Aspects of the Chemistry of Dehydromethionine

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Dehydromethionine [(1*R*,3*S*)-*S*-methylisothiazolidine-3-carboxylate] is shown to be a useful intermediate for the preparation of methyl-labelled methionines *via* its base-catalysed exchange in [$^2H_4$]methanol or methan[^{2}H]ol. However, it is not possible to effect stereoselective exchange of protons at C-5 of dehydromethionine using sodium[$^2H_3$]methoxide–[$^2H_4$]methanol. A complete analysis of the ^{1}H n.m.r. spectrum of dehydromethionine has been achieved by computer-assisted simulation and by comparison with the spectra of ^{2}H-labelled dehydromethionines. Dehydromethionine is converted, by treatment with aqueous sodium hydroxide, mainly into the (*S,S*)-sulphoxide of methionine. This result can be rationalised by postulating a trigonal bipyramidal intermediate having in-line OH and NH attached to sulphur. Syntheses of stereochemically distinct [3,4-$^2H_2$]methionines are described.

DEHYDROMETHIONINE (*S*-methylisothiazolidine-3-carboxylate) was first prepared by Lavine,[1] who correctly assigned its structure (without regard to relative stereochemistry) and reported its bacteriostatic properties.[2] An analysis of the crystal structure of the racemic compound revealed the carboxy-group to be on the opposite side of the ring to the *S*-methyl group.[3] The structure of the dehydromethionine derived from (*S*)-methionine is therefore that of compound (1), having the (1*R*,3*S*)-configuration.

Dehydromethionine is easily prepared by oxidising methionine, *e.g.* by iodine in methanol, and although this oxidation normally produces sulphoxides from sulphides, dehydromethionine arises because of intramolecular attack by the amino-group in a sulphonium iodide intermediate.[4] Dehydromethionine decomposes to 2-amino-4-methylsulphinylbutanoic acid (methionine sulphoxide) in aqueous alkali.[5] It is reduced to methionine by thiols in buffer solutions.[6] Dehydromethionine has been identified as an intermediate in the photo-oxygenation of methionine to methionine sulphoxide sensitised by Rose Bengal.[7] Surprisingly, refs. 1—7 are the complete literature of dehydromethionine, a unique heterocyclic compound.

When the crystal structure of dehydromethionine appeared, we were engaged in syntheses of stereospecifically labelled [3,4-$^2H_2$]methionines. The synthesis from (*E*)-[$^2H_2$]ethylene of a [3,4-$^2H_2$]methionine of uncertain relative configuration has been described.[8] We thought that dehydromethionine could be useful for the production of specifically labelled methionines. It was hoped that complete assignment of the ^{1}H n.m.r. spectrum of dehydromethionine would enable us to confirm the relative configurations of diastereoisomeric [3,4-$^2H_2$]methionines by converting them into the corresponding dideuteriodehydromethionines. Also, dehydromethionine would be expected to undergo exchange of its methyl group and C-5 protons. One of the C-5 protons might exchange faster than the other by analogy with 1-methylthiolanium iodide (2). In this compound, the protons *cis* to the *S*-methyl group exchange faster than those *trans*,[9] with a rate difference of between 12 : 1 and 28 : 1.[10]

We have also studied the stereochemistry of the conversion of dehydromethionine into methionine sulphoxide by aqueous alkali.[5]

RESULTS AND DISCUSSION

The ^{1}H *N.m.r. Spectrum of* (1R,3S)-*Dehydromethionine.*—The 220 MHz ^{1}H n.m.r. spectrum (Figure 1a) of (1*R*,3*S*)-dehydromethionine (1) in [$^2H_2$]water shows signals at δ 4.39, 3.8, 3.6, 2.8, and 2.5 (each 1 H, each m), and at δ 2.8 (s, methyl-H). The signal at δ 4.39 [(A) in Figure 1a] can be assigned to the proton α- to the carboxylate group [11] because of its multiplicity and

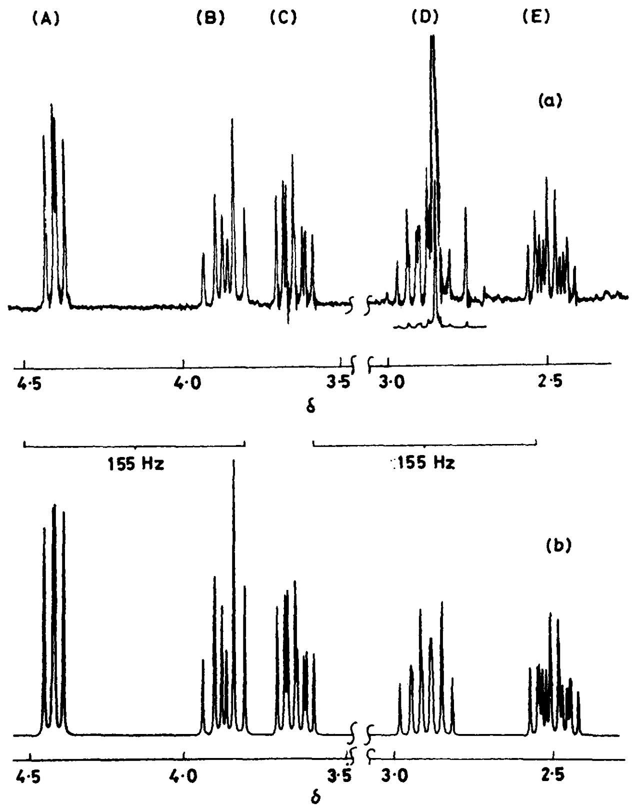
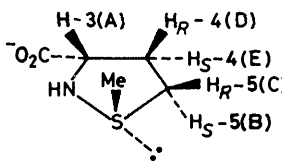


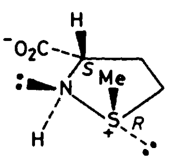
FIGURE 1 220 MHz ^{1}H N.m.r. spectrum of (1*R*,3*S*)-dehydromethionine: (a) observed spectrum; (b) calculated spectrum chemical shift, and the remaining four signals may be assigned to two geminal pairs. The signals at δ 3.8 (B) and 3.6 (C) are assigned as the pair attached to C-5,[12] and those at δ 2.8 (D) and 2.5 (E) as the pair attached to C-4.[13]

