Z Isomer: 10.100 (d, J = 8 Hz, C-29), 5.800 (d, J = 8 Hz, C-28), 3.330 (s, OCH_3), 1.152 (d, J = 6.9 Hz, C-26, 27), 1.019 (s, C-19), 0.965 (d, J = 6.5 Hz, C-21), 0.720 (s, C-18).

The following sequence of reactions was separately carried out on each isomer: The aldehyde (**11E** or **11Z**) (2 mg) in methanol (0.5 mL) was added to 10 μmol (6 mCi) of sodium borotritide (ICN) and stirred at room temperature for 5 min, when TLC analysis showed the reaction to be complete. The solvent was evaporated (N_2) and then passed through a short column (0.5 g) of silica gel in CH_2Cl_2. The resulting alcohol in 2 mL of CH_2Cl_2 was treated dropwise (over 30 min) with 15 mg of pyridinium chlorochromate in 1.5 mL of CH_2Cl_2. When a TLC monitor showed the reaction to be complete (40 min), the mixture was run through a short silica gel column (5 g, CH_2Cl_2), the aldehyde was divided into two portions, and each aliquot was subjected to the decarbonylation reaction.

When the preceding sequence was carried out with sodium borodeuteride, more than 99% of the recovered aldehyde retained the deuterium, which was shown to be the stereotopically pure E isomer by a clean NMR doublet centered at 5.35.

Deuterated E alcohol: ^{1}H NMR 5.35 (d, J = 7 Hz, C-28), 4.11-4.20 (m, C-29), 3.32 (s, OCH_3), 1.019 (s, C-19), 1.017 (d, J = 7 Hz, C-26, 27), 0.969 (d, J = 7 Hz, C-21), 0.714 (s, C-18). **Deuterated E aldehyde**: ^{1}H NMR 5.83 (s, C-28), 3.33 (s, OCH_3), 1.11 (d, J = 7 Hz, C-26, 27), 1.03 (s, C-19), 1.015 (d, J = 6 Hz, C-21), 0.730 (s, C-18). **Undeuterated alcohol (E:Z, 8:1)**: ^{1}H NMR 5.34 (t, C-28), 3.32 (s, OCH_3), 1.019 (s, C-19), 1.017 (d, J = 6.5 Hz, C-26, 27), 0.969 (d, J = 6.6 Hz, C-21).

The decarbonylation was carried out under argon in a sealed heavy-walled Wheaton glass reaction vial (2 mL) containing degassed dry (Na) toluene (0.3 mL), one portion of the above tritiated aldehyde, and 5 mg of tris(triphenylphosphine)rhodium(I) chloride (Aldrich). After heating to 120 °C for 1 h, the reaction was then cooled, extracted with methylene chloride, and passed twice through a short silica gel column with methylene chloride to remove any colored material. The isomethyl ether protecting group was removed in dioxane-water (1:1, 0.5 mL) by heating at 110 °C for 10 min with one crystal p-toluenesulfonic acid. Triethylamine (2 drops) was added, the solvents were evaporated, and the [28-^{3}H]-24-methylenecholesterol was purified by column chromatography (silica gel, CH_2Cl_2) to give (Z)-[28-^{3}H]-24-methylenecholesterol, 1.6 × 10^2 μCi/mg, and (E)-[28-^{3}H]-24-methylenecholesterol, 28 μCi/mg.

Incorporation of (E)- and (Z)-[28-^{3}H]-24-Methylenecholesterol into Sponge. Incorporations according to previously described[13,21] methods were performed by using 12.3 μCi of (E)-[28-^{3}H]-24-methylenecholesterol (**2E**) and 14 μCi of (Z)-[28-^{3}H]-24-methylenecholesterol (**2Z**) in duplicate on 14-g fragments of one specimen of *Xestospongia testudinaria*, living at a depth of 14 m at John Brewer Reef, Australia.

Incorporation of [6-^{3}H]-Fucosterol. The incorporation procedure was identical except that 20 μCi of [6-^{3}H]fucosterol was used on another specimen of *Xestospongia testudinaria* which was shown to have the same sterol content as the first specimen.

Isolation and Purification of Incorporated Sterols: Isofucosterol and Fucosterol. The sterol fraction of the sponge samples from the incubation experiments was obtained according to our standard procedures.[13] Reversed-phase HPLC chromatography in methanol isolated the isofucosterol-fucosterol fraction from most of the other sterols (Table I). Further HPLC using acetonitrile-methanol-ethyl acetate removed the contaminating cholesterol. Finally methanol-water (97:3), containing silver nitrate, separated the fucosterol from the isofucosterol. The RRTs of the isofucosterol and fucosterol (+cold carrier) were 85 and 95 min, respectively. The sterols were separated from the silver nitrate by concentration of the solvents, extraction with methylene chloride, and followed by drying through sodium chloride-silica gel (0.5 g).

The radioactivity for each peak remained unchanged after repeated chromatography. Purity of each peak (>99.5%) was established on cold material by capillary GLC (RRT fucosterol, 1.5; RRT isofucosterol, 1.6). Futhermore, when [6-^{3}H]fucosterol was incorporated into the sponge, our separation technique was shown to be >99.7% efficient in terms of the purification of isofucosterol. The NMR spectra (on cold material) showed each isomer to be totally free of the other by the different shifts of the C-28 protons: fucosterol ^{1}H NMR 5.19 (q, J = 7.5 Hz, 1 H), (C-28), 1.010 (s, C-19), 0.988 (d, J = 6.6 Hz, C-21), 0.979 (d, J = 6.9 Hz, C-26, 27), 0.687 (s, C-18); isofucosterol ^{1}H NMR 5.11 (q, C-28), 1.009 (s, C-19), 0.975 (d, J = 6.9 Hz, C-26, 27), 0.946 (d, J = 6.6 Hz, C-21), 0.683 (s, C-18).

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Registry No. 11a, 81256-55-5; **11b**, 81256-56-6; isofucosterol, 481-14-1; fucosterol, 17605-67-3; (E)-[28-^{3}H]-24-methylenecholesterol, 132564-87-5; (Z)-[28-^{3}H]-24-methylenecholesterol, 132564-88-6; 22-dehydrocholesterol, 34347-28-9; desmosterol, 313-04-2; 24-methylenecholesterol, 474-63-5; crinosterol, 17472-78-5; brassicasterol, 474-67-9; epiclerosterol, 105226-41-3; cholesterol, 57-88-5; cholestanol, 80-97-7; campesterol, 474-62-4; stigmasterol, 83-48-7; poriferasterol, 481-16-3; sitosterol, 83-46-5; clionasterol, 83-47-6.

Quadrone Structural and Synthetic Studies. Total Synthesis of Natural (-)-Quadrone, the (+)-Enantiomer, and the Racemate. Conformational Analysis, Circular Dichroism, and Determination of Absolute Stereochemistry

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Abstract: A concise (17 steps, 1.8% overall yield) and highly stereoselective synthesis of the architecturally novel, cytotoxic sesquiterpene (-)-quadrone (**1**) has been achieved, together with parallel constructions of the unnatural (+)-enantiomer and the racemate. The cornerstone of the strategy involved generation of the tricarbocyclic quadrone skeleton via an efficient, acid-promoted rearrangement of [4.3.2]propellane derivative **11**. Further elaboration of rearrangement product **14** furnished enone acid **3**, an advanced intermediate which had previously been converted to terrecyclic acid A (**2**) and thence to **1**. Detailed conformational analysis of quadrone, encompassing molecular mechanics calculations, 2D NMR studies, and X-ray crystallography, converged upon two energetically significant conformers (**D** and **E**; predicted ratio 97.5:2.5 at 25 °C). An empirical method for estimation of the sign and magnitude of $\Delta\epsilon$ indicated that strain effects and octant-dissignate contributions of the pseudoaxial α-hydrogens dominate the circular dichroism spectrum of (-)-**1**.

In 1978 Ranieri and Calton reported the structure of (-)-quadrone (**1**), an architecturally novel sesquiterpene isolated from fermentation broths of the fungus *Aspergillus terreus*.[1] The intriguing structure and reported cytotoxicity of **1** at once inspired

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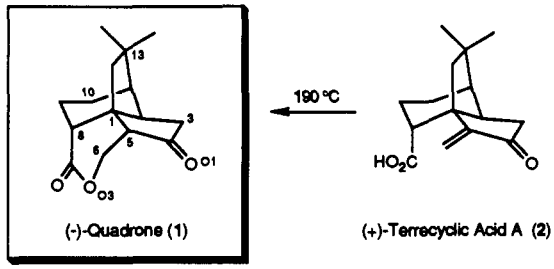
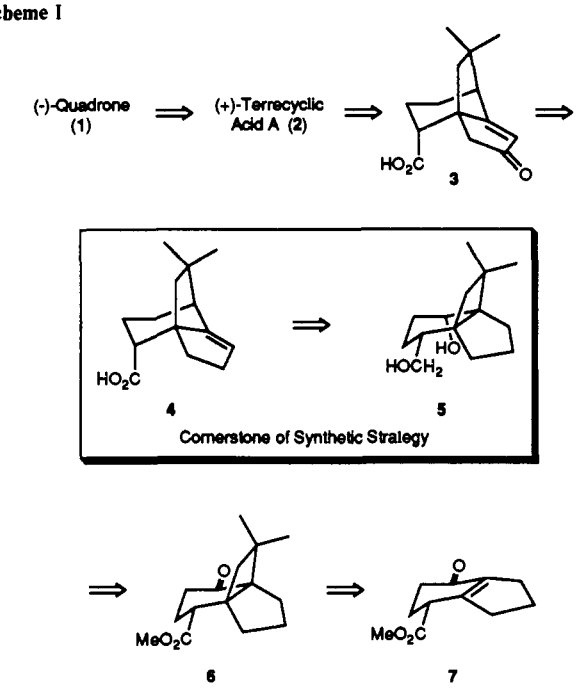
considerable synthetic activity, culminating in several elegant constructions of racemic quadrone. Indeed, during the past decade quadrone emerged as a focal point for the development and evaluation of numerous methods and strategies and as such ranked among the most significant contemporary targets for complex molecule synthesis.[2-4]



Further studies of *A. terreus* yielded the modestly cytotoxic congener (+)-terrecyclic acid A (**2**),[5,6] which interestingly had been prepared in racemic form by Danishefsky and co-workers en route to (±)-**1**.[2a] Along with the latter group,[2a] we had recognized that in vivo retro-Michael fragmentation of the lactone ring in **1** would generate **2** and that the resultant α-methylene cyclopentanone functionality, common to numerous antitumor sesquiterpenes, could account for the putative anticancer activity of quadrone.[7] Upon heating at 190 °C, natural (+)-**2** cyclized to furnish the isomeric (−)-**1**.[5b] However, the absolute configurations of **1** and **2** remained to be elucidated by our synthesis of (+)-quadrone,[8,9] in concert with a construction of (−)-terrecyclic acid A by Isoe.[10,11] In this full account, we describe a concise approach which has furnished natural (−)-quadrone and the (+)-antipode of high enantiomeric purity as well as the racemate. In addition, we present a detailed conformational analysis of **1**, involving molecular mechanics calculations in conjunction with 2D NMR studies and X-ray crystallography. An empirical method for estimation of Cotton effects for chiral cyclopentanones was employed for correlation of the quadrone circular dichroism spectrum with the structural features of the significant conformers.

