Communications to the Editor

[Chem. Pharm. Bull. 32(10)4213—4216(1984)]

CHEMICAL STRUCTURE OF A NEW METABOLITE ISOLATED FROM THE MYCELIUM OF *ASPERGILLUS TERREUS* VAR. *AUREUS*

Kyosuke Nomoto[a], Kousei Mizukawa[a], Yoshiaki Kato[b], Mayuri Kubo[b], Teruo Kamiki[c] and Yoshihiko Inamori*,[b]

Suntory Institute for Bioorganic Research[a], Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan, Osaka College of Pharmacy[b], Kawai, Matsubara-shi, Osaka 580, Japan, and Public Health Research Institute of Kobe City[c], Minatojimanakamachi, Chuo-ku, Kobe-shi, Hyogo 650, Japan

From the mycelium of *Aspergillus terreus* var. *aureus*, a new metabolite, gillusdin (1), was isolated. 1 was found to be 5,7-dichloro-5',6-dihydroxy-6'-methoxy-4-methyl-3,4-dioxospiro [benzofuran 2(3H),1'-[2,5] cyclohexadiene]-2-carboxylic acid methyl ester based on a ^{13}C-NMR long-range selective proton decoupling (LSPD) experiment and other spectral data. Unlike the metabolites so far isolated from *Aspergillus terreus*, 1 is a new substance which seems to be biosynthesized *via* deoxyerythrolaccin as an intermediate.

KEYWORDS ——— gillusdin; metabolite; mycelium; *Aspergillus terreus* var. *aureus*; ^{13}C-NMR long-range selective proton decoupling experiment; 5,7-dichloro-5',6-dihydroxy-6'-methoxy-4-methyl-3,4-dioxospiro [benzofuran 2(3H),1'-[2,5] cyclohexadiene]-2-carboxylic acid methyl ester; erdin; geodin; deoxyerythrolaccin

As previously reported, six metabolites were isolated from the culture broth of *Aspergillus terreus* var. *aureus*: emodin, questin, dihydrogeodin, sulochrin, a new compound, 2-(3-chloro-4-methyl-γ-resorcyloyl)-5-hydroxy-m-anisic acid methyl ester, and another compound the chemical structure of which has not yet been clarified.[1)]

We have now isolated a new metabolite, named gillusdin (1), from the mycelium of *Aspergillus terreus* var. *aureus*, and here describe its chemical structure as 1.

The naturally dried mycelium of *Aspergillus terreus* var. *aureus* gathered in the shaking culture[1)] and the stationary culture (medium of the shaking culture + 1% agar) was extracted with ethyl acetate. 1 was isolated from the extract by silica gel column chromatography using benzene-ethyl acetate as an eluent.

Gillusdin (1), $C_{17}H_{12}Cl_2O_8$ (high resolution mass spectrum: M^+ 413.9921, Calcd for $C_{17}H_{12}Cl_2O_8$: 413.9909), mp 269.5-273°C, $[\alpha]_D^{25} \pm 0$ (c=1 in DMSO), CD (c=0.03 ethanol) $\Delta\varepsilon^{25}$: 0 (250), -0.16 (220), -0.33 (210), -0.69 (200), was positive in the Beilstein reaction. Absorption maxima in the UV spectrum were observed at 251, 285 and 385 nm, and in the IR spectrum absorption bands were observed at 3400 (OH), 1725

(ester), 1680 and 1615 (C=O) cm^{-1}. The ^{1}H-NMR spectrum (δ ppm, DMSO-d_6) of 1 exhibited three singlets at 2.50, 3.43 and 3.71, indicating a methyl, a methoxyl and a methoxycarbonyl group, respectively, and a singlet signal due to olefinic proton at 6.08. Furthermore, the structure of 1 was analyzed by ^{13}C-NMR long-range selective proton decoupling (LSPD) experiments. The full assignments of the ^{13}C-NMR peaks of 1 are summarized in Table I.

Table I. ^{13}C-NMR and LSPD Experiments: Assignments and Coupling Constants for Gillusdin (1) (DMSO-d_6)

