Rapid Paper

Microbial Oxygenation of Organo-metallic Bissulfides Leading to Formation of Planar Chirality and C_2 Symmetry

Yoshimitsu YAMAZAKI and Kuniaki HOSONO

Fermentation Research Institute,
Agency of Industrial Science and Technology,
Tsukuba, Ibaraki 305, Japan

Received May 1, 1990

1,2-Bis(methylthiomethyl)ferrocene (**3**) was oxidized by *Corynebacterium equi* IFO 3730 to give monosulfoxide **4** in two diastereomeric forms with (1*S*,2*R*,*S*_S) and (1*S*,2*R*,*R*_S) configurations in a ratio of 4:1, while 1,1'-bis(methylthiomethyl)ferrocene (**5**) was oxidized by *Penicillium frequentans* IFO 5692 to (*R*)-monosulfoxide **6** and then preferentially to (*R*,*R*)-bissulfoxide **7**. Thus, the bacterial monooxygenase generated specific planar chirality in the metallocenic monosulfoxide, and the fungal enzyme formed C_2 symmetry in the bissulfoxide.

Asymmetric sulfoxides¹⁾ and chiral metallocene derivatives²⁾ are useful as chiral auxiliaries in organic synthesis. Fusion of both structural units is intriguing with respect to achieving higher stereoselectivity in their applications. Optically active sulfoxides can be conveniently prepared by microbial oxygenation of the precursor sulfides,³⁾ but little attention has been paid to the biooxidation of organometallic sulfides. Recently, we have reported that some kinds of microorganisms asymmetrically oxidized a metallocenic sulfide, (methylthiomethyl)ferrocene (**1**), to the (*R*)- or (*S*)-sulfoxide (**2**).^{4a)} Our continuing interest in the asymmetric bioconversion of organometallic compounds⁴⁾ prompted us to expand this reaction to the bissulfides in order to give metallocenes more complex molecular chirality.

A preliminary screening of 144 strains of yeasts, molds and bacteria showed that homoannular bissulfide **3** was oxidized mainly by coryneform bacteria (e.g., *Corynebacterium equi* IFO 3730 and *Arthrobacter simplex* IAM 1660). These strains were inactive or slightly

* PMR and/or MS data are consistent with each structure.

active for heteroannular bissulfide **5**, which was readily oxidized by such yeasts and molds as *Rhodotorula rubra* IFO 889 and *Penicillium frequentans* IFO 5692.

Homoannular bissulfide **3** was treated in shaken cultures of *C. equi* to give the dextrorotatory monosulfoxide (**4**) in 38% yield. Its PMR spectrum showed two singlets for the sulfinyl methyl protons (2.4H at δ 1.92 and 0.6H at δ 1.97), indicating that (+)-**4** was a mixture of two diastereomers in a ratio of 4:1.

To clarify this stereochemistry, a stereospecifically trideuteriated substrate, (1*S*,2*R*)-1-[methylthio($^2\text{H}_2$)methyl]-2-[(*S*)-methylthio($^2\text{H}_1$)methyl]ferrocene ($(^2\text{H}_3)$ -**3**), was prepared from (1*S*,2*R*)-1-[hydroxy($^2\text{H}_2$)methyl]-2-[(*S*)-hydroxy($^2\text{H}_1$)methyl]ferrocene (**8**)^{4b} and oxi-

dized by either the bacterium or NaIO_4 . The chemically prepared monosulfoxide ($(^2\text{H}_3)$ -**4**) showed 4 broad triplets ($J_{\text{HD}}=1.5$ Hz) for the methylene proton in the PMR spectrum (Fig. 1), indicating the occurrence of all possible stereoisomers. It may be reasonable to consider that the signal at the lowest frequency (δ 3.57) was due to one stereoisomer oxidized on the monodeuteriated side chain ($(1*R*,2*S*,*R}_S)*$ - $(^2\text{H}_3)$ -**4** or $(1*R*,2*S*,*S}_S)*$ - $(^2\text{H}_3)$ -**4**) and that at the highest frequency (δ 3.13) was due to another stereoisomer oxidized on the other side chain ($(1*S*,2*R*,*R}_S)*$ - $(^2\text{H}_3)$ -**4** or $(1*S*,2*R*,*S}_S)*$ - $(^2\text{H}_3)$ -**4**).

