CHRYMUTASINS: NOVEL-AGLYCONE ANTITUMOR ANTIBIOTICS FROM A MUTANT OF *Streptomyces chartreusis*

II. CHARACTERIZATION AND STRUCTURAL ELUCIDATION

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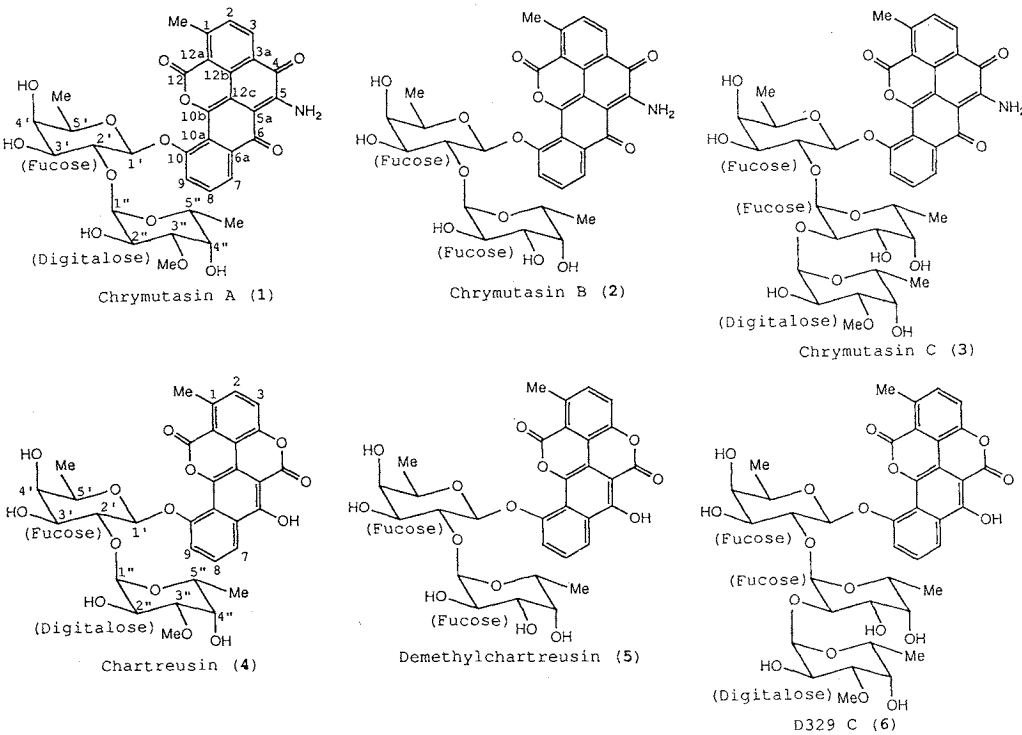
Chrymutasins A, B and C are glycosidic antibiotics produced by a mutant of the chartreusin producer-organism *Streptomyces chartreusis*. We report here the structure elucidation of these compounds. The sugar moieties involved were determined by comparison with the related chartreusins. The structure of the aglycone, the same in all three compounds, was elucidated by NMR, incorporation studies of labeled compounds and synthesis of derivatives. The chrymutasin aglycone differs from that of chartreusin by a single carbon and an amino group.

The chrymutasins[1] are novel antitumor antibiotics produced by *Streptomyces chartreusis* D329-185. Chrymutasins A (**1**), B (**2**) and C (**3**) are related compounds corresponding to chartreusin[2] (**4**), demethylchartreusin[3] (**5**) and the compound D329C[4] (**6**), respectively (Fig. 1). In the preceding paper[5] the

Fig. 1. Structures of chrymutasins, chartreusin and related compounds.



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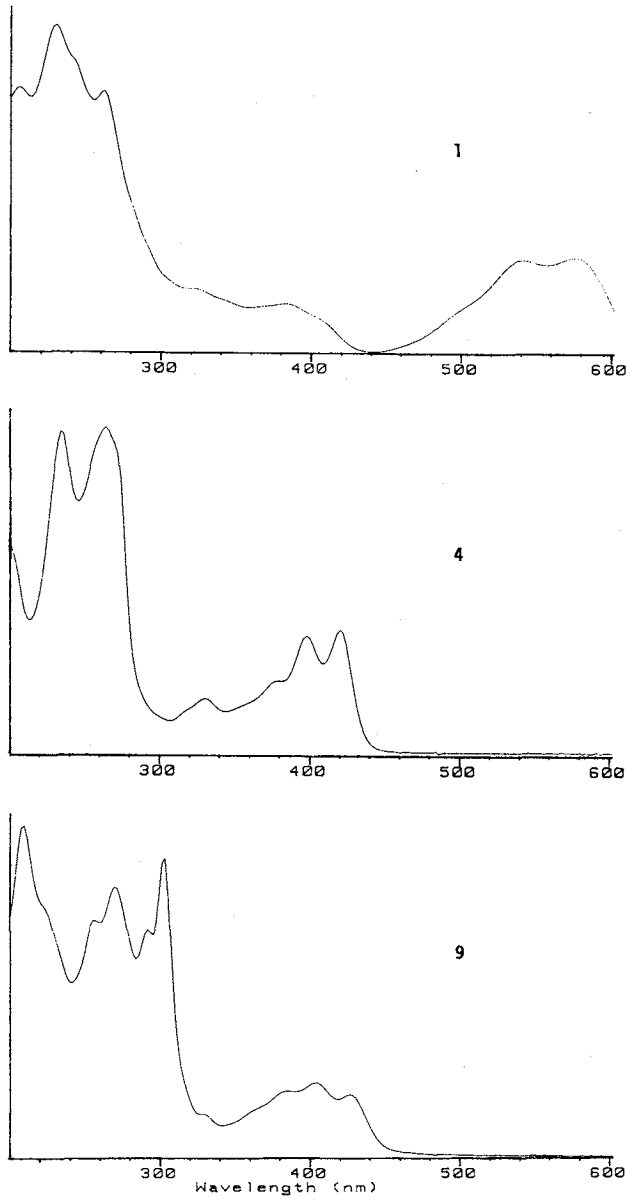
taxonomy, mutation, fermentation, isolation and biological activities of chrymutasins have been described. In this paper, we report the structure elucidation of the chrymutasins.

Results and Discussion

Compound **1**, $C_{33}H_{33}NO_{13}$, isolated as a violet powder, showed a characteristic UV absorption (Fig. 2). ^{1}H and ^{13}C NMR data are shown in Table 1. Other physico-chemical properties are shown in Table 2. The structure elucidation of the sugars and aglycone of **1** are described separately below. Comparison

Fig. 2. UV spectra of chrymutasin A (**1**), chartreusin (**4**) and compound **9**.



