Tetrahedron Vol. 41, No. 15, pp. 3037 to 3048, 1985
Printed in Great Britain.

0040-4020/85 $3.00 + .00

STRUCTURE OF AMAUROMINE, A NEW HYPOTENSIVE VASODILATOR PRODUCED BY AMAUROASCUS SP.

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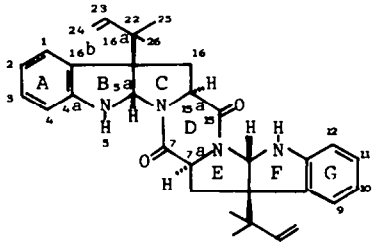
(*Received in Japan* 20 *March* 1985)

Abstract-The structure of amauromine, a novel alkaloid with potent vasodilating activity was determined chemically and spectroscopically to be (5aS,7aS,8aR,13aS,15aS,16aR)-8a,16a-bis-(1,1-dimethyl-2-propenyl)-5a,8,8a,13,13a,15a,16,16a-octahydro-pyrazino[1",2":1,5;4",5":1',5']dipyrrolo[2,3-b:2',3'-b']-diindole-7,15(5H,7aH)-dione.

Amauromine (1a) is a new alkaloid isolated from Amauroascus sp. No. 6237 in our screening program for new vasodilators from microbial metabolites using superfusion technique[1]. A pharmacological study has revealed that the vasodilating activity of amauromine originates in a calcium antagonism[2]. In the previous communication[3], we reported that amauromine is a dimeric indole alkaloid (1a) consisting of a hexahydropyrrolo[2,3-b]indole skeleton and possessing a reversed prenyl group at position 3 of the indole nucleus. The group of the indole alkaloids bearing the 1,1-dimethyl-2-propenyl substituent at the position 3 and 2 of the indole moiety, such as roquefortine and echinulin, has been rapidly expanding and the biosynthesis of those alkaloids has been extensively explored[4] in recent years. Among those indole alkaloids, several compounds which possess the reversed prenyl group at position 3a of 1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole nucleus have been isolated from microbial and marine sources during the last decade[5](Fig. 1). However, none of absolute stereostructure of these alkaloids have so far been elucidated. In the present paper, we describe a full account on the structure elucidation of amauromine including its absolute configuration.



Amauromine (1a)

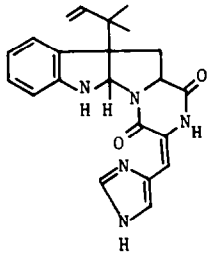
PLANE STRUCTURE OF AMAUROMINE

Amauromine (1a) forms colorless prisms from EtOH: m.p. 156-158°C, $[\alpha]_D^{23}$ -583° (c=1.0, $CHCl_3$). The molecular formula, $C_{32}H_{36}N_4O_2$, was established by elemental analysis and high resolution mass spectrum.[1] Amauromine reveals nuclear magnetically symmetrical features. Thus, in the ^1H and ^{13}C NMR spectra the signals due to half number of protons (18) and carbons (16) of the molecule were observed,

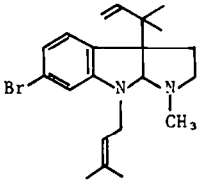
respectively. This symmetrical property was also recognized in the NMR spectra of tetrahydroamauromine (2) derived from 1a by catalytic hydrogenation. The ^{1}H NMR and ^{13}C NMR spectral data of 1a and 2 are summarized on Table 1. With the molecule of amauromine being a dimer as explained above, structure elucidation of amauromine was focused on the half part of the structure corresponding to the formula $C_{16}H_{18}N_2O$.

The presence of the 1,1-dimethyl-2-propenyl group in 1a was proved as follows. In the ^{1}H NMR of 1a, three signals at δ 5.98, 5.08, and 5.04 constituting an ABX system were assigned to the protons due to a terminal olefin. The assignment was verified by the fact that the ^{1}H NMR spectrum of 2 lacked the corresponding signals, while it revealed new signals at δ 1.44 and 0.81 attributable to an ethyl group. Two singlet signals at δ 1.08 and 0.98 due to methyl groups in the ^{1}H NMR spectrum of 1a were expected to be a geminal dimethyl group attached to an allylic carbon. This was corroborated by IR absorption bands at 1380 and 1365 cm^{-1} in 1a and two methyl proton signals resonated at a higher field (δ 0.93) in 2. Furthermore, in the EI mass spectra of 1a and 2, the base peaks at m/z 439 ($M^{+}-C_5H_9$) and m/z 441 ($M^{+}-C_5H_{11}$) were observed, respectively. In addition, signals at δ 143.5 (d), 114.3 (t), 40.7 (s), 22.8 (q) and 22.5 (q) in the ^{13}C NMR spectrum of 1a, were in excellent agreement with the carbon signals due to 1,1-dimethyl-2-propenyl group observed in aszonalenin[5] (6) and roquefortine[5] (5).

