treated with bromine in the presence of epichlorohydrin as an acid scavenger[7] to afford the 4-bromo-3-one **9**: λ_{max}^{film} 5.78 and 5.82 μ. Reaction of **9** with semicarbazide, followed by hydrolysis (aqueous pyruvic acid) of the intermediary α,β-unsaturated semicarbazone[8] and purification by preparative tlc on silica gel (1:9 ethyl acetate–hexane; continuous elution for 4 hr), gave *dl*-testosterone benzoate (**10**), mp 167–178° (homogeneous by tlc), in 45% yield overall from **4** (18% yield overall from trienynol **1**). A sample was recrystallized from absolute ethanol three times to give colorless plates: mp 184–185.5 (*Anal.* Found: C, 79.5; H, 8.2); $\lambda_{max}^{CHCl_3}$ 5.83, 6.01, 7.81, and 8.91 μ. The nmr spectrum[3] included singlets at δ 0.98 (3 H), 1.20 (3 H), and 5.75 (1 H, olefinic proton). This sample of racemic material was identical by nmr, solution ir, and tlc with an authentic, naturally derived specimen of testosterone benzoate.

Acknowledgment. We are indebted to the National Institutes of Health and the National Science Foundation for support of this research.

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(8) *Cf.* B. J. Magerlein, D. A. Lyttle, and R. H. Levin, *ibid.*, **20**, 1709 (1955).

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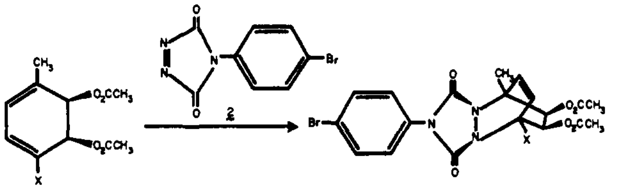
X-Ray Determination of the Absolute Stereochemistry of the Initial Oxidation Product Formed from Toluene by *Pseudomonas putida* 39/D

Sir:

Mammalian enzymes incorporate one atom of molecular oxygen into aromatic hydrocarbons with the resultant formation of arene oxides.[1] Subsequent enzymatic hydration produces trans diols.[2] A different oxidation pathway has been observed in bacteria. Both atoms of molecular oxygen are incorporated into aromatic hydrocarbons and cis diols are the first detectable oxygenated products.[3] However, assignments of cis stereochemistry by infrared spectrometry, by the Karplus equation, or by reaction rates with potassium triacetylosmate are not unambiguous.[4] We now report the absolute stereochemistry of the cis diol that is formed during the oxidation of toluene by *Pseudomonas putida* 39/D. This organism oxidizes several monocyclic aromatic compounds to cis diols.[5]

Toluene was oxidized to (+)-3-methyl-3,5-cyclohexadiene-1,2-diol by *Pseudomonas putida* 39/D and converted to the diacetate **1** as previously described.[5] Freshly sublimed (100° (0.1 mm)) 4-(*p*-bromophenyl)-1,2,4-triazoline-3,5-dione (**2**) in dry acetone (1:10 wt/wt) was added dropwise at 0° to an equimolar amount of **1** in dry acetone (1:10 wt/wt).[6] Throughout the course of addition, the deep red color of **2** was immediately discharged; upon addition of excess reagent, the red color remained.[7] The solvent was removed from the reaction mixture and the crude residue recrystallized two times from hexane–acetone (~1:10, v/v) to give a crystalline adduct in 70% yield: mp 174°; molecular ion mass calcd for $^{12}C_{19}{}^{1}H_{18}{}^{14}N_3{}^{16}O_6{}^{79}Br$, 463.0387; found, 463.0379. On the basis of the ir (5.7, 5.85, 6.9, 7.3, 12.1, 12.3, and 12.7 μ; Nujol mull–NaCl) and 60-MHz pmr (δ 1.9 (s, 1 H), 1.98 (s, 1 H), 5.4 (d of d, J = 8 Hz, 1 H), 6.4 (m, 2 H), 7.35 (d, J = 9 Hz, 2 H), 7.6 (d, J = 9 Hz, 2 H); saturated solution in acetone-d_6) spectral data, the product was assigned the Diels–Alder cycloaddition structure 8,9-diacetoxy-1-methyl-4-(*p*-bromophenyl)-2,4,6-triazatricyclo[5.2.2.0^{4,6}]undec-10-ene-3,5-dione (**3**).



