References and Notes

(1) G. Herzberg and J. Soosmith, *Can. J. Phys.*, **34**, 523 (1956); G. Herzberg, *Proc. R. Soc. London, Ser. A*, **262**, 291 (1961).
(2) R. W. Fessenden, *J. Phys. Chem.*, **71**, 74 (1967).
(3) L. Y. Tan, A. M. Winer, and G. C. Pimentel, *J. Chem. Phys.*, **57**, 4028 (1972).
(4) D. E. Milligan and M. E. Jacox, *J. Chem. Phys.*, **47**, 5146 (1967); M. E. Jacox, *J. Mol. Spectrosc.*, **66**, 272 (1977).
(5) J. Pacansky and J. Bargon, *J. Am. Chem. Soc.*, **97**, 6896 (1975).
(6) L. Golob, N. Jonathan, A. Morris, M. Okuda, and K. J. Ross, *J. Electron Spectrosc.*, **1**, 506 (1972); J. Dyke, N. Jonathan, E. Lee, and A. Morris, *J. Chem. Soc., Faraday Trans. 2*, **72**, 1385 (1976).
(7) T. Koenig, T. Balle, and W. Snell, *J. Am. Chem. Soc.*, **97**, 662 (1975); T. Koenig, T. Balle, and J. C. Chang, *Spectrosc. Lett.*, **9**, 755 (1976).
(8) F. A. Houle, privately communicated, 1977.
(9) P. Millie and G. Berthier, *Int. J. Quantum Chem.*, **2**, 67 (1968).
(10) F. Driessler, R. Ahlrichs, V. Staemmler, and W. Kutzelnigg, *Theoret. Chim. Acta (Berlin)*, **30**, 315 (1973).
(11) A. J. Duke, *Chem. Phys. Lett.*, **21**, 275 (1973).
(12) D. S. Marynick and D. A. Dixon, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 410 (1977).
(13) G. T. Surratt and W. A. Goddard III, *Chem. Phys.*, **23**, 39 (1977).
(14) C. E. Dykstra, M. Hereld, R. R. Lucchese, H. F. Schaefer III, and W. Meyer, *J. Chem. Phys.*, **67**, 4071 (1977).
(15) P. F. Zittel, G. B. Ellison, S. V. ONeil, E. Herbst, W. C. Lineberger, and W. P. Reinhardt, *J. Am. Chem. Soc.*, **98**, 3731 (1976); P. C. Engelking and W. C. Lineberger, *J. Chem. Phys.*, **65**, 4323 (1976).
(16) M. W. Siegel, R. J. Celotta, J. L. Hall, J. Levine, and R. A. Bennett, *Phys. Rev. A*, **6**, 607 (1972).
(17) H. Hotop and W. C. Lineberger, *J. Phys. Chem. Ref. Data*, **4**, 539 (1975).
(18) J. D. Swalen and J. A. Ibers, *J. Chem. Phys.*, **36**, 1914 (1962).
(19) Camille and Henry Dreyfus Teacher-Scholar.

G. Barney Ellison*
Department of Chemistry, University of Colorado Boulder, Colorado 80309

P. C. Engelking, W. C. Lineberger*[19]
Department of Chemistry, University of Colorado, and Joint Institute for Laboratory Astrophysics of the University of Colorado and National Bureau of Standards Boulder, Colorado 80309

Received January 3, 1978

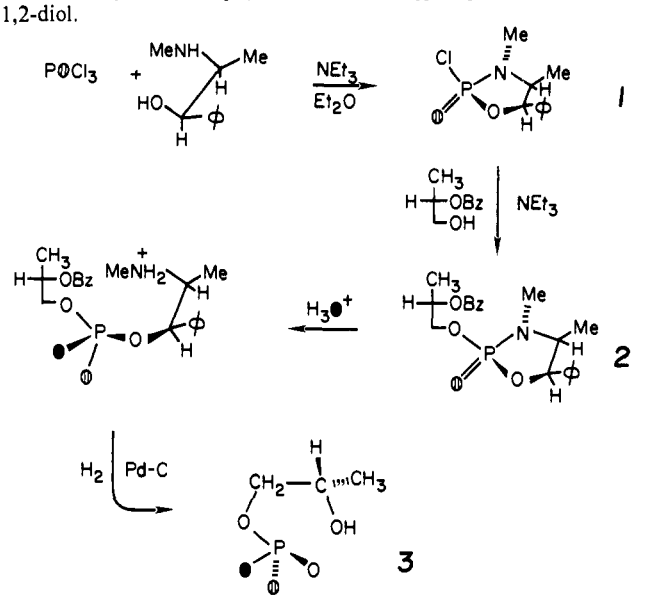
Chiral [^{16}O, ^{17}O, ^{18}O]Phosphate Monoesters. 1. Asymmetric Synthesis and Stereochemical Analysis of [1(*R*)-^{16}O, ^{17}O, ^{18}O]Phospho-(*S*)-propane-1,2-diol

