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FUNGITOXIC DIHYDROFURANOISOFLAVONES AND RELATED COMPOUNDS IN WHITE LUPIN, *LUPINUS ALBUS**

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Key Word Index—*Lupinus albus*; Leguminosae; dihydrofuranoisoflavones; lupinisoflavones A–F, pyranoisoflavones, prenylated isoflavones; 3′-*O*-methylorobol; fungitoxins.

Abstract—Chromatographic investigation of a methanolic extract of white lupin roots has revealed the presence of six new dihydrofuranoisoflavones (lupinisoflavones A–F). Three monoprenylated (3,3-dimethylallyl-substituted) isoflavones (wighteone, luteone and licoisoflavone A), two diprenylated isoflavones [6,3′-di(3,3-dimethylallyl)genistein (lupalbigenin) and 6,3′-di(3,3-dimethylallyl)-2′-hydroxygenistein (2′-hydroxylupalbigenin)] and two pyranoisoflavones (parvisoflavone B and licoisoflavone B) have also been isolated from the same source. In addition to genistein, leaf extracts of *L. albus* contain 3′-*O*-methylorobol which is presumed to be the precursor of lupisoflavone [5,7,4′-trihydroxy-3′-methoxy-6-(3,3-dimethylallyl)isoflavone]. Probable biogenetic relationships between the prenylated, and dihydrofurano- and pyrano-substituted isoflavones in roots and leaves of *L. albus* are briefly discussed.

INTRODUCTION

In an earlier paper [1] we reported the results of a quantitative and qualitative analysis of constitutive isoflavones in leaves and other parts of eight species belonging to the genus *Lupinus* (family Leguminosae; subfamily Papilionoideae; tribe Genisteae). Our attention focused particularly on the white lupin (*Lupinus albus* L., cv. Kievskij Mutant), and apart from genistein (**1a**) the leaves of this species yielded large quantities of 2′-hydroxygenistein (**1b**), wighteone (**2a**) and luteone (**2b**). A new prenylated isoflavone, lupisoflavone [5,7,4′-trihydroxy-3′-methoxy-6-(3,3-dimethylallyl)isoflavone (**3a**)] was also identified as a minor leaf constituent. The roots of *L. albus* were found to contain isoflavones **1a**, **1b**, **2a** and **2b** as well as alpinumisoflavone (**4a**), licoisoflavone B (**5**) and 6,3′-di(3,3-dimethylallyl)genistein (lupalbigenin, **6a**). It was clear, however, that white lupin roots also contained further isoflavone-like compounds which at the time could not be obtained in amounts sufficient for complete chemical characterisation.

In this paper we present data relating to a comprehensive reinvestigation of the root components of *L. albus* which has resulted in the successful identification of several rare or previously unrecognised dihydrofurano-, pyrano- and 3,3-dimethylallyl-substituted isoflavones. 3′-*O*-Methylorobol (**3b**) has also been detected for the first time as a constituent of *L. albus* leaves.

RESULTS AND DISCUSSION

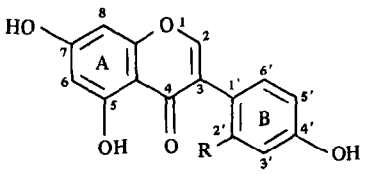
Leaves

Preparative TLC (PTLC) of a methanolic extract of white lupin leaves using the CAAm solvent system (see Experimental for details) afforded genistein (**1a**) admixed with small quantities (2.23 mg from 1 kg fresh leaves) of another related isoflavone. Both compounds were subsequently separated by PTLC in CM, and the identity of the new compound as 5,7,4′-trihydroxy-3′-methoxyisoflavone (3′-*O*-methylorobol, **3b**) was then determined by spectroscopic methods. Thus the methanolic UV maximum at 263 nm shifted bathochromically upon addition of sodium acetate (9 nm; C-7 hydroxyl) and aluminium chloride (10 nm; C-5 hydroxyl) whilst characteristic low-intensity MS signals resulting from retro-Diels–Alder fission of the molecule were apparent at *m/z* 153 (A-ring derived fragment with dihydroxylation) and *m/z* 148 (B-ring derived fragment with OH and OMe groups). The 3′ (OMe)/4′ (OH) substitution pattern of ring-B was readily deduced from the ^{1}H NMR spectrum as chemical shift values in acetone-d_6 (100 MHz) for 2′-H (δ7.26, *d*, J = 2.0 Hz), 5′-H (δ6.88, *d*, J = 8.1 Hz) and 6′-H (δ7.08, *dd*, J = 8.1 and 2.0 Hz) and the 3′-methoxyl group (δ3.89, *s*) were essentially identical with those recently noted for the comparably substituted isoflavone, lupisoflavone (**3a**) (δ7.25, *d*, J = 2.4 Hz, 2′-H; δ6.89, *d*, J = 8.3 Hz, 5′-H; δ7.06, *dd*, J = 8.3 and 2.4 Hz, 6′-H; δ3.89, *s*, 3′-OMe) [1]. 3′-*O*-Methylorobol (**3b**) has previously been obtained in a pure state from *Dalbergia inundata* [2] (Leguminosae) and two species of *Wyethia* [3, 4] (Compositae). It is also known to occur with the isomeric isoflavone pratensein (4′-*O*-methylorobol) in leaves and stems of several *Thermopsis* spp. (Leguminosae) [5] but the separation of these two compounds does not seem to have been achieved. The presence of **3b** in leaf extracts of *L. albus* is not altogether surprising as it is presumably a biosynthetic precursor of the 3,3-dimethylallyl-substituted isoflavone, lupisoflavone (**3a**).

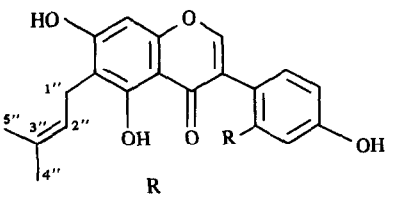
Roots

Isoflavones were extracted from chopped white lupin roots with methanol, and the extract was then frac-

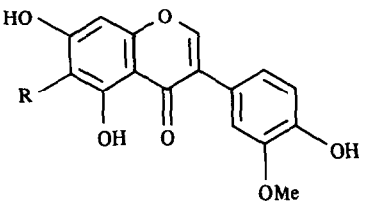
*"Antifungal Isoflavones in Lupins", Part 2. For Part 1 see ref [1].



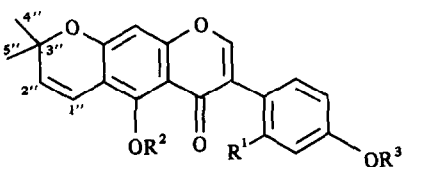
R
1a H
1b OH



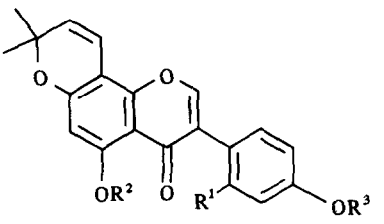
R
2a H
2b OH



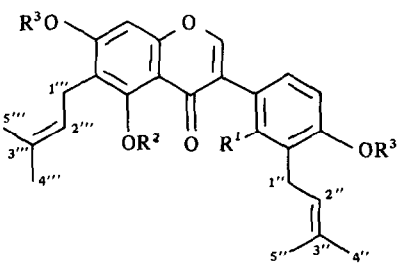
3a R = $CH_2CH = CMe_2$
3b R = H



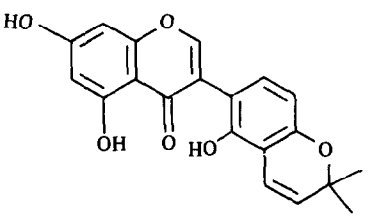
4a $R^1 = R^2 = R^3 = H$
4b $R^1 = H$, $R^2 = R^3 = Ac$
4c $R^1 = OH$, $R^2 = R^3 = H$
4d $R^1 = OMe$, $R^2 = H$, $R^3 = Me$
4e $R^1 = OMe$, $R^2 = R^3 = Me$
4f $R^1 = OAc$, $R^2 = R^3 = Ac$



