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IDENTIFICATION OF 4′,7-DIHYDROXY-2′,3′-DIMETHOXY ISOFLAVAN IN BEAN ROOTS

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ABSTRACT.—We have isolated and identified laxifloran (4′7-dihydroxy-2′3′-dimethoxy isoflavan) from the root of *Phaseolus vulgaris*, suggesting that there is an additional biosynthetic pathway besides those already proposed.

Eighteen isoflavonoids have now been identified as postinfectional metabolites of the French bean *Phaseolus vulgaris* L. (1). The majority of these compounds have been proposed as possible metabolic intermediates in the biosynthesis of the 2′,4′-(isoflavone nomenclature)-dioxygenated isoflavonoids phaseollin (**1**) or kievitone (**2**) (1,2). Continuation of our studies into the isoflavonoids of bean has led to the isolation and identification of the trioxygenated (B ring) isoflavonoid: 4′7-dihydroxy-2′3′-dimethoxy isoflavan (laxifloran) (**3**) from field-grown bean roots, suggesting the existence of an additional biosynthetic pathway to those already proposed.

Compound **3** was isolated from root extracts by silicic acid chromatography and reverse phase hplc. High resolution ms of **3** gave the elemental composition $C_{17}H_{18}O_5$ for the molecular ion. Preparation of the dimethyl ether **4** indicated the presence of two unsubstituted hydroxyl groups in the molecule. The major fragment ion at *m/z* 180, resulting from an RDA fragmentation with retention of charge on ring B (3) suggested an isoflavan isomeric with mucronulatol (**5**) or isomucronulatol (**6**), but because of the close similarities of published ms data on compounds **3, 5,** and **6** (4-9), unequivocal identification was considered not possible by ms alone (4).

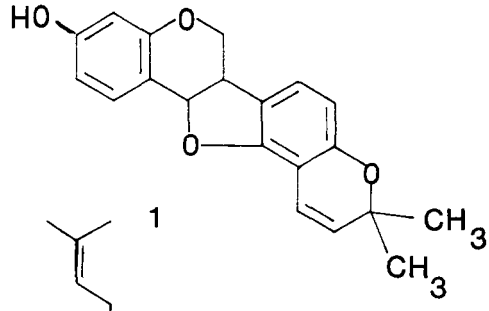
The 80 MHz ^{1}H-nmr spectrum of **3** in chloroform-d confirmed the basic isoflavan structure with two broad resonances at δ 2.85 and 2.75 (H-4), and additional multiplets at δ 3.9-4.3 (H-2) and δ 3.4-3.5 (H-3). The presence of an ABX coupling pattern in the aromatic region centred at δ 6.29, 6.32 and 6.86 provided confirmation of a C-7 hydroxyl substituent in Ring A (4,6,7,10).

The signals from two aromatic protons in ring B coincided to give an apparent singlet at δ 6.64 in chloroform-d, but were resolved in pyridine-d5 (δ 7.02, 6.90, J=8.5 Hz), and in acetone-d6 (δ 6.80, 6.65, J=8.5 Hz). From solvent-induced chemical shift experiments (pyridine-d5) on **3** and on substituted phenols, it was concluded that the hydroxyl group on ring B was *ortho* to one aromatic proton and *meta* to the other. The lack of a Nuclear Overhauser Effect (nOe) on aromatic proton signals upon irradiation of the two methoxyl signals confirmed the identification as **3**. The ^{1}H-nmr spectrum of **3** in acetone-d6 was in good agreement with that reported for dihydrohaginin A (**3**) (10).

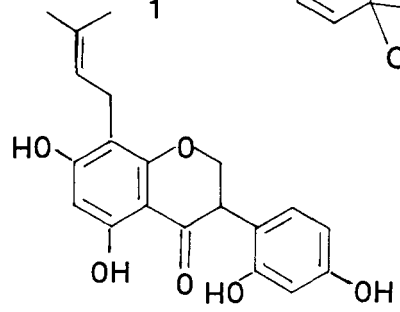
Until now, identification of **3** as a natural product has relied mainly on indirect evidence: in the initial report on *Lonchocarpus laxiflorus* wood (11), **3** was partially characterized in crude extracts, and then purified as the dimethyl ether. Assignment of the hydroxyl at the 4′ position was based both on ms and ir measurements on partially purified extracts and on biogenetic grounds. This proposal was later queried (4) inasmuch as chemically synthesized isomeric isoflavans **3, 5,** and **6** gave very similar fragmentation patterns by ms. Identification of **3** in diffusates of *Lablab niger* leaves (5) was based on uv

and ms data and on tlc differences from isomeric isoflavans **5** and **6** in the underivatized form, but on identical properties of **3**, **5**, and **6** following methylation (5). The present work offers positive identification of **3** as a natural product and confirms its presence in the Phaseoleae.

Although **3** is not found in sufficient concentration in the bean root to be considered important in chemical defences, its presence is, nevertheless, of biosynthetic interest, particularly in relation to the establishment of B ring oxygenation patterns. Current concepts of B ring oxygenation in pterocarpans and isoflavans have been shaped by red clover feeding experiments in which it was demonstrated that oxygenation at positions other than 2′ or 4′ occurred prior to the 2′-hydroxylation step (12). The general applicability of these results to other plants has not yet been rigorously tested. Extrapolation of the red clover observations to bean would imply that a previously undiscovered pathway of 3′,4′-oxygenated isoflavonoid intermediates exist in the bean plant, analagous to intermediates of other isoflavans. Alternatively, any evidence of 3′-oxygenation occurring after the 2′-hydroxylation step in bean tissues would imply that results ob-



1



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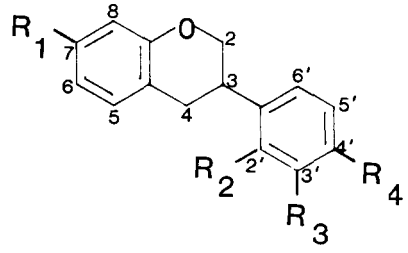

	R_1	R_2	R_3	R_4
3	OH	OMe	OMe	OH
4	OMe	OMe	OMe	OMe
5	OH	OH	OMe	OMe
6	OH	OMe	OH	OMe
7	OH	OH	chromenyl-O	

tained from red clover on isoflavanoid biosynthesis may not be applicable to other plants as well.

