Synthesis and Evaluation of Cyclobutylcarbinyl Derivatives as Potential Intermediates in Diterpene Biosynthesis

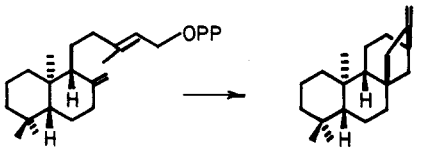
Robert M. Coates* and Han-Young Kang

Department of Chemistry, University of Illinois, Urbana, Illinois 61801

Received October 22, 1986

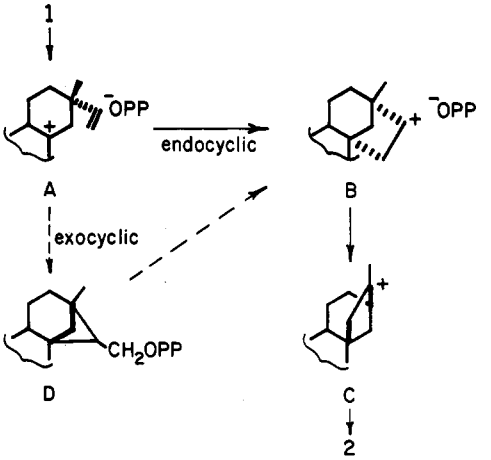
A new mechanism for the enzyme-catalyzed bicyclization of copalyl pyrophosphate (1) to kaurene (2) and related bridged perhydrophenanthrene-type diterpenes is considered. The key steps in the mechanism are an exocyclic vinyl group ring closure of the pimar-15-en-8-yl carbocation to a *D*-norbeyerane-15-methyl intermediate (D) and a subsequent ring expansion (A → D → B) instead of the usual endocyclic pimarenyl → beyeranyl cyclization (A → B). Beyeran-16-one (7), prepared in six steps from isosteviol methyl ester (**5b**), was converted to 16-diazobeyeran-15-one (10) via the 15,16-dione. Irradiation of the diazo ketone afforded an exo–endo mixture of *D*-norbeyerane-15-carboxylic acids or esters (11 and 12). The isomeric esters were separated by selective hydrolysis and reduced to the *exo*- and *endo*-cyclobutylcarbinols (13-OH and 14-OH). Acetolysis of the corresponding tosylates initiated a ring-expansion rearrangement to beyerene, kaurene, and isokaurene, as well as fragmentation to 7,15-, 8,15-, and 8(14),15-pimaradienes (Scheme III). However, the lack of incorporation of either [^{3}H]-13-OPP or [^{3}H]-14-OPP into kaurene upon incubation with a kaurene synthetase preparation from *Marah macrocarpus* ruled out the *exo*- and *endo*-cyclobutylcarbinyl pyrophosphates as free intermediates in the cyclization catalyzed by this enzyme.

The biosynthesis of the bridged perhydrophenanthrene-type diterpenes including beyerene, kaurene (2), and trachylobane[1] involves a novel enzyme-catalyzed bicyclization of the intermediate, copalyl pyrophosphate (1).[2] The usual mechanism proposed for this process is based upon the original Wenkert biogenetic pathway to the tri- and tetracyclic diterpenes.[3] In the case of kaurene, this consists of the following four steps: S_N' cyclization of 1 to the pimarenyl ion (A), vinyl group cyclization to the beyeranyl ion (B), Wagner–Meerwein rearrangement to the kauranyl ion (C), and termination by proton elimination to form the exocyclic methylene group.[4]



1 2

The lack of success in achieving cyclizations analogous to the pimarenyl → beyeranyl step (A → B) in model reactions with diterpene substrates[5-10] has, however, raised questions regarding the validity of this mechanism.[6,7] The apparent reluctance of vinyl group cyclizations to occur upon the C-8 position may be attributed to poor orbital overlap in the transition state for this endocyclic ring closure[12] and to the fact that a stable tertiary carbocation must be converted to a secondary one.



An alternative mechanism[13] can be conceived in order to avoid this violation of Baldwin's rules for ring closure.[12] An exocyclic bridging of the pimarenyl ion between C-8 and C-15 would give rise to a cyclobutylcarbinyl inter-

(1) The diterpenes and their derivatives described in this paper, with one exception (reference sample of pimara-8(14),15-diene) belong to the enantiomeric series having the 10α methyl configuration as shown in the structures. However, the *ent* descriptor is omitted from the names of the diterpenes in the discussion section for convenience. The complete systematic names with the *ent* descriptors are given in the headings in the Experimental Section. With this exception, the names and positional numbers used throughout this paper conform to the recommendations (*The Common and Systematic Nomenclature of Cyclic Diterpenes*, 3rd ed.; Oct 1968; Addenda and Corrigenda, Feb 1969) prepared by J. W. Rowe (Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, Madison, WI 53705).

(2) West, C. A. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, Chapter 7.

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

(4) For previous investigations from this laboratory on the stereochemistry of the enzyme-catalyzed cyclization, see: (a) Coates, R. M.; Cavender, P. L. *J. Am. Chem. Soc.* **1980**, *102*, 6358. (b) Coates, R. M.; Koch, S. C.; Hegde, S. *Ibid.* **1986**, *108*, 2762.

(5) For a review, see: Coates, R. M. *Progr. Chem. Org. Nat. Prod.* **1976**, *33*, 73.

(6) (a) Blunt, J. W.; Boyd, G. S.; Hartshorn, M. P.; Munro, M. H. G. *Aust. J. Chem.* **1976**, *29*, 987. (b) Blunt, J. W.; Ditzel, E. J.; Hartshorn, M. P.; Hickey, B. J.; Johnstone, P. K.; Munro, M. H. G.; Robinson, W. T. *Ibid.* **1981**, *34*, 2475. (c) Blunt, J. W.; Ditzel, E. J.; Hartshorn, M. P.; Sieng, L. H.; Munro, M. H. G.; Robinson, W. T. *Tetrahedron Lett.* **1981**, *22*, 1923.

(7) ApSimon, J. W.; Hall, S. F. *Can. J. Chem.* **1978**, *56*, 2156.

(8) (a) Shmidt, E. N.; Gatilov, Yu. V.; Osadchii, S. A.; Korchagina, D. V.; Bardina, N. M.; Polovinka, M. P.; Shevtsov, S. A.; Barkhash, V. A. *Zh. Org. Khim.* **1981**, *17*, 1553; *J. Org. Chem. USSR (Engl. Trans.)* **1981**, *17*, 1380. (b) Vlad, P. F. *Izv. Akad. Nauk MSSR, Ser. Biol. Khiml, Nauk* **1977**, 67.

(9) (a) Taran, M.; Delmond, B. *J. Chem. Soc., Chem. Commun.* **1984**, 716. (b) Delmond, B.; Taran, M.; Valade, J. *Tetrahedron Lett.* **1980**, *21*, 1339.

(10) An analogous vinyl group cyclization of the monocyclic model, 1,3-dimethyl-3-vinylcyclohexyl carbocation, has recently been realized: Coates, R. M.; Kang, H.-Y. *J. Chem. Soc., Chem. Commun.* **1987**, 232.

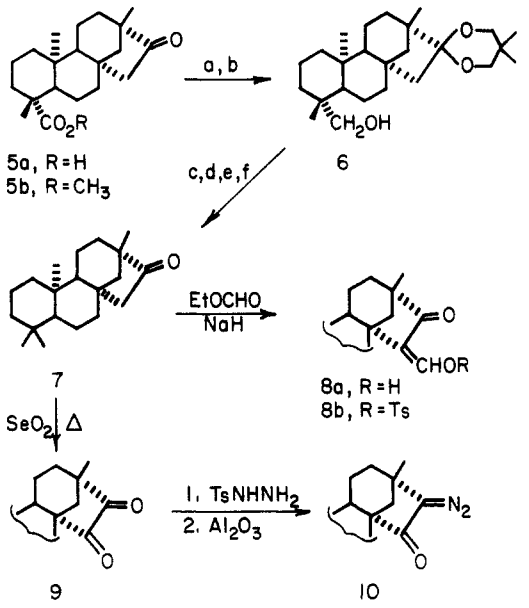
(11) Vinyl group cyclizations onto C-9 of pimaren-9-yl carbocations related to those that presumably occur in the biosynthesis of the tetracyclic diterpenes, aphidicolin and stemodin, have been reported.[8a,9a]

(12) (a) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734. (b) Baldwin, J. E.; Cutting, J.; Dupont, W.; Kruse, L.; Silberman, L.; Thomas, R. C. *Ibid.* **1976**, 736.

(13) This cyclobutylcarbinyl mechanism for the vinyl group cyclization was suggested to the senior author by Professor Duilio Arigoni, ETH, Zurich, Switzerland.

0022-3263/87/1952-2065$01.50/0

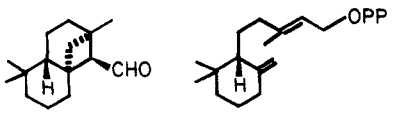
Scheme I



[a] Reagents: (a) 2,2-dimethylpropane-1,3-diol, TsOH; (b) $LiAlH_4$; (c) CH_3SO_2Cl, Et_3N; (d) PhSNa, DMF, Δ; (e) Li, NH_3; (f) H_3O^+.

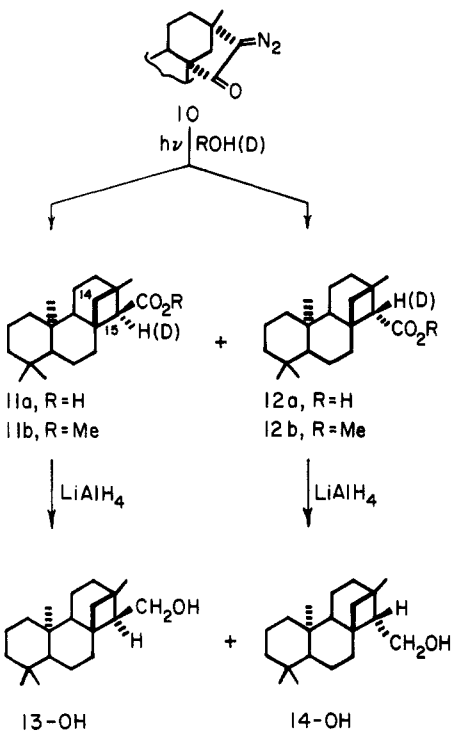
mediate, which could undergo ring expansion to same beyeranyl ion (A → D → B). The more favorable orbital overlap attainable in this 4-*exo-Trig* cyclization[12] is, however, offset to some degree by the strain energy of the cyclobutane ring and by the formation of a primary carbocation. The electronic destabilization could perhaps be diminished by tight ion pairing with the pyrophosphate anion enforced by the active site of the cyclase. Collapse of the ion pair at this point would give rise to cyclobutylcarbinyl pyrophosphate D, a new potential intermediate in the enzymatic cyclization.

