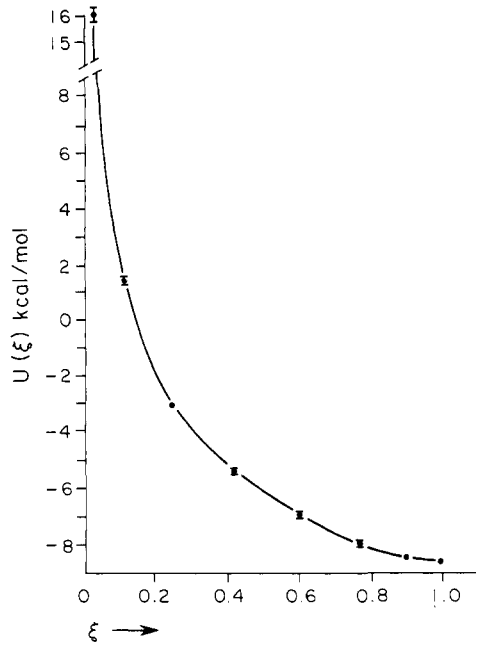


Figure 1. Calculated $U(\xi)$ vs. ξ for liquid water.

to ~1% within 500 K Metropolis steps. Each point required 100 K bytes of core and 4 h of computer time on the IBM 370/168 machine. A plot of $U(\xi)$ vs. ξ, including error bounds on each calculated point, is shown in Figure 1. The observed smoothness of the function can be understood by considering ξ as a temperature weighting factor and the ξ coordinate as a transcritical tieline. The integration over ξ was carried out by means of an 8-point Gaussian integration.[13]

The free energy was calculated directly from the integration over ξ and the entropy of the system was calculated using the free energy and the previously determined internal energy of the system. The results were found to be the following. Free energy: calculated, -4.31 ± 0.07; observed,[4] -5.74 kcal/mol. Entropy: calculated, -14.33 ± 0.09; observed[4] -13.96 cal/deg mol.

A comparison of the calculated and observed values shows the discrepancy between the calculated and observed free energy to be closer to that found for internal energy alone, and is thus ascribed to same reasons as enumerated above. The close accord between calculated and observed values of entropy indicates the statistical part of the problem to be well described under the assumption of pairwise additivity for this particular system, but further study on this point is required before any generalizations may be set forth.

Acknowledgment. This research was supported by NIH Grant 1-R01-NS12149 and Research Career Development Award (D.L.B.) 6T-K04-GM21281.

References and Notes

(1) O. Matsuoka, E. Clementi, and M. Yoshimine, *J. Chem. Phys.*, **64**, 1351 (1976). The MCY-CI potential function as used herein was augmented with an r^{-12} term joined smoothly to the short range region so that the function was defined on all intermolecular separations.
(2) G. C. Lie, E. Clementi, and M. Yoshimine, *J. Chem. Phys.*, **64**, 2314 (1976).
(3) S. Swaminathan and D. L. Beveridge, *J. Am. Chem. Soc.*, **99**, 8392 (1977). The calculated and observed values for U quoted in this reference differ by a factor of $3RT$ from those reported herein.
(4) G. N. Sarkisov, V. G. Dashevsky, and G. G. Malenkov, *Mol. Phys.*, **27**, 1271 (1974).
(5) J. C. Owicki and H. A. Scheraga, *J. Am. Chem. Soc.*, **99**, 7403 (1977).
(6) J. P. Valleau and G. M. Torrie in "Modern Theoretical Chemistry: Statistical Mechanics", Part A, B. J. Berne, Ed., Plenum Press, New York, N.Y., 1977. See also J. C. Owicki and H. A. Scheraga, *J. Phys. Chem.*, in press.
(7) J. G. Kirkwood, in "Theory of Liquids", B. J. Alder, Ed., Gordon and Preach, New York, N.Y., 1968.
(8) D. A. McQuarrie, "Statistical Mechanics", Harper and Row, New York, N.Y., 1976.
(9) For a related work on ion–water clusters, see M. R. Mruzik, F. F. Abraham, D. E. Schreiber, and G. M. Pound, *J. Chem. Phys.*, **64**, 481 (1976).
(10) N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller, and E. Teller, *J. Chem. Phys.*, **21**, 1087 (1953).
(11) F. Kohler, "The Liquid State", Crane Russack, 1972, Chapter 3.
(12) W. W. Wood in "Physics of Simple Liquids", H. N. V. Temperley, F. S. Rowlinson, and G. S. Rushbrooke, Ed., North-Holland Publishing Co., Amsterdam, 1968, pp 116–230.
(13) K. J. Nielsen, "Methods in Numerical Analysis", MacMillan, New York, N.Y., 1964.

