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STEREOSELECTIVE OXIDATION OF *GEM*-DISULPHIDES WITH *ASPERGILLUS NIGER*

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Abstract—A strain of *Aspergillus niger* was used to prepare optically active *gem*-disulphide S-oxides **2** and **3** and the (*s*)-*meso*-disulphoxide **4**. The structural assignment was made by intercorrelating the senses of NMR non-equivalence in pseudoasymmetric disulphoxides **4** and **5** of established configurations with the configurationally related monosulphoxides **2** and **3**. The enantiomeric composition and absolute configurations were determined using the chiral solvent NMR method. The stereoselectivity in the microbial oxidation was found to be independent of the configuration of the incipient chiral centre at carbon.

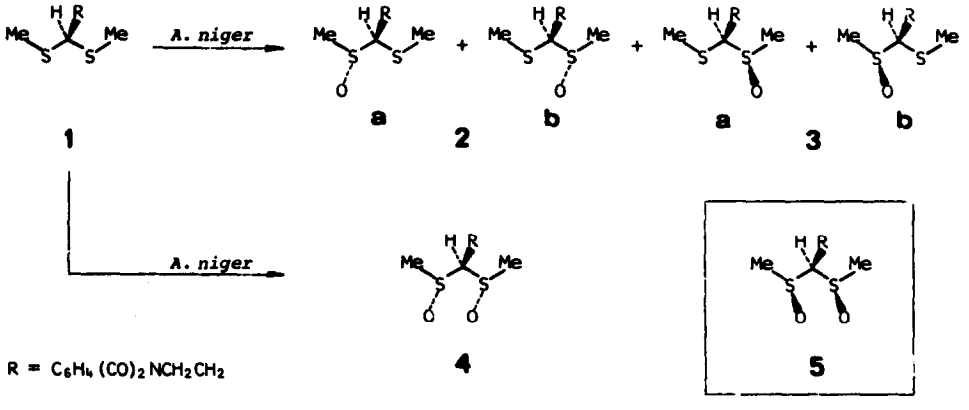
Studies on microbial metabolic processes in which O atom transfer to the sulphide occurs have shown that the stereopreference in the oxidation depends on the substrate[1] as well as on the strain and species.[2] These studies have been extended in the present work in which 1,1-bis-methylthio-3-phthalimidopropane[3] (**1**) was used as the substrate for microbial oxidation in order to study the influence of an incipient chiral centre at carbon on the stereopreference in the oxidation to sulphoxides.

Prochiral *gem*-disulphide **1** has two enantiotopic methylthio ligands, each containing two diastereotopic lone-pair sites at sulphur. The fermentation of **1** with *Aspergillus niger* NRRL 337 using the method similar to that described previously,[2] produced the diastereomeric mixture of 1 - methylsulphinyl - 1 - methylthio - 3 - phthalimidopropane (**2** and **3**, 44%), $[\alpha]_D^{22}+24.9°$ ($CHCl_3$). Sulphoxides were isolated by extraction with chloroform and chromatography, but the separation of diastereomers **2** and **3** failed. However, the information concerning the diastereomer ratios and configurations was derivable from the NMR spectrum of the mixture. The senses of non-equivalence (when discernible) for all protons within a given group of the configurationally related mono- and disulphoxides, **2**→**4**, and **3**→**5**, would be expected to be the same. Pseudoasymmetric (*s*)-*meso* **4** and (*r*)-*meso*-disulphoxide **5** of established configurations,[5] have been intercorrelated with the monosulphoxide pairs **2** and **3**, and the diastereomeric ratio **2**/**3** of about 60:40 was determined by integration of methylsulphinyl NMR signals at δ 2.70 and 2.53, respectively. The use of chiral solvent, (*R*)-(−) - 1 - phenyl - 2,2,2 - trifluoroethanol (**6**), allowed the direct determination of enantiomeric composition and empirical assignment of absolute configurations according to Pirkle *et al.*[6] As estimated by NMR (**6**, CCl_4) the ratio of signals ($SOCH_3$) at δ 2.66(**2a**)/2.64(**2b**) was 44:16, and the ratio at δ 2.49(**3a**)/2.47(**3b**) was 29:11.

The oxidation of **1** with (1*S*)-(+)-monopercamphoric acid at −20° proceeded with low optical result to yield the diastereomeric mixture **2**+**3** (88%), $[\alpha]_D^{22}-0.8°$ ($CHCl_3$), the enantiomeric composition being extremely sensitive to the experimental conditions. The diastereomeric ratio **2**/**3**, however, was identical to that obtained by oxidation with *A. niger*.

In a modified experiment, using the aerated fermentation vessel (Fig. 1), (*s*) - 1,1 - bis - (methylsulphinyl) - 3 - phthalimidopropane[5] (**4**, 16%) was the main oxidation product of *A. niger* NRRL 337, and diastereomeric monosulphoxides **2** and **3** were produced in a low yield (7%) showing no optical activity.

