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IDENTIFICATION AND STEREOCHEMISTRY OF THE PRODUCT OF 4-HPPD CATALYZED OXIDATION OF THE KETOACID OF METHIONINE

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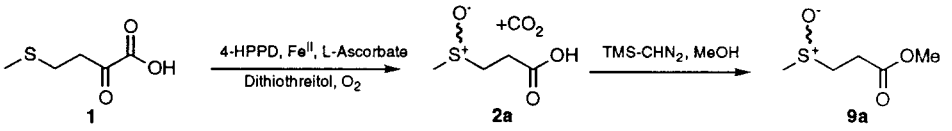
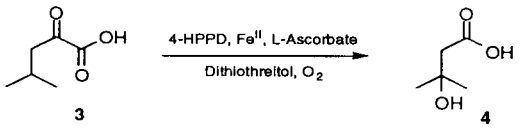
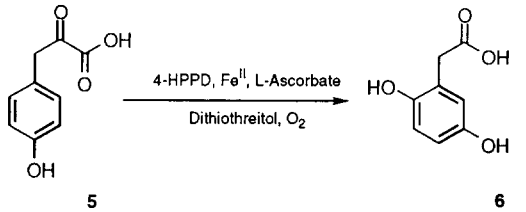
Abstract: 4-Hydroxyphenylpyruvate dioxygenase catalyzes the conversion of 2-oxo-5-thiahexanoic acid **1**, the ketoacid from methionine (Scheme 1) to an unequal mixture of 4-thiapentanoic acid-4-oxide enantiomers **2a**.



Scheme 1



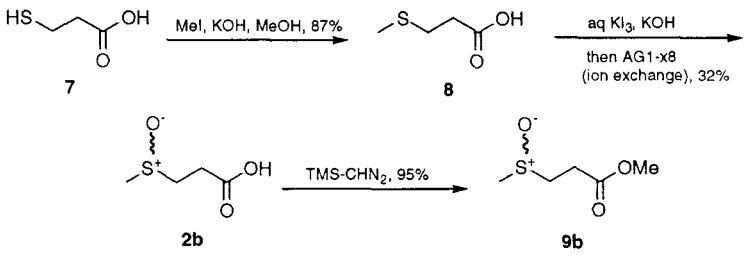
Scheme 2



Scheme 3

It has been known for some years that the enzyme responsible for the oxidative decarboxylation and hydroxylation of α-ketoisocaproate (KIC) **3** (Scheme 2) also catalyzes a reaction involving 2-oxo-5-thiahexanoic acid **1**[1], the ketoacid formed from transamination of methionine *in vivo*. We have since demonstrated that this enzyme is the same as 4-hydroxyphenylpyruvate dioxygenase (4-HPPD)[2,3], which converts 4-hydroxyphenylpyruvate **5** to homogentisate **6** (Scheme 3).

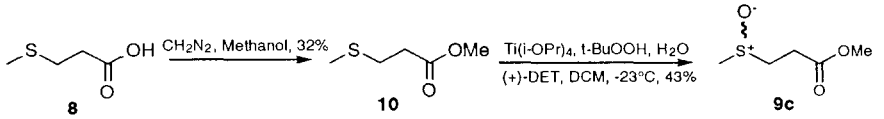
The original authors identified the product of the enzymic oxidation of **3** as the hydroxyacid **4** but were unable to establish the identity of the enzymic product from compound **1**. Our studies now demonstrate that it is the sulfoxide **2**.



Scheme 4

The racemic sulfoxide **2b** was made, *via* modification of a literature procedure,[4] in two steps from the commercially available acid **7** (Scheme 4) *via* oxidation of the thioether **8**. Anion exchange chromatography gave the racemic sulfoxide **2b** (28% yield from **7**). In practice, it was more convenient to work with the methyl ester so **2b** was treated with trimethylsilyldiazomethane and the product chromatographed on silica to afford the analytically pure sulfoxide **9b** (95%).

With an authentic sample of **2** in hand, the ketoacid **1** was incubated on a 10 mg scale with recombinant 4-HPPD (cloned from a rat liver cDNA library in this laboratory[3]) in the presence of oxygen and the cofactors iron(II), L-ascorbate and dithiothreitol (27°C, 0.2M Tris, 0.2M maleate, pH 6.5, 18h). After precipitation of the protein with acetone and centrifugation the supernatant was subjected to the same purification and derivatization protocol as the synthetic sample to afford the pure sulfoxide **9a**. This material was identical to the authentic standard **9b** by 500 MHz proton NMR spectroscopy ($CDCl_3$) and mass spectrometry (151 (MH^+) NH_3 chemical ionization). In addition, it was optically active. We did not observe the formation of any other products in the conversion of **1** to **2a**. In the absence of 4-HPPD but with the other cofactors and oxygen present no conversion of **1** to **2a** occurred.



