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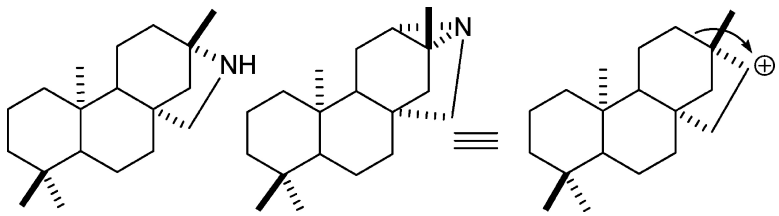

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16-Aza-*ent*-beyerane and 16-Aza-*ent*-trachylobane: Potent Mechanism-Based Inhibitors of Recombinant *ent*-Kaurene Synthase from *Arabidopsis thaliana*

Arnab Roy,[†,‡] Frank G. Roberts,[†,§] P. Ross Wilderman,[||] Ke Zhou,[||] Reuben J. Peters,[||] and Robert M. Coates*[,†]

Contribution from the Department of Chemistry University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801, and Department of Biochemistry, Biophysics, & Molecular Biology, Iowa State University, Ames, Iowa 50011

Received April 7, 2007; E-mail: rmcoates@uiuc.edu

Abstract: The secondary *ent*-beyeran-16-yl carbocation (**7**) is a key branch point intermediate in mechanistic schemes to rationalize the cyclic structures of many tetra- and pentacyclic diterpenes, including *ent*-beyerene, *ent*-kaurene, *ent*-trachylobane, and *ent*-atiserene, presumed precursors to >1000 known diterpenes. To evaluate these mechanistic hypotheses, we synthesized the heterocyclic analogues 16-aza-*ent*-beyerane (**12**) and 16-aza-*ent*-trachylobane (**13**) by means of Hg(II)- and Pb(IV)-induced cyclizations onto the Δ^{12} double bonds of tricyclic intermediates bearing carbamoylmethyl and aminomethyl groups at C-8. The 13,16-seco-16-norcarbamate (**20a**) was obtained from *ent*-beyeran-16-one oxime (**17**) by Beckmann fragmentation, hydrolysis, and Curtius rearrangement. The aza analogues inhibited recombinant *ent*-kaurene synthase from *Arabidopsis thaliana* (GST-rAtKS) with inhibition constants ($IC_{50} = 1 \times 10^{-7}$ and 1×10^{-6} M) similar in magnitude to the pseudo-binding constant of the bicyclic *ent*-copalyl diphosphate substrate ($K_m = 3 \times 10^{-7}$ M). Large enhancements of binding affinities ($IC_{50} = 4 \times 10^{-9}$ and 2×10^{-8} M) were observed in the presence of 1 mM pyrophosphate, which is consistent with a tightly bound *ent*-beyeranyl$^+$/pyrophosphate$^-$ ion pair intermediate in the cyclization−rearrangement catalyzed by this diterpene synthase. The weak inhibition ($IC_{50} = 1 \times 10^{-5}$ M) exhibited by *ent*-beyeran-16-*exo*-yl diphosphate (**11**) and its failure to undergo bridge rearrangement to kaurene appear to rule out the covalent diphosphate as a free intermediate. 16-Aza-*ent*-beyerane is proposed as an effective mimic for the *ent*-beyeran-16-yl carbocation with potential applications as an active site probe for the various *ent*-diterpene cyclases and as a novel, selective inhibitor of gibberellin biosynthesis in plants.

Introduction

The biogenetically related polycyclic diterpene hydrocarbons *ent*-beyerene, *ent*-kaurene, *ent*-trachylobane, and *ent*-atiserene (Figure 1, **1**−**4**) occur widely in plants, where they are likely intermediates in the biosynthesis of numerous functionalized and degraded natural products.[1,2] For example, *ent*-kaurene is a well-established intermediate in the multistep pathway to the gibberellin family of plant growth regulators[3] as well as the intensely sweet-tasting triglycoside stevioside (**14**) derived from 13-hydroxykaurenoic acid.[4] The related diterpene alcohols, *ent*-kauran-16α-ol and *ent*-atiseran-16α-ol, were recently found to be oviposition stimulants for the banded sunflower moth.[5]

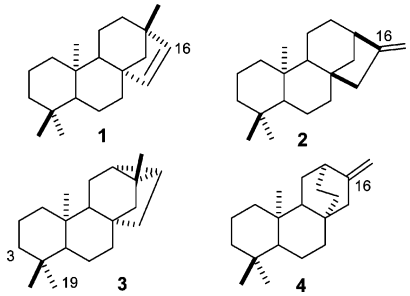


Figure 1. Structures of *ent*-beyerene, *ent*-kaurene, *ent*-trachylobane, and *ent*-atiserene diterpenes (**1**−**4**).

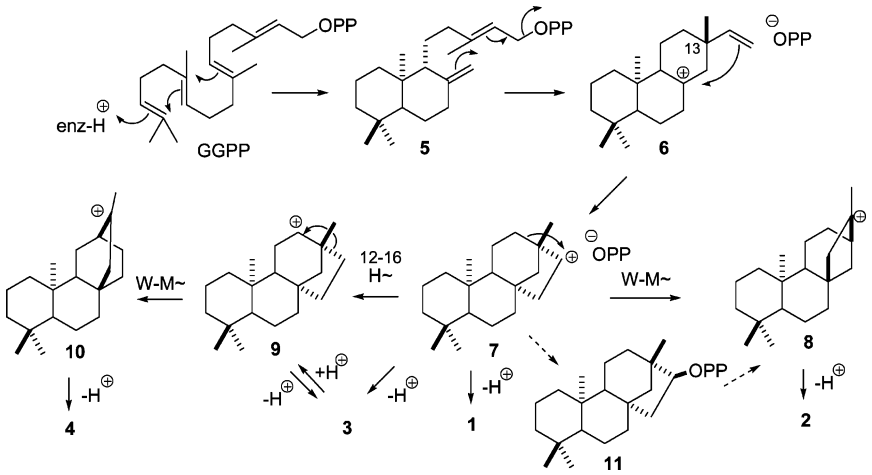
Recent reports document significant bioactivities of oxygenated diterpenes in mammalian cells. *ent*-Kauren-19-oic acid, an intermediate in gibberellin biosynthesis, exhibits anti-inflam-

[†] University of Illinois.
[‡] Present address: Albany Molecular Sciences, Hyderabad, India.
[§] Present address: Department of Chemistry, University of Chicago, Chicago, IL.
[||] Iowa State University.

(1) All beyerane, kaurane, and trachylobane derivatives prepared or discussed in this paper belong to the enantiomeric (ent) family of biogenetically related diterpenes. The ent prefix descriptor is attached to the chemical names (eg, *ent*-beyerane, *ent*-kaurane, *ent*-trachylbane) frequently in the text to remind the reader of the absolute configuration. Complete semisystematic names are given as headings in the Experimental Section and Supporting Information. For IUPAC nomenclature guidelines, see: Riguady, J.; Klesney, S. P. *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F, and H*; Pergamon: Oxford, 1979, and ref 2b.

(2) (a) Dev, S.; Misra, R. *CRC Handbook of Terpenoids: Diterpenoids*. Dev, S., Ed.; CRC Press: Boca Raton, FL, 1986; Vol. IV. (b) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids, Vol. 2, Di- and Higher Terpenoids*; Chapman & Hall: London, 1991. (c) Glasby, J. S. *Encyclopedia of the Terpenoids*. Wiley: Chichester, 1982; Vols. 1 and 2. (d) *Dictionary of Natural Products*; Buckingham, J., Ed.;Chapman and Hall: London, 1994. (e) Hanson, J. R. *Nat. Prod. Rep.* **2006**, *23*, 875−885 and preceding annual reviews in this series. (f) Buckingham, J. *Dictionary of Natural Products (on-line web edition)*; Chapman & Hall/CRC Press: London, 2002.

