Determination of the Absolute Configuration at the Sulfonium Center of *S*-Adenosylmethionine. Correlation with the Absolute Configuration of the Diastereomeric *S*-Carboxymethyl-(*S*)-methionine Salts

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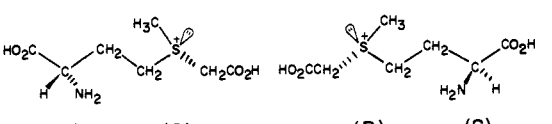
Abstract: The absolute configuration at the sulfonium center of naturally occurring *S*-adenosylmethionine has been determined. The diastereomeric mixture of *S*-carboxymethylmethionine salts from reaction of (*S*)-methionine with iodoacetic acid was resolved by crystallization of the polyiodides (probably pentaiodides) into (*R*)- and (*S*)-[((3*S*)-3-amino-3-carboxypropyl)-(carboxymethyl)methylsulfonium] salts, and these were converted into the 2,4,6-trinitrobenzenesulfonates. All four salts were characterized. The crystal structure of (*R*)-[((3*S*)-3-amino-3-carboxypropyl)(carboxymethyl)methylsulfonium] 2,4,6-trinitrobenzenesulfonate was studied by x-ray diffraction and the relative and absolute configurations at the two asymmetric centers were established. The crystals are triclinic belonging to the space group *P*1 and each unit cell contains two molecular ion pairs in closely similar conformations. The unit cell dimensions are a = 5.408 (2), b = 11.061 (3), c = 18.317 (3) Å; α = 105.06° (2), β = 91.50° (2), γ = 106.50° (2). The structure, including the absolute configuration, was determined and refined to R = 0.070. [$^{14}CH_3$]-*S*-Adenosylmethionine, prepared enzymatically, was cleaved successively by dilute alkali and by sodium periodate. On chromatography of the product, a strong peak of radioactivity was eluted at the retention time of (*S*)-[((3*S*)-3-amino-3-carboxypropyl)(carboxymethyl)methylsulfonium] ion, with minimal radioactivity at the retention time of the (*R*)-sulfonium diastereomer. The radioactivity cocrystallized with the 2,4,6-trinitrobenzenesulfonate of the (*S*)-sulfonium diastereomer and was lost almost completely on crystallization with the (*R*)-sulfonium diastereomer. It was deduced that natural *S*-adenosylmethionine is 5′-[((3*S*)-3-amino-3-carboxypropyl)methyl-(*S*)-sulfonio]-5′-deoxyadenosine; that is, it had the *S* configuration at sulfur.

This paper reports the determination of the absolute stereochemical configuration of *S*-adenosylmethionine.[2] This versatile and ubiquitous metabolic intermediate functions as the principal biological donor of methyl groups, as the source of the propylamine moieties of spermidine and spermine, and as the regulator of a variety of enzymatic reactions.[3] *S*-Adenosylmethionine has been recently implicated in the chemotactic behavior of bacteria.[4] *S*-Adenosylmethionine was discovered by Cantoni,[5] who on the basis of biochemical evidence proposed a structure[6] which was later confirmed by degradation[7] and by synthesis.[8] Attention was first directed to the configuration at the sulfonium center of *S*-adenosylmethionine by de la Haba et al.,[9] who prepared both the "natural" and "unnatural" sulfonium diastereomers, resolved them enzymatically, and measured their optical rotations.[10] Insofar as this matter has been examined,[9,11] the enzymatic synthesis and (with minor exceptions) the enzymatic utilization of *S*-adenosylmethionine are specific for that configuration at the sulfonium center which was designated (because of its lower specific optical rotation[9,10]) as (−)-*S*-adenosyl-L-methionine. However, some transfer reactions tolerate various structural and stereochemical alterations in the *methionine* moiety of this molecule.[9,12] Apart from its intrinsic interest for the mechanism of the formation of *S*-adenosylmethionine and its utilization, knowledge of the absolute configuration of the sulfonium center is relevant to the design of inhibitors of the synthesis of *S*-adenosylmethionine,[13] and to the inhibition of certain transmethylation reactions.[14]

The stereochemistry of sulfur compounds has been reviewed comprehensively in recent years.[15,16] The chirality of sulfonium compounds was first demonstrated independently by Smiles[17] and by Pope and Peachey.[18] Although a number of optical resolutions of sulfonium compounds have been reported in subsequent years, the absolute configuration of acyclic sulfonium compounds remained indeterminate until the recent report of Kelstrup, Kjaer, Abrahamsson, and Dahlén[19] who established the *S* configuration for (+)-ethylmethylpropylsulfonium 2,4,6-trinitrobenzesulfonate which was obtained by an unambiguous synthesis which relied on the stereochemical identification of an intermediate by x-ray diffraction. Specific chirality has also been recently assigned by NMR methods to cyclic sulfonium salts.[20]

Experimental Plan and Results

Since crystals of *S*-adenosylmethionine suitable for x-ray diffraction studies have not to our knowledge been obtained, we elected to degrade *S*-adenosylmethionine to *S*-carboxymethylmethionine in conditions designed to retain the stereochemical integrity at the sulfonium center.[21] The experimental plan required the separation and characterization of the two diastereomeric *S*-carboxymethylmethionine salts (**1** and **2**) and their crystallization in a form suitable for the determination of the relative and absolute configurations of the chiral centers by x-ray diffraction. It was thought, correctly, that crystallization would be easier with *S*-carboxymethylmethionine salts than with those of *S*-adenosylmethionine. Finally, it was necessary to identify the *S*-carboxymethylmethionine obtained by degradation of *S*-adenosylmethionine, with one of these two synthetic diastereomers.

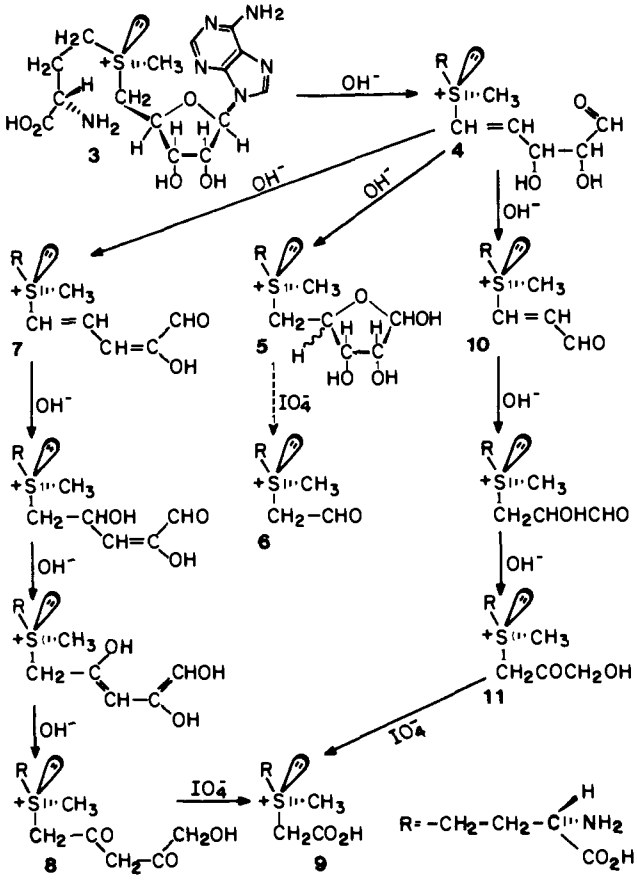


The synthesis of an amorphous diastereomeric mixture of *S*-carboxymethylmethionine iodides has been described by

Gundlach, Moore, and Stein,[21] who demonstrated the partial separation of the two diastereomers (designated isomers A and B from the order of their elution from the column) on an analytical scale by amino acid analyzer chromatography (Figure 1). Separation of the diastereomers on a preparative scale by column chromatography and recovery from the citrate-containing buffers proved to be difficult, and consequently we examined the fractional crystallization of various salts. Whereas crystalline Reineckate and picrolonate salts of isomer B were easily obtained, the corresponding salts of the A isomer could not be crystallized in acceptable yield because of their high solubilities in water. However, the stepwise addition of aqueous I_2–KI permitted clean fractional crystallizations of both diastereomers of *S*-carboxymethylmethionine polyiodide in a stereochemically pure form. The recrystallized polyiodides could then be converted to the more tractable 2,4,6-trinitrobenzenesulfonates, which were repeatedly crystallized from water.

