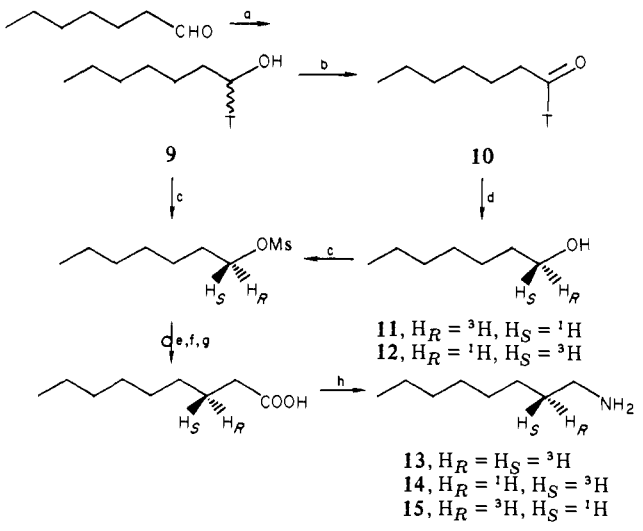
Scheme III[a]



[a] (a) $[^3H]KBH_4$. (b) PCC. (c) MsCl, Et_3N. (d) 9-BBN, (-)- or (+)-α-pinene. (e) *n*-BuONa, $CH_2(CO_2Et)_2$. (f) NaOH. (g) H^+, Δ. (h) HN_3.

lines of the doublet was about five times that of the singlet at the approximate center of the doublet corresponding to $[^{13}C,^{14}N]$-labeled molecules. One can therefore conclude that *n*-octylamine is incorporated into elaiomycin with retention of its nitrogen atom.[7]

The conversion of *n*-octylamine into elaiomycin leads to the formation of a cis double bond between C-1 and C-2 of the amine. The stereochemistry of hydrogen removal associated with the creation of this double bond has been elucidated by means of experiments with doubly labeled precursors. Samples of [1(*R*,*S*)-^{3}H]-, [1(*R*)-^{3}H]-, and [1(*S*)-^{3}H]-*n*-octylamine were synthesized by the route outlined in Scheme II. Reduction of *n*-octanal with tritiated borohydride yielded [1(*R*,*S*)-^{3}H]-*n*-octanol (**2**). The tritiated alcohol was converted to [1(*R*,*S*)-^{3}H]-*n*-octylamine (**6**) via formation of the mesylate, displacement with azide, and catalytic reduction. PCC oxidation[8] of **2** yielded [1-^{3}H]-*n*-octanal (**3**). Reduction of **3** with the adduct of 9-borabicyclononane and (-)- or (+)-α-pinene[9] was expected to give [1(*R*)-^{3}H]- and [1(*S*)-^{3}H]-*n*-octanol (**4**, **5**), respectively. The stereochemistry assigned to the alcohols **4** and **5** follows from literature precedents.[9,10] The chirally tritiated alcohols **4** and **5** were transformed into [1(*S*)-^{3}H]- and [1(*R*)-^{3}H]-*n*-octylamine (**7**, **8**) via mesylation, azide displacement, and reduction. On the basis of the assumption that the displacement step occurs with inversion of configuration, the chirally labeled alcohols **4** and **5** lead to [1(*S*)-^{3}H]- and [1(*R*)-^{3}H]-*n*-octylamine (**7**, **8**), respectively. Administration of the three forms of [1-^{3}H]-*n*-octylamine to *S. gelaticus* in conjunction with [1-^{14}C]-*n*-octylamine gave the results summarized in Table I (experiments 3-5). The data clearly reveals that *n*-octylamine is incorporated into elaiomycin with removal of the 1-*pro*-*R* hydrogen atom.