First-order analysis of the signals at δ 4.39, 3.8, and 3.6 leads to the following assignments:

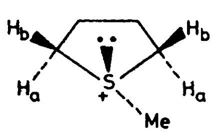
δ 4.39 (A) = dd; δ 3.8 (B) = ddd; δ 3.6 (C) = ddd, and J *ca.* 5.8 and 7.4 Hz for signal (A); J *ca.* 8.2 and 13.1 Hz for signal (B); and J *ca.* 5.0, 6.9, and 13.0 Hz for signal (C). J_{gem} *ca.* 13.8 Hz in signal (E) was obtained by measuring the width of the signal. There are four possible combinations of vicinal coupling constants: J_{AD} 5.8 or 7.4, J_{AE} 7.4 or 5.8, J_{BD} 8.2, J_{BE} 8.2, J_{CD} 5.0 or 6.9, and J_{CE} 6.9 or 5.0 Hz. Simulation of spectra with the aid of a computer gave the closest fit to the observed spectrum with J_{AD} 7.4, J_{AE} 5.9, J_{BD} 8.2, J_{BE} 8.2, J_{CD} 6.9, J_{CE} 5.0, J_{BC} 13.1, J_{DE} 13.9 Hz (*cf.* Figure 1b).

In the analysis of the ¹H n.m.r. spectra of 5-membered rings some authors[11] obtain a configurational assignment by applying the rule that, in 5-membered rings approaching planarity, $J_{trans} < J_{cis}$ generally holds (for a discussion of the validity of this approach for heterocycles with 5-membered rings see references 14 and 15). However, this approach fails for signal (B) because both J_{trans} and J_{cis} are 8.2 Hz. The conformation of the dehydromethionine (1) in crystals is an envelope with C-3 out of the plane defined by C-1, C-2, S, and N, and on the same side of this plane as the *S*-methyl group.[3] Assessment of vicinal coupling constants from dihedral angles in a Dreiding model of this conformation gave values inconsistent with the above. Therefore, compound (1) in solution may exist as two or more rapidly interconverting conformations.





(1)



(2)

FIGURE 2 Numbering scheme and H_R/H_S assignments for dehydromethionine

In analyses of the ¹H n.m.r. spectra of proline[11] and derivatives of proline,[16] performed by Pogliani *et al.*, a strong case is presented to suggest that the geminal non-equivalence of protons on the carbon atom β- to the carboxy-group is due to an anisotropic field effect from the carboxy-group on protons lying in its plane. They conclude that the proton in a *cis*-orientation to the carboxy-group appears at higher field to that in a *trans*-orientation. Hence, with dehydromethionine the proton H_S-4 (*cf.* Figure 2) gives rise to the resonance at δ 2.5, *i.e.* H_S-4 gives signal (E) and H_R-4 gives signal (D). Assuming that $J_{trans} < J_{cis}$ for signal (C) leads to the assignments H_R-5 gives (C) and H_S-5 gives (B) [N.B. J_{CD} (6.9 Hz) > J_{CE} (5.0 Hz)]. The complete assignment of the ¹H n.m.r. spectrum of the dehydromethionine (1) is given in the Table.

¹H N.m.r. spectral assignments for the dehydromethionine (1)

δ	J
(1) H-3 = 4.39 (A)	(1)-(2) = 7.4
(2) H_R-4 = 2.8 (D)	(1)-(3) = 5.9
(3) H_S-4 = 2.5 (E)	(1)-(4) = 0
(4) H_S-5 = 3.8 (B)	(1)-(5) = 0
(5) H_R-5 = 3.6 (C)	(2)-(3) = −13.9
	(2)-(4) = 8.2
	(2)-(5) = 6.9
	(3)-(4) = 8.2
	(3)-(5) = 5.0
	(4)-(5) = −13.1

Syntheses of Stereospecifically Labelled [3,4-$^2H_2$]*Methionines*.—The method of synthesis is analogous to that described for racemic [3,4-$^{13}C_2$]methionine.[17] [$^2H_2$]Acetylene was reduced stereospecifically to (*E*)- or (*Z*)-[$^2H_2$]ethylene ($C_2H_2{}^2H_2$), and the stereochemical purity of these ethylenes was checked by i.r. spectroscopy.[18] The ethylenes were then converted into 1-chloro-2-methylthioethanes *via* reaction with methanesulphenyl chloride at low temperature in dichloromethane. This reaction is a *trans*-addition involving a symmetrical cyclic intermediate,[19,20] and so the product obtained is a 1 : 1 mixture of enantiomers in each case [*i.e.* 1 part (1*R*,2*R*) + 1 part (1*S*,2*S*), from (*E*)-[$^2H_2$]ethylene and 1 part (1*R*,2*S*) + 1 part (1*S*,2*R*) from (*Z*)-[$^2H_2$]ethylene]. These chlorides were then condensed with the sodium salt of diethyl acetamidomalonate in ethanol to give labelled ethyl 2-acetamido-2-ethoxycarbonyl-4-(methylthio)butanoates.[21] This reaction was expected to proceed by an S_N1 pathway, *via* a thiiranium ion, giving products with the stereochemistry shown in Figures 3a and 3b. To prove this we set up a competitive reaction between 1-chloro-2-methylthioethane (14 mmol) and 1-chlorobutane (14 mmol) and the sodium salt of diethyl acetamidomalonate (24 mmol) in ethanol. The *sole* product was derived from 1-chloro-2-methylthioethane, *i.e.* no product from 1-chlorobutane was observed. Note that 1-chloro-2-methylthioethane reacts with potassium iodide–acetone (S_N2 conditions) only 1.5 times faster than 1-chlorobutane.[22] Hydrolysis of ethyl 2-acetamido-2-ethoxycarbonyl-4-(methylthio)butanoate in aqueous hydrochloric acid leads directly to racemic methionine.[21] The product isolated from a dideuteriated intermediate would be a mixture of four stereoisomers, (2*R*, 3*R*, 4*R*)-, (2*S*, 3*R*, 4*R*)-, (2*R*, 3*S*, 4*S*)-, and (2*S*, 3*S*, 4*S*)-[3,4-$^2H_2$]methionine, from the synthesis which starts from (*E*)-[$^2H_2$]ethylene; this will be abbreviated as rac-(3*R*,4*R*)methionine. From (*Z*)-[$^2H_2$]-ethylene a mixture of rac-(3*S*,4*R*)-[3,4-$^2H_2$]methionine and rac-(3*R*, 4*S*)-[3,4-$^2H_2$]methionine [abbreviated as rac-(3*R*,4*S*)-methionine] is obtained (see Figures 3a and 3b).