S-Adenosylmethionine is sensitive to cold dilute alkali, giving adenine and a product thought to be an *S*-(5′-deoxy-5′-ribosyl)methionine.[22] Baddiley and co-workers[23] showed in an analogous case that position 4 of the sugar moiety loses chirality during the cleavage and suggested that a mechanism which, in the case of *S*-adenosylmethionine (**3**, Scheme I),

.Scheme I. Degradation of *S*-Adenosylmethionine



would lead by way of the vinylsulfonium compound (**4**) to an inhomogeneous "pentosylmethionine" (**5**). Mamalis and Rydon[24] had demonstrated that vinylsulfonium ions are formed readily by base-catalyzed eliminations and that with excess of base these are, relatively slowly, hydrated to β-hydroxymethylsulfonium ions. Our original plan was to oxidize "pentosylmethionine" with periodate to the aldehyde (**6**) and then to use another oxidizing agent to convert this into the desired carboxylic acid. However, no compound with the properties of **6** was ever observed in periodate oxidation of alkali-treated *S*-adenosylmethionine; instead, a product having

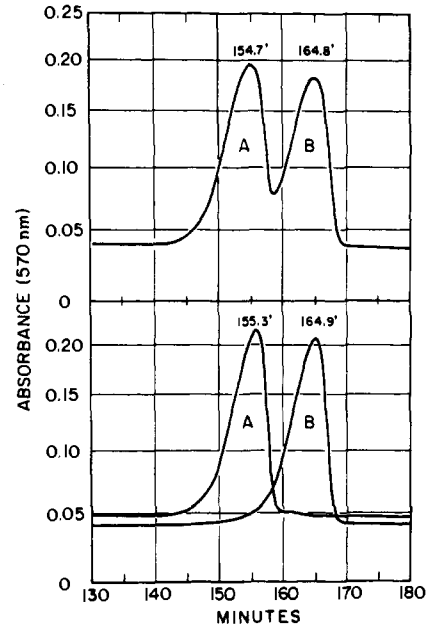


Figure 1. Chromatographic separation of diastereomers A and B of *S*-carboxymethylmethionine. The *lower* panel is a composite of parts of the amino acid analyzer ninhydrin color tracings obtained in two runs in which 0.5 μmol of the TNBS salts of isomer A and isomer B were chromatographed separately. The *upper* panel represents a chromatographic run in which a mixture of the isomers (0.5 μmol each) was chromatographed under identical conditions which are described in the text (citrate buffer, pH 2.70; 50 °C; 70 mL/h). The base lines for the two runs of the separate isomers have been displaced slightly for clarity in presentation. The aspartic acid peak (added as a standard) was eluted at 105–107 min in the three runs. Integration of the areas under the curves indicated that the ninhydrin color yields for the TNBS salts of isomer A and isomer B were 73.1 and 73.7%, respectively, of those obtained for an equimolar quantity of aspartic acid.

the chromatographic mobility of *S*-carboxymethylmethionine (isomer A) was regularly obtained in modest yield. Retrospectively, we attribute this good fortune to an alternative action of hydroxyl ion on the intermediate vinylsulfonium ion (**4**). The reaction leading to **5** is an addition of hydroxyl ion to the aldehyde carbonyl, followed by intramolecular cyclization.[23] A competing enolization of the aldehyde would tend to produce, by dehydration, the dienal (**7**); and hydration of this, followed by a prototropic shift of known type, could generate a 1,3-diketone (**8**). Such compounds are known[25] to be oxidized by periodate with cleavage and the normal product of such a reaction would be *S*-carboxymethylmethionine (**9**). Direct oxidation of **6** to **9** by periodate would be an unprecedented type of reaction for this reagent; it seems more likely that any aldehyde formed from **5** was destroyed by oxidative cleavage at the unexpectedly active[26] methylene group. An alternative, though in our view less probable, mode of formation of **9** would be retroaldol cleavage of **4** followed by base-catalyzed hydration and isomerization of the unsaturated aldehyde (**10**) to yield the ketol (**11**). The latter would be oxidized by periodate to **9**.

Because [$^{14}CH_3$]-*S*-adenosylmethionine, prepared enzymatically and therefore[9] having the "natural" configuration at sulfur, was readily available it could be used to demonstrate the formation of [$^{14}CH_3$]-*S*-carboxymethylmethionine (isomer A). Degradation of radioactive *S*-adenosylmethionine by alkali and periodate, followed by chromatography, gave a peak of radioactivity (Figure 2) eluted at the same time as carrier *S*-carboxymethylmethionine, isomer A. Radioactivity corresponding in elution time to isomer B was very small. The fractions of highest radioactivity were combined and two portions were separately mixed with the pure 2,4,6-trinitro-

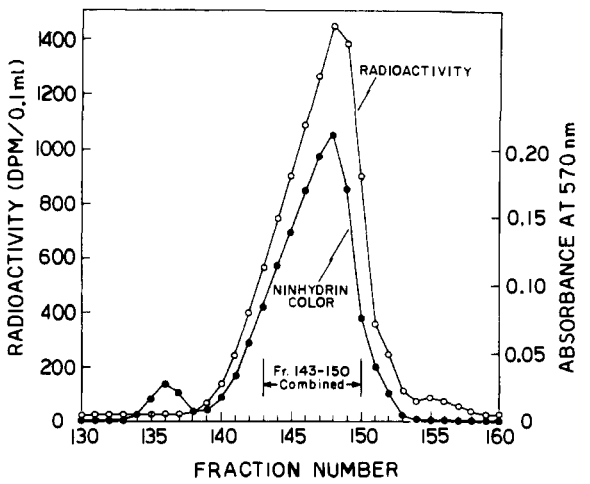


Figure 2. Presentation of split stream run on the amino acid analyzer column showing coincidence of carrier isomer A of *S*-carboxymethylmethionine with the radioactive degradation product of natural [$^{14}CH_3$]-*S*-adenosylmethionine. Flow rate of column was 70 mL/h of which 60 mL/h was collected in 1.0-mL fractions. The remaining effluent (10 mL/h) was diluted with buffer (60 mL/h) and then mixed in the analytical system with the ninhydrin reagent (35 mL/h). There is a small nonradioactive peak centered in fraction 136 (probably homoserine) and a small rise in radioactivity in the position corresponding to isomer B (fraction 155). A zero time control containing an equivalent amount of [$^{14}CH_3$]-*S*-adenosylmethionine was carried through the same procedures but not subjected to base degradation or periodate oxidation. On chromatography, less than 10 dpm per 0.1-mL aliquot of each fraction and no detectable ninhydrin-positive material was found in fractions 1–160. (*S*-Adenosylmethionine itself is retained on the column under these conditions).

Table I. Carrier Crystallization of Degradation Products of [$^{14}CH_3$]-*S*-Adenosylmethionine with Isomers A and B of *S*-Carboxymethylmethionine Trinitrobenzenesulfonate[a]

	Crystallization with Isomer A	Isomer B
Volume of aliquot	1.10 mL	0.30 mL
Total radioactivity in aliquot	98 200 dpm	26 800 dpm
Amount of carrier added	133.2 mg	75.8 mg
Specific activity of 1st crystals	690.8 dpm/mg	37.9 dpm/mg
Specific activity of 2nd crystals	680.2 dpm/mg	20.9 dpm/mg
Specific activity of 3rd crystals	685.5 dpm/mg	20.7 dpm/mg
Specific activity of dried residue of supernatant from 3rd crystals	649.1 dpm/mg	43.4 dpm/mg

[a] Fractions 143–150 (see Figure 2) were combined and reduced in volume under a vacuum (<40 °C) to 1.42 mL. This solution was divided into two aliquots (1.10 and 0.30 mL) and mixed with crystalline TNBS salts of isomer A and B in quantities indicated in the table. Each crystallization was done from warm water (65 °C). The product from each crystallization was collected, washed with a minimum of cold water, and dried under a vacuum to constant weight. Aliquots (2–4 mg) were weighed on a microbalance, dissolved in 1.0 mL of H_2O, and mixed with 10.0 mL of PCS fluor (Amersham/Searle, Chicago, Ill.). Radioactivity was determined in a liquid scintillation counter at ambient temperature at a wide channel setting. The counting efficiency was determined directly by addition of an internal standard of toluene-^{14}C. All counts were cumulated to a standard error of ±1%, and are corrected for background and efficiency (background 38.1 cpm).

benzenesulfonate salts of isomers A and B. Both these salts were then crystallized to constant radioactivity. From the specific radioactivities of the recrystallized salts (Table I) it can be calculated that 94% of the radioactivity in the combined eluates was present as isomer A and 6% as isomer B. Since the radioactive fractions corresponded in elution time to isomer A rather than B, the apparent presence of B might be attributable to a small amount of epimerization during isolation or crystallization.[27] In any case it is clear from Figure 2 that little radioactivity could have been present in the form of isomer B in the eluate from the column.

It does not seem possible to resist the conclusion that isomer A was the actual substance present in the radioactive peak; had the radioactivity been due to any other substance it would be expected to crystallize also with isomer B. *S*-Adenosylmethionine is therefore degraded by the procedure described to *S*-carboxymethylmethionine, isomer A, unless some radioactive impurity, chiral at sulfur and accounting for at least 10% of the radioactivity, was present in several specimens of *S*-adenosylmethionine and was the source of *S*-carboxymethylmethionine, isomer A.

