6. Fresh rat-liver preparations catalyse transaminations with L- α -amino acid- α -keto acid pairs, not involving glutamate- α -oxoglutarate or alanine-pyruvate.

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REFERENCES

- Awapara, J. (1949). *J. biol. Chem.* **178**, 113.
 Awapara, J. & Seale, B. (1952). *J. biol. Chem.* **194**, 497.
 Braunstein, A. E. (1939). *Enzymologia*, **7**, 25.
 Braunstein, A. E. (1947). *Advanc. Protein Chem.* **3**, 1.
 Brush, M. K., Boutwell, R. K., Barton, A. D. & Heidelberger, C. (1951). *Science*, **113**, 4.
 Cammarata, P. S. & Cohen, P. P. (1950). *J. biol. Chem.* **187**, 439.
 Cohen, P. P. & Hekhuis, G. L. (1941). *J. biol. Chem.* **140**, 711.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
 Copenhagen, J. H. jun., McShan, W. H. & Meyer, R. K. (1950). *J. biol. Chem.* **183**, 73.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.
 Frohman, C. E., Orten, J. M. & Smith, A. H. (1951). *J. biol. Chem.* **193**, 277.
 Gale, E. F. (1948). *Eiweiss-Forsch.* **1**, 145.
 Hird, F. J. R. & Rowsell, E. V. (1950). *Nature, Lond.*, **166**, 517.
 Keilin, D. & Hartree, E. F. (1936). *Proc. Roy. Soc. B*, **119**, 114.
 Knox, W. E. & LeMay-Knox, M. (1951). *Biochem. J.* **49**, 686.
 Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
 La Du, B. N. jun. & Greenberg, D. M. (1951). *J. biol. Chem.* **190**, 245.
 Maddock, S. & Svedberg, A. (1938). *Amer. J. Physiol.* **121**, 203.
 Neuberger, A. (1948). *Biochem. J.* **43**, 599.
 O'Kane, D. E. & Gunsalus, I. C. (1947). *J. biol. Chem.* **170**, 433.
 Quastel, J. H. & Witty, R. (1951). *Nature, Lond.*, **167**, 556.
 Rowsell, E. V. (1951). *Nature, Lond.*, **168**, 104.
 Rowsell, E. V. (1956). *Biochem. J.* **64**, 235.
 Rudman, D. & Meister, A. (1953). *J. biol. Chem.* **200**, 591.
 Schepartz, B. (1951). *J. biol. Chem.* **193**, 293.
 Svedberg, A., Maddock, S. & Drury, D. D. (1938). *Amer. J. Physiol.* **121**, 209.
 Van Slyke, D. D. & Meyer, G. M. (1913). *J. biol. Chem.* **16**, 213.
 Weil-Malherbe, H. (1936). *Biochem. J.* **30**, 665.

(+)-(S-Methyl-L-cysteine S-Oxide) in Cabbage

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For some years work in our laboratory has been aimed at identifying in plant juices compounds of low molecular weight containing residues of chemically bound amino acids which are set free by hydrolysis with acid (Synge, 1951; Synge & Wood, 1954; Ellfolk & Synge, 1955). Most of this work has been with grass extracts. However, we have cursorily examined extracts from a number of other plants. The results were generally not much different from those with grass, but with fractions from cabbage the treatment with hot acid led to a striking increase in the intensity of the spot in the position of valine on our two-dimensional paper chromatograms.

In order to isolate the parent substance from the unhydrolysed juice we first tried fractionation by electrical transport in a diaphragm cell (Synge, 1951). At pH 6-7 the substance migrated, though less readily than aspartic and glutamic acids, into the acetic acid compartment of the four-compartment cell, while in dilute acetic acid in the three-compartment cell it migrated into the cathode compartment, though less readily than the ordinary

neutral amino acids. Experiments were then made with paper ionophoresis. A sample strip from the paper was coloured with ninhydrin; the zones so located were cut out, eluted and the eluates hydrolysed. Our substance was associated with a ninhydrin-staining zone due, it seemed, to an ampholyte intermediate in behaviour between the dicarboxylic and neutral amino acids. On two-dimensional paper chromatograms, this material occupied position A (Fig. 2). Accordingly, to isolate the material in larger quantities we tried displacement chromatography on a cation-exchange resin (cf. Westall, 1950). The diffusate of the juice could be applied to the column without preliminary concentration and much anionic and neutral material removed by washing with water; on displacement with ammonia the substance emerged early from the column, contaminated chiefly with aspartic and glutamic acids, serine, threonine and glutamine. It was readily separated from these amino acids by partition chromatography with phenol-water. The product crystallized readily, and represented (best yield) 4.4% of the N of the cabbage diffusate (non-

protein N), 2.2% of the total N of the cabbage and 0.57% of the dry matter of the original cabbage.

The new substance had the elementary composition $C_4H_9O_3NS$. As we thought that we were dealing with a peptide, we were surprised to find that all the nitrogen reacted under the Van Slyke ninhydrin- CO_2 and nitrous acid procedures. Electro-metric titration confirmed the presence of equivalent amounts of ionizing groups having pK values 1.7 and 8.0 approx. (Fig. 1). Accordingly, it seemed that we were dealing with an α -amino monocarboxylic acid. The fact that its optical rotation was more positive in acidic than in neutral solution strongly suggested the *L* configuration at the α -C atom (see Neuberger, 1948). Paper chromatography showed that under the usual conditions of acid hydrolysis of proteins the compound was completely destroyed, giving a single spot at the valine position (*B*, Fig. 2). However, the new amino acid formed accounted for only 40–50% of the original nitrogen (ninhydrin- CO_2 reaction). The rest of the nitrogen was determinable as NH_3 . Some CO_2 was formed, suggestive of the formation of a keto acid (see below). We soon found that the resulting amino acid was not valine, but that it agreed in chromatographic behaviour both before and after various oxidative procedures (see below) with *S*-methylcysteine. In particular, oxidation with hydrogen peroxide caused re-formation of what seemed to be the compound originally present in the cabbage.