Carbon	δ ppm	OFF resonance	NOE decoupling	LSPD with NOE							Coupling constant
				CH_3	OCH_3	$COOCH_3$	H	$OH^{a)}$	$OH^{a)}$ 3'-H	CH_3 3'-H	
C-3	193.2	s	brs	s[b]				s[b]	s[b]	s[c]	$^4J_{CH}$ < 0.8 Hz
C-4'	180.5	s	m[d]					d	s		$^2J_{CH}$=1.5 Hz, J_{COH}=2.9, 3.7 Hz
2'-$\underline{C}OOCH_3$	170.2	s	dq			d		q	q	q	$^3J_{CH}$=4.4 Hz, J_{COCH}=4.4 Hz
C-6'	166.7	s	q		s						J_{COCH}=4.4 Hz
C-7a	165.3	s	s								
C-6	149.5	s	s								
C-5'	147.8	s	d					s	s	s	$^3J_{CH}$=4.4 Hz
C-4	143.6	s	q	s						s	$^2J_{CH}$=5.9 Hz
C-5	114.6	s	q	s						s	$^3J_{CH}$=4.4 Hz
C-2'	110.4	s	s								
C-3a	106.9	s	q	s						s	$^3J_{CH}$=4.4 Hz
C-3'	102.7	d	d								$^1J_{CH}$=167.8
C-7	91.8	s	m[e]					s	s		J_{COH}=2.2, 3.7 Hz
C-1'	85.9	s	d					s	s	s	$^3J_{CH}$=6.6 Hz
2'-$COOCH_3$	57.6	q	q								$^1J_{CH}$=148.0 Hz
6-OCH_3	51.1	q	q								$^1J_{CH}$=148.0 Hz
4-CH_3	18.4	q	q								$^1J_{CH}$=129.7 Hz

a) Since the broad signal due to 2H of hydroxyl group was observed at 8.40-8.95 ppm, the variation of signals on irradiation at 8.70 ppm are described.

b) A singlet of small half band width was observed as compared with the broad singlet of NOE decoupling.

c) The carbon signal in C-3 changed a sharp singlet on irradiation at the proton of the methyl group (18.4 ppm) attached to carbon (143.6 ppm) and proton attached to carbon (102.7 ppm).

d) On addition D_2O, the multiplet changed to a doublet.

e) On addition D_2O, the multiplet changed to a singlet.

s: singlet, d: doublet, q: quartet, brs: broad singlet, dq: double quartet, m: multiplet.

As shown in Table I, the proton attached to the carbon at 102.7 ppm was coupled to the carbons at 180.5, 147.8, 193.2, 85.9 ppm and to the carbonyl carbon (170.2 ppm) of the methoxycarbonyl group attached to carbon (110.4 ppm) with coupling constants of 1.5, 4.4, 0.8, 6.6 and 4.4 Hz, respectively. These findings showed the partial structure of 1 to be A, Fig. 1. The proton of the methyl group (18.4 ppm) attached to the carbon at 143.6 ppm was coupled to the carbons at 106.9, 193.2, 143.6 and

114.6 ppm with coupling constants of 4.4, 0.8, 5.9 and 4.4 Hz, respectively. These results indicated the partial structure of 1 to be B. The proton of the hydroxyl group attached to the carbon at 147.8 ppm was coupled to the carbon at 180.5 ppm with coupling constants of 2.9 and 3.7 Hz, respectively. Further, the proton of the methoxyl group (51.1 ppm) attached to the carbon at 166.7 ppm was coupled to the carbon at 166.7 ppm with a coupling constant of 4.4 Hz. These findings indicated the partial structure of 1 to be C. The proton of the hydroxyl group attached to the carbon at 149.5 ppm was coupled to the carbon at 91.8 ppm with coupling constants of 2.2 and 3.7 Hz, respectively. The signal of the carbon at 91.8 ppm appeared in a higher field than those of other benzene carbons. However, the signal of carbon (C-7) combined with the chlorine atom in griseofulvin[2,3] also appeared at 95.2 ppm in a high field. The signal of the carbon at 91.8 ppm was thus assigned to the carbon combined with the chlorine atom in the partial structure D.

