K. Fukumoto, *J. Am. Chem. Soc.*, **99**, 3461 (1977).
(22) T. Kametani, M. Kajiwara, and K. Fukumoto, *Tetrahedron*, **30**, 1053 (1974).
(23) T. Kametani, Y. Hirai, Y. Shiratori, K. Fukumoto, and F. Satoh, *J. Am. Chem. Soc.*, **100**, 554 (1978).
(24) K. L. Williamson, T. Howell, and T. A. Spencer, *J. Am. Chem. Soc.*, **88**, 325 (1966).
(25) D. K. M. Duc, M. Fetizon, and J.-P. Flament, *Chem. Commun.*, 886 (1972); *Tetrahedron*, **31**, 1897 (1975).
(26) G. Stork, A. Brizzolara, H. Landesman, J. Szmuskovicz, and R. Terrell, *J. Am. Chem. Soc.*, **85**, 207 (1963).
(27) C. Djerassi, R. R. Engle, and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

Microbial Stereodifferentiating Reduction in [2.2]Metacyclophane Derivatives[1,2]

Masao Nakazaki,* Hiroaki Chikamatsu, Yoshiki Hirose, and Toru Shimizu

Department of Chemistry, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka, 560 Japan

Received September 18, 1978

Incubation of (±)-1-oxo[2.2]metacyclophane (**9**) with *Rhodotorula rubra* gave a mixture of (+)-axial alcohol **10** and (−)-equatorial alcohol **11** both with remarkably high optical purity. The same high stereoselectivity was observed when *R. rubra* was incubated with 1,10-dioxo[2.2]metacyclophane (**12**), which was converted into (−)-axial-equatorial diol **14** via (−)-axial ketol **13**. *R. rubra* was also found to reduce (±)-[2.2]metacyclophane-4-aldehyde (**20**), affording a 13% yield of the (+)-4-hydroxymethyl derivative **21** with 11.7% optical purity.

We[1-3] have been studying the microbial reduction of bridged cyclic ketones with a constrained carbonyl group in a wide variation of molecular frameworks (**1–8**, Chart I); common features among these are a conformationally rigid structure as well as the absence of a cyclohexanone moiety fixed in a chair conformation. The latter feature seems to make these substrates particularly conspicuous among numerous cyclic ketones whose microbial reductions have been well documented.[4-7] Our study on the stereochemistry of their metabolites coupled with our observation of the marked enantiomeric selectivity exhibited by *Curvularia lunata* and *Rhodotorula rubra* toward gyrochiral[8] ketones with a carbonyl group located on the C_2 axis led us to propose a quadrant rule which predicts the stereochemistry of the metabolites, eventually providing information on the absolute configurations of their molecular frameworks.

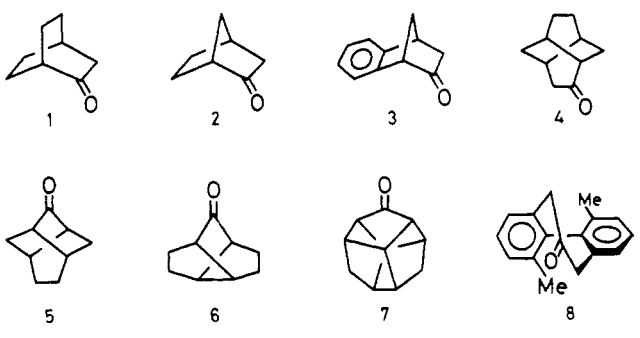
Among a number of C_2-ketones[9] studied in our laboratory,[3] the atropisomeric C_2-biphenyl ketone **8** will be noteworthy in two respects: (a) in contrast to the extremely high enantiomeric selectivity (90–100%) exhibited by *R. rubra, C. lunata* showed almost no enantiomeric selectivity toward this ketone **8**; and (b) this is the first axially chiral cyclic ketone on which microbiological reduction has ever been carried out.

These results prompted us to investigate the microbiological reduction of carbonyl compounds with planar chirality, and this paper describes microbial stereodifferentiating reduction of (±)-1-oxo[2.2]metacyclophane (**9**), 1,10-dioxo[2.2]metacyclophane (**12**), and (±)-[2.2]metacyclophane-4-aldehyde (**20**) with *R. rubra* and *Rhizopus arrhizus*.

Microbial Reduction of (±)-1-Oxo[2.2]metacyclophane (9) (Figure 1). Being a racemic ketone with C_1 symmetry, (±)-1-oxo[2.2]metacyclophane (**9**) (belongs to the C_1-ketone[9]) has four stereochemically distinguishable faces around the carbonyl plane, two for each enantiomer.

Corresponding to these faces, there arise four quadrant orientations,[2] C_1-1, C_1-2, C_1-3, and C_1-4 (Figure 1), for the racemic [2.2]metacyclophane ketone **9**, and the quadrant rule[2] tells us that *C. lunata* and *R. rubra* should favor C_1-1 orientation followed by C_1-4. Distinction between these two orientations is that while both have the larger carbonyl flanking groups (L) on the right side (+y direction), C_1-1 has the smaller part of the molecule in the lower quadrant, whereas C_1-4 has the larger part of the molecule in this lower section.

Chart I



Upon hydrogen delivery from the lower sections, C_1-1 and C_1-4 orientations are expected to furnish the diastereoisomeric 1-hydroxy[2.2]metacyclophanes **10** and **11**, respectively; the former possesses the *pR*,1*S* configuration with an axial hydroxyl group,[10] while the latter has the *pS*,1*S* configuration with the hydroxyl group in an equatorial orientation.[10]

This analysis can be summarized to predict the following: (a) the metabolite alcohol with an axial hydroxyl group must have the *pR*,1*S* configuration, while the metabolite with an equatorial hydroxyl group must have the *pS*,1*S* configuration; (b) since C_1-1 orientation is favored over C_1-4 orientation, the axial alcohol **10** will be the major reduction product to be isolated from the culture solution when incubation is terminated at the point where about 50% of the starting material is reduced; and (c) the recovered ketone will have the *pS* configuration corresponding to the unfavored orientations C_1-3 and C_1-4.

Although preliminary incubation tests on a small scale indicated that *Aspergillus tamarii, Fusarium solani, Rhizopus nigricans, Rhizopus formosaensis, Mucor javanicus, Curvularia lunata, Rhodotorula rubra,* and *Rhizopus arrhizus* were all capable of reducing the racemic ketone **9**, preparative scale incubations were conveniently carried out with *Rhodotorula rubra* and *Rhizopus arrhizus*.

