Notes

A NOVEL COMPOUND RELATED TO CHARTREUSIN FROM A MUTANT OF *Streptomyces chartreusis*

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In the screening of microbial metabolites active against tumors, we found that *Streptomyces chartreusis* D329, isolated from soil collected in Ōmiya City, Saitama Prefecture, Japan, produced chartreusin (**1**)[1].

Though **1** itself is not utilized clinically, its semi-synthesized derivatives[2] with high therapeutic activity against mouse tumor and elsamicins[3], natural products having the same aglycone but different sugars, are expected to be useful based on its antitumor activities and unique structures.

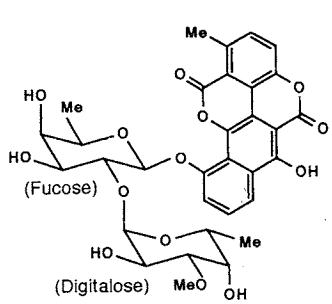
Therefore, we tried to mutate the *Streptomyces* with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in order to produce the novel antibiotics related to **1**. First, the fermentation broths from mutated strains were analyzed with TLC and UV (366 nm) exposure. Some broths, whose TLC showed to have a characteristic fluorescing spot with a different Rf value from that of **1**, were further analyzed with an HPLC system having a photodiode array. From the analysis of about 3,500 broths, we found a mutant strain (D329-185 strain, FERM BP-3269) which produced two chartreusin related compounds. One compound was identified as demethylchartreusin[4] by comparing its various spectral data, and the other, called D329C compound (**2**), showed UV and IR spectra similar to those of **1** but different spectral data from those of **1**.

This paper describes the fermentation, the isolation and structural determination of the novel **2**.

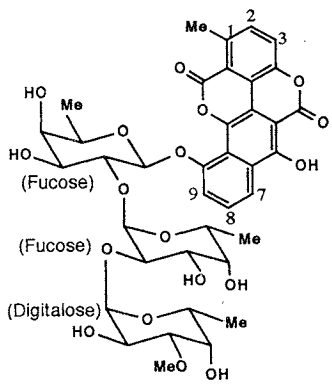
The *Streptomyces chartreusis* D329-185 strain was cultivated in 3 liters flasks containing 600 ml each of fermentation medium (dry yeast 1.0%, K_2HPO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, Mannitol 0.5%, $FeSO_4 \cdot 2H_2O$ 0.00001%, $MnCl_2 \cdot 4H_2O$ 0.00001%, $ZnSO_4 \cdot 7H_2O$ 0.00001%, $CuSO_4 \cdot 5H_2O$ 0.00001%, $CoCl_2 \cdot 6H_2O$ 0.00001%, adjusted to pH 8.0). The fermentation was carried out on a rotary shaker at 30°C for 8 days.

The fermentation broth described above was extracted with *n*-BuOH. The extract was concentrated to afford an oily residue, which was separated on ODS column chromatography (YMC AQ-type, YMC Co., Ltd., H_2O-acetonitrile = 7 : 3). The fractions containing **2** were collected, and were successively purified on a reverse phase HPLC (Waters Nova-pak HR C18 i.d. 25 × 100 mm) eluted with H_2O-acetonitrile (7 : 3). 19 mg of **2**

Fig. 1. Structures of chartreusin and D329C.



Chartreusin (**1**)



D329C (**2**)

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Table 1. Physico-chemical properties of D329C.

	D329C
Appearance	Yellow powder
Molecular formula	$C_{38}H_{42}O_{18}$
HRFAB-MS (m/z) $(M+Na)^+$	
Found:	809.2330
Calcd:	809.2269
$[\alpha]_D^{20}$ (c 0.5, MeOH)	−61.8°
UV λ_{max} nm (ε) in MeOH	235 (43,000), 264 (42,000), 332 (7,100), 376 (9,400), 399 (16,000), 422 (17,000)
IR ν_{max} (KBr) cm^{-1}	3440, 2940, 1700, 1380, 1260, 1240, 1080
MP (°C)	194~199
TLC Rf value[a]	
CH_2Cl_2 - MeOH (85:15)	0.27

[a] Silica gel TLC (Merck Art. No. 5715).

