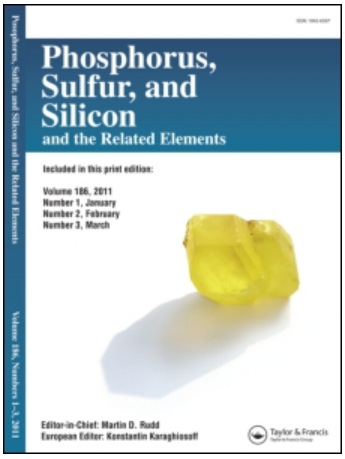

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

SYNTHESIS AND CHROMATOGRAPHIC PROPERTIES OF S-CARBOXYMETHYLHOMOCYSTEAMINE (2 CARBOXYMETHYL-3-AMINOPROPYL SULFIDE)

A. Rinaldi[a]; S. Dernini[a]; M. G. Pellegrini[a]; C. De Marco[a]
[a] Isituto di Chimica Biologica della Università di Cagliari Cagliari, Italy and ÎCattedra di Chimica Biologica della Facoltà di Scienze M.F.N. della Università di Roma, Roma, Italy

To cite this Article Rinaldi, A. , Dernini, S. , Pellegrini, M. G. and De Marco, C.(1978) 'SYNTHESIS AND CHROMATOGRAPHIC PROPERTIES OF S-CARBOXYMETHYLHOMOCYSTEAMINE (2 CARBOXYMETHYL-3-AMINOPROPYL SULFIDE)', Phosphorus, Sulfur, and Silicon and the Related Elements, 4: 1, 27 — 31
To link to this Article: DOI: 10.1080/03086647808079959
URL: http://dx.doi.org/10.1080/03086647808079959

Phosphorus and Sulfur
1978, Vol. 4, pp. 27-31

SYNTHESIS AND CHROMATOGRAPHIC PROPERTIES OF S-CARBOXYMETHYLHOMOCYSTEAMINE (2 CARBOXY-METHYL-3-AMINOPROPYL SULFIDE)

A. RINALDI, S. DERNINI, M. G. PELLEGRINI and C. DE MARCO

Isituto di Chimica Biologica della Università di Cagliari, Cagliari, Italy and
I^ Cattedra di Chimica Biologica della Facoltà di Scienze M.F.N. della Università di Roma, Roma, Italy

(Received May 23, 1977)

Details are reported for the synthesis of carboxymethylhomocysteamine starting either from homocysteamine and monochloroacetic acid, or from 3-brompropylamine and thioglycolic acid. CMHC was obtained in pure crystalline form, and with fairly good yield. The corresponding sulfoxide and sulfone have also been prepared in a crystalline form. These compounds may be separated by paper chromatography, and identified by specific reactions. On the Amino Acid Analyzer they may be easily separated from each other and from the homologous carboxymethyl-cysteamine, its sulfoxide and its sulfone, using the long column and the standard sodium citrate buffers at pH 3.25 and 4.25.

Recently we have demonstrated that cystathionamine, a sulfur diamine which may be regarded as the higher homolog of lanthionamine, is oxidatively deaminated by pig kidney diamine oxidase (E.C. 1436).[1,2] Preliminary data suggested that, as in the case of cystamine[3,4] and lanthionamine,[5] the first product of the oxidation of cystathionamine is the corresponding cyclized aminoaldehyde, which may undergo further degradation.

