mL). NaOH (2 N) was added to adjust pH to ~7, and then $AgNO_3$ (0.172 g, 1.01 mmol) in water (4 mL) was added. The mixture was stirred in the dark for 3 h at 2 °C, and the precipitate was filtered off, washed with ethanol and ether, and dried: 0.400 g (1.22 mmol, yield 80%); mp 150 °C dec; IR (Nujol) 1530, 1340 COO^-.

Preparation of Glucoside 22. The above silver salt (0.400 g, 1.22 mmol) was suspended in anhydrous benzene (20 mL) and (bromoacetoxy)-β-D-glucose. The mixture was stirred for 24 h in the dark. The precipitate was filtered off and the solvent removed. The residue was chromatographed on preparative TLC plates (eluent PE/EE, 25:75): two main bands (R_f ~0.4 and R_f ~0.2) were isolated. The less polar product (R_f ~0.4) was a mixture of acetylglucose and glucoside **22**. The more polar product (R_f ~0.2) was a 4:1 mixture of diastereomeric glucosides **22** which was recrystallized in ether–hexane: 0.065 g (0.18 mmol, yield 15%); mp 158–159 °C; IR 1755, 1745; NMR 2.02 (m, 12 H, OAc), 4.57 (s, 2 H, OCH_2Ph, minor isomer), 4.59 (s, 2 H, OCH_2Ph, major isomer), 3.3–5.5 (m, 10 H, CHO and OH), 5.74 (d, 1 H, H_α, J = 7.0), 6.11 and 6.50 (2 br s, 2 H, $C{=}CH_2$, major isomer), 6.03 and 6.44 (2 br s, 2 H, minor isomer); mass spectrum, m/e 354 (M^+ – 18), 331 (M^+ – aglycon part).

Anal. Calcd for $C_{26}H_{32}O_{13}$: C, 56.52; H, 5.79. Found: C, 56.53; H, 5.67.

Acknowledgment. Thanks are due to the Institut National de la Santé et de la Recherche Médicale (INSERM) for financial assistance (Contrat libre No. 77-1-9-073) and to DGRST for a fellowship (Allocation de Recherches) to J.P.C.

Registry No. **1**, 38965-80-9; **5**, 76299-53-1; **6**, 76299-54-2; **7**, 76299-55-3; **8** (R = Me), 993-88-4; **8** (R = Me), 20345-61-3; **9a**, 73756-09-9; **9b**, 73738-48-4; **9c**, 76299-56-4; **9d**, 73738-53-1; **9e**, 73738-55-3; **10a**, 73738-58-6; **10b** (isomer 1), 76319-65-8; **10b** (isomer 2), 76299-57-5; **10c**, 73738-84-8; **10d**, 73738-62-2; **10e**, 73738-64-4; **12a**, 73738-67-7; **12b**, 73738-65-5; **12c**, 76299-58-6; **12d**, 73738-71-3; **12e** (isomer 1), 76299-59-7; **12e** (isomer 2), 76299-60-0; **13a**, 73738-80-4; **13b** (isomer 1), 73738-74-6; **13b** (isomer 2), 73738-75-7; **13c**, 76299-61-1; **13d**, 76299-62-2; **13e**, 76299-63-3; **15**, 76299-64-4; **18**, 73738-72-4; **19**, 24923-78-2; **20** (R = CH_2Ph), 76299-65-5; **21** (R = CH_2Ph), 76299-66-6; **22** (isomer 1), 76299-67-7; **22** (isomer 2), 76299-68-8; **23**, 6919-96-6; **24** (R = CH_2Ph), 76299-69-9; 2-phenylpropionaldehyde, 93-53-8; $Ph(CH_3)C{=}CH(OAc)$ (isomer 1), 37973-51-6; $Ph(CH_3)C{=}CH(OAc)$ (isomer 2), 37973-52-7; $MeCH{=}CHOAc$, 3249-50-1; i-$PrCH{=}CHOAc$, 54779-59-8; MeCHCH(OAc)O (isomer 1), 76299-70-2; MeCHCH(OAc)O (isomer 2), 76319-66-9; i-PrCHCH(OAc)O, 76299-71-3; $C_5H_{11}CHCH(OAc)O$, 53662-41-2; $(CH_3)_2CHC(OAc)CHO$, 73738-47-3; $CH_3CH(OAc)CHO$, 22094-23-1; $C_2H_5CH(OAc)CHO$, 5921-90-4; $C_6H_5(CH_3)C(OAc)CHO$, 60860-35-7; $C_2H_5(C_2H_5)C(OAc)CHO$, 76299-72-4; $C_5H_{11}CH(OAc)CHO$, 22094-22-0; $CH_2(OAc)CHO$, 5371-49-3; 1,2-diacetoxy-1-ethoxyethane, 3100-09-2; ethyl vinyl ether, 109-92-2; phenacyl chloride, 98-88-4; β-hydroxy-α-methylene-γ-pentyl-γ-butyrolactone, 76299-73-5; α-(benzyloxy)acetaldehyde, 60656-87-3; $ClCH_2CH_2OCH_2Ph$, 17229-17-3; $(PhCH_2O)CH_2CH{=}C(COOC_2H_5)CH_2SPh$, 76299-74-6; $(PhCH_2O)CH_2CH{=}C(COOC_2H_5)CH_2OSPh$, 76299-75-7.

