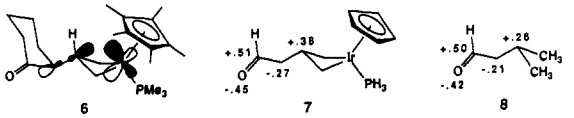
Chart I



6 7 8

oxygen to one metallacycle α-carbon in the equatorial conformation.

This new stereoelectronic effect can be attributed to a transannular interaction between an occupied metal spd hybrid orbital and the C_β–C2 σ^* orbital (cf., **6**, Chart I).[12] The polarization of the C_β–C2 bond is further delocalized into the carbonyl system by hyperconjugation with the CO π^* orbital.[13] Neither a statistically significant elongation of the Cβ–C2 bond nor a contraction of the C2–C1 bond, however, is observed in the crystal structure (Figure 1). Nonetheless, using the structurally determined bond distances, Fenske–Hall molecular orbital calculations[15] on model complex **7** show consistent metal-induced perturbations of the Mulliken atomic charges compared to the organic fragment **8**. Calculated orbital overlap populations are also consistent with the predicted polarization: the carbonyl and Cβ–C2 bonds are both weakened and the C2–C1 bond is strengthened compared to **8**. Finally, consistent with a transannular interaction, one occupied, substantially metal-centered frontier molecular orbital[16] (see structure **6**, Chart I) includes significant $\sigma^*_{C\beta\text{-}C2}$ character, confirming that this interaction is energetically reasonable and symmetry allowed.[17]

In summary, a novel transition-metal-mediated stereoelectronic effect has been identified for enolate-derived metallacyclobutane complexes, capable in favorable cases of controlling the conformation of the appended organic fragment. Further investigation of this interesting phenomenon is anticipated as complexes incorporating other metals, ancillary ligands, and substitution patterns are prepared.

Acknowledgment. We thank Dr. William E. Streib for the X-ray crystal structure determination, Dr. Feng Lin for assistance with NMR experiments, Dr. K. E. Gilbert and Prof. J. J. Gajewski for molecular modelling, Prof. E. R. Davidson and Dr. R. H. Cayton for instruction in MO calculations, and Profs. T. S. Widlanski and J. M. Takacs for helpful discussion. Financial support from the National Science Foundation and an American Cyanamid Faculty Award is gratefully acknowledged. We also thank Johnson Matthey, Inc. for a generous loan of precious metals.

Supplementary Material Available: Spectroscopic data, including selected decoupling, difference NOE, and *J*-resolved NMR spectra, for compounds **2–5**, descriptions of the calculational method and results, and details of the data collection and crystal structure solution for complex **2** (23 pages); tables of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

(12) Direct transannular interactions between *d* and π^* orbitals of β-oxometallacyclobutane complexes have been discussed: Kemmitt, R. D. W.; McKenna, P.; Russell, D. R.; Prouse, L. J. S. *J. Chem. Soc., Dalton Trans.* **1989**, 345 and references therein.

(13) As described, this effect is a doubly homologous version of the well-documented "α-effect" in α-metalla-enolate complexes, $L_nMCH_2C(O)R$, which show substantial shifts of the carbonyl absorption frequencies to lower energy.[14]

(14) Coates, G. E.; Green, M. L. H.; Wade, K. *Organometallic Compounds*; Butler & Tanner, Ltd.: London, 1968; Vol. 2, p S215. Recent examples: Engelbrecht, J.; Greiser, T.; Weiss, E. *J. Organomet. Chem.* **1981**, *204*, 79. Vicente, J.; Chicote, M. T.; Cayuelas, J. A.; Fernandez-Baeza, J.; Jones, P. G.; Sheldrick, G. M.; Espinet, P. *J. Chem. Soc., Dalton Trans.* **1985**, 1163. Burkhardt, E. R.; Doney, J. J.; Bergman, R. G.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 2022.

(15) Hall, M. B.; Fenske, R. F. *Inorg. Chem.* **1972**, *11*, 768. See the supplementary material for details.

(16) This metal orbital, qualitatively speaking, is the one previously involved in the central carbon to metal bond in the starting η^3-allyl cation **1**.

(17) Consistent with a metal-based electronic effect, replacement of the phosphine ligand with the π-acidic CO reduces the magnitude of the shift in ν_{CO} to about half that observed in complex **2**: Wakefield, J. B.; Stryker, J. M. Unpublished results.

