THE OCCURRENCE OF MUSTARD OIL GLUCOSIDES IN *LEPIDIUM HYSSOPIFOLIUM* DESV., *L. BONARIENSE* (L.), AND *CAPSELLA BURSA PASTORIS* (L.) MEDIC.*

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The annual crucifers, *Lepidium hyssopifolium* Desv. and *L. bonariense* (L.) (pepperwort or peppercress), and *Capsella bursa pastoris* (L.) Medic. (shepherd's purse) are common weeds of farm and pasture in south-east Queensland. These and other cruciferous weeds are frequently grazed by livestock during dry winter months and when ingested can impart objectionable flavours to the milk of dairy cattle, or to chicken and beef flesh.[1,2] The incidence of "weed taints" in milk and other dairy produce can be quite severe during unfavourable seasonal conditions and is of considerable economic concern to the dairy industry in Queensland.

Little is known of the chemical nature of the substances responsible for these taints, although it is commonly believed that the constituent mustard oil glucosides are in some way implicated.[1] Indeed, benzyl thiocyanate, which is a product of an enzymic degradation of glucotropaeolin, the constituent mustard oil glucoside of *Coronopus didymus* Sm., is known to be a substance responsible for the development of the *C. didymus* taint in milk.[3] Hussong *et al.*[4] and later Conochie[5] reported that the milk of cows ingesting *Lepidium* spp. had a high content of indole or skatole, which was thought to be responsible for the "faecal" odour present in such milk. We now wish to report the presence of glucotropaeolin in the seed-bearing portions of *L. hyssopifolium* and *L. bonariense* and the presence of the mustard oil glucoside sinigrin in *C. bursa pastoris*.

The seeds of *L. hyssopifolium* were found to contain *c.* 0·1% of glucotropaeolin, a yield comparable to those found in other *Lepidium* spp. examined elsewhere.[6] A second mustard oil glucoside, probably sinigrin, was shown to be present in small amount in the seeds of *L. bonariense*.

The occurrence of mustard oil glucosides in the *Lepidium* spp. examined here suggests that these constituents may contribute to the development of the *Lepidium* taint in meat and dairy produce in a manner analogous to the *Coronopus* taint. However, this contrasts to the reports that skatole and/or indole are responsible for the *Lepidium* taint in milk, and a further study of the role of these glucosides or their breakdown products in the development of the *Lepidium* taint is warranted.

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The presence of sinigrin in *C. bursa pastoris* is in contrast to the findings of Kjaer *et al.*[7] and Schultz *et al.*[8], but confirms the suggestion of Bodinus.[9] The concentration of sinigrin in *C. bursa pastoris* as found here is considerably lower than that of mustard oil glucosides in other cruciferous plants, however. This may indicate that the ability of this plant to impart off-flavours to the milk of grazing dairy cattle is lower than in other crucifers, in agreement with the observations of Conochie and others.[1]

Experimental

Analyses are by Mr J. Kriauciunas, University of Queensland. Melting points are corrected. Infrared spectra were determined on a Perkin–Elmer 21 instrument with sodium chloride optics. The "semi-micro" still contains 20 cm of packing as described by Bower and Cooke[10] and has a stillhead designed to minimize hold-up. Gas chromatography was carried out on a column, 4 ft by ¼ in., with 20% of Apiezon M on Gas Chrom. P support, operated at 170°; a hydrogen flame temperature detector was used.

(*a*) L. hyssopifolium

The seed-bearing portions of the fresh plants obtained near the University grounds (2·2 kg) were extracted with boiling methanol and the solvent almost completely removed from the extract under reduced pressure. The extract was partitioned between carbon tetrachloride (500 ml) and water (500 ml) and the aqueous portion concentrated by evaporation under reduced pressure.

