

## Chirality of the methylsulfinyl group is (R) in glucosinolates and (S) in native amino acids

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### Introduction

Cruciferous products from oilseed rape and cruciferous vegetables are known for their content of nutritional valuable and high value compounds as oils, proteins, dietary fibres, vitamins, antioxidants and other essential nutrients. It is also the odor, smell, taste, health promoting and antinutrient compound in or from cruciferous materials which give the characteristic properties associated with cruciferous products. Important in this connection is the structure and structure related properties of glucosinolates and glucosinolate derived components (Frandsen *et al.*, 2011, this volume). In total more than 140 different compounds are known (Bellostas *et al.* 2007) and of this group about 30 different glucosinolates are known as constituents of Brassica, rapeseed and cruciferous vegetables. In all of these plants or plant parts, glucosinolates co-occur with myrosinase isoenzymes (EC 3.2.1.147) and it give the basis for a great number of different products as result of both myrosinase catalysed reactions (Bellostas *et al.* 2007 b, Bellostas *et al.* 2009) and as result of non-enzymatic catalysed reactions (Bellostas *et al.* 2008, Buskov *et al.* 2000a, 2000b). The bioactivities and biological activities of the compounds are in most cases closely related to the actual structure or constitution and configuration (stereochemistry) of the compounds (Andersen *et al.* 2010). The structural variation following modification of glucosinolates after the steps in the basic glucosinolate biosynthesis (Hill *et al.* 2003, Bellostas *et al.* 2007a) comprise both the potential acylation of the thioglucose part and side chain modifications (Hill *et al.* 2003 and refs cites therein) including specific oxidations comprising productions of additional chiral centers. The biological effects related to chiral centers can be appreciable (Andersen *et al.* 2010), and the opportunities for analytical determinations of chirality resulting from methylsulfinyl groups in amino acids and glucosinolates- including glucoraphanin are the aim of the present work.

### Materials and methods

Materials and methods are described elsewhere; Isolation of amino acids (Egum and Sørensen, 1989), DNFB derivatisation of amino acids and MECC method (Sørensen *et al.* 1999, Determination of glucosinolates (Hill *et al.* 2003), synthesis of S-methylsulfinyls (Eggum and Sørensen, 1989), DTAB-MECC procedure for thiocyanate determination (Bjerggaard *et al.* 1996)

### Results and discussion

To investigate the possibilities for occurrence in the plants of methylsulfinylamino acids with both R and S configuration at the methylsulfinyl group, the synthetic diastereoisomeric mixtures were produced from L- methionine and L-S-methyl-cysteine were produced by oxidation with hydrogen peroxide. In both cases, a racemic mixture of sulfoxide with a 1:1 ratio between the (R) and (S) methylsulfinyl groups in the L-amino acids were produced as shown by <sup>1</sup>H-NMR the MECC and results (Figure 1).

The sulfoxides were ordinarily prepared from the sulphides with the theoretical amount of H<sub>2</sub>O<sub>2</sub> in aqueous solution (Eggum and Sørensen, 1989). When molecular excess of the amino acids was used some uncharged sulphides of the amino acids were left in the solution (Figure 1) and when molecular excess of H<sub>2</sub>O<sub>2</sub> was used some of the sulfoxide were transformed into sulfones (Figure 1).