4'a $R^1 = R^2 = R^3 = H$
4'b $R^1 = OH$, $R^2 = R^3 = H$
4'c $R^1 = OMe$, $R^2 = R^3 = Me$

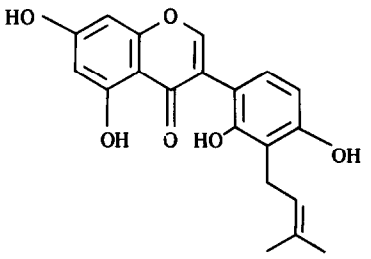


6a $R^1 = R^2 = R^3 = H$
6b $R^1 = OH$, $R^2 = R^3 = H$
6c $R^1 = OMe$, $R^2 = H$, $R^3 = Me$
6d $R^1 = OAc$, $R^2 = R^3 = Ac$

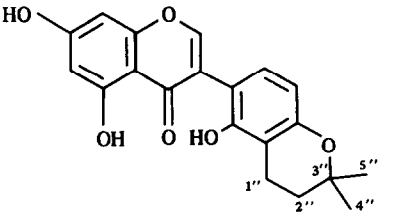


5

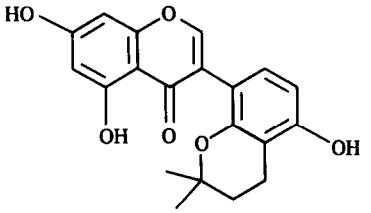
tionated by column chromatography on silica gel using principally benzene plus increasing amounts of ethyl acetate as the eluting solvent. The isoflavones in each fraction were separated and purified by PTLC as outlined in the Experimental. Fractions 6 (15% ethyl acetate in benzene) and 7 (30% ethyl acetate in benzene) contained the same three isoflavones, two of which were identified as licoisoflavone B (**5**) and lupalbigenin (**6a**) by chromatographic and spectroscopic comparison with authentic material previously obtained from *L. albus* [1]. The third



7a



7b



7c

compound (M^+ 422), which readily formed a tetraacetate (M^+ 590), was identified as 2'-hydroxylupalbigenin (**6b**) largely on the basis of its ^1H NMR spectrum. When compared with that of lupalbigenin [1] (**6a**), the ^1H NMR of **6b** was seen to differ only with respect to ring B where two *ortho*-coupled (J = 8.3 Hz) protons were apparent (*cf.* the ABX system of protons in ring B of **6a** [1]). Chemical shift values for these protons (δ6.50 and δ6.92) closely resembled those attributable in acetone-d_6 to 5'-H (δ6.51*/6.54 [6]) and 6'-H (δ6.93*/6.90 [6]) of licoisoflavone A (phaseoluteone) [6, 7] which possesses a B-ring with hydroxyl groups at C-2' and C-4', and a 3,3-dimethylallyl side chain at C-3'. The new *Lupinus albus* isoflavone is thus 5,7,2',4'-tetrahydroxy-6,3'-di(3,3-dimethylallyl)isoflavone (**6b**) (2'-hydroxylupalbigenin), a view entirely supported by the available MS and UV shift data. Although 2'-hydroxylupalbigenin is already known to occur in the roots of *L. angustifolius* (blue lupin) [8], its chemical and spectroscopic properties have not previously been reported.

Column fractions 8 and 9 (eluted with 30% and 40% ethyl acetate in benzene, respectively) contained a large number of isoflavones including genistein (**1a**), 2'-hydroxygenistein (**1b**), wighteone (**2a**) and luteone (**2b**). The identity of these four compounds was confirmed by direct (UV, MS, TLC) comparison with authentic *Lupinus*-derived samples [1]. However, the major component of both fractions was a prenylated tetrahydroxyisoflavone **7a** (M^+ 354) which ran above its isomer **2b** (luteone) on PTLC plates developed in CM, but below **2b** when the solvent system was changed to CAAm. Major MS fragments at m/z 311 $[M-43]^+$ and m/z 299 $[M-55]^+$, and ^1H NMR signals at δ1.65 (3H, *s*), 1.78 (3H, *s*), 3.44 (2H, *d*, J = 7.1 Hz) and 5.33 (1H, *br t*, J = *ca* 7.0 Hz) were consistent with the presence of a 3,3-dimethylallyl attachment. Two *meta*-coupled (J = 2.2 Hz) aromatic protons (δ6.35 and 6.50) attributable to 6-H and 8-H (compare corresponding values for **3b** δ6.29*, 6.42* and **5** δ6.35, 6.50 [1]) and a low-intensity MS fragment at m/z 153 suggested that ring A was dihydroxylated. This was confirmed by 6–11 nm bathochromic shifts of the 263 nm (methanol) UV maximum following addition of sodium acetate (C-7 hydroxyl) and aluminium chloride (C-5 hydroxyl). The side chain and two hydroxyl groups must therefore be located on ring B which also contains a pair of *ortho*-coupled protons. Significantly, acid-mediated cyclisation gave two isomeric pyranoisoflavones (**7b** and **7c**) in approximately equal quantities and assuming oxygenation at C-4', as in other reported *Lupinus* isoflavones [1, 9], this result allows **7a** to be correctly formulated as 5,7,2',4'-tetrahydroxy-3'-(3,3-dimethylallyl)isoflavone. In addition to *L. albus*, compound **7a** has also been isolated as licoisoflavone A from roots of Sinkiang licorice (*Glycyrrhiza* sp.) [7], and as 'phaseoluteone' from the fungus-inoculated pods of French bean (*Phaseolus vulgaris*) [6]. Spectroscopic data recorded for *Lupinus* licoisoflavone A were in very good agreement with those published for the French bean compound [6].

Apart from **7a** and compounds **1a**–**2b**, column fractions 8 and 9 also yielded a mixture of two other isoflavones which were eventually separated by PTLC in solvent system PEAa. The lower compound (R_f 0.18) was a new dihydrofuranoisoflavone for which we propose the common name lupinisoflavone A, whilst the material at higher R_f (0.21) proved to be identical with parvisoflavone B (**4c**). A major fragment at m/z 337 ($[M-15]^+$; base peak) in the MS of the latter *Lupinus* isoflavone (M^+ 352) indicated the possibility of a gem-dimethylpyrano substituent [10] (*cf.* MS data for alpinumisoflavone and licoisoflavone B [1]), and subsequent retro-Diels–Alder

*Data in this paper.

fission to afford an ion at *m/z* 203 [1, 11] allowed this side chain to be placed on ring A. Dihydroxylation of ring B, as in **1b** and **2b** [1], was evident from the minor MS fragment at *m/z* 134. Support for the 2′/4′-dihydroxy nature of ring B was provided by the ^{1}H NMR spectrum which revealed that the aromatic protons had chemical shifts and coupling patterns (δ6.49, incomplete doublet, 3′-H; δ6.45, *dd*, *J* = 8.3 and 2.4 Hz, 5′-H; δ7.13, *d*, *J* = 8.3 Hz, 6′-H) comparable with those of **2b** [12] (δ*ca* 6.5, incomplete doublet, 3′-H; δ6.44, *dd*, *J* = 8.9 and 2.4 Hz, 5′-H; δ7.12, *d*, *J* = 8.9 Hz, 6′-H). In addition to 2-H and the C-5 hydroxyl group, an isolated A-ring proton (δ6.41, *s*) was also apparent as were two olefinic protons (δ5.79, *d* and 6.69, *d*; *J* = 10 Hz) and two methyl groups resonating as a 6H singlet at δ1.47. Chemical shift values for the olefinic and methyl group protons were in close accord with those of alpinumisoflavone (**4a**; δ5.78/6.69 and 1.47, respectively) which possesses a gem-dimethylpyrano unit attached linearly (C-6/C-7) to ring A. As bathochromic shifts in the UV spectrum of **4c** were induced by aluminium chloride but not sodium acetate, it follows that here too the side chain must be cyclised to C-7 in either a linear (as in **4a**) or an angular (as in parvisoflavone A, **4′b**) disposition.