These UV spectra were measured on a HPLC system having a photodiode array with gradient (CH_3CN-H_2O, at neutral pH).

was made with the data for **4** (**4**, **5** and **6** were also isolated from fermentations of the same strain.).

Sugars

It was assumed that the sugars of **1** and **4** were the same by a direct comparison of the NMR data

Table 1. ^1H and ^{13}C NMR data for chrymutasins A, B and C.

Position	Chrymutasin A (**1**)			Chrymutasin B (**2**)		Chrymutasin C (**3**)	
	^1H (δ, Hz)[a]		^{13}C (δ)[b]	^1H (δ, Hz)[a]		^1H (δ, Hz)[c]	
Aglycone							
1			152.3 s				
2	7.33 d	8.0	132.2 d	7.34 d	8.1	7.34 d	8.1
3	8.43 d	8.0	133.5 d	8.47 d	8.1	8.54 d	8.1
3a			125.6 s				
4			179.0 s				
5			147.7 s				
5a			104.0 s				
6			182.3 s				
6a			135.0 s				
7	8.40 d	8.0	121.2 d	8.42 d	8.1	8.37 d	7.9
8	7.54 t	8.0	130.9 d	7.54 t	8.1	7.52 t	7.9
9	7.73 d	8.0	119.6 d	7.73 d	8.1	8.01 d	7.9
10			156.1 s				
10a			120.1 s				
10b			146.2 s				
12			159.1 s				
12a			118.5 s				
12b			134.0 s				
12c			106.9 s				
1-Me	2.79 s		23.9 q	2.80 s		2.78 s	
5-NH_2	9.1 br s			9.1 br s		9.4 br s	
	11.3 br s			11.3 br s		11.4 br s	
Sugar							
1′	5.75 d	7.6	101.2 d	5.72 d	7.5	6.07 d	7.6
2′	4.97 dd	7.6, 9.4	80.5 d	4.96 dd	7.5, 9.5	5.18 dd	7.6, 9.5
3′	4.27 dd	9.4, 3.4	74.1 d	4.21 dd	9.5, 3.3	4.29 dd	9.5, 3.5
4′	4.17 d	3.4	72.7 d	4.14 d	3.3	4.07 d	3.5
5′	4.07 q	6.4	71.9 d	4.04 q	6.4	3.92 q	6.4
5′-Me	1.55 d	6.4	17.1 q	1.53[e] d	6.4	1.42 d	6.4
1″	6.36 d	4.0	101.7 d	6.38 d	3.4	6.65 d	3.4
2″	4.51 dd	4.0, 10.0	69.1 d	4.40 dd	3.4, 9.5	4.71 dd	3.4, 10.0
3″	3.83 dd	10.0, 3.0	81.6 d	4.45 br dd	9.5, 2.7	4.76 dd	10.0, 3.0
4″	4.11 br s		69.5 d	4.10 br d	2.7	4.18 br d	3.0
5″	4.90 q	6.4	67.6 d	4.94 q	6.4	5.32 q	6.4
5″-Me	1.55 d	6.4	17.1 q	1.54[e] d	6.4	1.51 d	6.4
3″-OMe	3.35 s		56.9 q				
1‴						5.75 d	3.7
2‴						3.96 dd	3.7, 10.0
3‴						3.73 dd	10.0, 3.0
4‴						3.84 br d	3.0
5‴						4.45 q	6.8
5‴-Me						0.96 d	6.8
3‴-OMe						3.21 s	

[a] ^1H NMR: 400 MHz, pyridine-d_5, 50°C.
[b] ^{13}C NMR: 100 MHz, pyridine-d_5, 50°C.
[c] ^1H NMR: 400 MHz, pyridine-d_5, room temperature.
[e] Assignments may be reversed.

Table 2. Physico-chemical properties of chrymutasins A, B and C.

	Chrymutasin A	Chrymutasin B	Chrymutasin C
Appearance	Violet powder	Violet powder	Violet powder
Molecular formular	$C_{33}H_{33}NO_{13}$	$C_{32}H_{31}NO_{13}$	$C_{39}H_{43}NO_{17}$
HRFAB-MS (*m/z*)			
Mode	Positive	Negative	Positive
Found:	676.2039 $(M+Na+H_2)^+$	637.1791 $(M)^-$	820.2423 $(M+Na)^+$
Calcd:	676.2006	637.1796	820.2428
$[\alpha]_D^{20}$ (*c* 0.001, pyridine)	−100,000	−220,000	−24,000
UV λ_{max} nm (ε)			
in MeOH	228 (16,000), 243 (13,000, sh), 262 (12,000), 385 (2,600), 534 (5,200), 572 (5,600)	229 (20,000), 242 (18,000, sh), 262 (17,000), 385 (5,600), 534 (7,200), 570 (7,600)	231 (26,000), 243 (23,000, sh), 263 (22,000), 387 (4,800), 537 (9,600), 573 (10,000)
in 0.1 N NaOH-MeOH	215 (44,000), 249 (12,000), 270 (8,800), 365 (3,600), 675 (2,000)	216 (43,000), 242 (22,000), 270 (16,000), 365 (6,400), 680 (4,000)	216 (44,000), 243 (23,000), 265 (18,000), 365 (6,400), 670 (3,200)
IR ν_{max} (KBr) cm^{-1}	3430, 2940, 1730, 1590, 1300, 1080, 1060	3450, 2930, 1720, 1590, 1280, 1070	3430, 2920, 1730, 1590, 1290, 1070
MP (°C)	183~198	249~255	188~192
TLC Rf value[a]			
CH_2Cl_2-MeOH (85:15)	0.68	0.42	0.24
CH_2Cl_2-MeOH (90:10)	0.41	0.12	0.09
EtOAc-MeOH (80:20)	0.46	0.22	0.16

[a] Silica gel TLC (Merck Art. No. 5554).

of the sugar moieties of **1** with those of **4** (Figs. 3 and 4). In order to confirm this assumption, the trimethylsilyl derivatives of the sugars obtained from the hydrolysis of **1** and **4** were analyzed with GC-MS using similar methods[4]. These analyses show that the derivatives of **1** and **4** gave the same chromatograms and MS fragment patterns (Fig. 5). These results confirm that the sugars of **1** consisted of one molar equivalent of fucose and one equivalent of digitalose.

The connective order of the sugars was determined from the NOESY experiments (Fig. 6). In addition, this was supported by the NOEs of the tetraacetate derivative (**7**).

