The Glucosinolate of Land Cress (*Coronopus didymus*) and Its Enzymic Degradation Products as Precursors of Off-Flavor in Milk—A Review

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Land cress (*Coronopus didymus*) is a cruciferous weed which imparts a burnt, unclean flavor to milk when ingested by dairy cows. This off-flavor, unlike many weed taints, is intensified by heat treatment and cannot be eliminated from milk or cream by conventional vacuum pasteurization techniques. Land cress contains the glucosinolate, glucotropaeolin, from which benzyl cyanide, benzyl isothiocyanate, and benzyl thiocyanate are liberated enzymically when the plant is crushed. Benzyl methyl sulfide and benzyl mercaptan have been considered responsible for the off-flavor, the former having been isolated from cress-tainted cream. Benzyl thiocyanate appears to be a key intermediate in off-flavor formation. Complete elucidation of the chemical nature of the land cress off-flavor and its precursors has important practical value in the development of methods for control or removal of a taint which has caused significant economic loss to the dairy industries of several countries.

Certain feeds and weeds consumed by dairy cows have long been recognized for their contribution to the appearance of abnormal and undesirable flavors in milk and its products. Several general reviews of this subject are available (Babcock, 1938; Conochie, 1950; Parks, 1967; Strobel *et al.*, 1953). Onions, fermented silage, alfalfa, cabbage, turnips, rape, beet tops, musty hay, and distillers' grains impart off-flavors to milk, but these can usually be avoided by controlled feeding practices. Many varieties of weeds, when ingested by cows, impart taints to milk, and in some cases only minimal consumption of a weed is required to produce a strong off-flavor. The consumption of weeds by dairy cows is more difficult to control than the basic feeding regime. Nevertheless, the incidence of weed taints in milk can be greatly reduced by good pasture management practices.

Off-flavors imparted to milk are not necessarily of the same characteristics as the flavor of the consumed feed. In some cases chemical constituents of the plant are metabolically altered by the rumen microflora or the cow, prior to transfer into milk. For this reason a particularly offensive flavor may become evident in milk following ingestion of a plant which has relatively inoffensive odor and flavor characteristics. Alternatively, since chemical compounds are not all transferred from blood to milk to the same extent, a strong odor, emitted by an ingested plant, will not necessarily appear in the milk at organoleptically significant levels.

Feed and weed off-flavors cause a lowering of the flavor quality of beverage whole milk and considerable deterioration in the flavor of bland, high-fat products such as cream and butter. The level of many of these off-flavors can be greatly reduced in milk and cream by vacuum steam distillation (vacreation) and this technique is often employed by commercial buttermakers. Some weeds, however, impart to milk off-flavors which are not reduced, but intensified by subjecting milk or cream to such a process. This is the case with the burnt, unclean flavor which appears in the pasteurized milk from cows which have consumed land cress (*Coronopus didymus*). This weed, also known as twin cress or swine cress, has caused flavor problems in dairy products in New Zealand and Australia for many years, and recently has been reported as affecting milk flavor in some eastern states of the U.S.

The off-flavor imparted by land cress is not generally detectable in raw milk or cream, but is formed readily from an unknown precursor upon heat treatment during pasteurization or vacreation. Consumption of as little as 2 oz of this weed by a cow can result in the appearance of an off-flavor in pasteurized cream. For these reasons the problem has become more acute with the widespread use of bulk milk collection, since contamination in one supply may affect the bulk collection.

This paper reviews current knowledge regarding the nature of the off-flavor and its possible precursors in the land cress plant. Information pertinent to the formation and control of the off-flavor is also discussed.

THE LAND CRESS PLANT

Land cress, *Coronopus didymus* (L.) SM., is a cruciferous weed also known as hogs cress, swine cress, or twin cress. It is an annual or biennial plant which forms a disk-like mat of prostrate shoots radiating from the top of the tap root. Leaves (1 to 3 cm long) are alternate and deeply lobed. The flowers are minute, in slender auxiliary racemes, with white petals often missing. The finely wrinkled seed pods (3 mm diameter) are divided into two ovoid nutlets, each containing one seed. The plant is self-pollinating and the average output per plant is probably 1600 seeds, although up to 18,000 will be produced by large plants. The seeds are distributed by mud on hooves of animals, and by man, ants, and birds (Salisbury, 1961). Plants appear in early spring or autumn, especially in newly sown pasture, around water troughs, cattle yards, gateways, and in overgrazed pasture.

Land cress is indigenous to South America but is now prevalent in most dairying countries of the world. It was first recorded in Britain towards the end of the eighteenth century and reported by Davis (1940) as affecting milk flavor in England. It occurs widely in Australia, especially in the state of Queensland, where up to 20% of the butter produced is weed-tainted, the principal off-flavor being due to *C. didymus* (Armitt, 1968a). The weed has also been recorded in the U.S., being prevalent on the Atlantic coast and in southern

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states (Muenscher, 1955) and has recently been reported to be causing milk off-flavor in North Carolina (Gregory, 1964). Land cress was first recorded in New Zealand in 1846 and is common in the major dairying areas of the country.

Glucosinolate of Land Cress. *C. didymus* contains the glucosinolate, glucotropaeolin (Gray and Dolby, 1968), a member of a class of compounds, formerly known as mustard oil glucosides, which are characterized by their usual ability to undergo enzymic hydrolysis and produce isothiocyanates, sulfate, and glucose.