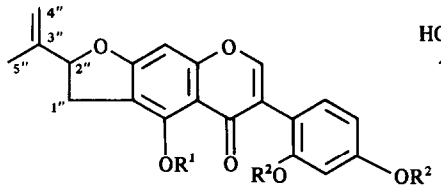
A means of differentiating between alpinumisoflavone (**4a**) and its synthetic isomer, **4′a** (now termed derrone [9]) was reported by Jackson *et al.* who observed that acetylation of the linear isoflavone (**4a**) resulted in a shift to higher field of the olefinic 1″-H (denoted 4″-H in ref. [13]) and a shift to lower field of the olefinic 2″-H (3″-H in ref [13]) in $CDCl_3$. For the angular isomer (**4′a**), however, both of the corresponding proton signals moved to lower field upon acetylation. More recently, this effect has been successfully employed in the identification of a *Millettia* (Leguminosae) seed isoflavone as the 3′-hydroxy derivative of alpinumisoflavone 4′-*O*-methyl ether instead of the alternative derrone analogue [11]. When the *Lupinus* isoflavone was fully acetylated, it was immediately clear from the ^{1}H NMR spectrum in acetone-d_6 that the 1″-H and 2″-H resonances had shifted to a higher and lower field, respectively (**4c** → **4f**: 1″-H, Δδ −0.07; 2″-H, Δδ +0.17). These movements were comparable with the chemical shifts noted for 1″-H and 2″-H of acetylalpinumisoflavone in acetone-d_6 (**4a** → **4b**: 1″-H, Δδ −0.06; 2″-H, Δδ +0.18). Moreover, the dimethyl ether (**4d**; the C-5 hydroxyl remains underivatized) of **4c** reacted slowly with Gibbs reagent to give a blue/green colour, a result which indicated that the position *para* to the C-5 hydroxyl group was unsubstituted. Lastly, the trimethyl ether of the *Lupinus* isoflavone had a UV spectrum (λ_{max}^{MeOH}: 268 nm) which more closely resembled that of compound **4e** (parvisoflavone B trimethyl ether; λ_{max}^{EtOH}: 267 nm) than of the angular isomer **4′c** (parvisoflavone A trimethyl ether: λ_{max}^{EtOH}: 258 and 263 nm) [14]. The presence of parvisoflavone B (**4c**) in *Lupinus* roots is thus confirmed. Although **4c** occurs, together with **4′b** (parvisoflavone A), in trunk wood of *Poecilanthe parviflora* [14] (Leguminosae), this is the first report of its presence in the tissues of a non-woody legume.

PTLC examination of combined column fractions 10 and 11 (eluted with 40–55% ethyl acetate in benzene), and combined fractions 12 and 13 (eluted with 55–70% ethyl acetate in benzene) yielded a total of five isoflavones (lupinisoflavones B–F) none of which seemed to have been isolated from other plant sources. All were purified to homogeneity by PTLC in CM, CAAm or PEAa, and some of their spectroscopic characteristics, together with those of lupinisoflavone A, are summarized in Table 1. From their UV, mass and ^{1}H NMR spectra it was clear that lupinisoflavones A–F were all closely related 5-hydroxyisoflavones containing either one (A, B, C, D) or two (E, F) cyclic isoprene-derived side chains. Whilst the present work was in progress, it was found that the fungus *Botrytis cinerea* could metabolise luteone (**2b**) to give various products including a substance identical with lupinisoflavone B, and the characterization of this compound as 2′-hydroxyerythrinin C (**9a**) is reported elsewhere [12]. Like **9a**, lupinisoflavones C–F each afforded MS fragments at $[M-59]^+$ and *m/z* 59 indicative of a hydroxyisopropyl-dihydrofuran substituent [15]. In contrast to **9a**, however, both lupinisoflavone C and D gave bathochromic UV shifts with sodium acetate (C-7 hydroxyl). As these compounds each have a C-5 hydroxyl group [see Table 1 for relevant UV ($AlCl_3$) and ^{1}H NMR data] and a pair of *meta*-coupled (*J* = *ca* 2.1 Hz) A-ring protons, it follows that the alkyl side chain must be attached to ring B. From their ^{1}H NMR spectra, it is clear that lupinisoflavones E and F both contain two hydroxyisopropyl-dihydrofuran attachments. Neither of these compounds gave a UV shift with sodium acetate (*cf.* **9a** [12]) and the presence of an isolated aromatic proton signal at δ6.38 (E) and 6.45 (F) strongly suggested that ring A was substituted as in lupinisoflavone B (**9a**). Linear

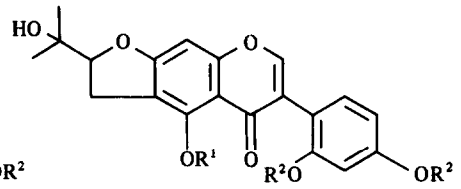
Table 1. Spectral properties of lupinisoflavones A–F

Compd. code	Molecular formula (HRMS)	UV λ_{max} (nm)			^{1}H NMR (δ, acetone-d_6)				MS *m/z* (rel. int.)		
		MeOH	$AlCl_3$	NaOAc	2-H	C-5-OH	aromatic proton* ring A	ring B	$[M]^+$	$[M-59]^+$	59
A	$C_{20}H_{16}O_6$	263	271	263	8.19	13.0	*s*	*o,m,om*	100	—	—
B	$C_{20}H_{18}O_7$	263	271	263	8.18	13.0	*s*	*o,m,om*	100	91	67
C	$C_{20}H_{18}O_6$	263	273	272	8.17	13.0	*m,m*	*o,m,om*	35	100	11
D	$C_{20}H_{18}O_7$	261	269	272	8.18	12.7	*m,m*	*o,o*	38	100	14
E	$C_{25}H_{26}O_7$	267	277	267	8.18	13.2	*s*	*o,m,om*	64	100	44
F	$C_{25}H_{26}O_8$	264	273	264	8.20	12.8	*s*	*o,o*	64	100	29

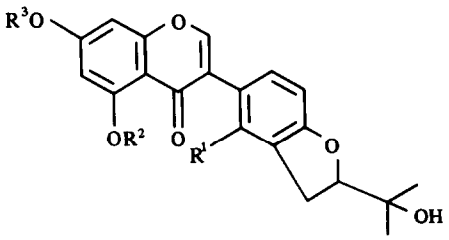
*Each aromatic proton is shown as: *s*, a singlet proton; *m*, a *meta*-coupled proton; *o*, an *ortho*-coupled proton and *om*, an *ortho-meta*-coupled proton.



