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FURANOACETYLENE AND ISOFLAVONOID PHYTOALEXINS IN *LENS CULINARIS*

DAVID J. ROBESON

Phytochemical Unit, Department of Botany, Plant Science Laboratories, University of Reading, U.K.

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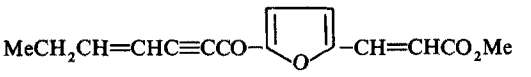
Key Word Index—*Lens culinaris*; *L. nigricans*; Vicieae; Leguminosae; lentil; phytoalexin; furanoacetylene; wyerone epoxide; variabilin.

Abstract—The ability of two *Lens* species to synthesise the furnaoacetylenes wyerone and wyerone epoxide, as well as the pterocarpan variabilin, has been demonstrated. This links *Lens* more close to *Vicia* than to the remaining genera of the tribe Vicieae.

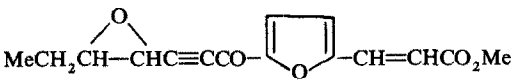
Recent studies of phytoalexin induction have proved to be of taxonomic as well as of phytopathological interest [1]. The majority of leguminous plants examined have been found to acumulate isoflavonoid compounds in response to fungal invasion [2]. An interesting anomaly however is the production of furanoacetylenic phytoalexins by *Vicia faba* L. (tribe Vicieae) [3]. If the recent recommendation [4] to exclude the genus *Cicer* L. from the Vicieae is accepted, the tribe then consists of the four genera *Pisum* L., *Lathyrus* L., *Vicia* L. and *Lens* Miller. It was therefore of interst to determine whether *Lens* showed a similar response to *Vicia* by producing furanoacetylenes as opposed to the more typical responses of *Pisum* and *Lathyrus* which accumulate the isoflavonoid phytoalexin pisatin [5].

Despite the economic importance of *Lens culinaris* Medik., comparatively little attention has been paid to the nature of its dynamic resistance to fungal attack. The technique employed for the examination of the seeds of *Lens* spp. (*Lens culinaris* Medik and *Lens nigricans* Godr.) was essentially that of Hargreaves *et al.* previously reported for phytoalexin studies on *V. faba* [6]. It has been successfully used for the detection of furanoacetylenic phytoalexins in a range of *Vicia* spp. [7]. Imbibed seeds, from which the testas have been removed, are immersed in a dense spore suspension of *Botrytis cinerea* Pers. before incubation on moist tissue paper in a damp chamber for 5 days at 22°. Control seeds are surface sterilised in 1% NaClO and rinsed in sterile de-ionised water ×3 prior to incubation as above. At the end of the incubation period inoculated seeds have normally developed brown or black, necrotic, limited lesions on the surface of the cotyledons. Et_2O extracts (×4) are bulked, dried and dissolved in a small vol. of EtOH for TLC (Merck Si gel G, F_{254}, 0.25 mm). When this technique was applied to *L. culinaris* and the developed chromatogram (*n*-hexane–Me_2CO, 2:1, then after drying $CHCl_3$–petrol (bp 60–80°), 2:1) was examined under long wavelength UV light, two deep blue fluorescent bands were present. The upper band ran opposite an authentic sample of wyerone to R_f 0.60 while the lower band, which was much more intense ran to R_f 0.52. The upper band was purified by TLC in $CHCl_3$–MeOH (50:1) and after elution in Et_2O–*n*-hexane (3:1). In each of the above solvent systems it had the same R_f (0.63 and 0.47 respectively) as an authentic sample of wyerone run on the same chromatogram. The lower band was also purified by TLC in $CHCl_3$–MeOH, 50:1 (R_f 0.62) then Et_2O–*n*-hexane (3:1) (R_f 0.42). It gave an orange colouration on a yellow background after spraying the chromatogram with picric acid reagent [8] which is specific for epoxides. UV and MS data confirmed the identity of the upper and lower bands as the furanoacetylenic ketoesters wyerone (**1**) and wyerone epoxide (**2**) respectively. The presence with wyerone of the dihydro derivative (**3**) previously reported as a contaminant of the natural product was apparent from the shift in the UV spectrum after treatment of the sample with piperidine, the relatively intense peak in the MS at *m/e* 260, and the *m/e* 258: *m/e* 151 intensity ratio [9].

