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Auripyrones A and B, Cytotoxic Polypropionates from the Sea Hare *Dolabella auricularia*: Isolation and Structures

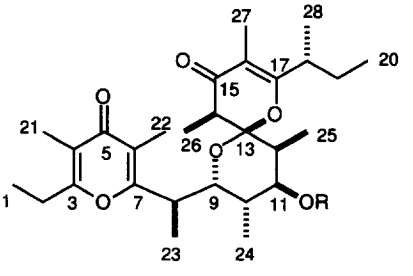
Kiyotake Suenaga, Hideo Kigoshi, and Kiyoyuki Yamada*

Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

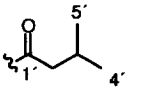
Abstract: Two new polypropionates, auripyrones A (**1**) and B (**2**), have been isolated as cytotoxins from the sea hare *Dolabella auricularia*. The gross structures of **1** and **2** were determined by spectroscopic analysis, and their relative stereochemistry except for the ester moiety of **2** was established on the basis of the NOESY data. Copyright © 1996 Elsevier Science Ltd

A number of bioactive peptides and other unique metabolites have been reported from the sea hare *Dolabella auricularia* (Aplysiidae) collected both in Western Indian Ocean[1] and in Japan.[2] We report here the isolation and structures of two new cytotoxic polypropionates, auripyrones A (**1**) and B (**2**), from Japanese specimens of this animal.

The MeOH extract of the internal organs of the sea hare *D. auricularia* (452 kg, wet wt), collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material, which exhibited cytotoxicity against HeLa S_3 cells with an IC_{50} of 1.2 μg/mL, was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to bioassay-guided fractionation using silica gel (i. toluene/EtOAc, EtOAc, and then EtOAc/MeOH, step gradient; ii. 2:1 hexane/acetone and then 9:1 acetone/MeOH), ODS silica gel (i. 70% aqueous MeOH to MeOH, linear gradient; ii. 80% aqueous MeOH to MeOH, linear gradient), and silica gel (15:1 $CHCl_3$/acetone and then 10:1 $CHCl_3$/acetone), successively, to afford a cytotoxic fraction (IC_{50} = 0.32 μg/mL). The fraction was further separated by reversed phase HPLC (i. Develosil ODS-HG-5, 65:35 MeCN–0.02 M NH_4OAc and then MeOH; ii. Develosil ODS-HG-5, 80% aqueous MeOH) to afford a mixture of auripyrones A (**1**) and B (**2**). Final purification by reversed phase HPLC (CHIRALCEL OD-R, 85% aqueous MeOH) yielded **1**[3] (1.0 mg, 2.2×10^{-7}% yield based on wet weight) and **2**[4] (1.7 mg, 3.8×10^{-7}% yield) as colorless crystals, respectively. Auripyrones A (**1**) and B (**2**) exhibited cytotoxicity against HeLa S_3 cells with IC_{50} values of



auripyrone A (**1**) R =



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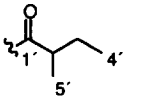


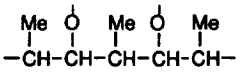
Table 1. NMR Data for auripyrones A (**1**) and B (**2**) in C_6D_6

