# Phytoalexins of Hyacinth Bean (*Lablab niger*)

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(Z. Naturforsch. **32 c**, 1018–1020 [1977]; received August 9, 1977)

Leguminosae, *Lablab*, Phytoalexins, Isoflavonoids, Benzofuran

Seven isoflavonoid phytoalexins have been isolated from the fungus inoculated hypocotyls of *Lablab niger* and identified as the isoflavone 2′-hydroxygenistein, the isoflavanones dalbergioidin and kievitone, the pterocarpan phaseollidin and the isoflavans demethylvestitol, isovestitol and laxifloran. Small quantities of the 2-arylbenzofuran, vignafuran, were also detected.

Phytoalexin production by the grain legume, hyacinth bean (*Lablab niger* Medik.; syn. *Dolichos lablab* L.) was first described by Smith [1] who found that antifungal material accumulated in spore suspensions of *Colletotrichum lindemuthianum* (race δ) when these were incubated in the seed cavities of detached pods. Only slight antifungal activity was associated with samples from pods treated with distilled water. More recently, an isoflavone of undetermined constitution was reported as a possible phytoalexin of hyacinth bean hypocotyls [2]. *Lablab niger* is widely cultivated as a vegetable in south-eastern Asia, India, Egypt and the Sudan where it is particularly important as a cover crop [3]: in view of its agricultural value, a detailed examination of the phytoalexins characteristic of hyacinth bean has now been undertaken. Eight compounds have been isolated from this species and their identification and antifungal properties are described below.

Excised, etiolated hypocotyls [4] were inoculated with a conidial suspension of the fungus *Helminthosporium carbonum* Ullstrup [4] ($5 \times 10^4$ spores/ml in 2% [w/v] aqueous glucose) and incubated (22 ± 2 °C; approx. 400 lx) for 48 h. Hypocotyl tissues directly beneath the inoculation sites were then removed and extracted with EtOH [5]. TLC (Si gel [2]; $CHCl_3$ : MeOH, 25 : 1) of these extracts afforded 5 major phenolic bands at approx. $R_F$ 0.66, 0.54, 0.36, 0.13 and 0.07; a minor phenolic band (approx. $R_F$ 0.31) was also observed. All the above zones were eluted (EtOH) and further purified (see Experimental) to give the following compounds: 2′-hydroxygenistein (**1**) (5,7,2′,4′-tetrahydroxyisoflavone), dalbergioidin (**2**) (5,7,2′,4′-tetrahydroxyisoflavanone), kievitone (**3**) (5,7,2′,4′-tetrahydroxy-8-isopentenylisoflavanone), phaseollidin (**4**) (3,9-dihydroxy-10-isopentenylpterocarpan), demethylvestitol (**5**) (7,2′,4′-trihydroxyisoflavan), isovestitol (**6**) (7,4′-dihydroxy-2′-methoxyisoflavan) laxifloran (**7**) (7,4′-dihydroxy-2′,3′-dimethoxyisoflavan) and the non-isoflavonoid benzofuran-derivative, vignafuran (**8**). None of the above compounds were isolated from the tissues of hypocotyls treated with deionised water [4].

Compounds **1** – **6** and **8** were identified by UV, TLC (5 solvent systems) and MS (**2** – **6**) comparison with authentic specimens. A sample of laxifloran (**7**) was not available for comparative purposes. However, the MS of **7** (see Experimental) was entirely consistent with its formulation as an isoflavan having a single A ring OH substituent and a trioxygenated B ring (one OH and two $OCH_3$ groups) [6]; indeed, the MS was essentially identical with those of mucronulatol (**9**) and isomucronulatol (**10**) [7, 8]. Methylation ($CH_2N_2$) gave a dimethyl ether (**11**) indistinguishable (UV, MS, TLC) from 7,2′,3′,4′-tetramethoxyisoflavan produced from **9** and **10**. Compound **7** did not react when sprayed with Gibbs reagent in marked contrast to **9** and **10** both of which afforded deep blue derivatives; the single B ring OH group of **7** can thus be assigned to C-4′ rather than C-2′ or 3′. Like other isoflavans with 4′-hydroxylation (*e. g.* 7,4′-dihydroxyisoflavan, **5** and **6**) compound **7** gives a predominantly orange derivative with diazotised *p*-nitroaniline; **9** and **10** both give a yellow colouration with this reagent. All the above data suggest that **7** is identical with the rare isoflavan laxifloran, a compound previously isolated only from roots of the leguminous African shrub, *Lonchocarpus laxiflorus* [6].

The concentration of compounds **1** – **8** in hypocotyl tissues inoculated with *H. carbonum* is shown in Table I. Isovestitol, laxifloran, phaseollidin and kievitone are clearly the major isoflavonoid phytoalexins of hyacinth bean with 2′-hydroxygenistein, dalbergioidin and demethylvestitol occurring as comparatively minor constituents. Rapid metabolism of the latter compounds to give kievitone (**1** → **2** → **3**) or isovestitol/laxifloran (**5** → **6** → **7**) presumably limits their accumulation.

