plexing agents, e.g., Newman's chiral aromatic nitro compound.[12] After three recrystallizations of the complex in benzene/ethanol, they obtained an isomer [$[\alpha]^{27}_D$ -123° (OP 3.3%)] in 22% yield.

Optically active PTrMA seems to be very useful for the resolution of many racemic compounds, particularly aromatic hydrocarbons, the resolution of which is rather difficult by the usual method.

Acknowledgment. We are grateful to Professor M. Nakazaki and Dr. K. Yamamoto in our department for providing us with valuable hydrocarbons. We also thank Daicel Chemical Industries Ltd. for giving us several racemic compounds.

(12) Newman, M. S.; Lednicer, D. *J. Am. Chem. Soc.* **1956**, *78*, 4765.

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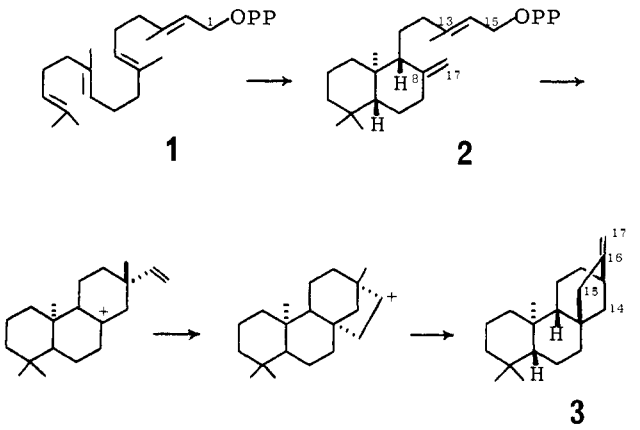
Stereochemistry of the Enzymatic Cyclization of Copalyl Pyrophosphate to Kaurene in Enzyme Preparations from *Marah macrocarpus*

Sir:

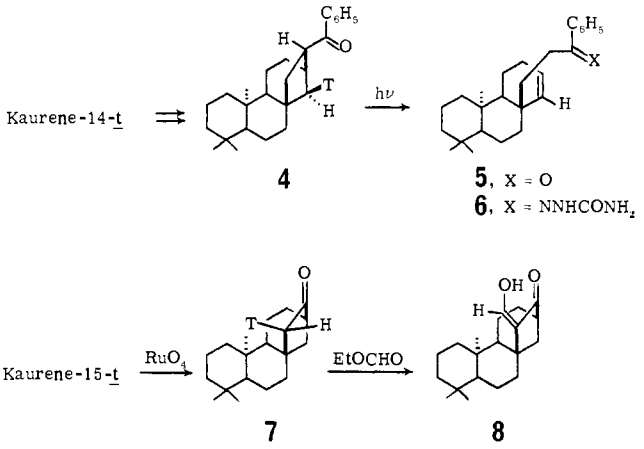
The biosynthesis of (-)-kaurene (**3**) is of considerable interest since this diterpene hydrocarbon is a key intermediate in the biosynthesis of the gibberellin plant growth regulating substances[1] and is a representative member of a biogenetically novel family of tetra- and pentacyclic diterpenoids.[2] The essential features of the commonly accepted biogenetic pathway to kaurene from geranylgeranyl pyrophosphate (**1**) shown in Scheme I were first proposed by Wenkert in 1955.[3] The results of tracer studies on gibberellin biosynthesis[1,4] are in accord with this pathway, and the intermediacy of copalyl pyrophosphate (**2**) has been established.[5] In this communication, we describe experiments which elucidate the stereochemistry of the cyclization of **2** to **3** with respect to C-15 and C-17 of the former in soluble enzyme preparations from *Marah macrocarpus*.[6]

Oxidation of (*R,S*)-geranylgeraniol-*1-t* (MnO_2, hexane, 0 °C)[7] followed by stereospecific reduction of the labeled aldehyde with liver alcohol dehydrogenase and NAD^+ (0.1 M PO_4 buffer, pH 7.5, 0.57 M C_2H_5OH, Tween 80, 30 °C, 19 h)[8] provided (*S*)-geranylgeraniol-*1-t* in 65% yield.[9] The corresponding pyrophosphates (43.4 and 9.8 mCi/mmol, respectively) of the *R,S* and *S* alcohols were prepared[7a] and separately incubated with a reconstituted lyophilisate isolated from the endosperm of immature *M. macrocarpus* seeds which is known to contain kaurene synthetase activity.[6] The incubation conditions were as follows: 0.1 M Tris buffer, pH 7.4, 0.01 M PO_4, 2 mM $MgCl_2$, 27.5 mg of lyophilisate/mL, 20 μM substrate, 30 °C, 8.5-25 h. Kaurene was isolated by extraction, purified by preparative TLC, diluted with ca. 100 mg of nonradioactive kaurene,[12] and crystallized to constant specific activity (20.0 and 7.16 μCi/mmol, respectively). The incorporation of radioactivity into kaurene was 31-49% in three incubations.