	auripyrone A (**1**)			auripyrone B (**2**)	
position	$^1H^a$	$^{13}C^b$	HMBC^c	$^1H^a$	$^{13}C^b$
1	0.89 t (7.3)	11.0 q		0.89 t (7.2)	10.8 q
2a	2.31 dq (15.0, 7.3)	24.7 t	H-1	2.31 dq (15.0, 7.2)	24.7 t
2b	2.09 dq (15.0, 7.3)			2.09 dq (15.0, 7.2)	
3		162.0 s	H-1, 2b, 21		162.0 s
4		118.1 s	H-21		118.1 s
5		178.4 s	H-21, 22		178.4 s
6		121.3 s	H-22		121.3 s
7		161.5 s	H-22, 23		161.5 s
8	2.75 dq (10.2, 7.0)	36.7 d	H-23	2.75 dq (10.2, 7.0)	36.7 d
9	3.98 dd (10.2, 2.2)	70.5 d	H-23, 24	3.98 dd (10.2, 2.3)	70.6 d
10	1.81 m	34.1 d	H-24	1.79 m	34.1 d
11	4.92 dd (3.3, 3.3)	75.2 d	H-24, 25	4.92 dd (3.6, 3.6)	75.2 d
12	1.83 m	31.7 d	H-25	1.84 dq (3.6, 7.0)	31.7 d
13		105.0 s	H-14, 25, 26		105.1 s
14	2.32 q (7.0)	44.5 d	H-26	2.32 q (7.0)	44.6 d
15		191.7 s	H-14, 26, 27		191.7 s
16		107.8 s	H-27		107.9 s
17		166.2 s	H-27, 28		166.2 s
18	2.26 ddq (6.6, 3.7, 6.6)	37.2 d	H-28	2.26 ddq (6.6, 3.7, 6.6)	37.2 d
19a	1.52 m	26.3 t	H-20, 28	1.50 m	26.4 t
19b	1.36 m			1.34 m	
20	0.84 t (7.3)	12.0 q		0.83 t (7.7)	12.0 q
21	1.99 s	9.7 q		1.98 s	9.7 q
22	2.03 s	10.7 q		2.03 s	10.7 q
23	0.73 d (7.0)	12.1 q		0.72 d (7.0)	12.1 q
24	0.63 d (7.0)	9.5 q		0.63 d (7.0)	9.4 q
25	0.77 d (7.0)	11.9 q		0.77 d (7.0)	11.8 q
26	1.12 d (7.0)	8.1 q		1.13 d (7.0)	8.2 q
27	1.65 s	9.0 q		1.66 s	9.0 q
28	0.99 d (6.6)	16.0 q		0.99 d (6.6)	15.9 q
1'		172.0 s	H-11, H-2'		175.9 s
2'	2.18-2.10 m^d	44.0 t	H-4', 5'	2.43 ddq (7.0, 7.0, 7.0)	41.6 d
3a'	2.19 m	26.4 d	H-4', 5'	1.75 m	27.4 t
3b'				1.43 m	
4'	0.91 d (6.2)	22.3 q	H-5'	0.88 t (7.7)	11.8 q
5'	0.90 d (6.6)	22.3 q	H-4'	1.16 d (7.0)	16.9 q

^{a} Recorded at 600 MHz. Residual C_6HD_5 as internal standard (δ 7.16). Coupling constants (Hz) are in parenthesis. ^{b} Recorded at 67.8 MHz. C_6D_6 as internal standard (δ 128.0). Multiplicity was based on the HSQC spectrum. ^{c} Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 Hz. ^{d} 2 H.

0.26 and 0.48 μg/mL, respectively.

Auripyrone A (**1**) has a molecular formula of $C_{33}H_{50}O_7$ as determined by the HRFABMS [*m/z* 581.3480 $(M + Na)^+$, Δ +2.6 mmu] and NMR data (Table 1). The presence of a γ-pyrone ring in **1** was indicated by the IR spectral data (1655 and 1595 cm^{-1})[5] together with the UV absorption band at 260 nm (ε 13000),[5] which was supported by the observation of the ^{13}C NMR signals at δ 178.4 (s), 162.0 (s), 161.5 (s), 121.3 (s), and 118.1 (s) that were typical of a fully substituted γ-pyrone.[6] The ^1H NMR signals at δ 2.03 (s, 3 H) and 1.99 (s, 3 H) were assigned to the two methyl groups at the β and β' carbons on the γ-pyrone ring

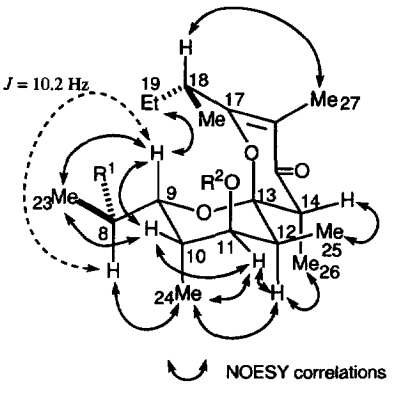


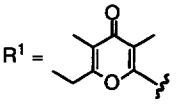
(I)

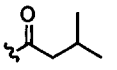
by the HMBC data. Detailed analysis of the ^1H-^1H COSY spectrum of **1** allowed construction of a partial structure (I) and four additional partial structures [CH_3CH_2-, -$CH(CH_3)$-, $CH_3CH_2(CH_3)CH$-, $(CH_3)_2CHCH_2$-]. Besides, auripyrone A (**1**) was shown to have one more methyl group, the signal of which was observed as a singlet (δ 1.65) in the ^1H NMR spectrum. This singlet together with four ^{13}C NMR signals at δ 191.7, 107.8, 166.2, and 9.0 indicated the presence of an α,β-unsaturated ketone bearing an α-methyl group and a β-ethereal oxygen function.

