1-Cyano-2,3-Epithiopropane as the Primary Sinigrin Hydrolysis Product of Fresh Cabbage

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ABSTRACT

Dichloromethane extracts of juices from fresh cabbages, including four known (Brutus, Galaxy, Bentley, Structon) and two unknown cultivars, were analyzed by GC-MS for the presence of sinigrin degradation products. Allyl isothiocyanate (AITC), which has been reported to be the important aroma compound of freshly disrupted cabbage was not detected in any of the dichloromethane extracts of cabbage juice. Instead, 1-cyano-2,3-epithiopropane, which is one of the isomers of AITC, was the primary volatile compound in all cabbage extracts. AITC was detected in relative trace concentration only when cabbage juice was injected into the GC, using a wide bore, packed column. Thus, the relative importance of AITC and 1-cyano-2,3-epithiopropane to the aroma of freshly disrupted cabbage should be considered.

Key Words: 1-cyano-2,3-epithiopropane, sinigrin, allyl isothiocyanate, cabbage

INTRODUCTION

HEWITT ET AL. (1956) witnessed the generation of cabbage flavor when they added an enzyme preparation from cabbage and mustard seeds into rehydrated dehydrated cabbage. The first report we found of the presence of allyl isothiocyanate (AITC) in cabbage, however, was made by Mackay and Hewitt (1959). They reported AITC was generated when they repeated the investigation of Hewitt et al. (1956) by adding a thioglucosidase (thioglucoside glucohydrolase, myrosinase) preparation from cabbage and mustard seeds into rehydrated dehydrated cabbage. AITC also has been reported by other scientists to be one of the important aroma compounds of fresh (Clapp et al., 1959; Bailey et al., 1961; Chin and Lindsay, 1993; Schwimmer, 1963) and heated (MacLeod and MacLeod, 1970) cabbage, along with other volatile sulfur compounds. The concentration of allylglucosinolate (sinigrin), the precursor of AITC, was reported to be 17-612 ppm (Van Etten et al., 1976). Four different volatile compounds (AITC, allyl cyanate, allyl thiocyanate and 1-cyano-2,3-epithiopropane) have been reported to be generated from myrosinase hydrolysis of sinigrin, depending on reaction conditions (Springett and Adams, 1988).

However, during the course of our investigations on the antibacterial activity of fresh cabbage juice (Kyung and Fleming, 1994a, b), we did not detect AITC. Instead, we tentatively identified a prominent GC peak as 1-cyano-2,3-epithiopropane based on GC retention time and MS characteristics (Kyung and Fleming, 1994b).

1-Cyano-2,3-epithiopropane has been reported to occur in disrupted, fresh cruciferous vegetables (Cole, 1975; 1976; Daxenbichler et al., 1977; Petroski and Tookey, 1982) and in aqueous suspensions of crambe seed flour (Luthy and Benn, 1979) or cabbage seed meal (Kaoulla et al., 1980), but not in white mustard seed or horseradish (Petroski and Tookey, 1982). Both AITC and 1-cyano-2,3-epithiopropane are generated from a common precursor, allylglucosinolate (sinigrin, Fig. 1), by the action of thioglucosidase. Cole (1975) indicated that different thioglucosidases produced different end products from the same substrate. Tookey (1973), however, isolated a protein in the seeds of *Crambe abyssinica*, which was required for the formation of cyanoepithioalkane. He termed it epithiospecifier protein (ESP). Following the discovery of ESP in crambe seeds, Petroski and Tookey (1982) isolated ESP from various cruciferous vegetables. Thioglucosidase enzyme alone produced only alkenyl isothiocyanates from alkenylglucosinolates, while the enzyme along with ESP generated the corresponding cyanoepithioalkane. White mustard seed and horseradish generated only isothiocyanate because they lacked ESP (Petroski and Tookey, 1982). Thioglucosidase from those plants, however, generated 1-cyano-2,3-epithiopropane from sinigrin when ESP obtained from cruciferous vegetables was added.

Our objective was to verify our hypothesis that 1-cyano-2,3-epithiopropane, and not AITC, is the primary sinigrin hydrolysis product in homogenized fresh cabbage.

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MATERIALS & METHODS

CABBAGE CULTIVARS (cvs.) Brutus, Galaxy, Bentley and Structon were obtained from Castle Harvester Company, Inc. (Seneca Castle, NY). Two unknown cultivars of cabbage and one of horseradish were purchased from local stores. AITC, thioglucosidase, and dichloromethane were purchased from Sigma Company (St. Louis, MO).

Preparation of juices from cabbage and horseradish

Juices from vegetables were extracted by an electrical centrifuge-type juice extractor (Braun, Germany) as described by Kyung and Fleming (1994a). Cabbage juice augmented with AITC (50 ppm) was made by adding AITC into a portion (50 mL) of cabbage juice.

