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A NEW ISOFLAVAN PHYTOALEXIN FROM LEAFLETS OF *LOTUS HISPIDUS*

JOHN L. INGHAM* and PAUL M. DEWICK†

* Phytochemical Unit, Department of Botany, University of Reading, Reading, RG6 2AS, U.K.;
† Department of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, U.K.

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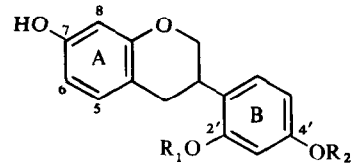
Key Word Index—*Lotus hispidus*; Leguminosae; Loteae; isoflavonoids; isoflavans; phytoalexins; phenolic compounds; antifungal activity; synthesis.

Abstract—A new isoflavonoid phytoalexin isolated from the fungus-inoculated leaflets of *Lotus hispidus* (hairy birdsfoot trefoil) has been identified as 5,4′-dimethoxy-7,2′-dihydroxyisoflavan (5-methoxyvestitol). Three known isoflavans (demethylvestitol, vestitol and sativan) are also produced by *L. hispidus*. The synthesis of 5-, 6- and 8-methoxyvestitol is described. Preparation of the pterocarpan analogues of 6- and 8-methoxyvestitol has allowed the structures of two additional legume phytoalexins to be unequivocally confirmed.

INTRODUCTION

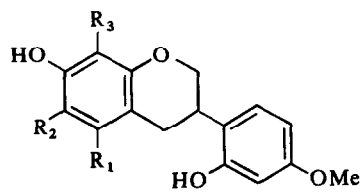
At least 12 isoflavan phytoalexins have been isolated from the fungus-inoculated tissues of species belonging to the Papilionoideae subfamily of the Leguminosae [1–3]. Isoflavans normally co-occur with the correspondingly oxygenated pterocarpans [2–5] although evidence for such an association in *Lotus* (tribe Loteae) and several related genera (e.g. *Anthyllis*, *Dorycnium*, *Hosackia* and *Tetragonolobus*) is currently lacking (Ingham, J. L., unpublished data; [6, 7]). The *Helminthosporium carbonum*-inoculated leaflets of *Lotus corniculatus* produce three biosynthetically related isoflavans which have recently been identified as demethylvestitol (7,2′,4′-trihydroxyisoflavan, **1**), vestitol (7,2′-dihydroxy-4′-methoxyisoflavan, **2**) and sativan (7-hydroxy-2′,4′-dimethoxyisoflavan, **3**) [6, 7]. **1** and **2** are also produced by *L. uliginosus* [7] whilst the allied species, *Tetragonolobus maritimus* (*L. siliquosus*) accumulates **1** together with isovestitol (7,4′-dihydroxy-2′-methoxyisoflavan, **4**) and the 2-arylbenzofuran phytoalexin, 6-demethylvignafuran [7, 8]. As part of a continuing chemosystematic survey of the Leguminosae (Ingham, J. L., unpublished data), we have investigated the phytoalexin response of a third *Lotus* species namely *L. hispidus* (hairy birdsfoot trefoil). Leaflet inoculation with conidial suspensions of *H. carbonum* [9, 10] led to the formation of compounds **1**–**3**; in addition, small quantities of 5-methoxyvestitol (5,4′-dimethoxy-7,2′-dihydroxyisoflavan, **5**) were also obtained from this plant. The isolation, purification and total synthesis of 5-methoxyvestitol (and its 6- and 8-methoxy isomers) are described in the present paper.