A Strategy for the Synthesis of (−)-Quadrone. Retrosynthetic analysis of the quadrone problem suggested the economic approach outlined in Scheme I.[12] The penultimate structure, terrecyclic acid A (**2**), was envisioned to arise via elaboration of enone acid **3**. Subsequent to the formulation of our strategy, the viability of **3** as a precursor of **2** was established by Danishefsky;[2a] ultimately this enone acid evolved as the common objective of many other synthetic designs as well.[13] In our scheme, the enone moiety of **3** would be secured via allylic oxygenation of **4**, the earliest

Scheme I



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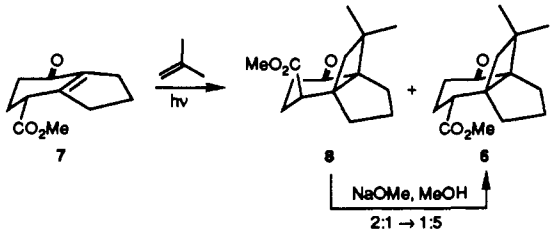
(13) See refs 2c–i, 2k, 2m, 2n, and 9. Moreover, an analogous enone was employed in all five published routes to descarboxylquadrone.[3a-e]

intermediate to embody the tricarbocyclic quadrone skeleton.

The cornerstone of our strategy entailed generation of **4** via acid-promoted rearrangement of a [4.3.2]propellane derivative such as **5**. Although many related propellane rearrangements had been documented previously, the unprecedented use of a hydroxyl group in a six-membered ring for initiation posed important stereo- and regiochemical questions which occasioned extensive studies in our laboratory (vide infra).[14] The hydroxyl group of **5** would in principle also accommodate the preparation of material in each enantiomeric series by separation of diastereomeric derivatives. Finally, ketone **6**, the direct antecedent of **5**, would arise via photochemical [2 + 2] cycloaddition of isobutylene to enone ester **7**.

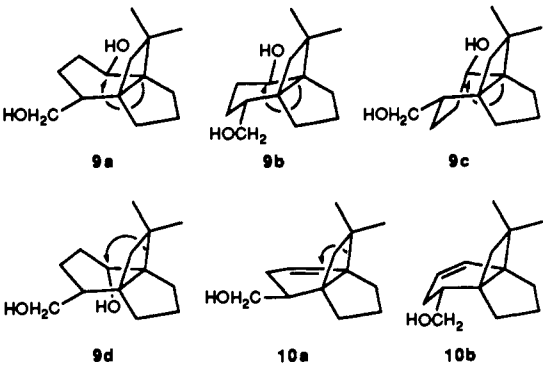
Notwithstanding the intensive efforts elsewhere directed toward quadrone synthesis, the sequence for carbocyclic ring assembly described herein (i.e., AC → ABC) remains unique. Other ingenious rearrangements have been employed to advantage in several more recent approaches;[2e,j,4e,d] of particular interest vis-ă-vis the current venture, alternative propellane rearrangements have served as key steps in three syntheses of (±)-descarboxylquadrone.[4b-d] The postulated biosynthesis of **1** involves a cascade of carbenium ion rearrangements, initiated by cyclization of a bicyclo[6.3.0]undecene-derived cation.[15,16] To date, however, no biomimetic approaches to the construction of quadrone have been described.

(±)-Quadrone: The Initial Synthetic Objective. Our point of departure thus became enone ester (±)-**7**, a material which fortuitously had served as an intermediate in our synthesis of modhephene.[17] The [2 + 2] photoaddition of isobutylene to **7** furnished predominantly the undesired syn keto ester **8** (2:1 ratio of **8** to **6**).[14] However, epimerization with sodium methoxide in

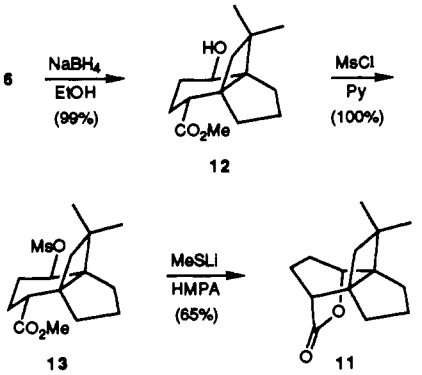


methanol furnished a mixture enriched in the anti isomer **6** (5:1 ratio), whereupon pure **6** was easily isolated by crystallization.

At this juncture, we began to investigate the key step in our strategy, the generation of the quadrone tricyclic framework via acid-promoted rearrangement of a suitable [4.3.2]propellane. For the initial phase of this study, six plausible quadrone precursors, the diastereomeric diols **9a–d** and diastereomeric alkenes **10a–b**, were readily prepared from **6** and **8**. As described earlier,[14] the rearrangements of these substrates, and of numerous related compounds, proceeded under strict stereoelectronic control: the central or peripheral cyclobutane σ-bond better aligned with the leaving group or alkene π-system invariably underwent initial migration. Thus, the syn[18] diols **9a** and **9b** and the anti isomer **9c** all suffered unwanted central bond shifts. Anti diol **9d** and syn alkene **10a** did rearrange exclusively via initial peripheral migration, as desired, but the intervention of secondary rearrangements precluded isolation of materials containing the requisite carbon skeleton. These five substrates are depicted in the presumed reactive conformations.[14b] Under the usual experimental conditions, anti alkene **10b** was inert.



Although the products of these reactions were not suitable for elaboration to quadrone, the results did point to a simple and effective tactic: acid-catalyzed scission of the anti ester linkage in **11** would be expected to induce the stereoelectronically favored peripheral bond migration and also to provide an internal nucleophile for carbenium ion capture, suppressing secondary rearrangement. For the preparation of **11**, we sought to exploit the ready availability of syn alcohol **12** by inverting the carbinol stereocenter concomitant with lactone formation. Thus, sodium borohydride reduction of propellanone **6**, directed by the *gem*-



dimethyl moiety,[19] was followed by exposure to methanesulfonyl chloride in pyridine, furnishing mesylate **13** quantitatively from **6**. Upon treatment with potassium superoxide in the presence of 18-crown-6,[20] the mesylate methyl ester did undergo demethylation and cyclization as desired, affording **11** in up to 70% yields; however, this procedure suffered from poor reproducibility. In contrast, reaction of **13** with lithium methanethiolate in HMPA[21] reliably gave lactone **11** in ca. 65% yield.[22] The structure of the putative rearrangement substrate was confirmed by lithium aluminum hydride reduction, which produced diol **9c**.

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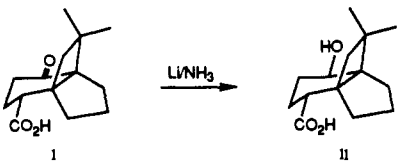
(18) The syn/anti designations for diols **9a–d** and related compounds describe the orientation of the secondary hydroxyl with respect to the C–CMe_2 peripheral cyclobutane bond. For alkenes **10a,b**, the syn or anti relationship of the latter bond to the hydroxymethyl substituent is indicated.

(19) Dissolving metal (Li/NH_3) reduction of keto acid **i** likewise furnished the syn[18] isomer **ii**.



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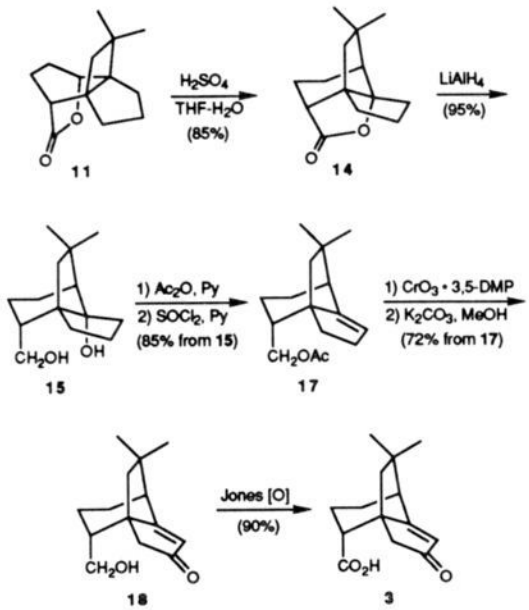
(21) Kelly, T. R.; Dali, H. M.; Tsang, W.-G. *Tetrahedron Lett.* **1977**, 3859–3860.

(22) Treatment of **13** with KO-*t*-Bu/H_2O/DMSO[22a] afforded small amounts of **11**, whereas CsOAc in DMF[22b] gave none of the desired material. Likewise, exposure of **ii**[19] to the DEAD protocol (diethyl azodicarboxylate/PPh_3)[22c] gave no lactone. See: (a) Gassman, P. G.; Hodgson, P. K. G.; Balchunis, J. *J. Am. Chem. Soc.* **1976**, *98*, 1275–1276. (b) Huffman, J. W.; Desai, R. C. *Synth. Commun.* **1983**, *13*, 553–557. (c) Mitsunobu, O. *Synthesis* **1981**, 1–28.

