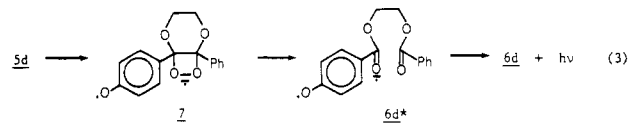
and dioxetanones.[1] The modest rate enhancements provided by the hydroxy and methoxy substituents are consistent with a biradical mechanism for the decomposition of these dioxetanes involving rate-limiting O–O bond homolysis.[15]

More significantly, we find that deprotonation of **5c** to give the phenoxide-substitued dioxetane **5d** dramatically changes the properties of **5** to those more typical of the biological intermediate **2a**. While **5c** has a half-life of 17 years at −30 °C, treatment of a solution of **5c** in toluene at −30 °C with the hindered base $(Me_3Si)_2MeCONa$ results in a flash of brilliant bluish luminescence. Rate constants for the decomposition of **5d** were measured by injection of 1 equiv of base in toluene into a prethermostated solution of **5c** (10^{-4} M in toluene) at temperatures from −35 to 2 °C. Control experiments have shown that the rates are unaffected by an excess of base but are reduced significantly with less than 1 equiv. Within experimental error, the following bases give rise to identical rates of decomposition for **5d**: $CsHCO_3$ with 24-crown-8, KOH or KO-*t*-Bu with 18-crown-6, and $(Me_3Si)_2MeCONa$ with 15-crown-5. An Arrhenius plot (Figure 1) gave an activation energy of 13.4 kcal/mol, with a calculated half-life at 25 °C of only 46 ms. *The relative rate of decomposition of* ***5d*** *vs.* ***5c*** *($k_{O^-/OH}$) at 25 °C is 4.4 × 10^6.*

There is also a significant increase in the singlet chemiexcitation efficiency upon deprotonation (Table I). Cleavage product **6d** is sufficiently fluorescent ($\phi_F = 0.002$)[16] so that $^1\phi_{CE}$ could be determined directly. As the phenoxide ion **6d** is chemically unstable in the presence of oxygen, both the fluorescence quantum yield of **6d** and the chemiluminescence of **5d** were measured in N_2-bubbled solutions.[17]



Intramolecular electron-transfer mechanisms have been proposed for the efficient chemiluminescence from dioxetanes bearing easily oxidized substituents.[18] Chemiluminescence has also been observed by Schuster[19] and Adam[20] from intermolecular electron-transfer reactions between peroxides and fluorescent hydrocarbons with low oxidation potentials. On the basis of these results, similar mechanisms have been suggested for the bioexcitation process in the firefly system.[18a,21] For the present case, we suggest that cleavage of dioxetane **5d** is initiated by the transfer of an electron from the phenoxide substituent to the peroxide σ^* orbital. Subsequent decomposition of intermediate **7** can yield directly a charge-transfer excited state of **6d**. The contrast between dioxetanes **5d** and **5a–c** together with the results of an earlier study of a related amino-substituted dioxetane[18a] now provides an explanation for the observations of White of the substituted luciferins **1a–e**. Further, a comparison of the efficiencies and stabilities of **5c** and **5d** prompts us to speculate about a possible control mechanism for the rapid flashing of the firefly involving initiation of luminescence by deprotonation of **2**.

Acknowledgment. Support from the U.S. Army Research Office is gratefully acknowledged.

Registry No. 4c, 81616-86-6; **5a**, 67592-95-4; **5b**, 81616-87-7; **5c**, 81616-88-8; **5d**, 81616-89-9; **6c**, 81616-90-2; **6d**, 81616-91-3; 2-phenyl-3-(4′-methoxyphenyl)-1,4-dioxene, 73260-63-6; 4-methoxybenzoin, 1889-84-5; ethylene glycol ditosylate, 6315-52-2.

(15) Richardson, W. H.; Montgomery, G. C.; Yelvington, M. B.; O'Neal, H. E. *J. Am. Chem. Soc.* **1974**, *96*, 7525–7532.

