The Stereochemical Course of Methyl Group Transfer Catalysed by the *S*-Methylmethionine: Homocysteine Transferase from Jack Beans

By GUNNAR GRUE-SØRENSEN, EBBE KELSTRUP, ANDERS KJÆR,* and JØRGEN ØGAARD MADSEN
(*Department of Organic Chemistry, The Technical University of Denmark, 2800 Lyngby, Denmark*)

Summary The (*pro-R*) methyl group of *S*-methyl-(*S*)-methionine is transferred to (*S*)-homocysteine with a stereoselectivity of 90% or more in a reaction catalysed by a transferase from jack beans.

S-METHYL-(*S*)-METHIONINE (MMT) (**1**), first reported as a natural product in 1954,[1] has since been encountered in a surprisingly wide selection of higher plants. Though its detailed biological function is poorly understood, the presence of enzymes in rat liver,[2] micro-organisms,[3] and plant seeds,[4–6] catalysing the essentially irreversible transfer of a methyl group from (**1**) to (*S*)-homocysteine (HCy) (**2**) (equation 1) is hardly accidental.

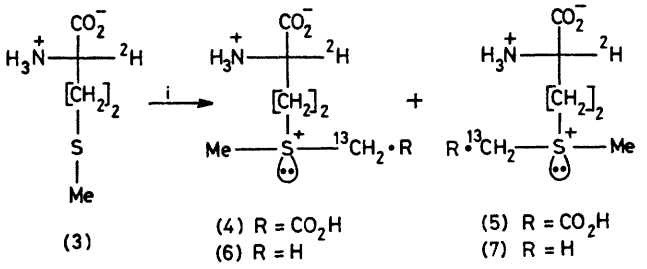
$$\underset{(\mathbf{1})}{[Me]_2\overset{+}{S}\cdot R} + \underset{(\mathbf{2})}{HS\cdot R} \longrightarrow 2\,MeS\cdot R + H^+ \qquad (1)$$

$$\{R = (S)\text{-}[CH_2]_2CH(NH_3^+)CO_2^-\}$$

MMT (**1**) contains diastereotopic methyl groups, transferable, in principle, to chiral or achiral acceptors with nonequal rates. The co-occurrence in jack beans (*Canavalia ensiformis*) of MMT[7] and an MMT : HCy-transferase[4] renders this material particularly relevant in a biological context. We have studied the stereochemical aspects of this reaction and report our results.

(*S*)-[2-^{2}H]Methionine (**3**) (96% ^{2}H), prepared by resolution[8] of the commercial racemate, on treatment with bromo[2-^{13}C]acetic acid (70% ^{13}C), afforded a *ca.* 1 : 1

mixture of the diastereomeric [2-^{2}H,*carboxymethyl*-1-^{13}C}-*S*-carboxymethyl-(*S*)-methionines, (**4**) and (**5**) (as bromides) (Scheme 1). These were converted into poly-



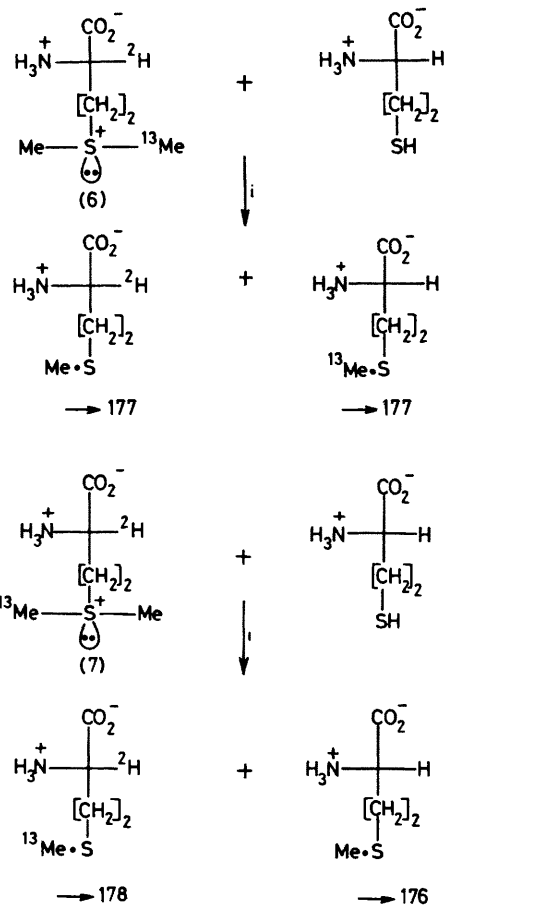
SCHEME 1. Reaction conditions (i) Br·$^{13}CH_2CO_2H$, H_2O, 20 °C, 43 h [in the case (**3**) → (**4**) + (**5**)].