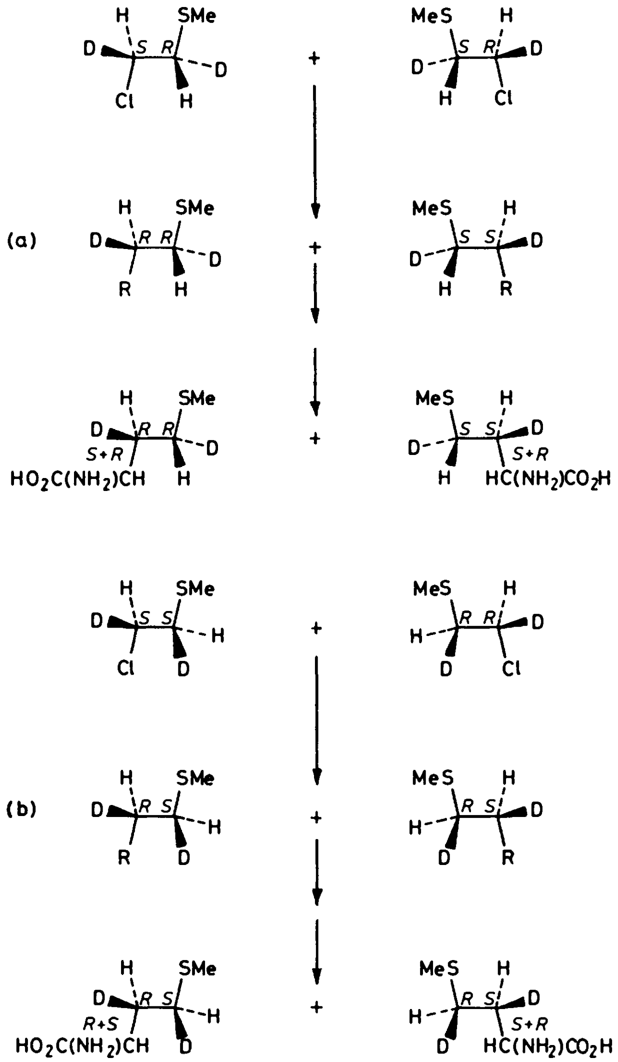


FIGURE 3 (a) Synthesis of rac-(3*R*,4*R*)-methionine. (b) Synthesis of rac-(3*R*,4*S*)-methionine (R = ethyl 2-acetamido-4-ethoxycarbonyl)

Conversion of [3,4-$^2H_2$]*Methionines into Dehydromethionines;* ^{1}H *N.m.r. Spectra of the Latter Compounds.*—The structures and stereochemistries of the dehydromethionines derived from these methionines are shown in Figure 4. It can be seen that the mixture of dideuteriodehydromethionines derived from rac-(3*R*, 4*R*)-methionine will show only *cis*-vicinal couplings and that from rac-(3*R*, 4*S*)-methionine will show only *trans*-vicinal couplings for the C(4)–C(5) bond.

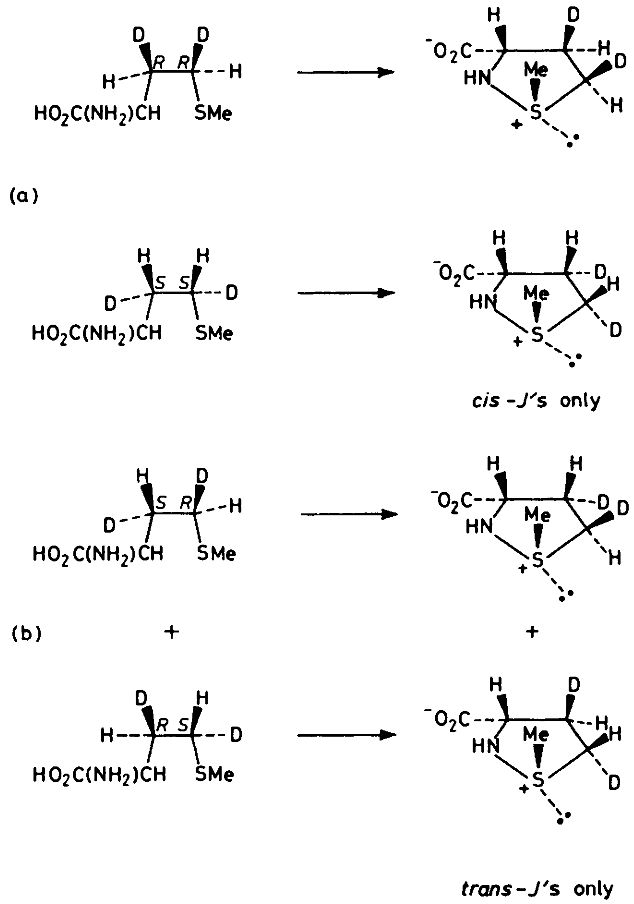


FIGURE 4 The stereochemistries of the dehydromethionines derived from (a) rac-(3*R*,4*R*)-, and (b) rac-(3*R*,4*S*)-methionine. (Only the materials derived from (*S*)-methionine are shown)

The 220 MHz ^{1}H n.m.r. spectrum of the mixture of the dideuteriodehydromethionines obtained from rac-(3*R*, 4*R*)-methionine shows doublets at δ 3.8 (B) (*J* 8.2 Hz), and 3.6 (C) (*J* 6.9 Hz) (in Figure 5) which are both *cis*-couplings. Decoupling by irradiating at δ 2.5 (E) caused the signal at δ 3.8 (B) to collapse to a singlet, whereas the signal at δ 3.6 (C) was unaffected (*cf.* Figure 6a). Irradiation at δ 2.8 (D) caused the signal at δ 3.6 (C) to collapse, whereas the signal at δ 3.8 (B) was unchanged, confirming the stereochemical homogeneity of the sample. Thus, J_{BE} is 8.2 Hz and is a *cis*-coupling, and J_{CD} is 6.9 Hz and is also a *cis*-coupling.

