Isolation and Structures of Two New Indoloditerpenes Related to Aflavinine from a Microsclerotium-Producing Strain of *Aspergillus flavus*[1)]

Koohei NOZAWA,[a] Setsuko SEKITA,[b] Masatoshi HARADA,[b] Shun-ichi UDAGAWA[b] and Ken-ichi KAWAI*,[a]

Faculty of Pharmaceutical Sciences, Hoshi University,[a] Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan and National Institute of Hygienic Sciences,[b] Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan. *Received August 31, 1988*

Along with paspalinine (3), aflatrem (4), aflavinine (5), and dihydroxyaflavinine (6), two new indoloditerpenes, monohydroxyaflavinine (7) and monohydroxyisoaflavinine (8), were isolated from the methylene chloride extract of a microsclerotium-producting strain of *Aspergillus flavus*, which has activity to produce aflatoxins. The structures of the above compounds were determined on the basis of spectroscopic investigations and X-ray crystal analyses of monohydroxyaflavinine (7) acetone solvate and monohydroxyisoaflavinine (8).

Keywords *Aspergillus flavus*; sclerotium; indoloditerpene; monohydroxyaflavinine; monohydroxyisoaflavinine; aflavinine; dihydroxyaflavinine; aflatrem; paspalinine; X-ray analysis

Recently we reported the isolation and the structural elucidation of emindoles DA (**1**)[2,3)] and SA (**2**),[2,4)] new-type indoloditerpenes, from *Emericella desertorum* SAMSON *et* MOUCHACCA and *Emericella striata* (RAI, TEWARI *et* MUKERJI) MALLOCH *et* CAIN, respectively. From *Aspergillus flavus* LINK: FR., which is a well known aflatoxin-producing fungus,[5,6)] two tremorgenic mycotoxins, paspalinine (**3**)[7)] (originally isolated from sclerotia of *Claviceps paspali* STEVENS *et* HALL[8)]) and aflatrem (**4**),[9,10)] were isolated recently. Aflavinine (**5**)[11)] and dihydroxyaflavinine (**6**)[7)] were also isolated from *A. flavus*. In a separate analysis of the culture medium and sclerotia of several sclerotium-producing strains of *A. flavus*, Wicklow and Cole[12)] reported that tremorgenic indoloditerpenes such as **4** and **6** were detectable only in the sclerotia of *A. flavus*.

In the course of a preliminary study on the distribution of aflatoxigenic fungi in northern Thailand, Tsuruta *et al.*[13)] found atypical sclerotigenic strains of *A. flavus* from soil in maize fields. Subsequently, Saito *et al.*[14)] reported the results of a more extensive survey on their geographic distribution in Thailand and a study on the aflatoxin productivity of 11 strains of the atypical *A. flavus* in comparison with that of typical strains. Our recent survey of aflatoxigenic fungi in commercial samples of Indonesian traditional medicines (native name, "Jamu"; made from herbs and spices) also resulted in the isolation of atypical *A. flavus*. All of these isolates were identified as atypical forms of *A. flavus* falling into the fifth group of Hesseltine *et al.*[15)] This fifth group was initially represented by three strains: one isolated from walnuts in U.S.A. and two from Nigerian peanuts. These strains differ from the typical *A. flavus* and *A. parasiticus* SPEARE by having profuse microsclerotia measuring about 300 μm in diameter. Interestingly, all the microsclerotium-producing strains of *A. flavus* are aflatoxigenic at very high levels and most of them accumulated large amounts of aflatoxin G_1 as well as aflatoxin B_1.[16)]

When six representatives of the microsclerotium-producing strains of *A. flavus* was grown on Czapek Yeast Autolysate agar (CYA)[17)] at 37 °C, they showed greatly increased sclerotium production. This cultural characteristic prompted us to search for tremorgenic indoloditerpenes in the microsclerotia of the atypical *A. flavus*. The above compounds (**3**—**6**) were detected on thin layer chromatography (TLC) of the methylene chloride extract of the sclerotia of all 6 strains. In the course of screening the above compounds, two new indoloditerpenes related to **5** and **6**, designated monohydroxyaflavinine (**7**) and monohydroxyisoaflavinine (**8**), were isolated from one of the above strains, strain IAF34, isolated from the seeds of *Pangium edule* REINW. collected in Indonesia.

Monohydroxyaflavinine (**7**), mp 161—162 °C, gave the molecular ion peak at *m/z* 421 in electron impact ionization mass spectrometry (EI-MS), and elemental analysis and high-resolution mass spectrometry (HR-MS) confirmed the molecular formula of **7** as $C_{28}H_{39}NO_2$. Violet coloration by van Urk's reagent[18)] suggested the presence of an indole moiety in **7**. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **7** is closely similar to that of aflavinine (**5**), except for the appearance of the signal at δ 4.04 (1H, dd), which was assigned to the proton attached to the carbon bearing the secondary hydroxy group, along with the signal at δ 4.49 (1H, br s), which corresponded to the signal at δ 4.48 in **5**. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **7** is also similar to that of **5**, except for the appearance of the signal due to carbon bearing the hydroxy group (δ 69.43) instead of one of the

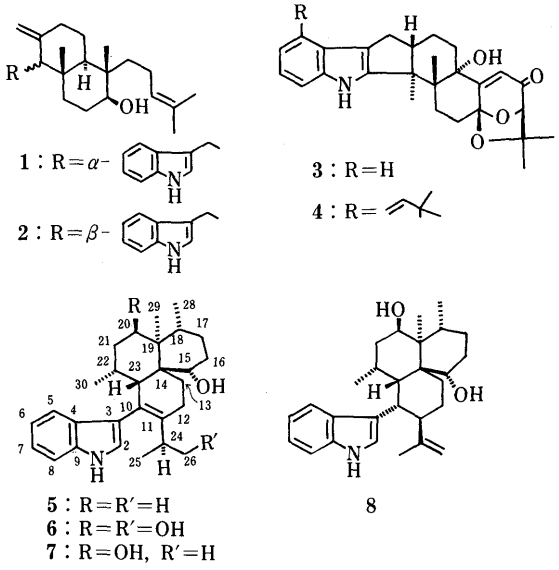

Chart 1

methylene carbons in **5**. The ^{1}H-NMR signals at δ 4.44 (1H, br s) and 3.99 (1H, br d) in dihydroxyaflavinine (**6**) were assigned to two protons attached to the carbons bearing the secondary hydroxy groups. From the above results, considering the molecular formula, it was suggested that monohydroxyaflavinine (**7**) was the monohydroxylated derivative of **5** at C-20.

