Gambieric Acids, New Potent Antifungal Substances with Unprecedented Polyether Structures from a Marine Dinoflagellate *Gambierdiscus toxicus*[1]

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From the dinoflagellate *Gambierdiscus toxicus*, we isolated gambieric acids A, B, C, and D which possess highly potent antifungal properties. The structures of gambieric acids A and B were elucidated from two-dimensional NMR data; they have novel brevetoxin-type structures consisting of nine contiguous ether rings (7/6/6/7/9/6/6/6/6) and one isolated tetrahydrofuran. Gambieric acids C and D are 3-methylhemiglutarates of gambieric acids A and B.

Marine dinoflagellates are attracting increased attention as a source of compounds with unique structures possessing desirable biological activity. Many of them are polyethers, which have become valuable reagents in biomedical research, e.g., okadaic acid,[2] maitotoxin,[3] brevetoxins,[4] and ciguatoxins.[5]

We previously discovered potent antifungal activity in many polyether toxins of dinoflagellate origin[6] and suspected that fungicidal substances are their common metabolites. Recent reports of antifungal compounds, goniodomins from *Alexandrium hiranoi* (*Goniodoma pseudogoniaulax*),[7] and of potent antineoplastic macrolides, amphidinolide A, B, and C from *Amphidinium* sp.[8] further demonstrated that dinoflagellates are a promising source of antieukaryotic compounds. As these substances may have potential medicinal value and also play a role in the marine ecosystem, we initiated a search for antimicrobial substances among phytoplankton. As a result, extremely potent antifungals, named gambieric acids, were detected in one strain of *Gambierdiscus toxicus*,[9] an epiphytic marine dinoflagellate well-known for its implication in ciguatera fish poisoning by producing ciguatoxins and maitotoxin.[10]

In this paper, we report isolation and structure elucidation, including stereochemistry, of gambieric acids A (1), B (2), C (3), and D (4).

Results and Discussion

Isolation. The acids were purified from the filtered medium of *G. toxicus* cultures. The filtrate (5000 L total) was passed through a column of polystyrene resin (Amberlite XAD-2). The retained antifungal substances were eluted with MeOH. Further purification by solvent partition and by column chromatography yielded gambieric acid A (1; 1.2×10^{-8}% by weight of the cultured medium), gambieric acid B (2; 0.3×10^{-8}%), and a mixture of gambieric acids C and D (3 and 4; 11.6×10^{-8}%). Activity was concentrated in the mixture, but the constituents were inseparable even by HPLC. Alkaline hydrolysis of the mixture yielded 1, 2, and 3-methylglutaric acid, which were used for the following structural studies.

Structure of Gambieric Acid A (1). The acid was obtained as a colorless amorphous solid. The molecular formula $C_{59}H_{92}O_{16}$ was determined by HR-FABMS. The IR spectrum (KBr) showed the presence of hydroxyl (3500 cm^{-1}) and carbonyl groups (1735 cm^{-1}).

Detailed analyses of ^1H–^1H COSY and 2D-HOHAHA spectra allowed us to deduce partial structures, encompassing H4–H18, H22–H34, H36–H39, and H41–H49. The H41–H49 fragment included two quaternary carbons. Cross-peaks on the COSY maps showed allylic couplings between H42/H57a,b, H44/H57a,b, H45/Me58, and H47/Me58, leading to assignments of all spin systems from H41 to H49.

Proton connectivities of 1 were interrupted by four quaternary carbons each bearing a methyl group. HMBC experiments clearly established connectivities around these quaternary carbons, because sensitivity of $J_{C,H}$ in ^1H-detected experiments like HMBC is in proportion to peak height of the ^1H-signal. Two- or three-bond ^1H–^{13}C couplings of angular methyls are the most suitable to see connectivity around an adjacent quaternary carbon. Prominent cross-peaks due to $^{2,3}J_{CH}$ couplings between Me52/C18, Me52/C20, Me53/C20, Me53/C22, Me55/C34, Me55/C36, Me56/C39, and Me56/C41 allowed us to assemble the four fragments into a single carbon chain (Figure 1).

Two-dimensional NMR data did not tell whether Me-50 was attached to C2 or C3; close chemical shifts of H2 and H3, as well as their large second-order couplings, made one of them *J*-coupled with Me50 via four bonds. HMBC experiments were not informative, because long-range $J_{C,H}$ via two and three bonds were indistinguishable. A one-dimensional HOHAHA experiment[11] solved the problem;

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(11) One-dimensional HOHAHA of 1 was measured at 400 MHz in C_5D_5N/CD_3OD (1:1) with increasing spin-locking time from 20 to 80 ms while Me-50 was being selectively excited by a long 180° pulse (50 ms).