Table 2. ^1H and ^{13}C NMR data of D329C.

	Position	^1H (δ, Hz)		^{13}C (δ)
Aglycone	2	7.30 d	8.3	132.8
	3	7.41 d	8.3	120.5
	7	8.36 br d	8.0	118.3
	8	7.59 t	7.2	128.1
	9	7.94 d	7.8	116.6
	1-Me	2.80 s		22.1
Sugar	1′	6.01 d	7.7	100.9
	2′	5.14 dd	7.7, 9.5	78.0
	3′	4.27 dd	9.5, 3.6	73.7
	4′	4.07 d	3.6	73.0
	5′	3.97 q	6.4	71.7
	5′-Me	1.44 d	6.4	17.0
	1″	6.59 d	3.6	96.9
	2″	4.59 dd	3.6, 10.0	73.8
	3″	4.66 dd	10.0, 3.4	70.0
	4″	4.11 d	3.4	73.5
	5″	5.20 q	6.6	67.2
	5″-Me	1.46 d	6.6	17.0
	1‴	5.60 d	3.7	96.4
	2‴	3.53 br d		68.5
	3‴	3.61 dd	9.8, 2.7	81.4
	4‴	3.78 d	2.7	69.0
	5‴	4.35 q	6.3	67.0
	5‴-Me	0.94 d	6.3	16.3
	3‴-OMe	3.58 s		56.7

^1H NMR: 400 MHz, pyridine-d_5, 60°C.
^{13}C NMR: 100 MHz, pyridine-d_5, 60°C.

was obtained from 10.2 liters of the fermentation broth. The mutant strain also produced both **1** and demethylchartreusin (for example, **2**: demethylchartreusin : **1** = 1 : 210 : 350, by HPLC quantitative analyses).

The UV and IR spectra of **2** were very similar to those of **1**. The FD-MS spectrum suggested that the molecular weight was 786, which was confirmed by HR-MS (Table 1).

^1H NMR of **2** is shown in Table 2.

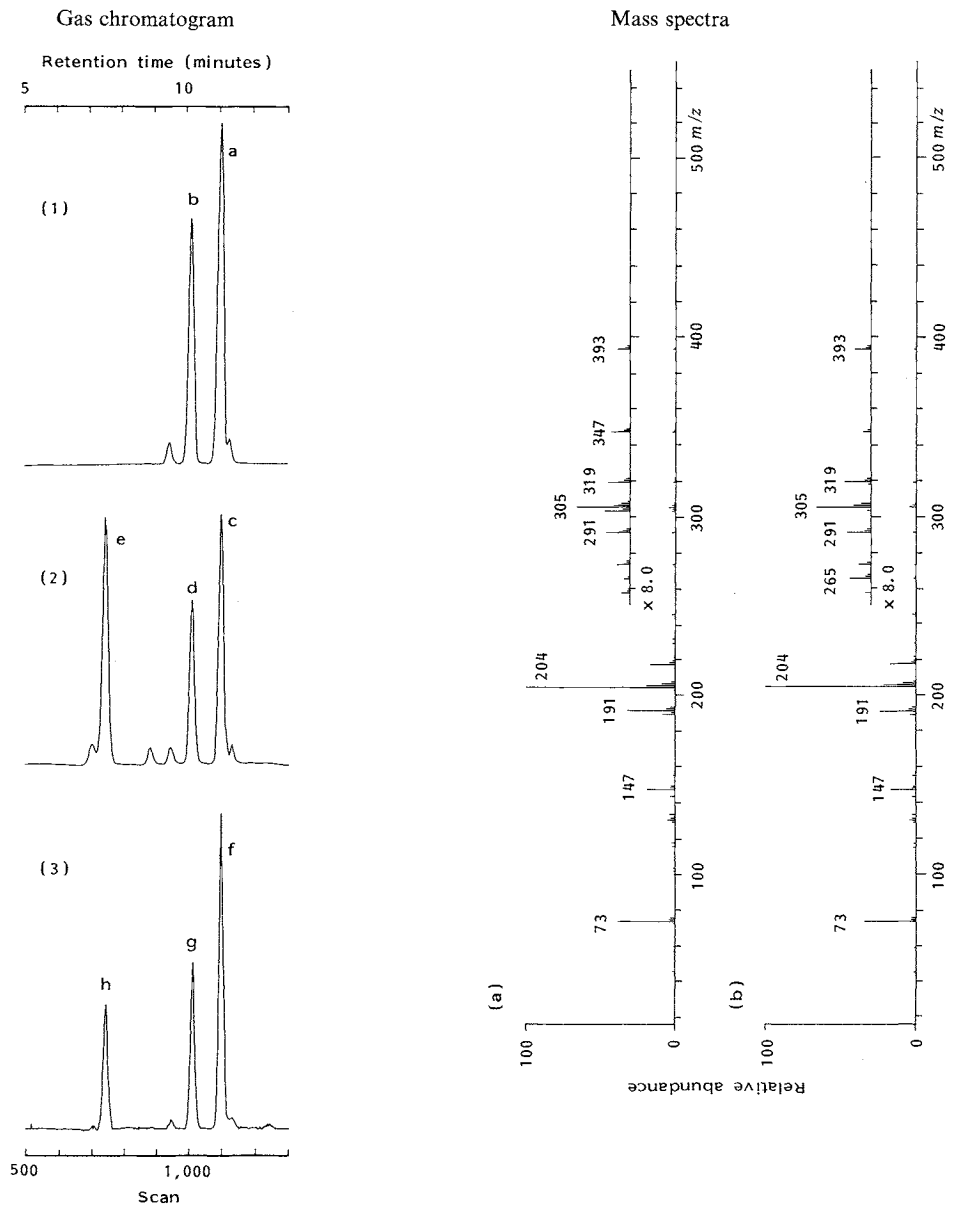
By detailed analysis of ^1H NMR (Table 2), COSY and ^{13}C NMR (Table 2) and by subtracting the molecular formula of **1** from that of **2**, we estimated that **2** had three sugars attached to chartarin[5] (aglycone of **1**).

