Syntheses of Metabolites of *S*-Carboxymethyl-L-cysteine and *S*-Methyl-L-cysteine and of Some Isotopically Labelled (^{2}H, ^{13}C) Analogues

Claus O. Meese[a]*, Dagmar Specht[b], and Ute Hofmann[a]

[a] Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstr. 112, D-7000 Stuttgart 50, FRG
[b] Abteilung Klinische Chemie, Robert-Bosch-Krankenhaus, Auerbachstr. 110, D-7000 Stuttgart 50, FRG

Received November 9, 1989

The chemical syntheses of human metabolites of *S*-carboxymethyl-L-cysteine (**3**) and *S*-methyl-L-cysteine (**12**) are described. The additional preparation of some ^{2}H- and ^{13}C-labelled isotopomers enabled the direct evaluation of the stabilities of **3** and **12** under physiological conditions and also facilitated the unambiguous assignments of the signals in the ^{13}C-NMR spectra of all compounds mentioned.

Synthese von *S*-Carboxymethyl-L-cystein- und *S*-Methyl-L-cystein-Metaboliten und einigen isotopenmarkierten (^{2}H, ^{13}C) Analoga

Die chemische Synthese der Humanmetabolite von *S*-Carboxymethyl-L-cystein (**3**) und *S*-Methyl-L-cystein (**12**) wird beschrieben. Durch zusätzliche Herstellung einiger ^{2}H- und ^{13}C-markierter Isotopomerer wird die direkte Abschätzung der Stabilität von **3** und **12** unter physiologischen Bedingungen und die zweifelsfreie Resonanzsignalzuordnung in den ^{13}C-NMR Spektren aller erwähnten Verbindungen ermöglicht.

According to previous investigations, the mucolytic agent *S*-carboxymethyl-L-cysteine (**3a**) is metabolised by the human organism in a complex manner involving predominantely *S*-oxidation or *N*-acetylation, respectively. In addition, its decarboxylation to furnish *S*-methyl-L-cysteine (**12a**), the further oxidation or *N*-acetylation of the latter, as well as the formation of bis(carboxymethyl) sulphide (thiodiglycolic acid; **23a**) also appear to play a part in the process[1-3]. As a consequence of the genetically conditioned polymorphism in the oxidative metabolism of this drug, compound **3a** was used as a test agent for the detection of reduced sulphoxidation in the population[4,5] for the purpose of explaining, for example, food intolerance[6], increased toxicities of D-penicillamine[7,8] and sodium gold thiomalate[9] as well as the pathogenesis of diseases of the motor neuron[10] and primary biliary cirrhosis[11] in the phenotype of subjects with suspected deficient sulphoxidation. Since both investigations of the human metabolism[3] as well as all phenotyping processes[4] are based on paper chromatographic methods and in light of the fact that serious discrepancies in comparison to other analytical methods have become apparent [12-14], a reinvestigation of the human metabolism of **3a** has become essential. The fundamental prerequisite for investigations of this type is the availability of metabolites which can be characterised unambiguously as well as of their stable-isotope labelled analogues which are indispensible for identification and quantification by mass and ^{13}C-NMR spectroscopic methods. The present publication is thus addressed to these synthetic aspects and the results may also be transferred directly to other cysteine conjugates.

For the purpose of direct ^{13}C-NMR investigations on the metabolism of **3a** and **12a** as well as estimations of their stabilities under physiological conditions (see below), the isotopomer **3b** was initially synthesised from the readily available starting materials **1b** and **2** (Scheme 1). By analysis of the ^{13}C-NMR spectrum of the highly enriched (>99% ^{13}C) isotopomer **3b**, it was shown that **3b** underwent slow cyclisation to form the lactam **4b** (see [15,16]) even at neutral pH and room temp. With the help of the same tracer technique it also became apparent that **3b** was autoxidised, to a very minor extent, by atmospheric O_2 (Fig. 1). These results allowed us to discount the contribution of these two non-enzymatic processes to the spectrum of metabolites obtained from **3a**.

This new procedure is worthy of note in that it permits, as a result of the ^{13}C-labelling in conjunction with the natural ^{13}C abundance (1.1%) of the remaining C-atoms, the direct, simultaneous, non-destructive, and quantitative registration of slowly proceeding reactions at less than the reaction level of 1 mol %. The subsequent products **4a** (racemic, see below), **5a**, and **6a** are preparatively accessible in high yields from the unlabelled precursors by acid-catalysed cyclisation[15,16] or controlled *S*-oxidation with H_2O_2[17], respectively.

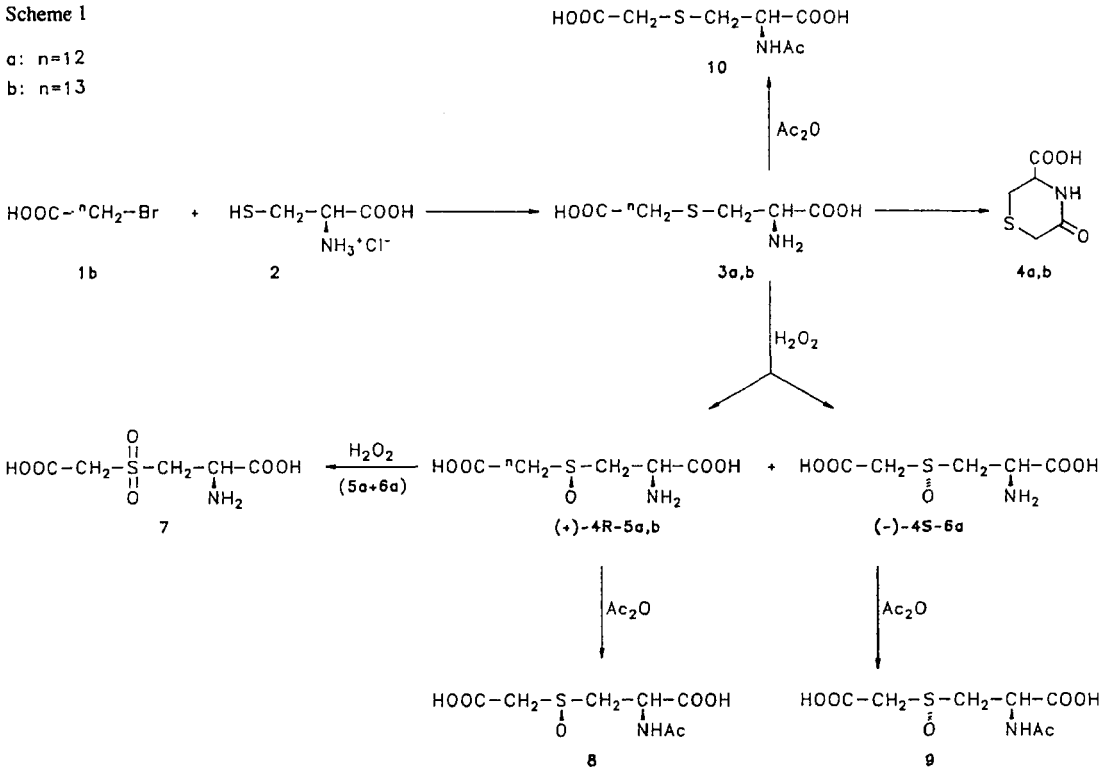
Excess oxidising agent and longer reaction times led to the sulphone **7**[18-20]. For comparison, the ^{13}C-analogue **3b** was also oxidised on a sub-millimole scale; after recrystallisation of the crude product, the practically isomerically pure sulphoxide (+)-4*R*-**5b** was isolated directly. After separation of the epimeric sulphoxides **5a** and **6b** by fractional crystallisation as described[17], the pure isomers as well as **3a** were converted into the corresponding mercapturates **8, 9,** and **10** by pH-controlled *N*-acetylation.

