Stereoselective Total Synthesis of (-)-Picrotoxinin and (-)-Picrotin

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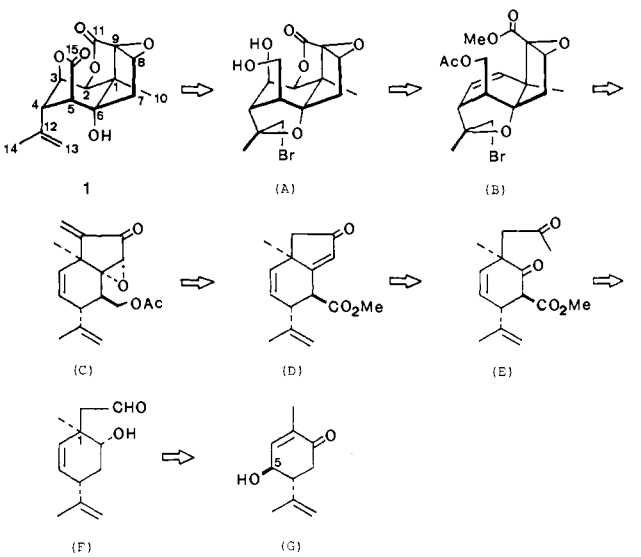
Abstract: The stereoselective total synthesis of (-)-picrotoxinin (**1**) and (-)-picrotin (**2**), starting from (+)-5β-hydroxycarvone (**3**), is described. Eight contiguous asymmetric centers on a cis-fused hydrindane ring system were stereoselectively prepared via three key transformations: (1) the stereospecific introduction of the C_1 quaternary center via a Claisen rearrangement; (2) a novel organoselenium-mediated reduction of epoxy ketone **13**; and (3) a stereospecific construction of glycidic ester **18**.

Picrotoxin, a plant toxin, was first isolated in 1811 by Boullay from the plant *Menispermum cocculus*.[1a] However, it required nearly 150 years for its structure to be established due to its intricate structure and a molecular compound composed of picrotoxinin (**1**), the toxic principle, and nontoxic picrotin (**2**).[2] (See Figure 1.) Picrotoxin (**1**) is one of the most toxic compounds of plant origin and has been known to act as a specific antagonist against GABA,[3] the suppressive nervous transmittal substance, and to inhibit a chloro ion channel from opening in vivo.[4] Today **1** is indispensable to neuropharmacological studies.[4] Related picrotoxanes such as coriamyrtin and tutin are known to have similar biological properties.[1b] These natural products are highly oxygenated cage molecules possessing a *cis*-hydrindane ring with sterically hindered γ-lactone ring(s), epoxide(s), and axially oriented isopropenyl and hydroxyl groups. Their unique structures and quite interesting physiological activities have elicited considerable attention from synthetic chemists.[5] The first total synthesis of (-)-picrotoxinin (**1**)[5a] and (-)-picrotin (**2**)[5b] was reported by Corey and Pearce in 1979 and 1980, respectively. Recently, Inubushi et al. achieved total synthesis of racemic coriamyrtin,[5c] while Yamada and co-workers have reported completed syntheses of (-)-**1**,[5d] (+)-coriamyrtin,[5d] (+)-tutin,[5e] and (+)-asteromurin A.[5e,f]

We report herein the highly stereoselective total synthesis of (-)-**1** and (-)-**2** starting from (+)-5β-hydroxycarvone.[6]

Synthetic Strategy. Picrotoxinin (**1**) and picrotin (**2**) contain eight contiguous asymmetric centers on a *cis*-hydrindane ring system in which two characteristic bridged γ-lactones are incorporated. The successful synthesis of these molecules depends on the regio- and stereoselective construction of these two γ-lactones since both **1** and **2** are readily transformed into the thermodynamically more stable δ-lactones bridged with C_2 hydroxyl and C_5 carboxyl groups upon treatment with base.[7] In order to solve this problem, we designed a synthetic strategy involving glycidic ester (B) as a key intermediate (Scheme I) since OsO_4 oxidation of *B* would regioselectively form the C_2-C_9 γ-lactone (A) due to the neighboring group participation of the C_9 ester group. The C_3-C_5 lactone would be assembled from lactone diol (A) via oxidative lactonization. The key intermediate glycidic ester (B) is derivable from epoxy enone (C), provided that the unprecedented chemoselective reduction of the epoxy ketone moiety coexisting with an enone function is feasible. Nevertheless, we were optimistic this challenging transformation could be achieved using an organoselenium reagent.[8] Finally, the stereospecific introduction of the quaternary center at C_1 could be achieved by taking advantage of the Claisen rearrangement of 5β-hydroxycarvone (G).

Scheme I[a]



[a] Skeletal numbering in the schemes corresponds to that of the picrotoxane skeleton (*Chem. Rev.* **1967**, *67*, 441).

Synthesis of Epoxy Enone 13. Our synthesis begins with (+)-5β-hydroxycarvone (**3**;[9] Scheme II) which was readily obtainable from (-)-carvone. Treatment of **3** with ethyl vinyl ether in the presence of mercuric acetate, followed by reduction with $LiAlH_4$ in ether at -78 °C, gave vinyl ether **4** in 92% yield, in which reduction of the C_2 ketone took place with a high degree of stereoselectivity (a 99:1 mixture of the desired α-alcohol **4** and

(1) (a) Boullay, P. F. G. *Ann. Chim. Phys.* **1811**, *80*, 209. For reviews on the picrotoxane substances, see: (b) Porter, L. A. *Chem. Rev.* **1967**, *67*, 441. (c) *Cyclopentanoid Terpene Derivatives*; Taylor, W. I., Battersby, A. R., Coscia, C. J., Ed.; Dekker: New York, 1969; Chapter 2, pp 147-201.

(2) (a) Conroy, H. *J. Am. Chem. Soc.* **1951**, *73*, 1889; **1952**, *74*, 491, 3046; **1957**, *79*, 1726, 5550. (b) Craven, B. M. *Tetrahedron Lett.* **1960**, *2*, 21.

(3) Olsen, R. N.; Ticku, M. K.; Miller, T. *Mol. Pharmacol.* **1978**, *14*, 381. Narabayashi, T. *Physiol. Rev.* **1974**, *54*, 813.

(4) Nistri, A.; Constanti, A. *Progress in Neurobiology*; Pergamon: New York, 1979; Vol. 13, pp 117-235. Aickin, C. C.; Deisz, R. A.; Lux, H. D. *Amino Acid Neurotransmitters*; Raven Press: New York, 1981; pp 301-307.

(5) (a) Corey, E. J.; Pearce, H. L. *J. Am. Chem. Soc.* **1979**, *101*, 5841. (b) *Tetrahedron Lett.* **1980**, *21*, 1823. (c) Tanaka, K.; Uchiyama, F.; Sakamoto, K.; Inubushi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4965; *Chem. Pharm. Bull.* **1983**, *31*, 1943, 1948, 1972. (d) Niwa, H.; Wakamatsu, K.; Hida, T.; Niiyama, K.; Kigoshi, H.; Yamada, M.; Nagase, H.; Suzuki, M.; Yamada, K. *J. Am. Chem. Soc.* **1984**, *106*, 4547. (e) Wakamatsu, K.; Kigoshi, H.; Niiyama, K.; Niwa, H.; Yamada, K. *Tetrahedron Lett.* **1984**, *25*, 3873; *Tetrahedron* **1986**, *42*, 5551. (f) Wakamatsu, K.; Niiyama, K.; Niwa, H.; Yamada, K. *Chem. Lett.* **1984**, 1763. (g) Miyashita, M.; Yoshikoshi, A. *Chem. Abstr.* **1983**, *100*, 210176w. (h) Trost, B. M.; Jebaratnam, D. J. *Tetrahedron Lett.* **1987**, *28*, 1611. For synthetic studies of picrotoxane alkaloids, see references cited in ref 5h.

(6) This work was in part presented at the 29th Symposium on the Chemistry of Natural Products, Sapporo, 1987, and the 16th International Symposium on the Chemistry of Natural Products (IUPAC), Kyoto, 1988.

(7) Meyer, R. J.; Bruger, P. *Chem. Ber.* **1898**, *31*, 2958. Horrmann, P. *Chem. Ber.* **1912**, *45*, 2090.

(8) (a) Miyashita, M.; Suzuki, T.; Yoshikoshi, A. *Tetrahedron Lett.* **1987**, *28*, 4293. (b) Miyashita, M.; Hoshino, M.; Yoshikoshi, A. *Tetrahedron Lett.* **1988**, *29*, 347.

(9) Miyashita, M.; Suzuki, T.; Yoshikoshi, A. *J. Org. Chem.* **1985**, *50*, 3377.

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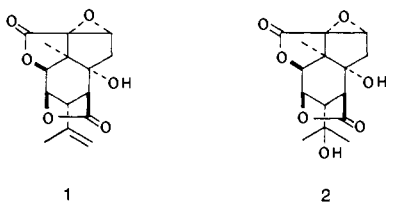
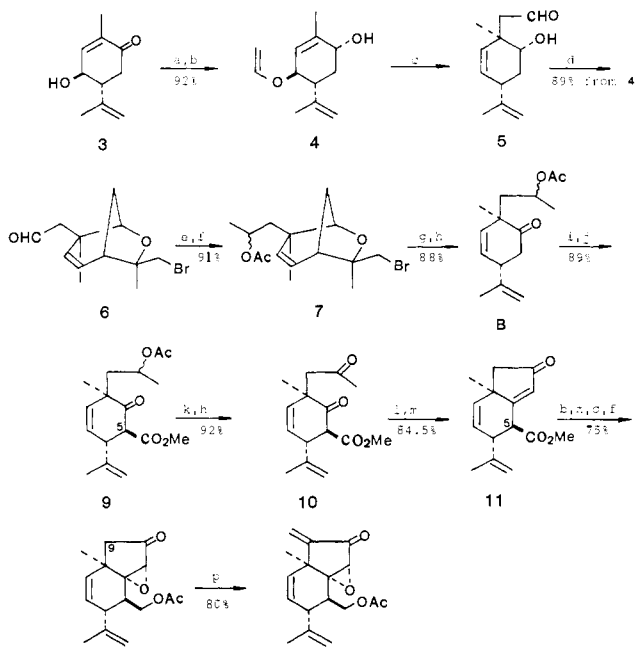


1 2

Figure 1.

