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TETRAHEDRON: *ASYMMETRY*

Biocatalytic and chemical preparation of all four diastereomers of methionine sulfoxide

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Abstract

Biocatalytic or chemical oxidations can be used in a complementary manner for the preparation of all four diastereomers of methionine sulfoxide with high diastereomeric purity in overall isolated yields of 20–55% from methionine. The N-phthaloyl derivatives of L- and D-methionine were selectively oxidised to the (S_SS_C) and (S_SR_C) sulfoxides respectively by biotransformation using the fungus *Beauveria bassiana* ATCC 7159. Hydrogen peroxide oxidation of the same materials gave mixtures from which the (S_SS_C) and (R_SR_C) isomers can be readily isolated by crystallisation. Chromatography of the residual material then afforded the (R_SS_C) and (S_SR_C) isomers. © 1998 Elsevier Science Ltd. All rights reserved.

Sulfoxides of the natural amino acids methionine and *S*-methylcysteine are involved in a variety of biological processes. Enzymatic *S*-oxidation and reduction of methionine have been implicated in the modulation of potassium channel function,[1] and these reactions are also involved in cellular response to oxidative stress conditions.[2] *S*-Methylcysteine sulfoxide, present in cabbage, possesses antibacterial properties,[3] and the activity of this and related compounds as substrates for sulfoxide lyase enzymes is responsible for the formation of volatile sulfur-containing compounds in rotting or damaged vegetable tissue.[4] The absolute stereochemical requirements, if any, for these processes is currently unknown, although the (S_SS_C) diastereomer has been reported as the naturally occurring form of methionine sulfoxide in *Phormia regina*.[5,6] This lack of stereochemical data is compounded by a scarcity of methods for the preparation of diastereomerically defined amino acid sulfoxides. The existing method for the preparation of single diastereomers of L-methionine sulfoxide involves a tedious and low-yield separation of picrate salts:[7] no procedures have been reported for the preparation of single diastereomers of other natural amino acid sulfoxides, or for the diastereoselective sulfoxidation of any of the natural thioether amino acids in useful diastereomeric excesses.

As part of our ongoing investigation into the formation of chiral sulfoxides by microbial biotransformation,[8,9] we have screened for the oxidation of N-phthaloyl-protected L- and D-methionines[10] by a variety of biocatalysts, and have selected *Beauveria bassiana* ATCC 7159 for