The credibility of this cyclobutylcarbinyl pathway is strengthened by the following literature precedents. Internal return of pyrophosphate anion is known to occur in enzyme-catalyzed allylic rearrangements and cyclizations associated with mono- and sesquiterpene biosynthesis.[14,15] An X-ray crystal analysis revealed that the marine sesquiterpene acanthodoral has a bridged cyclobutanecarboxaldehyde structure 3 analogous to D.[16] Acanthodorol might arise from *trans*-γ-monocyclofarnesyl pyrophosphate (4)[16,17] by S_N' and exocyclic vinyl group cyclizations analogous to those above, although other pathways can certainly be conceived. Solvolysis of *exo*- and *endo*-bicyclo[3.1.1]heptane-6-methyl tosylates leads to efficient ring expansion and formation of bicyclooctyl products.[18]



3 4

Scheme II



In this paper we report the synthesis of the *exo*- and *endo*-cyclobutylcarbinols corresponding to D, a study of the solvolytic rearrangements of the derived tosylates as a biogenetic model reaction, and the evaluation of the isomeric pyrophosphates as actual intermediates in the enzymatic cyclization of copalyl pyrophosphate to kaurene.

Synthesis of *D*-Norbeyerane-*exo*- and -*endo*-15-methanols (13-OH and 14-OH). The synthesis of the *exo*- and *endo*-cyclobutylcarbinols from beyeran-16-one (7) (Scheme I) was modeled after that used to prepare the simple bicyclic analogues, i.e., the bicyclo[3.1.1]heptane-6-methanols.[19] The available diterpene glucoside, stevioside, was extracted from leaves and stems of *Stevia rebaudiana* Bertoni[20] and its sugar residues were hydrolyzed with concomitant rearrangement of ring D according to literature procedures[21,22] to give isosteviol (**5a**, 2.3–3%), which was esterified with diazomethane. Protection of the ring D ketone of isosteviol methyl ester (**5b**) as the dimethyldioxane followed by reduction with lithium aluminum hydride afforded ketal alcohol **6** (78–89%).[23] The hydroxyl group at the hindered C-19 position was conveniently removed by lithium ammonia reduction of the corresponding phenylthio ether according to the procedures of Crossley and Dowell.[24] The known beyeran-16-one (7)[25] was liberated by hydrolysis of the ketal. Condensation of 7 with ethyl formate in the presence of sodium

(14) For reviews, see: (a) Croteau, R. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W., Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, Chapter 5. (b) Cane, D. E., ref 14a, Chapter 6. (c) Cane, D. E. *Tetrahedron* **1980**, *36*, 1109.

(15) For recent articles, see: (a) Cane, D. E.; Saito, A.; Croteau, R.; Shaskus, J.; Felton, M. *J. Am. Chem. Soc.* **1982**, *104*, 5831. (b) Cane, D. E.; Iyengar, R.; Shiao, M.-S. *Ibid.* **1981**, *103*, 914.

(16) Ayer, S. W.; Anderson, R. J.; Cun-heng, H.; Clardy, J. *J. Org. Chem.* **1984**, *49*, 2653.

(17) (a) Suzuki, K. T.; Nozoe, S. *Bioorg. Chem.* **1974**, *3*, 72. (b) Suzuki, K. T.; Suzuki, N.; Nozoe, S. *J. Chem. Soc. D* **1971**, 527.

(18) Wiberg, K. B.; Hess, B. A., Jr. *J. Am. Chem. Soc.* **1966**, *88*, 4433.

(19) Wiberg, K. B.; Hess, B. A., Jr. *J. Org. Chem.* **1966**, *31*, 2250.

(20) The plant material was purchased from Empresas Agro-Industriales, 25 de Mayo 993, Asuncion, Paraguay, through the agency of Mr. Luis Enrique de Gasperi.

(21) (a) Wood, H. B., Jr.; Allerton, R.; Diehl, H. W.; Fletcher, H. G., Jr. *J. Org. Chem.* **1955**, *20*, 875. (b) Ruddat, M.; Heftmann, E.; Lang, A. *Arch. Biochem. Biophys.* **1965**, *110*, 496. (c) Mosettig, E.; Beglinger, U.; Dolder, F.; Lichti, H.; Quitt, P.; Waters, J. A. *J. Am. Chem. Soc.* **1963**, *85*, 2305.

(22) (a) Coates, R. M.; Bertram, E. F. *J. Org. Chem.* **1971**, *36*, 2625. (b) Bertram, E. F., Ph.D. Thesis, University of Illinois, Urbana, 1970.

(23) This reaction sequence for converting isosteviol methyl ester to beyeran-16-one was developed earlier in our laboratory by David A. Ley: Ph.D. Thesis, University of Illinois, Urbana, 1976.

(24) Crossley, N. S.; Dowell, R. *J. Chem. Soc. C* **1971**, 2496.

(25) Kitahara, Y.; Yoshikoshi, A. *Tetrahedron Lett.* **1964**, 1771.

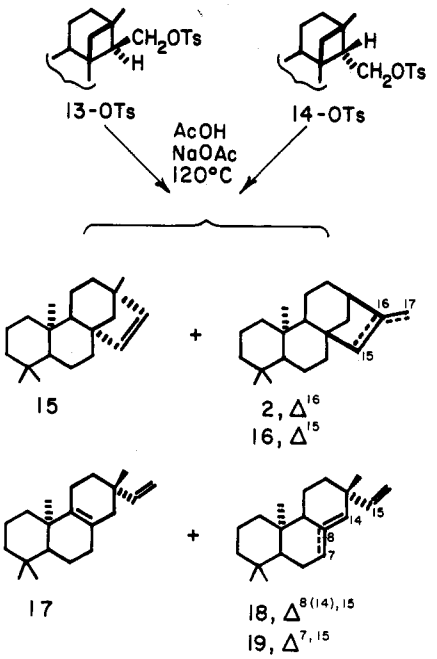
hydride, or sodium and potassium hydrides together,[26] in tetrahydrofuran (THF) at room temperature was sluggish and at best 44–49% yields of the α-hydroxymethylene ketone **8a** were realized. Attempts to effect diazo transfer[27] by reaction of **8a** with tosyl azide gave instead the enol tosylate **8b**. A small amount of 15-diazobeyeran-16-one (isomer of **10**) was obtained by direct condensation of **7** with 2,4,6-triisopropylbenzenesulfonyl azide with beyeranone according to the procedure of Lombardo and Mander.[28]

A satisfactory synthesis of the isomeric α-diazo ketone was achieved via beyerane-15,16-dione (**9**). Oxidation of **7** with selenium dioxide[29] in refluxing xylene for 42 h gave the orange α-diketone (**9**) in 83–94% yield. Condensation of **9** with tosylhydrazine in chloroform gave a single monotosylhydrazone, which underwent elimination of toluenesulfinic acid upon adsorption on basic alumina.[30] The resulting 16-diazobeyeran-15-one (**10**, 65–76%) was clearly different from the diazo ketone mentioned previously. Thus, condensation of **9** with tosylhydrazine occurred at the less hindered 16 position.

Photochemical Wolff rearrangement of **10** in aqueous dioxane effected ring contraction to the epimeric cyclobutanecarboxylic acids **11a** and **12a** (Scheme II). Esterification with diazomethane provided a 63:37 mixture of the exo and endo esters **11b** and **12b** (77% from **10**). In contrast, irradiation of **10** in methanol afforded **11b** and **12b** (64%) with a reversed isomer ratio (16:84). Similar solvent effects on the exo/endo product ratios from photochemical Wolff rearrangements have been reported.[31] Separation of the exo/endo isomers was accomplished by selective hydrolysis of the less hindered exo ester with 1.3 M sodium hydroxide in aqueous methanol at reflux temperature, extraction of the exo acid, and reesterification.

The stereochemistry of the carboxyl group at C-15 was readily assigned from the ^{1}H NMR spectra of **11b** and **12b**. The proton at C-15 in the more readily hydrolyzed ester (longer t_R on GC) appears as a doublet at δ 2.79 (^4J = 4.8 Hz). This splitting results from long-range W-coupling between the endo protons on the bridging carbons of the cyclobutane ring and is characteristic of bicyclo[*n*.1.1]alkanes bearing exo substituents on the 1-carbon bridge (^4J = 5.5–6.0 Hz for *n* = 3).[19,32] On the other hand, the ^{1}H NMR spectrum of the hydrolytically stable isomer exhibits a singlet at δ 2.01 for the α-proton at C-15. The assignments of these peaks to the protons α to the ester in **11b** and **12b** was verified by preparation of the α-deuterio esters via irradiation of **10** in deuterium oxide–dioxane and subsequent esterification and separation. The relevant peaks are absent from the ^{1}H NMR spectra of the resulting monodeuterated esters. The resonances for the exo and endo protons of the cyclobutane methylene group (C-14) of the exo ester were located by homonuclear decoupling. Irradiation at δ 2.79 caused the doublet of doublets at δ 1.05 (endo H at C-14, J = 8.8 Hz) to collapse to a doublet (J = 8.8 Hz). Irradiation at δ 2.10 collapsed the same multiplet to a doublet (J = 4.8 Hz). The 8.8-Hz geminal coupling constant is similar to data reported for bicyclo[3.1.1]heptanes (J_{gem} = 8.5–9.5 Hz).[19,32] Reduction of **11b** and **12b** with lithium aluminum hydride in ether afforded the desired exo and endo cyclobutylcarbinols, **13**-OH and **14**-OH, as crystalline solids in 85% yield. The corresponding tosylates, **13**-OTs and **14**-OTs, were prepared in the usual way.[33]

Scheme III



Solvolytic Rearrangements. The solvolysis of **13**-OTs and **14**-OTs was investigated as a chemical model reaction for the conversion of the cyclobutylcarbinyl pyrophosphate to the beyeranyl ion (D → B). Acetolysis of the exo and endo tosylates in acetic acid buffered with sodium acetate at 120 °C for 10–20 min gave mixtures of seven hydrocarbon products (86%), six of which were formed from both tosylates (Scheme III). The small acetate fractions (14%) contained many components and were not examined further.

Three of the six hydrocarbons common to both product mixtures were isolated in a state of high purity by column chromatography on silica gel and/or silica gel impregnated with silver nitrate. They were securely identified as beyerene (**15**), kaurene (**2**), and pimara-8,15-diene (**17**) by comparison of 360-MHz ^{1}H NMR spectra, mass spectra, and GC retention times with authentic samples,[34] or in the case of **17** with literature data.[35] Although isokaurene (**16**) could not be obtained free of beyerene, it was readily identified by a 360-MHz ^{1}H NMR spectrum of a 1:2 mixture. The assignment was confirmed by GC and MS comparisons with an authentic sample.[36] The identification of pimara-8(14),15-diene (**18**) is somewhat tentative, being based only upon retention time and GC/MS comparisons with an authentic sample of its enantiomer.[37]

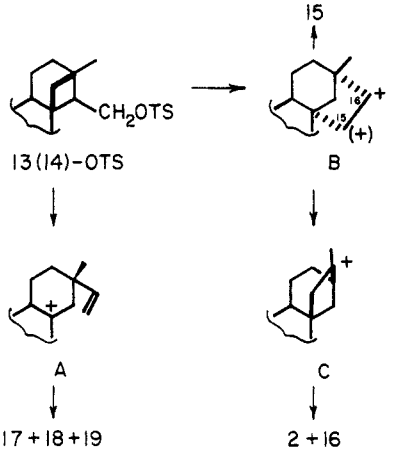
(26) Ruest, L.; Blouin, G.; Deslongchamps, P. *Synth. Commun.* **1976**, *6*, 169.
(27) (a) Regitz, M. *Synthesis* **1972**, 351. (b) Regitz, M.; Ruter, J.; Liedhegener, A. *Org. Synth.* **1971**, *51*, 86.
(28) Lombardo, L.; Mander, L. N. *Synthesis* **1980**, 368.
(29) Rabjohn, N. *Org. React. (N.Y.)* **1976**, *24*, 261.
(30) Muchowski, J. M. *Tetrahedron Lett.* **1966**, 1773.
(31) (a) Gibson, T.; Erman, W. F. *J. Org. Chem.* **1966**, *31*, 3028. (b) Meinwald, J.; Jensen, C. B.; Lewis, A.; Swithenbank, C. *Ibid.* **1964**, *29*, 3469.
(32) Wiberg, K. B.; Lowry, B. R.; Nist, B. J. *J. Am. Chem. Soc.* **1962**, *84*, 1594.
(33) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol. 1, p 1180.
(34) (a) The reference sample of beyerene was prepared by deoxygenation of beyer-15-en-19-ol (monogynol) as described above for **6**.[23] A sample of monogynol was kindly contributed by Professor C. A. West, University of California, Los Angeles, who in turn obtained it from Dr. S. Dev, National Chemical Laboratory, Poona, India. (b) The reference sample of (−)-kaurene was provided by the Abbott Laboratories, North Chicago, IL.
(35) (a) Dockerill, B.; Hanson, J. R. *J. Chem. Soc., Perkin Trans. 1* **1977**, 324. (b) Edwards, O. E.; Rosich, R. S. *Can. J. Chem.* **1968**, *46*, 1113.
(36) Isokaurene was isolated from a mixture of kaurene and isokaurene obtained by dehydration of kauran-16β-ol. See ref 4b and S. S. Canan, M.S. Thesis, University of Illinois, Urbana, 1984.