(16) Determined by adding 1 equiv of KO-*t*-Bu/18-crown-6 to a nitrogen-purged toluene solution of **6c** at 3 °C. ϕ_F for methyl *p*-hydroxybenzoate under the same conditions is 0.038.

(17) A referee suggested that singlet emission could result from triplet–triplet annihilation. However, the chemiluminescence efficiencies and rates are identical within experimental error in aerated solution, in the presence of 0.2 M 2,3-dimethyl-1,3-butadiene or under N_2 and Ar.

(18) (a) Zaklika, K. A.; Thayer, A. L.; Schaap, A. P. *J. Am. Chem. Soc.* **1978**, *100*, 4916–4918. (b) McCapra, F.; Beheshti, I.; Burford, A.; Hann, R. A.; Zaklika, K. A. *J. Chem. Soc., Chem. Commun.* **1977**, 944–946. (c) Nakamura, H.; Goto, T. *Photochem. Photobiol.* **1979**, *30*, 27–30. (d) Lee, C.; Singer, L. A. *J. Am. Chem. Soc.* **1980**, *102*, 3823–3829.

(19) Schuster, G. B. *Acc. Chem. Res.* **1979**, *12*, 366–373.

(20) Adam, W.; Cueto, O. *J. Am. Chem. Soc.* **1979**, *101*, 6511–6515.

(21) (a) McCapra, F. *J. Chem. Soc., Chem. Commun.* **1977**, 946–948. (b) Koo, J.-Y.; Schmidt, S. P.; Schuster, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 30–33. (c) Zaklika, K. A.; Kissel, T.; Thayer, A. L.; Burns, P. A.; Schaap, A. P. *Photochem. Photobiol.* **1979**, *30*, 35–44.

Stereochemistry of Casbene Biosynthesis

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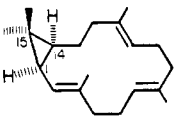
The isolation of casbene (**1a**) from a mixture of diterpene



1a

hydrocarbons produced by incubation of mevalonate (**2a**) or geranylgeranyl pyrophosphate (GGPP, **3a**) with an enzyme extract from castor bean (*Ricinus communis* L.) seedlings was reported by Robinson and West in 1970.[2] The structure proposed for casbene has been confirmed and its stereochemistry (1*R*, 14*S*) established by total synthesis from methyl (+)-*cis*-chrysanthemate.[3] Further studies by West and co-workers have shown that casbene is a phytoalexin for *R. communis*,[4] i.e., a fungal-elicited host metabolite that exhibits significant antifungal activity. In the context of the continuing interest on the mechanism and stereochemistry of the biosynthesis of cyclopropane-containing natural products,[5] we report experimental results that elucidate the stereochemistry of casbene biosynthesis.

Soluble enzyme extracts (S-150 fraction) were prepared from 2.5–3-day-old castor bean seedlings according to the procedures of Robinson and West.[2,6] In many germinations, the seedlings were inoculated with a spore suspension of *Rhizopus Stolonifer* 12 h before harvesting to enhance casbene synthetase activity.[4] Large-scale incubations of [2-^{14}C]mevalonic acid (**2b**) and ATP or of [1-$^3H_1$]- or [1-^{14}C]GGPP[7] with 300–700 mL of the S-150 enzyme preparations gave rise to the usual mixture of five diterpene hydrocarbons,[8] from which casbene was separated by column chromatography on silver nitrate impregated silica gel. The incorporation of radioactivity into casbene was typically 5–15% (200–800 μg) from mevalonate and 10–30% (100–300 μg)

(1) 3M Co. Fellow, 1977–1978; Eastman Kodak Fellow, 1978–1979; Merck and Company Fellow, 1979–1980.

