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APLYDILACTONE, A NOVEL FATTY ACID METABOLITE FROM THE MARINE MOLLUSC *APLYSIA KURODAI*

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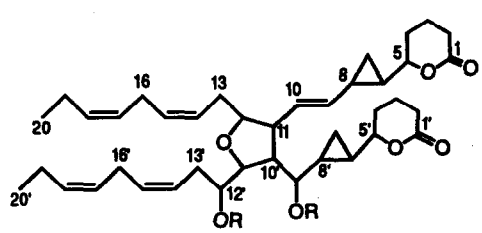
Summary: Aplydilactone (**1**), a new dimeric fatty acid metabolite having a phospholipase A_2 activating activity was isolated from the marine mollusc *Aplysia kurodai* and its planar structure was elucidated on the basis of spectral and chemical means.

During the course of our search for biologically significant marine natural products, we have isolated a novel dimeric fatty acid metabolite, aplydilactone (**1**), from the marine mollusc *Aplysia kurodai* and found that it exhibits a phospholipase A_2 activating activity. In this paper, we report the structural elucidation of the new metabolite on the basis of spectroscopic data and chemical degradation.

The marine mollusc *A. kurodai* (15.3 kg, wet weight) was collected at Yasuri-hama, Mie Prefecture, Japan. The EtOAc-soluble material from the methanolic extract was partitioned between 70% MeOH and CH_2Cl_2-CCl_4 (1:1) followed by partitioning of the CH_2Cl_2-CCl_4 (1:1) portion between 80% MeOH and CCl_4. The 80% MeOH portion was chromatographed four times on silica gel [i. EtOAc; ii. C_6H_6-acetone (5:1); iii. hexane-Et_2O-acetone (12:1:3); iv. $CHCl_3$-acetone (3:1)], and was further separated by reversed-phase HPLC (ODS, 85% MeOH) to give aplydilactone (**1**) (22.2 mg).

Aplydilactone (**1**), colorless oil, $[\alpha]_D^{27}$ -1.63° (*c* 1.00, $CHCl_3$), has a molecular formula, $C_{40}H_{58}O_7$, which was determined by high resolution desorption chemical ionization mass spectrometry (HRDCIMS) [*m/z* 651.4265 $(M+H)^+$, Δ +0.4 mmu]. ^1H NMR and ^{13}C NMR spectral data were assigned as shown in Table 1 by ^1H-^{13}C COSY experiment. The IR absorption bands at 3450 and 1725 cm^{-1} ($CHCl_3$) indicated the presence of hydroxyl and ester (or lactone) functions, respectively. Acetylation of **1** (Ac_2O, Py) afforded diacetate **2**[1] (^1H NMR, Table 1), while dimethyl ester **3**[2] (^1H NMR, Table 1) was obtained upon methanolysis of **1** (NaOMe, MeOH). These findings suggested **1** to be a diol dilactone. The presence of lactones in **1** was also supported by the ^{13}C NMR signal at δ 171.5 (2C, s). The ^1H NMR and ^{13}C NMR spectra of **1** further revealed the presence of five 1,2-disubstituted double bonds, six oxymethines, and two 1,2-disubstituted cyclopropane ring



1 : R = H
2 : R = Ac

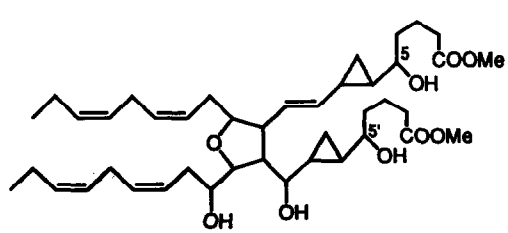


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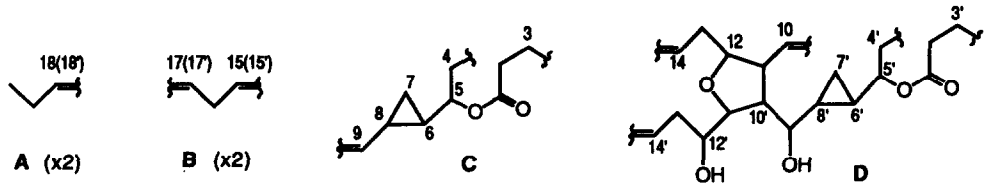
Table 1. NMR Spectral Data of Aplydilactone (1) and Derivatives 2 and 3 ($CDCl_3$).[a]

