period. Stirring was continued for 1 h at -78 °C. Without removal of the cooling bath, the solution was warmed to 10 °C over 1.5 h. The mixture was then poured into ethyl acetate, washed with ammonium chloride and brine, and dried over magnesium sulfate. Preparative thin-layer chromatography [1000-μm silica gel plate, elution with 20% ether–methylene chloride (v/v)] afforded 92 mg (53%) of a diastereomeric mixture of alcohols **34** isolated as a yellow foam: IR ($CHCl_3$) 3200–3600 (m, br), 1650–1750 (s, br) 1610 (s), 1580 (m, sh), 1200–1250 (s, br), 1140 (m), 830 (m) cm^{-1}; NMR (360 MHz, $CDCl_3$) δ 1.32–1.43 (2 s and d overlapping, 9 H), 2.61 (br s, 1 H), 2.95–3.08 (m, 1 H), 3.98–4.12 (m, 2 H), 4.20, 4.21 (2 br s, 1 H), 5.51, 5.54 (2 s, 1 H), 6.50 (d, J = 11 Hz, 1 H), 6.66–6.76 (m, 2 H), 7.26 (d, J = 11 Hz, 1 H), 7.51 (d, J = 11 Hz, 1 H); mass spectrum, m/e 344.1287 (M^+, calcd for $C_{19}H_{20}O_6$ 344.1260).

7-[[3-(4,5-Dihydro-5,5-dimethyl-4-oxo-2-furanyl)-2-butenyl]oxy]-2*H*-1-benzopyran-2-one (3, 35). To a solution of 119 mg (0.35 mmol) of **34** in 12 mL of benzene were added 84.4 mg (0.41 mmol) of dicyclohexylcarbodiimide and 40 mg (0.4 mmol) of purified cuprous chloride. The mixture was refluxed 26 h and then filtered through a pad of magnesium sulfate. Removal of the benzene in vacuo followed by preparative thin-layer chromatography [1000-μm silica gel plate, elution with 20% ether–methylene chloride (v/v)] afforded a 1:1 mixture of E and Z isomers of geiparvarin (30% based on recovered alcohol).

The E isomer (**3**; 14.5 mg, R_f 0.38) was recrystallized from methanol (mp 157–158 °C) and proved to be identical in all respects with geiparvarin, possessing the following spectral data: IR ($CHCl_3$) 2800–3000 (m), 1675–1710 (s, br), 1600 (s), 1550 (s), 1350–1400 (m), 1285 (m), 1180 (s), 1165 (s), 1130 (m), 1015 (m, br), 835 (s) cm^{-1}; NMR(360 MHz, $CDCl_3$) δ 1.42 (s, 6 H), 2.03 (d, J = 0.84 Hz, 3 H), 4.82 (d, J = 5.4 Hz, 2 H), 5.61 (s, 1 H), 6.17 (d, J = 9 Hz, 1 H), 6.64 (t, J = 5.4 Hz, 1 H), 6.70, 6.75 (s and d, J = 9 Hz, 2 H), 7.29 (d, J = 9 Hz, 1 H), 7.53 (d, J = 9 Hz, 1 H), NMR (60 MHz, $CDCl_3$) δ 1.40 (s, 6 H), 2.10 (d, J = 15 Hz, 3 H), 4.80 (d, J = 6 Hz, 2 H), 5.60 (s, 1 H), 6.25 (d, J = 9 Hz, 1 H), 6.70–7.00 (m, 3 H), 7.30 (d, J = 9 Hz, 1 H), 7.65 (d, J = 9 Hz, 1 H); mass spectrum, m/e 326.1168 (M^+, calcd for $C_{19}H_{18}O_5$ 326.1154) [lit.[5a] mp 160–161 °C; NMR[5b] (60 MHz, $CDCl_3$) δ 1.4 (s, 6 H), 2.1 (d, J = 1 Hz, 3 H), 4.87 (d, J = 6 Hz, 2 H), 5.6 (s, 1 H), 6.3 (d, J = 9.5 Hz, 1 H), 6.6–7.0 (m, 3 H), 7.3 (d, J = 9.5 Hz, 1 H), 7.61 (d, J = 9.5 Hz, 1 H).

The Z isomer (**35**; 17.3 mg, R_f 0.42) recrystallized from methanol as a fluffy white solid: mp 149–150 °C IR ($CHCl_3$) 2800–3000 (m), 1675–1720 (s, br), 1600 (s), 1550 (s), 1340–1400 (m), 1290 (m), 1175 (m), 1135 (m), 1020 (m, br), 840 (s) cm^{-1}; NMR (360 MHz, $CDCl_3$) δ 1.47 (s, 6 H), 2.06 (d, J = 1.5 Hz, 3 H) 5.02 (d, J = 4. 3 Hz, 2 H), 5.58 (s, 1 H), 6.08 (t, J = 4.3 Hz, 1 H), 6.26 (d, J = 9 Hz, 1 H), 6.78 (s, 1 H), 6.83 (d, J = 9 Hz, 1 H), 7.39 (d, J = 9 Hz, 1 H), 7.64 (d, J = 9 Hz, 1 H); mass spectrum, m/e 326.1143 (M^+, calcd for $C_{19}H_{18}O_3$ 326.1154).