Tests against the mycelial growth of *H. carbonum* [4] indicated that isovestitol ($ED_{50}$ 17 μg/ml) [5], phaseollidin ($ED_{50}$ 30 – 35 μg/ml) [9], demethylvestitol ($ED_{50}$ approx. 38 μg/ml) [5] and kievitone ($ED_{50}$ 52 μg/ml) were highly inhibitory to this microorganism; synthetic vignafuran (**8**) was also active giving an $ED_{50}$ value of between 15 and 20 μg/ml. Compounds **3**, **4**, and **8** are also inhibitory to a number of other fungi [10–12]. Mycelial growth tests

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Table I. Phytoalexin concentration ($\mu$g/g fr. wt.) in *H. carbonum*-inoculated hypocotyl tissues of hyacinth bean [a, b, c].

| Compound | Concentration |
|---|---|
| 2′-hydroxygenistein (**1**) | 36 |
| Dalbergioidin (**2**) | 29 |
| Kievitone (**3**) | 148 |
| Phaseollidin (**4**) | 170 |
| Demethylvestitol (**5**) | 47 |
| Isovestitol (**6**) | 372 |
| Laxifloran (**7**) | 181 |
| Vignafuran (**8**) | 5 |

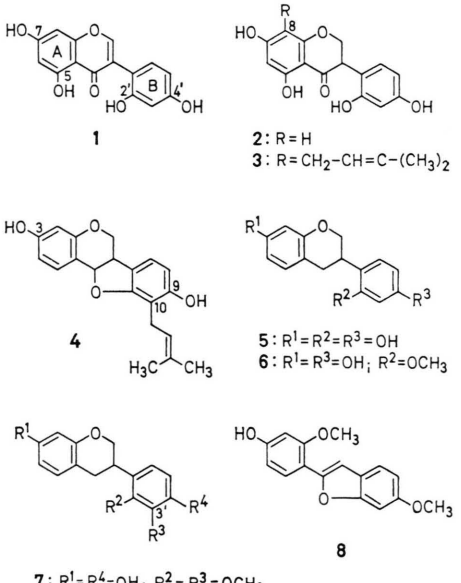
[a] Values are mean of two determinations involving tissue samples of 1.03 and 2.14 g fr. wt.

[b] Concentrations (determined after 48 h incubation) of **2**, **3**, **4**, and **8** are based on previously reported extinction coefficients (**2**, log $\varepsilon$=4.31 at 288 nm [19]; **3**, log $\varepsilon$=4.17 at 294 nm [20]; **4**, log $\varepsilon$=3.78 at 286.5 nm [11]; **8**, log $\varepsilon$=4.59 at 320 nm [12]). Values for **1**, **5**/**6**, and **7** are based respectively on log $\varepsilon$ for genistein (4.63 at 262 nm [2]), vestitol (3.62 at 285 nm [21]) and mucronulatol (3.62 at 282 nm [8]).

[c] Compounds **1**—**8** were absent from control hypocotyls.

were not undertaken for **1**, **2**, and **7**. However, in a TLC bioassay against *Cladosporium herbarum* Fr. [4] laxifloran (20, 30 and 40 $\mu$g) had antifungal activity comparable with that of isomucronulatol **10**, a phytoalexin of European licorice [7]. Dalbergioidin (30 $\mu$g) was also inhibitory to *C. herbarum* although 2′-hydroxygenistein was only slightly active even at a level of 50 $\mu$g.

2′-hydroxygenistein, phaseollidin, kievitone and vignafuran have previously been isolated as phytoalexins from *Phaseolus vulgaris* (**1**, **3**, **4**) [10, 11, 13], *P. lunatus* (**3**) [14], *Vigna unguiculata* (**3**, **4**, **8**) [12, 15] and *Psophocarpus tetragonolobus* (**4**) [9]. Like *L. niger*, all these species belong to the tribe Phaseoleae. **1** has also been isolated as a minor stem phytoalexin of the taxonomically related species, *Cajanus cajan* (Cajaneae) [2]. Other phytoalexins (*e. g.* phaseollin and phaseollinisoflavan) characteristic of *Phaseolus vulgaris* were not isolated from *L. niger*. Although compounds **2** and **5**—**7** have not previously been associated with any member of the tribe Phaseoleae, the isoflavans **5** and **6** are known to accumulate in the fungus-inoculated leaves of several species belonging to the tribe Loteae including *Tetragonolobus requienii* [5] and *Hosackia americana* (Ingham, unpublished data). In these species, however, isovestitol co-occurs with its isomer vestitol (7,2′-dihydroxy-4′-methoxyisoflavan) a compound not obtained from hyacinth bean; the pterocarpan medicarpin (probably the most common legume phytoalexin) was also absent from *L. niger* despite its formation by *V. unguiculata* [16]. MS analysis of *Lablab* phaseollidin failed to reveal the 3′-isopentenyl derivative of **6** ($M^+$ 340), which is produced by several lines of *V. unguiculata* [17] and which co-chromatographs with phaseollidin ($M^+$ 324) in the TLC solvents used to purify this latter substance. Dalbergioidin (an isoflavonoid from the leaves and heartwood of *Ougeinia dalbergioides*; tribe Desmodieae) [18] and laxifloran are reported for the first time as phytoalexins of the Leguminosae. Recent studies indicate that phytoalexins are also produced by other species (*e. g. Pachyrrhizus erosus*) closely related to *L. niger* and these compounds are now under active investigation.