8a $R^1 = R^2 = H$
lupinisoflavone A
8b $R^1 = H, R^2 = Me$
8c $R^1 = R^2 = Me$
8d $R^1 = R^2 = Ac$

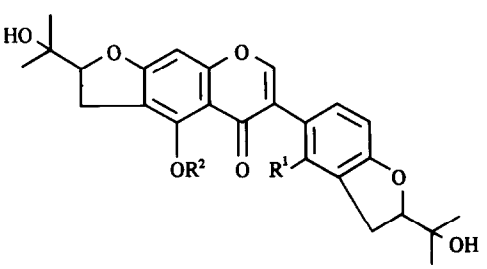


9a $R^1 = R^2 = H$
lupinisoflavone B
9b $R^1 = H, R^2 = Me$
9c $R^1 = R^2 = Me$



10a $R^1 = R^2 = R^3 = H$, lupinisoflavone C
10b $R^1 = R^2 = H, R^3 = Me$
10c $R^1 = H, R^2 = R^3 = Me$

11a $R^1 = OH, R^2 = R^3 = H$, lupinisoflavone D
11b $R^1 = OMe, R^2 = H, R^3 = Me$
11c $R^1 = OMe, R^2 = R^3 = Me$
11d $R^1 = OAc, R^2 = R^3 = Ac$



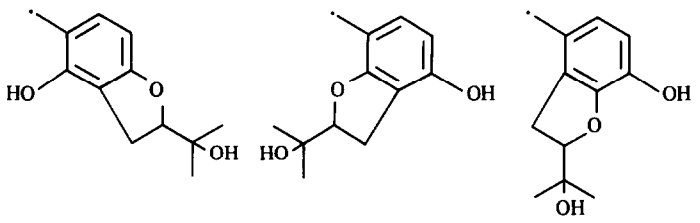
12a $R^1 = R^2 = H$, lupinisoflavone E
12b $R^1 = H, R^2 = Me$

13a $R^1 = OH, R^2 = H$, lupinisoflavone F
13b $R^1 = OMe, R^2 = H$
13c $R^1 = OMe, R^2 = Me$

attachment of the side chain to ring A was established by means of the Gibbs test. Thus lupinisoflavone E (**12a**) and the monomethyl derivative of lupinisoflavone F **13a** (but not the dimethyl ether, **13c**) consistently gave a blue/green colour on TLC plates treated with a chloroform solution of Gibbs reagent and then fumed with ammonia vapour. This result indicates that C-8 is unsubstituted in E and F [16], and hence the A-ring side chain is cyclised from C-6 to the oxygen atom at C-7.

Chemical shift data suggested that the B-ring of lupinisoflavone C was identical with that of lupinisoflavone E, and a similar relationship between lupinisoflavones D and F was also deduced from the relevant ^{1}H NMR spectra. In fact, lupinisoflavone D (M^+ 370) differs from lupinisoflavone C (M^+ 354) only by its possession of an extra hydroxyl substituent which must reside on ring B. Lupinisoflavone F (M^+ 454) and lupinisoflavone E (M^+ 438) are related in an exactly comparable fashion. The B-ring protons of lupinisoflavones C and E formed an ABX system, as in lupalbigenin [1], so if oxygenation at C-4′ is assumed, the cyclic hydroxyisopropyl-dihydrofuran unit must also be attached at C-3′. Structures **10a** and **12a** can therefore be assigned to lupinisoflavone C and lupinisoflavone E, respectively.

The B-ring protons of lupinisoflavones D and F appeared as *ortho*-coupled doublets at δ6.34 (D and F) and 7.01 (D)/7.02 (F) with J = 8.5–8.8 Hz. Because of this observation the ring B side chain was provisionally assigned to C-3′/C-4′ as in **10a** and **12a**, whilst the single hydroxyl group was placed at C-2′ by analogy with isoflavones **1b**, **2b**, **4c**, **5**, **6b**, and **7a** (see part structure **a**). Two other arrangements (**a**′ and **a**″) were also feasible, however, but these were effectively excluded on the basis of a Gibbs reaction. For comparative purposes, licoisoflavone A (**7a**) was cyclised by treatment with *p*-toluenesulphonic acid [3] (see Experimental) to give the model dihydropyranoisoflavones **7b** and **7c**, and these were also subjected to the Gibbs test. Both **7b** and **7c** were Gibbs positive but whereas the former gave an immediate deep blue colour, the latter reacted very slowly to afford a blue/green product. The difference between **7b** and **7c** with respect to colour and the rate of colour development is to be expected as **7c** contains only an H-bonded C-5 hydroxyl group capable of reacting with Gibbs reagent, whilst **7b** possesses both a C-5 hydroxyl and more importantly an unchelated hydroxyl substituent at C-2′. Other hydroxyl groups at C-7 (**7b**, **7c**) and C-4′ (**7c**) with substituted *para*-positions are not involved in the Gibbs reaction. When tested under identical conditions to **7b** and **7c**, lupinisoflavones D and F each rapidly afforded a deep blue product with Gibbs reagent (*cf.* the response of **7a**) thereby confirming that part structure **a** was correct. Note also that after methylation of the 2′-hydroxyl group



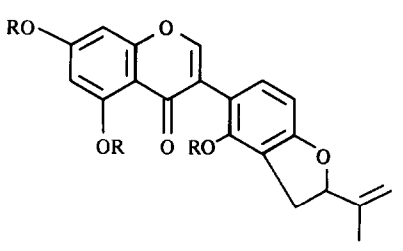
a **a'** **a''**

(**11a** → **11b**, and **13a** → **13b**; the C-5 hydroxyl is unaffected in both cases), the dimethyl ether of lupinisoflavone D (**11b**) and the monomethyl ether of lupinisoflavone F (**13b**) gave a Gibbs reaction indistinguishable from that of **7c**. Lupinisoflavone D therefore has structure **11a**, whilst lupinisoflavone F has structure **13a**.

Lupinisoflavone D (**11a**) and its trimethyl ether (**11c**) were treated with thionyl chloride in pyridine and the structures of the resulting dehydration products were confirmed by mass and ^{1}H NMR spectroscopy. For example, thionyl chloride dehydration of **11a** readily furnished two new isomeric isoflavones (DHY-1 and DHY-2) in the ratio of 4.6:1.0. A ^{1}H NMR study revealed that the major product (DHY-1; M^+ 352) differed from **11a** only with respect to the terminal section of the alkyl side chain. Thus the dimethylcarbinol absorption of **11a** was replaced in the ^{1}H NMR spectrum of DHY-1 by resonances attributable to an allylic methyl substituent (δ1.79, *s*) and an exomethylene (CH_2=) group (δ4.91 and 5.10, both *br s*). Comparable signals are given in $CDCl_3$ by the rotenoid, rotenone (δ1.77, Me; 4.90 and 5.06, CH_2=) [17] and in acetone-d_6 by the pterocarpan, apiocarpin (δ1.79, Me; δ4.87 and 5.15, CH_2=) [18] both of which have side chains ending with an isopropenyl unit. Isoflavone DHY-1 can therefore be assigned structure **14a**.