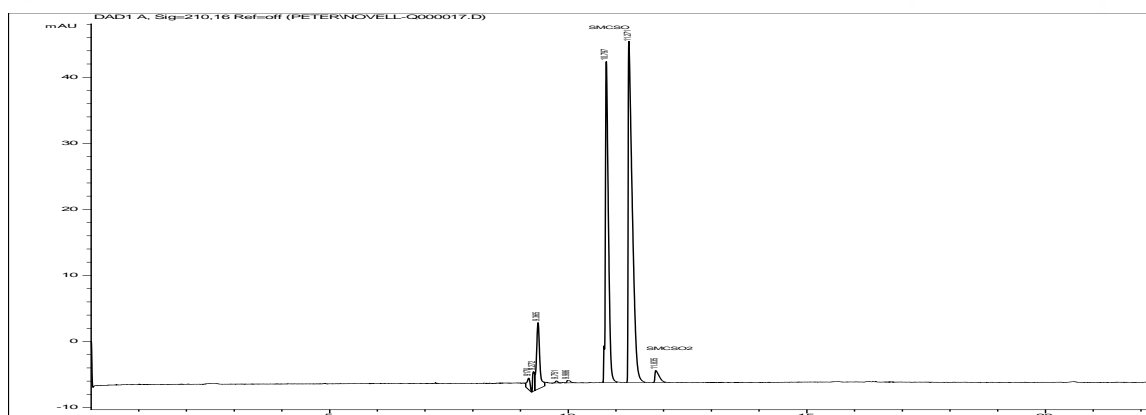


Figure 1. MECC in the cholate system of the synthetic mixtures of L-MCSO diastereoisomers in a mixture with L-S-methyl- cysteine (L-MC) (molecular deficit of H<sub>2</sub>O<sub>2</sub>) and of L-MCSO diastereoisomers in a mixture with the sulfone L-MCSO<sub>2</sub> (Molecular excess of H<sub>2</sub>O<sub>2</sub>).

As seen from figure 1, the two diastereoisomeric L-MCSOs (Figure 2) are well separated from each other in the developed MECC-cholate system, as well as from the parent amino acid (L-MC) and their oxidation products (L-MCSO<sub>2</sub>). We need, however, to know which of the two diastereoisomeric L-MCSO we have as the natural product and/or if both occur as natural products (figure 3)

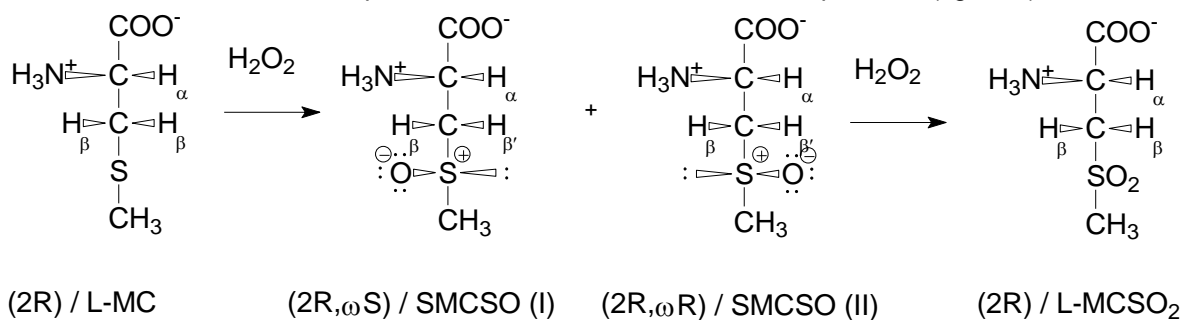


Figure 2. Structures of sulphides, sulfoxides and sulfones of sulphur containing amino acids.

The structure of the cysteine derivatives are shown for the L-isomers, which for cysteine is the (2R)-configuration of carbon nr 2, whereas the other 18 chiral (L)-amino acids, which can be incorporated in proteins, have (2S) configuration. If we then consider the chirality of the S-methylsulfinyl group, it is S-configuration in L-SMCSO(I) and R-configuration in L-SMCSO(II) (Figure 2). With L-methionine sulfoxide it gives then two corresponding diastereoisomers, where the L-carbon in L-Met has (2S) configuration and the methylsulfinyl groups have (S) configuration in (2S, ωS) L-MSO(I) and (R) configuration in (2S, ωR) L-MSO(II) (figure 4).