The simplest hypothesis was therefore that the new compound is a sulfoxide of *S*-methylcysteine, i.e. $CH_3.SO.CH_2.CH(NH_2).CO_2H$. Confirmation for this view was obtained, following Stoll & Seebeck (1948), by reduction with metabisulphite at room temperature. The reaction was allowed to proceed until there was no further change in optical rotation. *S*-Methyl-L-cysteine was isolated from the reaction mixture, which further confirmed the *L* configuration at the α -C atom.

We then undertook synthesis. Although we have found in the literature two reports of preparation of sulfoxides from *S*-methyl-L-cysteine by oxidation with hydrogen peroxide (Bentley, McDermott & Whitehead, 1951; Yurugi, 1954), no attempt to resolve the diastereoisomers resulting from the asymmetry introduced at the *S* atom seems to have been recorded. Lavine (1947) used fractional crystallization of picrates to resolve the diastereoisomeric sulfoxides of methionine; Stoll & Seebeck (1951*a*) resolved those of *S*-allylcysteine and *S*-propylcysteine by direct crystallization.

Following Stoll & Seebeck (1951*a*) we oxidized *S*-methyl-L-cysteine with aqueous hydrogen peroxide at room temperature and obtained a largely crystalline product. Fractional crystallization of this either directly or as picrate (after addition of

$\frac{1}{2}$ – $\frac{3}{4}$ mol.prop. of picric acid) led indeed to fractions having optical rotations greater and less than that of the starting material, but it was soon clear that, at best, much wasteful recrystallization would be necessary before the rotation of the natural material could be approached. Filter-paper chromatography with collidine–water showed some resolution, but was not very encouraging.

Accordingly, we tried resolution of the mixture by chromatography on a cation-exchange resin, in view of the results of Moore & Stein (1954*a*) with the diastereoisomeric sulfoxides of methionine. This was successful. The form of the elution curves suggested three zones (Fig. 3), of which the first two (I) were imperfectly resolved, and gave a poorly crystallizing product having strong negative rotation. The third zone (II) gave a well crystallizing product having the same optical rotation, both in water and in acid, as the natural material. Identity was further confirmed by elementary analysis and, conclusively, by a crystallographic comparison by Mr R. Hine and Dr D. Rogers, whose report is given in the Experimental section. The chromatographic results strongly suggest that the action of hydrogen peroxide on *S*-methylcysteine produces, besides the expected diastereoisomeric sulfoxides, a third substance also giving a colour with ninhydrin and closely similar to the (–)-sulfoxide in chromatographic behaviour. This might be homologous with the intermediate ‘dehydromethionine’ formed during the oxidation of methionine (Lavine, 1945, 1949). (+)-(S-Methyl-L-cysteine *S*-oxide) (MCS), on oxidation with hydrogen peroxide in presence of molybdate followed by paper chromatography (cf. Dent, 1948), gave a new spot in position *C* (Fig. 2). We assume this to be the sulphone; the relative positions of the spots in Fig. 2 are clearly analogous with those for the corresponding derivatives of methionine (Dent, 1948). However, *S*-methylcysteine and MCS are only very incompletely oxidized to sulphone by peroxide–molybdate, and mostly sulfoxide remains. MCS thus seems to be more stable to this reagent than the corresponding methionine derivative. With bromine water it is quite unstable; MCS readily consumed 3–4 atoms of bromine per molecule; paper chromatography of the reaction product showed complete destruction, with no formation of the sulphone or of any other amino acid.

MCS seems, judged by the optical rotation, to be stable to $N-HCl$ at room temperature. Hot HCl , as stated above, gives *S*-methylcysteine, ammonia, some CO_2 and an unpleasant cabbage-like odour. Vilkki (1954) noted a similar instability to hot acid of an amino acid isolated by him from onion and thought to be alliine. Among the decomposition products he recognized about 0.9 mol.prop. as cyst(e)ine and about 0.1 mol.prop. as equimolar

amounts of ammonia and pyruvic acid (see also Challenger & Hollingworth, 1954; Osono, Mukai & Tominaga, 1955). MCS does not seem to be stable to alkali at room temperature, judged by the irreversibility of the potentiometric titration with alkali. With hydriodic acid under the Zeisel conditions, Dr Cornforth reported only 0.3 mol.prop. of methyl iodide. Much further work is required to throw light on the reactivity of sulfoxide groups and their effects on the stability of amino acids when incorporated at different positions in the molecule.

It appears from the ionophoretic and potentiometric results now reported that the carboxyl group of MCS is somewhat stronger than that of ordinary α -amino acids. The same is presumably also true for the sulfoxides of methionine, and explains the early emergence from columns of cation-exchange resin. However, the resolution of diastereoisomeric sulfoxides on such columns is not explained by differences of pK of their carboxyl groups, and presumably depends on some other aspect of their interaction with the resin, as our attempts to resolve the mixed diastereoisomeric sulfoxides of *S*-methyl-L-cysteine by paper ionophoresis at low pH showed no differences of behaviour.

Our experiences with MCS in cabbage should serve as an object lesson against the common practice of describing indiscriminately as 'peptides' substances which give ninhydrin-staining spots on paper chromatograms and which disappear and are replaced by new spots when the material has been subjected to acid hydrolysis.