In order to confirm the structure of **7**, an X-ray structure analysis of **7** acetone solvate was undertaken. Crystals were grown as colorless prisms from acetone solution. The molecular structure of **7** acetone solvate is illustrated in Fig. 1. Therefore the relative structure of monohydroxyaflavinine was confirmed to be as depicted by **7**. The final atomic parameters for non-hydrogen atoms of **7** acetone solvate are shown in Table I. Bond lengths and angles for non-hydrogen atoms are shown in Tables II and III. These values are not significantly different from the expected ones. Based on the O(A)–O(31), O(31)–O(27), and O(27)–N(1) distances (2.803, 2.892, and 2.958 Å, respectively), O(A)---H–O(31)---H–O(27)---H–N(1) seem to be intermolecular hydrogen bonds. The molecules are packed together mainly through hydrogen bonding between two molecules of **7** and acetone in the crystals.

The molecular formula of monohydroxyisoaflavinine (**8**), which has a positive coloration (light green) with van Urk's reagent,[18] was confirmed as $C_{28}H_{39}NO_2$ by HR-MS. Therefore **8** is an isomer of monohydroxyaflavinine (**7**). The ^{1}H-NMR spectra of **7** and **8** are similar to each other, except for the appearance in **8** of the signals at δ 4.67 (1H, br s) and 4.80 (1H, br s), which were assigned to exomethylene protons of the double bond, and the appearance in **8** of the vinylic methyl group at δ 1.55 instead of a methine proton on carbon bearing two secondary methyl groups in **7**. In the ^{13}C-NMR spectrum of **8**, signals of one triplet sp^2 carbon and one doublet sp^3 carbon appeared and those of one singlet sp^2 carbon and one quartet sp^3 carbon were lost as compared with **7**. From the above results, it was suggested that monohydroxyisoaflavinine was a double bond isomer of **7** as shown in the structure **8**. In order to

TABLE I. Final Atomic Parameters for Non-hydrogen Atoms and Equivalent Thermal Parameters, with Estimated Standard Deviations in Parentheses, of Monohydroxyaflavinine (**7**)

Atom	x	y	z	B_{eq} (Å^2)
O(27)	0.4474 (4)	0.2039 (3)	0.5521 (5)	3.7
O(31)	0.1044 (4)	0.3513 (3)	0.5961 (6)	4.7
O(A)	0.9982 (8)	0.3780 (6)	0.8157 (11)	13.3
N(1)	0.5891 (5)	0.5600 (3)	0.8441 (7)	3.7
C(2)	0.5182 (6)	0.5098 (4)	0.8640 (8)	3.4
C(3)	0.5394 (6)	0.4480 (4)	0.8018 (8)	3.1
C(4)	0.6299 (6)	0.4590 (4)	0.7406 (8)	3.0
C(5)	0.6874 (6)	0.4160 (4)	0.6631 (8)	3.9
C(6)	0.7722 (8)	0.4436 (5)	0.6184 (10)	5.4
C(7)	0.8009 (8)	0.5128 (6)	0.6493 (11)	6.1
C(8)	0.7445 (7)	0.5571 (5)	0.7248 (10)	4.9
C(9)	0.6583 (6)	0.5305 (4)	0.7677 (8)	3.3
C(10)	0.4845 (6)	0.3806 (4)	0.7933 (8)	3.0
C(11)	0.5048 (6)	0.3230 (4)	0.8619 (8)	3.4
C(12)	0.4526 (6)	0.2534 (4)	0.8445 (8)	3.9
C(13)	0.3593 (6)	0.2581 (4)	0.7685 (8)	3.2
C(14)	0.3696 (5)	0.3086 (4)	0.6502 (7)	2.7
C(15)	0.4549 (6)	0.2806 (4)	0.5691 (7)	2.9
C(16)	0.4666 (6)	0.3154 (5)	0.4400 (8)	3.8
C(17)	0.3724 (6)	0.3149 (5)	0.3677 (8)	4.4
C(18)	0.2938 (6)	0.3523 (5)	0.4423 (8)	3.8
C(19)	0.2731 (6)	0.3125 (4)	0.5690 (8)	3.2
C(20)	0.1990 (5)	0.3532 (4)	0.6535 (8)	3.4
C(21)	0.2281 (6)	0.4300 (4)	0.6781 (9)	3.9
C(22)	0.3188 (6)	0.4309 (4)	0.7612 (9)	3.7
C(23)	0.3995 (6)	0.3843 (4)	0.7008 (8)	2.9
C(24)	0.5852 (7)	0.3229 (5)	0.9581 (8)	4.2
C(25)	0.5438 (9)	0.3219 (7)	1.0936 (10)	7.2
C(26)	0.6564 (8)	0.2610 (6)	0.9414 (12)	7.2
C(28)	0.2049 (7)	0.3618 (6)	0.3564 (9)	5.4
C(29)	0.2312 (6)	0.2372 (4)	0.5449 (9)	4.0
C(30)	0.2951 (7)	0.4160 (5)	0.9007 (9)	5.1
C(A1)	0.9603 (8)	0.4175 (7)	0.8875 (11)	7.5
C(A2)	0.8560 (10)	0.3926 (8)	0.9250 (14)	9.6
C(A3)	1.0022 (15)	0.4612 (11)	0.9686 (25)	21.4