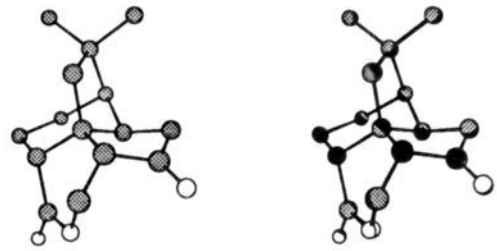
Scheme II



In the event, exposure of **11** to 40% aqueous sulfuric acid in THF at 50 °C for 1 h furnished a single product **14** in 85% yield (Scheme II). The latter structure was initially assigned on the basis of spectroscopic analysis and ultimately was secured by conversion to quadrone. To that end, lithium aluminum hydride reduction afforded crystalline diol **15** in 95% yield. The latter underwent selective monoacetylation upon treatment with acetic anhydride in pyridine (25 °C, 24 h), and exposure of the resultant acetoxy alcohol **16** to thionyl chloride and pyridine in dichloromethane (0 °C, 4 h) then furnished unsaturated acetate **17** in 85% yield overall from **15**. Allylic oxidation was effected by reaction with the chromium trioxide·3,5-dimethylpyrazole reagent[23] in dichloromethane at −35 °C for 1 h. Deacetylation with potassium carbonate in methanol gave crystalline hydroxy enone **18** in 72% yield from **17**. Finally, Jones oxidation[24] generated the requisite enone acid (±)-**3** in 90% yield; the latter material proved to be identical with an authentic sample.[25] As noted earlier, Danishefsky first converted (±)-**3** to racemic terrecyclic acid A (**2**) and thence to quadrone (**1**).[2a]

Conformational Analysis of Quadrone. As a prelude to the synthesis of homochiral material, we next sought to deduce the absolute configuration of natural (−)-quadrone by analysis of its cyclopentanone n → π* circular dichroism (CD) spectrum. Unfortunately, an incorrect conformational model led to an erroneous initial interpretation of the CD data.[8] This difficulty has prompted us to evaluate the conformations of **1** in some detail. Whereas the bridged polycyclic structure might seem a priori to inhibit significant conformational mobility, a Dreiding model revealed apparent variability and interdependence of the cyclopentanone and δ-valerolactone geometries as well as potential flexibility in the cyclohexane ring. These possibilities were then further explored via a multifaceted conformational analysis, involving molecular mechanics calculations in conjunction with X-ray crystallography and 2D NMR experiments.

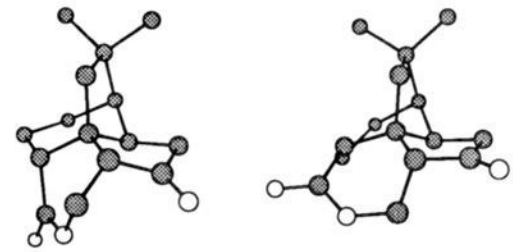
Single-crystal X-ray analysis of quadrone served as the starting point for more rigorous evaluation. Although the X-ray structure of (−)-**1** was communicated in 1978,[1b] the cyclopentanone conformation could not be discerned from the original report; furthermore, the atomic coordinates were neither published nor deposited. Thus, for the present work the coordinates were obtained from a participant in the earlier study.[26] The unit cell of crystalline (−)-**1** ($z = 8$) contains two unique but nearly identical conformers (**A** and **B**); an overlay (**C**) generated by least-squares minimization[27] established the very close correspondence of **A** and **B** (RMS deviation 0.034 Å).



A **C**

The observation of essentially a single conformer for crystalline (−)-**1** of course did not preclude significant conformational mobility in solution. Accordingly, conformational energies for quadrone were evaluated via molecular mechanics calculations, performed with the MM2 force field as supplied with version 2.5 of MACROMODEL.[28] Initial minimizations of **A** and **B** converged upon a single lower-energy structure. We then began a conformational search by means of the Monte Carlo method.[29] Starting with structure **A**, eight torsion angles were allowed to vary through ±90°, and four ring-closure bonds were defined with a window of 0.25–3.5 Å. Generation of 9522 conformations and partial minimization afforded three unique structures with energies 42.83, 45.02, and 48.09 kcal/mol and gradient rms values 0.006, 0.001, and 1.952, respectively. Upon further minimization to a gradient rms of 0.001, two unique conformations (**D** and **E**) remained.

The global minimum **D** ($E = 42.83$ kcal/mol) was identical with the structure obtained by minimization of X-ray conformers **A** and **B**, whereas **E** ($E = 45.02$ kcal/mol) differed significantly. Least-squares overlays[27] (e.g., **F**) verified the close similarities of the global minimum **D** with **A** and **B**. Key features of these three structures include a boat δ-valerolactone unit,[30] a twisted chair cyclohexane, and a distorted envelope ("folded") conformation of the cyclopentanone ring.[31] In marked contrast, **E**



D (E = 42.83 kcal/mol) **E** (E = 45.02 kcal/mol)

contains a slightly twisted boat cyclohexane ring, a twist ("bow tie") cyclopentanone conformation, and a distorted boat δ-valerolactone moiety, the latter almost a half-chair. The striking

(23) Salmond, W. G.; Barta, M. A.; Havens, J. L. *J. Org. Chem.* **1978**, *43*, 2057–2059.

(24) Bowden, K.; Heilbrow, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc.* **1946**, 39–45.

(25) We wish to thank Professor Andrew Kende for kindly providing an authentic sample of racemic **3**.

(26) See ref 1b. Complete X-ray coordinates and structure factors have been obtained from Molecular Structure Corporation, College Station, TX, and deposited in the Cambridge X-ray Structure Data Base.

(27) Kabsch, W. *Acta Crystallogr.* **1976**, *A32*, 922–923; **1978**, *A34*, 827–828.

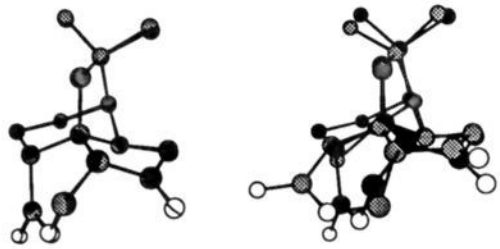
(28) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. Department of CHemistry, Columbia University, New York, NY 10027.

(29) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.

(30) For the parent δ-valerolactone, the half-chair and boat conformers are similar in energy. Importantly, the chair conformer is stereoelectronically disfavored. See: (a) Lambert, J. B.; TeVrucht, M. L. E. *Org. Magn. Reson.* **1984**, *22*, 613–615. (b) Philip, T.; Cook, R. L.; Malloy, T. B., Jr.; Allinger, N. L.; Chang, S.; Yuh, Y. *J. Am. Chem. Soc.* **1981**, *103*, 2151–2156. (c) Reference 32.

(31) Review of cyclopentanone conformational analysis: Fuchs, B. In *Topics in Stereochemistry*; Eliel, E. L.; Allinger, N. L., Eds.; Wiley: New York, 1978; Vol. 10, pp 1–94.

differences between the MM2 conformers are highlighted by the least-squares overlay[27] **G** (rms deviation 0.741 Å). Neglecting entropy contributions, the ratio of equilibrium populations of **D** and **E** is predicted to be 97.5:2.5 at 25 °C.



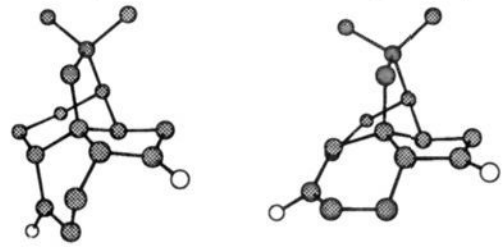
F
Least-squares
Overlay of A and D
A - ● D - ●

G
Least-squares
Overlay of D and E
D - ● E - ●

Further examination of the Dreiding model had suggested that a sterically advantageous chair form of the δ-valerolactone unit would favor the twist arrangement of the cyclopentanone ring. Given the potential significance of the latter feature in circular dichroism analysis (vide infra), this conformer was further evaluated via molecular mechanics. To this end, the dihedral angles of the lactone ring (C1–C5–C6–O3 and C1–C8–C7–O3) were constrained to values derived from the Dreiding model (43 and −49°, respectively) via V1 torsional energy increments of 1000 kcal/mol. Minimization then gave structure **H**, which proved to be significantly less stable (E = 49.31 kcal/mol) than **D** and **E**. Moreover, **H** was not a local energy minimum: elimination of the torsional constraints followed by minimization regenerated the global minimum **D**. The cyclopentanone moiety in **H** actually adopted a distorted envelope conformation, rather than a twist as suggested by the Dreiding model. Interestingly, intermediate-energy conformers containing twist cyclopentanone rings were formulated by MACROMODEL during the relaxation of **H** to **D**. The cyclohexane ring in H again was a chair.

H
(E = 49.31 kcal/mol)

The boatlike lactone moieties found in **D** and crystalline **1** and the flattened boat (nearly half-chair) lactone ring in **E** resemble the two stable conformations of the parent δ-valerolactone,[30] reflecting the stereoelectronic preference for coplanarity of the C–O–(CO)–C array.[32] We further probed the importance of the latter interaction via computational analysis of a cyclohexanone analogue of quadrone, created by replacing the lactone ring oxygen with a methylene group. A Monte Carlo search generated 4650 conformations, which upon partial minimization furnished three unique structures within 6 kcal/mol of the global minimum. Further minimization to a gradient rms of 0.001 then gave three conformers, all local minima. These closely resembled the corresponding structures calculated for quadrone, except for details attributable to the propensity of the cyclohexanone ring to adopt a chair conformation. Thus, structures **I** and **J** (E = 49.12 and 50.00 kcal/mol, respectively) were analogous to the calculated quadrone conformers **D** and **E**. The third cyclohexanone structure **K** embodied the chair–chair conformation suggested by the Dreiding model but shared the distorted envelope geometry predicted by MM2 for the cyclopentanone ring in **H**. Furthermore, **K** proved to be intermediate in energy between **I** and **J** (E = 49.31 kcal/mol), substantiating the influence of lactone stereoelectronic requirements upon the conformational energies for quadrone.



