Phytochemistry, 1975, Vol. 14, pp. 1389–1390. Pergamon Press. Printed in England.

STRUCTURE OF THE PHYTOALEXIN FROM SOYBEAN

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(*Received* 9 *June* 1974)

Key Word Index—*Glycine max*; Leguminosae; soybean; phytoalexin; phaseollin; hydroxyphaseollin.

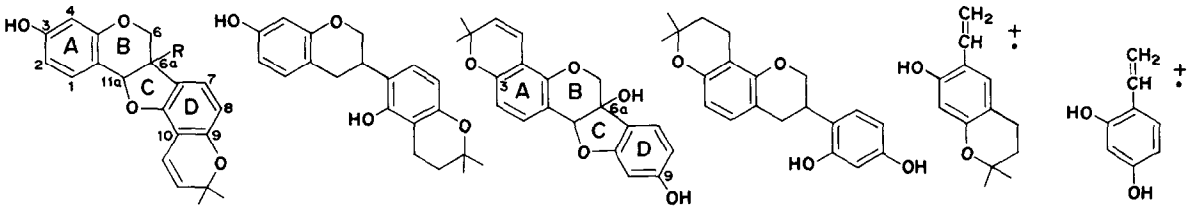
Abstract—The induced antifungal compound in soybean is shown to have been previously incorrectly identified by other workers as 6a-hydroxyphaseollin and a new structure is proposed.

In our studies on the metabolism of the phytoalexin phaseollin (**1**) by *Colletotrichum lindemuthianum* we have provided evidence [1] for the structure of a metabolite (metabolite 1) as 6*a*-hydroxyphaseollin (**2**). This is the same structure as that proposed by other workers [2] for an induced antifungal substance in soybean. However, as the spectral data reported for the two compounds were not identical, we decided to reisolate the soybean phytoalexin in order to make a direct comparison.

The cotyledons of freshly germinated soybean seeds were removed and treated with 5×10^{-3} M cupric chloride solution for several days. This treatment [3] induced the formation of an antifungal compound which was highly inhibitory to *Cladosporium cucumerinum* on silica plates and which after purification by TLC had spectral properties in full agreement with those reported [2] by Sims *et al.* In particular a λ_{max}(EtOH) was observed at 286 nm with shoulders at 291, 306 and 318 nm. Treatment with KOH induced a shift of the λ_{max} to 294 nm with a shoulder at 317 nm. This spectrum, however, was markedly different from that of metabolite 1 which had λ_{max}^{EtOH} 280 nm with shoulders at 286 and 314 nm and underwent a negligible shift with KOH.

The difference between the two compounds was confirmed by silica gel TLC (hexane–ethyl acetate–methanol, 60 : 40 : 1) when the soybean phytoalexin was found to have a lower R_f than metabolite 1. It also gave an orange colour with diazotized *p*-nitroaniline in contrast to the yellow colour obtained with metabolite 1. Nevertheless, it was clear that the compounds were structurally very similar, each having the same functional groups including a tertiary hydroxyl situated at C-6a as revealed by PMR data [1, 2] and by the ready loss of water on treatment with acid [1, 2].

A possible difference between the two compounds was the position of the 2,2-dimethylchromen ring. To investigate this, both compounds were dehydrated with formic acid to yield the corresponding pterocarpens which were then hydrogenated in ethanol over palladium/charcoal to provide the hexahydroderivatives. The derivative so obtained from metabolite 1 was found to be identical with the isoflavan (**3**) obtained by hydrogenolysis of phaseollin. The MS which exhibited major ions at *m/e* 326 (49%), 204 (100), 191 (59), 149



(1) R = H
(2) R = OH
(3)
(4)
(5)
(a)
(b)

(43), 148 (50), 147 (24), 135 (44) and 123 (25) was fully in accord with this structure, the most prominent ion (a) at *m/e* 204 arising from a retro-Diels-Alder fragmentation [4]. The MS of the isoflavan derived from the soybean phytoalexin exhibited peaks at *m/e* 326 (67%), 271 (17), 191 (100), 147 (25), 136 (60), 135 (59) and 123 (40). The ion *m/e* 204 was absent but was replaced by one at *m/e* 136, corresponding to fragment (b). From this evidence therefore the 2,2-dimethylchromen ring in the soybean phytoalexin should be attached to ring A rather than ring D.

The presence in the PMR spectrum ($CDCl_3$) of the soybean phytoalexin of two low field doublets (both J 8 Hz) at δ 2·78 and 2·84 implies that there are two protons in the molecule which are *meta* to two oxygen substituents [5] and are coupled only to a neighbouring *ortho* proton. This information is sufficient to locate the hydroxyl substituent of ring D at C-9 and if the assumption is made on biogenetic grounds that C-3 is oxygenated (all known pterocarpans carry an oxygen substituent at C-3 then the only feasible structure is **4**. This structure which has two *ortho* aromatic protons in ring A, one of which is *meta* to two oxygen containing substituents, is in full agreement with the PMR data. The isoflavan derived by dehydration and hydrogenolysis of soybean phytoalexin is then **5**. Structure **4** for the soybean phytoalexin does not appear to have been considered by Sims *et al* although it is totally consistent with their evidence.

Acknowledgements—We thank Professor R. L. Wain, F.R.S., for his advice and encouragement, Mr. R. Self and Mr. J. Eagles (Food Research Institute, Norwich) for MS and Mrs. Pat Twyman for technical assistance.

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