Tetrahedron Letters,Vol.25,No.41,pp 4673-4676,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

STRUCTURE OF AMAUROMINE, A NEW ALKALOID WITH VASODILATING ACTIVITY PRODUCED BY AMAUROASCUS SP.

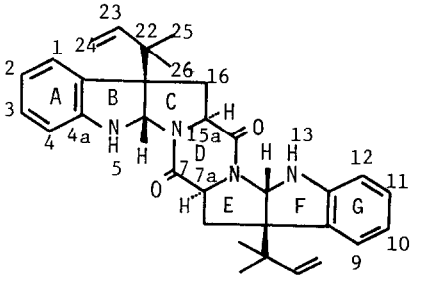
Shigehiro Takase, Yoshio Kawai, Itsuo Uchida,
Hirokazu Tanaka and Hatsuo Aoki

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co.,Ltd.
1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532, Japan

Summary : Structure of amauromine (1a), a novel alkaloid with potent vasodilating activity has been established by chemical and spectroscopic evidences.

Amauromine (1a) is a novel alkaloid with vasodilating activity, recently isolated from Amauroascus sp. No.6237 [1]. Herein we report the structure elucidation of this unique alkaloid possessing two reversed prenyl groups and a C_2 symmetry in the molecule.

Amauromine (1a), $C_{32}H_{36}N_4O_2$ (EI-High MS, M^+ obsd. 508.283, calcd. 508.284 and elementary analysis [2]), mp. 156-158°, $[\alpha]_D^{23}$ -583° (c=1.0, $CHCl_3$), forms colorless prism from EtOH. UV and IR spectral properties were λ_{max}^{EtOH} 245 (11,000), 300 (4,200) nm (ε) ; $\nu_{max}^{CHCl_3}$ 3420, 2970, 1660, 1600, 1420, 1380, 1365, 920 cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra measured in $CDCl_3$ indicated that the molecule is a dimer, because signals corresponding to half protons (18) and carbons (16) of amauromine molecule were observed in those spectra respectively. This property was also observed in the NMR spectra of tetrahydroamauromine (2) ($[\alpha]_D^{23}$ -553°) obtained by hydrogenation of amauromine over PtO_2. ^{1}H-NMR and ^{13}C-NMR spectral data of 1a and 2 are shown on Table 1. The presence of 1,1-dimethyl-2-propenyl group in 1a was proved by the comparison of signals assigned to positions 23, 24, 25, 26 in ^{1}H and ^{13}C-NMR of 1a and 2, together with IR absorption bands (1380, 920 cm^{-1}) in 1a and with mass fragmentation peaks [1a : m/z 439 (M^+-69), 2 : m/z 441 (M^+-71)]. Six signals at δ 149.9 (s), 128.8 (s), 128.7 (d), 124.7 (d), 118.6 (d) and 109.2 (d) in the ^{13}C-NMR spectrum of amauromine (1a) indicated the presence of indoline moiety [3]. Singlet signal at δ 5.74 in the ^{1}H-NMR spectrum of 1a, was assigned to the methine proton at position 5a by comparison with ^{1}H-NMR spectra of roquefortine [4] and aszonalenin [5].

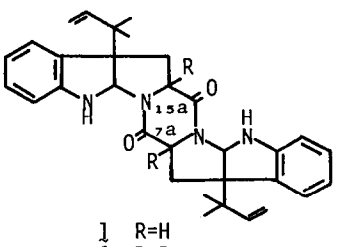


Amauromine 1a

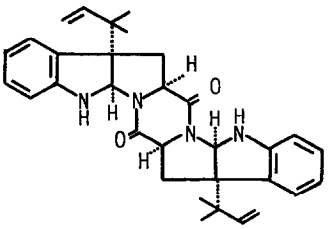
Table 1 NMR Data of 1a and 2.

Position	^{13}C NMR *		^{1}H NMR **	
	1a	2	1a	2
1	124.7(d)	124.9(d)	6.46(1H,d,J=3)	6.46(1H,d,J=8)
2	118.6(d)	118.6(d)	6.66(1H,t,J=3)	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			4.9 (1H,s)	4.9 (1H,s)
5a	77.1(d)	77.1(d)	5.42(1H,s)	5.46(1H,s)
7	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 $CDCl_3$		** 100 MHZ in $CDCl_3$	
	δ (multiplicity)		δ (multiplicity, coupling const. in HZ)	

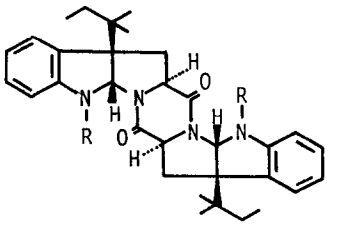
Strong IR absorption band at 1660 cm^{-1} and a signal at δ 166.1 (s) in the ^{13}C-NMR spectrum showed the presence of amide function in amauromine molecule. Hydrolysis of 1a, with 6N HCl at 110°C for 4 hours yielded L-tryptophan [6] as a degradation product. From these results, the plane structure of amauromine was deduced to be 1, which satisfies the following evidences. On treatment with Na_2CO_3 in refluxing CH_3OD, amauromine was converted to a dideutero compound (3) (M^+ 510), which lacked ^{1}H-NMR signal at δ 3.78 present in the spectrum of amauromine. The CH_2 doublet at δ 2.43 in amauromine simplified to a singlet in the ^{1}H-NMR spectrum of dideutero amauromine (3). Treatment of 3 on the same reaction condition using methanol instead of CH_3OD regenerated amauromine. Thus, signals at δ 3.78 (t, J=8 Hz) and δ 2.43 (d, J=8 Hz) in the ^{1}H-NMR spectrum of amauromine were assigned the protons at positions 15a and 16 respectively.