I
(E = 49.12 kcal/mol)

J
(E = 50.00 kcal/mol)

K
(E = 49.31 kcal/mol)

Finally, NMR analysis demonstrated a close correspondence of the global minimum **D** with the predominate solution structure of **1**. Calculated ^{1}H coupling constants for conformers **D** and **E** were secured via the NMR mode of MACROMODEL,[33] whereas experimental values were determined via one-dimensional decoupling, at 27 and at −54 °C, and two-dimensional, phase-sensitive COSY experiments. Successful simulation of the quadrone spectrum with the PANIC program confirmed the latter assignments. Correlation of the experimental coupling constants with those calculated for the global minimum **D** proved to be very good (R^2 = 0.955), whereas correlation of experimental couplings with the values for the MM2 minor conformer **E** was poor (R^2 = 0.015). As expected, the 500-MHz ^{1}H NMR spectrum of **1** showed no significant temperature dependence in the range −74 to 27 °C.

Circular Dichroism of Natural (−)-Quadrone. Chiroptical analysis comprises a powerful method for elucidating details of molecular structure.[34] In particular, the Cotton effect associated with the n → π* carbonyl transition of a chiral ketone (ca. 290 nm) often permits the assignment of absolute configuration via circular dichroism (CD).[35] Empirical interpretations of ketone CD spectra have evolved around the octant rule, first proposed in 1961.[36] Basic octant analysis correlates the Cotton effect contribution of each atom or group with its position, relative to the nodal planes of the carbonyl moiety and a third boundary surface bisecting the C=O bond;[37] these surfaces partition the space surrounding the chromophore into octants, alternately as-

(32) For studies of energy barriers to rotation about the O—(C=O) bond in simple esters, see: Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935–5943, and references cited therein.

(33) Cf.: Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.

(34) General references on chiroptical methods in organic chemistry: (a) Legrand, M.; Rougier, M. J. In *Stereochemistry: Fundamentals and Methods*; Kagan, H. B., Ed.; Thieme: Stuttgart, 1977; Vol. 2, pp 33–198. (b) Scopes, P. M. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: New York, 1975; Vol. 32, pp 167–265. (c) See also ref 35.

(35) For an excellent recent review, see: Kirk, D. N. *Tetrahedron* **1986**, *42*, 777–818.

(36) Moffitt, W.; Woodward, R. B.; Moscowitz, A.; Klyne, W.; Djerassi, C. *J. Am. Chem. Soc.* **1961**, *83*, 4013–4018.

(37) Both the shape and the significance of the third boundary surface have been extensively debated. However, these uncertainties do not affect the present analysis because the entire quadrone structure (apart from the ketone oxygen) clearly lies within the rear octants.

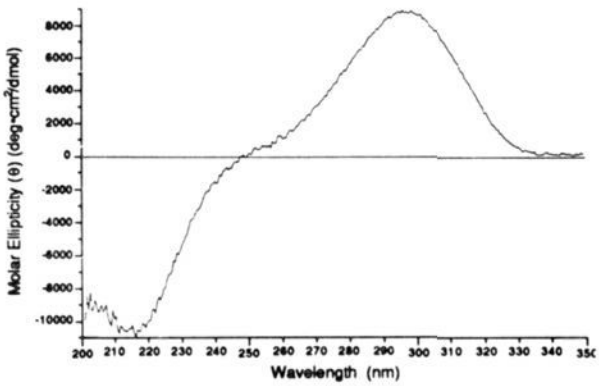


Figure 1. Experimental CD spectrum of (−)-quadrone at 25 °C in methanol.

signed as positive or negative. Extensive studies have led to increasingly sophisticated refinements of this approach.

Two factors generally complicate the interpretation of CD data for cyclopentanones, vis-à-vis the considerably simpler cyclohexanones:[35] (a) many cyclopentanone derivatives exhibit marked conformational mobility, and (b) significant conformations usually contain chiral cyclopentanone rings. The latter dissymmetry dictates explicit consideration of the Cotton effect contributions of the ring β-carbons and all α- and β-substituents, including hydrogens, for each conformer. For quadrone, the influence of the highly strained molecular framework and the lactone moiety must also be taken into account.

The experimental ambient-temperature CD spectrum of (−)-**1** in methanol (Figure 1) shows a single positive Cotton effect for the ketone carbonyl, centered at 298 nm with a moderately strong dichroic absorption ($\Delta\epsilon$) of +2.68. The second maximum, a negative band at 216 nm, corresponds to the lactone n → π^* transition. Correlation of δ-lactone Cotton effects with absolute configuration remains problematic.[35]

For circular dichroism analysis of the cyclopentanone moiety in (−)-quadrone, we relied primarily upon an empirical approach developed by Kirk for estimating the sign and magnitude of $\Delta\epsilon$.[38,39,35] A provocative feature of the scheme is the proposal, contravening the classical octant rule, that the absolute CD contributions of nearly all atoms and groups are in fact octant-dissignate.[40] C–H bonds, in particular, show markedly dissignate behavior;[38] other group contributions usually appear to be consignate only because they are less dissignate than the C–H bond increments they replace. The influence of dissignate C–H bonds may predominate via conformer stabilization; for example, the large dissignate effects of pseudoaxial α-hydrogens can account for the consignate behavior previously ascribed to the β-carbons in twisted cyclopentanone rings. Although not yet buttressed by rigorous theoretical justification, this interpretation has led to impressive success in estimation of dichroic absorption values.

We thus derived initial approximations of $\Delta\epsilon$ for the energetically significant MM2 conformers of quadrone (**D** and **E**) by using eq 1, as formulated by Kirk.[39] Treating the ketone moiety as an acetone derivative, the contribution of each α-substituent was calculated as a $\sin^2$ function of the O=C—C_α—$C(H)_\beta$ dihedral angle ω, multiplied by a factor k characteristic of the attached atom or group. The dihedral angles for **D** and **E** were

$$\Delta\epsilon = \sum(\pm)k_H \sin^2 \omega_H + \sum(\pm)k_C \sin^2 \omega_C \qquad (1)$$

generated by MACROMODEL. Standard k values were employed for the α-hydrogens ($k_H = -6.2$), the β-carbons of the ketone ring ($k_C = -1.9$ for C_β of a monocyclic cyclopentanone), and the α-alkyl group ($k_C = -4.6$, neglecting the influence of strain and of the lactone heteroatoms).[38,39]

The sign of the $k \sin^2 \omega$ contribution for each substituent was then determined by reference to the octant projections of the conformers (Figure 2), regarding each calculated increment as dissignate. This protocol furnished initial $\Delta\epsilon$ estimates for **D** and **E** of +0.77 and +2.71, respectively. The latter values, in conjunction with the ambient-temperature equilibrium proportions of **D** and **E** derived from the MM2 energies (97.5:2.5), led to a predicted $\Delta\epsilon$ for (−)-quadrone of +0.82, significantly smaller than the experimental measurement ($\Delta\epsilon$ = +2.68). Details of the calculation are provided as supplementary material.

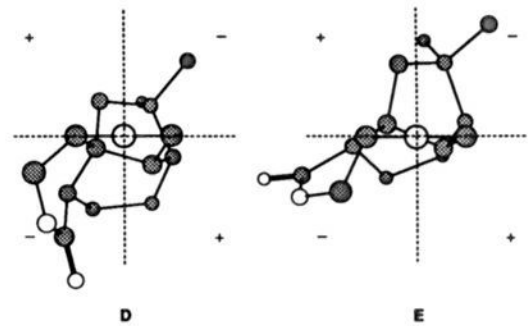


Figure 2. Octant projections of MM2 conformers D and E.

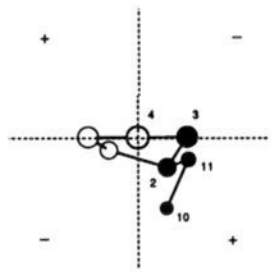


Figure 3. Octant projection of the C4–C10 zigzag of D.

Further analysis of the circular dichroism then entailed qualitative consideration of three other structural features: the cyclopentanone β-alkyl substituents, the lactone functionality, and the highly strained molecular architecture. With respect to the β-alkyl groups, we initially explored possible comparisons with analogous compounds; in this context, the tricarbocyclic quadrone skeleton can be regarded as a bridged, trans-fused hexahydroindan-2-one. Kirk has analyzed the CD spectra of several perhydroindanones,[41] but the presumed conformations of the [6,5]-bicyclic fragments of those structures differed significantly from the corresponding geometries in **D** and **E**. Nonetheless, the alkyl substituent contributions for quadrone could be assessed by recourse to more general correlations.

Maximal effects typically are observed for chains or ring fragments oriented as "primary zigzags," antiperiplanar bond paths formally originating at one lobe of a carbonyl carbon p orbital and oriented nearly equidistant from the carbonyl nodal planes.[42] A variety of cyclohexanone derivatives incorporate this geometry. However, inspection of the octant projections (Figure 2) as well as other views revealed that none of the β-alkyl chains in **D** or **E** is optimally disposed for interaction with the chromophore. The best β-alkyl zigzag comprises carbons 4, 3, 2, 11, and 10 in **D** (Figure 3) and occupies a positive octant; a modest consignate increment (or, equivalently, vis-à-vis the Kirk postulate, a diminished dissignate contribution) should slightly increase $\Delta\epsilon$ for that conformer.[35,42] The effects of the remaining β-alkyl groups should be both small and offsetting. These findings are in accord with the earlier conclusion that cyclopentanone β-substituents *generally exert little influence in circular dichroism, apart from* conformational effects.[38]

(38) Kirk, D. N. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2171–2177.
(39) Kirk, D. N. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2122–2148.
(40) The sign of an octant-consignate substituent contribution is in accord with the octant rule, whereas "anti-octant" behavior results in dissignate contributions.
(41) Kirk, D. N.; Klyne, W. *J. Chem. Soc., Perkin Trans. 1* **1976**, 762–779.
(42) Kirk, D. N.; Klyne, W. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1076–1103.

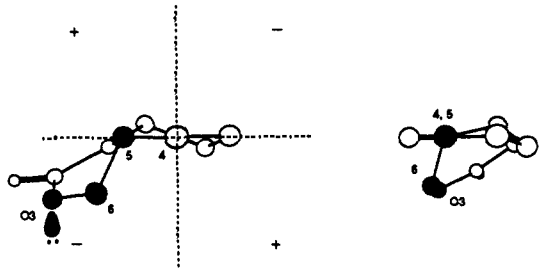


Figure 4. The lactone α-zigzag in E: octant projection and view along the C4–C5 bond axis.

