(14) NMR analysis indicated that ester **6** formed under these conditions did incorporate one atom of deuterium, as expected.
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Microbial Reduction of a Series of Substituted Benzils. Optical Properties and Nuclear Magnetic Resonance Spectra of Products

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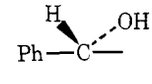
A series of para-substituted symmetric and unsymmetric benzils were reduced using *C. macerans* to yield the threo (*R,R*) diols of high optical purity and the (*S*)-benzoins with enantiomeric excesses of 20–30%. The absolute stereochemistry of the diols was established from CD measurements of the sign and magnitude of the 225-nm band and, in select cases, by chemical transformation to compounds of known configuration. The stereospecificity and/or high selectivity of these reductions are discussed. The proton NMR spectra of the isomeric erythro and threo diols were measured and assigned. Potential uses of coupling constants and chemical shifts to assign stereochemistry are discussed.

As part of a study on the stereochemical preferences of a mammalian enzyme, "hydrase", the absolute stereochemistries of several threo diols, obtained from enzymatic hydration of optically active substituted *cis*-stilbene oxides, were determined.[1] In order to examine aspects of the chemistry and spectroscopy of transformation products of these diols, we required a synthetic route capable of yielding reasonable quantities of these optically active compounds. The cis-substituted stilbene oxides had been prepared from the appropriate optically active mandelonitrile or mandelamide. This route could not be used to prepare optically active threo diols as the latter isomers were only minor products (formed in only 10–20%) in the hydride reduction of the intermediate, optically active benzoin.

One solution to this problem followed logically from our recent studies[2] on the stereospecific reductions of acetophenone and a series of substituted α-tetralone derivatives: the use of microbial reductions of substituted benzil derivatives to prepare the optically active threo diols. Prelog reported[3] that the reduction of benzil by *Curvularia falcate* yielded a mixture of erythro and threo (*S,S*) diols, in approximately equal amounts, as well as (*S*)-benzoin. In earlier studies Prelog et al. had formulated a rule,[4] shown in Figure 1, to account for the observed stereochemistry: if the ketone is placed with the larger group on the observer's left, the hydroxyl group formed is closer to the observer.

We first examined the reduction of benzil by *Cryptococcus macerans*, a microorganism that efficiently reduces acetophenone to (1*S*)-phenylethanol.[2] Microbiological reduction of benzil (**1a**) yielded (−)-(*S*)-benzoin (**2a**) and (+)-(1*R*,2*R*)-diphenylethanediol (**3a**) and only traces of the erythro isomer **4a**. The NMR spectrum of the crude extract was examined, in which the erythro and threo isomers showed easily distinguishable proton resonances for the protons on the benzylic carbons.[1] Although (*S*)-**2a** was formed in both our study and that reported by Prelog et al.,[3] there were two differences in our results. First, Prelog et al. obtained (−)-**3a** whereas (+)-**3a** formed with *C. macerans*, and second, appreciable quantities of the erythro isomer (**4a**) were obtained in their study while only traces were observed in our reduction. In addition, formation of (*S*)-**2a** and (*R,R*)-**3a** in our reductions was particularly perplexing because it was not apparent why the configuration about the hydroxyl-bearing carbons in the two compounds differed. In order to understand how or why this occurred, we investigated the mechanism of the reduction.

In order to establish that **2a** can be reduced to **3a**, racemic **2a** was examined under standard conditions as a substrate, and it was found to be efficiently converted to (*R,R*)-**3a** in greater than 50% yield by *C. macerans*. When unreduced **2a** was reisolated, it was found to be levorotatory, i.e., to contain an excess of the *S* enantiomer. These results require (*R*)-**2a** to be reduced much more easily than the *S* enantiomer, and since the (*R,R*) diol is obtained in greater than 50% yield, a mechanism for equilibrating *R* and *S* enantiomers exists. Since under our experimental conditions **2a** formed or recovered in these reductions is optically active, the rate of equilibration (racemization) is slower than the rate of reduction. These conclusions are incorporated in Scheme I which describes the course of these reductions. No conclusion as to the stereospecificity of the conversion of **1a** to **2a** can be made on the basis of our results. However, reduction of **2a** to **3a** is remarkable in the ability of the enzyme to reduce (*R*)-**2a** while effecting very little reduction of (*S*)-**2a**. Similar differences in the reduction rates of various substituted cyclohexanone derivatives were explained by Prelog as resulting from steric interferences between substituents on the substrate with the coenzyme on the enzyme surface.[5] Our results can be rationalized if the enzyme treats the phenyl group as the large substituent and the α-hydroxybenzyl



as the small one.