The constituent sugars of **2** and **3** (Fig. 1) were determined by GC-MS analysis of the trimethylsilyl derivatives of the sugars in a manner similar to that previously described (Fig. 5) and by detailed comparison of their NMR data (Tables 1 and 3; Figs. 3, 4, 7, 8 and 9). The ^1H NMR spectrum of the sugars of **2** (Fig. 7) was similar to that of **1** (Fig. 3) except for the 3″-proton of the fucose, because of the presence of a hydroxyl group rather than a methoxyl group of **1**. In the case of **3** (Fig. 8) and **6** (Fig. 9), the sugar resonances of the two spectra were similar except for 2‴-proton and 3‴-methoxyl group of the digitalose. This difference is attributable to the anisotropy effect for their different conformation. Analyzing NOEs of **6** (9-H↔1′-H, 2′-H↔1″-H, 1″-H↔1‴-H), 3‴-OMe (δ 3.58) seemed to be roughly located on the aglycone plane and 2‴-proton (δ 3.53) roughly placed over the plane[4]. On the other hand, two pairs of NOEs (9-H↔1′-H and 1″-H↔1‴-H) were observed in **3** but no NOE was between 2′-H and 1″-H. It was presumed that the digitalose in **3** was not near the aglycone. Therefore, 3‴-methoxyl group in **3** showed a higher chemical shift (δ 3.21) than that of **6** and 2‴-proton in **3** showed a lower one (δ 3.96) than that of **6**.

Fig. 3. ^{1}H NMR spectrum of chrymutasin A (**1**). (400 MHz, pyridine-d_5, 50°C).

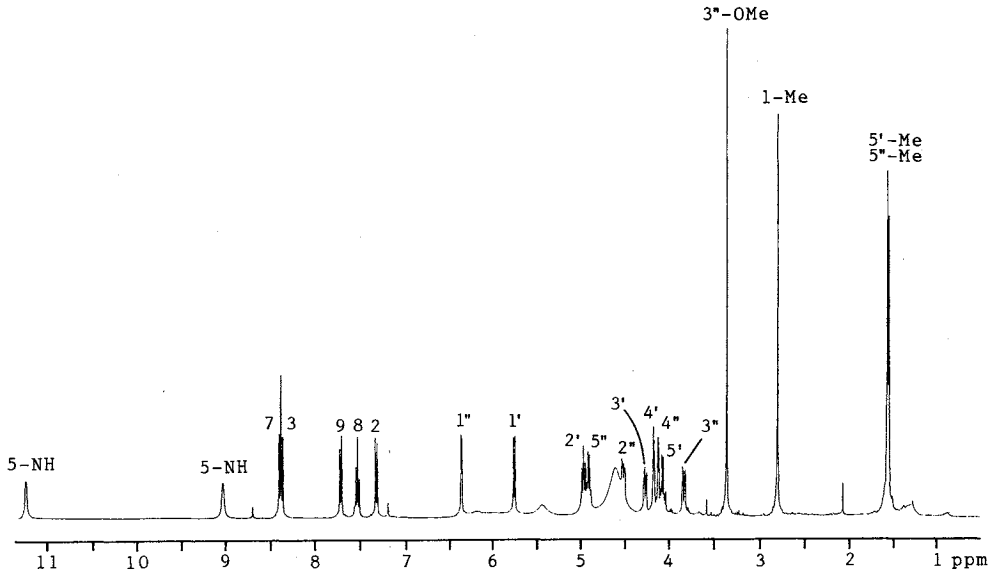
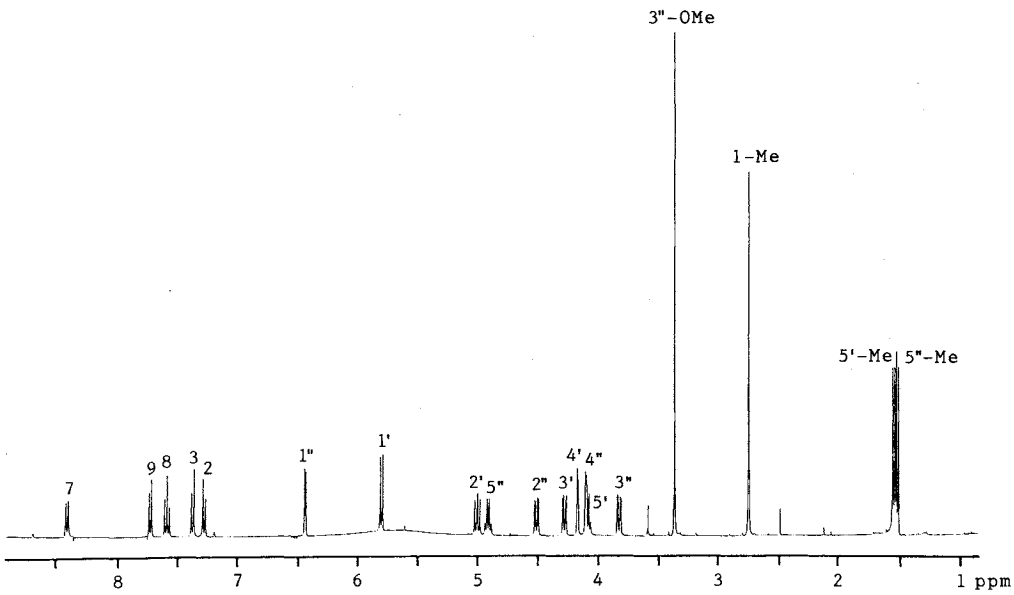


Fig. 4. ^{1}H NMR spectrum of chartreusin (**4**). (400 MHz, pyridine-d_5, 50°C).