In confirmation of the above, first trimethylsilyl derivatives (synthesized with a trimethylsilylation kit (TMS-HT, Tokyo Kasei Kogyo Co., Ltd.)) of commercially available fucose were analyzed with GC-MS (Fig. 2(1)). Qualitative analyses were carried out compared with MS fragment patterns, and quantitative ones were done compared with gas chromatogram peak area. Subsequently, the hydrolysis (1 N HCl, 100°C, 1 hour) products of **1** were converted to trimethysilyl derivatives under similar conditions. As a result of GC-MS analysis, three peaks were observed (Fig. 2(2)). The latter two peaks (α-anomer and β-anomer derivatives) had same retention times and MS fragment patterns to those of the authentic fucose derivatives. The former peak (α-anomer and β-anomer derivatives accidentally had the same retention time†) therefore was assigned as that of trimethylsilyl digitalose derivatives. The area of fucose derivatives in the gas chromatogram was equal to that of digitalose derivative. Consequently, it was confirmed that the sugars of **1** consisted of one molar equivalent of fucose and one molar equivalent of digitalose. In a reaction similar to that discussed above, two molar equivalent of fucose derivatives and one molar equivalent of digitalose derivative were obtained from **2** (Fig. 2(3)). On the other hand, the aglycone from **2** and that of **1** showed the same Rf value with several solvent systems on TLC.

3″-Position and 5″-position protons of the second

† Because the chromatograms (Fig. 2) are TIC (Total Ion Chromatogram), it is not quantitative in the strict sense. Accordingly, the exact quantitative analyses were carried out using also FID (Flame Ionization Detection) elsewhere. The FID analyses were performed under the below condition (column: 3% SE-30 on Chromosorb W, i.d. 3 mm × 1 m; carrier gas: N_2, 30.0 ml/minute; oven temperature: 130°C). Because of the TIC were measured using longer column than FID, high resolution charts were shown in Fig. 2. Although the α- and β-anomer of digitalose were not separated in these two condition, the two anomers could been separated using more higher resolution column.

Fig. 2. GC-MS chromatogram of sugar moiety derivatives.



(1) Trimethylsilyl-derivatives of authentic fucose, (2) trimethylsilyl-derivatives of the sugars from chartreusin, (3) trimethylsilyl-derivatives of the sugars from D329C.