Scheme II[a,b]



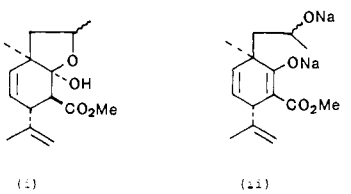
3 4 5 6 7 B 9 10 11 12 13

[a] (a) $CH_2{=}CHOCH_2CH_3$, $Hg(OAc)_2$; (b) $LiAlH_4$, Et_2O; (c) 185 °C, xylene; (d) NBS, CH_3CN; (e) CH_3Li, Et_2O; (f) Ac_2O, C_5H_5N, 4-(dimethylamino)pyridine, CH_2Cl_2; (g) Zn(Cu), NH_4Cl-H_2O, EtOH; (h) CrO_3-H_2SO_4, Me_2CO; (i) $LiN[Si(CH_3)_3]_2$, Et_2O, and then CO_2; (j) CH_2N_2-Et_2O, CH_2Cl_2; (k) NaH, THF, and then MeOH, DMF; (l) pyrrolidine, $PhCO_2H$, C_6H_6; (m) aqueous AcOH, AcONa, $CHCl_3$; (n) MnO_2, $CHCl_3$; (o) H_2O_2, 6 N NaOH, MeOH; (p) $(CH_2O)_n$, $[C_6H_5NH_2(CH_3)]O_2CCF_3$, THF. [b] See Scheme I, footnote a.

its β-epimer).[10] The Claisen rearrangement of **4** proceeded smoothly in refluxing xylene to afford hydroxy aldehyde **5**, which was treated with NBS in acetonitrile to yield bromo ether **6** in 89% overall yield. Homologation of the side chain by treatment with MeLi in ether followed by acetylation resulted in formation of a 1:1 mixture of acetates **7** in 91% yield.[11] The stereochemistry of the carbon bearing an acetoxyl group is of minor strategic importance since it is later transformed into a carbonyl carbon. Compound **7** was converted into keto ester **9**, via ketone **8**, in 78% overall yield in four steps: (1) reduction (Zn(Cu), EtOH); (2) Jones oxidation; (3) carbonation ($LiN(TMS)_2$, Et_2O, and then CO_2); (4) methylation (CH_2N_2). Interestingly, the newly introduced methoxycarbonyl group at C_5 was found to be a single stereoisomer and presumed to be β-oriented due to steric considerations. Subsequent conversion of **9** into diketone **10**, a precursor for cyclization, required ingenuity because usual conditions for hydrolysis of an acetate, e.g. K_2CO_3 in MeOH, led **9** exclusively to stable acetal (i; Figure 2), which obviously was resistant to subsequent oxidation. Formation of this byproduct was circumvented by the following sequential manipulations: (1) treatment of acetate **9** with NaH (3 equiv) in THF (room temperature, 2 h); (2) addition of MeOH (5 equiv) to form alkoxy enolate dianion (ii; Figure 2);[12] (3) direct Jones oxidation[13] of ii

(10) The ratio was determined by HPLC using a μ Porasil column (Waters) with CH_2Cl_2 as solvent.

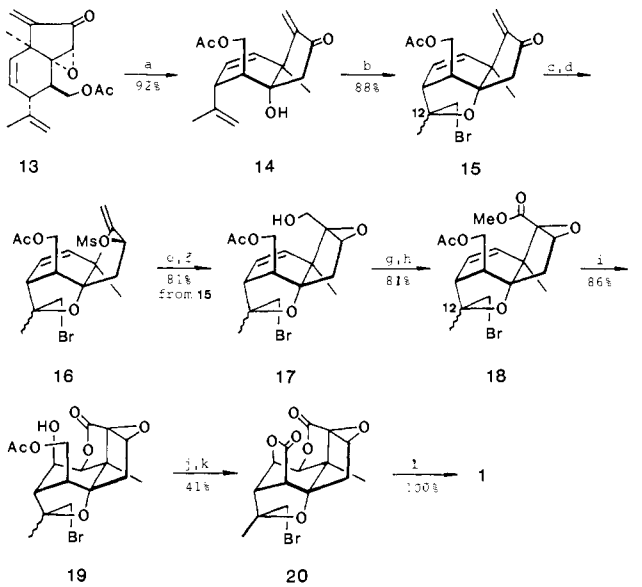
(11) The ratio was determined by ^1H NMR analysis.



(i) (ii)

Figure 2.

Scheme III[a,b]



13 14 15 16 17 18 19 20 1

[a] (a) $Na^+[PhSeB(OEt)_3]^-$, AcOH, EtOH; (b) NBS, THF; (c) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH; (d) MsCl, C_5H_5N; (e) OsO_4, C_5H_5N, and then aqueous $NaHSO_3$; (f) DBU, DMF; (g) CrO_3-C_5H_5N, CH_2Cl_2; (h) $NaClO_2$, NaH_2PO_4-H_2O, $(CH_3)_2C{=}CHCH_3$, *t*-BuOH, and then CH_2N_2-Et_2O; (i) OsO_4, C_5H_5N, and then H_2S, $CHCl_3$; (j) NaH, MeOH, and then AcOH; (k) PCC, CH_2Cl_2; (l) Zn(Cu), NH_4Cl-H_2O, EtOH. [b] See Scheme I, footnote a.

to produce diketone **10** as a single product in 92% yield.[14] The crucial cyclization of **10** was effected by the enamine-mediated annulation reaction[15] followed by hydrolysis of the resulting dienamine with aqueous AcOH in $CHCl_3$[16] providing the 9-norpicrotoxane skeleton **11** along with a small amount of β,γ-unsaturated isomer. Since the latter isomer was readily converted into **11** by treatment with Al_2O_3 in benzene, the cyclization of **10** to **11** was achieved in 84.5% overall yield. As expected, under these conditions, only the thermodynamically and stereochemically more stable isomer **11** possessing a β-equatorial methoxycarbonyl group was formed, as no trace of α-isomer was detected. Standard epoxidation of the enone **11** with alkaline hydrogen peroxide proved to be fruitless since enone **11** readily forms the enol form on contact with base. As a result, **11** was transformed in 75% yield into epoxy ketone **12** by the following four-step sequence: (1) reduction with $LiAlH_4$; (2) oxidation of the resulting diol with MnO_2; (3) epoxidation with alkaline hydrogen peroxide in MeOH; (4) acetylation. Note that the generation of single α-epoxide **12** is consistent with the well-known preference of the perhydroindenone systems to afford cis-fused compounds.[17] Methylenation at C_9 in **12** was

(12) Initial treatment of **9** with NaH generated the enolate anion of β-keto ester moiety, whose acetoxyl group was then attacked by sodium methoxide, generated by alcoholysis of excess NaH (2 equiv) with MeOH (5 equiv), to yield the dianion ii.

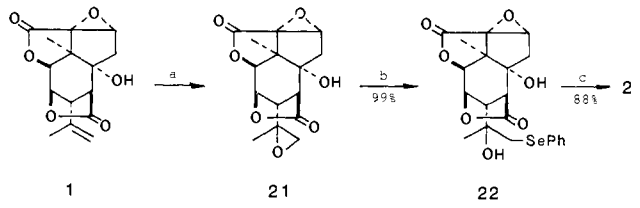
(13) A solution of dianion ii in THF was added dropwise to Jones reagent in acetone at 0 °C.

(14) The cyclic acetal (i) was not formed at all under these conditions.

(15) The cyclization of **10** did not occur under a variety of acidic or alkaline conditions due to its propensity to favor enol or enolate form on contact with acid or base.

(16) Hydrolysis of the dienamine in a homogeneous solution such as aqueous AcOH or aqueous AcOH in THF resulted in lower yields of the product (ca. 25–35%).

Scheme IV[a]



1 21 22

[a] (a) MCPBA, CH_2Cl_2; (b) $Na^+[PhSeB(OEt)_3]^-$, AcOH, EtOH; (c) Bu_3SnH, AIBN, $C_6H_5CH_3$.

effected by the modified Gras procedure[18] (paraformaldehyde, *N*-methylanilinium trifluoroacetate, THF) to give epoxy enone **13** in 80% yield. Thus, the key intermediate **13** having the complete carbocyclic skeleton of picrotoxinin was syntheisized from (+)-5β-hydroxycarvone (**3**) in 27.1% overall yield.

Total Synthesis of (−)-Picrotoxinin. Completion of picrotoxinin (**1**) from the epoxy enone **13** was achieved as illustrated in Scheme III.

The chemoselective reduction of the epoxy ketone **13** was efficiently performed with sodium (phenylseleno)triethoxyborate ($Na^+[PhSeB(OEt)_3]^-$)[19] in ethanol to give hydroxy ketone **14** in 92% yield, which was then treated with NBS in THF to afford a mixture of bromo ethers **15** (12*S* and 12*R*)[20] in 88% yield. It is noteworthy that the enone function in **13** was not affected at all under these conditions,[21] whereas the reduction of **13** with other reducing agents, e.g. zinc powder, gave the product in quite low yield (~5%), owing to the concomitant reduction of the enone function.

Enone **15** was stereospecifically transformed into epoxy alcohol **17** via allylic mesylate **16** as follows; reduction of **15** with $NaBH_4$ in the presence of cerium(III) chloride[22] and subsequent mesylation with methanesulfonyl chloride in pyridine yielded allylic mesylate **16** as an unstable oil, which was subjected to further oxidation. Oxidation of **16** with OsO_4 in pyridine occurred exclusively at the *exo*-olefin from the convex face to give the corresponding diol, which was then treated with DBU in DMF affording epoxy alcohol **17** as a single product in 81% overall yield from **15**, as a result of intramolecular S_N2 displacement. In turn, the epoxy alcohol **17** was converted into glycidic ester **18**[23] in 81% yield in two steps: (1) Collins oxidation;[24] (2) oxidation of the resulting aldehyde with $NaClO_2$[25] in aqueous *t*-BuOH followed by methylation with CH_2N_2.