For the synthesis of the ^{13}C-labelled analogue **12b** (Scheme 2), the *S*-methylation of **2** was optimised with regard to [^{13}C]-CH_3I (**11b**) by use of Li-salts which, in contrast to product **12b**, are very soluble in the solvents used (54% yield; Ref.[21]: 20%). The mild oxidations of the thioethers **12a** and **12b** with one equivalent of H_2O_2 gave rise to 39:61 mixtures of the extremely water-soluble sulphoxides **13** and **14** which, in the case of the unlabelled compounds **a**, could be completely separated by repeated fractional crystallisations. Even a slight excess of H_2O_2 resulted in a further oxidation to the sulphone **15** which could also be obtained in crystalline form. Although the direct

 ©VCH Verlagsgesellschaft mbH, D-6940 Weinheim, 1990 0365-6233/90/1212-0957 $ 3.50 + .25/0

Scheme 1

a: n=12
b: n=13



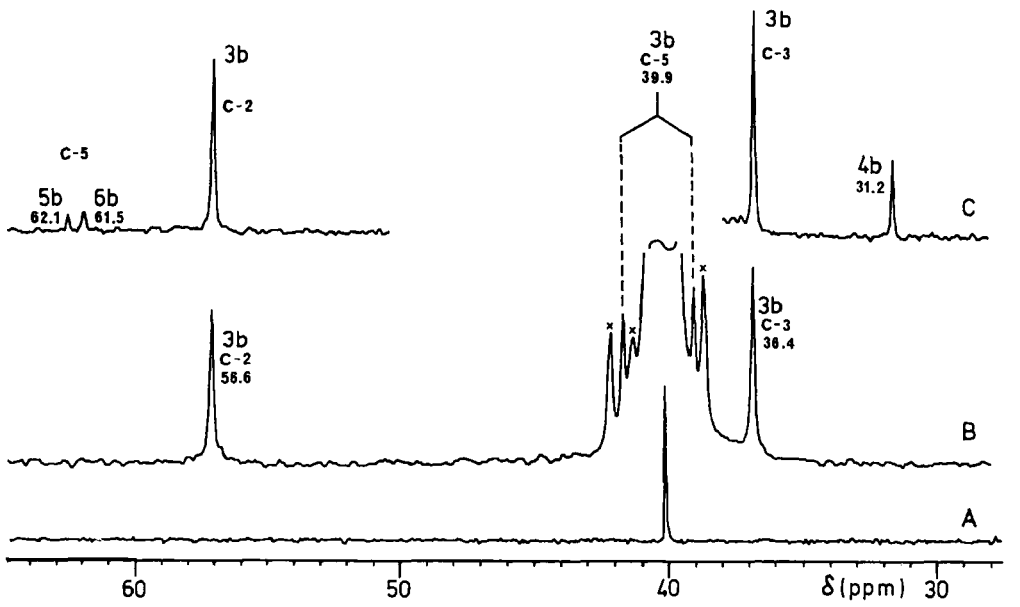
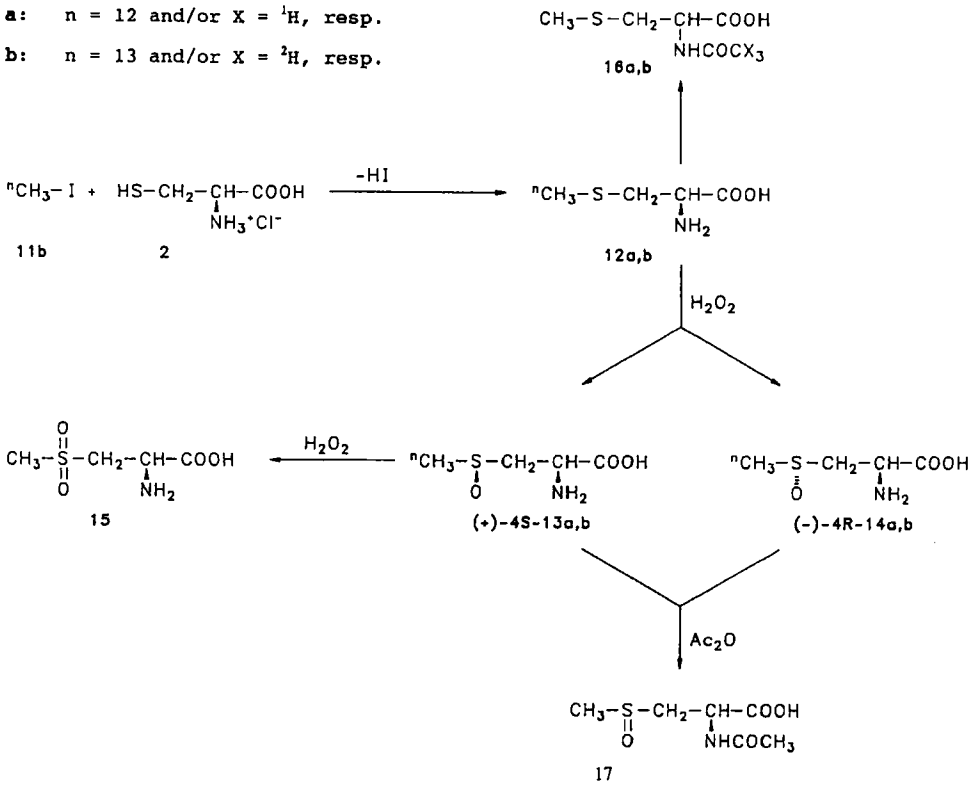


Fig. 1: Partial ^{13}C-NMR spectra of **3b**. **A**: 6 μmol·ml^{-1} phosphate buffer (pH = 7.0, see Experimental Part); 3700 scans; 39.9 ppm = resonance of S-$^{13}CH_2$ (C-5). **B**: 0.2 mmol·ml^{-1}, immediately after dissolution, 64 fold amplification, 58000 scans; x = spinning side bands; ^{2}J(C-5 [99% ^{13}C], C-6 [1.1% ^{13}C]) = 52 Hz. **C**: Repeat spectrum under measurement conditions **B** after 35 d at room temp. The signal at 31.2 ppm (resonance of the labelled C-atom) demonstrates the formation of the lactam **4b** (0.37% based on 1.1% C-3 of **3b**). The C-5 resonance of the epimeric *S*-oxides **5b** and **6b** at 62.1 and 61.5 ppm are indicative of 0.08% (referred to C-2 of **3b**) autoxidation, respectively.

reaction of acetic anhydride with **12a** furnished the completely racemised DL-**16a** (see Refs.[22,23]), L-**16a** was obtained in optically pure form (e.e. >95%) by careful control of the pH of the reaction mixture. Correspondingly, the epimeric sulphoxides were converted to the respective mercapturates **17** without loss of the chirality. For the detection of the chirality at C-2, the cysteine derivatives were converted to their methyl esters and the ^{1}H-NMR spectra

Scheme 2



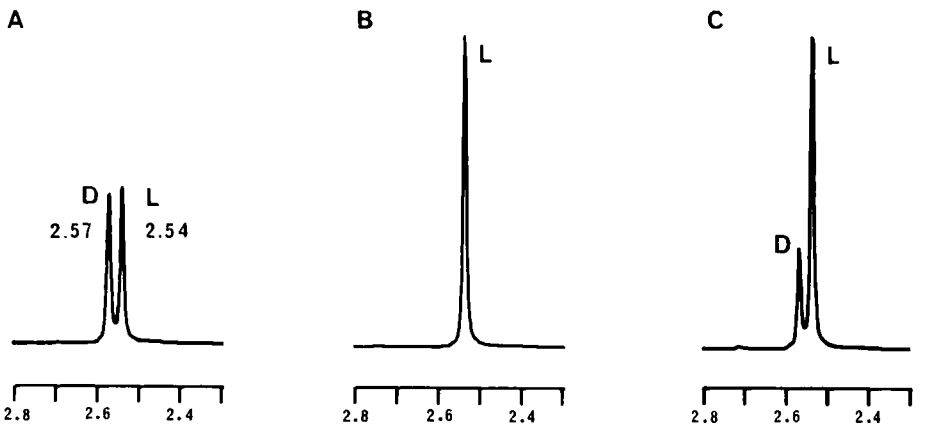
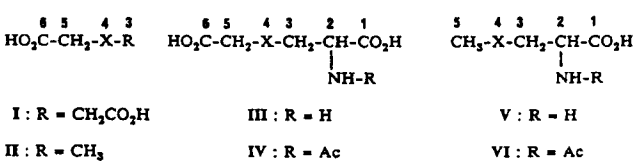


Fig. 2: *S*-Methyl resonances (^{1}H-NMR, δ scale [ppm], $CDCl_3$) of the methyl esters of the mercapturates DL-**16a** (**A**), L-**16a** (**B**), and, for configuration assignments, a mixture of DL- and L-**16a** (**C**) after addition of Eu(hfc)$_3$.

were recorded in the presence of the chiral shift reagent Eu(hfc)$_3$ (Fig.2 and Experimental Part).

The scattered reports that bis(carboxymethyl) sulphide (**23a**) can occur as a metabolite of sulphur-containing amino acids or various glutathione conjugates[24-29] prompted us to attempt preparations of the biosynthetic precursors and subsequent products of this dicarboxylic acid (Scheme 3).