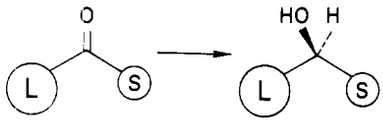


Figure 1.

It is appropriate at this point to comment on the difference between our results and those reported by Prelog et al.[3] Since the oxidoreductases that effect these reductions are a family of enzymes,[6] Prelog et al. used a purified enzyme in their studies, while we used a different microorganism and a whole cell preparation. However, these results in conjunction with an earlier study[2] on the configuration of a series of alcohols obtained by reduction of several substituted α-tetralone derivatives, indanone, and benzsuberone demonstrate that these microorganisms exhibit a consistent pattern of results which can be used to prepare products of the same configuration.

These microbial reductions potentially provide a simple procedure to prepare optically active threo diols, as the difficult separation of an equal mixture of erythro and threo isomers is not necessary. In order to demonstrate the utility of the method, and in order to further test an earlier suggestion[1] relating the sign of the CD band at 225 nm in these diols and their configuration, we have examined the effect of substituents on the aromatic ring on the course of the reduction.

The substituted benzils **1b, 1c, 1d,** and **1e** were studied as substrates. It is interesting to note that although two benzoins can form from each substituted benzil, only those resulting from reduction of the carbonyl adjacent to the unsubstituted ring were obtained. Their structures were assigned from a comparison of their ^{1}H NMR and mass spectra with the ones obtained from authentic material. The absolute stereochemistries of **2b, 2c,** and **2d** are known,[10-12] and in each case the benzoin isolated had the *S* configuration (see Table I). The optical purities of the isolated benzoins were 20 to 30%, suggesting that either the reductions were not stereospecific or, more probably, that some racemization occurred. These results clearly parallel those observed earlier in the reduction of **1a** and indicate that the presence of a para substituent does not affect the course of the reduction. The diols isolated in these reductions were also compared to those previously reported[1] (see Table I) and the results parallel those noted for **1a,** i.e., the (*R,R*) diol forms in yields of 95% or more, with only 5% of the erythro isomer. The CD data (see Table I) are in good agreement with those previously reported for these