Scheme 1



matory activity attributed to inhibition of the nuclear factor κB pathway in macrophages,[6a] and its 15-oxo derivative blocks mitotic chromosome movement through effects on a kinetochore protein.[6b] The lethal norkaurane glycoside disulfate, carboxyatractyloside, binds to the mitochondrial ADP/ATP transporter protein.[6c] The 3β-hydroxy derivative of trachylobane induces apoptosis in human leukemia cells at 50 μM concentration.[7]

The tetracyclic and pentacyclic structures of these diterpenes are biosynthesized from the bicyclic labdane precursor *ent*-copalyl diphosphate (**5**) through a series of cyclizations and rearrangements beginning with an S_N' ring closure that forms the C ring. (Scheme 1)[3a,8] Rotation of the C13 vinyl group in the resulting *ent*-pimaren-8-yl carbocation (**6**) and a second cyclization generate the *ent*-beyeran-16-yl ion (**7**). The four diterpene hydrocarbon end products are then formed from this key branch point intermediate by 1,2- and 1,3-proton eliminations (to **1** and **3**), by the Wagner−Meerwein rearrangement **7** → **8** and methyl−methylene elimination (to **2**), and by 1,3-hydride shift to the isomeric beyeran-12-yl ion **9**, Wagner−Meerwein rearrangement **9** → **10**, and proton elimination (to **4**). A covalent beyeranyl diphosphate (PP) **11** is another conceivable intermediate in the multistep mechanism.

This scheme is supported by the occurrence of similar biomimetic transformations,[9] by the results of isotope labeling experiments with crude enzyme extracts containing *ent*-kaurene synthase (KS) activity,[3a,8,10] and by the alteration of product outcomes with mutant KSs.[11] An alternative path to the atiserane diterpenes would involve ring opening of a free or enzyme-bound trachylobane intermediate.

Solublization and characterization of KS from the gibberellin-producing fungus *Gibberella fujikuroi* and various higher plants were reported in pioneering studies by West and co-workers.[8a,12] Separation of distinct *ent*-beyerene, *ent*-kaurene, and *ent*-trachylobane synthases in enzyme extracts from elicited seedlings of *Ricinus communis* has been recorded.[13] KS genes from the fungi *G. fujikuroi* and *Phaeosphaeria* Sp L487 and from the higher plants *Arabidopsis thaliana*, *Stevia rebaudiana*, *Cucuribita maxima* (pumpkin), rice (*Oryza sativa*), and moss have recently been identified and characterized.[4c,14]

Numerous aza analogues of postulated carbocation intermediates in terpene biogenetic mechanisms have been synthesized and evaluated as inhibitors of specific terpene synthases[15] or for other purposes, including sterol biosynthesis inhibitors and antifungal agents.[16] Although the aza analogue concept has been very useful for inhibitor design, the extent to which tetrahedral ammonium ions with their localized positive charges actually

(3) (a) MacMillan, J.; Beale, M. H. Diterpene Biosynthesis. In *Comprehensive Natural Products Chemistry*. *Isoprenoids Including Carotenoids and Steroids*; Cane, D. E., Ed.; Elsevier: Amsterdam, 1999; Vol. 2, Chapter 8, pp 217−243. (b) Mander, L. N. *Nat. Prod. Rep.* **2003**, *20*, 49−69.
(4) (a) Kinghorn, A. D. *Med. Aromat. Plants−Ind. Profiles* **2002**, *19* (Stevia), 1−17. (b) Geuns, Jan M. C. *Phytochemistry* **2003**, *64*, 913−921. (c) Richman, A. S.; Gijzen, M.; Starratt, A. N.; Yang, Z.; Brandle, J. E. *Plant J.* **1999**, *19*, 411−421.
(5) Morris, B. D.; Foster, S. P.; Grugel, S.; Charlet, L. D. *J. Chem. Ecol.* **2005**, *31*, 89−102.
(6) (a) Castrillo, A.; de las Heras, B.; Hortelano, S.; Rodríguez, B.; Villar, A.; Boscá, L. *J. Biol. Chem.* **2001**, *276*, 15854−15860. (b) Rundle, N. T.; Nelson, J.; Flory, M. R.; Joseph, J.; Th'ng, J.; Aebersold, R.; Dasso, M.; Andersen, R. J.; Roberge, M. *ACS Chem. Biol.* **2006**, *1*, 443−450. (c) Pebay-Peyroula, E.; Dahout-Gonzalez, C.; Kahn, R.; Trézéguet, V.; Lauquin, G. J-M.; Brandolin, G. *Nature* **2003**, *426*, 39−44.
(7) (a) Baccelli, C.; Block, S.; Van Holle, B.; Schanck, A.; Chapon, D.; Tinant, B.; Van Meervelt, L.; Morel, N.; Quetin-Leclercq, J. *Planta Med.* **2005**, *71*, 1036−1039. (b) For a review on the trachylobane diterpenes, see Fraga, B. M. *Phytochem. Anal.* **1994**, *5*, 49−56.
(8) (a) West, C. A. In *Biosynthesis of Isoprenoid Compounds*, Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1983; Vol 1, Chapter 7. (b) Davis, E. M.; Croteau, R. *Top. Curr. Chem.* **2000**, *209*, 53−95.
(9) (a) For a review see, Coates, R. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, J. W., Eds.; Springer Verlag: New York, 1976; Vol. 33, pp 73−230. (b) Coates, R. M., Kang, H.-Y. *J. Chem. Soc., Chem. Commun.* **1987**, 232−233.
(10) (a) Coates, R. M.; Cavender, P. L. *J. Am. Chem. Soc.* **1980**, *102*, 6358−6359. (b) Drengler, K. A.; Coates, R. M. *J. Chem. Soc., Chem. Commun.* **1980**, 856−857. (c) Coates, R. M.; Koch, S. C.; Hegde, S. *J. Am. Chem. Soc.* **1986**, *108*, 2762−2764.
(11) Xu, M.; Wilderman, P. R.; Peters, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7397−7401.
(12) (a) Duncan, J. D.; West, C. A. *Plant Physiol.* **1981**, *68*, 1128−1134. (b) Shen-Miller, J.; West, C. A. *Phytochemistry* **1985**, *24*, 461−464. (c) Railton, I. D.; Fellows, B.; West, C. A. *Phytochemistry* **1984**, *23*, 1261−1267.
(13) (a) Robinson, D. R.; West, C. A. *Biochemistry* **1970**, *9*, 70−79 and 80−89. (b) Spickett, C. M.; Ponnamperuma, A.; Abell, C. *Phytochemistry* **1994**, *37*, 971−973. (c) Frost, R. G.; West, C. A. *Plant Physiol.* **1977**, *59*, 22−29.
(14) (a) Kawaide, H.; Sassa, T.; Kamiya, Y. *J. Biol. Chem.* **2000**, *275*, 2276−2280, and refs cited. (b) Toyomasu, T.; Kawaide, H.; Ishizaki, A.; Shinoda, S.; Otsuka, M.; Mitsuhashi, W.; Sassa, T. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 660−664. (c) Yamaguchi, S.; Sun, T.; Kawaide, H.; Kamiya, Y. *Plant Physiol.* **1998**, *116*, 1271−1278. (d) Yamaguchi, S.; Saito, T.; Abe, H.; Yamane, H.; Murofushi, N.; Kamiya, Y. *Plant J.* **1996**, *10*, 203−213. (e) Xu, M.; Wilderman, P. R.; Morrone, D.; Xu, J.; Roy, A.; Margis-Pinheiro, M.; Upadhyaya, N. M.; Coates, R. M.; Peters, R. J. *Phytochemistry* **2007**, *68*, 312−326. (f) Hayashi, K.-i.; Kawaide, H.; Notomi, M.; Sakigi, Y.; Matsuo, A.; Nozaki, H. *FEBS Lett.* **2006**, *580*, 6175−6181.
(15) For reviews and leading references, see: (a) Narula, A. S; Rahier, A.; Benveniste, P.; Schuber, F. *J. Am. Chem. Soc.* **1981**, *103*, 2408−2409. (b) Rahier, A.; Taton, M.; Benveniste, P. *Biochem. Soc. Trans.* **1990**, 48−52, (c) Poulter, C. D. *Acc. Chem. Res.* **1990**, *23*, 70−77. (d) Abe, I.; Tomesch, J. C.; Wattanasin, S.; Prestwich, G. D. *Nat. Prod. Rep.* **1994**, *11*, 279−302.
(16) Burbiel, J.; Bracher, F. *Steroids* **2003**, *68*, 587−594.