Table I. Distribution of Identified Hydrocarbon Products from Acetolysis of *D*-Norbeyerane-*exo*- and -*endo*-15-methyl Tosylates (13-OTs and 14-OTs)[a,b]

tosylate	beyerene (15)	isokaurene (16)	kaurene (2)	pimaradienes 17	18	19
exo[c] (13-OTs)	52	27	8	6	3	4
endo[c] (14-OTs)	31	12	5	28	8	15

[a] Acetolysis conditions: 1 equiv of NaOAc, 120 °C, 20 min. [b] Determined by GC analysis on a 30-m DB-5 fused silica capillary column at 160 °C. [c] Two unidentified minor hydrocarbon products appeared in the GC chromatograms: t_R 32.1 min, ~1% (from exo); t_R 25.8 min, ~1% (from endo).

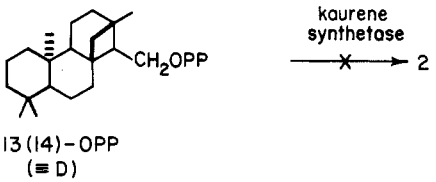
The structure of the sixth hydrocarbon as pimara-7,15-diene (**19**) was deduced from an ^1H NMR spectrum of a three-component mixture with beyerene and isokaurene. The ABX pattern for the vinyl group and the chemical shifts of the four quaternary methyl groups correspond to the literature values for its enantiomer.[38]



The distributions of the six identified products from the exo and endo tosylates are shown in Table I. Control experiments demonstrated that the four products for which authentic samples were available (**2**, **15**, **16**, and **18**) are stable to the acetolysis conditions. The similar ratios of the three tetracyclic products (**15**:**16**:**2**) and the three pimaradienes (**17**:**18**:**19**) indicate that each product group probably arises from a common carbocation precursor. The proposed biogenetic ring expansion to the beyeranyl ion is a major reaction pathway (87% from exo; 48% from endo). Kaurene and isokaurene are clearly formed by Wagner–Meerwein rearrangement of the beyeran-16-yl carbocation. However, beyerene might arise from either the beyeran-15- or -16-yl precursors. The similar ratio of the three products indicates that ring expansion of the epimeric tosylates gives either the same proportion of the two beyeranyl ions or exclusively the beyeran-16-yl ion. The greater extent of ring fragmentation from the endo tosylate (51% from endo, 13% from exo) is probably caused by relief of a steric interaction between the carbinyl carbon and the axial hydrogen at C-11. The predominant, if not exclusive, fragmentation pathway involves cleavage of the 8–15, rather than the 13–15, bond since the single unidentified component in both product mixtures was present to the extent of only at 1%. This regioselectivity may have its origin in bond angle distortions arising from fusion of the B ring onto the bicyclo[3.1.1]heptane moiety.

The principal differences between the acetolysis reactions of **13**-OTs and **14**-OTs and those of the corresponding bicyclo[3.1.1]heptane-6-methyl tosylates[18] are the occurrence of ring fragmentation and the absence of products formed via 12,16-hydride shift with the diterpene substrates. The fragmentation pathway is more favorable owing to alkyl substitution at the bridgehead position so that ring cleavage leads to a tertiary carbocation. The lack of facile 12,16-hydride shift from the beyeranyl carbocation (B) under kinetically controlled solvolysis conditions is attributable to the rigid chair conformation of the C-ring, which restrains the 12α proton to an equatorial position. Similarly solvolysis of beyeranyl derivatives under kinetically controlled conditions does not lead to 12,16-hydride shift.[39]

Experiments with Kaurene Synthetase. Tritium-labeled compounds were required for experiments to test whether either of the cyclobutylcarbinyl pyrophosphates is an intermediate in kaurene biosynthesis. Oxidation of **13**-OH and **14**-OH with pyridinium chlorochromate gave the corresponding aldehydes, which were reduced with sodium borotritide to the tritium-labeled alcohols. The pyrophosphates **13**-OPP and **14**-OPP were prepared by phosphorylation with phosphate ion and trichloroacetonitrile according to the procedure of Cramer and Rittersdorf[40a,b] and they were isolated by ion exchange chromatography.[41,42] A crude enzyme extract rich in kaurene synthetase activity was isolated from the endosperm of *Marah macrocarpus* seeds by differential centrifugation. The activity of the enzyme preparation was verified by incubation with [^3H]copalyl pyrophosphate ([^3H]-**1**), which resulted in incorporation of 31% of the radioactivity into kaurene. In contrast, incubation of either [^3H]-**13**-OPP or [^3H]-**14**-OPP with the enzyme extract lead to negligible incorporation (<1%) of tritium into kaurene.



The straight-forward, and most likely, conclusion is that neither of the cyclobutylcarbinyl pyrophosphates is an intermediate in this enzyme-catalyzed cyclization. However, the possibility that **13**-OPP (or **14**-OPP) is produced as a transient, enzyme-bound intermediate incapable of exchanging with exogenous pyrophosphate in solution cannot be excluded. For example, the cyclization of geranylgeranyl pyrophosphate catalyzed by kaurene synthetase from *Fusarium moniliforme* is known to occur with little or no exchange with exogenous copalyl pyrophosphate under some conditions.[41b] Another conceivable scenario is exocyclic ring closure of A to a cyclobutylcarbinyl intermediate that becomes covalently linked to a nucleophilic group on the enzyme, instead of the pyrophosphate ion.

(37) The authentic sample of pimara-8(14),15-diene was prepared from pimaric acid by esterification, reduction with lithium aluminum hydride, and deoxygenation as described above for **6** and beyer-15-en-19-ol.[23,34a] We are grateful to Dr. D. F. Zinkel, USDA Forest Products Laboratory, Madison, WI, for a sample of pimaric acid.

(38) Buckwalter, B. L.; Burfitt, I. R.; Felkin, H.; Joly-Goudket, M.; Naemura, K.; Saloman, M. F.; Wenkert, E.; Wovkulich, P. M. *J. Am. Chem. Soc.* **1978**, *100*, 6445.

(39) Coates, R. M.; Bertram, E. F. *J. Org. Chem.* **1971**, *36*, 3722.

(40) (a) Cramer, F.; Bohm, W. *Angew. Chem.* **1959**, *71*, 775. (b) Cramer, F.; Rittersdorf, W. *Tetrahedron* **1967**, *23*, 3015. (c) Edmond, J.; Popjak, G.; Wong, S.-M.; Williams, V. P. *J. Biol. Chem.* **1971**, *246*, 6254.

(41) (a) Sofer, S. S.; Rilling, H. C. *J. Lipid Res.* **1969**, *10*, 183. (b) Fall, R. R.; West, C. A. *J. Biol. Chem.* **1971**, *246*, 6913.

(42) The procedures for preparation and isolation of the pyrophosphates were previously used in this laboratory by D. A. Ley; see ref 23.

This "X-group mechanism"[44] would continue by ring expansion of the enzyme-linked cyclobutylcarbinyl intermediate in the same manner as the pyrophosphate.

In summary, this investigation has shown that *exo*- and *endo*-*D*-norbeyerane-15-methyl tosylates, 13-OTs and 14-OTs, undergo ring expansion and Wagner–Meerwein rearrangements in accord with the new mechanism for tetracyclic diterpene biogenesis presented above. However, since the corresponding cyclobutylcarbinyl pyrophosphates were not rearranged to kaurene by kaurene synthetase from *M. macrocarpus*, they are evidently not free intermediates in the cyclization of copalyl pyrophosphate catalyzed by this enzyme.

Experimental Section

General Aspects. Melting points were determined in either open-ended capillary tubes with a Thomas-Hoover melting point apparatus or on a Reichert hot stage microscope and are uncorrected. ^{1}H NMR spectra were obtained with one of the following: a Varian EM-390 (90 MHz), a Varian XL-200 (200 MHz), or a Nicolet NT-360 (360 MHz) spectrometer. ^{13}C NMR were determined with a Varian XL-200 (50.3 MHz) or a Nicolet NT-360 (90.5 MHz). IR spectra were obtained with a Perkin-Elmer 137 or a Nicolet 7199 FT-IR. Mass spectra were recorded on a Varian-MAT CH-5, 311A (GC/MS), or 731 mass spectrometer by Carter Cook and associates. Elemental analyses were performed by J. Nemeth and associates in the University of Illinois Microanalytical Laboratory. Optical rotations were obtained by using an Autopel III polarimeter.

Analytical gas chromatography was performed on a Varian Model 3700 instrument equipped with a flame ionization detector using helium as the carrier gas. The following standard operating conditions were used: 230 °C injector temperature, 340 °C detector temperature. Normal analyses were carried out by using the following columns: (A) 1.8 m × 6.4 mm glass packed with 3% OV-17 on 100/120-mesh Chromosorb Q or (B) 3.6 m × 6.4 mm glass column packed with 3% OV-17 on 100/120-mesh chromosorb Q. Analyses were performed isothermally at temperatures between 200 and 250 °C as appropriate. GC analyses of the solvolysis products were performed isothermally on a 30-m, DB-5 fused silica capillay column between 155 and 250 °C as appropriate (usually 160 °C).

Flash chromatography[45] was carried out on Woelm 32–64-μm silica packed in glass columns. Analytical thin-layer chromatography (TLC) was conducted on either Brinkman Polygram plastic plates precoated with 0.25 mm of silica gel GF-254 or on Merck glass plates precoated with 0.25 mm of silica gel 60 F-254. Preparative TLC was performed on Merck glass plates precoated with 2.0 mm of silica gel 60 F-254. Thin layer chromatograms were visualized with 5% phosphomolybdic acid reagent in 95% ethanol and/or UV light.

Specific activities (SA) were determined by liquid scintillation counting (LSC) on Tractor Analytical Beta Trac 6895 instrument. Either 6 g of 2,5-diphenyloxazole (PPO) in 1 L of toluene or "Aquasol" (supplied by New England Nuclear) was used as scintillation cocktail.

