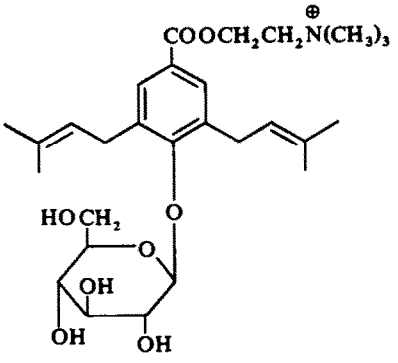
The basic compound (X) $C_5H_{14}ON$–$C_6H_2O_7N_3$ (as picrate) was proved to be choline by the comparison of m.p. (picrate) and IR spectrum with those of authentic sample.

The acidic compound, kurameric acid (XI) has a molecular formula $C_{23}H_{32}O_8 \cdot H_2O$ and exhibits the following properties: the existence of polyhydroxyl groups (3350 and 1120–980 (complicated absorptions) cm^{-1}) and benzoic acid moiety (1690 and 1605 cm^{-1}) are proved by the IR spectrum and in the UV spectrum, the absorptions of maxima at 243, 278, 288 mμ (ε = 13,000, 2300, 2000) in MeOH, about 235 (shoulder), 278, 288 mμ (δ = 12,500, 2300, 2000) in alkaline MeOH, provide the evidence that kurameric acid (XI) has the same chromophore as nervosinic acid (III). Moreover, the NMR spectrum displays two singlets at 1·63 (6H) and 1·73 ppm (6H) and a doublet at 4·02 ppm (4H) coupled to a broad triplet at 5·57 ppm (2H), having J = 7 c/s. These characteristic peaks indicate the presence of two side chains as being $—CH_2—CH=C(CH_3)_2$ in XI. This conclusion was supported by the NMR spectrum of hydrokurameric acid (XII), obtained from catalytic hydrogenation of XI, showing a doublet at 0·90 (12H, J = 5 c/s), a multiplet at 1·3–1·9 (6H) and a broad triplet at 3·20 ppm (4H, J = 7 c/s).

Further evidence for determining the structure of kurameric acid to XI has been derived from the results of acidic degradations of XI and XII.

* Japanese name is Zigabachiso.

Treatment of XI with 2N HCl gave D-glucose and two acidic compounds VII and VIII and while, hydrolysis of XII gave D-glucose and V. The value of molecular rotation of XI ($[M]_D = -7200$) supports the glycosidic bond at the C_1 position of glucose to be a β-linkage. From all the results described above, the complete structure of kuramerine can be represented as IX, which consists of choline (X) and kurameric acid (XI).

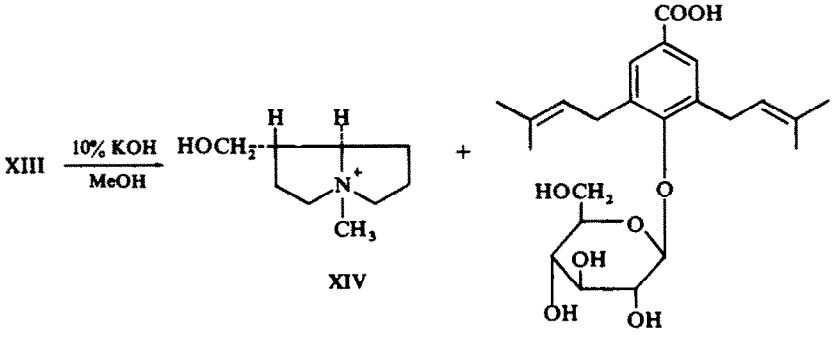


IX

Kumokirine

Kumokirine[5] (XIII), $C_{32}H_{48}O_8N.H_2O$–$C_6H_2O_7N_3$ m.p. 100–102° (as picrate), is a main alkaloid obtained from *Liparis Kumokiri* F. Maekawa* and shows the high p*Ka'* value (over 12) indicating that kumokirine (XIII) should be a quarternaly base. Comparison of the spectral data of kumokirine with those of kuramerine shows closely resemblance between them.

Alkaline hydrolysis of XIII gave one mole of a strongly basic compound and one mole of an acidic compound in good yield. The latter product is kurameric acid (XI), which was confirmed by the comparison of m.p. and IR spectrum with those of authentic sample.



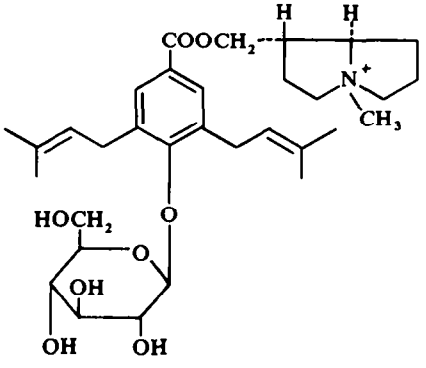
XIV XI

* Japanese name is Kumokiriso.

The basic compound, kumokiridine (XIV) $C_9H_{18}ON$–$C_6H_2O_7N_3$ (as picrate), shows the prominent signals at 3·39 (3H, s) and 3·88 ppm (2H, d, J = 4·5 c/s) in the NMR spectrum, which are indicative of $-\overset{|}{\underset{|}{\overset{+}{N}}}-CH_3$ and $CH-CH_2OH$ groups, respectively. Other broad and complicated signals at 1·90–2·60 (7H), 3·30–3·95 (4H) and 4·16 ppm (1H) suggest XIV having a pyrrorizidine ring.

Therefore, the structure of kumokiridine may be represented as XIV, which was supported by the evidence that pyrolysis of XIV gave a small amount of laburnine[6] (XVI), practically identical with an authentic sample by the IR and mass spectra. In fact, the compound prepared from laburnine and MeI was completely identical to kumokiridine (XIV) in m.p., IR spectrum and $[\alpha]_D$ value.

Accordingly, the submitted formula XIII for kumokirine would account for the all findings described above.



XIII

Malaxin

A new alkaloid, malaxin[7] (XV) was isolated from both *L. bicallosa* Schltr.* and its variant, *L. hachijoensis* Nakai† partly as a crystalline substance of malaxin-guanidine complex[8] and partly as an amorphous solid of malaxin.

This complex was obtained as a sulfate, $C_{27}H_{42}O_8N_4 . H_2O$–$H_2SO_4$ m.p. 180–182°; $[\alpha]_D^{20} = -33°$ (H_2O), which was completely decomposed even by chromatography on paper to give malaxin and guanidine in any solvent. Conversely, when free base of malaxin and guanidine sulfate were mixed in adequate ratio (about 1:1·5 mol. eq.), a crystalline of malaxin–guanidine complex was obtained in good yield.