Acknowledgment. It is a pleasure to acknowledge the support of this investigation by the National Institutes of Health (National Cancer Institute) through Grant No. CA-22807. In addition, we thank Mr. S. T. Bella of the Rockefeller University for the microanalyses and the Middle Atlantic Regional NMR Facility (NIH No. RR 542) at the University of Pennsylvania where the 220- and 360-MHz NMR spectra were recorded.

Registry No. 3, 36413-91-9; **5**, 18458-23-6; **6**, 16851-02-8; **13**, 55816-60-9; **14**, 75767-42-9; **15**, 75767-43-0; **16** (isomer 1), 74796-27-3; **16** (isomer 2), 74796-32-0; **17** (isomer 1), 74796-28-4; **17** (isomer 2), 74796-33-1; **18** (isomer 1), 74796-26-2; **18** (isomer 2), 74796-31-9; **19** (isomer 1), 74796-29-5; **19** (isomer 2), 74796-34-2; **20**, 75767-44-1; **21**, 74796-38-6; **22**, 74796-39-7; **23**, 74796-40-0; **24**, 74796-41-1; **25**, 74796-36-4; **26**, 74796-37-5; **27**, 74796-42-2; **28**, 74796-43-3; **31**, 93-35-6; **32**, 31005-03-5; **33**, 22919-21-7; **34** (isomer 1), 74796-30-8; **34** (isomer 2), 74796-35-3; **35**, 74796-44-4; 3-hydroxy-3-methyl-2-butanone, 115-22-0; propionaldehyde, 123-38-6; acetaldehyde, 75-07-0; benzaldehyde, 100-52-7; *trans*-cinnamaldehyde, 14371-10-9; allyl bromide, 106-95-6.

Microbial Stereodifferentiating Reduction and Absolute Configuration of 8-Deltacyclanone and 4-Brendanone.[1] An Application of the Quadrant Rule

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Incubation of (±)-8-deltacyclanone (**11**) and (±)-4-brendanone (**16**) with *Curvularia lunata* furnished a 9:7:1 mixture of (+)-ketone **11**, (−)-endo alcohol **12**, and exo alcohol **14** and a 2:1 mixture of (−)-ketone **16** and (−)-endo alcohol **17**, respectively. The CD spectra as well as the NMR spectra of these metabolites permitted assignment of their absolute configurations which were found to be compatible with those predicted on application of the quadrant rule to these racemic ketone substrates.

In previous papers,[2] we have reported the microbial stereodifferentiating reduction of various racemic C_2 ketones[3] including 9-*twist*-brendanone (**1**), 2-brexanone (**2**), D_3-trishomocubanone (**3**), and the biphenyl-bridged ketone (**4**) (Chart I) with *Curvularia lunata* and *Rhodotorula rubra* and summarized the results in a "C_2-ketone rule"[4]

Chart I

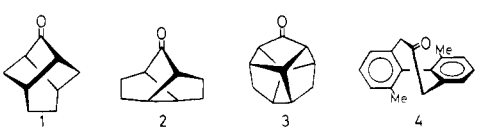
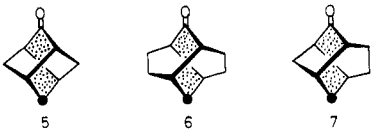


Chart II



which states that these microbes preferentially reduce the P–C_2 ketone enantiomers possessing the larger parts of

(1) Presented at the 41th Annual Meeting of the Chemical Society of Japan, Osaka, Apr 1980; Abstracts, Vol. II, p 1137.

(2) (a) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Nishino, M.; Murakami, H.; Asao, M. *J. Chem. Soc., Chem. Commun.* **1978**, 667–8. (b) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Nishino, M.; Murakami, H.; Asao, M. *J. Org. Chem.* **1979**, *44*, 4588–93.

(3) In this paper, ketones are conveniently classified according to their symmetry: C_s ketones belong to the C_s point group and have the plane of symmetry coincident with the carbonyl plane; C_2 ketones belong to the C_2 point group and have the C_2 axis coincident with the carbonyl axis; C_1 ketones have no symmetry element passing through the carbonyl axis.

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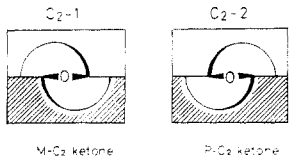
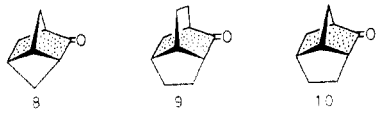
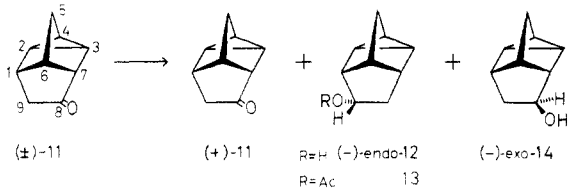


Figure 1. Two quadrant orientations (C_2-1 and C_2-2) for the enantiomers of C_2 ketones with *M* and *P* helicity.