Scheme III portrays the methods that were utilized to synthesize three forms of [2-^{3}H]-*n*-octylamine. [1(*R*,*S*)-^{3}H]-*n*-Heptanol (**9**) was prepared in standard fashion and converted to the corresponding mesylate. Alkylation of diethyl malonate with the labeled mesylate was followed by ester hydrolysis, decarboxylation, and Schmidt degradation to yield [2(*R*,*S*)-^{3}H]-*n*-octylamine (**13**). A similar reaction sequence was then applied to [1(*R*)-^{3}H]- and [1(*S*)-^{3}H]-*n*-heptanol (**11**, **12**) obtained by 9-BBN-α-pinene reduction of [1-^{3}H]-*n*-heptanal (**10**). If it is assumed that the malonate alkylation step proceeds with inversion of configuration, then alcohols **11** and **12** will be converted to [2(*S*)-^{3}H]- and [2(*R*)-^{3}H]-*n*-octylamine (**14**, **15**). The results of precursor incorporation experiments employing **13**-**15** are shown in Table I (experiments 6-8). The tritium to carbon-14 ratios of the labeled samples of elaiomycin isolated in these experiments indicate that *n*-octylamine is incorporated into the antibiotic with loss of the 2-*pro*-*R* hydrogen atom. It therefore follows that the $\Delta^{5,6}$-double bond of elaiomycin is generated by the syn removal of two hydrogen atoms. A priori, this dehydrogenation process could proceed either by a direct removal of two hydrogen atoms or by oxidation to an imine followed by tautomerization. A decision between these two alternatives will require additional investigation.

Acknowledgment. We thank the National Institutes of Health (Grant CA-25142) and the Robert A. Welch Foundation (Grant C-729) for support of these investigations.

Registry No. 1, 23315-05-1; **6**, 80106-32-7; **7**, 80183-03-5; **8**, 80183-04-6; **13**, 80106-33-8; **14**, 80183-05-7; **15**, 80183-06-8; sodium [1-^{14}C]-octanoate, 13095-58-4; [1-^{14}C]-*n*-octylamine, 80106-34-9.

(7) Other recent examples of the use of ^{13}C-^{15}N coupling in biosynthetic investigations include: (a) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707. (b) Pita Boente, M. I.; Kirby, G. W.; Robins, D. J. *J. Chem. Soc., Chem. Commun.* **1981**, 619. (c) Leete, E.; McDonnell, J. A. *J. Am. Chem. Soc.* **1981**, 103, 658. (d) Grue-Sorensen, G.; Spenser, I. D. *Ibid.* **1981**, *103*, 3208. (e) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *Ibid.* **1981**, *103*, 4639.

(8) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647.

(9) Midland, M. M.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1977**, *99*, 5211.

(10) (a) Parry, R. J. *J. Chem. Soc. Chem. Comm.* **1978**, 294. (b) Parry, R. J.; Trainor, D. A. *J. Am. Chem. Soc.* **1978**, *100*, 5243.

Synthesis and Spectroscopic Properties of a Novel Cofacial Chlorophyll-Based Dimer

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We wish to report the synthesis and unique spectroscopic properties of a novel, doubly linked, cofacial chlorophyll (Chl) dimer. Considerable effort has been directed toward the synthesis and characterization of covalently connected porphyrins and Chls.[1,2] The work on porphyrins is motivated by an interest in the chemical consequences of positioning two multivalent metal ions in well-defined proximity without intervening ligands.[1e] The synthesis of covalently connected Chls is stimulated by the considerable body of evidence that a special pair of Chls and bacteriochlorophylls serve as the primary electron donors in green plant (photosystem I) and bacterial photosynthesis, respectively,[3] or by an interest in the synthesis of rigid models for photosynthetic electron transfer. The characteristic spectral properties of the in vivo electron donors are a red shift and split CD for the Q_y absorption bands. These observations provide evidence of interchromophore resonance (exciton) interactions,[4] and are further supported by ESR and ENDOR data on the cation radical of the electron donor in bacterial systems.[3a,b]

The properties of three synthetic Chl dimers are compared in this paper (see Figure 1): the novel cofacial dimer, Mg_2-I, the doubly linked "hinged" dimer, Mg_2-II, prepared by Wasielewski et al.,[2d] and the original singly linked dimer, Mg_2-III, prepared by Boxer and Closs,[2a] whose Q_y band exhibits a large red shift

(1) (a) Schwarz, F. P.; Gouterman, M.; Muljiani, Z.; Dolphin, D. H. *Bioinorg. Chem.* **1972**, *2*, 1. (b) Collman, J. P.; Elliott, C. M.; Halbert, T. R.; Tovrog, B. S. *Proc. Natl. Acad. Sci. U.S.A* **1977**, *74*, 18. (c) Chang, C. K.; Kuo, M. S.; Wang, C. B. *J. Heterocycl. Chem.* **1977**, *14*, 943. (d) Kagan, N. E.; Mauzerall, D.; Merrifield, R. B. *J. Am. Chem. Soc.* **1977**, *99*, 5484. (e) Collman, J. P.; Denisevich, P.; Konai, Y.; Marrocco, M.; Koval, C.; Anson, F. C. *Ibid.* **1980**, *102*, 6027.