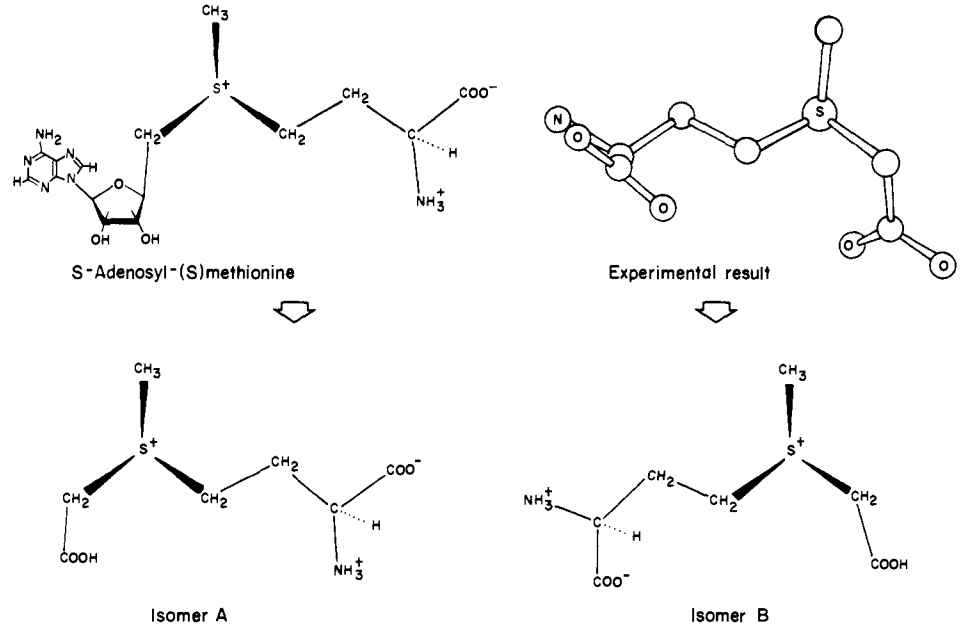
The x-ray crystallographic analysis of isomer B (see below) has shown this isomer to have the *R* configuration (**2**) at sulfur: it follows that isomer A has the *S* configuration (**1**), the two isomers having been formed in substantially equal yield from the same specimen of (*S*)-methionine.[28] Since no *inversion* (as opposed to racemization) of configuration at sulfur could conceivably have occurred during the degradation, the natural configuration at sulfur of *S*-adenosylmethionine must therefore be *S*, the stereochemistry being represented completely by the expression (**3**) (Scheme I). That part of the experimental plan involving the x-ray crystallographic analysis, and its relationship to the chemical studies, is shown in Scheme II.

X-ray crystallographic studies of the TNBS salt of isomer B gave results on two (*S*)-methionine-(*R*)-carboxymethylsulfonium cations and two TNBS anions in each unit cell. However, the bond lengths and angles in the two crystallographically independent cations are the same within experimental error. It is found that the carboxyl group of the carboxymethyl chain on the sulfonium pole is not ionized while the carboxyl group adjacent to the amino group is ionized. However, as discussed later, a very short hydrogen bond between the carboxyl and carboxylate groups may indicate some disorder in the hydrogen position, suggesting similar p*K* values for both groups.

Both (*S*)-methionine-(*R*)-carboxymethylsulfonium ions in each unit cell also have very nearly the same conformation. The atoms from the amino nitrogen atom to the methylene carbon atom of the carboxymethyl group assume a planar and extended conformation. The carboxylate, carboxyl, and methyl groups lie out of this plane. The sulfonium pole displays pyramidal geometry with nearly equal S–C bond lengths and with C–S–C angles that are significantly less than the ideal tetrahedral values of 109° 28′ in agreement with other studies of sulfonium compounds.[29]

The (*S*)-methionine-(*R*)-carboxymethylsulfonium cations are connected by hydrogen bonds between carboxylic acid and carboxylate groups of different cations. Each independent cation is hydrogen bonded to two other cations to form a helix running parallel to the *a* axis in the crystal. Two such ribbons form a double helix pattern as illustrated in Figure 4b. These hydrogen bonds are short and it appears that the hydrogen atoms are disordered along them. The $-NH_3^+$ group in each of the cations is hydrogen bonded to a sulfonate oxygen atom of a TNBS anion. The sulfonium poles of the cations have their shortest intermolecular contacts with *p*-nitro groups of the TNBS anions. The packing, illustrated in Figure 4a, shows layers of TNBS anions and layers of helices of cations. The hydrogen bonding to the amino groups hold these layers and helices together.

Scheme II. Diagram of the Absolute Configuration of Isomer B of *S*-Carboxymethylmethionine, and; by Analogy Isomer A and the Natural Form of *S*-Adenosyl-(*S*)-methionine



Experimental Section

Chemical Studies. Synthesis of *S*-Carboxymethylmethionine Iodide. The procedure was slightly modified from Gundlach, Moore, and Stein.[21] A solution of 4.0 g (21.5 mmol) of iodoacetic acid (recrystallized from benzene-petroleum ether) and 1.20 g (8.04 mmol) of recrystallized (*S*)-methionine[28] in 50 mL of distilled water was agitated gently for 15–17 h at 37–40 °C under a stream of nitrogen while protected from light. The reaction mixture was extracted with peroxide-free ether, filtered, and lyophilized as rapidly as possible while protected from light. The white (or very pale yellow) hygroscopic powder (2.46–2.55 g, 87–90% crude yield) was stored in a vacuum over P_2O_5 at −20 °C in the dark. Analysis on the amino acid analyzer revealed two incompletely resolved peaks (separated by 10 min), designated as diastereomers A and B, respectively, in order of their elution from the column (Figure 1). A 60-MHz NMR spectrum (100 mg/mL in D_2O): δ 2.75–2.10 (m, $CH_2CH(NH_2)CO_2^-$, 2), 2.99 (s, CH_3, 3), 3.90–3.43 (m, $-CH_2CH_2SCH_3$, 2), 4.19 (t, J = 2 Hz, $SCH_2CO_2^-$, 2), 4.65 (DHO).[26] The downfield shift of the protons α to the sulfonium pole in relation to the corresponding protons in (*S*)-methionine and other thioethers is characteristic for sulfonium salts generally.[30] On the basis of the analysis of Gundlach et al.,[21] a formula weight of 353.2 for the monohydrate was used in the following calculations.

Analytical and Preparative Chromatography. All chromatographic procedures were carried out on a Beckman Model 120 C amino acid analyzer.[31] The column (0.9 × 55 cm) of sulfonic acid ion exchange resin (UR 30) was operated at 50 °C with citrate buffer at a flow rate of 70 mL/h. The citrate buffer contained 0.05 M citrate and 0.2 M Na^+ and was adjusted with HCl to pH 2.70 ± 0.05. For analytical purposes, ninhydrin was added at 35 mL/h. The diastereomeric *S*-carboxymethylmethionine cations were incompletely resolved and appeared on the chart at 150–170 min (with different citrate buffer preparations), with a peak separation of 10 min, and a peak to valley ratio of about 4:1 with large sample loads (Figure 1). Aspartic acid was added as an internal standard. The two diastereomeric *S*-carboxymethylmethionine cations were eluted at 1.45 and 1.55 times, respectively, the elution volume of aspartic acid.

Split stream runs were conducted under identical conditions. Precisely 60 mL of the 70 mL/h of column eluate was diverted and collected in 1.0-mL fractions. The remaining eluate (10 mL/h) was diluted with citrate buffer (60 mL/h) by means of an auxiliary pump and fed into the regular ninhydrin analytical system. The delay between the appearance of a peak in the fraction collector and on the analyzer chart was precisely 8.5 min.

Separation of *S*-Carboxymethylmethionine Diastereomers as Polyiodides. Portions (20 mL) of 0.15 M aqueous solutions of crude *S*-carboxymethylmethionine iodide (mol wt 353.2) were maintained at 4 °C and successive additions of 1.0 M iodine[32] solutions were made at approximately 2-day intervals following the harvesting of each accumulated crop of crystals by filtration. The crystals were washed with small quantities of cold water. The amount of I_2 added initially was 0.5 molar equiv (i.e., 1.5 mmol of I_2) and each successive addition amounted to 0.1 molar equiv (0.3 mmol of I_2) up to 1.1 molar equiv of I_2. Black, shiny, pyramid-shaped crystals of predominantly isomer B polyiodide (contaminated by less than 10% of isomer A) were removed at each step of addition of I_2 between 0.5 and 1.1 molar equiv. At this point, 85% of isomer B had crystallized as judged by amino acid analysis of the supernatant fluid. The further addition of I_2 solution afforded either black, needle-shaped crystals of isomer A or a mixture of the two crystal forms. The needle-shaped A isomer crystals were removed and set aside for use as seed crystals in subsequent crystallizations. If the supernatant solution at 1.1 molar equiv of I_2 was seeded with crystals of isomer A, and the I_2 concentration raised in two steps to 1.25 and 1.40 molar equiv of I_2, needles of isomer A, essentially uncontaminated by isomer B, could be obtained in reasonable yield. Inspection of the crystals (needles or pyramids) was an excellent guide to the progress of the resolution. The total yield of dry isomer A was about 0.32 g (yield 25%) and that of isomer B was about 1.0 g (yield 85%). The yields are calculated on the basis of a mol wt of 842 for the pentaiodide (see below). The crystals were stored at −20 °C in a vacuum over P_2O_5 and protected from light.

Recrystallization of *S*-Carboxymethylmethionine Polyiodides. The polyiodides were suspended in water at 45 mg/mL for isomer A and at 120 mg/mL for isomer B. Each suspension was extracted exhaustively with many portions of benzene until the compound dissolved and neither phase was colored. The aqueous phase was filtered and traces of benzene were removed with a stream of N_2. The solution was cooled to 4 °C and 1.0 M iodine solution was dadded in portions until characteristic crystals of each isomer were obtained. Twice recrystallized polyiodides were analyzed. Anal. Calcd for $C_7H_{14}I_5NO_4S$: C, 9.98; H, 1.67; N, 1.66; S, 3.80; I, 75.29. Found for isomer A: C, 10.19; H, 1.59; N, 2.20; S, 3.36; I, 74.28, 74.17. Found for isomer B: C, 10.86; H, 1.66; N, 1.80; S, 4.34; I, 75.33, 74.86.

Although the results cannot be regarded as entirely satisfactory, the discrepancies may in part be ascribed to analytical difficulties resulting from the high iodine content. The analyses are more satisfactory for I_5^- (75.29% iodine) than I_3^- (64.64% iodine) or I_6^- (78.52% iodine). The indeterminate and variable nature of the stoichiometry of potassium polyiodides has been recognized for many years.[33] Chromatographic analyses showed that each recrystallized *S*-carboxymethylmethionine polyiodide contained less than 1–2% of the contaminating diastereomer.

