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Bisabolyl-Derived Sesquiterpenes from Tobacco 5-Epi-aristolochene Synthase-Catalyzed Cyclization of (2*Z*,6*E*)-Farnesyl Diphosphate

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Abstract: We report the structures and stereochemistry of seven bisabolyl-derived sesquiterpenes arising from an unprecedented 1,6-cyclization (cisoid pathway) efficiently catalyzed by tobacco 5-epi-aristolochene synthase (TEAS). The use of (2*Z*,6*E*)-farnesyl diphosphate as an alternate substrate for recombinant TEAS resulted in a robust enzymatic cyclization to an array of products derived exclusively (≥99.5%) from the cisoid pathway, whereas these same products account for ca. 2.5% of the total hydrocarbons obtained using (2*E*,6*E*)-farnesyl diphosphate. Chromatographic fractionations of extracts from preparative incubations with the 2*Z*,6*E* substrate afforded, in addition to the acyclic allylic alcohols (2*Z*,6*E*)-farnesol (6.7%) and nerolidol (3.6%), five cyclic sesquiterpene hydrocarbons and two cyclic sesquiterpene alcohols: (+)-2-epi-prezizaene (44%), (−)-α-cedrene (21.5%), (*R*)-(−)-β-curcumene (15.5%), α-acoradiene (3.9%), 4-epi-α-acoradiene (1.3%), and equal amounts of α-bisabolol (1.8%) and epi-α-bisalolol (1.8%). The structures, stereochemistry, and enantiopurities were established by comprehensive spectroscopic analyses, optical rotations, chemical correlations with known sesquiterpenes, comparisons with literature data, and GC analyses. The major product, (+)-2-epi-prezizaene, is structurally related to the naturally occurring tricyclic alcohol, jinkohol (2-epi-prezizaan-7β-ol). Cisoid cyclization pathways are proposed by which all five sesquiterpene hydrocarbons are derived from a common (7*R*)-β-bisabolyl$^+$/pyrophosphate$^-$ ion pair intermediate. The implications of the "cisoid" catalytic activity of TEAS are discussed.

Introduction

Tobacco 5-epi-aristolochene synthase (TEAS) from *Nicotiana tabacum* is a monomeric, 64 kDa sesquiterpene cyclase[1] that catalyzes the Mg^{2+}-dependent cyclization and rearrangements of the natural substrate (2*E*,6*E*)-farnesyl diphosphate ((*E*,*E*)-**1**, FPP) to primarily the bicyclic hydrocarbon (+)-5-epiaristolochene (**2**), the eremophilane precursor of the phytoalexin capsidiol (**3**) (Figure 1).[2] According to the general mechanism of catalysis attributed to all sesquiterpene synthases,[3] this multistep cyclization reaction pathway takes place by initial Mg^{2+}-assisted ionization of the allylic diphosphate, C1−C10 macrocyclization, and elimination into the cis-terminal methyl group to form the (*E*,*E*,*R*)-germacrene A intermediate.[4] Protonation of the germacrene A intermediate as the UD (boat-

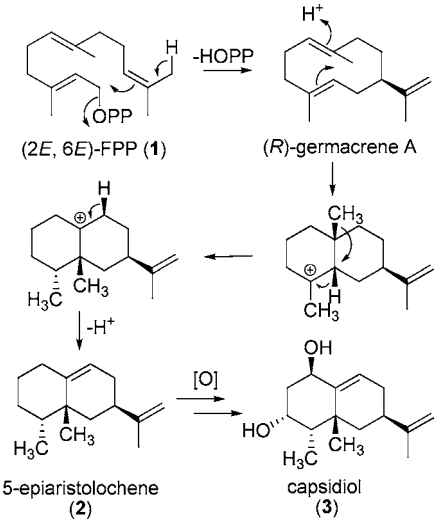

Figure 1. Biosynthesis of the phytoalexin capsidiol (**3**) in tobacco plants.

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(1) (a) Vogeli, U.; Freeman, J. W.; Chappell, J. *Plant Physiol.* **1990**, *93*, 182–187. (b) Rising, K. A.; Starks, C. M.; Noel, J. P.; Chappell, J. *J. Am. Chem. Soc.* **2000**, *122*, 1861–1866.
(2) (a) Whitehead, I. M.; Threlfall, D. R.; Ewing, D. F. *Phytochemistry* **1989**, *28*, 775–779. (b) Whitehead, I. M.; Ewing, D. F.; Threlfall, D. R.; Cane, D. E.; Prabhakaran, P. C. *Phytochemistry* **1990**, *29*, 479–482.
(3) Cane, D. E. *Chem. Rev.* **1990**, *90*, 1089–1103.

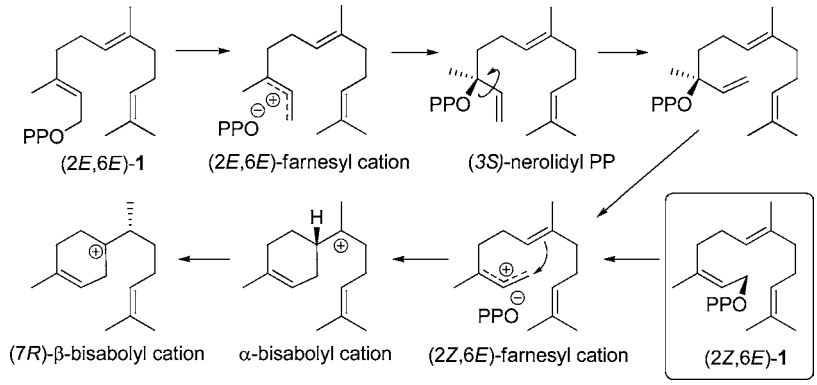


Figure 2. Formation of the β-bisabolyl cation from FPP isomers (2*E*,6*E*)-**1** and (2*Z*,6*E*)-**1**.

chair) conformer[5] leads to further cyclization and rearrangements terminated by 9β proton elimination accounting for the formation of the nonisoprenoid eremophilane structure **2** (Figure 1). The cryptic germacrene A synthase activity of recombinant TEAS is supported by site-directed mutagenesis,[1b] by the incorporation of a single deuteron at the 1β position of compound **2** from incubations in deuterated media,[4] and by the exclusive formation of 1-fluorogermacrene A as product from 6-fluoro-FPP.[6]

Using a combination of authentic standards, mass spectral libraries, and gas chromatography (GC) retention indexes, O'Maille et al. recently discovered that, in addition to the major products (+)-5-epiaristolochene (**2**) (78.9%), its $\Delta^{1(10)}$ isomer (−)-4-epieremophilene[7] (6.2%), and (*R*)-germacrene A (3.7%), incubations of (2*E*,6*E*)-FPP (**1**) with TEAS lead to 22 additional sesquiterpenes (11% of total products).[8] Among the identified minor products were (−)-α-cedrene (**5**), an isomer of (−)-prezizaene (**4**) and an acoradiene (**7**) (together accounting for 2.5% of the total hydrocarbon fraction). Interestingly and quite unexpectedly, the formation of these latter products supported a bifurcating mechanistic pathway comprised of cyclizations via the cisoid farnesyl cation. This process was proposed to occur by enzyme-mediated allylic rearrangement of the primary diphosphate (*E*,*E*)-FPP to its tertiary homologue (3*S*)-nerolidyl diphosphate (NPP), a putative neutral enzyme-bound intermediate, which is in turn further converted to the cyclic sesquiterpenes through S_N' cyclization involving the proximal C6−C7 double bond of FPP.[9,10] This unexpected mode of action displayed by TEAS, which initially gives rise to the α-bisabolyl carbocation (Figure 2), is analogous to the reaction mechanism hypothesized by Ruzicka to explain the formation of the terpinyl cation via a cis (neryl) allylic cation in the biogenesis of the cyclic monoterpenes.[9e,f,11] In the sesquiterpene series, this reaction mechanism has been invoked to account for the formation of β-macrocarpene,[12a] amorpha-4,11-diene,[12b,c] trichodiene,[13] cedranes such as isocedrol,[14] and many others.[15] Recently, epi-isozizaene, a newly described sesquiterpene, was proposed to arise via (3*R*)-NPP and the α-bisabolyl cation.[16]

In this paper we report the full structural and stereochemical characterization of the cisoid products generated by recombinant TEAS with the alternative substrate (2*Z*,6*E*)-FPP ((2*Z*,6*E*)-**1**).[17] Based on the stereochemistry of the cisoid products identified, we reason that the farnesyl chain of (2*Z*,6*E*)-**1** adopts a distinctive folding pattern differing from, but congruent with, that of the native substrate (2*E*,6*E*)-FPP (**1**) (Figure 1). Diphosphate ionization triggers an alternative reaction pathway (Figure