For this purpose, compounds **18** and **19** were first converted to 3-(carboxymethylthio)-2-oxopropanoic acid (**20a**). Borohydride reduction of **20a** gave the racemic lactic acid derivative **21a** in a smooth reaction. Since the methylene hydrogen atoms at C-3 of **20a** are acidic, they can be readily exchanged by D_2O (this can be followed directly by ^{13}C-NMR spectroscopy). Subsequent carbonyl reduction with borodeuteride resulted in the *in situ* generation of the $^2H_3$-labelled analogue **21b** which was used without further purification as the deuterated internal standard for mass spectrometric (ms) quantification of **21a** in biological samples[30,31]. The $^{13}C_4$-isotopomer **23b** of bis(carboxymethyl) sulphide was prepared from doubly labelled bromoacetic



Scheme 3

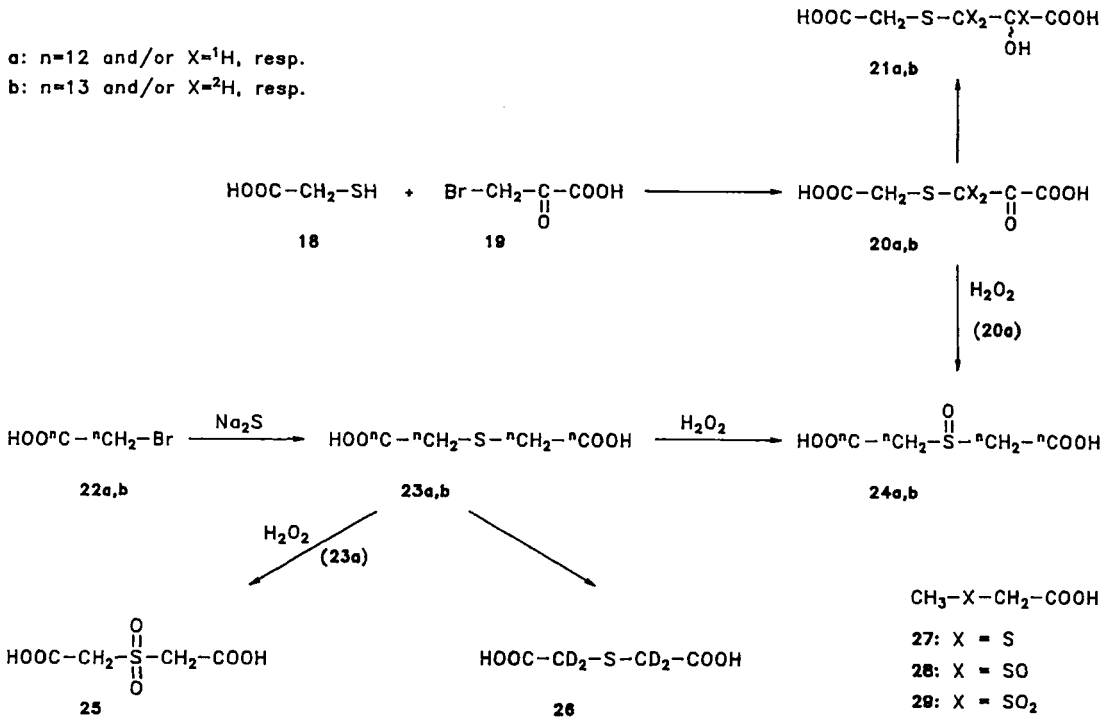


Table 1: Δδ Values (ppm) of the ^{13}C-NMR resonance signals of compounds of the structural types **I** to **IV** on going from thioethers (X = S) to sulphoxides (X = SO) and from sulphoxides (X = SO) to sulphones (X = SO_2)[a].

Structural Type (Cpd. No.)	C-6	C-5	C-3	C-2	C-1	
I (23a/24a)	-6.1	+20.7	-	-	-	
II (27/28)[b]	-6.5	+21.6	+21.6	-	-	
III (3a/5a,6a)	-6.5	+21.9	+17.8	-3.2	-0.4	SO
IV (10/8,9)[c]	-6.5	+21.5	+19.2	-4.6	-1.4	↑
V (12a/13a,14a)	-	+23.0	+18.9	-2.9	-0.8	S
VI (16a/17)[c]	-	+22.5	+20.0	-4.1	-1.7	
I (24a/25)	-3.5	+1.7	-	-	-	
II (28/29)[b]	-3.2	+1.0	+6.0	-	-	SO_2
III (5a,6a/7)	-3.5	+1.6	+2.2	-1.5	-1.3	↑
V (13a,14a/15)	-	+3.4	+1.3	-1.2	-0.2	
VI (17)[c,d]	-	+3.9	-0.5	±0	-0.6	SO

[a] Positive sign = shift to lower field; negative sign = shift to higher field. Average values given for epimeric sulphoxides.
[b] C-3 = CH_3S-.
[c] No significant shift effect (±0.2 ppm) at the C-atoms of the acetyl group.
[d] The *N*-acetyl derivative of **15** was prepared *in situ* (Experimental Part).

acid (**22b**) and sodium sulphide, as described for the unlabelled compound[32]. Subsequent oxidation of the thioethers **23a** and **23b** with H_2O_2 gave rise to the corresponding sulphoxides **24a**[33] and **24b**. ^{1}H-NMR

spectroscopy indicated that bis(carboxymethyl) sulphoxide (**24a**) was also formed spontaneously by oxidation of **20a** (see Experimental Part). Formation of the sulphone **25** only occurred with a large excess of H_2O_2; it was obtained more simply by oxidation with permanganate[34]. Deuterium was introduced into **23a** by repeated H/D-exchange. In contrast to earlier assumptions[27], the label in **26** is to a large extent stable towards retro-exchange so that this compound can be used as a stable-isotope standard for ms trace analysis of **23a**[30,31]. For reference purposes, 2-(methylthio)-acetic acid (**27**) was also oxidised to 2-(methylsulphinyl)-acetic acid (**28**) and 2-(methylsulphonyl)-acetic acid (**29**); it was further employed for the signal assignments of the above-mentioned compounds.

The shifts of the ^{13}C-NMR resonance signals obtained when thioethers of the structural types **I** to **IV** (thioether, X = S) are oxidised to the sulphoxides (X = SO) and when these, in turn, are oxidised to the sulphones ($X = SO_2$) are summarised in Table 1.

As expected, the signals of C-atoms in an α-position to a S-atom experience a strong shift to low field amounting to 17.8 to 23.0 ppm of transformation of S to SO as a result of the increase in electronegativity. As a consequence of the β-effect a constant shift to high field results for C-2 and, to a greater extent, for C-6. Signals shifts in the same sense were observed for the transformation SO to SO_2. However, since the electronegativity differences between sulphinyl and sulphonyl groups are smaller, the magnitudes of the Δδ values are lower. Consideration of the unequivocal ^{2}J couplings of the ^{13}C-labelled analogues and the characteristic reduction of the signal intensities of the ^{2}H-labelled isotopomers through coupling and the nuclear *Overhauser* effect, allowed all of the C-resonances in the proton decoupled ^{13}C-NMR spectra to be assigned.

Whereas the described unlabelled compounds represent indispensible reference compounds for the identification and quantification in biological material, the value of the isotopomers obtained either preparatively or generated *in situ* by H/D-exchange arises not only from their function as isotopically labelled internal standards for MS but also from the facts that they can be employed as starting materials for further metabolisation investigations (e.g. **3b, 12b**)[35] and that they make possible the unambiguous assignment of the signals in the ^{13}C-NMR spectra of other, structurally related compounds.

We are grateful to the *Robert-Bosch-Stiftung*, Stuttgart, for financial support, to Prof. Dr. *M. Eichelbaum* and Prof. Dr. *H. Wisser*, for fruitful discussions, and to Ms. *S. Holzer* and Ms. *S. Seefried* for assistance with the preparative work.