Scheme I

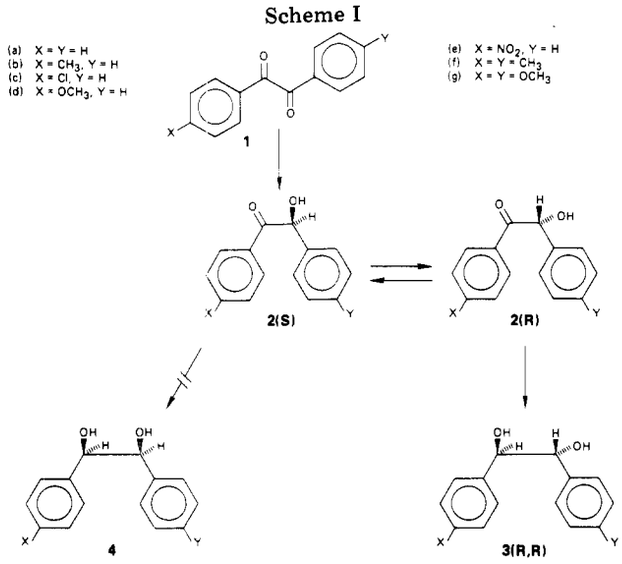


Table I. Microbiological Reductions of Benzils

Benzil	diol registry no.	diol yield, %	diol abs. stereochemistry	diol registry no.	diol $[\alpha]^{25}_D$ (EtOH) obsd	diol $[\alpha]^{25}_D$ (EtOH) reported[1]	diol CD $\theta(\lambda, nm)$ obsd	diol CD $\theta(\lambda, nm)$ reported[1]	benzoin yield, %	benzoin abs. stereochemistry	benzoin registry no.	benzoin $[\alpha]^{25}_D$ (EtOH) obsd	benzoin $[\alpha]^{25}_D$ (EtOH) reported	recovered benzil, %
1a	134-81-6	27	(*R,R*)	52340-78-0	+91° (*c* 1.11)	+92°	+37000 (219)	+37680 (219)	18	(*S*)	5928-67-6	+48.5° (*c* 0.907, acetone)	+120.5° (*c* 0.413, acetone[19]	50
1f	3457-48-5	25	(*R,R*)	66768-19-2	+107° (*c* 1.16)		+37600 (225)		11		1218-89-9			69
1g	1226-42-2	6	(*R,R*)	66768-20-5	+101° (*c* 1.15)		+28300 (232)		2		119-52-8			90
1c	22711-23-5	41	(*R,R*)	62086-76-4	+123° (*c* 1.40)	+125°	+53600 (222)	+50300 (222)	21	(*S*)	66768-22-7	+10.1° (*c* 1.100)	−45°[a]	31
1b	2431-00-7	38	(*R,R*)	62137-63-7	+100° (*c* 1.11)	+100°	+50600 (222)	+37000 (220)	19	(*S*)	66768-23-8	+20.3° (*c* 3.080)	+83°[1]	47
1d	22711-21-3	24	(*R,R*)	66768-21-6	+94° (*c* 1.32)		+33200 (226)		13	(*S*)	4984-91-2	+19.4° (*c* 0.660)	+80 (*c* 1.0, EtOH)[11]	60
1e	22711-24-6	2	(*R,R*)	62086-77-5	+97° (*c* 1.10)	+95°	+11000 (215)	+9850 (215)			4984-91-2			95

[a] Specific rotation reported for (*R*) enantiomer.

Table II. Microbiological Reductions of Benzoins

benzoin	registry no.	diol yield, %	diol abs. stereochemistry	diol $[\alpha]^{25}_D$ (EtOH)	recovered benzoin yield, %	recovered benzoin $[\alpha]^{25}_D$ (EtOH)	recovered benzoin abs. stereochemistry
(±)-benzoin	579-44-2	59	(*R*,*R*)	+90° (*c* 1.11)	38	+9.10° (*c* 1.11)	(*S*)
(±)-*p*-chlorobenzoin	66749-61-9	55	(*R*,*R*)	+120° (*c* 1.35)	27	+3.30° (*c* 0.23)	(*S*)
(±)-*p*-methylbenzoin	66749-62-0	46	(*R*,*R*)	+97° (*c* 0.99)	32	+13.3° (*c* 1.60)	(*S*)
(±)-*p*-methoxybenzoin	4842-37-9	31	(*R*,*R*)	+96° (*c* 0.766)	40	+15.3° (*c* 1.45)	(*S*)

compounds. Since the introduction of a *p*-methoxy substituent in benzil results in a greater perturbation of benzil's absorption spectrum than does either a *p*-methyl or a *p*-chloro substituent, it was not certain that the CD curves of **3d** would show the same positive bands (θ ~40 000) at ~225 nm observed for the other (*R*,*R*) diols. The CD band observed for **3d** has the same magnitude and position as the *p*-methyl and *p*-chloro compounds. While the result may be accidental, the consistency between the CD curves of these diols and those described for **3f** and **3g** suggests that a detailed theoretical model unifying these observations could be constructed.

In our proposed description of the reduction of benzil (Scheme I), the stereospecific (or highly stereoselective) reduction of (*R*)-**2a** critically controlled the configuration of the diol. Therefore, in addition to examining the products from substituted benzils, we also isolated the products when racemic monosubstituted benzoins (**2b, 2c,** and **2d**) were used as substrates. The results summarized in Table II demonstrate that as with (+)-**2a,** the recovered substituted benzoin in each case is enriched in the *S* enantiomer. The (*R*,*R*) diol is formed along with traces of the erythro compounds, which were not isolated. The pattern and selectivity noted here are completely consistent with the description (Scheme I) for the reduction of **2a** and **1a.** The observations are thus best rationalized using Prelog's concept[4] of "product specific enzymes". This concept enables one to assign the absolute stereochemistry of the diol (+)-**3e** as (*R*,*R*). The stereochemistry thus assigned agrees with that made using the "product stereospecific enzymatic hydration" of the *cis*-*p*-nitrostilbene oxide.[1]

