

# SOME CONSTITUENTS OF *APIUM LEPTOPHYLLUM* (D.C.) F. MUELL. IN RELATION TO TAINING OF MILK

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*Apium leptophyllum* (D.C.) F. Muell. (slender celery, wild carrot, or carrot weed) of the Umbellifereae family is a common annual weed of improved pastures and other cultivated areas in south-east Queensland.<sup>1,2</sup> The weed is one of several which are frequently grazed by livestock in the dry winter months and are claimed to impart an objectionable flavour to the milk of dairy cattle. However, conclusive evidence of tainting by *A. leptophyllum* is lacking. Little is known of the chemical nature of tainting substances although in the case of the cruciferous weeds, mustard-oil glucosides and their breakdown products are held responsible.<sup>3,4</sup>

Examination of *A. leptophyllum* plants from the Lockyer district of Queensland yielded no trace of mustard-oil glycosides. Steam distillation of fresh plants yielded 0.25% of essential oil possessing an odour reminiscent of crushed carrot leaves and consisting of thymol methyl ether ( $\approx 7\%$  by wt.), carvacrol methyl ether ( $\approx 9\%$ ), thymoquinol dimethyl ether (29%), 11 terpene hydrocarbons (totaling 45%), and not less than 10 minor constituents including probably thymol, carvacrol, and sesquiterpene hydrocarbons. The 11 terpene hydrocarbons were provisionally identified by gas chromatography as, in order of decreasing relative amount,  $\gamma$ -terpinene, *p*-cymene,  $\beta$ -pinene,  $\alpha$ -pinene, myrcene,  $\alpha$ -thujene, limonene, sabinene, *cis*- and *trans*-ocimene, and  $\beta$ -phellandrene.

The principal components of *A. leptophyllum* oil have been administered orally to dairy cattle to determine if they possess the ability to induce the formation of a taint in the milk or derived butter. The aromatic ethers failed to induce taint formation even when 15-ml quantities were administered, whereas a dose of 25 ml of terpene hydrocarbons (equivalent to the amount isolated from 22 kg of plants) produced a definite "feed" or eucalyptus-like taint in milk and butter, as judged by a panel of dairy produce graders.

The principal components were added directly to cream and butter to a level detectable by taste and submitted to the panel of graders for taint evaluation. The aromatic ethers produced an unfamiliar taint whereas that produced by 50 p.p.m. of the terpene hydrocarbons was readily associated with the smell of *A. leptophyllum*. However, the graders could not agree on whether this taint was similar to any of the weed taints commonly occurring in dairy produce.

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It can be concluded from these tests that the aromatic ethers would be unlikely to contribute to the formation of a taint in milk or butter obtained from cows ingesting *A. leptophyllum*, whereas ingestion of large quantities (c. 22 kg) of the plant could cause the development of a taint originating from the terpene hydrocarbon constituents.

### Experimental

#### *Absence of Mustard-Oil Glycosides*

Air-dried whole plants (1.3 kg) were extracted with refluxing methanol for 24 hr and the extract was concentrated to yield a residue which was partitioned between carbon tetrachloride and water. The concentrated aqueous layer was examined by paper chromatographic procedures<sup>5-7</sup> for the presence of mustard-oil glucosides, with negative results.

#### *The Essential Oil*

Fresh plants (c. 45 kg) were steam-distilled with cohobation for 72 hr to yield 105 ml of pale yellow oil. Gas chromatograms of the oil and of distilled fractions on Apiezon L, QF-1, and Carbowax 20M columns were consistent with the presence of thymol methyl ether, carvacrol methyl ether, thymoquinol dimethyl ether, a complex mixture of terpene hydrocarbons,<sup>8</sup> minor amounts of thymol and/or carvacrol, and sesquiterpene hydrocarbons which were not further investigated.

#### *Thymol Methyl Ether and Carvacrol Methyl Ether*

The distillate fraction of b.p. 91–92°/10 mm (Found: C, 80.2; H, 10.1; OCH<sub>3</sub>, 14.1. Calc. for C<sub>11</sub>H<sub>16</sub>O: C, 80.4; H, 9.8; 1 × OCH<sub>3</sub>, 18.9%) gave two incompletely resolved peaks (c. 90% purity) with the retention times of thymol and carvacrol methyl ethers on gas chromatography. The fraction showed infrared absorption bands at 2860, 1244, and 1040 cm<sup>-1</sup> (aryl methyl ether) and ultraviolet absorption maxima at 284, 280 (log  $\epsilon$  3.31), and 275.5 m $\mu$  in cyclohexane. The p.m.r. spectrum of the neat fraction showed a peak (3H) at 3.35  $\delta$  (from external tetramethylsilane) attributed to methoxyl protons; also shown were signals for three aromatic protons (6.77–6.1  $\delta$ ) and for a methyl and an isopropyl group attached to an aromatic ring.

Refluxing the oil with hydriodic acid in acetic acid for 1 hr yielded thymol as colourless needles from water, m.p. 51° (Found: C, 79.8; H, 9.5. Calc. for C<sub>10</sub>H<sub>14</sub>O: C, 80.0; H, 9.4%), undepressed by authentic thymol, m.p. 51°.

#### *Thymoquinol Dimethyl Ether*

The distillate fraction of b.p. 120–121°/10 mm (Found: C, 75.0; H, 9.8; OCH<sub>3</sub>, 27.3. Calc. for C<sub>12</sub>H<sub>16</sub>O<sub>2</sub>: C, 74.2; H, 9.3; 2 × OCH<sub>3</sub>, 31.9%) gave substantially (c. 90%) one peak on gas chromatography, showed infrared absorption bands at 2850, 1210, and 1048 cm<sup>-1</sup> (for aryl methyl ether), and ultraviolet absorption maxima at 292 and 290 m $\mu$  (log  $\epsilon$  3.58) in cyclohexane. The p.m.r. spectrum of the neat fraction showed signals for two aromatic protons as singlets at 3.52 and 3.62  $\delta$  (from external tetramethylsilane), two 3-proton singlets at 6.60 and 6.65  $\delta$ , and further signals for a methyl and an isopropyl group attached to an aromatic ring.

Refluxing the oil with hydriodic acid yielded thymoquinol as colourless prisms from water, m.p. 140.5–141.5° (Found: C, 72.4; H, 8.4. Calc. for C<sub>10</sub>H<sub>14</sub>O<sub>2</sub>: C, 72.3; H, 8.4%) which were benzoylated to yield colourless needles from ethanol, m.p. 142–143°. Thymoquinol and its dibenzoate melt at 141.5° and 141–142° respectively.

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