Microbial Stereodifferentiating Reduction of 1,6-Spiro[4.4]nonanedione, a Gyrochiral Diketone with Two Homotopic Carbonyl Groups

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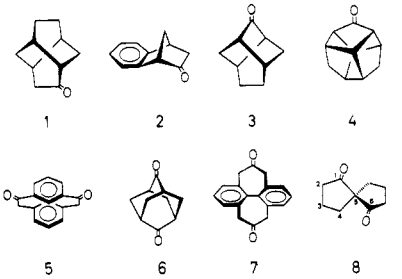
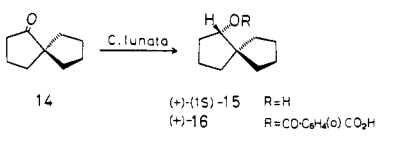
After a preliminary incubation of 1-spiro[4.4]nonanone (**14**) with *Curvularia lunata*, affording (+)-(1*S*)-alcohol **15** with 100% optical purity, (±)-1,6-spiro[4.4]nonanedione (**8**) was incubated with *C. lunata* for 8 h at 30 °C to yield a 34:30:36 mixture of (−)-(5*S*)-**8**, (+)-*trans*-(5*R*,6*S*)-ketol **9**, and (−)-*cis*-(5*R*,6*R*)-ketol **10** with respective 82%, 76%, and 6% optical purities. Incubation of (±)-*trans*-6-hydroxyspiro[4.4]nonan-1-one (**9**) furnished a metabolite mixture containing (−)-*trans*-(5*S*,6*R*)-**9**, (+)-*trans*,*trans*-(1*S*,5*R*,6*S*)-diol **11**, and (+)-*cis*,*trans*-(1*R*,5*S*,6*S*)-diol **12** with respective 56%, 80%, and 73% optical purities. Although a modified quadrant rule for C_1 ketones could explain these microbial stereoselectivities, serious perturbing effects from the unique spirane framework and the neighboring functional groups were observed.

Summarizing the stereodifferentiating aptitude of *Curvularia lunata* and *Rhodotorula rubra* in the microbial reduction of various cage-shaped ketones (e.g., **1** and **2**, Chart I) with C_1 symmetry, we have proposed a "quadrant rule" whose application in predicting the stereochemical course of the microbial reduction as well as in assigning the absolute configuration of the metabolites has been demonstrated in a wide variety of substrate ketones.[1] Prompted by this accomplishment, we then explored the stereochemistry of the microbial reduction of C_2 ketones[2] (e.g., **3** and **4**); accumulated stereochemical information in this field led us to propose a "C_2-ketone rule".[3]

Chart I



Scheme I



(1) (a) Nakazaki, M.; Chikamatsu, H. *Kagaku no Ryoiki* **1977**, *31*, 819–33. (b) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Hirose, Y. "Abstracts of Papers", 36th Annual Meeting of the Chemical Society of Japan, Osaka, Apr 1977; The Chemical Society of Japan: Tokoyo, 1977; No. II, p 1214. (c) Chikamatsu, H.; Asao M.; Nakazaki, M. *Ibid.*, p 1214. (d) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Hirose, Y. "Abstracts of the 26th IUPAC Congress"; Tokyo, Sept 1977; IUPAC Congress: Tokyo, 1977; p 63. (e) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Hirose, Y.; Shimizu, T.; Asao, M. *J. Chem. Soc., Chem. Commun.* **1978**, 668–70. (f) Nakazaki, M.; Chikamatsu, H.; Naemura, K.; Asao, M. *J. Org. Chem.* **1980**, *45*, 4432–40.

0022-3263/81/1946-1147$01.25/0

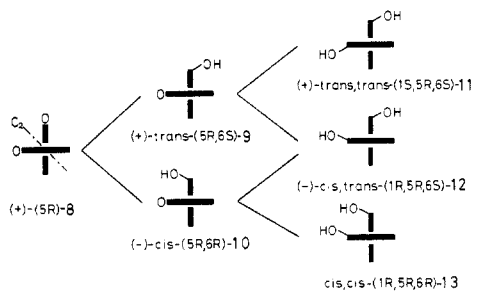
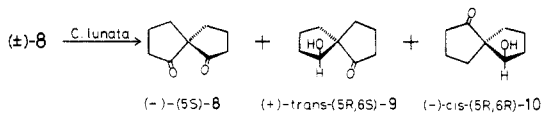


Figure 1. Generation of two diastereomeric ketols and three diastereomeric diols from (+)-(5*R*)-1,6-spiro[4.4]nonanedione (**8**).

Scheme II



An extension of this research to various diketones resulted in our incubation experiments of 1,10-dioxo[2.2]-metacyclophane (**5**),[4] 2,6-adamantanedione (**6**),[5] and the doubly bridged biaryl ketone **7**,[6] and in the present paper we report the stereochemistry in microbial reduction of another diketone, **8**, a spirodiketone possessing two carbonyl groups constrained in two cyclopentane rings which are orthogonal to each other.