All reagents and solvents were reagent grade and used without further purification unless specified otherwise. Technical grade ethyl acetate, hexane, and pentane used for column chromatography were distilled prior to use. Tetrahydrofuran (THF), and diethyl ether, when used as solvents for reactions, were freshly distilled from sodium–benzophenone ketyl. Dimethylformamide (DMF) was stored over 4-Å molecular sieves, and triethylamine was distilled before use. All solutions and buffers used in the biochemical procedures were prepared with twice-distilled, deionized water. Ethereal diazomethane was generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald), supplied by Aldrich Chemical company, by using the procedure provided on the container.

***ent*-16-Oxobeyeran-19-oic acid (5a, isosteviol)** was isolated from 1 kg of dried leaves and stems of *Stevia rebaudiana* Bertoni[20] according to a literature procedure[21b] used previously in these laboratories.[22] The crude crystalline material was purified by preadsorption on 70 g of 0.05–0.2-mm silica gel and elution with 2–3 L of 5:1 hexane–ethyl acetate. The yield of crude isosteviol was 23–30 g (2.3–3.0%).

Methyl *ent*-16-Oxobeyeran-19-oate (5b, Isosteviol Methyl Ester). A solution of 13.3 g (84 mmol) of isosteviol (**5a**) in 250 mL of ether was stirred as ethereal diazomethane was added until the deep yellow color persisted. After nitrogen evolution ceased, the solution was heated on a steam bath briefly to expel the excess diazomethane and was evaporated. Recrystallization from acetone provided 13.1 g (95%) of isosteviol methyl ester (**5b**): mp 202–204 °C (lit.[22a] mp 202–203 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.69, 0.98, 1.19 (3 s, 9 H, 3 CH_3), 2.18 (bd, J = 13 Hz, 1 H), 2.63 (dd, 1 H, J = 3.7, 19 Hz, CH_2CO), 3.64 (s, 3 H, OCH_3). Anal. Calcd for $C_{21}H_{22}O_3$: C, 75.86; H, 9.70. Found: C, 75.90; H, 9.54.

***ent*-16-[(2,2-Dimethyl-1,3-propanediyl)dioxy]beyeran-19-ol (6)** was prepared according to procedures developed by Ley.[23] A solution of 19.7 g (59 mmol) of isosteviol methyl ester (**5b**), 10.3 g (68 mmol) of 2,2-dimethyl-1,3-propanediol, and 0.05 g (0.3 mmol) of *p*-toluenesulfonic acid in 100 mL of benzene was heated at reflux for 32–34 h until the theoretical amount of water was collected in a Dean–Stark trap, and the reaction was judged to be complete by TLC. Evaporation of the solvent gave a thick syrupy oil, which crystallized to a white solid on standing. This material was used for the next step without further purification. A solution of the crude ketal in 50 mL of ether was added dropwise to a well-stirred suspension of 2.1 g (55 mmol) of lithium aluminum hydride in 50 mL of ether. The mixture was stirred for 2 h at room temperature and heated at reflux for 45 min. Stirring was continued as 2 mL of water, 2 mL of 15% sodium hydroxide solution, and 6 mL of water were added in succession.[46] The solution was filtered and the salts were washed thoroughly with ether. Evaporation gave an oil that solidified upon standing. Recrystallization from pentane gave 18.1 g (78% from **5b**) of ketal alcohol **6**: mp 174–179 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.70 (s, 3 H, CH_3), 0.92 (s, 6 H, 2 CH_3), 0.97, 1.28 (2 s, 6 H, 2 CH_3), 2.18 (dd, 1 H, J = 3, 13 Hz), 3.1–3.8 (m, 6 H).

***ent*-Beyeran-16-one (7).** The reductive removal of the C-19 hydroxyl group was carried out by the procedures of Crossley and Dowell.[24] A solution of 1.10 g (2.8 mmol) of ketal alcohol **6** and 3.5 g (35 mmol) of triethylamine in 50 mL of pentane was stirred at room temperature as 500 mg (4.4 mmol) of methanesulfonyl chloride was added. The solution was allowed to stir for about 2 h until the TLC spot for the starting ketal alcohol had disappeared. The solution was diluted with pentane and washed with ice–water, 10% hydrochloric acid, saturated sodium carbonate, and saturated sodium chloride. Evaporation of the dried solution gave the methanesulfonate as an oil, which was used in the following reaction without further purification.

To a solution of 3.65 g (27 mmol) of sodium thiophenoxide[47] in 7 mL of dry DMF was addded a solution of the methanesulfonate ester in 5 mL of DMF at room temperature. The resulting solution was heated at 110–120 °C for 16–18 h. The solution was poured into 2 N hydrochloric acid solution and extracted with ether. The ether layer was washed with water, 2 N sodium hydroxide, and water and dried ($MgSO_4$). Evaporation followed by drying under reduced pressure overnight yielded 1.70 g of crude product. The phenylthio ether could be purified by chromatography; however, it was used in the following reaction without purification.

To a stirred solution of 0.2 g (27 mmol) of lithium in 30 mL of liquid ammonia was added a solution of the crude phenylthio ether in 5 mL of THF. After 1 h at −33 °C, ethanol was added dropwise until the blue color was discharged. The ammonia was allowed to evaporate, and after 2 h the resulting mixture was diluted with water and extracted with hexane. The combined hexane extracts were washed with water, 5% hydrochloric acid, and saturated sodium chloride and then evaporated. The resulting solid material was purifed by flash chromatography with 10%

(43) (a) West, C. A.; Upper, C. D. *Methods Enzymol.* **1969**, *15*, 481. (b) Hirano, S. D. Ph.D. Thesis, University of California, Los Angeles, 1976. (c) Sherwin, P. F. Ph.D. Thesis, University of Illinois, Urbana, 1982.
(44) Cornforth, J. W. *Angew. Chem., Intern. Ed. Engl.* **1968**, *7*, 903.
(45) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(46) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; Vol. 1, p 584.
(47) Sheehan, J. C.; Daves, G. D., Jr. *J. Org. Chem.* **1964**, *29*, 2006.

ethyl acetate in hexane as eluent to give 0.84 g of the ketal.

A mixture of 0.84 g (2.2 mmol) of the ketal and 0.15 g (0.87 mmol) of *p*-toluenesulfonic acid in 25 mL of dioxane, 25 mL of water, and 15 mL of ether was stirred at room temperature for 24 h. The mixture was extracted with ether, washed with saturated sodium bicarbonate and saturated sodium chloride, and evaporated. Purification of the resulting solid material by flash chromatography with 10% ethyl acetate in hexane as eluent afforded 530 mg (64% from ketal alcohol **6**) of beyeranone **7** as a white solid which was sufficiently pure according to GC and TLC analyses to be used in the following reactions without further purification. Recrystallization from hexane gave the analytical sample: mp 103.5–104.5 °C (lit.[25] mp 102–103 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.82, 0.98 (2 s, 6 H, 2 CH_3), 0.89 (s, 6 H, 2 CH_3), 1.96 (d, J = 20 Hz, 1 H), 2.89 (dd, 1 H, J = 4, 19 Hz, CH_2C=O); ^{13}C NMR (50 MHz, $CDCl_3$) δ 222.9 (C=O). Anal. Calcd for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 83.02; H, 11.23.

***ent*-15-(Hydroxymethylene)beyeran-16-one (8a).** To a stirred suspension of 325 mg (50% in mineral oil, 9.6 mmol) of sodium hydride and 500 mg (1.73 mmol) of ketone **7** in 5 mL of tetrahydrofuran was added 0.65 mL (8 mmol) of ethyl formate (stored over potassium carbonate and distilled over phosphorous pentoxide before use) at room temperature under nitrogen. Stirring was continued for 24 h, and the progress of the reaction was monitored by GC and TLC. Six additional 0.65-mL (8 mmol) portions of ethyl formate were added during the course of reaction. The mixture was poured into ice-cold water, acidified with 1 N hydrochloric acid solution, and extracted with ether. The organic layer was separated, washed with saturated sodium chloride with water, and dried ($MgSO_4$). Evaporation and purification of the resulting solid material by flash chromatography with 5% ethyl acetate in hexane as eluent provided 218 mg (40%) of α-hydroxymethylene ketone **8a**. Sublimation at 110–120 °C (0.1 mmHg) furnished the analytical sample: mp 100–102 °C; $[\alpha]^{22.5}_D$ –23.4° (*c* 0.145, CCl_4); IR (CCl_4) 1680, 1610 cm^{-1}; ^{1}H NMR (90 MHz, CCl_4) δ 0.86 (s, 6 H, 2 CH_3), 0.91, 0.99 (2 s, 6 H, 2 CH_3), 7.00 (s, 1 H, =CHOH). Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.63; H, 10.20.

Formylation of 250 mg of beyeranone by a similar procedure with sodium hydride and 2–3 drops of potassium hydride dispersion in mineral oil[26] for 54 h gave 134 mg (49%) of **8a**.

***ent*-15-[[(*p*-Tolylsulfonyl)oxy]methylene]beyeran-16-one (8b).** A solution of 100 mg (0.32 mmol) of hydroxymethylene ketone **8a** and 64 mg (1.62 mmol) of triethylamine in 5 mL of dichloromethane was stirred and cooled with an ice–salt bath as 62 mg (0.32 mmol) of *p*-toluenesulfonyl azide[48] in 1 mL of dichloromethane was added. The resulting solution was stirred at room temperature for 4–5 h after which 2.5 mL of 2 N aqueous potassium hydroxide solution was added. After 15 min the solution was diluted with dichloromethane, washed with 1 N potassium hydroxide and water, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography with 5% ethyl acetate in hexane as eluent. No α-diazo ketone could be detected by IR analysis. A solution of the product in hexane deposited crystalline enol tosylate **8b** upon cooling in a refrigerator overnight. This compound appeared to be the major product on TLC, although the yield was not measured. Recrystallization from hexane gave the analytical sample: mp 126–129 °C; ^{1}H NMR (CCl_4, 90 MHz) δ 0.73 (2 s, 6 H, 2 CH_3), 0.82 (s, 6 H, 2 CH_3), 2.43 (s, 3 H, CH_3), 7.80 and 7.31 (AB d, 4 H, J = 9 Hz, aromatic CH), 7.36 (s, 1 H, =CHO). Anal. Calcd for $C_{28}H_{38}O_4S$: C, 71.50; H, 8.14; S, 6.81. Found: C, 71.66; H, 8.21; S, 6.85.

***ent*-15-Diazobeyeran-16-one** was prepared from 250 mg (0.87 mmol) of beyeranone (**7**) according to the procedure of Mander and co-workers.[28] 2,4,6-Triisopropylbenzenesulfonyl azide, mp 41–43 °C (lit.[49] mp 41–43 °C) was prepared by an *Organic Syntheses* procedure.[48] Purification of the product by flash chromatography with 5% ethyl acetate in hexane as eluent gave 35 mg (13%) of the diazo ketone as a viscous liquid. Subsequent preparations on a 100-mg scale provided 24% and 34% yields, after purification by preparative TLC. The product was contaminated with 2,4,6-triisopropylbenzenesulfonamide (ca. 10% estimated by NMR). The following spectral data were obtained for the diazo ketone: IR (KBr) 2035 (C=N_2), 1677 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.76, 0.81, 0.88, 1.03 (4 s, 12 H, 4 CH_3).