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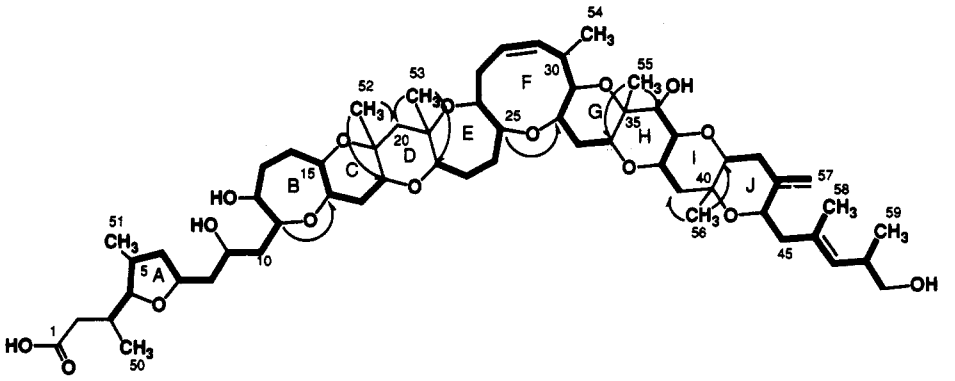
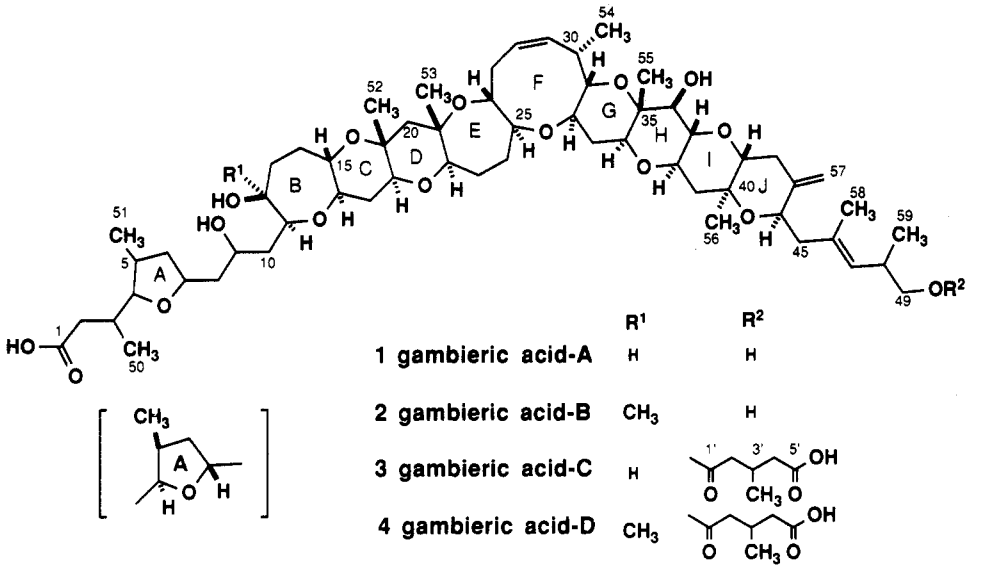


Figure 1. Two-dimensional NMR data used for structural elucidation of 1. The bold lines denote fragments assigned by ^1H–^1H COSY and 2D HOHAHA (TOCSY); arrows denote long-range correlations between methyl protons (tail) and a carbon (head) shown in HMBC experiments.

Chart I[a]



[a] For structures 1–4, relative stereochemistry is shown for rings A–J. Ring A in brackets shows orientation of the three substituents, but is not correlated with stereochemistry for rings A–J.

the magnetization generated by a selective 180° pulse at Me50 was transferred, during spin-locking, from Me50 to H4 through H3, but not through H2.

Presence of a carbonyl function in 1 was suggested by an IR band at 1735 cm^{-1}, although no ^{13}C NMR signal was observed in the carbonyl region. The ^{13}C NMR spectrum of the methyl ester of 1 clearly revealed a carbonyl carbon at 174.7 ppm. A possible reason for the absence of a carbonyl signal may be the exchange rate between monomeric and dimeric forms arising from hydrogen bonding of the carboxylic acid, which is within the NMR time scale.

Location of the carboxylic acid at C1 is based on the following reasons: chemical shifts of CH_2-2 (δ 2.04/2.35) are typical for α-methylene to a carbonyl group; also, methylation of the carboxylic acid brought out a C2 signal at 39.9 ppm. Broadening of a C2 signal in the 1D ^{13}C NMR spectrum of 1 as is the case with the carbonyl carbon (C1) also suggested that C2 must be near C1.

Number and location of hydroxyl groups were clarified by combined use of ^1H–^{13}C COSY and deuterium shifts observed in the ^{13}C NMR spectra. Four ^{13}C-signals assignable to C9, C12, C36, and C49 showed approximately –0.1 ppm shifts following deuterium replacement.