Reduction with *Rhodotorula rubra*. After a small scale trial incubation in which *R. rubra* was observed to reduce the (±)-ketone **9** completely into a mixture of diastereomeric alcohols within 15 min at 30 °C, 300 mg of the (±)-ketone **9** was incubated with *R. rubra* in 20 batches of 25 mL of culture medium for 45 h at 30 °C. Monitoring the process with silica

0022-3263/79/1944-1043$01.00/0

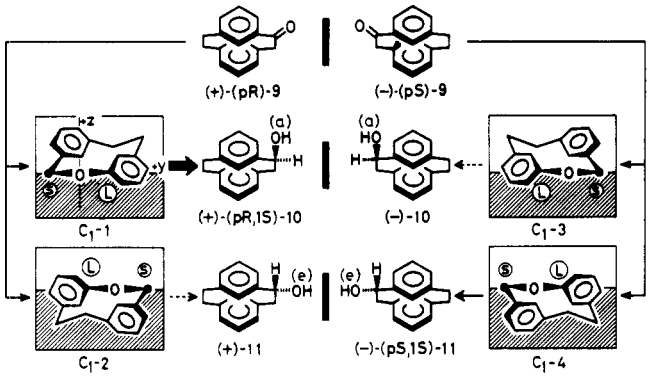


Figure 1. Schematic representation of the four quadrant orientations for (±)-1-oxo[2.2]metacyclophane (**9**). Substrate ketone molecules are orientated in a three-dimensional system with the carbonyl plane on the *xy* plane, the carbonyl axis coincident with the *x* axis, and the carbonyl oxygen pointing toward +*x* direction. *Curvularia lunata* and *Rhodotorula rubra* favor C_1-1 orientation with the larger carbonyl flanking group located on the +*y* side and the smaller part of molecule in the lower quadrants, followed by C_1-4 orientation with the larger carbonyl flanking group on the +*y* side but the larger part of molecule in the lower quadrant sections. Hydrogen delivery from the lower quadrants furnishes metabolite alcohols.

gel TLC revealed, after 30 h of incubation, instead of complete disappearance of the starting material, formation of a 1:1 mixture of two diastereomeric alcohols.

Preparative TLC led to separation of these alcohols to give the crude (+)-axial alcohol **10,** which was purified by sublimation in vacuo,[12] mp 138.5–139.5 °C, $[\alpha]^{26}_D$ +24.5° (optical purity 94%[13]), and the crude (−)-equatorial alcohol **11,** whose sublimation in vacuo yielded a sample melting at 150.5–151 °C, $[\alpha]^{26}_D$ −125.7° (optical purity 100%[13]).

Gschwend's study[11] on the optically active axial alcohol **10** and equatorial alcohol **11** combined with the X-ray crystallographic analysis carried out at CIBA-GEIGY[14] indicates the *pR*,1*S* and *pS*,1*S* configurations for our (+)-axial (**10**) and (−)-equatorial 1-hydroxy[2.2]metacyclophanes (**11**), respectively, in complete agreement with our prediction from the quadrant rule.

Reduction with *Rhizopus arrhizus*. Several exploratory experiments with *Rhizopus arrhizus* suggested that this microbe reduced the racemic ketone **9** with a moderate rate, and this prompted us to seek the optically active ketone **9** to be recovered from the incubation mixture when incubation was terminated at the point where about 50% of starting material had been consumed.

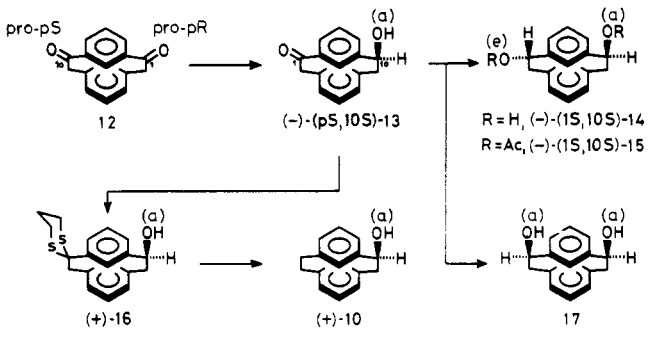
A "resting cell suspension" of *Rhizopus arrhizus* was incubated with 344 mg of the racemic ketone **9** in 28 batches of 25 mL of culture medium at 30 °C, and the incubation was terminated after 44 h, when TLC monitoring indicated about 40% of the starting ketone had been reduced.

Preparative TLC separated the recovered ketone and the diastereomeric alcohols, affording the (−)-ketone **9** (37% yield) as an oil with $[\alpha]^{26}_D$ −98.3° (optical purity 22%[13]), the crude (+)-axial alcohol **10,** which was sublimed in vacuo to melt at 137–138 °C, $[\alpha]^{26}_D$ +24.97° (optical purity 96%) (8.5% yield), and the crude (−)-equatorial alcohol **11,** whose purification via sublimation yielded a sample melting at 148–149 °C, $[\alpha]^{26}_D$ −110.3° (optical purity 88%) (1.1% yield).

Although its optical purity was found to be rather low,[15] the recovered (−)-ketone **9** with the *pS* configuration[14] again supports our prediction based on the quadrant rule.

Optical purities of the diastereomeric alcohols **10** and **11** combined with their yields enabled us to calculate their approximate relative rates of formation: (+)-**10**/(−)-**10**/(+)-**11**/(−)-**11** = 49:1:0.4:6. This relation again seems to support the quadrant rule, confirming that (a) the C_1-1 quadrant orientation is most favored followed by the C_1-4 orientation and (b) the (−)-ketone **9** with the *pS* configuration is to be recovered from the halfway terminated incubation mixture.

Scheme I



In contrast to the classical techniques for resolving the racemic ketone **9** indirectly,[11] which are tedious and require the expenditure of a large amount of time and materials, the microbial reduction approach discussed above is direct and conveniently carried out in laboratory scale, rendering this method to be the quickest research-scale method of preparing these 1-oxygenated [2.2]metacyclophanes of high enantiomeric and optical purity.

Microbial Reduction of 1,10-Dioxo[2.2]metacyclophane (12) (Scheme I). Belonging to C_s symmetry, 1,10-dioxo[2.2]metacyclophane (**12**) is composed of the enantiomeric molecular moieties, each corresponding to (+)-ketone **9** and (−)-ketone **9,** respectively.

This stereochemical characteristic about the diketone **12** coupled with the stereochemistry of the microbial reduction products of the racemic ketone **9** discussed above allows us to predict the sequence of the microbial reduction of the diketone **12** by *R. rubra* as well as the stereochemistry of its metabolites.