(4) Schenk, D. J.; Starks, C. M.; Manna, K. R.; Chappell, J.; Noel, J. P.; Coates, R. M. *Arch. Biochem. Biophys.* **2006**, *448*, 31–44.
(5) Faraldos, J. A.; Wu, S.; Chappell, J.; Coates, R. M. *Tetrahedron* **2007**, *63*, 7733–7742.
(6) Faraldos, J. A.; Zhao, Y.; O'Maille, P. E.; Noel, J. P.; Coates, R. M. *ChemBioChem* **2007**, *8*, 1826–1833.
(7) Zhao, Y.; Schenk, D. J.; Takahashi, S.; Chappell, J.; Coates, R. M. *J. Org. Chem.* **2004**, *69*, 7428–7435.
(8) O'Maille, P. E.; Chappell, J.; Noel, J. P. *Arch. Biochem. Biophys.* **2006**, *448*, 73–82.
(9) (a) Croteau, R.; Cane, D. E. In *Methods in Enzymology* (*Steroids and Isoprenoids)*; Law, J. H., Rilling, H. C., Eds.; Academic: New York, 1984; Vol. 110, pp 383−405. (b) Cane, D. E. In *Isoprenoids Including Carotenoids and Steroids*; Cane, D. E., Ed. (Volume 2 in Comprehensive Natural Products Chemistry; Barton, D., Nakanishi, K., Meth-Cohn, O., Eds.); Elsevier: Amsterdam, 1999; pp 155−215. (c) Cane, D. E. *Acc. Chem. Res.* **1985**, *18*, 220–226. (d) Cane, D. E. *Tetrahedron* **1980**, *36*, 1109–1159. (e) Wise, M. L.; Croteau, R. In *Comprehensive Natural Products Chemistry*; Cane, D. E., Ed. (Volume 2 in Comprehensive Natural Products Chemistry; Barton, D., Nakanishi, K., Meth-Cohn, O., Eds.); Elsevier: Amsterdam, 1999; pp 97−153. (f) Croteau, R. *Chem. Rev.* **1987**, *87*, 929–954.
(10) A referee raised the possibility that the small amount (3%) of 2,3-cisoid products formed from incubations with commercial (*E*,*E*)-FPP in the previous work (ref 8) might have been derived from contamination of the substrate with a small amount of (2*Z*,2*E*)-FPP. This possibility was rendered unlikely by 500 MHz analysis of (*E*,*E*)-FPP purchased later from the same commercial supplier in D_2O with and without spiking with pure (2*Z*,6*E*)-FPP. No signal could be detected by integrations in the regions of the well-separated CH_2OPP absorption (δ 4.365 ppm) or the C3 methyl singlet peak (δ 1.658 ppm) for the latter. We estimate that the maximum amount of the Z,E isomer present was ≤0.5−1%, considerably less than the 3% of products derived from the cisoid pathway.
(11) (a) Ruzicka, L.; Eschenmoser, A.; Hesser, H. *Experientia* **1953**, *9*, 357–367. (b) Ruzicka, L. *Proc. Chem. Soc.* **1959**, 341–360. (c) Ruzicka, L. *Pure Appl. Chem.* **1963**, *6*, 493–522.
(12) (a) Köllner, T. G.; Schnee, C.; Li, S.; Svatos, A.; Schneider, B.; Gershenzon, J.; Degenhardt, J. *J. Biol. Chem.* **2008**, *283*, 20779–20788. (b) Picaud, S.; Mercke, P.; He, X.; Sterner, O.; Brodelius, M.; Cane, D. E.; Brodelius, P. E. *Arch. Biochem. Biophys.* **2006**, *448*, 150–155. (c) Kim, S. H.; Heo, K.; Chang, Y. J.; Park, S. H.; Rhee, S. K.; Kim, S. U. *J. Nat. Prod.* **2006**, *69*, 758–756.
(13) Cane, D. E.; Ha, H.-J. *J. Am. Chem. Soc.* **1988**, *110*, 6865–6870, and references cited herein.
(14) Mercke, P.; Crock, J.; Croteau, R.; Brodelius, P. E. *Arch. Biochem. Biophys.* **1999**, *369*, 213–222.
(15) For an outline of numerous bisabolyl-derived sesquiterpenes and literature references, see: (a) Hong, Y. J.; Tantillo, D. J. *J. Am. Chem. Soc.* **2009**, *131*, 7999–8015.
(16) (a) Lin, X.; Hopson, R.; Cane, D. E. *J. Am. Chem. Soc.* **2006**, *128*, 6022–6023. (b) Lin, X.; Cane, D. E. *J. Am. Chem. Soc.* **2009**, *131*, 6332–6333.
(17) In a parallel submission, X-ray crystal structures of TEAS complexes with (2-*trans*,6-*trans*)- and (2-*cis*,6-*trans*)-2-fluoro-FPPs that reveal the binding of inert fluoro analogues are reported: Noel, J. P.; Dellas, N.; Faraldos, J. A.; Zhao, Y.; Hess, B. A., Jr.; Smentek, L.; Coates, R. M.; O'Maille, P. E. *ACS Chem. Biol.*, in press.

Table 1. Steady State Kinetic Parameters of TEAS Determined Using (2*Z*,6*E*)- and (2*E*,6*E*)-FPP (**1**) as Substrates

kinetic constants	(2*Z*,6*E*)-FPP[17]	(2*E*,6*E*)-FPP[a]
K_M (μM)	14.03 ± 2.59	8.40 ± 0.89
k_{cat} (min^{-1})	5.71 ± 0.08	2.50 ± 0.7
k_{cat}/K_M	0.41	0.30

[a] Values for (2*E*,6*E*)-FPP were previously reported. See ref 22.

2) leading to the formation of seven bisabolyl-derived sesquiterpenes through the intermediacy of a common (7*R*)-β-bisabolyl cation.

Results and Discussion

The synthesis (2*Z*,6*E*)-farnesyl diphosphate as its tris-ammonium salt (2*Z*,6*E*)-**1** has been recently reported.[18] In the present work, (2*Z*,6*E*)-**1** (≥99.5% purity by NMR analysis) was readily synthesized following the procedures developed by Meyers[19] and Poulter[20] starting from (2*Z*,6*E*)-farnesol.[21] Recombinant TEAS from *Nicotiana tabacum* was expressed and purified as previously described.[8]

Initial GC−MS analyses[22] of the pentane extracts obtained from analytical-scale incubations showed that TEAS catalyzes the cyclization of the alternative substrate (2*Z*,6*E*)-farnesyl diphosphate to at least 18 sesquiterpenes, three of them (**4**, **5**, and **6**) accounting for 82% of the total hydrocarbon mixture. The measured steady-state kinetic parameters of TEAS with the (2*Z*,6*E*)-**1** substrate (Table 1) indicate that TEAS possesses a slightly poorer Michaelis constant (K_M) for the 2*Z*-isomer of **1** when compared with its natural substrate (2*E*,6*E*)-FPP but exhibits a 2-fold higher turnover rate (k_{cat}) with the overall catalytic efficiency (k_{cat}/K_M) almost identical for the two substrates. The MS fragmentation patterns and GC retention times of the two major sesquiterpenes **4** (44%) and **5** (21.5%) obtained in the present work, were identical to those reported by O'Maille et al.[8] for isoprezizaene (2,5-diepi-prezizaene)[23] and (−)-α-cedrene (**5**), respectively.

Hydrocarbons **4** and **5** were identified as minor products (taken together, <2.5%) in a total of 22 alternative minor sesquiterpenes (11% total hydrocarbon fraction) catalytically produced by TEAS from incubations with the normal substrate (2*E*,6*E*)-farnesyl diphosphate.[8] Although the structural identity of sesquiterpene **5** was securely established by coincidence of GC retention time and MS comparison with commercially available (−)-α-cedrene using a chiral GC column, the major sesquiterpene **4** failed to coelute with an authentic sample of (−)-prezizaene[24] using the same chiral phase, proving that the latter and the TEAS-generated sesquiterpene **4** were different. However, on the basis of the identity of MS fragmentations, O'Maille et al[8] surmised that **4** must be a stereoisomer of prezizaene, and this product was designated isoprezizaene.[23]