The ^{1}H NMR data of the substituted diphenylethanediols and diacetates are summarized in Table IV. In several cases it was possible to measure the coupling constants of the threo diols and to compare these with those of the erythro compounds, prepared by hydride reduction of the appropriate benzil. In the limited examples available, the erythro diols showed a somewhat smaller (6.0 Hz) coupling than the corresponding threo isomer (7.5–8.0 Hz). The similarities in these values, however, make any stereochemical assignment on this basis hazardous. Several investigators have prepared cyclic derivatives, i.e., dioxolanes,[7] thiono carbonates,[8] etc., in order to distinguish erythro and threo configurations.

In addition to the unsymmetrically substituted benzil derivatives mentioned above, we also examined the reduction of *p*,*p*′-dimethyl- and *p*,*p*′-dimethoxybenzil (**1f** and **1g**). The diols isolated in each case were optically active and are therefore threo. The CD curves of **3f** and **3g** each exhibited positive bands whose magnitude and position were virtually identical with those of the (*R*,*R*) diols **3a, 3b, 3c,** and **3d.** While the correlations between the sign of the 225-nm band and the absolute stereochemistry of the compounds is empirical, the conclusion from the CD data and that employing the "product specificity" argument both require **3f** and **3g** to have (*R*,*R*) configurations. As one of our purposes was to test the relation between the sign of the CD band at 225 nm and the absolute stereochemistry of the diols, we did not wish to rely on the assignment of absolute stereochemistry based solely on the stereospecificity of these enzymatic reductions, and therefore elected to determine the absolute stereochemistry of **3f** by relating it to a compound of established absolute stereochemistry. The compound chosen for comparison was dimethyl (2*S*,3*S*)-diacetoxysuccinate, synthesized from (−)-tartaric acid. A sample of **3f** was acetylated and exhaustively ozonized and the resulting acids were methylated to yield (+)-dimethyl (2*S*,3*S*)-diacetoxysuccinate which after purification was identical with the (+)-dimethyl (2*S*,3*S*)-diacetoxysuccinate prepared from (−)-tartaric acid. The absolute stereochemistry of **3f** assigned by chemical transformation supports conslusions as to the stereospecificity of these microbial reductions as well as the relation between the sign of the 225-nm CD band and these diols' absolute stereochemistry.

Table III. Solvent Effects on the Chemical Shifts of the Benzylic Hydrogens (*p*,*p*′-Dimethyldiphenyl)ethanediol[a]

	CCl_4	$CDCl_3$	benzene-d_6	acetone-d_6	Me_2SO-d_6
erythro	4.60	4.75	4.66	4.74	4.50
threo	4.44	4.59	4.52	4.60	4.48
differences	0.16	0.16	0.14	0.14	0.02

[a] Concentration is 0.5 mg in 5 mL of solvent.

The three sets of symmetrically substituted erythro and threo diols allowed us to evaluate the possibility of assigning stereochemistry from the ^{1}H NMR chemical shifts of the benzylic protons. The NMR spectra were measured in several solvents in order to determine whether the polarity of the solvent affected chemical shift differences. The largest differences were observed in carbon tetrachloride and $CDCl_3$, and the results of these measurements are summarized in Table III. Chemical shifts of the benzylic protons of the erythro isomers were consistently at lower fields (0.2 ppm) than those of the threo isomers (see Table IV). The differences between isomers in each set arise from differences in the amounts of inter- and intramolecular hydrogen bonding and from different rotamer distributions. In polar solvents (Me_2SO) these differences are minimal; after acetylation the benzylic protons in both isomers have virtually identical chemical shifts.

Conclusion

Microbial reduction of a series of symmetric and unsymmetric para-substituted benzil and benzoin derivatives yields primarily the threo isomer in satisfactory yield and in high optical purity. The CD curves of these diols were determined and where the para substituent(s) does not severely perturb the absorption spectrum, the sign of the CD band at 225 nm *correlates* with the absolute stereochemistry of the diol. The ^{1}H NMR spectra of sets of erythro and threo isomers were determined; the benzylic proton of the erythro isomer in each set absorbs at lower field than that in the corresponding threo isomer.

