mixture[14] of **9**[15] and its anti isomer), and **10** (67% yield of a 27:1 mixture of **10**[15] and its anti[16] isomer), respectively (Scheme II). The formation of **8–10** demonstrates that a variety of substitution patterns can be tolerated by **1b** without major inhibition of the formal 2 + 4 cycloaddition[17] of the corresponding allyl cation to 1,3-cyclohexadiene. This bodes well for the broad generality of this reaction.

In summary, we have developed a very low temperature process for the addition of acetals of acrolein and its derivatives to 1,3-dienes. We believe that the underlying mechanistic concept associated with this facile cycloaddition reaction will find widespread usage. We are currently exploring a variety of applications of these principles to other systems.

Acknowledgment. We are indebted to the National Science Foundation and to the General Medical Institute of the National Institutes of Health for grants that supported this investigation.

(13) The two components of the mixture were separated by chromatography and the isomer ratio was based on the isolated yields of 78% for **8** and 7% for the epimer of **8**.
(14) The isomer ratio was determined by the use of ^{1}H NMR studies.
(15) The stereochemistries of **9** and **10** were assigned on the basis of difference NOE studies and shift-reagent studies, respectively.
(16) Trace amounts (3–4%) of an impurity could be detected in **10** by ^{1}H NMR. The peak positions that could be determined for this impurity were consistent with it having the structure of the anti isomer.
(17) It is interesting to note that the structure of **10** establishes the formal suprafacial nature of our cycloaddition reaction.

Biosynthesis of Brevetoxins. Evidence for the Mixed Origin of the Backbone Carbon Chain and the Possible Involvement of Dicarboxylic Acids

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The toxins found in the extremely deleterious red tide organism *Gymnodinium breve* (=*Ptychodiscus brevis*) possess unusual polycyclic ether structures examplified by brevetoxin A (**1**)[1] and brevetoxin B (**2**).[2] Although the ring systems of the two toxins differ significantly, the resemblance of the terminal four rings suggests their closely related biosynthesis. The methyl-substituted carbon chain backbone appeared to be biosynthesized by the straightforward elongation of acetate units and the introduction of methyl groups from methionine and/or propionate as seen with the biosynthesis of most polyketides and fatty acid derivatives. However, the feeding experiments conducted by the authors' group[3] and Nakanishi's group[4] using carbon-13-labeled acetates and methionine resulted in inexplicable labeling patterns in brevetoxin B (**2**), as summarized in Figure 1A. The puzzling finding prompted us to reexamine the entire ^{13}C NMR signal assignment[5] and repeat the experiments. Now we are able to present experimental data to explain what initially appeared to be irrational results.

(1) Shimizu, Y.; Chou, H. N.; Bando, H.; Van Duyne, G. D.; Clardy, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 514–515.
(2) Lin, Y. Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775.
(3) [1-^{13}C]Acetate, [2-^{13}C]acetate, [1,2-$^{13}C_2$]acetate, and [*methyl*-^{13}C]-methionine were used in a number of feeding experiments. The details of the experiments will be published elsewhere.
(4) Lee, M. S.; Repeta, D. J.; Nakanishi, K.; Zagorski, M. G. *J. Am. Chem. Soc.* **1986**, *108*, 7855–7856.
(5) The carbon signal assignment was made by a combination of hetero-COSY and exhaustive proton-decoupling experiments. The total proton and carbon assignment of signals in CD_2Cl_2 can be found in the supplementary material.

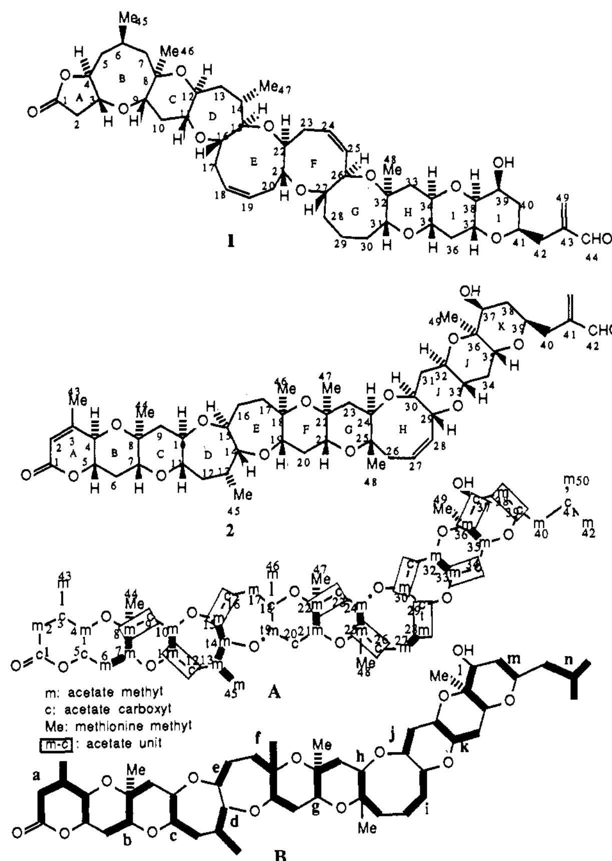

Figure 1. Structures of brevetoxin A (**1**) and brevetoxin B (**2**), the labeling patterns resulted from feeding experiments with carbon-13-labeled precursors (A) and assumed building blocks of **1** (B). The solid lines in (A) indicate the presence of couplings.