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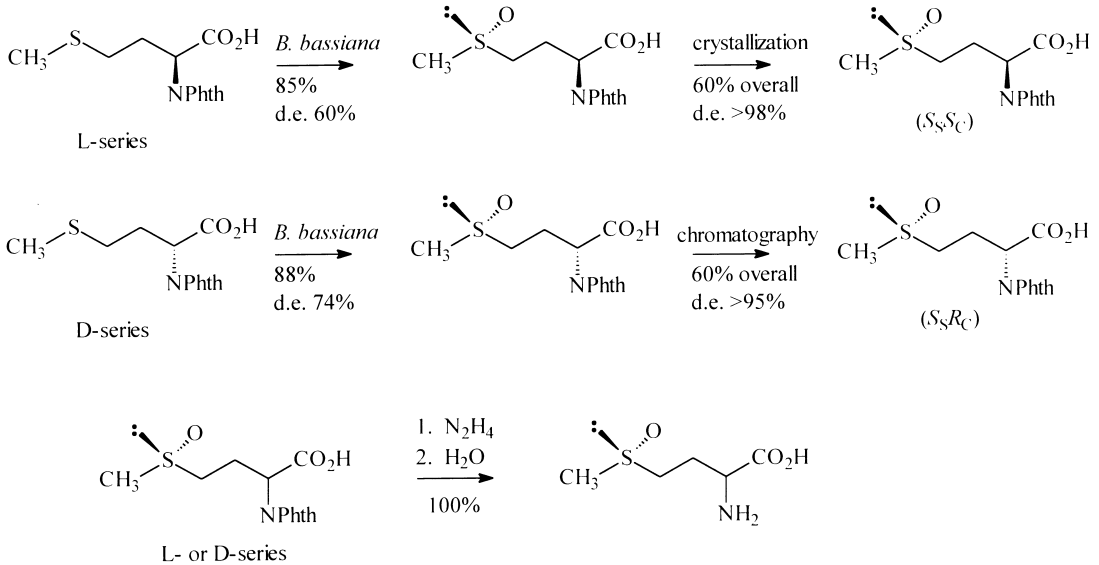
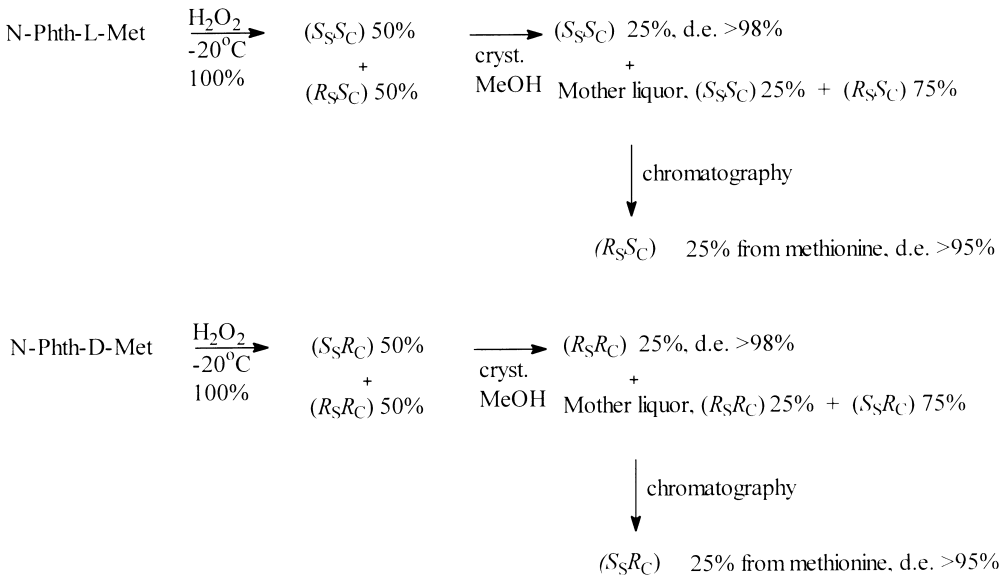
detailed examination.[11] The results of a 48 hour incubation of these substrates with *B. bassiana* are outlined in Scheme 1.[12]



Scheme 1. Preparation of the (*S*)-sulfoxides of L- and D-methionine by biotransformation of the N-phthaloyl derivatives using *Beauveria bassiana* ATCC 7159

(S_SS_C)-N-Phthaloylmethionine, d.e.>98%, mp 217–218°C, $[\alpha]_D$ +12.5 (EtOH) (reported[13] mp 215–217°C, $[\alpha]_D$ +13 (EtOH)) could be obtained directly in 60% isolated yield by methanol crystallisation[14] of the crude product obtained from biotransformation of N-phthaloyl L-methionine.[15] (S_SR_C)-N-Phthaloylmethionine, d.e.≥95%, mp 181–183°C, $[\alpha]_D$ +63 (MeOH), was also isolated in 60% yield by chromatography of the biotransformation product from N-phthaloyl D-methionine over silica gel, eluting with ethyl acetate followed by a 5% stepwise gradient elution from ethyl acetate to 30% methanol/70% ethyl acetate. In both cases, diastereomeric purity was assessed by ^{13}C NMR, in which the signals from carbons 2 to 4 and the *S*-methyl group were baseline-resolved in deuteromethanol solvent at 75 MHz, and configuration at sulfur was assigned following conversion to methionine sulfoxides, for which configurational data are available.[6] Both biotransformation products were readily converted to the corresponding amino acid sulfoxides in quantitative yields by standard treatment with hydrazine followed by aqueous work up, resulting in the overall route summarised in Scheme 1 for the conversion of L- or D-methionine to the *S*-sulfoxide. The products obtained in this way were (S_SS_C) methionine sulfoxide, mp 250–255°C (dec.), $[\alpha]_D$ +101 (c 0.5, water) (reported[5,7] mp 250–255°C, $[\alpha]_D$ +99.7, water) and (S_SR_C) methionine sulfoxide, mp 235–240°C (dec.), $[\alpha]_D$ +78 (c 0.5, water) (reported[5,7] for the (R_SS_C) enantiomer, mp 239°C, $[\alpha]_D$ −77, water).