GC condition (column: SPB-5, Supelco, Inc., i.d. 0.53 mm × 15 m; carrier gas: He, 10.0 ml/minute; oven temperature: 120°C for 6 minutes and heated 5°C/minute to 230°C).

Fig. 2. (Continued)

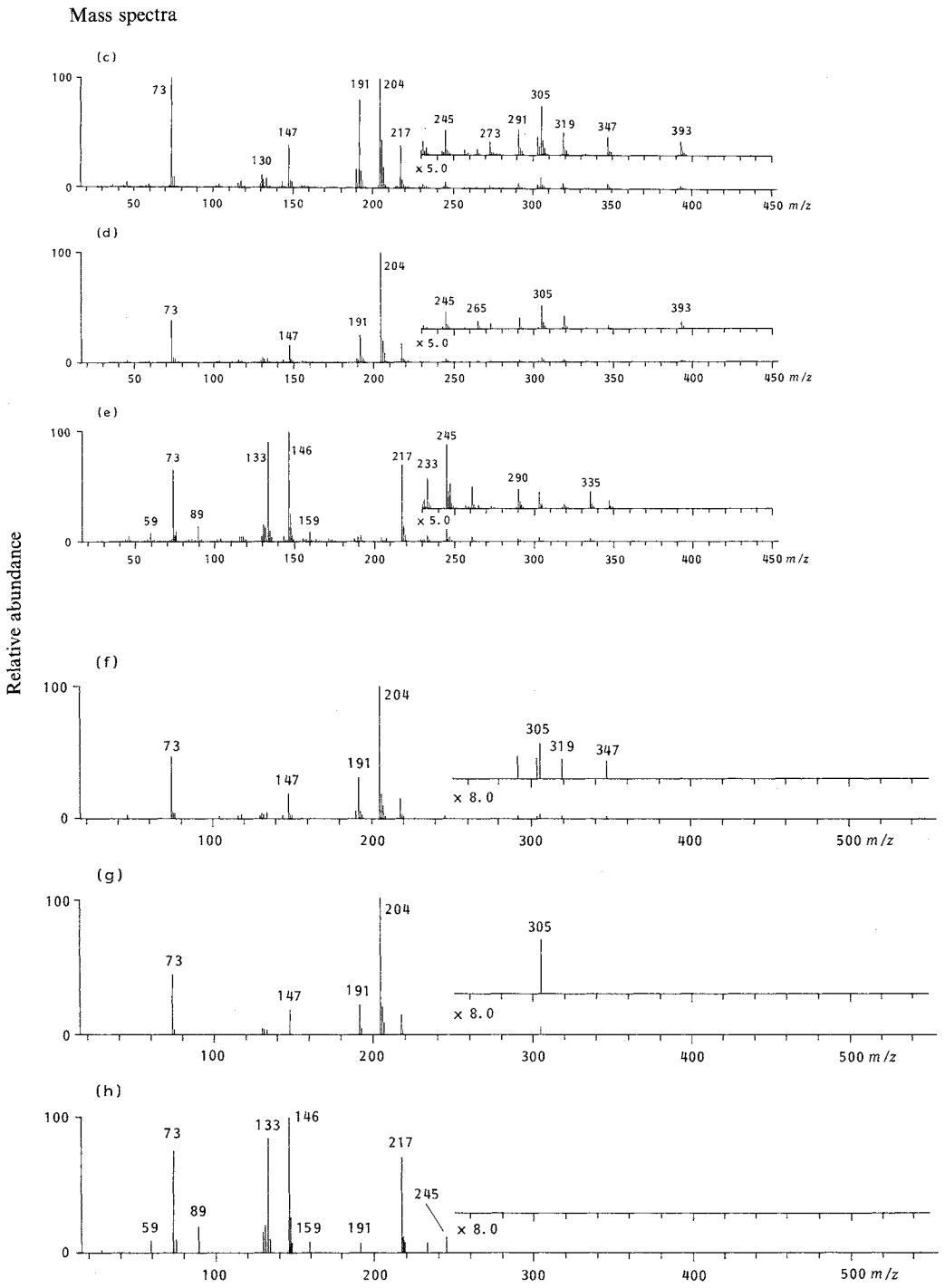
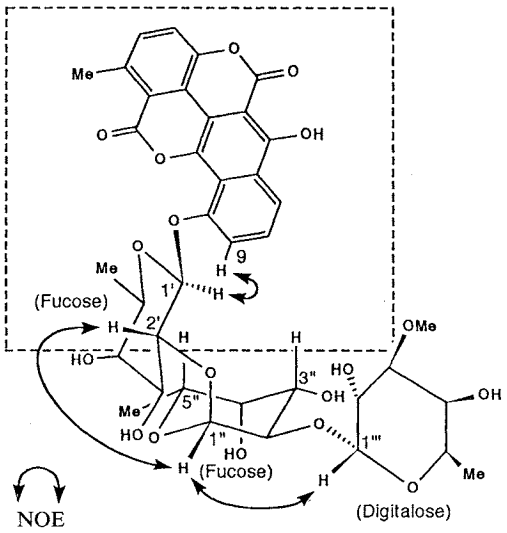