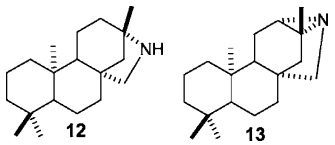


Figure 2. Structures of 16-aza-*ent*-beyerane (**12**) and 16-aza-*ent*-trachylobane (**13**), proposed aza analogue mimics for the *ent*-beyeran-16-yl ion **7** (Scheme 1), and a protonated *ent*-trachylobane (**3**), high-energy intermediates in the cyclization of *ent*-copalyl PP to *ent*-kaurene.

resemble presumably trigonal carbocations with their more dispersed charge is open to question. However, the protonated forms of 2-azabornane and the isomeric 15-azapimarenes are notable as effective mimics for high-energy, pyramidalized, secondary born-2-yl and pimaren-15-yl carbocation/PP·Mg ion pairs presumably generated at the active sites of bornyl diphosphate and abietadiene synthases.[17,18] Azabornane and other aza-terpenes have served as useful active site probes in X-ray crystallography of mono- and sesquiterpene cyclases.[19]

In this paper, we report the synthesis and characterization of 16-aza-*ent*-beyerane and 16-aza-*ent*-trachylobane (**12** and **13**, Figure 2), as well as the potential intermediate *ent*-beyeran-16-yl PP (**11**), and a preliminary kinetic evaluation of the compounds as mechanism-based inhibitors of recombinant KS from *A. thaliana*. The nitrogen heterocycle **12** is a novel aza-analogue of the high-energy, secondary *ent*-beyeranyl ion **7** in the mechanism in Figure 1, and 16-azatrachylobane (**13**) resembles a bridged ion (protonated cyclopropane) intermediate or transition state in the Wagner−Meerwein rearrangement step (**7** → **8**).

Results and Discussion

16-Aza-*ent*-beyerane and 16-Aza-*ent*-trachylobane. The two heterocyclic analogues (**12** and **13**) were synthesized from *ent*-beyeran-16-one (**16**) by cleavage of ring D, Curtius rearrangement to 13,16-seco-norcarbamates **20** and 13,16-seco-noramine **21**, and recyclizations onto nitrogen as outlined in Schemes 2−4. Multigram quantities of crude isosteviol (**15a**) were obtained from stevioside (**14**) by hydrolysis and pinacol-type rearrangement of the sweet *ent*-kaurane glycoside[20] using either *Stevia rebaudiana* plant material (2.4%) or, better yet, commercial stevia extract sweetener (24%). Tetracyclic ketone **16** was prepared from isosteviol methyl ester (**15b**) through deoxygenation of the sterically hindered axial carboxyl group at C4 in six steps according to previously described procedures: ketalization, $LiAlH_4$ reduction, mesylate formation, thiophenoxide displacement, Li/NH_3 reduction, and hydrolysis (60−62% yield).[21]

Cleavage of the D ring of *ent*-beyeranone was accomplished by Beckmann fragmentation of the oxime derivative (**17**). The oxime was formed as a single isomer in high yield (NH_2OH·HCl, pyr) and presumed to have the less sterically crowded E configuration. Fragmentation occurred readily upon reaction of the oxime with tosyl chloride (DMF, 25 °C),[22] giving rise to a 2.6:1 mixture olefinic nitriles **18a** and **18b**. Although the double bond isomers had very similar properties and polarities, both were obtained in pure, crystalline form by repeated chromatographies on silica gel. The broadened signal for the vinyl hydrogen (δ_H 5.33, $W_{1/2}$ = 9.5 Hz) in the proton NMR spectrum of the major isomer compared to the much sharper NMR resonance (δ_H 5.34, narrow q, J = 1 Hz, $W_{1/2}$ = 3 Hz) for the vinyl proton of the minor isomer was the basis for a preliminary assignment of the double bond positions shown. This assignment was confirmed by X-ray diffraction analysis of a single crystal of the major isomer (see the Supporting Information).

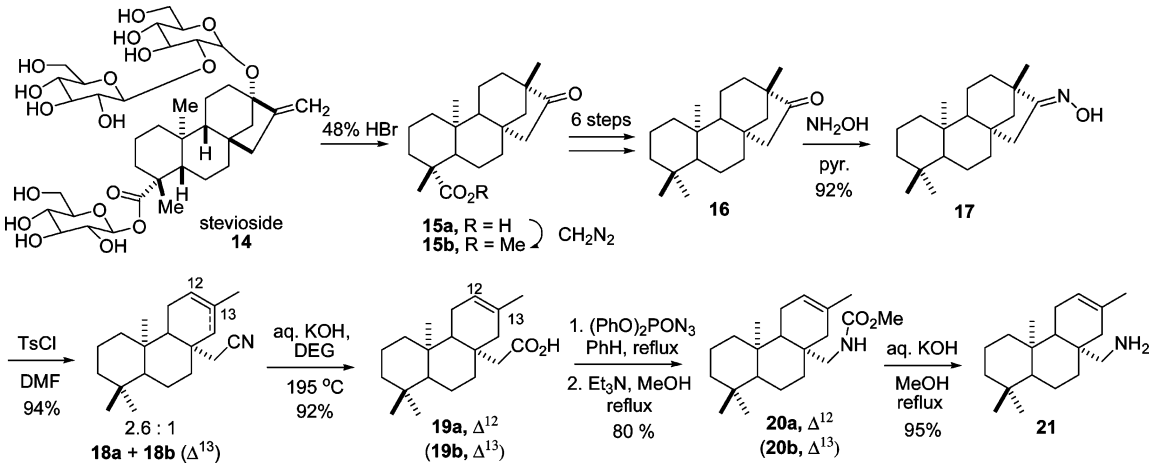
Further degradation to the required seco-noramine carbamates **20** was effected by Curtius rearrangements of the corresponding carboxylic acids **19a** and **19b** (Scheme 2). The reactions were conducted with the pure Δ^{12} and Δ^{13} isomers and with a 1:4 mixture enriched in the latter. Nitriles **18a** and **18b** were hydrolyzed to the corresponding 13,16-seco-acids (92%) by heating with aqueous KOH in diethylene glycol in a metal bomb at 195 °C. Curtius degradations of the acids with diphenylphosphoryl azide (Et_3N, PhH, reflux, 2 h)[23,24] gave the isocyanate intermediates that were converted to carbamates **20a** and **20b** (80 and 82%) by addition of methanol (MeOH, Et_3N, reflux, 15 h). The isomeric carbamates were readily distinguished by their vinyl hydrogen signals in the respective proton NMR spectra (**20a**, δ_H 5.38; **20b**, δ_H 5.08). Hydrolysis (KOH, aqueous MeOH, reflux, 8 h) of the Δ^{12} seco-norcarbamate (**20a**) provided the corresponding primary amine **21** in high yield.

Intramolecular mercury amidation of carbamate **20a** with $Hg(OAc)_2$ in THF[25a] at room temperature was quite slow and incomplete after 5 days. However, heterocyclization of **20a** (Scheme 3) proceeded smoothly with the more electrophilic trifluoroacetate reagent[25b] [$Hg(O_2CCF_3)_2$, THF, 25 °C, 16 h], and direct reduction of the intermediate mercurial with $NaBH_4$ (aqueous NaOH) furnished azabeyerane carbamate **23**. Cyclization of the Δ^{13} isomer (**20b**) with $Hg(OTFA)_2$ in THF proceeded with a similar rate, and $NaBH_4$ reduction afforded **23** with comparable efficiency. The organomercurial intermediate **22** was isolated from a separate run, and the crude, polar solid was characterized by its proton NMR spectrum (see below). Hydrolysis of the cyclized carbamate (KOH, aqueous 1,2-propylene glycol, reflux 18 h) provided azabeyerane (**12**).