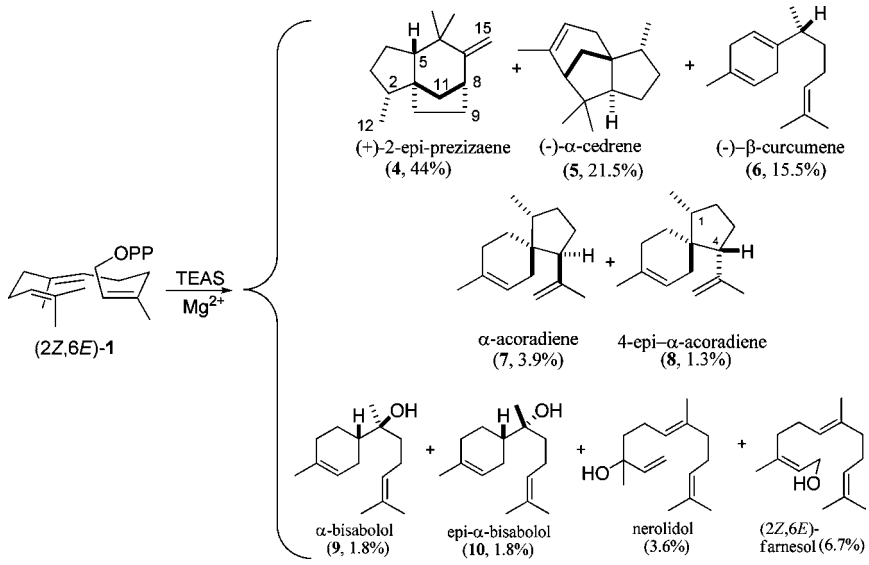
A preparative-scale incubation was carried out using 127 mg (310 μmol) of (2*Z*,6*E*)-**1** and a total of 18 mg of recombinant TEAS to accumulate sufficient material for chromatographic fractionations, NMR analyses, and optical rotation measurements of the major products (**4**, **5**, and **6**). Extraction of the incubation mixture with pentane and purification by silica gel chromatography yielded 27.3 mg (46% overall yield) of sesquiterpene hydrocarbons (86% of total product) and 4.4 mg (7% overall yield) of sesquiterpene alcohols (14% of total product) (Scheme 1). Further purification of the hydrocarbons by preparative TLC afforded three fractions for analyses by 500 MHz ^{1}H NMR spectroscopy. *Fraction 1* (17.6 mg) was a 2:1 mixture of compounds **4** and **5** respectively. *Fraction 2* (2.2 mg) was a 2:1:3:1 mixture of **4**, **6**, **7**, and **8** respectively, and *fraction 3* (4.0 mg) contained a single sesquiterpene (**6**), the purity of which was judged to be 95% by ^{1}H NMR peak integrations. Hydrocarbons **4** and **5** were obtained in pure form (>98%) by preparative TLC on $AgNO_3$-impregnated silica gel.

The structure and stereochemistry of the major TEAS-mediated cyclization product (**4**) were deduced using a combination of ^{1}H, ^{13}C, and NOE NMR analyses; comparison with literature values; and chemical correlation with the known jinkohol (**14**, Scheme 2). This sesquiterpene was obtained as an oil ($[\alpha]_D^{25}$ +26.8° ($CDCl_3$, *c* 0.1), Lit.[25] +10° ($CHCl_3$, *c* 0.6), and its molecular formula $C_{15}H_{24}$ is consistent with the molecular ion peak observed at *m*/*z* 204 by electron-impact ionization MS. The ^{1}H and ^{13}C NMR spectral data match well with literature data (500 and 125 MHz, $CDCl_3$) for semisynthetic (+)-2-epi-prezizaene,[25,26] and they show distinctive differences from those for prezizaene and 5-epi-prezizaene.[25−29] (See Supporting Information for more detailed discussion and comparisons.) Furthermore the relative stereochemistry was confirmed by NOE measurements.

The structure of the major product as 2-epi-prezizene (**4**) was independently verified by chemical conversion to the known sesquiterpene alcohol jinkohol (2-epi-prezizaan-7β-ol, **14**; see Scheme 2).[25,29] Thus, peracid (*m*-CPBA) epoxidation of *fraction 1* (2:1 mixture of **4** and **5**) led to an inseparable 5:1:1 mixture of two 2-epi-7,15-epoxyprezizane isomers (**11** and **12**) and a single cedrane epoxide (**13**). The latter was identified as 8α,9α-epoxy-cedrane based on its characteristic ^{1}H NMR resonances, by favorable comparisons with literature ^{1}H NMR values,[30] and by spectral comparisons with an authentic sample obtained by *m*-CPBA epoxidation of (−)-α-cedrene. $LiAlH_4$ reduction of the mixture of epoxides afforded pure alcohols **14**, **15**, and **16** after preparative TLC purification. Surprisingly epi-cedrol[30] was not detected in this case. The ^{1}H NMR (500 MHz) data of the major alcohol (**14**) were identical to the published data for jinkohol.[25,31]

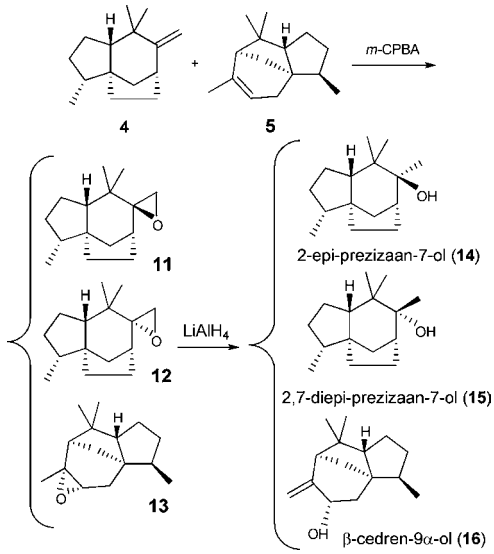
(18) Shao, Y.; Eummer, J. T.; Gibbs, R. A. *Org. Lett.* **1999**, *1*, 627–630.
(19) Collington, E. W.; Meyers, A. I. *J. Org. Chem.* **1971**, *36*, 3044–3045.
(20) Woodside, A. B.; Huang, Z.; Poulter, C. D. *Org. Synth* **1993**, *8*, 616–620.
(21) Zhao, Y., Ph.D Thesis, University of Illinois at Urbana-Champaign, 2005.
(22) O'Maille, P. E.; Chappell, J.; Noel, J. P. *Anal. Biochem.* **2004**, *335*, 210–217.
(23) The name isoprezizaene (2,5-diepi-prezizaene) was initially coined[8] to point out that the putative TEAS-generated prezizaene was not identical (GC−MS) to an authentic sample of (−)-prezizaene.[24]
(24) Vettel, P.; Coates, R. M. *J. Org. Chem.* **1980**, *45*, 5430–5432.
(25) Nakanishi, T.; Yamagata, E.; Yoneda, K.; Miura, I. *Phytochemistry* **1981**, *20*, 1597–1599.
(26) Sakurai, K.; Kitahara, T.; Mori, K. *Tetrahedron* **1990**, *46*, 761–774.
(27) Selvakumar, N.; Subba Rao, G. S. R. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3217–3223.
(28) Based on exhaustive ^{13}C NMR experiments, the structure of jinkohol, initially assigned as (+)-prezizaan-7β-ol,[25,31] has been recently revised as (+)-2-epi-prepizaan-7β-ol.[29] Thus, as pointed out by Weyerstahl and coworkers,[29] the synthetic hydrocarbon obtained by Nakanishi et al.,[25] as well as isoprezizaene reported by O'Maille et al.,[8] should be corrected to (+)-2-epi-prezizaene.
(29) Weyerstahl, P.; Marschall, H.; Splittgerber, U.; Wolf, D. *Flavour Fragr. J.* **2000**, *15*, 61–83.
(30) (a) Joseph-Nathan, P.; Santillan, R. L.; Gutierrez, A. *J. Nat. Prod.* **1984**, *47*, 924–933. (b) Joseph-Nathan, P.; Gutierrez, A.; Hernandez, J. D.; Roman, L. U.; Santillan, R. L. *J. Nat. Prod.* **1986**, *49*, 79–89.
(31) Nakanishi, T.; Yamagata, E.; Yoneda, K. *J. Chem. Soc., Perkin Trans. 1* **1983**, 601–604.

Scheme 1. Sesquiterpenes Produced by Incubations of TEAS with (2*Z*,6*E*)-FPP (**1**)[a]



[a] The % figures are estimates of the relative proportion of each product based on 500 MHz NMR integrations.

Scheme 2. Chemical Correlation of Cyclization Products **4** and **5** with the Known Compounds 8α,9α-Epoxycedrane (**13**), Jinkohol (2-Epi-prezizaan-7β-ol, **14**), and β-Cedren-9α-ol (**16**) by Epoxidation and $LiAlH_4$ Reduction



The identity of the second most abundant product as (−)-α-cedrene (**5**) was previously assigned by GC and MS comparisons of authentic (−)-α-cedrene with GC peak 2 of the product profile from (2*E*,6*E*)-FPP incubations.[8] This conclusion was further corroborated in the present work by ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectroscopy, favorable comparisons with literature data,[30] and chemical conversions to 8α,9α-epoxycedrane (**13**),[30] β-cedren-9α-ol (**16**),[32] and epi-cedrol[30] using the pure TEAS-generated product **5** and an authentic sample of (−)-α-cedrene. Furthermore the NMR spectra of the epoxides from both sources as well as their products from subsequent $LiAlH_4$ reactions were identical.