The 220 MHz ^{1}H n.m.r. spectrum of the mixture of dideuteriodehydromethionines obtained from rac-(3*R*, 4*S*)-methionine also showed two doublets, at δ 3.8 (B) (*J* 8.2 Hz), and 3.6 (C) (*J* 5 Hz) (Figure 7) which are both *trans*-couplings. In this case, decoupling by irradiating at δ 2.5 (E) caused the signal at δ 3.6 (C) to collapse to a singlet, whereas the signal at δ 3.8 (B) was unaffected (*cf.* Figure 6b). Irradiation at δ 2.8 (D) caused the collapse of the signal at δ 3.8 (B) to a singlet, and again no residual doublet or effect on other signals was observed. Thus, J_{BD} is 8.2 Hz and is a *trans*-coupling, and J_{CE} is 5.0 Hz, and is also a *trans*-coupling (*cf.* Figure 7). These results are fully consistent with the assignment of coupling constants and chemical shifts given in the Table.

Base-catalysed Exchange Reactions of Dehydromethionine.—Addition of an excess (25%) of sodium [^{2}H]-hydroxide to a solution of dehydromethionine in [$^2H_2$]-water gave a solution which, after 2 min at 37 °C,

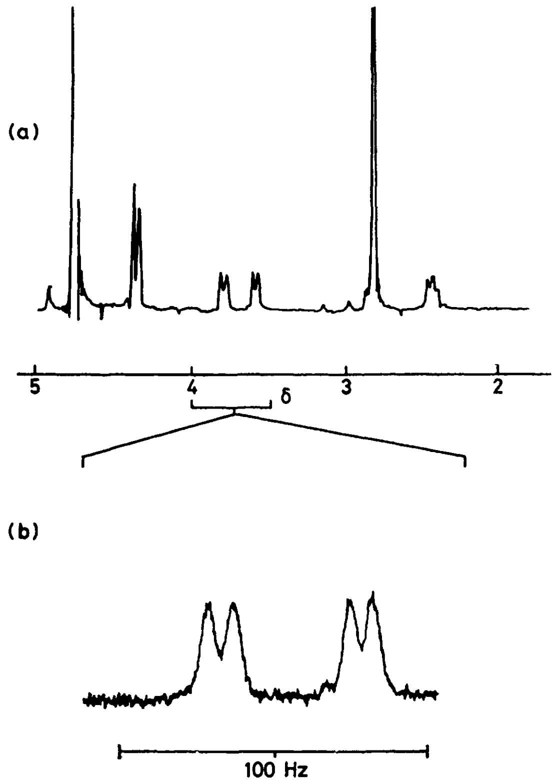


FIGURE 5 The 220 MHz ^{1}H n.m.r. spectrum of the dehydromethionine derived from rac-(3*R*,4*R*)-methionine (a) and expanded as shown (b)

contained no dehydromethionine (^{1}H n.m.r. analysis). By t.l.c. and comparison with reference spectra, the product was identified as methionine sulphoxide with *ca.* 70% deuterium in its methyl group. No further exchange of this methionine sulphoxide was observed over 24 h at 37 °C. Further experiments involving the addition of 0.1, 0.25, 0.50, and 1 mol equiv. of sodium [^{2}H]hydroxide to dehydromethionine solutions demonstrated that two reactions were occurring: the exchange of the methyl protons in dehydromethionine, and the stoicheiometric, irreversible reaction between NaO^{2}H and dehydromethionine giving methionine sulphoxide, which did not undergo further exchange. The partial deuterium incorporation observed in the methyl group of the methionine sulphoxide indicates that the rates of the two reactions are comparable. In all cases reactions were complete in under 2 min.

We reasoned that exchange in dehydromethionine without decomposition might be effected by an alkoxide ion in the corresponding alcohol, because the reaction leading to methionine sulphoxide requires eventual deprotonation of the attacking O^{2}H$^-$. Therefore, the exchange of dehydromethionine in [$^2H_4$]methanol, containing catalytic sodium [$^2H_3$]methoxide, was examined. Addition of 0.018 mol equiv. of sodium [$^2H_3$]methoxide to a solution of dehydromethionine in [$^2H_4$]methanol led to exchange of the methyl protons with $\tau_{\frac{1}{2}}$ of $\leqslant 3$ min at 37 °C. The rate of this reaction was monitored by ^{1}H n.m.r. spectroscopy of the methyl protons. A plot of log[H] (where H = % of protons present in the partially deuteriated species) *versus* time is shown in Figure 8. The values of [H] are corrected to allow for the fact that at equilibrium the dehydromethionine would contain 91% ^{2}H in its methyl group. The non-linearity of this plot is attributed to the consumption of base by its irreversible reaction with dehydromethionine (possibly caused by trace amounts of water) to give methionine sulphoxide. However, an estimate of $\tau_{\frac{1}{2}}$ for the reaction could be obtained.