Mihaly Mezei, S. Swaminathan, David L. Beveridge*
Chemistry Department
Hunter College of the City University of New York
New York, New York 10021
Received November 8, 1977

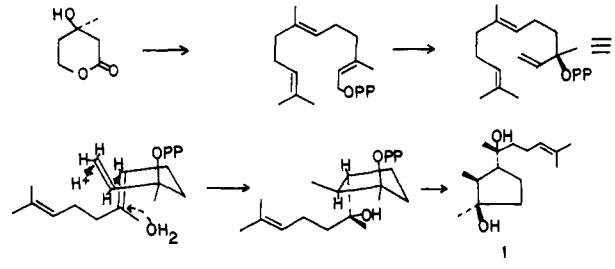
Conversion of Farnesyl and Nerolidyl Pyrophosphate to Cyclonerodiol by a Cell-Free Extract from *Gibberella fujikuroi*

Sir:

Biosynthetic incorporation experiments carried out in our laboratory[1] and that of Hanson[2] have established the mevalonoid origin of the fungal metabolite cyclonerodiol (**1**)[3] and suggested a biosynthetic pathway in which a molecule of water adds across the central double bond and the vinyl group of nerolidyl pyrophosphate, formed by the isomerization of farnesyl pyrophosphate. The proposed scheme (Scheme I) is further corroborated by the apparent conversion of farnesyl pyrophosphate to **1** by cultures of *Trichothecium roseum.*[2] On the other hand the failure of *T. roseum* to incorporate nerolidol itself has led the Sussex group to postulate an alternative cyclization mechanism involving the intermediacy of a cyclopropyl cation. We have independently observed that neither nerolidol, when fed to cultures of *Gibberella fujikuroi,* nor nerolidyl pyrophosphate, when administered to *T. roseum,* is incorporated into cyclonerodiol. On the assumption that the above negative results might be due in part to permeability problems with the intact organism, we have turned our attention to developing a suitable cell-free system. We report below the successful conversion of both nerolidyl and farnesyl pyrophosphates to cyclonerodiol by cell-free extracts of *G. fujikuroi.*[4]

The mycelium from a four-day-old culture of *G. fujikuroi,* ATCC 12616 (1 L), grown in the usual manner,[1,3] was harvested by filtration and washed with several volumes of cold distilled water, followed by 0.1 M sodium phosphate buffer, pH 7.6. The wet mycelium (52–57 g), suspended in 15 mL of phosphate buffer containing 2 mM DTE, was then passed through a precooled French Press under 10 000–15 000-psi pressure. After addition of 5 mL of phosphate/DTE buffer to the crushed cell mass, the suspension was centrifuged for 20 min at 15 000 g to remove cell debris. The supernatant fraction (S_{15}) was recentrifuged at 27 000 g for 60 min, the resulting cloudy S_{27} being filtered through a plug of glass wool to remove floating lipid. All centrifugations were carried out at 0–4 °C

