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NEW PTEROCARPAN PHYTOALEXINS FROM *LATHYRUS NISSOLIA*

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Key Word Index—*Lathyrus nissolia*; Vicieae; Leguminosae; grass vetch; isoflavonoids; pterocarpans; phenolic compounds; phytoalexins; antifungal activity; DDQ oxidation.

Abstract—In addition to 3-hydroxy-9-methoxypterocarpan (medicarpin), the fungus-inoculated phyllodes of *Lathyrus nissolia* produce two previously unreported isoflavonoid phytoalexins. These compounds have been identified as 3,9-dihydroxy-10-methoxypterocarpan (nissolin) and 3-hydroxy-9,10-dimethoxypterocarpan (methylnissolin).

INTRODUCTION

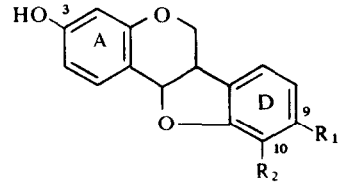
A recent survey of the genus *Lathyrus* [1] (Leguminosae–Papilionoideae; tribe Vicieae) has revealed the widespread occurrence of inducible fungitoxic compounds (phytoalexins) [2] including the known isoflavonoid derivatives, medicarpin (3-hydroxy-9-methoxypterocarpan, **1**), maackiain (3-hydroxy-8,9-methylenedioxypterocarpan), variabilin (3,9-dimethoxy-6a-hydroxypterocarpan) and pisatin (3-methoxy-6a-hydroxy-8,9-methylenedioxypterocarpan). However, although pisatin is a particularly common *Lathyrus* phytoalexin [1, 3], it could not be isolated from the fungus (*Helminthosporium carbonum*)-inoculated phyllodes of *L. nissolia* (grass vetch). Instead, this species produced **1** together with two previously undescribed pterocarpans for which the common names nissolin (**2**) and methylnissolin (**3**) are proposed. The chemical characterization of compounds **2** and **3** is reported in the present paper.

RESULTS AND DISCUSSION

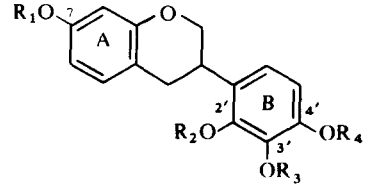
Nissolin (M^+ 286) formed a dimethyl ether (CH_2N_2) and was easily hydrogenated (Pd–C) to afford a dihydro derivative (7,2′,4′-trihydroxy-3′-methoxyisoflavan, **4**) with M^+ 288; methylation of **4** gave a product indistinguishable (UV, MS, TLC) from 7,2′,3′,4′-tetramethoxyisoflavan (**5**) [4]. As the A-ring (OH) and B-ring (2 OH; OMe) substituents of dihydronissolin could be deduced from its characteristic MS fragmentation pattern [5], the parent compound must either be 3,9-dihydroxy-10-methoxypterocarpan (**2**) or the known isomer, vesticarpan (3,10-dihydroxy-9-methoxypterocarpan, **6**), an extractive from the wood of *Machaerium vestitum* (tribe Pterocarpeae) [6] and *Platymiscium trinitatis* (tribe Lonchocarpeae) [7]. Structure **2** for the *Lathyrus* phytoalexin was confirmed when nissolin and authentic vesticarpan proved to be separable by Si gel TLC in $CHCl_3$–MeOH, 25:1 (**2**, R_f 0.47; **6**, R_f 0.37).

The second pterocarpan (methylnissolin, **3**) was clearly related to **2** and was provisionally identified as 9-*O*-methylnissolin; this structure was subsequently confirmed by DDQ oxidation of the licorice (*Glycyrrhiza glabra*) phytoalexin, isomucronulatol (7,2′-dihydroxy-3′,4′-dimethoxyisoflavan, **7**) [4] to give 3-hydroxy-9,10-dimethoxypterocarpan identical (UV, MS, TLC) with the natural product.

Diffusates from the *H. carbonum*-inoculated phyllodes of 4 *L. nissolia* assessions (Table 1) always contained substantial quantities of nissolin. In contrast, methylnissolin was often present in comparatively small amounts whilst medicarpin invariably occurred as a trace constituent. In leaf tissues underlying the inoculum droplets (accession Kw), nissolin reached a concentration of *ca* 640 μg/g fr. wt after 48 hr incubation. Attempts to detect methylnissolin in tissue extracts were unsuccessful, a fact which may reflect its rapid *in vivo* conversion to **2**. When subjected to TLC bioassay against spore germination of *Cladosporium herbarum*



1 R_1 = OMe; R_2 = H
2 R_1 = OH; R_2 = OMe
3 R_1 = R_2 = OMe
6 R_1 = OMe; R_2 = OH



4 R_1 = R_2 = R_4 = H; R_3 = Me
5 R_1 = R_2 = R_3 = R_4 = Me
7 R_1 = R_2 = H; R_3 = R_4 = Me

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Table 1. Concentration (μg/ml) of pterocarpan phytoalexins in 48 hr diffusates from fungus-inoculated phyllodes of 4 *L. nissolia* accessions*†

Accession	Medicarpin	Compound Nissolin	Methylnissolin
Fr	<5	37	22
Kw	<1	79	15
Na	<5	81	68
Ox	<5	72	29

* Concentrations were determined spectrophotometrically using the following extinction coefficients: **1**, $\varepsilon = 7762$ at 287 nm [18]; **2/3**, $\varepsilon = 3700$ at 288 nm for (+)-**6** [6].