1, X = H
4, X = Cl

3, X = H
5, X = Cl

2 was chosen for characterization of dihydrodiols for the following reasons: (1) the 1,2,4-triazoline-3,5-diones, as a class, are among the most reactive dienophiles known[8] permitting rapid cycloaddition to diacetates, thus minimizing the competitive, labile aromatization of these compounds *via* elimination of acetic acid; *e.g.*, attempted cycloaddition of *p*-chlorotoluenediol diacetate (**4**) and maleic anhydride gave only the *p*-chlorocresyl acetates; in contrast, the cycloaddition of **2** and **4** was complete within 20 min at 0° yielding **5**; (2) Diels–Alder cycloaddition, in the main, occurs cis with retention of configuration of the constituent atoms; thus the stereochemistry of the acetoxy methine carbons should not be affected;[9] and (3) the bromine atom incorporated in **2** permitted the determination *via* X-ray diffraction methods of the absolute stereochemistry of **3** as 8(*S*),9(*R*)-diacetoxy-1(*S*)-methyl-4-(*p*-bromophenyl)-2(*R*), 4, 6(*S*)-triazatricyclo[5.2.2.0^{4,6}]undec-10-ene-3,5-dione, and thus that of the parent toluene diol as 1*S*,2*R*.

Crystals of **3** are monoclinic, $P2_1$, a = 6.822, b = 24.381, and c = 5.950 Å, β = 94.40°, Z = 2. Using the 2167 observed reflections of 2223 measured with Cu Kα radiation, the structure was solved by the heavy atom method, after location of the bromine atom from a Patterson map. The structure has been refined, including all hydrogen atoms, to a conventional R value of 0.060 and a weighted R value of 0.060, omitting the

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(1) D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *Biochemistry*, **9**, 147 (1970).

(2) D. M. Jerina, J. W. Daly, and B. J. Witkop, *J. Amer. Chem. Soc.*, **89**, 5488 (1967).

(3) D. T. Gibson, *Crit. Rev. Microbiol.*, **1**, 199 (1971).

(4) (a) A. R. H. Cole and P. R. Jeffries, *J. Chem. Soc.*, 4391 (1956); (b) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963); (c) R. Criegee, R. Marchand, and H. Wannowius, *Justus Liebigs Ann. Chem.*, **550**, 99 (1942); (d) refer to references in B. R. Brown and H. A. H. MacBride, *J. Chem. Soc.*, 3822 (1964).

(5) D. T. Gibson, M. Hensley, H. Yoshioka, and T. J. Mabry, *Biochemistry*, **9**, 1626 (1970).

(6) Prepared by the method of J. C. Stickler and W. H. Pirkle, *J. Org. Chem.*, **31**, 3445 (1966), in 30% yield based on *p*-bromophenyl isocyanate.

(7) Upon exposure to light, solutions of **1** and excess **2** in acetone at ambient temperature soon discharge their color; on the other hand, such solutions kept in the dark retain their color for 2 or more days. I. Stevens, *et al.*, *J. Chem. Soc. C*, 1905 (1967), have observed rapid addition of refluxing acetone to 4-phenyl-1,2,4-triazoline-3,5-dione to yield the nominal 2-hydroxy-2-propene "ene" adduct.

(8) *Cf.* Stevens, *et al.*, cited in ref 7.

(9) For a discussion of Diels–Alder reaction stereochemistry, see C. H. Depuy and O. L. Chapman, "Molecular Reactions and Photochemistry," Prentice-Hall, Englewood Cliffs, N. J., 1972, p 140.