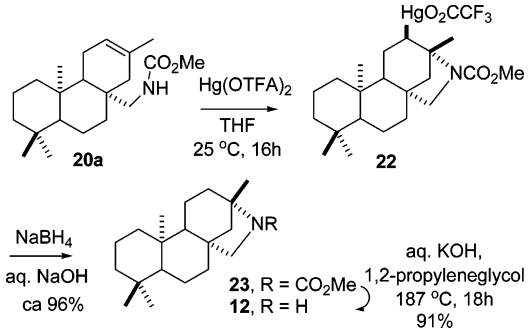
Conversion of seco-noramine **21** to 16-aza-*ent*-trachylobane **13** was accomplished by Nagata's intramolecular aziridination method (Scheme 4)[26,27] Oxidation of the primary amine with 1.4 equiv of $Pb(OAc)_4$ in benzene buffered heterogeneously with powdered K_2CO_3 (15 °C, 1 h) effected nitrenoid cyclization to the bridged aziridine in quantitative yield. Although azatrachylobane was unstable as a neat liquid at room temperature,

(17) Whittington, D. A.; Wise, M. L.; Urbansky, M.; Coates, R. M.; Croteau, R.; Christianson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15375−15380.
(18) (a) Peters, R. J.; Ravn, M. M.; Coates, R. M.; Croteau, R. *J. Am. Chem. Soc.* **2001**, *123*, 8974−8978. (b) Ravn, M. M.; Peters, R. J.; Coates, R. M.; Croteau, R. *J. Am. Chem. Soc.* **2002**, *124*, 6998−7006.
(19) Christianson, D. W. *Chem. Rev.* **2006**, *106*, 3412−3442.
(20) (a) Ruddat, M.; Heftmann, E.; Lang, A. *Arch. Biochem. Biophys.* **1965**, *110*, 496−499. (b) Mossetig, E.; Beglinger, U.; Dolder, F.; Lichti, H.; Quitt, P.; Waters, J. A. *J. Am. Chem. Soc.* **1963**, *85*, 2305−2309. (c) Wood, H. B., Jr.; Allerton, R.; Diehl, H. W.; Fletcher, H. G., Jr. *J. Org. Chem.* **1955**, *20*, 875−883.
(21) (a) Coates, R. M.; Kang, H.-Y. *J. Org. Chem.* **1987**, *52*, 2065−2074. (b) Crossley, N. S.; Dowell, R. *J. Chem. Soc. C* **1971**, 2496−2498.
(22) Mammato, D. C.; Eadon, G. A. *J. Org. Chem.* **1975**, *40*, 1784−1792.
(23) (a) Shioiri, T.; Ninomiya, K.; Yamada, S.-i. *J. Am. Chem. Soc.* **172**, *94*, 6203−6205. (b) Yamada, S.-i.; Ninomiya, K.; Shioiri, T. *Tetrahedron Lett.* **1973**, *14*, 2343−2346.
(24) De Kimpe, N.; Boeykens, M.; Tehrani, K. A. *J. Org. Chem.* **1994**, *59*, 8215−8219.
(25) (a) Harding, K. E.; Burks, S. R. *J. Org. Chem.* **1981**, *46*, 3920−3922. (b) Kocovsky, P. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; Wiley: Chichester, 1995; pp 3267−3270.
(26) Nagata, W.; Hirai, S.; Kawata, K.; Aoki, T. *J. Am Chem. Soc.* **1967**, *89*, 5045−5046.
(27) Portoghese, P. S.; Sepp, D. T. *Tetrahedron* **1973**, *29*, 2253−2256.

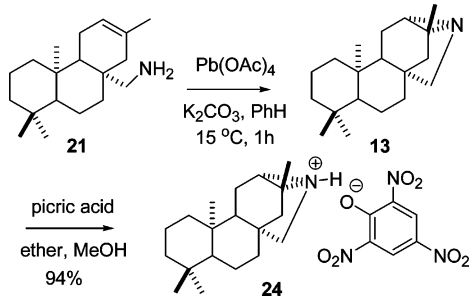
Scheme 2



Scheme 3



Scheme 4



presumably undergoing polymerization,[27] the aza analogue could be stored indefinitely in hexane solution at −20 °C. The picrate derivative (**24**) was formed readily in ether−methanol at 0 °C in 94% yield. The ORTEP for the X-ray crystal structure of the picrate salt is presented in Figure 3.

***ent*-Beyeran-16 exo-yl Diphosphate.** The beyeranyl$^+$ carbocation intermediate (**7**) shown in Scheme 1 is presumably accompanied by the PP·Mg counterion in the active site of the cyclase enzymes. If covalent bond formation occurred as it does in mono and sesquiterpene biosynthetic mechanisms,[28−30] the resulting *ent*-beyeranyl PP (**11**) would be a new, stable intermediate. Solvolysis of beyeranyl sulfonates in fact leads to bridge rearrangements and formation of kaurane and atiserane derivatives.[9a] Alternatively, if the beyeranyl$^+$ ion is tightly associated with the PP·Mg anion in the cyclase active site, beyeranyl PP might be an effective inhibitor and active site probe for diterpene synthases. These considerations and the availability of *ent*-beyeran-16-one (**16**) were incentives for the synthesis of **11** (Scheme 5) and experiments with KS.

Meerwein−Pondorff−Verley reduction of *ent*-beyeranone in a manner similar to that reported for isosteviol methyl ester (**15b**)[31] afforded an approximately 1:1 mixture of the known endo and exo isomers,[32] **25a** and **25b**, which were separated by three successive chromatographies on silica gel in yields of 30 and 36%. The physical and spectral properties agreed well with the literature values. Conversion of the exo isomer to the diphosphate was accomplished by the Cramer−Danilov phosphorylation method ($Bu_4NH_2PO_4$, CCl_3CN, CH_3CN, 25 °C, 20 min; NH_3, MeOH)[33,34] and fractionation on a Dowex column with NH_4HCO_2/MeOH eluent to separate **11** from the monophosphate and inorganic phosphates. *ent*-Beyeran-15 exo-yl PP was obtained as the NH_4 salt (24%) and characterized by its ^{1}H and ^{31}P NMR spectra.

Proton NMR Spectra. Most of the proton NMR spectra of the tetra- and pentacyclic diterpenes showed distinctive and well-separated signals for $H15_{exo}$ and $H15_{endo}$ with $\Delta\delta_H$ ranging from 0.84 to 1.08 ppm. (Table 1) In the cases of exo and endo beyeranols (**25a** and **25b**), the smaller chemical shift of the signals for the protons at C15 resulted in disappearance of $H15_{endo}$ into the broad envelope for the ring protons and only $H15_{exo}$ was readily observable. The consistently higher field positions of $H15_{endo}$ are attributable to the cumulative shielding influence of the C−C bonds in the B and C rings of the cyclic structures. In most cases, the resonance for $H15_{exo}$ displayed additional long-range coupling ($^4J_{HH}$ = 1.6−3.6 Hz), presumeably arising from the W relationship to the axial proton at C14.[35] It is interesting that the altered bond angles resulting from aziridine ring formation in azatrachylobane (**13**) reorient $H15_{endo}$ into position for W-coupling to $H14_{axial}$, and consequently, the

(28) Wise, M. L.; Croteau, R. In *Comprehensive Natural Product Chemistry*; Cane, D. E., Ed; Elsevier Science: Oxford, 1999; Vol. 2; pp 97−153.
(29) (a) Croteau, R.; Karp, F. *Arch. Biochem. Biophys.* **1979**, *198*, 512−522. (b) Croteau, R.; Shaskus, J. *Arch. Biochem. Biophys.* **1985**, *236*, 535−543.
(30) Cane, D. E. In *Comprehensive Natural Product Chemistry*; Cane, D. E., Ed; Elsevier Science: Oxford, 1999; Vol. 2; pp 156−200.
(31) (a) Wilcox, C. F.; Sexton, M., Jr.; Wilcox, M. F. *J. Org. Chem.* **1963**, *28*, 1079−1082. (b) Coates, R. M.; Bertram, E. F. *J. Org. Chem.* **1971**, *36*, 2625−2631.
(32) (a) Sobti, R. R.; Dev, S. *Tetrahedron Lett.* **1966**, *33*, 3939−3942. (b) Bell, R. A.; Ireland, R. E.; Mander, L. N. *J. Org. Chem.* **1966**, *31*, 2536−2542.
(33) Assink, B. K.; MS thesis, University of Illinois at Urbana−Champaign, 1999.
(34) (a) Danilov, L. L.; Mal'tsev, S. D.; Shibaev, V. N. *Soviet J. Bioorg. Chem.* **1988**, *14*, 712−714, 1287−1289. (b) Cramer, F.; Rittersdorf, W. R. *Tetrahedron* **1967**, *23*, 3015−3022.
(35) Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds*; Springer-Verlag: Berlin, 2000, pp 177−178.