Little is known about the function of glucosinolates in plants (Ettlinger and Kjaer, 1968) but the effects of their breakdown products on microorganisms and animals are widespread. In addition to the milk-tainting properties, nitriles derived from glucosinolates exhibit toxic effects on animals (Virtanen, 1963, 1965), while isothiocyanates reacting with thiols and amines have antibacterial, antifungal, and insecticidal properties (Das *et al.*, 1957; Lichtenstein *et al.*, 1964; Virtanen, 1965). Furthermore the antithyroid, goiter-inducing effects of isothiocyanates on mammals continue to draw attention (Ettlinger and Kjaer, 1968).

Glucosinolates are present in all investigated species of the cosmopolitan *Cruciferae* family, and in some species of *Capparidaceae*, *Moringaceae*, and *Resedaceae* which belong to the same order (*Rhoeadales*) as the *Cruciferae*. According to Guignard (1890), glucosinolates are localized mainly in the parenchymal tissue of the plant and in the embryo of the seed.

In contrast to most investigated species of the *Cruciferae* family, which usually contain more than one glucosinolate, *C. didymus* appears to contain only the benzylglucosinolate, glucotropaeolin (Gray and Dolby, 1968). The general structure for glucosinolates (Ettlinger and Lundeen, 1956) is illustrated in Figure 1. This structure differs from that previously postulated by Gadamer (1899) in that the side chain is attached to the carbon atom rather than the nitrogen atom, which is accommodated in an oxime-like arrangement. Ettlinger and Lundeen (1957) synthesized the benzylglucosinolate ion and conclusively demonstrated this compound to be identical with the glucosinolate isolated from garden nasturtium (*Tropaeolum majus*).

Isolation and Detection of Glucosinolates. The method for isolation of glucosinolates involves the disintegration of plant tissue after inactivation of the enzyme, extraction of the glucosinolate with water or aqueous alcohols, followed by purification and crystallization. Glucosinolates have been purified on ion exchange resins or by alumina adsorption chromatography (Kjaer, 1960).

Until recently, references to the occurrence of glucosinolates in higher plants were based on detection of the isothiocyanates liberated by enzymic hydrolysis, as no analytical technique existed for glucosinolate detection. Gadamer (1899) was able to postulate the presence of glucotropaeolin in garden cress (*Lepidium sativum*) from identification of benzyl isothiocyanate in the steam distillate of the crushed plant and benzyl cyanide in the steam distillate of the intact plant. Gadamer attributed the effect of crushing to cause contact between the enzyme myrosinase and the glucosinolate, thereby liberating benzyl isothiocyanate, whereas steam distillation of the intact plant caused fission of the glucosinolate resulting in formation of the nitrile. In a similar manner McDowall *et al.* (1947) postulated the presence of glucotropaeolin in land cress by identifying benzyl cyanide in the steam distillates of both the crushed and intact plant. Benzyl isothiocyanate was obtained from land cress by an indirect method (Gadamer, 1899) involving alcoholic extraction of the

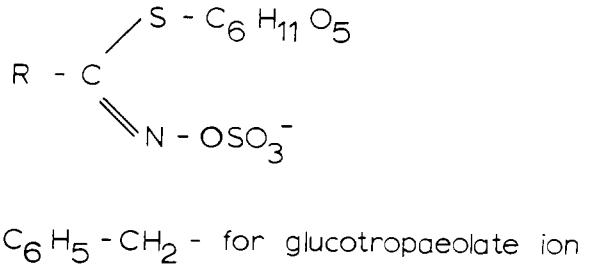

Figure 1. General structure for glucosinolates (Ettlinger and Lundeen, 1956)

glucosinolate and subsequent decomposition of its silver salt with sodium thiosulfate. Benzyl cyanide and benzyl isothiocyanate were identified by boiling points, refractive indices, and the formation of derivatives such as phenyl acetic acid and benzyl thiourea.

Schultz and Gmelin (1952, 1953) developed paper chromatographic methods for the separation of glucosinolates in crude plant extracts. This technique, combined with the paper chromatographic analysis of thioureas formed from isothiocyanates, has enabled confirmation of the occurrence of glucotropaeolin in *L. sativum* and *Lepidium ruderale* (Gmelin and Virtanen, 1959) and in *C. didymus* (Gray and Dolby, 1968).

Glucosinolates have more recently been separated by thin-layer chromatography on silica gel G (Wagner *et al.*, 1965); this procedure being more rapid than the 18 hr required for paper chromatographic analysis. Furuya (1965) has developed a gas-liquid chromatographic method for the separation of plant glycosides, which may find specific application for the separation of glucosinolates.

No studies have been undertaken to determine the amount of glucotropaeolin present in the land cress plant, although estimates have been made from the yields of enzymic cleavage products (Gray and Dolby, 1968; McDowall *et al.*, 1947). The glucosinolate content of land cress may show considerable variation depending on environmental factors and the stage of growth.

Enzymic Cleavage of Glucosinolates. When the land cress plant is crushed, it emits a sharp odor and, in the mouth, has a sharp burning taste. This feature is common to the closely related cruciferous species such as garden cress (*L. sativum*), water cress (*Nasturtium officinale*), white mustard (*Sinapis alba*), and black mustard (*Brassica nigra*). Enzymic cleavage of the glucosinolates in these plants to yield isothiocyanates (mustard oils), sulfate, and glucose is considered responsible for the pungent odor and taste of many species of *Cruciferae*. Garden cress and land cress are unusual, however, in that on enzymic fission of the glucosinolate, benzyl cyanide and benzyl thiocyanate are reported as breakdown products, in addition to the expected benzyl isothiocyanate (Gmelin and Virtanen, 1959; Gray and Walker, 1969, unpublished data; Park, 1965).