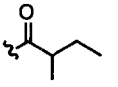
The HMBC data summarized in Table 1 allowed us to connect the partial structures and the functional groups described above, establishing all of the carbon-connectivities. The location of the acyloxy group [$(CH_3)_2CHCH_2COO$-] was determined to be at C11 by the ^1H NMR signal at δ 4.92 (H-11) and the HMBC correlation (H-11/C1'). The presence of a ketal group was deduced from a signal (δ 105.0) in the ^{13}C NMR spectrum; the ketal carbon must be connected to two ethereal oxygens at C9 and C17 to form a spiroketal structure on the basis of the molecular fomula and the degree of unsaturation. Thus, the gross structure of auripyrone A is represented by the formula **1**.

A plausible conformation of **1** with the selected NOESY correlations is shown in Figure 1. The vicinal spin-spin coupling constants ($J_{9,10}$ = 2.2 Hz, $J_{10,11}$ = 3.3 Hz, $J_{11,12}$ = 3.3 Hz) and the NOESY correlations (H-8/H-24, H-12/H-24) indicated that the tetrahydropyrane ring (C9–C13) had a chair conformation and that the four substituents at C9, C10, C11, and C12 of the tetrahydropyrane ring were equatorial, axial, axial, and equatorial, respectively (Figure 1). The large coupling constant ($J_{8,9}$ = 10.2 Hz) and the NOESY correlation (H-10/H-23) suggested that the substituent at C9 was in a rigid conformation and that the relative stereochemistry concerning C8 and C9 was 8*R**, 9*R** (Figure 1). The correlations (H-12/H-26, H-14/H-25) in the NOESY spectrum clarified the relative stereochemistry between C12 and C14 and further the stereochemistry of the spiroketal at C13 where both ketal oxygens were in axial positions. The NOESY correlations (H-9/H-19, H-18/H-27) disclosed the relative stereochemistry between C9 and C18. Thus, the relative stereochemistry of **1** was established as depicted in Figure 1.





auripyrone A (**1**) R^2 = 

auripyrone B (**2**) R^2 = 

Auripyrone B (**2**) has a molecular formula of $C_{33}H_{50}O_7$ as determined by the HRFABMS [*m/z* 581.3454 $(M + Na)^+$, Δ +0.0 mmu] and NMR data (Table 1), which is identical with that of **1**. The gross structure of **2** was determined by the 2D NMR technique in the same manner as described above for **1**. Thus, it was found that the structure of **2** was identical with that of **1** except for the acyloxy moiety: the acyloxy group [$(CH_3)_2CHCH_2COO$-] at C11 in **1** was replaced by a different acyloxy group [$CH_3CH_2(CH_3)CHCOO$-] in **2**. The relative stereochemistry of the carbon skeleton of **2** was also elucidated by the NOESY data (Figure 1) to be identical with that of **1**.

Auripyrones A (**1**) and B (**2**) are novel cytotoxic polypropionates containing a γ-pyrone ring and a spiroketal moiety. Structurally related polypropionates are siphonarins A and B[6b] and dihydrosiphonarins A and B,[6b] isolated from other marine molluscs.

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3. **1**: mp 172-176 °C (pentane); $[\alpha]^{26}_D$ +28 (*c* 0.083, $CHCl_3$); UV (MeOH) λ_{max} 260 (ε 13000), 220 (ε 9500) nm; IR ($CHCl_3$) 1725, 1655, 1620, 1595, 1460, 1385 cm^{-1}.
4. **2**: mp 126-128 °C (pentane); $[\alpha]^{26}_D$ +39 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} 260 (ε 14000), 217 (ε 6800) nm; IR ($CHCl_3$) 1725, 1655, 1620, 1595, 1460, 1385 cm^{-1}.
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