Chart III



Scheme I



molecule in upper right and lower left quadrants in their quadrant orientations (Figure 1).

Bisnoradamantanone (**5**) and 2-twistanone (**6**) (Chart II), however, have been outstanding exceptions to the C_2-ketone rule, both having been found to be immune against these microbial reduction.[6]

Inspection of their molecular models reveals an interesting common structural feature, a twist-boat cyclohexanone moiety (shown with dotting) with the C_2 symmetry axis coincident with the carbonyl axis.

Since this conspicuous feature is also shared by a C_1 ketone,[3] 2-*twist*-brendanone (**7**) which resists stubbornly the attack of the microbes, we were tempted to attribute their reluctance to undergo microbial reduction to the presence of the protruding methylene groups (shown with the closed circles) located on the C_2 symmetry axes.

This assumption has been born out by our recent preliminary incubation experiments[1] with *C. lunata* and *R. rubra* which clearly indicated that these microbes smoothly reduced their position isomeric tricyclic cage-shaped ketones, 2-norbrendanone (**8**), 2-isotwistanone (**9**), and 2-brendanone (**10**) (Chart III), all having a similar steric environment around the carbonyl group but lacking the protruding methylene group opposite to the carbonyl group.

These results prompted us to investigate the microbial reduction of the closely related tetra- and tricyclic cage-shaped ketones 8-deltacyclanone (8-tetracyclo[4.3.0.0^{2,4}.0^{3,7}]nonanone, **11**) and 4-brendanone (4-tricyclo[4.2.1.0^{3,7}]nonanone, **16**), and this paper describes their stereochemical behavior toward *C. lunata* and *R. rubra* which eventually led to assignment of their absolute configuration.

Results and Discussion

Microbial Reduction of 8-Deltacyclanone (11) (Scheme I). Monitoring by means of GLC indicated that

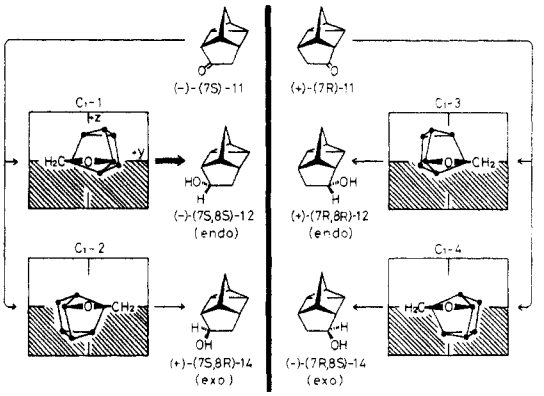


Figure 2. Four quadrant orientations for (±)-8-deltacyclanone (**11**).

C. lunata fairly rapidly reduced 8-deltacyclanone (**11**), affording a 9:7:1 mixture of the ketone **11**, the endo alcohol **12**, and the exo alcohol **14** after 32 h of incubation at 30 °C. After separation of the ketone fraction from the alcohol fraction via column chromatography over neutral alumina, the crude ketone was purified by preparative TLC (silica gel) followed by distillation to provide a 15% yield of the dextrorotatory ketone **11**, $[\alpha]_D$ +96.4°.

The reported characteristic NMR signals[7] (**CHOH**) of the endo alcohol **12** and the exo alcohol **14** allowed us to estimate a 7:1 ratio of the *endo*-**12** and the *exo*-**14** in the combined crude alcohol fraction, and their separation was attempted via rechromatography over neutral alumina.

Although we failed to isolate the pure exo alcohol **14** from the combined earlier pentane–ether eluates, our examination of the slowly moving zones was rewarded by isolation of a 17% yield of the pure (-)-endo alcohol **12**, $[\alpha]_D$ -29.8°.

Successful isolation of the (-)-exo alcohol **14** in pure state was finally achieved by carrying out incubation of (±)-**11** with *R. rubra* which afforded a 6:3:1 mixture of **11**, *endo*-**12**, and *exo*-**14** after the mixture was shaken for 48 h at 30 °C. Column chromatography over alumina provided a specimen of the (+)-ketone **11** (32% yield, $[\alpha]_D$ +82.5°), and the combined crude alcohol fractions containing **12** and **14** in a ratio of 2:1, as evidenced by NMR assay, was rechromatographed over alumina. The earlier eluates were combined to furnish a 6% yield of (-)-*exo*-**14** ($[\alpha]_D$ -4.76°) which was followed by intermediate fractions and then by slow-moving fractions containing pure (-)-*endo*-**12**: $[\alpha]_D$ -45.8°; 12% yield.