The results strengthen the conclusion that the optical activity of sulphoxides **2** and **3** obtained by microbial oxidation from the prochiral *gem*-disulphide **1** arises mainly from the asymmetric transfer of oxygen in the



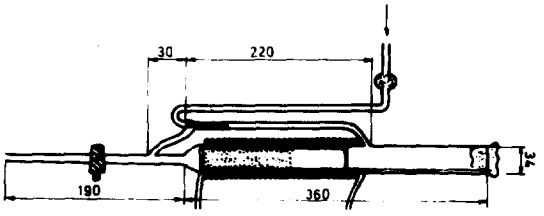


Fig. 1.

initial stage. The configuration of the incipient chiral centre at carbon showed no directive effect in the oxidation with *A. niger*: approximately the same enantiomeric ratio **2a/2b** and **3a/3b** revealed the (*R*)-stereopreference in the conversion to diastereomeric sulphoxides. The diastereomeric ratio **2/3**, however, reflects the relative thermodynamic stabilities of the two, as assessed by comparison with the results of chemical oxidations. NMR spectra of analogous *gem*-disulphides, reported earlier,[7] show similar shielding characteristics, and the predominant (*R,S*)/(*S,R*) diastereomeric pair in the mixture can be determined in a simple way from the signs of differences in the signal positions of constitutionally equivalent protons.

The over-oxidation of **1** in the fermentation with *A. niger* afforded the (*s*)-*meso*-disulphoxide **4** providing the first example of a diastereospecific microbial oxidation into a pseudoasymmetric *gem*-disulphoxide. It is noteworthy that no sulphones were detected among the products obtained by oxidation with *A. niger*. Combined recoveries of sulphide and sulphoxides in fermentations were from 30–50%, showing that other reactions are occurring. It is likely that under the reaction conditions the CO equivalent groups in monosulphoxides **2** and **3** as well as in disulphoxide **4**, could be further degraded to various sulphur-free products.

Although a number of techniques have been developed for the preparation of optically active sulphoxides,[4] only two methods were appropriate for the preparation of optically active *gem*-disulphide *S*-oxides.[8,9] The present investigation has shown that these chiral carbonyl equivalent groups could also be prepared by oxidation in the presence of *Aspergillus niger*. Studies aimed at the conversion to optically active sulphoxides in this series are at present in progress in our laboratory.

EXPERIMENTAL

M.ps are uncorrected. The IR spectra were taken on a Perkin–Elmer 257 spectrometer. ^{1}H NMR spectra were recorded on a Varian T-60 spectrometer. Chemical shifts are given in δ units and coupling constants are expressed in Hz (s, singlet; d, doublet; t, triplet; q, quartet; umc, unresolved multiplet centre). Tlc was performed on Silica Gel HF_{254} (E. Merck) and spots developed with 1% $KMnO_4$ solution. Organic sulphides and sulphoxides were detectable with this reagent as intense yellow spots on pink background. In preparative tlc plates (40 × 30 cm) of Silica Gel PF_{254} (E. Merck), layer 1.0 mm, were used. Light petroleum refers to the fraction b.p. 40–60°.

Asperigillus niger NRRL 337 subcultured in Zagreb over 15 yr,[2] was maintained on wort agar slants at +4°. Inoculum for the fermentation was prepared by suspending the microbial sporae in sterile deionized water containing Tween 80, and with these suspensions seeding 2 Kolle flasks with the wort agar slope. The sporae resulting from the incubation for 5–7 days at 28° were used to inoculate fifty 500 ml Erlenmeyer flasks (5×10^7 sporae/ml) containing 100 ml each of a sterile medium (3% sucrose, 0.001% ferrous sulphate, 0.05% magnesium sulphate, 0.05% potassium chloride, 0.4% dipotassium phosphate, and 0.2% sodium nitrate at pH 4). After incubation for 48 hr on a rotary shaker (200 rpm) at 28° the mycelial pellets of *A. niger* NRRL 337 (about 1.5 mm thick) were obtained. The pellets were used in fermentation vessels filled with aforementioned nutrient broth. The stereoselectivity in the oxidation of phenyl benzyl sulphide with the pellets of *A. niger* NRRL 337 was in agreement with the results obtained previously.[2]

Oxidation of 1,1-*bis*-(*methylthio*)-3-*phthalimidopropane* **1** *with* Aspergillus niger