(2) (a) Robinson, D. R.; West, C. A. *Biochemistry* **1970**, *9*, 70–79. (b) Robinson, D. R.; West, C. A. *Ibid.* **1970**, *9*, 80–89. (c) Robinson, D. R. Ph.D. Thesis, University of California, Los Angeles, 1967.

(3) Crombie, L.; Kneen, G.; Pattenden, G.; Whybrow, D. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1711–1717.

(4) (a) Sitton, D.; West, C. A. *Phytochemistry* **1975**, *14*, 1921–1925. (b) Stekoll, M.; West, C. A. *Plant. Physiol.* **1978**, *61*, 38–45. (c) West, C. A. *Naturwissenschaften* **1981**, *68*, 447–457.

(5) (a) Presqualene pyrophosphate: Poulter, C. D.; Rilling, H. C. In "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Eds.; Wiley-Interscience: New York, 1981; Chapter 8. (b) Cycloartenol: Altman, L. J.; Han, C. Y.; Bertolino, A.; Handy, G.; Laungani, D.; Muller, W.; Schwartz, S.; Shanker, D.; deWolf, W. H.; Yang, F. *J. Am. Chem. Soc.* **1978**, *100*, 3235–3237. Blätter, W. A. Ph.D. Thesis, ETH, Zürich, 1978. (c) Δ^3-Carene: Akhila, A.; Banthorpe, D. V. *Phytochemistry* **1980**, *19*, 1691–1694. (d) Cyclopropane-containing fatty acids: Law, J. H. *Acc. Chem. Res.* **1971**, *4*, 199–203. Buist, P. H.; MacLean, D. B. *Can. J. Chem.* **1981**, *59*, 828–838; Obrecht, J. Ph.D. Thesis, ETH, Zürich, 1982.

(6) Drengler, K. A.; Coates, R. M. *J. Chem. Soc., Chem. Commun.* **1980**, 856–857.

(7) Typical conditions: 0.3 mM potassium mevalonate (6 μM GGPP), 3.1 mM ATP, 12 mM $MgCl_2$, 0.3 mM $MnCl_2$, 100 mM Tris–bicarbonate buffer, pH 7.3; 4 h at 30 °C.

(8) Trachylobane, kaurene, sandaracopimaradiene, beyerene, and casbene.

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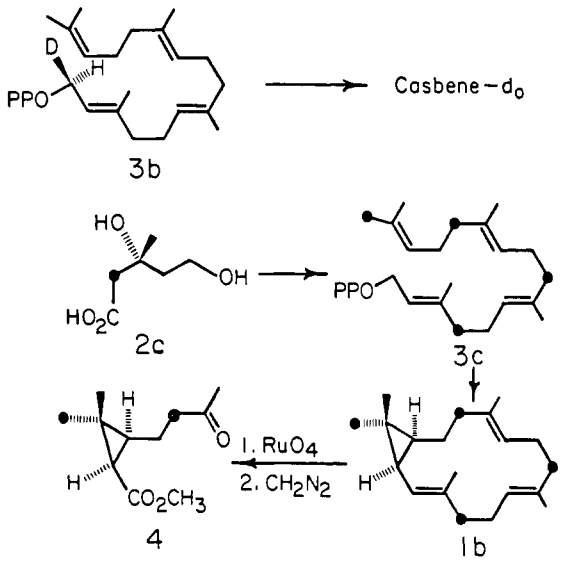
Scheme I

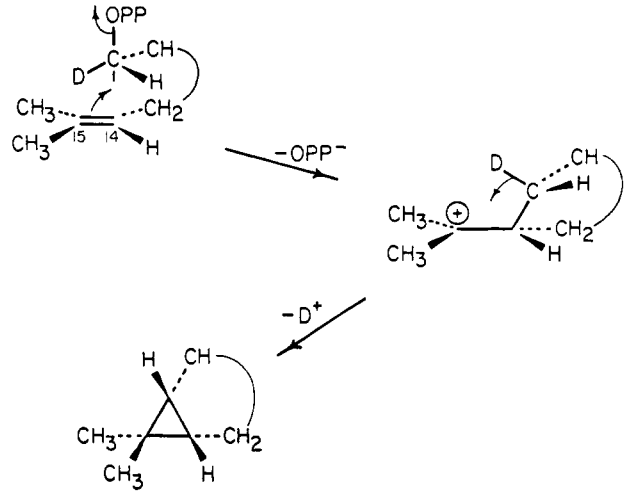


from GGPP. The identity of casbene was established by its position in the characteristic chromatographic elution profile,[2] its mass spectrum (GC/MS),[9] and its ^1H NMR spectrum.[9]