(2) (a) Boxer, S. G.; Closs, G. L. *J. Am. Chem. Soc.* **1976**, *98*, 5406. (b) Wasielewski, M. R.; Studier, M. H.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4282. (c) Wasielewski, M. R.; Smith, U. H.; Cope, B. T.; Katz, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 4172. (d) Wasielewski, M. R.; Svec, W. A.; Cope, B. T. *Ibid.* **1978**, *100*, 1961.

(3) (a) Norris, J. R.; Uphaus, R. A.; Crespi, H. L.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 625. (b) McElroy, J. D.; Feher, G.; Mauzerall, D. C. *Biochim. Biophys. Acta* **1972**, *267*, 363. (c) Wasielewski, M. R.; Norris, J. R.; Shipman, L.; Lin, C.-P.; Svec, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2957.

(4) Sauer, K. In "Bioenergetics of Photosynthesis"; Govindjee, Ed.; Academic Press: New York, 1975; pp 115-181.

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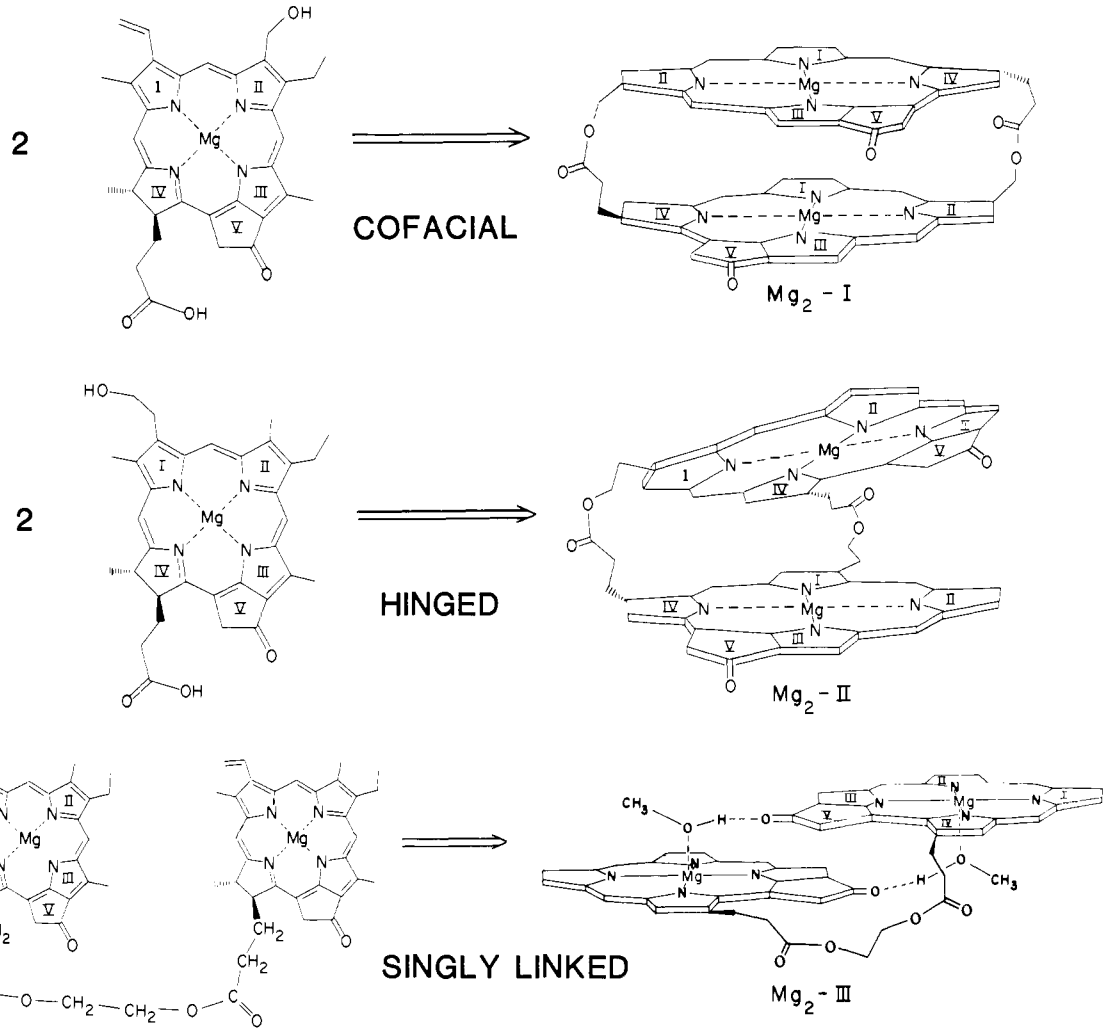