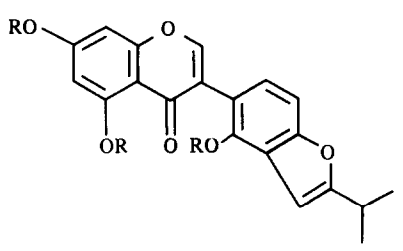
Side chain modification was also associated with the minor dehydration product (DHY-2; M^+ 352) which gave ^{1}H NMR signals indicative of an isopropyl group [19] (δ1.35, 6H, *d*, J = 6.8 Hz; 3.10, 1H, *d sep*, J = 6.8 and 1.0 Hz) and the β-proton of a benzofuran ring (δ6.61, *s*; compare the β-proton signal of glyceofuran, δ6.63, in acetone-d_6 [20]). Other proton resonances were comparable with those given by **11a** and hence isoflavone DHY-2 can be represented as the α-substituted benzofuran **15a**. In two earlier studies, it has been demonstrated that P_2O_5 dehydration of dalpanol [21] or villol [17], each with a side chain as in **11a**, yields a benzofuran derivative (isorotenone [21] or isovillosone [17]) analogous to **15a**. As expected, dehydration of lupinisoflavone D trimethyl ether (**11c**) afforded isoflavones **14b** and **15b** in a ratio of 3.5:1.0.

The final problem concerned the structure determination of lupinisoflavone A which, as mentioned earlier, was eluted in column fractions 8 and 9. Fortunately, identification of lupinisoflavone A was facilitated by preparation of the dehydroisoflavone **14a** (DHY-1; see above) as both compounds were found by ^{1}H NMR spectroscopy to contain an isopropenyl-dihydrofuran side chain. Signals for this substituent in the ^{1}H NMR spectrum of lupinisoflavone A were evident at δ1.79 (3H, *s*, allylic methyl, 5″-H_3), 2.98 (1H, *dd*, A part of ABX system, 1″-H_a), 3.42 (1H, *dd*, B part of ABX system, 1″-H_b), 5.47 (1H, *dd*, X part of ABX system, 2″-H) and 4.96/5.12 (both 1H, each *br s*, exomethylene, 4″-H_2). Corresponding chemical shift values for **14a** were as follows: δ1.79 (5″-H_3), 2.99 (1″-H_a), 3.39 (1″-H_b), 5.29 (2″-H) and 4.91/5.10 (4″-H_2). A low-intensity MS fragment at *m/z* 134 indicated that ring B was dihydroxylated. As signals due to the three B-ring protons were identical or almost coincident with those of **2b** [12], **4c**, **9a** and the luteone metabolites AF-1 (luteone hydrate), AF-2, BC-1 and BC-2 [12], it was considered that lupinisoflavone A also possessed hydroxyl groups at C-2′ and C-4′. The 263 nm UV (methanol) maximum of lupinisoflavone A shifted bathochromically in the presence of aluminium chloride (8 nm; C-5 hydroxyl) but not sodium acetate, and thus the side chain is cyclised *via* an oxygen atom to C-7. Upon methylation lupinisoflavone A yielded a phenolic (the C-5 hydroxyl remains underivatized) dimethyl ether (**8b**) which gave a blue/green colour when subjected on TLC plates to the Gibbs test. The side chain is therefore attached linearly to ring A, and hence lupinisoflavone A has structure **8a**. The isopropenyl-dihydrofuran side chain of **8a** has previously been associated with only one



14a R = H (DHY-1)

14b R = Me (MDHY-1)



15a R = H (DHY-2)

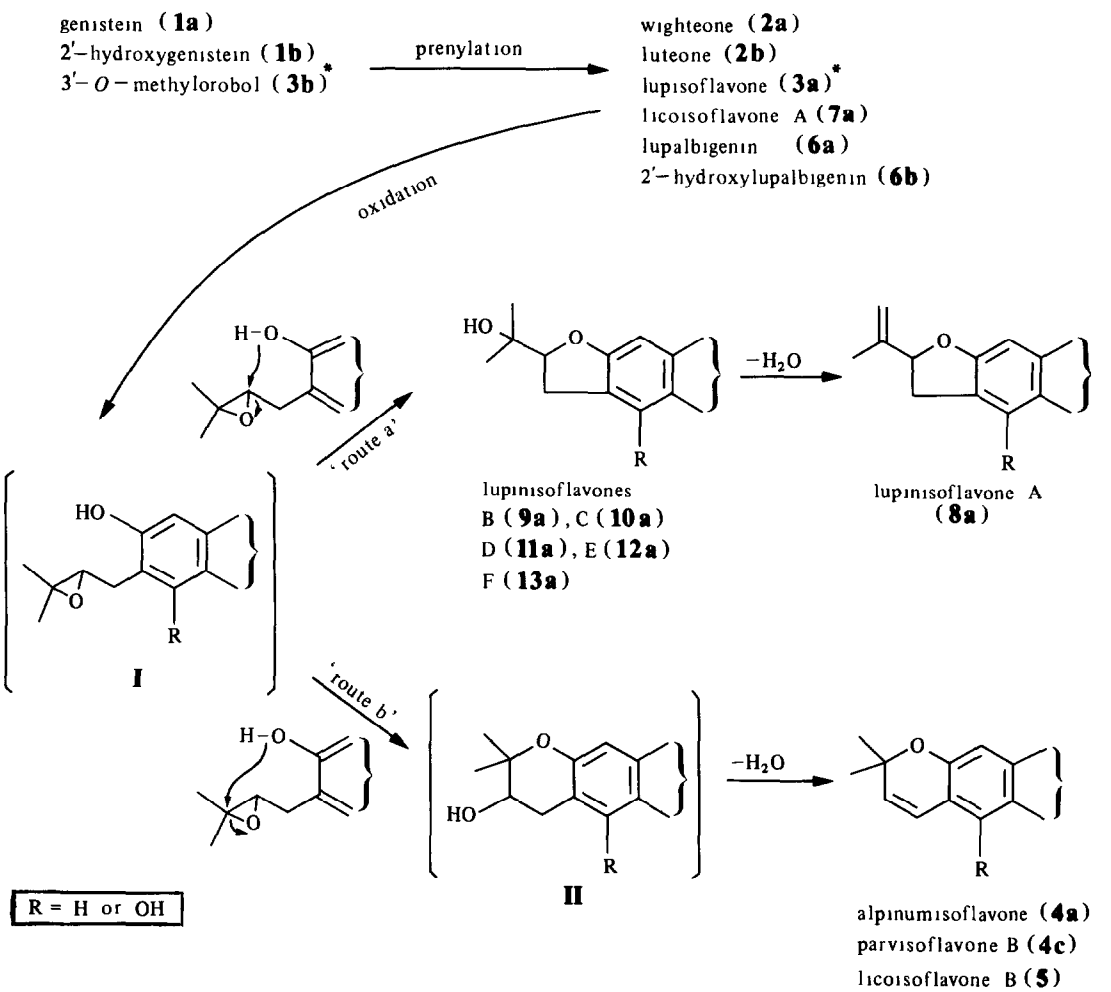
15b R = Me (MDHY-2)

other isoflavone, namely glabrescione A from seeds of *Derris glabrescens* (Leguminosae) [22]. However, it is commonly encountered in the rotenoid group of isoflavonoid compounds [9] (e.g. rotenone), and three pterocarpan phytoalexins (apiocarpin, glyceollin III and canescacarpin) [9] also possess this substituent.

All lupinisoflavones except B were found to be optically active but whereas lupinisoflavone A was dextrorotatory, compounds C–F were laevorotatory. The stereochemistry at C-2″ of lupinisoflavones A and C–F remains to be determined.

