Chrymutasins: a New Type of Aglycone Related to Chartreusin; Novel Antitumour Antibiotics from a Mutant of *Streptomyces chartreusis*

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The structure of chrymutasin A, B and C are determined from spectral studies, incorporation studies of ^{13}C-labelled sodium acetates and an aglycone derivative.

Having found that *Streptomyces chartreusis* D329 produced chartreusin, a glycosidic antitumour antibiotic, we tried to mutate the strain D329 with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in order to obtain other antibiotics related to chartreusin. Among the related compounds to **1**, elsamicin A[2] (related natural product) and IST-622[3] (one of the semi-synthesized derivatives) have been clinically explored in phase studies.

From the analyses of mutant products, we found a mutant strain (D329-185 strain, FERM BP-3269) which produced demethylchartreusin **2**[4] and D329C compound **3**.[5] As a result of further analysis of the other products in the fermentation broth of the mutant strain, three novel compounds, named as chrymutasin A **4**, B **5** and C **6** were isolated. The results are unique from that of the parent strain. As an example of isolation from *ca.* 10 l fermentation broth, **4** (97 mg), **5** (12 mg) and **6** (0.5 mg) were isolated by a combination of silica gel and ODS chromatography.

Compound **4**, $C_{33}H_{33}O_{13}N$ {HR FAB-MS (pos.): *m/z* found 676.2039 $(M + Na + H_2)^+$, calc. 676.2006}, a violet powder, showed characteristic UV absorption {λ_{max} nm (ε) in MeOH: 228 (16000), 243 (13000, sh.), 262 (12000), 385 (2600), 534 (5200), 572 (5600)}. Selected ^{1}H and ^{13}C NMR data are shown in Table 1. It was assumed that the sugars of **4** and **1** were the same by comparison of their NMR data. The results of GC-MS analyses of TMS (trimethylsilyl) derivatives of the sugars of **4** and **1** showed the same chromatograms and MS fragment patterns. These results confirmed that the sugars of **4** consisted of one fucose and one digitalose. The attachment position (C-10) and sequence (aglycone-fucose-digitalose) were determined by NOESY experiments.‡

The constituent sugars of **5**§ and **6**¶ were determined (shown in Table 2) with GC-MS analysis of the TMS derivatives of sugars in a manner similar to that previously discussed and in detailed comparison of NMR data with those of **2** and **3**, respectively.

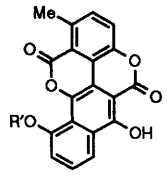
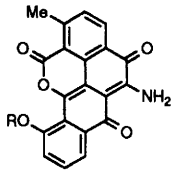
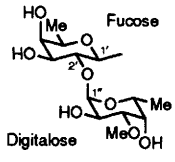
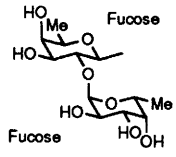
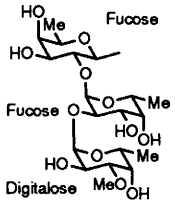
The aglycones from **4**, **5** and **6** had the same retention time on HPLC,‖ and thereby it was confirmed that these three compounds had the same aglycone, named as chrymutin (**7**, 5-amino-10-hydroxy-1-methyl-12*H*-11-oxabenzo[*def*]chrysene-4,6,12-trione). Many quaternary carbons were present in the aglycone. Some carbons, bonding to ^{1}H through $^1J_{C-H}$, $^2J_{C-H}$ and $^3J_{C-H}$ were revealed easily (by C–H COSY, C–H long range COSY), but other carbons were not assigned by normal NMR techniques. A previous report[6] has described that chartarin (aglycone of **1**) had been biosynthesized from polyketide as an early biosynthetic intermediate. In order to determine the aglycone structure, ^{13}C-labelled **4** was prepared from the fermentation broth by adding [1-^{13}C], [2-^{13}C] and

Table 1 ^{1}H and ^{13}C NMR data[a] of chrymutasin A

Position	Chrymutasin A **4** ^{1}H(δ, Hz)			^{13}C(δ)	
Aglycone					
1				152.3	s
2	7.33	d	8.0	132.2	d
3	8.43	d	8.0	133.5	d
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	7.54	t	8.0	130.9	d
9	7.73	d	8.0	119.6	d
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
5-NH_2	9.1	br s			
	11.3	br s			

[a] ^{1}H NMR: 400 MHz, [$^2H_5$]pyridine, 50 °C; ^{13}C NMR: 100 MHz, [$^2H_5$]pyridine, 50 °C.

Table 2 Structures of chrymutasins and related compounds

Sugars (R or R')	Aglycone: 	Aglycone: 
	Chartreusin **1**	Chrymutasin A **4**
	Demethyl-chartreusin **2**	Chrymutasin B **5**
	D329C **3**	Chrymutasin C **6**
H	Chartarin	Chrymutin **7**

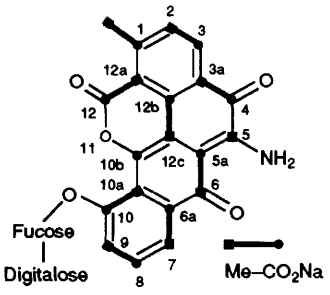


Fig. 1 Labelling pattern of **4**

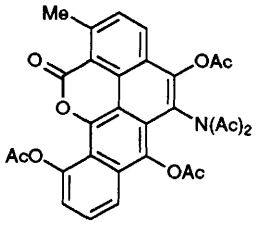


Fig. 2 Reductive acetylated derivative (**8**) of **7**

[1,2-$^{13}C_2$] sodium acetates, respectively. From the ^{13}C NMR spectrum, nine pairs of ^{13}C–^{13}C bonded directly from the labelled acetate and are summarized in Fig. 1. Furthermore, on account of LSPD (long-range selective proton decoupling) experiments from N–*H*, $^2J_{C-D}$ coupling and isotopic shift in ^{13}C NMR with D_2O addition, all aglycone carbons were unambiguously determined and assigned.

Because of both very poor solubility of **7** in many organic solvents and confirmation of the quinone in **7**, a reductive acetylated derivative **8** was synthesized and confirmed by ^1H NMR and MS** analysis.

Compound **4** showed the strongest cytotoxicities among **4**, **5** and **6**, and hence antitumour activities of **4** *in vivo* were assayed. Compound **4** showed that the T/C (survival time of test group/that of control one ×100) value was 173% (Meth A fibrosarcoma cells and **4** were intraperitoninally given to mice; dose of **4**: 20 mg kg^{-1} × 4 days). However, the T/C of **1** was 150% for similar conditions.

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Footnotes

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‡ Two pairs of protons with the NOEs were shown. δ 7.73 (aglycone 9-position) ↔ 5.75 (1′-position in fucose); δ 4.97 (2′-position in fucose) ↔ 6.36 (1″-position in digitalose).

§ $C_{32}H_{31}O_{13}N$; HR FAB-MS (neg.): *m/z* found 637.1791 (M^-), calc. 637.1796.

¶ $C_{39}H_{43}O_{17}N$; HR FAB-MS (pos.): *m/z* found 820.2423 $(M + Na)^+$ calc. 820.2428.

‖ Column: Shiseido, Co., Ltd, CAPCELL PAK C_{18}, SG120 Å, 5 μm, φ4.6 × 150 mm; mobile phase: MeCN–H_2O (1:1); flow rate: 1 ml min^{-1}; retention time: 8.3 min.

** $C_{30}H_{23}NO_{10}$; HR EI-MS: *m/z* found 557.1333 $(M)^+$, calc. 557.1322; ^1H NMR ($CDCl_3$, 400 MHz, room temp.) 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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