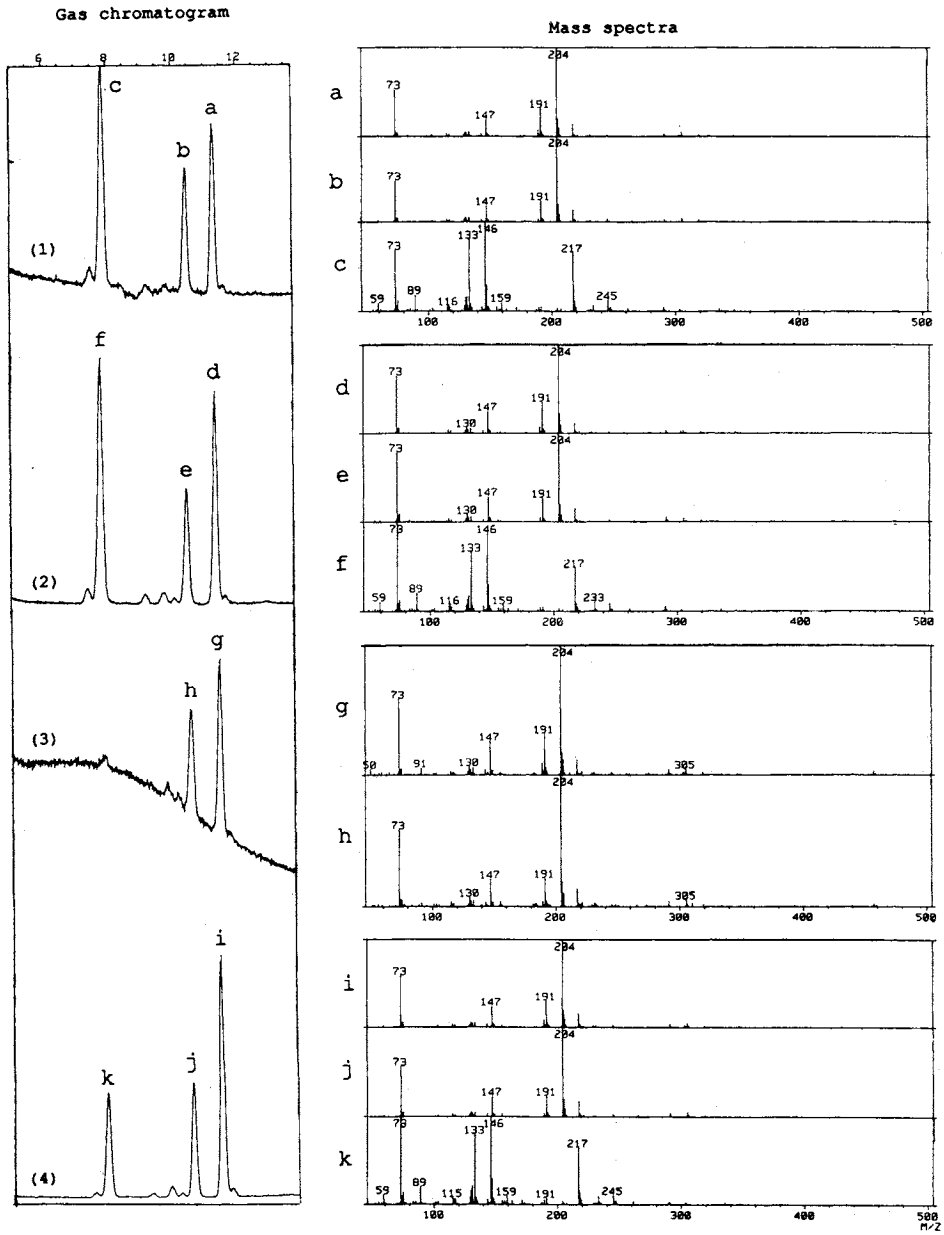


Aglycone

The similar UV spectra (Table 2) of **1**, **2** and **3** immediately suggested that these compounds had the same chromophore. The aglycones from **1**, **2** and **3** had the same retention time on HPLC, thereby confirming that these three compounds had the same aglycone, chrymutin (**8**).

Some experiments, which determined the aglycone structure and the assignments of the ^{13}C NMR, are described below. Since **8** was poorly soluble in most NMR solvents, the NMR analysis of the aglycone

Fig. 5. GC-MS of sugar derivatives.



(1) Trimethylsilyl-derivatives of fucose and digitalose from chartreusin. (2) Trimethylsilyl-derivatives of the sugars from chrymutasin A (**1**). (3) Trimethylsilyl-derivatives of the sugars from chrymutasin B (**2**). (4) Trimethylsilyl-derivatives of the sugars from chrymutasin C (**3**).

moiety was carried out with **1**. The complete decoupling, DEPT, and ^{13}C-^{1}H HETCOR data revealed the direct connection of five aromatic protons to five carbons and all of the assignments of the sugar carbons (Fig. 10). The ^{13}C-^{1}H long range COSY (8 Hz) permitted the assignments of fourteen of the twenty aglycone carbons (Fig. 11).

Two broad peaks (δ 9.3 and 11.3) in the ^1H NMR spectra were coupled to each other (by COSY) and disappeared with addition of D_2O. HR-MS revealed the presence of one nitrogen atom, and **1** was basic property, therefore, the presence of a NH_2 group was assumed.

In addition to the data described above, the carbon skeleton of the aglycone was anticipated to be closely related to chartarin[6], thus a proposed aglycone structure was arrived at (Fig. 10). It was shown that two carbons (δ 179.0 and 104.0) were coupled to two amino protons using LSPD (long-range selective proton decoupling) experiments (Figs. 10 and 12). Two carbons (δ 147.7 and 106.9) were uncoupled to any protons, and consequently, the LSPD experiment could not be applied.

In order to determine the assignments of the remaining four carbons (δ 159.1, 147.7, 146.2 and 106.9), the incorporation of ^{13}C labeled precursors was tried. A previous report[7] has demonstrated that chartarin was biosynthesized *via* a polyketide pathway. First incorporation of ^{13}C in **4** by cultivation of the mutant strain with sodium [1-^{13}C]acetate was verified. Subsequently, singly and doubly ^{13}C labeled **1** were isolated from fermentations as previously described[5] for unlabeled **1**. Results of the ^{13}C NMR experiments are shown in Table 4. Nine pairs of ^{13}C-^{13}C direct connections from labeled acetate are summarized in Fig. 13. ^{13}C-^{13}C direct couplings indicated the assignments of C_4 (δ 179.0), C_{12} (δ 159.1) C_{12c} (δ 106.9) and C_{5a} (δ 104.0).

For the remaining two carbons (δ 147.7, 146.2), $^2J_{C-D}$ (^{13}C-N-*D*) coupling and an isotope shift (about 0.5 ppm) to high field of the carbon at δ 147.7 appeared with the addition of D_2O (Fig. 14). Therefore, the carbon at δ 147.7 was directly connected to the NH_2 group, and assignment of the

Fig. 6. NOEs of chrymutasin A (**1**).

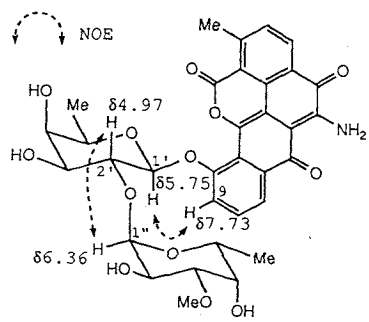


Table 3. ^{13}C NMR data for chrymutasins A, B and C. (100 MHz, pyridine-d_5, room temperature).