Since the *pro-pR* carbonyl group[16] of the diketone **12** corresponds to the carbonyl part of the (+)-ketone **9,** this *pro-pR* carbonyl group is expected to be preferentially reduced by the microbe to furnish the axial ketol **13** with the *pS*,10*S* configuration. Then reduction of the remaining carbonyl group corresponding to the (−)-ketone **9** will come next, providing the (1*S*,10*S*)-axial-equatorial diol[19] **14** as the final product.

These predictions were confirmed by isolation of the reduction products and elucidation of their stereochemistry to be discussed below.

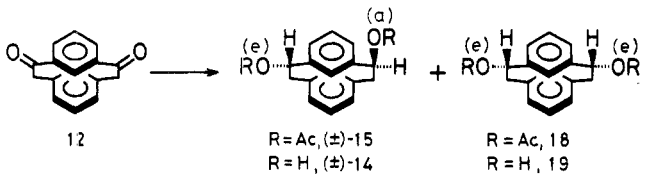
Test-scale exploratory incubations with *R. rubra* were carried out, and TLC monitoring of the reaction process revealed that a 10-h incubation at 30 °C gave a 6:1 mixture of the ketol **13** and the diol **14.** After a 24-h incubation this ratio became 2.3:1, and the diol **14** was the sole metabolite to be isolated from a 48-h incubation mixture.

R. rubra was grown at 30 °C for 48 h in eight batches of 200 mL of culture medium, eight aliquots of the meso diketone **12** (1.04 g) dissolved in 40 mL of ethanol were added to the culture solutions, and the incubation was continued for 10 h at 30 °C on a shaker. Preparative TLC of the reaction mixture afforded the (−)-ketol **13** (54.6% yield), mp 151–152.5 °C, $[\alpha]^{28}_D$ −400.5°, and the (−)-diol **14** (11.3% yield), mp 178.5–179.5 °C, $[\alpha]^{28}_D$ −87.6°,[12] both with practically 100% optical purity.[13]

(−)-(*pS*,10*S*)-10-Hydroxy-1-oxo[2.2]metacyclophane (13). The *pS*,10*S* configuration was assigned to the (−)-ketol **13** on the basis of the following observations.

The (−) Cotton effect exhibited by the (−)-ketol **13** (λ_{max} 321 nm, $[\theta]$ −3.30 × 10⁴) can be compared with the (−) Cotton effect of the (−)-ketone **9**[14] (λ_{max} 318 nm, $[\theta]$ −3.67 × 10⁴) due

Scheme II



to n → π* transition, indicating the *pS* chirality for the (−)-ketol **13** with the *pro-pS* carbonyl group of the starting diketone **12** remaining intact during the earlier stage of the microbial reduction.

Information on the configuration around the C-10 asymmetric center came from an inspection of the NMR spectrum of **13**, which showed the C-10 proton signal as a triplet centered at δ 5.18 (J = 3 Hz). Comparison of this with the spectra of the (+)-axial alcohol **10** and the (−)-equatorial alcohol **11**, each showing distinct peaks due to the C-1 proton at δ 5.24 (triplet, J = 3 Hz) and 4.28 (double doublet, J = 4 and 10 Hz), respectively, clearly assigns the axial conformation to the C-10 hydroxyl group of the ketol **13**.

This view was conclusively supported by the formation of the (+)-axial alcohol **10**, mp 139–139.5 °C, $[\alpha]^{31}_D$ +26.15°, on Raney nickel desulfurization of the (+)-dithioacetal alcohol **16**, $[\alpha]^{32}_D$ +16.8°, prepared from the (−)-ketol **13** with 1,3-propanedithiol and boron trifluoride etherate.

(−)-(1*S*,10*S*)-1,10-Dihydroxy[2.2]metacyclophane (14). Optical activity observed in metabolite diol **14** obviously excludes symmetrical structures with axial-axial[19] **17** or equatorial-equatorial[19] **19** configuration from its candidates, leaving the axial-equatorial[19] diol as its possible structure.

This asymmetric character demonstrates itself in its NMR spectrum, exhibiting C-1 and C-10 protons signals at δ 5.25 and 4.22, respectively,[20] as well as in the NMR spectrum of the (−)-diacetate **15**, mp 87.5–88.5 °C, $[\alpha]^{28}_D$ −91.0°, which shows two methyl signals discretely at δ 1.94 and 2.16. Although this conclusion was confirmed by comparison of their NMR spectra with those of their racemic modifications prepared by $LiAlH_4$ reduction of the diketone **12**[21] (Scheme II), conclusive evidence proving the 1*S*,10*S* configuration was to be found in $LiAlH_4$ reduction of the intermediate metabolite (−)-(*pS*,10*S*)-ketol **13**. Preparative TLC of the reaction mixture yielded, besides the axial-axial diol **17** (24% yield), mp 169–170 °C, the (−)-diol **14** (55% yield), mp 177–178.5 °C, $[\alpha]^{28}_D$ −83.3°.

The stereochemistry of these metabolites of the meso diketone **12** not only confirmed our prediction, but also presented the first established example of microbial enantioselectivity with respect to the plane of prochirality. It may also be worthy to note that the (−)-diol **14** with 1*S*,10*S* configuration can be looked at as a composite structure made up of units of (+)-**10** and (−)-**11**, reflecting the meso structure of 1,10-dioxo[2.2]metacyclophane (**12**).

Microbial Reduction of (±)-[2.2]Metacyclophane-4-aldehyde (20) (Scheme III). Reduction of (±)-[2.2]metacyclophane-4-aldehyde (**20**) with *R. rubra* demonstrated another microbial stereodifferentiation among enantiomers with planar chirality.

The (±)-aldehyde **20** was incubated with *R. rubra* for 48 h at 30 °C, and preparative TLC of the reduction products afforded a 13% yield of (+)-4-(hydroxymethyl)[2.2]metacyclophane (**21**), mp 82–83 °C, $[\alpha]^{28}_D$ +2.93° (ethanol) (optical purity 11.7%[22]).

Contrary to the ketones whose carbonyl groups are constrained in conformationally rigid molecular frameworks, application of the quadrant rule to the enantiomers **20** requires information on the most probable conformation around the aldehyde group.

