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Chaetochromin, a Bis(naphthodihydropyran-4-one) Mycotoxin from *Chaetomium thielavioideum*: Application of ^{13}C-^{1}H Long-rang Coupling to the Structure Elucidation

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Details of the separation of the metabolites (I—V) of *Chaetomium thielavioideum* are presented. The structure of a new mycotoxin named chaetochromin was studied by physical method (ultraviolet, infrared, ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), and mass spectrum) and it was identified as the bis(naphthodihydropyran-4-one (II). The assignment of the ^{13}C-NMR signals was largely achieved with the aid of the two- and three-bond spin-spin couplings between ^{13}C and ^{1}H.

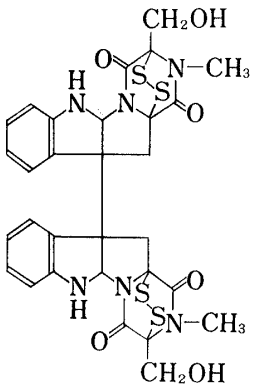
Keywords——*Chaetomium thielavioideum*; chaetochromin; bis(naphthodihydropyran-4-one); mycotoxin; cytotoxicity; mutagenicity; ^{1}H-NMR; ^{13}C-NMR; ^{13}C-^{1}H long-range coupling

During the course of surveys on mycotoxin production by *Chaetomium* spp. and related fungi (Pyrenomycetes), strains identified as *C. thielavioideum* Chen were found to exhibit strong cytotoxicity.[2)] As the causative agents, chaetocin (I) [an antibiotic substance first isolated from *C. minutum* Krzemieniewska and Badura[3)]], a new phenolic compound (II) of mp 222—224°, sterigmatocystin (III) [a well-known carcinogenic mycotoxin[4)]], and its *O*-methyl ether (IV)[5)] were isolated.[2)]

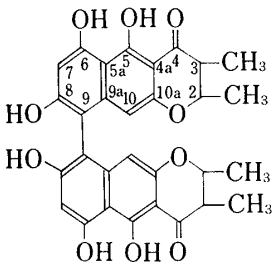
This paper presents the experimental details of the separation of the metabolites from one strain (NHL 2829) of the species isolated from soil obtained from Thailand, and describes the structural elucidation of the new phenolic mycotoxin (II).

Chromatographic separation of dichloromethane and methanol extracts of the fungal culture on polished rice grains afforded the metabolites (I—IV), ergosterol, eugenitin (V) [a chromone derivative first isolated from *Eugenia caryophyllata* Thunb. (Myrtaceae)[6)] and then from the lichen fungal symbiont of *Lecanora rupicola* Zahlbr.[7)] and a fungus, *Cylindrocarpon* sp.[8)]], and anthranilic acid.[9)] As shown in Table I, cytotoxicity to HeLa cells was observed in the cases of chaetocin (I), the new phenolic compound (II), and sterigmatocystin (III) among the isolated metabolites,[2)] while mutagenicity in the *Salmonella*/microsome test was seen only with III and the methyl ether (IV).[10)] Acute toxicity tests with mice showed that chaetocin (I) and the new phenolic (II) are lethal at the level of 10 mg/kg upon intraperitonial administration.[11)] Thus the mold is quite noteworthy in that it produces a variety of cytotoxic, mutagenic, and acutely toxic substances.

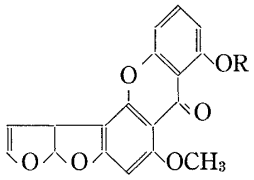
1) Location: *Kamiyoga-1-chome, Setagaya-ku, Tokyo 158, Japan.*
2) S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori, and M. Umeda, *Can. J. Microbiol.*, **25**, 170 (1979).
3) D. Hauser, H.P. Weber, and H.P. Sigg, *Helv. Chim. Acta*, **53**, 1061 (1970).
4) T. Hamasaki and Y. Hatsuda, "Mycotoxins in Human and Animal Health," ed. by J.V. Rodricks, C.W. Hesseltine, and M.A. Mehlman, Pathotox Publishers, Il., 1977, pp. 597—607.
5) H.J. Burkhardt and J. Forgacs, *Tetrahedron*, **24**, 717 (1968).
6) H. Schmid, *Helv. Chim. Acta*, **32**, 813 (1949).
7) C.H. Fox and S. Huneck, *Phytochemistry*, **8**, 1301 (1969).
8) R.G. Coombe, H.I.C. Lowe, and T.R. Watson, *Aust. J. Chem.*, **25**, 875 (1972).
9) E.W. Bassett and S.W. Tanenbaum, *Experientia*, **14**, 38 (1958).
10) Private communication from Drs. M. Ishidate and H. Yoshikawa, and Miss R. Miyata of this Institute.
11) Private communication from Dr. K. Ohtsubo, Tokyo Metropolitan Institute of Gerontology.