Scheme I



Scheme II



The samples of kaurene-*14-t* were converted to the exo phenyl ketone **4** (mp 166 °C)[13] in five steps,[14] and the latter upon irradiation to low conversion (Rayonet reactor, *t*-C_4H_9OH, 3 h) afforded unsaturated ketone **5** (mp 96-97 °C, 20-30%), the semicarbazone (**6**, mp 196-197 °C) of which was recrystallized to constant specific activity (Scheme II). Whereas **4** (5.09 μCi/mmol) derived from the *R,S* pyrophosphate gave rise to **6** retaining 59% of the original radioactivity, **4** (2.1 μCi/mmol), originating from the *S* substrate, produced **6** which had lost 99% of the tritium label (0.02 ± 0.005 μCi/mmol). Since Norrish II photofragmentation reactions occur via intramolecular γ-hydrogen transfer,[15] it follows that the tritium in kaurene biosynthesized

(1) For a recent review, see: P. Hedden, J. MacMillan, and B. O. Phinney, *Annu. Rev. Plant Physiol.*, **29**, 149-192 (1978).

(2) For a review, see: R. McCrindle and K. H. Overton in "Rodd's Chemistry of Carbon Compounds", 2nd ed., S. Coffey, Ed., Elsevier, Amsterdam, 1969, Vol. II, Part C, pp 373-402.

(3) E. Wenkert, *Chem. Ind. (London)*, 282 (1955).

(4) J. R. Hanson, *Fortschr. Chem. Org. Naturst.*, **29**, 395 (1971).

(5) (a) I. Shechter and C. A. West, *J. Biol. Chem.*, **244**, 3200 (1969); (b) R. R. Fall and C. A. West, *ibid.*, **246**, 6913 (1971).

(6) C. D. Upper and C. A. West, *J. Biol. Chem.*, **242**, 3285 (1967); M. O. Oster and C. A. West, *Arch. Biochem. Biophys.*, **127**, 112 (1968).

(7) (a) R. M. Coates, R. A. Conradi, D. A. Ley, A. Akeson, J. Harada, S.-C. Lee, and C. A. West, *J. Am. Chem. Soc.*, **98**, 4659 (1976); (b) R. M. Coates, D. A. Ley, and P. L. Cavender, *J. Org. Chem.*, **43**, 4915 (1978).

(8) (a) D. E. Gregonis and H. C. Rilling, *Biochemistry*, **13**, 1538 (1974); A. R. Battersby, J. Staunton, and H. R. Wiltshire, *J. Chem. Soc., Perkin Trans. 1*, 1156 (1975); (b) we are grateful to R. A. Conradi for developing this procedure.

(9) Although the stereospecificity of liver alcohol dehydrogenase for delivery of hydrogen to the *re* face of citral and farnesal has been established,[10] the stereochemistry of (*S*)-geranylgeraniol-*1-d* prepared by the same procedures was independently verified by the NMR method of Gerlach and Zagalak.[11]

(10) C. Donninger and G. Ryback, *Biochem. J.*, **91**, 11 P (1964); K. H. Overton and F. M. Roberts, *ibid.*, **144**, 585 (1974); K. Imai and S. Marumo, *Tetrahedron Lett.*, 4401 (1974).

(11) H. Gerlach and B. Zagalak, *J. Chem. Soc., Chem. Commun.*, 274 (1973).

(12) We are grateful to Drs. A. O. Geiszler and M. A. Nyman, Abbott Laboratories, North Chicago, Illinois, for providing the natural (-)-kaurene used in this research.

(13) The IR and NMR spectra of all compounds described in this work were fully consistent with the structure shown or implied. Only key data are cited. Satisfactory combustion analyses were obtained for all new, crystalline compounds reported.

(14) The following sequence of reactions afforded phenyl ketone **4** in 75% overall yield: (i) epoxidation (*m*-$ClC_6H_4CO_3H$, $CHCl_3$, 0 °C); (ii) rearrangement to *endo*-kauran-17-al (BF_3·Et_2O, C_6H_6, 25 °C); (iii) Grignard addition (C_6H_5MgBr, ether, reflux); (iv) oxidation (Collins reagent, CH_2Cl_2, 25 °C); (v) epimerization ($NaOC_2H_5$, C_2H_5OH, 25 °C, 2 h).