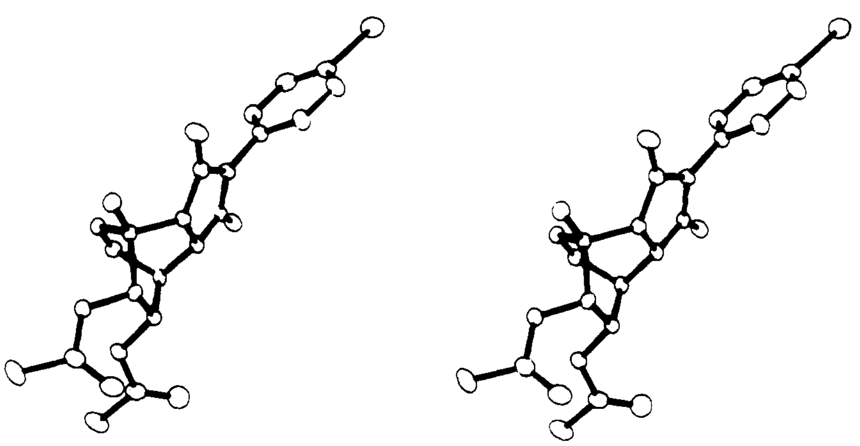

Figure 1. Stereoscopic view of **3**.

imaginary component, $\Delta f''$, of the bromine atom, and making no anomalous dispersion corrections for carbon, oxygen, or hydrogen.

The absolute configuration was determined from the X-ray diffraction data by two methods. In the first of these, each enantiomer was refined, by least-squares methods, to convergence, after inclusion of the $\Delta f''$ correction to the scattering factor of Br. These refinements resulted in R values of 0.057 for the $8S,9R$ structure and 0.061 for the $8R,9S$ structure. Using Hamilton's tables of significance points for R-value ratios,[10] the $8R,9S$ structure may be rejected above the 99.5% confidence level; thus, we conclude that the $8S,9R$ structure (Figure 1) is the correct one.

In the second method, intensity ratios were calculated for Bijvoet pairs, assuming the $8S,9R$ structure and including the bromine $\Delta f''$ term. For several Bijvoet pairs for which ratios quite different from 1.0 were calculated, and for which the intensity was sufficiently large to ensure small random counting error, intensity ratios were determined experimentally, using the same crystal for which the original intensity data had been collected. The results of these measurements are shown in Table I. The uniform agreement of calculated and observed ratios in the direction of deviation from 1.0 again confirms the correctness of the assumed structure, $8S,9R$.

Table I. Calculated and Observed Intensity Ratios for Some Bijvoet Pairs

h	k	l	$I(hkl)/I(\bar{h}\bar{k}\bar{l})$ Calcd[a]	$I(hkl)/I(\bar{h}\bar{k}\bar{l})$ Obsd[b]
0	12	0	1.22	1.35
1	12	0	1.32	1.40
2	8	0	0.74	0.86
3	12	0	1.25	1.27
4	6	0	1.22	1.22
1	14	1	0.72	0.88
2	11	1	0.79	0.91
0	7	1	0.66	0.81
2	7	2	0.80	0.93
1	2	1	0.49	0.59
0	4	4	1.26	1.25
1	3	−2	1.29	1.27

[a] Calculated with coordinates for the $8S,9R$ structure and $\Delta f''$ for Br = 1.46. [b] Based only on counting statistics, the estimated error in observed ratios does not exceed 2%.

The final structure, including the determined absolute stereochemistry, is shown in Figure 1. There are no unusual bond distances, bond angles, or conformational features. A description of the structure determination and refinement, as well as details of molecular geometry, will be published later (R. E. D. and A. G.). The absolute stereochemistry of the toluenediol has also been determined by chemical and spectrophotometric studies.[11]

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(11) H. Ziffer, D. M. Jerina, D. T. Gibson, and V. M. Kobal, *J. Amer. Chem. Soc.*, submitted for publication.

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Secondary Deuterium Isotope Effects in Pericyclic Reactions. The Thermal Rearrangement of Dideuteriobiscyclopropylidene

Sir:

For some time now we have been intrigued by the observation in our laboratory[1] and others[2] of a normal isotope effect in the product-forming destruction of resonance-stabilized biradicals. Such an effect in an apparently bond-forming process was of obvious mechanistic significance, since simple bond formation is generally expected to give rise to an *inverse* secondary deuterium isotope effect.

In a recent paper, we proposed that such isotope effects derive from *rotation* of a methylene group of a

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