With the key compound **18** in hand, we set about the final task, which was the regio- and stereoselective formation of two γ-lactones. Oxidation of the *endo*-olefin in **18** with OsO_4 was expected to occur selectively from the concave (β) face since molecular models suggested that the α-side of the double bond was tightly shielded by the bromomethyl or methyl group at C_{12}. Indeed, **18** was oxidized with OsO_4 in pyridine (7 days) yielding the single osmate, which was treated with hydrogen sulfide in $CHCl_3$ resulting in formation of lactone **19** in 86% yield.[26] After hydrolysis of the acetoxyl group in **19** with sodium methoxide in MeOH, the resulting diol was subjected to oxidative lactonization in an attempt to lead to dilactone **20**. However, oxidative lactonization of this diol was not effective at all with the well-known lactonizing agents such as silver carbonate on Celite (Fetizon reagent),[27] ruthenium complex ($RuH_2(PPh_3)_4$),[28] autoxidation (PtO_2, O_2),[29] etc., probably owing to the sterically hindered diol. Finally, pyridinium chlorochromate (PCC)[30] was found to effect this particular transformation. Thus, treatment of the diol with PCC in CH_2Cl_2 gave dilactone **20** as a crystalline compound[31] in 41% yield, which was identical with (−)-β-bromopicrotoxinin[2b,7] derived from natural **1**.[32] Reduction of **20** with zinc copper couple in EtOH yielded synthetic (−)-picrotoxinin (**1**) in nearly quantitative yield, which was identical with natural **1**[32] (IR, ^1H NMR, $[\alpha]_D$, and chromatographic comparison).

Total Synthesis of (−)-Picrotin. (−)-Picrotin (**2**) was efficiently synthesized from (−)-picrotoxinin (**1**) in three steps (Scheme IV).

Oxidation of (−)-**1** with MCPBA in CH_2Cl_2 gave a 5:2 mixture of epimeric epoxides **21**, which was treated with $Na^+[PhSeB(OEt)_3]^-$ in ethanol[8] producing a diastereomeric mixture of hydroxy selenide **22** in 99% overall yield for the two steps. Reduction of **22** with tributyltin hydride[33] in toluene furnished (−)-picrotin (**2**) in 88% yield. The synthetic compound was identical with an authentic sample in all respects (IR, ^1H NMR, $[\alpha]_D$, and chromatographic comparison).

In summary, a stereoselective synthesis of (−)-**1** and (−)-**2** was achieved wherein all of eight contiguous asymmetric centers on a *cis*-hydrindane skeleton were constructed with high stereoselectivity. The total yield of (−)-**1** and (−)-**2** from **3** was 5.0% and 4.3%, respectively.

Experimental Section

General Experimental Procedure. All reactions requiring anhydrous conditions were run in flame-dried glassware under an argon atmosphere. Melting points were determined on a Mitamura Riken MP-A melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco A-3 spectrophotometer as liquid film unless otherwise noted. ^1H NMR spectra were measured at 90 MHz on a JEOL FX-90Q spectrometer in $CDCl_3$. High-resolution mass spectra were recorded on a JEOL JMS-DX 300 instrument. Optical rotations were measured on a Jasco DIP-181 polarimeter. High-pressure liquid chromatography (HPLC) was performed on a Waters ALC/GPC-244 instrument, equipped with a μ Porasil column. Merck silica gel 60 (230–400 mesh) was employed for flash column chromatography. Macherey-Nagel precoated silica gel G-25F UV254 plates (0.25 mm) were used for thin-layer chromatography (TLC) and Merck silica gel 60 (70–230 mesh) for preparative thin-layer chromatography. Elemental analyses were performed by the laboratory (N. Sato, T. Naganuma, and S. Hirabuki) of this institute.

(1*R*,4*S*,5*R*)-4-(Ethenyloxy)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol (4). A mixture of (+)-5β-hydroxycarvone (**3**)[9] (1.0 g, 6.0 mmol), ethyl vinyl ether (freshly distilled from sodium, 80 mL), and mercuric acetate (450 mg, 1.4 mmol) was stirred at reflux (55–60 °C) under an argon atmosphere for 24 h. Every 24 h, 300 mg (1.1 mmol) of mercuric acetate was added and stirring was continued under reflux for 3 days. The cooled reaction mixture was treated with acetic acid (80 μL)[34] and stirred for another 3 h at room temperature. The mixture was diluted with an equal volume of hexane and washed two times with 5%

(17) Velluz, L.; Valls, J.; Nomine, G. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 181–200.

(18) Disanayaka, B. M.; Weedon, A. C. *Synthesis* **1983**, 952. The source of formaldehyde used in the original procedure has been found to be paraformaldehyde and not trioxane as reported: Gras, J. E. *Tetrahedron Lett.* **1978**, *24*, 2111.

(19) The reagent can be readily prepared by the reduction of diphenyl diselenide with $NaBH_4$ in ethanol by the Sharpless protocol: Sharpless, K. B.; Lauer, R. F. *J. Am. Chem. Soc.* **1973**, *95*, 2697. The structure of the reagent was recently determined as a sodium (phenylseleno)triethoxyborate complex.[8b]

(20) The stereochemistry and ratio of these isomers were not determined at this stage because their ^1H NMR spectra and TLC behavior were superimposable.

(21) Michael addition of $PhSe^-$ to the α,β-methylene ketone was not observed under the conditions. It has been established that this reduction proceeds via an α-substitution process: Miyashita, M.; Hoshino, M.; Suzuki, T.; Yoshikoshi, A. *Chem. Lett.* **1988**, 507.

(22) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

(23) The ratio of 12*S* and 12*R* was shown to be 5:2 by ^1H NMR analysis.

(24) Collins, J. C.; Hess, W. W.; Frank, F. J. *Tetrahedron Lett.* **1968**, 3363.

(25) Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888. Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175, 4825.

(26) Although the product was diastereomeric mixture regarding C_{12}, the ratio could not be determined by ^1H NMR analysis.

(27) Fetizon, M.; Golfier, M. *C. R. Seances Acad. Sci. Ser. C* **1968**, *267*, 900. Fetizon, M.; Golfier, M.; Louis, J.-M. *Tetrahedron* **1975**, *31*, 171.

(28) Harris, R. O.; Hota, N. K.; Sadavoy, L.; Yuen, J. M. C. *J. Organomet. Chem.* **1973**, *54*, 259. Ishii, Y.; Osakada, K.; Ikariya, T.; Saburi, M.; Yoshikawa, S. *J. Org. Chem.* **1986**, *51*, 2034.

(29) Fried, J.; Sih, J. C. *Tetrahedron Lett.* **1973**, 3899.

(30) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647. Corey, E. J.; Ishiguro, M. *Ibid.* **1979**, 2745.

(31) The product was a 95:5 mixture of 12*S* and 12*R*.

(32) Natural picrotoxinin (**1**) and picrotin (**2**) are commercially available (Aldrich) as picrotoxin, which can easily be separated into **1** and **2** by silica gel chromatography.

(33) Clive, D. L. J.; Chittattu, G.; Wong, G. K. *J. Chem. Soc., Chem. Commun.* **1978**, 41. Nicolaou, K. C.; Gasic, G. P.; Barnette, W. E. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 293.

(34) Church, R. F.; Ireland, R. E.; Marshall, J. A. *J. Org. Chem.* **1966**, *31*, 2526.

aqueous KOH, three times with water, and finally with saturated brine. After removal of the solvent, the residue was purified by florisil column chromatography (benzene) to afford 1.06 g (92%) of keto vinyl ether: ^{1}H NMR 6.68 (m, 1 H), 6.38 (dd, 1 H, J = 14.4, 7.2 Hz), 4.84–4.92 (m, 2 H), 4.62 (dm, 1 H, J = 9.7 Hz), 4.31 (dd, 1 H, J = 14.4, 2.1 Hz), 4.12 (dd, 1 H, J = 7.2, 2.1 Hz), 2.92 (ddd, 1 H, J = 12.6, 9.7, 5.4 Hz), 2.54 (dd, 1 H, J = 12.6, 12.6 Hz), 2.44 (dd, 1 H, J = 12.6, 5.4 Hz), 1.70–1.90 (m, 6 H); IR 2924, 1691, 1680, 1638, 1620, 1195, 1110, 900 cm^{-1}. The product was submitted to the next reduction. $LiAlH_4$ (209 mg, 5.5 mmol) was added to a solution of the keto vinyl ether (1.06 g, 5.5 mmol) in dry ether (50 mL) at −78 °C, and the mixture was stirred for 40 min at the same temperature. Again $LiAlH_4$ (95 mg, 2.5 mmol) was added, and stirring was continued at −78 °C for an additional 30 min. The mixture was warmed to 0 °C, and the excess hydride was decomposed by the slow addition of wet ether followed by water and filtered. Removal of the solvent in vacuo afforded 1.07 g (100%) of **4**, which was analyzed by HPLC using CH_2Cl_2 as solvent to show that the product was a 99:1 mixture of **4** and its β-epimer. **4**: ^{1}H NMR 6.37 (dd, 1 H, J = 14.0, 6.8 Hz), 5.57 (q, 1 H, J = 1.6 Hz), 4.87 (m, 2 H), 4.29 (dd, J = 14.4, 1.8 Hz), 4.48–4.02 (m, 2 H), 4.04 (dd, 1 H, J = 6.5, 1.8 Hz), 2.6–1.3 (m, 4 H), 1.84 (br s, 3 H), 1.80 (d, 3 H, J = 1.6 Hz); IR 3340, 2940, 2920, 1633, 1618, 1198, 1056, 985, 900 cm^{-1}. Anal. Calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.13; H, 9.04.

