be devoid of any intrinsic stereochemistry, in some cases stereochemistry can be enforced by conventional methods. In this context, substrate **10**, with a conformationally locked six-membered ring, gave essentially complete equatorial incorporation of the allyl unit upon reaction with allyltri-*n*-butylstannane, and mannose derivative **12** was found to yield exclusively the product expected from approach of allyltri-*n*-butylstannane to the least hindered face of the intermediate radical. The lyxose derivative **14** likewise affords a single allylated product (as a mixture of anomers) whose stereochemistry has not yet been rigorously confirmed. More detailed investigations of factors controlling stereochemistry, as well as applications in natural product synthesis, are in progress and will be reported in due course.

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Enzymatic Cyclization of Geranyl Pyrophosphate to Bornyl Pyrophosphate. Role of the Pyrophosphate Moiety

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Within the last several years cell-free investigations of the biosynthesis of both monoterpenes and sesquiterpenes have led to a clearer understanding of the mechanisms by which the acyclic precursors geranyl and farnesyl pyrophosphate are converted to cyclic metabolites.[2] As a result, a coherent picture has begun to emerge for the critical cyclization processes[3] that lie at the heart of terpenoid biogenetic theory.[4] Of particular importance has been the recent demonstration that an enzyme system from *Salvia officinalis* (sage) catalyzes the conversion of geranyl pyrophosphate (**1**) to *d*-bornyl pyrophosphate (**2**).[5] The recognition that the enzymatic cyclization product is itself a pyrophosphate ester provides a unique opportunity to examine experimentally the role of the pyrophosphate moiety in the isomerization–cyclization process. We report below oxygen-18 labeling studies that establish a remarkably tight coupling of the pyrophosphate and terpenoid partners that exists within the enzyme active site and results in an essentially complete lack of positional isotope exchange[6] of the pyrophosphate ester oxygen atom during the conversion of geranyl to bornyl pyrophosphate.

Scheme I

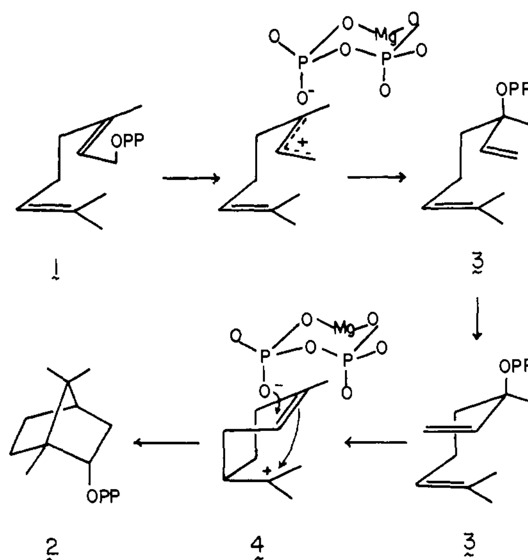

Table I. Conversion of [1-$^3H_2$,α-^{32}P]Geranyl Pyrophosphate and [1-$^3H_2$,β-^{32}]Geranyl Pyrophosphate to *d*- and *l*-Bornyl Pyrophosphate and Determination of the $^3H/^{32}P$ Ratio of the Derived Monophosphate[a]

	$^3H/^{32}P$	
geranyl pyrophosphate[b]	*d*-bornyl phosphate (sage)	*l*-bornyl phosphate (tansy)
[1-$^3H_2$,α-^{32}P]-, 9.4[c]	9.5	9.6
[1-$^3H_2$,β-^{32}P]-, 10.5[d]	522	482

[a] A minimum of 3.5 × 10^5 dpm of ^3H were recovered in each product, of which 25% was counted. Data are half-life corrected. [b] The location of the ^{32}P in each substrate was verified by enzymatic hydrolysis to the corresponding monophosphate with apyrase (Del Campo, G.; Puente, J.; Valenzuela, M. A.; Cori, O. *Biochem. J.* 1977, *167*, 525) and by hydrogenation (Jacobson, H. I.; Griffin, M. J.; Jensen, E. V. *J. Am. Chem. Soc.* 1957, *79*, 2068), and subsequent acid hydrolysis to 3,7-dimethyloctyl phosphate, followed by purification of these products by TLC. [c] Specific activity 150 Ci ^3H/mol. [d] Specific activity 300 Ci ^3H/mol.