TABLE II. Bond Lengths (Å) for Monohydroxyaflavinine (**7**) with Estimated Standard Deviations in Parentheses

O(27)–C(15)	1.455 (10)	O(31)–C(20)	1.456 (11)
O(A)–C(A1)	1.186 (18)	N(1)–C(2)	1.384 (11)
N(1)–C(9)	1.378 (11)	C(2)–C(3)	1.365 (12)
C(3)–C(4)	1.438 (11)	C(3)–C(10)	1.482 (12)
C(4)–C(5)	1.404 (12)	C(4)–C(9)	1.427 (12)
C(5)–C(6)	1.378 (14)	C(6)–C(7)	1.398 (15)
C(7)–C(8)	1.397 (15)	C(8)–C(9)	1.382 (13)
C(10)–C(11)	1.333 (12)	C(10)–C(23)	1.543 (12)
C(11)–C(12)	1.508 (12)	C(11)–C(24)	1.517 (13)
C(12)–C(13)	1.536 (12)	C(13)–C(14)	1.578 (12)
C(14)–C(15)	1.560 (11)	C(14)–C(19)	1.602 (11)
C(14)–C(23)	1.573 (11)	C(15)–C(16)	1.523 (12)
C(16)–C(17)	1.523 (13)	C(17)–C(18)	1.526 (14)
C(18)–C(19)	1.562 (13)	C(18)–C(28)	1.551 (14)
C(19)–C(20)	1.567 (12)	C(19)–C(29)	1.550 (12)
C(20)–C(21)	1.520 (13)	C(21)–C(22)	1.544 (13)
C(22)–C(23)	1.563 (13)	C(22)–C(30)	1.539 (14)
C(24)–C(25)	1.546 (16)	C(24)–C(26)	1.541 (16)
C(A1)–C(A2)	1.583 (20)	C(A1)–C(A3)	1.323 (29)

TABLE III. Bond Angles (°) for Monohydroxyaflavinine (**7**) with Estimated Standard Deviations in Parentheses

C(2)–N(1)–C(9)	108.6 (7)	N(1)–C(2)–C(3)	110.4 (8)
C(2)–C(3)–C(4)	106.7 (7)	C(2)–C(3)–C(10)	129.9 (8)
C(4)–C(3)–C(10)	123.5 (7)	C(3)–C(4)–C(5)	133.3 (8)
C(3)–C(4)–C(9)	106.9 (7)	C(5)–C(4)–C(9)	119.8 (8)
C(4)–C(5)–C(6)	118.6 (8)	C(5)–C(6)–C(7)	121.0 (10)
C(6)–C(7)–C(8)	121.6 (10)	C(7)–C(8)–C(9)	117.8 (9)
N(1)–C(9)–C(4)	107.4 (7)	N(1)–C(9)–C(8)	131.3 (8)
C(4)–C(9)–C(8)	121.1 (8)	C(3)–C(10)–C(11)	123.3 (8)
C(3)–C(10)–C(23)	113.6 (7)	C(11)–C(10)–C(23)	123.1 (8)
C(10)–C(11)–C(12)	122.2 (8)	C(10)–C(11)–C(24)	121.6 (8)
C(12)–C(11)–C(24)	116.2 (7)	C(11)–C(12)–C(13)	115.3 (7)
C(12)–C(13)–C(14)	111.9 (7)	C(13)–C(14)–C(15)	107.7 (6)
C(13)–C(14)–C(19)	112.0 (6)	C(13)–C(14)–C(23)	107.2 (6)
C(15)–C(14)–C(19)	111.4 (6)	C(15)–C(14)–C(23)	106.7 (6)
C(19)–C(14)–C(23)	111.4 (6)	O(27)–C(15)–C(14)	110.3 (6)
O(27)–C(15)–C(16)	108.7 (6)	C(14)–C(15)–C(16)	115.6 (7)
C(15)–C(16)–C(17)	110.8 (7)	C(16)–C(17)–C(18)	111.2 (8)
C(17)–C(18)–C(19)	111.0 (8)	C(17)–C(18)–C(28)	109.1 (8)
C(19)–C(18)–C(28)	114.2 (8)	C(14)–C(19)–C(18)	109.0 (7)
C(14)–C(19)–C(20)	105.9 (7)	C(14)–C(19)–C(29)	111.5 (7)
C(18)–C(19)–C(20)	112.3 (7)	C(18)–C(19)–C(29)	111.4 (7)
C(20)–C(19)–C(29)	106.7 (7)	O(31)–C(20)–C(19)	110.6 (7)
O(31)–C(20)–C(21)	109.8 (7)	C(19)–C(20)–C(21)	112.5 (7)
C(20)–C(21)–C(22)	109.1 (8)	C(21)–C(22)–C(23)	110.8 (7)
C(21)–C(22)–C(30)	111.5 (8)	C(23)–C(22)–C(30)	116.6 (8)
C(10)–C(23)–C(14)	112.4 (7)	C(10)–C(23)–C(22)	108.8 (7)
C(14)–C(23)–C(22)	116.9 (7)	C(11)–C(24)–C(25)	110.2 (8)
C(11)–C(24)–C(26)	113.8 (8)	C(25)–C(24)–C(26)	109.9 (9)
O(A)–C(A1)–C(A2)	112.9 (12)	O(A)–C(A1)–C(A3)	127.1 (17)
C(A2)–C(A1)–C(A3)	115.4 (15)		

