Isoflavonoid Phytoalexins from Leaflets of *Dalbergia sericea*

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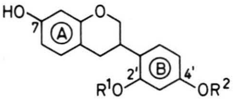
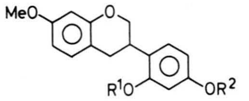
The fungus-inoculated leaflets of *Dalbergia sericea* produce several isoflavonoid phytoalexins including the known pterocarpans, medicarpin and maackiain, and the isoflavan, vestitol. A fourth, previously undescribed, phytoalexin has been identified as 7-methoxy-2′,4′-dihydroxyisoflavan (neovestitol) by comparison with synthetic material.

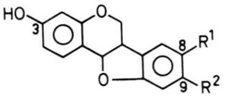
Simple 7,2′,4′-oxygenated isoflavans accumulate in the fungus-inoculated tissues of many papilionate legumes where they appear to function as defensive compounds (phytoalexins) [1, 2]. Vestitol (7,2′-dihydroxy-4′-methoxyisoflavan, **1**) is the most common isoflavan phytoalexin [3] and occurs widely in the Papilionoideae subfamily of the Leguminosae; in contrast, the related compound, isovestitol (7,4′-dihydroxy-2′-methoxyisoflavan, **2**), the three possible monomethyl ethers of **1** and **2** (sativan, **3**; isosativan, **4**; and arvensan, **5**) and demethylvestitol (7,2′,4′-trihydroxyisoflavan, **6**) have a more restricted distribution being found principally in temperate legume tribes such as the Loteae and Trifolieae [3–6]. It has recently been possible to investigate the phytoalexin response of several species belonging to *Dalbergia* (tribe Dalbergieae), a relatively large (approx. 100 species) tropical/subtropical genus of trees, shrubs and climbers. During the course of this work, it was found that the fungus (*Helminthosporium carbonum*)-inoculated leaflets of the Himalayan species, *D. sericea*, produced substantial quantities of **1** together with a previously undescribed isoflavan (**7**) for which the common name, *neovestitol*, is proposed. This paper describes the isolation, purification and identification of neovestitol as 7-methoxy-2′,4′-dihydroxyisoflavan.



1: $R^1 = H$; $R^2 = Me$
2: $R^1 = Me$; $R^2 = H$
3: $R^1 = R^2 = Me$
6: $R^1 = R^3 = H$



4: $R^1 = H$; $R^2 = Me$
5: $R^1 = Me$; $R^2 = H$
7: $R^1 = R^2 = H$
10: $R^1 = R^2 = Me$



8: $R^1 = H$; $R^2 = OMe$
9: $R^1 = R^2 = O{-}CH_2{-}O$

Extraction and TLC purification of fungus-induced leaf diffusates (see *Experimental* and [7, 8]) gave the known phytoalexins, vestitol (**1**), medicarpin (3-hydroxy-9-methoxypterocarpan, **8**) and maackiain (3-hydroxy-8,9-methylenedioxypterocarpan, **9**); all three compounds were identified by comparison (UV, MS, Co-TLC) with authentic material [4, 5, 9–11]. In addition to compounds **1**, **8** and **9**, the diffusates also contained large amounts (see below) of a fourth component (**7**) which proved to be highly fungitoxic (minimum detection limit, < 5 μg) when subjected to a TLC bioassay against spore germination of *Cladosporium herbarum* [12]. This compound – which reacted immediately when TLC plates were sprayed with either diazotised *p*-nitroaniline (orange/yellow) or Gibbs reagent/aqueous Na_2CO_3 (purple/blue) – had M^+ 272 (cf. **1** and **2** [4, 10]) and could be methylated (CH_2N_2; 5 min; CH_2Cl_2/MeOH, 1 : 1) [13] to yield a product indistinguishable (UV, MS, Co-TLC) from 7,2′,4′-trimethoxyisoflavan (**10**) [4]. Partial diazomethane methylation (30 sec; CH_2Cl_2/MeOH, 1 : 4) afforded a mixture of **10** and isosativan (**4**). Together with the prominent MS fragments at *m/e* 137, 136 and 123 [14, 15] (see *Experimental*), the above data indicate that **5** is a simple isoflavan substituted with one OCH_3 (A-ring; C-7) and two OH (B-ring; C-2′/4′) groups. This was confirmed by catalytic hydrogenation of synthetic 7-methoxy-2′,4′-dibenzyloxyisoflavone [16] to give (±)-7-methoxy-2′,4′-dihydroxyisoflavan (neovestitol) identical (UV, MS, Co-TLC) with the natural product. *Dalbergia sericea* is currently the only known plant source of neovestitol; the other *Dalbergia* species examined in these laboratories (*e. g. D. latifolia, D. retusa* and *D. sissoo*) characteristically produce **1** and **8**, and occasionally **9**.