Belonging to the C_2 point group, 1,6-spiro[4.4]nonanedione (**8**) is a gyrochiral molecule with two homotopic carbonyl groups. Besides this rather unusual stereochemistry, **8** seems to promise an interesting substrate in the microbial reduction with the following features: (a) its rigid structure with two functional groups in a well-defined relative geometry should allow us to have an insight on the limitation of our proposed quadrant rule which had been deduced mainly from working on cage-shaped monoketones; (b) the stereochemistry of **8** as well as its reduction products (Figure 1) has been well established.[7-9]

Results and Discussion

Microbial Reduction of 1-Spiro[4.4]nonanone (14, Scheme I). Since there has seemed to be no paper reporting the microbial reduction of any spiroketone, we first incubated 1-spiro[4.4]nonanone (**14**) with *C. lunata*. GLC monitoring indicated that 24 h of incubation at 30 °C afforded a 1:1 mixture of **14** and the alcohol **15**, and ether extraction followed by column chromatography (Al_2O_3) gave a 33% yield of (+)-**15**, whose $[\alpha]_D$ +39.8° was found to be unchanged on purification via the phthalate **16** (mp 115 °C; $[\alpha]_D$ +107°), indicating its almost 100% optical purity. This conclusion was further supported by the fact that no anisochronous enantiomer shift was observed in its NMR spectrum with addition of Eu(facam)$_3$.[10,11]

R. rubra was found rather sluggish in reducing **14**, and a 17% yield of (+)-**15** ($[\alpha]_D$ +33.0°, optical purity 83%) was isolated from the culture solution after 48 h of incubation at 30 °C.

Prelog's rule[1f,12] predicts that the spiroketone **14**, belonging to simple C_s ketones,[2] should yield the corresponding alcohol with an *S* configuration, and this was supported by Solladie's experiment[13] which demonstrated that (+)-**15** has the *S* configuration.

Partial Reduction of (±)-1,6-Spiro[4.4]nonanedione (8, Scheme II). Our small-scale test incubation of (±)-**8** with *C. lunata* revealed that the ethereal extract of the

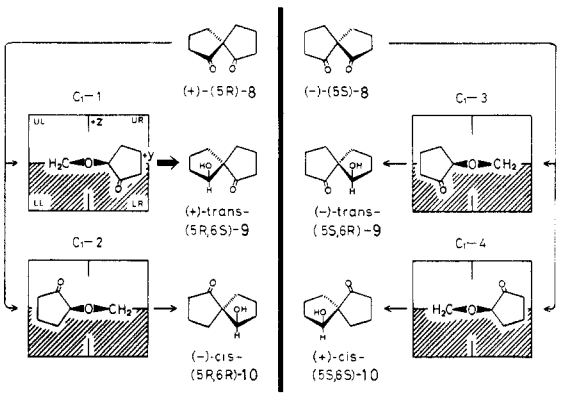


Figure 2. Schematic representation of the four quadrant orientations for (±)-1,6-spiro[4.4]nonanedione (**8**).

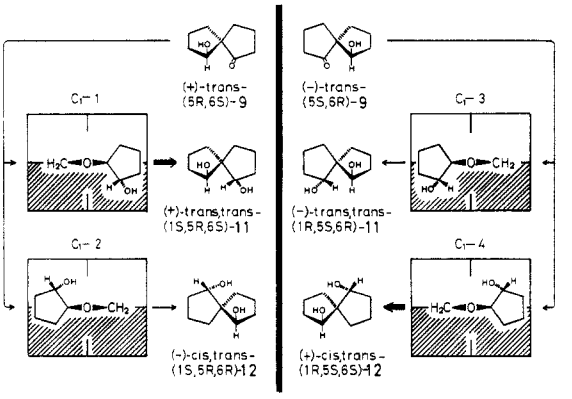


Figure 3. Schematic representation of the four quadrant orientations for (±)-*trans*-6-hydroxyspiro[4.4]nonan-1-one (**9**).

(2) 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.

(3) (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.

(4) Nakazaki, M.; Chikamatsu, H.; Hirose, Y.; Shimizu, T. *J. Org. Chem.* **1979**, *44*, 1043–8.

(5) (a) Chikamatsu, H.; Murakami, H.; Nakazaki, M. "Abstracts of Papers", 36th Annual Meeting of the Chemical Society of Japan, Osaka, Apr 1977; The Chemical Society of Japan: Tokyo, 1977; No. II, p 1216. (b) Nakazaki, M.; Chikamatsu, H.; Nishino, M.; Murakami, H. *J. Org. Chem.*, following paper in this issue.

(6) (a) Nakazaki, M.; Chikamatsu, H.; Nishino, M.; Asao, M. "Abstracts of Papers", 41st Annual Meeting of the Chemical Society of Japan, Osaka, Apr 1980; The Chemical Society of Japan: Tokyo, 1977; No. II, p 1137. (b) Ref 5b.

(7) Relative configurations of the *trans*-ketol **9**, the *cis*-ketol **10**, the *trans,trans*-diol **11**, the *cis,trans*-diol **12**, and the *cis,cis*-diol **13**: (a) Cram, D. J.; Steinberg, H. *J. Am. Chem. Soc.* **1954**, *76*, 2753–7. (b) Hardegger, E.; Maeder, E.; Semarne, H. M.; Cram, D. J. *Ibid.* **1959**, *81*, 2729–37.

(8) Absolute configuration of the diketone **8**: (a) Gerlach, H. *Helv. Chim. Acta* **1968**, *51*, 1587–93. (b) Harada, N.; Ochiai, N.; Takeda, K.; Uda, H. *J. Chem. Soc., Chem. Commun.* **1977**, 495–7.

(9) For the absolute configuration of the *trans,trans*-diol **11** see ref 8a.

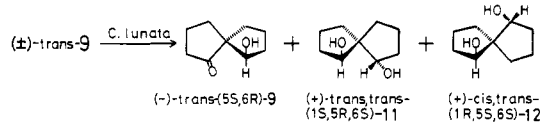
(10) Eu(facam)$_3$ = tris[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]europium(III).