The treatment of $(^2\text{H}_3)$ -**3** with *C. equi* gave dextrorotatory monosulfoxide (+)- $(^2\text{H}_3)$ -**4** in 34% yield. Its PMR spectrum showed two

Fig. 1. PMR Spectra in Benzene- d_6 of $(^2\text{H}_3)$ -**4** Prepared by Chemical Oxidation (whole spectrum and expanded inset a) and by Microbial Oxidation (inset b).

Assignments for the numbered signals: 1, $-\text{SCH}_3$; 2 and 3, $-(\text{SO})\text{CH}_3$; 4-7, $-\text{C}^2\text{HHS}-$; 8 and 9 (t, $J=2.5$ Hz), H-4; 10 and 11, unsubst. ring; 12, H-3; 13 and 14, H-5. Chemically prepared $(^2\text{H}_3)$ -**4** contained all the stereoisomers illustrated in the figure. According to the conclusion in the text, signal 4 (δ 3.13) represents $(1*S*,2*R*,*S}_S)*$ - $(^2\text{H}_3)$ -**4**, and signal 5 (δ 3.31) $(1*S*,2*R*,*R}_S)*$ - $(^2\text{H}_3)$ -**4**. The intensities of signals 4, 5, 6 and 7 are in the ratio of 3:2:2:3 (integral curves omitted). This fact indicates that the stereoisomers for signals 4 and 7 are an enantiomeric pair and those for signals 5 and 6 are another enantiomeric pair, after neglecting the deuterium atoms. Therefore, signals 7 and 6 represent $(1*R*,2*S*,*R}_S)*$ - $(^2\text{H}_3)$ -**4** and $(1*R*,2*S*,*S}_S)*$ - $(^2\text{H}_3)$ -**4**, respectively. Signals 2, 8, 10 and 14 are due to major stereoisomers ($(1*S*,2*R*,*S}_S)*$ - and $(1*R*,2*S*,*R}_S)*$ - $(^2\text{H}_3)$ -**4**), and signals 3, 9, 11 and 13 are due to minor stereoisomers ($(1*S*,2*R*,*R}_S)*$ - and $(1*R*,2*S*,*S}_S)*$ - $(^2\text{H}_3)$ -**4**). Signals 1 and 12 are not split by the isomerism. The conserved stereochemistry in the substitution of **8** is suggested by ref. 6.

sulfinyl methyl singlets (2.5H at δ 1.92 and 0.5H at δ 1.97) similarly to (+)-**4**, but for the methylene proton, only two broad triplets at δ 3.13 and 3.31 (Fig. 1b). This fact indicates that (+)-($^2\text{H}_3$)-**4** was composed of two stereoisomers, and that they were in a diastereomeric relationship (not enantiomeric) even if the deuteriated asymmetric center is left out of consideration. Since the major stereoisomer corresponded to the signal at δ 3.13, it has either the (1*S*,2*R*,*R*_{*S*}) or (1*S*,2*R*,*S*_{*S*}) configuration.

We then took the PMR spectrum of (+)-($^2\text{H}_3$)-**4** in the presence of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE). The spectrum of the methyl protons is depicted in

Fig. 2b together with that recorded for chemically prepared ($^2\text{H}_3$)-**4** (Fig. 2a). The configurational assignments about the asymmetric sulfur atom given in Fig. 2a are based on the solvation model for sulfoxides with TFAE and the shielding effect by the anthryl group.^{3b,4a)} A comparison between Fig. 2b and Fig. 2a reveals the absolute configuration of the sulfinyl group to be (*S*) for the major stereoisomer of (+)-($^2\text{H}_3$)-**4**, and (*R*) for the minor one. The whole configuration of the former proves to be (1*S*,2*R*,*S*_{*S*}). The aforementioned diastereomeric relationship between the major and minor stereoisomers coupled with the (*R*) sulfinyl configuration for the minor stereoisomer determined its configuration to be (1*S*,2*R*,*R*_{*S*}). Thus, the planar chirality was the same for both isomeric components.

A. simplex gave (1*S*,2*R*,*R*_{*S*})-**4** (70%) and (1*S*,2*R*,*S*_{*S*})-**4** (17%) as the predominant products in the oxidation of **3**. It can be concluded that the bacterial monooxygenases were not very stereoselective in forming a chiral center about the sulfur atom, but that their enantiotopic differentiation between two side chains was considerable so as to generate a single planar chirality in the metallocenic sulfoxide.