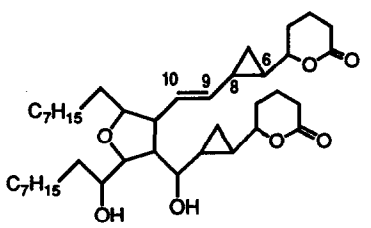
	1		2	3
No.	^1H	^{13}C[b]	^1H	^1H
1		171.5 s		
2	2.46 m	29.4 t	2.46 m	2.34 t (7.6)
	2.56 m		2.54 m	
3	1.80 m	18.4 t[c]	1.80 m	1.60 m
	1.94 m		1.92 m	
4	1.65 m	27.6 t[d]	1.66 m	1.75 m
	2.00 m		2.00 m	
5	3.81 m	82.9 d	3.84 ddd (10.4, 7.3, 3.1)	3.01 ddd (7.6, 7.6, 5.5)
6	1.10 m	24.7 d (157)	1.13 m	0.92 m
7	0.69 m	10.3 t (160)	0.70 m	0.56 ddd (8.8, 5.0, 5.0)
				0.65 m
8	1.50 dddd (8.0, 7.2, 5.0, 5.0)	18.9 d (160)	1.50 dddd (8.5, 7.9, 5.0, 5.0)	1.39 m
9	5.34 m	132.8 d[e]	5.43 m	5.17 dd (15.2, 8.4)
10	5.48 m	131.8 d[e]	5.54 dd (15.6, 9.8)	5.25 - 5.55 m
11	2.76 m	48.7 d	2.71 ddd (9.8, 5.2, 3.4)	2.80 m
12	3.82 m	81.2 d	3.69 m	3.92 dt (6.1, 7.3)
13	2.17 m	29.4 t	2.20 m	2.21 m
	2.33 m		2.33 m	
14, 15	5.27 - 5.55 m	126.2 d[e], 127.1 d[e]	5.25 - 5.50 m	5.25 - 5.55 m
16	2.80 m	25.7 t[f]	2.79 br t (5.8)	2.80 m
17, 18	5.27 - 5.55 m	127.7 d[e], 130.2 d[e]	5.25 - 5.50 m	5.25 - 5.55 m
19	2.07 dq (7.5, 7.5)	20.4 t	2.07 m	2.07 dq (7.5, 7.5)
20	0.97 t (7.5)	14.2 q	0.97 t (7.6)[g]	0.97 t (7.5)[h]
1'		171.5 s		
2'	2.46 m	29.4 t	2.46 m	2.34 t (7.5)
	2.56 m		2.54 m	
3'	1.80 m	18.2 t[c]	1.80 m	1.60 m
	1.94 m		1.92 m	
4'	1.70 m	27.5 t[d]	1.63 m	1.75 m
	2.04 m		1.95 m	
5'	3.64 ddd (10.9, 7.9, 3.0)	84.2 d	3.67 m	2.86 ddd (8.7, 7.4, 5.0)
6'	1.08 m	21.1 d (158)	1.09 m	0.84 m
7'	0.48 ddd (8.2, 5.0, 5.0)	6.6 t (159)	0.44 ddd (8.5, 5.0, 5.0)	0.46 ddd (8.1, 5.0, 5.0)
	0.69 m		0.70 m	0.65 m
8'	1.08 m	22.6 d (157)	1.11 m	0.92 m
9'	3.28 dd (6.0, 6.0)	73.5 d	4.58 dd (7.8, 7.8)	2.91 dd (9.2, 4.6)
10'	2.26 ddd (7.3, 6.0, 6.0)	52.0 d	2.08 m	2.32 m
11'	3.93 dd (7.3, 2.3)	81.8 d	3.75 dd (6.4, 2.1)	3.97 dd (8.3, 2.0)
12'	3.81 m	71.4 d	4.88 dt (7.2, 2.1)	3.82 br dd (6.5, 6.5)
13'	2.42 m	32.4 t	2.47 m	2.43 m
			2.53 m	
14',15'	5.27 - 5.55 m	126.2 d[e], 127.1 d[e]	5.25 - 5.50 m	5.25 - 5.55 m
16'	2.80 m	25.6 t[f]	2.83 br t (7.2)	2.80 m
17',18'	5.27 - 5.55 m	129.5 d[e], 131.7 d[e]	5.25 - 5.50 m	5.25 - 5.55 m
19'	2.07 dq (7.5, 7.5)	20.4 t	2.07 m	2.07 dq (7.5, 7.5)
20'	0.97 t (7.5)	14.2 q	0.96 t (7.5)[g]	0.98 t (7.5)[h]
Ac			2.04 s, 2.15 s	
OMe				3.67 s