Preparation of Both Diastereomers of *S*-Carboxymethylmethionine Trinitrobenzenesulfonate. Isomer B may be obtained directly from the diastereomeric mixture of *S*-carboxymethylmethionine iodides. A 0.2 M aqueous solution of the latter when mixed with 0.5 molar equiv of 1 M TNBS and cooled to 4 °C provided yellow needles of the TNBS derivatives of isomer B. The more soluble TNBS salt of isomer

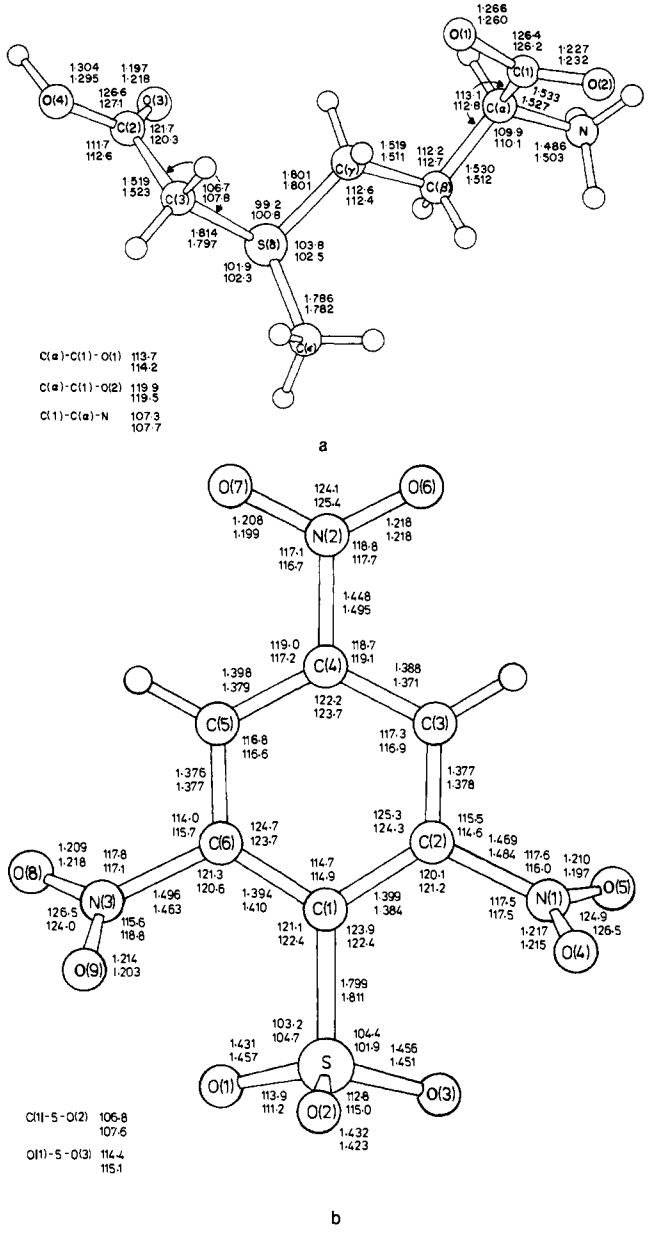


Figure 3. Bond angles and distances for (a) the cation of *S*-carboxymethylmethionine (isomer B), and (b) the 2,4,6-trinitrobenzenesulfonate anion. The duplicate values refer to experimental results for each of the two ions contained in each unit cell.

A could not be easily obtained from these solutions, since there was a tendency for the isomers to emerge from solution as mixed crystals. Moreover, the TNBS salts do not differ so remarkably in crystal form as the polyiodides, and this makes the visual monitoring of the crystallization procedure more difficult. The TNBS salt of isomer A crystallizes as elongated platelets.

TNBS salts of diastereomers A and B were readily prepared from the corresponding pure polyiodides of these isomers. This procedure was mandatory for the preparation of pure isomer A TNBS. Suspensions of the polyiodides in water were converted to the monoiodides by benzene extraction as described above. The solutions (0.2 M) were treated with 1.0 M TNBS. For isomer B, 0.5 molar equiv of 1.0 M TNBS was added, and, on cooling, needles of diastereomerically pure TNBS salt of isomer B were obtained. For isomer A, the addition of TNBS was usually stepwise and 0.4, 0.6, and 0.9 molar equiv of TNBS were added, successively. Isomer A separated as elongated platelets of greater than 98% diastereomeric purity.

Both TNBS salts of isomer A and isomer B could be recrystallized from water warmed to 65 °C. Isomer A was almost 2.5 times more soluble than isomer B. Twice recrystallized salts were analyzed.

Anal. Isomer A ($C_{13}H_{16}N_4O_{13}S_2 \cdot H_2O$). Calcd: C, 30.12; H, 3.49; N, 10.81; S, 12.37. Found: C, 30.05; H, 3.19; N, 10.37; S, 12.19.

Table II. Crystal Data for Isomer B of (*S*)-Carboxymethylmethionine Trinitrobenzenesulfonate

Formula: $2(C_6H_2N_3O_9S^-) \cdot 2(C_7H_{14}NO_4S^+)$ Mol wt: 500.43
Space group: $P1$
Crystal system: triclinic
Cell dimensions:
a = 5.408 (2) Å b = 11.061 (3) Å c = 1.8317 (3) Å
α = 105,06 (2)° β = 91.50 (2)° γ = 106.50 (2)°
λ(Cu Kα) = 1.5418 Å
d_{calcd} (x ray) = 1.649 g cm^{-3}
μ(Cu Kα) = 30.5 cm^{-1}

Table III. Anomalous Scattering Data for Isomer B of (*S*)-Carboxymethylmethionine Trinitrobenzenesulfonate[a]

h	k	l	I_{hk} (I_+)	$\sigma(I)$	$I_{\bar{h}\bar{k}\bar{l}}$ (I_-)	$\sigma(I)$	I_+/I_- Obsd	I_+/I_- Calcd
0	2	−3	1944	98	722	37	2.69	2.77
0	2	4	1193	55	2079	57	0.57	0.68
0	3	−4	4088	144	2978	99	1.37	1.32
0	2	−9	533	22	699	24	0.76	0.71
1	−10	5	1580	37	1210	28	1.31	1.36
1	−6	−4	710	32	961	36	0.74	0.68
1	−5	−4	3069	105	2301	83	1.33	1.27
1	−5	−2	2080	73	1317	48	1.58	1.76
1	−4	−2	2433	98	3512	130	0.69	0.64
1	−2	10	771	34	1163	40	0.66	0.69
1	−1	0	30912	901	31056	903	0.99	0.84
1	1	0	32568	920	40752	1022	0.80	0.85
1	3	1	4147	153	5192	223	0.80	0.74
1	−2	−2	17880	691	21672	754	0.83	0.85
2	−3	5	5840	230	4416	158	1.32	1.47

[a] Intensities are listed on a relative scale. This table shows that in all cases the sign of $(I_+ - I_-)$ agrees with that calculated when the absolute configuration is *S* at the amino acid asymmetric carbon atom and *R* at the sulfur atom.

Isomer B ($C_{13}H_{16}N_4O_{13}S_2$). Calcd: C, 31.20; H, 3.22; N, 11.20; S, 12.82. Found: C, 31.35; H, 3.26; N, 10.91; S, 12.77.

Isomer A had $[\alpha]^{25}D$ +17.4° (*c* 0.541, 5 N HCl); isomer B, $[\alpha]^{25}D$ +4.5° (*c* 0.869, 5 N HCl), +14.1° (*c* 0.913, 5 N HCl).

Degradation of [$^{14}CH_3$]-*S*-Adenosylmethionine to *S*-Carboxymethylmethionine Trinitrobenzenesulfonate. The degradation was carried out in a final volume of 4.0 mL containing 10 μmol of *S*-adenosylmethionine (4.0 μCi). The initial pH of 2.7 was adjusted to 12.0 by addition of 140 μL of 1 N NaOH and maintained at 25 °C with stirring, under a stream of N_2, for exactly 10 min. The pH was then lowered to 4.0 by addition of 25 μL of 5 N HCl, followed by addition of 80 μL of 0.5 M $NaIO_4$ (40 μmol). The pH began to fall and was maintained at 4.0 by addition of 0.1 N NaOH with an automatic titrator. After 10 min of periodate oxidation at 25 °C, approximately 1.0 mL (one-fourth) of the reaction mixture was removed for further processing. This aliquot was cooled to 4 °C, and excess IO_4^- waprecipitated by addition of 200 μL (20 μmol) of 0.1 M $BaCl_2$. The precipitate was removed by centrifugation. No radioactivity was lost from the solution. Nine-tenths of the sample was mixed with 2 μmol of pure diastereomer A of *S*-carboxymethylmethionine trinitrobenzenesulfonate and 2 μmol of aspartic acid. The mixture was adjusted to pH 1 with HCl and applied to the amino acid analyzer column and chromatographed in a split stream run. The total radioactivity applied to the column was 1.35 × 10^6 dpm. Aliquots (0.1 mL) of the collected fractions (1.0 mL) were counted. The radioactivity and the ninhydrin trace from the amino acid analyzer are shown in Figure 2. Approximately 10% of the radioactivity applied to the column was eluted in the region of isomer A (Figure 2). The peak of radioactivity was found in fraction 148 which corresponds to the ninhydrin peak at 156.5 min on the ninhydrin chart. (The 8.5-min delay has been compensated for in Figure 2). Fractions 143–150 inclusive were combined and their volume reduced to 1.42 mL on a rotary evaporator (<40 °C) under a vacuum. This material was divided into two portions: 1.10 mL