Since 1840, when the classical cleavage of glucosinolates to isothiocyanates was first recognized, there has been much interest in the mode of action, localization, and distribution of the enzyme, myrosin. Lepage (1846) reported that a large variety of *Cruciferae* seeds contained myrosin and Guignard (1890) demonstrated the enzyme to be localized in particular cells (idioblasts) of the plant. This is in contrast to the glucosinolates which appear to be distributed throughout the parenchymal tissue. Hence, in accordance with the distribution of substrate and enzyme, no reaction takes place until the plant tissue is disintegrated.

Neuberg and Wagner (1926) and Neuberg and Schoenbeck

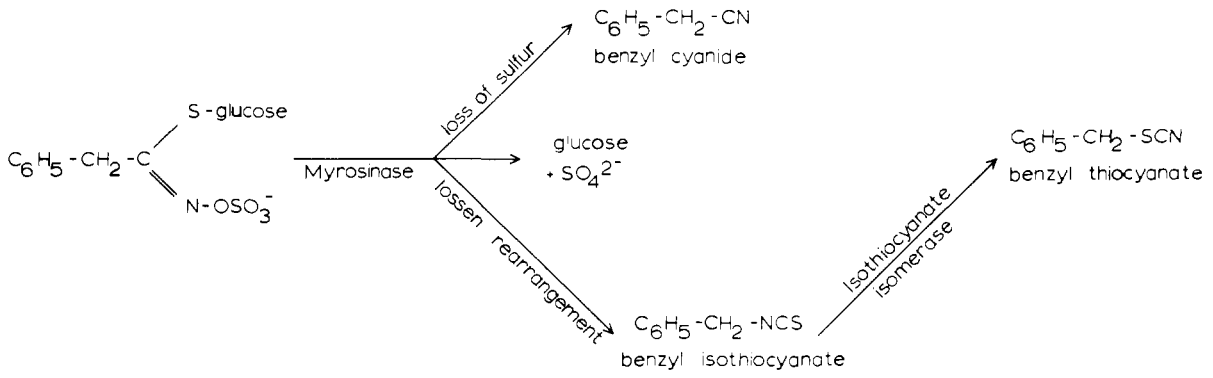

Figure 2. Proposed pathway for enzymic cleavage of the benzylglucosinolate in crushed seeds of garden cress (Virtanen, 1965)

(1933) reported separation of myrosin into thioglycosidase and sulfatase fractions which cleaved the thioglycoside and sulfate linkages, respectively. The term "myrosinase" was then adopted for the enzyme, which was believed to be heterogeneous until Ettlinger and Lundeen (1957), on proving the correct structure of the glucosinolates, suggested that liberation of sulfate would occur by Lossen rearrangement following enzymic cleavage of the thioglycosidic bond. This view was supported by Nagashima and Uchiyama (1959), who provided evidence that myrosinase is a single enzyme. These workers also demonstrate that the enzyme is markedly activated by L-ascorbate and suggested that the activating effect is due to reduction of disulfide bonds in the enzyme. Mabry (1960), on the other hand, reported that the activating effect of ascorbate appeared to correlate with vitamin C activity.

Further studies on myrosinase by Ettlinger and Thompson (1962) showed that ascorbate-activated enzymic cleavage was specific to glucosinolates and demonstrated hydrolysis of desulfonated glucosinolates suggesting β-thioglucosidase activity. They use the term glucosinolase to describe the enzyme and describe the formation of a thiohydroxamate intermediate during enzymic cleavage which undergoes Lossen rearrangement to yield isothiocyanate and sulfate.

The work of Gaines and Goering (1960, 1962) revived the controversy of whether myrosinase is a single enzyme. They reported separating, on DEAE cellulose, myrosulfatase and thioglucosidase components of myrosinase from defatted seeds of yellow mustard (*Brassica juncea*). The myrosulfatase activity was determined using sinigrin (allylglucosinolate) and oxime sulfonates as substrates. Calderon *et al.* (1966) repeated this study but could find no component specific for the hydrolysis of oxime sulfonates.

Tsuruo *et al.* (1967) separated myrosinase, obtained from yellow mustard seeds, into two distinct fractions by chromatography on TEAE cellulose at pH 8.5, but were unable to demonstrate separate thioglucosidase and sulfatase activity in these fractions. They concluded that the fractions represented two closely related species of myrosinase. Furthermore Tsuruo and Hata (1967) demonstrated that oxidation or reduction of ascorbic acid did not influence its activating effect on myrosinase. Ettlinger *et al.* (1961) showed that myrosinase could be activated by analogs of ascorbic acid which lack reducing ability. They suggest that there may exist two enzymes, only one of which is activated by ascorbate.

Tsuruo and Hata (1968a,b,c) have continued their studies on the mode of action of myrosinase and the interaction of ascorbate with the enzyme. They propose an effector site for ascorbate and point out an apparent resemblance of myrosinase to β-glucosidases. Based on the model for β-glucosidases by Pigman (1944) these workers suggest a schematic model demonstrating the interaction of ascorbate with the proposed aglycon and glycon substrate sites on the enzyme surface.