The lactone O-acyl moiety should impart a small dissignate contribution via inductive electron withdrawal; the negative octant orientation would result in positive $\delta\Delta\epsilon$ increments for both conformers.[35] In **E**, however, a nonbonding electron pair on the ring oxygen extends a primary zigzag (Figure 4); this geometry could permit slight through-bond electron donation, attenuating the dissignate contribution or even causing weakly consignate behavior.[43] The lactone ring oxygen in **D** does not lie on a primary zigzag.

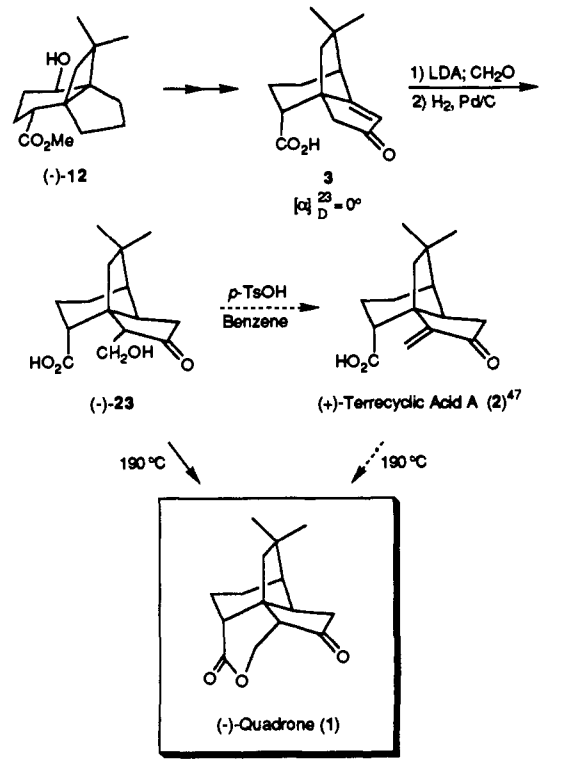
Strained bonds generally make more consignate (or, following Kirk, less dissignate) contributions to the Cotton effect than their unstrained counterparts.[39] The quadrone molecular framework indubitably incorporates considerable strain, generated primarily by the two-carbon bridge spanning the trans-fused hydrindanone moiety. Adjustments of the estimated $\Delta\epsilon$ values for **D** and **E** must take into account the effects of strain both upon substituent contributions and on the cyclopentanone ring itself. Significant changes in substituent increments typically result when strained bonds comprise part of a primary zigzag.[39] Thus, strain in the C(4)–C(10) β-alkyl chain in **D** and the lactone α-appendage in **E** should cause modestly increased and decreased dichroic absorption values for the respective conformers.

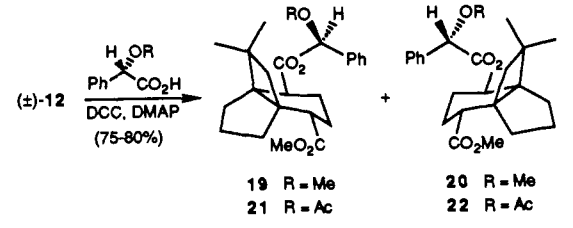
More importantly, the calculated $\Delta\epsilon$ values can also be corrected for the enhanced strain in the cyclopentanone moiety by using more positive k_C values for the ring β-carbons in eq 1.[39] Severe C(2) bond angle distortions (i.e., angles of 100 and 124° in **D**, 99 and 122° in **E**) reveal that C(2), the tertiary β-carbon, bears more strain than the bridgehead β-carbon, C(1), in both **D** and **E**. A larger positive k_C increment for C(2), in conjunction with the values of $\sin^2\omega$ for carbons 1 and 2 and the signs of the respective octants, will increase $\Delta\epsilon$ for both conformers and for (−)-**1**. Although a general quantitative correlation of k_c values with strain energies remains elusive, earlier analyses of polycyclic ketones suggest that strain could account for the magnitude of the Cotton effect observed for quadrone.[39]

In summary, estimates of $\Delta\epsilon$ for **D** and **E** calculated via eq 1 were dominated by octant-dissignate contributions of the pseudoaxial α-hydrogens, in accord with the conclusions set forth by Kirk.[35,38] Qualitative assessments indicate that additional structural features, in particular the strained polycyclic architecture, also contribute significantly to the dichroic absorption observed for (−)-quadrone. We note that this analysis correctly correlates the absolute configuration of (−)-**1** with the positive sign of the cyclopentanone Cotton effect.

Total Synthesis of (+)- and (−)-Quadrone: Determination of Absolute Configuration. The construction of nonracemic quadrone began with the resolution of tricyclic hydroxy ester **12**. Our approach to the latter problem was inspired by a report of Whitesell, who employed mandelate esters for preparative alcohol resolutions.[44] Treatment of (±)-**12** with (*S*)-(+)-*O*-methylmandelic acid,[45] dicyclohexylcarbodiimide, and 4-(dimethylamino)pyridine catalyst afforded diastereomeric esters **19** and **20** (ratio 1.4:1) in 78% yield, admixed with unreacted, partially resolved **12**. Derivatization with (*S*)-(+)-*O*-acetylmandelic acid,[46] under similar conditions, gave a comparable mixture of **21** and **22**. Each pair of diastereomers could be separated by flash chromatography, but the *O*-methyl compounds proved more amenable to preparative work. Removal of the chiral auxiliary was effected by treatment of **19** and **20** with sodium methoxide in methanol (40 °C, 12 h), affording alcohols (+)-**12** and (−)-**12**, respectively. The absence of mandelate racemization during methanolysis was demonstrated by GC monitoring of unreacted esters.

Scheme III





The absolute configurations of (+)- and (−)-**12** emerged from single-crystal X-ray analysis of **21** (Figure 5), in conjunction with the known *S* configuration of the mandelate moiety and the liberation of (+)-**12** from **21** by methanolysis.

Our first synthesis of enantiomerically pure quadrone employed the more abundant (+)-enantiomer of **12**. Following the sequence outlined above, (+)-**12** was converted to nonracemic **3**. Surprisingly, the latter material had negligible optical rotation, although the melting point (183–187 °C) differed markedly from that of the racemate (142–146 °C). Further transformations as in the racemic series then furnished the unnatural (+)-enantiomer of quadrone. The levorotatory terrecyclic acid A [(−)-**2**] generated from (+)-**23** was of course also enantiomeric with the natural material.[47] These experiments, together with the contemporaneous

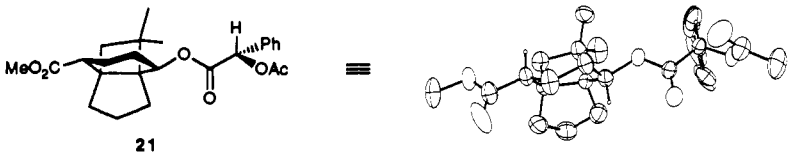
(43) Cf.: axial vs equatorial 3-acetoxyadamantanones: Snatzke, G.; Eckhardt, G. *Tetrahedron* **1970**, *26*, 1143–1155. See also ref 35.

(44) Whitesell, J. K.; Reynolds, D. *J. Org. Chem.* **1983**, *48*, 3548–3551. Inasmuch as various *O*-methyl- and *O*-acetylmandelate esters had been previously employed to advantage in our laboratory, the use of simple mandelates (i.e., containing unprotected hydroxyl groups) was not explored in the present study.

(45) Bonner, W. A. *J. Am. Chem. Soc.* **1951**, *73*, 3126–3132.

(46) Breitholle, E. G.; Stammer, C. H. *J. Org. Chem.* **1974**, *39*, 1311–1312.

(47) Both enantiomers of **23** were individually converted to quadrone by heating at 190 °C. Terrecyclic acid A was independently generated from **23** (by exposure to *p*-TsOH in benzene) only in the unnatural series.



21

Figure 5. ORTEP plot for 21.

efforts by Isoe,[10] first revealed the absolute configurations of quadrone and terrecyclic acid A.

In similar fashion, the hydroxy ester (−)-**12** furnished natural (−)-quadrone (Scheme III). The transformation of (−)-**12** to (−)-**1** proceeded via intermediates (+)-**13**, (+)-**11**, (−)-**14**, (−)-**15**, (−)-**17**, (−)-**18**, and (−)-**23**. As in the unnatural series, enone acid **3** was optically inactive. Synthetic (−)-**1** proved to be spectroscopically indistinguishable from an authentic sample of the natural product.[48]

Experimental Section[49]

Hydroxy Ester (±)-12. Sodium borohydride (200 mg, 5.3 mmol) was added to a stirring solution of keto ester **6** (380 mg, 1.52 mmol) in 20 mL of anhydrous ethanol. The mixture was stirred overnight at room temperature, and then excess reducing agent was destroyed by the addition of 1 N NaOH. After evaporation of most of the ethanol, the solution was extracted with dichloromethane. Drying over magnesium sulfate and concentration gave 380 mg (99%) of (±)-**12**, suitable for further transformation. Crystallization of a small sample from ether/hexane gave analytically pure material: mp 66–68 °C; IR ($CHCl_3$) 3600 (w), 3500 (br, w), 2940 (s), 2860 (m), 1725 (s), 1155 (m), 1040 (br, m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (s, 3 H), 1.48 (s, 3 H), 1.20–2.00 (m, 12 H), 2.40 (dt, 1 H, J = 14, 5 Hz), 2.86 (dd, 1 H, J = 13, 3 Hz), 3.55–3.75 (m, 1 H), 3.61 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 175.2, 80.3, 53.9, 51.0, 50.7, 47.5, 45.9, 40.3, 35.3, 33.8, 33.0, 28.9, 27.5, 26.0, 22.7. Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.48; H, 9.62.

