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Note

Thin-layer chromatography of S-methylcysteine sulphoxide (kale anaemia factor) in the presence of common amino acids

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Brassicas, and especially kales, are a potentially valuable source of green winter feed for ruminant animals, but their use is limited by the incidence of kale poisoning, a severe anaemia which has been reported after feeding with rape, cabbage, brussel sprouts, swedes and kale[1–4]. The amino acid S-methylcysteine sulphoxide (SMCO) has been implicated as the primary toxin in the cases of kale[5] poisoning, although other S-alkylcysteine sulphoxides may also be present in the feedstuffs. In order to be able to expand the potential of brassicas as animal food, it is necessary to breed new lines with low SMCO content and have available suitable analytical techniques for the assessment of their toxicity.

The analysis of SMCO in animal foodstuffs is complicated by the necessity to be able to separate SMCO from the commonly occurring abundant amino acids. Morris and Thompson[6] used two-dimensional paper chromatography to determine the SMCO content of turnips, cabbage and several other feedstuffs. Paper chromatography was similarly used by Mae *et al.*[7] in a study of SMCO in Chinese cabbage. Separation by electrophoresis on silica gel impregnated glass-fibre sheet[8] and ion-exchange chromatography have also been suggested. A simple method for the approximate determination of SMCO, utilizing thin-layer chromatography (TLC) on cellulose, has been proposed by Matheson and Moir[9] but the layer stability problems associated with the use of cellulose TLC plates favour the development of a separation based on silica layers. In this paper we report the development of such a system, applicable to the estimation of the SMCO content of common vegetable crops.

EXPERIMENTAL

All solvents were of analytical reagent grade unless otherwise stated.

S-Methylcysteine sulphoxide (SMCO) was prepared from S-methylcysteine (SMC) (Aldrich, Gillingham, Great Britain) as follows. To a stirred solution of SMC (1 g SMC dissolved in 5 ml of 15% (v/v) hydrochloric acid) was slowly added 0.85 ml of hydrogen peroxide (30%) and the resulting orange solution was heated on a steam bath for 10 min. The yellow product was purified by passing the solution through a cation-exchange resin column (Dowex 50W-X8, H^+ form, Dow Chemical, Midland, Mich., USA) until the pH of the eluent was four. The SMCO was then eluted from the column with 0.5 *M* ammonia solution and the eluent was evaporated to dryness

using a rotatory evaporator. The product was precipitated from the minimum quantity of water by the addition of acetone. The oily off-white precipitate was recrystallized from the minimum quantity of water, containing a small volume of ethanol, to give white crystals (yield 55%).

Amino acid solutions (1 mg/ml) were prepared by dissolving chromatographic grade acids (BDH, Poole, Great Britain) in 10% (v/v) aqueous *n*-propanol or, for the more insoluble acids, in 10% (v/v) hydrochloric acid.

TLC plates (layer thickness *ca.* 0.5 mm) were prepared in the laboratory from silica gel G (E. Merck, Darmstadt, G.F.R.). The plates were oven dried and then deactivated by standing in the laboratory for at least two days prior to use. The amino acid spots were visualized by spraying with ninhydrin solution (0.3 g ninhydrin dissolved in 3% (v/v) acetic acid). After drying the plates at 110° for 10 min, the amino acids are visible as pink-purple spots (except proline and hydroxyproline which give yellow spots).

RESULTS AND DISCUSSION

Of the many solvent systems investigated, two systems showed particular promise and were optimized for the separation of SMCO from other amino acids. Maximum resolution using isopropanol–acetic acid–water mixture, was obtained with a solvent composition 70:7.5:22.5 (v/v). With this solvent mixture SMCO was resolved from all studied amino acids except proline with which slight overlap was experienced. These two amino acids are however readily distinguished by the colours and colour yields produced on visualization with ninhydrin. An alternative solvent system using a mixture of methylene chloride, formic acid, dimethyl sulphoxide and water allows control of the SMCO mobility by altering the proportion of dimethyl

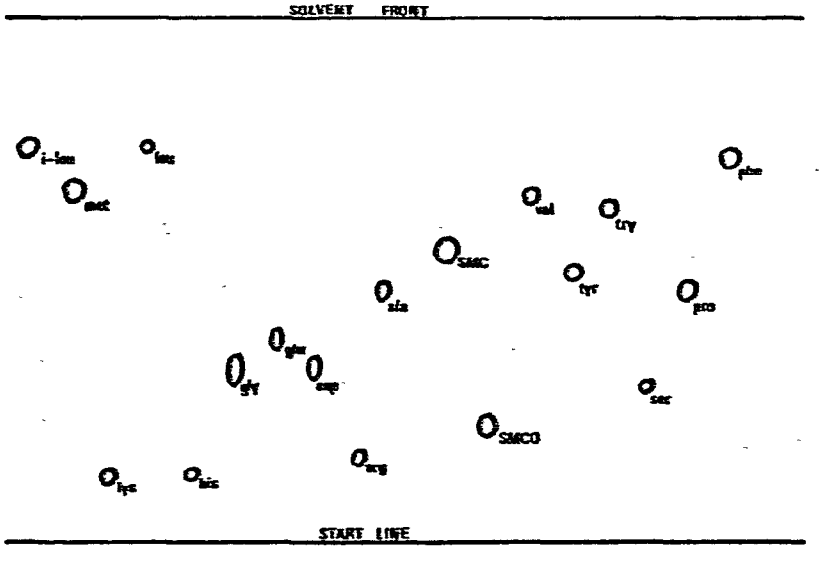

Fig. 1. Migration of amino acids on deactivated silica gel using methylene chloride–formic acid–dimethyl sulphoxide–water (65:7.5:22.5:5) as solvent system.

sulphoxide in the mixture. Optimum solvent composition, yielding complete resolution of SMCO from the other amino acids was obtained using a methylene chloride: formic acid: dimethyl sulphoxide: water ratio of 65:7.5:22.5:5 (v/v). An example chromatogram, obtained using this solvent system, is shown in Fig. 1. The R_F values of SMCO and the other studied amino acids, with the two solvent systems, are given in Table I.

TABLE I

R_F VALUES OF AMINO ACIDS

Solvent 1: methylene chloride–dimethyl sulphoxide-water-formic acid (65:22.5:5:7.5). Solvent 2: isopropanol–water–acetic acid (70:22.5:7.5).

Amino acid	R_F	
	Solvent 1	*Solvent 2*
SMCO	0.20	0.30
SMC	0.52	0.56
Ala	0.42	0.50
Arg	0.15	0.15
Asp	0.32	0.39
Glu	p.36	0.53
Gly	0.31	0.42
His	0.11	0.13
i-Leu	0.75	0.64
Leu	0.74	0.67
Lys	0.12	0.12
Met	0.67	0.67
Pro	0.47	0.33
Ser	0.30	0.66
Thr	0.37	0.47
Phe	0.73	0.59
Try	0.64	0.49
Tyr	0.52	0.70
Val	0.63	0.57

CONCLUSIONS

TLC on deactivated silica gel, using a methylene chloride–formic acid–dimethylsulphoxide–water solvent system provides good resolution of SMCO from other naturally occurring amino acids and is sufficient for the analysis of SMCO in animal foodstuffs. The one-dimensional separation allows the simultaneous estimation of the SMCO content of several samples in one TLC run. For the screening of large numbers of plant samples, extensive sample preparation is to be avoided and complex plant extracts must therefore be analysed. The ninhydrin colour reaction provides a sensitive detection system, with sufficient selectivity for amino acids, to allow the determination of SMCO in such extracts.

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