Scheme III

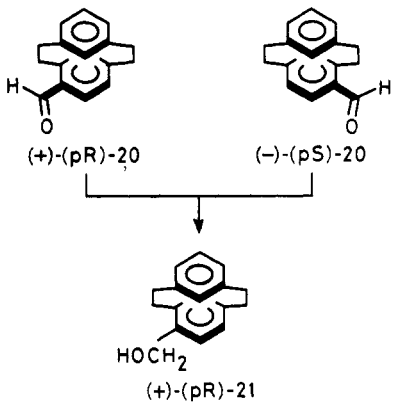
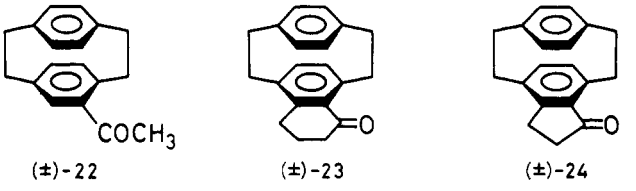


Chart II



An inspection of the molecular model indicates that the most comfortable conformation would have the carbonyl oxygen atom as remote as possible from the nearest [2.2] bridges, and at the same time would have the carbonyl plane coplanar with the plane of the adjacent benzene ring.

The (*pR*)-aldehyde **20** with this conformation can assume the most favorable C_1-1 quadrant orientation (Figure 1), eventually yielding the 4-hydroxymethyl derivative **21** enriched in the (*pR*) enantiomer.

These reasonings automatically assign the *pR* configuration both to the (+)-alcohol **21** and its precursor, the (+)-aldehyde **20**.

Incidentally, this assignment is found to be opposite to the one proposed by Schlögl.[23] We have additional experimental evidence which supports our present view by correlating 1-oxygenated [2.2]metacyclophanes with known absolute configurations with optically active [2.2]metacyclophane-4-carboxylic acid, and this will be the subject of our coming paper.

It seems pertinent to note here our so far sterile attempts to find microorganisms capable of reducing the carbonyl groups of various [2.2]paracyclophane derivatives (**22**,[24] **23**,[25] and **24**,[26] Chart II).

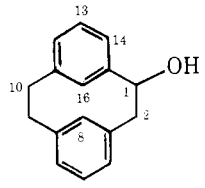
We have tentatively attributed this failure to the expected disturbing effect of their [2.2] bridges extruding in the front quadrants (Figure 1).

Optical Purities and Absolute Rotations of 1- and 1,10-Oxygenated [2.2]Metacyclophanes. Table I summarizes the enantiomers differential shifts (ΔΔδ) observed in the (±)-axial **10**, the (±)-equatorial **11**, and the (±)-diacetate **15** with addition of a chiral shift reagent Eu(facam)$_3$.[27–29]

Application of these fairly large differential shifts to our specimens of the (+)-axial alcohol **10**, $[\alpha]^{31}_D$ +26.15°,[30] the (−)-equatorial alcohol **11**, $[\alpha]^{26}_D$ −125.7°, and the (−)-diacetate **15**, $[\alpha]^{28}_D$ −91.0°, enabled us to estimate their enantiomeric ratios which eventually revealed, within experimental error, their 100% optical purity.

Chemical correlation of the (−)-diacetate **15**, $[\alpha]^{28}_D$ −91.0° (optical purity 100%), with the (−)-diol **14**, $[\alpha]^{28}_D$ −87.6°, and transformation of the (−)-ketol **13**, $[\alpha]^{28}_D$ −400.5°, via the dithioacetal **16**, to the (+)-axial alcohol **10**, $[\alpha]^{31}_D$ +26.15° (optical purity 100%), indicated also 100% optical purity for our specimens of the (−)-diol **14** and (−)-ketol **13**.

Table I. Enantiomer Differential Shifts ($\Delta\Delta\delta$) Observed in (±)-Axial 10, (±)-Equatorial 11, and (±)-Diacetate 15 with Addition of Eu(facam)$_3$



sample	molar ratio[b]	$\Delta\Delta\delta$, ppm[a] C-1 H	C-2 H(e)[c]	C-2 H(a)[c]	C-13 H	C-14 H	C-16 H
(±)-**10**	1:0.23	0.10	0.62	0.16		0.24	0.22
(±)-**11**	1:0.22	0.25			0.03	0.08	
(±)-**15**	1:0.30			0.12 (–OCOCH$_3$)[d]			

[a] In about 2% CCl_4 solution. [b] The molar ratios are expressed as sample/Eu(facam)$_3$. [c] e = equatorial; a = axial. [d] Corresponding to the singlet at δ 2.16 in the normal spectrum.

Table II. Absolute Rotations ($[\alpha]_D$) of 1- and 1,10-Oxygenated [2.2]Metacyclophanes

(*pR*)-**9**	(*pR*,1*S*)-**10**	(*pS*,1*S*)-**11**	(*pS*,10*S*)-**13**	(1*S*,10*S*)-**14**
+446°	+26.15°	−125.7°	−400.5°	−87.6°

Lastly, Gschwend[11] reported conversion of the (−)-equatorial alcohol **11** with $[\alpha]^{25}_D$ −123.8° into the (−)-ketone **9**, $[\alpha]^{25}_D$ −439.3°, and this coupled with our absolute rotation of $[\alpha]_D$ −125.7° for (−)-**11** assigned $[\alpha]_D$ −446° to the absolute rotation of the (−)-(*pS*)-ketone **9**.

Table II tabulates the Ingold–Cahn–Prelog notations[31] for the optically active 1- and 1,10-oxygenated [2.2]metacyclophanes discussed in this paper with their absolute rotation values.

Experimental Section

Melting points are uncorrected. NMR spectra were determined on a JNM-NH-100 and a JNM-C-60HL with Me_4Si as an internal standard (δ = 0). Coupling constants are expressed in hertz, s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, and m = multiplet. Unless specified otherwise, optical rotations all refer to $CHCl_3$ solutions, and 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 FID using a 1 m × 3 mm column of 10% Carbowax 20M on Chromosorb W. Preparative TLC was carried out with silica gel 60 $PF_{254+366}$ (Merck).

The cultures of *Rhizopus arrhizus* and *Rhodotorula rubra* were obtained from the Institute for Fermentation, Osaka, Japan, and were identified by their IFO Catalog serial numbers IFO 6155 and IFO 0889, respectively.

The culture medium[32] for these microorganisms was prepared by dissolving glucose (30 g), KH_2PO_4 (1 g), corn-steep liquor (10 g), $MgSO_4 \cdot H_2O$ (0.5 g), $NaNO_3$ (2 g), $FeSO_4 \cdot 7H_2O$ (0.02 g), K_2HPO_4 (2 g), and KCl (0.5 g) in 1000 mL of tap water and was sterilized at 122–123 °C for 15 min before incubation.