The stereochemistry of casbene biosynthesis at C-1 was revealed by experiments with deuterium-labeled GGPP (Scheme I). Incubation of a mixture of (*R*,*S*)-[1-$^2H_1$]- and (*R*,*S*)-[1-$^3H_1$]-GGPP[6,10] with the S-150 enzyme extract afforded casbene that had lost exactly half (50–51 ± 2% in three separate runs) of the deuterium label according to GC/MS analyses.[11] In contast, casbene biosynthesized from a mixture of (*S*)-[1-$^2H_1$]- and (*S*)-[1-$^3H_1$]GGPP[6,10] lost virtually all (99–100 ± 1% in three runs) of the deuterium. It is therefore apparent that the *pro-S* hydrogen at C-1 is stereospecifically eliminated in this enzymatic cyclopropanation reaction.

The stereochemical course of casbene biosynthesis at C-15 was elucidated by means of carbon-13 labeling. Incubation of [2-^{13}C]mevalonic acid (**2c**, 79% enriched)[12] with the S-150 cyclase preparation gave rise to [4,8,12,20-$^{13}C_4$]casbene (**1b**, ca. 300 μg): ^{13}C NMR (C_6D_6) δ 29.0 (q, CH_3), 39.8 (t, 2, CH_2), 40.7 (t, 1, CH_2). Oxidation of the ^{13}C-labeled casbene (ca. 600 μg) with ruthenium tetroxide–sodium periodate in acetone at room temperature,[13] esterification with diazomethane, and purification by column chromatography gave keto ester **4** (116 μg, 32%): ^{13}C NMR (C_6D_6) δ 28.79 (trans ring CH_3), 43.17 (CH_2CO).[14] Authentic samples of this keto ester were obtained by ozonolysis[15] of Δ^2-carene[17] and by diazomethane esterification of the corresponding keto acid prepared by cyclopropanation of the ethylene ketal of 6-methyl-5-hepten-2-one with ethyl diazoacetate and hydrolysis.[18] The ^{13}C NMR spectrum (C_6D_6) of unlabeled cis keto ester exhibited 11 peaks, 4 of which were assigned to the methyl groups by off-resonance decoupling: δ 14.26 (q, cis ring CH_3), 28.42 (q, $COCH_3$), 28.79 (q, trans ring CH_3), 50.76 (q, OCH_3). The assignment of the signal at δ 14.26 to the cis ring methyl group was proven by stereospecific synthesis of keto ester labeled with deuterium at this position.[19] As a consequence of the stereochemistry of the isopentenyl–dimethylallyl pyrophosphate isomerization,[20] the *pro-E* terminal methyl group of GGPP (**3c**) would become labeled with carbon-13. It follows that the intramolecular cyclopropanation reaction is suprafacial on the *re*, *re* face of the 14,15 double bond of GGPP.

Scheme II



A hypothetical mechanism for the formation of the cyclopropane ring of casbene is depicted in Scheme II[21] and is based upon the following assumptions: (1) the process occurs via carbonium ion intermediate(s), as is widely accepted for other terpene biogenetic pathways,[21–23] (2) the initial alkylation takes place with Markovnikov orientation and inversion of stereochemistry analogous to the prenyl transferase reaction,[20] (3) a least-motion mechanism involving proton (deuteron) loss from the plane of the incipient three-membered ring is favored. Within the framework of the foregoing assumptions the stereochemistry of the alkylation/γ-elimination process may be characterized as a "Markovnikov/syn" cyclopropanation.[24] It is of interest to note that the intermolecular cyclopropanation in the biosynthesis of presqualene pyrophosphate is also "Markovnikov/syn",[5a] albeit on the opposite (i.e., 2-*re*, 3-*si*) face of the 2,3 double bond of farnesyl pyrophosphate producing a trans-substituted cyclopropane ring.