The isomers comprising the minor diastereomeric components of the biotransformation products, the (R_SS_C) and (R_SR_C) stereoisomers, were conveniently isolated following hydrogen peroxide oxidation of N-phthaloylmethionines as outlined in Scheme 2. Although this oxidation shows no diastereoselectivity, the enantiomeric (S_SS_C) and (R_SR_C) forms, obtained by oxidation of N-phthaloyl L- and D-methionine respectively, can be easily separated by crystallisation from methanol,[16] and the respective mother liquors then processed by silica gel chromatography as described above. Conversion of the resulting (R_SS_C) and (R_SR_C) N-phthaloyl methionine sulfoxides to the amino acid sulfoxides was then performed as outlined in Scheme 1. The products obtained in this way were (R_SR_C) methionine sulfoxide, mp 250–255°C (dec.), $[\alpha]_D$ −100.2 (c 0.6, water) and (R_SS_C) methionine sulfoxide, mp 235°C (dec.), $[\alpha]_D$ −78 (c 0.5, water).



Scheme 2. Hydrogen peroxide oxidations of N-phthaloyl L-methionine and N-phthaloyl D-methionine

Biocatalytic or chemical oxidations can therefore be used in a complementary manner for the preparation of all four diastereomers of methionine sulfoxide with high diasteromeric purity in overall isolated yields of 20–55% from methionine, depending on the target isomer. Preliminary data[17] suggest that these procedures are equally applicable to the corresponding ethionine derivatives, and further studies involving these and other naturally occurring amino acids are in progress.

Acknowledgements

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11. The application of this microorganism for chiral sulfoxidation has not hitherto been reported, but its use was suggested to us by preliminary studies using *B. bassiana* for the oxidation of benzyl methyl sulfides: Holland, H. L.; Reese, P. B.; Brown, F. M. Unpublished data.
12. *Beauveria bassiana* ATCC 7159 was grown in 1 L Erlenmeyer flasks at 27°C, 180 rpm, in a medium consisting of glucose (10 g), and cornsteep liquor (20 g) per L of tap water, adjusted to pH 4.85. After 72 hours growth, the fungus was harvested by filtration and resuspended in a volume of distilled water equal to that of the original medium. A solution of substrate (750

mg) in ethanol (30 mL) was then added to achieve a final concentration of 0.75 g substrate per L of water. Biotransformation was allowed to proceed for a further 48 hours, at which time conversion to sulfoxide was complete. No sulfone products were detected. The fungus was then removed by filtration, the medium pH adjusted to 3.0, and the medium then extracted with dichloromethane (continuous extraction for 96 h). The residue obtained by evaporation of the extract was treated with decolourising carbon in chloroform solution, and then either crystallised or chromatographed as described in the text.

13. Christensen, B. W.; Kjær, A. *J. Chem. Soc., Chem. Commun.* **1969**, 934.
14. The crude biotransformation product after decolourisation (3.5 g from 4 g substrate) was dissolved in hot methanol (30 mL). The volume of the solution was reduced to ca 15 mL by boiling, and the solution then allowed to cool to room temperature, yielding 2.56 g of product.
15. This and all products subsequently referred to were fully characterised by mp, ^{1}H NMR, ^{13}C NMR, MS and rotation data.
16. The crude oxidation product (2.95 g) was dissolved in 10 mL of boiling methanol, and the solution allowed to cool to room temperature, yielding 1.3 g of ($S_S S_C$) or ($R_S R_C$) product, d.e. ca 80–85%. One recrystallisation from methanol (10 mL) then afforded 0.85–0.9 g of diastereomerically pure material.
17. Trial experiments indicate that biotransformation of N-phthaloyl L- and D-ethionines by *B. bassiana* proceeds in high yield to give sulfoxides with d.e.s of 86% and 76%, respectively.