Yurugi (1954) obtained circumstantial evidence for the occurrence of *S*-methylcysteine *S*-oxide in *Allium* spp. (see below). Substance '23', isolated by Westall (1955) from human urine, had similar properties to MCS except that it failed to crystallize and its conversion into the brown spot '6A' by peroxide-molybdate was complete. On this basis '6' and '6A' could be *S*-methylcysteine and the sulphone respectively.

Several other sulfoxides have already been recognized in nature. Of these, the only well-authenticated amino acid is alliin, (+)-(*S*-allyl-L-cysteine *S*-oxide), which occurs free in garlic. Work with alliin has been reviewed by Stoll & Seebeck (1951*b*; see also Stoll & Jucker, 1955; Yurugi, 1954). There have been numerous reports of the natural occurrence of methionine sulfoxide, but these have been mostly based on paper chromatography, and no isolation or stereoisomeric identification seems to be on record. Schmid & Karrer (1948*a, b, c*) have isolated the sulfoxide-isothiocyanate 'sulphoraphene' and the corresponding nitrile from radish seeds (see also Kjaer & Gmelin, 1955). Zwergal (1951) isolated the next higher homologous sulfoxide-nitrile from kohlrabi. These compounds

have obvious relations with the sulphone-isothiocyanates cheirolin and erysolin, also found in cruciferous plants. Reichstein (1936; cf. Reichstein & Goldschmidt, 1936) isolated bis-(β -hydroxyethyl) sulfoxide from adrenal glands, but this may have been an artifact of the alkaline hydrolysis used in the isolation. Of the sulfoxides of biotin, the (+)-sulfoxide was isolated by Melville (1954; cf. Melville, Genghof & Lee, 1954) from milk, although he did not demonstrate the absence from the milk of the (-)-sulfoxide. Both isomers were formed by oxidation of biotin with hydrogen peroxide. The (-)-sulfoxide has been found in culture filtrates of *Aspergillus niger* from which the (+)-sulfoxide was shown to be absent (Wright & Cresson, 1954; Wright, Cresson, Valiant, Wolf & Folkers, 1954; Wright & Driscoll, 1954).

Since in several of these natural sulfoxides the S atom is a centre of asymmetry, it is important that their configurations should be established. This has not yet been done for any sulfoxide, synthetic or natural. Mr Hine and Dr Rogers are now undertaking an X-ray structural analysis of MCS which, if successful, should relate the configuration at the S atom with that at the α -C atom, which is known, thus superseding the proposal of Schmid & Karrer (1948*c*) to use sulphoraphene as the configurational reference compound for asymmetric sulfoxides.

We have examined a number of other cruciferous plants for the possible presence of MCS, but have found evidence for it only in some (see Table 1). Cruciferous vegetables are well known to contain much organic sulphur (e.g. Dahlen, 1874; Salkowski, 1914*b*); using Dahlen's figures for cabbage (0.41–0.48 % organic S on dry matter) we can account for about a quarter of this as MCS.

As to the metabolic role of MCS in the plant, one may speculate on its relationship to the sulphonium compound *S*-methylmethionine found, in much smaller amount, in cabbage by McRorie *et al.* (1954), and in asparagus by Challenger & Hayward (1954). MCS may likewise give rise to methanethiol and related volatile compounds commonly described as having the odour of decaying cabbage. The evolution of methanethiol and H_2S on cooking some cruciferous vegetables was reported by Niemann (1893) (cf. Rubner, 1893). Rubner gives much information on the occurrence and properties of mercaptans which has escaped notice in Beilstein's *Handbuch*. Nakamura (1925) described the isolation of methanethiol from roots of *Raphanus sativus*. Zwergal (1951) obtained H_2S and mercaptan by steam distillation of kohlrabi. MCS could conceivably also act as precursor for the CH_3 -S group in sulphoraphene and related compounds and for the $=C=S$ moiety of the isothiocyanate radical in mustard oils, which may be formed by addition of such a group

to decarboxylated amino acids (cf. Barger & Coyne, 1928; Kjaer, 1954). Yurugi (1954) and Yurugi, Matsuoka & Togashi (1954) have investigated the reaction of alline and similar compounds with thiamine, to form allithiamine, etc. This reaction is catalysed by an enzyme present in garlic. Their enzyme preparations gave the methyl analogue of allithiamine when *S*-methylcysteine *S*-oxide and thiamine interacted in its presence. As the same compound was formed when crude preparations from numerous *Allium* spp. acted on thiamine, it can be inferred that MCS as well as alline was present in these.

Any relationship of MCS to the antibiotic or antithyroid properties of cabbage remains to be investigated.

Salkowski (1914*a, b*; 1917) noticed abnormal occurrence of thiosulphate in the urine of rabbits fed exclusively on cabbage. He showed that the precursor could be extracted from the cabbage with boiling water, and was destroyed by hot hydrochloric acid, so it could well have been MCS. MCS might also be the precursor of mercaptan found in the urine after cruciferous vegetables had been eaten (Rubner, 1893). Salkowski (1914*a*) found that mercaptan distillable from the urine of his cabbage-fed rabbits appeared, along with S and H₂S, only after the urine had been heated with hydrochloric acid. This may indicate that MCS was present in the urine as such.

A preliminary account of some of this work was communicated to the Biochemical Society (Synge & Wood, 1955). Morris & Thompson (1955) published a note on the isolation from turnip of MCS, with parallel evidence in support of the proposed structure.