Sir:

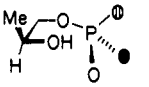
To discover the stereochemical consequences of phosphoryl transfer reactions involving phosphate monoesters, we have devised *a general method for establishing whether retention or inversion at phosphorus occurs in these processes.* To make a phosphate monoester chiral at phosphorus, we use the three stable isotopes of oxygen, ^{16}O, ^{17}O, and ^{18}O. We report here the synthesis of [1(*R*)-^{16}O, ^{17}O, ^{18}O]phospho-(*S*)-propane-1,2-diol (**3**) by a general route, and an independent evaluation of the configuration of the phosphoryl group in this compound.

Synthesis. The synthesis of **3** is outlined in Scheme I. Reaction of [^{17}O]-$POCl_3$[1] with (−)-ephedrine gave two epimeric chloro adducts **1**,[2] which reacted with 2-benzyl-(*S*)-propane-1,2-diol[3] to give the two phosphoramidate diesters **2** in 9:1 ratio. This reaction goes with retention at phosphorus.[2] The predominant isomer (shown in Scheme I) was isolated in 70% yield (based on $POCl_3$) after chromatography on silica gel. The phosphoramidate **2** was ring opened in $H_2{}^{18}O$ by an in-line pathway.[2,4] The resulting diester was debenzylated by catalytic hydrogenolysis[5] to give **3** in 70% yield (based on **2**). The route

Scheme I. Synthesis of [1(*R*)-^{16}O, ^{17}O, ^{18}O]phospho-(*S*)-propane-1,2-diol.

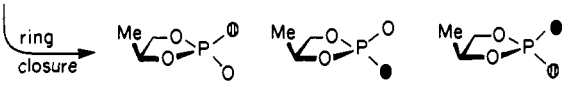


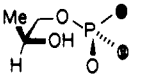
Scheme II. Sets of 1,2-cyclic phosphates derived by "in-line" ring closure of 1(*R*)-phospho-(*S*)-propanediol (**3**) and of 1(*S*)-phospho-(*S*)-propanediol (**4**).



(**3**)

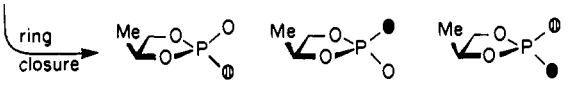
1-R-phospho-S-propanediol





(**4**)

1-S-phospho-S-propanediol



shown in Scheme I should be a general one for the preparation of chiral phosphate esters from appropriately protected precursors.[6]

Stereochemical Analysis. It is very unlikely that any physical technique would be capable of discriminating between the (*R*) and (*S*)-[^{16}O, ^{17}O, ^{18}O]phosphate monoesters themselves, and so the following path was adopted. The ester **3** is cyclized to the 1,2-cyclic phosphate,[7] losing ^{16}O, ^{17}O, or ^{18}O equally,[8] producing an equimolar mixture of *three* species. Since the carbon skeleton is also chiral (at C-2), these three cyclic phosphates are epimers (at phosphorus) of the three species that would derive from a phosphoryl group of the opposite configuration. Scheme II shows these relationships. Methylation[9] of the cyclic phosphate occurs on either of the exocyclic oxygens, and gives the two ('syn' and 'anti') diastereoisomeric cyclic methyl esters that can be separated chromatographically.[10] Scheme III illustrates the three isotopic variants of the 'syn' cyclic triester derived from an (*R*)-phosphoryl group, alongside the corresponding set that derives from an (*S*)-phospho compound. These two sets of cyclic triesters give identical mass spectra,[8] with parent peaks at 153, 154, and 155 (corresponding to the species containing $^{17}O + {}^{16}O$, $^{16}O + {}^{18}O$,