Malaxin‡ (XV) $C_{26}H_{37}O_8N$, is a tretiary amine ($pKa' = 9·8$ in 66% MeOH; Dragendorff test, positive) and has strong bands of —OH groups at 3400 and 1130–980

* Japanese name is Yuukokuran.

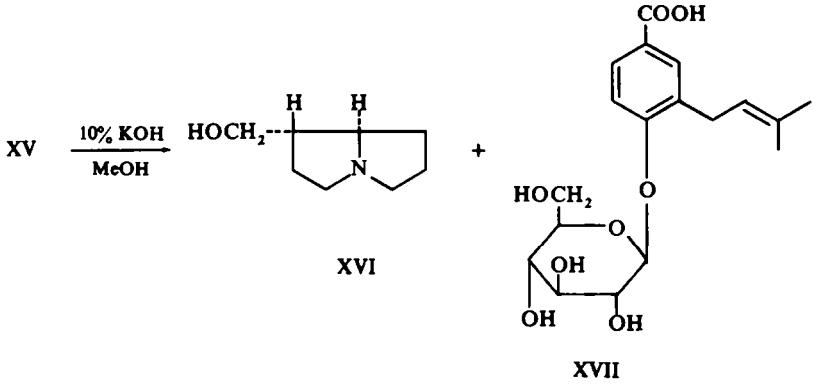
† Japanese name is Shimasasabaran.

‡ We published the structure of bicallosine (named by us to this alkaloid) at the 20th annual meeting of Chemical Society of Japan, 31 March, 1967 (Tokyo, Japan). At the short time later, the same structure was reported by B. Lüning *et al.* to malaxin which is an alkaloid obtained from *Malaxis congesta* Comb. nov. in *Orchidaceae*. Our sample was identical to malaxin in IR, UV and mass spectra.

(complicated bands) cm^{-1} and bands attributable to the chromophore of benzoic acid ester at 1713 and 1605 cm^{-1} in the IR spectrum (Fig. 8).

Treatment of malaxin with 10% KOHaq gave one mole of a basic compound (XVI), $C_8H_{15}ON$, $pKa' = 10{\cdot}5$ (50% MeOH) and one mole of an acidic compound (XVII), $C_{18}H_{24}O_8 \cdot H_2O$; $pKa' = 6{\cdot}1$ (66% MeOH).

The fragment pattern of XVI in the mass spectrum was completely agreed with that of previously described lindelofidine (II) (Fig. 1), but the m.p. and the IR spectrum of the picrate did not correspond with those of II.



The structure of the basic compound could be a diastereoisomer of lindelofidine, i.e. laburnine itself. In fact, the m.p. of its picrate (172–173°) and $[\alpha]_D^{20}$ value (+17°, MeOH) are in good agreement with those reported.[7]

The acidic compound (XVII), malaxinic acid, has strong bands of —OH groups

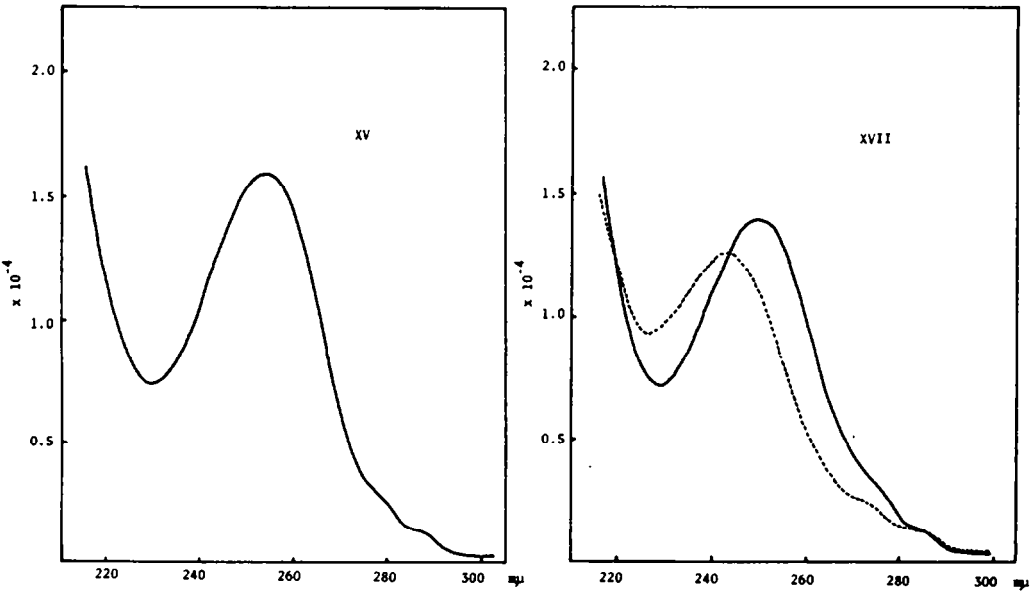
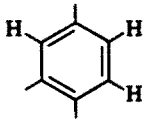


FIG. 4 The UV spectra of XV and XVII. ——— MeOH, - - - - - alkaline MeOH.

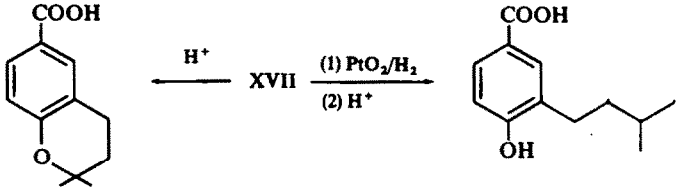
at 3400 and 1130–980 (complicated bands) cm^{-1} in the IR spectrum, suggesting a glycoside. The UV spectra of malaxin and malaxinic acid are depicted in Fig. 4, comparison of both of which reveal that XVII has a carboxyl function in conjugation with the same chromophore as XV.

The NMR spectrum of XVII indicates the presence of two Me groups attached to a double bond (1·74 ppm, 6H, broad s), one allylic methylene (3·41 ppm, 2H, d, J = 7·5 c/s) and one vinyl proton (5·42 ppm, 1H, tr, J = 7·5 c/s). These signals suggest that malaxinic acid has one $—CH_2—CH=C(CH_3)_2$ part as a side chain.

Further, three aromatic protons were observed, one of which was a singlet peak at 7·80 ppm and the other two were coupled peaks each other centered at 7·16 and 7·80 ppm (d, J = 9 c/s). The spectral evidence led to the following partial structure:



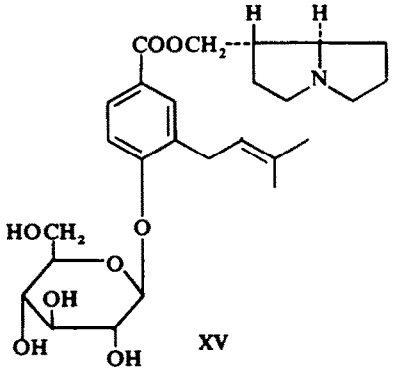
The relative positions of a carboxyl and 2-isopentenyl groups to the benzene protons were verified by the following results. On acid hydrolysis, XVII gave D-glucose and 2,2-dimethyl-6-carboxychroman (XVIII), identified with an authentic sample. On the other hand, the dihydro derivative of XVII afforded D-glucose and 3-(2-isopentanyl)-4-hydroxy-benzoic acid (XIX), identical with authentic samples under the same acidic conditions.



