Scheme III

<table>
<thead>
<tr>
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<tr>
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<td>b</td>
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<td></td>
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<td>H = H</td>
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Scheme IIIa

(0) (a) [3H]KBH₄, (b) PCC. (c) MeCl, Et₃N. (d) -BBr₂, + or (-)-α-pinene. (e) n-BuONa, CH₂CO₂Et, . (f) NaOH. (g) H⁺, Δ. (h) HN₃.

lines of the doubl et was about five times that of the singlet at the approximate center of the doubl et corresponding to [1C,14N]-labeled molecules. One can therefore conclude that n-oc tylamine is incorporated into elaiomycin with retention of its nitrogen atom. The conversion of n-oc tylamine 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)-H]-, [1(R)-H]-, and [1(S)-H]-n-oc tylamine were synthesized by the route outlined in Scheme II. Reduction of n-octanol with tritiated borohydride yielded [1(R,S)-H]-n-octanol (2). The tritiated alcohol was converted to [1(R,S)-H]-n-oc tylamine (6) via formation of the mesylate, displacement with azide, and catalytic reduction. PCC oxidation of 2 yielded [1-3H]-n-octanal (3). Reduction of 3 with the adduct of 9-borabicyclononane and - or (+)-α-pinene was explored to give [1(R)-H]- and [1(S)-H]-n-octanal (4, 5), respectively. The stereochemistry assigned to the alcohols 4 and 5 follows from literature precedents. The chirally tritiated alcohols 4 and 5 were transformed into [1(S)-H]- and [1(R)-H]-n-oc tylamine (7, 8) via mesylation, azide displacement, and reduction. On the basis of the assumption that the displacement step occurs with inversion of configur ation, the chirally labeled alcohols 4 and 5 lead to [1(S)-H]- and [1(R)-H]-n-oc tylamine (7, 8), respectively. Administration of the three forms of [1-3H]-n-oc tylamine to S. gelat uis in conjunction with [1-14C]-n-oc tylamine gave the results summarized in Table I (experiments 3-5). The data clearly reveals that n-oc tylamine 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 [2-3H]-n-oc tylamine. [1(R,S)-H]-n-Heptanol (9) was prepared in standard fashion and converted to the corre sponding mesylate. Alkylation of diethyl malonate with the labeled adduct of 9-borabicyclononane and (-)-α-pinene was employed to yield [2(R,S)-H]-n-oc tylamine (13). A similar reaction sequence was then applied to [1(R)-H]- and [1(S)-H]-n-Heptanol (11, 12) obtained by 9-BBN-α-pinene reduction of [1-3H]-n-Heptanol (10). If it is assumed that the malonate alkylation step proceeds with inversion of configur ation, then alcohols 11 and 12 will be converted to [2(R)-H]- and [2(R)-H]-n-oc tylamine (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-oc tylamine is incorporated into the antibiotic with loss of the 2-pro-R hydrogen atom. It therefore follows that the Δ₂⁺-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.


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

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Received September 23, 1981

We wish to report the synthesis and unique spectroscopic properties of a novel, doubly linked, cofacial chlorophyll (Chi) dimer. Considerable effort has been directed toward the synthesis and characterization of covalently connected porphyrins and Chls. 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. 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, 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 Qₐ absorption bands. These observations provide evidence of interchromophore resonance (exciton) interactions, and are further supported by ESR and ENDOR data on the cation radical of the electron donor in bacterial systems. The properties of three synthetic Chi dimers are compared in this paper (see Figure 1): the novel cofacial dimer, Mg₂-I, the doubly linked "hinged" dimer, Mg₂-II, prepared by Wasielewski et al., and the original singly linked dimer, Mg₃-III, prepared by Boxer and Coss, whose Qₐ band exhibits a large red shift.


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). Reduction of IV with NaCN-BH3 yielded alcohol V, which was hydrolyzed to give free acid VI. Compound VI was coupled with itself in a double esterification to give the cofacial dimer free base H4.IS7 Mg was inserted by exchange from magnesium etioporphyrin in toluene at 105 °C to give Mg2-I and Mg2-II were prepared as previously described.6d Monomers H2-VII and H2-VIII were prepared from the corresponding 7-methyl esters of the alcohols at positions 3a

(7) Typically 100 mg of 1-methyl-2-chloropyridinium iodide was combined with 10 mg of VI in dry CH2Cl2, 100 μL of triethylamine was added, and the mixture was stirred at 50 °C for 2-3 h. The product was purified by TLC.


(10) The interplanar separation may be as large as 5 Å but is likely to be about 3.5 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.; Schmitto, E. R.; Ibers, J. A. J. Am. Chem. Soc. 1981, 103, 516. This is supported by upfield shifts in the NMR spectrum of H4-I for all protons in the vicinity of ring II (α, β, 3a, and 4a) relative to monomer H2-VII.
structure of hinged Mg-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-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 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-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 angle2b), with most of the oscillator strength in the higher energy component13 (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.

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

(iii) "Folded" Singly Linked Mg-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 width of the ESR spectrum of Zn-VII⁺ is 5.9 G, while that of Zn-III⁺ is 8.5 G, as expected for equal delocalization of the transitions.

<table>
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Figure 2. Structures of synthetic intermediates.
hole over both macrocycles in Zn2-I+. The fluorescence lifetime of H4-I is 8 ns, compared with 7.5 ns for monomer H2-VII (CH2Cl). The ESR spectra of the photoexcited triplet states of H4-I and H2-VII or Zn-I and Zn-VII are nearly indistinguishable. 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 H4-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 GR23533, 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.

Biosynthesis of Streptonigrin from [U-13C6]-D-Glucose. Origin of the Quinoline Quinone1,2

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Since the introduction of the use of [1,2-13C2]acetate to study terpenoid and polyketide metabolism, precursors doubly labeled with carbon-13 have been used to investigate a host of complex biosynthetic problems. 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 13C 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 13C-13C 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. used a variation of the doubly labeled acetate technique in which uniformly 13C-labeled glucose ([U-13C6]glucose) was used as an in vivo precursor of [1,2-13C2]-acetyl-CoA, leading to the demonstration of the mevalonoid origin of pentenalactone and its precursor pentalenic acid, the apparent precursor of numerous families of natural products, has been the subject of intensive investigations 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.

We expected that the utilization of [U-13C6]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-phenylpyruvic acid moiety is derived from tryptophan (2)—a shikimate metabolite—via a putative 6-carbon intermediate. These studies had failed, however, to implicate any known pathway in the formation of the remaining, quinoline, portion of 1. We have now obtained evidence from a single sample of [U-13C6]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.

(8) We have used [U-13C6]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.


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.

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 quartet, resulting from the central carbon atom. The quartet 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 13C. 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 13C-13C decoupling experiments.

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 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.

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(10) (a) The doublets contributing to lines 2 and 4 of a quartet 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 JAB ≈ Jac. 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 experiments at high resolution—usually in the fast resolution of the [1,1-13C2]methylene group. (c) The center resonance of the quartet would be superimposed on both the natural abundance signal and an enhanced signal due to indirect enrichment by competing pathways.

