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MINOR PTEROCARPINOIDS OF SOYBEAN

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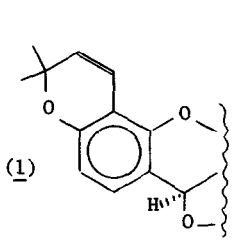
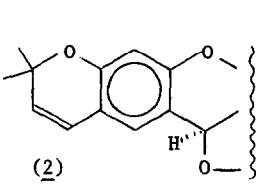
Seedlings of soybean (Glycine max (L.) Merr) respond to fungal infection or chemical injury ($CuCl_2$ etc.) with rapid production of antifungal compounds.[1,2] The three major phytoalexins have been characterised as the isomeric 6a-hydroxypterocarpans glyceollins I-III (1-3).[3,4] Two further members of this rare group have now been isolated from copper-treated cotyledons, their structures and biological activity giving further information on the biosynthesis of the glyceollins and their role in resistance.

Ethanol extracts from treated cotyledons were fractionated by chromatography on Sephadex LH-20 and subsequently by HPLC on Partisil. A minor non-crystalline pterocarpinoid metabolite, glyceollin IV [$C_{21}H_{22}O_5$(M^+=354.1468), λ_{max}(EtOH) 286 nm] was isolated in conjunction with the major glyceollins. The presence of a 6a-hydroxypterocarpan nucleus in this compound was deduced from its facile dehydration with formic acid to give a pterocarpene (M^+336, λ_{max}339,357 nm) and from the diagnostic ^{1}H n.m.r. spectrum (Table). This included a typical AB quartet ($H_{6,6'}$) at 4.01δ(J=11.4 hz) showing long-range coupling of the low-field hydrogen to H_{11a} at 5.20δ. The ABX pattern of aromatic signals (6.2-7.2δ) corresponded exactly with the pattern observed for ring D of (3). The two remaining singlet aromatic signals defined the positions of substituents in ring A as being present at C-2 and C-3. Methoxyl and 1,1-dimethylallyl groups were also identified from the spectral data, the latter group being assigned to C-2 on biogenetic considerations and by comparison with (3). The positive maximum (291 nm) in the CD spectrum of the metabolite permitted assignment of the 6aR,11aR configuration and hence defined the complete structure of glyceollin IV as (4).


(1)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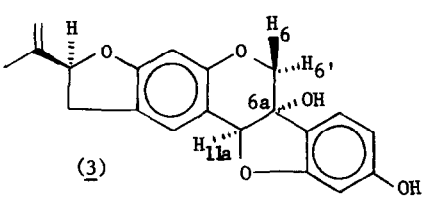
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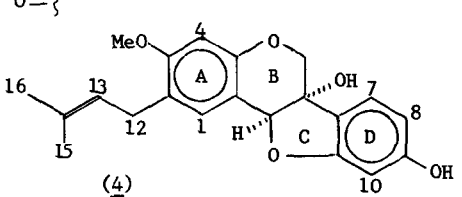
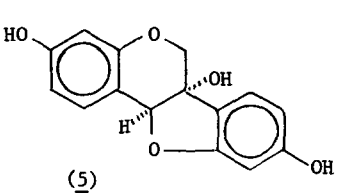

(4)


(5)

A more polar metabolite was also obtained from the extract of treated cotyledons [$C_{15}H_{12}O_5$ (M^+=272.0691), λ_{max} 282,287 nm]. The 6a-hydroxypterocarpan nucleus was again defined by dehydration with formic acid to give the corresponding pterocarpene (M^+=254, λ_{max} 335,350 nm) and by the ^1H n.m.r. spectrum of the metabolite. The presence of two phenolic hydroxyl groups was confirmed by methylation with diazomethane. Formic acid dehydration of this product again gave a pterocarpene (M^+=282, λ_{max} 335,350 nm). The aromatic hydroxylation pattern was defined by the n.m.r. spectrum (Table) of the metabolite. The exact coincidence of one group of ABX signals with those observed in glyceollins I-IV permitted their assignment to the hydrogens of ring D. Finally, the CD curve of the metabolite (positive maximum at 287 nm) completed definition of its structure as (6aR,11aR)-3,6a,9-trihydroxypterocarpan (5).

The antifungal activity of (4) is comparable to that of the other glyceollins in the Cladosporium plate bioassay, but the unsubstituted pterocarpan (5) is inactive. Whether activity is a function of polarity or of metabolic stability of the molecules is uncertain. Typical yields of (4) and (5) from $CuCl_2$-treated cotyledons were 0.8 and 7.0 μg/g fresh weight respectively, though neither compound has yet been detected in hypocotyls following inoculation with Phytophthora megasperma var. sojae. The possibility that (5) may be a precursor of the glycollins, as proposed by Keen et al (1972),[5] is currently being tested.

TABLE ^1H n.m.r. data for phytoalexins (3), (4) and (5)[a]

Proton	(3) δ	(3) J(Hz)	(4)[b] δ	(4)[b] J(Hz)	(5) δ	(5) J(Hz)
H_1	7.27s	-	7.18s	-	7.30d	8.3
H_2	-	-	-	-	6.55q	8.4/2.5
H_4	6.27s	-	6.40s	-	6.32d	2.3
H_6	4.05d	11	4.01d	11.4	4.02d	11.6
$H_{6'}$	4.15d	11	4.13d	11.4	4.16d	11.6
H_7	7.23d	8	7.20d	8.6	7.20d	8.3
H_8	6.46q	8;2	6.42q	8.2;2.1	6.42q	8.1;2.2
H_{10}	6.26d	2	6.25d	2.1	6.24d	2.0
H_{11a}	5.30s[c]	-	5.20s[c]	-	5.26s[c]	-

(a) Spectra measured in $(CD_3)_2CO$ on a JEOL PFT-100 spectrometer at 99.54 MHZ;
(b) Additional signals at 1.72 (6H,s,CH_3-15/16), 3.25 (2H,d(7.2Hz),H-12), 3.79 (3H,s,OCH_3), 5.24 (1H,t(7Hz),H-13)δ;
(c) Broad singlet.

References

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