(11) (a) McCreary, M. D.; Lewis, D. W.; Wernick, D. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1974**, *96*, 1038–54. (b) Goering, H. L.; Eikenberry, J. N.; Koermer, G. S.; Lattimer, C. J. *Ibid.* **1974**, *96*, 1493–501.

(12) (a) Acklin, W.; Prelog, V.; Schenker, F.; Serdarevic, B.; Walter, P. *Helv. Chim. Acta* **1965**, *48*, 1725–46. (b) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119–30. (c) Kieslich, K. "Microbial Transformations of Non-Steroid Cyclic compounds"; Georg Thieme Verlag: Stuttgart, 1976; p 24. (d) Perlman, D. "Applications of Biochemical Systems in Organic Chemistry"; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Part 1, p 71.

(13) Christol, H.; Duval, D.; Solladie, G. *Bull. Soc. Chim. Fr.* **1968**, 4151–6.

Scheme III



culture solution was a fairly complicated mixture containing, besides the recovered 8, two ketols and three diastereomeric diols (Figure 1), and this forced us to follow the process stepwise by interupting the incubation at a suitable stage.

Monitoring the process by means of GLC, we terminated the incubation after 8 h of shaking of the mixture at 30 °C when the culture solution was shown to contain a 34:30:36 mixture of the recovered ketone 8, the *trans*-ketol **9**, and the *cis*-ketol **10**.

The ethereal extract was chromatographed over silica gel, and, on elution with pentane–$CHCl_3$, the (-)-diketone 8 (10% yield, $[\alpha]_D$ -111.3°) came out first followed by the (-)-*cis*-ketol **10** (9.5% yield, $[\alpha]_D$ -1.6°) and the *trans*-ketol **9** (10% yield, $[\alpha]_D$ +99.2°) in that order.

While Jones oxidation[14] of both these ketols, (+)-*trans*-**9** and (-)-*cis*-**10**, back to the (+)-diketone **8** assigned their 5*R*,6*S* and 5*R*,6*R* configurations, adoption of Gerlach's maximal $[\alpha]_D$ -135°[15] reported for the (-)-diketone **8** as the absolute rotation allowed us to estimate the optical purity of metabolites: 82%, 76%, and 5.6% for (-)-**8**, (+)-*trans*-**9**, and (-)-*cis*-**10**, respectively.[17]

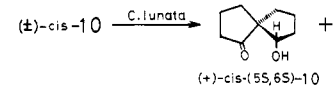
Inspection of Figure 2, which illustrates the four possible quadrant orientations for the enantiomers of the spirodiketone **8**, indicates that the (+)-*trans*-ketol **9** obtained with a high optical purity corresponds to the most favored C_1-1 orientation which is characterized by having the larger carbonyl flanking group in the right side (+*y* direction) and the other carbonyl group in the lower section. This preferential attack of (+)-(5*R*)-diketone **8** should account for the recovery of the enantiomeric (-)-diketone **8** with a remarkably high optical purity.

The newly discovered favorable effect of the polar group in the lower section, which will be encountered again in the microbial reduction of (±)-*trans*-ketol **9** (vide infra and Figure 3), seems to warn us that this effect ought to be taken into consideration with proper care in predicting the course of microbial reduction of substrates with a polar functional group close to the carbonyl reaction center.

A rather discouraging result found in a test incubation of (±)-**8** with *R. rubra* which furnished a mixture of all racemic **8**–**10** prevented us from pursuing further incubation experiments with this microbe.

It seems pertinent to point out here, besides these stereochemical interests, that this microbial reduction with *C. lunata* presents a convenient and practical single-step, research-scale method for preparing both enantiomers of the spirodiketone **8** with high optical purity.

Scheme IV



Microbial Reduction of (±)-*trans*-6-Hydroxyspiro[4.4]nonan-1-one (9, Scheme III). To trace further the metabolic pathway of the spirodiketone **8**, we next incubated the intermediate reduction product, the (±)-*trans*-ketol **9**, with *C. lunata* at 30 °C. The incubation was terminated after 72 h of shaking of the mixture when GLC monitoring indicated the formation of a 63:19:18 mixture of the *trans*-ketol **9**, the *trans,trans*-diol **11**, and the *cis,trans*-diol **12** in the culture solution, and column chromatography (SiO_2) of the ethereal extract afforded the (-)-*trans*-(5*S*,6*R*)-ketol **9** ($[\alpha]_D$ -73.5°), the (+)-*trans,trans*-(1*S*,5*R*,6*S*)-diol **11**[9] ($[\alpha]_D$ +48.5°), and the (+)-*cis,trans*-diol **12** ($[\alpha]_D$ +46.7°).

Since, among these metabolites, the absolute configuration of the *cis,trans*-diol **12** had been left unreported,[18] Jones oxidation of a specimen of this diol **12** ($[\alpha]_D$ +46.7°) was carried out to yield the diketone **8** ($[\alpha]_D$ -98.9°) and assign its 1*R*,5*S*,6*S* configuration as well as its 73% optical purity.[8,15,17]

This information together with the reported chiroptical properties of **9** and **11** indicates that the incubation of (±)-ketol **9** with *C. lunata* furnishes the (-)-*trans*-ketol **9** (18% yield), the (-)-*trans,trans*-diol **11** (6% yield), and the (-)-*cis,trans*-diol **12** (6% yield) with respective 56%,[19] 80%,[20] and 73% optical purities.[19]

Figure 3 illustrates the four quadrant orientations for (±)-*trans*-ketol **9**. Although the seemingly most favorable orientation C_1-1 with the polar hydroxyl group in the lower section would explain both the formation of the (+)-*trans,trans*-diol **11** with high optical purity and the recovery of the (-)-*trans*-ketol **9** with the enantiomeric molecular framework, isolation of the (+)-*cis,trans*-diol **12** in a comparable yield and optical purity seems to suggest that the C_1-1 superiority demonstrated in simple C_1 monoketones wanes in this case because of the unique stereochemistry of the spirane framework as well as a strong interference from a nearby hydroxyl group.