0002-7863/78/1500-2558$01.00/0

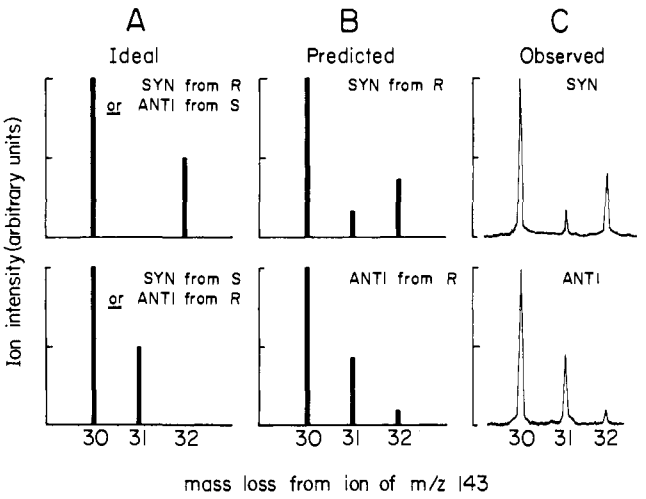
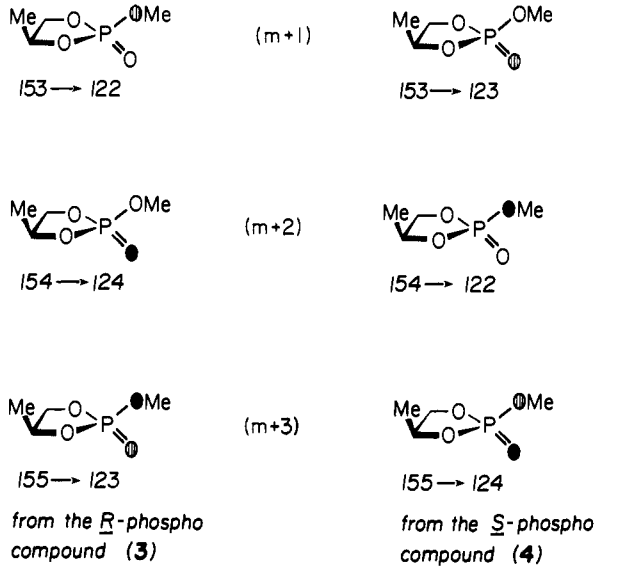


Figure 1. Metastable ion (linked scan) spectra for the $(m + 3)$ ions at m/z 143 which come from the "syn" and "anti" cyclic triesters derived from **3**: A, simplified spectra predicted for the fragmentation of the ion at m/z 143; B, predicted spectra as in A, taking into account the measured isomeric purity of the "syn" and "anti" cyclic triesters,[10] the measured enantiomer excess of the starting diol,[3] and the known contributions from the natural abundance of ^{13}C and ^{2}H; C, observed spectra.

Scheme III. Species that comprise the 'syn' cyclic triesters that derive from 1(*R*)-phospho-(*S*)-propanediol (**3**) or from 1(*S*)-phospho-(*S*)-propanediol (**4**).



and $^{18}O + {}^{17}O$) and daughter peaks at 122, 123, and 124 (corresponding to the loss of [^{16}O]-, [^{17}O]-, and [^{18}O]formaldehyde). To distinguish between the two sets of triesters shown in Scheme III, *we must relate the daughter ions to their parents*. For example, the $(m + 3)$ ion at 155 from the set of triesters derived from an (*R*)-phosphoryl group will lose 32 ([^{18}O]formaldehyde) giving a daughter ion at 123, whereas the $(m + 3)$ ion from the set derived from an (*S*)-phosphoryl group will lose 31 ([^{17}O]formaldehyde) to give a daughter ion at 124. Analogous arguments apply to the other two members of the sets (see Scheme III). The parent-daughter relationships can be established by metastable ion mass spectrometry: we have chosen to apply the linked-scan method.[11]

In practice, no suitable metastable fragmentation exists for the cyclic triesters shown in Scheme III[12] even under collisional activation.[13] However, reaction of the cyclic triester with methanol[14] gives 1- and 2-(dimethylphosphoryl)-(*S*)-propane-1,2-diol with no loss of isotopic label, which have similar mass spectra containing intense peaks due to the labeled ions: $[(MeO)_2P(^{i}O)^{j}OMe]^{+}\cdot$. These ions[15] lose formaldehyde in a strong metastable transition that is uncomplicated by alternative fragmentations or by isotope scrambling. *The fragmentation of these ions expresses all the stereochemical information contained in the families of cyclic compounds from which they derive.* Knowing the stereochemistry of ring closure[16] and the identity ('syn' or 'anti') of the diastereoisomers of the cyclic triester,[17] the absolute configuration of the original phosphate monoester can be assigned.