(1*S*,4*R*,7*R*)-7-(Bromomethyl)-4,7-dimethyl-6-oxabicyclo[3.2.1]oct-2-ene-4-acetaldehyde (6). A solution of **4** (4.20 g, 21.7 mmol) in xylene (140 mL) was heated at 185 °C (bath temperature) for 36 h with stirring under an argon atmosphere. The solvent was removed in vacuo, and the crude hydroxy aldehyde **5** obtained was dissolved in acetonitrile (130 mL) and then treated with NBS (7.7 g, 43.4 mmol) at 0 °C under argon. After it was stirred for 1 h at the same temperature, the mixture was diluted with ether–hexane (1:1, 300 mL) and washed three times with half-saturated brine. The aqueous washes were extracted once with ether–hexane (1:1, 100 mL). The combined organic extracts were washed with saturated brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by florisil column chromatography (AcOEt–hexane (1:5)) to afford 5.3 g (89%) of **6**: ^{1}H NMR 9.79 (t, 1 H, J = 2.9 Hz), 6.04 (ddd, 1 H, J = 9.7, 6.8, 1.3 Hz), 5.51 (dd, 1 H, J = 9.7, 2.0 Hz), 4.06 (dd, 1 H, J = 5.6, 2.2 Hz), 3.53 (d, 1 H, J = 9.9 Hz), 3.34 (d, 1 H, J = 9.9 Hz), 2.32 (t, 2 H, J = 2.9 Hz), 2.10–2.60 (m, 2 H), 1.97 (d, 1 H, J = 11.2 Hz), 1.42 (s, 3 H), 1.22 (s, 3 H); IR 2730, 1720, 1060, 993, 900 cm^{-1}. Anal. Calcd for $C_{12}H_{17}O_2Br$: C, 52.76; H, 6.27; Br, 29.25. Found: C, 52.48; H, 6.44; Br, 29.67.

1-[(1*S*,4*R*,7*R*)-7-(Bromomethyl)-4,7-dimethyl-6-oxabicyclo[3.2.1]-oct-2-en-4-yl]-2-propanol Acetate (7). MeLi (1.25 M solution in ether, 39 mL, 48.7 mmol) was added to a solution of the aldehyde **6** (5.3 g, 19.4 mmol) in ether (150 mL) at 0 °C under argon. After 1 h in the cold, the reaction was quenched by the slow addition of saturated NH_4Cl. The ether solution was washed twice with water, and aqueous washes were extracted with ether. The combined organic layers were washed with saturated brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by flash chromatography (AcOEt–hexane (1:3)) to afford 5.13 g (91%) of alcohols as a 1:1 epimeric mixture: ^{1}H NMR 5.97 and 5.90 (br d each, 1 H in total, J = 9.5 Hz), 5.58 and 5.39 (dd each, 1 H in total, J = 9.5, 2.2 Hz), 4.25 and 3.90 (dd each, 1 H in total, J = 5.4, 2.0 Hz), 4.19–3.84 (m, 1 H), 3.53 (d, 1 H, J = 9.5 Hz), 3.34 (d, 1 H, J = 9.5 Hz), 2.54–1.26 (m, 6 H), 1.43 (s, 3 H), 1.19 and 1.18 (d each, 3 H in total, J = 6.5 Hz), 1.12 and 1.09 (s each, 3 H in total); IR 3410, 2960, 2920, 1053, 983 cm^{-1}. Anal. Calcd for $C_{13}H_{21}O_2Br$: C, 53.99; H, 7.32; Br, 27.63. Found: C, 54.27; H, 7.62; Br, 27.43. A mixture of the above alcohols (5.13 g, 17.7 mmol), acetic anhydride (4.53 g, 44.4 mmol), pyridine (3.6 mL, 44.4 mmol), 4-(dimethylamino)pyridine (216 mg, 1.77 mmol), and dry CH_2Cl_2 (60 mL) was stirred for 3.5 h at room temperature. The mixture was diluted with ether (200 mL) and washed three times with water, saturated brine, and dried ($MgSO_4$). Removal of the solvent in vacuo afforded 5.90 g (100%) of acetates **7**: ^{1}H NMR 5.98 and 5.90 (ddd each, 1 H in total, J = 9.7, 2.9, 1.6 Hz), 5.36 and 5.33 (dd each, 1 H in total, J = 9.7, 1.8 Hz), 5.22–4.84 (m, 1 H), 4.03 and 3.92 (dd each, 1 H in total, J = 5.6, 2.0 Hz), 3.53 (d, 1 H, J = 9.5 Hz), 3.34 (d, 1 H, J = 9.5 Hz), 2.53–1.24 (m, 5 H), 2.02 and 2.00 (s each, 3 H in total), 1.41 and 1.40 (s each, 3 H in total), 1.20 (d, 3 H, J = 6.5 Hz), 1.05 (s, 3 H); IR 2970, 1738, 1730, 1240, 1122, 1053, 988, 898 cm^{-1}. An analytical sample was prepared by distillation: bp 120 °C (bath temperature, 0.8 mmHg). Anal. Calcd for $C_{15}H_{23}O_3Br$: C, 54.39; H, 7.00; Br, 24.12. Found: C, 54.07; H, 7.11; Br, 23.91.

(2*R*,5*S*)-2-(2-Acetoxypropyl)-2-methyl-5-(1-methylethenyl)-3-cyclohexen-1-one (8). To a solution of **7** (4.03 g, 12.2 mmol) in ethanol (80 mL) was added a mixture of zinc copper couple (12.6 g, 97.4 mmol), NH_4Cl (2.6 g, 48.7 mmol), and H_2O (0.5 mL), which was well-agitated beforehand by a spatula, and the resulting mixture was vigorously stirred at reflux for 50 min. The cooled mixture was diluted with hexane (80 mL) and filtered. Evaporation of the solvents left an oil, which was purified by flash chromatography (AcOEt–hexane (1:3)) to afford 3.07 g (100%) of hydroxy acetates as a 1:1 epimeric mixture: ^{1}H NMR 5.48 (dd, 0.5 H, J = 9.4, 2.0 Hz), 5.44 (br s, 1 H), 5.34 (br d, 0.5 H, J = 9.4 Hz), 5.30–4.82 (m, 1 H), 4.73 (br s, 2 H), 4.01–3.64 (m, 1 H), 2.91 (dd, 1 H, J = 11.2, 6.5 Hz), 2.3–1.3 (m, 5 H), 2.04 and 1.97 (s each, 3 H in total), 1.70 (br s, 3 H), 1.23 and 1.22 (d each, 3 H in total, J = 6.2 Hz), 0.98 and 0.96 (s each, 3 H in total); IR 3450, 3027, 1735, 1711, 1642, 1250, 1072, 1040, 893 cm^{-1}. Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.30; H, 9.86. The product was subjected to the next oxidation. To a solution of the hydroxy acetates (3.07 g, 12.2 mmol) in acetone (80 mL) was added dropwise Jones reagent (6.5 mL) at 0 °C, and the mixture was stirred for an additional 20 min. The mixture was poured into cold water (200 mL) and thoroughly extracted with CH_2Cl_2. The combined extracts were washed with half-saturated brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was flash chromatographed (AcOEt–hexane (1:6)) to give 2.68 g (88%) of keto acetate **8** as a 1:1 mixture regarding the acetoxyl group: ^{1}H NMR 5.[illegible] (dd, 0.5 H, J = 11.9, 3.2 Hz), 5.66 (d, 0.5 H, J = 1.8 Hz), 5.64 (s, H), 5.57 (dd, 0.5 H, J = 11.9, 2.0 Hz), 5.06–4.78 (m, 1 H), 4.76 (b 2 H), 3.34–2.96 (m, 1 H), 2.80–2.36 (m, 2 H), 2.35 (dd, 0.5 H, J = 14.[illegible], 10.1 Hz), 2.00 (dd, 0.5 H, J = 14.6, 3.6 Hz), 1.96 and 1.94 (s, each, 3 H in total), 1.78 (d, 0.5 H, J = 14.6 Hz), 1.72 (br s, 3 H), 1.45 (dd, 0.5 H, J = 14.6, 3.1 Hz), 1.19 and 1.15 (d each, 3 H in total, J = 6.3 Hz), 1.15 (s, 3 H); IR 2955, 1736, 1714, 1643, 1244, 1040, 900 cm^{-1}. Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.04; H, 8.99.

Methyl (1*S*,3*R*,6*R*)-3-(2-Acetoxypropyl)-3-methyl-6-(1-methylethenyl)-2-oxo-4-cyclohexene-1-carboxylate (9). To a solution of lithium hexamethyldisilazide prepared from hexamethyldisilazane (2.94 g, 18.2 mmol) and BuLi (1.6 M in hexane, 11.4 mL, 8.2 mmol) in dry ether (73 mL) was added dropwise a solution of **8** (2.68 g, 10.7 mmol) in ether (10 mL) at −70 °C over 3 min under argon. After the solution was stirred for 2 h at −70 °C, carbon dioxide was bubbled into the mixture for 2.5 h at the same temperature. The solution was warmed to 0 °C, kept for 20 min to expel excess carbon dioxide, and then extracted three times with 25% aqueous $NaHCO_3$ and two times with water. The combined aqueous extracts were acidified with 5% HCl in an ice bath, and the resulting white turbid solution was thoroughly extracted with CH_2Cl_2. The extracts were then treated with ethereal diazomethane at 0 °C. Removal of the solvent in vacuo afforded 2.29 g of keto esters **9** as a 3:2 diastereomeric mixture. On the other hand, 743 mg of **8** was recovered from the original ether layer, and this material was again subjected to the above carbonation reaction, giving 640 mg of keto ester **9**. Total amount of **9** was 2.93 g (89%): ^{1}H NMR 5.62 (dd, 0.6 H, J = 9.9, 4.0 Hz), 5.46 (d, 0.4 H, J = 3.6 Hz), 5.41 (br s, 0.4 H), 5.27 (ddd, 0.6 H, J = 9.9, 1.7, 1.1 Hz), 5.10–4.80 (m, 1 H), 4.75 (br s, 2 H), 3.80–3.63 (m, 1 H), 3.71 and 3.69 (s each, 3 H in total), 2.69 (d, 0.6 H, J = 0.4 Hz), 2.67 (d, 0.4 H, J = 0.6 Hz), 2.44 (dd, 0.6 H, J = 14.6, 10.4 Hz), 2.16 (dd, 0.4 H, J = 14.6, 3.6 Hz), 1.95 and 1.80 (s each, 3 H in total), 1.62 (d, 0.4 H, J = 14.6 Hz), 1.61 (t, 3 H, J = 1.1 Hz), 1.35 (dd, 0.6 H, J = 14.6, 2.7 Hz), 1.29 and 1.28 (s each, 3 H in total), 1.16 and 1.14 (d each, 3 H in total, J = 6.2 Hz); IR 2975, 1740, 1680, 1648, 1610, 1248, 1234, 1200, 1048, 840 cm^{-1}. Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85. Found: C, 66.48; H, 7.94.