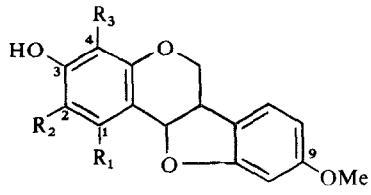
1 $R_1 = R_2 = H$
2 $R_1 = H$; $R_2 = Me$
3 $R_1 = R_2 = Me$
4 $R_1 = Me$; $R_2 = H$

RESULTS AND DISCUSSION

MS analysis of **5** gave M^+ 302 (corresponding to $C_{17}H_{18}O_5$) and prominent fragments at *m/e* 150 (base) and 137, indicative of an isoflavan having the same B-ring substituents (OH; OMe) as vestitol (**2**) [11–12]. The presence of two A-ring substituents (also OH and OMe, the former being assigned to C-7 by analogy with **1**, **2** and **3**) was deduced from the observed fragments at *m/e* 165 and 153 [11, 12]. As expected, compound **5** readily formed both a dimethyl ether (M^+ 330) and a diacetoxy derivative (M^+ 386). In view of its co-occurrence with compounds **1** and **2**, the new isoflavan (**5**) was provisionally identified as either 5-, 6- (**6**) or 8-methoxyvestitol (**7**). When these isomers were synthesized via $Tl(NO_3)_3$ rearrangement of suitable benzyloxy-chalcones and subsequent catalytic hydrogenation of the resulting isoflavones (see Experimental and [8, 14, 15]), the *Lotus* phytoalexin proved to be identical (UV, MS, co-TLC) with 5,4′-dimethoxy-7,2′-dihydroxyisoflavan. Synthetic and natural **5** were easily distinguished from 6- and 8-methoxyvestitol by TLC in $CHCl_3$–MeOH, 50:1 (**5**, R_f 0.19; **6**/**7**, R_f 0.29). 5-Methoxyvestitol is only the second



5 $R_1 = OMe$; $R_2 = R_3 = H$
6 $R_1 = R_3 = H$; $R_2 = OMe$
7 $R_1 = R_2 = H$; $R_3 = OMe$



8 $R_1 = R_3 = H$; $R_2 = OMe$
9 $R_1 = R_2 = H$; $R_3 = OMe$
10 $R_1 = OMe$; $R_2 = R_3 = H$

5-oxygenated isoflavan to be isolated as a natural product, the other being licoricidin [16] from *Glycyrrhiza glabra* (tribe Astragaleae). Its formation by a member of the Loteae is particularly interesting because, in terms of isoflavonoid biosynthesis, several of the genera comprising this tribe appear to be characterized by their exclusive production of simple isoflavan phytoalexins. Repeated examination of *L. hispidus* has failed to reveal any compounds belonging to the other recognized isoflavonoid groups.

Of the 4 isoflavans produced by *L. hispidus*, only vestitol (95–143 μg/ml diffusate) has been found to accumulate in quantities significantly greater than its ED_{50} value (17 μg/ml) [7] as determined against mycelial growth of *H. carbonum* (cf. **1**, 6–11 μg/ml, ED_{50} *ca* 38 μg/ml; **3**, 4–9 μg/ml, ED_{50} 10 μg/ml) [7, 10]. Diffusate concentrations of 5-methoxyvestitol (13–22 μg/ml based on $\log \varepsilon = 3.63$ at 281 nm for **2** [6]) were also slightly lower than the ED_{50} value (*ca* 30 μg/ml) recorded for this compound.

Despite recent comprehensive surveys (Ingham, J. L., unpublished data), neither 6- nor 8-methoxyvestitol are known to act as phytoalexins in the Leguminosae. However, the corresponding pterocarpans (2,9-dimethoxy-3-hydroxypterocarpan (**8**) and 3-hydroxy-4,9-dimethoxypterocarpan (**9**)) have been isolated from the fungus-inoculated tissues of *Pisum sativum* [17] and *Trifolium cherleri* (Ingham, J. L., unpublished data), respectively. Both compounds were initially identified largely on the basis of spectroscopic data and as yet a comparison with synthetic material has not been undertaken. Simple 2′-hydroxylated isoflavans can be readily converted to the analogous pterocarpans by oxidation using DDQ [18, 19]. When this procedure was applied to 6- and 8-methoxyvestitol, the resulting products were indistinguishable (UV, MS, co-TLC) from naturally-occurring **8** and **9**. Pterocarpan **8** was also obtained in lower yield by $NaBH_4$ reduction of the corresponding 2′-hydroxyisoflavone [15]. Compounds **8** and **9** could be separated from each other and from 1,9-dimethoxy-3-hydroxypterocarpan (**10**) (obtained by DDQ oxidation of **5**) by Si gel TLC in $CHCl_3$–MeOH, 50:1 (**8**, R_f 0.73; **9**, R_f 0.68; **10**, R_f 0.36).