Figure 1 shows the metastable ion spectra predicted for the fragmentation of the $(m + 3)$ ions of $[(MeO)_2P(^{i}O)^{j}OMe]^{+}\cdot$ that come from the products of the methanolysis of the 'syn' and 'anti' cyclic triesters derived from **3** (nota bene footnote 18), along with the observed spectra.[19] It is clear that the phosphate ester **3** *is* of one configuration, that it contains 91 ± 8%[20] enantiomer excess of *R*, and that the stereoanalytical method allows the configuration to be determined.[21]

Using procedures of definable stereochemistry, we intend to transfer chiral phosphoryl groups to propanediol for stereochemical analysis as described. The generality of both the synthesis and the analysis will therefore allow the definition of the stereochemical course of reactions of phosphate esters in both chemistry and enzymology.

Acknowledgments. We are indebted to Dr. George Orr for seminal suggestions, to Professor F. McLafferty and Frank Bockhoff for advice, encouragement, and practical help, to Dr. G. Dudek for helping to implement the linked-scan system, and to the National Institutes of Health for support.

References and Notes

(1) The [^{17}O]-$POCl_3$ contained 47% ^{17}O, 26% ^{16}O, and 27% ^{18}O.
(2) D. B. Cooper, C. R. Hall, J. M. Harrison, and T. D. Inch, *J. Chem. Soc., Perkin Trans. 1*, 1969 (1977). Intermediates and products were characterized by ^{31}P and ^{1}H NMR and by mass spectrometry.
(3) 78% enantiomer excess.
(4) Trifluoroacetic anhydride (0.5 equiv) in excess $H_2{}^{18}O$ (97% atom excess), room temperature. Quenched after 1 min with excess solid ammonium bicarbonate.
(5) H_2–Pd/C, 2 atm, 12 h. The resulting ester was purified by ion-exchange chromatography and crystallized as the dicyclohexylammonium salt.
(6) Thus unlabeled *sn*-glycerol 3-phosphate was synthesized analogously by coupling the 1,2-dibenzyl ether of *sn*-glycerol to the unlabeled chlorophosphoramidate **1**.
(7) Diphenylphosphorylimidazole (1 equiv) and diisopropylethylamine (1 equiv) in CH_2Cl_2, room temperature, overnight.
(8) Isotope effects are too small to be of practical significance.
(9) Freshly distilled dry diazomethane in ether/acetonitrile (1:1 v/v), 15 min.
(10) High-pressure liquid chromatography on Corasil II (Waters Associates) in dry ether, room temperature. Fractions were analyzed by GLC: the 'syn' isomer contained 15% 'anti' and the 'anti' isomer contained 5% 'syn'.
(11) M. J. Lacey and C. G. Macdonald, *Org. Mass Spectrom.*, **12**, 587 (1977).
(12) Specifically labeled P=^{18}O and P—^{18}OMe species showed extensive isotopic scrambling in all potentially useful ions deriving from the cyclic triester.
(13) K. Lausen and H. Schwarz, *Angew. Chem., Int. Ed. Engl.*, **15**, 509 (1976).
(14) Methanol–triethylamine (4:1 v/v), −78 °C, 12 h. These conditions avoid problems of OMe exchange (see, e.g., F. H. Westheimer, *Acc. Chem. Res.*, 1, 70 (1968)).
(15) Use of specifically labeled P=^{18}O and P—^{18}OMe species defines the structure of the $[(MeO)_2P(^{i}O)^{j}OMe]^{+}\cdot$ ion and demonstrates the exact equivalence of the three OMe groups. This ion is formed (intramolecularly) and fragments with no oxygen isotopic scrambling, from both the 1- and the 2-(dimethylphosphoryl)-(*S*)-propane-1,2-diol. This was checked by the independent synthesis of the labeled and unlabeled 1- and 2-phosphate esters.
(16) By analogy to the known stereochemical course of the ring closure effected by diethyl phosphorochloridate (D. A. Usher, D. I. Richardson, and F. Eckstein, *Nature (London)*, **228**, 663 (1970)), this is presumed to be an "in-line" process.
(17) W. G. Bentrude, J. H. Hargis, N. A. Johnson, T. B. Min, P. E. Rusek, H.-W. Tan, and R. A. Wielesek, *J. Am. Chem. Soc.*, **98**, 5348 (1976), and references cited therein.
(18) There are two different sets of labeled ions of $[(MeO)_2P(^{i}O)^{j}OMe]^{+}\cdot$ that are produced from the 'syn' cyclic triesters from an (*R*)- and the 'syn' cyclic triesters from an)*S*)-phosphoryl group (see Scheme III). These two sets of ions *are identical with* those produced from the 'syn' and the 'anti' cyclic triesters that derive from a phosphoryl group of *one* configuration. This means that distinguishing between the 'syn' and 'anti' triesters that come from a phosphoryl compound of one configuration is *exactly equivalent* to distinguishing between the two 'syn' triesters from phosphoryl groups of opposite configuration.