Experimental Section

General Procedure. Melting points were determined using a hot-stage apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian HR-220-MHz instrument using

Table IV. A Comparison of the NMR Spectra of Erythro- and Threo-Substituted 1,2-Diphenylethanediols and Diacetates

erythro	registry no.	benzylic H (δ)	J_{AB}, Hz	threo	registry no.	benzylic H (δ)	J_{AB}, Hz	diff
4a	579-43-1	4.83		**3a**	38270-73-4	4.60		0.23
4a diacetate	6316-82-1	6.09		**3a** diacetate	66749-68-6	6.05		0.04
4b	51343-94-3	4.75	6.0	**3b**	51343-95-4	4.57	7.7	0.18
		4.77				4.59		0.18
4b diacetate	66749-63-1	6.08	6.0	**3b** diacetate	66769-44-6	6.03	8.3	0.05
		6.10				6.05		0.05
4c	66768-18-1	4.81		**3c**	66768-15-8	4.56	7.6	0.25
						4.62		0.19
4c diacetate	66749-64-2	6.04	5.8	**3c** diacetate	66749-55-1	6.01		0.03
		6.05						0.04
4d	66749-65-3	4.74	5.9	**3d**	66749-56-2	4.63	8.2	0.11
		4.76				4.66		0.10
4d diacetate	66749-66-4	6.03	6.0	**3d** diacetate	66749-57-3	6.01	8.3	0.02
		6.07				6.03		0.04
4f	5173-29-5	4.75		**3f**	66749-58-4	4.59		0.16
4f diacetate	66749-67-5	6.04		**3f** diacetate	66749-59-5	6.03		0.01
4g	39090-30-7	4.75		**3g**	42565-21-9	4.60		0.15
4g diacetate	39090-32-9	6.03	6.0	**3g** diacetate	66769-45-7	6.01	8.5	0.02
		6.07				6.03		0.04

FT technique; chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard, with coupling constants (J) in hertz. Optical rotations and circular dichroism spectra were recorded on a Cary 60 spectropolarimeter. Chemical ionization mass spectra were taken with a Hitachi RMS-4 instrument. Microanalyses were performed by the Microanalytical section of NIH. Preparative and analytical TLC work was performed on plates coated with Kieselgel silica gel F-254.

Compounds **1b, 1c,** and **1d** were prepared via intermediates **2b, 2c,** and **2d** by a modification of the procedure by McKenzie,[9] as illustrated by **1b.**

***p*-Methylbenzoin (2b).** A solution of mandelonitrile (6.65 g) in 100 mL of ether was added dropwise to an ether solution (100 mL) of the Grignard reagent made from *p*-bromotoluene (34.2 g) and Mg (4.86 g). The solution was refluxed for 1.5 h under N_2 and was then poured into ice–water (200 g) containing 10 mL of concentrated HCl. The aqueous phase was immediately extracted with ether and the aqueous solution was then treated with an additional 10 mL of concentrated HCl. The solution was stirred overnight at room temperature, at which time the *p*-methylbenzoin precipitated. The crystals were separated by filtration and recrystallized from 95% aqueous ethanol: 18.2 g (41%); mp 98 °C (lit.[10] mp 99 °C); ^{1}H NMR (in $CDCl_3$) δ 2.34 (3 H, s), 4.55 (1 H, broad s), 5.91 (1 H, s), 7.18 (2 H, d, J = 8.9 Hz), 7.23–7.34 (5 H, m), 7.82 (2 H, d, J = 8.9 Hz).

***p*-Chlorobenzoin (2c).** A sample of **2c** was prepared from 1-bromo-4-chlorobenzene as above in 38% yield: mp 89.5 °C (lit.[11] mp 91 °C); ^{1}H NMR (in $CDCl_3$) δ 4.50 (1 H, d, J = 6.0 Hz), 5.89 (1 H, d, J = 6.0 Hz), 7.29–7.43 (5 H, m), 7.39 (2 H, d, J = 8.5 Hz), 7.86 (2 H, d, J = 8.5 Hz).

