

AMAUROMINE[†], A NEW VASODILATOR
TAXONOMY, ISOLATION AND CHARACTERIZATION

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Amauromine is a new alkaloid with vasodilating activity obtained from the culture broth of *Amauroascus* sp. No. 6237. Its molecular formula was determined to be C₃₂H₃₈N₄O₂ on the basis of elementary analysis and high resolution mass spectroscopic measurement. It has low toxicity in mice.

In the course of our screening program for new vasodilators among microbial metabolites using superfusion technique,¹⁾ a strain of *Amauroascus* sp. No. 6237 was found to produce the highly potent new vasodilator, designated amauromine. In this paper, the taxonomy of the producing organism, fermentation, isolation procedures, chemical and biological properties of amauromine are described.

Taxonomic Studies on Strain No. 6237

Strain No. 6237 was isolated from decayed wood obtained from the foot of Mt. Rokko, Hyogo Prefecture, Japan.

Macroscopic and Microscopic Observations

At 30°C growth on YpSs agar (Bacto Emerson YpSs agar, Difco) was ample but slow, attaining 2.0 cm in diameter in 7 days and about 3.0 cm in diameter in 10 days (Table 1). Pale lemon-yellow aerial hyphae develop within a few days. A 14~30 day old culture has a covering of thin, lemon-yellow aerial hyphae with ascocarps arranged in a zonate manner. Colony margins are submerged and colorless, while the reverse is unfurrowed and pale yellow (Table 2).

Table 1. Diameter of strain No. 6237 colony.

Medium	Diameter of colony (mm)		
	4 days	7 days	10 days
Potato - dextrose agar	12.5	22.5	28.5
Czapek - Dox agar	9	13	18
Malt agar	5.5	11	16
YpSs agar	12	22	31

Table 2. Cultural characteristics of strain No. 6237.

Medium	Growth	Aerial mycelium	Reverse side of colony	Soluble pigment
Potato - dextrose agar	Abundant	Pale yellow to brown, cottony	Pale yellow to brown	None
Czapek - Dox agar	Poor	White	Colorless	None
Malt agar	Good	White	Orange	None
YpSs agar	Abundant	White to gray	Pale yellowish orange	None
Oatmeal - yeast extract agar	Abundant	Pale brown to brown	Yellow orange	None
Bennett agar	Abundant	White to yellowish orange	Orange	None

[†] Amauromine was originally designated as WF 6237 (FR-900220).

Table 3. Morphological characteristics of strain No. 6237.

	Size	Morphology	Color
Peridium	0.5~2 mm	Spheroid	Yellow
Ascus	12~14×16~20 μ m	Clavate	Colorless
Ascospore	4~6 μ m	Spheroid, spiny	Yellow orange

Table 4. Media used for production of amauromine.

Seed medium (%)		Production medium (%)	
Corn starch	2.0	Glucose	2.0
Soy bean meal	1.0	Corn steep liquor	0.25
Dry yeast	1.0	Dry yeast	0.25
Corn steep liquor	1.0	Cotton seed meal	0.5
Glucose	0.5	Wheat germ	0.5
pH adjusted to 7.0		pH adjusted to 7.0	

Ascocarps are produced abundantly on oatmeal agar, Bennett agar and potato-dextrose agar. Prominent dense knots of aerial hyphae develop into ascocarps. Mature ascocarps are completely enclosed with several layers of thin-walled, undifferentiated hyphae, fulfilling the requirements for peridium. As shown in Table 3, the asci are borne in a nearly spherical, lanose, yellow peridium, 0.5~2 mm in diameter. They develop in clusters, are broadly clavate, rarely spherical, 12~14×16~20 μ m in size, and usually 8-spored. The ascospores are spherical, thick-walled, orangish yellow, reticulate-spiny and 4~6 μ m in diameter. Arthroconidia are formed in old cultures on several media.

Physiological Properties

This strain can grow at temperatures ranging from 14 to 38°C with optimum growth at 22~33°C and it can grow at pH 5~10 but the optimum is at pH 6~8.

From comparing the above-mentioned characteristics with the published descriptions^{2,3,4)} of various fungi, strain No. 6237 is considered to belong to the genus *Amauroascus* Schroeter. This strain has been deposited in the American Type Culture Collection as ATCC 20595 and in the Fermentation Research Institute, Agency of Industrial Science and Technology (Japan) under the number of FERM-P 5364.

Fermentation

Seed flasks containing 100 ml of the seed medium were inoculated with spores from the slant culture of strain No. 6237 and incubated at 30°C for 3 days. A 30-liter jar fermentor containing 15 liters of production medium was inoculated with 400 ml of the mature seed broth. The compositions of the seed and the production media are shown in Table 4. The fermentation was carried out at 30°C with an air flow of 20 liters per minute and agitation of 200 rpm. The amauromine production reached a maximum after 72 hours of cultivation and the yields were 20~30 μ g/ml.