XVIII XIX

Thus, the complete structure of malaxinic acid was represented as XVII. The strong levorotation of XVII indicates the conformation of the glycosidic bond to be a β-linkage, which is also supported by the coupling constant of the C_1 acetal proton of the glucose, having J = 6·0 c/s.

Now, from the all findings described above, the structure of malaxin could be described as XV, an ester compound of XVI and XVII.

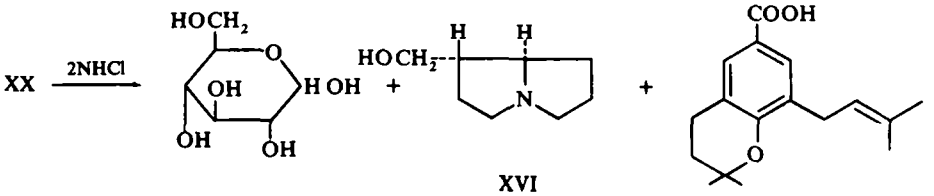


XV

Auriculine

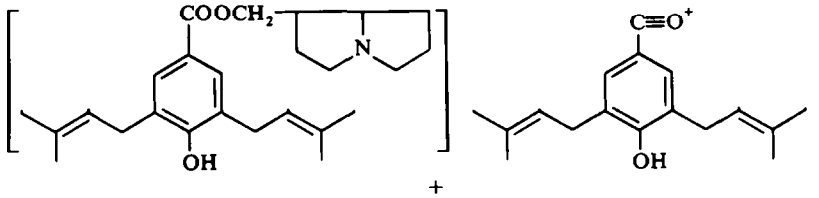
From *L. auriculata* Blume,* a new alkaloid, auriculine[8] (XX) was isolated as a picrate. As the plant is rare, only a small amount of the sample was obtained and furthermore, the crystallization and the determination of the molecular formula by elemental analysis or mass spectrometory were unsuccessful, but the structure of auriculine may be deduced by the following observations. Resemblance of the UV (λ_{max}^{MeOH} 247 mμ) and the IR (ν_{max}^{KBr} (cm^{-1}) 3400, 1715, 1605, 1100–980 (complicated bands)) spectral properties to the other Liparis alkaloids described above, suggests that auriculine (XX) probably has the same chromophore as *p*-glycosylbenzoic acid ester.

In fact, it gave glucose, laburnine (XVI) and VII by treatment with 2N HCl at 100° for several hrs.

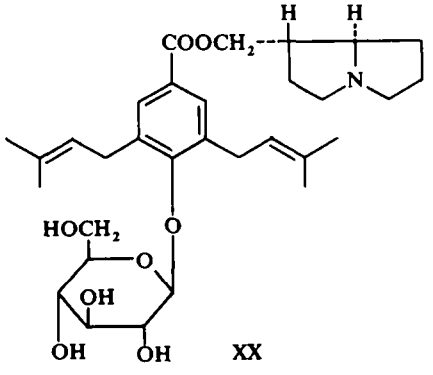


XVI VII

From these findings and the presumption that XX is similar to other Liparis alkaloids, auriculine should be constructed from one mole of glucose, nervogenic acid (V) and laburnine (XVI). Because, from the neutrallization equivalent or comparison in the absorption intensity of the picrate at 355 mμ with a picrate of an authentic amine having no absorption itself, the mol wt of XX was calculated at about 540.



m/e 397 XXI + *m/e* 257 XXII



XX

* Japanese name is Giboushiran, which is one of a very rare plants in genus Liparidinae in Japan.

The fragment peaks at *m/e* 397 and 257, which are interpreted by the ion of XXI and XXII, respectively, provided the evidence of the structure XX for auriculine.

EXPERIMENTAL

Unless otherwise noted, all m.ps are uncorrected. The spectra were recorded on the following instruments: IR spectra, Nihon-Bunko DG-402 and IR-S; all spectra were measured in the state of KBr tablet; UV spectra, Beckman DK-2 and Perkin-Elmer 202; NMR spectra, Varian Associates spectrometer A-60 and Nihon-Denshi JNMC-60 H; only prominent peaks are cited; the chemical shifts are given in ppm relative to the internal TMS; s, singlet; d, doublet; t, triplet; m, multiplet; coupling constants are given in c/s; mass spectra, Hitachi Model RMU-6D mass spectrometer. Optical rotations were determined with a JASCO ORD UV-5 spectropolarimeter. The p*Ka*'s values were measured by dissolving a few mg of samples in the mixture of 0·02N HCl (1 ml) and MeOH (1 or 2 ml) and titrating it with 0·1N KOH by means of Radiometer TTT-1 pH meter with an automatic recorder. For column chromatography, Toyo-Roshi cellulose powder (100 mesh) and Mallinckrodt silic acid (100 mesh) were used. For paper chromatography, Toyo-Roshi NO-51 and NO-51 A (40 × 40, 60 × 60) were employed.

Isolation of nervosine (I). Fresh materials of *L. nervosa* Lindl. (3 kg) were cut into small pieces and soaked in boiling MeOH (2 l) for 10 min and then the extracting flask was cooled to room temp. After standing for 2 days, the dark green MeOH extracts were collected by decantation. The residue was crushed into fine pieces and then re-extracted with MeOH (2 l) for one day at room temp. The combined MeOH extracts were concentrated under reduced press to 100 ml. This residual extract was washed with ether several times for the sake of removing chlorophyll and other water insoluble substances.

To the resulting brown water layer, a sat aqueous soln of picric acid was added and the ppt was recrystallized repeatedly from MeOH to give pure nervosine picrate, 4·5 g, m.p. 130–131°. (Found: C, 54·00; H, 6·47; N, 6·07. $C_{36}H_{53}O_{12}N.H_2O$—$C_6H_3O_7N_3$ requires: C, 53·72; H, 6·23; N, 5·97%).