Resolution of 12 via (*S*)-(+)-Mandelate Derivatives. A solution of (±)-**12** (7.33 g, 29.1 mmol), (*S*)-(+)-*O*-methylmandelic acid (4.83 g, 29.1 mmol), and 4-(dimethylamino)pyridine (355 mg, 2.91 mmol) in dichloromethane (200 mL) was treated with dicyclohexylcarbodiimide (5.99 g, 29.1 mmol) dissolved in dichloromethane. A white precipitate appeared almost immediately. After stirring for 48 h, the solvent was evaporated. Chromatography on silica gel separated the product mandelates from starting hydroxy ester, now partially resolved {$[\alpha]^{25}_D$ −16.1° (*c* 2.24, $CHCl_3$)}. The diastereomeric mandelates were then isolated by preparative HPLC (10% ethyl acetate/hexane), affording 8.53 and 11.7 g (20.23 g total, 78%) of the less polar and more polar products **20** and **19**, respectively, as oils. **20**: $[\alpha]^{25}_D$ +32.3° (*c* 1.06, $CHCl_3$); IR ($CHCl_3$) 3010 (m), 2940 (s), 2860 (m), 1730 (s, br), 1450 (m), 1195 (s), 1110 (m), 1020 (m), 590 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (s, 3 H), 1.32 (s, 3 H), 1.12–2.0 (m, 11 H), 2.20 (dt, 1 H, J = 13.2, 5.1 Hz), 2.80 (dd, 1 H, J = 11.0, 7.0 Hz), 7.35 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 174.2, 170.2, 136.5, 128.4, 127.0, 82.9, 81.6, 57.2, 52.5, 51.0, 50.3, 47.6, 45.7, 39.9, 35.1, 33.7, 29.0, 28.7, 26.8, 25.8, 22.1; high-resolution mass spectrum, m/z 400.2220 (M^+, calcd for $C_{24}H_{32}O_5$ 400.2250). **19**: $[\alpha]^{25}_D$ +33.7° (*c* 0.62, $CHCl_3$); IR ($CHCl_3$) 3010 (m), 2940 (s), 2860 (m), 1735 (s, br), 1450 (m), 1195 (s), 1110 (m), 1020 (m), 780 (m), 710 (m), 690 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.42 (s, 3 H), 1.29 (s, 3 H), 1.1–2.1 (m, 12 H), 2.81 (dd, 1 H, J = 13.1, 3.2 Hz), 3.37 (s, 3 H), 3.59 (s, 3 H), 4.67 (s, 1 H), 4.82 (dd, 1 H, J = 11.9, 6.6 Hz), 7.36 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 174.6, 170.3, 136.5, 128.5, 127.4, 82.9, 81.8, 77.6, 76.9, 76.3, 57.1, 52.4, 51.0, 50.5, 47.6, 45.7, 39.8, 34.9, 33.6, 29.7, 28.6, 26.1, 25.7, 22.4.

The use of (*S*)-(+)-*O*-acetylmandelic acid in the above procedure afforded a similar mixture of diastereomers **21** and **22**. The more polar major isomer **21** was isolated by chromatography on silica gel (ethyl acetate/hexanes, 1:14), followed by recrystallization from ether/hexanes: mp 118–120 °C; $[\alpha]^{25}_D$ +25.6° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3010 (m), 2950 (w), 1740 (s, br), 1220 (s), 1200 (s) cm^{-1}; ^1H NMR ($CHCl_3$) δ 0.40 (s, 3 H), 1.29 (s, 3 H), 1.1–2.1 (br m, 12 H), 2.16 (s, 3 H), 2.81 (dd, 1 H, J = 13.2, 3.2 Hz), 3.59 (s, 3 H), 4.82 (dd, 1 H, J = 11.8, 6.6 Hz), 5.88 (s, 1 H), 7.36 (m, 5 H); high-resolution mass spectrum m/z 428.2199 (M^+, calcd for $C_{25}H_{32}O_6$ 428.2199). The sample employed for X-ray diffraction analysis was recrystallized from dichloromethane/hexanes. Anal. Calcd for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 70.10; H, 7.41.

The chiral auxiliary was removed by treatment of the *O*-methylmandelates **19** and **20** with sodium methoxide (20 equiv) in dry methanol for 12 h at 40 °C, quantitatively affording the corresponding hydroxy esters (+)- and (−)-**12**, respectively. Analysis of unreacted starting material by gas chromatography indicated that no epimerization had occurred. (+)-**12**: $[\alpha]^{25}_D$ +24.2° (*c* 1.0, $CHCl_3$). (−)-**12**: $[\alpha]$ −26.2° (*c* 1.09, $CHCl_3$). Methanolysis of **21** similarly furnished (+)-**12**.

Mesylate 13. To a stirred solution of hydroxy ester (±)-**12** (500 mg, 1.98 mmol) and 4-(dimethylamino)pyridine (5 mg) in 10 mL of pyridine was added methanesulfonyl chloride (455 mg, 3.96 mmol). The resulting solution was stirred for 20 h, diluted with water, and extracted with dichloromethane. The combined organic phases were dried over magnesium sulfate and concentrated, affording 647 mg (99%) of mesylate (±)-**13**, as a white solid suitable for further transformation. Recrystallization of a small sample from hexane gave crystalline material: mp 96–97 °C; IR ($CHCl_3$) 3020 (w), 2940 (br, s), 2860 (m), 1730 (s), 1360 (s), 1330 (s), 1175 (s), 910 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (s, 3 H), 1.45 (s, 3 H), 1.0–2.3 (m, 11 H), 2.4 (dt, 1 H, J = 12, 7 Hz), 2.88 (dd, 1 H, J = 13, 3 Hz), 2.98 (s, 3 H), 3.62 (s, 3 H), 4.64 (dd, 1 H, J = 13, 7 Hz); ^{13}C NMR ($CDCl_3$) δ 174.2, 89.4, 52.8, 51.1, 50.2, 48.5, 45.7, 40.3, 38.8, 35.3, 33.8, 31.3, 28.9, 26.7, 25.7, 22.4; high-resolution mass spectrum, m/z 330.1505 (M^+, calcd for $C_{16}H_{26}O_5S$ 330.1501). Anal. Calcd for $C_{16}H_{26}O_5S$: C, 58.15; H, 7.93. Found: C, 58.32; H, 7.98.

Mesylates (+)- and (−)-**13** were prepared similarly. (−)-**13**: $[\alpha]^{25}_D$ −3.72° (*c* 1.02, $CHCl_3$); mp 97–99 °C. (+)-**13**: $[\alpha]^{25}_D$ +4.43° (*c* 1.94, $CHCl_3$); mp 97–99 °C.

Lactone 11. Method 1. Mesylate (±)-**13** (34 mg, 0.1 mmol) was added to a stirred solution of potassium superoxide (36 mg, 0.4 mmol) in dimethyl sulfoxide and dimethoxyethane (1:1, 3 mL) under argon. The disappearance of starting material was monitored by thin-layer chromatography (20% ethyl acetate/hexane). After ca. 30 min, the reaction mixture was diluted with water and extracted with ether. The combined extracts were concentrated, and the resulting oil then was partitioned between pentane and water. The aqueous layer was extracted with pentane, and the combined organic layers were dried over magnesium sulfate. Careful evaporation of solvent gave 16 mg (70%) of (±)-**11**, which solidified on standing.

Method 2. A solution of lithium methanethiolate (0.5 g, 9.3 mmol) in hexamethylphosphoramide (3.5 mL) was stirred under argon for 10 min, and mesylate (±)-**13** (500 mg, 1.5 mmol) was added. The resulting mixture was stirred 20 min further and quenched with 1 N HCl. The aqueous layer was extracted with dichloromethane, and the combined extracts were dried over magnesium sulfate and concentrated. Chromatography on silica gel (10% ethyl acetate/hexane) afforded 214 mg (65%) of (±)-**11**. Recrystallization from ether/hexane afforded an analytically pure sample: mp 113–115 °C; IR ($CHCl_3$) 3000 (w), 2960 (s), 2860 (w), 1740 (br, s), 1005 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (s, 3 H), 1.19 (s, 3 H), 1.34–2.5 (m, 12 H), 2.56 (m, 1 H), 4.54 (m, 1 H); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 176.4, 82.2, 54.4, 44.0, 43.7, 39.2,

(48) We thank Dr. Matthew Suffness (National Cancer Institute) for providing an authentic sample of natural (−)-quadrone.

(49) **Materials and Methods**: All solvents were reagent grade. Anhydrous ethanol was used as received. Ether, dimethoxyethane (DME), and tetrahydrofuran (THF) were distilled from sodium; dimethylsulfoxide, methylene chloride, pyridine, and hexane were distilled from calcium hydride. Organolithium reagents were obtained from Aldrich and standardized by titration with diphenylacetic acid. Gas chromatography was performed on a Hewlett-Packard 5790A instrument fitted with a Hewlett-Packard Ultra-1 (cross-linked methyl silicone) fused-silica capillary column (0.2 mm i.d., 25 m). Merck precoated silica gel plates (250 μ) with fluorescent indicator were used for analytical TLC. Merck silica gel 60 (particle size 0.04–0.063 mm) was employed for flash chromatography. High-pressure liquid chromatography (HPLC) was performed with a Waters Associates Prep LC/System 500 with silica gel columns. Melting points were obtained on either a Thomas-Hoover Unimelt apparatus or a Thomas Model 40 Micro Hot Stage apparatus and are corrected. Infrared spectra were recorded on a Perkin-Elmer Model 283B spectrophotometer. NMR spectra were measured with a Bruker WP-250 FT (250 Mhz) spectrometer. ^{13}C chemical shifts are reported in δ values (parts per million) relative to chloroform (δ $CHCl_3$ = 77.0). High-resolution mass spectra were obtained with a VG Micromass 7070H high-resolution chemical ionization spectrometer connected to a Kratos DS-50-S data system. Microanalyses were performed by the Rockefeller University Microanalytical Laboratories under the direction of Mr. S. T. Bella. Single-crystal X-ray data were collected with an Enraf-Nonius CAD-4 automatic diffractometer equipped with an incident-beam graphite-crystal monochromator.

(50) Chen, C. G.; Yang, J. T. *Anal. Lett.* **1977**, *10*, 1195–1207.

33.3, 32.3, 32.2, 28.3, 27.9, 26.7, 25.0, 18.3; high-resolution mass spectrum, m/z 220.1446 (M^+, calcd for $C_{14}H_{20}O_2$ 220.1460). Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.32; H, 9.15. Found: C, 76.53; H, 9.05.