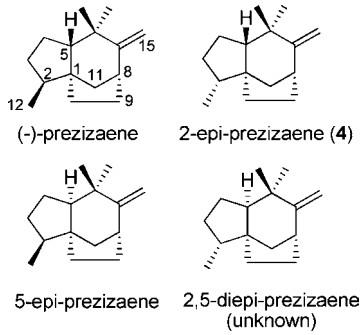


Figure 3. Structures of (−)-prezizaene and its stereoisomers.

Purification of *fraction 3* by preparative TLC on $AgNO_3$-impregnated silica gel led to the isolation of the third most abundant hydrocarbon from (2*Z*,6*E*)-FPP in pure form as a colorless oil: $[\alpha]_D^{25}$ −20° ($CDCl_3$, *c* 0.1), [Lit.[33] for (−)-β-curcumene, ORD $[\alpha]_D$ −6.6°, solvent unspecified]. The 500 MHz ^{1}H NMR spectrum of this component revealed the presence of three downfield sp^2-hybridized protons, four doubly allylic hydrogens, three allylic hydrogens, and four resonances for methyl groups in excellent agreement with those reported for synthetic (±)-β-curcumene.[34] Thus the structure of this component is assigned as (−)-β-curcumene (**6**). (See Supporting Information for NMR data and more detailed discussion.)

^{1}H NMR (500 MHz, $CDCl_3$) analysis revealed that *fraction 2* contained, in addition to **4** and **6** (∼40%), bicyclic hydrocarbons **7** and **8** (∼60%) in an approximate 3:1 ratio. Key individual signals for the latter components were identified by subtraction of peaks for the former, by their 3:1 integration ratios, and by comparisons with the ^{1}H NMR data reported for α-acoradiene (**7**)[35] and 4-epi-α-acoradiene (**8**).[36] The diagnostic peaks in this case were those arising from the ring vinyl protons, the isopropenyl $=CH_2$, the two allylic CH_3 groups, and the secondary CH_3 group.

The absolute configurations of the three major products were assigned by optical rotation measurements, and their enantio-

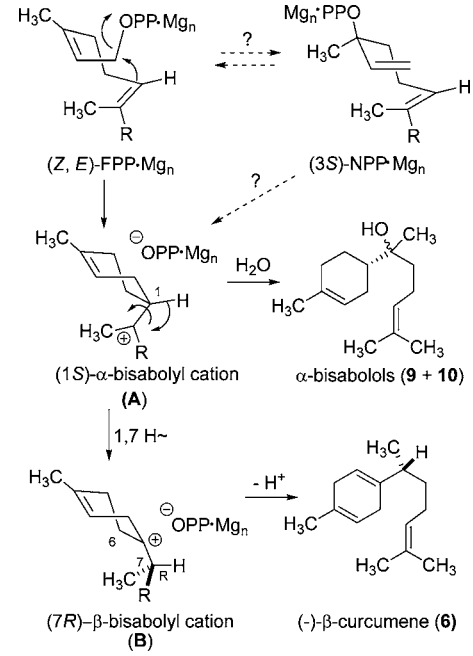
(32) Tomi, F.; Bighelli, A.; Casanova, J. *Spectrosc. Lett.* **1993**, *26*, 1661–1871.
(33) Andersen, N. H.; Syrdal, D. D. *Phytochemistry* **1970**, *9*, 1325–1340.
(34) Ryan-Zilenovski, J. S.; Hall, S. S. *Synthesis* **1979**, 698–699.
(35) Tashiro, T.; Kurosawa, S.; Mori, K. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 663–670.
(36) Kurosawa, S.; Bando, M.; Mori, K. *Eur. J. Org. Chem.* **2001**, *439*, 5–4399.

meric purities were evaluated by chiral GC analyses. Although the signs of the optical rotations of (+)-2-epi-prezizaene (**4**), (−)-α-cedrene (**5**), and (−)-*β*-curcumene (**6**) are consistent with the literature and their common absolute configurations (Scheme 1), the magnitudes deviate considerably ($[\alpha]_D$ +26.8° vs +10°; −23° vs −92°; −20° vs −6.6° or +27 for the enantiomer. See Experimental Section for references). While these variations may well be attributable to different chemical purities and other factors, a direct comparison of optical rotations for (−)-α-cedrene from the TEAS incubation and an authentic sample of the sesquiterpene determined with the same polarimeter, cell, and conditions ($[\alpha]_D$ −23° vs −42°) indicates an enantiomer ratio of at least 2*R*/2*S* = 77/23.

Further indication of the high enantiopurity of products **4**, **5**, and **6** was their elution as single, symmetrical peaks in high-resolution GC analyses on a *β*-cyclodextrin capillary column (dimethyl form) noted for its capacity to separate volatile terpene hydrocarbons and alcohols.[37−39] Therefore, it seems likely that (+)-2-epi-prezizaene (**4**) and (−)-*β*-curcumene (**6**) were formed with similarly high levels of enantioselectivity, the optical rotations notwithstanding. We assume that the mechanistically related α-acoradiene and 4-epi-α-acoradiene (**7** and **8**) share the same R configuration at the tertiary methyl groups and that the α-bisabolol epimers **9** and **10** possess the 6*S* configuration shown in Scheme 1.

Mechanistic pathways consistent with the products formed by the catalytic action of TEAS on (2*Z*,6*E*)-FPP are outlined in Schemes 3 and 4. We propose that (2*Z*,6*E*)-FPP (**1**) binds and reacts in the TEAS active site in the anti, endo conformation commonly proposed for monoterpene and sesquiterpene synthases that effect 1,6-cyclizations to terpinyl and bisabolyl cations.[9] Chemically induced cyclizations of related linalyl and neryl derivatives also take place predominantly from the same anti, endo conformation.[40] Furthermore, it seems reasonable to assume that the polyene chain of (2*Z*,6*E*)-FPP adopts a helical chirality similar to that of its E,E isomer with the 6,7 double bond coiled beneath the plane of the allylic diphosphate moiety (Scheme 3). In this orientation, the initially formed cisoid (2*Z*,6*E*)-farnesyl$^+$/OPP$^-$ ion pair would undergo cyclization on the face opposite to the departing pyrophosphate anion and generate the α-bisabolyl cation **A** with the proposed 1*S* configuration. In fact the X-ray crystal structure of wt TEAS with the inert fluoro analogue (2-*cis*,6-*trans*)-2-fluoroFPP bound in the active site shows the same 6re face proximal to C1.[17] The same stereochemistry would arise if the (2*Z*,6*E*)-farnesyl$^+$/OPP$^-$ ion pair collapsed to enzyme-bound (*S*)-nerolidyl diphosphate ((3*S*)-NPP) in the anti, endo conformation. (*R*)- and (*S*)-NPP are often proposed as key intermediates in sesquiterpene synthase reactions proceeding through the bisabolyl ions.[12−14,16] Reaction of the cisoid (2*Z*,6*E*)-farnesyl$^+$/OPP$^-$ and the (1 *S*)-α-bisabolyl$^+$/OPP$^-$ ion pairs with a water molecule would generate (2*Z*,6*E*)-farnesol, nerolidol, and the (1*S*)-bisabolol epimers **9** and **10** identified as alcohol products.

Scheme 3. Proposed Mechanism for the TEAS-Catalyzed Cyclization of (2*Z*,6*E*)-FPP·Mg_n Complex in the Anti, Endo Conformation to (−)-*β*-Curcumene (**6**), the α-Bisabolol Epimers (**9** and **10**), and the (7*R*)-*β*-Bisabolyl/PP Ion Pair Intermediate (**B**)



A second critical step is the 1→7 hydride shift of H1 onto the 7re face of the α-bisabolyl ion (**A**), thus generating the *β*-bisabolyl intermediate (**B**) with the 7*R* stereochemistry. A regioselective proton elimination from C6 would lead to the monocyclic product (*R*)-(−)-*β*-curcumene (**6**). We propose that association of the carbocation intermediates **A** and **B** with the pyrophosphate anion on the re faces may be a stereochemical determinant. Alternatively, the 1→7 hydride shift might occur indirectly by proton transfers to and from the pyrophosphate anion via a neutral *γ*-bisabolene intermediate.[41] Further cyclizations onto the 10,11 double bond positioned in exo and endo orientations as shown in Scheme 4 would occur on the unshielded si face of the (7*R*)-*β*-bisabolyl ion **B** to generate the isomeric acorenyl ions **C** (1*R*, 4*R*, 5*S*) and **D** (1*R*, 4*S*, 5*S*), precursors to the minor products, 4-epi-α-acoradiene and α-acoradiene (**8** and **7**), by proton eliminations from the methyl groups.