To a soln of pure nervosine picrate (1 g) in a mixture of MeOH and acetone (1 : 1, 50 ml), about 100 ml water and 1 ml 1N HCl were added under cooling in a ice bath. The picric acid was removed by extracting with Et_2O several times until the yellow color of the extracts almost disappeared and then the resulting water layer was neutrallized by treatment with Amberlite IR-4B resin (free amine type). After the resin was removed, the filtrate was treated with norit-A and concentrated *in vacuo* to an almost colorless solid of nervosine HCl. When in the previous experiment, Amberlite IRA-400 resin (OH type) was used, free base of nervosine was obtained as an amorphous material. All attempts at crystallization of this amorphous solid with several solvents were failed. On measurements of the physical data and chemical experiments were used this amorphous nervosine, m.p. 102–107° (dec), Dragendorff test, positive; Fehling and Tollens tests, both negative; $[\alpha]_D^{20}$ = 12·8° (*c* 4, MeOH); p*Ka'* = 10·0 (66% MeOH); IR spectrum, Fig. 5; UV spectrum, see Fig. 2.

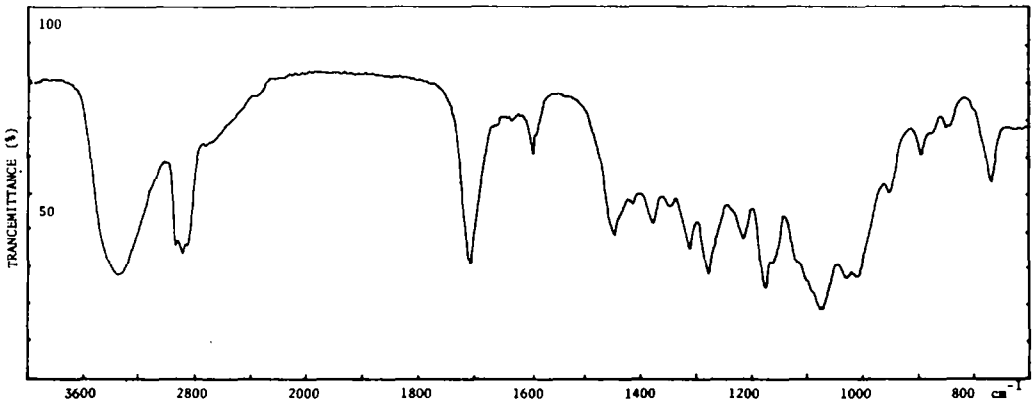


FIG. 5 The IR spectrum of I (KBr).

Alkaline hydrolysis of nervosine (I). To a soln of I (1·52 g) in MeOH (100 ml) was added 10% KOH aq (10 ml) and after refluxing for 2 hr, the mixture was slightly acidified with 0·2N HCl under cooling. On standing in an ice bath, a crystalline ppt appeared and was recrystallized from acetone as colorless needles of III, 850 mg, m.p. 168–169°; $[\alpha]_D^{20} = -14°$ (*c* 4, MeOH); *pKa'* = 5·80 (66% MeOH); IR bands at 3400, 1690, 1605, 1590, 1275, 1190, 1125, 1105, 1085, 1045, 1020 cm^{-1}; N7R signals (in pyridine) at 1·66 (6H, s), 1·73 (6H, s), 3·92 (2H, d, J = 7 c/s), 3·5–4·5 (10H, m), 4·88 (1H, t, J = 6 c/s), 5·3 (2H, br. d, J = 6 c/s), 5·56 ppm (2H, t, J = 7 c/s); UV spectrum, Fig. 2. (Found: C, 57·23; H, 7·39. $C_{28}H_{40}O_{12}.H_2O$ requires: C, 57·33, H, 7·22%).

The filtrate was made basic (pH, about 12) with Na_2CO_3 and the solvent evaporated completely. From the residual solid, a slightly brown oil was obtained by extraction with cold EtOH. The oily product (II) was purified by formation of a picrate, recrystallization of which gave yellow long needles of II, 690 mg, m.p. 193–194° (from MeOH); $[\alpha]_D^{20} = 73°$ (*c* 3, MeOH, free base); *pKa* = 10·5 (50% MeOH); mass spectrum, Fig. 1. (Found: C, 45·30; H, 5·07; N, 14·95. $C_8H_{15}ON$—$C_6H_3O_7N_3$ requires: C, 45·40; H, 4·90; N, 15·13%).

p-Nitrobenzoate of II. To a soln of II (50 mg) in dry pyridine (20 ml), *p*-nitrobenzoyl chloride (100 mg) was added under cooling. The mixture was allowed to stand over night at room temp and then left in a vacuum dessicator (containing conc H_2SO_4 as drying reagent) for one day.

The residue in the flask was extracted with $CHCl_3$ and the extracts were evaporated. The residue was chromatographed on silicic acid using $CHCl_3$–MeOH (95:5) as the eluting solvent. *p*-Nitrobenzoate of II was obtained from the second fraction, 40 mg, m.p. 213–215° (HCl salt); IR bands at 1718, 1605, 1522, 1345, 1278, 710 cm^{-1}; p*Ka'* = 10·1 (50% MeOH); mass, *m/e* 290 (M^+).

Oxidation of II *with* CrO_3. A soln of II (400 mg) in dil H_2SO_4 (0·1 ml of conc H_2SO_4 in 2 ml H_2O) was added carefully to a cooled soln of CrO_3 (0·25 g) in dil H_2SO_4 (0·2 ml of conc H_2SO_4 in 3 ml H_2O) and the mixture was warmed for a few min on a water bath. When reduction of CrO_3 was complete, a second charge of the oxidizing mixture of CrO_3 (0·25 g) in dil H_2SO_4 (0·2 ml of conc H_2SO_4 in 3 ml H_2O) was added. The soln was next heated on a boiling water bath for one day. The chromium ion and sulfuric acid was separated from the mixture by adding $Ba(OH)_2$ aq and then powdered dry ice was added until the soln became neutral. The ppt was removed and washed twice with H_2O. The combined filtrate was evaporated under reduced press to give an oil which was poured on a column packed with cellulose powder (20 g). The solvent used for elution was the following mixture: MeOH, benzene, isoamyl alcohol and 4% NH_4OH aq (35:35:17·5:12·5). Crystallization of the main fraction as a picrate gave yellow long needles of the amino acid corresponding to II, 300 mg, m.p. 220–221° (corrected); p*Ka'* = 4·05, 10·5 (50% MeOH); mass, *m/e* 155 (M^+), 127, 110, 83. (Found: C, 43·40; H, 4·53; N, 14·92. $C_8H_{13}O_2N$–$C_6H_3O_7N_3$ requires: C, 43·75; H, 4·20; N, 14·58%).