Microbial Reduction of (±)-1-Oxo[2.2]metacyclophane (9) with *Rhodotorula rubra*. The substrate (±)-ketone **9** was prepared following Gschwend's procedure,[11] mp 79.5–80 °C (lit.[11] mp 79–81 °C).

Twenty 100-mL Erlenmeyer flasks, each containing 25 mL of the culture medium, were inoculated with *R. rubra* and incubated at 30 °C for 24 h on a thermostated shaker. After 15 mg of the substrate ketone **9** dissolved in 0.5 mL of ethanol was added to each of the flasks, incubation was maintained for 45 h at 30 °C.

The combined culture solutions were suction filtered through a layer of Hyflo Super Cel which was extracted with acetone. Ether extraction of the filtrate followed by concentration gave 190 mg of the metabolites, which was combined with 28 mg of the extract obtained from the acetone extraction of the cells.

TLC ($CHCl_3$ elution) of the extract indicated complete absence of the substrate ketone **9** with formation of two metabolites with R_f 0.38 and 0.30, respectively, which were separated by preparative TLC.

Extraction of the fraction with R_f 0.38 afforded 83 mg (27.6% yield) of a crystalline solid which was sublimed in vacuo (120–125 °C, 5 mm) to furnish 52.4 mg of (+)-(*pR*,1*S*)-1-hydroxy[2.2]metacyclophane (**10**) (17.5% yield): mp 138.5–139.5 °C; $[\alpha]^{26}_D$ +24.5° (*c* 0.875) (optical purity 94%) (lit.[11] mp 137–138 °C, $[\alpha]^{25}_D$ +22.4°); NMR (60 MHz, $CDCl_3$) δ 1.8 (s, 1 H, –OH), 2.10 (d, *J* = 8 Hz, 2 H, C-9 H and C-10 H axial), 2.35 (q, *J* = 3 and 12 Hz, 1 H, C-2 H axial), 3.11 (d, *J* = 8 Hz, 2 H, C-9 H and C-10 H equatorial), 3.13 (q, *J* = 3 and 12 Hz, 1 H, C-2 H equatorial), 4.25 (br s, 1 H, C-16 H), 4.59 (br s, 1 H, C-8 H), 5.24 (t, *J* = 3 Hz, 1 H, C-1 H), 6.9–7.4 (m, 6 H, aromatic H).

Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.50; H, 7.15.

Extraction of the fraction with R_f 0.30 afforded 71.1 mg (23.7% yield) of a crystalline solid which was sublimed in vacuo (120–125 °C, 5 mm) to give 50 mg of (−)-(*pS*,1*S*)-1-hydroxy[2.2]metacyclophane (**11**) (16.7% yield): mp 150.5–151 °C; $[\alpha]^{26}_D$ −125.7° (*c* 0.805) (optical purity 100%) (lit.[11] mp 152–153 °C, $[\alpha]^{26}_D$ −123.8°); NMR (60 MHz, $CDCl_3$) δ 2.05 (d, *J* = 8 Hz, 2 H, C-9 H and C-10 H axial), 2.26 (d, *J* = 11 Hz, 1 H, C-2 H axial), 2.55 (br s, 1 H, –OH), 3.10 (d, *J* = 8 Hz, 2 H, C-9 H and C-10 H equatorial), 3.30 (q, *J* = 4 and 11 Hz, 1 H, C-2 H equatorial), 4.20 (s, 1 H, C-16 H), 4.26 (s, 1 H, C-8 H), 4.28 (q, *J* = 4 and 10 Hz, 1 H, C-1 H), 7.0–7.5 (m, 6 H, aromatic H).

Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.54; H, 7.17.

Microbial Reduction of (±)-9 with *Rhizopus arrhizus*. Twenty-eight 100-mL Erlenmeyer flasks, each containing 25 mL of the culture medium, were inoculated with *R. arrhizus* and incubated for 42 h at 30 °C on a shaker. The mycelia collected from each of the flasks were washed with 1/15 N Sørensen's phosphate buffer (pH 7) and suspended again in 25 mL of the same buffer solution with 1 g of glucose in a 100-mL Erlenmeyer flask. To each of these twenty-eight flasks was added a solution of ~12 mg of (±)-**9** in 0.5 mL of ethanol, and the incubation was maintained for 44 h at 30 °C. A similar procedure to the one described for *R. rubra* afforded 460 mg of the metabolites as a yellow oil whose TLC indicated the presence of the isomeric alcohols **10** and **11** and the recovered ketone **9**.

Preparative TLC separated these components, affording the following: (a) 126.1 mg of the (−)-ketone **9** (37% yield), $[\alpha]^{26}_D$ −98.3° (*c* 0.31) (optical purity 22%) (lit.[11] mp 110.5–111 °C, $[\alpha]^{26}_D$ −439.3°), which resisted crystallization upon further purification by preparative TLC; (b) 41.1 mg of the (+)-alcohol **10** (12% yield), which was sublimed in vacuo to weigh 29.1 mg (8.5% yield), mp 137–138 °C, $[\alpha]^{26}_D$ +24.97° (*c* 0.865) (optical purity 96%) (Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.38; H, 7.35.); and (c) 20.3 mg of the (−)-alcohol **11** (6% yield), which was purified by further preparative TLC followed by sublimation in vacuo to weigh 3.9 mg (1.1% yield), mp 148–149 °C, $[\alpha]^{26}_D$ −110.26° (*c* 0.195) (optical purity 88%) (Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.20.).

Microbial Reduction of 1,10-Dioxo[2.2]metacyclophane (12) with *R. rubra*. The substrate diketone **12** was prepared following Lehner's procedure,[33] mp 143.5–144.5 °C (lit.[33,34] mp 144–144.5 °C).

After eight batches of 25 mL of culture medium inoculated with *R. rubra* were incubated for 48 h at 30 °C, these cultures were added separately to eight 500-mL Erlenmeyer flasks containing 200 mL of the culture medium and incubation was maintained for another 48

h at 30 °C. Aliquots of the diketone **12** (1.04 g) in 40 mL of ethanol were added to these flasks, and incubation was continued for 10 h at 30 °C on a shaker. Working up in the usual way via ether extraction afforded 1.162 g of a mixture of metabolites as a pale yellow oil whose GLC analysis indicated the complete absence of the substrate diketone **12** with formation of the ketol **13** and the diol **14** in a ratio of 6:1. The metabolic product was divided into two parts, 589 mg of which was absorbed on a silica gel plate and eluted with $CHCl_3$–MeOH (20:1) to furnish 306 mg of the ketol **13** as an oil (R_f 0.46) and 78 mg of the diol **14** as a solid (R_f 0.22).

