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DOLICHINS A AND B, TWO PTEROCARPANS FROM BACTERIA-TREATED LEAVES OF *DOLICHOS BIFLORUS*

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Key Word Index—*Dolichos biflorus*; Leguminosae; horsegram; isoflavonoids; pterocarpans; *Pseudomonas pisi*.

Abstract—Two minor isoflavonoids isolated from bacteria-inoculated leaves of *Dolichos biflorus* have been identified as the 6a*R*; 11a*R*; 2′*R* and 6a*R*; 11a*R*; 2′*S* isomers of 3,9-dihydroxy-10-(2′-hydroxy-3′-methyl-3′-butenyl)pterocarpan.

INTRODUCTION

The horsegram, *Dolichos biflorus* L., is known to produce a variety of isoflavonoid phytoalexins following leaflet infiltration with cell suspensions of the incompatible bacterium, *Pseudomonas pisi* [1]. These compounds include isoflavonoids previously obtained from other sources (e.g. 2′-hydroxygenistein, dalbergioidin, kievitone and phaseollidin) as well as a new isoflavanone recently shown to be 5,7,4′-trihydroxy-2′-methoxyisoflavanone (isoferreirin) [1]. Further examination of *Pseudomonas*-inoculated *Dolichos* leaves has now revealed small quantities of a hitherto undescribed material ('dolichin'), the nature of which is described in this report.

RESULTS AND DISCUSSION

Leaves of *D. biflorus* were infiltrated with cells of *P. pisi* [1] and induced material subsequently extracted as described elsewhere [2]. Si gel TLC (hexanes–EtOAc–MeOH,* 60:40:1) gave 'dolichin' (R_f 0.41) admixed with the coumestan, psoralidin (3,9-dihydroxy-2-isopentenylcoumestan). After elution (EtOH) and further TLC purification ($CHCl_3$–Me_2CO–NH_4OH, 65:35:1), the 'dolichin' band (R_f 0.32; cf. psoralidin, R_f 0.13) was finally chromatographed in *n*-pentane–Et_2O–HOAc (75:25:6, × 6) to yield two phenolic compounds termed dolichin A (upper zone) and dolichin B (lower zone) respectively. Neither compound was detected in extracts of leaves treated with distilled H_2O.

In EtOH and EtOH + NaOH, the dolichins had UV maxima virtually indistinguishable from those of *Dolichos*-derived phaseollidin [(−)-6a*R*; 11a*R*-3,9-dihydroxy-10-isopentenylpterocarpan, **1**]. In both cases, however, MS analysis (see Experimental) gave the M^+ at *m/e* 340, a feature which suggested that these compounds were monohydroxy derivatives of **1**; moreover, the presence of major MS fragments at M − 70 and 71 instead of M − 55 and 56 as in phaseollidin (the latter ion representing loss of isobutene from an isopentenyl unit) [3] clearly indicated that the additional oxygen was attached to the sidechain rather than to one of the aromatic (A/D) or heterocyclic (B/C) rings.

This view was confirmed by examination of ^{1}H NMR (360 MHz) spectra of the two compounds (Table 1). Both spectra exhibited signals for aromatic and heterocyclic ring protons corresponding with those observed for phaseollidin (**1**). However, the remaining signals (attributable to protons in a C-10 sidechain), though similar for the two new compounds, were very different from those of **1**. Dolichin A gave only a single Me signal at 1.80 ppm (characteristic of an allylic methyl group), whilst two one-proton singlets at 4.72 and 4.88 ppm could be assigned to geminal olefinic protons, possibly resulting from double bond rearrangement in an initially-formed γ,γ-dimethylallyl sidechain. Corresponding signals were

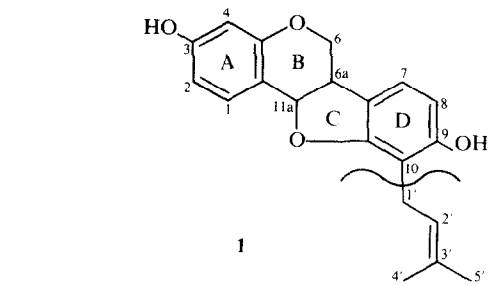
Table 1. ^{1}H NMR data for phaseollidin, dolichin A and dolichin B*

