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FROM THE DINOFLAGELLATE *ALEXANDRIUM HIRANOI*

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Key Word Index—*Alexandrium hiranoi*; dinoflagellate; biosynthesis; goniodomin A; polyether macrolide; TCA cycle; polyketide.

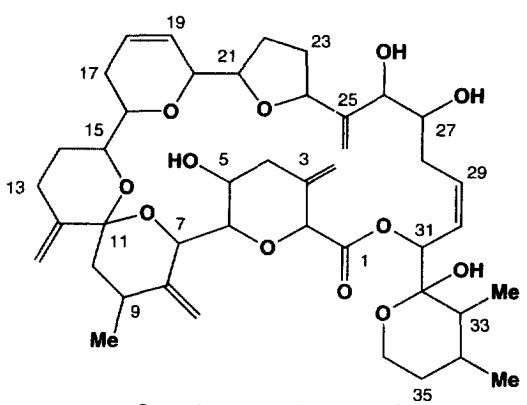
Abstract—A biosynthetic study of polyether macrolide goniodomin A(**1**), isolated from the dinoflagellate *Alexandrium hiranoi*, was carried out by feeding sodium [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate, and [*methyl*-^{13}C]-methionine. Considering the labelling patterns in a few compounds such as brevetoxins or dinophysistoxins (DTX), it is suggested that the TCA cycle participates, or single carbon deletion processes occur in the biosynthesis of goniodomin A.

INTRODUCTION

Recently, it is known that some polyether metabolites of marine dinoflagellates, such as brevetoxins, okadaic acids and dinophysistoxins (DTX), are biosynthesized through a unique pathway [1–6]. Chou and Shimizu [1] proposed that this pathway is different from a normal polyketide pathway and is closely related to TCA cycle and terpenoid metabolism. Similar precursors with normal polyketide pathway are suggested for okadaic acid [3] and DTX-1 [4]. Wright *et al.* [6] proposed a different pathway for DTX-4, that is the normal polyketide pathway with a single carbon deletion process. In the course of our studies on biologically active substances from microalgae, we found that the dinoflagellate *Alexandrium hiranoi* K & F (formerly *Goniodoma pseudogoniaulax*) [7, 8] had a potent antifungal activity, and isolated the polyether macrolide goniodomin A(**1**) as the antifungal principle [9]. In this paper, we report the biosynthetic origin of goniodomin A.

RESULTS AND DISCUSSION

Some feeding experiments using *A. hiranoi* fed with ^{13}C-labelled compounds were carried out to examine the biosynthetic pathway of goniodomin A. First, *A. hiranoi* was cultured with sodium [1-^{13}C]-acetate. The ^{13}C NMR spectra including DEPT of ^{13}C-labelled goniodomin A showed 13 carbons, noted as "c" in Fig. 1 and Table 1, were enriched with sodium [1-^{13}C]-acetate (1, 4, 6, 9, 12, 14, 19, 21, 23, 26, 28, 30 and 33). This fact suggests that these carbons were of acetate carbonyl origin. A signal at δ 27.5 was also enhanced but it was impossible to determine the position of enriched carbon in this experiment because two carbons, C13 and C17, had the same chemical shift at δ 27.5. A feeding experiment with sodium [2-^{13}C]-acetate indicated that **1** had 24 carbons originating from acetate methyl (2, 3, 3=CH_2, 5, 7, 8, 8=CH_2, 10, 11, 12=CH_4, 15, 16, 18, 20, 22, 24, 25, 25=CH_2, 27, 29, 31, 32, 33-Me and 34). These carbons were noted as 'm' in Fig. 1 and Table 1. There were two enhanced signals, but those could not be assigned in



Goniodomin A (**1**)

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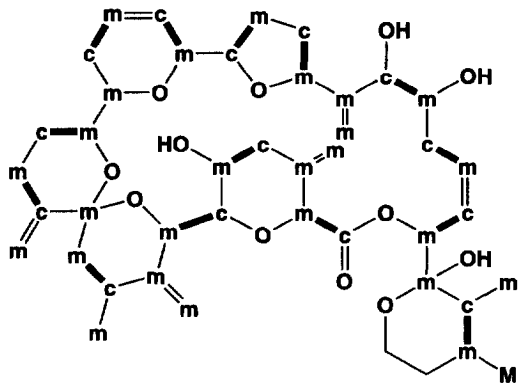


Fig. 1. The labeling pattern of goniodomin A. m: acetate methyl, c: acetate carbonyl, M: methionine methyl, bold bond: acetate unit.