EXPERIMENTAL

Mass and UV spectra were determined as previously described [20].

Isolation of compounds **1**–**3** *and* **5**. Leaves of *Lotus hispidus* Desf. were collected from an established plant growing at the Royal Botanic Gardens, Kew, England. Individual leaflets were treated with droplets of de-ionized H_2O (control) or spore suspensions of *Helminthosporium carbonum* and the resulting diffusates (48 hr) extracted (EtOAc) as described elsewhere [9, 10]. Si gel TLC ($CHCl_3$–MeOH, 50:1) [20] of the extracts gave **3** (R_f 0.59), **2**/**5** (R_f 0.19) and **1** (R_f 0.03). Compounds **1** and **3** were eluted (EtOH) and purified as previously reported [7]. Eluates of the **2**/**5** zone were chromatographed in *n*-pentane–Et_2O–HOAc (75:25:3, ×3) to afford **2** (upper zone) and **5** (lower zone) as well-separated bands. Isoflavans **1**–**3** were firmly identified by TLC comparison (3 solvent systems) with authentic material [6, 7]. MS and UV data for **1**, **2** and **3** as lit. [6, 7, 10, 13]. There was no evidence to suggest that extracts of *H. carbonum*-induced diffusates contained isovestitol (**4**) [7] or any other fungitoxic *O*-heterocyclic compounds. Isoflavans **1**–**3** and **5** were not isolated from control diffusates.

5,4′-Dimethoxy-7,2′-dihydroxyisoflavan (**5**). Diazotized *p*-nitroaniline, orange/yellow, Gibbs reagent, deep blue. λ_{max}^{EtOH} nm: 212 (100%), 230 sh (50%), 276 sh (14%), 280 (15%), 286 sh (13%); $\lambda_{max}^{EtOH+NaOH}$ nm: 210 (100%), 244 sh (22%), 291 (13%). MS *m/e* (rel. int.): 302 (M^+; 29), 165 (6), 164 (6), 154 (19), 153 (78), 151 (29), 150 (100), 149 (64), 148 (6), 137 (19), 123 (6), 121 (20), 107 (5). DiMe ether (CH_2N_2) (R_f 0.80, $CHCl_3$–CCl_4, 3:1) λ_{max}^{EtOH} nm: 215 (100%), 228 sh (83%), 275 sh (16%), 278 (17%), 284 sh (14%). MS *m/e* (rel. int.): 330 (M^+; 14), 179 (4), 178 (4), 166 (3), 165 (12), 164 (100), 152 (4), 151 (15), 150 (4), 149 (40), 135 (6), 121 (15). Diacetate (Py–Ac_2O) (R_f 0.78, $CHCl_3$) λ_{max}^{EtOH} nm: 211 (100%), 227 sh (69%), 275 (9%), 281 sh (7%). MS *m/e* (rel. int.): 387 (2), 386 (M^+; 7), 345 (3), 344 (13), 303 (1), 302 (7), 301 (6), 192 (26), 177 (3), 165 (10), 164 (7), 154 (6), 153 (46), 152 (8), 151 (26), 150 (100), 149 (22), 137 (22), 121 (10).