***p*-Methoxybenzoin (2d).** A sample of **2d** was prepared from *p*-bromoanisol as above in 35% yield: mp 109 °C (lit.[12] mp 108–109 °C); ^{1}H NMR (in $CDCl_3$) δ 3.80 (3 H, s), 4.66 (1 H, broad s), 5.89 (1 H, s), 6.85 (2 H, d, J = 8.9 Hz), 7.26–7.32 (5 H, m), 7.90 (2 H, d, J = 8.9 Hz).

Oxidation of Benzoins to Benzils. A solution of **2b** (1.6 g) in pyridine (5 mL) was added to a previously prepared solution of $CuSO_4 \cdot 5H_2O$ (4.48 g) in a mixture of pyridine (5 mL) and water (10 mL). Air was bubbled through this solution, while it was refluxed overnight. The solvent was next removed in vacuo, water (50 mL) was added, and the solution was concentrated again. The residue was extracted into ethyl acetate and the extract was washed with 5% HCl in water and dried over Na_2SO_4. The solvent was removed in vacuo and the residue purified by column chromatography over silica gel to yield 1.32 g (84% yield) of **1b**, crystallized from hexane mp 30 °C (lit.[13] mp 30 °C).

The above procedure was also used to prepare **1c** in 82% yield, mp 70 °C (lit.[14] mp 70 °C). and **1d** in 81% yield, mp 52 °C (lit.[15] mp 52–54 °C).

A sample of **1e** was prepared as described by Womack[16] in 38% yield, mp 142 °C (lit. mp 142 °C).

Preparation of *erythro*-*p*-Methyldiphenylethanediol (4b). To a slurry of $LiAlH_4$ (760 mg) in 20 mL of dry ether was added **1b** (1.12 g) and the solution was stirred overnight. The reaction mixture was decomposed using 10% NaOH and worked up as usual to yield 1.1 g of a product whose NMR spectrum indicated that it consisted of **4b** and **3b** in a 5:1 ratio. The product (550 mg) was warmed at 50 °C with acetic anhydride (10 mL) in pyridine (3 mL) overnight. Water (50 mL) was added and the acetic acid–pyridine–water mixture was removed in vacuo. The residue was extracted into ether, dried over Na_2SO_4, and concentrated to yield the acetates of **4b** and **3b,** which were separated by thick-layer chromatography on silica gel to yield the acetate of **4b** (480 mg, 67% yield) and **3b** (70 mg, 10% yield). The NMR spectra are summarized in Table IV.

Saponification of the diacetate of **4b** in methanol (10 mL) containing water (2 mL) and KOH (200 mg) was effected by refluxing for 2 h. The solvent was removed in vacuo and the residue extracted into ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The pure erythro diol, 184 mg (81% yield), was obtained by thick-layer chromatography on silica gel, mp 106 °C (recrystallized from 50% ethanol). Anal. Calcd for $C_{15}H_{12}O_2$: C, 78.95; H, 7.02. Found: C, 78.81; H, 7.15. The NMR spectra of **4b** and the other erythro diols **4c, 4d, 4e, 4f,** and **4g** prepared by essentially the same sequence of procedures are summarized in Table IV. Physical properties and analytical data are given in Table V.

Microbial Reduction of Benzils. Benzil (1a). A 1-L Erlenmeyer flask containing 250 mL of a sterile solution of 6% glucose, 4% peptone, 4% yeast extract, and 4% malt extract was inoculated with a culture of *C. macerans*. The flask was shaken at 30 °C for 2 days and to the optically dense culture 100 mg of benzil (**1a**) was added. Shaking was continued for 7 days. The suspension was then extracted three times with 250-mL portions of ethyl acetate. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Analysis of the crude concentrate by NMR indicated a 3:2 ratio of **3a** to **2a.** In addition ~5% (relative to the threo diol) of the erythro isomer was detected. The mixture was separated by thick-layer chromatography (silica gel, ethyl acetate:hexane (25:75)) to yield the threo diol **3a** (27 mg), benzoin (**2a**) (18 mg), and unreacted benzil (**1a**) (50 mg). Two crystallizations from 50% EtOH provided pure threo diol **3a,** mp 147 °C (lit.[1] mp 147 °C). The NMR, CD, $[\alpha]^{25}_D$ data, and yield of this sample as well as those of the other threo diols are summarized in Tables I and II, respectively. The above benzoin **2a** fraction was recrystallized from 50% EtOH to give a pure sample, mp 134 °C (lit.[9a] (*S*)-benzoin mp 132 °C). The NMR spectrum of this compound was identical with that of racemic material. The $[\alpha]^{25}_D$ data of this sample and the other optically active benzoins obtained from microbial reductions are summarized in Table II.