The nature of myrosinase and the manner in which it catalyzes the cleavage of glucosinolates to yield isothiocyanates, sulfate, and glucose has therefore received considerable attention recently. Much less, however, is understood regarding the formation of nitriles and thiocyanates from the glucosinolates of certain species of *Cruciferae*, *e.g.*, garden cress (*L. sativum*), and land cress (*C. didymus*).

Nitriles can be formed either by an enzymic or nonenzymic mechanism. Ettlinger and Thompson (1962) and Ettlinger *et al.* (1961) have investigated the nonenzymic formation of allyl cyanide from sinigrin. At pH less than 5, nitrile formation proceeds from the thiohydroxamate intermediate, in preference to the Lossen rearrangement which yields isothiocyanates. On the other hand, Virtanen (1965) considers the formation of benzyl cyanide in crushed seeds of garden cress to be enzymic. The formation of benzyl cyanide in garden cress occurs over a wide range of pH both acid and alkaline.

An important development in the study of the enzymic breakdown of glucosinolates was the discovery by Gmelin and Virtanen (1959) of the formation of allyl thiocyanate from sinigrin in *Thlaspi arvensi* and benzyl thiocyanate from glucotropaeolin in *L. ruderale* and *L. sativum*. This observation has since been supported in *C. didymus* (Gray and Dolby, 1968; Park, 1965). Saarivirta and Virtanen (1963) and Virtanen (1962) have shown that this reaction is enzymic and have postulated that an isomerase is responsible for the conservation of isothiocyanate to thiocyanate. They observed that not all related species have the ability to produce the thiocyanate and that although benzyl thiocyanate is produced in the crushed seeds of *L. sativum*, none is produced in the crushed green leaves of the plant.

A scheme illustrating the enzymic breakdown of the benzylglucosinolate in the crushed seeds of *L. sativum* is shown in Figure 2 (Virtanen, 1965). Because benzyl isothiocyanate, benzyl cyanide, and benzyl thiocyanate are also produced in crushed land cress, it is suggested that a similar sequence of reactions occurs in this plant. These three products have all been considered as possible precursors of the land cress off-flavor in milk.

Detection of Glucosinolate Breakdown Products. The enzymic hydrolysis of glucosinolates can be followed by determination of substrate or any of its products. Only methods related to detection of isothiocyanates, thiocyanates, and cyanides are, however, reviewed.

If the isothiocyanates are chemically stable, steam distillation is a convenient method of isolation. From aqueous distillates the isothiocyanates can be solvent extracted or converted to thiourea derivatives with alcoholic ammonia. The paper chromatographic method developed for the separation of thioureas by Kjaer and Rubinstein (1953) has been employed for the identification of many naturally derived isothiocyanates. In many instances a spectrophotometric assay based on the intense absorption shown by thioureas has been used for quantitative determinations (Kjaer *et al.*, 1953).

More recently gas-liquid chromatography has been employed for the analysis of glucosinolate breakdown products. Kjaer and Jart (1957) reported the separation of synthetic alkyl isothiocyanates having one to five carbon atoms while Lichtenstein *et al.* (1962) used gas-liquid chromatography to identify 2-phenyl isothiocyanate from turnip extracts. The isothiocyanates from rape seed have been separated by gas-liquid chromatography (Youngs and Wetter, 1967) and Binder (1969) has used this technique for the separation of a mixture of synthetic isothiocyanates. Mixtures of benzyl cyanide, benzyl isothiocyanate, and benzyl thiocyanate have been separated on butanediol-succinate and polypropylene glycol columns using flame ionization and argon tetrode detectors (Wahlroos and Saarivirta, 1964). The extreme sensitivity of the electron capture detector to isothiocyanates and thiocyanates has made it extremely useful in this field (Gray and Dolby, 1968; Lichtenstein *et al.*, 1964; Park, 1969) especially when used in combination with a flame ionization detector (Oaks *et al.*, 1964).

NATURE OF LAND CRESS OFF-FLAVOR

Several studies have demonstrated the absence or low intensity of the land cress off-flavor in raw milk or cream and the apparent formation or intensification of the defect only after pasteurization (Forss, 1951; McDowall *et al.*, 1947). It appears therefore that the land cress off-flavor is developed by the effect of heat, either by formation from a component with a low flavor threshold, or by liberation from a state in which it is loosely bound to some other component of the milk. McDowall *et al.* (1951a) demonstrated that the conditions of time and temperature employed during commercial pasteurization or vacuum pasteurization are sufficient to develop the off-flavor to an undesirable level if the milk or cream contains the potential for cress off-flavor formation. The defect itself has been variously described as sharp, biting, burnt, cress, pungent, unclean, scorched, or herby.

As a result of their early studies of land cress plant breakdown products, McDowall *et al.* (1951b) considered that benzyl cyanide and/or benzyl isothiocyanate may be responsible for the land cress off-flavor or may constitute its precursors in the raw milk. They established, however, that these two compounds, when added to cream, do not reproduce the typical land cress off-flavor in either cream or butter, before or after heat treatment. In addition, these two compounds, unlike the land cress off-flavor, are readily removed from cream by vacuum steam distillation. From these observations, and because the typical land cress off-flavor was absent from the milk, cream and butter from cows fed garden cress (*L. sativum*), which also contains the benzylglucosinolate, glucotropaeolin, McDowall *et al.* (1951b) concluded that glucotropaeolin was not the sole source of the land cress off-flavor compound(s) in milk.