***ent*-15,16-Beyeranedione (9)** was prepared by adaption of literature procedures.[29] A suspension of 800 mg (7.2 mmol) of selenium dioxide and 2.00 g (6.9 mmol) of **7** in 140 mL of xylene and stirred and heated at reflux. Stirring was continued for about 42 h until all the starting material was consumed. The progress of the reaction was followed by GC. Four or five 400-mg (3.6 mmol) portions of selenium dioxide were added during the course of reaction. The dark brown suspension was filtered through Celite. Evaporation of the filtrate and purification by flash chromatography with 7% ethyl acetate in hexane as eluent furnished 1.8 g (85%) of diketone **9** as a crystalline orange solid. Subsequent preparations on 500-mg (two runs) and 2.00-g scales gave 73%, 83%, and 94% yields, respectively. Recrystallization from pentane gave the analytical sample as orange needles: mp 215–219 °C; $[\alpha]^{22.5}_D$ –208 °C (*c* 0.51, $CHCl_3$); IR (KBr) 1752, 1732 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.74, 0.86, 0.90, 1.14 (4 s, 12 H, 4 CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 210.7, 209.3 (C=O); mass spectrum (70 eV), *m/e* (rel intensity) 302 (M^+, 6.9), 246 (60), 231 (51), 136 (100), 108 (43), 95 (42), 93 (37), 81 (51), 55 (42), 41 (59), 28 (52). Anal. Calcd for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.44; H, 9.95.

***ent*-16-[2-(*p*-Tolylsulfonyl)-1,1-diazenediyl]beyeran-15-one.** A solution of 1.79 g (5.9 mmol) of diketone **9** and 1.19 g (97%, 6.2 mmol) of (*p*-tolylsulfonyl)hydrazine in 200 mL of chloroform was stirred at room temperature for 30 h until the starting material had disappeared completely by TLC. Evaporation and flash chromatography with 10% ethyl acetate in hexane as eluent furnished 2.41 g (ca. 100 %) of the tosylhydrazone as a greenish, syrupy liquid that contained residual solvent: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.70, 0.84, 0.86, 1.07 (4 s, 12 H, 4 CH_3), 2.42 (1 s, 3 H, $ArCH_3$), 7.81 and 7.30 (AB d, 4 H, J = 8.1 Hz, aromatic CH), 12.09 (s, 1 H, NH).

***ent*-16-Diazobeyeran-15-one (10).** To solution of 2.41 g (5.9 mmol) of the tosylhydrazone in 200 mL of dichloromethane was added 100 g of aluminum oxide (Activity I, basic).[30] The mixture was stirred at room temperature for 62 h until all the starting material had reacted. Filtration of the alumina, evaporation of the filtrate, and purification by flash chromatography with 5% ethyl acetate in hexane as eluent yielded 1.47 g (79%) of a greenish yellow crystalline solid **10**. Subsequent preparations on 88-mg, 397-mg, and 2.34-g scales gave 82%, 70%, and 61% yields, respectively. Recrystallization from pentane furnished the analytical sample: mp 130–152 °C dec; $[\alpha]^{22.5}_D$ –75° (*c* 0.70, $CHCl_3$); IR (KBr) 2034 (C=N_2); 1677 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.84, 0.86, 0.87, 1.27 (4 s, 12 H, 4 CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) 203.5 (C=O). Anal. Calcd for $C_{20}H_{30}N_2O$: C, 76.39; H, 9.62; N, 8.91. Found: C, 76.14; H, 9.76; N, 8.83.

Methyl *ent*-*D*-Norbeyerane-*exo*- and -*endo*-15-carboxylate (11b and 12b). Method A. Irradiation in Water–Dioxane. A solution of 900 mg (2.9 mmol) of diazo ketone **10** in 60 mL of *p*-dioxane and 10 mL of water was placed in a 3 × 30 cm quartz test tube and deoxygenated with a slow stream of argon for 10 min. The quartz tube was suspended outside of the immersion well and was irradiated with a 450-W Hanovia mercury lamp for 8 h after which no UV active spot remained on a TLC plate. Nitrogen evolution was observed. Most of the dioxane and some water were removed by rotary evaporation. The remaining aqueous solution was acidified with 5% hydrochloric acid and extracted with ether. The combined ether extracts were washed with water, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography with 10% ethyl acetate in hexane as eluent, providing 685 mg (2.3 mmol, 79%) of carboxylic acid as a white solid. Esterification with diazomethane in ether and purification by flash chromatography with 10% ethyl acetate in hexane as eluent furnished 697 mg (77% from **10**) of a 63:37 mixture of **11b** and **12b** as an oily liquid.

Method B. Irradiation in Methanol. A solution of 193 mg (0.61 mmol) of diazo ketone **10** in 15 mL of methanol in a 1.6 × 30 cm quartz tube was deoxygenated with argon and irradiated with a 450-W Hanovia mercury lamp for 50 min as described in method A above. The course of the irradiation was checked every

(48) Regitz, M.; Hocker, J.; Liedhegener, A. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 179.

(49) Harmon, R. E.; Wellman, G.; Gupta, S. K. *J. Org. Chem.* **1973**, *38*, 11.

10 min by TLC. Evaporation of the colorless solution and purification of the residue by flash chromatography gave 125 mg (64%) of **11b** and **12b** as a viscous liquid that was a 16:84 mixture of exo and endo isomers by GC analysis.

C. Separation of Epimers. To 50 mL of a solution of sodium hydroxide–methanol–water (prepared by dissolving 3.75 g of sodium hydroxide in 60 mL of methanol and 15 mL of water) was added 697 mg (2.2 mmol) of the esters **11b** and **12b** (a 63:37 mixture of two epimers) with the aid of a minimum amount of ether. The mixture was stirred and heated at reflux for 14 h. The progress of the reaction was followed by GC. The peak height for the ester decreased and a new tailing peak for the carboxylic acid increased. After the hydrolytic separation was complete, the solution was acidified with 10% hydrochloric acid and extracted with ether. The combined ether extracts were washed with water, dried ($MgSO_4$), and evaporated. Purification by flash chromatography with 8% ethyl acetate in hexane as eluent provided 202 mg (29%) of unreacted endo ester as a solid (**12b**) and 388 mg (58%) of exo acid **11a** as a white solid: ^1H NMR ($CDCl_3$, 360 MHz) δ 0.82, 0.85, 0.97, 1.04 (4 s, 12 H, 4 CH_3), 2.01–2.11 (m, 1 H), 2.83 (d, 1 H, J = 4.7 Hz, $CHCO_2H$). The acid was converted to exo ester **11b** with diazomethane in ether. Sublimation of the esters at ca. 70 °C (0.01 mm) provided the analytical samples as white solids. The properties of the exo ester **11b** are as follows: mp 69–72 °C; $[\alpha]^{21.5}_D$ –27° (c 0.60, $CHCl_3$); IR (CCl_4) 1733 (C=O), 1158, 1142 (COC) cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.82, 0.84, 0.95, 0.98 (4 s, 12 H, 4 CH_3), 1.05 (dd, 1 H, J = 8.8 Hz and 4.8 Hz, endo-H at C-14), 1.91–1.85 (m, 1 H), 2.10 (d, 1 H, J = 8.8 Hz, exo-H at C-14), 2.79 (d, 1 H, J = 4.8 Hz, $CHCO_2CH_3$), 3.68 (s, 3 H, CO_2CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 174.3 (CO_2CH_3); mass spectrum (70 eV), m/e (relative intensity) 318 (M^+, 38), 303 (37), 244 (33), 137 (84), 123 (75), 95 (75), 81 (94), 69 (90), 55 (83), 41 (100). Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.17; H, 10.67. The assignments for the endo and exo protons at C-14 in the ^1H NMR spectrum are based upon homonuclear decoupling experiments (see Discussion section).

The properties of the endo ester **12b** are as follows: mp 69–71 °C; $[\alpha]^{22.5}_D$ +32° (c 0.60, $CHCl_3$); IR (CCl_4) 1733 (C=O), 1178, 1158 (COC) cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.73, 0.82, 0.85, 0.99 (4 s, 12 H, 4 CH_3), 1.90–1.95 (m, 1 H), 2.01 (s, 1 H, $CHCO_2CH_3$), 2.14–2.20 (m, 1 H), 3.62 (s, 3 H, CO_2CH_3); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 174.3 (CO_2CH_3); mass spectrum (70 eV), m/e (relative intensity) 318 (M^+, 22), 303 (61), 258 (32), 243 (46), 135 (51), 95 (65), 81 (75), 69 (87), 55 (78), 41 (100), 28 (81). Anal. Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.12; H, 10.70.

Methyl [15-^{2}H]-*ent*-*D*-Norbeyerane-*exo*- and -*endo*-15-carboxylates. Irradiation of 24 mg (0.076 mmol) of diazo ketone **10** in 8 mL of p-dioxane (distilled over lithium aluminum hydride before use) and 3 mL of deuterium oxide as described in the preceding procedure (method A) for **11b** and **12b** followed by esterification with diazomethane in ether provided 13 mg (56%) of a 51:49 mixture of the epimeric esters by GC analysis. The isomers were separated by selective hydrolysis and the deuteriated exo acid which was reesterified with diazomethane. The ^1H NMR spectra of the exo and endo esters show clearly the absence of the peaks for the α-protons at δ 2.79 and 2.01, respectively. Deuterium incorporations of 90% and 84% were observed for the exo and endo ester based on the intensity of the molecular ion in their MS.

***ent*-*D*-Norbeyerane-*exo*-15-methanol (13-OH).** A suspension of 80 mg (2.1 mmol) of lithium aluminum hydride in 10 mL of ether was stirred vigorously as a solution of 544 mg (1.7 mmol) of exo ester **11b** in 40 mL of ether was added. The mixture was stirred for 1.5 h at room temperature after which 0.1 mL of water, 0.1 mL of 15 sodium hydroxide solution, and 0.3 mL of water were added in succession.[46] The salts were removed by filtration and washed well with ether. Evaporation of the filtrate and purification of the residue by flash chromatography with 10% ethyl acetate in hexane as eluent gave 423 mg (85%) of the exo alcohol **13-OH** as a white solid: mp 77–81 °C; $[\alpha]^{22.5}_D$ +15° (c 0.55, $CHCl_3$); IR (KBr) 3400 (b, OH), 1012 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.82, 0.84, 0.85, 0.95 (4 s, 12 H, 4 CH_3), 1.80–1.86 (m, 1 H), 2.03–2.07 (m, 1 H), 3.78–3.93 (m, 2 H, CH_2OH); mass spectrum (10 eV), m/e (rel intensity) 290 (M^+, 28), 275 (60), 272 (43), 257 (42), 232 (40), 137 (100) 109 (61), 95 (57). Anal. Calcd for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.93; H, 11.51.

***ent*-*D*-Norbeyerane-*endo*-15-methanol (14-OH)** was prepared by reduction of 53 mg (0.17 mmol) of the endo ester **12b** with 5 mg (0.13 mmol) of lithium aluminum hydride in 20 mL of ether for 1 at room temperature as described in the preceding procedure. The yield was 43 mg (85%) of the endo alcohol **14-OH** as a white solid: mp 143–146 °C; $[\alpha]^{22.5}_D$ +18° (c 0.56, $CHCl_3$); IR (KBr) 3360 (br, OH), 1015 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.82, 0.84, 0.86, 0.99 (4 s, 12 H, 4 CH_3), 3.64–3.82 (m, 2 H, CH_2OH); mass spectrum (10 eV), m/e (rel intensity) 290 (M^+, 17), 275 (52), 259 (100), 257 (36), 149 (34), 137 (62), 123 (33), 109 (44), 95 (43), 81 (40). Anal. Calcd for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.53; H, 11.64.