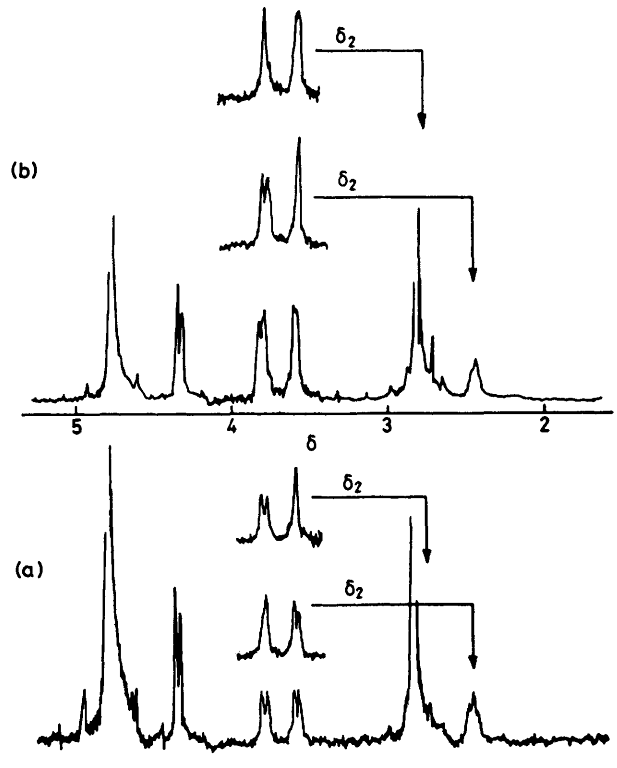


FIGURE 6 The effect of decoupling at the frequencies indicated on the ^{1}H n.m.r. spectra of the dehydromethionines derived from (a) rac-(3*R*,4*R*)-, and (b) rac-(3*R*,4*S*)-methionine

At higher concentrations of base (0.05, 0.10, and 0.50 mol equiv.) exchange of the methyl protons was too fast to be followed by ^{1}H n.m.r. spectroscopy. At these higher concentrations of base, no exchange of protons at C-5 could be detected (90 MHz ^{1}H n.m.r. spectroscopy) during 96 h. Instead the dehydromethionine decomposed at varying rates to methionine sulphoxide and methionine, identified by ^{1}H n.m.r. spectroscopy and t.l.c., until all of the base present had been consumed.

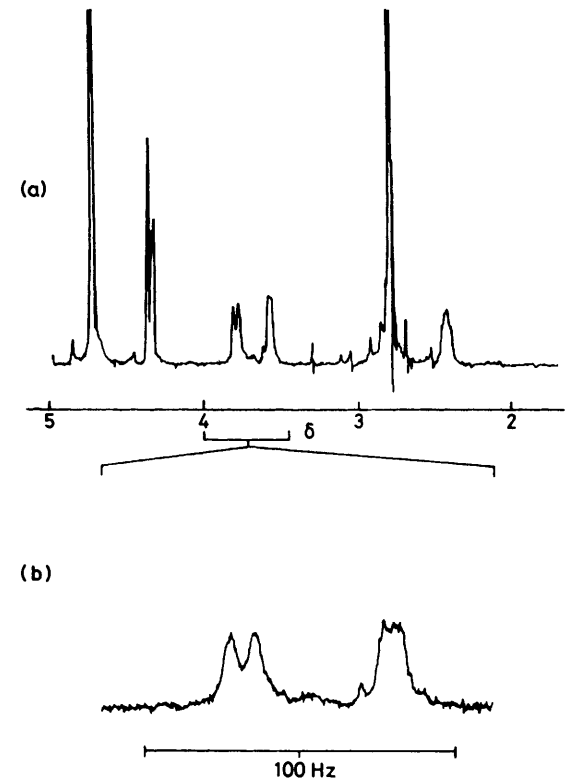


FIGURE 7 The 220 MHz ^1H n.m.r. spectra of the dehydromethionines derived from rac-(3*R*,4*S*)-methionine (a) and expanded as shown (b)

Thus, owing to the instability of dehydromethionine in basic media, we were unable to observe any exchange at C-5.

All the above reactions in [$^2H_4$]methanol were very sensitive to trace amounts of water ($NaOC^2H_3 + H_2O \longrightarrow NaOH + C^2H_3OH$, and then NaOH reacts

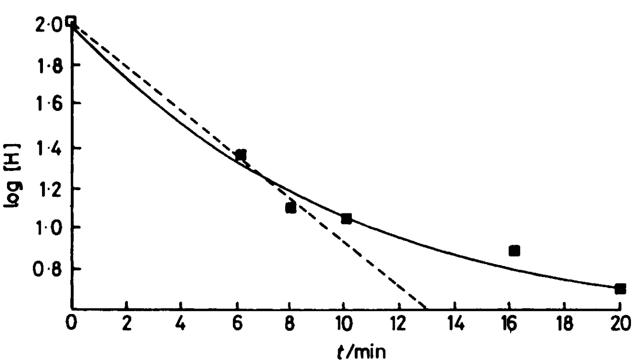


FIGURE 8 Qualitative kinetic plot of log[H] for dehydromethionine in [$^2H_4$]methanol (H = % unexchanged exchangeable hydrogen at time *t*) (1.8 mol % base present; 37 °C)

irreversibly with dehydromethionine), *i.e.* for the exchange which is represented in Figure 8, the amount of water necessary to consume effectively all the base would be 6×10^{-6} mol or 0.1 mg. However, the exchange of dehydromethionine in methan[^2H]ol at low base concentrations is useful for preparing methyl labelled methionines.[23] The stereochemical implication of the stoicheiometric conversion of dehydromethionine to methionine sulphoxide is described below.

The rate of the exchange of the methyl group in dehydromethionine in both water and [$^2H_4$]methanol is very fast, *cf.* the rate of exchange of 1-methylthiolanium iodide.[12] To compare directly the rates of methyl exchange for dehydromethionine and 1-methylthiolanium iodide, we studied the latter's exchange under conditions

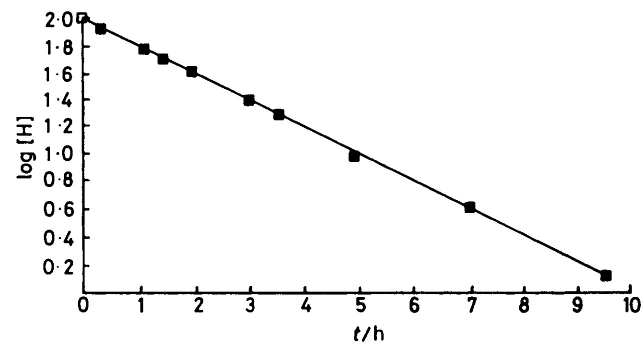


FIGURE 9 Qualitative kinetic plot of log[H] for 1-methylthiolanium iodide in [$^2H_4$]methanol (1.8 mol % base present; 37 °C)

identical with those used for dehydromethionine. The results indicate (Figure 9) that in [$^2H_4$]methanol containing 0.02 mol equiv. of sodium [$^2H_3$]methoxide the rate constant for exchange ($\tau_{\frac{1}{2}}$ 90 min) of the methyl protons of 1-methylthiolanium iodide is *ca.* 1/30th that of the methyl protons in dehydromethionine. Strict first-order kinetics are followed by the thiolanium compound (2) because no irreversible reaction between base ($NaOC^2H_3$ or NaO^2H) and 1-methylthiolanium