## *Experimental*

Mass and UV spectra were determined as previously described [2].

*Isolation and purification of compounds* **1**—**7**. Si gel TLC ($CHCl_3$ : MeOH, 25 : 1) of hypocotyl extracts (EtOH) gave six fluorescence quenching bands at approx. $R_F$ 0.66 ((B-1), 0.54 (B-2), 0.36 (B-3), 0.31 (B-4), 0.13 (B-5), and 0.07 (B-6). The above zones were eluted (EtOH) and purified (Si gel TLC) as follows, a) B-1, $CHCl_3$ (2 X) gave **8**, b) B-2, $CHCl_3$ (2 X) **4** (upper zone) and **7** (lower zone), c) B-3, *n*-pentane : $Et_2O$ : HOAc (PEA) (75 : 25 : 3, 3 X) **6**, d) B-5, PEA (75 : 25 : 6, 4 X) **3** (upper zone) and **1** (lower zone), e) B-6, PEA (75 : 25 : 6, 4 X) **5** (upper zone) and **2** (lower zone). Purification of B-4 (PEA, 75 : 25 : 3, 3 X) afforded traces of an isoflavonoid-like compound which was not identified. When sprayed with diazotised *p*-nitroaniline, vignafuran (**8**) gave a

purple/brown colouration; all the other compounds afforded orange (**2** – **7** and B-4) or orange/yellow (**1**) derivatives with this reagent.

*Compounds* **1** – **6** *and* **8**. MS and UV maxima as lit.[2, 5, 10, 12, 19, 20]; all were indistinguishable (TLC) from authentic specimens.

*7,4′-dihydroxy-2′,3′-dimethoxyisoflavan* (**7**) *(laxifloran)*. $\lambda_{max}$ (nm): EtOH 211, 228 sh, 282, 290 sh; EtOH + NaOH 213, 245, 294; MS (rel. int.) 303 (11), 302 ($M^+$; 52), 181 (12), 180 (100), 179 (9), 169 (5), 168 (37), 167 (34), 165 (24), 153 (9), 152 (9), 151 (23), 147 (15), 137 (9), 135 (26), 134 (9), 133 (24), 123 (24), 111 (10), 107 (16). Dimethyl ether **11** ($CH_2N_2$) ($R_F$ 0.53, $CHCl_3$ : $CCl_4$, 1 : 1) UV and MS as lit[7]. Diacetate (Py-$Ac_2O$) ($R_F$ 0.60, $CHCl_3$) $\lambda_{max}$ (nm) : EtOH 209, 268 sh, 275, 282; MS (rel. int.) 386 ($M^+$; 15), 345 (5), 344 (28), 303 (8), 302 (49), 301 (5), 181 (14), 180 (100), other fragments as given for **7**. Laxifloran (**7**) could be distinguished from the isomeric isoflavans, mucronulatol (**9**) and isomucronulatol (**10**) by TLC in $C_6H_6$ : MeOH, 9 : 1 (**7**/**9**, both $R_F$ 0.51; **10**, $R_F$ 0.57) and PEA, 75 : 25 : 3 (**7**, $R_F$ 0.28; **9**, $R_F$ 0.14; **10**, $R_F$ 0.30).

*Note Added in Proof:* Traces of genistein (5,7,4′-trihydroxyisoflavone) have recently been isolated from the *H. carbonum*-inoculated hypocotyls of *L. niger*. On TLC plates developed in $CHCl_3$ : MeOH (25 : 1) genistein was located immediately below B-4; additional purification (PEA, 75 : 25 : 3, 3×) gave the pure isoflavone indistinguishable (UV, TLC) from an authentic sample. Genistein presumably functions as the biosynthetic precursor of **1**.

The author thanks G. Ibrahim for seeds of *L. niger*, R. W. Butters (Tate and Lyle Ltd.) for MS analyses and L. Farkas, D. A. Smith, J. A. Bailey and P. M. Dewick for samples of **2**, **3**, **4**, and **8** respectively. Financial assistance from the Science Research Council is also gratefully acknowledged.

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