***ent*-*D*-Norbeyerane-*exo*- and -*endo*-15-methyl *p*-toluenesulfonates (13-OTs and 14-OTs)** were prepared by the method of Schleyer.[33] A solution of 102 mg (0.35 mmol) of **13-OH** in 2 mL of pyridine was cooled in an ice bath, and 131 mg (0.69 mmol) of p-toluenesulfonyl chloride was added. The resulting solution was kept in a refrigerator overnight. The mixture was poured into 15 mL of ice–water with stirring and extracted with two portions of ether. The combined ethereal extracts were washed twice with cold 50% hydrochloric acid and water, dried (K_2CO_3-Na_2SO_4), and evaporated to give a 125 mg (80%) of the exo tosylate (**13-OTs**). A TLC analysis and the ^1H NMR spectrum verified the purity of the partially crystalline product: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.75, 0.77 (2 s, 6 H, 2 CH_3), 0.81 (s, 6 H, 2 CH_3), 2.46 (s, 3 H, $ArCH_3$), 4.20–4.25 (m, 2 H, CH_2OTs), 7.82 and 7.35 (ABd, 4 H, J = 8.4 Hz, aromatic CH).

The endo tosylate **14-OTs** was prepared by reaction of 44 mg (0.15 mmol) of endo alcohol **14-OH** in 4 mL of pyridine and 58 mg (0.31 mmol) of p-toluenesulfonyl chloride as described in the preceding procedure to provide 55 mg (82%) of the endo tosylate (**14-OTs**) as a white solid. The purity of the endo tosylate was verified by a TLC analysis and the ^1H NMR spectrum: ($CDCl_3$, 200 MHz) δ 0.66, 0.78, 0.82, 0.91 (4 s, 12 H, 4 CH_3), 2.46 (s, 3 H, $ArCH_3$), 4.11–4.18 (m, 2 H, CH_2OTs), 7.79 and 7.35 (AB d, 4 H, J = 8.3 Hz, aromatic CH).

Acetolysis of *ent*-*D*-Norbeyerane-*exo*-15-methyl *p*-Toluenesulfonate (13-OTs). A typical run was performed by the following procedure. A solution of 98.3 mg (0.221 mmol) of **13-OTs** in 5 mL of acetic acid buffered with 18 mg (0.22 mmol) of sodium acetate was stirred and heated at 120 °C for 20 min. The cooled solution was poured into 5 mL of cold water and the product was extracted with two portions of pentane and one portion of ether. The combined organic extracts were washed with water, saturated sodium bicarbonate, and water and dried ($MgSO_4$). The oily liquid (60.2 mg) remaining after evaporation was separated into three fractions by flash chromatography with 5% ethyl acetate in hexane:hydrocarbon fraction, 40.8 mg (68%); acetate fraction, 8.3 mg (11%); and recovered tosylate, 7.9 mg (8%). Capillary GC analysis (160 °C) of the hydrocarbon fraction showed seven discrete peaks all of which exhibited a molecular ion at m/e 272 in GC/MS analysis. The relative proportions of the hydrocarbons was determined from the GC chromatogram and the data are presented in Table I.

Identification of the products was accomplished by a combination of the results from several runs. The two major components in the hydrocarbon fraction were identified from a 10-min acetolysis with 120 mg (0.27 mmol) of tosylate **13-OTs**. In this case the flash chromatography provided three hydrocarbon fractions. The first was 8:1 mixture of the most abundant and the second most abundant products by GC analysis. The ^1H NMR spectrum and GC retention time by coinjection of the major component corresponded exactly with those of authentic beyerene (**15**, see below). The three hydrocarbon fractions were then combined and subjected to flash chromatography on 9 g of 5% silver nitrate impregnated silica gel. The separation was effected by elution with 40% hexane in dichloromethane (fractions 1–50), dichloromethane (fractions 50–80), and 50% dichloromethane in ethyl acetate (fractions 80–110). The 5-mL fractions were analyzed by TLC, GC, and ^1H NMR. Almost all of the hydrocarbon products appeared in four fractions (90–93). One fraction was a 2:1 mixture of beyerene and the second most abundant hydrocarbon. This product was identified as isokaurene (**16**) on the basis of comparisons of the ^1H NMR spectrum (see below) of the 2:1 mixture, GC retention time by coinjection, and GC/MS data with those of authentic isokaurene.[36] In addition, a small amount

of pure beyerene was isolated from a fraction that was a 78:22 mixture of beyerene and isokaurene. Selective epoxidation with a ca. 1 mg of *m*-chloroperoxybenzoic acid in 1–2 mL of dichloromethane converted all of isokaurene and some beyerene to the corresponding more polar epoxides, which allowed separation of pure beyerene by chromatography. The ^{1}H NMR spectrum obtained from this purified beyerene is identical with that of the authentic sample.

The other components were identified from the 20-min acetolysis described above. The hydrocarbon fraction (40.8 mg) was subjected to flash chromatography on 10 g of 5% silver nitrate impregnated silica gel with a similar elution gradient to that described above. A total of 100 5-mL fractions was collected and analyzed by TLC and GC.

Pure kaurene (**2**) appeared in fractions 41–47. The ^{1}H NMR and mass spectra as well as GC retention time by coinjection are identical with those of authentic kaurene.[34b] Some of the chromatographic fractions contained a compound that was pure by GC analysis. The structure of this product is assigned as *ent*-pimara-8,15-diene (**17**) based on comparison of its ^{1}H NMR and mass spectra with the corresponding data for its enantiomer.[35] The identification of *ent*-pimara-8(14),15-diene (**18**) is based upon direct GC/MS comparisons and coincidence of GC retention times with those of an authentic sample of its enantiomer (see below). Unfortunately the small amount of this product prevented its isolation in pure form; consequently GC and GC/MS comparisons were carried out with the unseparated hydrocarbon fraction. The sixth hydrocarbon is tentatively assigned as *ent*-pimara-7,15-diene (**19**) on the basis of analyses of a chromatographic fraction, which was a mixture of beyerene, isokaurene, and **19** (43:33:22 ratio by GC analysis). The ^{1}H NMR spectrum exhibits four methyl groups that correspond reasonably well with the values reported for its enantiomer.[38]

The following ^{1}H NMR spectral data were obtained in chloroform-*d* at 360 MHz, the GC/MS analyses were run at 70 eV, and the GC retention times were measured on a 30-m DB-5 capillary column at 160 °C (split ratio, 58:1; column flow rate, 1.0 mL He per min. The MS data of the products corresponds to those of the authentic samples with ±5 relative intensity units in most cases.

***ent*-Beyerene (15)**: t_R 27.4 min; ^{1}H NMR δ 0.74, 0.82, 0.86, 0.99 (4 s, 12 H, 4 CH_3), 5.69, 5.45 (AB d, 2 H, J = 5.7 Hz); mass spectrum, m/e (rel intensity) 272 (M^+, 60), 257 (19), 229 (7), 201 (5), 187 (10), 159 (14), 148 (39), 135 (87), 134 (100), 122 (52), 106 (62), 105 (61), 93 (67).

***ent*-Isokaurene (16)**: t_R 34.6 min; ^{1}H NMR δ 0.79, 0.84, 1.02, 1.72 (4 s, 12 H, 4 CH_3), 5.09 (s, 1 H, vinyl H); mass spectrum, m/e (rel intensity) 272 (M^+, 19), 257 (22), 244 (7), 229 (10), 187 (10), 175 (8), 163 (23), 119 (29), 106 (67), 94 (100).

***ent*-Kaurene (2)**: t_R 40.5 min; ^{1}H NMR υ 0.81, 0.85, 1.02 (3 s, 9 H, 3 CH_3), 2.63 (br s, 1 H), 4.73, 4.79 (2 s, 2 H, C=CH_2); mass spectrum, m/e (rel intensity) 272 (M^+, 48), 257 (100), 229 (52), 231 (26), 201 (15), 187 (22), 175 (21), 161 (27), 147 (54), 125 (64), 105 (69), 91 (75), 81 (68), 69 (88), 55 (65), 41 (86).

***ent*-Pimara-8,15-diene (17)**: t_R 28.1 min; ^{1}H NMR δ 0.84, 0.88, 0.93, 0.95 (4 s, 12 H, 4 CH_3), 4.85–4.94 (d of 3-line m, AB part of ABX, 2 H, vinyl CH_2), 5.81 (dd, 1 H, J = 17.5, 10.7 Hz, vinyl H); mass spectrum, m/e (rel intensity) 272 (M^+, 30), 257 (100), 230 (8), 215 (7), 201 (8), 187 (27), 175 (20), 161 (49), 133 (22), 119 (23), 105 (35), 69 (35), 41 (42).

***ent*-Pimara-8(14),15-diene (18)**:[53] t_R 29.1 min; mass spectrum, m/e (rel intensity) 272 (M^+, 22), 257 (33), 204 (4), 187 (5), 175 (6), 161 (7), 148 (12), 137 (100), 136 (40), 123 (25), 105 (18), 95 (28), 91 (23), 81 (34).

***ent*-Pimara-7,15-diene (19)**: t_R 31.1 min; ^{1}H NMR δ 0.79, 0.86, 0.90, 0.96 (4 s, 12 H, 4 CH_3); mass spectrum, m/e (rel intensity) 272 (M^+, 31), 257 (50), 230 (16), 187 (15), 175 (10), 161 (17), 148 (97), 133 (43), 124 (42), 119 (52), 109 (100), 105 (59), 41 (56).

Acetolysis of *ent*-Norbeyerane-*endo*-15-methyl *p*-Toluenesulfonate (14-OTs). A solution of 70.8 mg (0.159 mmol) of the endo tosylate in 5 mL of acetic acid buffered with 18 mg (0.22 mmol) of sodium acetate was heated at 120 °C for 20 min. Isolation of the product as described in the preceding procedure, and flash chromatography with 5% ethyl acetate in hexane as eluent provided 22.2 mg (51%) of hydrocarbon fraction, 4.6 mg (9%) of acetate fraction, and 10.9 mg (15%) of starting tosylate. Capillary GC analysis (160 °C) of the hydrocarbon fraction showed seven significant peaks. The retention times of six of the seven peaks are identical with those observed in the acetolysis of the exo tosylate. The hydrocarbon fraction was separated by flash chromatography on silver nitrate impregnated silica gel and the resulting fractions were analyzed by the same procedure as described in the preceding procedure. The same six diterpene hydrocarbons were identified by coincidence of GC retention times with coinjected authentic samples. The identifications of beyerene, isokaurene, kaurene, and *ent*-pimara-8,15-diene (**17**) were confirmed by obtaining ^{1}H NMR spectra of pure or mixed fractions as described above. The relative proportions of the hydrocarbons were determined from the relative area of the GC peaks and the data are presented in Table I.