Gambieric Acids: Unprecedented Potent Antifungal Substances Isolated from Cultures of a Marine Dinoflagellate *Gambierdiscus toxicus*

Hiroshi Nagai

Central Research Institute
Taiyo Fishery Co., Tsukuba 300-42, Japan

Koichiro Torigoe, Masayuki Satake, Michio Murata, and Takeshi Yasumoto*

Faculty of Agriculture
Tohoku University, Sendai 981, Japan

Hiroshi Hirota

Faculty of Science, The University of Tokyo, Tokyo 113, Japan

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Marine dinoflagellates produce many polyether compounds of chemical and biomedical importance, e.g., okadaic acid,[1] maitotoxin,[2] brevetoxins,[3] and ciguatoxins.[4] As many of them inhibit the growth of fungi,[5] we screened marine phytoplankton for new antifungal metabolites. Potent antifungal substances were found in one strain of *Gambierdiscus toxicus*,[6] an epiphytic species implicated in ciguatera as the source of maitotoxin and ciguatoxins.[7] While these toxins were retained in the algal cells during culture, the antifungals were released into the medium.[6] Activity-guided purification led to the discovery of four new polyethers, designated gambieric acid, A, B, C, and D (GA-A, GA-B, GA-C, and GA-D). Their property of inhibiting the growth of *Aspergillus niger* was of unprecedented potency, exceeding that of amphotericin B by a factor of 2×10^3.[8] In this communication we report the structures of GA-A (**1**) and GA-C (**2**), which are novel ladder-shaped polyethers.

G. toxicus (GIII strain), isolated in the Gambier Islands, French Polynesia, was cultured in a seawater medium enriched with ES-1 nutrients[9] at 25 °C for 38 days. The medium (5000 L), free of algal cells, was passed through a column of Amberlite XAD-2. The crude antifungal compounds retained on the column were eluted with MeOH. Purification of the eluate was carried out by solvent partition and column chromatography.[10] Three active constituents were obtained: GA-A (0.6 mg), GA-B (0.15 mg), and a mixture of GA-C and GA-D (5.8 mg). The major activity resided in the mixture, but GA-C and GA-D were inseparable

(1) Murakami, Y.; Oshima, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fish.* **1982**, *48*, 69–72.

(2) Yokoyama, A.; Murata, M.; Oshima, Y.; Iwashita, T.; Yasumoto, T. *J. Biochem.* **1988**, *104*, 184–187.

(3) (a) Lin, Y.-Y.; Risk, M.; Ray, S. M.; Engen, D. V.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775. (b) Shimizu, Y.; Chou, H.-N.; Bando, H.; Duyne, G. V.; Clardy, J. *J. Am. Chem. Soc.* **1986**, *108*, 514–515.

(4) Murata, M.; Legrand, A. M.; Ishibashi, Y.; Yasumoto, T. *J. Am. Chem. Soc.* **1989**, *111*, 8929–8931.

(5) Nagai, H.; Satake, M.; Yasumoto, T. *J. Appl. Phycol.* **1990**, *2*, 305–308.

(6) Nagai, H.; Satake, M.; Murata, M.; Yasumoto, T. In *Toxic Marine Phytoplankton*; Graneli, E. et al., Eds.; Elsevier: New York, 1990; pp 385–390.

(7) Yasumoto, T.; Nakajima, I.; Bagnis, R.; Adachi, R. *Bull. Jpn. Soc. Sci. Fish.* **1977**, *43*, 1021–1026.

(8) GA-A, GA-B, and a mixture of GA-C and GA-D inhibited the growth of *A. niger* at 10, 20, and 10 ng/disk, respectively, by the paper disk method, while amphotericin B and okadaic acid were inhibitory at doses of 20 and 10 μg/disk, respectively. GA-A at a dose of 1 mg/kg showed no toxicity against mice upon an interaperitoneal injection. Cytotoxicity (IC50) of the mixture of GA-C and GA-D against mouse lymphoma L5178Y cells was 1.1 μg/mL when monitored by [^{3}H]thymidine incorporation.

(9) Provasoli, L. In *Proceedings of the U.S.-Japan Conference Held at Hakone*; September 12–15; Watanabe, A., Hattori, A., Eds.; Tokyo, 1966; pp 63–75.

(10) The crude antifungals eluted from the XAD-2 column were suspended in H_2O and extracted with Et_2O and then with 1-butanol. The 1-butanol extract was successively chromatographed on the following columns with solvents as indicated: Toyopearl HW-40 (Tosoh), CH_3OH/H_2O (1:1); Develosil ODS (Nomura Chem.), CH_3OH/H_2O (1:1, 7:3, 1:0); Develosil ODS-7, CH_3CN/H_2O (9:1); Develosil ODS-5, CH_3CN/H_2O (9:1); Develosil 60-5, $CHCl_3/CH_3OH/H_2O$ (200:10:1).