First we observed that the prolonged incubation with carbon-13-labeled acetate led to the random but differential labeling of carbons in **2**. In attempts to pulse feed acetate by a brief exposure of the organism to a large dose of methyl-^{13}C-labeled acetate, we isolated **2**, whose ^{13}C NMR spectrum shows carbon–carbon spin–spin couplings with only selected carbons.

In a typical experiment, the organism was grown in NH-15 medium[6] in Fernbach flasks (2 L × 10) under 4000–5000-lux illumination. Eleven days after inoculation, each flask was fed with [2-$^{13}C_1$]CH_3COONa (100 mg), chloramphenicol (5 mg), and streptomycin (10 mg). After 48 h, the culture was harvested, and a total of 1.1 mg of pure **2** was isolated according to the previously established procedure.[7] The ^{13}C NMR spectrum of the isolated toxin[8] showed enrichment with all the carbons which were found to be of acetate methyl origin in the previous experiments, but most significantly, the signals for the carbon fragments C6–C7–C8, C10–C11, C45–C13–C14–C15, C21–C22, C24–C25, C27–C28, C32–C33, and C35–C36 showed distinct splittings due to spin–spin couplings between the adjacent carbons. No such randomization was observed when carboxyl-labeled acetate was fed under a similar condition.[8] *The observed couplings indicate that those carbon fragments were constructed by rather confined carbon–carbon linkage formation between methyl originated carbons during the concentrated pulse feeding.* Furthermore, feeding experiments with [1,2-$^{13}C_2$]acetate indicate that

(6) Wilson, W. B.; Collier, A. *Science* (*Washington, D.C.*) **1955**, *121*, 394–395.
(7) Chou, H. N.; Shimizu, Y.; Van Duyne, G. D.; Clardy, J. C. In *Toxic Dinoflagellates*; Anderson, D. M., White, A. W., Baden, D. G., Eds.; Elsevier/North Holland: New York, 1985, pp 305–308.
(8) The ^{13}C NMR sppctra were obtained in CD_2Cl_2 on a Bruker WM500 instrument at 125 MHz with SW-29 500 Hz, AQ = 0.5 s, and PW = 5 μs. The numbers of scans (NS) are 45 816 and 12 404 for the samples from the methyl- and carboxyl-^{13}C acetate feedings, respectively. The spectra are provided in the supplementary material along with the assignment.

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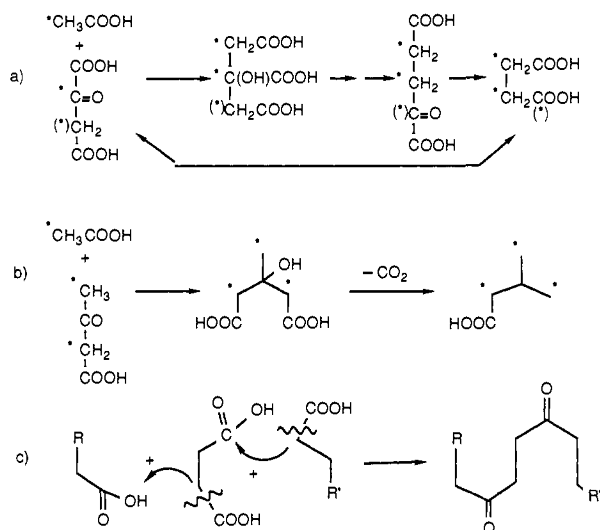
Scheme I



