Tetrahedron Letters No. 41, pp 4175 - 4178, 1972. Pergamon Press. Printed in Great Britain.

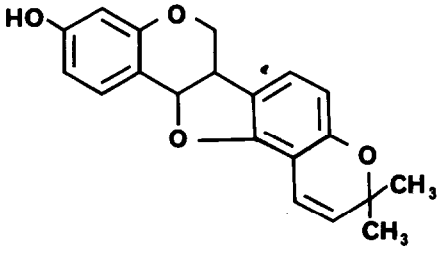
STRUCTURES OF THREE NEW ISOFLAVANOIDS FROM PHASEOLUS VULGARIS INFECTED WITH TOBACCO NECROSIS VIRUS

R.S. Burden, J.A. Bailey and G.W. Dawson

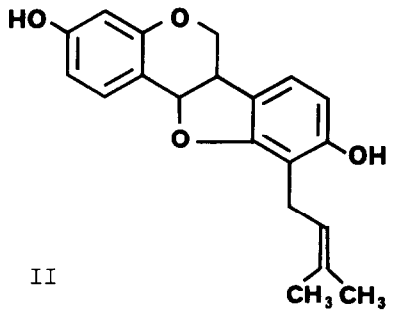
A.R.C. Plant Growth Substance and Systemic Fungicide Unit, Wye College (University of London), Ashford, Kent.

(Received in UK 11 August 1972; accepted for publication 7 September 1972)

Phaseollin (I) is a phytoalexin first isolated from fungal-infected French bean (1). Recently it was also found in tissue infected with tobacco necrosis virus (2). We now report the isolation and identification of three other isoflavanoids from this latter source. As all three compounds are inhibitory towards several fungi and are also produced in bean tissue infected with the fungus Colletotrichum lindemuthianum (3), they too may be regarded as phytoalexins.



I

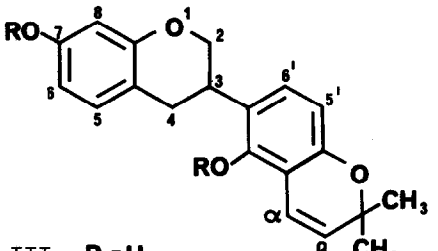


II

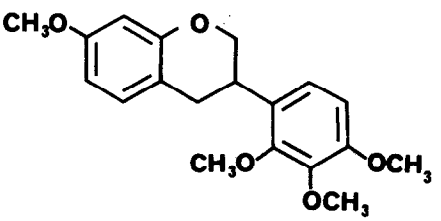
The first compound, $C_{20}H_{20}O_4$, had λmax(EtOH) 281.5 nm (log ε 3.76) and 287 (3.80), νmax ($CHCl_3$) 3350, 1628, 1607 cm^{-1}, M^+ 324, and gave an orange colour with diazotised nitroaniline but a negative Gibbs test. The major fragment ion in the mass spectrum was at m/e 268 (M^+-56) while two phenolic hydroxyls were indicat-

ed from the formation of a diacetate M^+408 with fragment ions at $^m/_e$ 366 (M^+-42), 324 (M^+-42-42) and 268 (M^+-42-42-56). Hydrogenolysis of phaseollin with sodium and ethanol afforded a major product identical to the new substance. Structure (II) was hence indicated, the ortho-(3,3-dimethylallyl)-phenol explaining the facile loss of isobutene in the mass spectrum (4).

While this work was in progress, Perrin, Whittle and Batterham characterised (5) a new antifungal compound 'phaseollidin' from French bean. Phaseollidin appears to be identical with the above substance.