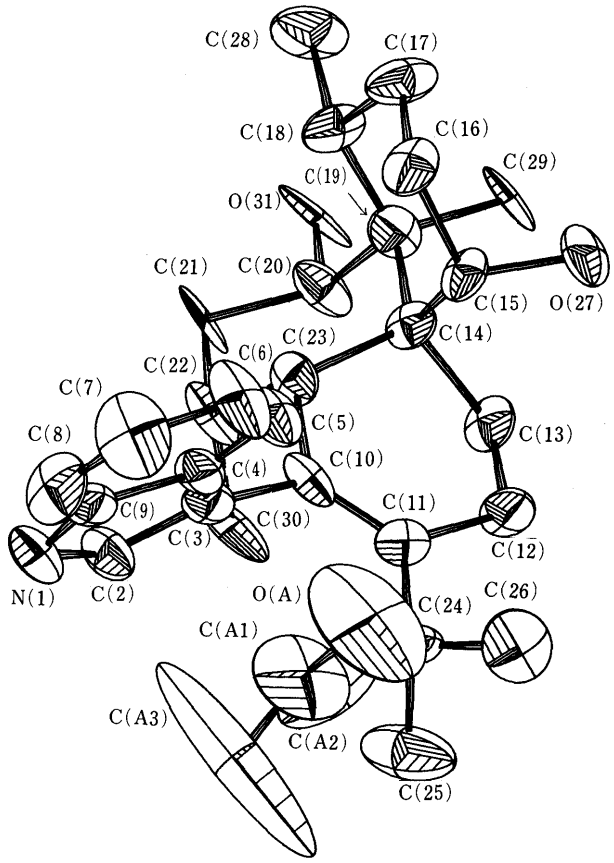

Fig. 1. Perspective View of the Crystal Structure of Monohydroxyaflavinine (**7**) Acetone Solvate with Thermal Ellipsoids at 50% Probability

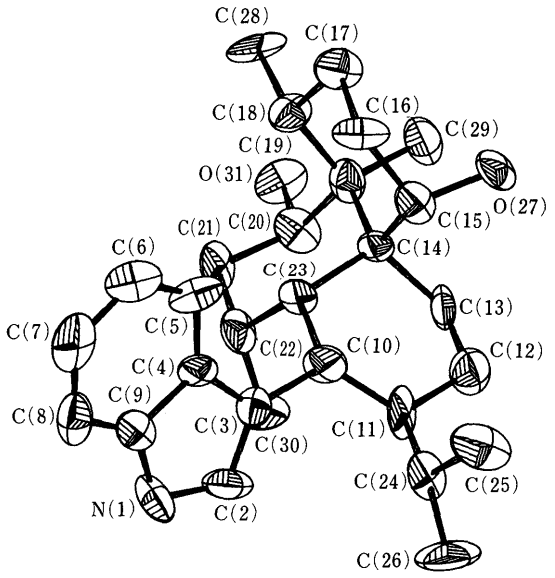

Fig. 2. Perspective View of the Crystal Structure of Monohydroxyisoaflavinine (**8**) with Thermal Ellipsoids at 50% Probability

confirm this assumption,[1]H-NMR decoupling experiments were performed. When the signal at δ 3.66 (1H, dd, J = 12.8, 5.5 Hz) was irradiated, the signal at δ 2.68 (1H, br t, J = 5.5 Hz) changed into a doublet and the signal at δ 3.17 (1H, m) was sharpened. The signals at δ 3.66 and 1.64 (1H, m) changed into a doublet (J = 12.8 Hz) and a triplet-like signal, respectively, when the signal at δ 2.68 was irradiated.

TABLE IV. Final Atomic Parameters and Equivalent Thermal Parameters for Monohydroxyisoaflavinine (**8**) with Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq} (Å^2)
O(27)	0.1143 (6)	0.2351 (5)	0.5527 (7)	4.5
O(31)	0.4614 (5)	0.3661 (5)	0.4111 (9)	4.2
N(1)	0.0907 (8)	0.3008 (6)	−0.1911 (9)	4.3
C(2)	0.1274 (10)	0.2522 (8)	−0.0943 (12)	4.3
C(3)	0.0997 (9)	0.2795 (7)	0.0206 (11)	3.4
C(4)	0.0396 (8)	0.3478 (7)	−0.0025 (11)	2.9
C(5)	−0.0156 (9)	0.3972 (7)	0.0775 (14)	4.4
C(6)	−0.0729 (11)	0.4562 (8)	0.0197 (15)	5.4
C(7)	−0.0750 (10)	0.4656 (8)	−0.1145 (15)	5.4
C(8)	−0.0191 (10)	0.4158 (8)	−0.1952 (13)	4.5
C(9)	0.0361 (9)	0.3592 (7)	−0.1376 (12)	3.9
C(10)	0.1238 (10)	0.2493 (7)	0.1488 (12)	3.9
C(11)	0.1345 (12)	0.1622 (8)	0.1651 (13)	5.3
C(12)	0.1734 (11)	0.1323 (8)	0.2916 (13)	4.9
C(13)	0.2439 (10)	0.1853 (7)	0.3618 (11)	3.6
C(14)	0.2111 (8)	0.2742 (7)	0.3645 (10)	2.6
C(15)	0.1090 (9)	0.2777 (7)	0.4324 (12)	3.7
C(16)	0.0741 (9)	0.3616 (8)	0.4573 (13)	4.3
C(17)	0.1504 (9)	0.4109 (8)	0.5334 (13)	4.4
C(18)	0.2442 (9)	0.4149 (7)	0.4540 (13)	3.6
C(19)	0.2876 (9)	0.3288 (7)	0.4352 (12)	3.5
C(20)	0.3802 (8)	0.3306 (8)	0.3477 (12)	3.8
C(21)	0.3604 (9)	0.3715 (8)	0.2205 (13)	3.9
C(22)	0.2888 (9)	0.3226 (7)	0.1438 (12)	3.7
C(23)	0.1944 (8)	0.3046 (7)	0.2225 (11)	3.0
C(24)	0.0679 (11)	0.1108 (8)	0.0960 (13)	5.3
C(25)	−0.0308 (11)	0.0942 (10)	0.1610 (15)	6.0
C(26)	0.1102 (14)	0.0446 (9)	0.0263 (17)	7.4
C(28)	0.3126 (10)	0.4738 (8)	0.5186 (15)	4.9
C(29)	0.3202 (10)	0.2928 (8)	0.5660 (13)	4.6
C(30)	0.3410 (9)	0.2521 (7)	0.0795 (14)	4.4