this experiment. The signal at δ 27.5 was also enhanced with [2-^{13}C]-acetate, as in the case of the feeding experiment with [1-^{13}C]- acetate. The 9-Me and 34-Me carbons which had a same chemical shift at δ 20.0 were enriched with sodium [2-^{13}C]-acetate. Furthermore, *A. hiranoi* was fed with sodium [1, 2-$^{13}C_2$]-acetate to determine the positions of C_2 units that originate from the same acetate unit. The result of the 1D- and 2D-INADEQUATE [10, 11] spectra of isolated labelled goniodomin A confirmed the positions of 14 intact acetate units noted as "—" in Fig. 1 and Table 1. Since the signal for C13, which was not assigned by the feeding experiment of [1-^{13}C]-acetate, showed a cross-peak due to coupling with C12 originating from acetate carbonyl, C13 was shown to originate from acetate methyl. C17 was determined to originate from acetate carbonyl because the signal for C17 showed a cross-peak due to coupling with C18.

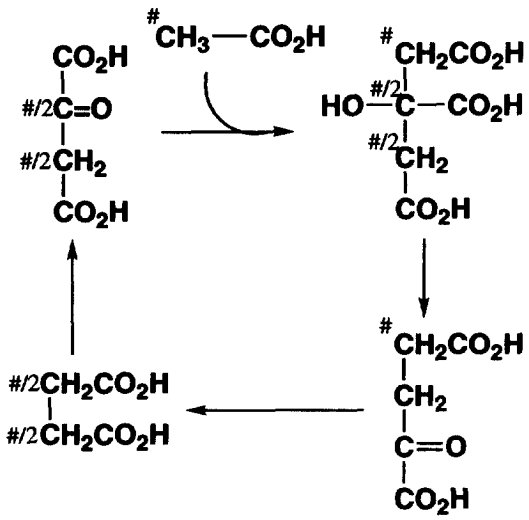
Chou and Shimizu [1] proposed that the biosynthesis of brevetoxins is closely related to the TCA cycle and terpenoid metabolism. Yasumoto and Torigoe [3] and Norte *et al.* [4] suggested a similar intervention of such precursors in a normal polyketide pathway to explain the biosynthesis of okadaic acid and DTX-1, respectively. From this point of view, the biosynthetic origin of goniodomin A could be explained as follows.

Two moieties (C17–C22, C26–C31) are presumed to be biosynthesized by a normal polyketide process because the labelling patterns are constructed from a succession of acetate units. Chou and Shimizu [1] suggested that two "m-m" moieties and three "m-m-m" moieties are presumed to be derived from the intermediates in the TCA cycle. Acetate is condensed with oxaloacetate to form citrate in the cycle. In the first cycle, C2 or C3 of the newly formed succinate will be originated from acetate methyl (Scheme 1). As a result of the "new" succinate, the "new" citrate formed by condensation of acetate with oxaloacetate in the second cycle will possess "m-m-m" labelling patterns. Thus the two "c—m-m" moieties (C9–C11, C14–C16) could be derived from succinate and the three "c—m-m-m" moieties (1/2/3/3=CH_2, 6/7/8/8=CH_2, 23/24/25/25=CH_2) from a tricarboxylic acid like citrate. Two intact acetate units (4/5,

Table 1. Isotope incorporation results based on the ^{13}C NMR data of goniodomin A

Position	Intensity ratio (labelled/unlabelled)[a] [1-^{13}C]-acetate	[2-^{13}C]-acetate	J_{CC}(Hz) [1,2-$^{13}C_2$]-acetate	Assignment[b]
1	4.1	0.9	68	c
2	1.2	2.2	68	m
3	1.0	1.9	—	m
3=CH_2	1.0	2.3	—	m
4	3.5	1.3	37	c
5	0.6	2.1	36	m
6	3.3	0.9	48	c
7	0.6	2.0	48	m
8	1.3	1.8	—	m
8=CH_2	1.4	2.5	—	m
9	3.3	1.0	32	c
9-Me	(1.1)[c]	(2.0)[c]	—	m
10	1.3	2.0	32	m
11	1.3	1.8	—	m
12	3.0	1.0	41	c
12=CH_2	1.2	2.1	—	m
13	(2.3)[d]	(1.8)[d]	(41)[d]	m
14	2.8	1	35	c
15	0.9	2.2	35	m
16	1.1	3.0	—	m
17	(2.3)[d]	(1.8)[d]	(41)[d]	c
18	1.1	2.2	40	m
19	3.5	1.1	44	c
20	0.9	2.4	44	m
21	4.4	0.9	34	c
22	1.3	2.2	34	m
23	3.5	0.9	35	c
24	0.7	1.9	35	m
25	1.4	2.1	—	m
25=CH_2	0.8	2.2	—	m
26	3.2	1.0	38	c
27	1.0	2.4	38	m
28	3.5	1.0	43	c
29	1.5	2.2	43	m
30	3.3	1.2	50	c
31	0.9	1.7	49	m
32	1.1	2.4	-	m
33	3.4	1.1	33	c
33-Me	1	2.6	—	m
34	1.3	2.4	33	m
34-Me	(1.1)[c]	(2.0)[c]	—	M
35	1.2	1.1	—	—
36	1.2	1.4	—	—