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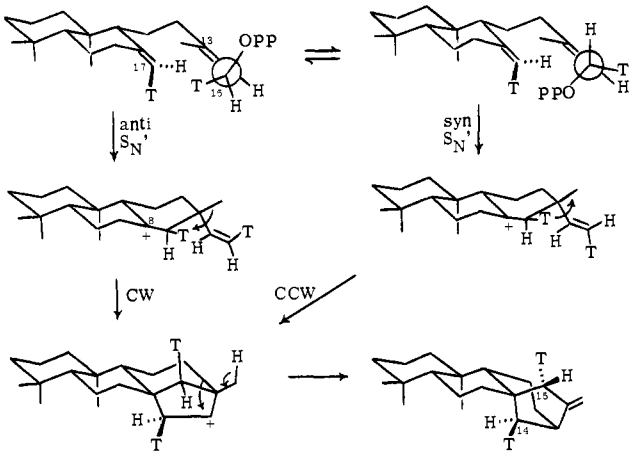
Scheme III



from (*S*)-**1**-*1-t* resides in the 14β position and that C-15 of **2** has undergone net inversion of configuration in the enzymatic cyclization.[16]

Copalyl pyrophosphate (**2**) labeled with tritium at C-17 in the anti-(*E*) configuration was prepared in order to reveal the stereochemistry of the cyclization, at this position. Methyl copalate[17] was converted to (*E*)-17-bromocopalol in three steps (32%),[18] and the corresponding tetrahydropyranyl ether was metallated with *n*-butyl- or *tert*-butyllithium (1:1 ether/THF, -20 to -30 °C, 1 h). Hydrolysis of the lithium derivative with tritiated water or deuterium oxide (-30 to 25 °C) followed by methanolysis to remove the protecting group afforded (*E*)-copalol-*17-t* and (*E*)-copalol-*17-d* (70-90% d_1): NMR ($CDCl_3$) δ 4.46 (s, 1 H, *syn*-C=CH_2), 4.76 (s, 0.1-0.3 H, *anti*-C=CH_2).[19,20] (*E*)-Copalyl pyrophosphate-*17-t* (0.65 mCi/mmol) was prepared[7a] and incubated with the enzyme extract from *M. macrocarpus* in four separate runs (0.5 M PO_4 buffer, pH 6.6, 2 mM $MgCl_2$, 5.5 mg of lyophilisate/mL). The kaurene-*15-t* was isolated, diluted, and recrystallized (10-27% incorporation, 0.36-0.45 μCi/mmol).

The location of the label in kaurene-*15-t* was determined by stereoselective exchange of the exo hydrogen at C-15 in 17-norkauranon-16-one (**7**). Base-catalyzed deuterium exchange (1 M NaOD, 1:2.7 v/v D_2O/dioxane, 22 °C) for 1 day and 19.5 days gave deuterated ketones having $d_0/d_1/d_2$ ratios of 3:97:0 and 3:73:24, respectively. Thus, the exo hydrogen underwent exchange ca. 200 times faster than the endo.[21] The exo assignment of the deuterium in **7**-d_1 was confirmed by reduction with sodium borohydride to 17-norkauran-16α-ol-*15*β-*d*: NMR ($CDCl_3$) δ 4.27 (br t, 1 H, J = ~5 Hz, C*H*OH). Norkauranone-*15*β-*t* was prepared in a similar manner, and the half-life for loss of radioactivity by exchange (1 M NaOH, 1:2.7 v/v H_2O dioxane, 22 °C) was estimated to be about 11 h. That is, one 17-h exchange decreased the radioactivity by 61-66%, and two such 17-h exchanges resulted in the loss of 84-94% of the tritium.

Oxidation (RuO_2, $NaIO_4$, H_2O/CCl_4/acetone, 25 °C) of the four biosynthetic samples of kaurene-*15-t* gave **7** (mp 111 °C, 43-55%, 0.27-0.57 mCi/mmol), following crystallization and sublimation. However, when subjected to one or two 17-h exchanges under the standard conditions, the recovered norkauranone-*15-t* retained essentially all [(92-111) ± (2-3%)] of its radioactivity. That the tritium was indeed situated in the endo position at C-15 was confirmed by condensation of **7** with ethyl formate (NaH, THF, 25 °C) to give the α-hydroxymethylene ketone **8** (mp 130-131 °C), which had lost (81-95) ± 7% of the radioactivity. We conclude that bond formation between C-13 and C-17 in the enzymatic cyclization of **2** occurs on the *si* (β) face of C-17.

Although these labeling experiments are sufficient to define the stereochemistry of the formation of ring C, there remain two stereochemically distinct pathways according to the biogenesis in Scheme I that would be consistent with overall inversion at C-15 (Scheme III). Thus, the combinations of either anti-S_N' attack of C-17 on C-13 of the allylic pyrophosphate with a clockwise (positive) rotation[22] of the vinyl group or syn-S_N' attack with counterclockwise (negative) rotation establish the *R* configuration in kaurene-*14-t*, it being assumed that bonding at C-8 occurs to the leading (i.e., *re*) face of the rotating vinyl group.