EXPERIMENTAL

General methods

Unless otherwise stated, N was determined by the micro-Kjeldahl procedure, C and H by Dipl. Chem. Pascher, Bonn, and S at the microanalytical laboratory of the National Institute for Medical Research. All evaporations were done *in vacuo* below 40°. Analyses were on samples dried to constant wt. over H₂SO₄ and soda-lime *in vacuo* at room temp. Optical rotations were measured in a 0.5 dm. tube. Two-dimensional filter-paper chromatography of amino acids was as described by Synge (1951), except that treatment with H₂O₂ was omitted when not specified here.

Isolation of MCS from cabbage

Isolations have been made from cabbages acquired from different sources at different times of year with essentially similar results. We give here details of our largest preparation, done when we had most experience and got highest yield.

Three freshly picked large cabbage heads grown on the Duthie Experimental Stock Farm of this Institute (29 November 1954) weighed 5 kg. They were chopped,

treated with ether and pressed (cf. Synge, 1951) to give 1.72 l. of pale-yellow juice containing 1.92 g. N. This was dialysed in cellophane against 10 l. of distilled water (saturated with CHCl₃) for 5 days at 0–2°. The diffusate (9.6 l.) contained 1.50 g. N. The original cabbage had total N, 2.4 % of dry matter. The fresh cabbage contained 7.5 % of dry matter (drying to const. wt. at 105°). Assuming even distribution of diffusible N between bag contents and diffusate, 95 % of the N expressed in the ether–water treatment was diffusible and, if the water remaining in the press cake contained the same concentration of diffusible N as the expressed juice, diffusible N represented about 50 % of the total N of the cabbage.

On diffusate from a different cabbage sample, carboxyl-N determinations had corresponded to 62 % of the diffusible N, rising to 74 % after hydrolysis with 6*N*-HCl for 24 hr. at 105° (procedure of Synge, 1951).

Three cylindrical columns (each 38 cm. × 5.4 cm.) were packed with Zeo-Karb 215 (Permutit Co. Ltd., London, W.4) cation-exchange resin (H⁺ form) in water, and coupled in series with short lengths of narrow tubing (cf. Westall, 1950). Diffusate (9.5 l.) was passed through the columns, followed by water (4 l.). The effluent was discarded. Aqueous NH₃ (0.15*N*) was then used for development, and the effluent was tested at intervals for colour on heating with ninhydrin. Colour was noted as soon as a light-brown zone reached the bottom of the chromatogram. Two further columns of fresh resin (9 cm. × 1.2 cm. and 7 cm. × 0.8 cm. respectively) were then connected in series to the bottom of the third column and development with NH₃ was continued, the effluent being collected in 20 ml. fractions as soon as it was ninhydrin-positive. Paper chromatography on small samples showed in fraction 1, aspartic acid; 2–6, aspartic acid, glutamic acid, MCS, serine; 7–19, same as 2–6 with threonine and glutamine; 20–60, threonine, glutamine, asparagine, valine, proline, leucine, etc. Fractions 2–19 were pooled and weighed 2.5 g. after evaporation to dryness.

Kieselguhr (Hyflo-Supercel, Johns-Manville Co., London) (90 g.) was well mixed with 60 ml. of the aqueous phase of the system phenol–water (prepared from redistilled A.R. phenol), made to a slurry with phenol-rich phase and packed in a chromatographic column (5.4 cm. diameter) with the procedure and special tool of Howard & Martin (1950). The column was washed with 300 ml. of phenol-rich phase, which removed some yellow material from the kieselguhr. The fraction from the Zeo-Karb column was then transferred (15 ml. of phenol-rich phase being used in all) to the kieselguhr column; development proceeded with phenol-rich phase in a cool room (15°) without special temperature control. The effluent became ninhydrin-positive after 265 ml. had emerged (from beginning of placing amino acids on column). Fractions (3 ml.) were then collected, of which, on paper chromatography of small samples, the first ten showed only MCS; the next six showed traces of MCS, also glutamine; the next five, glutamine only. The first ten fractions were pooled and evaporated to dryness, water being added repeatedly, so that aqueous phase was always present during the evaporation. Yield, 706 mg. of pale-pink crystals. This residue was dissolved in water and decolorized with 50 mg. of charcoal. The charcoal was filtered off and washed well with water. Filtrate and washings were evaporated to small volume, and brought to crystallization by addition of ethanol at 0°. The resulting crystals of MCS were filtered off, washing with cold ethanol (yield, 658 mg.).

Properties of MCS isolated from cabbage

MCS began to turn brown above 140° and had m.p. (uncorr.) 167–168° (decomp. with much browning and frothing); $[\alpha]_D^{21} + 118^\circ$ in water (*c*, 1.5); $[\alpha]_D^{24} + 168^\circ$ in *N*-HCl (*c*, 1.5). (Found: C, 32.1; H, 6.0; N, 9.3; S, 21.4%. $C_4H_5O_2NS$ requires C, 31.8; H, 6.0; N, 9.3; S, 21.2%.) In the Van Slyke amino-N determination (nitrite, 4 min. reaction time) 96% of the N reacted as amino N at 18°. In the ninhydrin- CO_2 determination (Van Slyke, Dillon, MacFadyen & Hamilton, 1941; modification of Synge, 1951; pH 4.7, reaction vol. 3 ml.) 92% of the N reacted as carboxyl N. All our preparations had a faint but characteristic odour of cabbage.

Potentiometric titration. This is plotted in Fig. 1 and shows agreement with the theoretical curve for a compound having two ionizing groups (*pK* 1.7 and 8.0 respectively) in the formula weight 151. There was irreversible consumption of alkali at the higher pH values, suggesting decomposition.

