The Structure of Desmocarpin, a Pterocarpan Phytoalexin from Desmodium gangeticum

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Ethyl acetate extracts of diffusates from the fungus-inoculated leaflets of Desmodium gangeticum have been found to contain six isoflavonoid phytoalexins including the isoflavones genistein and 2'-hydroxygenistein, and the isoflavonanes dalbergioidin, diphysolone and kievitone. These known phytoalexins occur together with a new antifungal isoflavonoid (desmocarpin) for which the structure (--)-(6αR; 11αR)-1,9-dihydroxy-3-methoxypterocarpan is proposed.

Work undertaken by Purushothaman et al. [1, 2] has revealed that three 'complex' laevorotatory pterocarpons (gangetin, gangetinin and desmodin) occur constitutively in roots of the papilionate legume Desmodium gangeticum DC., a species used medicinally in parts of India and Nepal [3]. Apart from their presence in apparently healthy plants, however, it is now widely recognised that 'simple' and/or 'complex' pterocarpons may accumulate rapidly in the tissues of many papilionate legumes as a defense against invading fungi and bacteria. These and various other inducibly-formed isoflavonoids are commonly referred to as phytoalexins [4, 5], and we were anxious to determine if species of the hitherto unexamined genus Desmodium could respond to fungal invasion by producing one or more compounds of this type. We report here on the isoflavonoid phytoalexin response of D. gangeticum.

As in previous studies involving legume phytoalexins, the drop-diffusate technique [4, 5] was used to routinely isolate antifungal material from the excised, fungus (Helminthosporium carbonum Ullstrup)-inoculated leaflets of D. gangeticum. Si gel TLC (CHCl₃-MeOH, 20:1) of an ethyl acetate extract of the diffusate from fungus-treated leaflets afforded several compounds which reacted with diazotised p-nitroaniline reagent [6] to give predominantly yellow or orange colours, and with Gibbs reagent [7, 8] to give either blue or purple-blue products. Elution and further Si gel TLC of these phenolic substances as described in the Experimental section eventually yielded pure 5,7,4'-trihydroxyisoflavone (genistein, 1), 5,7,2',4'-tetrahydroxyisoflavone (2'-hydroxygenistein, 2), 5,7,2',4'-tetrahydroxyisoflavonane (dalbergioidin, 3), 5,7,2',4'-tetrahydroxy-6-(3,3-dimethylallyl)isoflavanone (diphysolone, 4) and 5,7,2',4'-tetrahydroxy-8-(3,3-dimethylallyl)isoflavanone (kievitone, 5). Compounds 1–5 have all previously been found as phytoalexins in other species belonging to the subfamily Papilionoideae of the Leguminosae [4, 5, 9], and their identification was readily accomplished by UV and TLC comparison with authentic samples. Both 1 and 3 are also known to occur constitutively in two legumes (Lespedeza cyrtobotrya, 1+3 and Ougeinia dalbergioides, 3) very closely allied to D. gangeticum [4].

As well as the above mentioned compounds, fungus-induced diffusates invariably contained substantial quantities of a new laevorotatory isoflavonoid (desmocarpin) which we have now identified as 1,9-dihydroxy-3-methoxypterocarpan (6). When bioassayed against Cladosporium herbarum Fr. [8] using the thin-layer plate procedure developed by Homans and Fuchs [10], desmocarpin (20–25 μg) gave a prominent inhibition zone (approx. 80 mm²) similar in area to that afforded by comparable amounts of diphysolone or kievitone. Diffusates from control (H₂O-treated) leaflets were generally devoid of phytoalexin-like material, although they...
occasionally were found to contain traces (<1 μg/ml) of a substance chromatographically indistinguishable from genistein (1). No evidence was obtained to indicate that the root pterocarpans gangetin, gangetinin and desmodin [1, 2] were produced as phytoalexins by the H. carbonum-inoculated leaflets of D. gangeticum.

The identity of desmocarpin ([M]+ 286) as a monomethoxylated pterocarpan was immediately evident from its ¹H NMR spectrum. This revealed a single 3H methoxyl resonance at δ 3.72, and signals at δ 5.62d, 4.21dd, 3.54t and 3.45m attributable respectively to the heterocyclic ring protons H-11a, H-6eq, H-6ax and H-6a. Virtually coincident δ  

![Chemical structures](image.png)

Table I. Possible structures of some minor fragments observed in the mass spectrum of desmocarpin and its dimethyl ether [22, 23].