[a] ^1H NMR and ^{13}C NMR spectra were recorded at 500 MHz and at 67.8 MHz, respectively. Coupling constants, J_{H-H} and $^1J_{C-H}$ (in Hz), are given in parentheses.

[b] Multiplicities were determined by INEPT experiments.

[c-h] Values bearing the same superscript may be interchanged.





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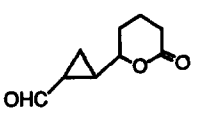

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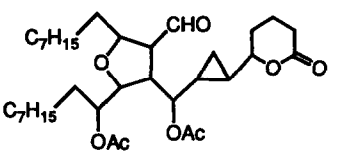

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systems. Intensive studies of the ^{1}H-^{1}H COSY spectra of **1** and diacetate **2** revealed the presence of four partial structures **A** - **D** in **1**. The positions of two hydroxyl groups in the partial structure **D** were determined by acetylation shifts observed at H-9' (δ 3.28 → 4.58) and H-12' (δ 3.81 → 4.88) on acetylation of **1** to **2**. On the other hand, upfield shifts of the signals due to H-5 (δ 3.81 → 3.01) and H-5' (δ 3.64 → 2.86) were observed upon methanolysis of **1** to **3**, indicating that the hydroxyl functions at C-5 and C-5' were acylated to form two lactones in the partial structures **C** and **D**. Since six of the seven oxygen atoms of **1** were accounted for by the two hydroxyl and two lactone functions, the remaining one oxygen atom was assigned to an ethereal function, which linked two oxymethines [H-12 (δ 3.82) and H-11' (δ 3.93)] to form a tetrahydrofurane ring system in the partial structure **D**. The substitution patterns of two cyclopropane ring systems were determined by the vicinal coupling constants of the cyclopropane ring protons, J_{cis} of which are generally larger than J_{trans}.[3] The methine proton (H-8) at δ 1.50 (dddd, J = 8.0, 7.2, 5.0, 5.0 Hz) in the partial structure **C** was coupled to the methylene protons (H-7) at δ 0.69 with the coupling constants J_{cis} = 8.0 Hz and J_{trans} = 5.0 Hz and to the methine proton (H-6) at δ 0.69 with J_{trans} = 5.0 Hz. In the partial structure **D** one of the methylene protons (H-7') at δ 0.48 (ddd, J = 8.2, 5.0, 5.0 Hz) was coupled to the two methine protons (H-6' and H-8') at δ 1.08 (2H, m) with J_{cis} = 8.2 Hz and J_{trans} = 5.0 Hz. These findings suggested that both of two 1,2-disubstituted cyclopropane ring systems had *trans* stereochemistry. Since these partial structures **A** - **D** contained all carbon, hydrogen, and oxygen atoms of **1**, the remaining problem was the connectivity of the ten sp^2 carbons and the four methylene carbons (C-3, C-4, C-3', and C-4'). Owing to the overlap of signals for the vinyl (δ 5.27 - 5.55) and methylene (δ 1.60 - 2.10) protons in the ^{1}H NMR spectrum of **1**, the connectivity of these partial structures could not be clarified from the ^{1}H-^{1}H COSY experiment. In order to obtain further structural information on **1**, chemical degradation of **1** was performed.