Ionic mobility at different pH values. This was studied by filter-paper ionophoresis in 0.16*M* acetic acid (pH 3 approx.) and in 0.04*M* sodium phosphate buffer (pH 7.1). Technical details were as given by Ellfolk & Synge (1955). *N*-2,4-Dinitrophenylethanolamine was used as marker of electro-endosmosis (cf. Mould & Synge, 1954). In the phosphate buffer (10 v/cm. for 4 hr.) 100 μ g. of DL-alanine had not migrated, whereas 100 μ g. of MCS had migrated 3.5 cm. towards the anode relative to the marker. In the acetic acid (20 v/cm. for 4 hr.) the alanine spot had migrated 10 cm. and the MCS spot 6 cm. towards the cathode relative to the marker. The amino acids were detected by spraying with ninhydrin.

Filter-paper chromatography. MCS gave a single spot at position *A* (Fig. 2), giving a rather pinker colour than the common amino acids with ninhydrin on papers imperfectly dried after the 'collidine' run.

Behaviour on heating in acid. MCS (5 mg.) was heated for 24 hr. in 1 ml. of 6*N*-HCl in a sealed evacuated tube at 105°. CO_2 was determined on opening the tube after connecting it to the Van Slyke apparatus (cf. Synge, 1951), and amounted to 0.066 mol./atom of N taken. The solution smelt strongly of foul cabbage. It was evaporated repeatedly to dryness with additions of water. The residue was dissolved in water and samples were analysed. (a) The ninhydrin- CO_2 reaction (as above) gave carboxyl N, 42% of N taken. (b) By the Conway microdiffusion method (cf. Synge, 1951) was found NH_3 -N, 58% of N taken. (c) Filter-paper chromatography showed a single spot at position *B* (Fig. 2), coinciding on a mixed chromatogram with authentic *S*-methyl-L-cysteine (see below). The material also behaved in the same way as *S*-methyl-L-cysteine under the treatments with H_2O_2 described in the following paragraph.

Behaviour of MCS and *S*-methyl-L-cysteine with oxidizing agents. (a) *Hydrogen peroxide alone.* Acting on *S*-methyl-L-cysteine under the conditions described below for synthesis of MCS, H_2O_2 completely destroyed the starting material and gave a product which on paper chromatography gave a spot in position *A* (Fig. 2) coinciding on mixed chromatograms with MCS but, unlike MCS, showing a tendency to doubling in the collidine direction.

(b) *Hydrogen peroxide with molybdate.* MCS or *S*-methyl-L-cysteine (0.2 mg.) was dissolved in 20 μ l. of 0.02% (w/v) ammonium molybdate; 10 μ l. of 30% (w/v) H_2O_2 was added

and the solution kept at room temperature for 1 hr. Subsequent paper chromatography gave closely similar results with either substance—a strong spot at position *A* and a weaker brownish pink spot at position *C* (Fig. 2). The latter was judged to be the sulphone.

(c) *Bromine water.* MCS (3.05 mg.) was dissolved in 2 ml. of 0.0119*N*-HCl, 2 ml. of 1% (w/v) aqueous Br_2 was added, the flask stoppered and the mixture kept for 50 min. at 20°. The first drops of Br_2 solution were decolorized immediately. A blank experiment was done side by side. 40% (w/v) aqueous KI (1 ml.) was added to both flasks. Titration with thiosulphate indicated that the MCS had consumed 0.0662 mg. equiv. of bromine, i.e. 3.28 atoms of Br/mol. of MCS. A sample of MCS treated with Br_2 under the same conditions and then evaporated to dryness showed no ninhydrin-staining spots on filter-paper chromatography.

Fig. 1. Potentiometric titration. MCS (24.18 mg.) was dissolved in 5 ml. of water and titrated at 14–17° with exclusion of CO_2 by means of a glass electrode connected to a Cambridge pH meter (calomel reference electrode), which had been calibrated with standard buffers at pH 4.0 and 9.2 (checked at end of run). The consumption of acid and alkali is plotted after subtracting the amounts required to attain same pH in a blank titration on 5 ml. of water. ●, Initial titration with 0.116*N*- H_2SO_4 to pH 2.1; ○, back titration with 0.126*N*-NaOH to pH 12 (at this pH the soln. was faintly cloudy—mixture kept for 30 min.); □, titration with 0.116*N*- H_2SO_4 from pH 12 to pH 6. Curve is theoretical for amphotyle having equiv.wt. 151, *pK* values 1.7 and 8.0.

Fig. 2. R_F values on two-dimensional paper chromatograms (see text). *A*, MCS; *B*, *S*-methylcysteine; *C*, supposed *S*-dioxide of *S*-methylcysteine. Positions of some common amino acids are included for reference.

Reduction to S-methyl-L-cysteine of MCS from cabbage. MCS (50 mg.) and sodium metabisulphite (100 mg.) were dissolved in 0.5 ml. of air-free distilled water and transferred to a polarimeter tube. The solution had initially a strong positive rotation which fell, settling in the course of 3 weeks to a small negative value. The solution was then washed out of the tube with water, 0.15 ml. of 10N-HCl was added, and the mixture evaporated to dryness. The residue was extracted with three portions of hot ethanol, and the combined filtered extracts were evaporated to dryness. This residue was dissolved in 0.5 ml. of water, 75 μ l. of pyridine was added, and the mixture brought to crystallization by partial evaporation with additions of ethanol. Yield, 19 mg. of colourless crystals. Chromatography with and without admixture of authentic S-methyl-L-cysteine gave a single spot at position B (Fig. 2). Combustion revealed 8.5% of ash. This was assumed, from the manner of preparation, to be due to NaCl, and the following analytical data and optical rotation have been corrected by making allowance for the original presence of 11% of salt. [Found: C, 35.6; H, 6.7; N (Dumas, by Pascher), 10.3; S (by Pascher), 22.9%. Calc. for $C_4H_9O_2NS$: C, 35.6; H, 6.7; N, 10.4; S, 23.7%.] $[\alpha]_D^{27} - 34^\circ$ in water (c, 0.8). du Vigneaud, Loring & Craft (1934) record for S-methyl-L-cysteine $[\alpha]_D^{27} - 31.2^\circ$ and -32.0° in water (c, 1).