Tertiary carbocations **C** and **D** undergo a third TEAS-mediated electrophilic cyclization involving the same C7−C8 double bond (acorane skeleton numbering) but leading to two different tricyclic skeletons. According to MM2 molecular models, C6 and C7 are almost equidistant (within <0.5 Å) with respect to the trigonal C12 of both acorenyl carbocations **C** and **D**, and therefore, both positions of the C7−C8 double bond

(37) (a) Schürig, V. *Trends Anal. Chem.* **2002**, *21*, 647–661. (b) König, W. A.; Rieck, A.; Hardt, I.; Gehrcke, B.; Kubeczka, K.-H.; Muhle, H. *J. High Resolut. Chromatogr.* **1994**, *17*, 315–320, and references cited herein.

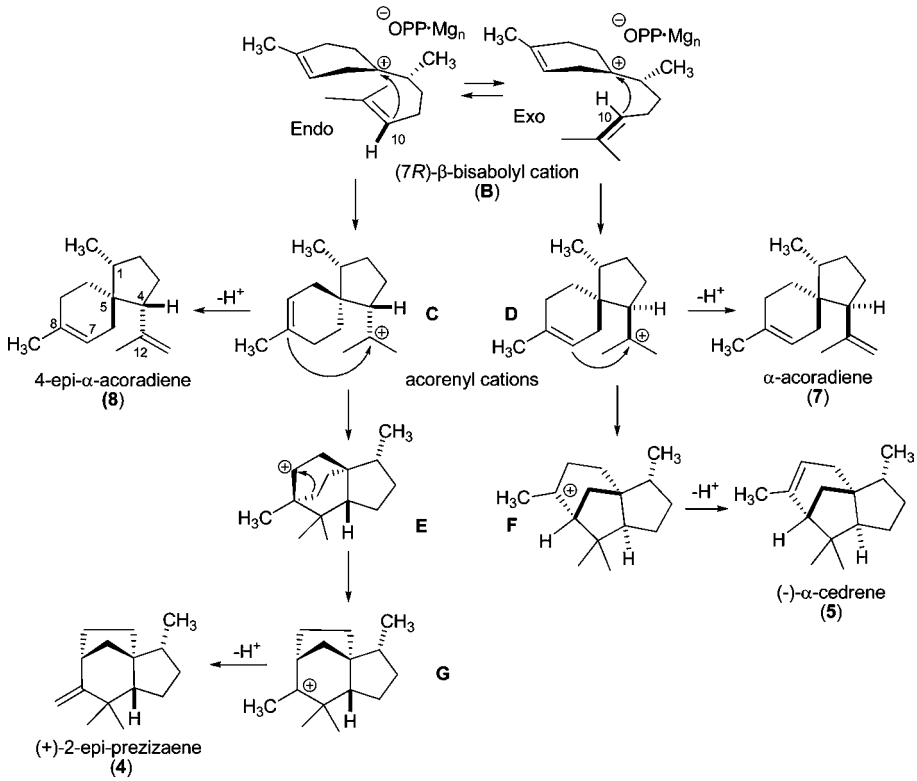
(38) The suitability of the column and conditions for our purposes was demonstrated by separation of (*R*)-, (*S*)-, and (*R*/*S*)-limonenes. Moreover, the effective separation of (+)- and (-)-α-cedrene was shown by GC analysis of a mixture of four sesquiterpenes containing (±)-α-cedrene and (±)-2-epi-α-cedrene as the major components. The mixture was obtained by acid-catalyzed cyclization of (±)-nerolidol.[39] 500 MHz NMR analysis of the mixture indicated a 50:50:7:18 ratio of (±)-2-epi-α-cedrene, (±)-2-α-cedrene, and probably the related (±)-α-funebrene and (±)-2-epi-α-funebrene. The separation of this mixture into four pairs of GC peaks was a further demonstration of effectiveness of the cyclodextrin column, and co-injection with (−)-α-cedrene from the TEAS incubation verified the high enantiomeric purity of this enzymatic product.

(39) Andersen, N. H.; Syrdal, D. D. *Tetrahedron Lett.* **1972**, *13*, 2455–2458.

(40) (a) Poulter, C. D.; King, C.-H. R. *J. Am. Chem. Soc.* **1982**, *104*, 1420–1422. (b) Poulter, C. D.; King, C.-H. R. *J. Am. Chem. Soc.* **1982**, *104*, 1422–1424. (c) Godtfredsen, S.; Obrecht, J. P.; Arigoni, D. *Chimia* **1977**, *31*, 62–63. (d) Winstein, S.; Valkanas, G.; Wilcox, C. F. *J. Am. Chem. Soc.* **1972**, *94*, 2286–2290.

(41) Hong, Y. J.; Tantillo, D. J. *Org. Lett.* **2006**, *8*, 4601–4604.

Scheme 4. Proposed Mechanism for the TEAS-Catalyzed Cyclization of the Common (7*R*)-β-Bisabolyl/PP Ion Pair Intermediate (**B**) to Bi- and Tricyclic Products **7**, **8**, **4**, and **5**



should be equally accessible to the internal C12 electrophile. However, while carbocation **D** proceeds to the relatively stable tertiary tricyclic cation **F** by ring closure onto the C7 position of the C7−C8 double bond, carbocation **C** undergoes an anti-Markovnikov mode of cyclization involving the less nucleophilic terminus (C8) of the same double bond. This C8−C12 ring closure generates a high energy secondary carbocation **E**, presumably to avoid forming a highly strained *trans*-bicyclo[3.3.0]octane moiety (18.4 kcal/mol)[42] that would arise from a Markovnikov orientation. High energy intermediate **E** is, in turn, stabilized through a Wagner−Meerwein rearrangement to tertiary carbocation **G**. Alternatively, theoretical calculations have located a transition structure between carbocations **C** and **G** in the gas phase that would allow a direct cyclization **C** → **G**, thus bypassing the localized secondary ion **E**.[15,17] The final TEAS-mediated proton eliminations from cations **G** and **F** provide the major hydrocarbons **4** and **5**.

Conclusions

The efficiency and stereospecificity of the TEAS-catalyzed reactions of (2*Z*,6*E*)-FPP are remarkable considering that the "natural" substrate for this cyclase is the (2*E*,6*E*)-isomer. While enzyme-catalyzed cyclizations of the former, as well as the related monoterpene neryl diphosphate, have been frequently reported,[9b,e] the 1,6-cyclizations catalyzed by these types of terpene synthases with their 2,3-*E* substrates are proposed to proceed by initial allylic rearrangements to enzyme-bound nerolidyl and linalyl diphosphate intermediates that can then undergo energetically accessible 2,3-σ bond rotations to assume the required cisoid conformations capable of 1,6 electrophilic cyclizations.

Thus, in these cases (2*Z*,6*E*)-FPP and neryl diphosphate serve as unnatural substrates but ones which closely resemble the conformations of the natural NPP and LPP intermediates in shape and reactivity. In contrast, the major catalytic pathway that TEAS utilizes to transform its (2*E*,6*E*)-FPP substrate begins with a 1,10 electrophilic cyclization onto the terminal double bond to form the (*E*,*E*)-germacrene A intermediate (ca. 97%, Scheme 1) upon proton loss. However, the recent careful analytical identification of acoradiene, 2-epiprezizaene, and α-cedrene as minor products formed catalytically by TEAS using (2*E*,6*E*)-FPP indicates a small amount of leakage (ca. 3%) into the cisoid mechanistic pathway.[8,10] The absence of (2*E*,6*E*)-farnesol in the alcohol products obtained using (2*Z*,6*E*)-FPP as substrate suggests that little isomerization (≤ca. 0.5%) back into the more widely observed 2,3-transoid pathway occurs with this unnatural substrate.

The interesting correlation of progress along the proposed reaction pathways with the relative proportions of alcoholic to olefinic products formed may be dynamically linked to the closure of the hydrophobic active site entrance. This conformational change from open (inactive) to closed (active), triggered by the formation of the ternary $[Mg^{2+}]_3$-PPi-enzyme complex, is accompanied by insertion of catalytically essential residues and sequestration of bound intermediates away from an exchangeable solvent. Whereas (2*Z*,6*E*)-farnesol and nerolidol arise from water capture of the initial cisoid allylic$^+$/OPP$^-$ ion pair, no detectable amounts of proton elimination occur at this early stage to form any of the three possible farnesene isomers. Similarly the α-bisabolyl$^+$/OPP$^-$ (**A**) ion pair undergoes hydration in competition with the 1,7-hydride shift, but no elimination takes place to form any of the five possible bisabolene isomers. Once the β-bisabolyl$^+$/OPP$^-$ (**B**) ion pair stage is reached, all products are formed by proton eliminations.