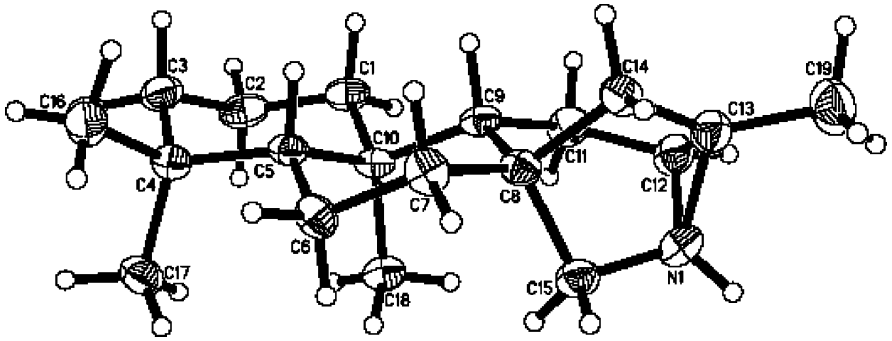


Figure 3. ORTEP diagram from the X-ray crystal structure of 16-aza-*ent*-trachylobane picrate salt (**24**). The picrate counterion is omitted.

Scheme 5

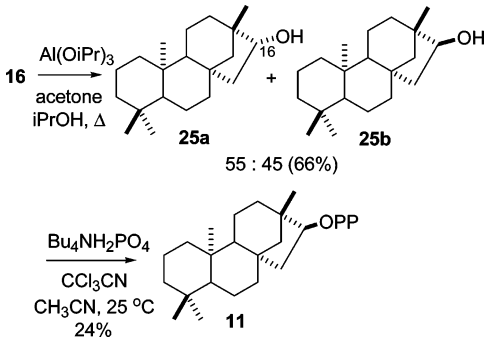
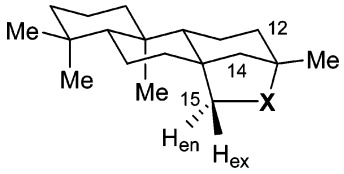


Table 1. ^{1}H NMR Chemical Shifts (δ_H), Chemical Shift Differences ($\Delta\delta_H$, Exo − Endo), Multiplicities (m), and Coupling Constants (*J*) for the Endo and Exo Protons at C15 of Various *ent*-Beyerane Derivatives



compd no.	X	H15 δ_H (ppm)	$\Delta\delta_H$ (ppm)	m	*J* (Hz)
16	C═O	endo 1.75	0.93	d	18.6
		exo 2.68		dd	18.6, 3.6
19	C═NOH	endo 1.94	1.08	d	18.4
		exo 3.02		dd	19, 3.2
23	NCO_2Me	endo[a] 2.90	0.99a	d	11
		endo[b] 2.97	1.01b	d	11
		exo[a] 3.89		dd	11, 2
		exo[b] 3.98		dd	11, 2
12	NH	endo 2.56	0.84	br d	10.8
		exo 3.40		br d	10.8
13	N	endo 2.28	0.92	dd	12, 1.6
		exo 3.20		d	11.6
25a	CHOH	endo ND[c]	ND[c]	ND[c]	ND[c]
	(endo OH)	exo 1.85		ddd	14.3, 4.8, 2.6
25b	CHOH	endo ND[c]	ND[c]	ND[c]	ND[c]
	(exo OH)	exo 2.59		ddd	14.3, 7.6, 2.4

[a] Major rotamer. [b] Minor rotamer. [c] ND = not determined.

additional coupling appears in the lower field resonance. The W relationship between $H15_{endo}$ and $H14_{axial}$ is apparent in the ORTEP in Figure 3.

The proton NMR spectra of the tricyclic and tetracyclic carbamates **20a** and **23** show doubling of several peaks (OMe, vinyl protons, and, in the case of **23**, the C13 bridge head methyl and CH_2N), clearly indicating mixtures of rotamers about the $N-CO_2Me$ bonds. The rotamer ratios determined from the integrals of the OCH_3 singlets were 11:1 for **20a** and 1.7:1 for **23**. The data for the distinctive peaks for $H15_{exo}$ and $H15_{endo}$ in the spectrum of **23** are presented in Table 1. Noticeably broadened doublets at 1.98 and 2.16 ppm in the spectrum are assigned to the equatorial protons at C12 in the minor and major rotamers on the expectation of a deshielding influence of the proximal carbamate π electrons.

Table 2. Internal Bond Lengths and Bond Angles within the Aziridinium Ring of Picrate Salt **24**

bond lengths (Å)		bond angles (deg)	
C12−C13	1.472	C12−C13−N1	60.7
C12−N1	1.510	C13−C12−N1	61.1
C13−N1	1.516	C12−N1−C13	58.2

The proton NMR spectrum for the mercurial intermediate (**22**) in the heterocyclization reaction (Scheme 3) was quite similar to that of the cyclic carbamate product **23**, except for the appearance of two additional broad singlets at 3.37 (~0.65H, $W_{1/2}$ = 7−8 Hz) and 3.56 ppm (0.35H, $W_{1/2}$ = 7−8 Hz). Additional signals confirming a 1.8:1 mixture of rotamers were apparent for $H15_{endo}$ and $H15_{exo}$, the methoxy singlets, the C13 bridgehead methyls, and other peaks. A chemical shift increment of $\Delta\delta_H$ 1.2−1.4 ppm for the substitution $CH_2 \rightarrow CHHgX$ was estimated from literature data[36] and provided the basis for assignment of the broad singlets to $CHHgO_2CCF_3$. The $W_{1/2}$ values of the rotamer peaks for $CHHgO_2CCF_3$ are consistent with overlapping equatorial/axial and axial/axial couplings arising from interaction of equatorial H12α with the adjacent C11 protons. Hence, the HgO_2CCF_3 group must be axial, as would be expected from trans diaxial addition of $Hg(O_2CCF_3)_2$ across the 12,13 C═C in the mercury-induced cyclization (**20a** → **22**).

X-ray Crystal Structures. An X-ray diffraction analysis of azatrachylobane picrate salt (**24**) was carried out to confirm the structure and to obtain bonding parameters for the charged aziridinium ring (Figure 3 and Table 2). A crystallographic determination was also conducted on methyl *ent*-trachyloban-19-oate (see **3** and the Supporting Information) to ascertain comparative dimensions of the cyclopropane ring in the pentacyclic diterpene. The two C−N bond lengths (1.510 and 1.516 Å) for the aziridine are quite similar and also of comparable magnitude to those of the cyclopropane ring bonds (1.50, 1.51, and 1.52 Å). In contrast, the C12−C13 bond length in the aza analogue salt is considerably shorter (1.472 Å), while the C12−N1−C13 interior bond angle (58.2°) is constricted com-

(36) (a) Kitching, W.; Atkins, A. R.; Wickham, G.; Alberta, V. *J. Org. Chem.* **1981**, *46*, 563−570. (b) Coxon, J. M.; Steel, P. J.; Whittington, B. I.; Battiste, M. A. *J. Org. Chem.* **1989**, *54*, 1383−1391. (c) Coxon, J. M.; Steel, P. J.; Whittington, B. I. *J. Org. Chem.* **1990**, *55*, 4136−4144.

pared to the two C−C−N bond angles (60.7° and 61.1°) and to the almost equal bond angles within the cyclopropane ring of methyl trachyloban-19-oate (59.5°, 59.9°, and 60.2°).