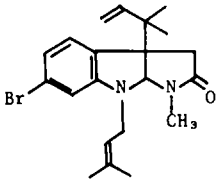
In the ^{13}C NMR spectrum of 1a, six signals at δ 149.9(s), 128.8(s), 128.7(d), 124.7(d), 118.6(d), and 109.2(d) due to the aromatic carbons of amauromine were observed, which closely resemble those of indoline[6], indicating the presence of indoline moiety in 1a. A strong IR absorption band at 1660cm^{-1} and a ^{13}C NMR signal at δ 166.1(s) in 1a showed the presence of an amide function in the amauromine molecule. Then, acid hydrolysis (6N HCl, 110°C, 4hr) of 1a was conducted, resulting in the isolation of L-tryptophan as a degradation product. This finding not only assisted the above speculation of the presence of indoline moiety in 1a, but also led us to assign the proton signal at δ 3.78 (1H, t, J=8) in the ^{1}H NMR



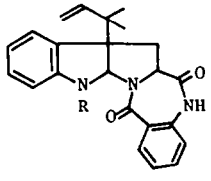
Roquefortine(5)



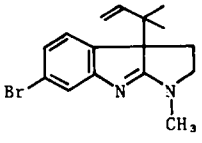
Flustramine A



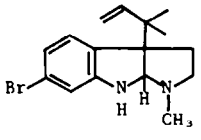
Flustramide A



R=H Aszonalenin(6)
R=Ac LL-S490β



Flustramine C



Dihydroflustramine C

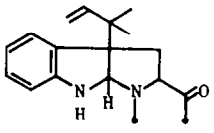
Fig. 1. Natural products[5] possessing 1,1-dimethyl-2-propenyl group at position 3a of hexahydropyrrolo[2,3-b]indole nucleus.

Table 1. NMR Data of 1a and 2.

Position	^{13}C NMR *		^{1}H NMR **	
	1a	2	1a	2
1	124.7(d)	124.9(d)	6.46(1H,d,J=8)	6.46(1H,d,J=8)
2	118.6(d)	118.6(d)	6.66(1H,t,J=8)	6.70(1H,t,J=8)
3	128.7(d)	129.4(d)	6.96-7.10(2H,m)	6.96-7.12(2H,m)
4	109.2(d)	109.1(d)		
4a	149.9(s)	150.0(s)		
5			4.92(1H,s)	4.94(1H,s)
5a	77.1(d)	77.1(d)	5.42(1H,s)	5.46(1H,s)
15	166.1(s)	166.3(s)		
15a	60.3(d)	60.5(d)	3.78(1H,t,J=8)	3.82(1H,t,J=8)
16	35.2(t)	35.0(t)	2.43(2H,d,J=8)	2.46(2H,d,J=8)
16a	61.8(s)	63.5(s)		
16b	128.8(s)	128.5(s)		
22	40.7(s)	37.9(s)		
23	143.5(d)	29.0(t)	5.98(1H,dd,J=17,11)	1.44(2H,q,J=7)
24	114.3(t)	8.4(q)	5.04(1H,d,J=17)	0.81(3H,t,J=7)
			5.08(1H,d,J=11)	
25	22.5(q)	21.2(q)	0.98(3H,s)	0.93(3H,s)
26	22.8(q)	21.3(q)	1.08(3H,s)	0.93(3H,s)
	* 25 MHz in $CDCl_3$ δ (multiplicity)		** 100 MHz in $CDCl_3$ δ (multiplicity, coupling const. in Hz)	

spectrum of 1a to the α-methine proton of α-amino acid function. On irradiation of a doublet signal at δ 2.43 (2H, J=8), the above triplet signal was changed to a singlet, indicating that the three protons at δ 3.78 and 2.43 constitute an A_2X pattern. The assignment of these three proton signals in the ^{1}H NMR spectrum of 1a was verified by the following chemical evidence. On treatment with Na_2CO_3 in refluxing CH_3OD, amauromine was converted to a dideutero compound (4) (M^+ 510), which lacked the ^{1}H NMR signal at δ 3.78 present in the spectrum of amauromine. On the other hand, the CH_2 doublet at δ 2.43 in amauromine simplified to a singlet at the same chemical shift in the ^{1}H NMR spectrum of 4. Treatment of dideuteroamauromine (4) on the same reaction conditions using methanol instead of CH_3OD regenerated amauromine. From these results, the methylene group due to the proton signal at δ 2.43 in amauromine is possibly attached to the position 3 of the indoline nucleus which is comprised of quaternary carbon and bears the 1,1-dimethyl-2-propenyl group. A broad singlet signal at δ 4.92 in the ^{1}H NMR spectrum of 1a was assigned to the NH proton since it was exchangeable with D on addition of D_2O. The remaining unknown ^{1}H NMR signal at δ 5.42(1H,s) was assignable to N-CH-N(CO)- as the chemical shift is in good agreement with the reported value for comparable systems such as aszonalenin[5] (6) (δ 5.57) and roquefortine[5] (5) (δ 5.70).

The above discussions led us to deduce the structure corresponding to the half part ($C_{16}H_{18}N_2O$) of the amauromine molecule to be as depicted in 3, which consisted of a hexahydropyrrolo[2,3-b]indole skeleton. The validity of the proposed structure 3 was supported by the following facts. The chemical shifts and multiplicities of the carbons for the half part of the amauromine molecule in the ^{13}C NMR spectrum were almost superimposable to those of the corresponding signals of the roquefortine molecule[5]. Furthermore, high resolution mass spectrum of amauromine showed fragmentation peaks at m/z 130.0663 (C_9H_8N requires 130.0657) and 157.0782 ($C_{10}H_9N_2$ requires 157.0766) which could be assigned to the ions 7 and 8, respectively, as in the mass spectrum of roquefortine[7]. Therefore, the plane



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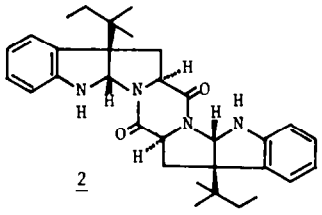

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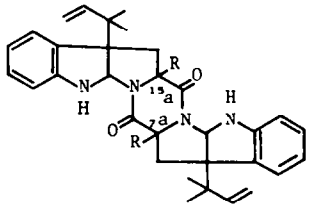
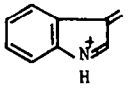