iodides and separated as such, essentially as described recently.[9] The mono-2,4,6-trinitrobenzenesulphonate (TNBS-salt) derived from the least soluble polyiodide and possessing the longest retention time (by amino acid analyser chromatography) was recently shown, by *X*-ray diffraction, to contain the C_SS_R-sulphonium ion (**5**).[9]†

Decarboxylation of the sulphonium acids (**4**) and (**5**), under conditions mild enough to exclude epimerisation by pyramidal inversion (bis-TNBS salt, hexamethylphosphoramide, 20 °C, 1 h; then pyridine, 60 °C, 15—17 min), followed by ion exchange operations [IR-120/Na^+, elution with NH_3; (i) HCl; (ii) TNBSH, 4 H_2O, 0 °C], afforded C_SS_R- and C_SS_S-[2-^{2}H, *methyl*-^{13}C]-*S*-methylmethionine, (**6**) and (**7**), respectively, as bis-TNBS salts (Scheme 1).‡

Each diastereomer, (**6**) and (**7**) (as the chloride), was subjected to transmethylation with HCy as the acceptor (equation 1) and with an enzyme extract, prepared from jack bean meal as described (buffer extraction, fractional precipitation with ammonium sulphate, dialysis),[5] as the catalyst. Only one enzyme, variously characterised, utilizing either HCy or MMT as substrates, has previously been demonstrated to be present in such an extract.[5] Methionine and its isotopic modifications, formed in the reactions, were isolated (ion exchange resin), converted into bis-trimethylsilyl derivatives, and analysed by g.l.c.–mass spectrometry. Intensity measurements were performed on the base peak arising from the fragment ions MeS·-$[CH_2]_2$·CH : $NH^+Si[Me]_3$, rather than on the low-intensity molecular ions§ (Scheme 2). The isotope distribution in the two series is presented in the Table.

The observed intensities (Table), in conjunction with the otherwise established ^{2}H content (95%) of the diastereomers (**6**) and (**7**), permit calculation, in each case, of the fraction of molecules (2*x*) donating a specific methyl group to HCy.¶



SCHEME 2. (*S*)-Methionine species produced in the enzyme-catalysed transfer of the (*pro-R*) methyl group [^{13}Me in (**6**), ^{12}Me in (**7**)] in MMT to HCy. The figures 176—178 denote the *m/e*-values of the predominant fragment ions deriving from the bis(trimethylsilyl)-derivatives of the methionine species. (i) Performed with a jack bean enzyme, essentially as described,[5] employing a large excess of (*RS*)-homocysteine as the acceptor molecule.

Thus, it was found, after correction for an established 5% contamination of each diastereomer with the other, that the (*pro-R*)^{13}C-methyl group of (**6**) was transferred to an extent of 95%; when (**7**) served as a donor, the (*pro-R*)^{12}C-methyl group was transferred to an extent of 96%. Hence the observed stereoselectivity in the two series amounted to 90 and 92%, respectively.