Microbial Reduction of (±)-*cis*-6-Hydroxyspiro[4.4]nonan-1-one (10, Scheme IV). The disturbing effect of a nearby hydroxyl group should be expected to be more serious in the *cis*-ketol **10** which has the hydroxyl group much closer to the carbonyl reaction center than does its *trans*-diastereomer **9**, and this conclusion was supported by recovery of the (+)-*cis*-ketol **10** (34% yield) with discouragingly low optical purity (17%)[19] from a culture solution of *C. lunata* incubated with (±)-**10** for 48 h at 30 °C.

Although examination of the diol fraction afforded a 10% yield of a product with $[\alpha]_D$ +21.3°, difficulties encountered in separating the diastereomeric diols **12** and **13** and observed isomerization of the *cis,cis*-diol **13** into the *cis,trans*-diol **12** during GLC analysis forced us to refrain from further exploration in this direction.

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

(15) Optical resolution of the (±)-spiroketone **8** has been reported from two laboratories. Gerlach[8a] obtained (-)-*trans,trans*-diol **11** through column chromatography of the diastereomeric bis[(-)-camphanates] prepared from the (±)-*trans,trans*-diol **11**, and oxidized the resulting (-)-*trans,trans*-diol **11** to a specimen of (-)-diketone **8** ($[\alpha]_D$ -135°) while Shingu and co-workers[16] secured a sample of the (+)-diketone **8** ($[\alpha]_D$ +135°) which they obtained through chromatography of the diastereomeric camphanates prepared from (±)-*cis*-ketol **10**. Their procedures of optical resolution involving chromatography, when coupled with their eventual preparation of the samples of the diketone **8** with same optical rotation via two different routes, should justify our adopting $[\alpha]_D$ -135° as the absolute rotation of (-)-**8**.

(16) Kuritani, H.; Iwata, F.; Sumiyoshi, M.; Shingu, K. *J. Chem. Soc., Chem. Commun.* **1977**, 542–3.

(17) These data automatically assign maximum rotations $[\alpha]_D$ +130.5°, -28.6°, and +64° for (+)-*trans*-ketol **9**, (-)-*cis*-ketol **10**, and (+)-*cis,trans*-diol **12**, respectively.

(18) Harada et al.[8b] determined the absolute configuration of the (-)-bis[*p*-(dimethylamino)benzoate] of the *cis,trans*-diol **12**, but the specific rotation of the diol **12** itself was not reported.

(19) Calculated from our reported maximum rotation.[17]

(20) Calculated from the reported maximum rotation $[\alpha]_D$ +61° (*c* 0.3, EtOH) for (+)-**11**.[21]

(21) Gerlach, H.; Müller, W. *Helv. Chim. Acta* **1972**, *55*, 2277–86.

Experimental Section[22]

Our general procedure for microbial incubation and extraction of the metabolites has been described elsewhere.[3b] The cultures of *Curvularia lunata* and *Rhodotorula rubra* used in following experiments were obtained from the Institute of Fermentation, Osaka, Japan, and were identified by their IFO catalog numbers, IFO 6288 and IFO 0889, respectively.

Microbial Reduction of 1-Spiro[4.4]nonanone (14). The substrate ketone **14** was prepared by the method of Cram et al.[7a]: bp 90–92 °C (23 mm); n^{25}_D 1.4742 [lit.[7a] bp 90 °C (22 mm); n^{25}_D 1.4737].

(a) Incubation with *C. lunata*. A total of 1 g of the ketone **14** was incubated at 30 °C for 24 h in eight batches (8 × 200 mL of culture media). The metabolite mixture (1.05 g) containing **14** and **15** in a ratio of 49:51 (GLC analysis) was taken up in *n*-pentane and chromatographed on 20 g of alumina.

Elution with *n*-pentane gave 350 mg of the recovered ketone **14**, bp 115 °C (31 mm). Further elution with *n*-pentane–ether (10:1) afforded 331 mg (33% yield) of (+)-1-spiro[4.4]nonanol (**15**): bp 120 °C (25 mm); $[\alpha]^{22}_D$ +39.8° (*c* 1.5, benzene); optical purity 100% [lit.[13] $[\alpha]^{25}_D$ +32.0° (*c* 0.507, benzene)].

Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 76.37; H, 11.53.

(b) Incubation with *R. rubra*. A total of 500 mg of the ketone **14** was incubated at 30 °C for 48 h in four batches (4 × 200 mL of culture media). The crude metablite mixture (480 mg) containing **14** and **15** in a ratio of 62:38 was chromatographed on alumina to give 160 mg of the recovered ketone **14** and 87 mg (17.4% yield) of (+)-**15**: bp 95–100 °C (23 mm); $[\alpha]^{23}_D$ +33.0° (*c* 0.705, benzene); optical purity 83%.