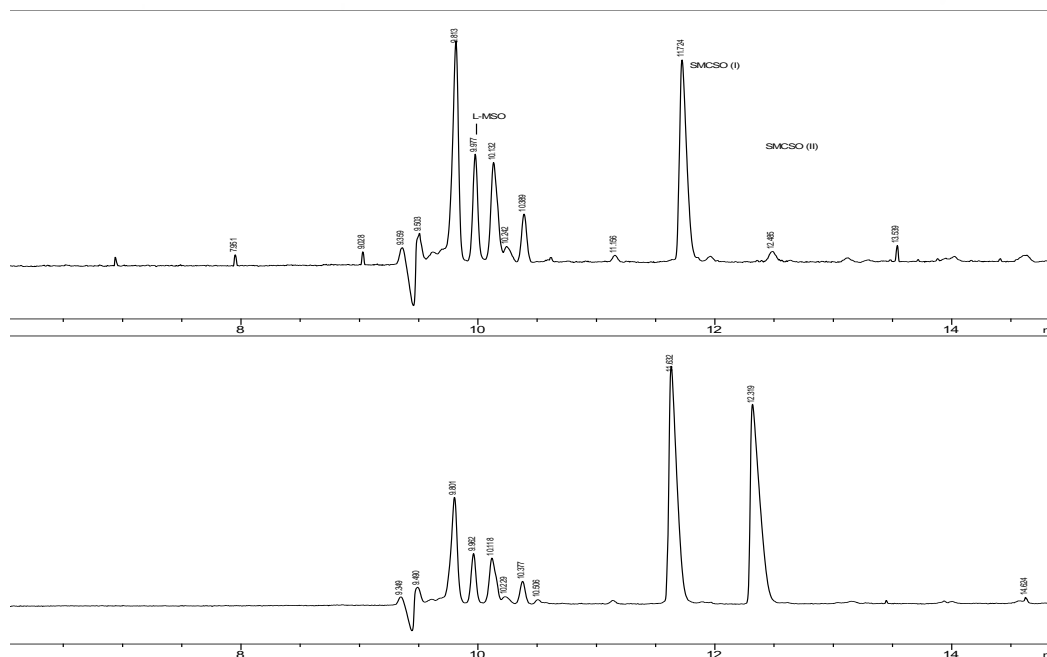


Figure 3. MECC-cholate system used to separate the broccoli sulfoxides from other sulphur containing amino acids which occur in florets of broccoli

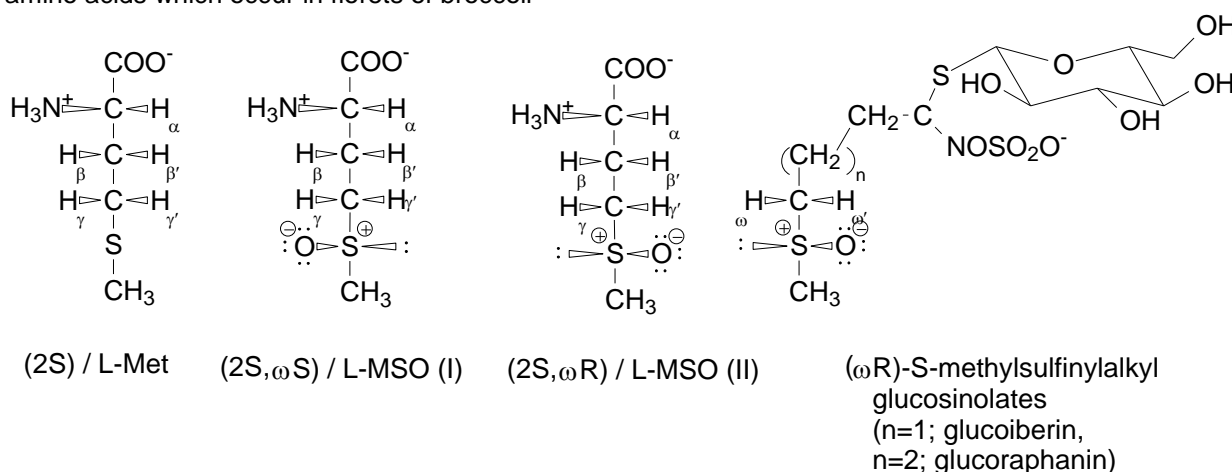


Figure 4. Structures of L-Met and its sulfoxides compared with structures of methylsulfinylalkylglucosinolates with the known (R)-configuration

Confirmations of the structures shown in figures 2 and 4 have been obtained from the NMR spectra of the individual compounds. For the glucosinolates MECC and NMR have also been used to confirmation of the (ωR) configuration for all of the S-methylsulfinylalkylglucosinolates, shown for n=1 to n=9 (Figure 4; except for n=6). The native (2R,ωS) SMCSO(I) present in and isolated from broccoli has been found to account for 95% of the diastereoisomers (2R)-S-methylcysteinesulfoxide (SMCSO(I)) in broccoli. This is as found for the stereospecific S-oxidation of S-carbonylmethyl-L-cysteine by the female Wistar rat enzyme (Boonyapiwat *et al.*, 2004) It is however, interesting to note, that broccoli produce some few percent of (2R,□R)-SMCSO in addition to the quantitatively dominating stereoisomeric (2R,□S)-SMCSO. The production of (□R)-S-methylsulfinylalkyl glucosinolates in broccoli and other cruciferous plant are in agreement with previously reported findings (Dalgaard *et al.*, 1977, Eggum *et al.*, 1989 and refs cited therein). The ability of cruciferous enzymes to produce 2-hydroxysubstituted glucosinolates both with (2R) and (2S)-configuration (Bellostas *et al.*, 2007b), and co-occurrence of the two diastereoisomers in different relative amounts is well documented.

### Acknowledgement

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