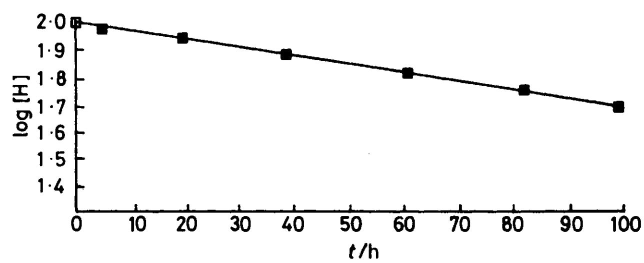


FIGURE 10 Qualitative kinetic plot of log[H] for 1-methylthiolanium iodide in [$^2H_2$]water (1.8 mol % base present; 37 °C)

iodide occurs. The rate of exchange for compound (2) was also examined under similar conditions in [$^2H_2$]water containing sodium [^2H]hydroxide (Figure 10). Here, the $\tau_{\frac{1}{2}}$ observed was almost 100 h, indicating a difference in rates between methanol and water of *ca.* 60-fold.

The Preparation of Optically Active Methionine Sulphoxide from Dehydromethionine.—Lavine reported[24] the oxidation of L-methionine to methionine sulphoxide by a number of reagents, including alcoholic iodine. In this case, sulphoxide was produced which he assayed as (+)-L-methionine sulphoxide [(*S*)-methionine-(*S*)-sulphoxide],[25] and he identified dehydromethionine as an intermediate in this very slow reaction (165 h). In the light of the crystal structure of dehydromethionine,[3] we considered that treatment of (1*R*,3*S*)-dehydromethionine [derived from (S) methionine] with base should lead to (*S*)-methionine (*S*)-sulphoxide [6,25] *via* the mechanism shown in Figure 11. Attack of OH^-

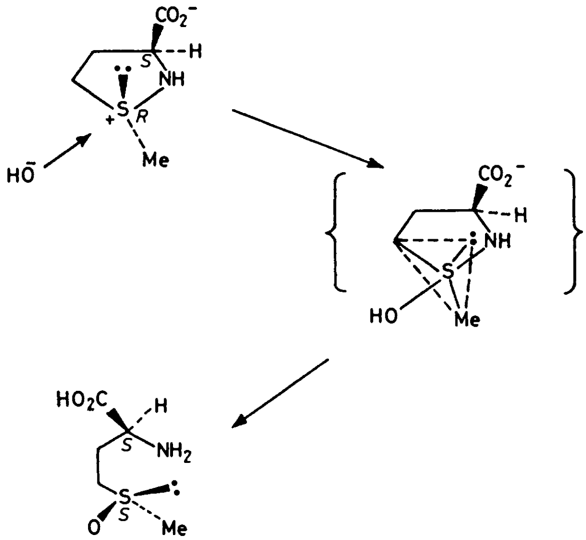


FIGURE 11 A possible mechanism for the production of (*S*)-methionine (*S*)-sulphoxide from (1*R*,3*S*)-dehydromethionine

along the axis of the S–N bond would lead to a trigonal bipyramidal intermediate with the maximum number of electronegative substituents in apical positions. The existence of intermediates of this nature is now well supported.[26,27] The intermediate shown (*cf.* Figure 11) would not be expected to undergo Berry pseudorotation [28] before decaying by fission of the apical S–N bond to (*S*)-methionine (*S*)-sulphoxide.

As reported above, the stoicheiometric conversion of dehydromethionine into methionine sulphoxide by sodium [^{2}H]hydroxide in [^{2}H]water takes *ca.* 2 min with 1 mol equiv. of sodium [^{2}H]hydroxide. To obtain methionine sulphoxide in a pure state, dehydromethionine was treated with aqueous lithium hydroxide and, after neutralisation, the addition of a large excess of acetone led to the precipitation of methionine sulphoxide, while lithium chloride remained in solution (*cf.* reference 24). This gave methionine sulphoxide (pure by t.l.c. and ^{1}H n.m.r. spectroscopy) in yields of 80—85% and with $[\alpha]_D^{24}$ +120° (*c* 1.8 in 1N-HCl), compared with the highest reported value for (*S*)-methionine (*S*)-sulphoxide of +131°.[25,29]

The discrepancy in our value for the optical rotation could be due to the presence of (*S*)-methionine (*R*)-sulphoxide, (*R*)-methionine (*R*)-sulphoxide, (*R*)-methionine (*S*)-sulphoxide, or a mixture of these materials. (*S*)-methionine (*R*)-sulphoxide ($[\alpha]_D^{25}$ −57.6° [25]) would arise if (1*S*,3*S*)-dehydromethionine were an impurity in (1*R*,3*S*)-dehydromethionine and was cleaved by OH^- attack along its S–N axis. Alternatively, this sulphoxide could arise from (1*R*,3*S*)-dehydromethionine *via* attack by OH^- along the axis of an S–C bond. To give the observed optical rotation, the composition of the isolated sulphoxide would be 94.1% (*S*)-methionine (*S*)-sulphoxide and 6% (*S*)-methionine (*R*)-sulphoxide. If the latter arose from (1*S*, 3*S*)-dehydromethionine, this would account for 6% of the dehydromethionine samples used. However, no evidence of diastereoisomeric (1*S*,3*S*)-dehydromethionine could be found in any of the samples prepared (220 MHz ^{1}H n.m.r. analysis). If (*R*)-methionine were an impurity in the commercial (*S*)-methionine used, it would give mainly (1*S*, 3*R*)-dehydromethionine on oxidation. Cleavage of this material by a similar mechanism to that shown in Figure 11 would give (*R*)-methionine (*R*)-sulphoxide ([α] −131°). Hence, the composition of the isolated methionine sulphoxide isolated would be 96% (*S*)-methionine (*S*)-sulphoxide and 4% (*R*)-methionine (*R*)-sulphoxide.