Experimental Part

Melting points (not corrected): Electrothermal instrument. - Elemental analyses: Institute for Organic Chemistry, University of Stuttgart; in the case of ^{13}C-labelled compounds, the ^{12}C values were calculated on the basis of the relative molecular mass of the isotopomer. - MS: Hewlett-Packard 5985 A mass spectrometer and mass selective detector MSD 5970; sample introduction after appropriate derivatisation via coupled GC; electron ionisation energy (EI) = 70 eV. - ^{1}H-NMR (80 MHz) and ^{13}C-NMR (20 MHz, proton broad band decoupled): Bruker WP 80 FT. When not stated otherwise, the ^{13}C-NMR spectra were recorded in 1M aqueous phosphate buffer (pH 7; from 53.4 g KH_2PO_4 and 108.2 g $Na_2HPO_4 \cdot 2H_2O$ in 1000 ml of solution) in the presence of 5 vol-% D_2O (as lock substance) and CH_3CN as internal standard [$\delta(CH_3)$ = 3.70 ppm] referred to sodium 3-(trimethylsilyl)-d_4-propanoate [$\delta(CH_3)$ = 0.00 ppm]. Acidic substances were neutralised with one equivalent of N NaOH prior to addition of the buffer; concentration of the ^{13}C-labelled compounds = 6 μmol·ml^{-1}, otherwise 0.2 to 0.3 mmol·ml^{-1}. For ^{1}H-NMR measurements, the substances were dissolved in methanol and treated with CH_2N_2 in Et_2O to form the corresponding methyl ester; the resultant substance was taken up in $CDCl_3$ (internal standard: TMS, δ = 0.00 ppm) and the spectra recorded. Tris[3-(heptafluoropropyl-hydroxymethylene)-*d*-camphorato]europium, Eu(hfc)$_3$, (Aldrich No. 16,474-7) was used as the chiral shift reagent. - Polarimetry: Jasco DIP 360. - TLC: Merck silica gel 60 F_{254}, Alufolien 5 x 7.5 cm; the eluent system n-butanol/acetic acid/water (3 + 1 + 1) was prepared freshly each weak. Detection: I_2-vapour chamber or spraying with 2% ninhydrin/ethanol (140°). - Recrystallisations of thioethers and all reactions with mercapto compounds were under argon. - Exchange experiments: D_2O (Merck no. 2919, enrichment >99.75%). All temp. values in °C.

S-Carboxy-[^{13}C]-methyl-L-cysteine (**3b**)

To a cooled (10-15°), vigorously stirred mixture of freshly distilled (bp 106-107°/17 torr, mp. 46-47°) 2-[^{13}*C*]-bromoacetic acid (**1b**; 7.8 g, 56 mmol; Promochem No. CLM-724, >99 atom-% ^{13}C) and L-cysteine hydrochloride (**2**; 10.5 g, 67 mmol) in 60 ml degassed H_2O are added dropwise within 35 min 49 ml 7 N NaOH. The mixture was allowed to stand at room temp. for 1 h and then treated with 100% glacial acetic acid (200 ml). The fine white crystals obtained after standing at 0°C for 18 h were filtered, washed with ice/H_2O and EtOH, and dried *in vacuo* over NaOH. The crude product (13.9 g) was recrystallised from boiling H_2O (420 ml), washed with ice/H_2O and EtOH, and dried *in vacuo* over P_2O_5 to furnish pure **3b**; yield 6.1 g (61%); mp. 191° (decomp.) [Ref.[2] 192-193° (decomp.), Ref.[24] 202-203° (decomp.), Ref.[27] 175-176° (decomp.)]. - $C_4{}^{13}CH_9NO_4S$ (180.2) Calcd. C 33.3 H 5.03 N 7.8 S 17.8 Found C 33.5 H 5.20 N 7.9 S 17.9. - TLC: R_f = 0.33. - $[\alpha]_D^{23}$ = -3.3° (c = 1.0, N HCl), $[\alpha]_D^{21}$ = -31.9° (c = 1.0, 1 M phosphate buffer, pH = 7.0). - ^{13}C-NMR: δ (ppm) = 39.9 ($^{13}CH_2$, C-5); natural abundance: 180.5 (d; ^{2}J = 52 Hz, C-6), 176.0 (C-1), 56.6 (C-2), 39.9 (d; ^{2}J = 52 Hz, C-5), 36.4 (C-3). On standing of the sample at room temp., three new signals were observed and their intensities increased with time. The signal at 31.2 ppm has to be assigned to the labelled C atom (S-$^{13}CH_2$-CO) of the slowly formed (0.1% after 5 d, 0.37% after 35 d, 0.55% after 49 d) lactam **4b**; the resonances with approximately equal intensities at 62.1 and 61.5 ppm demonstrated the presence of traces of the autoxidation products **5b** and **6b** (after 35 d, 0.08%, see Fig. 1).

3-Oxo-2,3,5,6-tetrahydro-4H-1,4-thiazine-5-carboxylic Acid (**4a**)

Lactamisation of **3a** as described gave rise to colourless crystals; mp. 187-188° (Ref.[16] 182-183°, Ref.[15] 183-185°). - ^{1}H-NMR ($CDCl_3$, methyl ester, AB system for H-6): δ (ppm) = 3.00 (dd; J_{AB} = 13.5 Hz, J (H-5/H-6$_A$) = 4.4 Hz, 1H, H-6$_A$), 3.10 (dd; J_{AB} = 13.5 Hz, J (H-5/H-6$_B$) = 7.6 Hz, 1H, H-6$_B$), 3.33 (s; H-2), 3.83 (s; 3H, OCH_3), 4.39 (ddd; J = 7.6 Hz, 4.4 Hz, 2.3 Hz, 1H, H-5), 6.39 (br. s; 1H, NH). The 1:1 splitting of the methoxy group signal (3.96 and 3.98 ppm) after addition of Eu(hfc)$_3$ demonstrated that **4a** was obtained in a completely racemised form. - ^{13}C-NMR: δ (ppm) = 179.1 (CO_2H), 173.1 (CONH, C-3), 61.1 (CH, C-5), 31.3 (CH_2, C-2), 30.4 (CH_2, C-6). - $C_5H_7NO_3S$ (161.2) Calcd. C 37.3 H 4.38 N 8.7 S 19.9 Found C 37.3 H 4.46 N 8.7 S 19.9.

S-Oxidation of Compounds **3a** *and* **3b**

The epimeric sulphoxides **5a** and **6a** were obtained by *S*-oxidation of **3a** with H_2O_2 [17] and separated by fractional crystallisation. - TLC: R_f = 0.12

(+)-*4R*-**5a**: ^{13}C-NMR: δ (ppm) = 175.3 (CO_2H, C-1), 173.9 (CO_2H, C-6), 62.1 (CH_2, C-5; complete H/D exchange after 5 h at room temp. in D_2O), 54.2 (CH_2, C-3), 53.6 (CH, C-2).

(-)-*4S*-**6a**: ^{13}C-NMR: δ (ppm) = 175.8 (CO_2H, C-1), 173.9 (CO_2H, C-6), 61.5 (CH_2, C-5; complete H/D exchange after 5 h at room temp. in D_2O), 54.3 (CH_2, C-3), 53.3 (CH, C-2).

4(R)-S-Carboxy-[^{13}C]-methyl-L-cysteine S-Oxide [(+)-(4*R*)-**5b**]

A stirred suspension of **3b** (94 mg, 0.52 mmol) in 0.6 ml H_2O at room temp. was treated portion-wise with solid $NaHCO_3$ (42 mg, 0.50 mmol). After addition of 0.20 ml 30% aqueous H_2O_2 the mixture was allowed to stand at room temp. for 18 h; the pH was then adjusted to 2 to 3 with N aqueous HCl. The mixture was then cooled (+4°) and seeded with a trace of the isomeric mixture **5a/6a**. After 24 h at +4°, the mother liquor was pipetted off and the precipitated crystals were washed several times with ice/H_2O. The product was recrystallised from a minimum of hot H_2O and dried *in vacuo* over P_2O_5 to give colourless crystals having 96% isomeric purity (^{13}C-NMR); yield 32 mg (31%); mp. 180° (decomp.). - ^{13}C-NMR: δ (ppm) = 62.2 ($^{13}CH_2$, C-5, 96% 4*R*), 61.5 ($^{13}CH_2$, C-5, 4% 4*S*). - $C_4{}^{13}CH_9NO_5S$ (196.2) Calcd. C 30.6 H 4.62 H 7.1 S 16.3 Found C 30.8 H 4.63 N 6.9 S 16.2.