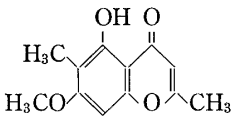
I: chaetocin



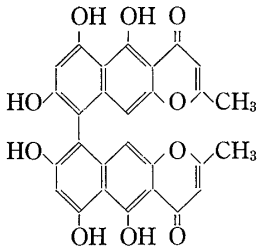
II: chaetochromin



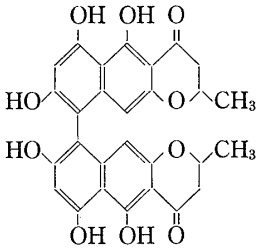
III: R=H sterigmatocystin
IV: R=CH_3 O-methyl-sterigmatocystin



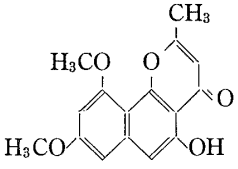
V: eugenitin



VI: ustilaginoidin A



VII: cepharochromin



VIII: flavasperone

TABLE I. Cytotoxicity[2] and Mutagenicity[10] of the Metabolites of *Chaetomium thielavioideum*

Compound	Cytotoxicity to HeLa Cells[a] (μg/ml)								Mutagenicity in *Salmonella typhimurium*[b]			
	100	32	10	3.2	1.0	0.32	0.1	0.032	TA100		TA98	
									−S-9	+S-9	−S-9	+S-9
Chaetocin (I)	4	4	4	4	4	4	3	1	—	—	—	—
Chaetochromin (II)	4	4	4	3.5	0				—	—	—	—
Sterigmatocystin (III)	4	4	4	4	2	0			8	650	3	54
O-Methylsterigmatocystin (IV)	4	4	2	1	0				22	565	—	34
Eugenitin (V)	0	0	0	0	0				—	—	—	—

a) The degree of toxicity is estimated on a scale ranging from 0 (no cellular damage) through 4 (complete cytolysis); see Ref. 2).
b) Number of revertant colonies per μg of the metabolite. Spontaneous revertant colonies were not included.

The structure elucidation of the new phenolic mycotoxin (II), designated chaetochromin in view of the proposed structure, will be discussed next, chiefly on the basis of the nuclear magnetic resonance (NMR) spectral data.

Chaetochromin (II), yellow needles of mp 222—224°, $[\alpha]_D$ +634° (in $CHCl_3$), M^+ 546.154 *m/e* ($C_{30}H_{26}O_{10}$, by high resolution mass spectrometry), showed λ_{max}^{MeOH} 235, 280, 292, 328 and 415 nm, similar to that of 4,5,4′,5′-tetrahydroxy-1, 1′-dinaphthyl,[12] and ν_{max}^{KBr} 3400 (broad,

12) D.C. Allport and J.D. Bu'Lock, *J. Chem. Soc.*, **1958**, 4090.

Table II. ^{1}H-NMR Chemical Shifts (δ) and Coupling Constants (Hz) of Chaetochromin (in $CDCl_3$)

		Remarks
2-CH_3	1.40, 3H, d, $J=6$	*a*)
2-H	4.16, 1H, dq, $J=11, 6$	*a*)
3-CH_3	1.23, 3H, d, $J=7$	*b*)
3-H	2.59, 1H, dq, $J=11, 7$	*b*)
5-OH	15.20, 1H, s	
6-OH	9.57, 1H, s	
7-H	6.44, 1H, s	*c*)
8-OH	5.83, 1H, s	*c*)
10-H	5.95, 1H, s	

a, *b*) The coupling was confirmed by decoupling experiments.
c) Irradiation at 8-OH caused a nuclear Overhauser effect at 7-H (8±3%).