Methyl (1*S*,3*R*,6*R*)-3-Methyl-6-(1-methylethenyl)-2-oxo-3-(2-oxopropyl)-4-cyclohexene-1-carboxylate (10). NaH (60%, 1.08 g, 27.1 mmol) was added to a solution of **9** (2.78 g, 9.02 mmol) in dry THF (70 mL), and the mixture was stirred for 2 h at room temperature. After addition of MeOH (1.82 mL, 45.1 mmol), stirring was continued for 1 h, DMF (10 mL) was added, and the mixture was further stirred for 2 h at room temperature. The reaction mixture was added dropwise to a cooled solution of Jones reagent (12 mL) in acetone (100 mL) at 0 °C. After 15 min, the mixture was poured into cold water and extracted with CH_2Cl_2 three times. The combined organic extracts were washed with water and saturated brine, dried ($MgSO_4$), and concentrated in vacuo. The residual oil was purified by flash chromatography (AcOEt–hexane (1:5)) to afford 2.19 g (92%) of diketone **10**: ^{1}H NMR 5.62 (dd, 1 H, J = 10.0, 3.7 Hz), 5.44 (ddd, 1 H, J = 10.0, 0.9, 0.9 Hz), 4.75 (q, 2 H, J = 1.1 Hz), 3.84–2.66 (m, 1 H), 3.72 (s, 3 H), 3.10 (d, 1 H, J = 15.7 Hz), 2.76 (d, 1 H, J = 0.9 Hz), 2.47 (d, 1 H, J = 15.7 Hz), 2.06 (s, 3 H), 1.66 (t, 3 H, J = 1.1 Hz), 1.34 (s, 3 H); IR 1720, 1675, 1643, 1610, 1282, 1220, 1028 cm^{-1}. Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.97; H, 7.84.

Methyl (4*S*,5*R*,7a*R*)-7a-Methyl-5-(1-methylethenyl)-2-oxo-1,4,5,7a-tetrahydro-2*H*-indene-4-carboxylate (11). A mixture of **10** (2.68 g, 10.1 mmol), benzoic acid (1.61 g, 13.2 mmol), pyrrolidine (2.54 mL, 30.5 mmol), and benzene (100 mL) was heated at 110 °C (bath temperature) for 9 h. The cooled mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ (60 mL). To this $CHCl_3$ solution was added a mixture of AcONa (8.28 g, 101 mmol), AcOH (60 mL), and water (60

mL), and the resulting heterogeneous mixture was stirred at 60 °C for 14 h. The reaction mixture was poured into cold water, and the $CHCl_3$ layer was separated. The aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were washed with water, aqueous $NaHCO_3$, water, and saturated brine and concentrated. The residual oil was purified by flash chromatography using AcOEt–hexane (1:5) as eluent, giving 1.82 g of **11** and its β,γ-unsaturated isomer (404 mg). The latter compound (404 mg) was converted into **11** (283 mg) by treating with neutral alumina (Woelm Activity I, 2.0 g) in benzene (30 mL) at 60 °C followed by purification by preparative TLC (AcOEt–hexane (2:5)). The total amount of **11** was 2.10 g (84.5%): $[\alpha]^{22}_D$ −185.2° (*c* = 0.86, $CHCl_3$); ^{1}H NMR 5.88 (d, 1 H, *J* = 1.5 Hz), 5.79 (dd, 1 H, *J* = 9.7, 2.2 Hz), 5.43 (dd, 1 H, *J* = 9.7, 2.2 Hz), 4.89 (q, 2 H, *J* = 1.2 Hz), 3.76 (s, 3 H), 3.70 (dd, 1 H, *J* = 10.5, 1.5 Hz), 3.47 (ddd, 1 H, *J* = 10.5, 2.2, 2.2 Hz), 2.39 (s, 2 H), 1.75 (t, 3 H, *J* = 1.2 Hz), 1.38 (s, 3 H); IR 1740, 1712, 1620, 1270, 1220, 1160 cm^{-1}. Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 73.40; H, 7.27.

(3*R*,3a*R*,4*R*,5*R*,7a*R*)-4-(Acetoxymethyl)-3,3a-epoxy-1,3,3a,4,5,7a-hexahydro-7a-methyl-5-(1-methylethenyl)-2*H*-inden-2-one (12). $LiAlH_4$ (639 mg, 16.8 mmol) was added to a solution of **11** (1.38 g, 5.61 mmol) in dry ether (130 mL) at 0 °C. After 20 min the excess hydride was decomposed by the slow addition of wet ether followed by water and filtered. Removal of the solvent left an oil (1.25 g), which was dissolved in $CHCl_3$ (60 mL) and treated with active MnO_2 (4.9 g, 56.1 mmol). After the solution was stirred for 2 days under an oxygen atmosphere, 4.9 g (56.1 mmol) more of MnO_2 was added and stirring was continued for 1 day. Again MnO_2 (4.9 g, 56.1 mmol) was added, and the resulting mixture was further stirred for an additional 1 day. The mixture was filtered through a pad of Celite by the aid of AcOEt, and the filtrate was concentrated in vacuo. Purification by flash chromatography (AcOEt–hexane (1:1)) afforded 1.04 g (85%) of a hydroxy enone: mp 70–71 °C (ether–hexane); $[\alpha]^{22}_D$ −260.4° (*c* = 0.5, $CHCl_3$); ^{1}H NMR 6.04 (br s, 1 H), 5.78 (dd, 1 H, *J* = 9.5, 1.5 Hz), 5.34 (dd, 1 H, *J* = 9.5, 1.5 Hz), 4.92 (br s, 2 H), 4.09–3.68 (m, 2 H), 2.90–2.80 (m, 2 H), 2.37 (s, 2 H), 2.06 (dd, 1 H, *J* = 5.2, 5.2 Hz), 1.76 (br s, 3 H), 1.37 (s, 3 H); IR 3400, 1705, 1674, 1610, 895 cm^{-1}. Anal. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 76.85; H, 8.47. To a solution of the hydroxy enone (135 mg, 0.62 mmol) in MeOH (4 mL) were added hydrogen peroxide (30%, 0.20 mL, 1.86 mmol) and 6 N NaOH (0.41 mL, 2.48 mmol) at 0 °C. After it was stirred for 15 min, the mixture was diluted with AcOEt (20 mL) and washed with half-saturated brine containing sodium thiosulfate. The aqueous washes were extracted once with AcOEt, and the combined organic layers were washed with saturated brine, dried ($MgSO_4$), and concentrated. The crude epoxy alcohol obtained was dissolved in CH_2Cl_2 (4 mL) followed by addition of a catalytic amount of 4-(dimethylamino)pyridine, acetic anhydride (189 mg, 1.86 mmol), and pyridine (0.23 mL, 2.8 mmol). After 2 h the reaction mixture was diluted with AcOEt (20 mL) and washed with water and saturated brine. Evaporation of the solvents left an oil, which was purified by preparative TLC (AcOEt–hexane (1:2)) affording 150 mg (88%) of **12** as crystals: mp 62–63 °C (hexane); $[\alpha]^{22}_D$ −55.6° (*c* = 0.5, $CHCl_3$); ^{1}H NMR 5.60 (dd, 1 H, *J* = 10.1, 2.5 Hz), 5.42 (dd, 1 H, *J* = 10.1, 2.0 Hz), 4.91 (q, 1 H, *J* = 1.4 Hz), 4.81 (br s, 1 H), 4.12 (dd, 1 H, *J* = 11.7, 4.2 Hz), 3.94 (dd, 1 H, *J* = 11.7, 3.2 Hz), 3.49 (s, 1 H), 3.12 (ddd, 1 H, *J* = 10.8, 2.5, 2.0 Hz), 2.64 (ddd, 1 H, *J* = 10.8, 4.2, 3.2 Hz), 2.33 (d, 1 H, *J* = 17.8 Hz), 2.04 (d, 1 H, *J* = 17.8 Hz), 2.04 (s, 3 H), 1.69 (dd, 3 H, *J* = 1.4, 0.7 Hz), 1.37 (s, 3 H); IR 1740, 1238, 1040, 900 cm^{-1}. Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.54; H, 7.30. Found: C, 69.77; H, 7.60.

(3*R*,3a*R*,4*R*,5*R*,7a*S*)-4-(Acetoxymethyl)-3,3a-epoxy-1,3,3a,4,5,7a-hexahydro-7a-methyl-1-methylene-5-(1-methylethenyl)-2*H*-inden-2-one (13). According to the procedure of the Gras method,[18] a mixture of **12** (11 mg, 0.004 mmol), paraformaldehyde (12 mg, 0.4 mmol), *N*-methylanilinium trifluoroacetate (26.5 mg, 0.12 mmol), and dry THF (1.3 mL) was heated at 90 °C (bath temperature) for 5 h under argon. The cooled reaction mixture was poured into water and thoroughly extracted with CH_2Cl_2. The combined extracts were washed with water and saturated brine, dried ($MgSO_4$), and evaporated in vacuo. The crude oil was purified by preparative TLC (AcOEt–hexane (1:2)) affording 9.2 mg (80%) of **13** as an oil: $[\alpha]^{22}_D$ −97.5° (*c* = 0.49, $CHCl_3$); ^{1}H NMR 6.18 (s, 1 H), 5.53 (dd, 1 H, *J* = 10.1, 2.0 Hz), 5.48 (s, 1 H), 5.39 (dd, 1 H, *J* = 10.1, 1.8 Hz), 4.92 (q, 1 H, *J* = 1.5 Hz), 4.83 (br s, 1 H), 4.14 (dd, 1 H, *J* = 11.5, 4.3 Hz), 3.98 (dd, 1 H, *J* = 11.5, 2.7 Hz), 3.70 (s, 1 H), 3.16 (ddd, 1 H, *J* = 11.2, 2.0, 1.8 Hz), 2.62 (ddd, 1 H, *J* = 11.2, 4.3, 2.7 Hz), 2.05 (s, 3 H), 1.70 (dd, 3 H, *J* = 1.5, 0.8 Hz), 1.43 (s, 3 H); IR 2950, 1732, 1723, 1640, 1230, 1038, 945, 903 cm^{-1}; MS (*m*/*z*). Calcd for $C_{17}H_{20}O_4$ (M^+): 288.1362. Found: 288.1374.