Location of ether linkages was clarified by HMBC and NOESY experiments. Detection of $^3J_{CH}$ couplings due to H11/C16 and H25/C32 in HMBC established ether linkage in rings B and F (Figure 1). Ether bonds in rings C, D, E, H, and J were confirmed by NOEs between angular protons or between an angular proton and a singlet methyl: H15–Me52, H18–H22, H26–Me53, H34–H38, and H44–Me56 (Figure 2).

The ether bonds of rings G and I were clarified by ^1H signal shapes; each signal of ring G revealed couplings typical of a substituted tetrahydropyran: $^3J_{H32,H33a} = 12$ Hz; $^3J_{H32,H33b} = 5$ Hz; $^2J_{H33a,33b} = 11$ Hz; $^3J_{H33a,H34} = 12$ Hz; and $^3J_{H33b,H34} = 5$ Hz. The ether linkage of ring I was not assignable by NOESY because of total overlap of a cross-peak arising from NOE between H37/H41 and H32/H34 (Table I). Both sets gave rise to significant NOEs. One-dimensional NOE difference spectra at –25 °C showed a triplet ($J = 10$ Hz), which did not coresond to H32 but to H37, when irradiating at δ 3.11 (both H34 and H41 were irradiated). Double diaxial couplings between H36/H37 and H37/H38 should make H37 a triplet, while H32 should give a double triplet due to its double diaxial couplings and an axial–equatorial coupling.

Nine ether rings, three olefins, and one carboxylic acid account for 13 of 14 unsaturations deduced from the molecular formula, thus leaving one unsaturation unassigned. Deuterium shift experiments revealed C4 and C7 to be ether-bearing carbons. Their location and the unsaturation number of the molecular suggested that they form a tetrahydrofuran ring (ring A). Moreover, chemical shifts of C4 (δ 86.4) and H7 (δ 4.40) were deshielded significantly

Table I. ^{13}C and ^{1}H NMR Assignments of Gambieric Acid A (1) and Gambieric Acid B (2)

	gambieric acid A (multiplicity)		gambieric acid B	
position	^{13}C[a]	^{1}H[b]	^{13}C	^{1}H
1				
2	40.8, t	2.35, 2.04	40.8	2.36, 2.08
3	33.6, d	2.08	33.5	2.10
4	86.4, d	3.48 (3, 9)	86.5	3.49
5	36.9, d	2.14 (m)	36.9	2.18
6	42.8, t	1.67 (2 H)	42.9	1.65 (2 H)
7	75.2, d	4.40 (m)	75.0	4.46
8	45.7, t	1.71, 1.50 (4, 11, 12)	45.6	1.74, 1.45
9	68.5, d	4.16 (m)	68.9	4.22
10	45.1, t	1.82 (2 H)	41.1	1.91, 1.88
11	86.5, d	3.73 (5, 6, 6)	88.3	3.60
12	75.6, d	3.84 (4, 4, 5)	75.6	
13	30.6, t	1.87, 1.80	41.5	1.93, 1.75
14	28.7, t	2.01, 1.68	29.7	1.93, 1.75
15	76.0, d	3.43	77.1	3.42
16	83.5, d	3.41	86.4	3.30
17	34.2, t	2.10, 1.53	34.0	2.21, 1.60
18	82.9, d	3.03 (5, 12)	83.4	3.10
19	74.1, s		74.5	
20	55.8, t	1.97 (12), 1.76 (12)	55.8	1.99, 1.78
21	77.2, s		77.3	
22	86.4, d	3.34 (4, 11)	86.6	3.39
23	25.4, t	1.84, 1.63	25.4	1.86, 1.63
24	32.7, t	2.08, 1.59	32.6	2.13, 1.65
25	86.4, d	3.26	86.4	3.30
26	78.8, d	3.78 (5, 5, 9)	78.8[d]	3.79
27	33.8, t	2.85 (5, 11, 12), 1.91 (5, 5, 12)	33.8[c]	2.84, 1.91
28	129.0, d	5.78 (5, 11, 11)	128.9	5.77
29	135.9, d	5.39 (11, 11)	135.9	5.35
30	33.7, d	3.08 (m)	33.7[c]	3.07
31	75.5, d	3.43 (7, 12)	75.6	3.43
32	85.3, d	3.22 (5, 12, 12)	85.3	3.22
33	34.4, t	2.27 (5, 5, 11), 1.68 (11, 12, 12)	34.4	2.32, 1.68
34	80.8, d	3.11 (5, 12)	80.8	3.12
35	78.8, s		78.8	
36	78.9, d	3.66 (10)	78.9[d]	3.65
37	85.3, d	3.23 (10, 10)	85.3	3.22
38	78.6, d	3.43	78.6[d]	3.45
39	45.3, t	2.11, 1.57	45.4	2.12, 1.55
40	74.8, s		74.8	
41	84.2, d	3.11 (5, 12)	84.2	3.10
42	36.7, t	2.42 (5, 13), 2.32 (12, 13)	36.8	2.41, 2.32
43	147.7, s		147.7	
44	70.4, d	4.18 (7, 9)	70.5	4.18
45	43.7, t	2.40 (7, 14), 2.13 (9, 14)	43.4	2.40, 2.13
46	135.0, s		135.0	
47	131.5, d	4.95 (9)	131.5	4.97
48	37.3, d	2.58 (m)	37.3	2.59
49	68.7, t	3.37, 3.34	68.7	ca. 3.36 (2 H)
50	19.4, q	1.14 (8)	19.5	1.21
51	15.1, q	0.83 (7)	15.1	0.85
52	17.8, q	1.18 (s)	17.9	1.20
53	19.6, q	1.17 (s)	19.7	1.19
54	16.8, q	0.97 (7)	16.8	0.97
55	12.0, q	1.25 (s)	12.0	1.25
56	17.1, q	1.26 (s)	17.1	1.27
57	111.3, t	4.83 (br s), 4.76 (br s)	111.3	4.82, 4.76
58	17.9, q	1.62 (s)	17.9	1.62
59	18.5, q	0.92 (7)	18.6	0.93
			Me on C12 25.8	1.14