Hydronervosinic acid (IV). To a soln of III (190 mg) in EtOH (30 ml), PtO_2 (20 mg) was added. Hydrogenation was performed at room temp for 3 hr. The catalyst was removed and the filtrate was evaporated under reduced press to give a colorless solid, recrystallization of which from acetone gave needles of IV, 175 mg, m.p. 187–189°; IR bands at 3400, 1690, 1605, 1085, 1020 cm^{-1}; UV spectrum, see Fig. 2; NMR signals (in pyridine) at 0·97 (12H, d, J = 5 c/s), 1·3–1·9 (6H, m), 3·17 (4H, br. t, J = 7·5 c/s), 3·5–4·6 (10H, m), 4·90 (1H, t, J = 6 c/s), 5·35 (2H, br. d, J = 6 c/s) ppm. (Found: C, 57·22; H, 7·91. $C_{28}H_{44}O_{12}.H_2O$ requires: C, 56·93; H, 7·85%).

Hydrogenervogenic acid (V). To a soln of IV (100 mg) in dioxan (6 ml), 2N HCl (6 ml) was added and the mixture was refluxed for 2 hr. After dilution with H_2O (30 ml), the mixture was extracted with three 20 ml portions ether. The ethereal extracts were washed with H_2O and sat NaCl aq and then dried over Na_2SO_4.

Removal of the solvent gave a product, which gave colorless needles of V by recrystallization from n-hexane, 40 mg, m.p. 138–139°; IR bands, at 3520, 3200, 2400, 1673, 1605, 1420, 1390, 1375, 1260, 1190cm^{-1} NMR spectrum, Fig. 3; mass, *m/e* 278 (M^+), 261, 234, 222 (s), 177, 165 (v. s). (Found: C, 73·31; H, 9·34. $C_{17}H_{26}O_3$ requires: C, 73·34; H, 9·41%).

Nervogenic acid (VI). To a soln of III (150 mg) in dioxan (6 ml), 1N H_2SO_4 (3 ml) was added and the mixture was heated at 85 ± 2° for 1·5 hr. After cooling, the mixture was diluted with H_2O (30 ml) and extracted with three 20 ml portions of Et_2O. The ethereal extracts were washed with H_2O and sat NaCl aq and then dried over Na_2SO_4. Evaporation of the solvent gave a crystalline solid which was chromatographed on silicic acid (5 g) using $CHCl_3$ as the eluent to afford colorless needles of VI, 50 mg, m.p. 96–97° (from pet ether); IR bands at 3500, 1670, 1605, 1325, 1290, 1260, 1185 cm^{-1}; UV absorption, max at 258 mμ (ε = 12,000) in MeOH, 291 mμ (ε = 17,000) in alkaline MeOH; mass, *m/e* 274 (M^+), 257, 230, 219, 203, 175, 159; NMR spectrum, Fig. 3. (Found: C, 74·13; H, 7·78. $C_{17}H_{22}O_3$ requires: C, 74·42; H, 8·08%).

Preparation of VII *and* VIII. A soln of III (100 mg) in dioxan (5 ml) was mixed with 2N HCl (5 ml). After refluxing for 3 hr, the cooled mixture was diluted with H_2O (30 ml) and extracted with three 20 ml portions $CHCl_3$. The $CHCl_3$ extracts were washed with H_2O, sat NaCl aq and dried over Na_2SO_4. Removal of the solvent under reduced press gave an oily residue, which was chromatographed on silicic acid (10 g). $CHCl_3$ eluted VIII, 34 mg, m.p. 120–121° (from n-hexane); p*Ka'* = 6·25 (66% MeOH); IR bands at 3200–2400, 1670, 1605, 1595, 1205, 1155, 1120 cm^{-1}; UV absorption, max at 265 mμ (ε = 13000) in MeOH, 255 mμ (ε = 11,500) in alkaline MeOH; NMR signals ($CDCl_3$) at 1·37 (6H, s), 1·76 (6H, s), 1·83 (2H, t, *J* = 7 c/s), 2·84 (2H, t, *J* = 7 c/s), 3·31 (2H, d, *J* = 7·5 c/s), 5·32 (1H, t, *J* = 7·5 c/s), 7·76 (2H, s) ppm; mass, *m/e* 274 (M^+), 219, 203, 177, 163. (Found: C, 74·22; H, 8·13. $C_{17}H_{22}P_3$ requires: C, 74·42; H, 8·08%).

The second fraction gave VIII, 20 mg, m.p. 170–171° (from n-hexane–Et_2O); p*Ka'* = 6·24 (66% MeOH); IR bands at 3300, 3200–2400, 1675, 1605, 1388, 1375, 1205, 1155, 1120 cm^{-1}; UV absorption, max at 265 mμ (ε = 14,000) in MeOH, 255 mμ (ε = 11,700) in alkalin*L* MeOH; NMR signals (CD_3COCD_3) at 1·25 (6H, s), 1·37 (6H, s), 1·50–1·95 (4H, m), 2·50–3·00 (4H, m), 7·64 (2H, s) ppm; mass, *m/e* 292 (M^+), 274 219, 203, 177, 163. (Found: C, 69·43; H, 8·31. $C_{17}H_{24}O_4$ requires: C, 69·83; H, 8·25%).

Identification of the sugar components. The aqueous layers, separated from the organic solv used for extracting the aglycones in the above experiments, were treated with Amberlite IR-4B resin (free amine type). After the resin had been removed, the filtrate was evaporated under reduced press. The residual oil was dissolved in a small amount of water and chromatographed on a paper using the following mixture: n-BuOH, AcOH, H_2O (4:1:1); phenol, H_2O (5:1); n-BuOH, EtOH, H_2O (4:1:5, upper layer); AcOEt, AcOH, H_2O (3:1:3, upper layer). Two spots corresponding to glucose and arabinose were detected by comparison with authentic samples. The development was carried out by the alkaline $AgNO_3$ reagent.

Formation of disaccharide (nervosiose). A soln of III (10 mg) in dioxan (2 ml) was diluted with 0·1N HCl (1 ml) and the mixture was warmed at 82–83° from 1·5 hr. The mixture was chromatographed on a paper with the mixture of n-BuOH, AcOH, H_2O (4:1:1).

Three spots were detected by development with the alkaline $AgNO_3$ reagent. The spot having the lowest R_f value was the disaccharide (nervosiose) which was proved by further acid hydrolysis to give glucose and arabinose.

Reduction of the disaccharide with $NaBH_4$ *and detection of sugar components.* To an aqueous soln (1 ml) of the disaccharide obtained above, $NaBH_4$ (10 mg) was added and allowed to stand over night at room temp. The excess of $NaBH_4$ was decomposed with 2N HCl cautiously, and further 0·5 ml of the excess was added to this mixture. The acidified mixture was warmed at 95° for 1·5 hr and then all the solvent and HCl were evaporated completely under reduced press.

The residue was dissolved in H_2O (20 ml) and poured on to a deionizing column (30 cm × 1 cm), followed by washing the column with H_2O (100 ml). The eluate was evaporated and the residue was chromatographed on paper.

Alkaline $AgNO_3$ and HIO_3 benzidine reagents were used for detection of the sugar components.