1 R=H

4 R=D



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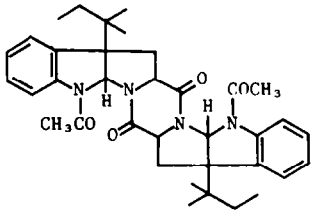

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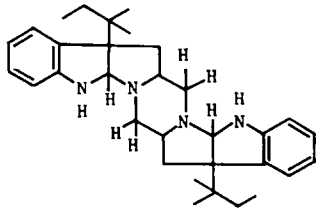

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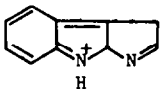

8

structure of amauromine was deduced to be 1, which corresponded to the dimer of the half structure 3. The deduced structure 1 for amauromine satisfied the following data. Tetrahydroamauromine (2) afforded a diacetate (9) on treatment with acetic anhydride in pyridine and a dideoxo compound (10) by aluminum hydride reduction. In the ^{1}H NMR spectra measured in $CDCl_3$ of 2, 9, and 10, the signal due to H-5a (δ 5.46 in 2) was observed at a lower field (δ 6.07) in 9 on account of N(5)-acetylation and at a higher field (δ 4.63) in 10 owing to the reduction of the amide carbonyl function. The crucial proof of the plane structure 1 for amauromine was obtained from the following study on the stereochemistry of amauromine.

ABSOLUTE STRUCTURE OF AMAUROMINE

Since amauromine possesses both the nuclear magnetically symmetrical feature as a dimer and the large specific rotation, a C_2 symmetry axis must exist on the center of the 2,5-dioxopiperazine ring (D ring) in the amauromine molecule. The fact that L-tryptophan was isolated by acid hydrolysis of amauromine proved both the absolute configurations at 7a and 15a to be S. A NOE-enhancement (9%) of the H-5a signal upon irradiation of the H_3-25 (or 26) signal (δ 0.98) showed the cis-fusion of B and C rings (E and F rings). As a result of these evidence and discussions, the absolute structure of amauromine is limited to be either 1a or 1b. Based on the following ground, we concluded that the absolute structure of amauromine is the one depicted as 1a.

In the ^{1}H NMR spectra of amauromine and tetrahydroamauromine (2), H-15a resonates at δ 3.78 and 3.82, respectively. Hydrogenation of amauromine over PtO_2 in acetic acid under 4 atmospheric pressure provided the perhydrocompound 11* as a major product (50%). In the ^{1}H NMR spectrum of 11 the signal due to H-15a appeared at δ 4.33, which was down-field shifted by 0.51 ppm from that of 2. This

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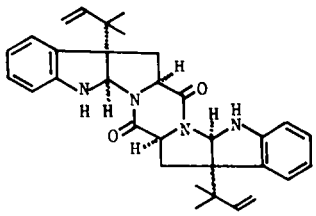
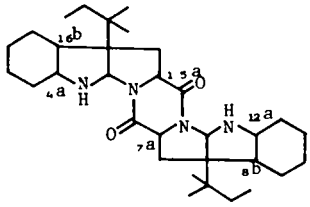
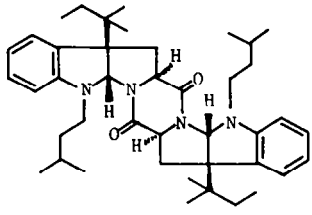
* Stereochemistry at positions 4a, 8b, 12a, 16b of this compound remains undefined.



1b



11



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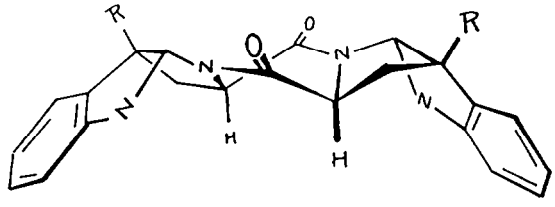
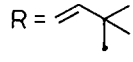
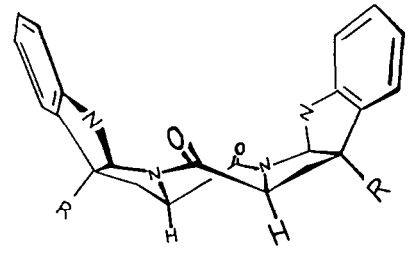


1a



R =

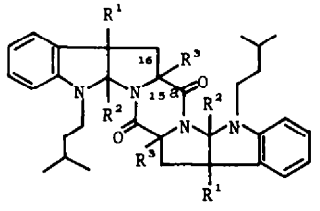


1b

Fig. 2. Proposed conformations of 1a and 1b based on the Dreiding model study.

observation can be accounted as follows. In the molecules of amauromine and tetrahydroamuromine (2), the resonances of H-15a take place at a higher field because of an anisotropic effect due to the aromatic ring A (G). This anisotropic effect is only explainable on the molecular model of 1a for amauromine (Fig. 2).

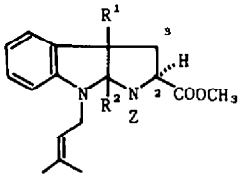
With the validity of the foregoing discussion on the chemical shift of H-15a, we proposed a plan to synthesize the analogous compounds 13 and 14, in order to compare their physical data with those of the amauromine derivative 12, which was derived from amauromine by treatment with prenyl bromide, followed by catalytic reduction. The syntheses of 13 and 14 were carried out as follows. Treatment of Z-L-tryptophan methyl ester[8] with prenyl bromide in the presence of $NaHCO_3$ in acetone produced two compounds 15 (29%) and 16 (22%), which were hydrogenated on Pd black in EtOAc, followed by treatment with di-tert-butyl dicarbonate to give 17 and 18, respectively. Stereochemistry of these four compounds was determined by comparison of the ^1H NMR spectra with those of the compounds 19 and 20 synthesized by Hino et al[9] (Table 2). After hydrolysis of the methyl ester, 15 was converted to the succinimide active ester by the conventional manner. Removal of the Z group by catalytic reduction over Pd black provided the dimeric compound 13 accompanying