Isolation Procedure

The flow diagram of the isolation procedure described below is shown in Fig. 1. Since vasodilating activity was found in both mycelium and broth filtrate, equal volume of acetone was added to the culture broth (75 liters), allowed to stand at room temperature overnight and then filtered. The filtrate was concentrated to 3 liters under reduced pressure, extracted with ethyl acetate (3 liters×2) and the extract was concentrated to dryness under reduced pressure and applied to a column of silica

Fig. 1. Isolation procedure of amauiromine.

Whole broth (75 liters)
 + Me₂CO (75 liters), allowed to stand overnight at room temp, filtration
 Filtrate
 concentrated to aq solution (3 liters)
 Extraction with EtOAc
 concentrated to dryness
 Silica gel column
 eluted with C₆H₆ - EtOAc (10: 1)
 Silica gel column
 eluted with 2% CH₃OH - CHCl₃
 Recrystallized from EtOH
 Amauiromine (1.35 g) as colorless prisms

Table 5. Physico-chemical properties of amauiromine.

		Amauiromine
Appearance		Colorless prisms
MP		156~158°C
[α] _D ²³ (c 1.0, CHCl ₃)		-583°
UV (EtOH) nm (ε)		245 (11,000), 300 (4,200)
IR (CHCl ₃) cm ⁻¹		3420, 2970, 1660, 1600, 1420, 1380, 1365, 920
Molecular formula		C ₃₂ H ₃₆ N ₄ O ₂
<i>Anal</i>		
Found		C 74.00, H 7.18, N 10.64
Calcd for C ₃₂ H ₃₆ N ₄ O ₂ · ½H ₂ O		C 74.25, H 7.20, N 10.82
MS	Obsd	508.2825
	Calcd	508.2840

gel (1.2 liters). After washing with *n*-hexane (3 liters), benzene (3 liters), the active substance was eluted from the column with a mixture of benzene - ethyl acetate (10: 1). The active fractions (800 ml) were dried *in vacuo* to give an oil (2.08 g) which was subjected to silica gel column chromatography (silica gel 1.0 liter, eluant: methanol - chloroform (2: 98)) to afford 1.5 g of crude powder. Recrystallization from ethanol gave pure amauiromine (1.35 g) as colorless prisms.

Physico-chemical Properties

The physico-chemical properties of amauiromine are summarized in Table 5.

Solubility: Amauiromine is soluble in chloroform, acetone, pyridine, sparingly soluble in methanol, ethanol, benzene, ether and insoluble in *n*-hexane and water.

Color Reactions: Positive in sulfuric acid, iodine vapor, ferric chloride-potassium ferricyanide and potassium permanganate tests and negative in ferric chloride and ninhydrin tests. The chemical structure of amauiromine will be described in succeeding papers.⁵⁾

Biological Properties

Screening and isolation of microbial products from fermentation broth was conducted by using the superfusion method reported previously.⁶⁾

Briefly, a male Sprague-Dawley rat 8~10 weeks of age was stunned and exsanguinated. The thoracic aorta was removed quickly and spiral strips of 2 mm width and 50 mm length were prepared. The tension of aortic strips was measured isometrically by means of force displacement transducer coupled to a polygraph.

The aortic strips were superfused with Tyrode solution containing KCl (30 mM) or norepinephrine (1.5×10^{-7} M) which produced approximately 500 mg of contracting tension.

The vasodilating property of amaumine was characterized by comparing its activity on rat aortic strips contracted by KCl (50 mM) and norepinephrine (5×10^{-7} M).

The intensity of relaxation activity was standardized with papaverine (1×10^{-4} M) which produced 100% of dilation.

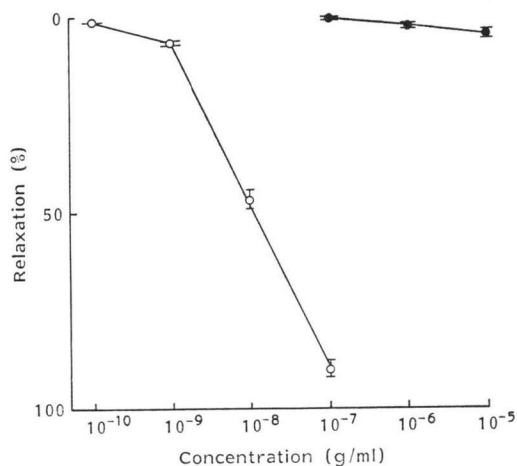
As shown in Fig. 2, the concentration which produced 50% relaxation of rat aortic strip contracted with KCl (50 mM) was 1.15×10^{-8} g/ml, but only slight relaxation was detected by the preparation contracted with norepinephrine at the extremely higher concentration of amaumine (1×10^{-5} g/ml).

According to the criteria proposed by RAHWAN,⁷⁾ it is relevant to classify amaumine as a novel calcium entry blocker.

Further details of pharmacological characterization concerning amaumine will be published in succeeding papers.⁸⁾

The LD₅₀ value of amaumine when given intraperitoneally to ddY mice was greater than 200 mg/kg.

Fig. 2. Effect of amaumine on KCl and norepinephrine induced contraction of isolated rat aorta. ○ KCl, ● norepinephrine.



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