

A Revised Structure for the Phytoalexin Cajanol

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Structure Revision

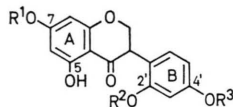
Peroxide oxidation of cajanol ethyl ether afforded the B-ring derived product, 2-methoxy-4-ethoxybenzoic acid. Formation of this compound means that the structure of cajanol, a major phytoalexin from *Cajanus cajan*, must be revised to 5,4'-dihydroxy-7,2'-dimethoxyisoflavanone.

Several isoflavonoid phytoalexins are known to accumulate in the etiolated stems of pigeon pea, *Cajanus cajan* (Leguminosae; subfamily Papilionoideae; tribe Cajaneae) following inoculation with the fungus, *Helminthosporium carbonum* [1]. These compounds have been identified [1] as the isoflavones, genistein (5,7,4'-trihydroxy-), 2'-hydroxy-genistein (5,7,2',4'-tetrahydroxy-) and cajanin (5,2',4'-trihydroxy-7-methoxy-) and the isoflavanone, cajanol; the latter phytoalexin was originally formulated as 5,2'-dihydroxy-7,4'-dimethoxyisoflavanone (1).

On chromatograms sprayed with diazotised *p*-nitroaniline, cajanol (M^+ 316; $C_{17}H_{16}O_6$) immediately gives a bright orange/yellow colouration. In contrast, with Gibbs reagent [2, 3] the expected dark blue indophenol appears slowly, an observation not in accord with an unsubstituted position *para* to the proposed 2'-hydroxyl function. Thus, the 2'-hydroxy isoflavanone, ferreirin (3) (5,7,2'-trihydroxy-4'-methoxyisoflavanone) rapidly affords an intense blue derivative when subjected to the Gibbs test [1]. The abovementioned results suggest that in the B-ring of cajanol, the hydroxyl group may be located at C-4' (and the methoxyl at C-2') rather than C-2' as previously reported [1]. The A-ring assignments (C-5 OH and C-7 OCH_3) – which were made on the basis of established spectral characteristics [4] – and the 2',4'-oxygenation pattern of ring B are not in dispute.

Further studies on the structure of cajanol have recently been facilitated by its isolation in relatively large quantities from the $CuCl_2$ -treated stems (ap-

prox. 20 mg cajanol/kg fresh wt) and roots (approx. 60 mg/kg fresh wt) of *C. cajan* (see Experimental). Ethylation of the chromatographically pure isoflavanone and subsequent H_2O_2 oxidation afforded a B-ring derived product indistinguishable by UV, MS and Co-TLC (in 5 solvent systems) from a synthetic specimen of 2-methoxy-4-ethoxybenzoic acid. Formation of this acid allows the B-ring OCH_3 and OH groups of cajanol to be unequivocally located at C-2' and C-4' respectively. Cajanol is thus 5,4'-dihydroxy-7,2'-dimethoxyisoflavanone (2) and not 5,2'-dihydroxy-7,4'-dimethoxyisoflavanone (1) as reported by Ingham [1]. As expected, peroxide oxidation of 5-hydroxy-7,2'-diethoxy-4'-methoxy isoflavanone (7,2'-di-O-ethylferreirin) gave 2-ethoxy-4-methoxybenzoic acid identical (UV, MS and TLC) with an authentic sample. The 2-ethoxy-4-methoxy substituted acid could be readily distinguished from the cajanol-derived isomer by comparative TLC (see Experimental).



- 1: $R^1=R^3=CH_3$; $R^2=H$
- 2: $R^1=R^2=CH_3$; $R^3=H$
- 3: $R^1=R^2=H$; $R^3=CH_3$
- 4: $R^1=R^2=R^3=H$
- 5: $R^1=R^3=H$; $R^2=CH_3$

Phytoalexin surveys in the Papilionoideae subfamily of the Leguminosae [5] suggest that cajanol is of exceptionally rare occurrence. Indeed, apart from *C. cajan*, this isoflavanone has only been obtained from the *H. carbonum*-inoculated hypocotyls of Florida velvet bean, *Mucuna (Stizolobium) deeringianum* (tribe Erythrineae) where it co-occurs with various other isoflavonoids including genistein, 2'-hydroxygenistein, dalbergioidin (4) (5,7,2',4'-tetrahydroxyisoflavanone) and a third isoflavanone provisionally identified as 5,7,4'-trihydroxy-2'-methoxyisoflavanone (5) (isoferreirin). In *M. deeringianum*, the latter compound might represent the immediate biosynthetic precursor of cajanol.