chelated and non-chelated hydroxyls), 1630 (strong absorption, hydrogen-bonded carbonyl groups), 1610, and 1582 (aromatic rings) cm^{-1}. Its ^{1}H-NMR spectrum showed only 13 protons and the ^{13}C-NMR showed 15 carbons (Tables II and III, respectively). The observations suggest that chaetochromin is a symmetric dimer of hydroxynaphthalene. Among the three hydroxy groups in the half of the molecule, one (δ 15.20 ppm) is strongly hydrogen-bonded to the carbonyl group (ν_{max}^{KBr} 1630 cm^{-1}), while the second (δ 9.57 ppm) is also chelated and the third (δ 5.83 ppm) appears to be almost free from hydrogen-bonding. The ^{1}H-NMR spectrum also showed the presence of two secondary methyl groups, which are adjacent to each other. This was confirmed by decouplings of the methyl and methine protons; the methyl group at δ 1.40 ppm and the methin group at δ 4.16 ppm form a secondary methyl group and are adjacent to an oxygen function. The other methyl group at δ 1.23 ppm and the methine group at δ 2.59 ppm are adjacent to an aromatic ring or a carbonyl group (Table II). These observations suggested that chaetochromin has a 2,3-dimethyldihydropyran-4-one moiety or a 3,4-dimethyldihydropyran-2-one moiety. The two signals due to the aromatic protons (δ 5.95 and 6.44 ppm) are not coupled; a high field shift of the former proton is noticeable. The nuclear Overhauser effect is observed between the non-chelated hydroxyl proton and the aromatic proton at δ 6.44 ppm (Table II).

The ^{13}C-NMR spectrum showed the signals of one carbonyl carbon, two aromatic carbons bonded to a hydrogen, eight quaternary aromatic carbons, among which four are assumed to be bonded to oxygen functions, and two each of methyl and methine carbons in one half of the molecule (Fig. 1 and Table III). The presence of four aromatic carbon atoms bearing oxygen functions favours a dihydropyran-4-one moiety rather than a dihydropyran-2-one moiety in the molecule. These considerations lead to a symmetric tetramethylhexahydroxybis(naphthodihydropyran-4-one) structure for chaetochromin. However, at this stage it was not clear whether chaetochromin has a linear fusion between the naphthalene and pyran-4-one moieties similar to that in ustilaginoidins (VI)[13] and cepharochromin (VII),[14,15] in which the fusion and the position of the dimeric linkage were established by synthetic and correlation reactions, or an angular fusion similar to that in flavasperone (VIII)[16] ((a), (b), or (c) in Chart 1),

13) S. Shibata, A. Ohta, and Y. Ogihara, *Chem. Pharm. Bull.*, **11**, 1174 (1963); S. Shibata, Y. Ogiwara, and A. Ohta, *ibid.*, **11**, 1179 (1963); S. Shibata and Y. Ogiwara, *Tetrahedron Lett.*, **1963**, 1777; *idem*, *Chem. Pharm. Bull.*, **11**, 1576 (1963); E. Morishita and S. Shibata, *Chem. Pharm. Bull.*, **15**, 1765, 1772 (1967).
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16) B.W. Bycroft, T.A. Dobson, and J.C. Roberts, *J. Chem. Soc.*, **1962**, 40; B.W. Bycroft and J.C. Roberts, *ibid.*, **1963**, 4868; S. Ghosal, K. Biswas, and D.K. Chakrabarti, *J. Agr. Food Chem.*, **27**, 1347 (1979).

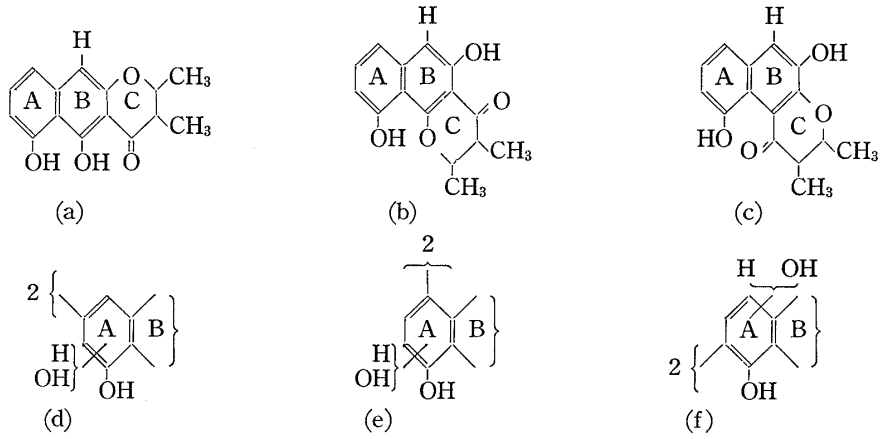

Chart 1

TABLE III. ^{13}C-NMR Chemical Shifts (δ) and ^{13}C-^{1}H Coupling Constants (Hz) of Chaetochromin

