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THE STRUCTURE OF PHASEOLIN

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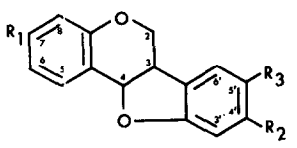
Phaseolin, a new antifungal compound has recently been isolated from French Bean (Phaseolus vulgaris L.) (1). It appears to be produced following fungal inoculation, and its biological activity indicates that it has the properties of a phytoalexin (2).

Phaseolin, $C_{20}H_{18}O_4$, m.p. 177-178°, $[\alpha]_{578} = -145°$ is a phenol and contains no other hydroxylic groups. Methoxyl, carbonyl and carboxyl groups are absent. Kuhn-Roth oxidation of phaseolin corresponds with 0.82 $C.CH_3$ groups. The ultraviolet (1) and infrared spectra of phaseolin are similar to those of homopterocarpin (3), for which structure I ($R_1 = OCH_3$, $R_2 = OCH_3$, $R_3 = H$) has been firmly established both by degradative methods and by synthesis (4). Consideration of the analysis for phaseolin together with this similarity of ultraviolet and infrared spectra suggested that both substances contain the same, chromanocoumaran, ring system which also occurs in pisatin (5), a phytoalexin isolated from the Garden Pea (Pisum sativum L.) (6) and in several other substances isolated from plants of the Leguminosae, sub-family Papilionaceae.

Detailed analysis of the NMR spectra (in $CDCl_3$) of phaseolin and homopterocarpin (Table 1) confirm this provisional assumption, and indicate that phaseolin has structure II.



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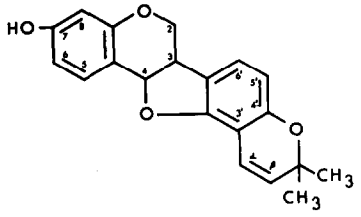

II

TABLE 1

NMR Spectra of Phaseolin and Homopterocarpin

Phaseolin

Position of peaks in p.p.m.	No. of protons	Remarks
2.48, 2.62	1	Doublet, J = 8.4 c.p.s.
2.92, 3.06	1	Doublet, J = 8.2 c.p.s.
3.33–3.66	4	Multiplet
4.20	1	Singlet (rounded)
4.30, 4.47	1	Doublet, J = 10.3 c.p.s.
4.42, 4.53	1	Doublet, J = 6.7 c.p.s.
5.68–6.03	1	Multiplet
6.33, 6.43, 6.48	2	3 peaks
8.57	3	Singlet, sharp
8.61	3	Singlet, sharp

Homopterocarpin

Position of peaks in p.p.m.	No. of protons	Remarks
2.53, 2.68	1	Doublet, J = 8.5 c.p.s.
2.88, 3.03	1	Doublet, J = 8.9 c.p.s.
3.30–3.68	4	Multiplet
4.52, 4.64	1	Doublet, J = 6.2 c.p.s.
5.75–6.13	1	Multiplet
6.32	3	Singlet, sharp
6.34	3	Singlet, sharp
6.41, 6.52, 6.57	2	3 peaks, partly screened by methoxyl signals

The NMR spectrum of phaseolin which gives a total proton count of 18, shows a gem-dimethyl group, τ = 8.57, 8.61 in the expected position for methyls of a 2,2-dimethylchromene system (7,8,9). (Compare τ = 8.51, 8.55 for munetone (7), τ = 8.55 for sericetin (8) and τ = 8.54 for jacareubin trimethyl ether (10)). Similarly, the doublet, centre τ = 4.39, J = 10.3 c.p.s. can be assigned to the β proton of a 2,2-substituted chromene system (7,8,9). (Compare munetone, a bis-dimethylchromene derivative with doublets having centres at 4.25 and 4.33 and coupling constants of 10 c.p.s. (7); sericetin τ = 4.40, J = 10 c.p.s. (8) and jacareubin trimethyl ether τ = 4.41, J = 10 c.p.s. (10)). The signal for the chromene α-proton lies in the aromatic region, and is discussed later. The somewhat diffuse peak at τ = 4.20 is assigned to the phenolic proton. This peak moves to higher τ values when the temperature is raised, and it disappears when D_2O is added. The doublets with centre τ = 4.48, J = 6.7 c.p.s. (1 proton) for phaseolin and τ = 4.58, J = 6.2 c.p.s. (1 proton) for homopterocarpin are assigned to H_4 in each case. (Compare doublet, centre τ = 4.52 for H_4 in pterocarpin (11)). Similarly, the three peaks (2 protons), centre τ = 6.41 for phaseolin and centre, τ = 6.50 for homopterocarpin must be due to the pair of protons on C_2. (Compare edulin τ = 6.4 for C_2 (12)). The existence of this splitting pattern indicates clearly that unlike pisatin, which has a hydroxyl on C_2, phaseolin has protons on C_2, C_3 and C_4 as is found also in pterocarpin, homopterocarpin, edulin (12) and trifolirhizin (13). The signal due to the two protons on C_2 is split by the proton on C_3, which in turn gives rise to the multiplet τ = 5.68 to 6.03 for phaseolin and τ = 5.75 to 6.13 for homopterocarpin. The corresponding peaks in the NMR spectra of pterocarpin (11) and edulin (12) are located in similar positions and show similar splitting.