The antifungal properties of 17 of 18 *Lupinus* isoflavones (compound **4a** was not examined) were determined by means of TLC plate bioassays [23] using *Cladosporium herbarum* Fr. (isolate AHU 9262) as the test fungus. Results, given in decreasing order of activity, were as follows: luteone (**2b**), licoisoflavone A (**7a**) ≥ wighteone (**2a**) > parvisoflavone B (**4c**), licoisoflavone B (**5**) > lupisoflavone (**3a**), lupinisoflavone A (**8a**) > lupinisoflavone B (**9a**), lupinisoflavone C (**10a**) > genistein (**1a**), 2′-hydroxygenistein (**1b**), 3′-*O*-methylorobol (**3b**), lupalbigenin (**6a**), 2′-hydroxylupalbigenin (**6b**), lupinisoflavones D (**11a**), E (**12a**) and F (**13a**).

The probable biogenetic relationships of the white lupin isoflavones detected during this and earlier investigations are summarized in Scheme 1. Only three oxygenation patterns seem to be characteristic of the simple isoflavones from *L. albus*, these being 5,7,4′ (**1a**), 5,7,2′,4′ (**1b**) and 5,7,3′(OMe),4′ (**3b**). Direct enzymatic prenylation [24] of the isoflavone nucleus at C-6 and/or C-3′ is presumably the route to compounds **2a**, **2b**, **3a**, **6a**, **6b** and **7a**. Depending on the enzyme(s) subsequently involved, cyclisation of the prenyl (3,3-dimethylallyl) side chain to an adjacent hydroxyl group at C-7 or C-4′ could lead to all the pyrano- and dihydrofurano-substituted isoflavones shown in Scheme 1. Note that although chemically feasible, cyclisation of the prenyl side chain to the C-5 or C-2′ hydroxyl groups has not been shown to occur in *L. albus*, and this presumably reflects the highly specific nature of the enzyme(s) concerned. It is possible that a transitory epoxide (I, or its B-ring equivalent) may be formed during the cyclisation process, and in amorphigenin biosynthesis the intermediates dalpanol (with a side chain as in lupinisoflavones B–F) and rotenone (side chain as in lupinisoflavone A) are thought to originate from such a compound [25, 26]. As an alternative to 'route a'



Scheme 1. Probable biogenetic relationships of white lupin isoflavones. With the exception of **3a** and **3b** (both marked with an asterisk), all the above isoflavones are known to occur in white lupin roots. Isoflavones **1a**–**3b** have also been isolated from leaf extracts of white lupin. I and II are possible intermediates.

(Scheme 1), an isoflavone epoxide may cyclise ('route b') to yield a hydroxydimethyldihydropyrano derivative (II, or its B-ring equivalent) from which **4a**, **4c** and **5** might arise by dehydration. In this respect, it is interesting to note that in *Psoralea corylifolia* the prenylated coumestan [9], psoralidin, is known to co-occur with its epoxyprenyl (psoralidin oxide) [27] and dihydroxydimethyl-dihydropyrano (corylidin) [28] analogues, both of which could originate in a manner comparable with that illustrated in Scheme 1.

EXPERIMENTAL

Mps were determined by the micro hot-plate method and are uncorrected.

General methods. TLC: Analytical and preparative TLC (PTLC) separations were carried out using Merck pre-coated Silica Gel 60 plates (F-254; layer thickness, 0.25 mm or 0.5 mm). The developing solvent systems were as follows: (a) CM = $CHCl_3$–MeOH (although a ratio of 50:2 was normally employed, the solvent ratio was occasionally changed to 50:1 or 50:3–4 depending on the polarity of the isoflavone(s) under investigation); (b) CAAm = $CHCl_3$–Me_2CO–conc. NH_3–H_2O (70:60·2); and (c) PEAa = *n*-pentane–Et_2O–HOAc (75:25:4). Isoflavones were eluted from chromatograms with EtOAc. Detection of isoflavones on developed TLC or PTLC plates was by inspection under long (365 nm) and short (254 nm) wavelength UV lights, and by the characteristic colours formed with Gibbs reagent *Gibbs test* [16]. A soln of purchased 2,6-dichloroquinone-4-chlorimide (Gibbs reagent) in $CHCl_3$ (0.2%, w/v) was sprayed on developed TLC/PTLC plates which were then immediately fumed with the vapour from dilute (*method a*) or concentrated (*method b*) ammonia water Any compound which within seconds gave a deep blue or purple–blue colour using *method a* was denoted Gibbs (+) rapid, whereas the gradual (30 sec–1 min) development of a dark blue or blue–green colour, even with *method b*, resulted in the designation Gibbs (+) slow. If a colour did not develop after several min using *method b*, the compound concerned was considered to be Gibbs (−). The response of model compounds such as genistein (**1a**), 2'-hydroxygenistein (**1b**), wighteone (**2a**) and luteone (**2b**), and their methyl ethers, was also determined for comparative purposes. *Determination of antifungal activity*: Bioautography on TLC plates as reported by Homans and Fuchs [23] was used to determine the antifungal activity of each isoflavone isolated from *L. albus* roots and leaves (see also ref. [12]). *Methylation* [29]: (a) *Partial methylation* An excess of CH_2N_2 in CH_2Cl_2 was added to a soln of the appropriate isoflavone (1–2 mg) in MeOH (1 ml) and CH_2Cl_2 (4 ml). The reaction mixture was maintained at room temp for 4 hr, or stored overnight at *ca* 4°, and the solvent was then removed under red. pres. Silica gel PTLC (CM, 50:1) of the residue afforded predominantly the partially methylated isoflavone (CH_2N_2 treatment does not readily affect the H-bonded C-5-OH group) although very small quantities of the corresponding permethyl ether were sometimes obtained. (b) *Complete methylation* (permethylation). Fully methylated isoflavones were routinely prepared by refluxing (2 hr) an Me_2CO soln of the hydroxylated starting material with Me_2SO_4 and K_2CO_3 [1]. After removal of Me_2CO and K_2CO_3, the product was purified by PTLC in CM (50:1). *Acetylation*: The isoflavone (2–10 mg) was dissolved in pyridine (0.1 ml) and Ac_2O (0 1 ml), and heated in a sealed tube at 100° for 3 hr. The reaction mixture was then diluted with EtOAc to 25–30 ml, washed successively with dil HCl, 5% $NaHCO_3$ and brine, and the organic layer dried (Na_2SO_4) before being concd *in vacuo*. Silica gel PTLC of the concentrate (CM, 50:1–2) afforded the acetate derivative in good yield.

Plant material. Seeds of *Lupinus albus* L. cv. Kievskij Mutant (generously supplied by Prof. W. Williams, Dept. Agricultural Botany, University of Reading, England) were sown on June 5th 1982 at the Experimental Farm of the Faculty of Agriculture, Hokkaido University, Sapporo. The plants were harvested (Sept. 5th) after 12 weeks growth and immediately divided into leaf (4 kg, fr. wt) and root (3.7 kg, after washing and air-drying overnight) material. Stems and all other plant parts were discarded.

