A NEW ANTITUMOR ANTIBIOTIC PRODUCT, DEMETHYLCHARTREUSIN

ISOLATION AND BIOLOGICAL ACTIVITIES

YOSHIYUKI AOYAMA, TAIJI KATAYAMA, MASASHI YAMAMOTO,
HISAKI TANAKA and KENJI KON

Biochemistry Research Laboratory,
Central Research Institute, Ishihara Sangyo Kaisha, Ltd.,
2-3-1 Nishi-shibukawa, Kusatsu, Shiga 525, Japan

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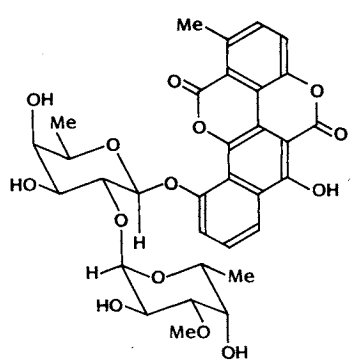
A new antitumor antibiotic, 3″-demethylchartreusin was isolated from the culture broth of *Streptomyces chartreusis*, as a minor component of crude chartreusin. It is structurally related to chartreusin, containing same aglycone of chartreusin, but different sugar moieties. 3″-Demethylchartreusin exhibits some potent inhibitory activities against murine tumors.

Chartreusin (**1**) is a *Streptomyces*-produced antibiotic that was originally reported in 1953.[1] It has significant antitumor effects against murine L1210, P388 leukemias, and B16 melanoma. When this drug was given iv, it was rapidly eliminated through the bile and therefore showed no activity by this route.[2] Related derivative of chartreusin, elsamicins which were isolated by KONISHI *et al.*,[3] have higher solubility and slower elimination than chartreusin. These results indicate the possibility to discover other soluble derivatives. So we started our investigation to search for more potent derivatives from culture broths of *Streptomyces chartreusis*. In this paper, we describe the identification, purification and biological activity of a compound related to chartreusin from *Streptomyces chartreusis*.

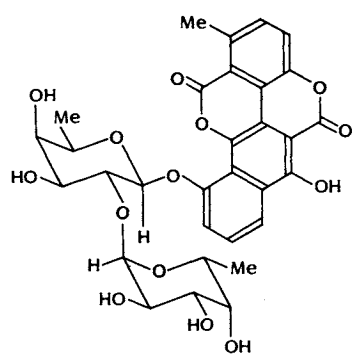
Fermentation

Spores from a slant culture of the producing organism (*Streptomyces chartreusis* IFO 1275) were inoculated into a 50-ml test tube. The culture medium (15 ml) consisted of lactose 4%, soybean powder 2%, corn steep liquor 2%, and $CaCO_3$ 5%, the pH being adjusted to 7.0 before sterilization. The test tube was then incubated at 28°C while being shaken at 200 rpm for 3 days. Then 15 ml of cultured broth was transferred into a 2-liter flask containing 500 ml of a culture medium having the same composition

Fig. 1. Structure of chartreusin and 3″-demethylchartreusin.



Chartreusin (**1**)



3″-Demethylchartreusin (**2**)

as above. The flask was then incubated at 28°C on a rotary shaker at 100 rpm for 3 days. Then 1 liter of cultured broth was transferred into a 50-liter jar fermenter containing 35 liters of a culture medium having the same composition as above. The 50-liter jar fermenter was run at 250 rpm at 28°C with aeration of 35 liters/minute for 7 days.

After the fermentation was carried out, the pH of culture broth (70 liters) was adjusted to 5.5~6.0 and stored overnight. Crude products were then salted out. The broth was then separated by using a Sharpless type centrifuge (Kokusan No. 4A) to yield 15 kg mycelial cake from the supernate.