C22–C23, C25–C26, C28–C29, C33–C34, and C36–C37 come from acetate units. The best known examples of bond formation between acetate methyl and primarily methyl-derived carbons are a condensation with oxaloacetate to form citrate and a condensation with glyoxalate (which can be formed from isocitrate by the action of isocitrate lyase) to form L-malate. In both cases, the newly formed connectivity will appear in C2 and C3 of succinate (Scheme I, path a).[9] Thus it seems to be reasonable to assume that the three carbon fragments c, g, h, i, k, and l (Figure 1B) are derived from succinate directly or indirectly. Similarly the four-carbon unit, b, can be of α-keto glutarate, whose C2, C3, and C4 are derived from acetate methyl carbon. The connectivity seen with the four-carbon sequence C45–C13–C14–C15 may be explained by the condensation of propionate to acetate, since the labeling of the acetate methyl group often appears in all three carbons of a propionate-derived fragment.[10] Thus all the mysterious labeling patterns can be interpreted by asssuming the limited involvement of the organic acids metabolism. Regarding the other unusual building blocks a, f, and n, the labeling patterns obtained in acetate feeding experiment are in accordance with the formation of 3-hydroxyl-3-methylglutarate from acetate and acetoacetate, the first step in the isoprenoid biosynthesis (Scheme I, path b).

All these observations seem to suggest a new type of polyketide formation involving dicarboxylic acids, in which a Claisen-type condensation occurs to the α-position of the second carboxylic function with a loss of carboxyl group (Scheme I, path c).[11] The step involves very possibly activation with CoA binding and carboxylation as with the case of polyketide formation in general. Mixed polyketides are actually not new. However, the involvement of unusual fatty acids are mostly at the terminal units,[12] and inside the chains, the condensation is limited to the α-position of rather common monocarboxylic acids.

Attempts to prove the above hypothesis by feeding the putative precursors including [2,3-$^{13}C_2$]succinate have been so far unsuccessful. The failure is not surprising, because the rejection or lack of activation of certain exogenous metabolites by organisms, especially dinoflagellates, is very common and frequently experienced by the authors' group in biosynthetic studies with other dinoflagellates.

Acknowledgment. We are grateful to Professor Koji Nakanishi, Columbia University, for providing us with unpublished data and to Professor David Cane, Brown University, and Professor Heinz G. Floss, The Ohio State University, for precious suggestions. This research was supported by the National Institutes of Health, Grants GM-24425 and GM-28754, which is greatly appreciated. We are also thankful to the Yale University NMR Facility for NMR service.

Supplementary Material Available: Table of carbon-13 and proton chemical shifts and assignment for compound **2** and the 125-MHz ^{13}C NMR spectra of brevetoxin B derived from methyl- and carboxyl-^{13}C-labeled acetate (2 pages). Ordering information is given on any current masthead page.

(9) After one round through TCA cycle, C3 of oxaloacetate should carry one-half of the labeling from the incorporated acetate. Thus one may expect a 2:1 isotope ratio in C2 and C3 of succinate formed in the next round. The rather even enrichment observed in the spectrum can be explained by the quickly declining uptake and utilization of acetate by the organism during the incubation.

(10) The information was provided by Professor David Cane.

(11) An alternative explanation may be the condensation to C3 of pyruvate as seen in the biosynthesis of chlorothricin (Lee, J. J.; Lee, J. P.; Keller, P. J.; Cottrell, C. E.; Chang, C. J.; Zahner, H.; Floss, H. G. *J. Antibiot.* **1986**, *39*, 1123–1134.). If that is the case, pyruvate has to come from the oxidative decarboxylation of malate to account for the labeling pattern.

(12) For example: Chen, T. S. S.; Chang, C. J.; Floss, H. G. *J. Am. Chem. Soc.* **1981**, *103*, 4565–4568. Parry, R. J.; Mafoti, R. *J. Am. Chem. Soc.* **1986**, *108*, 4681–4682.

Characterization of Six-Coordinate Ferryl Protoheme by Resonance Raman and Optical Absorption Spectroscopy

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Ferryl species (Fe^{IV}=O) have been postulated in the catalytic cycle of cytochrome *c* oxidase,[1] as the oxygen donating species in cytochrome P-450,[2] and as intermediates in the reactions of catalases and peroxidases.[3] Given the diverse chemistry catalyzed by these various enzymes, heme pocket modulation of the chemical reactivity of the Fe^{IV}=O unit seems likely. Resonance Raman detection of the ν(Fe=O) in various protein species and model compounds supports this notion. In a comparison of ferryl peroxidase species,[4] ferryl myoglobin,[5] and five- and six-coordinate heme model compounds,[6,7] the frequency of ν(Fe=O) varies by $\sim$85 cm^{-1} (see Table I). This is in strong contrast to ν(Fe–O_2) which varies by only $\sim$10 cm^{-1} in protein species and heme model compounds.[8] In addition, there are distinct differences in the optical spectra of the various ferryl protein species.[9-11] Previously

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(6) (a) Bajdor, K.; Nakamoto, K. *J. Am. Chem. Soc.* **1984**, *106*, 3045–3046. (b) Proniewicz, J. M.; Bajdor, K.; Nakamoto, K. *J. Phys. Chem.* **1986**, *90*, 1760–1766.

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