Control Experiments. Solutions of 3–5 mg of beyerene (**15**), isokaurene (**16**), and pimaradiene (**18**) and 20 mg of kaurene (**2**) in 2–5 mL of acetic acid buffered with 1 equiv of sodium acetate were heated at 120 °C for 20 min. The hydrocarbon was reisolated as described for the acetolysis of **13**-OTs and analyzed by capillary GC (160 °C). No significant changes were observed.

***ent*-Beyerene (15).** An authentic sample of this diterpene was obtained by deoxygenation of 84 mg of monogynol[34a] in a manner similar to that described above for converting **6** to **7**. Purification of the organic soluble product from the lithium–ammonia reduction by flash chromatography on silica gel with hexane as eluent gave 11 mg (14% over three steps) of beyerene as an oil, presumably contaminated with some residual solvent. The purity of the authentic beyerene sample was verified by GC analysis. The ^{1}H NMR and mass spectra are identical with the major hydrocarbon isolated from acetolysis of **13**-OTs and the data are presented above. The ^{1}H NMR spectral data correspond to the literature values.[50]

Pimara-8(14),15-diene (18). Esterification of 150 mg of pimaric acid[37] with excess diazomethane in ether followed by reduction with 18 mg (0.47 mmol) of lithium alumninum hydride in 10 mL of ether (see preparation of **6**) afforded 113 mg (78%) of pimara-8(14),15-dien-18-ol. Without purification the white solid (63 mg) was deoxygenated according to the procedures detailed above for the conversion of **6** to **7**. The overall yield for the three-step sequence was 16 mg (27%). The purity of the pimara-8(14),15-diene reference sample was established by TLC and GC analyses. The mass spectrum corresponds well with that of the product with a retention time of 29.1 min in the hydrocarbon fraction from the acetolysis of **13**-OTs and the MS data are given above. The reported ^{1}H NMR spectral data[35b] for pimara-8(14),15-diene agree with the following data from the ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) of the product: δ 0.73, 0.84, 0.88, 0.99 (4 s, 12 H, 4 CH_3), 4.8–5.0 (m, 2 H, CH=CH_2), 5.12 (br, 1 H, vinyl H at C-14), 5.73 (dd, 1 H, J = 16.8, 10.8 Hz, CH=CH_2).

***ent*-*D*-Norbeyerane-*exo*-15-carboxaldehyde.** A suspension of 203 mg (0.94 mmol) of pyridinium chlorochromate and 16.1 mg (0.20 mg) of sodium acetate in 10 mL of dichloromethane was stirred at room temperature as a solution of 183 mg (0.63 mmol) of **13**-OH in 10 mL of dichloromethane was added. The black mixture was stirred for 2 h after which it was passed through a short Florisil column with the aid of ether. Evaporation and purification by flash chromatography with 5% ethyl acetate in hexane provided 111 mg (61%) of the exo aldehyde as a white solid: mp 50–60 °C; IR (KBr) 1712 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.80, 0.84, 0.93, 1.02 (4 s, 12 H, 4 CH_3), 1.98–2.11 (m, 1 H), 2.12 (d, 1 H, J = 9.6 Hz, exo-H at C-14), 2.63 (t, 1 H, J = 5.6 Hz, CHCHO), 10.18 (d, 1 H, J = 6.4 Hz, CHO); mass spectrum (70 eV), m/e (rel intensity) 288 (M^+, 41), 273 (66), 244 (21), 149 (28), 137 (78), 123 (64), 121 (52), 109 (55), 95 (71), 81 (82), 69 (69), 55 (70), 41 (100); exact mass calcd for $C_{20}H_{32}O$ m/e 288.2455, found 288.2453. Anal. Calcd for $C_{20}H_{32}O$: C, 83.27; H, 11.18. Found: C, 82.87; H, 11.34.

***ent*-*D*-Norbeyerane-*endo*-15-carboxaldehyde** was prepared by oxidation of 24.3 mg (0.084 mmol) of **14**-OH with pyridinium chlorochromate as described in the preceding procedure (1 h reaction time). The yield was 20.3 mg (84%) of the endo aldehyde as a transparent film-like material. Attempts to obtain a satisfactory elemental analysis were unsuccessful. The ^{1}H NMR

(50) Robinson, D. R.; West, C. A. *Biochemistry* **1970**, *9*, 70.

($CDCl_3$, 360 MHz) spectral data for the endo aldehyde are as follows: δ 0.75, 0.81, 0.85, 1.03 (4 s, 12 H, 4 CH_3), 1.89–1.95 (m, 1 H), 1.98 (d, 1 H, J = 2.7 Hz, C*H*CHO), 2.03–2.09 (m, 1 H), 9.90 (d, 1 H, J = 3.0 Hz, CHO).

***ent*-*D*-Norbeyerane-*exo*-15-[1-^{3}H]methanol ([^{3}H]-13-OH).** A solution of 112 mg (0.39 mmol) of the exo aldehyde in 5 mL of ethanol was stirred and cooled at 0 °C as 22 mg (0.59 mmol, 206 mCi, SA = 347.8 mCi/mmol) of sodium borotritide was added. The mixture was stirred at 0 °C for 2 h after which 5 mL of saturated sodium chloride was added. The product was extracted with four portions of ether and the combined ethereal extracts were washed with water and saturated sodium chloride, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography with 10% of ethyl acetate in hexane as eluent. The product was diluted with 119 mg (0.41 mmol) of cold 13-OH and purified again by flash chromatography. The yield of the labeled exo methanol [^{3}H]-13-OH as a solid was 159 mg (68%). The radiochemical yield was 1.70 mCi (4.4%) and the specific activity was 16.6 mCi/mmol. The chemical purity was checked by GC analysis (>97% pure). TLC assay after scraping and counting indicated that all (100%) of the radioactivity was located in the same region as the authentic alcohol.

***ent*-*D*-Norbeyerane-*endo*-15-[1-^{3}H]methanol ([^{3}H]-14-OH).** A solution of 72 mg (0.25 mmol) of the endo aldehyde in 5 mL of ethanol was stirred and cooled at 0 °C as 3.5 mg (0.093 mmol, 32.3 mCi, SA = 347.8 mCi/mmol) of sodium borotritide was added in one portion, followed by 0.1 mL of 5% (w/v) methanolic potassium hydroxide. After 1 h, 1.0 mg of additional sodium borotritide was added and this was followed by 36.8 mg (0.97 mmol) of unlabeled sodium borohydride added 1 h later. After 30 min at room temperature, 2–3 mL of saturated sodium chloride solution was added. Isolation and purification as described above gave 41.3 mg (57%) of the labeled endo alcohol [^{3}H]-14-OH. The radiochemical yield was 41.4 mCi (7.4%) and the specific activity was 21.6 mCi/mmol. The chemical purity of the endo alcohol was established by TLC analysis.

***ent*-*D*-Norberyerane-*exo*- and -*endo*-15-[^{3}H]methyl Pyrophosphates ([^{3}H]-13-OPP and [^{3}H]-14-OPP).** The phosphorylation was carried out by the method of Cramer and Rittersdorf[40a,b] as modified by Popjak and co-workers.[40c,42] Bis(triethylammonium) hydrogen phosphate was prepared as reported[51] by using 100% phosphoric acid.

A solution of 41.3 mg (0.142 mmol, 3.07 mCi, specific activity 21.6 mCi/mmol) of the endo alcohol [^{3}H]-14-OH in 128 μL (1.28 mmol) of distilled trichloroacetonitrile was stirred at room temperature as a suspension of 128 mg (0.43 mmol) of bis(triethylammonium) hydrogen phosphate in 35 mL of acetonitrile was added over 5 h. The solution was allowed to stir for 24 h after which the solvent was evaporated. The residue was suspended in 20 mL of 2% concentrated ammonium hydroxide in methanol and centrifuged to remove the inorganic salts. The supernatant was placed on a Dowex 1-X8 column (formate form), which was packed to a height of 10–12 cm in a 1 × 50 cm column and eluted with a linear gradient of 0.053 M to 0.43 M ammonium formate in total of 400 mL of methanol[41a] while collecting 6-mL fractions. The fractions containing the monophosphate (10–20) and the pyrophosphate (34–44) were identified by liquid scintillation counting (LSC), combined, and evaporated under reduced pressure keeping the temperature below 30 °C. The mixture of ammonium pyrophosphate and ammonium formate was dissolved in 10 mL of 0.01 M aqueous ammonia and placed on a 12 cm × 1 cm Aberlite XAD column. The Amberlite column was previously washed six times with 0.01 M aqueous ammonia and 0.01 M methanolic ammonia and was packed with 0.01 M aqueous ammonia. The ammonium formate was removed first by elution with 30 mL of 0.01 M aqueous ammonia, a solution of 4 mL of 0.01 M aqueous ammonia and 2 mL of 0.01 M methanolic ammonia, a solution of 4 mL of 0.01 M methanolic ammonia and 2 mL of 0.01 M aqueous ammonia, and finally 30 mL of 0.01 M methanolic ammonia. Fractions (ca. 6 mL) were collected and analyzed by LSC. The appropriate fractions were combined and the solvent was evaporated, keeping the temperature below 30 °C. The residue was dissolved in 2.0 mL of 1.0 N aqueous ammonia and the solution was lyophilized to give 13.5 mg (19%) of the endo pyrophosphate ammonium salt, [^{3}H]-14-OPP (ca, 0.75 mCi, 24% radiochemical yield). Although the specific activity calculated from these data is 28 mCi/mmol, the actual value is presumed to be the same as the starting alcohol, i.e., 21.6 mCi/mmol. The pyrophosphate was stored as a solution in 2.0 mL of 1 M aqueous ammonia in a freezer.

The exo pyrophosphate [^{3}H]-13-OPP was prepared by the same procedure from 51.5 mg (0.177 mmol, 2.94 mCi, SA = 16.6 mCi/mmol) of [^{3}H]-13-OH except that the addition time was 3 h. Isolation as described above provided 18.3 mg (21%) of the pyrophosphate (0.428 mCi; 14.5% radiochemical yield) as a powder after lyophilization. The somewhat low specific activity estimated from these data is attributed to the presence of a small amount of ammonium formate. The actual specific activity is presumed to be the same as the starting alcohol, i.e., 16.6 mCi/mmol.

The purity of the exo pyrophosphate was assayed by TLC analysis on cellulose[52] and by phosphatase cleavage back to the original alcohol. The 0.1-mm cellulose plate was developed with 2-propanol/chloroform/acetonitrile/ammonium bicarbonate (5.5:2:1:1.5 (v/v/v/v)) to a distance of 8 cm. The pyrophosphate appeared at R_f 0.43 following visualization with ferric chloride and sulfosalicyclic acid solutions.[52] Approximately 95% of the radioactivity was found in the pyrophosphate zone by scraping and counting. The exo pyrophosphate (0.030 μmol, 0.52 μCi) was incubated in a solution of 23.5 μL of bacterial alkaline phosphatase (Sigma, 2.35 unit) in 0.5 mL of buffer solution (50 mM TRIS, 50 mM potassium bicarbonate, and 10 mM magnesium chloride; pH 7.4) for 23 h at 30 °C. The enzyme was denaturated by addition of 0.5 mL of methanol and the mixture was extracted three times with petroleum ether–benzene (9:1) solution. The combined organic solution was applied to a TLC plate (ca. 10 cm), which was developed with 20% ethyl acetate in hexane. The alcohol region (R_f 0.2 to 0.4) was scraped from the plate and counted: 0.40 μCi, 80%.