The oily ketol crystallized upon addition of a small amount of benzene, yielding 286.1 mg of (−)-(*pS*,10*S*)-10-hydroxy-1-oxo[2.2]-metacyclophane (**13**) (54.6% yield): mp 151–152.5 °C; $[\alpha]^{28}_D$ −400.5° (*c* 0.445) (optical purity 100%); IR (KBr) 3500, 1690 cm^{-1}; MS (M^+) *m/e* 238 (calcd for $C_{16}H_{14}O$, 238); CD (*c* 1.19 × 10^{-4}, isooctane) [θ] (nm) −3.30 × 10^4 (321); NMR (100 MHz, $CDCl_3$) δ 4.64 and 4.96 (each s, each 1 H, C-8 H and C-16 H), 5.18 (t, *J* = 3 Hz, 1 H, C-10 H).

Anal. Calcd for $C_{16}H_{14}O$: C, 80.64; H, 5.92. Found: C, 80.64; H, 6.00.

The crude diol **14** was purified by preparative TLC followed by sublimation in vacuo (115–125 °C, 0.5 mm) to yield 59.7 mg of (−)-(1*S*,10*S*)-1,10-dihydroxy[2.2]metacyclophane (**14**) (11.3% yield): mp 178.5–179.5 °C; $[\alpha]^{28}_D$ −87.6° (*c* 0.515), $[\alpha]^{28}_D$ −58.2° (*c* 1.11, acetone) (optical purity 100%); NMR (100 MHz, $(CD_3)_2CO$) δ 4.22 and 4.68 (each s, each 1 H, C-8 H and C-16 H), 4.22 (m, 1 H, C-10 H axial), 5.25 (t, *J* = 3 Hz, 1 H, C-1 H equatorial).

Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.68; H, 6.76.

Diacetate **15**, prepared from (−)-**14** by acetylation with acetic anhydride and pyridine, was purified by sublimation in vacuo: mp 87.5–88.5 °C; $[\alpha]^{28}_D$ −91.0° (*c* 1.32); NMR (100 MHz, $CDCl_3$) δ 1.94 (s, 3 H, $-OCOCH_3$), 2.16 (s, 3 H, $-OCOCH_3$).

Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.05; H, 6.22. Found: C, 73.98; H, 6.17.

$LiAlH_4$ Reduction of 1,10-Dioxo[2.2]metacyclophane (12). A solution of 500 mg of diketone **12** in 10 mL of THF was added dropwise to a stirred suspension of $LiAlH_4$ (200 mg) in 20 mL of THF at 0 °C. The reaction mixture was stirred at room temperature for 3.5 h, decomposed with dilute H_2SO_4, and then extracted with ether. The ether extract was washed with water, dried over $MgSO_4$, and concentrated to give 487 mg of a crystalline product which was acetylated with a mixture of 2.5 mL of acetic anhydride and 5 mL of pyridine. After standing at room temperature for 12 h, the reaction mixture was poured into ice water and extracted with ether. The ethereal layer was washed with water and dried over $MgSO_4$. Removal of the solvent gave 618 mg of a crystalline residue which was recrystallized from methanol.

The fraction less soluble in methanol gave 265 mg of the acetate **18** (41.9% yield): mp 169.5–170 °C; NMR (100 MHz, $CDCl_3$) δ 2.20 (s, 6 H, two $-OCOCH_3$), 4.25 and 4.34 (each s, each 1 H, C-8 H and C-16 H), 5.14 (dd, *J* = 4 and 10 Hz, 2 H, C-1 H and C-10 H).

Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.05; H, 6.22. Found: C, 73.79; H, 6.26.

The acetate **18** (150 mg) was hydrolyzed with 15 mL of 5% KOH–MeOH, and the resulting diol **19** was recrystallized from methanol followed by sublimation in vacuo to yield 97.1 mg of equatorial-equatorial diol **19**: mp 198–199 °C; NMR (100 MHz, $(CD_3)_2CO$) δ 4.2 (m, 2 H, C-1 H and C-10 H), 4.07 and 4.29 (each br s, each 1 H, C-8 H and C-16 H).

Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.68; H, 6.76.

The acetate more soluble in methanol gave 313 mg of **15** (49.4% yield): mp 99–99.5 °C; NMR (100 MHz, $CDCl_3$) δ 1.94 (s, 3 H, $-OCOCH_3$), 2.16 (s, 3 H, $-OCOCH_3$), 4.30 and 4.58 (each br s, each 1 H, C-8 H and C-16 H), 5.15 (dd, *J* = 4 and 10 Hz, C-10 H axial), 6.04 (t, *J* = 3 Hz, C-1 H equatorial).

Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.05; H, 6.22. Found: C, 73.90; H, 6.29.

Hydrolysis of the diacetate **15** furnished the (±)-axial-equatorial diol **14**, which was purified by recrystallization from methanol followed by sublimation in vacuo to weigh 99.1 mg: mp 199–200 °C; NMR (100 MHz, $(CD_3)_2CO$) δ 4.22 and 4.68 (each br s, each 1 H, C-8 H and C-16 H), 4.2 (m, 1 H, C-10 H axial), 5.25 (t, *J* = 3 Hz, 1 H, C-1 H equatorial).

Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.88; H, 6.86.

$LiAlH_4$ Reduction of (−)-10-Hydroxy-1-oxo[2.2]metacyclophane (13). A solution of 70 mg of the (−)-ketol **13** in 7 mL of dry THF was added dropwise to a suspension of 150 mg of $LiAlH_4$ in 30 mL of dry THF with cooling in an ice bath. After being stirred for 4 h at room temperature, the mixture was decomposed with dilute H_2SO_4 and extracted with ether. The ether extract was washed with 5% $NaHCO_3$ and water and then dried over $MgSO_4$. Removal of the solvent gave a residue (67 mg) which was absorbed on a silica gel plate and eluted with $CHCl_3$–MeOH (100:7). From the band with R_f 0.58, there was isolated 39 mg of (−)-axial-equatorial diol **14** (55% yield): mp 177–178.5 °C; $[\alpha]^{28}_D$ −83.3° (*c* 0.45).

Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.74; H, 6.62.

The band with R_f 0.51 afforded 17 mg of meso-axial-axial diol **17** (24% yield): mp 169–170 °C; NMR (100 MHz, $(CD_3)_2CO$) δ 4.28 and 5.38 (each br s, each 1 H, C-8 H and C-16 H), 5.3 (m, 2 H, C-1 H and C-10 H).