Forss (1951) attempted to identify the major element of the land cress off-flavor. In summarizing the characteristic features of the defect, he stated that it is intensified by heating, especially at temperatures above 80° C, is little affected by vacuum treatment of hot milk or cream, and is eliminated or greatly reduced in intensity by treating the milk or cream with sodium hypochlorite. Forss was able to isolate benzyl disulfide from fresh land cress plants and benzyl mercaptan from partially decomposed plants, and suggested that the latter is a major contributor to the land cress off-flavor in dairy products. He noted that when benzyl mercaptan is added to cream and butter at concentrations as low as 1 part per billion, the flavor is very reminiscent of the natural land cress off-flavor. Furthermore, sodium hypochlorite removes the flavor of benzyl mercaptan from cream as effectively as it eliminates the land cress off-flavor.

Benzyl disulfide and benzyl mercaptan, when fed to cows, do not produce an off-flavor in the milk, although benzyl disulfide is apparently reduced within the animal as both the urine and feces had a strong odor of benzyl mercaptan. It is not clear, therefore, how benzyl mercaptan might enter the milk. Forss (1951) suggested that an unknown precursor is secreted into the milk and this compound produces benzyl mercaptan when the milk is heated.

McDowall (1965) and Walker (1965) studied the vapor-liquid equilibrium relationships for benzyl mercaptan and found it to be not readily steam-distillable from cream because of its preferential solubility in the fat rather than the serum. This is also a feature of the natural off-flavor.

The suggestion that benzyl mercaptan is responsible for the land cress off-flavor was subsequently enhanced by the discovery of Gmelin and Virtanen (1959) that the cruciferous plants, *L. sativum* and *L. ruderale*, yield varying amounts of benzyl thiocyanate when crushed. It seemed possible that if benzyl thiocyanate is secreted into the milk, this compound may constitute the unknown precursor for benzyl mercaptan formation as suggested by Forss (1951). Indeed, Park (1965) and Gray and Dolby (1968) reported on the appearance of benzyl thiocyanate in extracts of crushed plants and seeds of land cress. Furthermore, the latter isolated benzyl thiocyanate and benzyl isothiocyanate from the rumen digesta of cows fed land cress.

Some confusion as to the role of benzyl thiocyanate arises when reports of the addition of this compound to milk and cream are examined. In the first such study Park (1965) claims that addition of 0.16 ppm of benzyl thiocyanate to milk produces a detectable off-flavor and 4 ppm imparts a strong flavor to the milk and a burning flavor, characteristic of the typical land cress off-flavor, to the cream. He alludes, however, to the possibility of a contribution by benzyl mercaptan which may be formed from benzyl thiocyanate by reduction during pasteurization. Subsequently, Gray and Dolby (1968) claimed that addition of 1 ppm of benzyl thiocyanate to cream was necessary to produce a detectable cress-like off-flavor. After further trials, however, they report that concentrations of 0.005 to 0.01 ppm of benzyl thiocyanate in raw cream are sufficient to produce a level of off-flavor in butter which is comparable to that in naturally tainted butter. Both groups report producing the land cress off-flavor in milk by drenching a cow with 1 gram or more of benzyl thiocyanate. In a recent publication, however, Park *et al.* (1969) revised their opinion of the off-flavor imparted by benzyl thiocyanate and now claim it to be similar, but not identical, to the natural land cress off-flavor in milk and cream.

The recent experimental work described by Park *et al.* (1969) represents the first attempt yet to isolate those trace compounds from milk which may be responsible for the typical land cress off-flavor. A herd of cows was grazed on a pasture heavily infested with land cress and the raw milk

obtained during this period was considered to have a strong typical land cress off-flavor. By high vacuum distillation and concentration of the distillates, fractions were obtained from the skim milk, butter oil, and buttermilk which had odors very reminiscent of the natural off-flavor. By a combination of gas chromatography and mass spectrometry, these fractions were found to contain benzyl isothiocyanate, benzyl cyanide, benzyl methyl sulfide, indole, and skatole. Benzyl isothiocyanate, benzyl cyanide, indole, and skatole were not considered by these workers to be major contributors to the natural land cress defect, based on comparison of levels calculated in milk with average flavor threshold values, and on opinions from expert graders. Benzyl methyl sulfide, on the other hand, was considered to impart a flavor to milk, cream, and butter which is "indistinguishable" from the natural off-flavor. The flavor thresholds of benzyl methyl sulfide in milk and butter oil were found to be 1 part in 10^8 and 1 part in 10^7, respectively, and this compound was isolated from the contaminated butter oil at a level of approximately 1 part in 10^6. Benzyl methyl sulfide must, therefore, be regarded as a prime candidate responsible for the land cress off-flavor.

Since benzyl methyl sulfide has not been isolated from extracts of crushed land cress plants or seeds, Park *et al.* (1969) have postulated that it is formed enzymically by methylation of benzyl thiocyanate within the animal. This reaction may occur directly or with prior reduction of benzyl thiocyanate to benzyl mercaptan.

Neither benzyl thiocyanate nor benzyl mercaptan was identified in the distillates obtained from the contaminated milk fractions. Park *et al.* (1969) admit, however, to some difficulty in detecting benzyl mercaptan in the gas chromatography-mass spectrometry system at the 1 to 2 μg level. Considering the very low flavor threshold of benzyl mercaptan in milk, *i.e.*, approximately 1 part in 10^9 (Forss, 1951), it is conceivable that the system was not capable of detecting levels of this compound which are significant from a flavor standpoint. In addition, none of the milk fractions investigated were heated above 50° C. In view of the suggestion that land cress off-flavor is intensified or modified by heat treatment, especially above 80° C, it is possible that changes in the nature or relative composition of the flavor compounds may have been observed had the milk fractions been preheated to the higher temperature.