In the course of these studies we had to investigate the possible production of homocysteamine during the reaction. A feasible way was to trap it with monoiodoacetic acid, searching then for the corresponding S-carboxymethyl derivative. The alkylation with monoiodoacetic acid had already been used to demonstrate the production of cysteamine and thiocysteamine during the oxidative deamination of cystamine.[6,7]

$H_2NCH_2CH_2CH_2SCH_2CH_2NH_2$	cystathionamine
$H_2NCH_2CH_2CH_2SH$	homocysteamine
$H_2NCH_2CH_2CH_2SCH_2COOH$	carboxymethyl-homocysteamine
$H_2NCH_2CH_2SCH_2COOH$	carboxymethyl-cysteamine

Abbreviations: CMHC: carboxymethylhomocysteamine; CMHC-SO: carboxymethylhomocysteamine sulfoxide; CMHC-SO_2: carboxymethylhomocysteamine sulfone; CMC: carboxymethylcysteamine; CMC-SO: carboxymethyl-cysteamine sulfoxide; CMC-SO_2: carboxymethylcysteamine sulfone.

To test the production of carboxymethyl-homocysteamine we needed the pure compound, and therefore we attempted to prepare it. In the present note we report the details for the synthesis of S-carboxymethylhomocysteamine, CMHC, and its sulfoxide and sulfone, together with some chromatographic data useful for their identification and differentiation from homologous compounds.

METHODS AND RESULTS

CMHC was prepared by two different methods, (a) by reaction of homocysteamine with monochloroacetic acid, (b) by reaction of 3-brompropylamine with thioglycolic acid.

Thioglycolic acid was obtained from Fluka; 3-brompropylamine · HBr from K & K Labs. Melting points were determined in a Kofler apparatus, and were uncorrected. NMR spectra were recorded in a JEOL C-60-HL spectrometer; the compounds were dissolved in D_2O at a concentration of *ca.* 5%; as an external standard hexamethyldisiloxane, HMDS, was used. Paper chromatographic analyses were performed on Whatman No. 1 paper sheets, in the solvents specified below. Ion-exchange chromatography was performed on a Bio Cal 200 Amino Acid Analyzer. The long column, 54 x 0.9 cm, filled with Aminex A 6 resin from Bio Rad, was used. The column temperature was 50°C; the buffer flow rate 80 ml/hr, the ninhydrin flow rate 40 ml/hr. The

standard 3 mm light path cell was used. The column was equilibrated with the standard 0.2 M sodium citrate buffer pH 3.25 and then eluted with this buffer, followed by the standard 0.2 M sodium citrate buffer pH 4.25.

Carboxymethylcysteamine, its sulfoxide and sulfone were prepared as previously described.[8]

a *CMHC from Homocysteamine and Monochloroacetic Acid*

Homocysteamine is prepared immediately before use from 3-brompropylamine and potassium hydrosulfide and, without isolation from the reaction mixture, it is allowed to react with monochloroacetic acid at alkaline pH.

In a 100 ml round bottomed flask 1.1 g (5 mmoles) of 3-brompropylamine · HBr dissolved in 5 ml methanol is added with 0.3 g (5.3 mmoles) of KOH dissolved in 5 ml methanol. A precipitate of KBr is immediately formed. To the suspension a solution of 0.3 g KOH in 5 ml methanol, previously gassed with H_2S until decoloration of phenolphthalein, is added. The flask is connected to a water condenser, and the suspension is refluxed for half an hour, then cooled and filtered through a sintered glass funnel to eliminate KBr. The filtrate, acidified with conc. HBr, is taken to dryness in a rotary evaporator. The oily residue is taken up in few ml of methanol, filtered from a further precipitate of KBr, diluted to double volume with water and placed in a water bath at 60°C, under continuous bubbling of nitrogen. Monochloroacetic acid, 0.55 g (5.5 mmoles) is now added, and the solution is made alkaline to phenolphthalein by dropwise addition of conc. ammonia. After 10 min. the nitroprusside test for -SH groups becomes negative, indicating that all the homocysteamine has reacted. The nitroprusside test, performed in the presence of KCN to detect S-S groups, reveals the presence of only traces of homocystamine.