Carbon atom	Solvent: Acetone-d_6 (1) δ[a]	^{1}J (C, H) Hz	^{2}J (C, H and C, OH) Hz	^{3}J (C, H and C, OH) Hz	Acetone-d_6 + D_2O (2) δ[a]	$CDCl_3$ (3) δ	(1)−(3) $\Delta\delta$	(2)−(1) $\Delta\delta$
2	79.1 (Dm)	141.6 (2-H)	*ca.* 3.0 (2-CH_3)		79.3 (Dm)	78.3	0.8	0.2
3	46.7 (Dm)	127.0 (3-H)	4.3 (3-CH_3)		47.2 (Dm)	46.2	0.5	0.5
4	201.9 (S)				202.0 (S)	200.8	1.1	0.1
4a	102.3 (Sdd)			*ca.* 5.0 (5-OH) 5.5 (10-H)	102.5 (Sd)	102.0	0.3	0.2
5	165.5 (br. S)				165.0 (br. S)	164.3	1.2	−0.5
5a	105.7 (Sm)			nd[b] (5-OH) nd (6-OH) 5.8 (7-H) 5.8 (10-H)	105.5 (Sdd)	105.4	0.3	−0.2
6	160.8[c] (Sdd)		3.9 (6-OH) 3.7 (7-H)		160.5[c] (Sd)	159.9	0.9	−0.3
7	100.9 (Ddd)	160.5 (7-H)		6.0[c] (6-OH) 7.0[c] (8-OH)	100.6 (D)	100.0	0.9	−0.3
8	161.6[c] (Sdd)		*ca.* 3.0 (8-OH) 3.1 (7-H)		161.6[c] (Sd)	160.4	1.2	0
9	106.4 (Sm)			4.3 (7-H) 4.8 (8-OH) 4.3 (10-H)	107.6 (Sdd)	102.2	4.2	1.2
9a	143.3 (S)				143.3 (S)	142.1	1.2	0
10	100.1 (D)	162.4 (10-H)			100.1 (D)	99.4	0.7	0
10a	156.5 (Sd)			3.7 (2-H)	156.6 (Sd)	156.2	0.3	0.1
2-CH_3	19.8 (Qm)	127.0 (2-CH_3)	3.1 (2-H)	1.8 (3-H)	20.2 (Qm)	19.7	0.1	0.4
3-CH_3	10.0 (Qd)	126.0 (3-CH_3)	4.3 (3-H)		10.5 (Qd)	9.8	0.2	0.5

a) br=broad, D and d=doublet, m=multiplet, S and s=singlet, Q=quartet. Capital letters refer to the pattern resulting from directly bonded ^{13}C-^{1}H couplings and small letters to that from ^{13}C-^{1}H couplings over more than one bond.
b) nd=not detected.
c) May be interchanged.

and it was also unclear whether the substitution pattern of the non-chelated hydroxyl groups and the dimeric linkage on the aromatic ring A is (d), (e), or (f) in Chart 1.

^{13}C-NMR spectroscopy is a powerful tool in studying the structures of a great variety of complex aromatic compounds. In aromatic systems the long-range couplings between ^{13}C and ^{1}H are usually in the order $^{1}J \gg {}^{3}J > {}^{2}J > {}^{4}J$ and the full utilization of these couplings has recently been shown to be the most useful approach to making signal assignments for such compounds.[17–19] Applying this method, the structures of xanthomegnin and the related fungal pigments, dimeric naphthodihydropyran-2-ones, were revised to the linear forms from the previously suggested angular structures.[17] Thus, precise examination of the ^{13}C-NMR spectrum of chaetochromin was attempted. The assignments were derived from proton noise decouplings (Fig. 1), gated decouplings (Fig. 2), D_2O addition (Fig. 2), and long-range selective proton decouplings (Fig. 3). Addition of D_2O affected not only the three signals of hydroxyl-substituted carbons (C-5, C-6, and C-8) but also the signals at δ 100.9 ppm, δ 105.7 ppm and δ 106.4 ppm (Fig. 2). The doublet of double doublets at δ 100.9 ppm changed to a doublet, so that an aromatic carbon carrying a hydrogen exists between two carbons bearing hydroxyl groups on the aromatic ring A (C-7) (Chart 1). Both of the two aromatic protons in ^{1}H-NMR appear as singlets. Accordingly, the proton on the ring carbon must be *ortho*, the free hydroxyl group must be *meta*, and the dimeric bonding site must be *para* to the weakly hydrogen-bonded hydroxyl group (C_6-OH). On the other hand, the signal at δ 100.1 ppm of the other