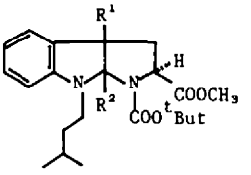


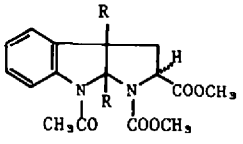
13 R^1= , R^2= —H, R^3= ·····H

14 R^1= , R^2=R^3= ·····H

21 R^1= , R^2= ·····H, R^3= —H







15 R^1= , R^2= —H 17 R^1= , R^2= —H 19 R= —H

16 R^1= ····· , R^2= ·····H 18 R^1= ····· , R^2= ·····H 20 R= ·····H

Table 2. Comparison of ^{1}H NMR data of compounds 15, 16, 17, 18, 19, and 20.

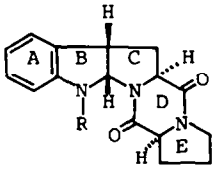
	^{1}H NMR(δ)	
	H-2	C(2)-$COOCH_3$
15	4.24	3.48
16	4.68	3.28
17	4.16	3.70
18	4.60	3.32
19	4.0	3.63 or 3.72
20	4.59	3.11

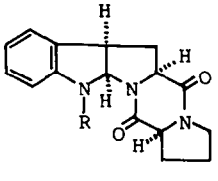
the reduction of the olefinic side-chains. The overall yield of 13 from 15 was 45%. In the same manner 14 was synthesized from 16 in 15% yield. The low yield of 14 is probably due to a difficulty of coupling for dimerization owing to the strained molecule of 14. In the ^{1}H NMR spectra, the signal of H-15a in 13 was observed at a higher field (δ 4.11) than that in 14 (δ 4.38). This is explainable by the aforementioned anisotropic effect. The $[\alpha]_D$, CD and ^{1}H NMR data of the amauromine derivative 12 are shown in comparison with those of the synthetic compounds 13 and 14 in Table 3. The physical data of 12 resemble those of 13 and are markedly different from those of 14. This fact enabled us to conclude that compound 12 possesses the same absolute configurations as those of compound 13. The following experimental results are quite consistent with this assignment for the stereochemistry of 12. When 12 and 13 were treated with Na_2CO_3 in refluxing methanol, no reaction took place as in the case of amauromine. However, by the same treatment 14 was converted to a new compound 21 ($[\alpha]_D^{23}$ +444°), which turned out to be the antipode of 13 because the IR and NMR spectra of the two compounds were identical.

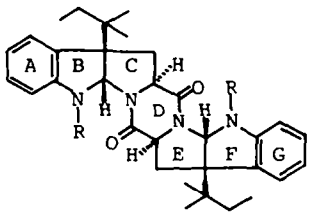
Table 3. $[\alpha]_D$, CD and ^1H NMR data of 12, 13, and 14.

	$[\alpha]_D$	CD [θ] (nm)	^1H NMR (δ) H-15a	^1H NMR (δ) H_2-16
12	-470.8°	-125,000(255) - 36,800(311)	4.06(1H,dd,J=6,11)	2.56(1H,dd,J=6,11) 2.28(1H,dd,J=11,11)
13	-433.3°	-100,000(254) - 23,400(307)	4.11(1H,dd,J=6,11)	2.66(1H,dd,J=6,11) 2.10(1H,dd,J=11,11)
14	+202.4°	- 32,600(225) + 35,900(255) - 2,600(313)	4.38(1H,t,J=9)	2.37(2H,d,J=9)

Table 4. CD data of tetrahydroamauromine (2), known related compounds 22, 24 and their N-acetylated derivatives 9, 23, 25.







CD [θ] (nm)		
22 R=H - 17300(249)	24 R=H + 13200(244)	2 R=H - 78000(243)
23 R=Ac + 60200(245.5)	25 R=Ac - 82300(236)	9 R=Ac + 105000(249)

Further support for the conclusion on the absolute configuration of amauromine (1a) was obtained by comparison of the CD data of the amauromine derivatives 2 and 9 with those of the related compounds which have the same ring system with the A, B, C, D, and E rings of amauromine (Table 4). The compounds 22, 23, 24, and 25 were synthesized starting from L-tryptophan and L-proline by Hino et al, and the stereochemistry of 23 was established by X-ray analysis[10]. The CD data shown in Table 4 suggest that 2 and 9 have the same type of absolute configuration at the BC ring junctions with those of 22 and 23, respectively.

In conclusion, the absolute structure of amauromine has been determined to be shown as 1a. Recently we achieved a biogenetic-like total synthesis of amauromine[11], and the details will be reported in due course.

EXPERIMENTAL

Melting points were taken using a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were measured on a Hitachi 220A double beam spectrophotometer, the maxima are given in nm (extinction ε). IR spectra were measured on a JASCO A-102 infrared spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL MH-100 or JEOL JNM FX-270 and ^{13}C NMR spectra were recorded on a JEOL PFP-100. The chemical shifts are given in ppm (δ) relative to internal TMS, coupling constants (J) in Hz and multiplicities are indicated by the usual symbols. Optical rotations were determined on a JASCO DIP-140 polarimeter, using a 10cm-microcell. CD curves were recorded on a JASCO J-20C automatic recording spectropolarimeter in MeOH unless otherwise stated. Low-resolution and high-resolution electron impact mass spectra (HRMS) were measured on a JEOL JMS-D300 and secondary ion mass spectra were measured with a Hitachi M-80 mass spectrometer. Medium pressure LC was performed by using Lobar pre-packed column (Merck), F.M.I. pump and UV detector UVILOG-10V (Yamazen). Preparative tlc (PLC) was carried out using pre-coated silica gel 60F_{254} plates (Merck, Art 5744).