Chrymutasin A ^{13}C (δ)	Chrymutasin B ^{13}C (δ)	Chrymutasin C ^{13}C (δ)	Chrymutasin A ^{13}C (δ)	Chrymutasin B ^{13}C (δ)	Chrymutasin C ^{13}C (δ)
182.0	182.2	182.3	100.9	101.0	96.6
178.8	179.0	179.3	81.6	80.5	96.3
158.9	159.0	158.9	80.2	74.1	81.3
156.1	156.2	155.2	74.2	73.5	77.9
152.2	152.2	152.1	72.7	72.6	73.63
147.8	147.9	147.9	71.9	71.9	73.56
145.9	146.0	145.9	69.4	71.9	73.4
134.9	134.9	135.0	69.0	70.5	72.9
133.7	133.9	134.3	67.4	67.6	71.6
133.4	133.4	133.4	56.8	24.0	69.9
132.2	132.1	132.0	24.1	17.2	68.7
130.8	130.9	130.7	17.2	17.1	68.6
125.3	125.4	125.7	17.1		67.1
120.9	121.0	121.4			66.9
119.8	119.8	120.7			56.5
119.1	119.2	120.2			24.1
118.3	118.4	118.8			17.2
106.8	106.8	107.2			17.0
103.7	103.8	104.1			16.5
101.8	102.1	100.5			

Fig. 7. ^{1}H NMR spectrum of chrymutasin B (**2**). (400 MHz, pyridine-d_5, 50°C).

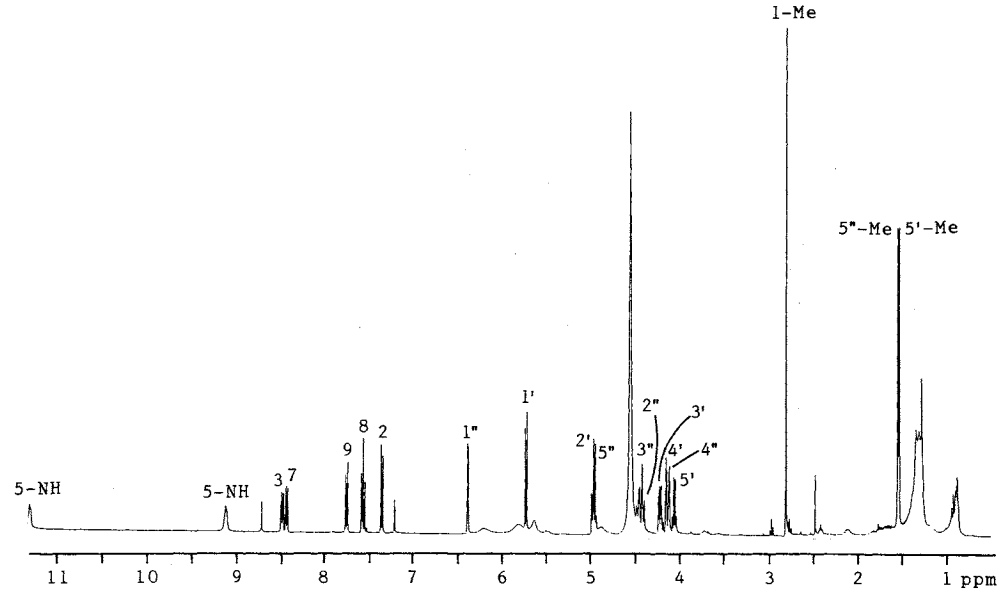
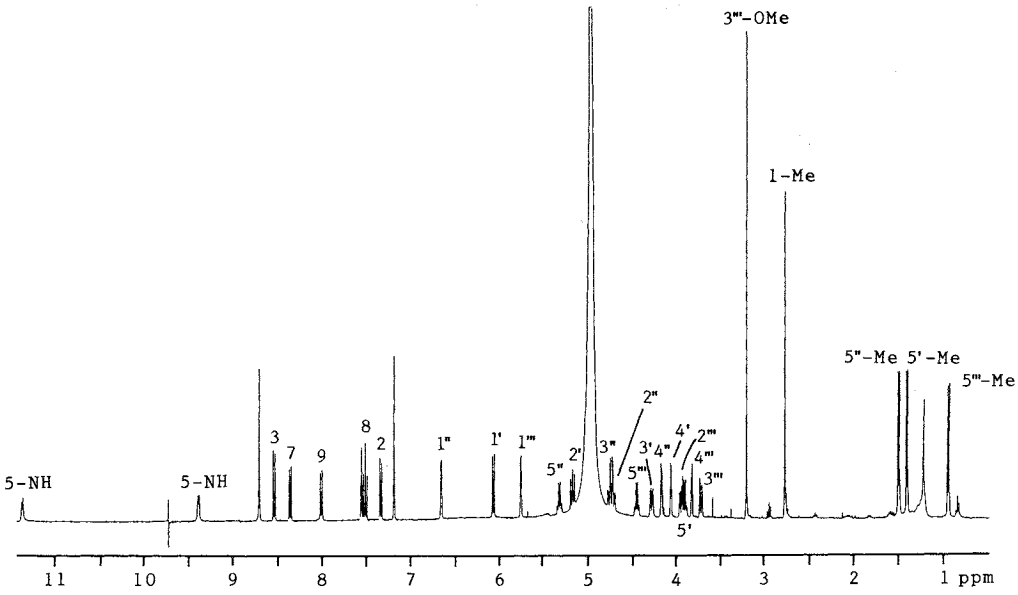


Fig. 8. ^{1}H NMR spectrum of chrymutasin C (**3**). (400 MHz, pyridine-d_5, room temperature).



remaining carbon (δ 146.2) to the C_{10b} position was made.

The presence of a quinone had been presumed from the UV and ^{13}C NMR spectra of **1**, therefore, an attempt to synthesize the acetate of the reduction products of **1** and **8** was made (Scheme 1). Compound **7**, having been acetylated on only the four sugar hydroxyl groups, was obtained from **1** under mild acetylation conditions. On the other hand, yellow compound **9**, which was the reductive acetylation product of **1**, was obtained upon addition of a catalytic amount of zinc powder. Compound **9** was also synthesized

Fig. 9. ^{1}H NMR spectrum of D329C (**6**). (400 MHz, pyridine-d_5, 60°C).