Scheme I



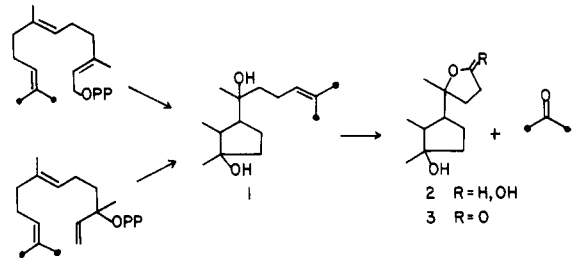
0002-7863/78/1500-3256$01.00/0

Table I. Conversion of Labeled Substrates to Cyclonerodiol

Substrate, dpm (nmol)	**1**, dpm (nmol)	**1**-$(DNB)_2$,[a] dpm/mmol	Acetone semicarbazone, dpm/mmol	**3**, dpm/mmol
[12,13-^{14}C]-FPP, 2.14×10^6 (4600)	2.03×10^4 (43)	1.99×10^5	1.98×10^5	Inactive
[12,13-^{14}C]-NPP, 6.48×10^6 (11 100)	4.99×10^4 (85)	4.27×10^5	4.18×10^5	Inactive
[12,13-^{14}C]Nerolidol, 4.70×10^5 (900)	[9.1×10][b] (<0.1)	$<7.5 \times 10^3$		

[a] Prepared from **1** after dilution with inactive carrier. [b] Maximum value based on **1**-$(DNB)_2$.

Scheme II



and the extract was kept at ice-bath temperatures. Extracts were freshly prepared just prior to use and typically contained 6–8 mg of protein/mL as determined by the method of Lowry.[5]

Extracts were assayed for cyclonerodiol synthesizing activity in the following manner. A 20-mL stoppered tube containing 10–15 mL of crude enzyme, 0.10 mL of 0.1 M magnesium chloride, and 0.6 mg of [1-$^3H_2$]farnesyl pyrophosphate[9] (9.48×10^6 dpm) was incubated at 26 °C for 4 h. The reaction was stopped by addition of 10–15 mL of acetone, and the solution was extracted with ether after filtration through Celite. Carrier cyclonerodiol (2–3 mg) was added to the concentrated ether extract which was then purified by TLC. Typically the ether extract contained 50% of the initial radioactivity, 1% being found as cyclonerodiol and 25% in a zone of nonpolar material corresponding to C_{15} alcohols. The activity of the cyclonerodiol could be confirmed by conversion to the corresponding bis(dinitrobenzoate),[3] and recrystallization to constant activity. Incubation with a boiled enzyme preparation yielded <0.4% ether-extractable material and inactive cyclonerodiol. The extracts were markedly unstable, all cyclonerodiol synthesizing activity being lost after 2–3 h at 27, 1 h at 30, or storage overnight at 4 °C.

The specificity of labeling was established by incubating [12,13-^{14}C]-FPP[11] with the crude enzyme extract from a 2-L culture. The resulting cyclonerodiol was diluted with inactive carrier and a portion converted to the bis(dinitrobenzoate). The remainder was treated with osmium tetroxide–sodium periodate to give acetone, isolated as the semicarbazone, and the hemiacetal **2**, which was converted to the corresponding crystalline lactone **3** by Jones oxidation[3] (Scheme II). The semicarbazone, when recrystallized to constant activity, was found to contain >99% of the activity of the parent bis(dinitrobenzoate), while **3** was inactive (Table I).

Repetition of the incubation with [12,13-^{14}C]nerolidyl pyrophosphate[12] gave cyclonerodiol in 0.8% yield. Degradation as above showed that 98% of the label resided in the terminal isopropylidene group, as expected. Finally, incubation of [12,13-^{14}C]nerolidol, dilution with inactive cyclonerodiol, and recrystallization of the derived bis(dinitrobenzoate), indicated that the conversion, if any, was <0.01%.

Although nerolidol is a well-known sesquiterpene alcohol which has been isolated from the essential oils of numerous higher plants,[14] there is very little experimental evidence concerning the metabolic role of nerolidol. An early suggestion that nerolidyl pyrophosphate might be a precursor of squalene was disproved by Rilling.[15] By analogy to a number of in vitro chemical cyclization experiments, nerolidyl pyrophosphate has been suggested as a possible intermediate in the formation of six-membered rings from farnesyl pyrophosphate,[16] but this hypothesis has never been substantiated either by enzymatic experiments or by feedings to whole cells. The study reported here is the first example of the enzymatic conversion of nerolidyl pyrophosphate to cyclized material. The intact incorporation of nerolidyl pyrophosphate into cyclonerodiol further supports the biosynthetic scheme elaborated in Scheme I.[17] The failure to utilize nerolidol as the free alcohol suggests that cyclization precedes pyrophosphate hydrolysis. Furthermore the location of label in **1** derived from FPP corroborates Hanson's earlier results which were based on $^3H/^{14}C$ ratios and whole cell feedings.[2] Finally, it should be noted that the availability of a suitable cell-free system provides an opportunity for determining the stereochemistry of formation of nerolidyl pyrophosphate itself from farnesyl pyrophosphate. The results of such a study will be communicated in the near future.