(c) Purification of (+)-Alcohol 15 via Hydrogen Phthalate 16. A mixture of (+)-alcohol **15** (200 mg, $[\alpha]^{22}_D$ +39.8°) and phthalic anhydride (250 mg) in 3 mL of pyridine was heated at 100 °C for 4 h. The solution was cooled and diluted with dilute H_2SO_4 to give a crystalline material which was collected by filtration and washed with water. The crude phthalate **16** [404 mg; mp 113–114 °C; $[\alpha]^{20}_D$ +102° (*c* 0.42, EtOH)] was recrystallized from MeOH to give 366 mg of phthalate **16** [mp 115–116 °C; $[\alpha]^{24}_D$ +107° (*c* 0.77, EtOH)] which was unchanged by further recrystallization [lit.[13] mp 115 °C; $[\alpha]^{25}_D$ +107° (*c* 0.521, EtOH)].

Anal. Calcd for $C_{17}H_{20}O_4$: C, 70.81; H, 6.99. Found: C, 70.85; H, 6.90.

A solution of hydrogen phthalate (340 mg, $[\alpha]^{20}_D$ +107°) in absolute ether (20 mL) was added dropwise to a suspension of $LiAlH_4$ (380 mg) in absolute ether (50 mL) and stirred at room temperature for 7 h. After decomposition of the reaction mixture with water, the ether layer was separated, washed with dilute Na_2CO_3, dried ($MgSO_4$), and then concentrated. The residual oil was taken up in *n*-pentane and chromatographed on 10 g of alumina. Elution with *n*-pentane–ether (10:1) afforded the crude alcohol which was distilled to give 113 mg of (+)-**15**: bp 120 °C (32 mm); $[\alpha]^{25}_D$ +39.6° (*c* 0.95, benzene).

Anal. Calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 76.43; H, 11.42.

Microbial Reduction of 1,6-Spiro[4.4]nonanedione (8). The racemic substrate diketone **8** was prepared by the method of Gerlach et al.;[21] mp 39–42 °C (lit. mp 39–41 °C,[21] mp 37–37.5 °C[7b]).

(a) Incubation with *C. lunata*. The racemic diketone **8** (1 g) was incubated in eight batches of *C. lunata* culture (8 × 200 mL) for 8 h at 30 °C. GLC monitoring of the metabolite extract indicated a 34:30:36 ratio of the diketone **8**, the *trans*-ketol **9**, and the *cis*-ketol **10**.

The crude extract (870 mg) was taken up in *n*-pentane and chromatographed on 17 g of silica gel. Elution with *n*-pentane–$CHCl_3$ (1:1) gave diketone fractions followed by *cis*-ketol fractions, and final elution with $CHCl_3$ afforded *trans*-ketol fractions.

The crude diketone (165 mg) was purified by preparative TLC (developed with $CHCl_3$) followed by sublimation in vacuo [55 °C (4 mm)] to give (–)-1,6-spiro[4.4]nonanedione (**8**): 100 mg (10% yield); mp 58–62 °C; $[\alpha]^{30}_D$ –111.3° (*c* 0.76, cyclohexane); optical purity 82% [lit.[8a] $[\alpha]_D$ –135° (*c* 2.2, cyclohexane)].

Anal. Calcd for $C_9H_{12}O_2$: C, 71.02; H, 7.95. Found: C, 71.05; H, 8.03.

The crude *trans*-ketol (135 mg) was purified by preparative TLC (developed with $CHCl_3$) followed by distillation to afford (+)-*trans*-6-hydroxyspiro[4.4]nonan-1-one (**9**): 102 mg (10% yield); bp 125 °C (4 mm); n^{24}_D 1.4963; $[\alpha]^{33}_D$ +99.2° (*c* 0.99, EtOH); optical purity 76% [lit.[23] (±)-**9** bp 97–100 °C (0.6 mm); n^{20}_D 1.4999].

Anal. Calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.07; H, 9.29.

p-Nitrobenzoate of (+)-**9**: mp 89–90 °C (from EtOH–H_2O); $[\alpha]^{29}_D$ +157.6° (*c* 0.97, $CHCl_3$) (lit. (±)-*p*-nitrobenzoate mp 91.5–92 °C,[7b] 89–91 °C[23]).

Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.43; H, 5.58; N, 4.71.

The crude *cis*-ketol (175 mg) was purified by preparative TLC (developed with $CHCl_3$) followed by distillation to give (–)-*cis*-6-hydroxyspiro[4.4]nonan-1-one (**10**): 97 mg (9.5% yield); bp 95 °C (4 mm); n^{24}_D 1.4940; $[\alpha]^{30}_D$ –1.6° (*c* 0.539, EtOH); optical purity 5.6% [lit.[23] (±)-**10** bp 80–82 °C (0.8 mm); n^{26}_D 1.4895].

Anal. Calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.22; H, 9.34.

p-Nitrobenzoate of (–)-**10**: mp 76–77 °C (from EtOH–H_2O); $[\alpha]^{29}_D$ 0° (*c* 2.2, $CHCl_3$) [lit. (±)-*p*-nitrobenzoate mp 86.5–87 °C;[7b] 75–77 °C[23]].

Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.44; H, 5.59; N, 4.65.

(b) Jones Oxidation of the (+)-*trans*-Ketol 9. The (+)-*trans*-ketol **9** (55 mg, $[\alpha]^{33}_D$ +99.2°) was dissolved in 3 mL of acetone and treated with 0.5 mL of 8 N Jones reagent[14] at 0 °C. The routine work up gave a crystalline product which was sublimed in vacuo [55 °C (5 mm)] to afford (+)-1,6-spiro[4.4]nonanedione (**8**): 37 mg; mp 57–61.5 °C; $[\alpha]^{23}_D$ +102.7° (*c* 0.75, cyclohexane); optical purity 76%.