A working model for the formation of *d*-bornyl pyrophosphate from geranyl pyrophosphate, consistent with available experimental data[2,5,7] and based on closely related chemical models,[8] is illustrated in Scheme I. According to this mechanism, geranyl pyrophosphate (**1**) is first isomerized to its tertiary allylic isomer, linalyl pyrophosphate (**3**).[9] Ionization of the cisoid conformer of **3** and electrophilic attack on the 6,7-double bond would generate a transient α-terpinyl cation (**4**). Subsequent electrophilic attack on the newly formed cyclohexene double bond and capture of the resultant carbocation by the pyrophosphate anion generate bornyl pyrophosphate with the observed stereochemistry. During the course of these reasonable, but as yet hypothetical, transformations the inorganic pyrophosphate moiety released by the ionization of the geranyl substrate may become more or less free of its cationic

(1) Fellow of the Alfred P. Sloan Foundation, 1978–1982; National Institutes of Health Research Career Development Award, 1978–1983.

(2) For recent comprehensive reviews of monoterpene and sesquiterpene biosynthesis, respectively, see: (a) Croteau, R. In "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Ed.; Wiley: New York, 1981; pp 225–282. (b) Cane, D. E. *Ibid.*, pp 283–374.

(3) For a recent review see: Cane, D. E. *Tetrahedron* **1980**, *36*, 1109.

(4) (a) Ruzicka, L. *Pure Appl. Chem.* **1963**, *6*, 493; *Proc. Chem. Soc.* **1959**, *7*, 82. (b) Hendrickson, J. B. *Tetrahedron* **1959**, *7*, 82. (c) Parker, W.; Roberts, J. S.; Ramage, R. *Q. Rev. Chem. Soc.* **1967**, *21*, 331.

(5) Croteau, R.; Karp, F. *Arch. Biochem. Biophys.* **1979**, *198*, 512; **1977**, *184*, 77. Croteau, R.; Karp, F. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 440.

(6) Rose, I. A. *Adv. Enzymol.* **1979**, *50*, 361.

(7) (a) Croteau, R.; Felton, M. *Arch. Biochem. Biophys.* **1981**, *207*, 460. Gambliel, H.; Croteau, R. *J. Biol. Chem.* **1982**, *257*, 2335. (b) Cane, D. E.; Swanson, S.; Murthy, P. P. N. *J. Am. Chem. Soc.* **1981**, *103*, 2136. (c) Suga, T.; Shishibori, T.; Morinaka, H. *J. Chem. Soc., Chem. Commun.* **1980**, 167. (d) Gotfredsen, S. E. *Diss. ETH Zurich No. 6243*, described in ref 2b and in ref 3, pp 1150–1151. (e) Arigoni, D. *Pure Appl. Chem.* **1975**, *41*, 219. (f) Anastasis, P.; Freer, I.; Gilmore, C.; Mackie, H.; Overton, K.; Swanson, S. *J. Chem. Soc., Chem. Commun.* **1982**, 268.

(8) (a) Gotfredsen, S.; Obrecht, J. P.; Arigoni, D. *Chimia* **1977**, *31*, 62. Weinstein, S.; Valkanas, G.; Wilcox, C. F. *J. Am. Chem. Soc.* **1972**, *94*, 2286. Stephan, K. *J. Prakt. Chem.* **1898**, *58*, 109. (b) Poulter, C. D.; King, C.-H. R. *J. Am. Chem. Soc.* **1982**, *104*, 1420, 1422.

(9) Linalyl pyrophosphate itself has been shown to serve as a substrate for bornyl pyrophosphate synthetase. Cf. ref 2a and 12.