Tetrahydroamauromine (2). A solution of amauromine (500mg) in ethanol (50ml) was hydrogenated over PtO_2 (140mg) at room temp. for 4hr under 1 atm pressure of H_2. The mixture was filtered and the filtrate was concentrated to dryness in vacuo to leave an oil which was chromatographed on a silica gel column using chloroform as eluent. The product fractions were combined and concentrated to dryness to give a powder. Crystallization from ethanol gave 400mg of 2 as colorless prisms, m.p. 172-175°; $[\alpha]_D^{23}$ -553°(C=1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm(ε) 243(13,500), 300(4,800); CD [θ] -78,000(243nm), -14,000(300nm); IR ($CHCl_3$) 3420, 2960, 1660, 1600, 1420 cm^{-1} ; ^{1}H NMR and ^{13}C NMR, see Table 1 ; MS m/z 512(M^+),441,371,157,130 ; HRMS m/z 512.3152($C_{32}H_{40}N_4O_2$ requires 512.3153). (Found: C, 73.68; H, 7.95; N, 10.65. $C_{32}H_{40}N_4O_2 \cdot 1/2\ H_2O$ requires: C, 73.67; H, 7.92; N, 10.74 %).

Acid Hydrolysis of Amauromine (1a). A suspended mixture of amauromine (400mg) in 6N HCl (20ml) was heated at 110°C for 4hr under argon atmosphere. The cooled reaction mixture was concentrated to dryness in vacuo to give a residue, which was extracted with water leaving an insoluble residue (220mg). The extract was neutralized with 5N NaOH and applied to a column of Diaion HP-20. Elution with 50% MeOH gave a residue (62mg), which was purified by cellulose column chromatography, eluted with wet butanol to afford 45mg of a pure compound identical with L-tryptophan in TLC, IR, ^{1}H NMR, SI-MS and CD, CD [θ] (0.1N HCl)+18,600(225nm). L-tryptophan, CD [θ] (0.1N HCl)+18,400(225nm); D-tryptophan, CD [θ] (0.1N HCl)-18,600(225nm).

7a,15a-Dideuteroamauromine (4). Sodium carbonate (40mg) was added to a solution of 1a (40mg) in CH_3OD (4ml) and the mixture was refluxed for 2hr. The cooled mixture was diluted with ethyl acetate, washed with water followed by brine, dried over $MgSO_4$. Removal of the solvent gave an oil which was purified on PLC (benzene-ethyl acetate 10:1) to yield 40mg of 4 as a powder, $[\alpha]_D^{23}$ -556°(C=1.0,MeOH); UV λ_{max}^{MeOH} nm (ε) 243(11,750), 299(4,500); IR($CHCl_3$) 3420,2970,1660,1605,1420,1380,1365,920cm^{-1} ; ^{1}H NMR(100MHz,$CDCl_3$) δ 6.96-7.12(4H,m), 6.70(2H,t,J=8Hz), 6.50(2H,d,J=8Hz), 6.02(2H,dd,J=17 and 11Hz), 5.42(2H,s), 5.12(2H,d,J=11Hz), 5.08(2H,d,J=17Hz), 5.00(2H,broad s), 2.44(4H,s), 1.12(6H,s), 1.00(6H,s); MS m/z 510(M^+),441,373, 158,130.

Regeneration of 1a from 4. Sodium carbonate (40mg) was added to a solution of 4

(40mg) in methanol (4ml) and the mixture was refluxed for 2hr. The mixture was cooled and diluted with ethyl acetate, washed with water, brine, dried over $MgSO_4$ and evaporated to dryness. Purification of the residue by PLC (benzene-ethyl acetate 10:1) afforded 35mg of a colorless powder which was identical in all respects ($[\alpha]_D$,UV,IR,NMR,MS,co-tlc) with amauromine (1a).

5,13-Diacetyltetrahydroamauromine (9). Acetic anhydride (1ml) was added to a solution of 2 (50mg) in pyridine (1ml) and the mixture was heated at 100° for 7hr. The cooled reaction mixture was concentrated to dryness in vacuo to leave an oil which was subjected to PLC (benzene-ethyl acetate 10:1) to give 44mg of 9 as an oil, $[\alpha]_D^{23}$ -165°(C=2.0,$CHCl_3$); UV λ_{max}^{MeOH} nm(ε) 245(16,900), 275(2,700), 283(2,300); CD +105,000(249nm), -9,000(280nm); IR($CHCl_3$) 2960,1660,1590,1380 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 7.86(2H,d,J=8Hz), 7.00-7.32(6H,m), 6.07(2H,s), 3.84(2H, dd, J=11 and 7 Hz), 2.60(6H, s), 2.36-2.70(4H, m), 1.32(4H, q, J=7Hz), 0.95(12H, s), 0.83(6H, t, J=7Hz); MS m/z 596(M^+), 554, 512, 130.