Synthesis of (±)*-5-methoxyvestitol*. 2,4-Dihydroxy-6-methoxyacetophenone [21] (1.3 g) and BzCl (0.91 g) in DMF (50 ml) were stirred at 60° with dry K_2CO_3 (10 g) and dry KI (1 g) for 1.5 hr. The mixture was then diluted with H_2O, and the product filtered off and recrystallized from EtOH to yield 4-benzyloxy-2-hydroxy-6-methoxyacetophenone (1.6 g), mp 87–88°, lit. 90–91° [22]. This acetophenone (0.5 g) and 2-benzyloxy-4-methoxybenzaldehyde (0.5 g) in EtOH (15 ml) were stirred at room temp. for 48 hr with KOH (5 g) in H_2O (5 ml). The mixture was diluted with H_2O, extracted with EtOAc (×2), the extracts washed with H_2O (×2) and evaporated to yield 2,4′-dibenzyloxy-2′-hydroxy-4,6′-dimethoxychalcone (0.6 g) which was recrystallized from $CHCl_3$–MeOH, mp 117–122°. The chalcone (0.5 g) was acetylated by treatment with dry Py (10 ml) and Ac_2O (1 ml) (room temp., overnight), and the reaction mixture then poured into H_2O and extracted with EtOAc (×2). The extracts were washed with 5% HCl (×2) and H_2O (×2) and evapd. The acetate was then dissolved in $CHCl_3$ (25 ml) and MeOH (50 ml), and stirred (room temp.) for 3 hr with $Tl(NO_3)_3 \cdot 3H_2O$ (0.5 g). The reaction mixture was neutralized with NaOH in MeOH, concd, diluted with H_2O and extracted with $CHCl_3$ (×2). The $CHCl_3$ extracts were evapd, then stirred with KOH (1 g) in MeOH (100 ml) for 1 hr, neutralized (conc HCl) and immediately acidified with 10% HCl (20 ml) and heated under reflux for 2 hr. After concn, the mixture was poured into H_2O, extracted with EtOAc (×2) and the extracts evapd. 5,4′-Dimethoxy-7,2′-dibenzyloxyisoflavone (0.24 g) was isolated by Si gel TLC (C_6H_6–EtOAc–MeOH–petrol (60–80°), 6:4:1:3) and crystallized from MeOH, mp 117–120°. ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 3.74 (3H, *s*, OMe), 3.87 (3H, *s*, OMe), 5.0 (2H, *s*, O-C<u>H</u>$_2$-Ph), 5.07 (2H, *s*, O-C<u>H</u>$_2$-Ph), *ca* 6.5 (4H, *m*, H-3′, -5′, -6, -8), 7.22 (5H, *s*, O-CH_2-*Ph*), 7.28 (1H, *d*, $J = 9$ Hz, H-6′), 7.35 (5H, *s*, O-CH_2-<u>Ph</u>), 7.70 (1H, *s*, H-2). This isoflavone (40 mg) in HOAc (10 ml) was hydrogenated over Pd/C (10%, 25 mg). Work-up and TLC (C_6H_6–EtOAc–MeOH–petrol (60–80°), 6:4:1:6) yielded (±)-5-methoxyvestitol as a gum. Synthetic and natural 5-methoxyvestitol were indistinguishable by UV, MS and TLC in $CHCl_3$–MeOH, 50:1 (R_f 0.19), *n*-pentane–Et_2O–HOAc, 75:25:3 (R_f 0.16) and C_6H_6–MeOH, 9:1 (R_f 0.23).

1,9-*Dimethoxy*-3-*hydroxypterocarpan* (**10**). DDQ oxidation of **5** (*ca* 1 mg) [19] gave **10** (*ca* 0.8 mg) in high yield (R_f 0.36, $CHCl_3$–MeOH, 50:1). Diazotized *p*-nitroaniline, orange/yellow; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 215 (100%), 233 sh (52%), 282 sh (23%), 286 (24%), 292 sh (21%); $\lambda_{max}^{EtOH+NaOH}$ nm: 214 (100%), 254 (24%), 285 (16%), 292 sh (15%). MS *m/e* (rel. int.): 301 (16), 300 (M^+; 100), 286 (7), 285 (46), 185 (5), 177 (6), 164 (7), 162 (7), 161 (9), 149 (5), 148 (11), 139 (5), 137 (5).