Experimental

Mass and UV spectra were determined as previously described [1]. All chromatographic separations were undertaken using Merck, pre-coated Si gel TLC plates (F 254; layer thickness, 0.25 mm).

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Induction of cajanol

Locally purchased seeds of pigeon pea (*Cajanus cajan* [L.] Millsp.) were germinated as previously described [1], sown in moist vermiculite and grown (darkness, 24 °C) for 15–20 days. The etiolated stems (200–300 cm) were then cut into short (approx. 10 cm) lengths and placed in clear plastic boxes containing $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ($5 \times 10^{-3}\text{M}$) in 0.05% aqueous Tween 20 to a depth of 3–5 mm. Roots of *C. cajan* were washed with de-ionised H_2O to remove the vermiculite and then immersed in aqueous CuCl_2 as described above. Stems and roots were incubated [1] for 5 or 6 days prior to extraction of cajanol.

Isolation and purification of cajanol

After incubation, the faintly brown stems (approx. 50 g) were macerated in warm MeOH (60 °C; 300 ml), filtered by suction and the fibrous mat re-extracted. The combined filtrates were reduced (*in vacuo*, 40 °C) to about 15 ml, diluted with de-ionised H_2O (250 ml) and shaken ($\times 3$) with equal volumes of EtOAc. The pooled organic fractions were reduced to dryness and the residue chromatographed in CHCl_3 : MeOH (50 : 1) to afford cajanol as a brown fluorescent band at R_F 0.60. This zone was eluted (MeOH) and re-chromatographed (*n*-pentane : Et_2O : HOAc, 75 : 25 : 3, $\times 3$) to give the pure isoflavanone (1–1.5 mg). Root tissues (approx. 150 g) were treated as described above except that 600 ml MeOH were used for the initial extraction. Final yields of cajanol varied from 8 to 11 mg. Only traces of cajanol were obtained from stems and roots incubated in aqueous Tween 20.

5,4'-dihydroxy-7,2'-dimethoxyisoflavanone (I) (cajanol)

λ_{max} (nm): MeOH 214 (100%), 229 (94%), 287 (85%), 336 sh (13%); NaOH 215 (100%), 244 (43%), 287 (38%), 356 (24%); NaOAc, Borate and AlCl_3 as lit. [1]; MS as lit. [1] 4'-acetox derivative (Py-Ac₂O) (R_F 0.80, CHCl_3). λ_{max} (nm): MeOH 215 (100%), 229 (84%), 288 (78%), 334 sh (10%); AlCl_3 312, 368; MS (rel. int.) 358 (M^+ ; 8), 316 (7), 192 (16), 168 (7), 167 (95), 166 (15), 151 (8), 150 (100), 135 (32), 107 (22).

Ethylation of cajanol

Cajanol (10 mg), dry Me_2CO (5 ml), anhydrous K_2CO_3 (1 g) and diethyl sulphate (15 μl) were re-

fluxed (approx. 60 °C) for 2 h. After removal of K_2CO_3 (by centrifugation) and Me_2CO (*in vacuo*, 40 °C), the residue was chromatographed (CHCl_3) to give impure 5-hydroxy-7,2'-dimethoxy-4'-ethoxyisoflavanone (4'-O-ethylcajanol) at R_F 0.71. Elution and additional TLC in *n*-pentane : Et_2O : HOAc (75 : 25 : 1) afforded about 9 mg of the pure isoflavanone (R_F 0.59).

4'-O-ethylcajanol

λ_{max} (nm): MeOH 212 (100%), 229 (90%), 259 sh (34%), 286 (81%), 338 sh (13%); NaOH 214 (100%), 247 sh (24%), 287 (25%), 356 (6%); AlCl_3 272, 310, 368; AlCl_3 + HCl 270 sh, 307, 366; addition of NaOAc and Borate did not affect the MeOH spectrum; MS (rel. int.) 344 (M^+ ; 11), 179 (23), 178 (100), 165 (3), 163 (5), 150 (5), 149 (7), 135 (12), 107 (8). Diazotised *p*-nitroaniline, yellow/orange; Gibbs reagent, dark blue (colour developing over several min); Fluorescent brown under long wavelength UV light.