Heteroannular bissulfide **5** was oxidized by a liquid culture of *P. frequentans* for 24 hr to give monosulfoxide (–)-**6** in 75% yield and bissulfoxide (–)-**7** in 3% yield. The absolute configuration of the sulfinyl group was (*R*) for (–)-**6** and (*R,R*) for (–)-**7** as determined by the PMR method. The structure of (*R,R*)-**7** has *C*₂ symmetry. This method also showed that both products were enantiomerically pure (96% *e.e.* for (–)-**6** and >97% *e.e.* for (–)-**7**), but that the latter was contaminated by the meso form (21%; see Fig. 2d). However, the yield of (–)-**7** was increased to 47% in a prolonged reaction (48 hr), and its recrystallization from ethyl acetate gave (*R,R*)-(–)-**7** with 95% purity.

The stereochemistry of these products and (*R*)-(–)-**2**^{4a)} formed by *P. frequentans* implies that the enantioselectivity in this fungal oxygenation process of one sulfide group was not affected by the presence of the other sulfide

Fig. 2. PMR Signals for the Sulfinylmethyl Group of 8 mm ($^2\text{H}_3$)-**4** (a), (+)-($^2\text{H}_3$)-**4** (b), ($\pm$)-**7** (c) and (–)-**7** (d) in the Presence of 25 mM TFAE in Benzene-*d*₆.

Subscripts I and II in (a) represent the major and minor diastereomeric components, respectively.

group in the molecule, but its degree decreased once one group was oxidized. The same tendency was found in the oxidation of **5** by *R. rubra*, as the optical purity of (–)-**6** was good (89% *e.e.*), whereas (–)-**7** was a mixture of (*R,R*)-**7** (97% *e.e.*) and (*R,S*)-**7** in a ratio of 3:4.

This work is the first to report how the planar chirality and C_2 symmetry in metallocenes are formed by microbial oxygenation. The products, (+)-**4**, (–)-**6** and (–)-**7**, are potentially useful as new chiral bidentate ligands for the chemistry of complex.

Experimental

Physical constants and spectroscopic data were obtained with the same instruments as those used previously.⁴⁾ The substrates (**3**, (²H₃)-**3** and **5**)* were prepared from 1,2-bis(hydroxymethyl)ferrocene,^{4b)} **8**,^{4b)} and 1,1'-bis(hydroxymethyl)ferrocene,⁵⁾ respectively, essentially according to the literature.⁶⁾ The authentic sulfoxides (**4**, **6** and **7**)* were prepared by NaIO₄ oxidation of **3** and **5** at 4°C in MeOH.⁷⁾

Initial screening. The microorganisms were mainly from IFO and IAM culture collections. Substrate **3** or **5** (2 mg in 20 μ l DMF) was added to each culture (8 ml, 24-hr old) in YM^{4c)} or nutrient medium⁸⁾ and shaken at 30°C for 48 hr before ether extraction and TLC analysis.

Microbial oxidation of 3. *C. equi* was grown in the nutrient medium (80 ml per 500-ml conical flask) with shaking (170 rpm) at 30°C for 16 hr. Forty milligrams of **3** (in 120 μ l DMF) were added to 6 cultures in equal portions. After shaking for 15 hr, the cells were removed by centrifugation and washed with 70% EtOH/H₂O (50 ml $\times$ 2). The supernatant and washings were combined, saturated with NaCl, and extracted with EtOAc (500, 300 and 200 ml). After drying over Na₂SO₄, the combined EtOAc layer was concentrated to give an oil, which was chromatographed on a silica gel column (1.2 $\times$ 18 cm), eluting with EtOAc and 5–10% MeOH/EtOAc. The yellow fraction was evaporated to afford 16 mg (38% yield) of 1-(methylsulfinylmethyl)-2-(methylthiomethyl)ferrocene (**4**) as a yellow oil, $[\alpha]_D^{25} + 46^\circ$ ($c=0.5$, EtOH), MS m/z : 322.0099 (M^+); calc. C₁₄H₁₈FeOS₂ = 322.0149, PMR δ (benzene-*d*₆): 1.76 (3H, s, –SCH₃), 1.92 (2.4H, s, –SOCH₃), 1.97 (0.6H, s, –SOCH₃), 3.12–3.36 (3.2H, m, –CH₂S– and –CH₂SO–), 3.60 (0.8H, d, $J=13.5$ Hz, –CHHSO–), 3.85 (0.8H, t, $J=2.5$ Hz, H-4), 3.87 (0.2H, t, $J=2.5$ Hz, H-4), 3.91 (4H, s, unsubst. ring), 3.94 (1H, s, unsubst. ring), 3.99 (1H, m, H-3), 4.08 (0.2H, m, H-5) and 4.12 (0.8H, m, H-5).