(9) Mass spectrum, *m/e* (rel intensity) 272 (M^+, 14), 136 (54), 121 (100), 107 (71), 93 (92); ^1H NMR (360 MHz, C_6D_6) δ 0.19 (t, 1 H, *J* = 10 Hz), 0.98 (s, 3 H), 1.06 (s, 3 H), 1.52 (s, 3 H), 1.61 (s, 3 H), 1.66 (s, 3 H), 1.95 (m, 2 H), 2.07–2.30 (m, 10 H), 5.05 (m, 2 H), 5.15 (m, 1 H).

(10) Coates, R. M.; Cavender, P. L. *J. Am. Chem. Soc.* **1980**, *102*, 6358–6359.

(11) The selective ion monitoring technique was used. The total area for each *m/e* value was measured and compared so as to avoid isotopic fractionation during GC/MS analyses.

(12) (a) Tanabe, M.; Peters, R. H. *Org. Synth.* **1981**, *60*, 92–101. (b) We are grateful to P. Roach-Vettel and S. Rhee for preparing [2-^{13}C]mevalonic acid.

(13) (a) Branca, S. J.; Smith, A. B. *J. Org. Chem.* **1977**, *42*, 1026–1030. (b) Berkowitz, L. M.; Rylander, P. N. *J. Am. Chem. Soc.* **1958**, *80*, 6682–6684.

(14) Keto ester **4** from casbene was identified as the (−) enantiomer[3,15] from NMR spectra in the presence of the chiral solute, (*R*)(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol,[16] which induces nonequivalence of the four methyl groups and one cyclopropane proton of (±)-**4**. This assignment confirms the (1*R*,14*S*) stereochemistry of casbene.[3]

(15) Cocker, W.; St. J. Lauder, H.; Shannon, P. V. R. *J. Chem. Soc., Perkin Trans. 1* **1975**, 332–335.

(16) Pirkle, W. H.; Hoover, D. J. In "Topics in Stereochemistry"; Eliel, E. L., Ed.; in press.

(17) Brunetti, P.; Fringuelli, F.; Taticchi, A. *Gazz. Chim. Ital.* **1977**, *107*, 433.

(18) (a) Chopra, A. K. Ph.D. Thesis, Queen Mary College, University of London, 1976. We are grateful to Professor G. P. Moss for providing details of the cyclopropanation procedure. (b) Owen, J.; Simonsen, J. L.; *J. Chem. Soc.* **1932**, 1424–1429; (c) Owen, J.; Simonsen, J. L. *Ibid.* 1223–1225.

(19) The *pro-Z* methyl group of 6-methyl-5-hepten-2-one ethylene ketal was labeled with deuterium by the following five steps: (a) O_3, CH_2Cl_2, C_5H_5N; (b) $CD_3C(=PPh_3)CO_2CH_3$, CH_2Cl_2; (c) AlH_3, ether; (d) NCS, CH_3SCH_3, CH_2Cl_2; (e) $LiBEt_3H$, THF. The deuterium-labeled ketal was converted to a chromatographically separable mixture of the cis and trans keto esters as described in the text.

(20) Poulter, C. D.; Rilling, H. C. In "Biosynthesis of Isoprenoid Compounds"; Porter, J. W., Spurgeon, S. L., Eds.; Wiley-Interscience: New York, 1981; Chapter 4.

(21) For a discussion of mechanisms for cyclopropane ring formation in terpene biogenesis see: Coates, R. M. *Prog. Chem. Org. Nat. Products* **1976**, *33*, 73–230.