Extraction and fractionation of leaf and root isoflavones. Leaves: Isoflavones were removed from the upper and lower surface of white lupin leaves by washing, for a total time of 3 min, with three equal vols of MeOH as previously described [1]. The combined methanolic washings (20 l.) were filtered by suction, reduced *in vacuo* to near dryness, and the oily brown residue then diluted to about 400 ml with more MeOH. A portion of the resulting soln (100 ml, corresponding to 1 kg fresh leaves) was concd to 20 ml, and extracted with 3 × 50 ml, EtOAc–C_6H_6 (2:1). The extracts were combined and reduced to dryness to give a pale yellow solid (*ca* 600 mg) which was taken up in EtOAc (10 ml) and chromatographed (silica gel PTLC) using the CAAm solvent system. A broad fluorescence-quenching (254 nm) band opposite the marker of authentic genistein was removed and eluted with EtOAc to give a colourless soln of **1a** (genistein) + **3b** (3'-*O*-methylorobol). Fluorescence-quenching bands corresponding to the markers of other known isoflavones (e.g. wighteone, luteone and 2'-hydroxygenistein) were not eluted. Compounds **1a** and **3b** were separated by PTLC in CM, 50:1 (**1a**, R_f 0.22; **3b**, R_f 0.37) and then finally purified by further PTLC using PEAa. Fresh white lupin leaves (1 kg) yielded 2.2 mg of **3b** and *ca* 3 mg of **1a**. *Roots*: The finely chopped roots were covered with MeOH (6 l.) and allowed to stand at room temp for 4 days. At this point the MeOH was decanted, and the above extraction procedure was repeated × 4 with 90% MeOH. The combined MeOH extracts (30 l.) were filtered by suction, concd under red pres. to about 500 ml, and shaken with EtOAc (6 × 400 ml) after first being adjusted to pH 3.0 with 2 N HCl. The extracts were again combined and washed (× 2) with one-fifth vols of 5% aq $NaHCO_3$, after which the organic layer was dried and reduced in volume to near dryness. EtOAc (70 ml) was then added to the sticky residue (*ca* 35 g) and the resulting suspension was adsorbed onto silica gel powder (Wako Gel C-200; 100 g). Following removal of the EtOAc *in vacuo*, the gel coated with root extract was transferred to a column of Wako Gel (200 g) settled in C_6H_6. Root isoflavones and other constituents were then eluted with benzene, benzene plus increasing amounts of EtOAc, or EtOAc alone Eluates (250 ml per fraction) were collected as follows: Fractions (Fr) 1 and 2, C_6H_6; Fr 3 and 4, 5% EtOAc in C_6H_6; Fr 5 and 6, 15% EtOAc in C_6H_6; Fr 7 and 8, 30% EtOAc in C_6H_6; Fr 9 and 10, 40% EtOAc in C_6H_6; Fr 11 and 12, 55% EtOAc in C_6H_6; Fr 13 and 14, 70% EtOAc in C_6H_6; Fr 15 and 16, EtOAc. Data relating to the number and, whenever possible, identity of lupin isoflavones were provided by preliminary TLC screening of small samples drawn from each column fraction using the CM, CAAm or PEAa solvent systems.

Fractions 1–5 and 14–16 were apparently devoid of isoflavones. However, fractions 6 and 7 contained large quantities of the same three isoflavones. These two fractions were therefore combined and concd *in vacuo* to give a pale brown oil which was taken up in 90% MeOH (200 ml). This soln was washed twice with *n*-hexane (150 ml) to remove any fatty substances. The MeOH layer was greatly reduced in volume and the residue was dissolved in EtOAc (15 ml). A portion (*ca* 1/6) of the solution was chromatographed (PTLC) in CAAm to yield two fluorescence-quenching bands at R_f 0.77–0.89 (band 1) and 0.37–0.45 (band 2).

Band 1 was eluted, and upon re-PTLC in CM afforded lupalbigenin (**6a**; R_f 0.50) and 2'-hydroxylupalbigenin (**6b**; R_f 0.42). Band 2, which consisted of only one impure component, was eluted and chromatographed (multiple development PTLC) to yield licoisoflavone B (**5**). The amounts of **5**, **6a**, and **6b** in *L. albus* roots were estimated to be 32, 165 and 297 mg/kg respectively.

A considerable number of related isoflavones were found in fractions 8 and 9 which were therefore combined and reduced to dryness. The residue (7.2 g) was dissolved in EtOAc (*ca* 20 ml), adsorbed on Wako Gel (30 g) as described above, and transferred to a column of Wako Gel (100 g) settled in C_6H_6. The charged column was first washed with 10% EtOAc in C_6H_6 (250 ml) and the isoflavone components of fractions 8 and 9 were then eluted (75 ml per fraction, FFr) as follows: FFr 1' and 2', 15% EtOAc in C_6H_6; FFr 3' and 4', 20% EtOAc in C_6H_6; FFr 5' and 6', 25% EtOAc in C_6H_6; FFr 7' and 8', 30% EtOAc in C_6H_6. Fractions 1' and 8' were low in isoflavones and were not further examined. Concn of both fraction 2' and fraction 3' afforded crude licoisoflavone A (**7a**; 590 mg). Recrystallization from *n*-pentane–EtOAc yielded 400 mg of pale yellow prisms as a 1:1 complex with EtOAc which melted at 63–65°. Lit., **7a** from licorice roots, mp 111–113° [7]; synthetic **7a**, mp 120–122° [30]. PTLC in CAAm of the mother liquor from fraction 3' afforded wighteone (**2a**; R_f 0.53; 65 mg) and a mixture of parvisoflavone B (**4b**) and lupinisoflavone A (**8a**) at R_f 0.43 (29 mg). These two isoflavones were successfully separated by multiple development PTLC in PEAa (**4b**, upper zone, 10 mg; **8a**, lower zone, 16.6 mg). Concentration of fractions 4' and 5' gave crude crystalline luteone (**2b**; 200 mg). Genistein (**1a**) was detected chromatographically in the mother liquors. Upon concentration, both fractions 6' and 7' deposited slightly impure crystals of 2'-hydroxygenistein (**1b**; 310 mg). On the basis of the above data, 1 kg of root material would be expected to yield 84 mg of **1b**, 18 mg of **2a**, 54 mg of **2b**, 3 mg of **4b**, 108 mg of **7a** and 5 mg of **8a**. The amount of genistein (**1a**) in *L. albus* root was not determined.

Fractions 10 and 11 from the main column were combined and concentrated. PTLC of *ca* 1/6 of this concentrate in CM (50:3) gave lupinisoflavone B (**9a**; R_f 0.32), C (**10a**; R_f 0.48) and D (**11a**; R_f 0.41). Isoflavones **9a** and **10a** were purified by re-PTLC in CM, and lupinisoflavone D (**11a**) was additionally chromatographed in CAAm (R_f 0.35) and PEAa (R_f 0.65) prior to spectroscopic examination. The amounts of **9a**, **10a**, and **11a** in 1 kg lupin roots were calculated to be 16, 19 and 111 mg, respectively.

Lastly, column fractions 12 and 13 were also combined and concd under red. pres. Preliminary TLC examination (CM, 50:3) indicated the presence of only two major isoflavones, namely lupinisoflavone E (**12a**; R_f 0.70) and lupinisoflavone F (**13a**; R_f 0.54). Both compounds were isolated in quantities sufficient for chemical and spectroscopic studies by PTLC of *ca* 1/6 of the above mentioned concentrate in CAAm (**12a**; R_f 0.66 and **13a**; R_f 0.28) and CM (25·2). Yields per kg of root material were 16 mg of lupinisoflavone E and 43 mg of lupinisoflavone F.