Isolation and Purification

Mycelial cake (15 kg) was extracted twice with 18-liter volumes of a chloroform-methanol (2:1) mixture. After removal of the insoluble fraction, the combined extracts were concentrated *in vacuo* to a small portion. Methanol was added again to the resulting suspension which was vigorously stirred for 15 minutes at 50~60°C and stored at 4°C overnight. After filtration, the precipitate was washed with a ether-hexane (1:3) mixture, and dried *in vacuo* to give crude chartreusin containing some minor components. The crude chartreusin (10 g) was dissolved in chloroform, applied on a column of Silica gel 60 (70~230 mesh; E. Merck Co.) that was developed successively with chloroform, and chloroform-methanol mixtures of the following compositions (100:1, 80:1, 60:1, 40:1, 10:1, 5:1). The fractions containing a minor component were collected, and concentrated *in vacuo* to afford a crude product, which was recrystallized from a chloroform-methanol mixture to give pure 3″-demethylchartreusin (96 mg) as yellow powder.

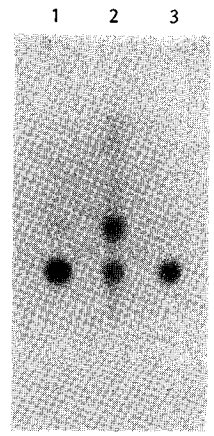
Physico-chemical Properties

The physico-chemical properties of 3″-demethylchartreusin are summarized in Table 1. The Rf value of this compound was clearly different from that of chartreusin. 3″-Demethylchartreusin was readily soluble in dimethyl sulfoxide, dimethylacetamide, dioxane and pyridine. On the other hand, it was slightly less soluble in hexane or ether.

Table 1. Physico-chemical properties of 3″-demethylchartreusin and chartreusin.

	3″-Demethylchartreusin	Chartreusin
Nature	Yellow powder	Yellow powder
MP (°C)	251~254	184~187
TLC		
Rf value ($CHCl_3$-MeOH, 8:1)	0.20	0.47
UV		
λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	235.8 (578), 264.7 (595), 399.1 (245)	235.5 (583), 265.8 (557), 399.1 (282)
Anal Calcd for	$C_{31}H_{30}O_{14}$: C 59.42, H 4.79	$C_{32}H_{32}O_{14}$: C 60.00, H 5.00
Found:	C 57.9, H 4.6	C 57.6, H 5.2
FD-MS *m/z* (positive)	626 (M), 480, 334	640 (M), 480, 334

Fig. 2. TLC of chartreusin's and 3″-demethylchartreusin's degradation products.



1; Fucose, 2; chartreusin's degradation products, 3; 3″-demethylchartreusin's degradation products. Solvent; benzene-methanol-acetic acid (3:1:1).

Chemical Degradation

A solution of 3″-demethylchartreusin (100 mg) in dioxane (12 ml) was refluxed with 3 N sulfuric acid (1 ml) for 30 minutes. The resulting solution was concentrated and then diluted with water. The aglycone precipitated as yellow needles which were filtered off. Supernatant was analyzed by TLC as shown in Fig. 2. The sugar moiety of chartreusin on TLC showed two spots, but that of 3″-demethylchartreusin showed only one spot. The aglycone of 3″-demethylchartreusin was identified by ^{1}H NMR (400 MHz in dimethyl sulfoxide), as identical with an authentic sample obtained from chartreusin.

NMR Spectra of 3″-Demethylchartreusin

NMR spectra were recorded on a Jeol JNM-GSX-400 spectrometer. Dimethyl sulfoxide (contained 5% D_2O) was used as solvent. Chemical shifts are given in δ values with tetramethylsilane as an internal standard.

All proton and carbon peaks were assigned as shown in Table 2. This minor compound is structurally related to chartreusin. It contains the common aglycone of chartreusin, but different sugar moieties. The ^{13}C NMR spectrum showed 31 carbons. The sugar moieties were quite similar to those of chartreusin except for one missing carbon. The distinctive singlet methoxy group in chartreusin could not be observed by ^{1}H and ^{13}C NMR. Thus the structure of this minor component was determined to be as shown in Fig. 1.