The solution is then percolated through a 2 x 8 cm column of Dowex 50-H^+, the column is washed with water and then eluted with 1 N NH_4OH collecting fractions of 5-7 ml. Aliquots of 10 μl of the first 4-6 alkaline fractions are dried on paper and tested with ninhydrin or with the iodoplatinate reagent.[9] The fractions giving positive reaction are pooled and taken to dryness in a rotary evaporator at 70°C. The oily residue is added with absolute ethanol and taken to dryness again, repeating this step once or twice until a crystalline residue is obtained. About 300 mg of CMHC are so obtained (44% of theory). For recrystallization it was dissolved in boiling ethanol by dropwise addition of water, filtered and allowed to cool in the refrigerator. White, birefringent, prismatic crystals were so obtained, which were washed with absolute ethanol and dried with ether. Melting point was 206-209°C. The elemental analysis gave the following results: calculated for $C_5H_{11}NO_2S$, C 40.26, H 7.38, N 9.39%; found C 40.14, H 7.24, N 9.25.

b *CMHC from 3-Brompropylamine and Thioglycolic Acid*

To a solution of 1.1 g (5 mmoles) of 3-brompropylamine HBr in 2 ml H_2O 0.3 ml of thioglycolic acid (5 mmoles) and 2 ml of 5 N KOH are added. The solution, which must now be alkaline to phenolphthalein, is placed in a water bath at 50°C under bubbling of nitrogen. After about 20 min. the nitroprusside test for the -SH groups becomes negative, indicating that all the thioglycolic acid has reacted. The solution is percolated through a 2 x 8 cm column of Dowex 50-H^+, and then one proceeds as described for method (a). About 450 mg of CMHC were obtained by this procedure (59% of theory). After recrystallization from water-ethanol the melting point and the analytical data were the same as for CMHC prepared by method (a).

CMHC-SO, Carboxymethylhomocysteamine Sulfoxide

To 150 mg (1 mmoles) of CMHC dissolved in 0.4 ml H_2O, 0.34 ml (3 mmoles) of 30% H_2O_2 is added dropwise. The solution is allowed to stand at room temperature for half an hour; then 5 ml ethanol is added before placing in the refrigerator. After a few hours the precipitate is collected by centrifugation, washed with absolute ethanol and dried with ether, 110 mg of white crystalline product are obtained. After recrystallization from water-ethanol CMHC-SO melts at 192-195°C. The elemental analysis gave the following results: calculated for $C_5H_{11}NO_3S$, C 36.36, H 6.66, N 8.48%; found C 36.20, H 6.90, N 8.21%.

CMHC-SO_2, Carboxymethylhomocysteamine Sulfone

10 mg of ammonium molybdate are added to 0.3 ml perchloric acid and 1.5 ml H_2O. The suspension is boiled for 3 min, then filtered and added to 150 mg (1 mmoles) of CMHC; 0.6 ml 30% H_2O_2 (7 mmoles) are added dropwise within 10 min. and the solution is then placed in a water bath at 60°C for 30 min. It is then cooled, neutralized with ethanolamine and 5 ml ethanol is added, crystallization immediately starts.