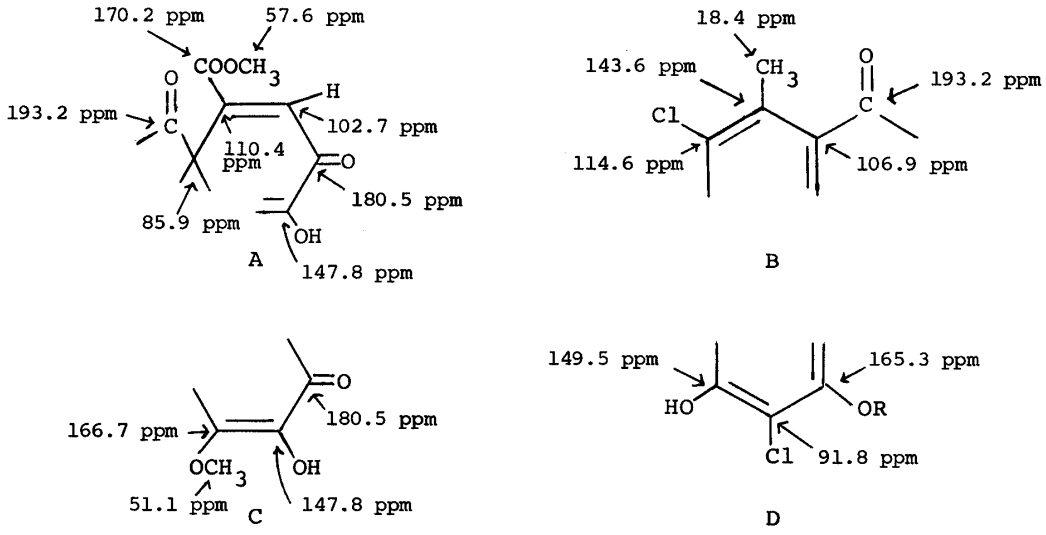


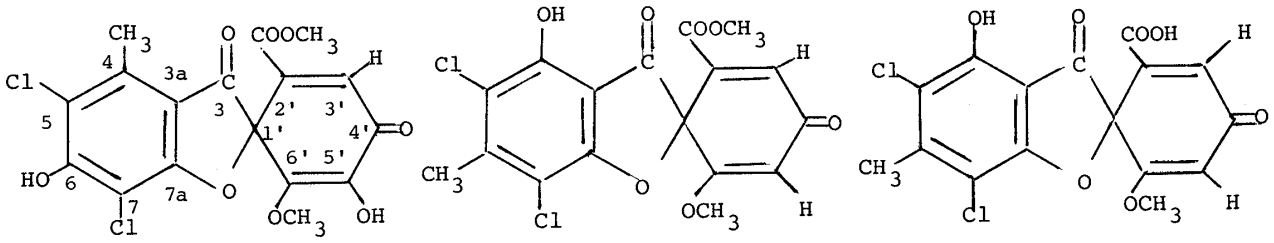
Fig. 1. The Partial Structures Indicated by ^{13}C-NMR Long-Range Selective Proton Decoupling Experiments

The nuclear overhauser effect was observed between the methyl group and the hydrogen atom attached to the carbon at 102.7 ppm. 1 was acetylated with pyridine-acetic anhydride to yield a diacetate $C_{21}H_{16}Cl_2O_{10}$ and methylated with diazomethane to yield a monomethyl ether $C_{18}H_{14}Cl_2O_8$. One of the hydroxyl groups was not methylated because of the strong hydrogen bond to carbonyl group (monomethyl ether, IR: 3400).

From these data, it was deduced that the chemical structure of 1 was 5,7-dichloro-5',6-dihydroxy-6'-methoxy-4-methyl-3,4'-dioxospiro [benzofuran 2(3H),1'-[2,5] cyclohexadiene]-2-carboxylic acid methyl ester with a spiran skeleton like erdin[4] and geodin[4] previously isolated from *Aspergillus terreus*. As shown in Fig. 2, the substituents attached to C-4 and C-6 of 1 were reversed compared with erdin and geodin. In this respect, the chemical structure of 1 is considerable interest. It suggests that 1 is biosynthesized *via* deoxyerythrolaccin[5] as the intermediate. Studies on the biosynthesis of 1 are in progress.

1 did not show any antifungal or antibacterial activities. On the other hand, geodin and erdin which have the same skeleton as 1 showed strong antifungal activity.

The relationship between antifungal activities and chemical structure, however, is not clear. Further studies on the activity-structure relationship and the stereochemistry of C-1' are in progress.



gillusdin (1) geodin erdin

Fig. 2. Chemical Structures of Gillusdin (1) and Relative Compounds Isolated from *Aspergillus terreus* sp.

ACKNOWLEDGEMENT The authors express their gratitude to Professor Mitsugi Kozawa, Osaka College of Pharmacy, for valuable advice. They also thank Dr. Hajime Komura, Suntory Institute for Bioorganic Research for the measurement of the CD spectrum.

REFERENCES AND NOTES

1) Y. Inamori, Y. Kato, M. Kubo, T. Kamiki, T. Takemoto and K. Nomoto, Chem. Pharm. Bull., 31, 4543 (1983).
2) S.G. Levine, R.E. Hicks, H.E. Gottlieb and E. Wenkert, J. Org. Chem., 40, 2540 (1975).
3) Y. Sato, T. Oda and S. Urano, Tetrahedron Lett., 1976, 3971.
4) C.T. Calam, P.W. Clutterbuck, A.E. Oxford and H. Raistrick, Biochem. J., 41, 458 (1947).
5) A. Yagi, K. Makino and I. Nishioka, Chem. Pharm. Bull., 22, 1159 (1974).

(Received July 11, 1984)