Titration of III *with* HIO_4. Titration of III with periodic acid was carried out in phosphate buffer (pH 6·1) by the usual method.[9] For a 30 min, about 1 mole equiv of iodate was consumed and after about 20 hr, a further one mole was consumed.

Full methylation of III. To a soln of III (200 mg) in dioxan (5 ml), 30% NaOH aq (1·5 ml) was added and then Me_2SO_4 (0·5 ml) was added dropwise with stirring.

After standing over night at room temp with stirring, the reaction mixture was heated at 80–90° for 30 min and then poured into water and extracted with CH_2Cl_2. The combined extracts were washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave a slightly yellow oil. The same procedure described above was repeated twice to give mainly one oily product of fully methylated nervosinic acid (III).

Methanolysis of fully methylated III. The oily product obtained above was dissolved in 1N absolute methanolic HCl and the soln was refluxed for 12 hr. The cooled soln was neutrallized with silver carbonate, filtered and the filtrate was concentrated under reduced press to give a syrup which was analysed on VPC as follows: a Hitachi Type K-53 Gas Chromatograph was employed, 2 m column, diethylene glycol succinate, 10% on Celite 545, at 210°, 20 ml of N_2/min.

The results were as follows: two peaks, which were detected at 7·2 and 9·4 min were identical not to α and β methyl-2,3,6-tri-O-methylglucopyranosides, but to α and β methyl-3,4,6-tri-O-methylglucopyranosides by the comparison with authentic samples.

Kuramerine (IX). Crude kuramerine picrate (2·7 g) was obtained from the fresh plants of *L. Krameri* French et. Sav. (2·3 kg) by the same method previously described as nervosine. Recrystallization from

EtOH gave fine needles of kuramerine picrate, m.p. 105–107°. (Found: C, 54·58; H, 6·47; N, 7·25. $C_{28}H_{44}O_8N$–$C_6H_2O_7N_3$ requires: C, 54·40; H, 6·17; N, 7·46%).

Kuramerine HCl salt was also obtained by the same method described above, using Amberlite IR-4B resin (free amine type). Crystallization of IX HCl salt was unsuccessful; Dragendorff test, positive; Fehling and Tollens tests, both negative; $[\alpha]_D^{20} = -19·7°$ (*c* 3, MeOH)· p*Ka'* = over 12; IR spectrum, Fig. 6; UV absorptions, maxima at 248, 280, 290 mμ (ε = 14,000, 2300, 2000) in MeOH.

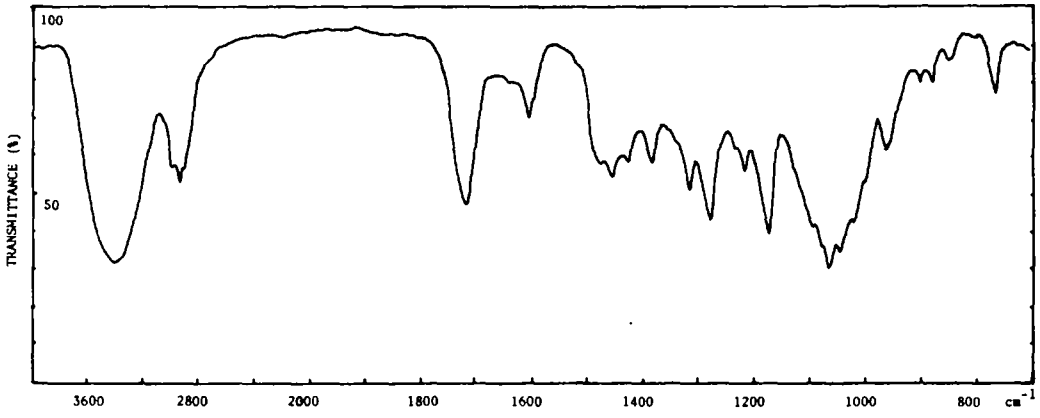


FIG. 6 The IR spectrum of IX HCl salt (KBr).

Alkaline hydrolysis of IX. To a soln of IX HCl salt (100 mg) in MeOH (5 ml), 10% KOH aq (3 ml) was added and the mixture was refluxed for 2 hr. The reaction mixture was diluted with H_2O (15 ml) and then acidified slightly with 2N HCl (pH 3–4).

On standing in an ice bath, a ppt appeared and was recrystallized from 80% EtOH as colorless needles of XI, 67 mg, m.p. 103–105°; $[\alpha]_D^{20} = -16·5°$ (*c* 4, MeOH); p*Ka'* = 6·1 (66% MeOH); IR bands at 3350, 1690, 1605, 1275, 1180, 1100, 1075, 1040, 1010 cm^{-1}; UV absorptions, maxima at 243, 278, 288 mμ (ε = 14,000, 2300, 2000) in MeOH, 235 (sh), 278, 288 mμ (ε = 12,500, 2300, 2000) in alkaline MeOH; NMR signals (in pyridine) at 1·36 (6H, s), 1·73 (6H, s), 4·02 (4H, d, *J* = 7 c/s), about 4·00 (1H, m) 4·31 (5H, br. m), 5·40 (1H, br. d, *J* = 6 c/s), 5·57 (1H, t, *J* = 7 c/s) ppm. (Found: C, 60·43; H, 7·40. $C_{23}H_{32}O_8.H_2O$ requires: C, 60·78; H, 7·54%).

From the filtrate, choline was obtained as picrate, 40 mg.

Hydrokurameric acid (XII). A mixture of XI (30 mg) and PtO_2 (5 mg) in EtOH (15 ml) was stirred for 3 hr under H_2 gas. The catalyst was removed and the filtrate was evaporated under reduced press to give a colorless solid of XII, 28 mg, m.p. 99–102°; IR bands at 3350, 1690, 1605, 1290, 1180, 1100–980 (complicated absorptions); NMR signals (in pyridine) at 0·90 (12H, d, *J* = 5 c/s), 1·3–1·9 (6H, m), 3·20 (4H, br. t, *J* = 7 c/s), 3·88 (1H, m, 430 (5H, br. m), 5·30 (1H, br. d, *J* = 6 c/s).

Acid degradation of XII. A soln of XII (100 mg) in dioxan (5 ml) was mixed with 2N HCl (5 ml) and the mixture was refluxed for 2 hr. After cooling, the mixture was diluted with H_2O (15 ml) and extracted with three 20 ml portions of $CHCl_3$ and the extract was washed with H_2O, sat NaCl aq and dried over Na_2SO_4. Evaporation of the solvent gave a light brown solid which was chromatographed on silicic acid (5 g) using the mixture of $CHCl_3$ and MeOH. First fraction was recrystallized from n-hexane to give colorless needles which were identical with VII, 40 mg. The second fraction proved to be VIII by IR spectra, 15 mg.

