New Pterocarpinoid Phytoalexins of Soybean

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Summary Structures have been assigned to two new antifungal pterocarpinoids identified in cupric chloride-treated and pathogen-infected soybean plants.

SEEDLINGS of soybean [*Glycine max* (L.) MERR.] synthesise antifungal compounds in response to infection by *Phytophthora megasperma* Drechs. var. *sojae* or on treatment with cupric chloride.[1] One phytoalexin has recently been assigned the revised structure (**1**).[2] We report the absolute configuration of this compound and the structures of two isomeric pterocarpinoid phytoalexins, produced in similar quantities by soybeans (var. Amsoy 71).

Metabolites extracted from cupric chloride-treated cotyledons were purified by chromatography on Sephadex LH-20 ($CHCl_3$) and by h.p.l.c. on 10 μm Partisil (propan-2-ol–hexane, 3:97). Three major antifungal components were isolated and characterised. The first compound (M^+ 338·1157) was identified by ^{1}H n.m.r. spectroscopy (Table) as (**1**). On the basis of the large negative trough in its o.r.d. spectrum {$[\Phi]_{242}$ (EtOH) −100,000} we assign the (6a*R*, 11a*R*) configuration[3] to this compound.

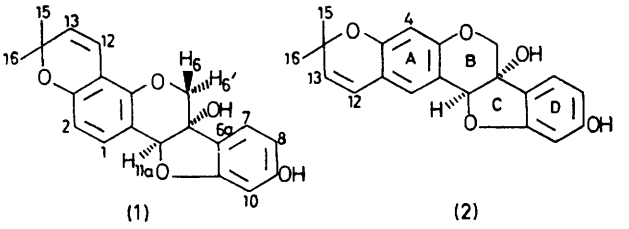
TABLE. ^{1}H n.m.r. data for phytoalexins (**1**), (**2**), and (**5**).[a]

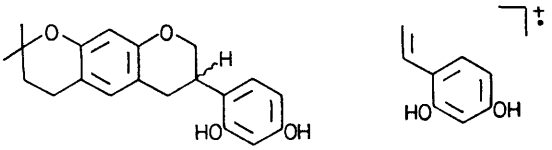
	(1)		(2)		(5)	
Proton	δ	*J*/Hz	δ	*J*/Hz	δ	*J*/Hz
H-1	7·24d	8·5	7·15s	—	7·27s	—
H-2	6·47d	8·5	—	—	—	—
H-4	—	—	6·21s	—	6·27s	—
H-6	4·12d	12	4·04d	12	4·05d	11
H-6′	4·32d	12	4·13d	12	4·15d	11
H-7	7·22d	8	7·21d	8	7·23d	8
H-8	6·43q	8; 2	6·43q	8; 2	6·46q	8; 2
H-10	6·25d	2	6·25d	2	6·26d	2
H-11a	5·27s[b]	—	5·25s[b]	—	5·30s[b]	—
H-12	6·53d	10	6·41d	10	3·05[c]	16; 8
					3·42[c]	16; 9
H-13	5·65d	10	5·65d	10	5·26[c]	8
H-15	1·36s[d]	—	1·37s[d]	—	4·91s[b]	—
					5·08s[b]	—
H-16	1·39s[d]	—	1·40s[d]	—	1·77s[d]	—

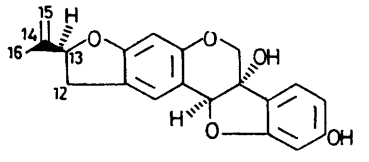
[a] Spectra measured in $(CD_3)_2CO$ relative to internal reference Me_4Si, on a JEOL PFT-100 spectrometer at 99·54 MHz. [b] Broad singlet. [c] ABX system. [d] 3-Proton singlet.

The second compound crystallised from toluene {m.p. 89—93 °C; $C_{20}H_{18}O_5$ (M^+ 338·1155); λ_{max} (EtOH) 227 (ε 38,500), 285 (8700), 307 (6200), and 318 (5800) nm; $[\Phi]_{243}$ (EtOH) −78,000; ν_{max} (KBr) 3420, 3225, 1625, 1615, and 1585 cm^{-1}} and was assigned structure (**2**). Comparison of spectra and the mass spectrometric fragmentation pattern with those of (**1**) suggested the presence of a 6a-hydroxypterocarpan ring system. This was confirmed by its ready conversion into an anhydro-derivative [M^+ 320; λ_{max} (EtOH) 232, 274, 348, and 366 nm] by formic acid treatment, and by the ^{1}H n.m.r. spectrum of (**2**) (Table) which contains an AB quartet showing W-coupling of the low field doublet (H-6′) to a benzylic proton (H-11a). Decoupling experiments defined the aromatic substitution pattern and the presence of a 2,2-dimethylpyran ring. The attachment of this heterocyclic system to ring A rather than to ring D was deduced from the isoflavan derivative (**3**), prepared by hydrogenation/hydrogenolysis of the anhydro-derivative of (**2**).[2] The ^{1}H n.m.r. spectrum of (**3**) showed a shift of only the ABX aromatic system on formation of the phenoxide with KOH, while its mass spectrum contained the characteristic retro-Diels–Alder cleavage ion (**4**) (*m/e* 136).[4]

The third phytoalexin crystallised from aqueous ethanol {m.p. 149—53 °C; $C_{20}H_{18}O_5$ (M^+ 338·1146); λ_{max} (EtOH) 287 (ε 9400) and 292 (9600) nm; $[\Phi]_{245}$ (EtOH) −79,000; ν_{max} (KBr) 3425, 3225, 1620, and 1610 cm^{-1}}. Spectral properties, dehydration, and conversion into the corresponding isoflavan established the molecule as a 2,3-substituted 6a,9-dihydroxypterocarpan. The structure of the side chain was deduced from the ^{1}H n.m.r. spectrum (Table) which contained signals assigned to an ABX system containing *gem*-benzylic protons, a single methyl group attached to an sp^2 carbon atom, and a 1,1-disubstituted olefin. The phytoalexin was thus assigned structure (**5**). The configuration at C-13 was determined by examination of the c.d. spectrum of the osmate esters at *ca.* 474 nm.[5] After treatment of (**5**) with OsO_4–pyridine, a positive Cotton effect was observed ($[\theta]_{max}$ +3860 °) whereas treatment of rotenone, which contains the same prenyl side-chain of defined *R* configuration,[6] resulted in a negative Cotton effect ($[\theta]_{max}$ −5800 °). Together with the negative trough in the o.r.d. spectrum this establishes the (6a*R*,11a*R*,13*S*) configuration of (**5**).







In a typical experiment the isomers (**1**), (**2**), and (**5**) were isolated in yields of 16, 7, and 17 μg/g fresh weight of cotyledons. The three compounds are also produced in response to infection of hypocotyls by *P. megasperma* var. *sojae* and possess similar activity against this organism (ED_{50} *ca.* 60 μg ml^{-1}). It is interesting to note that no significant amount of the C-4 prenylated isomer of (**5**) has been detected.

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