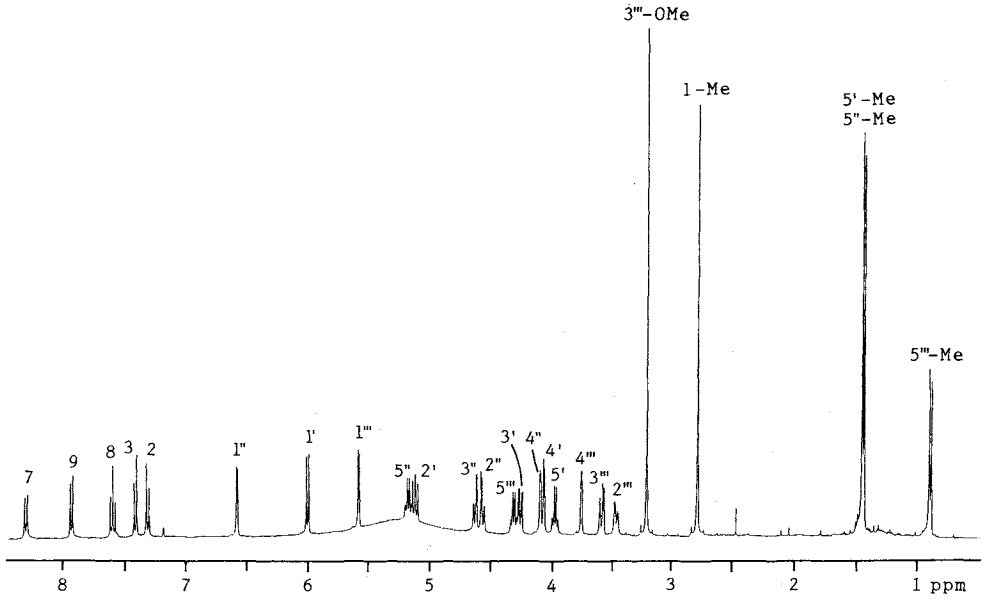


Fig. 10. Summary of observed ^{13}C-^{1}H coupling (^{13}C-^{1}H COSY and LSPD) for the aglycone of chrymutasin A (**1**).

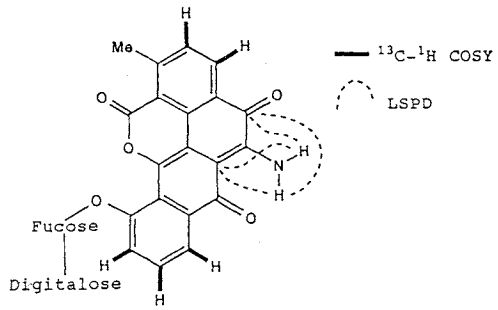
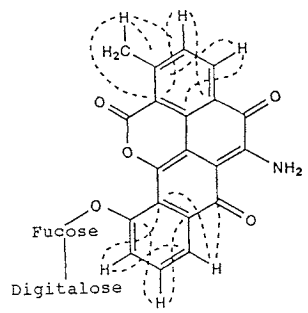


Fig. 11. Summary of observed ^{13}C-^{1}H coupling (^{13}C-^{1}H long-range COSY, 8 Hz) for the aglycone of chrymutasin A (**1**).



under heated acetylation conditions (80°C) from **1** or reductive acetylation conditions from **7**. The alkaline hydrolysis product of **9** showed the same retention time as that of **1** on HPLC analysis. Although good spectral data for **8** could not be obtained, the structure of **10** (the reductive acetate of **8**) was confirmed by ^{1}H NMR and MS spectra.

Therefore, the structure of **8** was confirmed as previously described. The complete structures of **1**, **2** and **3** are shown in Fig. 1.

Experimental

General Procedures

High and low resolution MS were recorded on a JEOL JMS-SX102 mass spectrometer. ^{1}H and ^{13}C NMR data were taken on a Varian VXR-400 and a Bruker AM-500 spectrometers. Optical rotations were measured on a HORIBA SEPA-200 polarimeter. IR spectra were recorded on a Hitachi 270-30 infrared spectrophotometer. Melting points were measured on a Yanako micromelting point ap-

Fig. 12. LSPD experiment of chrymutasin A (1).

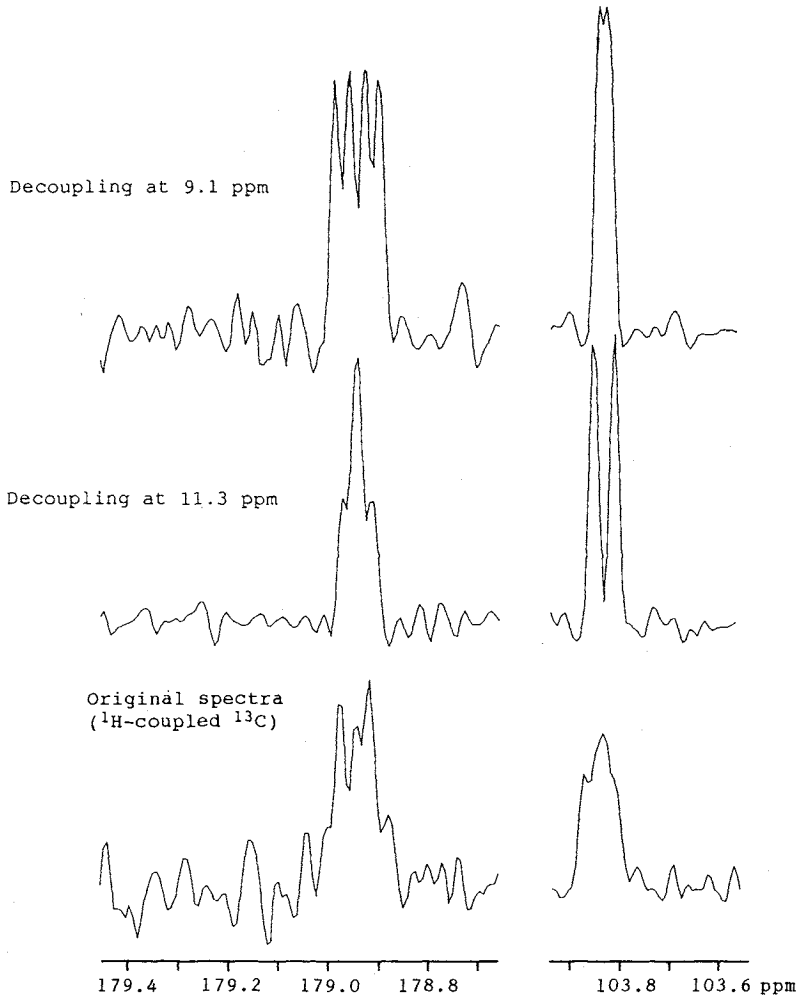


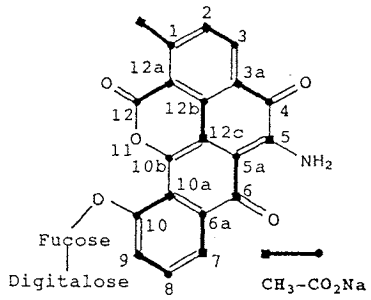
Table 4. Isotopic enrichment in chrymutasin A from cultures containing singly and doubly ^{13}C labeled acetates.