Fig. 3. Proposed comformation of D329C.



D329C (**2**)

fucose appeared in somewhat lower field in ^{1}H NMR. Pyridine-d_5 was used in the detailed ^{1}H NMR analyses, because of broad and unanalizable ^{1}H NMR signals of **2** in methanol-d_4 and DMSO-d_6.

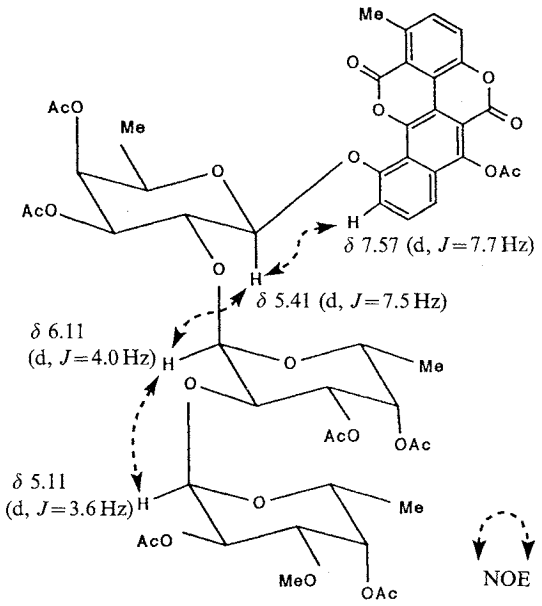
Generally speaking, the low field shifts were attributable to anisotropic effect of the pyridine-d_5 and the aglycone. In detail, the 3″-position proton (δ 3.84, dd, J=10.0, 6.8 Hz) and the 5″-position proton (δ 4.92, q, J=6.4 Hz) are present in ^{1}H NMR spectrum (50°C, pyridine-d_5) of **1**. The addition of one sugar causes change conformation, therefore, the lower chemical shift on 3″-position and 5″-position in **2** was observed. In consideration of a conformation suggested from NOE's of **2**, the 3″- and 5″-position protons are roughly located in the aglycone plane (dotted square in Fig. 3) and affected by the anisotropy effect from the aglycone.

In order to confirm the number of hydroxyl groups, we synthesized D329C acetate. ^{1}H NMR and FD-MS spectra (m/z 1,080 M^+) of the hepta-acetate supported the structure shown in Fig. 1. The analysis of NOESY of the acetate confirmed the order of the three sugars which is shown in Fig. 4.

The *vitro* cytotoxity of **2** showed IC_{100}=100 μg/ml (against W98 Vac cells). Although **2** showed no antitumor activities and no acute toxicities *in vivo* against Meth A (ip-ip) for max dose 400 mg/kg, higher dose assay was not carried out. On the other hand, **1** showed antitumor activities (T/C 150%, Meth A, ip-ip, 20 mg/kg).

Fig. 4. Connective order of sugars.

^{1}H NMR (400 MHz, $CDCl_3$, 45°C).



In addition to **2**, the mutant strain seems to produce some other compounds related to **1**; therefore, the analysis of the unknown compounds is also being investigated.

References

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