Lactones (+)- and (−)-**11** were prepared similarly. (−)-**11**: $[\alpha]^{25}_D$ −69.04° (*c* 0.73, $CHCl_3$); mp 134–135 °C. (+)-**11**: $[\alpha]^{25}_D$ +71.6° (*c* 2.26, $CHCl_3$); mp 134–135 °C.

Rearranged Lactone 14. Lactone (±)-**11** (40 mg, 0.18 mmol) was dissolved in tetrahydrofuran (8 mL), and 40% sulfuric acid (2 mL) was added. The resulting solution was heated at 50 °C for ca. 1 h, whereupon no starting material could be detected by thin-layer chromatography (20% ethyl acetate/hexane). The solution was cooled, neutralized with 1 N NaOH, and extracted with dichloromethane. The combined extracts were dried over magnesium sulfate and concentrated, furnishing 41 mg of crude product. Preparative thin-layer chromatography then afforded 34 mg (85%) of (±)-**14** as a clear oil: IR ($CHCl_3$) 2950 (s), 2920 (s), 2860 (m), 1760 (br, s), 945 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.09 (s, 3 H), 1.12 (s, 3 H), 1.20–2.30 (m, 13 H), 2.59 (m, 1 H).

Lactones (+)- and (−)-**14** were prepared similarly. (+)-**14**: $[\alpha]^{25}_D$ +4.66° (*c* 0.73, $CHCl_3$). (−)-**14**: $[\alpha]^{25}_D$ −4.35° (*c* 2.92, $CHCl_3$).

Diol 15. A stirred solution of lactone (±)-**14** (20 mg, 0.09 mmol) in tetrahydrofuran (3 mL) was treated with ca. 5 mg of lithium aluminum hydride. After heating to 50 °C for 3 h, the excess reducing agent was decomposed by the careful addition of water. Extraction with dichloromethane, drying over magnesium sulfate, and concentration afforded 19 mg (93%) of (±)-**15** as a white solid: IR ($CHCl_3$) 3600 (w), 3400 (br, w), 3010 (s), 2950 (m), 1220 (s), 1205 (s), 780 (s), 710 (s), 660 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (s, 3 H), 1.17 (s, 3 H), 1.3–2.2 (m, 13 H), 2.44 (m, 1 H), 3.50 (dd, 1 H, J = 12, 5 Hz), 3.88 (apparent d, 1 H, J = 11 Hz); ^{13}C NMR ($CDCl_3$) δ 101.6, 74.7, 62.1, 53.8, 45.7, 45.6, 39.9, 37.2, 34.3, 32.9, 27.2, 26.6, 25.3, 23.9; high-resolution mass spectrum, m/z 224.1767 (M^+, calcd for $C_{14}H_{24}O_2$ 224.1770).

Diols (+)- and (−)-**15** were prepared similarly. (+)-**15**: $[\alpha]^{25}_D$ +66.7° (*c* 0.76, $CHCl_3$); mp 115–116 °C. (−)-**15**: $[\alpha]^{25}_D$ −66.4° (*c* 1.72, $CHCL_3$); mp 115–116 °C.

Unsaturated Acetate 17. Diol (±)-**15** (19 mg, 0.08 mmol) was dissolved in pyridine (3 mL), and excess acetic anhydride was added. After stirring for 24 h at room temperature, the pyridine was evaporated. The resulting mixture was taken up in ether and washed with water and brine. Drying over magnesium sulfate and concentration afforded the crude hydroxy monoacetate (±)-**16**: IR ($CHCl_3$) 3610 (w), 3600 (br, w), 2990 (m), 2950 (br, s), 1725 (s), 1385 (m), 1365 (m), 1260 (br, s), 1020 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (s, 3 H), 1.15 (s, 3 H), 2.01 (s, 3 H), 1.34–2.26 (m, 14 H), 2.43 (m, 1 H), 4.29 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 171.1, 88.8, 68.5, 55.7, 53.2, 44.3, 40.4, 34.9, 34.2, 31.1, 28.4, 22.7, 21.7, 21.5, 21.1; high-resolution mass spectrum, m/z 266.1837 (M^+, calcd for $C_{16}H_{26}O_3$ 266.1875).

Acetates (+)- and (−)-**16** were prepared similarly. (+)-**16**: $[\alpha]^{25}_D$ +85.2° (*c* 4.95, $CHCl_3$). (−)-**16**: $[\alpha]^{25}_D$ −99.1° (*c* 1.1, $CHCl_3$).

A solution of (±)-**16** (24 mg, 0.08 mmol) in dichloromethane (1 mL) was cooled to 0 °C and treated with excess pyridine and thionyl chloride. After stirring at 0 °C for 4 h, the mixture was diluted with water and extracted with ether. The combined organic layers were washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine and dried over magnesium sulfate. Concentration afforded 19 mg (85%) of unsaturated acetate (±)-**17**: IR ($CHCl_3$) 2940 (br, s), 2870 (s), 1730 (s), 1460 (m), 1395 (m), 1370 (m), 1265 and 1240 (br, s), 1030 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (s, 3 H), 1.09 (s, 3 H), 1.14–2.00 (m, 10 H), 2.02 (s, 3 H), 2.47 (m, 2 H), 4.00 (apparent t, 1 H, J = 10 Hz), 4.18 (dd, 1 H, J = 12, 4 Hz), 5.12 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 171.1, 153.3, 114.0, 64.6, 58.7, 53.4, 46.5, 45.8, 40.2, 35.9, 35.8, 33.2, 25.1, 23.3, 21.8, 21.0; high-resolution mass spectrum, m/z 248.1757 (M^+, calcd for $C_{16}H_{24}O_2$ 248.1770).

In similar fashion, unsaturated acetates (+)- and (−)-**17** were prepared. (+)-**17**: $[\alpha]^{25}_D$ +46.4° (*c* 1.62, $CHCl_3$). (−)-**17**: $[\alpha]^{25}_D$ −53.3° (*c* 2.91, $CHCl_3$).

Hydroxy Enone 18. A suspension of chromium trioxide (1.13 g, 11.3 mmol) in dichloromethane (10 mL) was cooled to −20 °C, and 3,5-dimethylpyrazole (1.09 g, 11.3 mmol) was added. After stirring for 20 min at −20 °C, a solution of unsaturated acetate (+)-**17** (140 mg, 0.57 mmol) in dichloromethane was introduced. The mixture was allowed to stand in a freezer at −35 °C overnight and then was treated with 5 N NaOH (5 mL) for 2 h at 0 °C. The reaction mixture was extracted with ether, and the organic phases were dried over magnesium sulfate. Removal of solvent afforded crude enone acetate [IR 1735, 1700, 1640 cm^{-1}]. Exposure of this material to ca. 5 equiv of potassium carbonate in methanol/water (5:1), followed by silica gel chromatography (ethyl acetate/hexanes, 1:1), afforded 188 mg (72%) of hydroxy enone (+)-**18** as a white solid: mp 132–133 °C; $[\alpha]^{25}_D$ +43.0° (*c* 0.67, $CHCl_3$); IR ($CHCl_3$) 3610 (w), 3400 (br), 3000 (m), 2960 (s), 2940 (s), 2870 (m), 1695 (s), 1635 (s), 905 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (s, 3 H), 1.22 (s, 3 H), 1.4–2.34 (m, 9 H), 2.41 (br s, 1 H), 3.28 (dd, 1 H, J = 11, 7 Hz), 3.46 (dd, 1 H, J = 12.4, 5 Hz), 5.78 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 210.3, 192.0, 121.0, 60.9, 52.4, 50.3, 48.7, 48.3, 48.2, 40.0, 39.9, 32.9, 24.7, 22.9, 20.3; high-resolution mass spectrum, m/z 220.1457 (M^+, calcd for $C_{14}H_{20}O_2$ 220.1463).

Enones (±)- and (−)-**18** were prepared similarly. (−)-**18**: $[\alpha]^{25}_D$ −40.3° (*c* 0.74, $CHCl_3$); mp 131–132 °C.

Enone Acid 3. A stirred solution of hydroxy enone (±)-**18** (8.0 mg, 0.04 mmol) in freshly distilled acetone (2 mL) at 0 °C was treated with excess Jones reagent. After 5 min, excess isopropyl alcohol was added, and the resulting green solution was diluted with water and extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated. Preparative thin-layer chromatography (5% acetic acid/dichloromethane) afforded 7.5 mg (90%) of (±)-**3** as a white solid, identical with an authentic sample kindly provided by Professor Kende: mp 138–140 °C; IR ($CHCl_3$) 3500–2500 (br, m), 1705 (s), 1635 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (s, 3 H), 1.22 (s, 3 H), 1.38 (d, 1 H, J = 13.6 Hz), 1.85 (d, 1 H, J = 13.6 Hz), 1.80–2.22 (m, 4 H), 2.17 (d, 1 H, J = 18.4 Hz), 2.46 (br s, 1 H), 2.75 (d, 1 H, J = 18.4 Hz), 2.91 (d, 1 H, J = 5 Hz), 5.85 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 210.2, 190.0, 176.8, 122.5, 51.9, 51.6, 50.2, 48.6, 47.9, 40.3, 32.9, 25.8, 23.3, 21.7; high-resolution mass spectrum, m/z 234.1264 (M^+, calcd for $C_{14}H_{18}O_3$ 234.1256).

Samples of **3** were prepared similarly from (+)- and (−)-**18**. From (+)-**18** (unnatural absolute configuration): $[\alpha]^{25}_D$ 0° (*c* 4.25, $CHCl_3$); mp 183–187 °C. From (−)-**18** (natural absolute configuration): $[\alpha]^{25}_D$ 0° (*c* 1.27, $CHCl_3$); mp 185–187 °C.

(+)- and (−)-Quadrone (1). As described by Danishefsky[2a] for the racemic material, each enantiomer of **3** was converted to hydroxy keto acid **23**. (+)-**23**: mp 103–106 °C (hexane/ethyl acetate); $[\alpha]^{25}_D$ + 2.88° (*c* 0.66, $CHCl_3$). Upon heating at 190 °C,[2a] (+)- and (−)-**23** furnished (+)- and (−)-quadrone (**1**), respectively. Yields were comparable to those reported. (+)-Quadrone: mp 176–177 °C; $[\alpha]^{25}_D$ +49.8° (*c* 0.67, EtOH). (−)-Quadrone (synthetic): mp 184.5–185 °C; $[\alpha]^{25}_D$ −49.3° (*c* 0.43, EtOH). (−)-Quadrone (natural): mp 183–184 °C (lit.[1b] mp 185–186 °C); $[\alpha]^{25}_D$ −52.7° (*c* 0.59, EtOH). Mixture melting points: (a) (+)- and (−)-quadrone, 135–136 °C (lit.[2a] mp for racemic quadrone: 140–142 °C); (b) synthetic and natural (−)-enantiomers, 182–183 °C. Synthetic and natural (−)-quadrone also were identical in all other respects.