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Table II. Conversion of [1-^{18}O,8,9-^{14}C]Geranyl Pyrophosphate to *d*-Bornyl Pyrophosphate by Sage Enzyme (A) and *l*-Bornyl Pyrophosphate by Tansy Enzyme (B)

expt	geranyl pyrophosphate	recovered geraniol	bornyl benzoate[a]
A (^{14}C, dpm/μmol)	7.92×10^5 [b]	$7.3 \pm 0.5 \times 10^5$ [c]	*d*-, $(5.7 \pm 0.3) \times 10^5$
A (^{18}O %)	67.9 ± 0.4 [d]		*d*-, 52.4 ± 1.4 [e,f]
B (^{18}O %)	68.2 ± 4.4 [d]	67.9 ± 2.5 [g,h], 68.9 ± 5.3 [g,i]	*d*-, 63.8 ± 2.8 [e]

[a] Obtained by benzoylation of crude terpene alcohol extract and purification by HPLC. [b] Determined on the derived geranyl benzoate. [c] Geraniol benzoate obtained by benzoylation of crude terpene extract and purification by HPLC. [d] Determined on geraniol obtained by phosphatase hydrolysis of geranyl pyrophosphate. [e] Determined on parent peak cluster, *m/e* 260:258. [f] Similar values were obtained by measurement of the *P*-benzoyl fragments at *m/e* 153 and 155. [g] Geranyl Me$_3$Si obtained by hydrolysis and silylation of HPLC-purified geranyl benzoate derived from crude terpene alcohol extract. [h] Based on parent peak cluster, *m/e* 228:226. [i] Based on P-15 cluster, *m/e* 213:211.

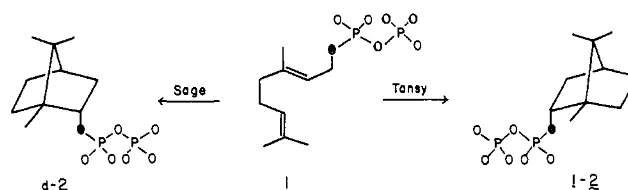
partner, depending on the precise course of the reaction.

Proof that the two ends of the pyrophosphate moiety do not become equivalent during the formation of bornyl pyrophosphate was provided by labeling each phosphorus atom. Samples of [1-^{3}H$_2$,α-^{32}P]- and [1-^{3}H$_2$,β-^{32}P]geranyl pyrophosphate[10] were separately incubated with phosphatase-free preparations[11] of *d*- and *l*-bornyl pyrophosphate synthetases, obtained from *Salvia officinalis* (sage)[5] and *Tanacetum vulgare* (tansy),[12] respectively, and the resulting bornyl pyrophosphate was isolated and hydrolyzed to bornyl phosphate. As summarized in Table I, both the *d*- and *l*-bornyl phosphates derived from [1-^{3}H$_2$,α-^{32}P]geranyl pyrophosphate exhibited unchanged ^{3}H/^{32}P ratios, whereas bornyl phosphate from β-^{32}P-labeled precursor contained less than 2% of the original ^{32}P label.

Further insight into the role of the pyrophosphate moiety required the preparation of ^{18}O-labeled substrates. To this end [1-^{18}O]geraniol,[13] mixed with a small quantity of [8,9-^{14}C]geraniol[15] as internal standard, was converted to [1-^{18}O,8,9-^{14}C]geranyl pyrophosphate (67.9% ^{18}O atom) by standard methods.[10b,16,17] The specific activity (7.92×10^5 dpm/μmol) of this sample was determined by analysis of the corresponding HPLC-purified geranyl benzoate.

For preparative-scale experiments with *d*-bornyl pyrophosphate synthetase, 105000*g* supernatants from whole leaf homogenates of *S. officinalis* were prepared as previously described,[5] treated with XAD-4 polystyrene resin to remove endogenous terpenoids,[18] and then dialyzed before use against 5 mM sodium phosphate, pH 6.2, containing 5% sucrose, 5×10^{-4} M dithioerythritol, and 10 mM MgCl$_2$. Aliquots (1.0 mL, containing ca. 1 mg of protein)

Scheme II



were incubated in screw-capped vials at 30 °C for 3 h with 3.5×10^{-5} M [1-^{18}O,8,9-^{14}C]geranyl pyrophosphate. At the end of the incubation period borneol and geraniol released from the corresponding pyrophosphates by endogenous phosphatases were extracted with redistilled pentane, and the concentrated extract was benzoylated. A control experiment[19] confirmed that hydrolysis of bornyl pyrophosphate takes place, as expected,[20] with P–O bond cleavage, thereby demonstrating that the alcoholic oxygens of recovered geraniol and borneol correspond to the ester oxygen of their respective pyrophosphate derivatives.