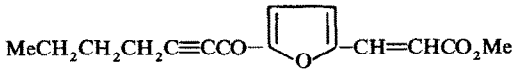
The zone on the original chromatogram between R_f 0.25–0.33 was eluted and chromatographed (TLC, $CHCl_3$–MeOH, 50:1) to afford a quenching band (short $\lambda_{254\,nm}$ UV) at R_f 0.50 identified from TLC, UV and MS data [10, 11] as variabilin 6a-hydroxy-3,9-dimethoxypterocarpan (**4**). Variabilin (= homopisatin) was also detected in diffusates from detached leaflets



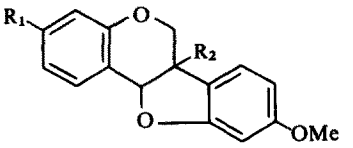
(1)



(2)



(3)



(4) R_1 = OMe, R_2 = OH

(5) R_1 = OH, R_2 = H

using the standard drop-diffusate technique [2] in which a conidial suspension of *Helminthosporium carbonum* Ullstrup acted as the inducing agent, and in MeOH extracts of etiolated epicotyls treated with $HgCl_2$ solution (5×10^{-4} M). Variabilin was previously known as a constituent of *Dalbergia variabilis* [12] and has been reported to occur in infected red clover leaves [10].

The exact role and relative importance of the various phytoalexins in restricting fungal invasion of the lentil is not known and more detailed quantitative studies are required; such studies may be complicated by the presence of other, as yet unidentified, antifungal compounds in extracts of infected tissue. It is interesting that in the cotyledons of *L. culinaris* wyerone epoxide occurs in greater concentrations than wyerone, a reversal of the situation in *V. faba* [3, 6]. Provisional investigations indicate, however, that the concentration of wyerone epoxide present in infected *Lens* cotyledons is significantly lower than that reported for *V. faba* [3] and that wyerone occurs only in comparatively trace amounts in the lentil.

When the cotyledons of *Pisum sativum* L. cv Dorina and *Lathyrus sativus* L. cv Canberra City were challenged with spore suspensions of *B. cinerea* and extracts of infected tissue subjected to TLC as described above furanoacetylenic phytoalexins were not detected. Instead pisatin accumulated to relatively high concentrations (1015 and 454 μg/g fr. wt respectively).

The multiple phytoalexin response of *Lens* is clearly different to any that has previously been described but it is similar to that of *V. faba* which is known to produce small amounts of the isoflavonoid medicarpin (**5**) [13] in addition to the furanoacetylenic phytoalexins. The relationship of *Lens* to *Vicia* and/or *Lathyrus* has long been a subject of controversy. No evidence has yet been found for the occurrence of pisatin in *Vicia* or *Lens* and although certain *Lathyrus* spp. produce variabilin and/or medicarpin as a minor component of their phytoalexin response [7] the apparently rare ability to synthesise the furanoacetylenes wyerone and wyerone epoxide links *Lens* and *Vicia* very closely and at the same time distinguishes them from *Pisum* and *Lathyrus*. Thus it would appear that there is a distinct dichotomy as regards phytoalexin induction within the tribe Vicieae.

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ESSENTIAL OIL FROM CHINESE DRUG 'CAODOUKOU', THE SEEDS OF *ALPINIA KATSUMADAI*

YASUHISA SAIKI*, YOHKO ISHIKAWA†, MITSUO UCHIDA† and SEIGO FUKUSHIMA†

*Faculty of Pharmaceutical Sciences, Kôbe-Gakuin University, Tarumi-ku, Kôbe 673 Japan;
†Shizuoka College of Pharmacy, Shizuoka-shi 422 Japan

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Chinese drug 'caodoukou' has been used as a remedial agent for malaria, for cancer-like symptoms of the stomach and throat and for nausea associated with pregnancy (1). This drug was regarded as originating from the seeds of *Alpinia katsumadai* Hayata a zingiberaceous plant native in Hainan Island of southern China (1).

Steam distillation of the drug affords an essential oil in about 1.5% yield. GLC (SE 30) of the oil showed the presence of 22 components. Three main components were isolated and identified as 1,8-cineole, α-humulene and *trans, trans*-farnesol by comparison with their spectral data.

By GC–MS of 8 other constituents were identified as