Anal. Calcd for $C_9H_{12}O_2$: C, 71.02; H, 7.95. Found: C, 71.09; H, 7.97.

(c) Jones Oxidation of the (–)-*cis*-Ketol 10. Oxidation of the *cis*-ketol **10** (18 mg, $[\alpha]^{30}_D$ –1.6°) in the same way as described above afforded 11.5 mg of the (+)-diketone **8**: mp 39–42 °C; $[\alpha]^{26}_D$ +7.6° (*c* 0.49, cyclohexane); optical purity 5.6%.

Anal. Calcd for $C_9H_{12}O_2$: C, 71.02; H, 7.95. Found: C, 71.12; H, 7.96.

Microbial Reduction of (+)-*trans*-6-Hydroxyspiro[4.4]-nonan-1-one (9). (a) Preparation of the Racemic Substrate Ketol 9. A 78:23 mixture[7a] (8.3 g) of the (±)-*trans*-ketol **9** and the (±)-*cis*-ketol **10** was chromatographed on silica gel, and elution with *n*-hexane–$CHCl_3$ (1:1) afforded the *trans*-ketol **9** in slow-moving fractions. The crude *trans*-ketol **9** (5.9 g), whose GLC indicated 6% contamination from the *cis*-ketol **10**, was fractionally distilled in vacuo to give a specimen (2.5 g) with a trace of the *cis*-ketol **10**: bp 96–96.5 °C (0.3 mm); n^{14}_D 1.4960 [lit.[23] bp 97–100 °C (0.6 mm); n^{20}_D 1.4999].

(b) Incubation with *C. lunata*. A total of 2 g of the racemic substrate **9** was incubated at 30 °C for 72 h in 16 batches (16 × 200 mL of culture media). The crude metabolite mixture (1.43 g) containing the *trans*-ketol **9**, the *trans,trans*-diol **11**, and *cis,trans*-diol **12** in a ratio of 63:19:18 (GLC analysis) was dissolved in *n*-hexane–$CHCl_3$ (2:1) and chromatographed on 20 g of silica gel.

Elution with 1200 mL of $CHCl_3$ afforded 465 mg of the ketol which was distilled to give the (–)-*trans*-ketol **9**: 359 mg (18% yield); bp 120–140 °C (20 mm); $[\alpha]^{20}_D$ –73.5° (*c* 1.19, EtOH); optical purity 56%.

Anal. Calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.25, H, 9.34.

p-Nitrobenzoate of (–)-**9**: mp 90–91 °C (from EtOH–H_2O); $[\alpha]^{20}_D$ –73.5° (*c* 1.19, EtOH).

Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.39; H, 5.63; N, 4.57.

(22) Melting points are uncorrected. Optical rotations were measured with a JASCO DIP-SL polarimer. GLC analyses were performed on a JGC-20K equipped with a FID and using 2 m × 3 mm i.d. columns of 10% Carbowax 20M on Chromosorb W and 15% silicone DC QF-1 on Uniport B. Preparative TLCs were carried out with Merck silica gel 60 $PF_{254+366}$. Woelm active alumina (neutral, activity III) and Merck silica gel 60 (70–230 mesh) were used for column chromatography.

(23) Weinges, K.; Bähr, W.; Rao, M. P. *Justus Liebigs Ann. Chem.* **1971**, *753*, 100–5.

Further elution with 800 mL of $CHCl_3$–MeOH (100:1) gave the crude *cis,trans*-diol **12** followed by the *trans,trans*-diol **11**.

The crude *cis,trans*-diol **12** (210 mg) was purified by preparative TLC [developed with $CHCl_3$–MeOH (100:3)] followed by distillation to afford the (+)-*cis,trans*-diol **12**: 110 mg (5.5% yield); bp 120–140 °C (10 mm); $[\alpha]^{20}_D$ +46.7° (*c* 0.75, EtOH); optical purity 73% [lit.[7b] (±)-**12** mp 43–43.5 °C].

Anal. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 68.74; H, 10.38.

Bis(*p*-nitrobenzoate) of (+)-**12**, mp 183–184 °C (from AcOEt–EtOH) [lit.[7b] (±)-bis(*p*-nitrobenzoate) mp 192–192.5 °C).

Anal. Calcd for $C_{23}H_{22}N_2O_8$: C, 60.79; H, 4.88; N, 6.17. Found: C, 60.89; H, 4.85; N, 6.06.

The crude *trans,trans*-diol **11** (120 mg) was sublimed in vacuo [95 °C (0.08 mm)] to give the (+)-*trans,trans*-doil **11**: 110 mg (5.5% yield); mp 126–129 °C; $[\alpha]^{20}_D$ +48.5° (*c* 0.54, EtOH); optical purity 80% [lit. mp 133–134 °C, $[\alpha]_D$ –53° (*c* 0.3, EtOH);[5a] mp 131–132 °C, $[\alpha]_D$ +61° (*c* 0.3, EtOH)[21]].

Anal. Calcd for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 68.91; H, 10.11.

(c) Jones Oxidation of the (+)-*cis,trans*-Diol 12. The (+)-*cis,trans*-diol **12** (35.5 mg) was dissolved in 5 mL of acetone and treated with 0.4 mL of 8 N Jones reagent at 0 °C. The crude product was sublimed in vacuo [50 °C (5 mm)] to afford 12 mg of the (–)-diketone **8**: mp 59–61 °C; $[\alpha]^{20}_D$ –98.9° (*c* 0.33, cyclohexane); optical purity 73%.