Anal. Calcd for $C_{16}H_{16}O_2 \cdot H_2O$: C, 74.39; H, 7.02. Found: C, 74.31; H, 7.03.

Conversion of (−)-10-Hydroxy-1-oxo[2.2]metacyclophane (13) into (+)-1-Hydroxy[2.2]metacyclophane (10). The other portion (579 mg, vide supra) of the metabolite products of the diketone **12** was dissolved in 8 mL of acetic acid and treated with 350 mg of 1,3-propanedithiol containing 40 mg of BF_3 etherate. After standing for 3 days at room temperature, the reaction mixture was treated with 2 mL of acetone to remove the excess propanedithiol and was kept at room temperature for 1 day. The mixture was poured into ice water and extracted with ether. The ether extract was washed with 5% $NaHCO_3$ and water and then dried over $MgSO_4$. Evaporation of the solvent gave 849 mg of a pale yellow oil whose preparative TLC afforded 56 mg of the (−)-diol **14**, mp 178–179 °C, $[\alpha]^{30}_D$ −84.6° (*c* 0.505), and 415 mg of the (+)-dithioacetal alcohol **16** as an oil: $[\alpha]^{32}_D$ +16.8° (*c* 0.85); NMR (100 MHz, $CDCl_3$) δ 5.30 (t, *J* = 3 Hz, 1 H, C-1 H equatorial), 4.15 and 5.82 (each br s, each 1 H, C-8 H and C-16 H).

A solution of 100 mg of (+)-**16** in 8 mL of ethanol was stirred with "0.7 mL" of Raney nickel (a sedimented suspension in ethanol) for 1 h at room temperature. The mixture was filtered, and the solvent was evaporated in vacuo to give 44 mg of a crystalline product which was sublimed in vacuo (125 °C, 25 mm) to give 35.8 mg of (+)-axial alcohol **10** in 52.4% yield: mp 139–139.5 °C; $[\alpha]^{31}_D$ +26.15° (*c* 0.803). Its IR spectrum was identical with that of (+)-**10** obtained from microbial reduction of (±)-**9** with *R. rubra*.

Anal. Calcd for $C_{16}H_{16}O$: C, 85.68; H, 7.19. Found: C, 85.19; H, 7.26.

Microbial Reduction of (±)-[2.2]Metacyclophane-4-aldehyde (20) with *R. rubra*. The substrate (±)-aldehyde **20** was prepared by the reaction of 4-(bromomethyl)[2.2]metacyclophane with sodium 2-propanenitronate in ethanol,[35] mp 105–108 °C (lit. mp 100–105[23] and 98–100 °C[36]).

Four batches of 25 mL of culture medium were inoculated with *R. rubra* and incubated for 48 h at 30 °C. These four batches of culture were separately transferred into four 500-mL Erlenmeyer flasks containing 200 mL of the culture medium, and incubation was maintained for another 48 h at 30 °C. After aliquots of 500 mg of the aldehyde **20** dissolved in 20 mL of ethanol were added to these culture solutions, incubation was continued for 48 h at 30 °C. The combined culture mixtures were worked up in the usual manner via ether extraction. The ether extract was washed with dilute $NaHCO_3$ and then with water, dried over Na_2SO_4, and concentrated to afford 110 mg of an oil, which was purified by preparative TLC (developed with $CHCl_3$) to give 65 mg of 4-(hydroxymethyl)[2.2]metacyclophane (**21**) (13% yield): mp 82–83 °C; $[\alpha]^{28}_D$ +2.93° (*c* 1.59, ethanol) (optical purity 11.7%[22]) [lit.[23] mp 75–80 °C, $[\alpha]^{20}_D$ +15° (ethanol)].

Anal. Calcd for $C_{19}H_{18}O$: C, 85.59; H, 7.51. Found: C, 85.67; H, 7.61.

Registry No.—(±)-**9**, 40143-99-5; (−)-**9**, 40017-50-3; (±)-**10**, 68907-17-5; (+)-**10**, 68907-11-9; (±)-**11**, 68907-18-6; (−)-**11**, 40017-51-4; **12**, 68907-12-0; (−)-**13**, 68876-10-8; (±)-**14**, 68926-56-7; (−)-**14**, 68926-54-5; (±)-**15**, 68907-15-3; (−)-**15**, 68876-11-9; (+)-**16**, 68876-12-0; *meso*-**17**, 55894-62-7; **18**, 68907-13-1; **19**, 68926-55-6; (±)-**20**, 68907-14-2; (+)-**21**, 41048-00-4; 4-(bromomethyl)[2.2]metacyclophane, 68907-16-4.

References and Notes

(1) A part of the preliminary account of this research was presented before the 26th IUPAC Congress, Sept 8, 1977, Tokyo, Abstracts, p 63.
(2) Preliminary communication: M. Nakazaki, H. Chikamatsu, K. Naemura, Y. Hirose, T. Shimizu, and M. Asao, *J. Chem. Soc., Chem. Commun.*, 668 (1978).
(3) M. Nakazaki, H. Chikamatsu, K. Naemura, M. Nishino, H. Murakami, and M. Asao, *J. Chem. Soc., Chem. Commun.*, 667 (1978).
(4) V. Prelog, *Pure Appl. Chem.*, **9**, 119 (1964).
(5) R. Bentley, "Molecular Asymmetry in Biology", Vol. 2, Academic Press, New York, N.Y., 1970, pp 22–50.
(6) C. J. Sih and J. P. Rosazza, "Applications of Biochemical Systems in Or-