A total of 3.88 μmol of [1-^{18}O,8,9-^{14}C]geranyl pyrophosphate was processed over a period of several weeks by a series of such incubations. The combined crude terpene benzoates, containing ca. 0.2 μmol *d*-bornyl benzoate, were separated and purified by HPLC. The identity of the purified bornyl benzoate was confirmed by 250-MHz ^{1}H NMR analysis of an 11-μg sample. As summarized in Table II, the recovered *d*-[2-^{18}O,8,9-^{14}C]bornyl benzoate contains 52.4 ± 1.4% ^{18}O based on mass spectrometric measurement of the molecular ion peaks. This figure corresponds to 72 ± 3% ^{18}O if corrected for endogenous dilution which has lowered the ^{14}C specific activity of recovered bornyl benzoate. Since complete equilibration of the three proximal oxygen atoms would have reduced the original ^{18}O enrichment of 67.9% to a value of 22.6% at C-2 of borneol, it is clear that the isomerization–cyclization of [1-^{18}O]geranyl pyrophosphate involves essentially no positional oxygen isotope exchange.

The availability of *l*-bornyl pyrophosphate synthetase from tansy[12] provided an unusual opportunity to corroborate the results obtained with the sage enzyme in a system which converts the same precursor, geranyl pyrophosphate, to a product of identical structure but opposite enantiomeric configuration. Accordingly a second sample of [1-^{18}O,8,9-^{14}C]geranyl pyrophosphate (68.2% ^{18}O) was incubated in lots of 3.5×10^{-2} μmol with 1-mL aliquots of the 105000*g* supernatant of whole leaf homogenates of *T. vulgare* under conditions identical with those described above for sage enzyme. In this manner a mixture containing ca. 0.25 μmol of *l*-bornyl benzoate and 0.75 μmol of recovered geranyl benzoate was obtained and purified by HPLC. The purest geranyl benzoate fractions were pooled and converted to [1-^{18}O]geranyl trimethylsilyl ether for ^{18}O analysis. As summarized in Table II, both the recovered geraniol and the product *l*-borneol have ^{18}O enrichments essentially unchanged from that of the [1-^{18}O]geranyl pyrophosphate precursor.

The above experiments clearly demonstrate that during the enzymatic conversion of geranyl pyrophosphate to either *d*- or *l*-bornyl pyrophosphate the original pyrophosphate ester oxygen of the precursor is the exclusive source of the pyrophosphate ester oxygen of the product (Scheme II). Such tight restriction on the motion of the transiently generated inorganic pyrophosphate moiety is unexpected and has interesting implications for the mechanism illustrated in Scheme I. The observed lack of oxygen scrambling contrasts with the equilibration of the three proximal

(10) (a) Croteau, R.; Karp, F. *Arch. Biochem. Biophys.* **1976**, *176*, 734. (b) Cramer, F.; Boehm, W. *Angew. Chem.* **1959**, *71*, 755. Cornforth, R. H.; Popjak, G. *Methods Enzymol.* **1969**, *15*, 539. (c) Dugan, R. E.; Rasson, E.; Porter, J. W. *Anal. Biochem.* **1968**, *22*, 249. (d) Michelson, A. M. *Biochim. Biophys. Acta* **1964**, *91*, 1.

(11) Croteau, R.; Karp, F. *Arch. Biochem. Biophys.* **1979**, *198*, 523.

(12) Croteau, R. In "Biochemistry of Isopentenoids in Plants"; Nes, W. D.; Fuller, G.; Tsai, L. S., Eds.; Marcel Dekker: New York, in press.

(13) Prepared by reacting geranyl bromide with sodium [^{18}O$_2$]acetate[14] in dimethylformamide followed by mild base hydrolysis (K$_2$CO$_3$, methanol) of the purified geranyl [1-^{18}O,acyl-^{18}O]acetate.

(14) Hutchinson, C. R.; Makune, C. T. *J. Labelled Compd. Radiopharm.* **1976**, *8*, 571.

(15) Prepared from the THP-ether of geranyl trisnoraldehyde and [^{14}C-methyl]isopropylidene triphenylphosphorane. Cf. ref 16.

(16) Cane, D. E.; Iyengar, R.; Shiao, M.-S. *J. Am. Chem. Soc.* **1981**, *103*, 914.