IIIa R = H
b R = CH_3

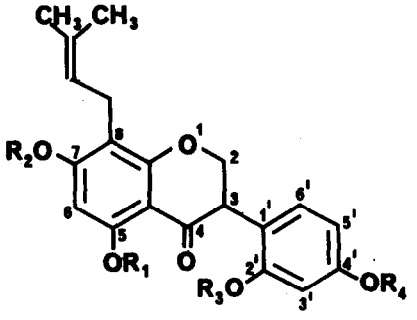


IV

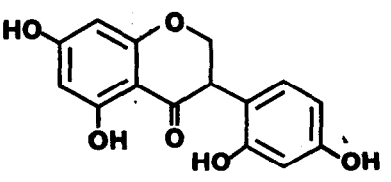
The second compound $C_{20}H_{20}O_4$, had λmax 280nm (4.01) and 310sh (3.27), νmax 3350, 1625, 1600cm^{-1} and gave an orange DNA reaction and a deep blue colour with Gibbs reagent. A dimethyl ether, M^+352, was obtained with diazomethane and an examination of the nmr spectrum ($CDCl_3$, 100 MHz) of this derivative leads to its formulation as the isoflavan (IIIb). Thus the protons at C-2 are non-equivalent and appear as a quartet τ5.77 (J_1=10, J_2=4) and a triplet τ6.09 (J=10) (compare the corresponding protons in laxifloran dimethyl ether (IV) (6) at 5.7,q,J_1=10, J_2=3 and 6.02,t,J=10). H-3 gives rise to a multiplet at τ6.3-6.7 while the protons at C-4 appear as a broad doublet τ7.11 and 7.20 (compare 7.06 and 7.16). The gem-dimethyls appear at τ8.62 (compare phaseollin (I) (1) 8.57,8.61) and the β proton of the 2,2-dimethylchromene gives a doublet at τ4.41 with J=10 (compare 4.39, J=10.3). Two of the aromatic protons appear as doublets (J=8) centred at 3.09 and 3.29 and are assigned to the protons H-5 and H-6′. They are respectively coupled to protons H-6 and H-5′ which appear with the chromene α proton in a multiplet at 3.50 -

3.66. H-8 resonates at τ 3.43 and exhibits the expected meta splitting (J=2).

The new substance is hence identified as phaseollinisoflavan (IIIa) and its mass spectrum fully supports this structure with prominent ions at $^{m}/e$ 309 (M^{+}-15) and $^{m}/e$ 187, the latter involving a retro-Diels-Alder fragmentation of the ion at $^{m}/e$ 309.



Va $R_1,R_2,R_3,R_4 = H$
b $R_1 = H; R_2,R_3,R_4 = CH_3$
c $R_1,R_2,R_3,R_4 = COCH_3$



VI

The third anti-fungal compound* is now formulated as the new isoflavanone (Va) and has been assigned the trivial name kievitone derived from the cultivar Kievitsboon Koekoek from which it was first obtained. Kievitone, $C_{20}H_{20}O_6$, M^{+}356, gave an orange DNA reaction and a purple-blue Gibbs test. It had λmax 293nm (log ε 4.22) and 330sh(3.58). Bathochromic shifts to 337 and 314nm induced by sodium acetate and aluminium trichloride respectively, indicated free hydroxyls at C-7 and C-5 (7). In the infrared spectrum, the chelated carbonyl appeared at 1640cm^{-1}. Methylation with diazomethane afforded a trimethylether (Vb), M^{+}398, νmax 1640cm^{-1} but acetylation gave mainly the tetraacetate (Vc), M^{+}524, νmax 1700cm^{-1}.

In the nmr spectrum (CD_3COCD_3, 100 MHz), kievitone exhibited signals from 4 aromatic protons. One appeared as a sharp singlet at τ 4.02 while two appeared as an ortho-pair (J=8) at τ 3.13 and 3.74. The higher field proton was also

*Smith and Bateman have isolated an identical substance from fungus-infected bean (personal communication).

meta-coupled (J=2) to the remaining proton at τ3.61. Two possible substitution patterns for ring B are suggested by these couplings but as the chemical shifts are almost identicalto those observed (τ3.09, 3.58, 3.69 in CD_3COCD_3) for dalbergioidin (VI) (8), the 1',2',4'-substitution is favoured. A 3,3-dimethylallyl side chain was diagnosed from the typical resonances at τ 8.40, 8.32 (gem-dimethyl), 6.82,d,J=7 (methylene) and 4.86,t,J=7 (olefinic proton). The protons at C-2 and C-3 appeared as a complex multiplet τ5.40 - 5.96 in agreement with the positions observed for the corresponding protons in other isoflavanones (8).

The 3,3-dimethylallyl group was shown to be located at C-8 by the negative Gibbs test of kievitone trimethylether (Vb) and the formation of only one 2,2-dimethylchroman isomer, M^+ 356, λmax 293, λmax (alkali) 293, λmax ($AlCl_3$) 315nm, on treatment of kievitone with acid.

We thank Professor Sir D.H.R. Barton and Dr. L. Phillips (Imperial College) for nmr facilities, Mr. R. Self (A.R.C. Food Research Institute) for mass spectrometry and Dr. B.J. Deverall and Professor R.L. Wain for their helpful advice.

References

1. Dawn R. Perrin, Tetrahedron Letters, 1964, 29.
2. J.A. Bailey and J.L. Ingham, Physiol. Pl. Path., 1971, 1, 433.
3. J.A. Bailey and R.S. Burden. In preparation.
4. J.A. Ballantine, D.J. Francis, C.H. Hassall and J.L.C. Wright, J.Chem.Soc. (C), 1970, 1175.
5. Dawn R. Perrin, C.P. Whittle and T.J. Batterham. Tetrahedron Letters, 1972, 1673.
6. A. Pelter and P.I. Amenechi. J.Chem.Soc.(C), 1969, 887.
7. T.J. Mabry, K.R. Markham and M.B. Thomas. 'The Systematic Identification of Flavanoids', Springer-Verlag, p.169-171.
8. L. Farkas, A. Gottsegen, M. Nógrádi and S. Antus. J.Chem.Soc.(C), 1971, 1994.