In general, literature data for aziridine and its derivatives, if unperturbed by electron-withdrawing substituents on nitrogen, show somewhat longer C−C bonds than their C−N counterparts while both are notably shorter than the analogous bonds in acyclic amines.[37−39] Both theoretical calculations[40] for the parent aziridine and aziridinium ion and X-ray crystal structures for 8-oxa-3-azatricyclo[3.2.1.$0^{2,4}$]octane derivatives[41] indicate shorter C−C ring bonds and longer C−N ring bonds in the protonated forms, in line with the data for **24**. These small distortions seem to indicate relatively little carbocation character at the aziridinium carbons, a conclusion similar to that reached for para-substituted aryl aziridinium ions, based on molecular orbital calculations and ESCA results.[42]

Inhibition of *ent*-Kaurene Synthase. Genes encoding KS have been cloned from several plant species, and the catalytic activities of full-length proteins, i.e., including the putative *N*-terminal plastidial targeting sequence, were verified in assays run with recombinant bacterial extracts.[4c,14c−14e] In the present work, KS from *A. thaliana* (AtKS) was heterologously expressed as a pseudomature protein (without the first 41 *N*-terminal residues) fused to glutathione-*S*-transferase (GST) in *Escherichia coli*. The resulting GST-rAtKS fusion protein was purified by affinity chromatography and was immediately utilized in enzymatic assays. Incubation of *ent*-copalyl PP (**5**) with GST-rAtKS yielded the expected *ent*-kaurene (**2**) product, as verified by GC−MS-based comparison to an authentic sample. Kinetic runs were conducted at 10 nM protein concentration according to recently published procedures.[11] Reactions were initiated by addition of [15-^{3}H]*ent*-copalyl PP (**5**),[14e] and [14-^{3}H]-*ent*-kaurene was isolated by extraction and analyzed by liquid scintillation counting.[11,43] (Table 3)

Studies with native KS partially purified from wild cucumber (*Marah macrocarpus*) seeds demonstrated that ring rearrangement occurs during the enzyme-catalyzed cyclization of *ent*-copalyl PP.[10a] Stereoelectronic considerations dictate that the ring D rearrangement cannot be concerted with the preceding cyclization **6** → **7** to the beyeranyl$^+$ intermediate. Thus, there must be a localized secondary beyeranyl carbocation intermediate **7** in the mechanism which undergoes a thermodynamically favorable rearrangement to the tertiary kauranyl carbocation **8** (Scheme 1). One possibility for "stabilization" of the energetically unfavorable secondary carbocation would be covalent bond formation with the PP·Mg anion released in the initiating ionization of *ent*-copalyl PP (**5**), producing an *exo*-beyeranyl PP intermediate (**11**). Inhibition studies demonstrated that **11** apparently does bind in the GST-rAtKS active site, although it appears to be a relatively weak competitive inhibitor with $IC_{50} = 10\ \mu M$, when compared with the pseudo-binding constant of the enzyme for the native substrate (**5**, $K_m = 0.3\ \mu M$). Incubation of GST-rAtKS with saturating amounts of **11** (50 μM) did not result in the formation of any detectable kaurene, apparently ruling out a role for the tetracyclic PP, at least as a free intermediate, in the KS-catalyzed cyclization reaction.

Table 3. Pseudo-Binding Constant (K_m) for [15-^{3}H]*ent*-copalyl PP (**5**) with GST-rAtKS and Inhibition Constants (IC_{50}) for *ent*,*exo*-Beyeran-16-yl PP, 16-Aza-*ent*-beyerane, and 16-Aza-*ent*-trachylobane (**11**, **12**, and **13**)[a] in the Absence and Presence Inorganic PP

substrate/ inhibitor	K_m or IC_{50} (M)	IC_{50} (+PP_i)[b] (M)
5	$3 \pm 1 \times 10^{-7}$	−
11	$1 \pm 0.3 \times 10^{-5}$	−
12	$1 \pm 0.4 \times 10^{-7}$	$4 \pm 1 \times 10^{-9}$
13	$1 \pm 0.2 \times 10^{-6}$	$2 \pm 0.5 \times 10^{-8}$

[a] Incubation conditions: 10 nM GST-rAtKS + 1.5 μM *ent*-CPP (**5**). [b] Inhibition by PP_i alone was negligible at 1 mM concentration.

Inhibition studies with 16-azabeyerane (**12**) and 16-azatrachylobane (**13**) were conducted to evaluate the capability of the azaditerpene analogues to inhibit the normal cyclization reaction and, hence, to estimate the extent to which **12** and **13** mimic secondary carbocation (**7**) and a bridged nonclassical carbocation (not shown), respectively, on the way to the tertiary *ent*-kauranyl ion **8** (Scheme 1, Table 3). The two azaditerpenes are clearly bound in the active site, as indicated by their competitive inhibition effects [**12** with an $IC_{50} = 0.1\ \mu M$ and **13** with an $IC_{50} = 1\ \mu M$, both similar to the 0.3 μM K_m for *ent*-copalyl PP (**5**)]. Intriguingly, extended incubation of **13** with led to increased inhibition, suggesting that it may covalently modify GST-rAtKS (i.e., irreversible inhibition), and the IC_{50} values reported here should be regarded as upper limits on the actual affinity of the binding interaction between **13** and GST-rAtKS. The binding of these heterocyclic analogues was also assayed in the presence of inorganic pyrophosphate (PP_i) to mimic the presence of the PP_i anion resulting from ionization of **5**. The addition of 1 mM PP_i, which does not inhibit GST-rAtKS alone, led to a large increase in the ability of **12** to bind to, and to inhibit, GST-rAtKS, with $IC_{50} = 4$ nM, corresponding to a 25-fold increase in affinity. Given that the assays contain 10 nM GST-rAtKS, this low IC_{50} value indicates that the true affinity is subnanomolar, much as observed previously for abietadiene synthase.[18] Similar binding enhancement was also observed with **13**, which exhibited an $IC_{50} = 20$ nM in the presence of 1 mM PP_i. These synergistic effects presumably signify formation of a beyeranyl$^+$ carbocation PP·Mg ion pair intermediate in the KS-catalyzed cyclization reaction mechanism (i.e., as drawn for **7** in Scheme 1).

The less significant synergy exhibited by **13** with PP_i might reflect the presumably lower free energy profile for the mimicked trachylobanyl bridged carbocation intermediate (secondary−tertiary) relative to the preceding beyeranyl secondary carbocation. However, particularly given the very similar

(37) (a) Pearson, W. H.; Lian, B. W.; Bergmeier, S. C. In *Comprehensive Heterocyclic Chemistry II*; Katritsky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Elsevier: Oxford, 1996; Vol. 1A, pp 1−60. (b) Rai, K. M.; Hassner, A. In *Comprehensive Heterocyclic Chemistry II*; Katritsky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Elsevier: Oxford, 1996; Vol. 1A, pp 61−96.
(38) (a) Lwowski, W. In *Comprehensive Heterocyclic Chemistry*; Katritsky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. 7, pp 1−16. (b) Padwa, A.; Woolhouse, A. D. In *Comprehensive Heterocyclic Chemistry*; Katritsky, A. R., Rees, C. W., Eds.; Pergamon: Oxford, 1984; Vol. 7, pp 47−93.
(39) Selected X-ray crystal structures of aziridines: (a) Chinnakali, K.; Fun, H.-K.; Sriraghavan, K.; Ramakrishnan, V. T.; Razak, I. A. *Acta Crystallogr.* **1998**, *C54*, 1299−1301. (b) Dyakonenko, V. V.; Shiskin, O. V.; Zbruev, A. V.; Desenko, S. M. *Acta Crystallogr.***2005**, *E61*, o667−o668. (c) Cheikh, R. B.; Chaabouni, R.; Kallel, A. *Acta Crystallogr.* **1992**, *C48*, 283−286. (d) Ko, T.-M.; Olansky, L.; Moncrief, J. W. *Acta Crystallogr.* **1975**, *B31*, 1875−1878.
(40) Cremer, D.; Kraka, E. *J. Am. Chem. Soc.* **1985**, *107*, 3800−3810.
(41) Pinkerton, A. A.; Gallacher, A. C.; Radil, M.; Kunze, A.; Allemann, S.; Vogel, P. *Acta Crystallogr.* **1993**, *B49*, 328−334.
(42) Crist, D. R.; Turujman, S. A.; Hashmall, J. A. *J. Heterocycl. Chem.* **1991**, *28*, 1993−1995.
(43) Peters, R. J.; Flory, J. E.; Jetter, R.; Ravn, M. M.; Lee, H.-J.; Coates, R. M.; Croteau, R. B. *Biochemistry* **2000**, *39*, 15592−15602.