From the aqueous layer, glucose was detected by chromatography on paper.

Kumokirine (XIII). From the plants of *L. Kumokiri* F. Maekawa (0·8 kg), kumokirine picrate was isolated by the same method previously described, 2·0 g, m.p. 100–102 (from MeOH). (Found: C, 55·49; H, 6·35; N, 6·78. $C_{32}H_{48}O_8N.H_2O$–$C_6H_2O_7N_3$ requires: C, 55·60; H, 6·39; N, 6·83%).

Kumokirine HCl salt is an amorphous material; Dragendorff test, positive; Fehling and Tollens tests, both negative; $[\alpha]_D^{20} = -23·4°$ (*c* 4, MeOH); p*Ka'* = over 12; IR spectrum, Fig. 7; UV absorptions, maxima at 247, 280, 290 mμ (ε = 14,000, 2300, 2000) in MeOH.

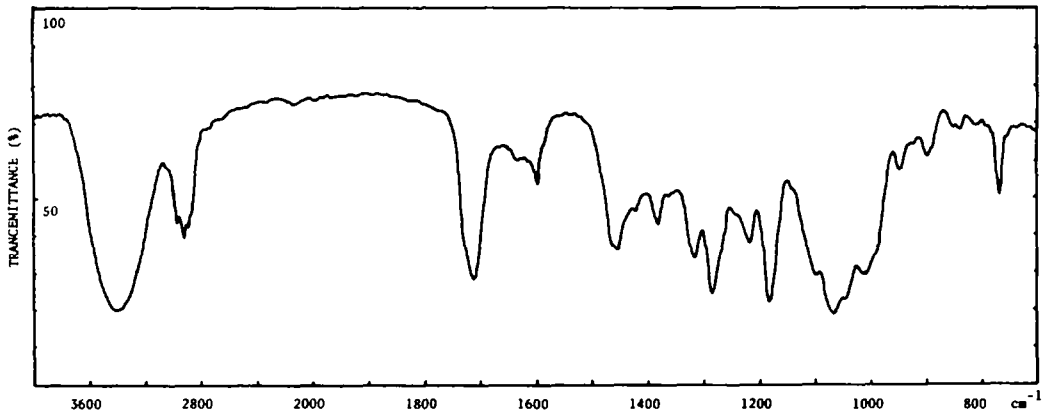


FIG. 7 The IR spectrum of XIII HCl salt (KBr).

Alkaline hydrolysis of XIII. A soln of XIII HCl salt (50 mg) in MeOH (3 ml) was mixed with 10% KOH aq (2 ml) and the mixture was refluxed for 2 hr. After cooling, the mixture was diluted with H_2O (10 ml) and acidified with 2N HCl (pH 3–4). The ppt was collected and recrystallized from EtOH as colorless needles, 30 mg, m.p. 103–105°. This product was proved to be XI by the IR spectra.

The filtrate was neutrallized with Na_2SO_4 and then dried completely. The MeOH extracts of the residue was concentrated and again dissolved in small volume of EtOH (0·5 ml). To this soln, sat picric acid in EtOH was added. Recrystallization of the ppt gave long yellow needles of the picrate of XIV, 24 mg, m.p. 247–248°; $[\alpha]_D^{20} = 12°$ (c 3, MeOH, as HCl salt). (Found: C, 46·50; H, 5·44; N, 14·66. $C_9H_{18}ON$–$C_6H_2O_7N_3$ requires: C, 46·87; H, 5·25; N, 14·58%).

Pyrolysis of XIII HCl *salt.* The HCl salt of XIII (30 mg) was heated in a vacuum sealed tube at 250–300° for 10 min. After cooling, the brown oil was dissolved in MeOH and the insoluble substances were filtered off and the filtrate concentrated. The residue gave 3 spots on a paper chromatography, one of which was separated by chromatography on paper to give laburnine (3 mg) being identical with an authentic sample by the IR (as picrate) and mass spectra.

Isolation of malaxin (XV) *and its guanidine complex.* Cut small pieces of *L. bicallosa* Schltr. or *L. hachijoensis* Nakai (2 kg) were soaked in boiling MeOH (2 l), which was allowed to stand for 2 days at room temp. The mechanolic extract was collected by decantation and the residual plants were crushed into fine pieces with a mixer and re-extracted with MeOH for one day at room temp. The combined MeOH extracts were concentrated under reduced press up to about 100 ml volume. The remainder was washed with Et_2O

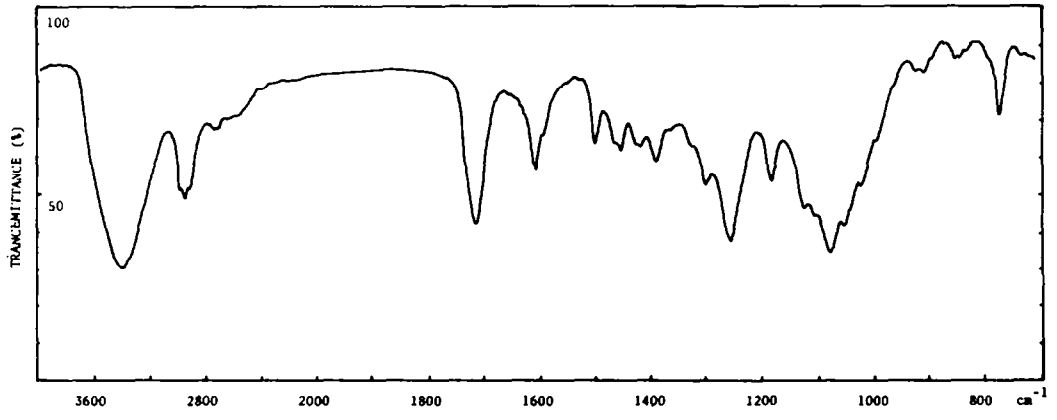


FIG. 8 The IR spectrum of XV (KBr).

several times. To the resulting water layer, excess of a sat soln of $NH_4[Cr(NH_3)_2(SCN)_4]$–H_2O aq at 60° was added and the mixture was cooled in an ice bath. The ppt was collected and dissolved in about 100 ml acetone. The acetone soln of the Reinecke salt was filtered and to the filtrate, a warmed sat soln of Ag_2SO_4 aq was added until the reddish color disappeared and the ppt was removed by centrifugation.

The supernatant was evaporated under reduced press to give a light brown semisolid which is partly crystalline, when a small amount of cold MeOH was added (about 50 ml). The insoluble material was collected and recrystallized from 90% MeOH to give prisms of malaxin-guanidine complex, 960 mg, m.p. 180–182°; $[\alpha]_D^{20} = -33°$ (*c* 2, H_2O); IR bands at 3400, 3200 (sh), 1710, 1660 (strong), 1605, 1245, 1115, 1070, 1040, 1020 cm^{-1}. (Found: C, 48·29; H, 7·13; N, 8·23. $C_{27}H_{42}O_8N_4.H_2O$–H_2SO_4 requires: C, 48·64; H, 6·95; N, 8·40%).