Synthesis and resolution of the S-oxides of S-methyl-L-cysteine: comparison of synthetic and natural MCS

S-Methyl-L-cysteine was prepared from L-cysteine by the Na-NH₃-methyl iodide procedure of du Vigneaud *et al.* (1934) and had $[\alpha]_D^{23} - 32^\circ$ in water (c, 1.0). This product (0.63 g.) was dissolved in 7.7 ml. of water, 0.55 ml. of 30% (w/v) H₂O₂ was added and the mixture kept 24 hr. at room temp. with occasional shaking. The solution was then evaporated to dryness. The colourless, largely crystalline residue had $[\alpha]_D^{19} - 16^\circ$ in water (c, 1.0), and was freely soluble in water. It showed in all cases the same behaviour as described above for MCS from cabbage on filter-paper ionophoresis, filter-paper chromatography, heating with acid and treatment with oxidizing agents, except for the tendency to give a double spot on filter-paper chromatography with collidine (see above).

Resolution of the mixture was effected chromatographically on Dowex 50-X4 as processed to AG50-X4 (200-400 mesh/in. H⁺ form) by Bio-Rad Laboratories, 800 Delaware, Berkeley, California. The general procedure of Moore & Stein (1954a) was followed. After the resin had been washed with HCl and NaOH as described, it was made into a column (90 cm. $\times$ 2 cm.) and equilibrated with the developing sodium citrate buffer, made up as for the pH 2.2 buffer of Moore & Stein (1954a, Table 1), except that 85 ml. of HCl was used instead of 80 ml. and the final volume was 6.65 l. instead of 5 l. (pH 2.13). When the pH of the effluent had reached that of the inflowing buffer, 75 mg. of the sulfoxide mixture dissolved in 5 ml. of 0.1N-HCl was placed on the column and development started at 20 ml./hr. No special temperature control was used; 5 ml. fractions were collected. One-tenth of each fraction was used for the ninhydrin spectrophotometric determination as described by Moore & Stein (1954b), except that an EEL portable colorimeter with 570 m μ . filter (Evans Electroselenium Ltd, Harlow, Essex) was used. Fig. 3 shows the results. The form and relative breadth of the peaks was the same in five different experiments; it seems that at least two different

Fig. 3. Chromatography on Dowex 50-X4 of mixture obtained by oxidizing S-methyl-L-cysteine with aqueous hydrogen peroxide (eluting buffer, pH 2.13). For details see text.

components must be present in the first peak. The remainders of the fractions comprising peak I (1260-1520 ml.) and peak II (1530-1620 ml.) were pooled separately. The amino compounds present were freed from buffer constituents by passing the solution through a column of Zeo-Karb 215 (H⁺ form) (30 g./100 ml. buffer), washing with water till the effluent had pH 4 and displacing the amino compound with 0.15N-NH₃, as in isolating MCS from cabbage. The minimum necessary NH₃ was allowed to emerge and the solution was evaporated without delay. Nevertheless, slight discoloration and contamination resulted from having the amino acid in contact with Zeo-Karb 215 in an ammoniacal environment, and some alternative desalting procedure would be preferable. Peak I yielded 30 mg. of crystals embedded in syrup, $[\alpha]_D^{22} - 79^\circ$ in water (c, 1.0). This fraction was not further studied. Peak II yielded 36 mg. of highly crystalline product: $[\alpha]_D^{26} + 118^\circ$ in water (c, 1.0); $[\alpha]_D^{20} + 172^\circ$ in N-HCl (c, 1.0). In the HCl, the specific rotation fell within 2 hr. to $+148^\circ$, at which value it remained constant for 24 hr. We attribute this change to reaction with traces of reducing material from the buffer constituents or resins. [Found: C, 31.9; H, 6.0; N, 9.4; S, 21.0% (dried at 56° *in vacuo*). $C_4H_9O_2NS$ requires C, 31.8; H, 6.0; N, 9.3; S, 21.2%.]

In addition to the comparisons already described the synthetic (+)-(S-methyl-L-cysteine S-oxide) was further compared with the material from cabbage as follows. The synthetic and natural products behaved identically on two-dimensional paper chromatograms individually and in mixture. The synthetic product had m.p. 167-168° (decomp.), not altered by admixture of the natural product. Synthetic and natural products were recrystallized by slow addition of ethanol to concentrated aqueous solutions, and the crystals submitted to Mr R. Hine and Dr D. Rogers of the Department of Physics, University College, Cardiff, who reported as follows (9 January 1956):

'Single crystals of both the natural and the synthetic materials were photographed with X-rays using stationary-film and Weissenberg techniques. The dimensions and intensities of the reciprocal lattices were found to be identical and, as the synthetic material had already been resolved by Synge & Wood to give the form corresponding optically with the natural material, the identity of the products is established.

'The material crystallizes in clusters of very thin irregular plates or laths which exhibit parallel extinction, the long axis (a) of the laths being slow.

Table 1. *Distribution of S-methylcysteine S-oxide in some cruciferous plants*

With the exception of cabbage, cauliflower and kale (where the heads were used) the whole plant was taken for analyses.