structures of **12** and **13**, it seems more likely that the reduced affinity of **13** relative to **12** (in either the absence or presence of PP_i) is a function of the lower basicity of the aziridine nitrogen (parent aziridinium ion p$K_a \sim 8.0$)[44] relative to that for the bridged pyrrolidine in **12** (pyrrolidinium ion pK_a = 11.3).[44] Thus azabeyerane would exist exclusively in the ammonium form in the incubation medium at pH 7.4, and it is likely bound to the enzyme in the protonated state, as seems to be the case for azabornane in the active site of bornyl PP synthase.[17] By contrast, the proportion of the bridged aziridine in **13** existing in the binding-competent, protonated state would be much lower. Since aziridinium ions are known to act as electrophiles in solution,[37,38,45] there is a distinct possibility that the protonated form of **13** reacts slowly with an active site nucleophile, thus inactivating the enzyme and explaining the increased inhibition observed in extended incubations mentioned above.

The relatively weak affinity observed for *ent*-beyeranyl PP (**11**) implies that the beyeranyl$^+$ carbocation and the PP·Mg anion leaving group are further separated in the KS active site than a typical covalent C−O bond length (1.42 Å).[46] This disparity presumably arises from separation of the PP·Mg anion and the hydrocarbon moieties of **5** following ionization by changes in their relative positions and/or orientations. From previous structural studies it seems most likely that absence of covalent bond formation reflects repositioning of the hydrocarbon moiety, since the bound PP·Mg counterions occupy a relatively constant position in various terpene synthase−substrate analogue cocrystals.[19]

Conclusions

The lack of any detectable conversion of *ent*-beyeran-16-yl PP (**11**) to *ent*-kaurene in incubations with *ent*-kaurene synthase appears to exclude this secondary PP as a free intermediate in the enzyme-catalyzed bicyclization of *ent*-copalyl PP (**5**). The enhanced affinities of 16-aza-*ent*-beyerane and 16-aza-*ent*-trachylobane for KS in the presence of inorganic PP, in comparison to the binding of the bicyclic substrate, indicate that the heterocycles behave as transition state inhibitors and are good candidates for active site probes for this key enzyme associated with gibberellin phytohormone biosynthesis in plants. In addition, the strong synergy exhibited by these compounds with inorganic PP suggests substantial stabilization of the corresponding intermediates, in particular beyeran-16-yl$^+$, by ion pairing with the PP·Mg released in the initial ionization of the *ent*-copalyl PP substrate. Similar findings have been reported with other terpene synthases,[17,18] suggesting that the use of the charged PP·Mg complex as a counterion to stabilize proximal secondary carbocation intermediates may be a relatively common enzymatic mechanism utilized by these enzymes. However, such counterion stabilization seems to be limited to carbocations located relatively close to the original PP position, as indicated by the weaker synergy exhibited by distal aza analogues and structural studies.[17,19] Indeed, it has been suggested that the PP·Mg anion may drive reactions toward localization of the carbocation intermediates close to the negative charge, particularly in the absence of other stabilizing influences in the active site.[11] Finally, the fact that *ent*-beyeran-16-yl PP does not appear to be a free intermediate further emphasizes the tight control KS must exert over the ionized carbocation intermediates and PP·Mg anion within its active site to prevent recombination of the closely associated ion pair.

Experimental Section (see Supporting Information for procedures and data for other compounds)

Methyl 16-*ent*-Azabeyerane-16-carboxylate (23). A literature procedure[25a] was followed with modification. A solution of commercially available mercuric trifluoroacetate[25b] (65 mg, 0.15 mmol) and carbamate **20a** (44 mg, 0.132 mmol) in 3 mL of THF was purged with nitrogen, covered with aluminum foil, and stirred at room temperature for 16 h. The reaction was judged to be complete by TLC analysis (R_f 0.22, 1:4 EtOAc:petroleum ether). A solution of $NaBH_4$ (6 mg, 0.14 mmol) in 0.1 mL of 2.5 M NaOH was added dropwise. After another 8 h, aqueous Na_2CO_3 (1 mL) was added, and stirring was continued for an additional 4 h. The suspension was concentrated to remove THF, and the residue was extracted with ether (2 × 20 mL). The combined ethereal extracts were washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated. The resulting solid material (42 mg, 96%) showed the following properties for a 1.7:1 mixture of N−CO_2Me rotamers: TLC R_f 0.23 (1:9 EtOAc:hexane); FTIR ($CHCl_3$) ν_{max} 3017, 2950, 1682, 1449, 1387 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.93 (s, ca. 1.9 H, CH_3), 0.94 (s, ca. 1.1H CH_3), 1.34 (s, ca. 1.1H, CH_3), 1.20−1.72 (m, 12.5 H), 1.44 (s, 1.9H, CH_3), 1.98 (br d, ∼0.4 H, $J \cong 8$ Hz, H12α), 2.16 (br d, ∼0.6 H, $J \cong 8$ Hz, H12α), 2.90 (app d, ∼0.6 H, $J \cong 10$ Hz, H15 endo), 2.97 (app d, ∼0.4 H, $J \cong 11$ Hz, H15 endo), 3.64 (s, ∼1.9 H, CO_2CH_3), 3.66 (s, ∼1.1 H, CO_2CH_3), 3.89 (app dd, ∼0.6 H, $J \cong 11$, 2 Hz, H15 exo), 3.98 (app dd, ∼0.4H, $J \cong 11$, 2 Hz, H15 exo). The crude product was hydrolyzed to azabeyerane (**12**) without further purification.

16-Aza-*ent*-beyerane (12). The carbamate hydrolysis conditions were modeled after a literature procedure.[47] A mixture of **23** (44 mg, 0.13 mmol), 1,2-propylene glycol (1 mL), water (0.1 mL), and KOH (336 mg, 6 mmol) was heated at reflux for 18 h, cooled to room temperature, diluted with water (5 mL), and extracted with ether (3 × 15 mL). The combined organic extracts were washed with water (1 × 10 mL) and dried ($MgSO_4$). Evaporation of the solvent afforded azabeyerane (**12**, 33 mg, 91%) as a clear oil: TLC R_f 0.22 (10:89:1 EtOAc:hexane:triethylamine); $[\alpha]^{25}_D$ −10.6 (c = 2.5, $CHCl_3$); FTIR (neat) ν_{max} 3312, 2922, 1699, 1454 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (s, 3H, CH_3), 0.78−0.88 (m, 2H), 0.85 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.05−1.26 (m, 4H), 1.16 (s, 3H, CH_3), 1.33−1.71(m, 12H), 2.56 (d, 1H, J = 10.8 Hz, $H15_{endo}$), 3.40 (d, 1H, J = 10.8 Hz, $H15_{exo}$); ^{13}C NMR (125.64 MHz, $CDCl_3$) δ 15.1, 18.6, 20.3, 20.4, 20.6, 22.0, 22.1, 26.9, 33.3, 33.9, 37.9, 39.1, 39.8, 39.9, 42.1, 45.5, 55.3, 56.1, 56.5; HRMS (ESI) m/z calcd for $C_{19}H_{34}N$ $(M + H)^+$ 276.2691, found 276.2687.