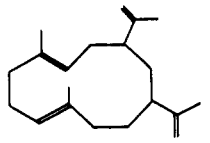
(19) Analysis of the $(m + 1)$ and $(m + 2)$ ions is consistent with the assignment from the $(m + 3)$ ion shown here.
(20) Corrected for the enantiomer excess of the propanediol, the cross-contamination of 'syn' and 'anti' diastereoisomers, and the natural abundance of ^{13}C and ^{2}H. The largest contribution to the error is in the estimate of the cross-contamination of the 'syn' and 'anti' isomers; the metastable ion spectra are precise to ±3%.
(21) Methods of ring closure other than that reported here have been less satisfactory, giving apparent values for the enantiomer excess of 9% (using thermal closure of the open dimethyl triester) and 40% (using dipyridyl disulfide/triphenylphosphine in dioxane, on **3**).

Steven J. Abbott, Stephen R. Jones
Steven A. Weinman, Jeremy R. Knowles*
Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Received January 9, 1978

Cubitene: An Irregular Twelve-Membered-Ring Diterpene from a Termite Soldier

Sir:

The oily defensive secretions released from the frontal glands of *Cubitermes* soldiers (Isoptera: Termitidae: Termitinae) are known to contain uncharacterized diterpene hydrocarbons.[1] These compounds are used in conjunction with attacks by the termites' mandibles,[2] a characteristic mode of defense used by *soldats faucheurs* ("reaping soldiers"). [3]Although the defensive effectiveness of these *Cubitermes* secretions has not been studied in detail, a possible role of another nontoxic hydrocarbon mixture has been suggested in the case of a *Macrotermes* soldier, which possesses analogous defensive behavior.[4] In this communication, we report the structure of cubitene, one of the major constituents of the frontal gland secretion of soldiers of the East African termite *Cubitermes umbratus* Williams.[5] Cubitene (**1**)[6] proved to possess a novel cyclododeca-1,5-diene skeleton resulting from an irregular joining of isoprene units.



I

Cubitene was isolated from the hexane extract of *C. umbratus* soldier heads (~75 μg/soldier) by chromatography over Florisil followed by preparative GLC.[7] Its low resolution EI mass spectrum exhibited major fragments at *m/e* 67, 68, 81, and 93; the CI-MS (CH_4 reagent gas) showed a weak $(M + 1)^+$ peak at *m/e* 273. High resolution mass spectroscopy indicated the molecular formula of cubitene to be $C_{20}H_{32}$ (M^+, *m/e* 272.2496; calcd, 272.2504). Catalytic hydrogenation of 0.5 mg of cubitene (Pd/C) gave a mixture of at least three perhydro derivatives. The CI mass spectra of each of these showed $(M - 1)^+$ peaks at *m/e* 279, consistent with their formulation as substituted cycloalkanes of the composition $C_{20}H_{40}$. Cubitene is therefore a monocyclic hydrocarbon, with four centers of unsaturation. The ^1H NMR spectral data revealed the presence of two 1,1-disubstituted double bonds (δ 4.75, br s, 4 H) and two additional olefinic protons (4.95, br m, 2 H). Four methyl groups attached to double bonds were observed at δ 1.55 (s, 3 H), 1.56 (s, 3 H), 1.64 (s, 3 H), and 1.77 (s, 3 H). No other diagnostically useful signals appeared in the ^1H NMR spectrum. However, the ^{13}C NMR spectrum of cubitene allowed the recognition of two trisubstituted double bonds (δ 134.4, 132.5, 127.5, 123.1) and confirmed the presence of two terminal methylene groups (δ 150.0, 149.4, 110.7, 109.0). The remaining twelve resonances (δ 40.9, 40.7, 39.9, 37.0, 31.3, 30.9, 28.0, 24.8, 22.6, 18.3, 15.1, and 14.3) were unassigned.