Species	Common name	Date and place of obtaining*	Result
<i>Alyssum saxatile</i> L.	Golden tuft	18. viii. 55 B	-
<i>Arabis albida</i> Stev.		18. viii. 55 B	-
<i>Aubrieta deltoidea</i> (L.) DC.		18. viii. 55 B	-
<i>Barbarea vulgaris</i> R.Br.	Winter cress or yellow rocket	18. viii. 55 B	-
<i>Brassica campestris</i> L.	Turnip	20. ix. 55 D	+
<i>B. oleracea</i> L.	Cabbage	See text	+
	Cauliflower	8. vi. 55 P	+
	Kale	20. ix. 55 D	+
<i>Capsella bursa-pastoris</i> L.	Shepherd's purse	7. vi. 55 B	+
<i>Cardamine hirsuta</i> L.	Hairy bitter cress	18. viii. 55 B	-
<i>Cheiranthus cheiri</i> L.	Wallflower	7. vi. 55 B	+
<i>Cochlearia officinalis</i> L.	Scurvy grass	19. vi. 55 E	-
<i>Hesperis matronalis</i> L.	Dame's violet	18. viii. 55 B	-
<i>Iberis sempervirens</i> L.	Candytuft	7. vi. 55 B	-
<i>Lepidium sativum</i> L.	Cress	24. viii. 55 P	-
<i>Lunaria annua</i> L.	Honesty	18. viii. 55 B	-
<i>Matthiola incana</i> (L.) R.Br.	Stock	24. viii. 55 B	-
<i>Nasturtium officinale</i> R.Br.	Water cress	25. viii. 55 P	-
<i>Raphanus sativus</i> L.	Radish	30. viii. 55 P	-
<i>Sinapis alba</i> L.	White mustard	18. viii. 55 B	+
<i>Sisymbrium officinale</i> (L.) Scop.	Hedge mustard	19. viii. 55 B	-

* B, Botanic Garden, University of Aberdeen; D, Duthie Experimental Stock Farm, Rowett Institute; E, Estuary of River Dee, Aberdeen; P, purchased commercially. +, MCS detected; -, MCS absent (see text).

'The crystals are orthorhombic with a unit cell having the dimensions: $a = 5.2 \pm 0.05 \text{ \AA}$; $b = 7.4 \pm 0.1 \text{ \AA}$; $c = 16.3 \pm 0.1 \text{ \AA}$.

'The systematically absent spectra indicate uniquely the space group $P2_12_12_1$ and, assuming 4 molecules per unit cell, the calculated density is 1.54 g.cm.^{-3} (obs. 1.56 g.cm.^{-3}).

Tests for MCS in cruciferous plants

Juice expressed after ether treatment was placed in the specimen compartment of a four-compartment diaphragm cell (Synge, 1951) and desalting was effected at pH 6 until current had fallen to 10 mA; desalting was not continued for long enough to remove much aspartic or glutamic acid from the specimen compartment. An amount of desalted juice corresponding to 200 $\mu\text{g.}$ of N in the original juice was subjected to two-dimensional filter-paper chromatography. To a duplicate sample MCS ($1.85 \mu\text{g.}$ of N) was added as a control. The results are given in Table 1. In every case where MCS is noted as absent from the original juice, a new spot was easily seen at position A (Fig. 2) in the control. The negative results therefore indicate that considerably less than 1% of the N of the juice (predominantly non-protein N) was MCS. Positive results cannot be taken as conclusive evidence for the presence of MCS, as many other amino compounds are known to occupy similar positions on the chromatograms.

SUMMARY

1. A new amino acid has been isolated from cabbage. It occurs free, accounting for more than 4% of the non-protein N. Chemical study indicated that it is one of the diastereoisomeric sulfoxides of *S*-methyl-L-cysteine.

2. (+)-(*S*-Methyl-L-cysteine *S*-oxide) (MCS) was isolated by ion-exchange chromatography from the reaction products of *S*-methyl-L-cysteine with

hydrogen peroxide, and shown to be identical with the amino acid from cabbage. The identification included a preliminary crystallographic study by Mr R. Hine and Dr D. Rogers, who are embarking on structural studies aimed at determining the absolute configuration at the sulphur atom.

3. The behaviour of MCS with oxidizing agents and under the conditions of acid hydrolysis of proteins showed some unexpected features.

4. A number of cruciferous plants have been examined for possible occurrence of MCS.

5. Relationships of MCS with other naturally occurring S compounds are discussed. Reference is made to nineteenth-century data on occurrence and properties of mercaptans which have escaped notice in Beilstein's *Handbuch*.

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REFERENCES

- Barger, G. & Coyne, F. P. (1928). *Biochem. J.* **22**, 1417.
 Bentley, H. R., McDermott, E. E. & Whitehead, J. K. (1951). *Proc. Roy. Soc. B*, **138**, 265.
 Challenger, F. & Hayward, B. J. (1954). *Chem. & Ind.* p. 729.
 Challenger, F. & Hollingworth, H. D. (1954). *Chem. & Ind.* p. 463.
 Dahlen, H. W. (1874). *Landw. Jb.* **3**, 321, 723.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.