Figure 1. Chemical structures and schematic molecular conformations for the dimers described in the text.

to about 700 nm when the macrocycles are encouraged to interact (the "folded" form, as illustrated) by the addition of appropriate ligands.

The synthesis of the cofacial dimer begins with pyromethylpheophorbide *b* (IV, Figure 2).[5] Reduction of IV with NaCNBH$_3$[6] yielded alcohol V, which was hydrolzed to give free acid VI. Compound VI was coupled with itself in a double esterification to give the cofacial dimer free base H$_4$-I.[7] Mg was inserted by exchange from magnesium etioporphyrin in toluene at 105 °C to give Mg$_2$-I.[8] Mg$_2$-II and Mg$_2$-III were prepared as previously described.[2a,d] Monomers H$_2$-VII and H$_2$-VIII were prepared from the corresponding 7-methyl esters of the alcohols at positions 3a or 2b, respectively, by coupling to propionic acid.

The electronic absorption and CD spectra of the three types of dimers are compared with their respective monomers in Figure 3. The MCD spectra of Mg$_2$-I and Mg$_2$-II (not shown) are essentially identical with their respective monomers, indicating no change in the assignment of the Q_x and Q_y transitions. Unlike the metalloporphyrins, the transition dipole moments of the Chls have well defined orientations in the macrocycle plane due to the much lower symmetry of the chlorin.[9] Furthermore, unlike most of the cofacial diporphyrins prepared to date, a *single* stereoisomer is present for the synthetic Chl dimers because of the preexisting stereochemistry at position 7 in the precursor. These characteristics, combined with the geometric variations in these compounds, lead to the extraordinary diversity of optical properties shown in Figure 3.

Inspection of a molecular model of the cofacial dimer shows that the macrocycles are parallel, and the angle between the *y* axes is about 40 ± 5°. The centers may be offset as much as 3.5 Å, as the rings move closer to maximize π–π interactions.[10] The

(5) Strain, H. H.; Svec, W. A. In "The Chlorophylls"; Vernon, L. P., Seeley, G. R., Eds.; Academic Press: New York, 1966; Chapter 2.

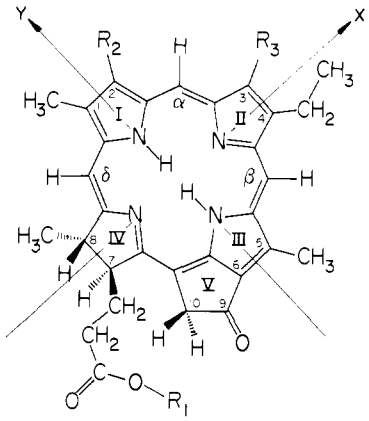
(6) Boxer, S. G.; Bucks, R. R. *J. Am. Chem. Soc.* **1979**, *101*, 1883.

(7) Typically 100 mg of 1-methyl-2-chloropyridinium iodide was combined with 10 mg of VI in dry CH_2Cl_2, 100 μL of triethylamine was added, and the mixture was stirred at 50 °C for 2–3 h. The product was purified by TLC. The mass spectrum of H$_4$-I was obtained in negative ion D/CI mode by using NH_3 as the reagent gas and gave a parent ion of 1064 (expected for H$_4$-I, 1064). The 360-MHz ^{1}H NMR spectrum of H$_4$-I contains a single peak for each type of proton, the CH_2 protons at position 3 are diasteriotopic, and the highest field central NH proton is shifted 1.5 ppm upfield relative to the monomer H$_2$-VII. This compares with a shift for this proton of 0.75 ppm in H$_4$-II relative to H$_2$-VIII, reflecting the greater ring overlap in the cofacial dimer.