***p*-Methylbenzil (1b).** The microbial reduction of **1b** and separation of **1b, 2b,** and **3b** was carried out as described for **1a.** The threo diol **3b** was recrystallized from 50% EtOH, mp 97 °C (lit.[1] 97 °C). The isolated benzoin **2b** was recrystallized from 95% EtOH, mp 99 °C (lit.[1] mp 99 °C). the NMR and mass spectrum of this sample were identical with those of racemic material.

***p*-Chlorobenzil (1c).** Compound **1c,** synthesized as described above, was reduced by *C. macerans* in the same way as **1a.** The crude mixture was separated by thick-layer chromatography (silica gel, ethyl acetate:hexane (25:75)) to yield **1c, 2c,** and **3c.** The threo diol **3c** was recrystallized from 95% MeOH, mp 99 °C (lit.[1] mp 99 °C). The benzoin **2c** was recrystallized from 50% EtOH, mp 92 °C (lit.[1] (*R*)-benzoin,

Table V. Physical Properties, Analytical Data, and Molecular Ion (Mass Spectrum)

compd	mp, °C	solvent for crystallization	anal. data		molecular ion
			calcd	obsd	
4a	134	EtOH–H_2O	C 78.50	78.51	
			H 6.54	6.48	
4c	131	MeOH	C 67.61	67.70	
			H 5.23	5.19	
			Cl 14.29	14.20	
4d	135	$CHCl_3$–petroleum ether	C 73.77	73.67	244
			H 6.56	6.71	
4e	123	EtOH–H_2O	C 64.86	64.77	
			H 5.02	5.10	
			N 5.40	5.38	
4f	128	hexane	C 79.34	79.21	
			H 7.44	7.58	
4g	164	EtOH–H_2O	C 70.07	70.11	274
			H 6.57	6.54	

mp 91 °C). The NMR and mass spectrum of this sample were identical with those of racemic material.

***p*-Methoxybenzil (1d).** Compound **1d,** synthesized as described above, was reduced by *C. macerans* and the crude microbial reduction mixture was separated by thick-layer chromatography (silica gel, ethyl acetate:hexane (3:7)) to yield **1d, 2d,** and **3d.** The threo diol **3d** was purified by vacuum distillation, 120–121 °C (0.1 mmHg). Anal. Calcd for $C_{15}H_{16}O_3$: C, 73.77; H, 6.56. Found: C, 73.69; H, 6.62; MS 244 (M^+), 137, 107. The benzoin **2d** was recrystallized from 50% EtOH, mp 101 °C (lit.[11] (*R*)- and (*S*)-benzoin mp 102–103 °C). The NMR and mass spectrum of this sample were identical with those of racemic material.

***p*-Nitrobenzil (1e).** The crude microbial reduction mixture obtained from *C. macerans* was separated by thick-layer chromatography (silica gel, ethyl acetate:hexane (1:1)) to yield **1e** and **3e.** The threo diol **3e** was recrystallized from 50% EtOH, mp 112 °C (lit.[1] mp 112 °C).

***p,p'*-Dimethylbenzil (1f).** The crude microbial reduction mixture obtained from *C. macerans* was separated by thick-layer chromatography (silica gel, ethyl acetate:hexane (3:7)) to yield **1f** and **3f.** The threo diol **3f** was recrystallized from hexane, mp 110 °C. Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.34; H, 7.44. Found: C, 79.29; H, 7.56; MS 242 (M^+), 121.

***p,p'*-Dimethoxybenzil (1g).** The threo diol **3g** was separated by thick-layer chromatography as described for **1a** and was recrystallized from hexane, mp 123 °C. Anal. Calcd for $C_{16}H_{18}O_4$: C, 70.07; H, 6.57. Found: C, 70.16; H, 6.48.