Anal. Calcd for $C_9H_{12}O_2$: C, 71.02; H, 7.95. Found: C, 71.01; H, 7.85.

Microbial Reduction of (±)-*cis*-6-Hydroxyspiro[4.4]nonan-1-one (10) with *C. lunata*. The racemic ketol **10** was prepared by the method of Carruthers et al.:[24] bp 115 °C (7 mm); n^{24}_D 1.4928 [lit.[24] bp 112 °C (0.05 mm)].

A total of 1 g of the racemic ketol **10** was incubated at 30 °C for 48 h in eight batches (8 × 200 mL of culture media). GLC of the crude metabolite extract (720 mg) indicated its constitution containing the recovered ketol **10** and diols **12** plus **13** in a ratio of 72:28.

The mixture was taken up in *n*-hexane–$CHCl_3$ (2:1) and chromatographed on 25 g of silica gel. Elution with 150 mL of $CHCl_3$ afforded the (+)-*cis*-ketol **10**: 335 mg (34% yield); bp 110 °C (4 mm); $[\alpha]^{26}_D$ +4.8° (*c* 1.5, EtOH); optical purity 17%.[19]

Anal. Calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.07; H, 9.29.

p-Nitrobenzoate of (+)-**10**, mp 77–78 °C (from EtOH–H_2O).

Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.36; H, 5.65; N, 4.62. Found: C, 63.31; H, 5.61; N, 4.68.

Further elution with 200 mL of $CHCl_3$ afforded 107 mg of a mixture of the *cis,trans*-diol **12** and *cis,cis*-diol **13**: bp 130 °C (4 mm); $[\alpha]^{27}_D$ +21.3° (*c* 0.85, EtOH).

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Registry No. (±)-**8**, 39746-33-3; (–)-**8**, 21932-23-0; (+)-**8**, 36551-90-3; (±)-*trans*-**9**, 76215-54-8; (–)-*trans*-**9**, 76248-64-1; (–)-*trans*-**9** *p*-nitrobenzoate, 76248-65-2; (+)-*trans*-**9**, 76248-66-3; (+)-*trans*-**9** *p*-nitrobenzoate, 76248-67-4; (±)-*cis*-**10**, 65427-11-4; (–)-*cis*-**10**, 76248-68-5; (–)-*cis*-**10** *p*-nitrobenzoate, 76248-69-6; (+)-*cis*-**10**, 76248-70-9; (+)-*cis*-**10** *p*-nitrobenzoate, 76248-71-0; (+)-*trans,trans*-**11**, 39746-37-7; (+)-*cis,trans*-**12**, 65167-79-5; (+)-*cis,trans*-**12** bis(*p*-nitrobenzoate), 76232-16-1; *cis,cis*-**13**, 76318-77-9; **14**, 14727-58-3; (+)-**15**, 21945-22-2; (+)-**16**, 21945-21-1.

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Microbial Stereodifferentiating Reduction of 2,6-Adamantanedione and Hexahydrodibenzoheptalene-5,11-dione, Diketones with Two Homotopic Carbonyl Groups on a C_2 Symmetry Axis

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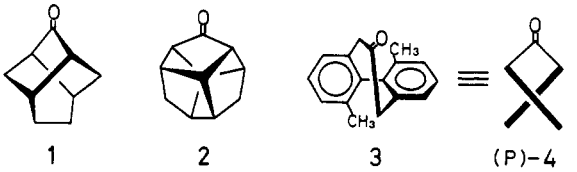
The microbial stereodifferentiating aptitude toward diketones with two homotopic carbonyl groups on a C_2 symmetry axis was studied. Incubation of 2,6-adamantanedione (**6**) with *Curvularia lunata* yielded, via the ketol **10**, (–)-(*R*)-2,6-adamantanediol (**11**), and incubation with *Rhodotorula rubra* converted the (±) doubly bridged biphenyl diketone **7** into a mixture of the recovered (+)-(*R*)-diketone **7**, the (+)-(*R*)-ketol **18**, and the (–)-(*S*)-*cis*-diol **19**. These stereoselectivities were analyzed to test the proposed "C_2-ketone rule".

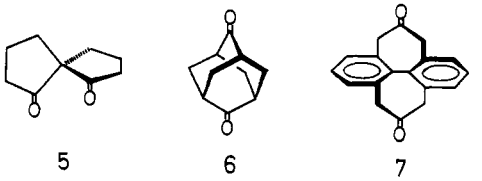
Analysis of the stereodifferentiating aptitude of *Curvularia lunata* and *Rhodotorula rubra* toward various C_2 ketones[1] [e.g., 9-*twist*-brendanone (**1**), 2-trishomocubanone (**2**), and the bridged biphenyl ketone **3**, Chart I] has led us to summarize their enantiomer selectivity in a "C_2-ketone rule"[2] which states that these microbes preferentially reduce the enantiomer with *P* helicity[3] (Figure 1).

Chart I



1 2 3 (P)-4

Chart II



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These findings together with our continuing interests in the stereochemistry of gyrochiral molecules[4] prompted

(1) 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.

(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) As the quadrant projection formulas (Figure 1) indicate, the enantiomer with *P* helicity corresponds to that possessing the larger parts of molecule in the upper right and lower left quadrants.

0022-3263/81/1946-1151$01.25/0