(8) This is a modification of the procedure given by: Corwin, A. H.; Wei, P. E. *J. Org. Chem.* **1962**, *27*, 4285. Both Mg-H$_2$-I and Mg$_2$-I can be prepared and are readily identified by their absorption, NMR, and CD (vide infra) spectra. All standard Mg-insertion methods failed. See, e.g.: (a) Baum, S. J.; Burnham, B. F.; Plane, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *52*, 1439. (b) Falk, H.; Hoornaert, G.; Isenring, H.-P.; Eschenmoser, A. *Helv. Chim. Acta* **1975**, *58*, 2347. (c) Wasielewski, M. R. *Tetrahedron Lett.* **1977**, 1373.

(9) The absolute orientations of the transition dipole moments can be determined in single crystals of chlorophyllide–apomyoglobin complexes: (a) Boxer, S. G.; Wright, K. A. *J. Am. Chem. Soc.* **1979**, *101*, 6791. (b) Wright, K. A.; Boxer, S. G. *Biochemistry* **1981**, *20*, 7546. (c) Boxer, S. G.; Kuki, A.; Wright, K. A.; Katz, B. A.; Xuong, N. H. *Proc. Natl. Acad. Sci. U.S.A.*, in press.

(10) The interplanar separation may be as large as 5 Å but is likely to be about 3.5–4 Å, due to π–π interactions. This was found for the structure of a diporphyrin linked by a six-atom chain. See: Collman, J. P.; Chong, A. O.; Jameson, G. B.; Oakley, R. T.; Rose, E.; Schmittou, E. R.; Ibers, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 516. This is supported by upfield shifts in the NMR spectrum of H$_4$-I for all protons in the vicinity of ring II (α, β, 3a, and 4a) relative to monomer H$_2$-VII.



Compound	R_1	R_2	R_3
IV	$-CH_3$	$-CH=CH_2$	$-CHO$
V	$-CH_3$	$-CH=CH_2$	$-CH_2OH$
VI	$-H$	$-CH=CH_2$	$-CH_2OH$
VII	$-CH_3$	$-CH=CH_2$	$-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$
VIII	$-CH_3$	$-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_2-CH_3$	$-CH_3$
IX	$-CH_3$	$-CH=CH_3$	$-CH_3$

Figure 2. Structures of synthetic intermediates.

structure of hinged Mg_2-II is less well defined because there is considerable flexibility in the connection. However, for small opening angles of the hinge, the y axes of the monomers are at approximately 90° to each other and are perpendicular to the vector joining the macrocycle centers. The y axes of the monomers comprising "folded" Mg_2-III are nearly antiparallel, on the basis of an extensive NMR analysis.[2a]

Given these structures, we can rationalize the spectral shifts and CD data in Figure 3 by considering the resonance (exciton) coupling between the degenerate Q_y transition dipole moments of the monomers comprising each dimer.[11] This treatment predicts the energy and intensity difference between the two exciton components and the signs of their equal and opposite rotational strengths in the CD spectrum.[12]

(i) Cofacial Mg_2-I. The two components are predicted to be separated by about 700 cm^{-1} (about 30 nm, the exact value depends critically on the interplanar separation and precise angle[10]), with most of the oscillator strength in the higher energy component[13] (8:1, leading to a blue shift); the rotational strengths of these components should be large, equal and opposite in sign,[14] with the band at lower energy positive.