(3a*R*,4*R*,5*R*,7a*S*)-4-(Acetoxymethyl)-1,3,3a,4,5,7a-hexahydro-3a-hydroxy-7a-methyl-1-methylene-5-(1-methylethenyl)-2*H*-inden-2-one (14). According to the procedure of Sharpless,[19] $NaBH_4$ (74 mg, 1.93 mmol) was added in batches to a mixture of $(PhSe)_2$ (300 mg, 0.96 mmol) and ethanol (5 mL) at room temperature. After evolution of hydrogen ceased, the faint yellow solution of $Na^+[PhSeB(OEt)_3]^-$ obtained was cooled to 0 °C, to which AcOH (7.1 μL, 0.13 mmol) was added. The resulting mixture was then added to a solution of **13** (185 mg, 0.64 mmol) in ethanol (5 mL) under argon, and stirring was continued for 5 min at room temperature. The mixture was diluted with AcOEt (50 mL) and washed twice with half-saturated brine, and aqueous washes were extracted once with AcOEt. The combined organic layers were concentrated in vacuo leaving a yellow oil, which was purified by flash column chromatography (AcOEt–hexane (1:1)) to afford 171 mg (92%) of hydroxy ketone **14** as crystals: mp 148 °C; $[\alpha]^{26}_D$ −303.7° (*c* = 1.0, $CHCl_3$); ^{1}H NMR 6.15 (s, 1 H), 5.35 (s, 1 H), 5.34 (s, 2 H), 4.92 (q, 1 H, *J* = 1.5 Hz), 4.88 (br s, 1 H), 4.49 (dd, 1 H, *J* = 11.8, 3.1 Hz), 4.13 (dd, 1 H, *J* = 11.8, 4.8 Hz), 2.91 (d, 1 H, *J* = 10.7 Hz), 2.63 (d, 1 H, *J* = 18.1 Hz), 2.59 (s, 1 H, –OH), 2.32 (d, 1 H, *J* = 18.1 Hz), 2.19 (ddd, 1 H, *J* = 10.7, 4.8, 3.1 Hz), 2.07 (s, 3 H), 1.70 (br s, 3 H), 1.36 (s, 3 H); IR (KBr) 3360, 1736, 1720, 1638, 1378, 1246, 1060, 752 cm^{-1}. Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.43; H, 7.69.

(3*R*,5a*S*,8a*R*,9*R*)-2-(Bromomethyl)-2,5a-dimethyl-6-methylene-7-oxo-2,3,5a,6,7,8-hexahydro-3,8a-methano-8a*H*-cyclopent[*b*]oxepin-9-methanol Acetate (15). NBS (457 mg, 2.57 mmol) was added to a solution of **14** (171 mg, 0.59 mmol) in dry THF (13.5 mL) at 0 °C under argon. After it was stirred for 30 min in the cold, the mixture was diluted with AcOEt (50 mL) and washed with half-saturated brine containing $NaHSO_3$ and half-saturated brine. Aqueous washes were extracted with AcOEt. The combined organic layers were concentrated in vacuo, and the residue oil was purified by flash chromatography (AcOEt–hexane (1:3)) to provide 192 mg (88%) of **15** as a 5:2 diastereomeric mixture: ^{1}H NMR 6.04 (d, 1 H, *J* = 7.2 Hz), 5.96 (br d, 1 H, *J* = 7.2 Hz), 5.92 (s, 1 H), 5.16 (s, 1 H), 4.14 (dd, 1 H, *J* = 11.5, 6.2 Hz), 3.94 (dd, 1 H, *J* = 11.5, 8.7 Hz), 3.60 (d, 1 H, *J* = 9.0 Hz), 3.40 (d, 1 H, *J* = 9.4 Hz), 2.92–2.50 (m, 2 H), 2.90 (d, 1 H, *J* = 18.9 Hz), 2.50 (d, 1 H, *J* = 18.9 Hz), 1.96 (s, 3 H), 1.54 (br s, 3 H), 1.19 (s, 3 H); IR 1738, 1642, 1370, 1230, 1028, 732 cm^{-1}; MS (*m*/*z*). Calcd for $C_{17}H_{21}O_4{}^{79}Br$ (M^+): 368.0624. Found: 368.0633.

(3*R*,5a*S*,7*S*,8a*R*,9*R*)-2-(Bromomethyl)-7-[(methylsulfonyl)oxy]-2,5a-dimethyl-6-methylene-2,3,5a,6,7,8-hexahydro-3,8a-methano-8a*H*-cyclopent[*b*]oxepin-9-methanol Acetate (16). $NaBH_4$ (19 mg, 0.48 mmol) was added in batches to a mixture of **15** (176 mg, 0.48 mmol) and $CeCl_3 \cdot 7H_2O$ (178 mg, 0.48 mmol) in MeOH (7 mL) at 0 °C. After 10 min the reaction was quenched with saturated NH_4Cl solution. The mixture was poured into cold water and extracted with AcOEt, and the organic layers were combined. Evaporation of the solvent in vacuo afforded an allylic alcohol as an oil, which was used for the next reaction without purification: ^{1}H NMR 5.96 (br s, 1 H), 5.91 (br d, 1 H, *J* = 2.0 Hz), 5.16 (d, 1 H, *J* = 2.2 Hz), 4.93 (d, 1 H, *J* = 2.2 Hz), 4.75 (ddd, 1 H, *J* = 10.5, 5.4, 2.9 Hz), 4.38 (dd, 1 H, *J* = 13.0, 2.2 Hz), 4.24 (dd, 1 H, *J* = 13.0, 1.4 Hz), 3.55 (d, 1 H, *J* = 9.2 Hz), 3.33 (d, 1 H, *J* = 9.2 Hz), 2.68–2.40 (m, 3 H), 2.63 (dd, 1 H, *J* = 15.1, 5.4 Hz), 2.06 (s, 3 H), 1.78 (dd, 1 H, *J* = 15.1, 2.9 Hz), 1.48 (s, 3 H), 1.05 (s, 3 H); IR 3500, 1735, 1658, 1370, 1238, 1036, 738 cm^{-1}; MS (*m*/*z*). Calcd for $C_{17}H_{23}O_4{}^{79}Br$ (M^+): 370.0780. Found: 370.0803.

Methanesulfonyl chloride (0.26 mL, 3.34 mmol) was added to a solution of the crude allylic alcohol (ca. 0.48 mmol) in pyridine (7 mL) at 0 °C. After it was stirred for 12 h at room temperature, the mixture was diluted with AcOEt (40 mL), and washed with water (3 × 20 mL) and saturated brine. Removal of the solvent in vacuo gave **16** as an unstable oil, which was immediately subjected to the next oxidation: ^{1}H NMR 5.99 (br s, 1 H), 5.95 (s, 1 H), 5.57 (dd, 1 H, *J* = 9.9, 2.5 Hz), 5.23 (d, 1 H, *J* = 2.2 Hz), 5.08 (d, 1 H, *J* = 2.2 Hz), 4.28 (dd, 1 H, *J* = 11.5, 4.4 Hz), 4.01 (dd, 1 H, *J* = 11.5, 9.0 Hz), 3.56 (d, 1 H, *J* = 9.4 Hz), 3.36 (d, 1 H, *J* = 9.4 Hz), 3.09 (s, 3 H), 2.74–2.46 (m, 2 H), 2.73 (dd, 1 H, *J* = 15.8, 9.9 Hz), 2.15 (dd, 1 H, *J* = 15.8, 2.5 Hz), 2.05 (s, 3 H), 1.49 (s, 3 H), 1.05 (s, 3 H); IR 1734, 1663, 1360, 1240, 1172, 1040, 738 cm^{-1}.

(1*R*,2a*R*,5*R*,7a*R*,7b*R*,8*R*)-4-(Bromomethyl)-4,7a-dimethyl-1a,2,5,7a-tetrahydro-2a,5-methano-2a*H*-oxireno[3,4]cyclopent[1,2-*b*]oxepin-7b(4*H*),8-dimethanol 8-Acetate (17). A mixture of the crude allylic mesylate **16** (ca. 0.48 mmol) and OsO_4 (182 mg, 0.72 mmol) in pyridine (3 mL) was stirred for 33 h at room temperature in the dark. Aqueous $NaHSO_3$ (10%, 4 mL) was added, and the mixture was stirred for an additional 1 h. The mixture was poured into cold water and extracted with CH_2Cl_2 (3 × 20 mL). The extracts were washed with water and saturated brine and concentrated in vacuo affording 250 mg of a diol mesylate: ^{1}H NMR 5.92 (br s, 1 H), 5.88 (br s, 1 H), 5.09 (dd, 1 H, *J* = 10.1, 4.8 Hz), 4.48–3.58 (m, 6 H), 3.57 (d, 1 H, *J* = 9.5 Hz), 3.40 (d, 1 H, *J* = 9.5 Hz), 3.10 (s, 3 H), 2.74 (dd, 1 H, *J* = 16.2, 10.1 Hz), 2.74–2.44 (m, 2 H), 2.10 (s, 3 H), 2.09 (dd, 1 H, *J* = 16.2, 4.8 Hz), 1.48 (s, 3 H), 1.18 (s, 3 H); IR 3450, 1732, 1348, 1235, 1172, 1030, 757 cm^{-1}. In turn, a mixture of the crude diol mesylate (ca. 0.48 mmol) and DBU

(0.36 mL, 2.38 mmol) in DMF (5 mL) was stirred at 70 °C for 40 min under argon. The reaction mixture was cooled to 0 °C and partitioned between aqueous NH_4Cl solution and AcOEt–hexane (4:1). The aqueous layer was extracted with AcOEt–hexane (4:1), and the organic layers were combined and washed with water and saturated brine. Evaporation of the solvent in vacuo left an oil, which was purified by flash chromatography (AcOEt–hexane (1:1)) to give 150 mg (81% overall yield from **15**) of crystalline **17**: mp 108–112 °C; ^1H NMR 6.00 (br s, 1 H), 5.95 (br d, 1 H, J = 1.8 Hz), 4.29 (dd, 1 H, J = 11.7, 1.6 Hz), 4.11 (d, 1 H, J = 11.7 Hz), 3.96 (dd, 1 H, J = 12.8, 5.8 Hz), 3.54 (dd, 1 H, J = 12.8, 6.7 Hz), 3.49 (d, 1 H, J = 9.4 Hz), 3.47 (d, 1 H, J = 2.9 Hz), 3.29 (d, 1 H, J = 9.4 Hz), 2.68–2.40 (m, 2 H), 2.13 (d, 1 H, J = 7.2 Hz), 2.07 (s, 3 H), 1.94 (dd, 1 H, J = 7.2, 2.9 Hz), 1.69 (dd, 1 H, J = 6.7, 5.8 Hz, -OH), 1.48 (s, 3 H), 1.06 (s, 3 H); IR 3450, 1738, 1235, 1040, 900, 836, 730 cm^{-1}. Anal. Calcd for $C_{17}H_{23}O_5Br$: C, 52.72; H, 5.98; Br, 20.63. Found: C, 52.43; H, 5.92; Br, 20.97.