Neovestitol appears to be the major isoflavonoid phytoalexin produced by leaflets of *D. sericea* attaining a concentration (based on $\log \varepsilon = 3.62$ at 285 nm for **1** [9]) of between 50 and 64 μg/ml diffusate within 48 h of inoculation. Although vestitol also accumulates in significant quantities (44–53 μg/ml), neither medicarpin (*ca.* 1 μg/ml) nor maackiain (< 0.5 μg/ml) occur in more than trace amounts.

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Leaf tissues beneath the inoculum droplets contain **1** and **7** at concentrations of *ca.* 450 and 900 μg/g fresh wt. respectively. Compounds **1** and **7**–**9** were either absent from control samples or were present at levels insufficient for satisfactory UV measurement.

Experimental

Unless otherwise stated, MS/UV analyses and all chromatographic separations were carried out as previously described [10, 17, 18].

Induction, isolation and purification of compounds **1** *and* **7**–**9**. Seeds of *Dalbergia sericea* G. Don (supplied by the Forest Research Institute, Dehra Dun, India) were germinated (70°; John Innes No. 1 compost) and the resulting plants grown (75°; 7000 lx; 16 h daylength) for approx. 6 months prior to inoculation of detached leaflets with spore suspensions of *Helminthosporium carbonum* [7, 8]. Control leaflets were treated with droplets of de-ionised H_2O. Si gel TLC ($CHCl_3$: MeOH, 20 : 1) of 48 h diffusate extracts (EtOAc) gave **8**+**9** (R_F 0.69), **1** (R_F 0.32) and **7** (R_F 0.20). After elution (EtOH), compounds **8** and **9** were separated by TLC in *n*-pentane : Et_2O : HOAc (75 : 25 : 3, ×3) [19]; this solvent system was also used to purify **1** and **7**. Inoculated leaf tissues were excised and extracted (EtOH) as outlined elsewhere [4]. Si gel PLC ($CHCl_3$: MeOH, 10 : 1, layer thickness, 0.5 mm) gave **1** and **7** at R_F 0.45 and 0.38 respectively. Both compounds were eluted and further purified as described above. Pterocarpans **8** and **9** could not be isolated from tissue extracts. There was no evidence to suggest that *D. sericea* produced other isoflavonoid phytoalexins such as isovestitol (**2**), isosativan (**4**) and arvensan (**5**).

7-Methoxy-2′,4′-dihydroxyisoflavan (**7**) (*neovestitol*). Diazotised *p*-nitroaniline, orange/yellow; Gibbs reagent, purple/blue. λ max (nm) EtOH 212 (100%), 227 sh (65%), 281 sh (30%), 284 (31%), 289 (28%); EtOH + NaOH 215 (100%), 246 sh (8%), 286 sh (6%), 291 (7%), 302 sh (5%). MS (rel. int.) 273 (2), 272 (M^+; 17), 150 (6), 149 (12), 148 (9), 138 (10), 137 (100), 136 (34), 135 (14), 123 (11), 121 (18), 109 (6), 108 (5), 107 (17). *4′-O-methyl ether* (**4**) (CH_2N_2) (R_F 0.05, $CHCl_3$: CCl_4, 3 : 1) Diazotised *p*-nitroaniline, yellow; Gibbs reagent, deep blue. UV (EtOH and EtOH + NaOH) as lit. [19]. MS (rel. int.) 287 (8), 286 (M^+; 39), 151 (10), 150 (100), 149 (33), 148 (14), 138 (11), 137 (78), 121 (17). *Dimethyl ether* (**10**) (R_F 0.55, $CHCl_3$: CCl_4, 3 : 1) UV (EtOH) and MS as lit. [4, 8]. *Diacetate* (Py-Ac_2O) (R_F 0.64, $CHCl_3$) λ max (nm) EtOH 212 (100%), 228 sh (72%), 275 sh (14%), 280 (17%), 283 (18%), 289 (15%). MS (rel. int.) 356 (M^+; 3), 315 (2), 314 (10), 273 (2), 272 (14), other fragments as given for **7**.

Synthesis of **7**.7-Methoxy-2′,4′-dibenzyloxyisoflavone was prepared as previously described [16]. This isoflavone (2 mg), HOAc (2 ml) and Pd/C (10%; 10 mg) were shaken with H_2 for *ca.* 6 h (room temp., 1 atm.). Work up and Si gel TLC ($CHCl_3$: MeOH, 10 : 1) gave 7-methoxy-2′,4′-dihydroxyisoflavan (*ca.* 1.2 mg). UV and MS as reported for the natural product. The synthetic and *Dalbergia*-derived isoflavans were inseparable when co-chromatographed (Si gel TLC) in $CHCl_3$: MeOH, 20 : 1 (R_F 0.20), *n*-pentane : Et_2O : HOAc, 75 : 25 : 3 (R_F 0.23), Et_2O : *n*-hexane (R_F 0.55) and C_6H_6 : MeOH, 9 : 1 (R_F 0.36).

Acknowledgements

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