Table IV. Refined Atomic Parameters for Isomer B of *S*-Carboxymethylmethionine Trinitrobenzenesulfonate[a]

	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
				A. Amino Acid (First Molecule)					
O(1)	0.1328 (8)	0.6792 (4)	0.8015 (2)	6.12 (16)	4.84 (14)	4.59 (16)	2.96 (15)	2.51 (14)	2.31 (16)
O(2)	0.2601 (7)	0.5343 (3)	0.8452 (2)	3.42 (12)	4.09 (13)	4.48 (15)	1.85 (12)	1.29 (11)	1.54 (13)
O(3)	−0.3851 (8)	1.0177 (4)	0.9036 (3)	3.67 (15)	4.16 (14)	5.96 (20)	0.57 (14)	−0.21 (14)	2.25 (16)
O(4)	−0.1712 (7)	1.1947 (4)	0.8683 (3)	3.81 (15)	5.30 (15)	5.59 (18)	1.19 (14)	−0.66 (14)	2.83 (17)
C(1)	0.1220 (9)	0.6049 (4)	0.8442 (3)	3.29 (17)	2.77 (16)	2.77 (18)	0.88 (15)	0.33 (14)	0.35 (15)
C(2)	−0.1916 (10)	1.1027 (5)	0.9023 (3)	3.55 (18)	3.49 (17)	3.52 (20)	1.43 (17)	−0.08 (15)	0.77 (17)
C(3)	0.0671 (10)	1.1139 (5)	0.9421 (3)	3.47 (18)	2.80 (16)	3.38 (19)	0.99 (15)	0.36 (15)	0.80 (16)
N	−0.1152 (9)	0.4969 (4)	0.9322 (3)	4.92 (18)	2.36 (14)	5.54 (23)	0.79 (14)	2.84 (19)	0.91 (17)
C(α)	−0.0892 (9)	0.6076 (5)	0.8983 (3)	2.63 (16)	2.65 (16)	3.55 (20)	0.45 (14)	0.41 (14)	0.48 (16)
C(β)	−0.0284 (10)	0.7370 (5)	0.9612 (3)	4.43 (20)	2.72 (17)	2.97 (18)	0.91 (16)	1.01 (16)	0.57 (16)
C(γ)	−0.0061 (10)	0.8520 (5)	0.9284 (3)	4.27 (20)	2.86 (17)	3.10 (19)	1.22 (17)	0.55 (16)	0.28 (16)
S(δ)	−0.0006 (2)	0.9999 (1)	0.9997 (1)	3.77 (4)	2.81 (3)	2.94 (4)	1.46 (3)	0.56 (3)	0.65 (3)
C(ε)	0.3033 (11)	1.0443 (5)	1.0559 (3)	4.82 (22)	3.42 (19)	3.38 (22)	1.52 (20)	−0.58 (17)	0.26 (18)
				B. Amino Acid (Second Molecule)					
O(1′)	1.4191 (8)	1.1803 (4)	0.8004 (3)	3.47 (15)	4.61 (15)	5.90 (18)	0.24 (13)	−1.27 (15)	2.51 (17)
O(2′)	1.6679 (8)	1.3580 (4)	0.7740 (3)	3.16 (15)	3.43 (14)	5.89 (20	0.13 (13)	−0.52 (14)	1.38 (15)
O(3′)	0.4546 (8)	0.8463 (4)	0.6937 (3)	2.37 (14)	2.12 (12)	3.56 (18)	1.46 (13)	1.15 (13)	1.34 (15)
O(4′)	0.4973 (9)	0.6725 (4)	0.7272 (3)	5.76 (18)	4.01 (14)	5.91 (18)	2.41 (15)	3.12 (17)	2.54 (17)
C(1′)	1.4624 (9)	1.2699 (4)	0.7675 (3)	2.79 (17)	2.37 (15)	3.60 (20)	0.62 (15)	0.29 (15)	0.31 (15)
C(2′)	0.5598 (10)	0.7624 (5)	0.6934 (3)	3.00 (18)	3.09 (18)	3.46 (20)	−0.03 (15)	1.10 (16)	0.91 (16)
C(3′)	0.7884 (10)	0.7548 (5)	0.6475 (3)	3.22 (18)	2.18 (15)	3.97 (21)	0.64 (15)	0.83 (16)	0.90 (16)
N′	1.3022 (9)	1.3878 (4)	0.6903 (3)	4.39 (19)	2.39 (14)	4.91 (21)	1.25 (15)	0.18 (16)	0.97 (16)
C(α′)	1.2265 (9)	1.2683 (4)	0.7191 (3)	2.73 (17)	2.59 (15)	3.35 (18)	0.79 (14)	0.47 (14)	1.04 (16)
C(β′)	1.1305 (10)	1.1456 (5)	0.6531 (3)	4.12 (21)	2.75 (16)	3.13 (19)	0.73 (16)	0.05 (16)	0.86 (16)
C(γ′)	1.0255 (11)	1.0229 (5)	0.6784 (3)	4.20 (21)	2.59 (17)	3.02 (19)	0.83 (17)	0.51 (16)	0.49 (16)
S(δ′)	0.8493 (3)	0.8836 (1)	0.6012 (1)	3.11 (4)	2.37 (3)	3.23 (4)	0.69 (3)	0.14 (3)	0.74 (3)
C(ε′)	1.0986 (12)	0.8530 (6)	0.5442 (3)	5.15 (24)	4.81 (23)	3.33 (22)	2.02 (22)	1.35 (19)	1.06 (21)
				C. TNBS (First Molecule)					
S(t)	0.4623 (2)	0.4894 (1)	0.0820 (1)	4.54 (4)	4.49 (4)	3.85 (4)	2.47 (4)	1.65 (3)	1.55 (4)
C(1t)	0.2684 (8)	0.5530 (4)	0.1514 (3)	2.66 (15)	3.36 (15)	2.89 (16)	1.21 (14)	−0.07 (13)	0.92 (15)
C(2t)	0.3154 (8)	0.6868 (4)	0.1874 (3)	2.57 (15)	3.05 (14)	3.49 (17)	0.76 (13)	−0.04 (13)	1.55 (15)
C(3t)	0.1859 (9)	0.7357 (4)	0.2462 (3)	3.47 (18)	2.72 (15)	3.10 (18)	0.97 (15)	0.05 (14)	0.45 (15)
C(4t)	−0.0049 (9)	0.6455 (5)	0.2704 (3)	3.39 (18)	3.05 (16)	3.31 (19)	1.00 (15)	0.55 (15)	0.27 (15)
C(5t)	−0.0623 (10)	0.5100 (5)	0.2383 (3)	3.55 (18)	3.10 (17)	4.19 (22)	1.08 (16)	1.03 (16)	0.64 (17)
C(6t)	0.0755 (9)	0.4694 (4)	0.1796 (3)	2.86 (16)	2.58 (15)	4.16 (21)	0.74 (14)	0.82 (15)	0.67 (16)
N(1t)	0.5104 (8)	0.7856 (4)	0.1620 (3)	3.34 (16)	3.29 (14)	4.65 (19)	0.85 (13)	0.96 (14)	1.47 (15)
N(2t)	−0.1539 (9)	0.6937 (5)	0.3295 (3)	5.18 (19)	4.06 (18)	5.19 (22)	1.60 (17)	2.33 (18)	0.71 (18)
N(3t)	−0.0034 (9)	0.3237 (5)	0.1458 (3)	3.23 (16)	3.29 (16)	7.35 (28)	1.03 (14)	1.52 (18)	0.02 (18)
O(1t)	0.4621 (7)	0.3687 (3)	0.0970 (3)	3.86 (13)	3.87 (13)	7.42 (21)	2.13 (12)	1.92 (14)	2.02 (16)
O(2t)	0.3362 (10)	0.4729 (6)	0.0087 (3)	9.70 (25)	9.19 (25)	3.48 (17)	5.31 (28)	1.23 (16)	1.16 (20)
O(3t)	0.7140 (7)	0.5895 (4)	0.0989 (3)	3.76 (13)	4.38 (14)	8.21 (22	1.68 (12)	2.66 (15)	3.16 (18)
O(4t)	0.4721 (9)	0.7920 (4)	0.0975 (3)	5.87 (19)	6.15 (16)	5.78 (19)	2.17 (16)	1.68 (16)	3.75 (20)
O(5t)	0.6947 (10)	0.8558 (5)	0.2071 (3)	5.21 (22)	5.89 (21)	7.26 (26)	−1.37 (18)	0.28 (20)	1.55 (21)
O(6t)	−0.0926 (9)	0.8112 (4)	0.3607 (3)	6.50 (19)	3.80 (15)	5.34 (21)	1.94 (15)	1.73 (16)	0.28 (15)
O(7t)	−0.3320 (11)	0.6133 (5)	0.3451 (4)	11.07 (24)	4.65 (23)	13.31 (33)	0.96 (20)	9.30 (38)	0.58 (26)
O(8t)	0.0095 (11)	0.2567 (4)	0.1876 (4)	10.85 (28)	4.03 (16)	11.33 (32)	3.33 (20)	6.05 (29)	3.75 (26)
O(9t)	−0.0828 (8)	0.2847 (5)	0.0787 (4)	3.56 (15)	5.26 (19)	9.21 (33)	1.34 (16)	−0.28 (18)	−3.51 (23)
				D. TNBS (Second Molecule)					
S′(t)	0.7039 (3)	0.3533 (2)	0.5299 (1)	4.45 (4)	6.26 (5)	3.22 (4)	3.33 (5)	0.80 (3)	1.00 (4)
C(1′t)	0.9055 (8)	0.2964 (5)	0.4597 (3)	2.81 (15)	3.69 (16)	2.68 (17)	1.66 (15)	−0.15 (12)	0.21 (14)
C(2′t)	1.1046 (10)	0.3818 (5)	0.4354 (3)	3.64 (18)	3.02 (17)	3.95 (22)	1.27 (17)	0.04 (15)	−0.05 (17)
C(3′t)	1.2450 (10)	0.3433 (5)	0.3768 (3)	3.66 (19)	3.11 (17)	5.03 (24)	0.88 (16)	1.12 (18)	0.77 (18)
C(4′t)	1.1804 (9)	0.2115 (4)	0.3411 (3)	3.60 (16)	3.04 (16)	3.44 (18)	1.28 (15)	1.20 (14)	0.79 (16)
C(5′t)	0.9842 (10)	0.1182 (5)	0.3603 (3)	3.78 (18)	2.79 (16)	3.18 (18)	0.68 (15)	0.24 (14)	0.68 (15)
C(6′t)	0.8543 (9)	0.1628 (4)	0.4201 (3)	3.20 (17)	3.02 (16)	2.81 (17)	0.53 (14)	0.08 (13)	0.89 (15)
N(1′t)	1.1868 (10)	0.5251 (5)	0.4732 (4)	4.77 (19)	2.86 (17)	9.49 (34)	1.51 (17)	2.75 (23)	−0.67 (21)
N(2′t)	1.3350 (10)	0.1637 (5)	0.2797 (3)	5.24 (19)	4.08 (18)	4.38 (21)	1.57 (16)	2.03 (17)	0.63 (17)
N(3′t)	0.6517 (8)	0.0618 (4)	0.4409 (3)	3.51 (16)	4.25 (17)	3.90 (17)	0.97 (14)	0.58 (13)	1.55 (15)
O(1′t)	0.4558 (8)	0.2502 (4)	0.5114 (3)	3.61 (15)	6.24 (19)	7.24 (22)	1.74 (15)	2.21 (15)	2.60 (19)
O(2′t)	0.8228 (10)	0.3656 (6)	0.6028 (3)	9.09 (20)	10.37 (26)	2.80 (15)	6.02 (29)	−0.03 (14)	0.52 (19)
O(3′t)	0.7017 (7)	0.4762 (4)	0.5158 (2)	4.49 (13)	4.85 (14)	5.10 (18)	3.08 (15)	0.34 (12)	0.50 (15)
O(4′t)	1.2412 (10)	0.5605 (6)	0.5417 (4)	5.18 (19)	7.42 (24)	9.96 (35)	3.41 (22)	−2.23 (22)	−5.10 (30)
O(5′t)	1.2009 (12)	0.5945 (5)	0.4321 (5)	13.75 (30)	3.98 (18)	16.44 (44)	3.87 (22)	10.38 (44)	3.32 (32)
O(6′t)	1.5295 (10)	0.2442 (5)	0.2703 (4)	6.51 (20)	5.25 (21)	10.49 (32)	1.07 (18)	5.20 (27)	1.07 (25)
O(7′t)	1.2600 (9)	0.0501 (4)	0.2443 (3)	6.80 (20)	3.88 (16)	4.63 (18)	1.84 (16)	1.97 (16)	0.11 (15)
O(8′t)	0.4671 (9)	−0.0015 (5)	0.3934 (3)	3.98 (17)	6.40 (22)	5.96 (22)	−0.61 (16)	−0.79 (16)	1.17 (19)
O(9′t)	0.6816 (9)	0.0420 (5)	0.5016 (3)	5.54 (18)	7.40 (18)	5.06 (18)	1.49 (16)	0.44 (16)	3.87 (21)