(42) Chang, S.-J.; McNally, D.; Shary-Tehrany, S.; Hickey, S. M. J.; Boyd, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 3109–3118.

The lack of hydration at this stage indicates the sequestration of carbocations **B**−**G** away from the surrounding aqueous environment, and the anchoring of the pyrophosphate anion with its associated water molecules at the active site entrance distant from the reactive carbocation intermediates.

It is evident that product selectivity is under kinetic control, despite potential thermodynamic factors that might have been expected to bias product formation.[43] Thus, proton elimination from the β-bisabolyl$^+$/OPP$^-$ ion pair **B** occurs at C6 producing the nonconjugated 1,4-diene (−)-β-curcumene (**6**) without any detectable elimination from C2 which would lead to the more stable conjugated 1,3-diene isomer (γ-curcumene). Similarly, the reaction channel from the 4-epi-acorenyl carbocation **C** to the tricyclic 2-epi-prezizaene (**4**, 44%) predominates over the acorenyl cation **D** cyclization to α-cedrene (**5**, 22.5%), despite the substantially greater stability of the tertiary cedryl cation **F** over the secondary allocedryl carbocation **E**. Since chemically induced cyclizations of α-acoradiene and related spiro[4.5]spirodecane sesquiterpenes occur readily under acidic conditions to generate α-cedrene, and allocedryl formate and sulfonate derivatives of allocedrol rearrange to zizaane sesquiterpenes,[44] it is clear that the primary catalytic functions of TEAS in the bridging cyclizations of acorenyl carbocations **C** and **D** are to lengthen the residence time of intermediates in the encapsulated active site and to minimize the extent of premature proton elimination.

The high stereoselectivity with which the initial 1,6-cyclization occurs to produce the (1*S*)-α-bisabolyl$^+$/OPP$^-$ ion pair **A** follows from the helicity of the anti, endo conformation of (2*Z*,6*E*)-FPP (Scheme 3). However, the reason for the predominance of the 1,7 hydride shift to the re face of C7 (**A**→**B**) is not so obvious. A 120° clockwise (CW) rotation about the C1−C7 bond shown in Scheme 3 is required to align the carbocation orbital for a suprafacial hydride shift, whereas a least motion 60° counter-clockwise (CCW) rotation would enable suprafacial hydride migration to the 7-si face, generating the 7*S* configuration. We speculate that the 120° CW rotation leading to a rotamer of **A** (not shown) can adopt a more compact conformation which more closely conforms to the shape and size of the UD conformer of the (*E*,*E*,*R*)-germacrenyl$^+$ ion produced in the TEAS-catalyzed cyclization of the natural (2*E*,6*E*)-FPP substrate. That is, an examination of molecular models indicates that the axial C7−C12 side chain may be folded underneath the cyclohexenyl ring to a position that brings C10 close to an imaginary carbon atom (C4′) attached to C3 (bisabolyl position numbers) of the 120° CW rotamer of **A**, whereas the minimal approach distance of C10 to C4′ in the 60° CCW rotamer would be much greater. Hence, evidently the contours of the TEAS active site enforce a bias in favor of the 120° CW rotameric form leading to a hydride shift onto the 7-re face, and the resulting stereochemistry at the methine position in **B** is carried through the remainder of the deprotonation and cyclization steps.

The numerous products catalytically produced by TEAS acting upon the "natural" (2*E*,6*E*)-FPP substrate classify this sesquiterpene synthase as yet another example of a multiproduct sesquiterpene cyclase.[45] One contributing factor to this diversity of reaction channels is the apparently small energy difference separating conformations of the central and terminal isoprene units of the substrate, as deduced recently from X-ray crystal structures of TEAS complexes with 2-fluoro FPP analogues.[17] However, despite the plurality of both potential and observed products, TEAS exhibits remarkable control of both cisoid and transoid cyclization pathways to produce predominantly a few stereochemically complex bicyclic and tricyclic products.

The existence of the "cisoid" catalytic activity of TEAS may well be a vestige of an ancestral synthase that produced primarily bisabolyl-derived sesquiterpenes, or a latent function that might be enhanced by future adaptation under specific evolutionary selection. In fact, a (2*Z*,6*Z*)-FPP synthase from the wild tomato species *Solanum habrochaites* has recently been characterized, and a co-occurring protein that catalyzes the cisoid cyclization of the Z,Z substrate to a series of bicyclic sesquiterpenes was identified.[46] Although to our knowledge no prenyl transferases specific for (2*Z*,6*E*)-FPP have been discovered from plant sources, an enzyme producing the Z,E isomer for polyprenol biosynthesis in *Mycobacterium tuberculoisis* is known.[47]

TEAS may be able to exploit the low natural levels (3−14%) of (2*Z*,6*E*)-FPP generated by FPP synthases to produce a different array of sesquiterpenes in vivo.[48] For example, if the concentration of (2*E*,2*E*)-FPP was depleted by its diversion into biosynthesis of phytosterols, farnesylated proteins, or diterpenes, TEAS would be capable of utilizing (2*Z*,6*E*)-FPP in its place. Thus, the interesting question is raised whether the cisoid cyclization products in Scheme 1 might serve as "backup phytoalexins," or their biosynthetic precursors, in pathogen-stressed tobacco plants.

Experimental Section

Incubation of (2*Z*,6*E*)-Farnesyl Diphosphate with Recombinant TEAS. The procedure recently reported for a large scale preparative incubation of 6-fluoro-FPP with recombinant TEAS was followed.[6] Thus, (2*Z*,6*E*)-FPP (127 mg, 0.31 mmol) was incubated with purified recombinant TEAS (18 mg, 0.026 mg/mL) in 700 mL of buffer solution (200 mM Tris-HCl (pH 7.5), 40 mM $MgCl_2$ in a 1:1 ratio). The final reaction solution was overlaid with pentane (15 mL), sealed with parafilm, and stirred for 19 h at room temp in a constant temp bath. Extraction of the mixture with HPLC-grade pentane (3 × 100 mL) afforded, after evaporation of the solvent and filtration through a short silica gel pipet column with pentane as eluent, 27.3 mg (46% yield) of hydrocarbons. Further elution with Et_2O gave a polar fraction (4.4 mg, 7% yield) composed of sesquiterpene alcohols. Purification of the latter by preparative TLC on silica gel afforded, in addition to (2*Z*,6*E*)-farnesol (1.8 mg, 48% of alcohol fraction), an inseparable mixture (2.0 mg, 52% of alcohol fraction) of the tertiary alcohols nerolidol, α-bisabolol (**9**), and epi-α-bisabolol (**10**) in a 2:1:1 ratio, respectively, as evidenced by ^{1}H NMR spectroscopy.

α-Bisabolol (9). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (broad s, 1H, H-2), 5.09 (m, 1H, H-12), 1.68 (broad s, 3H, Me-13

(43) Vedula, S. L.; Rynkiewicz, M. J.; Pyun, H.-J.; Coates, R. M.; Cane, D. E.; Christianson, D. W. *Biochemistry* **2005**, *44*, 6153–6163.

(44) For an overview of these biomimetic transformations, see: Coates, R. M. *Biogenetic-type Rearrangements of Terpenes*; Herz, W., Grisebach, H., Kirby, J. W., Eds.; Progress in the Chemistry of Organic Natural Products; 1976: Vol. 33, pp 73−230, esp pp 151−155.

(45) (a) Steele, C. L.; Lewinsohn, E.; Croteau, R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4164–4168. (b) Steele, C. L.; Bohlmann, J.; Crock, J.; Croteau, R. *J. Biol. Chem.* **1998**, *273*, 2078–2089.

(46) Sallaud, C.; Rontein, D.; Onillon, S.; Jabés, F.; Duffé, P.; Giacalone, C.; Thoraval, S.; Escoffier, C.; Herbette, G.; Leonhardt, N.; Causse, M.; Tissier, A. *Plant Cell* **2009**, *21*, 301–317.

(47) (a) Schulbach, M. C.; Mahapatra, S.; Macchia, M.; Barontini, S.; Papi, C.; Minutolo, F.; Bertini, S.; Brennan, P. J.; Crick, D. C. *J. Biol. Chem.* **2001**, *276*, 11624–11630. (b) Schulbach, M. C.; Brennan, P. J.; Crick, D. C. *J. Biol. Chem.* **2000**, *275*, 22876–22881.