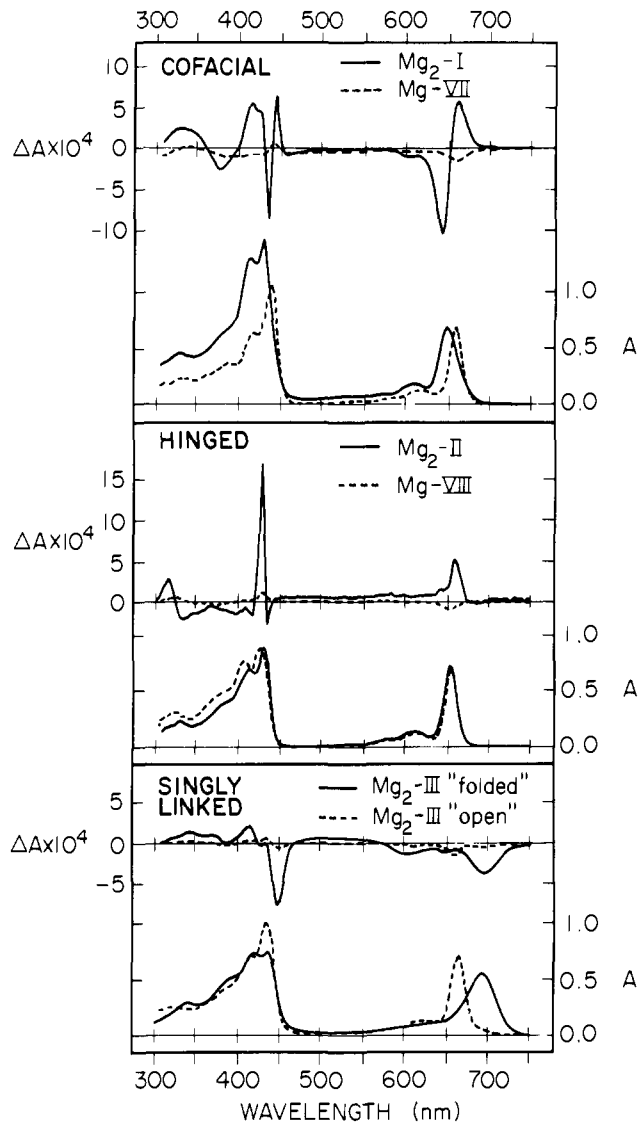


Figure 3. Comparisons of the absorption and CD spectra for the monomers (---) and the dimers (—) shown in Figure 1 and described in the text (1-cm pathlength). The spectra of the cofacial and hinged dimers and their respective monomers were obtained in CH_2Cl_2 with 0.5 M ethanol (the optical densities were identical for λ_{max} in the red Q_y transition). The spectra of the "folded" singly linked dimer was obtained in toluene with 0.5 M ethanol, and the "open" form is the same sample after pyridine was added (10% by volume). Note that the scales for all compounds are identical.

(ii) Hinged Mg_2-II. Since the transition moments are perpendicular, the Q_y transition should not be split (no shift) and any excitonic components in the CD should cancel.[14]

(iii) "Folded" Singly Linked Mg_2-III. Since the transition dipole moments are nearly antiparallel, the two components are predicted to be separated by >400 cm^{-1} (>20 nm), with nearly all of the oscillator strength in the lower energy component (>100:1, leading to a red shift); the rotational strength should be nearly zero.

Inspection of Figure 3 shows that for each case the observed spectra are consistent with these predictions. The important conclusion of this analysis is that the dipole coupling model is shown to be valid for very different Chl dimers with well-defined geometries and interdipole distances which are only several times the lengths of the transition dipole moments themselves (~1 Å).

We have collected a body of pertinent spectroscopic data on the cofacial dimer, which will be discussed in detail, along with photochemical properties, in a subsequent paper. The peak-to-peak line wiidth of the ESR spectrum of Zn_2-$I^{+\cdot}$ is 5.9 G, while that of Zn-$VII^{+\cdot}$ is 8.5 G, as expected for equal delocalization of the

(11) Tinoco, I., Jr. *Radiat. Res.* **1963**, *20*, 133.

(12) The dipole strength of pyrochlorophyllide *a* is 21.6 D^2. See: Shipman, L. L. *Photochem. Photobiol.* **1977**, *26*, 287. Given the uncertainties in the precise molecular structure and the true dipole strength of the monomers comprising these dimers, we restrict our analysis to ranges of relative intensities and splittings. Environmental shifts are neglected. See, e.g.: Shipman, L. L.; Cotton, T. M.; Norris, J. R.; Katz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1791.

(13) On close inspection of Figure 3A it is apparent that the line shape of the Q_y transition for cofacial Mg_2-I has a shoulder at lower energy as predicted, whereas the monomer Mg-VII does not.