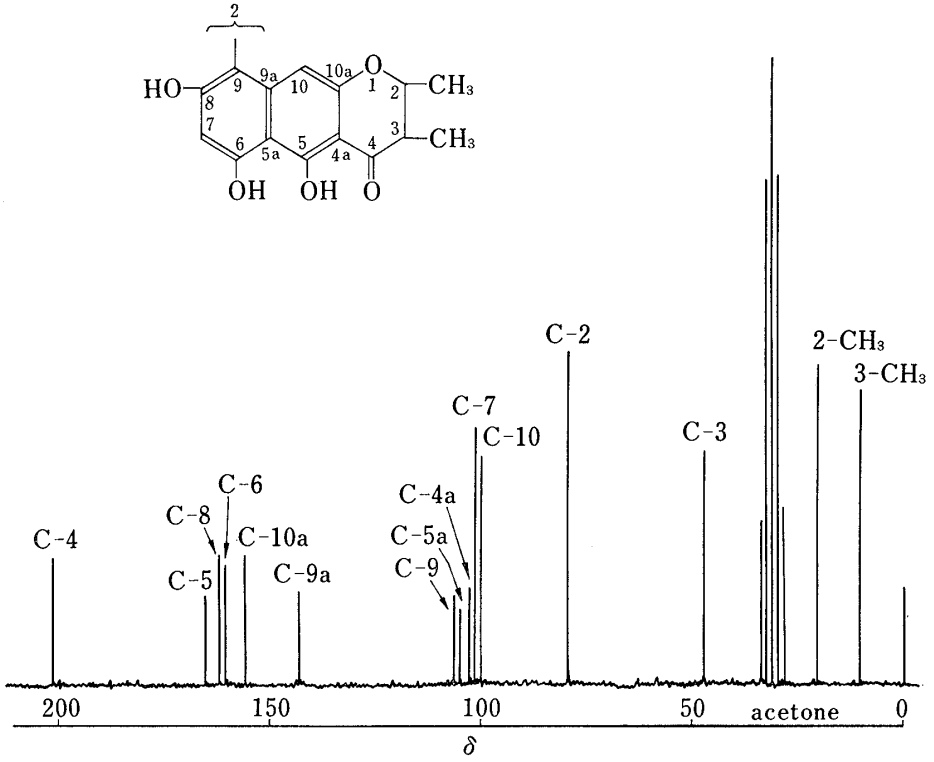

Fig. 1. The Proton-noise-decoupled ^{13}C-NMR Spectrum of Chaetochromin in CD_3COCD_3

17) T.J. Simpson, *J. Chem. Soc.*, Perkin I, **1977**, 592; G. Höfle and K. Röser, *Chem. Commun.*, **1978**, 611; A. Zeeck, P. Russ, H. Laatsch, W. Loeffler, H. Wehrle, H. Zähner, and H. Holst, *Chem. Ber.*, **112**, 957 (1979).
18) C.-J. Chang, *Lloydia*, **41**, 17 (1978).
19) G. Höfle, *Tetrahedron*, **33**, 1963 (1977); P.S. Steyn, P.L. Wessels, and W.F.O. Marasas, *ibid.*, **35**, 1551 (1979); P.S. Steyn, R. Vleggaar, P.L. Wessels, R.J. Cole, and De B. Scott, *J. Chem. Soc.*, Perkin I, **1979**, 451.