Synthesis of (±)-6-*methoxyvestitol*. 2,4-Dibenzyloxy-5-methoxyacetophenone [23] (0.5 g) and 2-benzyloxy-4-methoxybenzaldehyde (0.5 g) in EtOH (25 ml) were stirred (room temp., overnight) with KOH (3 g) in H_2O (3 ml). The supernatant liquid was decanted and the precipitated gum washed with a little EtOH and taken up in EtOAc (50 ml). This soln was washed with H_2O and then evapd to give 2,2',4'-tribenzyloxy-4,5'-dimethoxychalcone as a gum (0.75 g). Without further purification, the chalcone (0.75 g) was dissolved in $CHCl_3$ (20 ml) and MeOH (40 ml), and stirred (room temp., 1 hr) with $Tl(NO_3)_3.3H_2O$ (0.6 g). The mixture was concd, treated with H_2O and then extracted with $CHCl_3$ (×2). The $CHCl_3$ extracts were evapd, then treated at 80° for 2 hr with HOAc (30 ml) and conc HCl (10 ml). The reaction mixture was poured into H_2O, extracted with EtOAc (×2), the extracts washed successively with aq. $NaHCO_3$ (×2) and H_2O, and then evapd. TLC purification (C_6H_6–EtOAc–MeOH, 6:4:1) of the product gave 6,4'-dimethoxy-7,2'-dihydroxyisoflavone (105 mg) which was crystallized from $CHCl_3$, mp 199°. ^1H NMR (60 MHz, $CDCl_3/(CD_3)_2CO$, TMS): δ 3.74 (3H, *s*, OMe), 3.85 (3H, *s*, OMe), 6.43 (1H, *dd*, J = 8, 2 Hz, H-5'), 6.49 (1H, *d*, J = 2 Hz, H-3'), 6.95 (1H, *s*, H-8), 7.07 (1H, *d*, J = 8 Hz, H-6'), 7.53 (1H, *s*, H-5), 8.11 (1H, *s*, H-2), 9.40 (1H, *br*, OH). Hydrogenation of this isoflavone (50 mg) in HOAc (10 ml) over Pd/C (10%, 50 mg) gave, after work-up and Si gel TLC (C_6H_6–EtOAc–MeOH–petrol (60–80°), 6:4:1:6, then $CHCl_3$–*iso*-PrOH, 10:1), (±)-6-methoxyvestitol (**6**) as a gum.

6,4'-*Dimethoxy*-7,2'-*dihydroxyisoflavan* (**6**). Diazotized *p*-nitroaniline, yellow/orange; Gibbs reagent, deep blue. λ_{max}^{EtOH} nm: 212 (100%), 227 sh (70%), 281 sh (33%), 288 (39%), 294 sh (35%), 298 sh (32%), 305 sh (23%); $\lambda_{max}^{EtOH+NaOH}$ nm: 215 (100%), 244 sh (11%), 301 (12%). MS *m/e* (rel. int.): 303 (6), 302 (M^+; 50), 166 (9), 165 (25), 164 (20), 153 (27), 151 (17), 150 (100), 149 (31), 138 (14), 137 (52), 133 (18), 121 (15). DiMe ether (R_f 0.59, $CHCl_3$–CCl_4, 1:1) λ_{max}^{EtOH} nm: 210 (100%), 227 sh (63%), 280 sh (32%), 286 (34%), 293 sh (32%), 303 sh (25%). MS *m/e* (rel. int.): 331 (6), 330 (M^+; 39), 179 (16), 178 (17), 177 (5), 166 (8), 165 (12), 164 (84), 163 (5), 152 (16), 151 (96), 150 (8), 149 (79), 138 (9), 135 (7), 128 (5), 122 (5), 121 (100), 119 (13). Diacetate (R_f 0.57, $CHCl_3$) λ_{max}^{EtOH} nm: 210 (100%), 224 (93%), 277 sh (29%), 284 (33%), 293 (34%). MS *m/e* (rel. int.): 386 (M^+; 1), 344 (10), 302 (3), 167 (9), 165 (11), 164 (8), 153 (6), 151 (5), 150 (33), 149 (52), 138 (6), 137 (21), 133 (8), 121 (11); base peak at *m/e* 43.

2,9-*Dimethoxy*-3-*hydroxypterocarpan* (**8**). DDQ oxidation of **6** (*ca* 0.8 mg) gave **8** (*ca* 0.5 mg; R_f 0.73, $CHCl_3$–MeOH, 50:1). Diazotized *p*-nitroaniline, yellow; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 210 (100%), 232 sh (28%), 288 sh (23%), 293 (25%), 303 sh (14%); $\lambda_{max}^{EtOH+NaOH}$ nm: 216 (100%), 252 (7%), 296 (10%). MS as lit. [17].