In the aromatic region for phaseolin (in $CDCl_3$), the doublet, centre $\tau = 2.55$, $J = 8.4$ c.p.s. in phaseolin is assigned to H_5. (Compare $\tau = 2.61$, $J = 8.5$ c.p.s. for H_5 in homopterocarpin; $\tau = 2.59$, $J = 8.5$ c.p.s. in pterocarpin (11) and $\tau = 2.64$, $J = 8.7$ c.p.s. in pisatin (14). Similarly, the doublet centred at $\tau = 2.99$, $J = 8.2$ c.p.s. in phaseolin is assigned to $H_{6'}$. (Compare $\tau = 2.96$, $J = 8.9$ c.p.s. in homopterocarpin). These low values are to be expected because in both cases they are in positions meta to two oxygen-substituents (11), and in turn support the conclusion from biogenetic grounds that the O atoms are located in positions 7 and 4'.

Further analysis of the aromatic region of phaseolin required the use of another solvent system to secure adequate resolution. Figure 1 shows the analysis of the aromatic region of phaseolin in acetonitrile after deuteration. The values obtained are listed in Table 2 along with those for homopterocarpin in deuterochloroform.

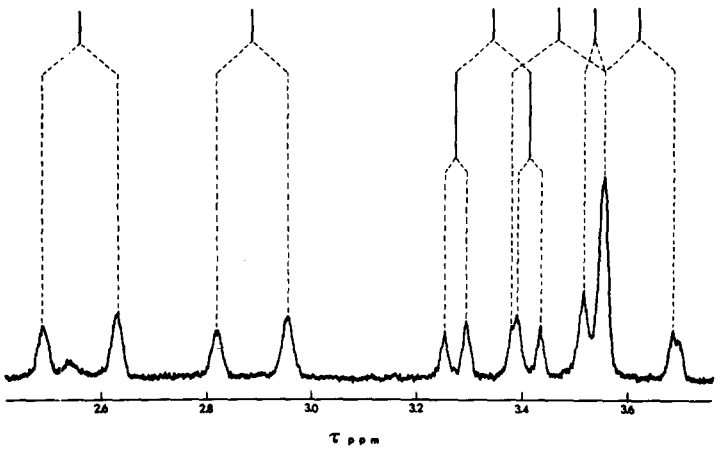

FIG. 1
NMR Spectrum of Phaseolin

TABLE 2

Comparison of the Aromatic Regions of the NMR Spectra of Phaseolin and Homopterocarpin

Phaseolin (in acetonitrile)			Homopterocarpin (in deuterochloroform)		
p.p.m. (centre)	J c.p.s.	Assign-ment	p.p.m. (centre)	J c.p.s.	Assign-ment
2.56	8.5	H_5	2.61	8.5	H_5
2.89	8.1	H_6,	2.96	8.9	H_6,
3.35	2.5, 8.4	H_6	3.40	2.4, 8.3	H_6
3.48	10.4	α			
3.55	2.3	H_8	3.51	2.1	H_3, or H_8
			3.56	2.4	H_8 or H_3,
3.64	8.1	H_5,	3.60	2.3, 8.8	H_5,

α α-chromenyl

In phaseolin, three aromatic protons show ortho coupling only, one proton exhibits ortho and meta coupling while the remaining proton shows meta coupling only. (Para coupling was not detected). These observations require a substitution pattern of the type shown in II where the protons H_5, H_5, and H_6, show ortho coupling only (J = 8.5, J = 8.1 and J = 8.1 c.p.s. respectively) and H_6 shows both ortho and meta splitting (J = 8.4, 2.5 c.p.s. respectively). The corresponding H_6 values are τ = 3.35 for pisatin (14) and τ = 3.40 for pterocarpin (14). The proton H_8 exhibits meta splitting only, and the τ value 3.55 is in good agreement with the published values for H_8 in other chromanocoumarans. (Compare τ = 3.54 for pisatin (14); τ = 3.53 for pterocarpin (14)).

The assignment of the proton τ = 3.48, to the α- chromenyl position accords with similar values in other 2,2-dimethylchromenyl systems.

(Compare τ = 3.32 in jacareubin trimethyl ether (10)).

This analysis indicates that phaseolin is closely related to the aglycone (I R_1 = OH, R_2 R_3 = OCH_2O) of the antifungal substance trifolirhizin (13) isolated from red clover (Trifolium pratense L.), and some other legumes (15,16). This aglycone differs only in having a methylenedioxy group in the 4', 5' - position instead of the dimethylchromenyl residue in the 3', 4' - position. Spectrophotometric determinations in aqueous solutions of the pK_a values of phaseolin and the aglycone of trifolirhizin gave values of 9.13 $\pm$ 0.04 and 9.18 $\pm$ 0.04 (at approx. 20^0 and I = 0.01). Similarly the spectra of the anions in ethanol included new peaks at 250 mμ (log ε = 4.17) and 249 mμ (log ε = 4.11) (13) respectively. The close agreements of pK and spectral values confirm that the phenolic groups in both substances are similarly located with respect to other substituents in the molecules and hence that the phenolic hydroxyl in phaseolin is correctly located on C_7.

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