8a,16a-Bis(1,1-dimethylpropyl)-5,5a,7,7a,8,8a,13,13a,15,15a,16,16a-dodecahydropyrazino[1",2":1,5;4",5":1',5']dipyrrolo[2,3-b:2'3'-b']diindole (7,15-dideoxotetrahydroamauromine 10). Lithium aluminum hydride (34mg) was added portionwise to a solution of aluminum chloride (40mg) in dry diethyl ether (20ml) at 5°C, and then the mixture was stirred at room temp. for 40min. To this mixture was added dropwise a solution of tetrahydroamauromine (2) (150mg) in dry diethyl ether (5ml) at 5°C under nitrogen atmosphere and stirred for 30mn at the same temp. After the excess reagent was destroyed by careful addition of water, the reaction mixture was diluted with ethyl acetate, washed with water followed by brine, dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The resulting oil was subjected to medium pressure LC (silica gel, Lobar size A, MeOH-$CHCl_3$ 3:97) to give 72mg of 10 as a colorless oil, $[\alpha]_D^{23}$ -207°(C=1.3, $CHCl_3$); UV λ_{max}^{MeOH} nm(ε) 245(15,000),301(5,400); CD [θ] -87,000(247nm),-12,000(296nm); IR($CHCl_3$) 3420,2950,1600,1480,1460 cm^{-1} ; ^1H NMR(270MHz,$CDCl_3$) δ 7.10 (2H,d,J=8Hz), 6.98(2H,t,J=8Hz), 6.64(2H,t,J=8Hz), 6.47(2H,d,J=8Hz), 4.63(2H,s), 4.08(2H,s,exchangeable), 2.90(2H,dd,J=10.5 and 3.2Hz), 2.72(2H,m), 2.58(2H,dd,J=10.5 and 5.4Hz), 2.20(2H,dd,J=11.2Hz and 11.2Hz), 1.73(2H,dd,J=11.2 and 4.0Hz), 1.17-1.50(4H,m), 1.00(6H,s), 0.93(6H,s), 0.82(6H,broad t,J=7Hz) ; MS m/z 484(M^+),413,130.

8a,16a-Bis(1,1-dimethylpropyl)-1,2,3,4,4a,5a,8,8a,8b,9,10,11,12,12a,13,13a,15a,16,16a,16b-eicosahydropyrazino[1",2":1,5;4",5":1',5']dipyrrolo[2,3-b:2',3'-b']-diindole-7,15(5H,7aH)-dione (perhydroamauromine 11). Amauromine (70mg) was hydrogenated with PtO_2 (160mg) in acetic acid (0.7ml) for 3hr under 4 atm pressure of H_2. The mixture was filtered and the filtrate was concentrated to dryness in vacuo. The resulting oil was purified by PLC (MeOH-$CHCl_3$ 5:95) to give 37mg of the major product 11 and 9mg of the minor product as oils. Major product 11, $[\alpha]_D^{23}$ -174°(C=1.4,$CHCl_3$); UV end absorption; IR($CHCl_3$) 3300,2920,1660,1400 cm^{-1} ; ^1H NMR(270MHz,$CDCl_3$) δ 5.38(2H,s), 4.33(2H,dd,J=10 and 6Hz), 3.40(2H,m), 3.00(2H,dd,J=15 and 6 Hz), 2.30(2H,s,exchangeable), 1.20-1.92(24H,m), 1.00 (12H,s), 0.80(6H,t,J=7Hz); MS m/z 524(M^+), 495,453,427,164; HRMS m/z 524.4104 ($C_{32}H_{52}N_4O_2$ requires 524.4093). Minor product, $[\alpha]_D^{23}$ -155°(c=0.9,$CHCl_3$) ; UV end absorption; IR($CHCl_3$) 3300,2920,1660,1400 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 5.43(1H,s), 5.18(1H,s), 4.14-4.50(2H,m), 3.33-3.53(2H,m), 2.70-3.37(2H,m), 2.37(2H,s,exchangeable), 1.17-2.17(24H,m), 0.67-1.03(18H) ; MS m/z 524(M^+),495,453,427,164 ; HRMS m/z 524.4128($C_{32}H_{52}N_4O_2$ requires 524.4093).

5,13-Diisopentyltetrahydroamauromine (12). To a solution of 2 (130mg) in N,N-dimethylformamide (5ml) was added potassium carbonate (180mg) and 1-bromo-3-methyl-2-butene (0.4ml, available from Tokyo kasei). The mixture was stirred at 50°c for 2hr. The cooled mixture was diluted with ethyl acetate, washed with water followed by brine and dried over $MgSO_4$. Removal of the solvent gave an oil which was purified by PLC ($CHCl_3$). The purified compound was hydrogenated over PtO_2 (30mg) in ethanol (10ml) under 1 atm pressure of H_2. The reaction mixture was filtered and the filtrate was evaporated in vacuo to give an oil which was subjected to PLC (Et_2O-CCl_4 1:9) to afford 105mg of 12 as an oil, $[\alpha]_D^{23}$ -470.8°(C=0.8,$CHCl_3$) ; UV λ_{max}^{EtOH} nm(ε) 254(19,000), 311(5,500) ; CD [θ] see Table 3 ; IR($CHCl_3$) 2960,1655,1600,1430 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 7.07(2H,d,J=8Hz), 7.05(2H,t,J=8Hz), 6.59(2H,t,J=8Hz), 6.26(2H,d,J=8Hz), 5.80(2H,s), 4.06(2H,dd,J=11 and 6Hz), 3.20-3.46(4H,m), 2.56(2H,dd,J=11 and 6Hz), 2.28(2H,dd,J=11 and 11Hz), 1.22-1.70(10H,m), 0.86-0.92(24H), 0.80(6H,t,J=7Hz) ; MS m/z 652(M^+), 581,130 ; HRMS m/z 652.4737($C_{42}H_{60}N_4O_2$ requires 652.4719).