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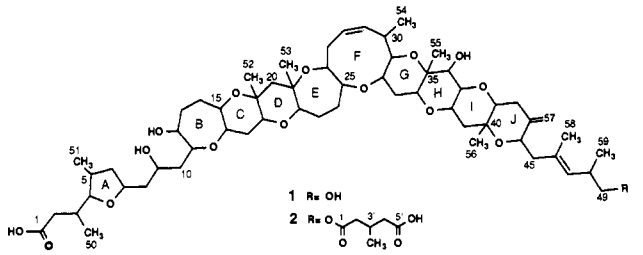
even by HPLC. Negative FABMS of the mixture suggested molecular weights of 1184 for GA-C and 1198 for GA-D. Hydrolysis of the mixture in a methanolic NaOH solution yielded GA-A (4.1 mg), GA-B (0.9 mg), and 3-methylglutaric acid, which was identified by ^{1}H NMR and FABMS data.[11] Thus, GA-C and GA-D apparently were 3-methylglutarate hemiesters of GA-A and GA-B, respectively. Structural studies were carried out mainly on GA-A and GA-C.

GA-A (**1**) was obtained as a white amorphous solid: $[\alpha]^{20}_D$ +33° (*c* 0.488, CH_3OH); UV (MeOH) $\lambda_{max} < 210$ nm; IR (KBr) 3500, 1735 cm^{-1}; HR-FABMS $[M + Na]^+$ *m/z* 1079.6330 (1079.6280 calcd for $[C_{59}H_{92}O_{16}Na]^+$). A carboxylic acid, suggested by the IR band at 1735 cm^{-1}, was proven to be present by measuring the IR, FABMS, and ^{13}C and ^{1}H NMR spectra of the methylated product,[12] although no corresponding signal was observed in the ^{13}C NMR spectrum of **1**.



Detailed analyses of ^{1}H-^{1}H COSY and 2D-HOHAHA spectra[13] allowed us to deduce partial structures H4-H18, H22-H34, and H36-H39, and H41-H49. The location of Me-50 was unassignable due to overlapping of the ^{1}H NMR signals and large second-order couplings between H2 and H3. One-dimensional HOHAHA experiments[14] solved the problem; magnetization was transferred from Me-50 to H4 through H3 upon irradiation at Me-50.

HMBC spectra[15] clarified the connectivities around the quaternary carbons by giving cross peaks due to $^{2,3}J_{CH}$ couplings between C52/H18, C52/H20, C53/H20, C53/H22, C55/H34, C55/H36, C56/H39, and C56/H41. Eventually, a remaining carboxyl carbon could be connected to C2; chemical shifts of H_2-2 (δ 2.04/2.35) are typical for an α-methylene of a carbonyl group.

The number and location of hydroxyl groups were clarified on the basis of deuterium shifts observed on ^{13}C NMR signals.[16] Detection of $^3J_{CH}$ couplings in the HMBC experiment revealed ether linkages of H11-C16 (B ring) and H25-C32 and C25-H32 (F ring). The presence of rings C, D, E, H, and J was confirmed by NOESY measurements,[17] which showed NOEs between angular protons or between an angular proton and a singlet methyl. The presence of rings G and I was clarified on the basis of proton coupling constants[18] and NOEs between H37 and H41 (1D NOE difference spectra at -25 °C). We could not obtain direct evidence of an ether linkage between C4 and C7 in ring A. The five-membered ring was deduced from the deuterium shift experiment. Moreover, the chemical shifts of C4 (δ 86.4) and H7 (δ 4.40) were deshielded significantly in comparison with those of an acyclic system, presumably due to the steric effect of the five-membered ring.

These results led us to **1** as the planar structure for GA-A. Assignments of ^{1}H and ^{13}C NMR signals of **1** are given in the supplementary material. The structure of GA-C (**2**) was readily determined by detailed comparison of the 1D and 2D NMR spectra with those of **1**.[19] GA-A is unique in possessing an isolated ring in its terminal chain in addition to a continuous chain of fused rings. The absence of ciguatoxins in this GII1 strain indicates the biosynthetic versatility of this organism. It is conceivable that these extremely potent antifungal metabolites released from the cells may act as repellents against other epiphytic microorganisms.