TABLE V. Bond Lengths (Å) for Monohydroxyisoaflavinine (**8**) with Estimated Standard Deviations in Parentheses

O(27)–C(15)	1.460 (15)	O(31)–C(20)	1.438 (16)
N(1)–C(2)	1.408 (17)	N(1)–C(9)	1.372 (17)
C(2)–C(3)	1.350 (18)	C(3)–C(4)	1.452 (17)
C(3)–C(10)	1.480 (18)	C(4)–C(5)	1.416 (19)
C(4)–C(9)	1.434 (17)	C(5)–C(6)	1.418 (22)
C(6)–C(7)	1.420 (22)	C(7)–C(8)	1.427 (21)
C(8)–C(9)	1.372 (19)	C(10)–C(11)	1.504 (21)
C(10)–C(23)	1.563 (18)	C(11)–C(12)	1.523 (22)
C(11)–C(24)	1.465 (23)	C(12)–C(13)	1.520 (20)
C(13)–C(14)	1.584 (17)	C(14)–C(15)	1.582 (17)
C(14)–C(19)	1.593 (17)	C(14)–C(23)	1.597 (16)
C(15)–C(16)	1.535 (18)	C(16)–C(17)	1.568 (19)
C(17)–C(18)	1.543 (20)	C(18)–C(19)	1.601 (18)
C(18)–C(28)	1.537 (21)	C(19)–C(20)	1.576 (18)
C(19)–C(29)	1.572 (18)	C(20)–C(21)	1.534 (19)
C(21)–C(22)	1.525 (19)	C(22)–C(23)	1.575 (17)
C(22)–C(30)	1.558 (19)	C(24)–C(25)	1.550 (23)
C(24)–C(26)	1.369 (24)		

Moreover the signal at δ 3.66 changed into a doublet (J = 5.5 Hz) when the signal at δ 3.17 was irradiated. These results confirmed that the above four signals at δ 3.66, 3.17, 2.68, and 1.64 were assigned to 10-H, 11-H, 23-H, and 22-H, respectively, and therefore the structure of monohydroxyisoaflavinine, including the relative stereochemistry at C-10, C-11, C-22, and C-23, was assumed to be as shown in **8**.

In order to confirm the stereochemistry of **8**, an X-ray structure analysis of **8** was undertaken. Crystals were grown

TABLE VI. Bond Angles (°) for Monohydroxyisoaflavinine (**8**) with Estimated Standard Deviations in Parentheses

C(2)–N(1)–C(9)	109.3 (1.1)	N(1)–C(2)–C(3)	109.9 (1.2)
C(2)–C(3)–C(4)	106.9 (1.1)	C(2)–C(3)–C(10)	129.0 (1.2)
C(4)–C(3)–C(10)	124.1 (1.1)	C(3)–C(4)–C(5)	133.5 (1.1)
C(3)–C(4)–C(9)	107.2 (1.0)	C(5)–C(4)–C(9)	119.2 (1.1)
C(4)–C(5)–C(6)	118.1 (1.3)	C(5)–C(6)–C(7)	121.2 (1.4)
C(6)–C(7)–C(8)	120.8 (1.4)	C(7)–C(8)–C(9)	117.3 (1.3)
N(1)–C(9)–C(4)	106.7 (1.1)	N(1)–C(9)–C(8)	129.6 (1.2)
C(4)–C(9)–C(8)	123.5 (1.2)	C(3)–C(10)–C(11)	118.1 (1.2)
C(3)–C(10)–C(23)	112.3 (1.1)	C(11)–C(10)–C(23)	118.7 (1.2)
C(10)–C(11)–C(12)	117.8 (1.3)	C(10)–C(11)–C(24)	118.3 (1.4)
C(12)–C(11)–C(24)	117.0 (1.4)	C(11)–C(12)–C(13)	116.7 (1.2)
C(12)–C(13)–C(14)	113.4 (1.1)	C(13)–C(14)–C(15)	107.4 (0.9)
C(13)–C(14)–C(19)	112.3 (0.9)	C(13)–C(14)–C(23)	109.7 (0.9)
C(15)–C(14)–C(19)	111.0 (0.9)	C(15)–C(14)–C(23)	106.3 (0.9)
C(19)–C(14)–C(23)	110.0 (0.9)	O(27)–C(15)–C(14)	109.1 (0.9)
O(27)–C(15)–C(16)	109.5 (1.0)	C(14)–C(15)–C(16)	113.0 (1.0)
C(15)–C(16)–C(17)	112.2 (1.1)	C(16)–C(17)–C(18)	108.2 (1.1)
C(17)–C(18)–C(19)	109.9 (1.1)	C(17)–C(18)–C(28)	107.8 (1.1)
C(19)–C(18)–C(28)	115.2 (1.1)	C(14)–C(19)–C(18)	110.4 (1.0)
C(14)–C(19)–C(20)	106.1 (1.0)	C(14)–C(19)–C(29)	111.6 (1.0)
C(18)–C(19)–C(20)	111.0 (1.0)	C(18)–C(19)–C(29)	111.0 (1.0)
C(20)–C(19)–C(29)	106.6 (1.0)	O(31)–C(20)–C(19)	111.8 (1.0)
O(31)–C(20)–C(21)	110.5 (1.1)	C(19)–C(20)–C(21)	111.9 (1.1)
C(20)–C(21)–C(22)	109.0 (1.1)	C(21)–C(22)–C(23)	111.5 (1.0)
C(21)–C(22)–C(30)	110.7 (1.1)	C(23)–C(22)–C(30)	117.4 (1.0)
C(10)–C(23)–C(14)	110.9 (1.0)	C(10)–C(23)–C(22)	112.0 (1.0)
C(14)–C(23)–C(22)	115.8 (0.9)	C(11)–C(24)–C(25)	116.3 (1.4)
C(11)–C(24)–C(26)	117.3 (1.4)	C(25)–C(24)–C(26)	125.4 (1.4)