The aqueous concentrate was chromatographed on paper with n-butanol, ethanol, and water (4 : 1 : 4), and the dried papers (5 min at 105°) treated with ammoniacal silver nitrate or silver nitrate–potassium dichromate.[6] A large brown spot, *R*(glucotropaeolin) 1·0, indicated the presence of glucotropaeolin in the extract.

The aqueous concentrate was adjusted to pH 6·8 by the addition of a 1M sodium citrate solution, followed by a myrosinase preparation (5 ml) from yellow mustard powder[11] and ascorbic acid (5 mg). This mixture was incubated at room temperature for 24 hr. During this period a sharp, slightly lachrymatory odour developed in the extract. The digested extract was steam-distilled with cohobation of the aqueous layer, using an oil trap containing benzene (2 ml) to collect any steam-volatile product. The residual aqueous material was mixed with a barium chloride solution, yielding a heavy white precipitate of barium sulphate, which did not appear prior to digestion with myrosinase. The clear filtrate was chromatographed on paper, using the procedure described above. A new spot appeared at *R*(glucose) 1·0, reacting only to the ammoniacal silver nitrate spray reagent.

The benzene-soluble steam distillate was mixed with methanol which had been saturated with ammonia (10 ml), stoppered, and held overnight. This yielded, after three crystallizations from ethyl acetate, colourless rhombs of benzyl thiourea, m.p. 161·5–163° (Found: C, 58·0; H, 6·3; N, 16·4. Calc. for $C_8H_{10}N_2S$: C, 57·8; H, 6·1; N, 16·9%). This demonstrated that benzyl isothiocyanate, sulphate, and glucose were released by the incubation of the extract with myrosinase, consistent with the presence of glucotropaeolin.[6]

(*b*) L. bonariense

The seed-bearing portions of fresh plants obtained near Lowood, Qld., were extracted whole in the same manner as the *L. hyssopifolium* seeds above, and the aqueous concentrate thus obtained was examined by paper chromatography, as before. Two spots appeared, reacting to both spray reagents: one, the stronger of the two, *R*(glucotropaeolin) 1·0, and the other at *R*(glucotropaeolin) 0·30.

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(c) C. bursa pastoris

Fresh plants, obtained at Moggill, Qld., were extracted as for *L. hyssopifolium* and the resultant aqueous concentrate examined by paper chromatography, incubated with the myrosinase preparation, followed by application of the tests for sulphate and glucose as described above. A faint but definite spot appeared in the aqueous concentrate at R(glucotropaeolin) 0·30 (sinigrin), and a positive test was obtained for the presence of sulphate and glucose in the concentrate, after incubation with myrosinase.

Further fresh plants (80 kg) were steam-distilled in a batchwise operation (10 kg lots), using an oil trap containing diethyl ether, for 16 hr periods. The combined ether extracts were concentrated by careful removal of solvent through a column of Fenske helices (10 cm) to a small volume (10 ml). This concentrate was fractionated through the semi-micro still to yield eight fractions of b.p. range 36–105°. Fraction 8, b.p. 79–105° (1 ml), n_D^{25} 1·3949, was shown by gas chromatography to contain two principal components. The infrared spectrum of this fraction as a liquid smear showed absorption bands at 2255 (C≡N), 1645, 1420, 988, and 918 cm^{-1} (vinyl), attributed to the component of interest and a strong band at 3480 cm^{-1} (hydroxyl) from the other component (ethanol).

Fraction 8 (0·8 ml) was mixed with mercaptoacetic acid and hydrogen chloride in the manner described by Condo *et al.*[12] for the characterization of nitriles as their α-iminoalkyl mercaptoacetic acid hydrochlorides. A derivative was obtained, m.p. 63° (dec.), which behaved identically with the corresponding derivative of allyl cyanide, b.p. 118·5°, n_D^{25} 1·4039, obtained by synthesis from allyl bromide.[13] Allyl cyanide is a well-known product of the decomposition of sinigrin during steam distillation from plant material, having been isolated by Will and Korner from oil of mustard in 1863.[14]

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