[a] $^{13}CD_3OD$ as 49.8 ppm (C_5D_5N–CD_3OD (1:1), 100 MHz). [b] CD_2HOD as 3.31 ppm (C_5D_5N–CD_3OD (1:1), 400 MHz). [c,d] Denotes each assignment is interchangeable.

in comparison with those of an acyclic system, presumably due to the steric constraint of the five-membered ring. These results led us to the two-dimensional structure of gambieric acid A (1).

Stereochemistry of Gambieric Acid A. The gambieric acids were the fifth groups possessing brevetoxin-type polyether skeletons (brevetoxin A, brevetoxin B, yessotoxin, and ciguatoxins). In all four preceding types the ether rings without exception are trans-fused. The same seemed to be the case with gambieric acids. NOESY experiments and coupling constants indicate that ether rings B–J are trans-fused (Figure 2).

Interproton couplings of 6-membered rings revealed that all rings are in the chair conformation (see Table I).[12] By MM2 calculations the distance between angular protons

(12) Vicinal diaxial ^{3}J between angular protons in trans-fused tetrahydropyran is 8.5–10 Hz. Diaxial and axial equatorial ^{3}J between an angular proton and the adjacent methylene are 9.5–10.0 and 4.0–4.5 Hz respectively.

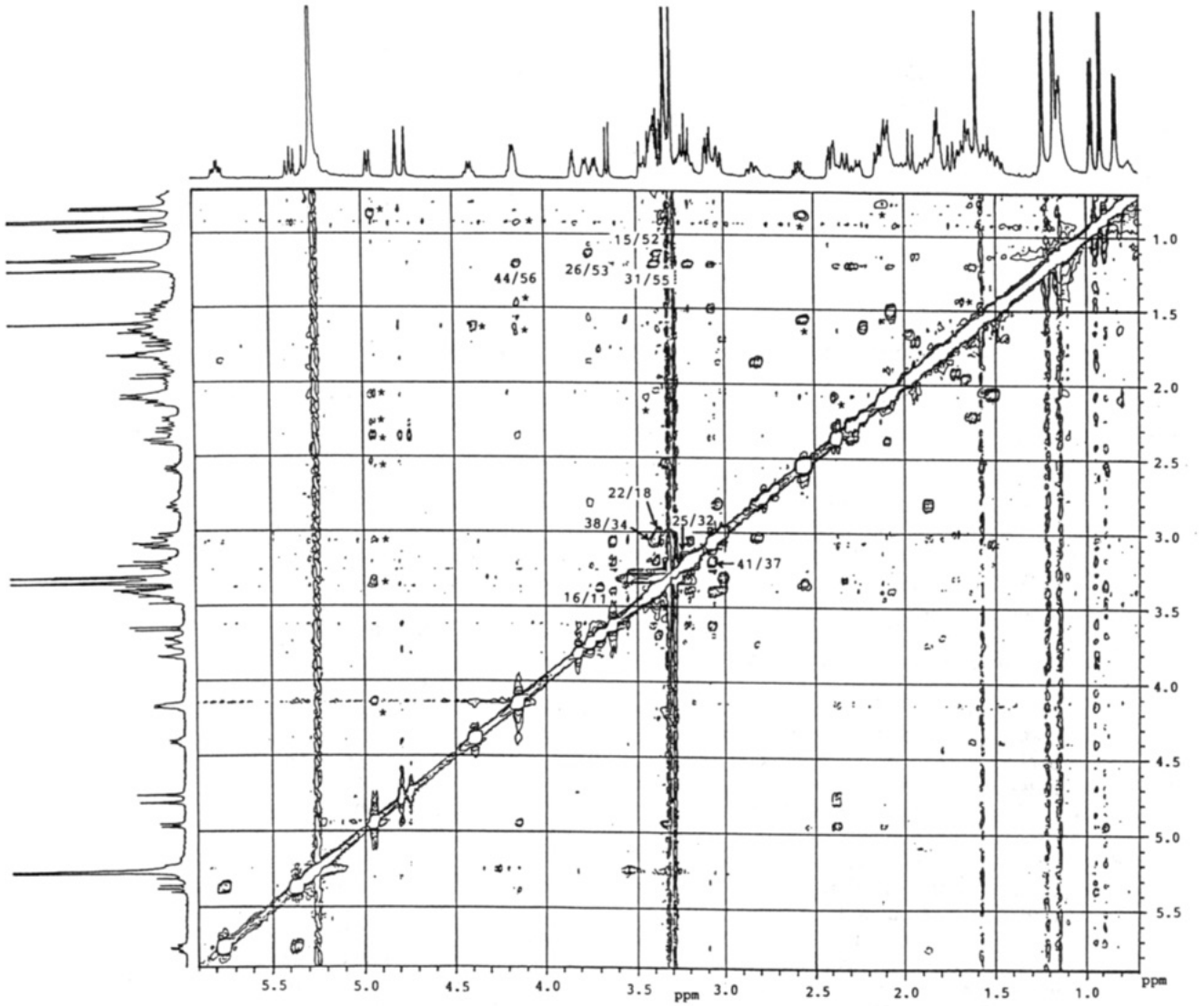


Figure 2. NOESY spectrum of 1. The spectrum was measured in CD_3OD–C_5D_5N (1:1) at 500 MHz (Bruker AM-500) with mixing time of 130 ms. Asterisks denote positive cross-peaks, while others including diagonal peaks are observed as negative contours.

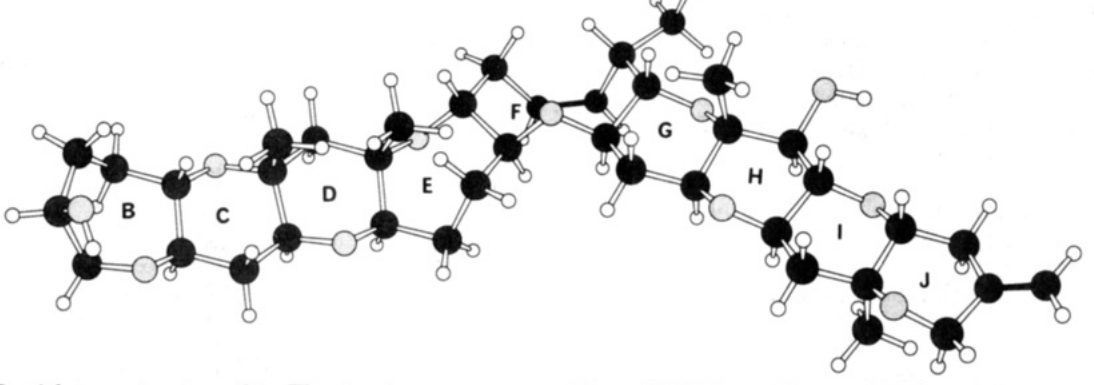


Figure 3. Partial stereostructure of 1. The structure was composed from NMR data with use of MM2 and Chem3D.