[a] Positional parameters are listed as fractions of cell edges. Anisotropic temperature factors are expressed as $\exp[-\frac{1}{4}(h^2a^{*2}B_{11} + k^2b^{*2}B_{22} + l^2c^{*2}B_{33} + 2hka^*b^*B_{12} + 2hla^*c^*B_{13} + 2klb^*c^*B_{23})]$ and isotropic temperature factors as $\exp(-B \sin^2\theta/\rho^2)$ with B values given in Å^2. Estimated standard deviations are listed in parentheses with respect to the last digits given for any parameter.

Table V. Conformation and Packing in Ions of Isomer B of *S*-Carboxymethylmethionine Trinitrobenzenesulfonate

	First molecule	Second molecule
A. Angles between Planes[k]		
C(α),C(1),O(2),O(1)/C(α),C(1),N	11.6°	4.1°
/C(2),C(3),O(3),O(4)	52.4	55.7
/S(δ),C(3),C(γ),C(β),C(α)	52.7	49.6
C(2),C(3),O(3),O(4)/C(α),C(1),N	55.8	56.7
/S(δ),C(3),C(γ)	81.3	79.5
/S(δ),C(3),C(γ),C(β),C(α)	76.4	74.8
S(δ),C(3),C(γ),C(β),C(α)/C(α),C(1),N	47.8	49.1
C(1t),C(2t),C(3t),C(4t),C(5t),C(6t)/N(1t),O(4t),O(5t)	63.5	52.5
/N(2t),O(6t),O(7t)	5.6	9.4
/N(3t),0(8t),O(9t)	58.8	65.5
B. N···O and O···O Hydrogen Bonds		
N···O(4′)[a]	2.908 Å	
O(2′t)[b]	2.927	
O(2)[a]	2.929	
N′···O(2t)[c]	2.894	
O(2′)[d]	2.936	
O(4)[e]	3.164	
O(1)···O(4′)	2.431	
O(1′)···O(4)[f]	2.457	
C. S^+···N and S^+···O Distances Less Than 4 Å		
S(δ)···O(9t)[g]	3.267 Å	
O(3)[h]	3.786	
O(8t)[g]	3.839	
N(3t[g]	3.878	
O(4t)[c]	3.912	
S(δ′)···O(9′t)[b]	3.115	
O(3′)[h]	3.820	
O(4′t)[j]	3.986	

[a] 1 + x, 1 + y, z. [b] x, 1 + y, z. [c] x − 1, y, 1 + z. [d] x − 2, y − 1, z. [e] x, y − 1, z. [f] x + 2, y, z. [g] x, 1 + y, 1 + z. [h] 1 + x, y, z. [j] x − 1, y, z.

(98 200 dpm) received 133.2 mg of the TNBS salt of isomer A, whereas 0.30 mL (26 800 dpm) received 75.8 mg of the TNBS salt of isomer B. Three crystallizations from warm water (65 °C) were carried out for each isomer, and the specific radioactivities of each crop were determined, as well as those of an aliquot of the dried mother liquor from the final crystallization. Constant specific activity was achieved quickly for both isomers (Table I).

II. Crystallographic Studies. X-Ray Diffraction Data Collection. All studies were carried out on crystals of the TNBS salts of isomer B of (*S*)-carboxymethylmethionine since they were of better quality than those from isomer A (which were not suitable for x-ray studies). The crystal data are given in Table II. Although the crystals were found to be twinned, it was possible to obtain single crystals by careful dissection. Three-dimensional x-ray diffraction data were collected on a Syntex automated diffractometer with Ni-filtered Cu Kα radiation using a highly oriented graphite crystal monochromator. It was necessary to utilize three different crystals since the crystals showed a sudden, marked anisotropic decay after 40–45 h of exposure to x rays. The data from each crystal were reduced to structure amplitudes by application of Lorentz and polarization factors and then corrected for absorption using the method of Coppens.[34] The data from the three crystals were then scaled to a common scale by the method of Hamilton, Rollet, and Sparks[35] and combined into a single data set consisting of 7438 reflections (3719 unique reflections and their Friedel-related pairs). This set represents all the data available out to $(\sin\theta)/\lambda = 0.603$ Å^{-1} ($2\theta = 138°$). Of this set, those reflections with $I/\sigma(I) < 1.0$, where I is the measured intensity and $\sigma(I)$ is derived from counting statistics, were considered unobserved. There was 6812 data points with $I_{meas} \geq \sigma(I)$.

Structure Determination and Refinement. The structure was solved by Patterson superposition techniques, using the S-S vectors in the Patterson maps, and by successive structure factor calculations of Fourier syntheses. The 64 nonhydrogen atoms were then refined with isotropic thermal parameters by full-matrix least-squares methods to $R = 0.10$ where $R = \Sigma|kF_{obsd} - F_{calcd}|/\Sigma kF_o$. Further refinement with anisotropic thermal parameters for the nonhydrogen atoms was carried out by a blocked full-matrix least-squares method. A difference map was then computed which revealed the positions of the hydrogen atoms with the exception of the amino hydrogen atoms. Also some disorder of hydrogen atoms along the two carboxylic acid hydrogen bonds was found. The parameters of the nonhydrogen atoms were then refined further with idealized hydrogen atom parameters held constant. The hydrogen atom positions were recomputed after each cycle. The thermal parameters assigned to the hydrogen atoms were the equivalent isotropic temperature factors of the atoms to which the hydrogen atoms are bound. The final $R = 0.70$ and the final weighted residual $R_w = 0.078$ ($R_w = [\Sigma w(kF_o - F_c)^2/w(kF_o)^2]^{1/2}$).