H_2O_2 oxidation of 4'-O-ethylcajanol

The above compound (2 mg), aqueous KOH (10%; 3 ml), EtOH (0.5 ml) and H_2O_2 (30%; 0.2 ml added in 20 μl portions over 1 h) were stirred (50 °C \pm 2 °C) for 90 min. The mixture was then acidified (2 N HCl; pH 3), extracted ($\times 3$) with equal volumes EtOAc and the organic fractions pooled and reduced to dryness. TLC (CHCl_3 : MeOH, 50 : 1) of the residue gave a broad, fluorescence-quenching band (R_F 0.44) opposite the marker of authentic 2-methoxy-4-ethoxybenzoic acid (*cf.* authentic 2-ethoxy-4-methoxybenzoic acid, R_F 0.56). Traces of unchanged 4'-O-ethylcajanol were detected (diazotised *p*-nitroaniline) at R_F 0.90. Further TLC purification (*n*-pentane : Et_2O : HOAc, 75 : 25 : 3) of the R_F 0.44 fraction gave 2-methoxy-4-ethoxybenzoic acid (R_F 0.32; *cf.* 2-ethoxy-4-methoxybenzoic acid, R_F 0.46) indistinguishable (UV, MS, TLC) from synthetic material.

2-methoxy-4-ethoxybenzoic acid

λ_{max} (nm) EtOH: 214 (100%), 254 (81%), 289 (46%); MS (rel. int.) 196 (M^+ ; 70), 179 (5), 167 (7), 151 (65), 139 (37), 138 (11), 123 (8), 122 (25), 121 (100), 108 (10). The acid derived from 4'-O-ethylcajanol co-chromatographed with authentic material in the following solvents: (a) CHCl_3 : CCl_4 (3 : 1), R_F 0.11 (b) C_6H_6 : MeOH (9 : 1), R_F 0.27

and (c) Et₂O : *n*-hexane (3 : 1), *R_F* 0.20. Corresponding *R_F* values for 2-ethoxy-4-methoxybenzoic acid were 0.23, 0.43 and 0.36.

Ethylation of ferreirin

Ferreirin (12 mg) was ethylated using the procedure described for cajanol. TLC purification (CHCl₃ : CCl₄, 3 : 1) of the reaction products gave about 10 mg of 5-hydroxy-7,2'-diethoxy-4'-methoxyisoflavanone (7,2'-di-O-ethylferreirin) (*R_F* 0.25) together with small quantities (about 1.5 mg) of 5,2'-dihydroxy-7-ethoxy-4'-methoxyisoflavanone (7-O-ethylferreirin) (*R_F* 0.06).

7-O-ethylferreirin

λ_{\max} (nm): MeOH 215 (100%), 229 sh (95%), 287 (90%), 340 sh (15%); NaOH 218 (100%), 245 sh (53%), 289 (61%), 352 (34%); AlCl₃ 274 sh, 311, 368; AlCl₃ + HCl 274 sh, 309, 368; addition of NaOAc and Borate did not affect the MeOH spectrum; MS (rel. int.) 330 (M⁺; 24), 182 (8), 181 (100), 178 (27), 163 (10), 153 (16), 151 (7), 150 (47), 149 (11), 148 (5), 124 (5), 121 (5). Diazotised *p*-nitroaniline, bright yellow; Gibbs reagent, deep blue (colour developing rapidly); Fluorescent orange/brown under long wavelength UV light.

7,2'-di-O-ethylferreirin

λ_{\max} (nm): MeOH 214 (100%), 228 (93%), 287 (87%), 338 sh (16%); NaOH 216 (100%), 246 sh

(52%), 287 (68%), 357 (23%); AlCl₃ 274 sh, 312, 370; AlCl₃ + HCl 274 sh, 309, 370; addition of NaOAc and Borate did not affect the MeOH spectrum; MS (rel. int.) 358 (M⁺; 15), 179 (13), 178 (100), 177 (5), 165 (6), 164 (5), 163 (56), 150 (15), 149 (32), 135 (5), 121 (5). Diazotised *p*-nitroaniline, pale yellow; Gibbs reagent, weak blue; Fluorescent orange/brown under long wavelength UV light.

H₂O₂ oxidation of 7,2'-di-O-ethylferreirin

The above isoflavanone (5 mg) was treated with H₂O₂ as described for 4'-O-ethylcajanol. TLC purification of the product (see oxidation of 4'-O-ethylcajanol for solvents and *R_F* values) gave 2-ethoxy-4-methoxybenzoic acid indistinguishable (UV, MS, TLC) from an authentic sample.

2-ethoxy-4-methoxybenzoic acid

λ_{\max} (nm): EtOH 215 (100%), 254 (78%), 289 (35%); MS (rel. int.) 196 (M⁺; 33), 163 (20), 152 (7), 151 (19), 150 (100), 135 (6), 122 (48), 107 (23). For comparative TLC data see 2-methoxy-4-ethoxybenzoic acid.

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