on both sides of tetrahydropyran (rings D, H, and I) was estimated to be 0.23 nm; the value is well within the range to produce prominent NOEs. The distance between a methyl carbon at an angular position and an angular proton was approximately the same as that between protons. NOEs around ether linkage of rings C, D, G, H, I, and J were observed as cross-peaks in NOESY (Figure 2) or ROESY experiments, thus indicating that the rings are trans-fused.

The fusion mode of seven- or nine-membered rings (rings B, E and F) was readily assignable on the basis of NOEs. In the NOESY experiment cross-peaks due to H11/H16, Me53/H26, and H25/H32 (Figure 2) supported trans-fusion around rings B, E, and F. As the conformation of these medium-sized rings is important for assigning orientation of substituents, MM2 calculations were carried out to deduce their stable conformers. The two seven-membered rings B and E appear to take a twisted chair conformation as shown in Figure 3 as was the case with the related polyethers.

Ring F, a 5-oxonene, often appears in this group of compounds, e. g., brevetoxin A or ciguatoxins. NOE experiments[13] coupled with MM2 calculations revealed that conformation of ring F was different from that of brevetoxin A;[4b] in Figure 3 the olefinic carbons, C28 and C29, of 1 are anti to H26/H31 while those of crystalline brevetoxin A could be either syn or anti.[4c] A possible reason for this difference is that pseudoequatorial orientation of the C54 methyl stabilizes the anticonformer. Conversely, the methyl would come too close to one of the $H_2$27 protons in the syn conformer. Ciguatoxin has a similar oxonene moiety differing only by the lack of an allyl methyl; the conformational change of the ring is believed to cause extreme broadening of ^1H and ^{13}C NMR signals. Both ^1H and ^{13}C NMR signals arising from ring F of 1, however, were observed without significant peak broadening. The methyl substitution may therefore affect the exchange rate or population between conformers.