as colorless prisms from methanol solution. The molecular structure of **8** is illustrated in Fig. 2. Therefore the relative structure of monohydroxyisoaflavinine was confirmed to be as depicted as **8**. The final atomic parameters are shown in Table IV. Bond lengths and angles are shown in Tables V and VI. These values are not significantly different from the expected ones. Based on the O(31)–O(27) and O(27)–N(1) distances (2.755 and 2.935 Å, respectively), O(31)---H---O(27)---H---N(1) seem to be intermolecular hydrogen bonds. The molecules are packed together mainly through the hydrogen bonding between molecules of **8**.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 spectrometer. Infrared (IR) and ultraviolet (UV) spectra were recorded on a JASCO IR-810 spectrometer and a Hitachi 124 spectrometer, respectively. EI-MS were obtained on a JEOL JMS-D 300 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 spectrometer at 399.78 MHz and 100.43 MHz, respectively, using tetramethylsilane as an internal standard. The coupling patterns are indicated as follows: singlet = s, doublet = d, triplet = t, quartet = q, multiplet = m, and broad = br. Column chromatography was performed using Kieselgel 60 (Art. 7734; Merck). Low-pressure liquid chromatography (LPLC) was performed with a Chemco Low-Prep pump (81-M-2) and a glass column (150 × 10 mm) packed with silica gel CQ-3 (30—50 μ; Wako). High-performance liquid chromatography (HPLC) was performed with a Nihon Seimitsu NSP-800-18 pump equipped with a Senshu Pak silica-4301-N column (10 × 300 mm) at the flow rate of 4.8 ml/min. TLC was conducted on precoated Kieselgel 60 F_{254} plates (Art. 5715; Merck). Spots on TLC were detected under UV light, and/or by spraying van Urk's reagent.[18]

Isolation of Indoloditerpenes *Aspergillus flavus*, strain IAF34, was cultivated at 34 °C for 3 weeks in 190 Petri dishes (i.d. 90 mm) containing 25 ml per dish of melted Czapek-Yeast Autolysate agar.[17] The fresh sclerotia, freed as far as possible from hyphae and agar substrate, were collected and extracted with methylene chloride at room temperature. The evaporated extract (1.2 g) was separated by column chromatography into two fractions. The more polar fraction, eluted with acetone, was crystallized from methanol to give dihydroxyaflavinine (**6**) (mp 253—255 °C, 164 mg). The less polar fraction, eluted with chloroform–acetone (100 : 1—50 : 1, v/v), was purified by LPLC with benzene–ethyl acetate (25 : 1, v/v) to give aflavinine (**5**) (mp (102—104 °C, 6 mg), aflatrem (**4**) (mp 222—224 °C, 40 mg), paspalinine (**3**) (2 mg), and ergosterol (120 mg) in that order. The last eluate was recrystallized from MeOH to give crystals (mp 236—239 °C, 45 mg), which were composed from two compounds. Therefore, these crystals were further purified by repeated LPLC with hexane–ethyl acetate (3 : 1, v/v) to obtain monohydroxyaflavinine (**7**) (12 mg). The later fraction was purified by HPLC with hexane–ethyl acetate (4 : 1, v/v) to obtain pure monohydroxyisoaflavinine (**8**) (2 mg).

Monohydroxyaflavinine (7) Colorless prisms from acetone, mp 161—162 °C, $[\alpha]_D^{24}$ +64°; $[\alpha]_{365}^{24}$ +303° (c = 0.08, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3470 sh, 3420, 3310 (OH, NH), 1705 (acetone). UV λ_{max}^{MeOH} nm (log ε): 224 (4.63), 277 sh (3.93), 283 (3.96), 291 (3.93). EI-MS m/z: 421.2979 (M^+, 421.2979 for $C_{28}H_{39}NO_2$, 100), 403 ($M-H_2O$, 17), 130 (22). *Anal.* Calcd for $C_{28}H_{39}NO_2 \cdot C_3H_6O$: C, 77.62; H, 9.46; N, 2.92. Found: C, 77.63; H, 9.56; N, 2.98. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, d, J = 7.3 Hz, Me), 0.96 (3H, d, J = 6.7 Hz, Me), 1.01 (3H, d, J = 6.7 Hz, Me), 1.16 (3H, d, J = 7.3 Hz, Me), 1.20—1.33 (3H, m), 1.29 (3H, s, –CH(OH)Me), 1.51 (1H, m), 1.71—2.00 (4H, m), 2.03—2.25 (4H, m), 2.48 (1H, br d, J = 4.9 Hz, 23-H), 2.59 (1H, qq, J = 7.3, 6.7 Hz, 24-H), 4.04 (1H, dd, J = 16.0, 3.5 Hz, 20-H), 4.49 (1H, br s, 15-H), 6.91 (1H, d, J = 2.5 Hz, 2-H), 7. 09 (1H, ddd, J = 8.6, 6.7, 1.2 Hz, 6-H), 7.20 (1H, ddd, J = 7.9, 6.7, 1.2 Hz, 7-H), 7.38 (1H, br d, J = 7.9 Hz, 8-H), 7.41 (1H, br d, J = 8.6 Hz, 5-H), 8.04 (1H, br s, NH). ^{13}C-NMR (DMSO-d_6) δ: 13.16 (q), 19.07 (q), 19.21 (q), 19.93 (t), 20.50 (q), 21.53 (q), 27.22 (t), 29.69 (d), 29.77 (t), 30.14 (t), 30.29 (d), 30.86 (d), 35.37 (t), 38.13 (d), 42.86 (s), 43.96 (s), 67.93 (d), 69.43 (d), 111.39 (d), 116.53 (s), 118.26 (d), 118.40 (d), 120.60 (d), 121.82 (d), 125.41 (s), 126.89 (s), 135.83 (s), 139.59 (s).