Thus, partial hydrogenation of **1** (H_2, PtO_2) afforded the octahydro derivative **4**,[4] $C_{40}H_{66}O_7$ [HRDCIMS *m/z* 659.4878 $(M+H)^+$, Δ -0.9 mmu]. The ^{1}H NMR spectrum and ^{1}H-^{1}H COSY experiment of **4** revealed that one *trans* double bond [H-9: δ 5.64 (1H, dd, J = 15.5 and 7.9 Hz), H-10: δ 5.86 (1H, dd, J = 15.5 and 9.9 Hz)]

remained in **4**, implying that the partial structures **C** and **D** in **1** were connected through the *trans* double bond. The UV spectrum of **1** [204 nm (ε 20,000) in CH_3CN] supported the presence of the vinyl cyclopropane moiety (from C-6 to C-10).[5] The octahydro derivative **4**, after acetylation (Ac_2O, Py), was subjected to oxidative cleavage (i. OsO_4, THF, Py; ii. $NaIO_4$, EtOH, H_2O) to afford two aldehydes **5**[6] and **6**[7]. The structure of **5**, $C_9H_{12}O_3$ [HRCIMS *m/z* 169.0843 $(M+H)^+$, Δ -2.1 mmu], was easily determined by the ^1H NMR spectrum together with decoupling and NOE experiments. The IR absorption band at 1730 cm^{-1} supported the presence of δ-lactone in **5**. The formation of **5** having a δ-lactone moiety revealed the connectivity of C-3 ~ C-4 and C-3' ~ C-4' to form two δ-cyclopropyl-δ-lactones in the partial structures **C** and **D**. The ^1H NMR spectrum of **6** was much simpler than that of **1** and the ^1H-^1H COSY spectrum of **6** strongly supported the 2,3,4,5-tetrasubstituted tetrahydrofurane ring system of **1**. The remaining problem was connectivities of the eight sp^2 carbons in **1**. Since the cross peaks were observed between the allylic methylene protons [H-13: δ 2.17 (1H, m) and 2.33 (1H, m), H-13': δ 2.42 (2H, m)] and the bis-allylic methylene protons [H-16 and H-16': δ 2.80 (4H, m)] in the ^1H-^1H COSY spectrum of **1**, C-15 and C-15' of the partial structures **B** should be connected to C-14 and C-14' of the partial structure **D**, respectively, and therefore each of the partial structures **A** was linked to C-17 and C-17', respectively. The *cis* nature of the four double bonds at C-14, C-14', C-17, and C-17' in **1** was indicated by the chemical shifts observed in the ^{13}C NMR spectrum for the signals of the two bis-allylic methylenes (C-16 and C-16': δ 25.6 and 25.7).[8] In conclusion, the structure of aplydilactone is established to be the formula **1**.

Aplydilactone (**1**) seems to be biosynthesized from two eicosapentaenoic acids via an unsymmetrical dimerization and oxidative cyclizations to form lactones and cyclopropanes . The isolation of cyclopropane-containing fatty acid lactones from marine sources is quite rare[3,9] and **1** is the first example of the dimeric fatty acid metabolite. Aplydilactone (**1**) exhibited the activity of activating phospholipase A_2 *in vitro* (about two-fold at the concentration of 50 mM), which is an important enzyme for the prostaglandin biosynthesis.