Methyl (1*R*,2a*R*,5*R*,7a*R*,7b*S*,8*R*)-8-[(Acetyloxy)methyl]-4-(bromomethyl)-4,7a-dimethyl-1a,2,5,7a-tetrahydro-2a,5-methano-2a*H*-oxireno[3,4]cyclopent[1,2-*b*]oxepin-7b(4*H*)-carboxylate (18). The Collins reagent[24] (209 mg, 0.81 mmol) was added to a solution of **17** (63 mg, 0.16 mmol) in dry CH_2Cl_2 (6 mL) at room temperature. After 5 min an equal amount of the reagent (209 mg, 0.81 mmol) was added, and stirring was continued for 10 min. The mixture was then diluted with CH_2Cl_2 (6 mL) and passed through a short silica gel column by the aid of AcOEt. Evaporation of the solvents from the eluate left 57 mg (90%) of an aldehyde, which was used for the next oxidation: ^1H NMR 8.92 and 8.90 (s each, 1 H in total), 6.36 and 6.29 (d each, 1 H in total, J = 9.7 Hz), 6.10–5.74 (m, 1 H), 4.36 and 4.29 (d each, 1 H in total, J = 11.5, 9.9 Hz, respectively), 4.19 and 4.13 (d each, 1 H in total, J = 9.9, 11.5 Hz, respectively), 3.87–3.74 (m, 1 H), 3.69 and 2.58 (d each, 1 H in total, J = 5.0 Hz), 3.49 (d, 1 H, J = 9.5 Hz), 3.31 (d, 1 H, J = 9.5 Hz), 2.92–2.42 (m, 1 H), 2.22 and 2.16 (d each, 2 H in total, J = 3.2 Hz), 2.05 (s, 3 H), 1.47 (s, 3 H), 1.10 and 1.06 (s each, 3 H in total); IR 1735, 1238, 1028, 838, 730 cm^{-1}.

A solution of $NaClO_2$[25] (122 mg, 1.35 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (117 mg, 0.75 mmol) in water (1.5 mL) was added dropwise to a stirred solution of the aldehyde (57 mg, 0.15 mmol) and 2-methyl-2-butene (0.73 mL, 6.9 mmol) in *t*-BuOH (3.7 mL) at room temperature over 5 min. After it was stirred for 4 h, the mixture was cooled to 0 °C and an ethereal diazomethane solution (0.1 M solution, 4 mL, 0.4 mmol) was added. After 15 min at 0 °C the mixture was partitioned between AcOEt (30 mL) and water (15 mL), and the aqueous phase was extracted with AcOEt. The organic layers were combined and washed with saturated brine and concentrated in vacuo giving an oily residue, which was purified by preparative TLC (AcOEt–hexane (1:2)) to afford 55 mg (90%) of **18** as an oil: ^1H NMR 6.26 and 6.18 (d each, 1 H in total, J = 9.7 Hz), 6.01–5.70 (m, 2 H), 4.40 (dd, 1 H, J = 11.7, 2.0 Hz), 4.23 (d, 1 H, J = 11.7 Hz), 3.86–3.72 (m, 1 H), 3.73 (s, 3 H), 3.70 and 2.56 (dd each, 1 H in total, J = 5.4, 2.0 Hz, respectively), 3.50 (d, 1 H, J = 9.2 Hz), 3.30 (d, 1 H, J = 9.2 Hz), 2.91–2.40 (m, 1 H), 2.16 and 2.09 (d each, 2 H in total, J = 4.0 Hz), 2.06 (s, 3 H), 1.47 (s, 3 H), 1.12 and 1.09 (s each, 3 H in total); IR 1738, 1238, 1040, 908, 740 cm^{-1}; MS (m/z). Calcd for $C_{18}H_{23}O_6{}^{79}Br$ (M^+): 414.0679. Found: 414.0684.

(1a*R*,2a*R*,4*S*,5*S*,6*R*,6a*S*,8a*S*,8b*R*,9*R*)-4-(Bromomethyl)-8-oxo-1a,2,5,6,6a,8b-hexahydro-6-hydroxy-4,8b-dimethyl-4*H*-2a,5-methano-8*H*-1,3,7-trioxacyclopenta[*ij*]cycloprop[*a*]azulene-9-methanol Acetate (19). A mixture of **18** (35 mg, 0.84 mmol), OsO_4 (36 mg, 0.14 mmol), and pyridine (0.6 mL) was stirred at room temperature for 7 days in the dark. The solvent was evaporated in vacuo, and the black residue was dissolved in CH_2Cl_2 (1 mL), which was then chromatographed on a short silica gel column. After eluting the unchanged starting material with AcOEt–hexane (3:1, 50 mL), elution with AcOEt–MeOH (3:1, 80 mL) afforded an osmate ester as a black oil, which was dissolved in $CHCl_3$ (4 mL) and treated with hydrogen sulfide for 8 min. The reaction mixture was filtered through a pad of Celite by the aid of AcOEt. After evaporation of the solvents in vacuo, the residual oil was purified by a Florisil column (AcOEt as eluent) to give 30 mg (86%) of **19** as an oil: ^1H NMR 4.83 (d, 1 H, J = 8.5 Hz), 4.71 (br t, 1 H, J = 8.5 Hz), 4.68 (dd, 1 H, J = 12.2, 7.5 Hz), 4.07 (dd, 1 H, J = 12.2, 7.5 Hz), 4.01–3.88 (m, 1 H), 3.74 (m, 1 H), 3.39 (d, 1 H, J = 10.6 Hz), 3.27 (d, 1 H, J = 10.6 Hz), 3.10–2.72 (m, 1 H), 2.72–2.30 (m, 1 H), 2.53 (br d, 1 H, J = 7.0 Hz), 2.13 (dd, 1 H, J = 7.0, 1.4 Hz), 2.10 (s, 3 H), 1.49 (s, 3 H), 1.14 (s, 3 H); IR 3460, 1790, 1740, 1380, 1268, 1245, 1156, 934, 760 cm^{-1}.

(-)-Bromopicrotoxinin (20). NaH (60%, 13.1 mg, 0.33 mmol) was added to a solution of **19** (13.6 mg, 0.032 mmol) in MeOH (2 mL) at 0 °C under argon. After 1 h at 0 °C, the reaction was quenched with AcOH (33 μL, 0.59 mmol). The solvent was evaporated in vacuo, and the residue was dissolved in AcOEt and filtered to remove sodium acetate. Removal of the solvent in vacuo left a colorless oil, crude diol, which was immediately subjected to the next oxidation.

PCC[30] (14 mg, 0.066 mmol) was added to a solution of the above diol (ca. 0.032 mmol) in dry CH_2Cl_2 (2 mL) under argon. After the solution was stirred for 30 min, 14 mg more of PCC was added and the stirring was continued for an additional 3 h. The mixture was diluted with AcOEt (5 mL) and passed through a short silica gel column. After evaporation of the solvents in vacuo, the crystalline residue was purified by preparative TLC (AcOEt–$CHCl_3$ (2:9)) affording 5.0 mg (41.1%) of (-)-**20** as colorless crystals:[31] mp 256 °C dec; $[\alpha]^{19}_D$ -126° (c = 0.21, $CHCl_3$). The IR, ^1H NMR, and TLC behavior of (-)-**20** proved identical with those of (-)-β-bromopicrotoxinin[7] prepared from natural picrotoxinin.[32]

(-)-Picrotoxinin (1). To a solution of bromopicrotoxinin **20** (5 mg, 0.013 mmol) in EtOH (1.5 mL) was added a mixture of zinc copper couple (8.7 mg, 0.067 mmol), NH_4Cl (1.7 mg, 0.033 mmol), and H_2O (50 μL), which was well-agitated beforehand by a spatula, and the resulting mixture was vigorously stirred at reflux for 30 min. The mixture was cooled, and inorganic materials were filtered off. The filtrate was partitioned between CH_2Cl_2 and water, and the aqueous phase was extracted twice with CH_2Cl_2. The organic layers were combined and washed with saturated brine. Evaporation of the solvent in vacuo left crystals, which were purified by preparative TLC (benzene–AcOEt (1:1)) affording 3.8 mg (100%) of (-)-**1** as colorless crystals: mp 198–199 °C (needles from H_2O); $[\alpha]^{25}_D$ -6.7° (c = 0.19, $CHCl_3$). The IR, ^1H NMR, and TLC behavior of synthetic (-)-**1** proved identical in all respects with those of natural picrotoxinin.[32]

13-(Phenylseleno)picrotin (22). A mixture of picrotoxinin (137 mg, 0.47 mmol), MCPBA (80%, 202 mg, 0.94 mmol), and CH_2Cl_2 (5 mL) was stirred for 2.5 days at room temperature. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$, and half-saturated brine. The aqueous washes were extracted with CH_2Cl_2. The organic layers were combined and concentrated in vacuo giving 150 mg of crystals, which contained a diastereomeric mixture of epoxy picrotoxinin **21** along with a small amount of 3-chlorobenzoic acid. ^1H NMR analysis revealed that the ratio of the diastereomers was ca. 5:2. **21**: ^1H NMR 5.40 and 4.72 (dd each, 1 H in total, J = 5.4, 3.2 Hz), 4.83 (s, 1 H), 4.61 and 4.31 (d each, 1 H in total, J = 3.2 Hz), 3.70 (d, 1 H, J = 3.4 Hz), 3.43 and 3.32 (dd each, 1 H in total, J = 5.4, 4.3 Hz), 3.03 and 2.62 (d each, 1 H in total, J = 4.3 Hz), 2.99 (d, 1 H, J = 3.6 Hz), 2.85 (d, 1 H, J = 3.6 Hz), 2.84 (dd, 1 H, J = 15.5, 3.4 Hz), 2.14 and 2.11 (d each, 1 H in total, J = 15.5 Hz), 1.55 (s, 3 H), 1.37 (s, 3 H); IR (KBr) 3350, 1780, 1350, 1254, 1172, 734 cm^{-1}.