Monohydroxyisoaflavinine (8) Colorless prisms from methanol, mp 146—148 °C. $[\alpha]_D^{24}$ +56° (c = 0.10, MeOH). UV λ_{max}^{MeOH} nm (log ε): 226 (4.33), 283 (3.63), 291 (3.60). EI-MS m/z: 421.2985 (M^+, 421.2979 for $C_{28}H_{39}NO_2$, 63), 403 ($M-H_2O$, 9), 130 (100). ^{1}H-NMR ($CDCl_3$) δ: 1.05 (3H, d, J = 6.7 Hz, Me), 1.22 (2H, m), 1.27 (3H, s, Me), 1.32 (3H, d, J = 6.9 Hz), 1.55 (3H, br s, CH_2=C–Me), 1.64 (1H, m, 22-H), 1.68—2.03 (6H, m), 2.06—2.28 (3H, m), 2.68 (1H, br t, J = 5.5 Hz, 23-H), 3.17 (1H, m, 11-H), 3.66 (1H, dd, J = 12.8, 5.5 Hz, 10-H), 3.97 (1H, dd, J = 12.5, 2.9 Hz, 20-H), 4.67 (1H, br s, –C=CH_2), 4.80 (1H, br s, –C=CH_2), 4.84 (1H, br s, 15-H), 7.05 (1H, d, J = 1.8 Hz, 2-H), 7.11 (1H, ddd, J = 7.7, 6.7, 1.2 Hz, 6-H), 7.18 (1H, ddd, J = 7.3, 6.7, 1.2 Hz, 7-H), 7.34 (1H, br d, J = 7.3 Hz, 8-H), 7.52 (1H, br d, J = 7.7 Hz, 5-H), 7.92 (1H, br s, NH). ^{13}C-NMR (DMSO-d_6) δ: 13.36 (q), 18.17 (q), 19.28 (q), 22.32 (q), 24.18 (t), 27.08 (t), 27.24 (t), 29.92 (t), 30.56 (d), 30.88 (d), 33.85 (d), 37.62 (d), 38.29 (t), 42.58 (d), 43.66 (s), 45.31 (s), 66.05 (d), 69.48 (d), 110.80 (t), 111.36 (d), 114.56 (s), 117.29 (d), 118.14 (d), 120.51 (d), 123.30 (d), 126.93 (s), 135.83 (s), 149.87 (s).

Aflavinine (5) ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, d, J = 6.8 Hz), 0.83 (3H, d, J = 7.1 Hz), 0.97 (3H, d, J = 7.1 Hz), 0.99 (3H, s), 1.09 (3H, d, J = 7.3 Hz), 1.09—1.18 (2H, m), 1.78 (2H, br t, J = 14.9 Hz), 1.52—1.88 (5H, m), 1.95—2.14 (3H, m), 2.23 (2H, dd, J = 8.8, 3.9 Hz), 2.43 (1H, br d, J = 5.5 Hz), 2.59 (1H, qd, J = 6.8, 6.8 Hz), 4.48 (1H, br s), 6.89 (1H, d, J = 2.2 Hz), 7.09 (1H, br t, J = 8.0 Hz), 7.19 (1H, br t, J = 8.0 Hz), 7.37 (1H, br d, J = 8.0 Hz), 7.43 (1H, br d, J = 8.0 Hz), 8.03 (1H, br s, NH).

Dihydroxyaflavinine (6) ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, d, J = 7.0 Hz), 1.00 (3H, d, J = 6.7 Hz), 1.17 (3H, d, J = 7.3 Hz), 1.11 (1H, m), 1.17 (3H, d, J = 7.3 Hz), 1.27 (3H, s), 1.48 (1H, br d, J = 13.1 Hz), 1.98—1.82 (3H, m), 1.90 (1H, td, J = 12.8, 6.1 Hz), 1.70—2.17 (4H, m), 2.21 (1H, m), 2.35 (1H, m), 2.48 (1H, br d, J = 5.8 Hz), 2.61 (1H, d, J = 4.3 Hz), 2.66 (1H, qd, J = 7.0, 7.3 Hz), 3.36 (1H, ddd, J = 10.4, 7.6, 6.5 Hz), 3.54 (1H, ddd, J = 10.4, 7.9, 5.1 Hz), 3.99 (1H, br d, J = 12.8 Hz), 4.44 (1H, br s), 6.95 (1H, d, J = 2.1 Hz), 7.01 (1H, br t, J = 8.1 Hz), 7.11 (1H, br t, J = 8.1 Hz), 7.37 (1H, br d, J = 8.1 Hz), 7.41 (1H, br d, J = 8.1 Hz), 9.98 (1H, br s, NH).

X-Ray Structure Analysis of Monohydroxyaflavinine (7) Acetone Solvate Crystals of **7** were grown from acetone to yield **7** acetone solvate as colorless prisms, as described above.