Aglycone carbon	Position of label in precursor			Aglycone carbon	Position of label in precursor		
	[1,2-$^{13}C_2$] $^1J_{C\text{-}C}$ (Hz)	[1-^{13}C] Relative intensity[b]	[2-^{13}C] Relative intensity[b]		[1,2-$^{13}C_2$] $^1J_{C\text{-}C}$ (Hz)	[1-^{13}C] Relative intensity[b]	[2-^{13}C] Relative intensity[b]
Me	42.1	1.1	3.7	C-6a	60.8	2.3	1.2
C-1	41.9	3.6	1.4	C-9	56.2	1.1	2.9
C-2	56.2	1.1	3.0	C-8	56.2	4.0	1.2
C-3	56.1	4.4	1.4	C-10a	71.1	0.8	2.2
C-3a	60.2	0.7	2.0	C-10	70.7	2.6	1.0
C-4	60.0	2.0	1.0	C-10b	—	2.4	1.1
C-5	—	1.0	2.1	C-12a	73.4	1.0	3.0
C-6	58.5	0.8	3.0	C-12	73.4	2.2	1.0
C-5a	58.2	2.5	1.2	C-12c	53.4	1.0	2.7
C-7	60.5	0.8	2.8	C-12b	53.4	2.5	0.9

[a] ^{13}C NMR (100 MHz) was measured in pyridine-d_5 at 60°C.

[b] Each relative intensity was calculated taking an average signal height of sugar carbons as 1.0.

Fig. 13. Labeling pattern of **1**.

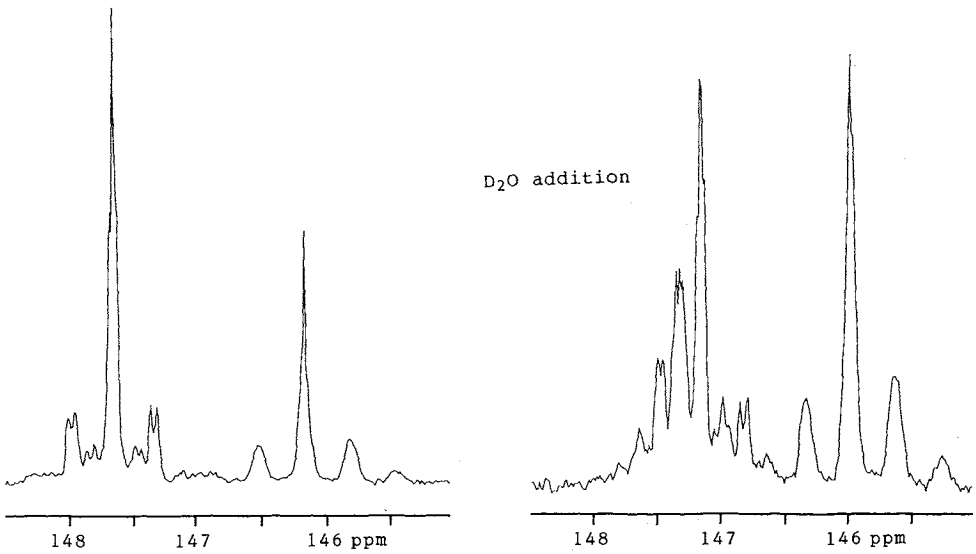


paratus MP-500D and are uncorrected. UV spectra were recorded using a Hitachi 200-20 spectrophotometer and a Hewlett Packard HP1090 Liquid chromatograph diode array spectrometer. Gas chromatograms were recorded on a Shimadzu GC-9A.

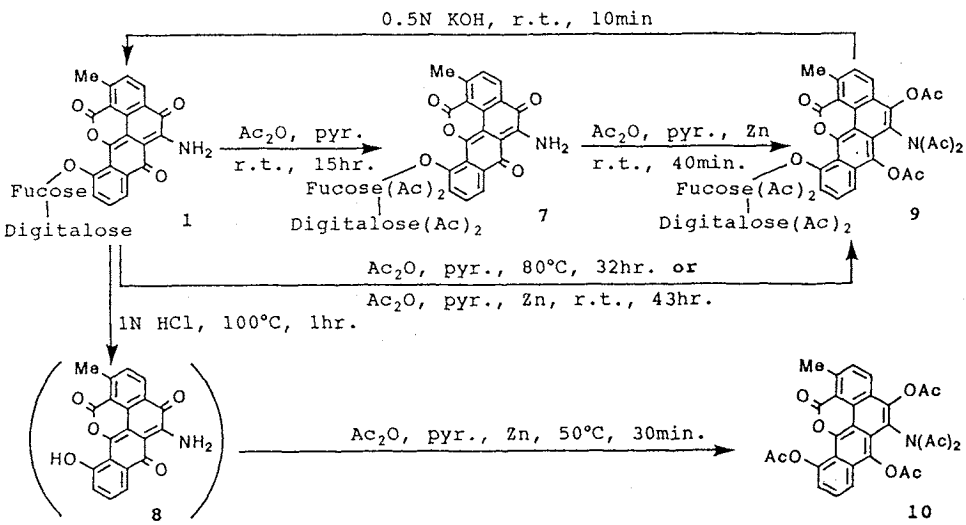
Analyses of the Sugars and the Aglycones of the Chrymutasins

Compound **1** (1.3 mg, 0.002 mmol) was stirred at 100°C in 0.2 ml 1 N HCl for 1 hour in a microscrew-capped tube. After being cooled to room

Fig. 14. $^{2}J_{C-D}$ coupling pattern and isotope shift of sodium [1,2-$^{13}C_2$]acetate incorporated chrymutasin A (**1**).



Scheme 1. Reductive acetylations of **1** and **8**.



temperature, the solution was filtered through filter paper and completely evaporated to dryness, followed by trimethylsililation with a commercially available kit (TMS-HT solution, consisted of trimethylchlorosilane, hexamethyldisilazane and pyridine, Tokyo Kasei Kogyo Co., Ltd.). The resulting reaction mixture solution was filtered using a 0.45 μm filter, and was analyzed using GC-EI-MS (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) for the MS fragment pattern and with GC (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) detected using FID for chromatogram integration.