For the early stages of refinement, the weights w were $1/\sigma^2(F)$ where $\sigma(F) = (F/2)\{\sigma^2(I)/I^2 + \delta^2\}^{1/2}$. δ is a measured instrumental uncertainty ($\delta = 0.05$) derived from the variation of the measured intensities of three periodically monitored standard reflections. In the final stages of refinement, the following weighting scheme was employed: $\sqrt{w} = 0$ when $F_o < 2.0$, $\sigma(F) = 1.1$ when $2.0 \geq F_o \geq 11.0$, and $\sigma(F) = 1.1 + 0.048\ (F_o - 11.0)$ when $F_o > 11.0$.

The absolute configuration of the structure was determined by comparing reflections h, k and $-h, -k, -l$. Table III contains the Bijvoet pairs of reflections used for this comparison, and Table IV is a listing of the final parameters for nonhydrogen atoms. The atomic scattering factors for sulfur, carbon, nitrogen, and hydrogen were those given in the International Tables,[36] and for hydrogen atoms, the values of Stewart, Davidson, and Simpson[37] were used. The anomalous dispersion corrections for sulfur, given by Cromer and Liberman,[38] were used. Computations were carried out on a PDP 11/40 and on the CDC 7600 at BNL using the Crysnet system,[39] X-RAY 76,[40] and ICRFMLS.[41] Diagrams were drawn with the program VIEW.[42] A table of observed and calculated structure factors may be obtained from the authors on request.

Description of the Structure. The numbering system for atoms, together with the bond distances and angles for the (*R*)-sulfonium

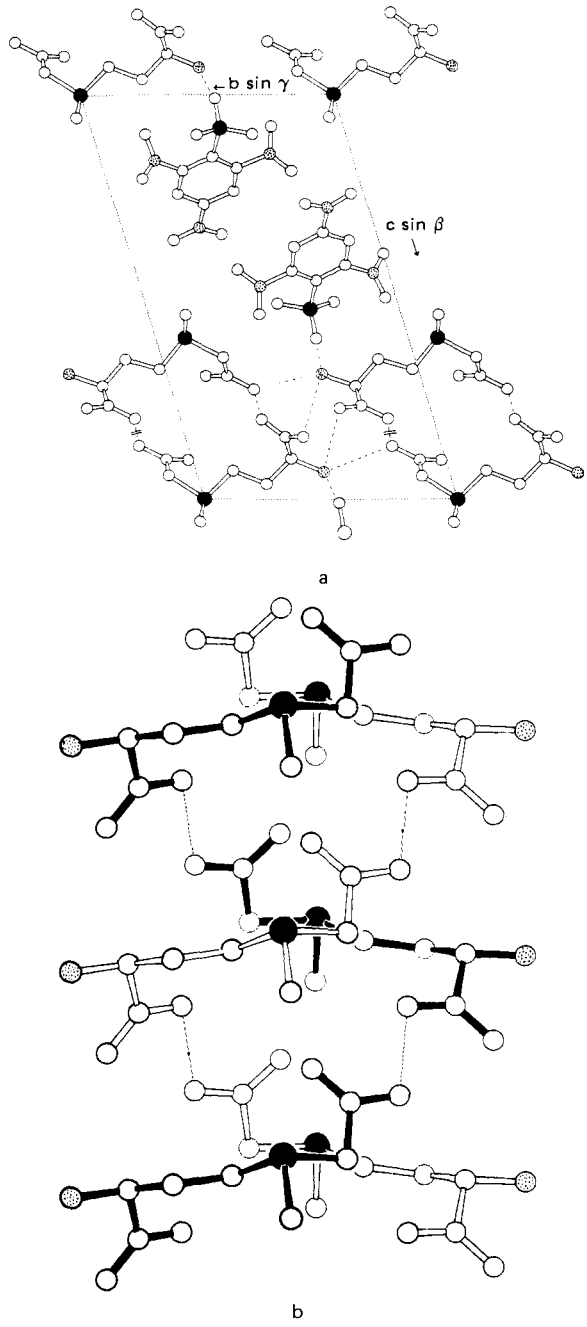


Figure 4. (a) Crystal packing of isomer B of *S*-carboxymethylmethionine cations and 2,4,6-trinitrobenzenesulfonate anions in the unit cell. (b) Double helical arrangement of amino acids in the crystal. Sulfur atoms are black and nitrogen atoms are stippled in both diagrams.

isomer of *S*-carboxymethylmethionine and the TNBS anion, are given in Figure 3. As shown, the bond lengths and angles in the two crystallographically independent pairs of cations and anions are the same within the experimental error. The bond lengths and angles for the (*S*)-methionine moieties are in good agreement with those reported by other investigators.[43] Some angles between planes in portions of the ions are given in Table Va, which describes, for instance, angles between the planes of the carboxyl groups and that of the backbone, as well as those of the other heteroatoms.

The bond lengths and angles of the two TNBS anions are also in agreement with previously reported values.[29,44] The benzene rings are planar in both ions and, while the *p*-nitro groups in the two anions are very nearly in the plane of the benzene ring, the *o*-nitro groups are twisted out of this plane (Table Va).

The crystal packing is illustrated in Figure 4a and the hydrogen bonding parameters are given in Table Vb. The hydrogen bond between O(1) and O(4) of another molecule is very short (2.431 and 2.457 Å). This hydrogen bonding results in the formation of a double helix, as shown in Figure 4b. The amino groups protrude from the helix and are hydrogen bonded to other parts of the crystal structure. From Table Vc it may be seen that the shortest interionic packing distances around sulfonium S (δ) are to O(9) of the TNBS anion.

Conclusion

In summary, we conclude from our combined chemical and crystallographic studies that the absolute configuration of the naturally occurring *S*-adenosyl-(*S*)-methionine is *S* at the sulfonium pole, i.e., viewed from where the sulfur atom obscures the lone electron pair, the adenosyl, the *S*-3-amino-3-carboxypropyl, and the methyl groups are arranged in counterclockwise order.

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References and Notes

(1) (a) Milstead Laboratory, Shell Research Limited; (b) The School of Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ, Sussex, United Kingdom; (c) The Johns Hopkins University School of Medicine; (d) Institute for Cancer Research.
(2) Nomenclature: "*S*-adenosylmethionine" denotes the "natural" stereoisomer, 5′-[((3*S*)-3-amino-3-carboxypropyl)methyl-(*S*)-sulfonio]-5′-deoxyadenosine, as shown in this paper. "*S*-carboxymethylmethionine" denotes either (*R*)- or (*S*)-[((3*S*)-3-amino-3-carboxypropyl)(carboxymethyl)methylsulfonium] ion, or a mixture of the two. TNBS is used for 2,4,6-trinitrobenzenesulfonic acid.
(3) S. K. Shapiro and F. Schlenk, Ed., "Transmethylation and Methionine Biosynthesis", University of Chicago Press, Chicago, Ill., 1964; F. Salvatore, Ed., "The Biochemistry of Methionine", Columbia University Press, in press.
(4) D. Aswad and D. E. Koshland, Jr. *J. Mol. Biol.*, **97**, 207–235 (1975); J. Adler, *Annu. Rev. Biochem.*, 44, 341–356 (1976).
(5) G. L. Cantoni, *J. Am. Chem. Soc.*, **74**, 2942–2943 (1952).
(6) G. L. Cantoni, *J. Biol. Chem.*, **204**, 403–416 (1953).
(7) J. Baddiley, G. L. Cantoni, and G. A. Jamieson, *J. Chem. Soc.*, 2662–2664 (1953).
(8) J. Baddiley and G. A. Jamieson, *J. Chem. Soc.*, 4280–4284 (1954); 1085–1089 (1955).
(9) G. de la Haba, G. A. Jamieson, S. H. Mudd, and H. H. Richards, *J. Am. Chem. Soc.*, **81**, 3975–3980 (1959).
(10) Two samples of enzymatically synthesized "(−)-*S*-adenosyl-L-methionine" had $[\alpha]^{25}_{D}$ +48.5 and +47.2°; "(+)-*S*-adenosyl-L-methionine" prepared by enzymatic resolution with guanidoacetate methyltransferase had $[\alpha]^{25}_{D}$ +57°.
(11) V. Zappia, C. R. Zydek-Cwick, and F. Schlenk, *Biochim. Biophys. Acta*, **178**, 185–187 (1969).
(12) V. Zappia, C. R. Zydek-Cwick, and F. Schlenk, *J. Biol. Chem.*, **244**, 4499–4509 (1969).
(13) A. W. Coulter, J. B. Lombardini, and P. Talalay, *Mol. Pharmacol.*, **10**, 293–304, 305–314 (1974); A. W. Coulter, J. B. Lombardini, J. R. Sufrin, and P. Talalay, *ibid.*, 10, 319–334 (1974).
(14) R. T. Borchard and Y. S. Wu, *J. Med. Chem.*, **19**, 1099–1103 (1976).
(15) P. H. Laur in "Sulfur in Organic and Inorganic Chemistry", Vol. 3, A. Senning, Ed., Marcel Dekker, New York, N.Y., 1972, p 91.
(16) A. Kjaer, *Tetrahedron*, **30**, 1551–1554 (1974).
(17) S. Smiles, *J. Chem. Soc.*, 77, 1174–1179 (1900).
(18) W. J. Pope and S. J. Peachey, *J. Chem. Soc.*, 77, 1072–1075 (1900).
(19) E. Kelstrup, A. Kjaer, S. Abrahamsson, and B. Dahlén, *J. Chem. Soc., Chem. Commun.*, 629–630 (1975).
(20) A. F. Cook and J. G. Moffatt, *J. Am. Chem. Soc.*, **90**, 740–746 (1968); G. K. Helmkamp, D. C. Owsley, and R. B. Harris, *J. Org. Chem.*, **34**, 2763–2766 (1969); K. K. Andersen, R. L. Caret, and I. Karup-Nielsen, *J. Am. Chem. Soc.*, **96**, 8026–8032 (1974).
(21) H. G. Gundlach, S. Moore, and W. H. Stein, *J. Biol. Chem.*, **234**, 1761–1764 (1959).
(22) L. W. Parks and F. Schlenk, *J. Biol. Chem.*, **230**, 295–305 (1958); *Arch. Biochem. Biophys.*, **75**, 291–292 (1958).
(23) J. Baddiley, W. Frank, N. A. Hughes, and J. Wieczorkowski, *J. Chem. Soc.*, 1999–2005 (1962).
(24) P. Mamalis and H. N. Rydon, *J. Chem. Soc.*, 1049–1065 (1955).
(25) G. F. Huebner, S. R. Ames, and E. C. Bubl, *J. Am. Chem. Soc.*, **68**, 1621–1628 (1946); R. H. Cornforth, J. W. Cornforth, and G. Popják, *Tetrahedron*, **18**, 1351–1354 (1962).
(26) The $SCH_2CO_2^-$ protons of *S*-carboxymethylmethionine salts were recently