1-Benzyl 2-methyl (2s,3as,8as)-3a,8-bis(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-1,2-dicarboxylate (15) and 1-benzyl 2-methyl (2s,3aR,8aR)-3a,8-bis(3-methyl-2-butenyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-1,2-dicarboxylate (16). To a solution of N^{α}-benzyloxycarbonyl-L-tryptophan methyl ester (15g) in acetone (10ml) was added sodium hydrogen carbonate (18.7g) and 1-bromo-3-methyl-2-butene (26ml). The mixture was stirred at room temp. overnight under argon atmosphere. The reaction mixture was diluted with water, extracted with ethyl acetate and the organic solution was washed with water, brine and then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave an oil which was chromatographed on a silica gel column. Elution with benzene-ethyl acetate (20:1) gave 6.0g of 15 and 4.4g of 16 as oils. Compound 15, $[\alpha]_D^{23}$ -155°(c=1.3, $CHCl_3$) ; UV λ_{max}^{MeOH} nm(ε) 252(10,200), 302(2,700) ; IR($CHCl_3$) 2920,1740,1700,1600,1410 cm^{-1} ; ^1H NMR (100MHz,$CDCl_3$) δ 7.32(5H,s), 6.96-7.20(2H,m), 6.74(1H,t,J=8Hz), 6.48(1H,d,J=8Hz), 5.48(1H,s), 4.92-5.24(4H,m), 4.24(1H,m), 4.20(1H,m), 3.80(1H,m), 3.48(3H,s), 2.08-2.60(4H,m), 1.76(3H,s), 1.66(6H,s), 1.56(3H,s) ; MS m/z 488(M^+),419,307,157,130,91 ; HRMS m/z 488.2676($C_{30}H_{36}N_2O_4$ requires 488.2677). Compound 16, $[\alpha]_D^{23}$ +80°(c=1.5,$CHCl_3$) ; UV λ_{max}^{MeOH} nm(ε) 254(10,700), 305(2,600) ; IR($CHCl_3$) 2920,1720,1700,1600,1410 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 7.30(5H,s), 6.92-7.12(2H,m), 6.60(1H,t,J=8Hz), 6.35(1H,d,J=8Hz), 5.42(1H,s), 4.96-5.32(2H,m), 5.12(2H,s), 4.68(1H,m), 3.88-4.20(2H,m), 3.28(3H,s), 2.20-2.50(4H,m), 1.68(9H,s), 1.54(3H,s) ; MS m/z 488 (M^+),419,307,157,130,91 ; HRMS m/z 488.2658($C_{30}H_{36}N_2O_4$ requires 488.2677).

1-tert-Butyl 2-methyl (2s,3aS,8aS)-3a,8-diisopentyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-1,2-dicarboxylate (17). Compound 15 (250mg) was hydrogenated with Pd black in ethyl acetate (10ml) for 2hr under 4 atm pressure of H_2. The mixture was filtered and the filtrate was concentrated to dryness in vacuo to leave an oil. The oil was dissolved in dry dichloromethane (15ml) and di-tert-butyl dicarbonate (170mg) was added thereto. The mixture was stirred for 1hr at room temp. and then concentrated in vacuo to give a residue which was subjected to medium pressure LC ($CHCl_3$) to give 159mg of 17 as an oil, $[\alpha]_D^{23}$ -106°(c=1.0,$CHCl_3$) ; UV λ_{max}^{MeOH} nm(ε) 254(9,500), 305(2,650) ; IR($CHCl_3$) 2950,1740,1690,1600,1380,1360,1160 cm^{-1}; ^1H NMR(100MHz,$CDCl_3$) δ 6.30-7.08(4H,m), 5.43(1H,s), 4.16(1H,m), 3.70(3H,s), 3.30-3.60(2H,m), 2.17-2.60(2H,m), 1.17-1.80(8H,m), 1.40(9H,s), 0.93(6H,d,J=7Hz), 0.80(6H,d,J=7Hz) ; MS m/z 458(M^+),402,345,130 ; HRMS m/z 458.3154($C_{27}H_{42}N_2O_4$ requires 458.3147).

1-tert-Butyl 2-methyl (2S,3aR,8aR)-3a,8-diisopentyl-1,2,3,3a,8,8a-hexahydropyrrolo [2,3-b]indole-1,2-dicarboxylate (18). Compound 18 (117mg) was prepared from 16 (250mg) by the same method as was 17 from 15. Compound 18, oil ; $[\alpha]_D^{23}$ +101°(C=1.1,$CHCl_3$) ; UV λ_{max}^{MeOH} nm(ε) 256(10,300), 310(2,700) ; IR($CHCl_3$) 2950,1740,1680,1600,1380,1160 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 6.88-7.12(2H,m), 6.56(1H,t,J=8Hz), 6.32(1H,d,J=8Hz), 5.44(1H,s), 4.60(1H,m), 3.32(3H,s), 3.30-3.54(2H,m), 2.18-2.40(2H,m), 1.16-1.80(8H,m), 1.48(9H,s), 0.96(6H,d,J=7Hz), 0.84(6H,d,J=7Hz) ; MS m/z 458(M^+),402,345,130 ; HRMS m/z 458.3161($C_{27}H_{42}N_2O_4$ requires 458.3147).