Proton	Phaseollidin	Dolichin A	Dolichin B
H-1	7.34*d*	7.33*d*	7.32*d*
H-2	6.56*q*	6.55*q*	6.55*q*
H-4	6.34*d*	6.35*d*	6.35*d*
H-6a/H-6ax	3.56*m*	3.55*m*	3.55*m*
H-6eq	4.24*q*	4.25*q*	4.24*q*
H-7	6.96*d*	7.01*d*	7.02*d*
H-8	6.40*d*	6.37*d*	6.38*d*
H-11a	5.46*d*	5.44*d*	5.46*d*
H-1′	3.25*d*	2.87*q*†	2.81*q*†
		3.01*q*	3.02*q*
H-2′	5.27*t*	4.32*q*	4.37*q*
H-4′	1.60*s*	4.72*s*	4.67*s*
		4.88*s*	4.82*s*
H-5′	1.73*s*	1.80*s*	1.76*s*

* Solvent, $(CD_3)_2CO$ except where indicated; chemical shifts are expressed as δ values (TMS reference).

† Signals quoted are chemical shifts in $CDCl_3$. In the $(CD_3)_2CO$ spectrum the peaks are obscured by solvent signals.

* A commercial mixture of hexane isomers (bp 68–70°) was used.



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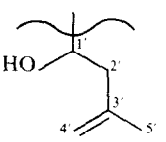

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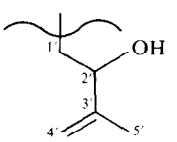

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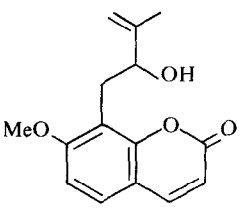

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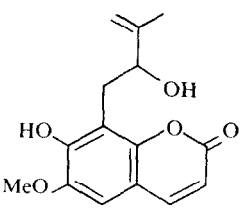

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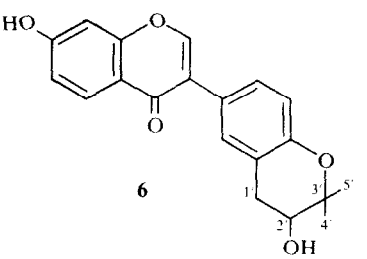

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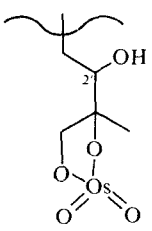

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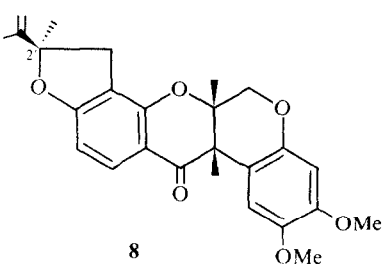

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also evident in the spectrum of dolichin B, leaving the additional oxygen function in each isomer to be assigned to either C-1′ (**2**) or C-2′ (**3**).

The remaining methylene and methine protons resonate at *ca* 2.9 and 4.3 ppm respectively in dolichins A and B. These shifts are in close agreement with those expected for 2′-hydroxylated isomers of type **3**, based on the reported values for auraptenol (**4**) [4] and cneorum-coumarin B (**5**) [5]. They also correlate well with predicted values based on the spectrum of psoralenol (**6**), in which the benzylic signal is observed at 2.92 ppm [6]. Allowing a net deshielding effect of 0.55 ppm for a secondary alcohol function, and introduction of an allylic double bond [7], the calculated chemical shift for C-2′ of **3** is *ca* 4.4 ppm, in close accord with the observed values. Similar calculations, based on hydroxylated phaseollin models [7,8] for H-1′ and H-2′ in the alternative structure **2**, indicate expected chemical shifts of *ca* 4.8 and 2.1 ppm respectively. Thus, the chemical shift data clearly support assignment of the sidechain hydroxyl groups of both dolichin A and B to C-2′.

Dolichins A and B are both strongly laevorotatory (see Experimental) permitting assignment of the 6a*R*; 11a*R* absolute configuration ([9] and refs. therein) and thus differ only in the absolute configuration at C-2′. This conclusion was confirmed by conversion of the two olefinic groups to the corresponding osmate esters (**7**) upon treatment with OsO_4 (see Experimental). The CD spectra of the derivatives at 470 nm (dolichin A, $[\theta]_{max} - 7930°$; dolichin B, $[\theta]_{max} + 5970°$) clearly indicated that the adjacent chiral centres had the opposite configuration. The sign of the CD curve for dolichin A was the same as that obtained with 2′*R* rotenone (**8**) ($[\theta]_{max} - 5850°$); this suggests that dolichin A also possesses the 2′*R* sidechain configuration and that dolichin B has the opposite (2′*S*) absolute stereochemistry. However, these assignments can only be provisional since they are based on a comparison with a cyclic sidechain system (**8**) which may or may not react with OsO_4 in the same manner as do the acyclic dolichins. As yet, the sidechain stereochemistry of the directly comparable model compounds, auraptenol (**4**) and cneorum-coumarin B (**5**), has not been determined [4,5].