S-Carboxymethyl-L-cysteine S,S-Dioxide (**7**)

S-Carboxymethyl-L-cysteine (**3a**; 4.5 g, 25 mmol) was stirred with 20 ml 30% aqueous H_2O_2 at 40° for 20 h. After work-up of a small aliquot (see below), the ^{13}C-NMR spectrum showed, in addition to the isomeric sulphoxides, about 30% reaction to give **7**. Further 20 ml H_2O_2 solution were added to the mixture which was then stirred at 40° for 8 days. The fine precipitate was separated by centrifugation, washed peroxide-free with H_2O, and dried *in vacuo* over P_2O_5; yield 2.8 g (53%). An analytically pure sample was obtained by recrystallisation from hot H_2O (160 ml·g^{-1}); mp. 180.5° [Ref.[20] 181-185°]. - $[\alpha]_D^{22}$ = -4.5° (c = 1.0, N NaOH). - ^{13}C-NMR: δ (ppm) = 174.2 (CO_2H, C-1), 170.4 (CO_2H, C-6; reduced signal intensity after H/D exchange at C-5), 63.4 (CH_2, C-5; disappeared after H/D-exchange), 56.4 (C-3; very slow H/D-exchange after 18 h at 80°C), 51.9 (C-2). - $C_5H_9NO_6S$ (211.2) Calcd. C 28.4 H 4.30 N 6.6 S 15.2 Found C 28.5 H 4.30, N 6.6 S 15.1.

4(R)-N-Acetyl-S-carboxymethyl-L-cysteine S-Oxide (**8**)

Compound **5a** (1.5 g, 7.7 mmol) was suspended in 10 ml ice/H_2O and the pH was adjusted to 7.0 with 4 N NaOH. With continued cooling at 0°, acetic anhydride (1.56 ml, 15.6 mmol) was slowly added dropwise together with 4 N NaOH (in order to maintain pH = 7.0). The solution was subsequently applied to a cation exchanger resin column (Bio-Rad AG 50W-X8®, 100-200 mesh, H^+ form). After elution with H_2O, fractions found to contain **8** by TLC analysis were combined and concentrated under vacuum. The colourless crystalline residue was dried *in vacuo* over NaOH and then recrystallised from 85 ml EtOH; yield 1.32 g (72%); mp. 157°. - TLC: R_f = 0.42. - $[\alpha]_D^{21}$ = +37.5° (c = 1.0, H_2O). - ^{13}C-NMR: δ (ppm) = 178.4 (CO_2H, C-1), 176.5 (CONH), 174.1 (CO_2H, C-6), 61.6 (CH_2, C-5), 56.4 (CH_2, C-3), 53.1 (CH, C-2), 24.9 ($\underline{C}H_3CO$). - $C_7H_{11}NO_6S$ (237.2) Calcd. C 35.4 H 4.67 N 5.9 S 13.5 Found C 35.5 H 4.76 N 5.8 S 13.3.

4(S)-N-Acetyl-S-carboxymethyl-L-cysteine S-Oxide (**9**)

As described for the preparation of **8**, compound **6a** (900 mg, 4.6 mmol) was treated with acetic anhydride. The colourless, amorphous, highly hygroscopic product separated after work-up [yield 1.13 g (>100%)] could not be obtained in analytical purity either by recrystallisation from various solvents or by formation of a salt with *N,N*-dicyclohexylamine (DCHA). - TLC: R_f = 0.42. - $[\alpha]_D^{25}$ = -57.1° (c = 1.0, H_2O). - ^{13}C-NMR: δ (ppm) = 178.7 (CO_2H, C-1), 176.9 (CONH), 174.1 (CO_2H, C-6), 61.6 (CH_2, C-5), 56.8 (CH_2, C-3), 52.7 (CH, C-2), 24.9 ($\underline{C}H_3CO$). - $C_7H_{11}NO_6S$ (237.2) Calcd. C 35.4 H 4.67 N 5.9 S 13.5 Found C 33.7 H 4.79 N 5.5 S 12.9.

N-Acetyl-S-carboxymethyl-L-cysteine (**10**)

The *N*-acetylation of **3a** (2.0 g, 11.2 mmol) with acetic anhydride (2.2 ml, 22.3 mmol) was performed as described for compound **8** in analogy to Ref.[16]. After cation exchange chromatography, the resultant colourless oil was repeatedly treated with toluene, concentrated *in vacuo*, and dried; yield 2.48 g (99%); oil. - TLC: R_f = 0.48. - ^{13}C-NMR: δ (ppm) = 180.6 (CO_2H, C-6), 179.9 (CO_2H, C-1), 176.7 (CONH), 57.5 (CH, C-2), 40.1 (CH_2, C-5), 37.4 (CH_2, C-3), 24.9 ($\underline{C}H_3CO$).

Mono-DCHA Salt of **10**

A solution of **10** (1.08 g, 4.87 mmol) in EtOH (4 ml) was treated with DCHA (0.97 ml, 4.87 mmol). The colourless crystals formed after a short time were filtered off, washed with a small amount of cold EtOH, recrystallised from 6 ml boiling EtOH, and dried under vacuum; yield 1.51 g (77%); mp. 165-166°. - $[\alpha]_D^{25}$ = -12.9° (c = 1.0, H_2O). - ^{13}C-NMR: δ (ppm) = 56.2, 31.9, 27.3, 26.8 (cyclohexyl C atoms), the remaining resonances were identical to those of **10**. - MS (70 eV) of dimethyl ester (M_r = 249): m/z (rel. int.) = 249 (10%, M^+), 217 (5, M^+ - 32), 190 (37, M^+ - 59), 176 (44), 158 (100), 148 (32), 126 (32), 117 (26). - MS (70 eV) of bis (tert.-butyldimethylsilyl) ester (M_r = 449): m/z (rel. int.) = 449 (3%, M^+), 434 (4, M^+ - 15), 392 (100, M^+ - 57), 333 (24), 186 (23), 149 (21), 147 (23), 144 (20), 116 (32). - $C_{19}H_{34}N_2O_5S$ (402.5) Calcd. C 56.7 H 8.52 N 7.0 S 8.0 Found C 56.8 H 8.67 N 7.0 S 8.1.

S-[^{13}C]-Methyl-L-cysteine (**12b**)

L-Cysteine hydrochloride (945 mg, 5.4 mmol, monohydrate; Aldrich C12, 180-0) was dissolved at 0° in a mixture of H_2O (4 ml), MeOH (0.5 ml) and solid LiOH (280 mg). With continued cooling and stirring, a solution of [^{13}C]-CH_3I (**11b**; 1.0 g, 7.0 mmol, Aldrich 27,718-5, 99.4 atom % ^{13}C) in 1 ml MeOH was added dropwise within 5 min. After the slightly exothermic reaction had subsided (about 10 min), the cooling bath was removed and the pH was adjusted to 6-6.5 with aqueous N HCl. The mixture was then warmed briefly, small amounts of cystine were removed by filtration, and 60 ml MeOH were added to the warm mixture. After 18 h at +4°, the pure white flakes were filtered, washed thoroughly with MeOH (20-30 ml), and dried *in vacuo*. The filtrate and washings were combined, made up to a volume of 150 ml with acetone, and allowed to stand. After 18 h, the second precipitate (114 mg) was worked-up as described. Both fractions were analytically, spectroscopically, and chromatographically pure; yield 394 mg (54%); mp. 241-245° (decomp.) [Ref.[21] 245° (decomp.)]. - TLC: R_f = 0.43. - ^{13}C-NMR: δ (ppm) = 17.5 ($^{13}CH_3S$). - **12a**: δ (ppm) = 17.5 (CH_3S), 37.4 (SCH_2), 56.1 (CH), 173.7 (CO_2H). - Even after 28 days standing at room temp., no autoxidation could be detected. - $C_3{}^{13}CH_9NO_2S$ (136.2) Calcd. C 35.3 H 6.66 N 10.3 S 23.5 Found C 35.3 H 6.64 N 10.0 S 23.5.

S-Oxidation of Compounds **12a** *and* **12b**

S-Methyl-L-cysteine (**12a**; 13.5 g, 0.10 mol) was suspended in 50 ml H_2O at 0°, stirred, and treated during 9 h with 11.5 ml 30% aqueous H_2O_2. The mixture was allowed to stand at room temp. for 18 h and then treated with 180 ml acetone. The separated oil was digested several times with a total of 150 ml acetone and then seeded with crystals of the isomeric mixture **13a/14a**. After a further 18 h, the resultant crystal mass was finely powdered, thorougly washed with acetone, and dried under vacuum; yield

13.5 g (89%); colourless crystals; mp. 161-163°. According to the ^{13}C-NMR spectrum the isomer ratio of **13a** to **14a** amounted to 39:61. The mixture of the epimeric sulphoxides was employed in the fractional crystallisation (see below). The isomers could not be separated by TLC. - TLC: R_f = 0.16.