ganic Chemistry", Part 1, J. B. Jones, C. J. Sih, and D. Perlman, Eds., Wiley, New York, N.Y., 1976, pp 71–78.
(7) J. B. Jones and J. F. Beck, ref 6, pp 287–357.
(8) This name is proposed to describe the symmetry of a shape which is chiral but asymmetric; cf. M. Nakazaki, K. Naemura, and H. Yoshihara, *Bull. Chem. Soc. Jpn.*, **48**, 3278 (1975).
(9) 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; and C_1-ketones have no symmetry element passing through the carbonyl axis.
(10) The axial and equatorial notations of hydroxyl groups in **10** and **11**, respectively, were given by Gschwend.[11]
(11) H. W. Gschwend, *J. Am. Chem. Soc.*, **94**, 8430 (1972).
(12) In order to minimize errors due to optical fractionation on repeated recrystallization, the optical purities and rotations cited in this paper are mostly for specimens purified by sublimation in vacuo.
(13) Based on their absolute values to be discussed in the subsequent section.
(14) K. Mislow, M. Brzechffa, H. W. Gschwend, and R. T. Puckett, *J. Am. Chem. Soc.*, **95**, 621 (1973).
(15) The reported facile racemization[11] ($t_{1/2}$ = 47.4 h at 25 °C) of the ketone **9** may be responsible for this rather low optical rotation.
(16) In meso diketone **12**, one of the enantiomeric ketone groups which leads to a *pR* compound when considered to be preferred to the other is termed *pro-pR* and the other termed *pro-pS*, as analogized to central prochiral compounds.[17,18]
(17) IUPAC tentative rules for the nomenclature of organic chemistry, Section E, *J. Org. Chem.*, **35**, 2849 (1970).
(18) K. R. Hanson, *J. Am. Chem. Soc.*, **88**, 2731 (1966).
(19) The axial-equatorial, axial-axial, and equatorial-equatorial notations of hydroxyl groups in **14**, **17**, and **19**, respectively, were given by Lehner et al.[20]
(20) C. Glotzmann, E. Haslinger, E. Langer, and H. Lehner, *Monatsh. Chem.*, **106**, 187 (1975).
(21) See Experimental Section.
(22) Calculated from the absolute rotation, $[\alpha]_D$ +25°, estimated by Schlögl et al.[23]
(23) B. Kainradl, E. Langer, H. Lehner, and K. Schlögl, *Justus Liebigs Ann. Chem.*, **766**, 16 (1972).
(24) D. J. Cram and N. L. Allinger, *J. Am. Chem. Soc.*, **77**, 6289 (1955).
(25) D. J. Cram, C. K. Dalton, and G. R. Knox, *J. Am. Chem. Soc.*, **85**, 1088 (1963).
(26) M. Nakazaki and K. Yamamoto, unpublished results.
(27) Eu(facam)$_3$ = tris(3-trifluoroacetyl-*d*-camphorato)europium(III).
(28) M. D. McCreary, D. W. Lewis, D. L. Wernick, and G. M. Whitesides, *J. Am. Chem. Soc.*, **96**, 1038 (1974).
(29) H. L. Goering, J. N. Eikenberry, G. S. Koermer, and C. J. Lattimer, *J. Am. Chem. Soc.*, **96**, 1493 (1974).
(30) This value corresponds to the (+)-axial alcohol **10** obtained from the (−)-ketol **13** via the dithioacetal alcohol **16**.
(31) R. S. Cahn, C. K. Ingold, and V. Prelog, *Angew. Chem., Int. Ed. Engl.*, **5**, 385 (1966).
(32) K. Kieslich; *Synthesis*, **1**, 120 (1969).
(33) H. Lehner, *Monatsh. Chem.*, **105**, 895 (1974).
(34) T. Hylton and V. Boekelheide, *J. Am. Chem. Soc.*, **90**, 6887 (1968).
(35) H. B. Hass and M. L. Bender, *J. Am. Chem. Soc.*, **71**, 1767 (1949).
(36) A. Maquestiau, Y. van Haverbeke, R. Flammang, M. Flammang-Barbieux, and N. Clerbois, *Tetrahedron Lett.*, 3259 (1973).

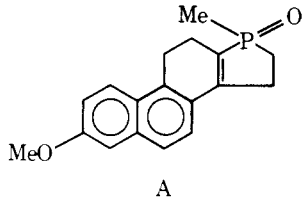
Synthesis of the Phosphasteroid System and of Potential Tricyclic Precursors by the McCormack Cycloaddition Method[1]

C. Symmes, Jr., and L. D. Quin*

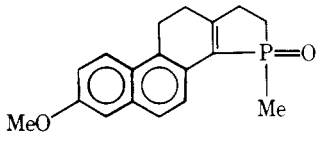
Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706

Received September 8, 1978

Cycloaddition of the diene 3,4-dihydro-7-methoxy-1-vinylphenanthrene and CH_3PCl_2 proceeded in good yield to give, after hydrolysis, a derivative with the tetracyclic steroid system containing phosphorus at the 17 position (A). The isomeric 2-vinyl compound also gave a tetracyclic derivative (a 15-phosphasteroid, B), but less readily. Hydrogenation of the remaining double bond in the 17-phosphasteroid was shown by ^{13}C and ^{31}P NMR spectral studies to proceed with high stereoselectivity, hydrogen entering cis with regard to the P-CH_3 group. Similar specificity was observed in the hydrogenation of the tricyclic phospholene oxides prepared in like manner from the 1- and 2-vinyl derivatives of 3,4-dihydro-6-methoxynaphthalene. These hydrogenated tricyclic compounds underwent smooth Birch reduction with lithium in a liquid NH_3–*tert*-butyl alcohol medium, giving enol ethers that were easily converted to ketones (nonconjugated) with an oxalic acid solution. The phosphoryl group was not attacked in the Birch reduction, although the same conditions applied to a related tricyclic phospholene sulfide cleanly removed sulfur to form a phosphine. The styrenoid double bond is also reduced when it is present in the tricyclic compounds.

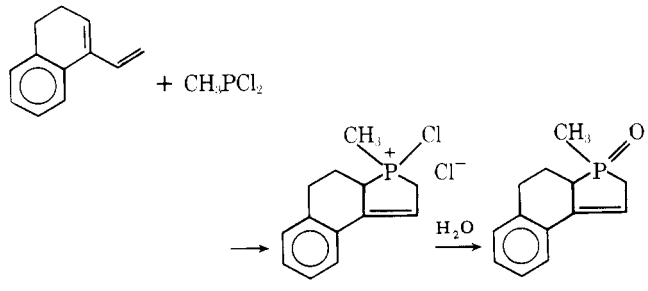


A



B

We recently showed[2] that 1-vinylcyclohexenes participate readily in the McCormack cycloaddition[3] with phosphorus(III) halides, making available a number of new bicyclic phosphorus compounds. An example of formation of a tricyclic phospholene oxide from a benzo derivative of the cyclohexene was included in this study. We recognized in this reaction the potential for producing compounds with the tetracyclic steroid system, of necessity having phosphorus in the D ring. Two approaches to such compounds were visualized: an ABC → ABCD route applying the cycloaddition to an appropriate naphthocyclohexene, and a BC → BCD → ABCD route, wherein a benzocyclohexene derivative (BC) would be used in the cycloaddition to form the tricyclic BCD structure, followed by annelation at ring B. These potential methods are illustrated in Scheme I for the synthesis of 17-phosphaster-



0022-3263/79/1944-1048$01.00/0