(48) Thulasiram, H. V.; Poulter, C. D. *J. Am. Chem. Soc.* **2006**, *128*, 15819–15823.

or Me-1), 1.65 (broad s, 3H, Me-13 or Me-1), 1.62 (broad s, 3H, Me-13 or Me-1), 1.11 (s, 3H, Me-10). The ^{1}H NMR spectrum and data are identical with those previously reported for the same compound at 250 MHz in $CDCl_3$.[49]

Epi-α-bisabolol (10). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.40 (broad s, 1H, H-2), 5.10 (m, 1H, H-12), 1.68 (broad s, 3H, Me-13 or Me-1), 1.65 (broad s, 3H, Me-13 or Me-1), 1.62 (broad s, 3H, Me-13 or Me-1), 1.14 (s, 3H, Me-10). The ^{1}H NMR spectrum and data are identical with those previously reported for the same compound at 250 MHz in $CDCl_3$.[49]

Purification of the hydrocarbon fraction by preparative TLC on silica gel using pentane as the developing solvent gave three fractions: *fraction 1* (17.6 mg, as a 2:1 mixture of sesquiterpenes **4** and **5**), *fraction 2* (2.2 mg, as a mixture of at least four minor hydrocarbons), and *fraction 3* (4.0 mg, ca. 95% *β*-curcumene (**6**)). Further purification of *fraction 1* (11.8 mg) and *fraction 3* by preparative $AgNO_3$-TLC[50] on silica (pentane) yielded pure (+)-2-epi-prezizaene (**4**, 7.4 mg), (−)-α-cedrene (**5**, 3.5 mg), and (−)-*β*-curcumene (**6**, 2.8 mg).

(+)-2-Epi-prezizaene (4). Colorless oil; $[\alpha]_D^{25}$ +26.8° ($CDCl_3$, *c* 0.1), Lit.[25] $[\alpha]_D^{25}$ +10° ($CHCl_3$ *c* 0.6); chiral GC (T_0 = 70 °C hold 3 min, ramp 5 °C/min to T = 160 °C min, injection port T = 180 °C): single peak at t_R 19.23 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.72 and 4.67 (ABq, J_{AB} = 1.9 Hz, $\Delta\upsilon$ = 23 Hz, 2H, H-15 and H-15′), 2.77 (dd, J = 6.5, 4.5 Hz, 1H, H-8), 1.89−1.80 (m, 1H), 1.79−1.72 (m, 2H), 1.65 (dd, J = 10.5, 4.5 Hz, 1H, H-11α), 1.63−1.49 (m, 4H), 1.38−1.32 (m, 2H), 1.21−1.13 (m, 1H), 1.08 (s, 3H, Me-6α or Me-6*β*), 1.06 (s, 3H, Me-6α or Me-6*β*), 1.05 (broad d, J = 10.5 Hz, 1H, H-11*β*), 0.86 (d, J = 7.0 Hz, 1H, Me-2α); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.4, 105.4, 59.1, 53.0, 47.3, 42.8, 39.1, 37.8, 32.0, 31.0, 29.9, 26.9, 21.4, 20.7, 14.3. The ^{1}H and ^{13}C NMR data are in excellent agreement with the values previously reported for synthetic (+)-2-epi-prezizaene.[25,29] LRMS (EI) *m*/*z* (rel. %) 204 (M^+, 21), 189 (42), 175 (12), 161 (30), 147 (20), 133 (100), 119 (42), 108 (49), 91 (70), 79 (21).

(−)-α-Cedrene (5). Colorless oil; $[\alpha]_D^{25}$ −23° ($CDCl_3$, *c* 0.1), Lit.[51] $[\alpha]_D^{25}$ −92° ($CHCl_3$ *c* 1); chiral GC (T_0 = 70 °C hold 3 min, ramp 5 °C/min to T = 160 °C min, injection port T = 180 °C): single peak at t_R 21.03 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.22 (broad s, 1H, H-9), 2.17 (d of sextets, J = 19.0, 2.5 Hz, 1H, H-10), 1.89−1.78 (m, 2H), 1.77−1.71 (m, 2H), 1.70−1.63 (m, 2H), 1.67 (d, J = 1.8 Hz, 3H, Me-8), 1.61−1.52 (m, 2H), 1.42−1.31 (m, 3H), 1.02 (s, 3H, Me-6α or Me-6*β*), 0.95 (s, 3H, Me-6α or Me-6*β*), 0.84 (d, J = 7.5 Hz, Me-2). The ^{1}H NMR spectrum was identical to that of an authentic sample of α-cedrene, $[\alpha]_D^{25}$ −42° ($CDCl_3$, *c* 0.1). For ^{1}H and ^{13}C NMR assignments and values, see ref 30a. LRMS (EI) *m*/*z* (rel. %) 204 (M^+, 55), 189 (5), 161 (23), 147 (10), 119 (100), 105 (26), 93 (30).

(−)-*β*-Curcumene (6). Colorless oil: $[\alpha]_D^{25}$ −20° ($CDCl_3$, *c* 0.1), Lit.[33] ORD $[\alpha]_D$ −6.6°, Lit.[52] $[\alpha]_D^{25}$ +27° for the enantiomer; chiral GC (T_0 = 100 °C hold 3 min, ramp 8 °C/ min to T = 200 °C min, injection port T = 180 °C): single peak at t_R 19.03 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.43 (broad s, 2H), 5.09 (m, 1H), 2.58 (broad s, 4H), 2.09 (sextet, J = 7.0 Hz, 1H), 1.89 (q, J = 7.5 Hz, 2H), 1.67 (s, 3H), 1.66 (d, J = 1.5 Hz, 3H), 1.58 (s, 3H), 1.44−1.38 (m, 1H), 1.31−1.23 (m, 2H), 0.99 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 131.3, 131.2, 124.8, 118.8, 117.8, 40.2, 35.0, 31.6, 26.6, 26.1, 25.7, 23.1, 19.6, 17.7. The ^{1}H NMR data are in excellent agreement with those published for synthetic (±)-*β*-curcumene.[34] LRMS (EI) *m*/*z* (rel. %) 204 (M^+, 23), 191 (5), 161 (17), 147 (10), 132 (13), 119 (100), 105 (37), 93 (53), 69 (24).

(−)-Prezizaene (Reference Sample).[24] ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.70 and 4.66 (ABq, J = 2 Hz, $\Delta\upsilon$ = 23 Hz, H-15 and H-15), 2.80 (app t, J_{app} = 5.5 Hz, 1H, H-8), 2.20−1.88 (m, 2H), 1.86−1.74 (m, 2H), 1.62−1.46 (m, 6H) 1.24 (d, J = 10.5 Hz, 1H), 1.12 (m, 1H), 1.11 (s, 3H, Me-6α or Me-6*β*), 1.07 (s, 3H, Me-6α or Me-6*β*), 0.87 (d, J = 7.2 Hz, 1H, Me-2α); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 105.5, 54.3, 53.5, 48.0, 41.1, 40.4, 37.6, 32.6, 32.2, 31.4, 29.9, 27.1, 22.8, 20.0. The NMR data correlate well with the literature data.[29]

Although *fraction* 2 was not further purified, ^{1}H NMR (500 MHz) analysis of the four-component mixture revealed that this fraction was composed of (+)-2-epi-prezizaene (**4**, 29%), (−)-*β*-curcumene (**6**, 14%), α-acoradiene (**7**, 44%), and 4-epi-α-acoradiene (**8**, 14%).

α-Acoradiene (7). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.332 (broad s, 1H, H-7), 4.823 (s, 1H, H-13), 4.624 (s, 1H, H-13), 1.725 (s, 3H, Me-8), 1.624 (s, 3H, Me-14), 0.863 (d, J = 6.8 Hz, 3H, Me-11. The partial ^{1}H NMR resonances recorded in the present work are in full agreement with the diagnostic signals recently reported for α-acoradiene.[35] LRMS (EI) *m*/*z* (rel. %) 204 (M^+, 21), 189 (9), 175 (5), 161 (21), 147 (21), 133 (12), 121 (56), 119 (100), 105 (35), 93 (44), 79 (24).

4-Epi-α-acoradiene (8). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.332 (broad s, 1H, H-7), 4.828 (s, 1H, H-13), 4.717 (s, 1H, H-13), 1.763 (s, 3H, Me-15), 1.614 (s, 3H, Me-12), 0.935 (d, J = 6.9 Hz, 3H, Me-11). The partial ^{1}H NMR resonances recorded in the present work are in full agreement with the diagnostic signals reported for 4-epi-α-acoradiene.[36] LRMS (EI) *m*/*z* (rel. %) 204 (M^+, 23), 189 (23), 175 (7), 161 (23), 147 (25), 133 (8), 121 (58), 119 (100), 105 (44), 93 (49), 79 (28).