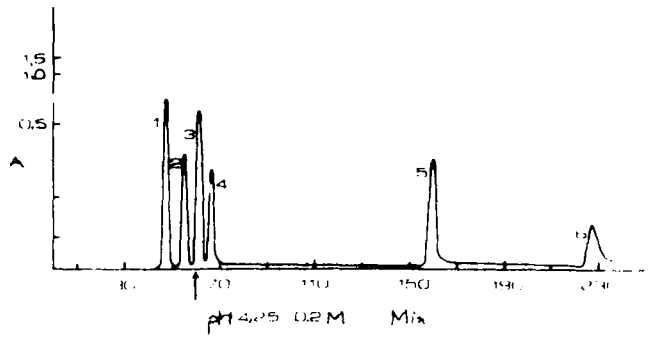

FIGURE 1 Elution profile of the compounds under study from the long column of the Amino Acid Analyzer. The column, 54 x 0.9 cm, filled with Aminex A 6 resin, was equilibrated with 0.2 M sodium citrate buffer pH 3.25 and eluted with this buffer for the first 60 min, then with the 0.2 M sodium citrate pH 4.25. Column temp. 50°C; buffer flow rate 80 ml/hr. Sample size 0.5 μmol. 1 = carboxymethylcysteamine sulfone, CMC-SO_2; 2 = carboxymethylhomocysteamine sulfone, CMHC-SO_2; 3 = carboxymethylcysteamine sulfoxide, CMC-SO; 4 = carboxymethylhomocysteamine sulfoxide, CMHC-SO; 5 = carboxymethylcysteamine, CMC; 6 = carboxymethylhomocysteamine, CMHC.

After a few hours in the refrigerator the crystals are collected by centrifugation, washed with absolute ethanol and dried with ether, 135 mg of white, birefringent crystals are obtained which, after recrystallization from water-ethanol, melted at 173-174°C. The elemental analysis gave the following results: calculated for $C_5H_{11}NO_4S$, C 33.15, H 6.07, N 7.73%; found C 31.72, H 6.28, N 7.41%.

NMR Spectra

The nmr spectra of CMHC, CMHC-SO and CMHC-SO_2 are reported in Figure 1. The signals were assigned to the methylenic groups as reported in Table I.

From these data it is evident that the resonances relative to the methylenic groups implicated in the thioether bonds undergo a progressive downfield shift in the sulfoxide and the sulfone, due to the deshielding effect of the -SO and even more of the $-SO_2$ group.

TABLE I

NMR data for CMHC, CMHC-SO, CMHC-SO_2. Compounds dissolved at ca. 5% in D_2O. External reference HMDS, δ (ppm) = 0. Chemical shifts are uncorrected for bulk magnetic susceptibility. Methylenic groups are indicated as in the following formula:

$$D_3N^+ - \underset{a}{CH_2} - \underset{b}{CH_2} - \underset{c}{CH_2} - S - \underset{d}{CH_2} - COO^-$$

Methylenic group	δ (ppm)			Relative intensity
	CMHC	CMHC-SO	CMHC-SO_2	
a triplet	3.43	3.43	3.48	2 H
b quintuplet	2.26	2.45	2.50	2 H
c triplet	2.98	3.34	3.78	2 H
d singlet	3.53	4.10	4.37	2 H

Paper Chromatography

On paper chromatograms CMHC, CMHC-SO and CMHC-SO_2 give well defined spots reactive to

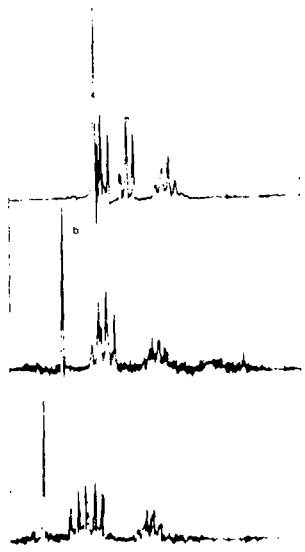

FIGURE 2 NMR spectra of carboxymethylhomocysteamine, (a) its sulfoxide, (b) and its sulfone, (c) recorded at 60 MHz in a JEOL C-60-HL spectrometer. Samples dissolved at 5% ca. in D_2O. External reference: HMDS, δ (ppm) = 0.

TABLE II

Rf values, x 100, of the compounds under study

Solvent system	CMHC	CMHC-SO	CMHC-SO_2	CMC	CMC-SO	CMC-SO_2
Phenol (water-saturated)	85	78	72	77	50	55
Collidine-Lutidine (1 : 1, water-saturated)	18	13	21	20	14	30
Butanol-Ethanol-Water (4 : 1 : 5, upper phase)	14	4	6	20	10	11
Butanol-Acetic Acid-Water (4 : 1 : 5, upper phase)	39	11	13	32	13	11
Butanol-Ammonia (butanol saturated with 3% ammonia)	8	2	2	8	2	4
Methanol-Pyridine-Water (80 : 4 : 20)	73	70	70	68	60	60
Reagent						
Ninhydrin	+	+	+	+	+	+
Iodoplatinate	+	±	−	+	±	−
KI + HCl	−	+	−	−	+	−

ninhydrin. CMHC is reactive also to iodoplatinate.[9] CMHC-SO reacts to iodoplatinate more slowly, and liberates iodine when sprayed with KI in HCl.[10] CMHC-SO_2 does not react to iodoplatinate nor to KI in HCl.