Incubation of [^{3}H]-13-OPP and [^{3}H]-14-OPP with Kaurene Synthetase from *Marah macrocarpus*. The enzyme extract was isolated from the endosperm of frozen seeds from immature fruit of *M. macrocarpus* according to the procedures of West and Upper[43a] as described by Hirano[43b] and Sherwin.[43c] The partially thawed endosperm was homogenized with a chilled Thomas homogenizer, the homogenate was filtered through chilled, moistened, 4-ply cheese cloth, and the filtrate was centrifuged at 27000 x *g* for 20 min at 0 °C. The supernatant was separated from the pellet and from a white insoluble uppermost layer and centrifuged at 48000 rpm (150000 x *g*) for 1 h at 4 °C. The supernatant was removed and lyophilized to give a light yellow solid. This lyophilized enzyme was stored in a refrigerator until use.

A series of three incubations was carried out with the following pyrophosphates: [15-^{3}H]copalyl pyrophosphate ([^{3}H]-1, prepared by Dr. S. V. Govindan and stored in 1 N ammonium hydroxide at −20 °C; 50 μL, 0.24 μmol, total radioactivity = 3.6 μCi, SA = 15.4 mCi/mmol), exo pyrophosphate [^{3}H]-13-OPP (50 μL, 0.25 μmol, total radioactivity = 0.16 mCi), endo pyrophosphate [^{3}H]-14-OPP (10 μL, 0.094 μmol, total radioactivity = 2.0 μCi). A typical incubation procedure is as follows: [^{3}H]Copalyl pyrophosphate (0.24 μmol) was incubated with 100 mg of lyophilized enzyme dissolved in 20 mL of 50 mM potassium hydrogen phosphate containing 2 mM manganese chloride (pH 6.6) for 2 h at 30 °C. The incubation was terminated by the addition of 10 mL of ethanol. The resulting mixture was extracted with hexane. The extracts were combined and concentrated to approximately 1 mL under a stream of nitrogen. The hexane solution was filtered through 0.5 g of silica gel packed in a disposable pipette with the aid of 10% benzene–hexane as eluent. After evaporation, the kaurene was further purified by chromatography in a disposable pipette (0.7 × 10 cm) packed with 10% silver nitrate impregnated silica gel to the height of 5 cm, using 10% benzene–hexane as eluent. The fractions (1 mL each) were an-

(51) Cornforth, R. H.; Popjak, G. *Methods Enzymol.* **1969**, *15*, 386.

(52) Davisson, V. J.; Woodside, A. B.; Poulter, C. D. *Methods Enzymol.* **1985**, *110*, 130.

(53) The MS data for 18 are those obtained by GC/MS analysis of 18 isolated from solvolysis of the endo tosylate. The GC/MS of 18 from the exo tosylate was contaminated somewhat with another product.

alyzed by LSC. After the appropriate fractions were collected, LSC showed 1.1 μCi (31%) incorporation. The exo and endo pyrophosphates ([^{3}H]-13-OPP and [^{3}H]-14-OPP) were incubated with kaurene synthetase, using the same procedure. After the column chromatographic separation, only negligible radioactivity (<100 cpm, <1%) was observed in the kaurene fraction in both cases.

Registry No. **2**, 562-28-7; **5a**, 27975-19-5; **5b**, 30217-41-5; **6**, 107540-93-2; **7**, 40140-46-3; **8a**, 107540-94-3; **8b**, 107540-95-4; **9**, 107540-96-5; **10**, 107540-97-6; **11a**, 107540-98-7; **11b**, 107540-99-8; **11b-d**, 107541-06-0; **12a**, 107597-53-5; **12b**, 107597-48-8; **12b-d**, 107597-51-3; **13**-OH, 107541-00-4; [^{3}H]-**13**-OH, 107541-08-2; **13**-OTs, 107541-01-5; [^{3}H]-**13**-OPP, 107541-09-3; **13**-al, 107541-07-1; **14**-OH, 107597-49-9; **14**-OTs, 107597-50-2; **14**-al, 107597-52-4; **15**, 3564-54-3; **16**, 36627-98-2; **17**, 21561-92-2; **18**, 19882-10-1; **19**, 107541-02-6; **29**, 107541-03-7; pimaric acid, 127-27-5; 2,2-dimethyl-1,3-propanediol, 126-30-7; *ent*-15-diazobeyeran-16-one, 107541-04-8; *ent*-16-[2-(*p*-tolylsulfonyl)-1,1-diazenediyl]beyeran-15-one, 107541-05-9; kaurene synthetase, 9055-64-5.

Theoretical Calculation of Effects of Steric Hindrance on Rates of Esterification and of Acid-Catalyzed Hydrolysis[1]

DeLos F. DeTar,* Seyhun Binzet, and Prashanth Darba

Department of Chemistry, The Florida State University, Tallahassee, Florida 32306

Received December 8, 1986

We report the calculation of rates of esterification and of acid-catalyzed hydrolysis of esters of a set of acetic acids $R_1R_2R_3CCOOH$ having R_1 equal to H, Me, Et, *i*-Pr, and *t*-Bu. The log *k*(rel) are represented as a LFER of relative gas-phase enthalpies of activation calculated by molecular mechanics. The tetrahedral intermediate was used as a model of the transition state on the way to the tetrahedral intermediate. The purpose of the study has been twofold, to gain a better understanding of the role of steric effects in acyl transfer reactions involving highly hindered substrates and to develop further the principles that underlie the use of molecular mechanics as a tool for reliable prediction of rates. The FSE values are transferable and also permit comparisons of steric effects among different esters. The predicted rate constants exhibit curious patterns; the more highly substituted tri-*tert*-butylacetic acid is predicted to react some 10000 times faster than isopropyldi-*tert*-butylacetic acid; both are predicted to react very slowly.

Molecular mechanics has been shown to be a useful tool for the estimation of relative rate constants of certain types of reactions both in the gas phase and in solution. Log *k* is correlated in a LFER expression with the steric energy (SE) of the reactant or with ΔSE between a model of the transition state and the reactant.[1] The correlations of relative rate constants have been quite successful in most cases.[5-37]

The ability to calculate relative rate constants reliably is of great importance, and molecular mechanics is becoming widely used in many laboratories to provide a clearer understanding of the role of steric factors in determining rates of reactions. Our interest has been to examine the underlying theoretical principles so that the calculations may be performed more reliably. We have been concerned with such questions as: Under what circumstances may differences of raw steric energies from a molecular mechanics calculation be taken as proportional to the gas-phase enthalpy of activation? In what respect is the gas-phase enthalpy of activation related to the free energy of activation in solution? How can we compare steric properties of reactants or of transition states?[3]

A practical goal of a molecular mechanics calculation is to estimate the difference of the steric components of the enthalpies of formation of conformer C1 of molecule M1

(1) Definitions: SE, raw steric energy as calculated by molecular mechanics. FSE, formal steric enthalpy.[2,3] Conformational labels are described in Appendix 1 and are illustrated in ref 4. LFER is linear free energy relationship; see, for example, eq 2 and 7-9.

(2) DeTar, D. F.; Binzet, S.; Darba, P. *J. Org. Chem.* **1985**, *50*, 2826.
(3) DeTar, D. F. *J. Org. Chem.*, previous paper in this issue.
(4) DeTar, D. F.; Binzet, S.; Darba, P. *J. Org. Chem.* **1985**, *50*, 5304.
(5) Westheimer, F. H.; Mayer, J. E. *J. Chem. Phys.* **1946**, *14*, 733.
(6) Westheimer, F. H. *J. Chem. Phys.* **1947**, *15*, 252.
(7) Westheimer, F. H. In *Steric Effects in Organic Chemistry*; Newman, M. S., Ed.; Wiley: New York, 1956, p 523.
(8) Dostrovsky, I.; Hughes, E. D.; Ingold, C. K. *J. Chem. Soc.* **1946**, 173.
(9) de la Mare, P. B. D.; Fowden, L.; Hughes, E. D.; Ingold, C. K.; Mackie, J. D. H. *J. Chem. Soc.* **1955**, 3200.
(10) Gleicher, G. J.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1967**, *89*, 582.
(11) Bingham, R. C.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1971**, *93*, 3189.
(12) Fry, J. L.; Engler, E. M.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1972**, *94*, 4628.
(13) DeTar, D. F.; Tenpas, C. J. *J. Am. Chem. Soc.* **1976**, *98*, 2456.
(14) DeTar, D. F.; Tenpas, C. J. *J. Am. Chem. Soc.* **1976**, *98*, 7903.
(15) DeTar, D. F.; Mcmullen, D. F.; Luthra, N. P. *J. Am. Chem. Soc.* **1978**, *100*, 2484.
(16) DeTar, D. F.; Luthra, N. P. *J. Am. Chem. Soc.* **1980**, *102*, 4505.
(17) DeTar, D. F. *J. Am. Chem. Soc.* **1981**, *103*, 107.
(18) DeTar, D. F. *Biochemistry* **1981**, *20*, 1730.
(19) Müller, P.; Perlberger, J. C. *J. Am. Chem. Soc.* **1976**, *98*, 8407.
(20) Perlberger, J. C.; Müller, P. *J. Am. Chem. Soc.* **1977**, *99*, 6316.
(21) Müller, P.; Blanc, J.; Lenoir, D. *Helv. Chim. Acta* **1982**, *65*, 1212.
(22) Müller, P.; Blanc, J.; Perlberger, J. C. *Helv. Chim. Acta* **1982**, *65*, 1418.
(23) Müller, P.; Blanc, J.; Mareda, J. *Chimia* **1985**, *38*, 389.
(24) Müller, P.; Mareda, J. *Helv. Chim. Acta* **1985**, *68*, 119.
(25) Müller, P. *Chimica* **1985**, *39*, 234.
(26) Harris, J. M.; Shafer, S. G.; Smith, M. R.; McManus, S. P. *Tetrahedron Lett.* **1979**, 2089.
(27) Carter, R. E.; Liljefors, T. *Tetrahedron* **1976**, *32*, 2915.
(28) Liljefors, T.; Carter, R. E. *Tetrahedron* **1978**, *34*, 1611.
(29) Farcasiu, D. *J. Org. Chem.* **1978**, *43*, 3878.
(30) Beckhaus, H. D.; Hellmann, G.; Rüchardt, C. *Chem. Ber.* **1978**, *111*, 72.
(31) Rüechardt, C.; Weiner, S. *Tetrahedron Lett.* **1979**, 1311.
(32) Winiker, R.; Beckhaus, H.-D.; Rüchardt, C. *Chem. Ber.* **1980**, *113*, 3456.
(33) Flam-ter Meer, M. A.; Beckhaus, H-D.; Peters, K.; Von Schnering, H. G.; Fritz, H.; Rüchardt, C. *Chem. Ber.* **1986**, *119*, 1492.
(34) Rüchardt, C.; Beckhaus, H.-D. *Top. Curr. Chem.* **1986**, *130*, 1.
(35) Schneider, H. J.; Thomas, F. *J. Am. Chem. Soc.* **1979**, *101*, 1424.
(36) Schneider, H. J.; Thomas, F. *J. Am. Chem. Soc.* **1980**, *102*, 1424.
(37) Gschwendtner, W.; Hoppen, V.; Schneider, H. J. *J. Chem. Res., Synop.* **1981**, 96.

0022-3263/87/1952-2074$01.50/0