Synthesis of (±)-8-*methoxyvestitol*. 4-Benzyloxy-2-hydroxy-3-methoxyacetophenone [24] (0.5 g) and 2-benzyloxy-4-methoxybenzaldehyde (0.5 g) in EtOH (25 ml) were stirred (room temp., 48 hr) with KOH (4 g) in EtOH (4 ml). The ppt. was filtered off, washed with H_2O and recrystallized from $CHCl_3$–MeOH to give 2,4'-dibenzyloxy-2'-hydroxy-3',4-dimethoxychalcone (0.57 g), mp 146–147°. This chalcone was acetylated as described above. The resulting acetate was dissolved in $CHCl_3$ (50 ml)–MeOH (50 ml) and stirred (room temp., 3 hr) with $Tl(NO_3)_3.3H_2O$ (0.5 g). The mixture was neutralized with NaOH in MeOH, filtered, and the filtrate concd, poured into H_2O and extracted with $CHCl_3$ (×2). The extracts were evapd, stirred with KOH (1 g) in MeOH (75 ml) for 1 hr, neutralized (conc HCl) and then acidified with 10% HCl (20 ml) and heated under reflux for 1 hr. After cooling, the mixture was concd, diluted with H_2O and extracted with EtOAc (×2). The extracts were then washed with H_2O and evapd to give 7,2'-dibenzyloxy-8,4'-dimethoxyisoflavone which was crystallized from MeOH (0.37 g), mp 103–104°. ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 3.74 (3H, *s*, OMe), 3.95 (3H, *s*, OMe), 5.0 (2H, *s*, O-C<u>H</u>$_2$Ph), 5.19 (2H, *s*, O-C<u>H</u>$_2$-Ph), 6.47 (2H, *m*, H-3', -5'), 6.98 (1H, *d*, J = 9 Hz, H-6 or -6'), 7.20 (1H, *d*, J = 9 Hz, H-6 or -6'), 7.20 (5H, *s*, O-CH$_2$-<u>Ph</u>), 7.32 (5H, *s*, O-CH$_2$-<u>Ph</u>), 7.88 (1H, *d*, J = 9 Hz, H-5), 7.88 (1H, *s*, H-2). This isoflavone (100 mg) in HOAc (10 ml) was hydrogenated over Pd/C (10%, 50 mg). Work-up and TLC (C_6H_6–EtOAc–MeOH–petrol (60–80°), 6:4:1:6) gave (±)-8-methoxyvestitol (**7**) (41 mg) which was recrystallized from MeOH, mp 200–202°.

7,2'-*Dihydroxy*-8,4'-*dimethoxyisoflavan* (**7**). Diazotized *p*-nitroaniline, yellow/orange; Gibbs reagent, deep blue. λ_{max}^{EtOH} nm: 216 (100%), 230 sh (78%), 277 sh (22%), 281 (24%), 286 sh (22%); $\lambda_{max}^{EtOH+NaOH}$ nm: 218 (100%), 244 sh (35%), 294 (21%). MS *m/e* (rel. int.): 303 (4), 302 (M^+; 30), 165 (7), 164 (16), 153 (17), 152 (8), 151 (16), 150 (100), 149 (19), 138 (12), 137 (58), 133 (20), 121 (12). DiMe ether (R_f 0.84, $CHCl_3$) λ_{max}^{EtOH} nm: 214 (100%), 231 sh (62%), 275 sh (21%), 279 (22%), 284 sh (21%). MS *m/e* (rel. int.): 331 (9), 330 (M^+; 36), 179 (16), 178 (32), 177 (6), 166 (13), 165 (12), 164 (55), 163 (6), 152 (16), 151 (100), 150 (8), 149 (66), 148 (5), 137 (6), 123 (8), 121 (38). Diacetate (R_f 0.54, $CHCl_3$) λ_{max}^{EtOH} nm: 208 (100%), 224 (69%), 272 sh (25%), 275 (26%), 280 sh (24%). MS *m/e* (rel. int.): 386 (M^+; 3), 345 (3), 344 (14), 303 (5), 302 (27), 165 (8), 164 (21), 153 (17), 152 (9), 151 (17), 150 (100), 149 (64), 148 (7), 138 (14), 137 (64), 133 (24), 123 (6), 122 (9), 121 (38).