(17) Popjak, G.; Cornforth, J. W.; Cornforth, R. H.; Ryhage, R.; Goodman, D. *J. Biol. Chem.* **1962**, *237*, 56. Kandutsch, A. A.; Paulus, H.; Levin, E.; Bloch, K. *Ibid.* **1964**, *239*, 2507. Holloway, P. W.; Popjak, G. *Biochem. J.* **1967**, *104*, 57. Epstein, W. W.; Rilling, H. C. *J. Biol. Chem.* **1970**, *245*, 4597.

(18) Loomis, W. D.; Lile, J. D.; Sandstrom, R. P.; Burbott, A. J. *Phytochemistry* **1979**, *18*, 1049.

(19) *d*-[G-^{3}H]Bornyl pyrophosphate (4.4×10^6 dpm/μmol) was incubated with the crude sage enzyme under the usual conditions in [^{18}O]water (72% atom). The [^{3}H]borneol thus liberated was extracted and converted to the benzoate as before. Analysis of the HPLC-purified material by GC–MS on an HP 9895 spectrometer at Yale University established the presence of less than 2% excess ^{18}O. We thank Richard Weber for his assistance in obtaining these spectra.

(20) Stein, S. S.; Koshland, D. E. *Arch. Biochem. Biophys.* **1952**, *39*, 229. Fernley, H. N. In "The Enzymes"; 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. 4, p 417. Ngan, H.-L.; Popjak, G. *Bioorg. Chem.* **1975**, *4*, 166. Mackie, H.; Overton, K. H. *Eur. J. Biochem.* **1977**, *77*, 101.

pyrophosphate oxygens during the closely related enzymatic isomerization of farnesyl to nerolidyl pyrophosphate, a result that has been interpreted in terms of an allylic cation–pyrophosphate anion pair.[16] While an equilibrium between geranyl and linalyl pyrophosphate may exist at the active site of bornyl pyrophosphate synthetase, the initial cyclization step involving formation of a new C–C bond is most likely irreversible. The absence of observable isotope scrambling in either *d*- or *l*-bornyl pyrophosphate implies that the cyclization step is fast compared to positional isotope exchange during the initial isomerization step, without giving any information as to the relative rates of ring closure compared to either forward or reverse isomerization.

Poulter has recently reported that [1-^{18}O]geranyl pyrophosphate reisolated from incubations with prenyl transferase has not undergone detectible scrambling, in spite of strong evidence for the generation of allylic cations at the enzyme active site.[21] In the bornyl pyrophosphate synthetase reaction, the observed lack of positional isotope exchange is all the more remarkable when one considers the transient generation of an α-terpinyl cation—pyrophosphate anion pair in which the charge separation is at least 3 Å. Further studies that address the stereochemical implications of Scheme I are in progress, and the results will be reported in due course.

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(21) Mash, E. A.; Gurria, G. M.; Poulter, C. D. *J. Am. Chem. Soc.* **1981**, *103*, 3927 and references cited therein.

On the Electronic Excited States of Model Chlorophyll

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The interaction of light with chlorophyll is one of the most important chemical reactions, for nearly all of life centers on photosynthesis. At the heart of this reaction is a magnesium chlorin complex, and it is the interaction of this complex with light that is the first step in photosynthesis. Since this molecular system is so widely used, it follows that the electronic structure of this molecule in its ground and excited states must possess some unique properties. In this paper we report on a preliminary investigation of the electronic structure of magnesium porphine in its ground and excited states as a first step in order to shed some light on these properties.

Methods. All calculations were of the restricted Hartree–Fock (RHF) type and were performed at the INDO level.

Closed-shell calculations were done by conventional RHF methods; open-shell calculations were performed by using an open-shell RHF procedure described elsewhere.[1]

Two types of parameter schemes were used: Pople type[2] for geometry optimization (INDO/1), and spectroscopic type[3] for spectra (INDO/S). The initial coordinates were taken from crystal X-ray data[4] subsequently symmetrized[5] and then optimized by using the INDO/1 method. The electronic spectra were calculated at the X-ray geometry and at the optimized geometry by performing selected singles-only configuration interaction calculations.

Excited states of interest were chosen from these CI calculations and calculated with the open-shell RHF formalism[1] by using both sets of parameters.