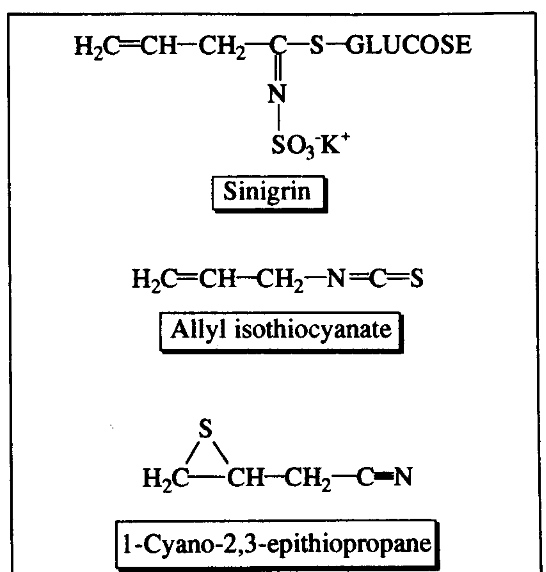

Fig. 1—Sinigrin and two of its hydrolysis products.

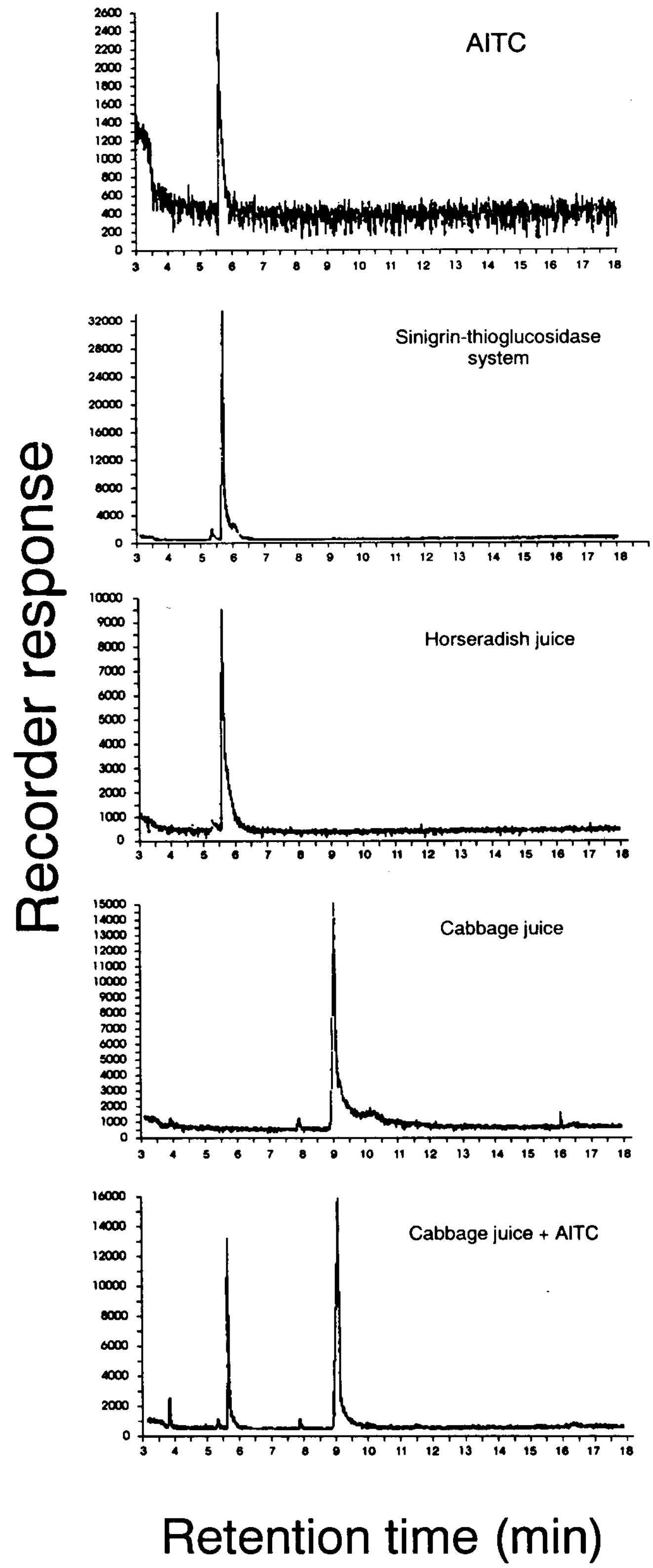

Fig. 2—Gas chromatograms of dichloromethane extracts of aqueous solutions. The analyses in Fig. 1 and 2 were obtained by combination GC/MS. A DB-5-coated, capillary column was used.