(22) (a) Ruzicka, L. *Proc. Chem. Soc. (London)* **1959**, 341–360. (b) Richards, J. H.; Hendrickson, J. B. "The Biosynthesis of Steroids, Terpenes, and Acetogenins"; W. A. Benjamin: New York, 1964.

(23) A classical tertiary carbonium ion is shown in Scheme II for simplicity. A bridged ion or an edge-protonated cyclopropane would also be satisfactory and equivalent for the present purposes.

(24) An "anti-Markovnikov/anti" cyclopropanation would, of course, also result in the loss of the *pro-S* hydrogen (deuterium) and would be an equally plausible mechanism, albeit presumably higher in energy. A "Markovnikov/anti" mechanism is excluded.

Acknowledgment. This research was supported in part by a grant (GM 13956) from the National Institutes of Health. High-field NMR spectra and GC/MS analyses were obtained with the aid of the University of Illinois NSF Regional Instrumentation Facility (NSF CHE 79-16100) and a grant from the National Cancer Institute (CA 11388), respectively. We are grateful to Professor Charles A. West, Department of Chemistry, University of California, Los Angeles, CA, for his advice and encouragement throughout this work and to Dr. H. Meier, Hofmann-La Roche, Basel, Switzerland, for a generous gift of (*E*,*E*)-farnesylacetone.

Registry No. 1a, 24286-51-9; **1b**, 81408-84-6; **4**, 73175-23-2; *cis*-**4**-d_3, 81408-85-7; *trans*-**4**-d_3, 81445-46-7; (-)-**4**, 81408-86-8; 6-methyl-5-hepten-2-one ethylene ketal, 3695-38-3.

Stereoselective Induction of Biomimetic Polyene Cyclizations[1] by Remote Chiral Centers. Effect of a Pro-C-7 Substituent in a Substrate Leading to Steroidal Products

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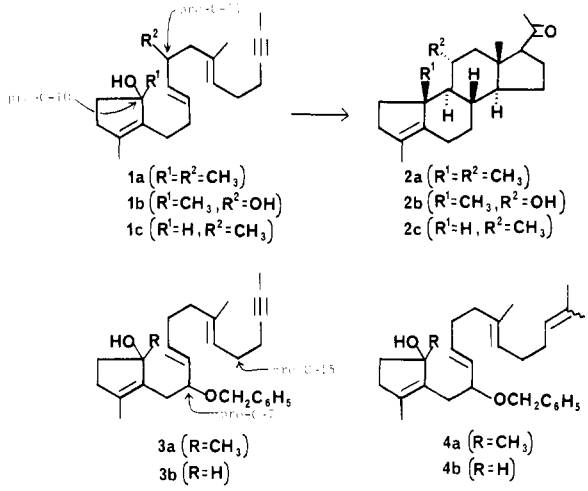
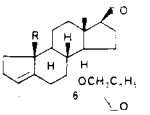
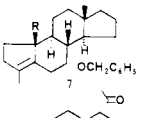
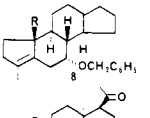
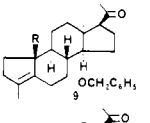
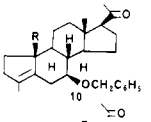
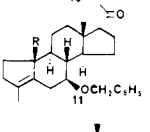
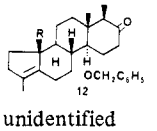
Ever since it was discovered that in the biomimetic cyclization of the substrates **1a** and **1b** the chiral center at *pro-C*-11 induced



ring closure so as to give preferentially the 11α-substituted products **2a** and **2b**, respectively[2]—a results that has led to the total asymmetric synthesis of a corticoid[3]—we have been intrigued by the possibility that a chiral center, even further removed from the initiating site of reaction might mediate diastereoselective cyclization. The case with a chiral center at *pro-C*-7, as in substrate **3a**, is particularly interesting for theoretical reasons (see below), and it also offered the potential of leading to some important steroids, e.g., spironolactone.