(14) It is evident that the exciton splitting for the Q_y transition of cofacial Mg_2-I is not perfectly conservative. A similar effect has been noted in dinucleoside phosphates (see: Bush, C. A.; Brahms, J. *J. Chem. Phys.* **1967**, *46*, 79) and other chiral aggregates. This is a consequence of nonconservative terms in the expression for the rotational strength of the dimer arising from coupled oscillator interactions with nondegenerate excited states of the other monomer comprising the dimer. The effect is very much in evidence in the CD spectra of the hinged and singly linked dimers (Figure 3), where the conservative terms vanish, and these effects dominate. We have described similar effects for strictly monomeric chlorophyllides in synthetic protein complexes,[9] and these effects should always be borne in mind when interpreting CD data for chlorophylls in natural systems.

hole over both macrocycles in Zn_2-I$^{+\cdot}$.[3a,b] The fluorescence lifetime of H_4-I is 8 ns, compared with 7.5 ns for monomer H_2-VII (CH_2Cl_2). The ESR spectra of the photoexcited triplet states of H_4-I and H_2-VII or Zn_2-I and Zn-VII are nearly indistinguishable.[15] Thus, it is evident that neither of these latter types of measurements distinguishes between monomers and dimers, in this case, although they have frequently been used to make this distinction in vivo.

Acknowledgment. We thank the Ribermag Corporation and Professor Djerassi for their generous assistance in obtaining the mass spectrum of H_4-I. This work was supported by NSF Grant PCM7926677. NMR spectra were obtained at the Stanford Magnetic Resonance Laboratory, supported by NIH and NSF grants RR00711 and GR23633, respectively. Fluorescence lifetimes were obtained at the Stanford Synchrotron Radiation Laboratory, supported by NIH Grant 01209-01. S.G.B. is a Sloan and Dreyfus Fellow.

(15) Measured in 2-MeTHF glasses at 80 K, 400–700-nm broadband excitation, 400-Hz light modulation.

Biosynthesis of Streptonigrin from [U-$^{13}C_6$]-D-Glucose. Origin of the Quinoline Quinone[1,2]

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Since the introduction of the use of [1,2-$^{13}C_2$]acetate to study terpenoid and polyketide metabolism,[5] precursors doubly labeled with carbon-13 have been used to investigate a host of complex biosynthetic problems.[6] This powerful method derives from the simple principle that two adjacent carbons simultaneously enriched in carbon-13 give rise to a pair of new coupled signals in the corresponding ^{13}C NMR spectrum. These coupled pairs appear as satellites about the natural abundance carbon signal, producing an easily recognized trio of resonances. Any intervening process which breaks an intact ^{13}C–^{13}C bond results instead in a simple enrichment of the appropriate sites in the resulting metabolite and a corresponding enhancement of the relevant natural abundance signals.

Recently Cane et al.[7] used a variation of the doubly labeled acetate technique in which uniformly ^{13}C-labeled glucose ([U-$^{13}C_6$]glucose) was used as an in vivo precursor of [1,2-$^{13}C_2$]-acetyl-CoA, leading to the demonstration of the mevalonoid origin of pentalenolactone and its precursor pentalenic acid by interpretation of the derived ^{13}C NMR spectra. Logically, this methodology should be applicable to the study of additional products of glucose metabolism.[8,9] Thus, the presence of a chain of three labeled carbon atoms derived intact from glucose should yield a characteristic pattern consisting of two trios, corresponding to each end of the chain, and a quintet, resulting from the central carbon atom. The quintet would arise from the superposition of a triplet corresponding to those species in which both neighboring carbons are labeled and a doublet resulting from those species in which either one or the other of the adjacent carbons is enriched with ^{13}C.[10] Similarly, a four-carbon unit could be recognized by the resulting pattern of trio–quintet–quintet–trio. Each of these various coupling relationships, illustrated schematically in Figure 1, is easily recognized by the characteristic coupling constants and should be directly verifiable by the appropriate homonuclear ^{13}C–^{13}C decoupling experiments.

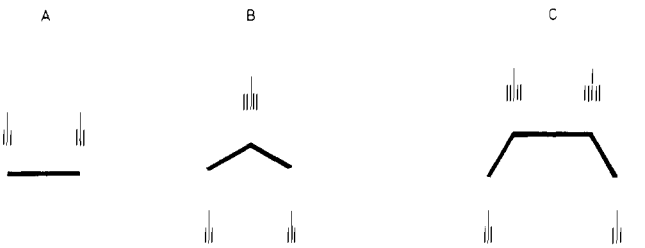

Figure 1. Schematic representation of carbon chains in which each carbon is enriched with carbon-13 and showing the expected NMR spin-coupled signal patterns. (A) A two-carbon unit; (b) a three-carbon unit; (C) a four-carbon unit.