Absolute Configuration and Optical Purity of the (+)-Ketone 11, the (-)-Endo Alcohol 12, and the (-)-Exo Alcohol 14. Belonging to the C_1 ketone[3] type, (±)-**11** has four quadrant orientations, C_1-1, C_1-2, C_1-3, and C_1-4 (Figure 2), two each for each enantiomer, and the proposed quadrant rule[8] indicates that both *C. lunata* and *R. rubra* favor the C_1-1 orientation, having the larger carbonyl flanking group on the right side (+*y* direction) and the smaller part of molecule in the lower quadrants, over the other orientations which are characterized by the stereochemistry having either the larger carbonyl flanking group on the left side or the larger part of molecule in the lower quadrants.

(4) Our recent study on crystalline horse liver alcohol dehydrogenase (HLADH)[5] has demonstrated that HLADH exhibits stereochemical characteristics completely opposite those of the "C_2-ketone rule" discovered in the microbial reduction.

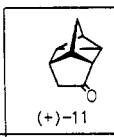
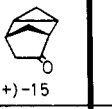
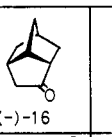
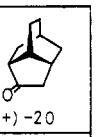
(5) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Sasaki, Y.; Fujii, T. *J. Chem. Soc., Chem. Commun.* **1980**, 626–7.

(6) A strain of *C. lunata* (IFO 6299) was found[2b] to exhibit a small activity toward **5**, furnishing a 20% yield of the (+)-ketone **5** with 3% optical purity after 10 days of incubation at 29 °C.

(7) Freeman, P. K.; Balls, D. M.; Blazevich, J. N. *J. Am. Chem. Soc.* **1970**, *92*, 2051–9.

(8) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Hirose, Y.; Shimizu, T.; Asao, M. *J. Chem. Soc., Chem. Commun.* **1978**, 668–70. Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Asao, M. *J. Org. Chem.* **1980**, *45*, 4432–40.

Table I. CD Spectra of (+)-8-Deltacyclanone (11), (+)-(1*R*)-*exo*-6-Tricyclo[3.2.1.0^{2,4}]octanone (15), (−)-4-Brendanone (16), and (+)-4-Isotwistanone (20)

	 (+)-11	 (+)-15	 (−)-16	 (+)-20
$[\theta]_{max}$	+ 1.4x10^4 [a]	+ 2.1x10^4	− 6.1x10^3 [a]	+ 8.9x10^3
$(\lambda)_{nm}$	(286.5)	(293)	(299)	(300)
(Solvent)	(CH_3OH)	(isooctane)	(CH_3OH)	(C_2H_5OH)

[a] These values are corrected to 100% optical purity according to their known optical purity.

Table II. Absolute Rotations of the Microbial Metabolites of (±)-8-Deltacyclanone (11) and (±)-4-Brendanone (16)

	 (+) -11	 (-)-endo-12	 (-)-exo-14	 (-) -16	 (-)-endo-17
$[\alpha]_{D,abs}$ [a]	290°	55°	9°	75°	36°

[a] Solvent $CHCl_3$.

Upon delivery of the hydrogen atom from the lower section, C_1-1 is expected to afford the (7*S*,8*S*)-endo alcohol **12** faster than the exo alcohol **14**. When incubation is terminated at a point where about 50% of the substrate ketone has been reduced, the quadrant rule predicts that (a) the recovered ketone **11** will have the 7*R* configuration, (b) the major metabolite will be the endo alcohol **12** with the 7*S*,8*S* configuration, corresponding to the most favored orientation C_1-1, and (c) the minor metabolite will be the exo alcohol **14** with the 7*R*,8*S* configuration, corresponding to C_1-4, the next favored orientation having the larger carbonyl flanking group on the right side but the larger part of molecule in the lower quadrants.

Comparison of the (+) Cotton effect exhibited by the recovered (+)-ketone **11** and that of closely related (+)-(1*R*)-*exo*-6-tricyclo[3.2.1.0^{2,4}]octanone (**15**)[9] both with the same homoconjugated cyclopropyl ketone structure (Table I) assigned the predicted 7*R* configuration to the (+)-ketone **11**, while respective chromic acid oxidation of (−)-*endo*-**12** and (−)-*exo*-**14** to enantiomeric (−)-**11** and (+)-**11** ultimately assigned their 7*S*,8*S* and 7*R*,8*S* configurations.

After satisfying ourselves that these stereochemistries of the metabolites were completely compatible with those predicted by the quadrant rule, we diverted our attention to an examination of their optical purity.

Addition of tris[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]europium(III) [Eu(facam)$_3$][10,11] to a CCl_4 solution of the acetate **13** ([α]$_D$ −44.2°) prepared from the endo alcohol **12** ([α]$_D$ −45.8°) split the CH_3CO signal into a doublet, and its integrated intensity indicated an enantiomer ratio 1:11, corresponding to 83% optical purity. This, coupled with the chromic acid oxidation of (−)-*endo*-**12** and (−)-*exo*-**14** to the optically active ketone **11** (see Experimental Section) enabled us to calculate the absolute rotations of these metabolites as tabulated in Table II.