Diastereomers of 1 - *methylsulphinyl* - 1 - *methylthio* - 3 - *phthalimidopropane* **2** *and* **3**. A soln of **1**[3] (0.03 g) in MeOH (2 ml) was added to each of twenty 500 ml Erlenmeyer flasks containing the pellets of *A. niger* NRRL 337. After incubation for 72 hr on a reciprocal shaker (110 m/min) at 28°, the product was extracted from the filtrate and mycelia with $CHCl_3$. The extracts were washed and dried, and the solvent removed *in vacuo*. Tlc in $CHCl_3$ showed two intense spots (R_f 0.05 and 0.49). The spot R_f value 0.49 was identical with **1**. The crude mixture (0.0579 g) was purified by preparative tlc in the same solvent. The starting *gem*-**1** (0.051 g, 3.5%) and still impure **2** and **3** (0.384 g) were obtained. Further purification of diastereomeric sulphoxides by tlc in $CHCl_3$–MeOH (9:1) afforded the mixture of **2** and **3** (0.277 g, 44%). Crystallization from CH_2Cl_2–light petroleum gave the analytical sample as colourless needles, m.p. 133°, $[\alpha]_D^{22}$ + 24.9° (*c*, 3.25, $CHCl_3$). ν_{max} (KBr) 1775, 1715, 1397, 1377, 1043, 1031, 870, 720, 714 cm^{-1}; NMR ($CDCl_3$), (*R,S*/*S,R*)-form **2a,b** (60%): δ 7.78 (4H, umc, *o*-C_6H_4), 4.03 (2H, t, J = 6.5, NCH_2), 3.60 (1H, *ca.* q, $J_{AX} + J_{BX}$ = 14, CH), 2.70 (3H, s, $SOCH_3$), 2.27 (3H, s, SCH_3), 2.2–1.7 (2H, um, CH_2) and (*R,R*/*S,S*)-form **3a,b** (40%): δ 7.78 (4H, umc, *o*-C_6H_4), 4.00 (2H, t, J = 7.0, NCH_2), 3.63 (1H, *ca* q, $J_{AX} + J_{BX}$ = 12, CH), 2.53 (3H, s, $SOCH_3$), 2.30 (3H, s, SCH_3), 2.2–1.7 (2H, um, CH_2). Enantiomeric composition (±2%) was determined and absolute configuration assigned according to Pirkle[6] from the relative peak heights of the methylsulphinyl resonances in CCl_4 after addition of (*R*)-**6**: δ 2.66 **2a** (44%), 2.64 **2b** (16%), 2.49 **3a** (29%), 2.47 **3b** (11%). Other proton resonances were either obscured or exhibited unresolvable shift differences. (Found: C, 52.76; H, 5.56; S, 21.69. $C_{13}H_{15}NO_3S_2$ requires: C, 52.50; H, 5.08; S, 21.56%).

Oxidation of **1** *with* Aspergillus niger *under extensive aeration.* s - 1,1 - *Bis* - (*methylsulphinyl*) - 3 - *phthalimidopropane* **4**

A soln of **1**[3] (0.1 g) in MeOH (5 ml) was added to the mycelial pellets of *A. niger* NRRL 337 collected from five Erlenmeyer flasks. The pellets were transferred to a net immerged into the fermentation vessel (Fig. 1) and nutrient broth was added to a volume of 200 ml. Air was introduced (120 l/hr) under aseptic conditions. After incubation for 72 hr at 28°, the filtrate and mycelia were extracted with $CHCl_3$. From four experiments carried out under the same conditions 0.285 g of crude product was obtained. Tlc in $CHCl_3$–MeOH (9:1) showed spots R_f 0.69 (**1**), R_f 0.63 (**2** and **3**), and the most intense spot R_f 0.54. Purification by preparative tlc in the same solvent system afforded **1** (0.036 g, 9%), optically inactive mixture of **2** and **3** (0.03 g, 7%), and still impure **4** (0.13 g). Recromatography and subsequent crystallization from CH_2Cl_2–light petroleum gave pure **4**[5] (0.065 g, 16%) as colourless plates, m.p. 158°. ν_{max} (KBr) 1715, 1397, 1043, 722 cm^{-1}; NMR ($CDCl_3$), δ 7.81 (4H, umc, *o*-C_6H_4), 4.03 (2H, t, J = 6.5, NCH_2), 3.83 (1H, t, J = 7.0, CH), 2.88 (6H, s, $SOCH_3$), 2.55 (2H, *ca* q, CH_2), (Found: C, 49.93; H, 5.28; S, 20.80. $C_{13}H_{15}NO_4S_2$ requires: C, 49.82; H, 4.82; S, 20.46%). This product and a sample of **4** prepared from **1** by oxidation with sodium metaperiodate[5] were identical in all respects.

Oxidation of **1** *with* (1S)-(+)-*monopercamphoric acid*

Diastereomeric-(−)-*monosulphoxides* **2** *and* **3**. (1*S*)-Monopercamphoric acid was prepared by oxidation of (+)-camphoric anhydride with sodium peroxide, acidification, and extraction with $CHCl_3$.[9] The titrated $CHCl_3$ soln of peroxy-acid (5.5 mequiv) was added dropwise to a stirred soln of **1** (1.41 g, 5 mmol) in $CHCl_3$ (50 ml) with the temp maintained at −20° until the oxidant had disappeared (iodimetric test). At the end of the reaction

(after 6–7 hr) the $CHCl_3$ soln was repeatedly extracted with a sat $NaHCO_3$aq, washed with water, and dried (Na_2SO_4). The removal of the solvent and crystallization from CH_2Cl_2–light petroleum gave **2** and **3** (1.3 g, 88%) as colourless needles, m.p. 135–7°, $[\alpha]_D^{22} - 0.8°$ (*c*, 4, $CHCl_3$). Diastereomeric ratio **2/3** was 60:40, as estimated by NMR ($CDCl_3$). At temps above −20° optically inactive products were obtained.

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