Preparation of an aqueous solution of AITC and sinigrin-thioglucosidase system

AITC standard was made by adding AITC into 50 mL of distilled water to make a final concentration of 50 ppm. Sinigrin (5 mg) and thioglucosidase (0.5 mg) were dissolved in 5 mL of 0.2M phosphate buffer (pH 6.2) and the mixture was kept at room temperature (≈23°C) for 30 min before extraction.

Dichloromethane extraction of volatiles

Aliquots of the preparations (50 mL each of aqueous solutions of AITC, cabbage juice, cabbage juice augmented with AITC and 5 mL each of sinigrin-thioglucosidase system and horseradish juice) were extracted with 5 mL of dichloromethane. The extracts were either analyzed immediately or stored at −83°C until analysis.

Identification of volatile compounds

Mass spectra of the isolated compounds were obtained by combination GC/MS (Model HP 5985B mass spectrometer and RTE VI data system, Hewlett-Packard, Palo Alto, CA). Volatiles in dichloromethane extracts were qualitatively analyzed. AITC and 1-cyano-2,3-epithiopropane were the compounds of primary interest. Portions of dichloromethane extract (2 or 3 µL) were injected for GC/MS analysis. The GC column (30 m capillary, J & W Scientific, Inc., Folsom, CA) was coated with DB-5 (0.25 µm thickness), and the oven temperature was programmed from 40 to 200°C at 5°C/min, with the initial temperature held 1 min. The carrier gas was He (1.5 mL/min). Samples were injected in the spitless mode. The injector port and detector were both at 150°C. Electron impact ionization, with an ionization potential of 70eV, was used and the mass range scanned was 40–200 daltons.

The volatile sulfur compounds were also analyzed under different conditions using a Shimadzu GC (Columbia, MD) with a column packed with 80–100 mesh Porapack P (Waters, Milford, MA). Two µL each of dichloromethane extract of cabbage juice, and of cabbage juice were directly injected. For headspace analysis, 1g of cabbage juice was weighed into 10 mL headspace glass vials (Tekmar Co., Cincinnati, OH). Juice samples were heated in a Tekmar 7000 Headspace Autosampler for 5 min at 100°C. The vials were pressurized to 103 kPa for 0.2 min and equilibrated for 0.2 min. The headspace was injected onto a 2 mL sampler loop for 0.5 min, equilibrated for 0.2 min, and the vapors from the loop were injected into the sampler line for 1 min. The sampler loop and line were held at 50°C, and vapors were collected with 34 kPa back pressure. The GC column was programmed from 105 to 225°C at 15°C/min, with initial temperature held for 0.5 min. A flame photometric detector was used. The carrier gas was N_2 (55 mL/min).

RESULTS & DISCUSSION

DICHLOROMETHANE EXTRACTS of cabbage showed one major peak (RT = 9.0 min), which had a longer retention time (RT) than authentic AITC (RT = 5.7 min; Fig. 2). No peak matching the RT of AITC appeared when dichloromethane extract was concentrated ≈50 times (data not shown). Authentic AITC added into cabbage juice showed the same RT as the AITC standard (Fig. 2). Extracts of both the sinigrin-thioglucosidase system and horseradish gave RT values identical to AITC (Fig. 2), confirming that AITC was produced as expected. There are reports that 1-cyano-2,3-epithiopropane has a longer RT than AITC (Cole, 1975; Daxenbichler et al., 1977; Kaoulla et al., 1980). The molecular ion at m/z 99 was the base peak, and the following losses are proposed to explain the observed fragment ions: loss of HCN (to give m/z 72), loss of $\cdot CH_2CN$ (to give m/z 59), and loss of $\cdot CH{=}S$ (to give m/z 54). These losses are consistent with the 1-cyano-2,3-epithiopropane structure. This structure also compared very well with published mass spectral data of 1-cyano-2,3-epithiopropane (Cole, 1975; Luthy and Benn, 1979; Kaoulla et al., 1980; Spencer and Daxenbichler, 1980; Springett and Adams, 1988). The latter compound has been reported in cruciferous vegetables (Cole, 1975; Daxenbichler et al., 1977; Luthy and Benn, 1979; Kaoulla et al., 1980; Spencer and Daxenbichler, 1980; Springett and Adams, 1988). When fresh cabbage was autolyzed, isothiocyanate peaks were essentially absent and nitriles, including 1-cyano-2,3-epithiopropane, were dominant (Daxenbichler et al., 1977). AITC, reported to be an important flavoring compound of fresh cabbage (Schwimmer, 1963; Chin and Lindsay, 1993), was not detected in our investigation. Also we did not detect AITC in our previous work (Kyung and Fleming, 1994b). Instead, we observed the presence of an unknown compound which had a mass spectrum matching 1-cyano-2,3-epithiopropane.