Examination of models indicates that while a 120° rotation suffices to bring the vinyl group into position below C-8 via the anti-CW pathway, a 240° rotation is required in the syn-CCW alternative. If the principle of least motion is considered relevant to an enzyme-catalyzed process such as this, the pathway involving initial anti-S_N' cyclization[23] may be the more likely, in which case the stereospecificity would be in accord with that found recently in the biosynthesis of rosenonolactone,[24] pleuromutilin,[25] and sandaracopimaradiene.[26]

Acknowledgment. We thank Professor Charles A. West, Department of Chemistry, University of California, Los Angeles, for providing the enzyme lyophilisate and for valuable advice on the enzymatic experiments. This research was supported in part by Grant GM-13956 from the National Institutes of Health.

(15) For example, see: (a) P. J. Wagner, *Acc. Chem. Res.*, **4**, 168 (1971); (b) F. D. Lewis, R. W. Johnson, and D. E. Johnson, *J. Am. Chem. Soc.*, **96**, 6090 (1974).

(16) Hanson and co-workers have previously shown that tritium from (5*S*)-mevalonic-*5-t* acid is found in the 14β position of kaurene biosynthesized with an enzyme preparation from *G. fujikuroi*. Thus, the stereochemistry of kaurene biosynthesis at this position is the same in *G. fujikuroi* and *M. macrocarpus*. See: R. Evans, J. R. Hanson, and L. J. Mulheirn, *J. Chem. Soc., Perkin Trans. 1*, 753 (1973).

(17) We are grateful to Dr. Raymond Zelnik, Department of Chemistry, Institute Butantan, São Paulo, Brazil, for supplying the copal resin from which copalic acid, as its methyl ester, was isolated: T. Nakano and C. Djerassi, *J. Org. Chem.*, **26**, 167 (1961); R. M. Coates, P. L. Cavender, and K. A. Drengler, unpublished results.

(18) (i) Bromine addition (Br_2, CH_2Cl_2, 3 equiv of C_5H_5N, 0 °C, 12 h); (ii) ester reduction (AlH_3, ether, -5 °C); (iii) dehydrobromination (KOH, 15:1 C_2H_5OH/H_2O, reflux 4.5 h).

(19) The C-17 vinyl proton appearing at higher field in the NMR spectra of related labd-13(17)-ene derivatives has been assigned the anti stereochemistry.[20] This assignment has been confirmed in our laboratory by analysis of the cyclopropane protons in the NMR spectrum of *ent*-8β,14β-methanopodocarpan-13α-ol-*18-d* prepared from copalol-*17-d*: K. A. Drengler, P. L. Cavender, and R. M. Coates, unpublished results.

(20) P. K. Grant and R. T. Weaver, *Tetrahedron*, **30**, 2385 (1974); R. A. Bell, M. B. Gravestock, and V. Y. Taguchi, *Can. J. Chem.*, **53**, 2869 (1975); P. Sundararaman and W. Herz, *J. Org. Chem.*, **42**, 806 (1977).

(21) The exo stereoselectivity of exchange is similar to that reported for methyl gibberate and bicyclo[2.2.1]heptan-2-one: see ref 16 and G. A. Abad, S. P. Jindal, and T. T. Tidwell, *J. Am. Chem. Soc.*, **95**, 6326 (1973).

(22) W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).

(23) For recent reviews, see: (a) D. E. Cane, *Tetrahedron*, **36**, 1109 (1980); (b) K. H. Overton, *Chem. Soc. Rev.*, **8**, 447 (1979).

(24) D. E. Cane and P. P. N. Murthy, *J. Am. Chem. Soc.*, **99**, 8327 (1977).

(25) D. Arigoni and H. Hasler, unpublished results; H. Hasler, Ph.D. Thesis, ETH, Zürich, 1979.

(26) R. M. Coates and K. A. Drengler, *J. Chem. Soc., Chem. Commun.*, in press.

(27) Biomedical Sciences Research Fellow, 1974-1975; Dow Chemical Corporation Fellow, 1975-1976.

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Nature of a Trimethylenemethane-Palladium Complex

Sir:

Delving into the structure and reactivity of trimethylenemethane (TMM) represents a continuing challenge.[1] Equally fascinating is the chemistry of the metal complexes of such reactive intermediates. The iron tricarbonyl complex of TMM has been shown

(1) For some recent studies, see: Platz, M. S.; Berson, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 2358. Duncan, C. D.; Corwin, L. R.; Davis, J. H.; Berson, J. A. *Ibid.* **1980**, *102*, 2350, and references cited therein. For a review, see: Berson, J. A. *Acc. Chem. Res.* **1978**, *11*, 446.

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