In addition, a small part of the precipitate (**8**) on the filter paper was dissolved in DMSO and analyzed by HPLC. (column: Shiseido Co., Ltd., Capcell Pak C_{18}, SG120 Å, 5 μm, i.d. 4.6 × 150 mm; eluent: CH_3CN-H_2O (1 : 1); Flow rate; 1 ml/minute; Retention time; 8.3 minute).

Compounds **2** and **3** were treated using the same procedure described above. The aglycones obtained from **2** and **3** showed the same retention time as that of **1**.

Labeled Compounds

Sodium [1-^{13}C]acetate (99% ^{13}C enriched), sodium [2-^{13}C]acetate (99%) and sodium [1,2-$^{13}C_2$]acetate (99%) were purchased from Aldrich Chemical Co.

Fermentation with Singly and Doubly Labeled Sodium Acetate

S. chartreusis D329-185 (FERM BP-3269) strain was cultivated in 5 × 3 liter bottles containing each 600 ml of fermentation medium (dry yeast 1.0%, K_2HPO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, mannitol 2.0%, $FeSO_4 \cdot 2H_2O$ 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). Sodium [1-^{13}C]acetate was dissolved in water at a concentration of 15 mg/ml, and the solution was filtered with a 0.45 μm filter in order to sterilize. Twenty ml aliquots of the solution per flask were added on 4, 7, 11 and 14 days after inoculation. The fermentation was carried out on a rotary shaker at 30°C for 19 days.

In the case of sodium [2-^{13}C]acetate, the strain was cultivated in 3 × 3 liter bottles each containing 600 ml of the same medium, the solution of sodium [2-^{13}C]acetate was added on 4, 7, 11 days (11 mg/ml, 20 ml per day), and the fermentation was carried out for 14 days.

In the case of sodium [1,2-$^{13}C_2$]acetate, the strain was cultivated in 3 × 3 liter bottles each containing 600 ml of the same medium, the solution of sodium [1,2-$^{13}C_2$]acetate was added on 4, 7, 11 days (11.5 mg/ml, 20 ml per day), and the fermentation was carried out for 16 days.

Preparation of Tetraacetate Derivative of **1**

In a typical example, **1** (3 mg, 0.005 mmol) was stirred in acetic anhydride (0.05 ml, 0.5 mmol) and pyridine (0.05 ml, 0.6 mmol) at room temperature for 15 hours. The resulting reaction mixture was evaporated as a toluene azeotrope. Crude **7** was purified by HPLC (UV detection at 570 nm) on a Capcell Pak C_{18} column (i.d. 6 × 250 mm), eluent: H_2O-CH_3CN (1 : 1), flow rate 1.5 ml/minute, retention time 32.8 minutes, to give pure **7** (2 mg, 53%). ^{1}H NMR ($CDCl_3$, 55°C) δ 2.22, 2.10, 2.07, 1.10 (each 3H, s, Ac); FD-MS *m/z* 819 (M^+).

Preparation of Reductive-acetate Derivative of **1**

In a typical example, acetic anhydride (0.5 ml, 5 mmol) and catalytic amount of zinc powder were added to **1** (9.7 mg, 0.015 mmol) in pyridine (0.5 ml, 6 mmol). After being stirred at room temperature for 43 hours, the reaction mixture was filtered through a 0.45 μm filter to remove the zinc powder and evaporated as a toluene azeotrope. Crude **9** was purified by preparative TLC (Merck, Art. 13895, *n*-hexane-acetone 6 : 4, 4 developments), to give pure **9** (7.4 mg, 50%) as a somewhat unstable (could be oxidized in air) yellow compound. **9**: $C_{49}H_{51}NO_{21}$; FAB-MS (positive): *m/z* 1028 $(M+K)^+$, 1012 $(M+Na)^+$, 990 $(M+H)^+$; (negative): *m/z* 989 (M^-); ^{1}H NMR (C_6D_6, 400 MHz, 50°C) δ 2.85, 2.33, 2.22, 2.08, 1.89, 1.72, 1.53, 1.34 (each 3H, s, Ac); UV λ_{max} nm at neutral pH: 258, 273, 292, 304, 383, 404, 427 (this UV spectrum was measured with a HPLC system having a photodiode array in order to eliminate the decomposed products from the pure sample, therefore, εs could not been calculated).

Compound **9** was also obtained from **1** or **7** in the following conditions: **1** was stirred in acetic

anhydride and pyridine at 80°C for 32 hours and purification.; **7** was stirred in acetic anhydride, pyridine, and a catalytic amount of zinc powder at room temperature for 40 minutes and purification.

Hydrolysis of **9**

1 N KOH 100 μl and a small amount of **9** in MeOH 100 μl were stirred at room temperature for 10 minutes. The reaction mixture was analyzed with TLC. The yellow spot (**9**) disappeared and a purple spot that had the same Rf value of **1** (Table 2) was newly detected. The new purple compound also showed the same retention time in comparison with **1** by HPLC analysis. (column: Shiseido Co., Ltd., Capcell Pak C_{18}, SG120 Å, 5 μm, i.d. 4.6 × 150 mm; eluent: CH_3CN - H_2O (3 : 7); flow rate: 1 ml/minute; retention time: 6.0 minutes).

Preparation of Reductive-acetate Derivative of **8** (the Aglycone of **1**)

Crude **1** (9.1 mg, 0.014 mmol) was hydrolyzed in 1 N HCl 2 ml at 100°C for 1.5 hours. The resulting precipitate was filtered, followed by being reductively-acetylated. Crude **8** was stirred in acetic anhydride and pyridine in addition of a catalytic amount of zinc powder at 50°C for 30 minutes. The crude **10** obtained was purified by preparative TLC (Merck, Art. 13895, CH_2Cl_2 - MeOH 99 : 1), to give pure **10** (2.7 mg, 35%) as a somewhat unstable yellow compound. **10**: $C_{30}H_{23}NO_{10}$; HREI-MS: *m/z* Found 557.1333 (M^+), Calcd. 557.1322; ^1H NMR ($CDCl_3$, 400 MHz, room temperature) δ 8.00 (1H, d, J= 8.2 Hz), 7.80 (1H, d, J=8.2 Hz), 7.78 (1H, dd, J=8.7, 1.2 Hz), 7.72 (1H, dd, J=8.7, 7.2 Hz), 7.41 (1H, dd, J=7.2, 1.2 Hz), 3.12 (3H, s), 2.74 (3H, s), 2.53 (3H, s), 2.48 (3H, s), 2.40 (6H, s).

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