4(S)-(+)-S-Methyl-L-cysteine S-Oxide [(+)-4*S*-**13a**]

A portion (2.25 g, 14.9 mmol) of the above obtained mixture **13a/14a** was dissolved with warming in 4 ml H_2O and treated with 10 ml MeOH. After 24 h large, clear rhombic crystals had separated. The mother liquor was separated and saved for the isolation of **14a** (see below). The obtained crystalline fraction (95% isomeric purity according to ^{13}C-NMR) was dissolved in 1 ml of warm H_2O and treated with 3 ml MeOH. After isolation and drying, fine white platelets were obtained; yield 278 mg (32%); mp. 171-173° (decomp). [Ref.[23] 160-165° (decomp.), Ref.[36] 167-168° (decomp.). - d.e. (^{13}C-NMR): >99%. - $[\alpha]_D^{22}$ = +125.8° (c = 2.0, H_2O) {Ref.[36] $[\alpha]_D^{21}$ = 118° (c = 1.5, H_2O), Ref.[23] $[\alpha]_D^{21}$ = +125° (c = 0.4, H_2O)}. - ^{13}C-NMR: δ (ppm) = 175.4 (CO_2H), 56.6 (CH_2), 53.9 (CH), 40.9 (CH_3). $C_4H_9NO_3S$ (151.2) Calcd. C 31.8 H 6.00 N 9.3 S 21.2 Found C 31.7 H 5.97 N 9.3 S 21.4. - The assignment of the absolute configuration was based on the optical rotation in analogy to Refs.[17,37].

4(R)-(-)-S-Methyl-L-cysteine S-Oxide [(-)-4*R*-**13a**]

The mother liquor from the fractional crystallisation of **13a** (see above) was warmed, treated with 10 ml MeOH, and allowed to stand at room temp. for 18 h. After removal of the supernatant liquid with a pipette, small amounts of **13a** were separated by hand and the major portion consisting of clusters of fine needles was recrystallised as described from 2.8 ml H_2O and 7.0 ml MeOH. Colourless crystals with an isomeric purity of 92% (^{13}C-NMR) were obtained; yield 700 mg (51%). An analytically pure sample was obtained by a further recrystallisation from the same solvent mixture; mp. 168-170° (decomp.) [Ref.[23] 165-170° (decomp.)]. - $[\alpha]_D^{22}$ = -122.2° (c = 1.5, H_2O) {Ref.[23] $[\alpha]_D^{21}$ = -110° (c = 1, H_2O)}. - ^{13}C-NMR: δ (ppm) = 175.5 (CO_2H), 56.1 (CH_2), 53.3 (CH), 40.2 (CH_3). - $C_4H_9NO_3S$ (151.2) Calcd. C 31.8 H 6.00 N 9.3 S 21.1 Found C 31.6 H 5.93 N 9.2 S 21.2.

4(RS)-S-[^{13}C]-Methyl-L-cysteine S-Oxide (**13b, 14b**)

Compound **12b** (100 mg, 0.73 mmol) was suspended in 0.5 ml H_2O at 0° and then treated with 0.15 ml 30% aqueous H_2O_2. After 3 days at +4°, TLC analysis of the mixture indicated complete conversion. The clear solution was then warmed to 40° and treated with 10 ml acetone. The mixture was cooled again to +4°, the supernatant liquid was removed, the separated oil was washed repeatedly, and then seed crystals of the epimeric mixture **13a/14a** were added. After 2 days at room temp. the crystalline mass was thoroughly digested with acetone and dried under vacuum; yield 99.4 mg (89%), colourless crystals; mp 157-158°. - ^{13}C-NMR: δ (ppm) = 40.9 ($^{13}CH_3$, 37% 4*S*), 40.2 ($^{13}CH_3$, 63% 4*R*). - $C_3{}^{13}CH_9NO_3S$ (152.2) Calcd. C 31.6 H 5.96 N 9.2 S 21.1 Found C 31.6 H 5.97 N 9.1 S 21.3.

S-Methyl-L-cysteine S,S-Dioxide (**15**)

By employing an excess of H_2O_2, the sulphone **15** was obtained as a crystalline product after fractionation; mp. 178-180° (decomp.). - TLC: R_f = 0.33. - $[\alpha]_D^{22}$ = -9.1° (c = 0.7, H_2O). - ^{13}C-NMR: δ (ppm) = 175.2 (CO_2H), 57.6 (CH_2), 52.4 (CH), 43.9 (CH_3). - $C_4H_9NO_4S$ (167.2) Calcd. C 28.7 H 5.42 N 8.4 S 19.2 Found C 28.8 H 5.44 N 8.4 S 19.2.

N-Acetyl-S-methyl-DL-cysteine (*DL*-**16a**)

A suspension of **12a** (10 g, 74 mmol) in 50 ml acetic anhydride was stirred at room temp. for 18 h, the excess of acylating agent was removed *in vacuo*, and the residue was treated three times with toluene followed by MeOH (100 ml each) with evaporation to dryness each time. Drying of the colourless crystals thus obtained and two recrystallisations from boiling MeOH gave **16**; yield 5.1 g (39%), mp 154°. - TLC: R_f = 0.62. - $[\alpha]_D^{21}$ = -1.3° (c = 0.74, H_2O). - ^1H-NMR ($CDCl_3$, methyl ester): δ (ppm) = 2.06 (s; 3H, CH_3CONH), 2.11 (s; 3H, CH_3S), 2.97 (d; J = 5.1 Hz, 2H, H-3), 3.78 (s; 3H, OCH_3), 4.84 (dt; J = 7.6 Hz, 5.1 Hz, 1H, H-2), 6.29 (br. s; 1H, NH). On addition of Eu(hfc)$_3$, low field shifts and 1:1 splitting of the *S*-methyl resonance were observed (see Fig. 2). - ^{13}C-NMR: δ (ppm) = 180.0 (CO_2H), 176.7 (CONH), 57.1 (CH), 38.8 (CH_2), 24.9 (CH_3CO), 17.7 (CH_3S). - MS (70 eV) of methyl ester (M_r = 191): m/z (rel. int.) = 191 (6%, M^+), 132 (100, M^+ - 59), 117 (26), 100 (36). - MS (70 eV) of tert.-butyldimethylsilyl ester (M_r = 291): m/z (rel. int.) = 291 (2%, M^+), 234 (35, M^+ - 57), 186 (100), 175 (81), 144 (34), 116 (21), 105 (11). - $C_6H_{11}NO_3S$ (177.2) Calcd. C 40.7 H 6.26 N 7.9 S 18.1 Found C 40.6 H 6.28 N 8.0 S 18.1.

N-Acetyl-S-methyl-L-cysteine (*L*-**16a**)

The pH-controlled reaction of **12a** (2.25 g, 16.6 mmol) with acetic anhydride (3.1 ml, 31.9 mmol) as described for **8** gave, after ion exchange chromatography, an oil which was evaporated several times with toluene and then dried under vacuum. The crystalline mass (2.51 g) obtained after a short time was finely powdered, suspended in 120 ml ethyl acetate, and stirred for 40 min at room temp. After filtration of the racemate, which is poorly soluble in this solvent[38], the filtrate was treated with n-hexane (about 50 ml) until turbidity just occurred and then allowed to stand at 0°. In order to complete the crystallisation, the mixture was diluted twice with 30 ml n-hexane and allowed to stand each time at -30° for 18 h. After decantation of the mother liquor, the colourless crystals obtained were dried under vacuum; yield 2.01 g (68%); mp 76-78° (Ref.[23] 82°, Ref.[38] 73-80°). - $[\alpha]_D^{21}$ = -30.1° (c = 1.0, H_2O) {Ref.[23] $[\alpha]_D^{17}$ = -33° (c = 1, H_2O), Ref.[38] $[\alpha]_D^{18}$ = -37.8° (c = 0.7, H_2O)}. - ^1H-NMR ($CDCl_3$, methyl ester): see DL-16a, after addition of Eu(hfc)$_3$, no splitting of the methyl resonance was observed (see Fig. 2; e.e. >95%). - $C_6H_{11}NO_3S$ (177.2) Calcd. C 40.7 H 6.26 N 7.9 S 18.1 Found C 40.6 H 6.23 N 7.7 S 17.9.

N-[$^2H_3$]-Acetyl-S-methyl-DL-cysteine (*DL*-**16b**)

The reaction of **12a** (1.5 g, 11 mmol) with [$^2H_6$]-acetic anhydride (1.7 ml, 16.7 mmol) as described above for **16a** gave rise to **16b**; yield 810 mg (40%); mp. 153-154°. - $[\alpha]_D^{22}$ = -0.4° (c = 1.0, H_2O). - ^1H-NMR ($CDCl_3$, methyl ester): see DL-**16a**, the resonance at 2.06 ppm was absent. - ^{13}C-NMR: see **16a**, the signal at 24.9 ppm (CD_3) was absent and the intensity of the resonance at 176.7 ppm (CONH) was greatly reduced.