The preparation of (*S*)-methionine (*S*)-sulphoxide from (*S*)-methionine *via* (1*R*, 3*S*)-dehydromethionine is not as stereochemically efficient as the resolution of the picrates [24,25] of methionine sulphoxide, or as the oxidation of methionine by gold(III) chloride.[29] However, it has the advantages of being rapid, experimentally simple, and of using readily available, cheap starting materials.

EXPERIMENTAL

^{1}H N.m.r. spectra were recorded with either (i) a Perkin-Elmer R-12 spectrometer operating at 60 MHz, or (ii) a Bruker WH90 spectrometer operating at 90 MHz, or (iii) a Perkin-Elmer R-34 spectrometer operating at 220 MHz. Optical rotations were measured with a Bendix NPL automatic polarimeter (Model 143D, using a 1.00 cm × 0.708 cm^2 cell). T.l.c. analyses were performed using Merck 5554 silica plates in one of the following solvent systems: (1) ammonia (*d* 0.886)–absolute ethanol (23 : 77); (2) dry methanol. Spots were visualised by spraying with ninhydrin and heating. Solutions of sodium methoxide in methanol (or deuteriomethanol) were prepared from a dry solvent and clean sodium in a dry box. Sodium was cleaned by dipping small, freshly cut pieces in a small sample of the solvent methanol. Aliquots of the bases were titrated against standard hydrochloric acid solutions (prepared from B.D.H. CVS ampoules) using phenolphthalein as indicator. TSS refers to sodium 3-trimethylsilylpropanesulphonate throughout. L-Methionine was obtained from B.D.H. and Fluka and possessed optical rotations in agreement with the highest literature values.

Preparation of [3,4-$^2H_2$]*Methionines*.—The procedure used was analogous to that described [17] for rac-[3,4-$^{13}C_2$]-methionines, but starting from either (*E*)- or (*Z*)-[$^2H_2$]-ethylene. [3,4-$^2H_2$]Methionines were obtained, after recrystallisation from aqueous ethanol, as brilliant white crystals. rac-(3*R*,4*S*)-Methionine (34%) (overall yield from [$^2H_2$]ethylene) m.p. 275—277 °C, δ (220 MHz; ^2H_2O/^{2}HCl, TSS) 2.12 (3 H, s), 2.25 (1 H, br, 2 × t), 2.7 (1 H, br d, *J* 4.8 Hz), and 4.28 (1 H, d), pure by t.l.c. (system 1, R_F 0.45); rac-(3*R*,4*R*)-methionine (30%) (overall yield from [$^2H_2$]ethylene), m.p. 275—278 °C, δ (220 MHz; ^2H_2O/

^{2}HCl, TSS) 2.12 (3 H, s), 2.25 (1 H, br, 2 × t), 2.7 (1 H, br d, *J* 7.3 Hz), and 4.28 (1 H, d), pure by t.l.c. (system 1).

Preparation of (1R,3S)-*Dehydromethionine.*—This material was prepared essentially as described in ref. 3, and was routinely purified by flash chromatography according to the method of ref. 30 (elution with methanol).

Preparation of [4,5-$^2H_2$]*Dehydromethionines.*—[4,5-$^2H_2$]-Dehydromethionines (*cf.* Figure 4) were prepared from rac-(3*R*,4*R*)-methionine and rac-(3*R*,4*S*)-methionine by the method of ref. 3. On a 100-mg scale it was convenient to purify the dehydromethionines by flash column chromatography using a 10-mm diam. column and eluting with methanol.

^{1}H *N.m.r. Analysis of* (1R,3S)-*Dehydromethionine and* [4,5-$^2H_2$]*Dehydromethionines.*—220 MHz ^{1}H N.m.r. spectra were recorded on samples of dehydromethionine (50 mg) in [^{2}H]water (99.8% ^{2}H; 0.5 cm^3) and referenced against TSS. The spectrometer was carefully tuned to achieve the best possible resolution by locking and tuning on the resonance of 2% t-butyl alcohol added to the samples. The broad nature of the signals at δ 3.8 and 3.6 in Figures 5, 6, and 7 arises from the unresolved ^{2}H–^{1}H couplings present in the signals. Spectra were computer simulated using a Nicolet NIC-80/S-7117-D (NMRCAL) computer programme.[31] Details of the theory and operation of the programme are presented in refs. 31 and 32. After simulation, a line width of 0.7 Hz was added to the calculated spectrum to enable plotting *via* the normal spectrometer plotting table.

The analysis of the ^{1}H n.m.r. spectrum of a single optical isomer of dehydromethionine, *i.e.* (1*R*, 3*S*)- derived from (*S*)-methionine, was performed, whereas the [4,5-$^2H_2$]-dehydromethionines were racemic [*i.e.* 1 : 1 (1*R*,3*S*)-(1*S*, 3*R*) derived from (*S*)- and (*R*)-methionines]. Therefore, the ^{1}H n.m.r. spectra of dehydromethionine derived from racemic methionine was also examined. The spectrum was identical with that of dehydromethionine derived from (*S*)-methionine. No significant differences existed in the ^{1}H n.m.r. spectra of crude samples, compared with samples which had been purified by flash column chromatography, and samples which had been so purified and then recrystallised.

Preparation of 1-*Methylthiolanium Iodide.*—Methyl iodide (24.2 g, 10.6 cm^3, 0.17 mol) was added dropwise to stirred tetrahydrothiophen (10 g, 10 cm^3, 0.11 mol) during 10 min at 0 °C. After 12 h at room temperature, the solid product was broken up, washed by decantation with ethanol (3 × 20 cm^3) and then ground with ethanol (100 cm^3). The resulting suspension was filtered at the pump and washed with ethanol (5 × 20 cm^3). Drying *in vacuo* gave 1-methylthiolanium iodide as a white solid, with a faint odour of tetrahydrothiophen. Two recrystallisations from ethanol–ether gave 1-methylthiolanium iodide as pure white odourless needles (19.5 g, 77%) which sublime at 190—192 °C, δ (^2H_2O, TSS) 2.38 (2 H, m), 2.87 (3 H, s), and 3.55 (2 H, m).