The summary of the present knowledge concerning the chemical nature of the off-flavor is now complete. Table I summarizes the compounds considered as contributors to the off-flavor, together with their approximate flavor threshold values in milk. Compounds 1 to 5 have been identified in extracts of crushed land cress plants but compound 5 only from partially decomposed plants. Compounds 1 to 4 impart off-flavors to milk but with characteristics which are considered unlike or not exactly like the natural land cress off-flavor. Compounds 5 and 6 may be considered at present as those most likely to be major contributors to the land cress off-flavor.

Table I. Compounds Possibly Involved in Milk Off-Flavor after Ingestion of Land Cress (*Coronopus didymus*)

	Compound	Formula	Approx. FTV (Milk)
1.	Benzyl isothiocyanate	R[a]—NCS	1 pp 10^7 [b]
2.	Benzyl cyanide	R—CN	1 pp 10^6 [b]
3.	Benzyl thiocyanate	R—SCN	1 pp 10^7 [c]
4.	Benzyl disulfide	R—S—S—R	?
5.	Benzyl mercaptan	R—SH	1 pp 10^9 [d]
6.	Benzyl methyl sulfide	R—S—CH_3	1 pp 10^8 [b]

[a] R = C_6H_5—CH_2—.
[b] Data for Park *et al.* (1969).
[c] Data of Park (1965).
[d] Data of Forss (1951).

DETECTION AND CONTROL OF THE LAND CRESS OFF-FLAVOR

At present no simple chemical tests are available for the detection of land cress off-flavor in raw milk or cream. The procedure employed to date has relied upon the ability of grading personnel to detect the off-flavor organoleptically. For this purpose several simple types of apparatus have been designed, so that small samples of milk or cream can be rapidly heated and evaluated for cress odor (McDowall *et al.*, 1947). During the period when milk and cream were transported to processing plants in cans, a sample from each can could, if necessary, be tested for cress off-flavor and any suspect supply could be easily segregated. Since the introduction of tanker collection, however, it is now possible for one farm supply of potentially tainted milk to contaminate the bulk collection.

For the reasons outlined above and because the land cress off-flavor cannot be removed from milk and cream by conventional means, the responsibility for controlling the off-flavor has fallen mainly to the supplier, who must attempt to eliminate the weed from pasture and fodder crops. Heavy stocking of pasture which leads to trampling and pugging, especially during wet periods, should be avoided, since land cress often appears in resulting bare patches. This can be overcome, however, by strip grazing, by the use of feeding platforms, or by indoor feeding of forage. Grass grub attack can also seriously weaken the sward and allow entry of land cress and other weeds, while heavy infestations often occur in newly sown spring and autumn pastures. Many grazing recommendations for the control of land cress taint have been made by Armitt (1968b).

Another method of control on the farm is concerned with limiting the intake of weed-infested forage by the milking cow, although this practice will not completely eliminate incidences of land cress off-flavor (Allo and McDowall, 1941; McDowall *et al.*, 1951c).

The growth of land cress in pastures and crops can be successfully inhibited with hormone-type herbicides. In young pasture, control is best achieved using methyl chlorophenoxy butyrate derivatives which do not affect the young clover plants, while in established pasture 2,4-dichlorophenoxy butyrate gives satisfactory control.

The land cress off-flavor cannot be easily removed from milk and cream. McDowall *et al.* (1951a) were unsuccessful in modifying the standard design of the vacuum pasteurizer to eliminate cress taint. Major (1969a,b), however, has described an ultra-high temperature nonvacuum pasteurizer which, at 340° F, is reported to reduce the level of land cress off-flavor in cream.

Cream contaminated with even moderate amounts of the cress off-flavor is unsatisfactory for buttermaking and is generally used for the production of butter oil. Major *et al.* (1962) have removed *Coronopus*, *Lepidium*, and *Rapistra* taints from butter oil by an electrolytically assisted, double refining process employing caustic soda and phosphoric acid.

DISCUSSION

Research completed up to the present time has not fully revealed either the origin or the nature of the land cress off-flavor in milk and its products. Indeed, many aspects of

the subject continue to be confused and sometimes even contradictory.

Land cress (*C. didymus*) and garden cress (*L. sativum*) both contain the benzylglucosinolate, glucotropaeolin, but the off-flavor appearing in milk after ingestion of land cress is considerably more objectionable and has different flavor characteristics than the off-flavor occurring in milk after ingestion of garden cress. Furthermore, Park (1969) has demonstrated that the fecal flavor which appears in milk following ingestion of peppercress (*Lepidium hyssopifolium*) is caused primarily by the presence of abnormally high levels of skatole and indole. *L. hyssopifolium* also contains glucotropaeolin (Park, 1967), yet Park demonstrated the absence of any glucotropaeolin degradation products in the milk after the consumption of this plant.