<table>
<thead>
<tr>
<th>Fragmenta</th>
<th>Desmocarpin (6)</th>
<th>Desmocarpin dimethyl ether (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Chemical structure" /></td>
<td>a: R = H (m/z 177)</td>
<td>a’: R = CH₃ (m/z 191)</td>
</tr>
<tr>
<td><img src="image.png" alt="Chemical structure" /></td>
<td>b: R = H (m/z 164)</td>
<td>b’: R = CH₃ (m/z 178)</td>
</tr>
<tr>
<td><img src="image.png" alt="Chemical structure" /></td>
<td>c: R = H (m/z 147)</td>
<td>c’: R = CH₃ (m/z 161)</td>
</tr>
<tr>
<td><img src="image.png" alt="Chemical structure" /></td>
<td>d: R = H (m/z 134)</td>
<td>d’: R = CH₃ (m/z 148)</td>
</tr>
</tbody>
</table>

a Information on the abundance of each ion fragment is given in the Experimental section.
values have also been reported for the corresponding protons of apiocarpin [11], a 1,9-dihydroxylated pterocarpan phytoalexin produced by *Apis tuberosa*. The aromatic (A/D) ring protons of 6 appeared as a pair of meta-coupled doublets (δ 5.98 and 6.17; J = 2.3 Hz; H-2 and H-4), and as an ABX system (J = 8.0 and 2.2 Hz), the latter being characterised by chemical shift values identical with those earlier assigned to H-7 (δ 7.13), H-8 (δ 6.36) and H-10 (δ 6.29) of apiocarpin [11].

In addition to the molecular ion (m/z 286) and a prominent fragment at M⁺–15 (m/z 271), the MS of desmocarpin exhibited signals of low intensity at m/z 177 (a), 164 (b), 147 (c) and 134 (d) (Table I) which suggested that one of the aromatic rings (considered to be D from the preceding ¹H NMR chemical shift data) was monohydroxylated (fragment ions c and d), and that the other possessed both an OH and an OCH₃ substituent (fragment ions a and b). Exactly comparable minor fragments (a’–d’) at m/z 191/178 and m/z 161/148 were also present in the MS of the non-phenolic dimethyl ether ([M]+ 314; 7) resulting from treatment of 6 with diazomethane [12]. Because pterocarpons are invariably oxygenated at C-3 and C-9 [4], it follows that desmocarpin must possess a C-9 OH group (cf. apiocarpin [11]) with the remaining meta-related substituents residing on ring A.

The 1-hydroxy-3-methoxy oxygenation pattern assigned to 6 was preferred over the isomeric 1-methoxy-3-hydroxy arrangement found in the *Psophocarpus* phytoalexin 1-methoxyphaseollidin [13] for two reasons. First, desmocarpin gave a dark blue colour on TLC plates sprayed with Gibbs reagent [7, 8], a result which indicated the presence of an unsubstituted position *para* to the phenolic A-ring OH group. Secondly, an NOE difference experiment showed that irradiation of the methoxyl group (δ 3.72) caused enhancement of both the H-2 and H-4 signals. No other protons were affected. When considered together, the above observations allow the OCH₃ group to be unambiguously located at C-3, and thus desmocarpin is 1,9-dihydroxy-3-methoxypterocarpan (6). Desmocarpin is strongly laevorotatory and therefore possesses the 6αR; 11αR absolute configuration [5].

The concentration of each phytoalexin in 48 h fungus-induced diffusates was determined spectrophotometrically using extinction coefficients previously published for genistein (ε = 42700 at 262 nm [14], compounds 1 and 2), dalbergioidin (ε = 20420 at 288 nm [15]), kievitone (ε = 16600 at 293 nm [16], compounds 4 and 5) and melilotocarpin B (4,9-dihydroxy-3-methoxypterocarpan, ε = 5888 at 283 nm [17], compound 6). The resulting values indicate that dalbergioidin (58 μg/ml diffusate) and desmocarpin (40 μg/ml) are the major phytoalexins produced by D. gangeticum, these being followed in decreasing order of abundance by kievitone (24 μg/ml), 2'-hydroxygenistein (14 μg/ml), diphysolone (11 μg/ml) and genistein (8 μg/ml).

