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Note

Determination of S-methyl cysteine sulfoxide in *Brassica* extracts by high-performance liquid chromatography*

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Brassica crops (*e.g.* turnip, swede, kale, cabbage, mustard) have been used over many centuries for both human and animal consumption. Species of *Brassica* contain toxic glucosinolates and (+)-S-methyl cysteine sulfoxide (SMCO)[1,2]; the latter compound is converted to toxic dimethyl disulfide by rumen microorganisms[2]. The disulfide has been shown to cause hemolytic anemia in cattle and sheep as a consequence of feeding on kales containing up to 1% of dry matter as SMCO[3]. Because of interest in investigating the levels of SMCO in *Brassicas* and its effect on cattle and sheep, several analytical procedures have been developed for measuring SMCO content in plant material[4–6]. These methods are based on ionic resin column chromatography, cellulose TLC, and electrophoresis. Separation and quantitation on an amino acid analyzer is very slow, and while other methods are faster, they are not specific or quantitative for (+)-SMCO. This article describes a fast, accurate analytical high-performance liquid chromatographic (HPLC) procedure for quantitating (+)-SMCO in plant materials. This method utilizes the same plant extraction procedure as used for glucosinolate analysis[7] and therefore lends itself to concurrent analysis of glucosinolates and SMCO.

EXPERIMENTAL

All chemicals and solvents used in this work were reagent grade; distilled water conductivity was 1.2 MΩ, or better. All solvents for HPLC were filtered through 0.45-μm filters immediately before use; no other special conditions or solvents were required to obtain optimum HPLC separations. The HPLC system consisted of Waters 6000A and M4500 pumps, a Waters U6K injector, a Waters Model 420 fluorescent detector (338 nm excitation, 425 nm emission) (Waters Assoc., Milford, MA, U.S.A.)**, and a Micromeritics Model 725 autoinjector (Micromeritics, Norcross, GA, U.S.A.). Samples were separated on a Supelcosil C_{18} column (Supelco, State College, PA, U.S.A.) using a S-100 computer-controlled HPLC system (Center Computer Consultants, State College, PA, U.S.A.) as described by Gustine and McCulloch[8]. Peak areas were calculated with the supplied algorithm.

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** Mention of a trade name does not imply an endorsement or recommendation by the USDA.

Amino acid standard mixture (protein hydrolysate) was purchased from Pierce (Rockford, IL, U.S.A.). SMCO standard was prepared from a reaction of S-methyl cysteine and hydrogen peroxide[9]; 0.6746 g of the compound was dissolved in 7 ml of distilled water, 1 ml of 35% hydrogen peroxide was added, and the reaction was allowed to proceed overnight (reaction mixture). (+)-SMCO was separated from other reaction products on a Dowex 50-X1 column, 50–100 mesh H^+ form (82 × 1.2 cm I.D., 0.2 *M* citrate). The combined (+)-SMCO fractions were passed through an identical column (H^+ form) and washed with deionized distilled water to remove citrate. The bound (+)-SMCO was eluted with 0.1 *N* ammonia, and then crystallized from water–ethanol (10:90, v/v).

Reaction mixture, (+)-SMCO, and amino acid standards were derivatized with *o*-phthalaldehyde (OPA) (Fluropa, Pierce, Rockford, IL, U.S.A.). Amino acid sample (10–50 μl) was added to 650–690 μl of methanol followed by addition of 200 μl of 0.5 *M* potassium borate (pH 10.4), and 100 μl of OPA reagent (20 μl ethanethiol and 20 mg OPA per ml methanol). Reaction was complete within 1 min. Swede (*B. napus* L. cv. Calder) extract was prepared as described by Gustine and Jung[7]; 20 μl of extract was derivatized as described above.

RESULTS AND DISCUSSION

Synge and Wood[9] demonstrated that treatment of S-methyl cysteine with hydrogen peroxide produced (−)- and (+)-SMCO, and that (+)-SMCO was the predominant naturally occurring isomer in cabbage. Thus the reaction mixture prepared for this study was first presumed to contain only (+)- and (−)-SMCO. However, analysis of the reaction mixture on an ion-exchange amino acid analyzer showed at least four products present, one of which co-chromatographed with authentic (+)-SMCO. Ion-exchange isolated, crystalline (+)-SMCO produced a single peak on the amino acid analyzer, and when co-chromatographed with the reaction mixture was well separated from the other three products. None of the four products was S-methyl cysteine. No attempt was made to isolate (−)-SMCO or to identify the other two products and therefore only the (+)-SMCO isomer was studied.

Initial attempts at separating OPA derivatives of (+)-SMCO and the other products in the reaction mixture from OPA-amino acid standards were unsuccessful using an acetonitrile–$Na_xH_xPO_4$ gradient and reversed-phase (RP) HPLC (data not shown). Under a variety of gradient conditions, not four, but one OPA derivative peak was observed and it was not separated from OPA-serine. Trial RP-HPLC separations of OPA derivatives of the four products in the reaction mixture with tetrahydrofuran–$Na_xH_xPO_4$ gradients indicated two peaks could be resolved, one that co-chromatographed with OPA–(+)-SMCO and one that apparently was composed of the other three products (Component 1, Fig. 1). The elution order of the two OPA-derivative peaks was determined by comparing them with the OPA derivative of authentic (+)-SMCO isolated by ion-exchange chromatography as described in the Experimental section. The separation of the OPA derivative of Component 1 and OPA–(+)-SMCO from OPA-histidine and OPA-serine by RP-HPLC is shown in Fig. 1. RP-HPLC separation of OPA–(+)-SMCO from OPA-histidine (Fig. 1) was not at baseline, but peak area comparisons of (+)-SMCO and histidine derivatives alone or in combination showed that accurate OPA–(+)-SMCO peak