(9) Lightner, D. A.; Beavers, W. A. *J. Am. Chem. Soc.* **1971**, *93*, 2677–84. Application of their proposed method qualitatively predicts a strong (+) Cotton effect of (+)-**11** which has stereochemistry similar to that of (+)-**15** around the homoconjugated cyclopropyl ketone system.

(10) McCreary, M. D.; Lewis, D. W.; Wernick, D. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1974**, *96*, 1038–54.

(11) Goering, H. L.; Eikenberry, J. N.; Koermer, G. S.; Lattimer, C. J. *J. Am. Chem. Soc.* **1974**, *96*, 1493–1501.

Scheme II

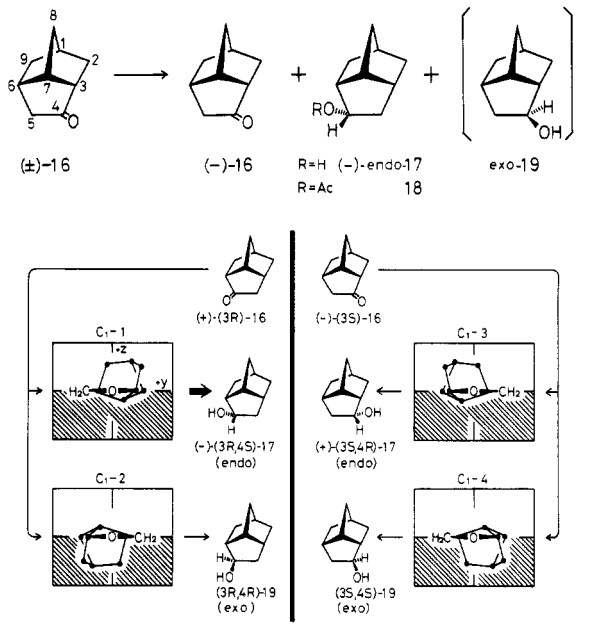


Figure 3. Four quadrant orientations for (±)-4-brendanone (**16**).

Taking into account these chiroptical informations, we now know that *C. lunata* yielded a 9:7:1 mixture of the (+)-ketone **11**, the (−)-endo alcohol **12**, and the exo alcohol **14** with respective 33%, 54%, and unknown optical purities, while *R. rubra* afforded a 6:3:1 mixture of (+)-**11**, (−)-*endo*-**12**, and (−)-*exo*-**14** with respective 29%, 83%, and 56% optical purities.

Microbial Reduction of 4-Brendanone (16) (Scheme II). Incubation of 4-brendanone (**16**) with *C. lunata* was terminated after 48 h of shaking at 30 °C when GLC monitoring indicated 33% of the substrate had been reduced to afford a 2:1 mixture of the recovered ketone **16** and an alcohol.

Ethereal extract of the culture solution was chromatographed over alumina, and the combined pentane eluates were sublimed in vacuo to furnished a 48% yield of the (−)-ketone **16**, [α]$_D$ −26.1°. Careful GLC examination of the alcohol fraction eluted with pentane–ether revealed no trace of the isomeric alcohol, and its sublimation in vacuo yielded the (−)-endo alcohol **17** ([α]$_D$ −31.0°, 20% yield) whose NMR signal (CHOH) centered at δ 4.25 (multiplet) again assigned its endo stereochemistry (vide supra).

Prompted by our above-mentioned experience with *R. rubra* which afforded the exo alcohol **14** in a fairly good yield, we carried out incubation of (±)-**16** with this microbe expecting to obtain the missing exo alcohol **19**. GLC examination of the isolated alcohol fraction detected a small amount (ca. 10%) of the exo alcohol **19**; however, the observed sluggish reduction rate forced us to abandon our attempt to isolate pure *exo*-**19**.

Absolute Configuration and Optical Purity of the (−)-Ketone 16 and the (−)-Endo Alcohol 17. Application of the quadrant rule (Figure 3) predicts that the isolated (−)-alcohol should be the endo diastereomer **17** with the 3*R*,4*S* absolute configuration, corresponding to the most favorable quadrant orientation C_1–1, while the recovered (−)-ketone should have the 3*S* configuration, corresponding to the less favored C_1-3 and C_1-4 orientations.

The latter conclusion concerning with the stereochemistry of the (−)-ketone **16** was born out by comparison of its CD spectrum, exhibiting a (−) Cotton effect, with the reported (+) Cotton effect of the closely related (+)-

(3*R*)-4-isotwistanone (**20**[12,13] Table I), and chromic acid oxidation of the (−)-endo metabolite alcohol **17** to the enantiomeric (+)-(3*R*)-ketone **16** supported its 3*R*,4*S* configuration and confirmed the quadrant rule's prediction.

Finally, it seems pertinent to describe here our efforts to estimate the optical purity of these metabolites from (±)-**16**.