Starting coordinates for the excited-state geometry optimizations were chosen to be the optimized ground-state coordinates. All coordinates are optimized by using a gradient technique and the Murtagh–Sargent method of function optimization.[6]

Results. It is felt that the important photochemically excited state is most likely a singlet.[7] We therefore report here the singlet spectrum of magnesium porphine at three different geometries by using singles-only CI and spectroscopic parameters. The geometries are X-ray crystal coordinates (X), optimized ground state (G), and optimized excited state (*). The spectra obtained are shown in Table I along with experimental values. Symmetry

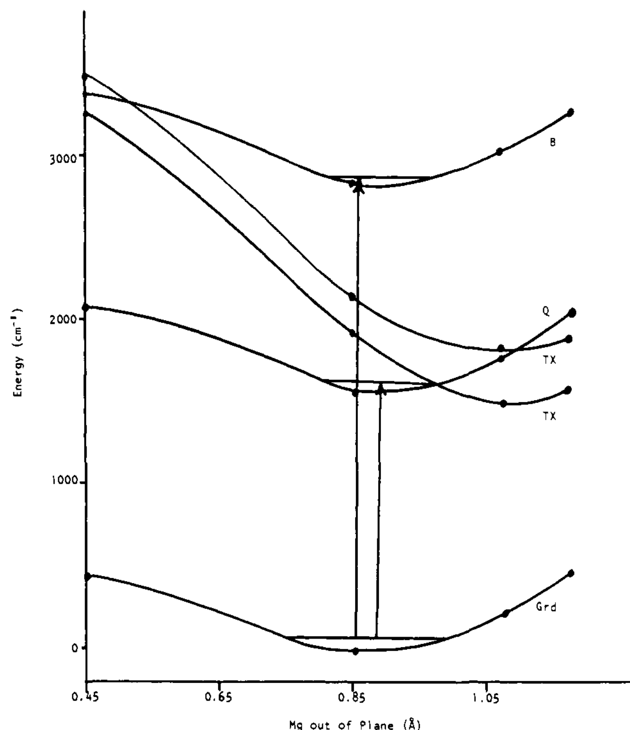

Figure 1. Lowest lying potential energy curves as a function of the Mg distance from the mean plane of the four chelating nitrogen atoms of porphin. Q is the state responsible for the visible band; B, for the Soret; and TX, the two porphin-to-Mg charge-transfer states.

Table I. Transition Energies Relative to Ground State[a] (cm^{-1})

band		geometry X (0.451)[b]	G (0.848)	* (1.08)	exptl[c]	ab initio[d]
Q	$^1E_1$	17424	15940	15524	17500	17100
CT	$^1A_2$	30745	19357	13042	forbidden	
Soret B	$^1E_1$	31099	28411	27884	24000	26200
CT	$^1B_1$	34871	21598	16231	forbidden	

[a] Singles-only CI relative to ground state at indicated geometry. [b] Numbers in parentheses are Mg distance from plane of nitrogen (Å). [c] Mg etioporphyrin (see ref 9). [d] Linearly fit to spectrum (see ref 8).

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(1) Edwards, W. D.; M. C. Zerner, to be submitted for publication. The method is similar to that of Davidson (Davidson, E. R. *Chem. Phys. Lett.* **1973**, *21*, 565–567) but allows for the consideration of degenerate states.
(2) Pople, J. A.; Beveridge, D. L.; Dobosh, P. A., *J. Chem. Phys.* **1967**, *47*, 2026–2033.
(3) (a) Ridley, J. E.; Zerner, M. C. *Theor. Chim. Acta* **1973**, *32*, 111–134. (b) Ridley, J. E.; Zerner, M. C. *Ibid.* **1973**, *42*, 223–236.
(4) Hoard, J. L.; Hamor, M. J.; Hamor, T. A. *J. Am. Chem. Soc.* **1963**, *85*, 2334–2335.
(5) Zerner, M. C.; Gouterman, M. *Theor. Chim. Acta* **1966**, *4*, 44–63.
(6) Murtagh, B. A.; Sargent, R. W. H. *Comp. J.* **1970**, *13*, 185–194.
(7) Baum, R. M. *Chem. Eng. News* **1982**, *June 14*, 21–25. Holten, D.; Hoganson, C.; Windsor, M. W.; Schenck, C. C.; Parson, W. W.; Migus, A.; Fork, R. L.; Shank, C. V. *Biochim. Biophys. Acta* **1980**, *592*, 461 and references therein.

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