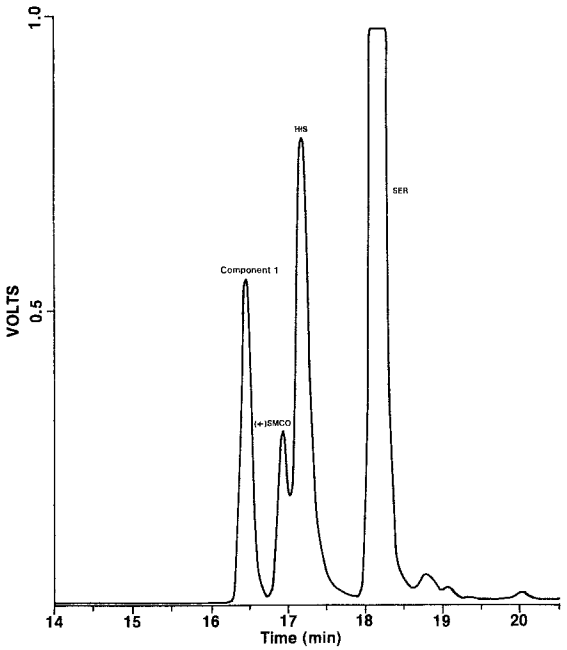

Fig. 1. Separation of amino acid standards from (+)-SMCO. Column: 250 mm × 4.6 mm I.D. Supelcosil LC-18; mobile phase: [tetrahydrofuran–water (55:45), 15 *M* $Na_xH_xPO_4$, pH 7.2]–(15 *M* $Na_xH_xPO_4$, pH 7.2) (25:75) convex gradient (Centre Computer Consultants S-100 HPLC controller, gradient No. 7) to 75:25 in 17 min; flow-rate 0.8 ml/min; sample size 17 μl (548 pmoles of histidine, 809 pmoles of serine, 34.2 pmoles (+)-SMCO; attenuation 2. Sample consisted of amino acid standard mixture and reaction mixture.

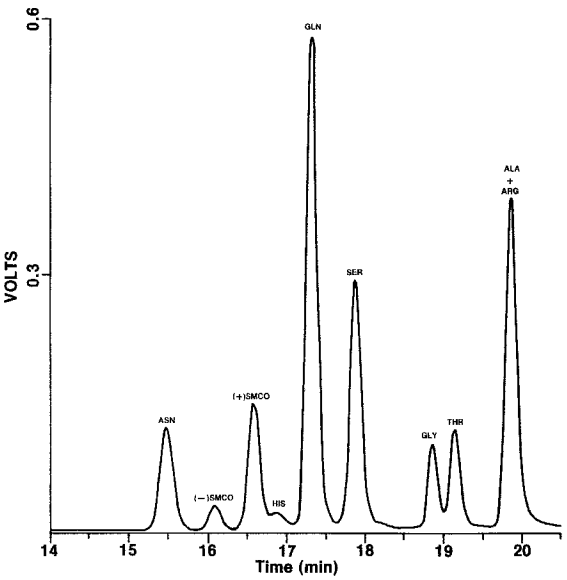

Fig. 2. Separation of OPA-amino acids in Calder swede extract. Experimental conditions same as in Fig. 1. (+)-SMCO 17.8 pmoles. The peak preceeding (+)-SMCO was tentatively identified as (−)-SMCO (see text).

areas were obtained in the presence of OPA-histidine. The SMCO derivative was well separated from other OPA-amino acid standards. Preparation of a standard curve for authentic (+)-SMCO produced a linear response out to 1.1 nmoles (r = 0.9997) using an attenuation setting of 4; sensitivity of detection can be increased 32-fold (about 30 pmoles) without loss of linearity by changing the detector attenuation to 128. The peak area for 286.7 pmoles of (+)-SMCO derivatized with OPA, obtained for seven separate determinations over a two-week period, was 6.17 ± 0.31 peak area units. The reaction of OPA and (+)-SMCO was complete within 1 min and no fluorescence decay was observed over a 12-h time period. Addition of 200 μl of the reaction mixture [containing 2 mg (+)-SMCO] to 1 g of Calder swede followed by extraction, resulted in an extraction recovery of 84.6% for (+)-SMCO. A portion of Calder swede extract was derivatized and the amino acids separated (Fig. 2); OPA–(+)-SMCO and a peak corresponding to the other three products of the reaction mixture were well resolved but OPA-histidine slightly overlapped OPA–(+)-SMCO. Ion-exchange amino acid analyzer analysis of the Calder swede extract demonstrated the presence of (+)-SMCO and a small peak eluting just before (+)-SMCO, but the other two components of the reaction mixture were absent, suggesting the small peak is (−)-SMCO and that small quantities of (−)-SMCO are present in Calder swede. Repeat RP-HPLC determinations of this and other *Brassica* extracts gave excellent reproducibility for (+)-SMCO with a coefficient of variation of less than ± 5%.

The RP-HPLC method described here is a fast, simple procedure for quantitative analysis of (+)-SMCO in plant extracts. Previous "fast" methods lacked sufficient resolution to separate and quantitate the (−)-isomer from the (+)-SMCO isomer[4,5,6]. This HPLC method (30 min/sample) is much faster than automated amino acid analyzer systems (120 min/sample). With automated pre-column OPA derivatization[10,11] now possible, (+)-SMCO and other amino acids can be analyzed at a rate of more than 40 samples per day.

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