EXPERIMENTAL

PLANT MATERIAL.—Field grown bean roots (*Phaseolus vulgaris* L. var. Fardenlosa) were collected in March 1979 and freeze-dried.

EXTRACTION AND FRACTIONATION.—A 2.8-kg sample was extracted with methanol in a Soxhlet apparatus. The extract was diluted with one-fifth volume of water and partitioned with petroleum ether (40-60°). The methanolic phase was dried *in vacuo*, the residue washed three times with chloroform and the washings chromatographed on silicic acid (Mallinckrodt CC7, 500 g, 5% water) with petroleum ether-ether (4:1) and (1:1) (v/v), followed by ether. Fractions containing phaseollinisoflavan (**7**) (as marker) and **3** (tlc, silica gel, chloroform-methanol, 19:1 (v/v), Rf. 0.4, 0.5, respectively) were rechromatographed on silicic acid with methylene chloride-methanol, 40:1 (v/v), then **3** purified by repeated reverse phase hplc (C_{18}-silica acid, methanol-water) to chromatographic homogeneity (tlc, silica gel, chloroform-methanol, 19:1, Rf 0.5; petroleum ether-ethyl acetate, 4:1, Rf 0.18; perlontype polyamide, methanol-water, 4:1, Rf 0.50; C_{18}-silicic acid, methanol-water 3:1, Rf 0.63). The yield of **3** was 0.25 mg, based on published extinction coefficients of **5** and **6** (6,7). Compound **7** was identified by comparison (uv, ms, tlc) with an authentic sample.

7,4′-DIHYDROXY-2′,3′-DIMETHOXYISOFLAVAN (**3**).—Uv λ max (MeOH) 213 nm (rel. int. 100), 225 (sh, 77), 281 (20), 290 (sh, 14); λ max (MeOH+NaOH) 219, 242 (sh), 291, effect reversible by HCl, no spectral changes with NaOAc or $AlCl_3$; ms: *m/z* (rel. int.) 302.1160 (M^+, 57) (calcd for $C_{17}H_{18}O_5$, 302.1153), 180 (100), 168 (43), 167 (40), 165 (28), 135 (30), 133 (32), 123 (26); ^{1}H-nmr (80 MHz, chloroform-d) δ 2.80 (2H, brd, *J*=8 Hz, H-4), 3.4-3.5 (1H, m, H-3), 3.9-4.3 (2H, m, H-2), 3.79 (3H, s, OCH_3); 3.85 (3H, s, OCH_3), 6.29 (1H, dd, *J* 2.5, 0.5 Hz, H-8), 6.32 (1H, dd, *J* 8.5, 2.5 Hz, H-6), 6.86 (1H. dd, *J* 8.5, 0.5 Hz, H-5), 6.64 (2H. s, H-5′ and H-6′). (Pyridine-d_5) δ 6.9 (1H, d, *J*=8.5 Hz, H-6′), 7.02 (1H, d, *J* 8.5 Hz, H-5′). (acetone-d_6) δ 2.8 (2H, m, H-4), 3.4 (1H, m, H-3), 3.83 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 4.1 (2H, m, H-2), 6.34 (1H, dd, *J* 2.5, 0.5 Hz, H-8), 6.37 (1H, dd, *J* 8.5, 2.5 Hz, H-6), 6.65 (1H, d, *J* 8.5 Hz, H-5′), 6.80 (1H, d, *J* 8.5 Hz, H-6′), 6.88 (1H, dd, *J* 8.5, 0.5 Hz, H-5).

7,2′,3′,4′-TETRAMETHOXY ISOFLAVAN (**4**).—This was prepared by reaction of **3** with CH_2N_2, ms: *m/z* (rel. int.) 330 (M^+, 13), 194 (100), 182 (30), 181 (25), 179 (35).

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LITERATURE CITED

1. M. D. Woodward, *Phytochemistry*, **19**, 921 (1980).
2. P.M. Dewick and M.J. Steele, *Phytochemistry*, **21**, 1599 (1982).
3. H. Audier, *Bull. Soc. Chim. Fr.*, 2892 (1966).
4. L. Farkas, A. Gottsegen, M. Nogradi, and S. Antus, *J. Chem. Soc. Perkin Trans.*, **I**, 305 (1974).
5. J.L. Ingham, *Z. Naturforsch., Teil C*, **32c**, 1018 (1977).
6. D.M.X. Donnelly, P.J. Keenan and J.P. Prendergast, *Phytochemistry*, **12**, 1157 (1973).
7. G.D. Manners and L. Jurd, *Phytochemistry*, **18**, 1037 (1979).
8. J.L. Ingham, *Phytochemistry*, **16**, 1457 (1977).
9. Ms data for **3** were at variance with those reported in ref. 10. Most of their data appears to deviate 1 atomic mass unit from reported m/z values (7).
10. T. Miyase, A. Ueno, T. Noro and S. Fukushima, *Chem. Parm. Bull.*, **28**, 1172 (1980).
11. A. Pelter and P.I. Amenichi, *J. Chem. Soc. C*, 887 (1969).
12. P.M. Dewick and D. Ward, *Phytochemistry*, **17**, 1751 (1978).

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