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Supplementary Material Available: Tables of ^{13}C and ^{1}H NMR assignments and 1D ^{1}H NMR, 1D and 2D HOHAHA, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, HMBC, and NOESY spectra of gambieric acid A (**1**) and NOESY (-20 °C) and NOE difference spectra (-25 °C) of the mixture of gambieric acid C (**2**, major) and gambieric acid D (13 pages). Ordering information is given on any current masthead page.

(11) 3-Methylglutaric acid: FABMS $(M - H)^-$ *m/z* 145; ^{1}H 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).

(12) **1** was treated with CH_2N_2 to give the methyl ester of **1**: IR (KBr) 1740 cm^{-1}; FABMS $(M + Na)^+$ *m/z* 1093. The ^{1}H and ^{13}C NMR spectra of the ester agreed well with those of **1**, except for signals due to C-1 and C-2: ^{13}C NMR (100 MHz, CD_3OD/C_5D_5N 1:1) δ 174.7 (C-1), 52.8 (CH_3O), 39.9 (C2); ^{1}H NMR (400 MHz, CD_3OD/C_5D_5N 1:1) δ 3.61 (3 H, s, CH_3O), 2.31 (1 H, d, *J* = 12 Hz, H-2), 1.97 (1 H, dd, *J* = 12, 4 Hz, H-2').

(13) ^{1}H-^{1}H COSY, 2D HOHAHA (mixing time 85 ms), and phase-sensitive ^{13}C-^{1}H COSY spectra were recorded on a GSX-400 (JEOL, 400 MHz) spectrometer in C_5D_5N/CD_3OD (1:1).

(14) The 1D HOHAHA spectrum of **1** was measured at 400 MHz in C_5D_5N/CD_3OD (1:1), with increasing duration of spin locking from 20 to 80 ms while Me-50 was being excited selectively with the use of a long 180° pulse (50 ms).

(15) HMBC of **1** was recorded on an AM-500 (Bruker, 500 MHz) spectrometer in C_5D_5N/CD_3OD (1:1). The experiment was optimized for J_{CH} of 8.3 Hz.

(16) Deuterium shifts were measured on the mixture of **2** (major) and GA-D by comparison of ^{13}C NMR signals of hydroxyl-bearing carbons between spectra measured in C_5D_5N/CD_3OD (1:1) and in C_5D_5N/CD_3OH (1:1). Significant shifts (0.08-0.12 ppm) were observed for C9, C12, and C36, indicating the presence of three hydroxyl groups.

(17) The NOESY spectrum of **1** was measured at 500 MHz with a mixing time of 130 ms in C_5D_5N/CD_3OD (1:1) at 20 °C. Both positive and negative NOEs were observed. NOESY spectrum of the mixture of **2** (major) and GA-D was recorded at 400 MHz with a mixing time of 150 ms in C_5D_5N/CD_3OD (1:1) at -20 °C. All NOEs were negative under these conditions. The spectra are available as supplementary material.

(18) The coupling constants of protons on ring G are typical for those of a substituted tetrahydropyran: $^{2,3}J_{H,H}$, H32/H33a, 12 Hz; H32/H33b, 5 Hz; H33a/H33b, 11 Hz; H33a/H34, 12 Hz; H33b/H34, 5 Hz.

(19) The location of the 3-methylglutarate ester in GA-C was determined on the basis of ^{1}H NMR chemical shifts of H_2-49 (3.80/3.93), which were significantly deshielded in comparison with those of **1** (δ 3.34/3.37).

Synthesis and Characterization of $C_{60}O$, the First Fullerene Epoxide

Kathleen M. Creegan, John L. Robbins, Win K. Robbins, John M. Millar, Rexford D. Sherwood, Paul J. Tindall, and Donald M. Cox*

Corporate Research Laboratories
Exxon Research and Engineering Company
Annandale, New Jersey 08801

Amos B. Smith, III,* John P. McCauley, Jr., David R. Jones, and Rex T. Gallagher

Department of Chemistry, the Monell Chemical Senses Center, and the Laboratory for Research on the Structure of Matter, University of Pennsylvania
Philadelphia, Pennsylvania 19104

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The isolation of C_{60} in preparatively useful quantities by Krätschmer et al.[1] has stimulated intensive efforts to generate and characterize functional derivatives. Many reactions of C_{60} furnish complex, inseparable mixtures of products,[2] and only a few dis-

(1) Krätschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. *Nature* **1990**, *347*, 354. Krätschmer, W.; Fostiropoulos, K.; Huffman, D. R. *Chem. Phys. Lett.* **1990**, *170*, 167.

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