***ent*-8β-Aminomethyl-13-methyl-12-podocarpene (21)**.[48] The hydrolysis was modeled after a literature procedure.[47] A solution of **20a** (150 mg, 0.45 mmol) and KOH (1 g, 17.85 mmol) in 1.8 mL of methanol and water (0.2 mL) was heated at reflux for 8 h, cooled to room temperature, diluted with water (8 mL), and extracted with ether (3 × 20 mL). The combined organic extracts were washed with water (2 × 10 mL) and dried ($MgSO_4$). Evaporation of the solvent afforded primary amine **21** (117 mg, 95%) as a clear oil: $[\alpha]^{25}_D$ −14 (c = 2.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75−0.98 (m, 2H), 0.81 (s,

(44) Dean, J. A. *Lange's Handbook of Chemistry*, 15th ed.; McGraw-Hill: New York, 1999; p 8.30.
(45) (a) Chuang, T.-H.; Sharpless, K. B. *Org. Lett.* **2000**, *2*, 3555−3557. (b) Crist, D. R.; Leonard, N. J. *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 962−974.
(46) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry Part A*, 4th ed; Kluwer Academic: New York, 2000; p 13.
(47) Angle, S. R.; Arnaiz, D. O. *Tetrahedron Lett.* **1989**, *30*, 515−518.
(48) The IUPAC nomenclature was followed according to which the α or β configuration of substituents in *ent*-diterpenes is that designated in the systematic name for the "normal" antipode. See Rigaudy, J.; Klesney, S. P. *Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F, and H*; Pergamon Press: Oxford, 1979; p 511.

3H, CH_3), 0.83 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 1.08−1.17 (m, 1H), 1.23−1.41 (m, 6H), 1.46−1.61 (m, 5H), 1.62 (s, 3H, CH_3 at C-13), 1.81−2.08 (m, 4H), 2.61 (dd, 1H, J = 13.5, 1.5 Hz, CH_2N), 2.70 (d, 1h, J = 13.5 Hz, CH_2N), 5.37 (br s, 1H, $W_{1/2}$ = ∼7 Hz, ═CH); ^{13}C NMR (100.57 MHz, $CDCl_3$) δ 15.9, 18.4, 18.6, 21.6, 22.4, 23.6, 33.4, 36.5, 37.1, 39.7, 40.3, 41.9, 42.6, 53.8, 57.2, 120.9, 131.1; HRMS (ESI) m/z calcd for $C_{19}H_{34}N$ $(M + H)^+$ 276.2691, found 276.2688.

16-Aza-*ent*-trachylobane (13). The aziridination conditions were modeled after a literature procedure.[27] A solution of amine **21** (100 mg, 0.36 mmol) in dry benzene (3 mL) was quickly added to a suspension of $Pb(OAc)_4$ (225 mg, 0.51 mmol), anhydrous K_2CO_3 (398 mg, 2.88 mmol), and dry benzene (2 mL) being stirred and cooled at 0 °C. The mixture was warmed to 15 °C and stirred for 1 h, after which 10% aqueous NaOH (10 mL, 10%) was added to quench the reaction. The product was extracted with ether (3 × 20 mL). The combined ethereal extracts were washed with water (2 × 15 mL) and brine (1 × 15 mL) and dried ($MgSO_4$). Evaporation of the solvent afforded aziridine **13** (94 mg, 94%) as a clear, unstable oil that decomposed within 5 h. A solution in hexane stored under N_2 at −20 °C remained unchanged for at least several weeks. Properties of **13**: $[\alpha]^{25}_D$ −5.5 (c = 2.0, $CHCl_3$); TLC R_f = 0.42 (1:4 EtOAc:hexane); ^1H NMR (400 MHz, $CDCl_3$):δ 0.66−0.85 (m, 2H), 0.77 (s, 3H, CH_3), 0.82 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.03−1.23 (m, 3H), 1.23 (s, 3H, CH_3 at C-13), 1.27−1.4 (m, ∼3H), 1.4−1.56 (m, ∼8H), 1.77 (br s, $W_{1/2}$ = 8 Hz, ∼1 H), 1.78−1.93 (16 line centrosym m, 2H), 2.28 (dd, 1H, J = 12, 1.6 Hz, CH_2N), 3.20 (d, 1H, J = 11.6 Hz, CH_2N); ^{13}C NMR (100.57 MHz, $CDCl_3$) δ 14.4, 18.0, 18.1, 20.2, 20.6, 21.7, 32.8, 33.4, 35.3, 37.8, 39.1, 40.3, 40.9, 41.9, 43.7, 48.6, 51.6, 55.2, 56.2.

Picrate Derivative of 16-Aza-*ent*-trachylobane. (24) A solution of **13** (30 mg, 0.11 mmol) in dry ether (2 mL) was added to a stirred solution of picric acid (0.11 mmol, 26 mg) in 1 mL of methanol at 0 °C. The picrate salt precipitated immediately. After 10 min the solvents were evaporated. Crystallization from 9:1 benzene and acetone furnished **24** as yellow crystals: yield, 52 mg (94%); mp 232−234 °C; $[\alpha]^{25}_D$ −28.6 (c = 1.28, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.74−0.85 (m, 2H), 0.79 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.08−1.31 (m, 2H), 1.34−1.45 (m, 3H), 1.48−1.71 (m, 5H), 1.63 (s, 3H, CH_3 at C-13), 1.83 and 1.91 (ABdd, 2H, J_{AB} = 13.6 Hz), 2.15−2.20 (16 line m, 2H), 3.01 (dd, 1H, J = 11.2, 2 Hz, CH_2N), 3.17 (bs, 1H, $W_{1/2}$ = ca. 7 Hz), 3.76 (d, 1H, J = 11.2 Hz, CH_2N), 8.85 (s, 2H, ArH), 10.79 (bs, 1H, NH); ^{13}C NMR (100.57 MHz, $CDCl_3$) δ 14.1, 17.06, 17.1, 17.7, 19.7, 21.6, 32.9, 33.2, 34.4, 37.8, 38.7, 40.0, 41.5, 44.7, 45.9, 49.5, 50.9, 53.3, 55.0, 126.5, 128.4, 141.7, 161.8.

Inhibition of *ent*-Kaurene Synthase. Assays for kinetic analysis were performed with purified GST-rAtKS, the concentration of which was determined using A_{280} and the calculated extinction coefficient ϵ = 172 850 M^{-1} cm^{-1}, prior to dilution in assay buffer (50 mM Hepes, pH 7.2; 10% (v/v) glycerol; 7.5 mM $MgCl_2$; 0.1 mg/mL α-casein; 5 mM DTT) to 10 nM. Reactions were run at room temperature, initiated by the addition of [15-^{3}H]*ent*-copalyl PP,[14e] allowed to react for 1 min, and terminated by the addition of KOH to 0.2 M and EDTA to 15 mM, as described.[43] The production of *ent*-kaurene was quantified by extracting the incubation solutions with hexanes and passing the organic extracts through short columns of $MgSO_4$ and silica gel to dry them and to remove any oxygenated diterpenoids resulting from nonspecific solvolysis prior to liquid scintillation counting.[43] Inhibition studies were carried out with saturating amounts of substrate (1.5 μM [15-^{3}H]-*ent*-copalyl PP) and varying amounts of inhibitor, and in the case of **13**, it was necessary to add substrate directly after inhibitor to detect any enzymatic activity. The resulting data were fit to an exponential equation using KaleidaGraph 4.0 (Synergy Software) to determine IC_{50} values as previously described.[18a]

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Supporting Information Available: General aspects and instrumentation; procedures and characterization data for **15a**−**20b**, **22**, **23**, **25a**, **25b**, and **11**; reproductions of NMR spectra; and ORTEP diagrams and tables of X-ray data for **18a**, methyl trachyloban-19-oate, and azatrachlobane picrate salt (**24**). This material is available free of charge via the Internet at http://pubs.acs.org.

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