(5aS,7aS,8aS,13aS,15aS,16aS)-5,8a,13,16a-Tetraisopentyl-5a,8,8a,13,13a,15a,16,16a-octahydropyrazino [1",2":1,5;4",5":1',5']dipyrrolo[2,3-b:2',3'-b']diindole-7,15(5H,7aH)-dione (13). To a solution of 15 (240mg) in methanol (3ml) was added 1N sodium hydroxide (0.6ml) and the mixture was stirred at room temp. overnight under argon atmosphere. After the reaction mixture was washed with n-hexane, the aq layer was acidified with 1N HCl, extracted with ether and dried over $MgSO_4$. Removal of the solvent gave an acid, $[\alpha]_D^{23}$ -106°(c=1.6,MeOH); UV λ_{max}^{MeOH} nm(ε) 253(10,000), 302(2,500) ; IR($CHCl_3$) 3200-2500,1700,1600,1410,1140 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 9.64(1H,broad s,exchangeable), 7.33(5H,s), 7.08(1H,t,J=8Hz), 6.97(1H,d,J=8Hz), 6.68(1H,t,J=8Hz), 6.44(1H,d,J=8Hz), 5.44(1H,s), 4.90-5.27(4H,m), 4.10-4.30(2H,m), 3.80(1H,m), 2.17-2.63(4H,m), 1.76(3H,s), 1.64(6H,s), 1.50(3H,s) ; MS m/z 474(M^+), 405,130,91. To a solution of the acid (225mg) in dioxane (20ml) was added N-hydroxysuccinimide (55mg) and N,N'-dicyclohexylcarbodiimide (98mg). The mixture was stirred at room temp. overnight. The reaction mixture was concentrated to dryness in vacuo and the residue was dissolved in ether and filtered. Evaporation of the solvent gave a succinimide ester (230mg), IR($CHCl_3$) 2930,1820,1780,1740,1710 cm^{-1}. The active ester (230mg) was hydrogenated with Pd black (50mg) in ethyl acetate (4ml) for 2hr under 4 atm pressure of H_2. The reaction mixture was filtered and the filtrate was concentrated in vacuo. Purification of the residue by medium pressure LC ($CHCl_3$) gave 13 (151mg) as an oil, $[\alpha]_D^{23}$ -433.3°(c=1.2,$CHCl_3$) ; UV λ_{max}^{EtOH} nm(ε) 254(20,400), 310(5,300) ; CD [θ]see Table 3 ; IR($CHCl_3$) 2950,1655,1600,1490 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 6.94-7.06(4H,m), 6.64(2H,t,J=8Hz), 6.30(2H,d,J=8Hz), 5.56(2H,s), 4.11(2H,dd,J=11 and 6Hz), 3.25-3.50(4H,m), 2.66(2H,dd,J=11 and 6Hz), 2.10(2H,dd,J=11 and 11 Hz), 1.10-1.80(16H,m), 0.76-0.92(24H) ; MS m/z 652(M^+), 595,581,130 ; HRMS m/z 652.4714($C_{42}H_{60}N_4O_2$ requires 652.4719).

(5aR,7aS,8aR,13aR,15aS,16aR)-5,8a,13,16a-Tetraisopentyl-5a,8,8a,13,13a,15a,16,16a-octahydropyrazino[1",2":1,5;4",5":1',5']dipyrrolo[2,3-b:2',3'-b']diindole-7,15(5H,7aH)-dione (14). Compound 14 (50mg) was prepared from 16 (250mg) according to similar manner to the preparation of 13 from 15. Compound 16 was transformed to an acid, $[\alpha]_D^{23}$ +90.5°(c=1.2,MeOH) ; UV λ_{max}^{MeOH} nm(ε) 256(113,00), 306(2,700) ; IR($CHCl_3$) 3200-2500,1700,1600,1400,1120 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 9.03(1H,broad s,exchangeable), 7.34(5H,s), 7.00(1H,t,J=8Hz), 6.92(1H,d,J=8Hz), 6.60(1H,t,J=8Hz), 6.40(1H,d,J=8Hz), 5.47(1H,s), 5.13(2H,s), 4.90-5.27(2H,m), 4.66(1H,broad t,J=6Hz), 3.90-4.07(2H,m), 2.40(4H,broad d,J=6Hz), 1.63(9H,s), 1.56(3H,s) ; MS m/z 474(M^+),405,130,91. The acid was converted to 14, +202.4°(C=0.88,$CHCl_3$) ; UV λ_{max}^{EtOH} nm(ε) 252(20,600), 303(5,100) ; CD see Table 3 ; IR($CHCl_3$) 2950,1675,1605,1480,1390 cm^{-1} ; ^1H NMR(100MHz,$CDCl_3$) δ 7.10(2H,t,J=8Hz), 6.97(2H,d,J=8Hz), 6.67(2H,t,J=8Hz), 6.38(2H,d,J=8Hz), 5.54(2H,s), 4.38(2H,t,J=9Hz), 3.36-3.48(4H,m), 2.37(4H,d,J=9Hz), 1.08-1.92(16H,m), 0.88(12H,d,J=7Hz), 0.84(6H,d,J=7Hz), 0.82(6H,d,J=7Hz) ; MS m/z 652(M^+),595,581,130 ; HRMS m/z

652.4746($C_{42}H_{60}N_4O_2$ requires 652.4719).

(5aR,7aR,8aR,13aR,15aR,16aR)-5,8a,13,16a-Tetraisopentyl-5a,8,8a,13,13a,15a,16,16a-octahydropyrazino[1",2":1,5;4",5":1',5']dipyrrolo[2,3-b:2',3',-b']diindole-7,15(5H,7aH)-dione (21). Sodium carbonate (30mg) was added to a solution of 14 (30mg) in methanol (3ml) and the mixture was refluxed for 12hr. The cooled reaction mixture was diluted with ethyl acetate, and the solution was washed with water, brine, dried over $MgSO_4$ and then evaporated to dryness. Purification of the residue by PLC (Et_2O-CCl_4 1:9) afforded 25mg of 21 as an oil, $[\alpha]_D^{23}$ +444.0° (C=1.2,$CHCl_3$). UV, IR, ^1H NMR, MS and TLC of 21 are identical with those of 13.

Acknowledgement-We are indebted to Prof. T. Shingu, Kobegakuin University, for NOE measurement of amauromine.

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