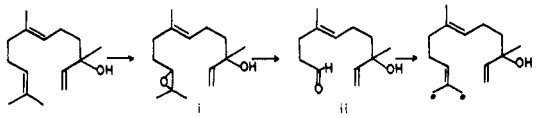
Acknowledgments. This work was supported by the National Science Foundation (PCM 74-07924) and by a grant from the Eli Lilly Co. Geranyl acetone was a generous gift of Hoffmann La Roche, Inc.

References and Notes

(1) D. E. Cane and M.-S. Shiao, *J. Am. Chem. Soc.*, **100**, 3203 (1978).
(2) R. Evans, J. R. Hanson, and R. Nyfeler, *J. Chem. Soc., Perkin Trans. 1*, 1214 (1976).
(3) S. Nozoe, M. Goi, and N. Morisaki, *Tetrahedron Lett.*, 1293 (1970); D. W. Pitel, G. P. Arsenault, and L. C. Vining, *J. Antibiot.*, **24**, 483 (1971); B. E. Cross, R. E. Markwell, and J. C. Stewart, *Tetrahedron*, **27**, 1663 (1971); J. R. Hanson, P. B. Hitchcock, and R. Nyfeler, *J. Chem. Soc., Perkin Trans. 1*, 1586 (1975).
(4) Cell-free systems from *G. fujikuroi* have been reported previously. A crude preparation from *G. fujikuroi* which catalyzes the conversion of mevalonate to (−)-kaurene has been described by Hanson.[5] This same enzyme system also converts gibberellin A_{12} aldehyde to the corresponding gibberellin A_{13} and A_{14} 7-aldehydes.[6] West has purified kaurene synthetase from *Fusarium moniliforme* (the imperfect stage of *G. fujikuroi*) some 170-fold.[7]
(5) R. Evans and J. R. Hanson, *J. Chem. Soc., Perkin Trans 1*, 2382 (1972).
(6) B. Dockerill, R. Evans, and J. R. Hanson, *J. Chem. Soc., Chem. Commun.*, 919 (1977).
(7) I. Shechter and C. A. West, *J. Biol. Chem.*, **244**, 3200 (1969); R. R. Fall and C. A. West, *ibid.*, **246**, 6913 (1971); T. R. Green and C. A. West, *Biochemistry*, **12**, 4720 (1974); T. R. Green, D. T. Dennis, and C. A. West, *Biochem. Biophys. Res. Commun.*, **64**, 976 (1975).
(8) O. A. Bessey, O. H. Lowry, and R. H. Love, *J. Biol. Chem.*, **180**, 755 (1949).
(9) Prepared by sodium [^{3}H]borohydride reduction of *trans,trans*-farnesal and formation of the pyrophosphate according to the literature procedure.[10]
(10) W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **245**, 4597 (1970); S. S. Sofer and H. C. Rilling, *J. Lipid Res.*, **10**, 183 (1969); A. A. Kandutsch, H. Paulus, E. Levin, and K. Bloch, *J. Biol. Chem.*, **239**, 2507 (1964); F. Cramer and W. Boehm, *Angew. Chem.*, **71**, 755 (1959); P. W. Holloway and G. Popjak, *Biochem. J.*, **104**, 57 (1967); G. Popjak, J. W. Cornforth, R. H. Cornforth, R. Ryhage, and D. J. Goodman, *J. Biol. Chem.*, **237**, 56 (1962).
(11) Synthesized from *trans,trans*-farnesol acetate by treatment with *m*-chloroperbenzoic acid to give the 10,11-epoxide which was cleaved to the trisnoraldehyde by successive treatment with perchloric acid and sodium

periodate. Reaction of the aldehyde with [*methyl*-^{14}C]isopropylidenetriphenylphosphorane and subsequent acetate hydrolysis gave [12,13-^{14}C]farnesol which was converted, as above, to the pyrophosphate.[10]