Acetylation of the (−)-endo alcohol **17** ($[\alpha]_D$ −31.0) afforded the (−)-acetate **18** ($[\alpha]_D$ −51.2°) whose 85% optical purity was assigned by observation of an anisochronous split of the CH_3CO signal in its NMR spectrum with the addition of Eu(facam)$_3$. This estimation eventually permitted us to calculate the absolute rotations of (−)-*endo*-**17** and (−)-ketone **16** as tabulated in Table II, indicating that *C. lunata* incubated with the (±)-ketone **16** furnished a 2:1 mixture of the (−)-ketone **16** and the (−)-endo alcohol **17** with respective 35% and 85% optical purity.

Experimental Section[14]

Our general procedure for microbial incubation and extraction of the metabolites has been described elsewhere;[2b] the cultures of *Curvularia lunata* and *Rhodotorula rubra* were obtained from the Institute of Fermentation, Osaka, Japan, and were identified by their IFO catalog serial numbers IFO 6288 and IFO 0889, respectively.

Microbial Reduction of (±)-8-Deltacyclanone (11). The racemic substrate ketone **11** was prepared by the method of Nickon et al;[15] bp 61–62 °C (2mm) [lit.[15] bp 88–90 °C (10 mm)].

(a) Incubation with *C. lunata*. A total of 1.0 g of the racemic ketone **11** was incubated at 30 °C for 32 h in eight batches (8 × 200 mL of culture media). The metabolite mixture (1.04 g) containing **11**, **12**, and **14** in a ratio of 53:41:6 (GLC analysis) was taken up in *n*-pentane and was chromatographed over 30 g of alumina.

Elution with *n*-pentane gave crude ketone fraction (480 mg) which was purified by preparative TLC followed by distillation to give 152 mg (15% yield) of (+)-8-delatacyclanone (**11**): bp 80–81 °C (6 mm); n^{23}_D 1.5058; $[\alpha]^{27}_D$ +96.4° (*c* 1.47, $CHCl_3$); optical purity 33% [lit.[7] $[\alpha]^{24}_{304}$ −90° (*c* 0.05, MeOH)]; CD (*c* 1.65 × 10^{-3} M, MeOH) $[\Theta]_{max}$ (λ) +4.73 × 10^3 (286.5 nm).

Anal. Calcd for $C_9H_{10}O$: C, 80.56; H, 7.51. Found: C, 80.18; H, 7.57.

Further elution with ether gave crude alcohol fraction (420 mg) containing **12** and **14** in a ratio of 88:12 (NMR analysis), which was rechromatographed over alumina. Elution with *n*-pentane–ether (10:1) gave 48 mg of a mixture of **12** and **14** (78:28): bp 95–115 °C (6 mm); $[\alpha]^{27}_D$ −20.5° (*c* 1.09, $CHCl_3$). Further elution with the same solvent gave 175 mg (17% yield) of pure (−)-*endo*-8-deltacyclanol (**12**): bp 95–115 °C (6 mm); n^{26}_D 1.518; $[\alpha]^{27}_D$ −29.8° (*c* 0.94, $CHCl_3$); optical purity 54%; NMR ($CDCl_3$, 60 MHz) δ 4.40 (m, 1 H, **CH**OH) [lit.[7] δ 4.40 (m, 1 H)].

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 78.69; H, 8.87.

(b) Incubation with *R. rubra*. A total of 2.2 g of the racemic ketone **11** was incubated at 30 °C for 48 h in 18 batches (18 × 200 mL of culture media) to give a metabolite mixture (2.6 g) containing **11**:**12**:**14** in a ratio of 60:29:11 (GLC analysis). The mixture was chromatographed over alumina to give 1.0 g of crude ketone fraction and 0.92 g of crude alcohol fraction.

The crude ketone was purified by preparative TLC followed by distillation to afford 0.7 g (32% yield) of (+)-8-deltracyclanone (**11**): bp 130 °C (16 mm); n^{18}_D 1.5137; $[\alpha]^{21}_D$ +82.5° (*c* 0.9, $CHCl_3$); optical purity 28.5%.

Anal. Calcd for $C_9H_{10}O$: C, 80.56; H, 7.51. Found: C, 80.30; H, 7.56.

The crude alcohol fraction containing **12** and **14** in a ratio of 2:1 (NMR analysis) was taken up in *n*-pentane, chromatographed over alumina (60 g), and eluted with *n*-pentane–ether (10:1). From fast-moving fractions there was obtained 185 mg of exo alcohol (GLC analysis) which was distilled to give 125 mg (6% yield) of (−)-*exo*-8-deltacyclanol (**14**): bp 120 °C (25 mm); $[\alpha]^{27}_D$ −4.76° (*c* 0.925, $CHCl_3$); optical purity 56.3%; NMR ($CDCl_3$, 60 MHz) δ 4.1 (dd, *J* = 2 and 6 Hz, 1 H, **CH**OH) [lit.[7] δ 4.01 (dd, *J* = 2 and 6.6 Hz)].

Anal.[16] Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 78.24; H, 8.98.