The precise origin of dolichins A and B remains obscure. Whilst both pterocarpans occur in bacteria-infiltrated leaves, they have not been isolated from fungus (*Phytophthora megasperma* f. sp. *glycinea*) inoculated *Dolichos* stems which actively synthesize phaseollidin and the other leaf phytoalexins described earlier [1]. Attempted abiotic elicitation (using aqueous ICH_2COONa, $CuCl_2$, $HgCl_2$ or $K_2Cr_2O_7$) of the various *Dolichos* isoflavonoids was generally unsuccessful, and

did not result in formation of either dolichin A or dolichin B. The dolichins may thus be bacterial metabolites of phaseollidin or, alternatively, may be specifically induced by *P. pisi* in *Dolichos* leaves. In this respect it should be noted that neither dolichin A nor dolichin B accumulated when phaseollidin (up to 100 μg/ml) was incubated (48 hr) in a liquid medium [10] with cells of *P. pisi*; bacterial growth was unaffected and the phytoalexin was recovered unchanged from the culture fluid. Additionally, there was no evidence to suggest that the prenylated isoflavanone, kievitone (a major *Dolichos* phytoalexin) underwent bacterial metabolism to give products comparable with either dolichin A or B. On balance, therefore, the dolichin isomers appear to be of plant origin although their presence as very minor induced leaf constituents, and their complete lack of antibacterial activity (as judged by TLC bioassays against *P. pisi* [1] at applied levels up to 50 μg) suggests that neither compound plays a significant role in restricting the *in vivo* development of *P. pisi*.

EXPERIMENTAL

Seeds of *Dolichos biflorus* L. (purchased from J. L. Hudson, Seedsman, Redwood City, California) were grown as previously described [1]. Dolichins A and B were isolated in approximately equivalent amounts from extracts of bacteria-inoculated leaves [1,2] as outlined under Results and Discussion.

Dolichin A. Diazotised *p*-nitroaniline, orange; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 212 (100%), 232 sh (45%), 282 (20%), 288 (22%), $\lambda_{max}^{EtOH+NaOH}$ nm: 215, 249, 298. MS *m/e* (rel. int.) 341 (18), 340 (M^+; 63), 323 (9), 322 (37), 321 (12), 308 (18), 307 (91), 305 (14), 279 (23), 271 (19), 270 (100), 269 (95), 268 (24), 267 (38), 185 (12), 161 (12), 149 (11), 148 (23), 147 (34), 137 (11), 135 (27), 134 (10), 123 (46). $[\alpha]_{589nm}$: $-265°$ (*ca* 0.5 mg in 1 ml MeOH; cf. *Dolichos* phaseollidin, $-203°$ (*ca* 0.8 mg in 1 ml MeOH)). ^{1}H NMR, see Table 1.

Dolichin B. Diazotised *p*-nitroaniline, orange; Gibbs reagent, no reaction. λ_{max}^{EtOH} nm: 213 (100%), 232 sh (60%), 282 (29%), 288 (32%); $\lambda_{max}^{EtOH+NaOH}$ nm: 216, 248, 298. MS *m/e* (rel. int.) 341 (15), 340 (M^+; 58), 323 (5), 322 (22), 321 (8), 308 (12), 307 (74), 305 (7), 279 (6), 271 (16), 270 (100), 269 (98), 268 (29), other fragments as given for dolichin A. $[\alpha]_{589nm}$: $-235°$ (*ca* 0.4 mg in 1 ml MeOH). ^{1}H NMR, see Table 1.

Preparation of osmate esters. A dry sample of dolichin A (420 μg, 1.24 μM) was dissolved in CH_2Cl_2 (61 μl) + pyridine (1.96 mg, 24.8 μM), and then treated with OsO_4 (345 μg, 1.36 μM) in CH_2Cl_2 (4.6 μl). After reaction for 30 min at room temp., the mixture was diluted with CH_2Cl_2 to a final vol. of 2.8 ml and the CD spectrum recorded on a Carey 61 spectrometer. Dolichin B (330 μg) was treated similarly using OsO_4 and pyridine in molar ratios of 1.1 and 20 respectively.

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