(12) *trans*-Nerolidol was obtained by addition of vinylmagnesium bromide in methylene chloride to *trans*-geranylacetone.[13] Epoxidation and cleavage to the trisnoraldehyde ii as above was carried out on the free alcohol. Reaction of ii with [*methyl*-^{14}C]isopropylidenetriphenylphosphorane gave



[12,13-^{14}C]nerolidol. The pyrophosphate was prepared in the usual manner.[10]

(13) O. Isler, R. Rueegg, L. Chopard-dit-Jean, H. Wagner, and K. Bernhard, *Helv. Chim. Acta*, **39**, 987 (1956).

(14) See Beilstein's "Handbuch der Organischen Chemie", 1, 464, I 241, II 513, III 2041.

(15) Sofer and Rilling.[10]

(16) D. Arigoni, *Pure Appl. Chem.*, **41**, 219 (1975); cf. N. H. Anderson and D. D. Syrdal, *Tetrahedron Lett.*, 2455 (1972); D. V. Banthorpe, B. V. Charlwood, and M. J. O. Francis, *Chem. Rev.*, **72**, 115 (1972); S. Gotfredsen, J. P. Obrecht, and D. Arigoni, *Chimia*, **31** (2), 62 (1977); C. D. Gutsche, J. R. Maycock, and C. T. Chang, *Tetrahedron*, **24**, 859 (1968); K. Stephan, *J. Prakt. Chem.*, **58**, 109 (1898); W. Rittersdorf and F. Cramer, *Tetrahedron*, **24**, 43 (1968); S. Winstein, G. Valkanas, and C. F. Wilcox, *J. Am. Chem. Soc.*, **94**, 2286 (1972).

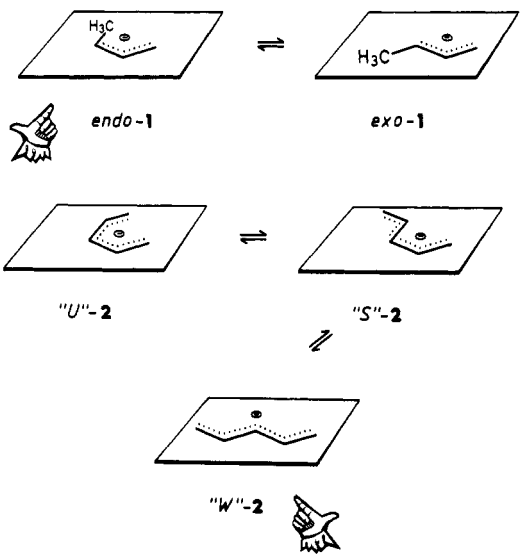
(17) The results obtained so far do not, of course, rigorously exclude the possibility that nerolidyl pyrophosphate might be isomerized back to farnesyl pyrophosphate which would then undergo conversion to **1** by an alternate pathway. This possibility is considered highly unlikely on grounds of mechanistic simplicity but can only be definitely ruled out by further experimentation.

David E. Cane,* Radha Iyengar
Department of Chemistry, Brown University
Providence, Rhode Island 02912
Received February 27, 1978