The same experiment as this was done with (²H₃)-**3** to give (+)-(²H₃)-**4** in 34% yield, $[\alpha]_D^{25} + 46^\circ$ ($c=0.5$, EtOH), MS m/z : 325.0347 (M^+); calc. C₁₄H₁₅²H₃FeOS₂ = 325.0337.* The oxidation of **3** by *A. simplex* gave (+)-**4** (45% yield) with $[\alpha]_D^{25} + 14^\circ$ ($c=0.5$, EtOH).

Microbial oxidation of 5. *P. frequentans* was grown in the YM medium (80 ml per flask) with shaking at 30°C for 31 hr. Bissulfide **5** (75 mg in 0.3 ml DMF) was added to 3 cultures in equal portions. After shaking for 24 hr, the cells were removed by filtration. The subsequent EtOAc extraction and chromatographic purification were done in the usual way to give 59 mg (75% yield) of monosulfoxide **6** as a yellow oil, $[\alpha]_D^{24} - 54^\circ$ ($c=2.3$, EtOH), MS m/z : 322.0169 (M^+); calc. C₁₄H₁₈FeOS₂ = 322.0149* and 2.8 mg (3% yield) of bissulfoxide **7** as yellow needles (EtOAc), mp 120–122°C, $[\alpha]_D^{24} - 141^\circ$ ($c=0.3$, EtOH), MS m/z : 338.0110 (M^+); calc. C₁₄H₁₈FeO₂S₂ = 338.0098.* In another run, the bioconversion was continued for 48 hr to give (–)-**6** and (–)-**7** in 11% and 47% yields, respectively. This specimen of (–)-**7** was twice recrystallized from EtOAc to afford pure (*R,R*)-(–)-**7** in an overall yield of 19% (from **5**), mp 129–130°C, $[\alpha]_D^{24} - 192^\circ$ ($c=0.67$, EtOH), PMR δ (benzene-*d*₆): 1.81 (6H, s, (–SOCH₃)₂), 3.11 (2H, d, $J=13.4$ Hz, (–CHHSO–)₂), 3.21 (2H, d, $J=13.4$ Hz, (–CHHSO–)₂), 3.83 (6H, m, ring protons) and 3.98 (2H, m, ring protons).

A similar treatment of **5** with *R. rubra* gave (–)-**6** in 40% yield, $[\alpha]_D^{24} - 51^\circ$ ($c=0.5$, EtOH), and (–)-**7** in 12% yield, $[\alpha]_D^{24} - 34^\circ$ ($c=0.5$, EtOH).

References

- 1) G. H. Posner, "The Chemistry of Sulphones and Sulphoxides," ed. by S. Patai, Z. Rappoport and C. J. M. Stirling, John Wiley & Sons Ltd., New York, 1988, p. 823.
- 2) T. Hayashi, *Pure Appl. Chem.*, **60**, 7 (1988).
- 3) (a) H. L. Holland, *Chem. Rev.*, **88**, 473 (1988); (b) Y. Okamoto, H. Ohta and G. Tsuchihashi, *Chem. Lett.*, **1986**, 2049.
- 4) (a) Y. Yamazaki and K. Hosono, *Biotechnol. Lett.*, **11**, 627 (1989); (b) Y. Yamazaki, M. Uebayasi and K. Hosono, *Eur. J. Biochem.*, **184**, 671 (1989); (c) Y. Yamazaki, M. Uebayasi, J. Someya and K. Hosono, *Agric. Biol. Chem.*, **54**, 1781 (1990); (d) Y. Yamazaki and K. Hosono, *Tetrahedron Lett.*, **31**, 3895 (1990).
- 5) A. Sonoda and I. Moritani, *J. Organometall. Chem.*, **26**, 133 (1971).
- 6) A. Ratajczak and B. Misterkiewicz, *J. Organometall. Chem.*, **91**, 73 (1975).
- 7) L. L. Replogle and J. R. Maynard, *J. Org. Chem.*, **32**, 1909 (1967).
- 8) Y. Yamazaki and H. Maeda, *Agric. Biol. Chem.*, **49**, 3203 (1985).