**Experimental**

**Plant and fungus material**

Plants of *Desmodium gangeticum* DC. were raised [18] from seeds supplied by the Forest Research Institute, Dehra Dun, India. Leaflets for phytoalexin studies were harvested at regular intervals [18] after the plants reached an age of approx. 12 weeks, all flower heads being periodically removed to encourage leaf production. Cultures of *H. carbonum* Ullistrup and *C. herbarum* Fr. were maintained as reported elsewhere [8].

**Isolation and purification of Desmodium phytoalexins**

Droplets of *H. carbonum* spore suspension [19] were applied to the lower surface of excised *D. gangeticum* leaflets and then incubated [20] for 48 h. The resulting faintly yellow, cloudy diffusate was extracted (×3) with equal volumes of EtOAc, and the combined organic fractions were reduced to dryness at 40 °C using a rotary evaporator. Si gel TLC (Merck, F-254, layer thickness 0.25 mm) of the residue in CHCl₃–MeOH (20:1) gave desmocarpin (6 (RF 0.34), genistein 1 (RF 0.29), 2'-hydroxygenistein 2 + diphysolone 4 (RF 0.19), kievitone 5 (RF 0.16) and dalbergioidin 3 (RF 0.12). After elution with MeOH (4 × 2.5 ml), the above compounds were purified by Si gel TLC as follows: 6, n-pentane–diethyl ether–glacial acetic acid (PEA), 75:25:6 (RF 0.19), and 1, 2 + 4, 3 and 5, PEA, 75:25:6: × 3. Phytoalexins 2 (lower zone) and 4 (upper zone) were readily separated by multiple development in the PEA system. A final TLC run in benzene–MeOH, 9:1 was sometimes required in order to completely free 2 (RF 0.21) and 6 (RF 0.30) from various very minor non-flavonoid contaminants. Compounds 1–5 were identified by UV and
TLC comparison with samples previously obtained from *Lupinus albus* (1 and 2 [18]), *Dolichos biflorus* (3 and 5 [21]) and *Diphysa robinioides* (4 [9]). TLC examination of extracts of diffusates from H2O-treated leaflets occasionally revealed traces of genistein but compounds 2—6 were not detected.

**1,9-Dihydroxy-3-methoxypterocarpan (6) (desmocarpin)**

Colour with diazotised p-nitroaniline reagent [6]. orange; colour with Gibbs reagent/aqueous Na2CO3 [7, 8], dark blue. UV: λmax, nm: MeOH 212 (100%), 236 sh (24%), 284 — 288 plateau (11%) or occasionally 286 (11%), 293 sh (10%); λmax, nm: MeOH + NaOH 209, 245 sh, 294. MS: m/z 287 (18%), 286 ([M]+; 100%), 285 (10%), 271 (M+ — 15; 18%), 226 (10%), 211 (12%), 177 (7%; a), 167 (14%), 164 (9%; b), 149 (50%), 147 (7%; c), 134 (8%; d). 1H NMR (acetone-d6; 250 MHz; TMS reference): δ 7.13 (1H, d, J = 8.0 Hz, H-7), 6.36 (1H, dd, J = 8.0, 2.2 Hz, H-8), 6.29 (1H, d, J = 2.2 Hz, H-10), 6.17 (1H, d, J = 2.3 Hz, H-4), 5.98 (1H, d, J = 6.4 Hz, H-2), 5.62 (1H, d, J = 10.5 Hz, H-11a), 4.21 (1H, approx. dd, J = 4.6 Hz, H-6eq), 3.72 (3H, s, OCH3), 3.54 (1H, approx. t, J = 10.5 Hz, H-6ax), 3.45 (1H, m, H-6a). [z]589nm = 250° (approx. 850 µg, based on ε = 5888 at 283 nm for melilotocarpan B [17], in 1 ml of MeOH). Dimethyl ether (7) (CH32N2; Rf 0.11 in CHCl3—CCl4, 2:1). Compound 7 did not give a colour on TLC plates treated with either diazotised p-nitroaniline reagent or Gibbs reagent/aqueous Na2CO3. UV: λmax, nm: 211 (100%), 235 sh (24%), 283—287 plateau (9%) or 285 (9%), 292 sh (7%). The MeOH spectrum of 7 was unaffected by aqueous NaOH. MS: m/z 315 (21%), 314 ([M]+; 100%), 313 (27%), 312 (9%), 300 (7%), 299 (M+ — 15; 17%), 191 (9%; a'), 178 (15%; b'), 162 (11%), 161 (10%; c'), 148 (17%; d').

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