For the 3,5-dinitrobenzoate: mp 104–106 °C; $[\alpha]^{29}_D$ +6.3° (*c* 5.1, $CHCl_3$).

Anal. Calcd for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.33; H, 4.27; N, 8.30.

The intermediate fractions giving 120 mg of a 1:1 mixture of **12** and **14** were followed by slow-moving fractions which afforded 255 mg (12% yield) of (−)-*endo*-8-deltacyclanol (**12**): bp 120 °C (25 mm); $[\alpha]^{29}_D$ −45.8° (*c* 0.96, $CHCl_3$); optical purity 83.2%.

Anal.[16] Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 78.12; H, 8.82.

For the 3,5-dinitrobenzoate: mp 109–110 °C; $[\alpha]^{29}_D$ −33.3° (*c* 0.61, $CHCl_3$).

Anal. Calcd for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.48; H, 4.34; N, 8.38.

(−)-*endo*-8-Deltacyclyl acetate (**13**): bp 120 °C (30 mm); $[\alpha]^{27}_D$ −44.2° (*c* 0.50, $CHCl_3$); optical purity 83.2%; NMR (CCl_4 100 MHz) δ 1.98 (s, 3 H, CH_3COO), 5.04 (m, 1 H, HCOAc).

Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.70; H, 7.81.

(c) Oxidation of the (−)-Endo Alcohol 12. Brown's reagent[17] (0.18 mL) was added to a chilled and stirred solution of the (−)-endo alcohol **12** (50 mg, $[\alpha]^{27}_D$ −45.8°) in 20 mL of ether over 5 min. The second 0.2 mL of the reagent was added in another 10 min. After 15 min, the ether layer was separated, washed with dilute $NaHCO_3$ and then water, and dried over $MgSO_4$. Removal of the solvent gave an oil which was purified by preparative TLC followed by distillation to give 30 mg of (−)-8-deltacyclanone (**11**): bp 120 °C (30 mm); $[\alpha]^{30}_D$ −241.0° (*c* 0.46, $CHCl_3$); optical purity 83.2%; CD (*c* 8 × 10^{-3} M, MeOH) $[\Theta]_{max}$ (λ) −1.13 × 10^4 (286.5 nm); UV (*c* 7.4 × 10^{-3} M, MeOH) λ_{max} 280 nm (ε 46.0).

Anal.[16] Calcd for $C_9H_{10}O$: C, 80.56; H, 7.51. Found: C, 78.47; H, 7.64.

For the 2,4-dinitrophenylhydrazone: mp 182.5–183 °C; $[\alpha]^{30}_D$ −79.4° (*c* 0.19, $CHCl_3$).

Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.38; H, 4.43; N, 17.85.

(d) Oxidation of the (−)-Exo Alcohol 14. Oxidation of 63 mg of the (−)-exo alcohol **14** ($[\alpha]^{27}_D$ −4.76°) was carried out in the same way described above to afford 35 mg of (+)-8-deltacyclanone (**11**): bp 120 °C (35 mm); $[\alpha]^{30}_D$ +163.2° (*c* 0.51, $CHCl_3$); optical purity 56.3%.

Anal.[16] Calcd for $C_9H_{10}O$; C, 80.56; H, 7.51. Found: C, 78.34; H, 7.53.

For the 2,4-dinitrophenylhydrazone: mp 186–187 °C; $[\alpha]^{31}_D$ +51.5° (*c* 0.19, $CHCl_3$).

Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.22; H, 4.50; N, 17.90.

Microbial Reduction of (±)-4-Brendanone (16). The racemic substrate ketone **16** was prepared by the method of Nickon et al;[15] mp 108–110 °C (in a sealed tube) (lit.[15] mp 120.5 °C).

(a) Incubation with *C. lunata*. The racemic ketone **16** (total 800 mg) was incubated at 30 °C for 48 h in eight batches (8 ×

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(14) Melting points are uncorrected. ^1H NMR spectra were determined on a JNM-NH-100 and a JNM-C-60-HL. Chemical shifts are reported as δ values in parts per million relative to internal Me_4Si (δ 0). Coupling constants (*J*) are reported in hertz; s = singlet, dd = double of doublets, and m = multiplet. Optical rotations were measured with a JASCO DIP-SL polarimeter. Circular dichroism (CD) spectra were determined on a JASCO J-40 spectropolarimeter. GLC analyses were performed on a JGC-20K equipped with an FID and using 2 m × 3 mm columns of 10% Carbowax 20M on Chromosorb W and of 15% silicone DC QF-1 on Uniport B. Preparative TLC and column chromatography were carried out with Merck silica gel 60 $PF_{254+366}$ and Woelm active alumina (neutral, activity III), respectively.

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200 mL of culture media). The crude metabolite mixture (840 mg) containing **16** and **17** in a ratio of 67:33 (GLC analysis) was taken up in *n*-pentane and chromatographed over 20 g of alumina.