The diastereoselectivity previously observed in the cyclization **1a** → **2a** as well as **1b** → **2b** is easily understood because the transition state required for production of the (undetected) 11β epimer involves a destabilizing 1,3-diaxial interaction between the C-19-methyl group at *pro-C*-10 and the substituent (R^2) at *pro-C*-11. This contention was confirmed by the fact that cyclization of substrate **1c**, lacking such a 1,3-diaxial interaction (no C-19 methyl) was not diastereoselective, forming ca. equal amounts of **2c** and its 11β epimer.[4]

Table I. Distribution (%) of Cyclization Products[a]

tetracyclic product	from 3a (R = CH_3)	from 4a (R = CH_3)	from 3b (R = H)	from 4b (R = H)
	61.5	49	59	48
	1.5	27.5	1	26
	12.5	5	17	6
	2	6.5	3.5	6
	9	4.5	2	2
	2	3.5	1	1
	8	0	8	0
unidentified products	3.5	4	8.5	11

[a] By GC on a 14-m SE 54 capillary column. Values are uncorrected for any differences in detector response.

In the cyclization of **3** and **4**, however, there are no obvious steric interactions that might lead to diastereoselectivity.[5] One possibility that comes to mind is that, if there were extensive coiling of the acyclic chain in the transition state (as for a totally concerted process), then the *pro*-equatorial β-substituent at *pro-C*-7 could have nonbonded interactions with the *pro-C*-15 methylene group. Such a hypothesis, however, seemed to be divergent with van Tamelen's evidence[6] that epoxide-initiated polyene cyclizations are not concerted beyond the stage of the formation of the first ring.[7] Indeed we were not optimistic about the *pro-C*-7 substituent problem until the emergence of some encouraging, although preliminary, results on the cyclization of **3b**.[8]

(1) For a recent paper in the series on biomimetic polyene cyclizations, see: Johnson, W. S.; Frei, B.; Gopalan, A. S. *J. Org. Chem.* **1981**, *46*, 1512-1513.

(2) (a) Johnson, W. S.; DuBois, G. E. *J. Am. Chem. Soc.* **1976**, *98*, 1038-1039. (b) Johnson, W. S.; Escher, S.; Metcalf, B. W. *Ibid.* **1976**, *98*, 1039-1041. (c) Compare: Johnson, W. S.; Lyle, T. A.; Daub, G. W. *J. Org. Chem.* **1982**, *47*, 161-163.

(3) Johnson, W. S.; Brinkmeyer, R. S.; Kapoor, V. M.; Yarnell, T. M. *J. Am. Chem. Soc.* **1977**, *99*, 8341-8343.

(4) Johnson, W. S.; Yarnell, T. M., unpublished work. See: T. M. Yarnell, Ph.D. Thesis, Stanford University, Stanford, CA, 1975.

(5) Note that the chiral center in the cyclopentenol moiety of **3** is expected to be lost, by dehydration, prior to cyclization; therefore, it may be ignored as a factor influencing the stereochemical course of the reaction (cf. footnote 6, ref 2a).

(6) van Tamelen, E. E.; James, D. R. *J. Am. Chem. Soc.* **1977**, *99*, 950-952.

(7) The concerted cyclization observed for formation of two rings (Bartlett, P. A.; Brauman, J. I.; Johnson, W. S.; Volkmann, R. A. *J. Am. Chem. Soc.* **1973**, *95*, 7502-7504) is a special case involving participation of an aromatic ring. It is not so surprising, therefore, that remote substituents in such systems have resulted in diastereoselective cyclizations: Broess, A. I. A.; van Vliet, N. P.; Zeelen, F. J. *J. Chem. Res., Synop.* **1981**, 20-21 and ref 1 cited therein. Macco, A. A.; Buck, H. M. *J. Org. Chem.* **1981**, *46*, 2655-2660 and ref 1 cited therein.

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