Crystal Data: $C_{28}H_{39}NO_2 \cdot C_3H_6O$; M = 479.70; orthorhombic; $P2_12_12_1$; a = 13.994 (21), b = 18.757 (26), c = 10.582 (12) Å; V = 2777.6 (65) Å^3; Z = 4; D_c = 1.148 g·cm^{-3}; $F(000)$ = 1048.

The diffraction intensities were collected from a monohydroxyaflavinine (**7**) acetone solvate crystal with dimensions of 0.6 × 0.5 × 0.2 mm on a Rigaku AFC-5 FOS four-circle diffractometer using CuK_α radiation monochromated by means of a graphite plate. A total of 1553 reflections were measured within a 2θ range of 100° as above the 3$\sigma(F)$ level. These were used in the solution and refinement of the structure.

Determination of the Structure: The structure was solved by the direct method using MULTAN 84[19] and refined by the block-matrix least-squares method. In the final refinement, anisotropic thermal parameters were used for non-hydrogen atoms. The contribution of hydrogen atoms of acetone was ignored. The final R factor was 0.065.[20]

X-Ray Structure Analysis of Monohydroxyisoaflavinine (8) Crystals of **8** were grown from methanol as colorless prisms, as described above.

Crystal Data: $C_{28}H_{39}NO_2$; $M=421.63$; orthorhombic; $P2_12_12_1$; $a=13.811$ (14), $b=17.083$ (29), $c=10.509$ (9) Å; $V=2479.3$ (53) Å^3; $Z=4$; $D_c=1.129\,g\cdot cm^{-3}$; $F(000)=920$.

The diffraction intensities were collected from a crystal of **8** with dimensions of $0.6\times0.5\times0.1$ mm on a Rigaku AFC-5 FOS four-circle diffractometer using CuK_α radiation monochromated by means of a graphite plate. A total of 1369 reflections were measured within a 2θ range of 100° as above the $3\sigma(F)$ level. These were used in the solution and refinement of the structure.

Determination of the Structure: The structure was solved by the direct method using MULTAN 84[19] and refined by the block-matrix least-squares method. In the final refinement, anisotropic thermal parameters were used. The contribution of hydrogen atoms was ignored. The final R factor was 0.099.[20]

Acknowledgement The authors are gateful to Mrs. M. Yuyama and Mrs. T. Ogata of our university for NMR and mass measurements and elemental analyses, respectively. This work was partially supported by the Japan Health Sciences Foundation.

References and Notes

1) Part XXVI in the series "Studies on Fungal Products." Part XXV: K. Nozawa, S. Udagawa, S. Nakajima and K. Kawai, *Phytochemistry*, **28**, 929 (1989).
2) K. Nozawa, S. Udagawa, S. Nakajima and K. Kawai, *J. Chem. Soc., Chem. Commun.*, **1987**, 1157.
3) K. Nozawa, S. Nakajima, K. Kawai and S. Udagawa, *J. Chem. Soc., Perkin Trans. 1*, **1988**, 1689.
4) K. Nozawa, M. Yuyama, S. Nakajima, K. Kawai and S. Udagawa, *J. Chem. Soc., Perkin Trans. 1*, **1988**, 2155.
5) R. J. Cole and R. H. Cox, "Handbook of Toxic Fungal Metabolites," Academic Press, New York, 1981, pp. 1—66.
6) W. B. Turner, "Fungal Metabolites," Academic Press, London, 1971, pp. 179—183.
7) R. J. Cole, J. W. Dorner, J. P. Springer and R. H. Cox, *J. Agric. Food Chem.*, **29**, 293 (1981).
8) T. Fehr and W. Acklin, *Helv. Chem. Acta*, **49**, 1907 (1966).
9) B. J. Wilson and C. H. Wilson, *Science*, **144**, 177 (1964).
10) R. T. Gallagher, J. Clardy and B. J. Wilson, *Tetrahedron Lett.*, **21**, 239 (1980).
11) R. T. Gallagher, T. McCabe, K. Hirotsu, J. Clardy, J. Nicholson and B. J. Wilson, *Tetrahedron Lett.*, **21**, 243 (1980).
12) D. T. Wicklow and R. J. Cole, *Can. J. Bot.*, **60**, 525 (1982).
13) O. Tsuruta, T. Goto, M. Saito, P. Siriacha, K. Panawas and D. Buangsuwon, *Proc. Assoc. Jpn. Mycotoxicol.*, **23**, 59 (1986).
14) M. Saito, O. Tsuruta, P. Siriacha, S. Kawasugi, M. Manabe and D. Buangsuwon, *Proc. Assoc. Jpn. Mycotoxicol.*, **24**, 41 (1986).
15) C. W. Hesseltine, O. D. Shotwell, M. Smith, J. J. Ellis, E. Vandegraft and G. Shannon, "Proceedings of the First U.S.-Japan Conference on Toxic Microorganisms," UJNR Joint Panels on Toxic Microorganisms and the U.S. Department of the Interior, Washington, D.C., 1970, pp. 202—210.
16) N. Narita, M. Suzuki, S. Udagawa, S. Sekita, M. Harada, N. Aoki, T. Tanaka, A. Hasegawa, S. Yamamoto, N. Toyazaki and Y. Matsuda, *Proc. Assoc. Jpn. Mycotoxicol.*, **27**, 21 (1988).
17) J. I. Pitt, "The Genus Penicillium and Its Teleomorphic States Eupenicillium and Taralomyces," Academic Press, London, 1979, p. 18.
18) E. Stahl and H. Kaldeway, *Hoppe-Seylers Z. Physiol. Chem.*, **323**, 182 (1961).
19) P. Main, G. Germain and M. M. Woolfson, "MULTAN 84. A Computer Program for the Automatic Solution of Crystal Structures from Diffraction Data," University of York, England, 1984.
20) Lists of F_o and F_c values, anisotropic thermal parameters, and atomic fractional parameters are available from one of the authors (K. K.) upon request.