To a solution of $Na^+[PhSeB(OEt)_3]^-$ (1.41 mmol) in EtOH (4 mL) containing AcOH (13 μL, 0.23 mmol), prepared from $(PhSe)_2$ (220 mg, 0.70 mmol) and $NaBH_4$ (53 mg, 1.41 mmol) in EtOH[19] as previously described, was added a solution of the above crude epoxy picrotoxinin in EtOH (6 mL) at room temperature under argon. After 30 min the mixture was partitioned between CH_2Cl_2 (40 mL) and saturated brine (15 mL). The aqueous phase was extracted with CH_2Cl_2. The combined organic layers were concentrated in vacuo giving yellow crystals, which were purified by preparative TLC (AcOEt–CH_2Cl_2 (1:2)) to afford 208 mg (99%) of crystalline **22** as a 5:2 diastereomeric mixture: ^1H NMR 4.94 and 4.80 (dd each, 1 H in total, J = 4.7, 3.6 Hz), 4.73 and 4.55 (d each, 1 H in total, J = 3.6 Hz), 4.33 (s, 1 H), 3.71 (d, 1 H, J = 3.2 Hz), 3.50 (d, 1 H, J = 1.6 Hz), 3.30 (d, 1 H, J = 11.5 Hz), 3.18 (d, 1 H, J = 11.5 Hz), 3.18 and 2.61 (d each, 1 H in total, J = 3.7 Hz), 3.05–2.78 (m, 1 H), 2.78 (dd, 1 H, J = 14.9, 3.2 Hz), 2.13 and 2.07 (d each, 1 H in total, J = 14.9 Hz), 1.41 (s, 3 H), 1.23 (s, 3 H); IR (KBr) 3350, 1778, 1254, 1160, 734, 690 cm^{-1}. Anal. Calcd for $C_{21}H_{22}O_7Se$: C, 54.20; H, 4.76. Found: C, 54.01; H, 5.00.

(-)-Picrotin (2). A mixture of **22** (208 mg, 0.46 mmol) and toluene (20 mL) was heated at 120 °C for 5 min to make a homogeneous solution, which was then cooled to 100 °C followed by addition of a catalytic amount of AIBN and tributyltin hydride (0.62 mL, 2.32 mmol). After being kept at 100 °C for 30 min, the reaction mixture was cooled to room temperature and the solvent was evaporated in vacuo. The residue was flash-chromatographed (AcOEt–CH_2Cl_2 (1:4)) to afford 126 mg (88%) of (-)-**2** as crystals: mp 253–254 °C (EtOH); $[\alpha]^{25}_D$ -69.9° (c = 1.07, EtOH). The IR, ^1H NMR, and TLC behavior of synthetic (-)-**2** were superimposable with those of natural picrotin.[32]

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Registry No. (-)-**1**, 17617-45-7; (-)-**2**, 21416-53-5; **3**, 90129-47-8; **3** keto vinyl ester derivative, 90056-28-3; **4**, 90056-29-4; **5**, 90056-30-7; **6**,

90056-31-8; **7** (isomer 1), 90129-48-9; **7** (isomer 2), 90056-32-9; **7** alcohol (isomer 1), 119637-88-6; **7** alcohol (isomer 2), 119717-48-5; **8** (isomer 1), 119619-14-6; **8** (isomer 2), 119717-34-9; **8** 1-hydroxy derivative, 119619-13-5; **9** (isomer 1), 119619-15-7; **9** (isomer 2), 119619-16-8; **10**, 119619-17-9; **11**, 119619-18-0; **11** β,γ-unsaturated derivative, 119619-19-1; **11** hydroxy enone derivative, 119619-20-4; **11** epoxy alcohol derivative, 119619-21-5; **12**, 119619-22-6; **13**, 119717-35-0; **14**, 119717-36-1; **15** (isomer 1), 119619-23-7; **15** (isomer 2), 119619-24-8; **15** 7-alcohol derivative, 119619-25-9; **16**, 119619-26-0; **16** diol mesylate derivative, 119637-89-7; **17**, 119619-27-1; **17** aldehyde derivative, 119619-28-2; **18**, 119619-29-3; **19**, 119619-30-6; **19** diol derivative, 119619-31-7; (-)-**20**, 20744-71-2; **21** (isomer 1), 119678-62-5; **21** (isomer 2), 119678-63-6; **22** (isomer 1), 119619-32-8; **22** (isomer 2), 119678-64-7; $Na^+[PhSeB(OEt)_3]^-$, 117268-79-8.

Squalene Synthetase. Inhibition by Ammonium Analogues of Carbocationic Intermediates in the Conversion of Presqualene Diphosphate to Squalene

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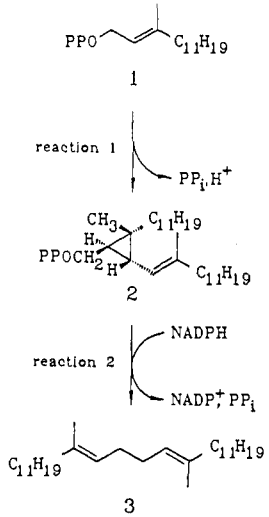
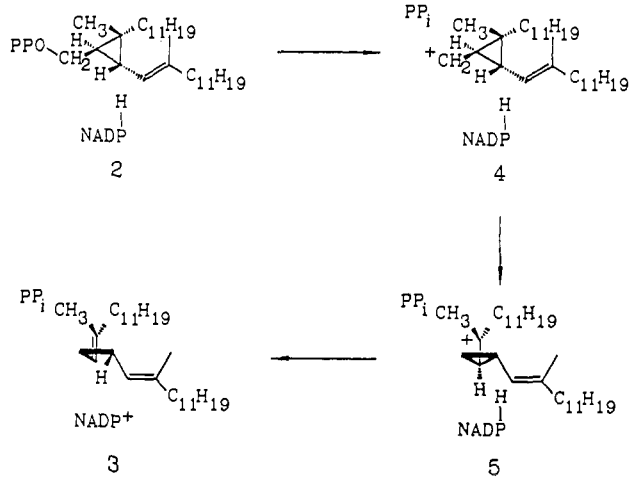
Abstract: Squalene synthetase (EC 2.5.1.21) catalyzes the formation of squalene (**3**) from farnesyl diphosphate (**1**) via presqualene diphosphate (**2**) in two steps. The mechanism of the rearrangement of **2** to **3** was studied with stable ammonium analogues **6** and **7** of primary and tertiary cyclopropylcarbinyl cations **4** and **5**, respectively, proposed as intermediates. In non-pyrophosphate-containing buffers, **6** and **7** were not inhibitors. However, the combination of **6** or **7** with PP_i produced potent synergistic inhibition of squalene synthesis from **1** and **2**. Amino acid **8**, an analogue in which a phosphonophosphate moiety was tethered to the amino group in **6**, was a potent inhibitor of squalene synthesis in pyrophosphate-free buffers. When synthesis of **2** and **3** from **1** was measured simultaneously in the presence of **8**, both rates were depressed in an identical manner. It was concluded that squalene synthetase has a single active site which catalyzes **1** → **2** and **2** → **3**. The mechanism of the second reaction is discussed.

Squalene synthetase (farnesyldiphosphate:farnesyldiphosphate farnesyl transferase, EC 2.5.1.21) catalyzes the formation of squalene from farnesyl diphosphate in two distinct steps.[1] In reaction 1, two molecules of farnesyl diphosphate (**1**) are condensed to form presqualene diphosphate (**2**), a prenyl transfer where the C1-C2 double bond of one farnesyl diphosphate serves as the prenyl acceptor for the farnesyl residue of the other.[2] Presqualene diphosphate is then converted to squalene (**3**) in reaction 2 by a rearrangement that cleaves the two newly formed cyclopropane bonds and joins the C1 carbons of the two original farnesyl residues to generate a 1′-1-fused isoprenoid.[3]



Scheme I. A Mechanism for Conversion of Presqualene Diphosphate to Squalene



In addition to conversion to squalene, whose sole fate is sterol synthesis, farnesyl diphosphate also serves as a primer for the prenyl transfers which generate 2,3-dehydrodolicyl diphosphate,[4] all-trans polyprenyl diphosphates for ubiquinone biosynthesis,[5] and perhaps the hydrophobic prenyl units involved in modification of nuclear proteins essential for cell division and maintenance of cellular morphology.[6] The 1′-1 condensation is the first pathway-specific reaction in sterol metabolism, and squalene synthetase

(1) Poulter, C. D.; Rilling, H. C. *Biosynthesis of Isoprenoid Compounds*; Porter, J. W.; Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 413-441.

(2) Poulter, C. D.; Rilling, H. C. *Biosynthesis of Isoprenoid Compounds*; Porter, J. W.; Spurgeon, S. L., Eds.; Wiley: New York, 1981; Vol. 1, pp 162-224.

(3) For a description of non-head-to-tail attachments in isoprenoids see: Poulter, C. D.; Marsh, L. L.; Hughes, J. M.; Argyle, J. C.; Satterwhite, D. M.; Goodfellow, R. J.; Moesinger, S. G. *J. Am. Chem. Soc.* **1977**, *99*, 3816-3823.

(4) Adair, W. L.; Cafmeyer, N.; Keller, R. K. *J. Biol. Chem.* **1984**, *259*, 4441-4446.

(5) Faust, J. R.; Goldstein, J. L.; Brown, M. S. *Arch. Biochem. Biophys.* **1979**, *192*, 86-99.

(6) Wolda, S. L.; Glomset, J. A. *J. Biol. Chem.* **1988**, *263*, 5997-6000.

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