4(RS)-N-Acetyl-S-methyl-L-cysteine S-Oxide (**17**)

The *N*-acetylation of the above described epimeric mixture (39:61) of the sulphoxides **13a/14a** (2.32 g, 15.4 mmol) with acetic anhydride (3.1 ml, 31 mmol) in analogy to the preparation of **8** gave a solid product after concentration. This solid was repeatedly treated with toluene and evaporated each time. Recrystallisation from EtOH (15 ml) gave rise to colourless crystals which were washed with the same solvent and then dried under vacuum; yield 1.58 g (54%); mp. 134-135°. - TLC: R_f = 0.32. - $[\alpha]_D^{21}$ = -93.5° (c = 1.0, H_2O). - ^1H-NMR ($CDCl_3$, methyl ester): δ (ppm) = 2.05 (s; 60%, 3H, CH_3CONH), 2.07 (s; 40%, 3H, CH_3CONH), 2.67 (s; 3H, CH_3SO), 3.25 (m; 2H, $SOCH_2$), 3.81 (s; 3H, OCH_3), 4.90 (m; 1H, CH), 6.8 (br. m; 40%, 1H, NH), 7.0 (br. m; 60%, 1H, NH). The *S*- and *O*-methyl resonances of the epimeric sulphoxides were also split after addition of Eu(hfc)$_3$; no signal splittings attributable to the occurrence of the D-form could be detected. - ^{13}C-NMR (40/60 isomeric mixture): δ (ppm) = 178.2/178.5 (CO_2H, C-1), 176.5/176.7 (CONH), 58.9/58.8 (CH_2, C-3), 53.2/52.8 (CH,

C-2), 40.2 (CH_3S, C-5), 24.8/24.9 ($\underline{C}H_3CO$). - $C_6H_{11}NO_4S$ (193.2) Calcd. C 37.3 H 5.74 N 7.3 S 16.6 Found C 37.3 H 5.75 N 7.1 S 16.4.

A second recrystallisation from EtOH gave a product with mp. 137-139° and $[\alpha]_D^{22}$ = -105.5° (c = 1.0, H_2O) in an isomer ratio of 25:75. After the spectra had been recorded, the sample was treated with 1 ml of 30% H_2O_2 solution, allowed to stand at room temp. for 5 d, and the spectra were recorded again. A clean conversion to *N*-acetyl-*S*-methyl-L-cysteine *S,S*-dioxide was observed. - ^{13}C-NMR: δ (ppm) = 177.7 (CO_2H, C-1), 176.8 (CONH), 58.3 (CH_2, C-3), 52.5 (CH, C-2), 44.1 (CH_3SO_2, C-5), 24.9 ($\underline{C}H_3CO$).

3-(Carboxylmethylthio)-2-oxopropanoic Acid (**20a**)

3-Bromo-2-oxopropanoic acid (**19**, 10.0 g, 59.9 mmol) was dissolved in 10 ml H_2O and then neutralised with 6.0 g $NaHCO_3$. The mixture was cooled (0°), stirred, and treated dropwise with thioglycolic acid (**18**; 4.17 ml, 59.9 mmol) in 60 ml 1 M Na_2CO_3. After 2 h at room temp., the mixture was again cooled to 0° and acidified with 11 ml 10 N HCl. The mixture was saturated with solid NaCl and extracted 13 times with 100 ml each of ethyl acetate. The combined extracts were evaporated to dryness to furnish a pale yellow solid (5.25 g) which decomposed on attempted recrystallisation; purity: approx. 80-90% (according to ^{1}H- and ^{13}C-NMR). - ^{13}C-NMR: δ (ppm) = 201.3 ($\underline{C}OCO_2H$, C-2), 179.7 (CO_2H, C-6), 171.3 ($CO\underline{C}O_2H$, C-1), 40.9 (CH_2, C-3; rapid H/D-exchange in D_2O, see below), 39.4 (CH_2, C-5).

An analytically pure sample in the form of the DCHA salt was obtained as follows: DCHA (0.40 ml, 4.0 mmol) in acetone (4 ml) was treated with a solution of **20a** (356 mg, 2 mmol) in EtOH (2 ml). The mixture was allowed to stand at 0° for 18 h whereupon the precipitated crystals were filtered and recrystallised from EtOH/acetone (6.5 ml + 7.0 ml) to give the bis(*N,N*-dicyclohexylammonium) salt of **20a** as colourless crystals; yield 340 mg (31%), mp. 204-205° (decomp.). - $C_{29}H_{52}N_2O_5S$ (540.6) Calcd. C 64.4 H 9.69 N 5.2 S 5.9 Found C 64.5 H 9.73 N 5.1 S 6.3.

DL-3-(Carboxymethylthio)-lactic Acid (**21a**)

A solution of crude dicarboxylic acid **20a** (2.0 g, 11 mmol) in 20 ml H_2O was adjusted to pH = 10 with 21 ml N NaOH and then treated portionwise with solid $NaBH_4$ (420 mg, 11 mmol). After 2 h at room temp., 10 g cation exchanger resin (Bio-Rad AG 50W-X8®, 100-200 mesh, H^+-form) were added. The mixture was allowed to stand for a short time, the resin was filtered under suction, and the filtrate was evaporated to dryness. The pale yellow, oily residue was taken up in ethyl acetate, concentrated, and dried in vacuum; yield quantitative. Although the ^{13}C-NMR spectrum did not reveal the presence of any impurities, neither the dicarboxylic acid **21a** nor its DCHA salt (amorphous solid, mp. 191°) could be obtained in analytically pure form. - ^{13}C-NMR: δ (ppm) = 182.3 (CO_2H, C-1), 180.9 (CO_2H, C-6), 74.3 (CH, C-2), 40.2 (CH_2, C-5), 39.9 (CH_2, C-3). - MS (70 eV) of tris (ten.-butyldimethylsilyl) derivative (M_r = 522): m/z (rel. int.) = 507 (3%, M^+ - 15), 465 (100, M^+ - 57), 305 (44), 259 (50), 189 (25), 147 (30). - MS (70 eV) of dimethyl ester trimethylsilyl ether derivative (M_r = 280): m/z (rel. int.) = 280 (9%, M^+), 249 (10, M^+ - 31), 248 (13, M^+ - 32), 221 (28, M^+ - 59); 205 (52), 161 (29), 158 (35), 147 (19), 89 (47), 73 (100), 59 (34).

In Situ Generation of Compounds **20b, 21b,** *and* **24a**

Compound **20a** (179 mg, 1.0 mmol) was dissolved in 1.7 ml N NaOH and 2.5 ml phosphate buffer (1 M, pH = 7.0) and then treated with 0.2 ml D_2O and 2 mmol CH_3CN. The ^{13}C-NMR spectrum exhibited two resonance signals of equal intensity at 40.9 (C-3) and 39.5 ppm (C-5). The mixture was then lyophilised, taken up in 2 ml D_2O, lyophilised again, and the residue dissolved in 3.5 ml D_2O containing 2 mmol CH_3CN. After 30 min at room temp., the ^{13}C-NMR spectrum exhibited an unchanged resonance signal for C-5 and almost complete loss of signal intensity for C-3 of **20b**. The mixture was then stirred at room temp. while being treated within 30 min with $NaBD_4$ (10 mg), allowed to stand for 30 min at room temp., and the spectrum recorded again. The disappearance of the carbonyl signal at 201.5 ppm and the low field shift of the C-5 signal (40.2 ppm) indicated complete reduction to **21b**.

[2, 3, 3-$^2H_3$]-DL-3-(Carboxymethylthio)-lactic Acid (**21b**)

MS (70 eV) of tris(tert.-butyldimethylsilyl) derivative (M_r = 525): m/z (rel.int.) = 510 (3%, M^+ - 15), 468 (100, M^+ - 57), 307 (64), 261 (49), 189 (27), 147 (43). On the basis of the natural isotopic abundance of **21a**, the following isotope composition was calculated: 1.9% $^2H_2$ (d_2), 98.1% $^2H_3$ (d_3).

A solution of **20a** (0.5 mmol), prepared as described above and buffered to pH = 7, was treated at room temp. with 0.2 ml 30% H_2O_2. After the slightly exothermic reaction had subsided the ^{13}C-NMR spectrum exhibited only two resonance signals at 174.5 and 60.9 ppm for the carboxy- and methylene-C atoms of **24a**, respectively.