aromatic carbon bearing hydrogen is coupled only with the directly bonded proton and is not affected by the phenolic protons. If chaetochromin has an angular fusion as shown by (b) or (c) in Chart 1, the signal at δ 100.1 ppm must exhibit a three-bond coupling of one hydroxyl group. Thus, an angular fusion of rings B and C was excluded and the carbon was assigned to C-10 in ring B of the linear form (a) in Chart 1. The multiplet signals at δ 106.4 ppm and δ 105.7 ppm changed to double doublets on D_2O addition, suggesting the carbons at three-bonds from the exchangeable protons, *i.e.* C-9 and C-5a. The choice between the two was made as follows: When the solvent was changed from acetone-d_6 to $CDCl_3$, the signal at δ 106.4 ppm showed a high field shift ($\Delta\delta$=4.2 ppm). Thus, this signal was assigned as C-9, where the two halves of the molecule are linked. Accordingly, the signal at δ 105.7 ppm was assigned to C-5a. The singlet signal at δ 143.3 ppm was assigned to C-9a, where there is no three-bond coupling between ^{13}C-^{1}H (Fig. 2).

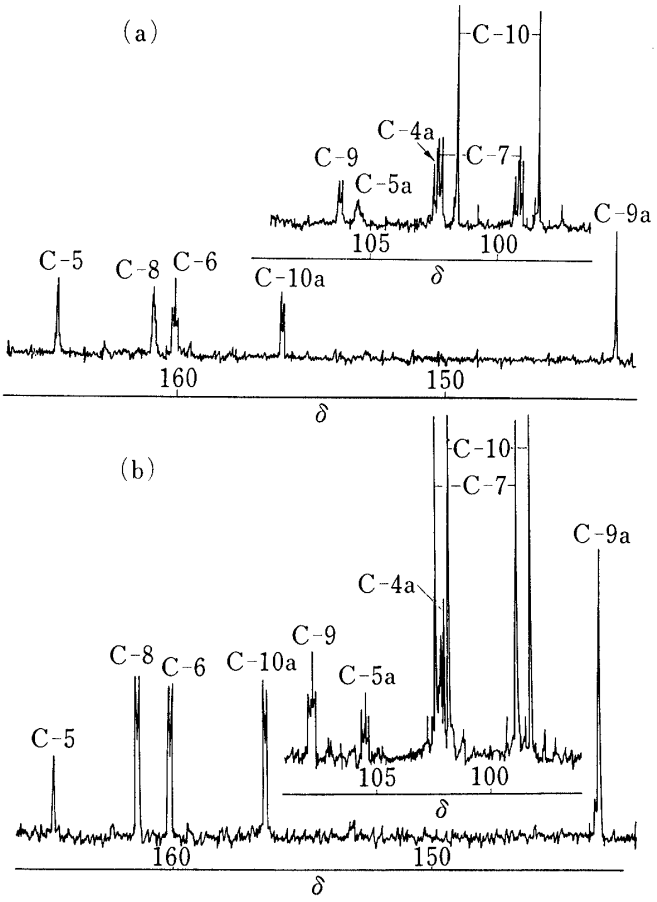

Fig. 2. The Aromatic Carbon Region of Chaetochromin in the Gated ^{1}H-Decoupling ^{13}C-NMR Spectra

(a); in CD_3COCD_3.
(b): after adding D_2O.

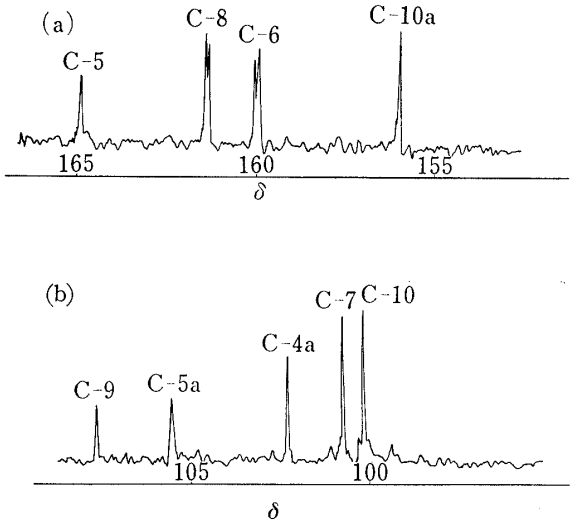

Fig. 3. The Long-Range Proton Decoupling ^{13}C-NMR Spectra of Chaetochromin (in $CD_3COCD_3+D_2O$)

(a) When the 2-H was irradiated, the signal of C-10a changed from a doublet to a singlet.
(b) When the aromatic protons were irradiated, the signals of C-10, C-7, C-4a, C-5a and C-9 changed to singlets.