Scheme 5

In order to determine the stereochemistry of **9a**, we applied the asymmetric oxidation of Kagan[5] to the thioether **10**, itself made by treatment of **8** with diazomethane (Scheme 5), using the (+) antipode of diethyl tartrate as the chiral inducer. In our hands, we obtained the analytically pure sulphoxide **9c** (14% from **8**) but with lower enantiomeric excess than claimed in the original paper. Both the enzymic and synthetic sulfoxides were analyzed by proton NMR spectroscopy in the presence of two equivalents of *R*-(-)-(dinitrobenzoyl)-α-methylbenzylamine[6] and the sulfinyl-methyl groups showed two resonances of unequal intensity. The bias was similar in magnitude when **9a** and **9c** were compared, but opposite in sense (Fig. 1). The same result was obtained

when the optical rotations and CD spectra were compared (Fig. 2). From Kagan's assignment of the absolute configuration[5], we conclude that the enzyme converts the ketoacid **1** to the sulfoxide **2a** with a slight bias (15% enantiomeric excess) in favour of the *S* isomer.

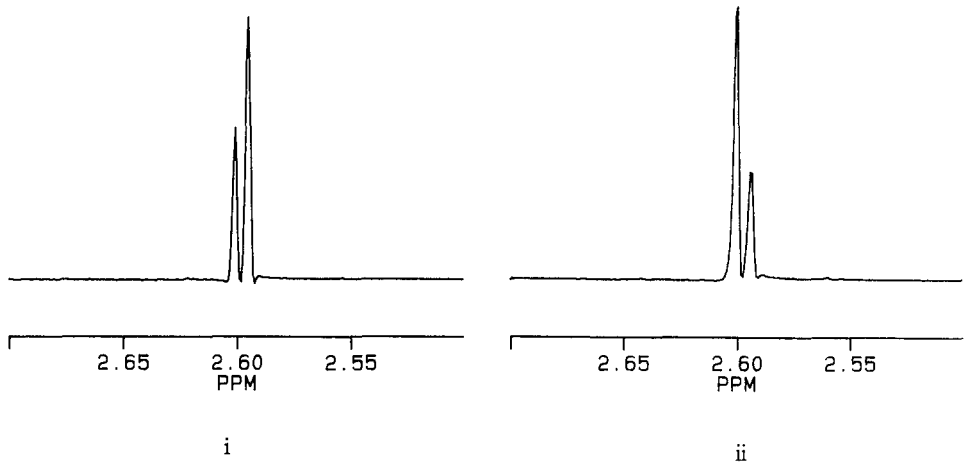


Figure 1 Partial proton NMR spectra of the sulfinyl-methyl resonances of (i) the enzymic sulfoxide **9a** and (ii) the synthetic sulfoxide **9c**

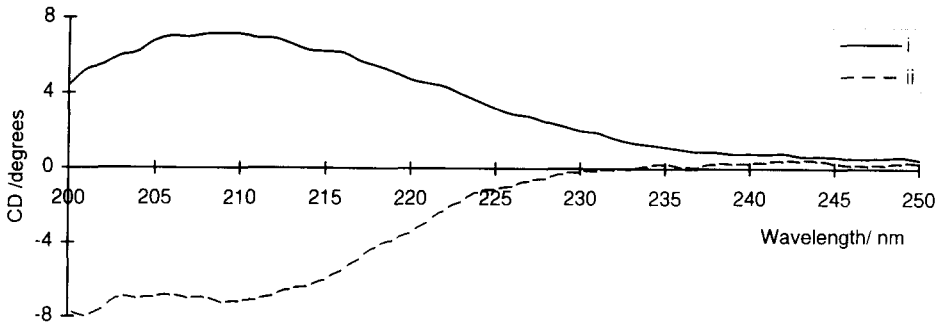


Figure 2 CD spectra of (i) the enzymic sulfoxide **9a** and (ii) the synthetic sulfoxide **9c**

In summary, we have established that 4-hydroxyphenylpyruvate dioxygenase oxidatively decarboxylates the ketoacid derived from methionine to an approximately *57: 43* mixture of *S* and *R* enantiomers of 4-thiapentanoic acid-4-oxide[7]. This corrects previous erroneous reports of the conversion in the literature[1].

Acknowledgements

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References

1) Sabourin, P. J.; Bieber, L. L. *J. Biol. Chem.*, **1982**, *257*, 7460 and references cited therein

2) Baldwin, J. E.; Crouch, N. P.; Fujishima, Y.; Lee, M.-H.; MacKinnon, C. H.; Pitt, J. P. N.; Willis, A. C. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1255

3) Baldwin, J. E.; Crouch, N. P.; Lee, M.-H.; MacKinnon, C. H.; Zhang, Z. H. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 1503

4) Doi, J. T.; Goodrow, M. H.; Musker, W. K. *J. Org. Chem.*, **1986**, *51*, 1026

5) Duñach, E.; Kagan, H. B. *Nouv. J. Chim.*, **1985**, *9*, 1

6) Deshmukh, M.; Duñach, E.; Juge, S.; Kagan, H. B. *Tet. Lett.*, **1984**, *25*, 3467

7) Pascal *et al* have demonstrated the 4-HPPD catalyzed oxidative decarboxylation of synthetic [(4-hydroxyphenyl)thio]pyruvate to [(4-hydroxyphenyl)sulfinyl]acetate, see Pascal, R.A.; Oliver, M.A.; Chen, Y.-C. J. *Biochemistry* **1985**, *24*, 3158

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