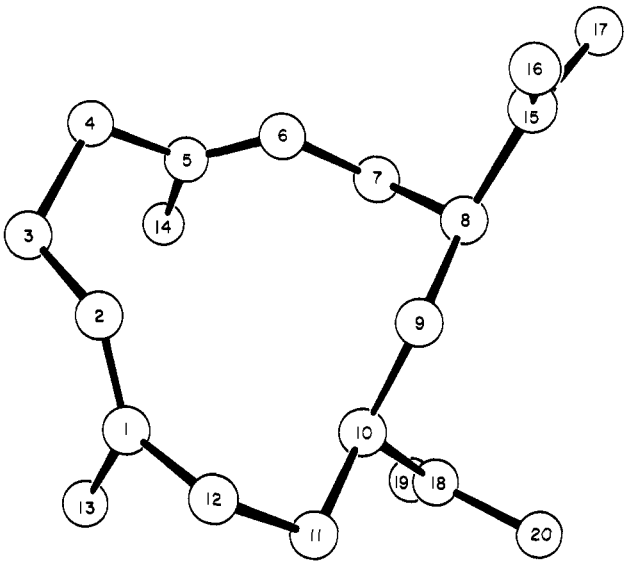


Figure 1. A computer-generated perspective drawing of cubitene (**1**). Hydrogens are omitted for clarity.

To further characterize cubitene, a small sample (0.1 mg) was ozonized[8] in CH_2Cl_2 at −78 °C. After reduction of the ozonide with triphenylphosphine, GC–MS analysis of the product mixture allowed the identification of 4-oxopentanal.[9] The mass spectrum of the product of highest molecular weight did not contain sufficient detail to permit an unambiguous structure assignment. Fortunately, at this stage in the investigation it was found that cubitene could be obtained crystalline (mp 33.5–34 °C) from cold methanol, permitting a single-crystal x-ray diffraction analysis.

Cubitene crystallized in the monoclinic crystal class with $a = 15.963$ (5), $b = 6.799$ (2), $c = 17.038$ (5) Å; $\beta = 96.69$ (2)°. Systematic extinctions combined with the known optical activity ($[\alpha]^{MeOH}_D$ +128° (*c* 0.76)) required space group *C*2. The limited amount of sample precluded a density measurement, but four molecules of composition $C_{20}H_{32}$ in this unit cell gave a reasonable hydrocarbon density of ~1.0 g/cm^3. All unique reflections with $\theta \leq 57°$ were collected on a four-circle diffractometer with graphite monochromated Cu Kα (1.54178 Å) x-rays. After correction for Lorentz, polarization, and background effects, 1058 (74%) reflections of the 1422 surveyed were judged observed ($F_o^2 \geq 3\sigma(F_o^2)$). The angular dependence of the scattering was removed as the reflection data were converted to normalized structure factors and solution via a multisolution weighted tangent formula approach attempted.[10,11] A plausible eight-atom fragment was located and the structure extended by tangent formula refinement.[12] Full-matrix least-squares refinements with anisotropic carbons and isotropic hydrogens have currently converged to a standard residual of 0.062 for the observed reflections.[13] A computer-generated perspective drawing is presented in Figure 1.

As can be seen from Figure 1, cubitene (**1**) is (1*E*,5*E*,8*S**, 10*R**)-1,5-dimethyl-8,10-bis(isopropenyl)cyclododeca-1,5-diene, a structure in which one isoprene unit is irregularly joined to three others. It appears to be the first example of a diterpene hydrocarbon based on a twelve-membered carbocyclic ring, and its biosynthesis poses interesting problems. One possibility would involve the initial coupling of farnesyl pyrophosphate to dimethylallyl pyrophosphate to give an irregular, acyclic diterpenoid, cyclization of which could lead directly to **1**.[14] A second, more indirect possibility is suggested by the observation that **1** co-occurs in *C. umbratus* with two cembrene derivatives.[15] In this context, the co-occurrence of the cembrene-related β-4,8,13-duvatriene-1,3-diol (**2**) and its frag-

0002-7863/78/1500-2560$01.00/0