Microbial Reduction of Benzoins. The reduction procedure and workup were essentially the same as those of the benzil derivatives. In each reduction (racemic **2a, 2b, 2c,** and **2d**), the corresponding (*R,R*) threo diol was formed and the recovered benzoin contained an excess of the *S* enantiomer. The NMR, CD, $[\alpha]^{25}_D$, and yield for these compounds are summarized in Tables II and IV, respectively.

Ozonolysis of (*R,R*)-*threo*-*p,p'*-Dimethyldiphenylethanediol Diacetate. (*R,R*)-*threo*-*p,p'*-Dimethyldiphenylethanediol diacetate was prepared by acetylation of (*R,R*)-*threo*-*p,p'*-dimethyldiphenylethanediol with acetic anhydride in pyridine in the usual manner. The resulting diacetate was purified by thick-layer chromatography on silica gel (ethyl acetate:hexane, 1:4), mp 61 °C, $[\alpha]^{25}_D$ −28.0° (*c* 2.47, EtOH).

A solution of the diacetate (82 mg) in acetic acid–dichloromethane (50 mL, 1:1) was ozonized at 0 °C using a stream of ozone (2–4%) from an Ozonator, Model 03V2. When the ozonolysis was complete, after 10 h, 2 mL of 30% hydrogen peroxide was added and the reaction mixture was stirred at 50 °C for 1 h. Unreacted hydrogen peroxide was decomposed with sodium sulfite and the solvent was removed in vacuo. Excess saturated aqueous sodium bicarbonate was added to the residue and the solution was extracted with hexane. The aqueous layer was then acidified with hydrochloric acid, saturated with sodium chloride, and extracted several times with ethyl acetate. The ethyl acetate extract was washed with saturated sodium chloride, dried over sodium sulfate, and concentrated in vacuo. The NMR of this crude reaction mixture showed that 2,3-diacetoxysuccinic acid was produced in ~90% yield. A solution of this residue in ether was then esterified with freshly prepared diazomethane. The ether was then removed; distillation of the residue (97 ~ 99 °C/0.2 mmHg) yielded a colorless oil which was crystallized from hexane to give a pure sample of (+)-dimethyl (2*S*,3*S*)-diacetoxysuccinate: 44 mg; 68% yield; mp 103 °C; $[\alpha]^{25}_D$ +21.9° (*c* 1.52, $CHCl_3$). The optical purity of this sample was 92%, as shown by comparison with an authentic sample prepared from (−)-tartaric acid.

A solution of (−)-(2*S*,3*S*)-tartaric acid (75 mg) in 10 mL of methanol containing 0.3 mL of concentrated HCl was refluxed for 1 h and the solvent was removed in vacuo. The residue was treated with acetic anhydride (10 mL) and pyridine (2 mL), heated for 1 h on a steam bath, and poured into water. The mixture was extracted with benzene, and the organic phase was washed with 10% HCl and water, dried over anhydrous sodium sulfate, and concentrated. The resulting crude mixture was separated by thick-layer chromatography (silica gel, ethyl acetate:hexane, 1:9) to provide the (+)-dimethyl (2*S*,3*S*)-diacetoxysuccinate in an overall yield of 89%, 116 mg, recrystallized from hexane: mp 103 °C; $[\alpha]^{25}_D$ +23.7° (*c* 1.52, $CHCl_3$); NMR (in $CDCl_3$) δ 2.18 (6 H, s), 3.80 (6 H, s), and 5.68 (2 H, s). Anal. Calcd for $C_{10}H_{14}O_8$: C, 45.80; H, 5.34. Found: C, 45.81; H, 5.29.

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Registry No.—*threo*-**3e,** 66768-16-9; *erythro*-**4e,** 66768-17-0; *p*-bromotoluene, 106-38-7; 1-bromo-4-chlorobenzene, 106-39-8; *p*-bromoanisol, 104-92-7; 2,3-diacetoxysuccinic acid, 66749-60-8; (+)-dimethyl (2*S*,3*S*)-diacetoxysuccinate, 6304-92-3; (−)-(2*S*,3*S*)-tartaric acid, 147-71-7.

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