The NOEs between H7/Me51 and H4/H5[14] clarified orientations of three substituents on the ring A although their stereochemistry was not correlated with those in rings B–J because of the acyclic part (C8–C10) residing between the rings A and B. The NOEs between H11/H12 indicated that β orientation of 12-OH. Equatorial orientation of 36-OH was also evidenced by NOEs between H34/H36 and H36/H38 and by $^3J_{H36,H37}$ (10 Hz) coupling, corresponding to diaxial interaction.

These results allowed us to deduce the relative stereochemistry of 1 except for the acyclic part of the molecule. A partial stereochemical view of 1 generated by MM2 and Chem3D is shown in Figure 3.

Structures of Gambieric Acids B, C, and D. The ^1H and ^{13}C NMR spectra of gambieric acid B (2) showed close similarity to those of 1. The molecular weight of 2 was m/z 1070, 14 mass larger than that of 1, suggesting an additional one-carbon unit. Conversion of a methine to a quaternary carbon by introduction of a new methyl at C12 was evidenced by interruption of connectivity H11–H12–H13 and absence of cross-peaks corresponding to H12 in ^1H–^1H COSY and 2D HOHAHA spectra. A new methyl singlet appeared at δ 1.14 in the ^1H NMR spectrum. On the HMBC spectrum of 2, the methyl signal gave cross-peaks versus three ^{13}C NMR signals at δ 41.5, 75.6 (quaternary), and 88.3, corresponding to C13, C12, and C11, respectively. These data unambiguously supported that the new methyl was at C12. NMR data (Table I) indicated that the rest of 2 was indistinguishable from 1, thus establishing the structure of gambieric acid B as 12-methylgambieric acid A.

The mixture of gambieric acids C (3) and D (4) exhibited the principal antifungal activity. Alkaline hydrolysis of the mixture yielded 3-methylglutaric acid as well as 1 and 2. 3-Methylglutaric acid was identified by comparing its ^1H NMR spectrum with that of an authentic specimen.[15] Detailed NMR analyses of the mixture revealed that no structural changes had occurred as a result of the alkali treatment, except for hydrolysis of the hemiester. Thus, 3 and 4 have structures in common with 1 and 2. In the ^1H and ^{13}C NMR spectra of the mixture, extreme broadening of the signals was observed in both termini of the molecules; those due to 3-methylglutarate virtually disappeared. A possible reason for the signal broadening is the slow exchange between monomeric and dimeric forms, as in the case of 1. Methylation of 3 and 4 with CH_2N_2 dramatically sharpened the broadened signals, as had been observed with 1.[16]

Location of the ester in 3 and 4 was determined by ^1H NMR chemical shifts. The chemical shifts of H_2-49 (δ 3.80/3.93) for the mixture of 3 and 4 were significantly deshielded in comparison with those of 1 (δ 3.34/3.37) and 2 (δ 3.36/3.36), thus indicating that the site of the ester was C49 of 3 and 4. Esterification at C49-OH was further evidenced by deuterium shift experiments of the mixture, in which the ^{13}C NMR signal of C49 was not shifted between C_5D_5N–CD_3OD (1:1) and C_5D_5N–CD_3OH (1:1).[1]

Several compounds consisting of fused polyether rings (brevetoxin type) have been reported so far. The gambieric acids are the first with an isolated ether ring in addition to contiguous fused ether rings. All members in this class are produced by dinoflagellates, except for yessotoxin whose origin is still in question.

The antifungal activity of gambieric acids is extremely potent; 1, 2, and the mixture of 3 and 4 inhibit the growth of *Aspergillus niger* at 10, 20, and 10 ng/disk. The potency exceeds that of amphotericin-B 2000-fold. To our knowledge, the gambieric acids represent the most potent antifungals known to date. Toxicity against mice or cultured mammalian cells was moderate,[17] which points to the potential of the acids as antifungal drugs.

From an ecological point of view, it is interesting to note that those epiphytic dinoflagellates release the antifungals from the cells, while retaining maitotoxin, which has no antimicrobial activity. Because of their poor solubility, gambieric acids may stay on the surfaces of the substrate near the dinoflagellates and exert an allelopathic function against other epiphytic organisms. Maitotoxin, on the other hand, may act as an antifeedant with its extreme toxicity toward higher animals.

Experimental Section

Spectral Measurements. FAB mass spectra were determined at an acceleration voltage of 3 kV with use of 3-nitrobenzyl alcohol as a matrix.

^1H NMR spectra (400 and 500 MHz) and ^{13}C NMR spectra (100 and 125 MHz) were measured in CD_3OD–C_5D_5N (1:1), except for CD_3OH–C_5D_5N (1:1) to observe deuterium shifts in ^{13}C NMR measurements.