References and Notes

1. **2**: IR ($CHCl_3$) 1730 cm^{-1}; DCIMS *m/z* 735 $(M+H)^+$, 675, 615, 465.
2. **3**: IR ($CHCl_3$) 3610, 3460, 1730 cm^{-1}; FABMS (*m*-nitrobenzyl alcohol as a matrix) *m/z* 715 $(M+H)^+$, 697, 679, 661, 643.
3. M. D. Higgs and L. J. Mulheirn, *Tetrahedron*, **37**, 4259 (1981).
4. **4**: IR ($CHCl_3$) 3425, 1720 cm^{-1}; DCIMS *m/z* 659 $(M+H)^+$, 631, 517, 499.
5. C. H. Heathcook and S. R. Poulter, *J. Am. Chem. Soc.*, **90**, 3766 (1968).
6. **5**: IR ($CHCl_3$) 1730, 1705 cm^{-1}; CIMS *m/z* 169 $(M+H)^+$, 151; ^1H NMR (270 MHz, C_6D_6) δ 0.43 (1H, ddd, *J* = 8.4, 6.3, 4.6 Hz), 0.78 (1H, ddd, *J* = 9.2, 4.6, 4.6 Hz), 0.75 - 1.05 (4H, m), 1.17 (1H, dddd, *J* = 9.2, 6.9, 6.3, 4.6 Hz), 1.56 (1H, dddd, *J* = 8.4, 4.6, 4.6, 3.9 Hz), 1.86 (1H, ddd, *J* = 17.4, 8.5, 6.7 Hz), 1.97 (1H, dddd, *J* = 17.4, 6.7, 5.3, 1.2 Hz), 2.93 (1H, ddd, *J* = 10.0, 6.9, 3.1 Hz), 8.96 (1H, d, *J* = 3.9 Hz).
7. **6**: IR ($CHCl_3$) 1730 cm^{-1}; DCIMS *m/z* 607 $(M+H)^+$, 579, 547, 519, 487; ^1H NMR (270 MHz, $CDCl_3$) δ 0.47 (1H, ddd, *J* = 8.6, 5.5, 5.5 Hz), 0.75 (1H, ddd, *J* = 9.4, 5.5, 5.2 Hz), 0.87 (3H, br t, *J* = 7.0 Hz), 0.88 (3H, br t, *J* = 7.0 Hz), 1.07 (2H, m), 1.25 (24H, m), 1.50 - 1.80 (6H, m), 1.92 (2H, m), 2.09 (3H, s), 2.16 (3H, s), 2.42 (1H, ddd, *J* = 17.6, 8.2, 6.7 Hz), 2.54 (1H, ddd, *J* = 17.6, 6.9, 6.9 Hz), 2.98 (1H, ddd, *J* = 8.9, 7.3, 4.0 Hz), 3.18 (1H, ddd, *J* = 6.9, 4.0, 2.0 Hz), 3.57 (1H, ddd, *J* = 10.7, 8.2, 2.8 Hz), 3.73 (1H, dd, *J* = 7.3, 2.0 Hz), 4.02 (1H, dt, *J* = 6.9, 7.3 Hz), 4.48 (1H, dd, *J* = 8.9, 8.9 Hz), 4.98 (1H, dt, *J* = 2.0, 7.3 Hz), 9.86 (1H, d, *J* = 2.0 Hz).
8. E. Wenkert, B. L. Buckwalter, I. R. Burfitt, M. J. Gasic, H. E. Gottlieb, E. W. Hagaman, F. M. Schell, and P. M. Wovkulich, in "*Topics in Carbon-13 NMR Spectroscopy*," ed by G. C. Levy, Wiley-Interscience, New York, 1976, Vol. 2, Chap. 2, pp 81-121.
9. H. Niwa, K. Wakamatsu, and K. Yamada, *Tetrahedron Lett.*, **30**, 4543 (1989).

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