The Rf values obtained in various solvent systems are reported in Table II, in comparison with the values for CMC, CMC-SO and CMC-SO_2.

Ion-exchange Chromatography

CMHC is eluted from the long column of the Amino Acid Analyzer by the standard sodium citrate buffer pH 4.25, it is well separated from CMC. CMHC-SO and CMHC-SO_2 are eluted by the pH 3.25 citrate buffer; they are well separated from each other as also from CMC-SO and CMC-SO_2. The elution profile of all these compounds is shown in Figure 2.

TABLE III

Elution times of the compounds under study from the long column of the Amino Acid Analyzer; color constants (C_{HW}) and $A_{440} : A_{570}$ ratios. Experimental conditions as in Figure 2.

Compound	Elution time min.	C_{HW}	$A_{440} : A_{570}$
CMC-SO_2	47	27	0.14
CMHC-SO_2	56	17	0.21
CMC-SO	62	28	0.21
CMHC-SO	67	14	0.25
CMC	159	24	0.23
CMHC	225	14	0.40

In Table III are reported the elution times, the color constants and the ratios $A_{440} : A_{570}$.

DISCUSSION

The data reported above show that CMHC may be easily prepared in pure crystalline form by the two methods suggested. Method (b) starting from brompropylamine and thioglycolic acid is simpler than method (a) and gives CMHC in slightly higher yield. The preparation of the sulfoxide and the sulfone is also quite simple.

The study of the chromatographic behavior of CMHC shows that it may be easily differentiated from the homologous CMC, either on paper or on the Amino Acid Analyzer. Therefore the alkylation with monochloro- or monoiodoacetic acid may be a useful and simple method for identification of homocysteamine and for its differentiation from cysteamine.

ACKNOWLEDGEMENTS

This work has been supported in part by a grant from the Consillio Nazionale delle Ricerche, Rome. The Authors are indebted to Dr. A. Lai of the Istituto di Chimica, Università di Cagliari, for the NMR spectra.

REFERENCES AND NOTES

1. M. R. Dessì, M. B. Fadda and M. G. Pellegrini, *Riun. Soc. It. Biochim.* (1974) Riva del Garda Com. No. 101.
2. A. Rinaldi, S. Dernini, M. B. Fadda and C. De Marco, *Ital. J. Biochem.* (1975) in press.

3. D. Cavallini, C. De Marco and B. Mondovì, *Biochim. Biophys. Acta* **24**, 353 (1957).
4. C. De Marco, *Proc. Symp. Chem. Biol. Aspects of Pyridoxal Catalysis*, Pergamon Press, 1963, p. 361.
5. C. De Marco, F. Riva and L. Bernini, *Boll. Soc. It. Biol. Sper.* **39**, 1981 (1963).
6. C. De Marco and G. Bombardieri, *Biochim. Biophys. Acta* **93**, 418 (1964).
7. C. De Marco, G. Bombardieri, F. Riva, S. Duprè and D. Cavallini, *Biochim. Biophys. Acta* **100**, 89 (1965).
8. C. De Marco, C. Crifò and A. Rinaldi, *Ital. J. Biochem.* **19**, 337 (1970).
9. G. Toennies and J. J. Kolb, *Anal. Chem.* **23**, 823 (1951).
10. D. Cavallini, C. De Marco and B. Mondovì, *J. Biol. Chem.* **234**, 854 (1959).