- du Vigneaud, V., Loring, H. S. & Craft, H. A. (1934). *J. biol. Chem.* **105**, 481.
- Ellfolk, N. & Synge, R. L. M. (1955). *Biochem. J.* **59**, 523.
- Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
- Kjaer, A. (1954). *Acta chem. scand.* **8**, 1110.
- Kjaer, A. & Gmelin, R. (1955). *Acta chem. scand.* **9**, 542.
- Lavine, T. F. (1945). *Fed. Proc.* **4**, 96.
- Lavine, T. F. (1947). *J. biol. Chem.* **169**, 477.
- Lavine, T. F. (1949). U.S. Pat. 2,465,461.
- McRorie, R. A., Sutherland, G. L., Lewis, M. S., Barton, A. D., Glazener, M. R. & Shive, W. (1954). *J. Amer. chem. Soc.* **76**, 115.
- Melville, D. B. (1954). *J. biol. Chem.* **208**, 495.
- Melville, D. B., Genghof, D. S. & Lee, J. M. (1954). *J. biol. Chem.* **208**, 503.
- Moore, S. & Stein, W. H. (1954a). *J. biol. Chem.* **211**, 893.
- Moore, S. & Stein, W. H. (1954b). *J. biol. Chem.* **211**, 907.
- Morris, C. J. & Thompson, J. F. (1955). *Chem. & Ind.* p. 951.
- Mould, D. L. & Synge, R. L. M. (1954). *Biochem. J.* **58**, 585.
- Nakamura, N. (1925). *Biochem. Z.* **164**, 31.
- Neuberger, A. (1948). *Advanc. Protein Chem.* **4**, 296.
- Niemann, F. (1893). *Arch. Hyg., Berl.*, **19**, 126.
- Osono, K., Mukai, I. & Tominaga, F. (1955). *Nagasaki Igakkai Zasshi*, **30**, 156. [Through *Chem. Abstr.* (1955). **49**, 8344].
- Reichstein, T. (1936). *Helv. chim. acta*, **19**, 29.
- Reichstein, T. & Goldschmidt, A. (1936). *Helv. chim. acta*, **19**, 401.
- Rubner, M. (1893). *Arch. Hyg., Berl.*, **19**, 136.
- Salkowski, E. (1914a). *Hoppe-Seyl. Z.* **89**, 485.
- Salkowski, E. (1914b). *Hoppe-Seyl. Z.* **92**, 89.
- Salkowski, E. (1917). *Biochem. Z.* **79**, 68.
- Schmid, H. & Karrer, P. (1948a). *Helv. chim. acta*, **31**, 1017.
- Schmid, H. & Karrer, P. (1948b). *Helv. chim. acta*, **31**, 1087.
- Schmid, H. & Karrer, P. (1948c). *Helv. chim. acta*, **31**, 1497.
- Stoll, A. & Jucker, E. (1955). *Modern Methods of Plant Analysis*, vol. 4, p. 689. Ed. by Paech, K. & Tracey, M. V. Berlin: Springer.
- Stoll, A. & Seebeck, E. (1948). *Helv. chim. acta*, **31**, 189.
- Stoll, A. & Seebeck, E. (1951a). *Helv. chim. acta*, **34**, 481.
- Stoll, A. & Seebeck, E. (1951b). *Advanc. Enzymol.* **11**, 377.
- Synge, R. L. M. (1951). *Biochem. J.* **49**, 642.
- Synge, R. L. M. & Wood, J. C. (1954). *Biochem. J.* **56**, xix.
- Synge, R. L. M. & Wood, J. C. (1955). *Biochem. J.* **60**, xv.
- Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A. & Hamilton, P. (1941). *J. biol. Chem.* **141**, 627.
- Vilki, P. (1954). *Acta chem. fenn. (Suomen Kemistilehti)*, **27B**, 21.
- Westall, R. G. (1950). *J. Sci. Fd Agric.* **1**, 191.
- Westall, R. G. (1955). *Biochem. J.* **60**, 247.
- Wright, L. D. & Cresson, E. L. (1954). *J. Amer. chem. Soc.* **76**, 4156.
- Wright, L. D., Cresson, E. L., Valiant, J., Wolf, D. E. & Folkers, K. (1954). *J. Amer. chem. Soc.* **76**, 4160, 4163.
- Wright, L. D. & Driscoll, C. A. (1954). *J. Amer. chem. Soc.* **76**, 4999.
- Yurugi, S. (1954). *J. pharm. Soc. Japan*, **74**, 502, 506, 511, 514, 519. [Through English summaries and *Chem. Abstr.* (1955). **49**, 8300-8302.]
- Yurugi, S., Matsuoka, T. & Togashi, M. (1954). *J. pharm. Soc. Japan*, **74**, 1017. [Through *Chem. Abstr.* (1955). **49**, 2032.]
- Zwergal, A. (1951). *Die Pharmazie*, **6**, 245.

The Separation, Micro-Estimation and Distribution of the Alkaloids of Hemlock (*Conium maculatum* L.)

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From the tissues of *Conium maculatum* the alkaloids coniine (I), *N*-methylconiine, γ -coniceine (II), conhydrine (III) and pseudoconhydrine (IV) have been isolated (Henry, 1949; Marion, 1950; Cromwell, 1955).

The alkaloids (III) and (IV) are present in very small amount and were first isolated from the residues from the separation of the major alkaloids of the group.

Methods for large-scale isolation and separation of the alkaloids have been described by Wolfenstein (1895) and Braun (1905). Farr & Wright (1904) have described titrimetric and gravimetric methods for the estimation of the total alkaloids (expressed as coniine) of hemlock tissues, and Madaus & Schindler (1938) have estimated the total alkaloid content of tissues during the growth and reproductive phases of the plant. Methods for the estimation of the individual alkaloids of the group do

not appear to have been described; the object of the present work was to develop methods for the separation and micro-estimation of each alkaloid of the group, and to use these methods for a study of the distribution of the alkaloids in the plant during the phases of growth and reproduction.