The mother liquor was concentrated and the residue was chromatographed on cellulose powder using a mixture of n-BuOH, AcOH, H_2O (4:1:1). The first eluate gave an amorphous solid of XV on evaporation of the solvent, 1·1 g. This did not crystallize m.p. 104–110°; p*Ka'* = 9·8 (66% MeOH); Dragendorff test, positive; Fehling and Tollens tests, both negative; IR spectrum, Fig. 8; UV spectrum, see Fig. 4. (High resolution of mass spectrum: Found: M^+, 491·245. $C_{26}H_{37}O_8N$ requires: 491·242).

Formation of malaxin-guanidine complex. To a soln of amorphous XV (50 mg) in MeOH (3 ml), a soln of guanidine sulfate (20 mg) in MeOH (3 ml) was added. On rubbing the walls of the flask with spatula for a few min, a ppt gradually appeared, and recrystallization gave a crystalline malaxin-guanidine complex, proved by comparison of the IR spectra.

The guanidine sulfate which was used, was one freshly precipitated by adding the mixture of acetone–Et_2O to the methanolic soln of guanidine HCl salt in the presence of excess H_2SO_4.

Alkaline hydrolysis of XV. A soln of XV (100 mg) in MeOH (5 ml) was mixed with 10% KOH aq (3 ml) and the mixture was refluxed for 2 hr. After cooling, the reaction mixture was diluted with 0·2N HCl until the soln became slightly acidic (pH 3–4) and was then cooled in an ice bath. The ppt was collected and recrystallized from AcOEt to give colorless needles of XVII, 60 mg, m.p. 124–126 ; $[\alpha]_D^{20} = -52$ (*c* 4, MeOH); p*Ka'* = 6·1 (66% MeOH); IR bands, at 3400, 1690, 1605, 1500, 1250, 1130–990 (complicated absorptions) cm^{-1}; UV spectrum, see Fig. 4; NMR signals (D_2O–DOK), at 1·74 (6H, s), 3·41 (2H, d, *J* = 7 c/s), 3·3–4·0 (6H, m), 5·16 (1H, d, *J* = 6 c/s), 5·42 (1H, br. t, *J* = 7 c/s), 7·16 (1H, d, *J* = 9 c/s), 7·80 (1H, s), 7·80 (1H, d, *J* = 9 c/s) ppm: mass, *m/e* 368 (M^+), 206, 191, 171, 155. (Found: C, 56·14; H, 6·51. $C_{18}H_{24}O_8.H_2O$ requires: C, 55·95; H, 6·78%).

The filtrate was made basic with $NaHCO_3$ and dried completely. MeOH extracts of the residue gave a light brown oil on evaporation of the solvent, and afforded a picrate of XVI, 52 mg, m.p. 172–173°; $[\alpha]_D^{20} = 17°$ (*c* 2, MeOH, as free base); p*Ka'* = 10·5 (50% MeOH); mass, *m/e* 141 (M^+), 124, 110, 83. (Found: C, 45·80; H, 4·89; N, 15·20. $C_8H_{15}ON$–$C_6H_3O_7N_3$ requires: C, 45·40; H, 4·90; N, 15·13%).

Formation of XVIII. To a soln of XVII (50 mg) in dioxan (3 ml), 2N HCl (2 ml) was added and the mixture was refluxed for 2 hr. The mixture was diluted with 10 ml H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O and dried over Na_2SO_4.

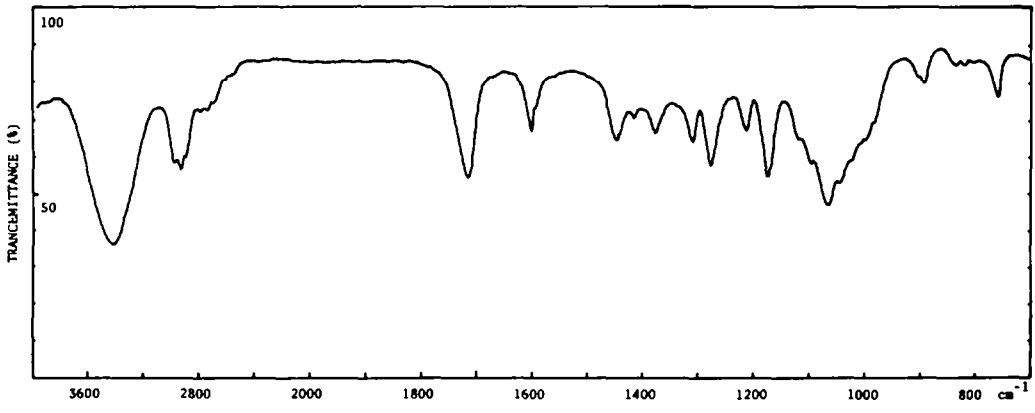


FIG. 9 The IR spectrum of XX (KBr).

After removal of the solvent, the crystalline solid was recrystallized from n-hexane to give needles of XVIII, 20 mg, m.p. 179–180°. From the aqueous layer, glucose was detected on paper chromatography.

Formation of XIX. In EtOH (20 ml), XVII (100 mg) was catalytically reduced with PtO_2 (20 mg).

After removal of the catalyst, the soln was concentrated under reduced press to give a colorless amorphous solid in quantitative yield. The soln of this product in dioxan (5 ml) was mixed with 2N HCl (2 ml) and the mixture was refluxed for 2 hr. The reaction mixture was poured into 50 ml water.

The ppt was recrystallized from 50% MeOH to give needles of XIX, 40 mg; m.p. 108–109 .

Auriculine (XX). Auriculine was isolated from *L. auriculata* Blume (34 g), as a picrate (20 mg). Auriculine (free base) is an amorphous material; $[\alpha]_D^{20} = -19{\cdot}1$ (*c* 2, MeOH); $pKa' = 9{\cdot}6$ (66% MeOH); IR spectrum, Fig. 9; UV absorption, max at, 247 mμ (MeOH); mass, *m/e* 397, 257, 212, 141, 83.

Degradation of XX. A soln of XX (8 mg) in 2N HCl (2 ml) was heated at 100° for 2 hr. The ppt was collected (2 mg), the mass spectrum of which was in good agreement with that of VII. The filtrate was dried completely to give light brown oil which was separated into glucose, laburnine (proved by the mass and IR spectral comparison), a small amount of XX and a unknown product by paper chromatography using the mixture of n-BuOH, AcOH, H_2O (4:1:1).

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