Culture of *G. toxicus*. *G. toxicus* (GII1 strain) isolated in the Gambier islands, French Polynesia, was cultured in seawater medium enriched with ES-1 nutrients[18] at 25 °C under illumination of 4000–8000 lx with 18-h light and 6-h dark cycles. After 38 days, the dinoflagellates were filtered and the filtrate was passed through an Amberlite XAD-2 column.

Isolation of Gambieric Acids. The column of Amberlite XAD-2 (Roehm & Haas, 80- × 400-mm i.d.) was first washed with H_2O (10 L) and then with MeOH (5 L). The methanolic eluate was evaporated and the residue suspended in H_2O and extracted first with diethyl ether and then with *n*-butanol. The crude

(13) NOEs between H26/Hβ27, Hβ27/Hβ30, and H30/H31 observed in NOESY spectrum indicated that ring F has the conformation shown in Figure 3. If ring F assumes the other conformation, e.g., C28═C29 *syn* to H26/H31, β protons of H27 and H30 assume pseudoequatorial orientation, which moves them out of NOE range.

(14) In the ROESY spectrum of 1, prominent ROEs were observed between H4/H5 and H7/Me51 but not between H4/Me51, indicating the α-orientation of H4 and β-orientations of H7 and Me51.

(15) Identification of 3-methylglutaric acid was done by negative FABMS, $(M - H)^-$ m/z 145, and ^1H NMR (400 MHz, CD_3OD) δ 2.39 (1 H, multiplet), 2.36 (2 H, multiplet), 2.19 (2 H, q, J = 8 Hz), 1.02 (3 H, d, J = 6 Hz).

(16) ^1H NMR signals due to the 3-methylglutarate of the methylated products derived from the mixture of 3 and 4 are as follows: ^1H NMR (400 MHz, CD_3OD–C_5D_5N (1:1)), δ 2.65 (1 H, multiplet, 3′), 2.55 (2 H, multiplet, 2′ and 4′), 2.38 (2 H, multiplet, 2′ and 4′), 1.07 (3 H, d, J = 7 Hz, Me-C3′).

(17) Both 1 and 2 at doses of 1 mg/kg showed no toxicity against mice upon interaperitoneal injection. Cytotoxicity (IC_{50}) of the mixture of gambieric acid C and D against mouse lymphoma L5178Y cells was 1.1 μg/mL when monitored by [^{3}H]thymidine incorporation.

(18) Provasoli, L. In *Proc. U.S.-Japan Conf. Held at Hakone*; Sept 12–15; Watanabe, A.; Hattori, A., Eds.; Tokyo, 1966; pp 63–75.

antifungals obtained in the *n*-butanol fraction was successively chromatographed over columns of Toyopearl HW-40 (Tosoh, 25- × 300-mm i.d.) with MeOH–H_2O (1:1) and Develosil ODS 15/30 (Nomura Chemicals, 10- × 40-mm i.d.) with MeOH–H_2O (1:1), MeOH–H_2O (7:3), and MeOH. The active substance obtained in the methanolic eluate was further purified by HPLC over reversed-phase columns of Develosil ODS-7 (10- × 250-mm i.d.) and Develosil ODS-5 (8- × 250-mm i.d.) with MeCN–H_2O (9:1). Further chromatography of the active fraction on normal-phase Develosil 60-5 (8- × 250-mm i.d.) with $CHCl_3$–MeOH–H_2O (200:10:1) yielded **1**, **2**, and the mixture of **3** and **4**. Each fraction gave a single spot on TLC; silica gel-60 (Merck) was developed with $CHCl_3$–MeOH–H_2O (90:10:1); R_f values for gambieric acid A (**1**), B (**2**), and the mixture of C (**3**) and D (**4**) were 0.32, 0.37, and 0.18, respectively.

Alkaline Hydrolysis of 3 and 4. The mixture of **3** and **4** (3.2 mg) was hydrolyzed with 0.8 mg of NaOH in 100 μL of MeOH–H_2O (9:1) at 60 °C for 1 h. The hydrolyzate, after being neutralized with dilute HCl, was extracted with EtOAc. Successive HPLC over Develosil ODS-5 (Nomura Chemicals, 8- × 250-mm i.d.) with MeCN–H_2O (9:1) and Develosil 60-5 (8- × 250-mm i.d.) with $CHCl_3$–MeOH–H_2O (200:10:1) yielded **1** (2.4 mg), **2** (0.5 mg), and 3-methylglutaric acid. Elution of the antifungal substances was monitored by a growth inhibition test against *A. niger*. The final amounts of the compounds used for determining structures were 7.0 mg of **1**, 2.3 mg of **2**, and 5.6 mg of the mixture of **3** and **4**.