3-*Hydroxy*-4,9-*dimethoxypterocarpan* (**9**). DDQ oxidation of **7** (*ca* 1.5 mg) gave **9** (*ca* 0.6 mg; R_f 0.68, $CHCl_3$–MeOH, 50:1). Diazotized *p*-nitroaniline, yellow; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 215 (100%), 232 sh (53%), 285 (27%), 292 sh (20%); $\lambda_{max}^{EtOH+NaOH}$ nm: 215 (100%), 256 (25%), 284 sh (18%), 287 (19%), 293 sh (17%). MS *m/e* (rel. int.): 301 (17), 300 (M^+; 100), 299 (14), 286 (6), 285 (42), 284 (7), 267 (11), 239 (6), 211 (5), 168 (5), 161 (12), 152 (7), 151 (6), 149 (16), 148 (33), 139 (7), 137 (5).

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REFERENCES

1. Ingham, J. L. (1979) *Proc. Int. Legume Conf. Kew* 1978, in press.
2. Harborne, J. B. and Ingham, J. L. (1978) in *Biochemical Aspects of Plant and Animal Coevolution* (Harborne, J. B., ed.) p. 343. Academic Press, London and New York.
3. VanEtten, H. D. and Pueppke, S. G. (1976) in *Biochemical Aspects of Plant–Parasite Relationships* (Friend, J. and Threlfall, D. R., eds.) p. 239. Academic Press, London and New York.
4. Ingham, J. L. (1978) *Biochem. Syst. Ecol.* **6**, 217.
5. Ingham, J. L. (1979) *Biochem. Syst. Ecol.* **7**, 29.
6. Bonde, M. R., Millar, R. L. and Ingham, J. L. (1973) *Phytochemistry* **12**, 2957.
7. Ingham, J. L. (1977) *Phytochemistry* **16**, 1279.
8. Ingham, J. L. and Dewick, P. M. (1978) *Phytochemistry* **17**, 535.
9. Higgins, V. J. and Millar, R. L. (1968) *Phytopathology* **58**, 1377.
10. Ingham, J. L. and Millar, R. L. (1973) *Nature* **242**, 125.
11. Pelter, A. and Amenechi, P. I. (1969) *J. Chem. Soc. C* 887.

12. Porter, Q. N. and Baldas, J. (1971) *Mass Spectrometry of Heterocyclic Compounds*, p. 87. Wiley-Interscience, London and New York.
13. Ingham, J. L. (1976) *Phytochemistry* **15**, 1489.
14. Farkas, L., Gottsegen, A., Nógrádi, M. and Antus, S. (1974) *J. Chem. Soc. Perkin Trans.* **1**, 305.
15. Dewick, P. M. (1977) *Phytochemistry* **16**, 93.
16. Shibata, S. and Saitoh, T. (1968) *Chem. Pharm. Bull. Tokyo* **16**, 1932.
17. Pueppke, S. G. and VanEtten, H. D. (1975) *J. Chem. Soc. Perkin Trans.* 1, 946.
18. Cornia, M. and Merlini, L. (1975) *J. Chem. Soc. Chem. Commun.* 428.
19. Robeson, D. J. and Ingham, J. L. (1979) *Phytochemistry* **18**, 1715.
20. Ingham, J. L. (1976) *Z. Naturforsch. Teil C* **31**, 504.
21. Dewick, P. M. (1975) *Phytochemistry* **14**, 983.
22. Jain, A. C., Jain, S. M. and Seshadri, T. R. (1972) *Indian J. Chem.* **10**, 581.
23. Dewick, P. M. (1978) *Phytochemistry* **17**, 249.
24. Khanna, R. H. and Seshadri, T. R. (1963) *Indian J. Chem.* **1**, 385.