Di-([$^{13}C_2$]-carboxymethyl) Sulphide (**23b**)

[$^{13}C_2$]-Bromoacetic acid was treated with Na_2S in aqueous solution and the mixture worked-up as described for the unlabelled reagent[32]. Recrystallisation from ethyl acetate/n-hexane produced colourless crystals with mp 125-127°. - ^{13}C-NMR: identical with that of **23a** except for the ^{2}J coupling constants: δ (ppm) = 180.6 (d; J = 54 Hz, CO_2H); 40.2 (d; J = 54 Hz, CH_2).

Di-([$^{13}C_2$]-carboxymethyl Sulphoxide (**24b**)

The reaction of **23b** (0.51 mmol) was carried out as described for the preparation of **24a**; yield of **24b** 61%, colourless crystals. - ^{13}C-NMR: identical with that of **24a** except for the ^{2}J coupling constants: δ (ppm) = 174.5 (d; J = 52 Hz, CO_2H), 60.9 (d; J = 52 Hz, CH_2). - Isotopic distribution (determined by MS of the bis(α-methylpentafluorobenzyl) ester[30]: 0.7% $^{13}C_2$, 99.3% $^{13}C_4$.

Dicarboxymethyl Sulphone (**25**)

Oxidation of **23a** with $KMnO_4$ as described[34] gave **25**; mp. 187° [Ref.[34] 182°]. - ^{13}C-NMR: δ (ppm) 171.0 (CO_2H), 62.6 (CH_2).

Di-(carboxy-[$^2H_2$]-methyl) Sulphide (**26**)

A solution of **23a** (4.5 g, 30 mmol) and NaOH (2.4 g, 60 mmol) in 20 ml D_2O was heated under reflux for 48 h. About 15 ml D_2O/H_2O were then distilled off, 15 ml fresh D_2O were added, and the mixture was heated to boiling for a further 68 h. The resultant solution was allowed to cool, the pH was adjusted to 2 by N aqueous HCl, and the product was extracted with ethyl acetate. The org. phase was concentrated, the residue was first recrystallised from a small amount of D_2O and then from CH_3CN; yield 2.46 g (53%); mp 124-125.5°. - $C_6{}^2H_6O_4S$ (156.2) Calcd. C 30.8 ^{2}H 7.74 S 20.5 Found C 31.0 ^{2}H 7.50 S 20.4. - The isotope distribution was determined by MS of the bis(α-methylpentafluorobenzyl) ester[30]: 0.2% $^2H_0$, 1.3% $^2H_1$, 9.1% $^2H_2$, 35.4% $^2H_3$, 54.0% $^2H_4$.

2-(Methylsulphinyl)-acetic Acid (**28**)

The oxidation of 2-(methylthio)-acetic acid (**27**) with H_2O_2 as described[37] gave rise to the title compound. - DCHA salt: mp. 152° (Ref.[37] 151°).

When an excess of the oxidising agent was used (18 h, 80-100°), 2-(methylsulphonyl)-acetic acid (**29**) was obtained. - ^{13}C-NMR of 2-(methyl-

thio)-acetic acid: δ (ppm) = 180.9 (CO_2H), 41.5 (CH_2), 17.9 (CH_3). - ^{13}C-NMR of 2-(methylsulphinyl)-acetic acid: δ (ppm) = 174.4 (CO_2H), 63.1 (CH_2), 39.6 (CH_3). - ^{13}C-NMR of 2-(methylsulphonyl)-acetic acid: δ (ppm) = 171.2 (CO_2H), 64.1 (CH_2), 43.6 (CH_3).

References

1 L.B. Turnbull, L. Teng, J.M. Kinzie, J.E. Pitts, F.M. Pinchbeck, and R.B. Bruce, Xenobiotica *8*, 621 (1978).
2 R.H. Waring, Xenobiotica *8*, 265 (1978).
3 R.H. Waring, Eur. J. Drug Metab. Pharmacokin. *5*, 49 (1980).
4 S.C. Mitchell, R.H. Waring, C.S. Haley, J.R. Idle, and R.L. Smith, Br.J. Clin. Pharmacol. *18*, 507 (1984).
5 S.C. Mitchell and R.H. Waring, Pharmac. Ther. *43*, 237 (1989).
6 G.K. Scadding, R. Ayesh, J. Brostoff, S.C. Mitchell, R.H. Waring, and R.L. Smith, Br. Med. J. *297*, 105 (1988).
7 G.S. Panayi, G. Huston, R.R. Shah, S.C. Mitchell, J.R. Idle, R.L. Smith, and R.H. Waring, Lancet I, *1983*, 414.
8 P. Emery, G. Huston, J.R. Idle, S.C. Mitchell, G.S. Panayi, R.L. Smith, and R.H. Waring, Br. J. Clin. Pharmacol. *18*, 286P (1984).
9 S.C. Mitchell, R. Ayesh, R.H. Waring, R.L. Smith, R. Madhok, H. A. Capell, R.H. Withrington, and M.H. Seifert, 3rd World Conf. Clin. Pharmacol. Ther., Stockholm (Sweden), July 27, 1986, Abstr. No. 1125.
10 G. Steventon, A.C. Williams, R.H. Waring, H.S. Pall, and D. Adams, Lancet II, *1988*, 644.
11 A.B. Olomu, C.R. Vickers, R.H. Waring, D. Clements, C. Babbs, T.W. Warnes, and E. Elias, N. Engl. J. Med. *318*, 1089 (1988).
12 D. Specht, D. Ratge, K.P. Kohse, C.O. Meese, M. Eichelbaum and H. Wisser, J. Clin. Chem. Clin. Biochem. *26*, 769 (1988).
13 C.O. Meese, D. Specht, U. Hofmann, P. Fischer, and M. Eichelbaum, Eur. J. Clin. Pharmacol. *36*, A 151 (1989).
14 D. Specht, K.P. Kohse, D. Ratge, C.O. Meese, M. Eichelbaum, and H. Wisser, Eur. J. Clin. Pharmacol. *36*, A 227 (1989).
15 A.F. Bradbury and D.G. Smyth, Biochem. J. *131*, 637 (1973).
16 H. Ozawa, Bull. Chem. Soc. Jpn. *36*, 920 (1963).
17 C.O. Meese, Arch. Pharm. (Weinheim) *320*, 473 (1987).
18 L. Goodman, L.O. Ross, and B.R. Baker, J. Org. Chem. *23*, 1251 (1958).
19 B.J. Finkle and E.L. Smith, J. Biol. Chem. *230*, 669 (1958).
20 C. Zervos and E. Adams, Int. J. Peptide Res. *10*, 1 (1977).
21 S.C. Mitchell, R.L. Smith, R.H. Waring, and G.F. Aldington, Xenobiotica *14*, 767 (1984).
22 V. Du Vigneaud and C.E. Meyer, J. Biol. Chem. *98*, 295 (1932).
23 E.A. Barnsley, Biochim, Biophys. Acta *90*, 24 (1964).
24 S. Yllner, Acta Pharmacol. Toxicol. *30*, 69 (1971).
25 S. Yllner, Acta Pharmacol. Toxicol. *30*, 257 (1971).
26 R.E. Hefner, P.G. Watanabe, and P.J. Gehrling, Ann. N.Y. Acad. Sci. *246*, 135 (1975).
27 T. Green and D.E. Hathway, Chem.-Biol. Interactions *11*, 545 (1975); ibid. *17*, 137 (1977).
28 G. Müller, K. Norpoth, and R. Eckard, Cancer Lett. *7*, 299 (1979).
29 A. Grupe and G. Spiteller, J. Chromatogr. *230*, 335 (1982).
30 U. Hofmann, S. Holzer and C.O. Meese, J. Chromatogr. *509*, 349 (1990).
31 U. Hofmann, M. Eichelbaum, S. Seefried and C.O. Meese, Drug. Metab. Dispos., submitted.
32 J.M. Lovén, Ber. Dtsch. Chem. Ges. *27*, 3059 (1894).
33 M. Gazdar and S. Smiles, J. Chem. Soc. *93*, 1833 (1908).
34 J.M. Lovén, Ber. Dtsch. Chem. Ges. *17*, 2817 (1884).
35 C.O. Meese and P. Fischer, Z. Naturforsch., in press.
36 R.L.M. Synge and J.C. Wood, Biochem. J. *64*, 252 (1956).
37 E.A. Barnsley, Tetrahedron *24*, 3747 (1968).
38 S. Blackburn, R. Consden, and H. Phillips, Biochem. J. *38*, 25 (1951).

[Ph768]