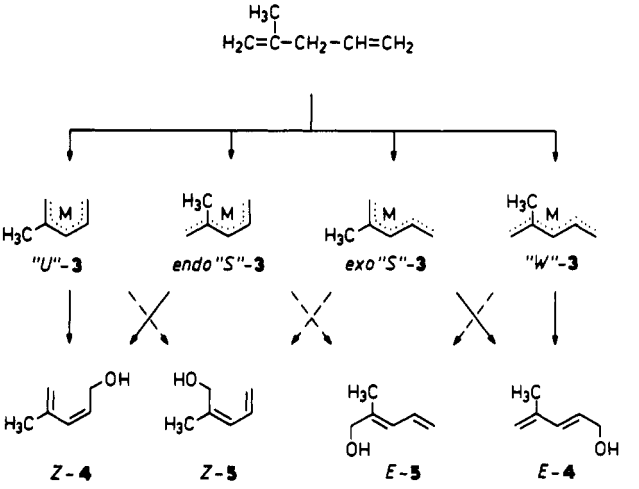
2-Methylpentadienyl- and 2,4-Dimethylpentadienylpotassium: First Examples of U-Shaped, though Open-Chain, Organometallics

Sir:

Whereas 2-alkenylpotassium compounds (e.g., butenylpotassium, derived from anion **1**) show a strong preference for the endo configuration,[1] a clear predominance of the most out-stretched, so-called "W" form was reported for pentadienyllithium (**2**-Li) in tetrahydrofuran[2] as well as pentadienylpotassium **2**-K) in liquid ammonia.[3] At equilibrium, the alternative "S"- (sickle-) and "U"-shaped structures were represented only by minor populations, if at all.



Scheme I



1-Methylpentadienyl metal (hexadienyl metal)[5,6] and 3-methylpentadienyl metal[6] compounds are also reported to exist preferentially as zigzag-like W anions.[7] In contrast, the ^{1}H NMR spectrum of 2-methylpentadienyllithium did not allow an unequivocal structure assignment, although it was tentatively attributed to a mixture of anions having the W form (W-**3**) and the sterically less hindered sickle form (that is, *exo*-S-**3**). Generally, the NMR approach will fail or lead to ambiguous conclusions, when applied to a multispin system of low symmetry or to a multicomponent mixture.[8] Under such circumstances, structure elucidation by chemical derivation is more reliable.

Of course, pentadienyl metal compounds or pentadienyl anions may react with an electrophile at any electron-rich site, that is, at any of three carbon atoms. Fortunately, the dimethoxyboration–oxidation sequence[9] was found to provide extreme regioselectivity. If there are terminal allylic positions available, they will be hydroxylated exclusively. Thus, no 2-methyl-1,4-pentadien-3-ol, the product derived from electrophilic attack to the central atom, is formed when 2-methyl-1,4-pentadiene was successively treated with the metalating agent (*sec*-butyllithium, butyllithium/potassium *tert*-butoxide,[10] or trimethylsilylmethylpotassium[11]), fluorodialkoxyborane, and alkaline hydrogen peroxide.[12] Still, a variety of hydroxylated derivatives has to be considered. Let us call the pentadienyl terminus adjacent to the methyl group the "head end" and the more distant terminal position the "tail end". Then every torsional isomer should give rise to one "head product" and one "tail product" (following the full-line arrow or, respectively, the broken-line arrow in Scheme I). Because of the steric bulk of the methyl group, the tail products should prevail slightly.

If the pentadienyl metal compound were present as a single torsional isomer, it would yeidl just one pair of such products, the configuration of which should disclose the shape of the organometallic precursor. Such a straightforward situation was met, when 2-methylpentadienyl*potassium* (**3**, M = K) was submitted to the dialkoxyboration–oxidation procedure at −78 °C. (*Z*)-4-Methyl-2,4-pentadien-1-ol ((*Z*)=**4**, 41%) and (*Z*)-2-methyl-2,4-pentadien-1-ol ((*Z*)-**5**, 22%) were identified as the almost exclusive products, the two corresponding *E* isomers being barely detectable (total yield 2%).[13] On the other hand, 2-methylpentadienyl*lithium* (**3**, M = Li) was converted into 10% (*Z*)-**4**, 12% (*Z*)-**5**, and 55% *E* isomers.[14] A similar product composition resulted from 2-methylpentadienylpotassium after keeping it for 24 h at −40 °C in the presence of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane ("Kryptofix 222").

Under the same conditions, both pentadienyllithium and

0002-7863/78/1500-3258$01.00/0