(−)-Terrecyclic Acid A (2). Following the Danishefsky procedure for (±)-**2**,[2a] (+)-**23** was converted to terrecyclic acid A.[47] The resultant (−)-**2** had the following characteristics in addition to those reported previously: mp 124–125 °C [lit.[5a] mp 122 °C for (+)-**2**]; [a]$^{25}_D$ −29° (*c* 0.5, EtOH) {lit.[9] $[\alpha]^{25}_D$ −28.0° (*c* 0.175, $CHCl_3$); lit.[5a] $[\alpha]^{20}_D$ +29.1° (*c* 4, EtOH)}.

NMR Analysis of Natural (−)-Quadrone. All spectra were acquired on a Bruker AM500 spectrometer. The sample was prepared by dissolving ca. 1 mg of natural (−)-quadrone in ca. 0.5 mL of methylene chloride-d_2. One-dimensional proton spectra were scanned at 300, 259, 239, 219, and 199 K. A 5000-Hz sweep width, an O1 frequency of 8510.59 Hz, and a 32K memory size afforded 0.305 Hz/p resolution. One-dimensional proton decoupling was carried out at 300 and 219 K by using the same parameters, with irradiation at 763.0, 856.0, 913.0, 949.8, 990.6, 1103.8, 1148.9, 1165.6, 1288.7, 1326.2, 2052.3, and 2239.9 Hz.

A phase-sensitive 2D COSY experiment with double quantum filtering was performed by using a sweep width in the f2 dimension of 1872.66 Hz. A 1K data set in the f2 dimension was collected, zero-filled to 2K, and subjected to sine-bell multiplication prior to Fourier transform. In the f1 dimension, 445 data sets were collected and likewise zero-filled to 2K. Sine-bell multiplication and 2D Fourier transform then gave a 2D data set with a digital resolution of ca. 1 Hz in the f2 dimension.

Proton resonances were assigned by using both the decoupling data and the COSY spectrum; chemical shifts were measured relative to the CH_2Cl_2 resonance at 5.32 ppm. Coupling constants were assigned via the 300 K 1D spectrum and confirmed by examining the antiphase nature of the appropriate 2D cross peaks. For the latter analysis we generated a second 2D spectrum, employing a Gaussian multiplier in both dimensions but no symmetrization.

To confirm the chemical shift and coupling constant assignments, the 300 K spectrum was simulated by using the PANIC program supplied with the 1985 version of the Bruker software. The spin system limitations of PANIC entailed division of the spectrum into three subsets of isolated spin systems, comprising the H1–H6, H7–H12, and H13–H14 protons. For each subset, the experimentally measured shifts and coupling constants were entered into the program, and a spectrum was calculated. The three spectra were then summed without iterative adjustment of the input data, affording satisfactory reproduction of the experimental spectrum as judged by visual comparison. Subsequent iterative refinement resulted in no significant alteration of the input values.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 4.48 (H6, dd, $J_{6,4}$ = 0.64 Hz, $J_{6,5}$ = 11.76 Hz), 4.10 (H5, dd, $J_{5,4}$ = 6.00 Hz, $J_{5,6}$ = 11.76 Hz), 2.65 (H7, d, $J_{7,8}$ = 6.86 Hz), 2.58 (H2, dd, $J_{2,1}$ = 14.24 Hz, $J_{2,3}$ = 17.25 Hz), 2.33 (H4, d, $J_{4,5}$ = 6.00 Hz), 2.30 (H3, dd, $J_{3,1}$ = 7.60 Hz, $J_{3,2}$ = 17.25 Hz), 2.21 (H9, dd, $J_{9,11}$ = 6.31 Hz, $J_{9,8}$ = 14.73 Hz), 1.98 (H13, d, $J_{13,14}$ = 14.38 Hz), 1.90 (H12, dd, $J_{12,10}$ = $J_{12,11}$ = 3.20 Hz), 1.83 (H8, dddd, $J_{8,7}$ = 6.86 Hz, $J_{8,10}$ = 7.25 Hz, $J_{8,11}$ = 13.22 Hz, $J_{8,9}$ = 14.73 Hz), 1.82 (H14, d, $J_{14,13}$ = 14.38 Hz), 1.72 (H1, dd, $J_{1,2}$ = 14.24 Hz, $J_{1,3}$ = 7.60 Hz), 1.70 (H10, ddd, $J_{10,12}$ = 3.20 Hz, $J_{10,8}$ = 7.25 Hz, $J_{10,11}$ = 13.22 Hz), 1.53 (H11, dddd, $J_{11,12}$ = 3.20 Hz, $J_{11,9}$ = 6.31 Hz, $J_{11,8}$ = $J_{11,10}$ = 13.22 Hz), 1.18 (H18-20, s), 1.12 (H15-17, s).

Circular Dichroism Spectrum of Natural (−)-Quadrone. A 1.00 mM solution was prepared by dissolving 1.326 mg of natural (−)-quadrone in 5.338 mL of methanol (Aldrich Chemical Co.; spectroscopic grade, used as received). The ambient-temperature (25 °C) CD spectrum was recorded on a Jasco J41 spectrometer, together with a blank spectrum of the solvent. The quadrone spectrum was corrected by subtraction of the solvent spectrum. The cuvette path length was 0.1 cm. The instrument was calibrated by using a 2.583 mM standard aqueous solution of camphorsulfonic acid. The latter solution furnished molar ellipticities (θ) of 7670 and −15 100 deg·cm^2/dmol at wavelengths of 291 and 192 nm, respectively, slightly lower than literature[50] values of 7800 and −15 600. Accordingly, the molar ellipticities measured for quadrone were further corrected by dividing by 0.9756. Dichroic absorption ($\Delta\epsilon$) values were calculated by using the relation $\theta = 3300\Delta\epsilon$.

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Registry No. (+)-**1**, 87480-01-1; (−)-**1**, 66550-08-1; (±)-**1**, 74807-65-1; (−)-**2**, 93219-11-5; **3** (natural isomer), 117557-24-1; **3** (unnatural isomer), 132831-32-4; (±)-**3**, 78739-64-7; (±)-**6**, 92216-07-4; (+)-**11**, 132831-34-6; (−)-**11**, 132831-33-5; (±)-**11**, 92096-28-1; (+)-**12**, 92216-09-6; (−)-**12**, 132831-35-7; (±)-**12**, 92096-26-9; (+)-**13**, 132831-37-9; (−)-**13**, 132831-36-8; (±)-**13**, 92096-23-6; (+)-**14**, 132831-38-0; (−)-**14**, 132831-39-1; (±)-**14**, 92096-24-7; (+)-**15**, 132751-50-9; (−)-**15**, 132831-40-4; (±)-**15**, 132831-41-5; **16** (natural isomer), 132831-42-6; **16** (unnatural isomer), 132751-51-0; (±)-**16**, 132831-43-7; (+)-**17**, 132831-44-8; (−)-**17**, 132831-45-9; (±)-**17**, 92096-25-8; (+)-**18**, 132831-46-0; (−)-**18**, 132831-47-1; (±)-**18**, 84057-44-3; **19**, 132751-52-1; **20**, 132831-48-2; **21**, 92096-27-0; **22**, 92216-08-5; (+)-**23**, 132831-49-3; (−)-**23**, 132831-50-6; (*S*)-PhCH(OMe)CO_2H, 26164-26-1; (*S*)-PhCH(OAc)CO_2H, 7322-88-5.

Supplementary Material Available: X-ray data for **1** and **21**, Chem3D coordinates for **D**, **E**, and **H–K**, and detailed $\Delta\epsilon$ calculations for **D** and **E** (26 pages). Ordering information is given on any current masthead page.

Biosynthesis of L-671,329, an Echinocandin-Type Antibiotic Produced by *Zalerion arboricola*: Origins of Some of the Unusual Amino Acids and the Dimethylmyristic Acid Side Chain

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Abstract: The biosynthesis of L-671,329, an antibiotic of the echinocandin class, was studied in *Zalerion arboricola* by stable isotope tracer techniques and high-field NMR spectroscopy. The organism incorporates DL-[2-^{13}C]tyrosine into the antibiotic with the label appearing as C-3 of the homotyrosine residue; the C-2 position of this residue can be labeled by [2-^{13}C]acetate. Thus, homotyrosine arises from tyrosine by a chain elongation mechanism involving condensation with acetate. [2-^{13}C]Acetate also labels all the even-numbered carbon atoms (C-2–C-14) of the myristic acid side chain. L-[$^{13}CH_3$]Methionine does not donate its methyl group to 3-hydroxy-4-methylproline but is the origin of both methyl moieties of the 10,12-dimethylmyristoyl side chain. L-[1-^{13}C]Proline is incorporated into only one of the two substituted proline residues, viz, 4-hydroxyproline. Label from L-[2-^{13}C]leucine enriches the 3-hydroxy-4-methylproline residue, suggesting that this proline moiety is formed by cyclization of leucine.

L-671,329 is an acylated cyclic hexapeptide antibiotic (**1**; Figure 1) produced by the fungus *Zalerion arboricola*.[1,2] It is a member of the echinocandin group of antifungal antibiotics, which also includes aculeacin and mulundocandin.[3] The peptide portions of these natural products contain threonine together with residues of the following unusual or nonprotein amino acids: 3,4-dihydroxyhomotyrosine, *trans*-4-hydroxyproline, 2,3-*trans*-3,4-*cis*-3-hydroxy-4-methylproline, and 4,5-dihydroxyornithine. The presence of 3-hydroxyglutamine and a 10,12-dimethylmyristoyl side chain distinguishes L-671,329 from other compounds in this class. Interest in L-671,329 recently intensified with the report

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