When the proton at C-2 was irradiated, the doublet signal at δ 156.5 ppm changed to a singlet, leading to assignment to C-10a (Fig. 3). The signal at δ 102.3 ppm was assigned to C-4a as the remaining quaternary aromatic carbon; this signal changed from a doublet to a singlet (upon addition of D_2O) on irradiation at the aromatic protons, but did not show any change on irradiation at the C-3 proton (Fig. 3). The signals of the two methyl and two methine groups were thus confirmed by selective proton decouplings and the data showed good agreement with those reported for 2,3-dimethylchromanone derivatives from *Calophyllum*

brasiliense Camb.[20] However, the selective proton decouplings at the C-2 and C-3 protons favored the *cis*-configuration of the 2,3-dimethyl groups, in contrast to the ^{1}H-NMR data, in which the C-2 and C-3 protons show a large coupling (J=11 Hz).

Based on these observations, especially the ^{13}C-^{1}H long-range coupling data, the assignment of all the ^{13}C atoms in the molecule of chaetochromin was established as shown in Table III. Based on these findings the structure (II) is put forward for chaetochromin. This structure (II) corresponds to the 3,3'-dimethyl derivative of cephalochromin (VII) from *Cephalosporium* sp.,[14,21] *Verticillium* sp.,[15] *Nectria viridescens* Booth, [22] and *N. flavoviridis* Wollenw.[22] Studies to confirm the stereochemistry of the C-9 dimeric site and the dimethyl groups at the 2- and 3-positions are in progress.

Experimental

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV), infrared (IR), and ^{1}H- and ^{13}C-NMR spectra were measured with Hitachi 200-10, Nihon Bunko DS-403G, JEOL C-60HL (60 MHz) and Varian XL100A (100 MHz), and JEOL FX 200 (50.15 MHz) machines, respectively. Chemical shifts are shown in ppm from Me_4Si added as an internal standard. Mass spectra were measured on a JEOL 01SG-2 high resolution mass spectrometer. $[\alpha]_D$ were measured with a Nihon Bunko DIP-180 machine.

Isolation of Metabolites from *Chaetomium thielavioideum*——Strain NHL 2829 was incubated in stationary culture on sterilized rice (10 kg) at 30° for 14 days.[2] The moldy rice was extracted twice with dichloromethane (20 l) for 24 hr each at room temperature. After concentration to 50 ml *in vacuo*, the precipitates (compound A, 3.5 g) formed were separated by filtration. The mother liquor was applied to a silica gel column using benzene and benzene-dichloromethane as developing solvents. From the first fraction eluted with benzene, a mixture of compounds B and C was obtained. From the next fraction eluted with benzene, compound D (370 mg) was obtained. From the benzene–dichloromethane (1:1) fraction, compound E (4.6 g) was obtained, and from the benzene–dichloromethane (1:2) fraction, compound F (30 mg). The mixture of compounds B and C was separated by preparative thin-layer chromatography (TLC) on precoated PLC plates (Silica Gel F_{254}, 2 mm, Merck) using dichloromethane–methanol (9.5:0.5) as the developing solvent. From the upper layer, compound B (1.7 g) was obtained and from the lower layer, compound C (5.7 g).

After extraction with dichloromethane, the moldy rice was extracted with methanol (20 l) for 24 hr at room temperature. The methanol extract was concentrated *in vacuo* and then extracted with ethyl acetate. After concentration, the ethyl acetate extract was dissolved in chloroform (100 ml) and the precipitates formed (*ca.* 200 mg) were separated by filtration. The mother liquor was applied to a silica gel column using dichloromethane as the developing solvent. From the first fraction, compound G (146 mg) was obtained. From the next fraction, compound E (10 mg) was obtained. The precipitates consisted largely of compound G.

Chaetochromin (II)——Compound E was recrystallized from benzene as yellow needles, mp 222—224°, $[\alpha]_D$ +634° (c=1.0, $CHCl_3$), MS m/e: 546.154 (M^+, Calcd for $C_{30}H_{26}O_{16}$: 546.152); UV λ_{max}^{EtOH} nm (log ε): 235 (4.64), 280 (4.67), 292 (4.73), 328 (4.18), 415 (4.02); IR ν_{max}^{KBr} cm^{-1}: 3400, 1630, 1610, 1582, 1440, 1380, 1343, 1145, 1134, 1082, 840; ^{1}H-NMR (Table II); ^{13}C-NMR (Table III).