Elution with 600 mL of *n*-pentane afforded the crude ketone **16** which was sublimed in vacuo to afford 381 mg (47.6% yield) of (−)-4-brendanone (**16**): mp 110–113 °C (in a sealed tube); $[\alpha]^{18}_D$ −26.06° (*c* 0.94, $CHCl_3$); optical purity 34.8%; CD (*c* 1.5 × 10^{-3} M, MeOH) $[\Theta]_{max}$ (λ) −2.13 × 10^3 (299 nm).

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.19; H, 8.79.

Further elution with 400 mL of *n*-pentane–ether (10:1) afforded the crude alcohol **17** which was sublimed in vacuo to afford 167 mg (20% yield) of (−)-*endo*-4-brendanol (**17**): mp 135–136 °C (in a sealed tube); $[\alpha]^{18}_D$ −31.0° (*c* 0.91, $CHCl_3$); optical purity 85.3%; NMR (CCl_4, 60 MHz) δ 4.25 (m, 1 H, CHOH).

Anal. Calcd for $C_9H_{14}O$: C, 78.21; H, 10.21. Found: C, 78.16; H, 10.13.

(−)-*endo*-4-Brendyl acetate (**18**): bp 110 °C (20 mm); $[\alpha]^{20}_D$ −51.2° (*c* 0.61, $CHCl_3$); optical purity 85.3%; NMR (CCl_4, 60 MHz) δ 1.93 (s, 3 H, CH_3COO), 4.98 (m, 1 H, HCOAc).

Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.89. Found: C, 73.16; H, 8.95.

(b) Oxidation of the (−)-Endo Alcohol 17. Brown's reagent[17] (0.36 mL) was added to a solution of the (−)-endo alcohol **17** (60 mg) in ether (25 mL) during 15 min at 0 °C. After an additional 15 min, the ether layer was worked up in the usual way to give the crude ketone (59.8 mg) which was taken up in *n*-pentane and chromatographed over alumina. The combined eluates were sublimed in vacuo [70 °C (20 mm)] to afford 43 mg of (+)-4-brendanone (**16**): mp 107–109 °C (in a sealed tube); $[\alpha]^{20}_D$ +63.8° (*c* 0.44, $CHCl_3$); optical purity 85.3%; CD (*c* 8.8 × 10^{-4} M, MeOH) $[\Theta]_{max}$ (λ) +4.8 × 10^3 (299 nm); UV (*c* 7.3 × 10^{-3} M, MeOH) λ_{max} 290 nm (ε 19.1).

Anal. Calcd for $C_9H_{12}O$: C, 79.37; H, 8.88. Found: C, 79.41; H, 8.82.

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Registry No. (±)-**11**, 75768-02-4; (±)-**11**, 75801-41-1; (−)-**11**, 29415-46-1; (−)-**11** DNP, 75801-42-2; (+)-**11** DNP, 75801-43-3; (−)-**12**, 75801-44-4; (−)-**12** 3,5-dinitrobenzoate, 75801-45-5; (−)-**13**, 75801-46-6; (−)-**14**, 75801-47-7; (−)-**14** 3,5-dinitrobenzoate, 75801-48-8; (+)-**15**, 75801-49-9; (±)-**16**, 75768-03-5; (−)-**16**, 75801-50-2; (+)-**16**, 75801-51-3; (−)-**17**, 75768-04-6; (−)-**18**, 75768-05-7; (+)-**20**, 37167-95-6.

(8-Quinolinesulfonyl)tetrazole: A New Type of Highly Efficient Coupling Agent for the Synthesis of Ribooligonucleotides by the Phosphotriester Approach[1]

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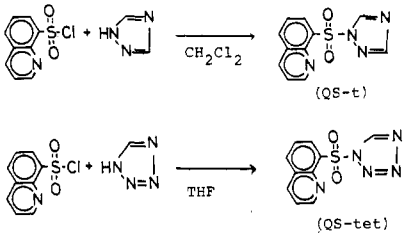
(8-Quinolinesulfonyl)Tetrazole (QS-tet) has been developed as a new type of highly efficient coupling agent in the synthesis of phosphotriester bonds. This reagent very rapidly completes the coupling reactions, and the yield is considerably higher than that obtained with conventional condensing agents.

The chemical synthesis of deoxyribonucleotides has developed sufficiently to produce long deoxyribooligonucleotides of defined sequence,[2] whereas because of the presence of the 2′-hydroxyl group, developments for the chemical synthesis of long ribooligonucleotides of defined sequence have been much slower. In our original studies on the synthesis of ribooligonucleotides by the phosphotriester approach, we developed 8-quinolinesulfonyl chloride (QS)[3] as a new type of coupling agent and the 5-chloro-8-quinolyl group (qcl)[4] as a very effective phosphate protecting group. However, QS is unsatisfactory for the synthesis of ribooligonucleotides containing the guanosine unit, owing to the liberation of hydrogen chloride during the coupling reactions, and the reactions are very slow (1–2 days).[5] Cramer et al.[6] and Narang et al.[7] have also re-

Scheme I



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