The above observations suggest either that glucotropaeolin is not the primary taint precursor in *C. didymus* or that the enzymic degradation of the glucosinolate in *C. didymus* yields different products or different proportions of similar products from those in *Lepidium* species. The first alternative is less likely, since glucotropaeolin would seem to be a very logical precursor of compounds such as benzyl mercaptan and benzyl methyl sulfide which have been considered as major contributors to the natural land cress off-flavor (Forss, 1951; Park *et al.*, 1969). The second alternative would appear to account more satisfactorily for the lack of a typical land cress off-flavor in milk after ingestion of *Lepidium* species. Saarivirta and Virtanen (1963) have demonstrated that benzyl thiocyanate is produced only in the crushed seeds of *L. sativum* and not in the crushed green parts of the plant. In *C. didymus*, however, benzyl thiocyanate production occurs in both the crushed green plant and in the crushed seeds (Gray and Dolby, 1968). For this reason the amount of benzyl thiocyanate liberated in the rumen of a cow which consumes land cress could be expected to be considerably greater than that amount liberated following ingestion of garden cress. If, as suggested by Park *et al.* (1969), benzyl thiocyanate does serve as the precursor of the land cress off-flavor, then this variation in the amount liberated during the crushing of plant tissue could account for observed differences in the nature of the off-flavors from the two plants.

One of the least well understood aspects of the land cress taint is its apparent development or intensification in milk and cream by the action of heat. The means by which the off-flavor is developed during the heating process will probably remain obscure until the exact nature of the tainting compound and its precursor are known. If, as suggested by Forss (1951), benzyl mercaptan is a major contributor to the off-flavor, one might speculate on its formation by reduction from benzyl thiocyanate or benzyl disulfide. The sulfhydryl groups which are activated during the heating of milk (Jenness and Patton, 1959) could act as the reducing agent for such a reaction. However, some doubt has recently arisen concerning this sequence of events, since Park *et al.* (1969) were unable to isolate benzyl thiocyanate, benzyl disulfide, or benzyl mercaptan from unheated but apparently land cress-tainted milk. Instead, their isolation of benzyl methyl sulfide from this milk has required a reevaluation of the nature of the off-flavor.

The land cress off-flavor is known to be greatly diminished in intensity when the milk or cream is treated with oxidizing agents such as sodium hypochlorite (Forss, 1951) or hydrogen peroxide (Conochie, 1950). Both benzyl mercaptan and benzyl methyl sulfide in milk would conceivably be oxidized by these reagents; the former to benzyl disulfide (Tarbell, 1961) and the latter to benzyl methyl sulfoxide (Barnard *et al.*, 1961). In any event, regardless of the exact nature of the tainting compound(s), hydrogen peroxide treatment may constitute a suitable method for reducing the land cress off-flavor in milk and cream, especially since any excess hydrogen peroxide could be destroyed with catalase (Roundy, 1961).

Complete elucidation of the chemical nature of the land cress off-flavor, its immediate precursor(s) in raw milk and its primary precursor(s) in the whole and crushed plant, is a challenging research problem. Hopefully, a commercially practical method for the removal of this weed taint from milk or cream will result from such research, thereby stemming the not inconsiderable economic loss hitherto experienced by the dairy industries of several countries.

LITERATURE CITED

Allo, A. V., McDowall, F. H., *N.Z. J. Agr.* **63,** 31 (1941).
Armitt, J. D., *Queensland Agr. J.* **94,** 2 (1968a).
Armitt, J. D., *Queensland Agr. J.* **94,** 96 (1968b).
Babcock, C. J., *J. Dairy Sci.* **21,** 661 (1938).
Barnard, D., Bateman, L., Cunneen, J. I., in "Organic Sulfur Compounds," Kharasch, N., Ed., pp. 229–47, Pergamon Press, London, 1961.
Binder, H., *J. Chromatogr.* **41,** 448 (1969).
Calderon, P., Pederson, C. S., Mattick, L. R., J. AGR. FOOD CHEM. **14,** 665 (1966).
Conochie, J., *Australian J. Dairy Technol.* **5,** 43 (1950).
Das, B. A., Kurup, P. A., Rao, P. L. N., *Indian J. Med. Res.* **45,** 191 (1957).
Davis, L. H., *Food Manuf.* **11,** 272 (1940).
Ettlinger, M. G., Dateo, G. P., Jr., Harrison, B. W., Mabry, T. J., Thompson, C. P., *Proc. Natl. Acad. Sci. U.S.A.* **47,** 1875 (1961).
Ettlinger, M. G., Kjaer, A., "Recent Advances in Phytochemistry," Vol. I, pp. 89–144, Appleton-Century-Crofts, New York, 1968.
Ettlinger, M. G., Lundeen, A. J., *J. Amer. Chem. Soc.* **78,** 4172 (1956).
Ettlinger, M. G., Lundeen, A. J., *J. Amer. Chem. Soc.* **79,** 1764 (1957).
Ettlinger, M. G., Thompson, C. P., "Studies of Mustard Oil Glucosides," Final Report, Contract DA 19-129-QM-1689, U.S. Army, Natick Laboratories, Massachusetts, Part II, 1962.
Forss, D. A., *Australian J. Appl. Sci.* **2,** 396 (1951).
Furuya, T., *J. Chromatogr.* **18,** 152 (1965).
Gadamer, J., *Ber. Deut. Chem. Ges.* **32,** 2335 (1899).
Gaines, R. D., Goering, K. J., *Biochem. Biophys. Res. Commun.* **2,** 207 (1960).
Gaines, R. D., Goering, K. J., *Arch. Biochem. Biophys.* **96,** 13 (1962).
Gmelin, R., Virtanen, A. I., *Acta Chem. Scand.* **13,** 1474 (1959).
Gray, I. K., Dolby, R. M., *N.Z. J. Dairy Technol.* **3,** 48 (1968).
Gregory, M. E., Food Sci. Extension Publication, N.C. State University, June, 1964.
Guignard, L., *J. Bot.* **4,** 385 (1890).
Jenness, R., Patton, S., "Principles of Dairy Chemistry," pp. 334–40, Wiley, New York, 1959.
Kjaer, A., *Fortschr. Chem. Org. Naturst.* **18,** 122 (1960).
Kjaer, A., Conti, J., Larsen, I., *Acta Chem. Scand.* **7,** 1276 (1953).
Kjaer, A., Jart, A., *Acta Chem. Scand.* **11,** 1423 (1957).
Kjaer, A., Rubinstein, K., *Acta Chem. Scand.* **7,** 528 (1953).
Lepage, P. N., *J. Chim. Med.* **2,** 171 (1846).
Lichtenstein, E. P., Morgan, D. G., Mueller, C. H., J. AGR. FOOD CHEM. **12,** 158 (1964).
Lichtenstein, E. P., Strong, F. M., Morgan, D. G., J. AGR. FOOD CHEM. **10,** 30 (1962).
Mabry, T. J., Ph.D. Thesis, Rice Institute, 1960.
McDowall, F. H., *J. Dairy Res.* **32,** 147 (1965).
McDowall, F. H., McDowell, A. K. R., Morton, I. D., Singleton, J. A., O'Dea, J. J., *N.Z. J. Sci. Technol.* **33,** 35 (1951a).
McDowall, F. H., Morton, I. D., McDowell, A. K. R., *N.Z. J. Sci. Technol.* **28,** 305 (1947).
McDowall, F. H., Morton, I. D., McDowell, A. K. R., *N.Z. J. Sci. Technol.* **33,** 30 (1951b).
McDowall, F. H., Singleton, J. A., O'Dea, J. J., *N.Z. J. Sci. Technol.* **33,** 51 (1951c).
Major, W. C. T., *Australian J. Dairy Technol.* **24,** 14 (1969a).
Major, W. C. T., *Australian J. Dairy Technol.* **24,** 18 (1969b).
Major, W. C. T., Cummings, D. A., Nichols, L. E., *Australian J. Dairy Technol.* **17,** 128 (1962).
Muenscher, W. C., "Weeds," 2nd ed., p. 241, The MacMillan Co., New York, 1955.
Nagashima, Z., Uchiyama, M., *Nippon Nogei Kagaku Kaishi* **33,** 114 (1959).
Neuberg, C., Schoenbeck, O. V., *Biochem. Z.* **265,** 223 (1933).
Neuberg, C., Wagner, J., *Biochem. Z.* **174,** 457 (1926).
Oaks, D. M., Hartmann, H., Dimick, K. P., *Anal. Chem.* **36,** 1561 (1964).