1 R=H
3 R=D

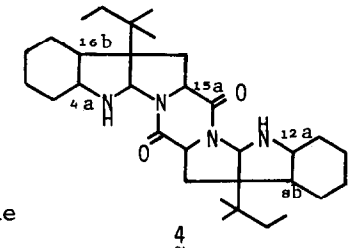


1b



2 R=H
5 R=

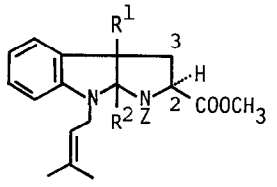
Stereochemistry of amauromine including absolute configuration was determined as follows. The fact that L-tryptophan was isolated by acid hydrolysis of amauromine proved both absolute configurations at 7a and 15a to be S. Irradiation at 25 (or 26)-Me produced 9 % of NOE at 5a-H, which shows cis relation between B and C rings (E and F rings). Since amauromine possesses nuclear magnetically symmetrical feature as a dimer and is optically active compound ($[\alpha]_D^{23}$ -583°), a C_2 symmetry axis must exist in its molecule. As a result of the foregoing discussions, the absolute structure of amauromine is restricted to be 1a or 1b. It was verified by the following experiments that 1a is the real structure of amauromine.

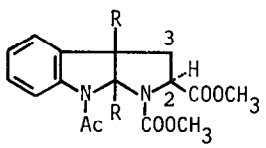


4

In the ^{1}H-NMR spectrum of amauromine, 15a-H resonates at δ 3.78 which is considered to be in rather high field because of the anisotropic effect due to the aromatic ring A (G). In fact, the corresponding proton (15a-H) of the perhydrocompound (4) [7] obtained by catalytic hydrogenation of amauromine (PtO_2, AcOH, 4 atm) was observed at δ 4.33. This anisotropic effect is explainable only on the dreiding model of the structure (1a). Z-L-tryptophan methyl ester was treated with prenyl bromide ($NaHCO_3$, acetone, r.t.) to give the compounds (6) (29 %) and (7) (22 %). Stereochemistry of 6 and 7 was determined by comparison of the ^{1}H-NMR spectra with those of the compounds (8) and (9) synthesized by Hino et al [8] (Table 2). Hydrolysis (1N NaOH) of 6 gave a carboxylic acid, which was converted to an active ester by conventional manner (DCC, HOSu), followed by catalytic reduction with Pd-black at 4 atm to yield a dimer (10) as a product. In the same manner 11 was synthesized from 7.

On treatment with prenyl bromide and K_2CO_3 in DMF at 50°C, followed by catalytic reduction (PtO_2, EtOH), amauromine was transformed into a N-alkylated derivative (5) (67 %). Comparison of $[\alpha]_D$, CD and ^{1}H-NMR spectra of 5, 10 and 11 (Table 3) lead us to conclude that compound (5) possesses the same absolute configurations with those of compound (10). When both 5 and 10 were treated in MeOH with Na_2CO_3, no reaction occured as in the case of amauromine, but by the same treatment 11 was changed into a new compound 12 ($[\alpha]_D^{23}$ = +444°), which turned out to be the antipode of 10 because IR and NMR spectra of the two compounds were identical. Consequently absolute structure of amauromine was proved to be shown as 1a.





6 R^1 =  , R^2 = —H

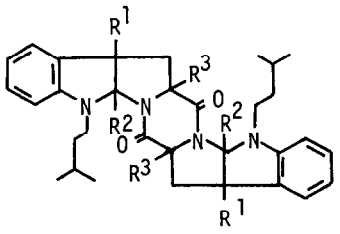
7 R^1 =  , R^2 = ····H

8 R = —H

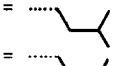
9 R = ····H

Table 2

	^{1}H - NMR (δ)	
	2-H	2-$COOCH_3$
6	4.2	3.5
7	4.68	3.28
8	4.0	3.63 or 3.72
9	4.59	3.11



10 R^1=  , R^2= H, R^3= ····H

11 R^1=  , R^2=R^3= ····H

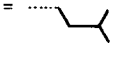
12 R^1=  , R^2= ····H, R^3= H

Table 3

	$[\alpha]_D$	CD [θ] (nm)	^{1}H - NMR (δ, $CDCl_3$) 15a - H	16 - H
5	-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)
10	-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)
11	+202.4°	- 32,600(225) + 35,900(255) - 2,600(313)	4.38(1H,t,J=9)	2.37(2H,d,J=9)

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

References and Notes

1) S. Takase, M. Iwami, T. Ando, M. Okamoto, K. Yoshida, H. Horiai, M. Kohsaka, H. Aoki and H. Imanaka, J. Antibiot. submitted for publication.

2) 1a : Anal. Found : C, 74.00 ; H, 7.18 ; N, 10.64,
Calcd. for $C_{32}H_{36}N_4O_2 \cdot 1/2\ H_2O$: C, 74.25 ; H, 7.20 ; N, 10.82 %

3) H. Fritz and T. Winkler, Helv. Chim. Acta, 59, 903 (1976)

4) P. M. Scott, Experientia, 140 (1976)

5) Y. Kimura, T. Hamasaki, H. Nakajima and A. Isogai, Tetrahedron Lett., 23, 225 (1982)

6) Absolute configuration of the isolated tryptophan was determined by measurement of CD spectrum.

7) The major product (50 %) isolated from hydrogenated mixture. Stereochemistry at positions 4a, 16b, 8b, 12a of 4 remains undefined.

8) M. Taniguchi and T. Hino, Tetrahedron, 37, 1487 (1981)

(Received in Japan 21 June 1984)