Chemical Correlations with Known Sesquiterpenes. Epoxidation of Fraction 1 (4 + 5). A solution of *fraction 1* (17.6 mg, 0.09 mmol, as a 2:1 mixture of **4** and **5**) in CH_2Cl_2 (2 mL) was allowed to react with *m*-CPBA (6.4 mg, 0.04 mmol) at room temp for 2 min. Saturated aq $NaHCO_3$ (1 mL) was added, and then the mixture was extracted with Et_2O (1 mL × 4). The ethereal extracts were dried over $MgSO_4$ and filtered through silica gel. Evaporation of the solvent with a stream of N_2 and purification by preparative TLC on silica gel using pentane as the developing solvent gave 11.8 mg of unreacted *fraction 1* (2:1 mixture of **4** and **5**) and a more polar fraction (3.7 mg) consisting of an inseparable 1:2 mixture of a single cedrane epoxide (**13**) and two 2-epi-7,15-epoxyprezizane isomers (**11** + **12**) in a 5:1 ratio. The former was identified as (8α,9α)-epoxycedrane (**13**) by its characteristic ^{1}H NMR resonances, by favorable comparisons with literature ^{1}H NMR values,[30] and by two identical *m*-CPBA epoxidation reactions using (a) a commercial sample of (−)-α-cedrene and (b) pure TEAS-generated **5** (see above) as starting materials. In addition, the ^{1}H NMR spectra of **13** obtained from experiments (a) and (b) were superimposable.

2-Epi-7*β*,15*β*-epoxyprezizaane (11, Major Isomer). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.70 (d, J = 4.5 Hz, 1H, H-15), 2.52 (d, J = 4.5 Hz, 1H, H-15), 1.03 (s, 3H, Me-6), 0.87 (d J = 6.0 Hz, 3H, Me-2), 0.70 (s, 3H, Me-6).

2-Epi-7α,15α-epoxyprezizaane (12, Minor Isomer). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.91 (d, J = 5 Hz, 1H, H-15), 2.45 (d, J = 5 Hz, 1H, H-15), 1.04 (s, 3H, Me-6), 0.86 (d J = 7.0 Hz, 3H, Me-2), 0.71 (s, 3H, Me-6). The ^{1}H NMR values obtained for epoxide **12** are similar but not identical to those published for 7α,15α-epoxyprezizaane.[29]

(8α,9α)-Epoxycedrane[30] (13). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.00 (d, J = 4.5 Hz, 1H), 1.93 (d, J = 14.7 Hz, 1H), 1.86 (d, J = 4.5 Hz, 1H), 1.81 (sextet, (d, J = 6.4 Hz, 1H), 1.77 (d, J = 12.0 Hz, 1H), 1.69−1.55 (m, 4H), 1.41 (sextet, J = 6.2 Hz, 1H), 1.42 (s, 3H), 1.29 (sextet, J = 6.2 Hz, 1H), 1.25 (ddd, J = 11.8, 4.5, 2.4 Hz, 1H), 1.42 (s, 3H), 1.18 (s, 3H), 1.00 (s, 3H), 0.80 (d, J = 7.1 Hz, 3H).

$LiAlH_4$ Reduction of the Mixture of Epoxides (11 + 12 + 13). To a solution of the epoxide mixture described above (**11** + **12** + **13**, 3.7 mg, 0.017 mmol) in dry THF (3 mL) was added

(49) Babin, D.; Fourneron, J.-D.; Julia, M. *Tetrahedron* **1981**, *37*, 1–7.
(50) Williams, C. M.; Mander, L. N. *Tetrahedron* **2001**, *57*, 425–447.
(51) Teisseire, P.; Plattier, M.; Wojnarowski, W.; Ourisson, G. *Bull. Soc. Chim. Fr.* **1966**, *9*, 2749–2756.
(52) Naves, Y. R. *Bull. Soc. Chim. Fr.* **1951**, 987–991.
(53) The synthesis of allylic alcohol **16** by $LiAlH_4$ reduction of epoxide **13** has been reported: Goryaev, M. I.; Tolstikov, G. A. *Zh. Obshch. Khim.* **1962**, *32*, 997–999.

$LiAlH_4$ (25.0 mg, 0.66 mmol) at room temp. The solution was heated at reflux for 2 h and then quenched by careful addition of 10% aq HCl. The mixture was extracted with Et_2O, and the extracts were dried over $MgSO_4$. Evaporation of the solvent with a stream of N_2 followed by preparative TLC on silica gel (10% EtOAc−hexane) afforded, in order of elution, pure alcohols **15**, **14**, and **16**.

2-Epi-prezizaan-7*β*-ol (14, Jinkohol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (dd, J = 5.5, 4.9 Hz, 1H, H-8), 1.86−1.76 (m, 2H), 1.69 (m, 1H, H-5), 1.67−1.60 (m, 2H), 157−1.47 (m, 6H), 1.38 (dd, J = 10.9, 4.6 Hz, 1H, H-11α), 1.29−1.22 (m, 2H), 1.18 (s, 3H, Me-7), 1.12 (m, 1H, H-3*β*), 0.91 (s, 3H, Me-6*β*), 0.90 (s, 3H, Me-6α), 0.84 (d J = 6.5 Hz, 3H, Me-2). The ^{1}H NMR data are identical to the published values for natural jinkohol,[25,31] the structure of which has been reassigned as 2-epi-prezizaan-7*β*-ol.[29]

2,7-Diepi-prezizaan-7α-ol (15). Partial ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.34 (d, J = 7, 2.4 Hz, 1H, H-8), 1.70 (dd, J = 10.5, 5.1 Hz, 1H, H-5), 1.25 (s, 3H, Me-7), 0.98 (s, 3H, Me-6), 0.84 (d, J = 6.6 Hz, 3H, Me-2), 0.75 (s, 3H, Me-6). The diagnostic ^{1}H NMR resonances recorded for this minor alcohol (**15**) are similar but not identical to the literature values for 7-epi-prezizaan-7α-ol and 5,7-diepi-prezizaan-7α-ol.[29]

***β*-Cedren-9α-ol (16).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.99 (t, J = 2.0 Hz, 1H, H-15), 4.75 (t, J = 2.0 Hz, 1H, H-15), 4.34 (ddt, J = 10.5, 9.2, 2.3 Hz, 1H, H-9), 2.35 (d, J = 4.1 Hz, 1H), 2.13 (ddd, J = 11.1, 7.5, 3 Hz, 1H), 1.87 (sextet, J = 6 Hz, 1H), 1.82−1.73 (m, 3H), 1.60−1.50 (m, 1H), 1.43 (sextet, J = 7.5 Hz, 1H), 1.33 (sextet, J = 7.5 Hz, 1H), 1.27−1.24 (m, 2H), 1.19 (dd, J = 11.7, 10.3 Hz, 1H), 0.97 (s, 3H, Me-6), 0.94 (s, 3H, Me-6), 0.87 (d, J = 7.1 Hz, 3H, Me-2); ^{13}C NMR (100 MHz, $CDCl_3$) 154.1, 106.5, 70.1, 60.4, 57.1, 54.9, 45.0, 44.9, 42.3, 41.7, 36.8, 26.7, 26.1, 25.9, 15.5. The allylic alcohol (**16**) was identified as *β*-cedren-9α-ol based on its characteristic ^{1}H NMR resonances and two independent syntheses through $LiAlH_4$ reductions of (a) an authentic sample of 8α,9α-epoxycedrane (**13**) and (b) pure epoxide **13** obtained by epoxidation of pure TEAS-generated **5**. The latter reactions afforded, in addition to epi-cedrol[30b] as the major product, small amounts of allylic alcohol **16**.[53] The ^{1}H NMR spectra of these authentic materials (**16**) were superimposable on that obtained for **16** when the mixture of epoxides (**11** + **12** + **13**) was used as starting material. In addition, the ^{13}C NMR resonances of alcohol **16** were identical to those recorded for commercial (Fluka) *β*-cedren-9α-ol.[32]

Epi-cedrol. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.92−1.85 (m, 2H), 1.76−1.64 (m, 4H), 1.62−1.48 (m, 5H), 1.42−1.37 (m, 1H), 1.36−1.30 (m, 1H), 1.32 (s, 3H), 1.30−1.24 (m, 1H), 1.14 (s, 3H), 1.01 (s, 3H), 0.85 (d, J = 7.2 Hz, 3H). The ^{1}H NMR values obtained for epi-cedrol are in good agreement with those recorded previously at 90 MHz.[30]

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Supporting Information Available: General aspects and instrumentation; procedures and characterization data for (2*Z*,6*E*)-**1**; protocol for expression and purification of TEAS; more detailed discussion and NMR data for the structure elucidations; reproductions of NMR and MS spectra. This material is available free of charge via Internet at http://pubs.acs.org.

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