Park, R. J., *Australian J. Chem.* **20,** 2799 (1967).
Park, R. J., *J. Dairy Res.* **36,** 31 (1969).
Park, R. J., *Nature* **207,** 640 (1965).
Park, R. J., Armitt, J. D., Stark, W., *J. Dairy Res.* **36,** 37 (1969).
Parks, O. W., in "The Chemistry and Physiology of Flavors," Schultz, H. W., Day, E. A., Libbey, L. M., Ed., pp. 297–300, Avi Publishing Co., Westport, Conn., 1967.
Pigman, W. W., *Advan. in Enzymol.* **4,** 41 (1944).
Roundy, Z. D., *Milk Prod. J.* **52,** 12 (1961).
Saarivirta, M., Virtanen, A. I., *Acta Chem. Scand.* **17** (Suppl. 1), S74 (1963).
Salisbury, E. J., "Weeds and Aliens," pp. 66–67, Collins, London, 1961.
Schultz, O. E., Gmelin, R., *Z. Naturforsch. B* **7,** 500 (1952).
Schultz, O. E., Gmelin, R., *Z. Naturforsch. B* **8,** 151 (1953).
Strobel, D. R., Bryan, W. G., Babcock, C. J., "Flavors of Milk," Publ. of U.S. Dept. Agr., Washington, D.C., 1953.
Tarbell, D. S., in "Organic Sulfur Compounds," Kharasch, N., Ed., pp. 97–102, Pergamon Press, London, 1961.
Tsuruo, I., Hata, T., *Agr. Biol. Chem.* (Tokyo) **31,** 27 (1967).
Tsuruo, I., Hata, T., *Agr. Biol. Chem.* (Tokyo) **32,** 479 (1968a).
Tsuruo, I., Hata, T., *Agr. Biol. Chem.* (Tokyo) **32,** 1420 (1968b).
Tsuruo, I., Hata, T., *Agr. Biol. Chem.* (Tokyo) **32,** 1425 (1968c).
Tsuruo, I., Yoshida, M., Hata, T., *Agr. Biol. Chem.* (Tokyo) **31,** 18 (1967).
Virtanen, A. I., *Arch. Biochem. Biophys.* (Suppl. 1), 200 (1962).
Virtanen, A. I., "Investigations in the Alleged Goitrogenic Properties of Milk," p. 1, Helsinki Biochemical Institute, 1963.
Virtanen, A. I., *Phytochemistry* **4,** 207 (1965).
Wagner, Von H., Horhammer, L., Nuffer, H., *Arzeim.-Forsch.* **15,** 453 (1965).
Wahlroos, O., Saarivirta, M., *Acta Chem. Scand.* **18,** 2191 (1964).
Walker, N. J., *J. Dairy Res.* **32,** 229 (1965).
Youngs, C. G., Wetter, L. R., *J. Amer. Oil Chem. Soc.* **44,** 551 (1967).

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