Gambieric acid A (1): white amorphous solid; $[\alpha]^{20}_D$ +33° (*c* 0.488, MeOH); UV (MeOH) max <210 nm; IR (KBr) 3500, 1735 cm^{-1}; HR-FABMS $[M + Na]^+$ *m/z* 1079.6330 (1079.6280 calcd for $[C_{59}H_{92}O_{16}Na]^+$); ^1H and ^{13}C NMR data are shown in Table I.

Gambieric acid B (2): white amorphous solid; UV (MeOH) max <210 nm; HR-FABMS $[M + Na]^+$ *m/z* 1093.6430 (1093.6440 calcd for $[C_{60}H_{94}O_{16}Na]^+$); ^1H and ^{13}C NMR data are shown in Table I.

Mixture of gambieric acids C (3) and D (4): white amorphous solid; UV (MeOH) max <210 nm; HR-FABMS obsd *m/z* 1185.6920 for gambieric acid C (**3**) (calcd for $C_{65}H_{101}O_{19}$ *m/z* 1185.6939); FABMS $[M(3) + H]^+$ 1185, $[M(3) + Na]^+$ 1207, $[M(3) + K]^+$ 1223, $[M(4) + H]^+$ 1199, $[M(4) + K]^+$ 1237.

Gambieric Acid A Methyl Ester. The methyl ester of **1** was prepared with use of CH_2N_2 diethyl ether solution: white solid; UV (MeOH) max <210 nm; IR (KBr) 3500, 1740 cm^{-1}; FABMS $[M + H]^+$ *m/z* 1071, $[M + Na]^+$ *m/z* 1093; ^1H NMR (400 MHz, CD_3OD–C_5D_5N (1:1)) δ 3.61 (3 H, S, MeO–), 2.31 (1 H, d, 12 Hz, H-2), 1.97 (1 H, dd, 12, 4 Hz, H-2′); ^{13}C NMR (100 MHz, CD_3OD/C_5D_5N) δ 174.7 (C1), 52.8 (MeO–), 39.9 (C2); the other signals of ^1H and ^{13}C NMR agreed well with those of gambieric acid A (**1**).

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Registry No. Gambieric acid A, 138434-64-7; gambieric acid B, 141363-65-7; gambieric acid C, 138458-89-6; gambieric acid D, 141363-66-8.

Supplementary Material Available: ROESY spectrum of gambieric acid A (**1**), HMBC, ^{13}C NMR (^1H broad band decoupling), ^1H–^1H HOHAHA (TOCSY), ^1H–^1H COSY of gambieric acid B (**2**), and ^1H–^1H COSY of a mixture of gambieric acid C and D. The other 2D data are available as supplementary material for the previously published communication[1] (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Synthesis of Optically Active β-Lactams by the Photolytic Reaction of Imines with Optically Active Chromium Carbene Complexes. 2. Synthesis of 1-Carbacephalothin and 3-ANA Relays

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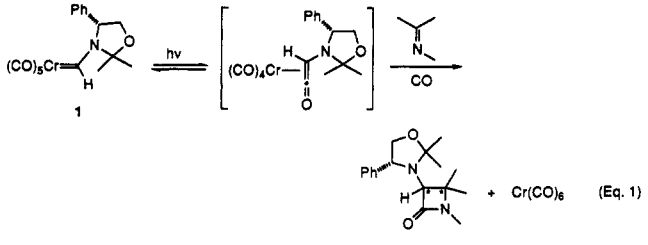
A relay (**3**) to optically active 1-carbacephalothin (**4**) was prepared in modest yield with high stereoselectivity by the photochemical reaction of optically active chromium carbene complex **1** with functionalized imine **2**. In contrast, the photochemical reaction of carbene complex **1** with imine precursors **15a,b** to the nocardicins was much less stereoselective.

Introduction

Recent studies in these laboratories have dealt with the synthesis of simple optically active β-lactams utilizing photochemical reactions of optically active chromium aminocarbene complexes with imines (eq 1).[1] This reaction proved highly stereoselective (>97% de) for *N*-benzylimines of acetaldehyde, *N*-benzylimidates, thiazolines, oxazines, thiazines, and simple 5- and 6-membered cyclic imines, but less so with (de 70%) symmetrical imines such as those of formaldehyde or acetone. The absolute stereochemistry of the position α to the carbonyl group was determined by the chiral auxiliary on the carbene complex ($R \rightarrow R,S \rightarrow S$) while the relative (cis/trans) stereochemistry was determined by the imine substrate. In marked contrast, reactions of **1** with *N*-benzylimines of benz-



(Eq. 1)

(1) (a) Hegedus, L. S.; Imwinkelried, R.; Alarid-Sargent, M.; Dvorak, D.; Satoh, Y. *J. Am. Chem. Soc.* **1990**, *112*, 1109. (b) For a review on the reactions of chromium aminocarbene complexes see: Schwindt, M. A.; Miller, J. R.; Hegedus, L. S. *J. Organomet. Chem.* **1991**, *413*, 143.

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