Identification of Ergosterol, Eugenitin, Chaetocin, Sterigmatocystin, *O*-Methylsterigmatocystin, and Anthranilic Acid——Compound B was recrystallized from benzene as colorless needles of mp 156°, MS m/e: 396.342 (M^+, Calcd for $C_{28}H_{46}O$: 396.340); UV λ_{max}^{EtOH} nm (log ε): 262 (3.94), 271 (4.06), 281 (4.07), 292 (3.84); IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1650, 1460, 1370, 1060, 1040, 970, 838, 800. Its identity with an authentic sample of ergosterol was confirmed by IR and TLC.

Compound C was recrystallized from benzene as colorless needles, mp 160° (lit. mp 161—162°,[6] mp 163—164°[8]), MS m/e: 220.0693 (M^+, Calcd for $C_{12}H_{12}O_4$: 220.0734); UV λ_{max}^{EtOH} nm (log ε): 231 (4.25), 255 (broad, 4.21), 291 (3.98), 330 (shoulder); IR ν_{max}^{KBr} cm^{-1}: 1655, 1620, 1580, 1440, 1400, 1340, 1300, 1212, 1180, 1132, 960, 840; ^{1}H-NMR (in $CDCl_3$) δ: 2.07 (3H, s), 2.33 (3H, s), 3.89 (3H, s), 6.00 (1H, s), 6.32 (1H, s). Its identity with an authentic sample of eugenitin (V) was confirmed by IR and TLC.

Eugenitin (10 mg) was dissolved in acetic anhydride (1 ml) containing one drop of sulfuric acid. The solution was heated for 10 min on a water bath and poured into ice-water. The precipitates were collected, washed with water, dried and recrystallized from benzene, mp 172° (lit.[8] mp 176—177°). IR ν_{max}^{KBr} cm^{-1}: 1753, 1648, 1609, 1445, 1380, 1362, 1340, 1215, 1122, 1080, 1040, 820.

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Compound A was recrystallized from methanol as colorless crystals of mp 238°, IR ν_{max}^{EtOH} cm^{-1}: 3520, 3350 (br.), 1670 (br.), 1603, 1470, 1352, 1052, 762. Its identity with an authentic sample of chaetocin (I) was confirmed by IR and TLC.[2)]

Compound D was recrystallized from benzene as pale yellow needles of mp 241—243°, IR ν_{max}^{KBr} cm^{-1}: 3340 (br.), 1630 (br.), 1590, 1485, 1230, 1126, 1092, 1062, 974, 844, 824. Its identity with an authentic sample of sterigmatocystin (III) was established by IR and TLC.[2)]

Compound F was recrystallized from methanol as colorless needles of mp 272—273°, $[\alpha]_D$ −327° (c=1.0, $CHCl_3$), MS m/e: 338.072 (M^+, Calcd for $C_{19}H_{14}O_6$: 338.076), IR ν_{max}^{KBr} cm^{-1}: 1644, 1619, 1593, 1473, 1277, 1100, 978, 813. Its identity with an authentic sample of *O*-methylsterigmatocystin (IV) was established by IR and TLC.[2,23)]

Compound G was recrystallized from benzene as colorless needles of mp 142°. *Anal.* Calcd for $C_7H_7NO_2$: C, 61.31; H, 5.15; N, 10.21. Found: C, 61.14; H, 5.15; N, 10.55. UV λ_{max}^{MeOH} nm (log ε): 214 (4.55), 246 (3.81), 330 (3.62); IR ν_{max}^{KBr} cm^{-1}: 3470, 3370, 3000, 2850, 2640, 1660, 1605, 1585, 1555, 1480, 1412, 1298, 1240, 1160, 913, 750; MS m/e: 137 (M^+). Its identity with an authentic sample of anthranilic acid was established by IR and TLC.

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23) The yield of *O*-methylsterigmatocystin (IV) depended on the incubation conditions; for example, when the strain (NHL 2827) was incubated at 23° for 3 weeks 435 mg/kg rice of the metabolite was produced, while at 30° for 2 weeks the yield was only 3 mg/kg rice.