On the basis of the consistent results we conclude: (i) that the (*pro-R*) methyl group in MMT is selectively transferred

† Amino acid analyser chromatography of the sparingly soluble bis-TNBS salts indicated that the specimens of (**4**) and (**5**) employed in the subsequent step were contaminated with about 7% of (**5**) and 3% of (**4**), respectively.

‡ The ^{13}C content of (**6**) was 69%, of (**7**) slightly higher; (**6**) and (**7**) were contaminated with about 5% of the other diastereomer (^{1}H n.m.r. spectroscopy).

§ Control experiments with [2-^{2}H]- and [*methyl*-^{13}C]-methionine derivatives served to ascertain that secondary isotope effects can be disregarded in the fragmentation.

¶ In the case of (**6**), the following relations obtain: $I^{176} = -0{\cdot}95cx + 50 \times 0{\cdot}95c - 50 \times 0{\cdot}95 - 50c + 100$; $I^{177} = 2 \times 0{\cdot}95cx - 100 \times 0{\cdot}95c + 50 \times 0{\cdot}95 + 50c$; $I^{178} = -0{\cdot}95cx + 50 \times 0{\cdot}95c$, where *c* denotes the enrichment factor for ^{13}C. From any two of these relations, values for *c* and 2*x* can be determined. An analogous set of relations are easily derived for (**7**).

TABLE

Bis-Me_3Si derivative of methionine from reaction of MMT-diastereomer		% Intensity, I^n, of fragment ions at $m/e = n$[a,b]		
	n	176[c]	177[d]	178[e]
C_SS_R[f] (**6**)		22 1	74 6	3 2
C_SS_S[g] (**7**)		47 8	22 4	29 8

[a] Corrected for natural abundance contributions [b] Average of two determinations [c] Deriving from the Me_3Si derivative of ^{12}C-^1H-methionine [d] Deriving from an unresolvable mixture of the Me_3Si derivatives of ^{13}C-^1H- and ^{12}C-^2H-methionine [e] Deriving from the Me_3Si derivative of ^{13}C-^2H-methionine [f] Containing *ca* 5% of (**7**) [g] Containing *ca* 5% of (**6**)

to homocysteine to the extent of 90% or more,** (ii) that isotope effects in the enzymic transfer are not decisive, in contrast with our recent finding in non-enzymic methyl group transfer between sulphur atoms [10,11]

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** The deviation from 100% may be attributable to experimental factors, such as less than full enantiomeric purity of the employed *S*-methyl-(*S*)-methionine, a slight, inadvertent epimerisation of the sulphonium centre, or a very minor contribution from a non-enzymic, non-specific transmethylation reaction

[1] R A McRorie, G L Sutherland, M S Lewis, A D Barton, M R Glazener, and W Shive, *J Amer Chem Soc*, 1954, **76**, 115
[2] S K Shapiro and D A Yphantis, *Biochim Biophys Acta*, 1959, **36**, 241, and references cited therein
[3] E Balish and S K Shapiro, *Arch Biochem Biophys*, 1967, **119**, 62, and references cited therein
[4] J E Turner and S K Shapiro, *Biochim Biophys Acta*, 1961, **51**, 581
[5] L Abrahamson and S K Shapiro, *Arch Biochem Biophys*, 1965, **109**, 376
[6] W A Dodd and E A Cossins, *Biochim Biophys Acta*, 1970, **201**, 461
[7] R C Greene and N B Davis, *Biochim Biophys Acta*, 1960, **43**, 360
[8] G P Wheeler and A W Ingersoll, *J Amer Chem Soc*, 1951, **73**, 4604
[9] J W Cornforth, S A Reichard, P Talalay, H L Carrell, and J P Glusker, *J Amer Chem Soc*, 1977, **99**, 7292
[10] G Grue-Sørensen, A Kjær, and E Wieczorkowska, *J C S Chem Comm*, 1977, 355
[11] G Grue-Sørensen, A Kjær, R Norrestam, and E Wieczorkowska, *Acta Chem Scand*, 1977, **B31**, 859