By way of example, the utility of such an approach can be readily envisioned for shikimic acid derived metabolites. Shikimic acid, the apparent precursor of numerous families of natural products, has been the subject of intensive investigations[11] and is now known to be derived from glucose by the combination of an intact four-carbon unit, erythrose 4-phosphate, and an intact three-carbon unit, phosphoenol pyruvate. However, studies of the biosynthesis of shikimate derived metabolites using singly labeled samples of glucose have frequently been difficult to interpret because of competition between alternative metabolic pathways which result in indirect labeling of numerous additional sites in the derived metabolites.[12]

We expected that the utilization of [U-$^{13}C_6$]glucose would be effectively transparent to scrambling processes while remaining opaque to the direct incorporation of intact biosynthetic units, regardless of the manner of their derivation from glucose. To test our proposal we have studied the antitumor antibiotic streptonigrin (**1**). The biosynthesis of this metabolite has been extensively investigated by Gould and his collaborators, who have shown that the 4-phenylpicolinic acid moiety is derived from tryptophan (**2**)—a shikimate metabolite—via a putative β-carboline intermediate.[13] These studies had failed, however, to implicate any known pathway in the formation of the remaining, quinoline, portion of **1**.[14] We have now obtained evidence from a single

(1) This is Part 5 in the series "The Biosynthesis of Streptonigrin".
(2) Reported at the 181st National Meeting of the American Chemical Society, Atlanta, GA, March 29–April 3, 1981.
(3) Career Development Awardee of the National Cancer Institute (CA 00627), 1979–1984.
(4) Career Development Awardee of the National Institute of Allergy and Infectious Diseases (AI 00318), 1978–1982; Fellow of the Alfred P. Sloan Foundation, 1978–1982.
(5) Tanabe, M.; Suzuki, K., *J. Chem. Soc., Chem. Commun.* **1974**, 445. McInnes, A. G.; Smith, D. G.; Walter, J. A.; Vining, L. C.; Wright, J. L. C. *Ibid.* **1974**, 282. Seto, H.; Sato, T.; Yonehara, H. *J. Am. Chem. Soc.* **1973**, *95*, 8461.
(6) For a review, see: McInnes, A. G.; Walter, J. A.; Wright, J. L. C.; Vining, L. C. In "Topics in Carbon-13 NMR Spectroscopy"; Levy, G. C., Ed.; Wiley-Interscience: New York, 1976; Vol. 2; pp 154–167.
(7) Cane, D. E.; Rossi, T.; Tillman, A. M.; Pachlatko, J. P. *J. Am. Chem. Soc.* **1981**, *103*, 1838.
(8) White has used [U-$^{13}C_6$]glucose in an investigation of thiamine biosynthesis in which the distribution of label was examined by mass spectrometry: White, R. H. *Biochemistry* **1978**, *17*, 3833.
(9) Rinehart has recently independently reported the use of [U-$^{13}C_6$]-glucose to confirm the shikimate origin of the C_7N unit of pactamycin and used homonuclear decoupling to confirm the observed labeling patterns: Rinehart, K. L., Jr.; Potgieter, M.; Delaware, D. L.; Seto, H. *J. Am. Chem. Soc.* **1981**, *103*, 2099.
(10) (a) The doublets contributing to lines 2 and 4 of a quintet arise because the precursor is not 100% labeled at each site and from competing scrambling processes which reduce the percentage of intact biosynthetic units. (b) The predicted quintet pattern is based on the assumption that $J_{AB} \sim J_{BC}$. For the more general case a more complex pattern of up to nine lines would be expected. Such patterns have in fact been observed in some cases by expansion at high resolution, as illustrated in the supplementary material. (c) The center resonance of the quintet would be superimposed on both the natural abundance signal and an enhanced signal due to indirect enrichment by competing pathways.
(11) Haslam, E. C. "The Shikimate Pathway"; Halsted Press: New York, 1974; Ganem, B. *Tetrahedron* **1978**, *34*, 3353.
(12) Cf.: Srinivasan, P. R.; Shigeura, H. T.; Sprecher, M.; Sprinson, D. B.; Davis, B. D. *J. Biol. Chem.* **1956**, *220*, 447. Hornemann, U.; Kehrer, J. P.; Eggert, J. H. *J. Chem. Soc., Chem. Commun.* **1974**, 1045. Haber, A.; Johnson, R. D.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 3541.
(13) Gould, S. J.; Chang, C. C. *J. Am. Chem. Soc.* **1980**, *102*, 1702.

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