The dichloromethane extracts of the sinigrin-thioglucosidase system and horseradish were devoid of a 1-cyano-2,3-epithiopropane peak but showed a major peak with RT and mass spectrum matching authentic AITC (Fig. 2 and 3). AITC added into cabbage juice, as well as that in an aqueous solution, was detected by GC-MS and showed matching RT (Fig. 2) and mass spectrum (Fig. 3) with that of authentic AITC. The mass spectrum of AITC matched well with that of AITC in published reports (Bailey et al., 1961; Kaoulla et al., 1980). These evidences suggest that sample handling, including extraction and analysis of AITC from cabbage juice was not causing loss of the AITC, nor forming 1-cyano-2,3-epithiopropane through isomerization.

When dichloromethane extract of Brutus cabbage was analyzed under different conditions with a wide bore column packed with Porapack P, recorder response for 1-cyano-2,3-epithiopropane and AITC were 99.3 and 0.12, respectively (data not shown). When fresh cabbage juice was directly injected into GC, 1-cyano-2,3-epithiopropane was one of the major peaks, with AITC showing only a trace concentration (data not shown).

Based on the GC-MS characteristics of authentic AITC and the volatiles from cabbage, horseradish, and sinigrin-thioglucosidase system, the primary hydrolysis product of sinigrin in freshly disrupted cabbage appears to be 1-cyano-2,3-epithiopropane, and AITC is probably present only in relatively small concentrations, if any, in freshly disrupted cabbage.

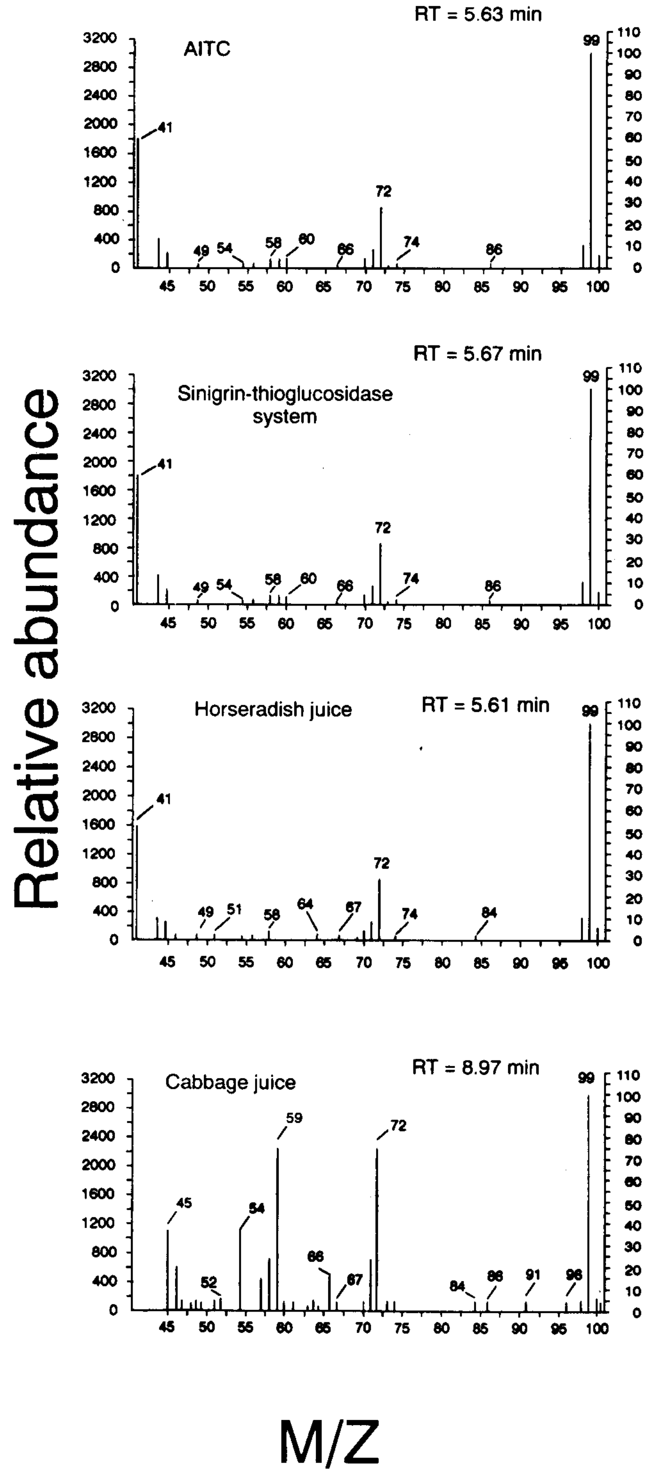

Fig. 3—Mass spectra of major compounds in dichloromethane extracts separated by GC.

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