† Key to accession sources: Fr = Botanic Garden, University of Frankfurt am Main, West Germany; Kw = Royal Botanic Gardens, Kew, England; Na = Botanic Garden, Nantes, France; Ox = Botanic Garden, University of Oxford (Magdalen College), England. Pterocarpans **1–3** were isolated from freshly collected phyllodes of the Kew accession; other accessions were obtained as seed and grown at Reading prior to phytoalexin induction.

Fr. [8, 9], nissolin and methylnissolin (20 μg) gave inhibition zones of 49 and 20 mm², respectively. The corresponding value for medicarpin was 42 mm². As yet, the fungitoxicity of **2** and **3** has not been tested against the mycelial growth of *H. carbonum*.

Although medicarpin is produced by the fungus-inoculated tissues of numerous temperate and tropical legumes (Ingham, J. L., unpublished results) including *Vicia faba* and several *Lathyrus* spp. [1, 10], neither nissolin nor methylnissolin has been encountered elsewhere in the Papilionoideae. Indeed, simple pterocarpans with C-10 oxygenation are exceptionally rare in the Leguminosae and prior to this investigation were known only as wood constituents of certain leguminous trees native to South America [6, 7, 11]. It is noteworthy, however, that a substance chromatographically indistinguishable from authentic orobol (5,7,3′,4′-tetrahydroxyisoflavone) has been found to occur constitutively in the leaves of *L. nissolia* and several other *Lathyrus* species (Grayer-Barkmeijer, R. J., personal communication); this rather uncommon isoflavone, which was initially isolated from the roots of *L. montanus* [12], is also oxygenated (OH) at the position corresponding to C-10 of **2** and **3**. Together with its apparent inability to produce pisatin, the formation, by *L. nissolia*, of pterocarpan derivatives (**2** and **3**) which are notably absent from other members of the genus, strongly suggests that this species occupies an isolated position within *Lathyrus* and fully supports its allocation to the monospecific section, *Nissolia*.

EXPERIMENTAL

Mass and UV spectra were determined as previously described [13].

Induction, isolation and purification of compounds **1**, **2** *and* **3**. (a) *Diffusates*. Excised phyllodes of *L. nissolia* were inoculated with a conidial suspension of *Helminthosporium carbonum* Ullstrup [9, 14, 15] and the resulting diffusate [14, 15] collected after *ca* 48 hr. Si gel TLC [13] ($CHCl_3$–MeOH, 50:1) of diffusate extracts (EtOAc) gave **1** + **3** and **2** at *ca* R_f 0.60 and 0.30 respectively. Compounds **1** and **3** were subsequently resolved by TLC in *n*-pentane–Et_2O–HOAc (PEA), 75:25:3 (**1**, R_f 0.54; **3**, R_f 0.32) or C_6H_6–EtOAc–*iso*-PrOH (BEP), 90:10:1 (**1**, R_f 0.60; **3**, R_f 0.44). **2** was also purified by Si gel TLC in either PEA (R_f 0.33) or BEP (R_f 0.36) prior to UV and MS analysis. Compound **1** was identified as medicarpin by comparison (UV, MS, TLC) with an authentic sample [16]. Diffusates from phyllodes treated with de-ionized H_2O did not contain detectable quantities of **1**, **2** or **3**. (b) *Tissues*. Phyllode tissues underlying the inoculum droplets were removed (No. 1 cork borer) and extracted (EtOH) as described elsewhere [17]. TLC ($CHCl_3$–MeOH, 25:1) of the extract afforded **2** (R_f 0.47) grossly contaminated with a yellow leaf pigment; chlorophyll zones were located above R_f 0.85. **2** was eluted (EtOH) and re-chromatographed (PEA, 75:25:3) to give the yellow pigment (R_f 0.14) and pure pterocarpan (R_f 0.33) as well separated bands. Compounds **1** and **3** could not be isolated from tissue extracts; markers of both phytoalexins co-chromatographed to R_f 0.73 ($CHCl_3$–McOH, 25:1).