[a] Intensity of each peak in the labelled **1** divided by that of the corresponding signal in the unlabelled **1**, normalized to give a ratio of 1 for an unenriched peak (33-Me for [1-^{13}C]acetate labelling and C14 for [2-^{13}C]acetate labelling). [b]m: acetate methyl, c: acetate carbonyl, M: methionine methyl. [c]9-Me and 34-Me completely overlapped each other. These values are calculated from the peak at δ 20.1. [d]C13 and C17 completely overlapped each other. These values are calculated from the peak at δ 27.5.



Scheme 1.

12/13) are derived from acetate directly. A methyl and a methylene group noted as "m" in Fig. 1 (9-Me, 12=CH_2) are presumed to be of acetate methyl origin. The methylations at C9 and C12 are by methyl groups of acetate units with subsequent loss of one C_1 unit. This type of methylation was observed in other polyethers from dinoflagellates [2, 4, 12] and bacterial metabolite [13].

Feeding of [*methyl*-^{13}C]-methionine showed enhancement of one carbon signal at δ 20.0.* The signal at δ 20.0 was also enhanced with sodium [2-^{13}C]-acetate. This fact indicated that one of two methyl carbons (9-Me and 34-Me) was of acetate origin and the other of methionine origin. The ^{1}H selective decoupling experiment (irradiation at δ 0.740; H34-Me) revealed that C34-Me was originated from the methionine methyl. Consequently, the origin of C9-Me was the acetate methyl.

Recently, Wright *et al.* [6] proposed that backbone carbons of DTX-4 are derived from polyketide without participation of other precursors. This pathway is also applicable to biosynthesis of goniodomin A. Six backbone carbons from the methyl groups of cleaved acetate units (3, 8, 11, 16, 25, 32) are considered to be derived from a single carbon deletion process [6]. The advantage of this proposal is that the isotope enrichment would be expected to be uniform, which is suggested from the enrichment data (Table 1), whereas this is unlikely when TCA intermediates are involved (Scheme 1).

In the biosynthesis of DTX-1 and 4 [5, 14], the starter unit is shown to be glycolate. Although there is no data to explain the biosynthetic origin of the remaining two methylene carbons (35 and 36), these carbons are likely of glycolate origin.

* Labelled/unlabelled ratio of signal at δ 20.0 to that of δ 25.4 (C14) was 5 up.

EXPERIMENTAL

Culture. Alexandrium hiranoi was isolated from the rock pool at Jogashima, Kanagawa Prefecture (Japan), and cultured in 10 l glass bottles containing Provasoli medium [nutritive salts mixture ($NaNO_3$ 350 mg, β-Na_2glycerophosphate 50 mg, Fe (as EDTA; 1:1 molar) 2.5 mg, PII metals 25 ml, vitamin B_{12} 10 μg, vitamin B_1 500 μg, biotin 5 μg, Tris 500 mg, H_2O 100 ml) 2 ml, 75% sea water 100 ml] [15] under illumination of 500 $\mu E^{-2}\,s^{-1}$ on a 12L-12D cycle at 25° for about 20 days. It was made axenic with an antibiotic mixt. (penicillin-G 50 mg $^{-1}$, polymixin-B 50 mg l^{-1}, neomycin 2.5 mg l^{-1}, streptomycin 50 mg l^{-1}, chloramphenicol 0.5 mg l^{-1}). All ^{13}C-labelled compounds (NaOAc, methionine) were added 3 days before harvesting and those concentrations were 50 mg l^{-1}.

Extraction and isolation of goniodomin A. The organism was collected by filtration and extracted with MeOH ×3. The extract was combined, concd to remove MeOH, and partitioned between H_2O and Et_2O. The organic layer was subjected to silica gel column chromatography with C_6H_6–EtOAc (4:1, 3:2). The C_6H_6–EtOAc (3:2) fr. was subjected to ODS cc with aq. MeOH. The 83% MeOH fr. was subjected to reversed-phase HPLC with 83% MeOH to yield goniodomin A(**1**); column CAPCELL PAK C_{18}, 250 × 10 mm; flow-rate 1.5 ml min^{-1}: detection UV (210 nm) and refractive index. The yield was about 2 mg from 10 l culture in each feeding experiment.

^{1}H and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra assignments of **1** were previously described [9]. The ^{13}C NMR data used in this study were acquired in $CDCl_3$ at 75 MHz. Chemical shifts are referenced to internal $CDCl_3$ at δ 77.0 for carbon.

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