Antitumor Activity of 3″-Demethylchartreusin

Antitumor activity of 3″-demethylchartreusin in mice was examined comparatively with that of chartreusin against P388 leukemia and B16 melanoma. P388 leukemia was inoculated ip into BDF_1 mice at 10^6 cells per mouse. B16 melanoma was inoculated ip into BDF_1 mice as a tumor homogenate (mix 1 g of tumor with 10 ml of 0.9% saline and homogenize), 0.5 ml per mouse. Test compounds were dissolved in 0.9% saline containing 5% dimethylacetamide and 4% Tween 80, and various doses of these drugs were administered ip on days 1, 4, and 7 (Q3D × 3) or once daily for 9 days (Q1D × 9) after tumor

Table 2. ^{1}H and ^{13}C NMR spectra of 3″-demethylchartreusin.

	Site	^{1}H (δ, Hz)	^{13}C (δ)
Aglycone	2	7.97 (d, 9.6)	133.1
	3	7.51 (d, 9.6)	120.8
	7	7.49 (d, 8.0)	116.9
	8	7.68 (t, 8.0)	128.4
	9	7.33 (d, 8.8)	114.3
Sugar	1′	5.32 (d, 4.4)	99.8
	2′	3.94 (br s)	78.1
	3′	3.64*	72.0
	4′	3.62*	71.2
	5′	6.91 (q, 6.4)	70.2
	5′-CH_3	1.23 (d, 6.4)	16.5
	1″	5.36 (d, 7.2)	99.3
	2″	3.25 (dd, 3.6, 9.6)	68.1
	3″	3.41 (dd, 3.0, 10.0)	69.8
	4″	3.37 (br s)	71.6
	5″	4.15 (q, 6.4)	56.9
	5″-CH_3	0.95 (d, 6.4)	16.4

* Overlap with DHO peak.

Table 3. Antitumor activity of chartreusin and 3″-demethylchartreusin.

Tumor/ schedule	Dose (mg/kg/day)	Life prolongation effects T/C (%)[a]	
		3″-Demethyl-chartreusin	Chartreusin
B16[b]	160	177	134 (toxic)
ip-ip	80	153	161
Q1D × 9	40	167	153
	20	146	146
	10	138	132
	5	123	111
P388[c]	160	160	160
ip-ip	80	170	157
Q3D × 3	40	160	160
	20	136	139
	10	136	136

[a] T/C was calculated as (Median survival times of treated mice/Median survival times of control mice) × 100.
[b] 5 mice with 1 group.
[c] 3 mice with 1 group.

implantation. Survival of the treated and non-treated animals was recorded daily for 45 days after the tumor implantation, and the median survival time (MST) was calculated for each test and control groups. The results are shown in Table 3. 3″-Demethylchartreusin showed almost the same activity as chartreusin. Thus the methoxy group in the sugar moiety is not essential for antitumor activity.

Table 4. Antibacterial activity of 3″-demethylchartreusin and chartreusin.

Test organism	MIC (μg/ml)	
	3″-Demethyl-chartreusin	Chartreusin
Bacillus subtillis PCI219	6.25	<1.56
Staphylococcus aureus 209P	12.5	12.5
Escherichia coli keio	>25	>25
Pseudomonas aeruginosa Homastus	>25	>25

Antibacterial Activity of 3″-Demethylchartreusin

The minimum inhibitory concentration (MIC) of 3″-demethylchartreusin and chartreusin was determined against several Gram-positive and Gram-negative bacteria species, by the serial two-fold agar dilution method. As shown in Table 4, 3″-demethylchartreusin and chartreusin were inactive against Gram-negative bacteria. However, they showed activity against Gram-positive bacteria. The anti-Bacillus activity of 3″-demethylchartreusin was 4 times lower than that of chartreusin.

Discussion

3″-Demethylchartreusin is a novel antitumor antibiotic produced by *Streptomyces chartreusis*, and exhibits inhibitory activities against murine tumor, which are comparable to chartreusin. The methoxy group in 3″-position is not essential for these activities. This new structure gave us useful informations,[4] and these findings encourage us to continue our investigations.

Addendum in Proof

The 3″-demethylchartreusin was already reported in patents by Kirin Brewary Co., Ltd.[5] and Ishihara Sangyo Kaisha, Ltd.[6] They were prepared from almost the same strain (*Streptomyces chartreusis*).

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