3,9-*Dihydroxy*-10-*methoxypterocarpan* **2** (*nissolin*). Diazotized *p*-nitroaniline, orange; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 212 (100%), 233 sh (50%), 277 sh (18%), 281 (20%), 287 (20%); $\lambda_{max}^{EtOH+NaOH}$ nm: 215, 251, 295; MS *m/e* (rel. int.): 287 (15), 286 (M^+; 100; $C_{16}H_{14}O_6$), 285 (30), 271 (30), 270 (20), 269 (11), 197 (8), 164 (13), 151 (14), 149 (7), 147 (17), 134 (12). *Di*Me *ether* (CH_2N_2) (R_f 0.50, $CHCl_3$–CCl_4, 1:1). λ_{max}^{EtOH} nm: 212 (100%), 233 sh (37%), 277 sh (10%), 280 (12%), 286 (13%); MS *m/e* (rel. int.): 315 (38), 314 (M^+; 100), 313 (33), 299 (41), 284 (22), 283 (10), 178 (8), 161 (17), 148 (11), 137 (9). *Diacetate* (Py–Ac_2O) (R_f 0.57, $CHCl_3$–CCl_4, 3:1). λ_{max}^{EtOH} nm: 210 (100%), 230 sh (32%), 278 (11%), 283 (12%); MS *m/e* (rel. int.): 371 (1), 370 (M^+; 10), 329 (3), 328 (22), 286 (100), 271 (7), 270 (4) 269 (3). Comparative data recorded for 3,10-dihydroxy-9-methoxypterocarpan **6** (vesticarpan) were as follows: diazotized *p*-nitroaniline, yellow/orange, Gibbs reagent, deep blue. λ_{max}^{EtOH} nm: 212 (100%), 233 (38%), 277 sh (10%), 281 (11%), 287 (9%); $\lambda_{max}^{EtOH+NaOH}$ nm: 212, 250, 291.

Hydrogenation of **2**. Nissolin (*ca* 1 mg), HOAc (10 ml) and 10% Pd–C (1 mg) were shaken with H_2 (room temp., 1 atm) for 16 hr. Catalyst and solvent were then removed and the residue chromatographed ($CHCl_3$–MeOH, 20:1) to afford 7,2′,4′-trihydroxy-3′-methoxyisoflavan **4** (dihydronissolin) at R_f 0.27. Diazotized *p*-nitroaniline, yellow/orange, Gibbs reagent, deep blue. λ_{max}^{EtOH} nm: 211 (100%), 230 sh (54%), 276 sh (21%), 282 (23%), 290 sh (18%); $\lambda_{max}^{EtOH+NaOH}$ nm: 212, 244, 294; MS *m/e* (rel. int.): 289 (9), 288 (M^+; 53), 167 (9), 166 (100), 165 (12), 154 (31), 153 (32), 151 (18), 147 (10), 136 (10), 135 (25), 134 (13), 133 (33), 123 (76), 107 (13). *Tri* Me *ether* (R_f 0.84, $CHCl_3$–CCl_4, 3:1). UV and MS as lit. [4]. The above Me ether co-chromatographed with authentic 7,2′,3′,4′-tetramethoxyisoflavan in $CHCl_3$–CCl_4, 3:1 and 1:1 (R_f 0.52).

3-*Hydroxy*-9,10-*dimethoxypterocarpan* **3** (*methylnissolin*). Diazotized *p*-nitroaniline, yellow; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 211 (100%), 232 sh (39%), 277 sh (13%), 281 (14%), 287 (13%); $\lambda_{max}^{EtOH+NaOH}$ nm: 215, 253, 287, 298 sh; MS *m/e* (rel. int.): 301 (12), 300 (M^+; 100; $C_{17}H_{16}O_5$), 285 (28), 270 (7), 269 (8), 238 (5), 185 (9), 151 (6), 147 (14), 135 (8). *Mono*Me *ether*. TLC, UV and MS data as given for dimethyl ether of **2**. *Monoacetate* (R_f 0.65, $CHCl_3$). λ_{max}^{EtOH} nm: 212 (100%), 232 sh (40%), 278 sh (13%), 285 (14%); MS *m/e* (rel. int.): 343 (5), 342 (M^+; 24), 300 (100), 299 (30), 285 (18).

Synthesis of **3**. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 1.5 mg) was dissolved in 1,4-dioxan (1.5 ml) and then added dropwise over 5 min to a soln of 7,2′-dihydroxy-3′,4′-dimethoxyisoflavan (2 mg) in dioxan (2 ml). After incubation (24°; 12 hr), the mixture was diluted (MeOH, 10 ml) and reduced to dryness (*in vacuo*, 40°). Si gel TLC of the residue ($CHCl_3$–

MeOH, 50:1) gave 3-hydroxy-9,10-dimethoxypterocarpan (*ca* 1.2 mg; R_f 0.58) together with unchanged starting material (*ca* 0.8 mg; R_f 0.48). The synthetic pterocarpan was indistinguishable from methylnissolin by UV, MS and co-TLC in $CHCl_3$–MeOH (50:1), PEA, 75:25:3 (R_f 0.33) and C_6H_6–MeOH, 9:1 (R_f 0.69).

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