Preparation of (S)-*Methionine* (S)-*Sulphoxide from* (S)-*Methionine* via (1R, 3S)-*Dehydromethionine.*—To a solution of (1*S*, 3*R*)-dehydromethionine (0.1 g, 0.67 mmol) in water (1.75 cm^3) was added saturated aqueous lithium hydroxide (0.25 cm^3 of a 3.3M-solution, 0.825 mmol). After leaving for 15 min at room temperature, the pH of the solution was adjusted to 7 by the addition of 2M-hydrochloric acid. Acetone (*ca.* 20 volumes) was slowly added, and the white solid which formed was allowed to settle for 20 min. The white crystalline precipitate was collected at the pump, washed with acetone (2 × 5 cm^3), and dried *in vacuo* to give (*S*)-methionine (*S*)-sulphoxide (0.9 g, 81%) as white crystals which darkened and sintered at 240—247 °C (in agreement with the literature [24]), pure by t.l.c. (system 1, R_F 0.29, and system 2, R_F 0.21), δ (^2H_2O, TSS) 2.3 (2 H, m), 2.7 (3 H, s), 3.0 (2 H, m), and 3.9 (1 H, t); $[\alpha]_D^{24}$ 120° (*c* 1.8 in 1M-HCl), and contained no Li$^+$ ions (flame test). The isomeric composition of this material is discussed in the text.

Exchange Reactions.—Reactions in [$^2H_4$]methanol were carried out under scrupulously anhydrous conditions, all manipulations being performed in a dry box, using very dry apparatus.

Exchange of (1R,3S)*dehydromethionine and* 1-*methylthiolanium iodide in* [$^2H_4$]*methanol.* In a dry box, sodium methoxide solution in [$^2H_4$]methanol (10 μl of a 0.625M-solution, 0.0063 mmol, 1.8 mol %) was added to a solution of (1*R*,3*S*)-dehydromethionine (0.05 g, 0.34 mmol) in [$^2H_4$]methanol (0.6 cm^3) and the solution was stored in a tightly capped n.m.r. tube (diam. 5 mm) at 37 °C. 60 MHz ^{1}H N.m.r. spectra were recorded at intervals, and integrated in order to compare the ^{2}H content of the methyl signal with that of non-exchanging C-4 protons. The results were corrected to allow for the equilibrium ^{2}H content of dehydromethionine being 91% and were then plotted as log[H%] present *versus* time. The results are depicted in Figure 8 and discussed in the text.

The rate of methyl exchange in 1-methylthiolanium iodide was studied under similar conditions. Thus, sodium methoxide in [$^2H_4$]methanol (12.5 μl of a 0.505M-solution, 0.0063 mmol, 1.8 mol %) was added to a solution of 1-methylthiolanium iodide (0.078 g, 0.34 mmol) in [$^2H_4$]methanol (0.6 cm^3). The tightly capped n.m.r. tube containing the solution was stored at 37 °C and 60 MHz ^{1}H n.m.r. spectra were recorded at intervals. The ^{2}H content of the methyl group was assayed by the comparison of integrals as above. Correction of these values to allow for the ^{2}H content at equilibrium gave the values of H% present, the logs of which are plotted *versus* time in Figure 9. This result is discussed in the text.

Exchange of 1-*methylthiolanium iodide in* ^2H_2O. To a solution of 1-methylthiolanium iodide (0.078 g, 0.34 mmol) in ^2H_2O (0.6 cm^3) was added sodium [^{2}H]hydroxide (NaO^{2}H) (9 μl of a 0.7M-solution, 0.0063 mmol, 1.8 mol %) in ^2H_2O, and the solution was stored as above. 60 MHz ^{1}H N.m.r. spectra were recorded, and integrated as above. Correction of the H% present thus obtained, to allow for the presence of 98% ^{2}H at equilibrium, gave the values of H% present, the logs of which are plotted *versus* time in Figure 10. This result is discussed in the text.

Attempts to exchange protons at C-5 *of dehydromethionine.* Solutions were made up as for the exchange of dehydromethionine in [$^2H_4$]methanol, described above, containing 0.05, 0.1, and 0.5 mol equiv. of sodium [$^2H_3$]methoxide (NaOC$^2H_3$). The samples were incubated at 37 °C, and 90 MHz ^{1}H n.m.r. spectra were recorded and integrated, at intervals. These spectra did not show any evidence for exchange at C-5 of dehydromethionine. Rather, from the ^{1}H n.m.r. spectra, and from t.l.c. run at the same time as the spectra were recorded, the decomposition of dehydromethionine to methionine and methionine sulphoxide was evident.

Exchange of dehydromethionine in ^2H_2O *and its reaction with* NaO^{2}H. Solutions of dehydromethionine (0.05 g, 0.34 mmol) in ^2H_2O (0.6 cm^3) were made up in the dry box, and NaO^2H added (addition of 9 µl, 45 µl, 112 µl, 224 µl, and 450 µl of a 0.7M-solution, *i.e.* 2, 25, 50, and 100 mol %). In each case, reaction was complete before a ^1H n.m.r. spectrum could be recorded (*ca.* 2 min). Each sample showed the presence of an amount of methionine sulphoxide (which was partially exchanged in the methyl group) corresponding to the amount of base added, and also partially exchanged residual dehydromethionine. In each case no further exchange, or conversion of dehydromethionine into methionine sulphoxide, was observed on storage at 37 °C for 12 h, indicating stoicheiometric consumption of NaO_2H. The presence of methionine sulphoxide was confirmed by t.l.c. The addition of successive 10 mol % aliquots of NaO^2H to the solution containing 10 mol % NaO^2H demonstrated stepwise stoicheiometric conversion of partially exchanged dehydromethionine into partially exchanged methionine sulphoxide until, with 1 mol equiv. of NaO^2H present, only partially exchanged methionine sulphoxide was present. This was confirmed by t.l.c. (system 2).

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