found [C. C. Q. Chin and F. Wold, *Arch. Biochem. Biophys.*, **167**, 448–451 (1975)] to undergo quite rapid exchange in deuterium oxide. A significant proportion of enol or enolate anion might therefore be expected to be present in aqueous solutions of the aldehyde **6**.
(27) Thermal racemization and solvolysis of optically active sulfonium compounds have been studied by several investigators: (a) M. P. Balfe, J. Kenyon, and H. Phillips [*J. Chem. Soc.*, 2554–2572 (1930)] concluded that racemization of (−)-ethylmethylphenacylsulfonium halides involved nucleophilic displacement by halide on carbon, followed by recombination to racemic sulfonium salt; (b) D. Darwisch and G. Tourigny [*J. Am. Chem. Soc.*, **88**, 4303–4304 (1966)] studied racemization of *tert*-butylethylmethylsulfonium perchlorate and concluded that loss of chirality proceeded by an internal inversion process since racemization occurs more rapidly than solvolysis; (c) R. Scartazzini and K. Mislow [*Tetrahedron Lett.*, 2719–2722 (1967)] concluded that racemization of 1-adamantylethylmethylsulfonium perchlorate was by pyramidal inversion; (d) D. Darwisch, S. H. Hui, and R. Tomlinson [*J. Am. Chem. Soc.*, **90**, 5631–5632 (1968)] concluded that racemization of several benzylethylmethylsulfonium perchlorates occurred by inversion but that in at least one case, the *p*-methoxybenzyl analogue, racemization was probably by heterolytic bond cleavage via an ion–neutral molecule pair; (e) A. Garbesi, N. Corsi, and A. Fava [*Helv. Chim. Acta*, **53**, 1499–1502 (1970)] provided strong evidence that stereomutation of cyclic sulfonium salts occured by pyramidal inversion at the sulfur atom. We found the *S*-carboxymethylmethionine isomers A and B not readily interconvertible, but a substance having the chromatographic properties of isomer A was produced by heating isomer B in 5 acetic acid at 60 °C; the half-life for interconversion appeared to be somewhat greater than 24 h.
(28) This sample of (*S*)-methionine had $[\alpha]^{25}_D$ +23.0° (*c* 1.255, 5 N HCl). S. M. Birnbaum, L. Levintow, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455–470 (1952), reported $[\alpha]^{25}_D$ +23.2° (*c* 2.0, 2 N HCl).
(29) W. Barnes and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **29**, 1868–1875 (1973); D. E. Zuccaro and J. D. McCullough, *Z. Kristallogr., Kristallgeom., Kristallphys., Kristallchem.*, **112**, 401–408 (1959); A. T. Christensen and E. Thom, *Acta Crystallogr., Sect. B*, **27**, 581–586 (1971). In this last reference one C–S bond is shortened as a result of delocalization of electrons with the adjacent ring system.
(30) M. C. Caserio, R. E. Pratt, and R. J. Holland, *J. Am. Chem. Soc.*, **88**, 5747–5753 (1966); C. Brown in "Sulfur in Organic and Inorganic Chemistry", Vol. 3, A. Senning, Ed., Marcel Dekker, New York, N.Y., 1972, pp 296–297.
(31) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190–1206 (1958).
(32) The 1.0 M I_2 solutions contained 15 g of KI (0.09 mol) and 12.7 g of I_2 (0.05 mol) in 50 mL solution in distilled water.
(33) H. W. Foote and W. C. Chalker, *Am. Chem. J.*, **39**, 561–567 (1908); H. L. Wells, H. L. Wheeler, and S. L. Penfield, *Z. Anorg. Allg. Chem.*, **1**, 442–455 (1892); G. S. Johnson, *J. Chem. Soc.*, **31**, 249–253 (1877).
(34) P. Coppens in "Crystallographic Computing", F. R. Ahmed, Ed., Munksgaard, Copenhagen, 1970, pp 255–270.
(35) W. C. Hamilton, J. S. Rollett, and R. A. Sparks, *Acta Crystallogr.*, **18**, 129–130 (1965).
(36) "International Tables for X-Ray Crystallography", Vol. III, Kynoch Press, Birmingham, England, 1962, pp 201–207.
(37) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175–3187 (1965).
(38) D. T. Cromer and D. Liberman, *J. Chem. Phys.*, **53**, 1891 (1970).
(39) H. J. Bernstein, L. C. Andrews, H. M. Berman, F. C. Bernstein, G. H. Campbell, H. L. Carrell, H. B. Chiang, W. C. Hamilton, D. D. Jones, D. Klunk, T. F. Koetzle, E. F. Meyer, C. N. Morimoto, S. S. Sevian, R. K. Stodola, M. M. Strongson, and T. V. Willoughby, "Crysnet—a Network of Intelligent Remote Graphics Terminals", Second Annual AEC Scientific Computer Information Exchange Meetings, Proceedings of the Technical Program, 1974, pp 148–158.
(40) J. M. Stewart, G. J. Kruger, H. L. Ammon, C. Dickinson, and S. R. Hall, The X-RAY System—Version of June 1972, Technical Report TR-192, Computer Science Center, University of Maryland, College Park, Md., 1972; J. M. Stewart, The X-RAY System—Update of 1976.
(41) P. K. Gantzel, R. A. Sparks, R. E. Long, and K. N. Trueblood, UCLALS4 Program in Fortran IV, 1969; H. L. Carrell, ICRFMLS, Modification of UCLALS4, 1975.
(42) H. L. Carrell, VIEW Program in Fortran, 1976.
(43) R. E. Stenkamp and L. H. Jensen, *Acta Crystallogr., Sect. B*, **31**, 857–861 (1975); K. Torii and Y. Iitaka, *ibid.*, **29**, 2799–2807 (1973).
(44) M. Meyers and K. N. Trueblood, *Acta Crystallogr., Sect. B*, **25**, 2588–2598 (1969).

Structural and Isotopic Effects in Hydrophobic Binding Measured by High-Pressure Liquid Chromatography. A Stable and Highly Precise Model for Hydrophobic Interactions in Biomembranes[1]

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Abstract: Highly reproducible measurements of partition ratios between aqueous solvents and hydrophobic high-pressure liquid chromatographic columns can be made, thus permitting this system to be used as a model for hydrophobic interactions in biomembranes. The partition ratios have different sensitivities to the addition of a methylene group onto alkyl chains, depending on the mole fraction of methanol added to the aqueous mobile phase. The sensitivity is highest for pure water, but appears simply to be the end of a continuum of hydrophobicity; pure water does not appear to be unique. The free energies of transfer per CH_2 group for these columns resemble those found for binding of alkanes into micelles. There appears to be a cooperative binding effect (fronting rather than tailing of peaks) with alkanes and long-chain carboxylic acids. It is possible to do accurate liquid–liquid chromatography experiments with hydrophobic columns coated with stearic acid, suggesting the possibility of experiments with other substances, including lipids. We have shown isotopic separation of deuterated and protiated palmitic acid and other molecules, and have been able to measure secondary deuterium isotope effects on hydrophobic binding, giving a probe of the nature of the hydrophobic effect.

We report herein our initial studies of hydrophobic effects on a variety of relatively simple molecules, including structural, solvent, and isotope effects, which have given highly reproducible partition constants between mobile and stationary phases.[1a]

The hydrophobic effect is an important structural feature of biological membranes.[2,3] Specific structural factors not found in bulk hydrophobic phases may be involved in membrane phenomena.[2-4] Hydrophobic interactions have been found to be important in determining the activities of a large number of physiologically active substances, and a quantitative linear free energy relationship involving octanol–water partition ratios has been found to have very significant predictive value for structure–activity correlations.[5-8] Chromatographic measures of hydrophobic effects have also been used for structure–activity correlations.[9]

Recently, high-pressure liquid chromatography, using hydrophobic columns consisting of silica particles coated with covalently attached 18-carbon *n*-alkyl chains, has been used to study hydrophobic effects and structure–activity relationships.[10-23]

The nature of the hydrophobic effect has been related to