Properties of the Lupinus *isoflavones and their chemical transformation products.* 3'-O-*Methylorobol* (**3b**) Colourless short needles, mp 214–216°. Lit. 218–220° [2]. MS *m/z* (rel. int.): 301 $[M+1]^+$ (9.7), 300 $[M]^+$ (100), 299 (3.0), 285 (2.3), 257 (2.2), 229 (2.0), 153 (17), 148 (3.2), 134 (3.0), 133 (2.4), 120 (3.5), 105 (1.7). UV λ_{max}^{MeOH} nm: 263, 292, 332; +NaOMe, 228 sh, 277, 327; +$AlCl_3$, 230 sh, 273, 313, 373; +NaOAc, 227 sh, 272, 327 (addition of solid boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 100 MHz): δ 3.89 (3H, *s*, C-3'-OMe), 6.29 (1H, *d*, J = 2.2 Hz, 6-H), 6.42 (1H, *d*, J = 2.2 Hz, 8-H), 6.88 (1H, *d*, J = 8.1 Hz, 5'-H), 7.08 (1H, *dd*, J = 8.1 and 2.0 Hz, 6'-H), 7.26 (1H, *d*, J = 2.0 Hz, 2'-H), 8.21 (1H, *s*, 2-H), 13.0 (*s*, C-5-OH).

2'-Hydroxylupalbigenin (**6b**). Gibbs test (+), rapid, purple–blue. Pale yellow prisms, mp 155–157°. MS *m/z* (rel. int.): 423 $[M+1]^+$ (15), 422 $[M]^+$ (100), 407 (1.6), 405 (1.1), 379 $[M-43]^+$ (32), 367 $[M-55]^+$ (53), 366 (21), 351 (50), 323 (63), 311 (41), 165 (25), 147 (4.5). UV λ_{max}^{MeOH} nm (log ε): 212 (4.66), 268.5 (4.50); +NaOMe, 226 sh, 280, 345; +$AlCl_3$, 215, 270, 310 sh, 363; +NaOAc, 276, 346 (addition of solid boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.65 and 1.76 (each 6H, both *s*, 4''-, 4'''-, 5''- and 5'''-H_3), 3.38 and 3.44 (each 2H, both *d*, J = 6.2 Hz, 1''- and 1'''-H_2), 5.31 (2H, *m*, 2''- and 2'''-H), 6.50 (1H, *d*, J = 8.3 Hz, 5'-H), 6.57 (1H, *s*, 8-H), 6.92 (1H, *d*, J = 8.3 Hz, 6-H), 8.16 (1H, *s*, 2-H), 12.8 (*s*, C-5-OH). *Trimethyl ether* (**6c**). Gibbs (+), quite slow, blue–green. Colourless plates, mp 169–171°. MS *m/z* (rel. int.): 465 $[M+1]^+$ (17), 464 $[M]^+$ (81), 421 (44), 410 (18), 409 (100), 393 (9.6), 365 (22), 233 (20). UV λ_{max}^{MeOH} nm: 213 sh, 266.5, 300 sh; +$AlCl_3$ (30 min after addition of the shift reagent): 213 sh, 272, 277 sh, 312, 365. *Tetraacetate* (**6d**). Colourless plates, mp 127–129°. MS *m/z* (rel. int.): 590 $[M]^+$ (7.7), 549 (23), 548 $[M-42]^+$ (100), 506 (51), 505 (30), 464 (20), 463 (53), 451 (13), 421 (15), 409 (16), 407 (23), 366 (14), 365 (59), 323 (17), 165 (23). UV λ_{max}^{MeOH} nm: 245, 251 sh, 300 sh, 310. ^{1}H NMR (acetone-d_6, 100 MHz): δ 1 65, 1.74 and 1.78 (6H, 3H and 3H, three *s*, 4''-, 4'''-, 5''- and 5'''-H_3), 2.08, 2.30, 2.33 and 2.37 (each 3H, four *s*, 4 × Ac), 3.23 and 3.31 (each 2H, both *br d*, J = *ca* 8 Hz, 1''- and 1'''-H_2), 5.02 (2H, *m*, 2''- and 2'''-H), 7.07 (1H, *d*, J = 8.3 Hz, 5'-H), 7.25 (1H, *d*, J = 8 3 Hz, 6'-H), 7.36 (1H, *s*, 8-H), 8.03 (1H, *s*, 2-H).

Parvisoflavone B (**4c**). Gibbs test (+), rapid, purple–blue. MS *m/z* (rel. int.): 352 $[M]^+$ (26), 338 (8.0), 337 $[M-15]^+$ (100), 203 (25), 169 (4.0). UV λ_{max}^{MeOH} nm: 228, 282, 290 sh; +NaOMe, 235 sh, 292–300 br, 350 sh, +$AlCl_3$, 230, 248 sh, 292 sh, 300, 350; +NaOAc, 231, 282, 290 sh. ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.48 (6H, *s*, 4''- and 5''-H_3), 5.79 (1H, *d*, J = 10 Hz, 2''-H), 6.41 (1H, *s*, 8-H), 6.45 (1H, *dd*, J = 8.3 and 2 4 Hz, 5'-H), 6.49 (1H, incomplete *d*, 3'-H), 6.69 (1H, *d*, J = 10 Hz, 1''-H), 7.13 (1H, *d*, J = 8.3 Hz, 6'-H), 8.17 (1H, *s*, 2-H), 13.2 (*s*, C-5-OH). *Dimethyl ether* (**4d**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 380 $[M]^+$ (15), 365 $[M-15]^+$ (100). UV λ_{max}^{MeOH} nm. 226, 282, 287 sh; +$AlCl_3$, 228, 250 sh, 293, 300 sh, 348. The MeOH spectrum of **4d** was unaffected by NaOAc. *Trimethyl ether* (**4e**). Gibbs test (−). MS *m/z* (rel. int.): 395 $[M+1]^+$ (6.1), 394 $[M]^+$ (82), 380 (13), 379 $[M-15]^+$ (100), 363 (2.9), 190 (5.4), 161 (5.2). UV λ_{max}^{MeOH} nm: 226, 258 sh, 268, 285 sh, 326. *Triacetate* (**4f**): MS *m/z* (rel. int.): 478 $[M]^+$ (1.5), 437 (11), 436 (81), 422 (17), 421 (100), 394 (5.3), 380 (11), 379 (92), 352 (3.5), 337 (17), 203 (22), 43 (65). ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.50 (6H, *s*, 4''- and 5''-H_3), 5.79 (1H, *d*, J = 10 Hz, 2''-H), 6.62 (1H, *d*, J = 10 Hz, 1''-H), 6.80 (1H, *s*, 8-H), 7.07 (1H, *d*, J = 2.2 Hz, 3'-H), 7.08 (1H, *dd*, J = 8.9 and 2.2 Hz, 5'-H), 7.40 (1H, *d*, J = 8.9 Hz, 6'-H), 8.05 (1H, *s*, 2-H, acetyl protons at 2.10, 2.28 and 2.33 (each 3H, all *s*).

Licoisoflavone A (**7a**). Gibbs test (+), rapid, purple–blue. Pale yellow prisms as a complex with EtOAc (1:1) from EtOAc–*n*-pentane, mp 63–65° (**7a** solidified after being heated around 70° melting at 112–113°, see ref. [7] MS *m/z* (rel. int.): 355 $[M+1]^+$ (6.3), 354 $[M]^+$ (73), 312 (3.5), 311 $[M-43]^+$ (49), 299 $[M-55]^+$ (100), 297 (29), 153 (6.8), 147 (3.1). UV λ_{max}^{MeOH} nm: 263, 300 sh; +NaOMe, 276, 327; +$AlCl_3$, 269, 311, 372; +NaOAc, 274, 330 (addition of solid boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.65 and 1.78 (each 3H, both *br s*, 4''- and 5''-H_3), 3 44 (2H, *d*, J = 7.1 Hz, 1''-H_2), 5.33 (1H, *br t*, J = *ca* 7 Hz, 2''-H), 6.35 (1H, *d*, J = 2.2 Hz, 6-H), 6.50 (1H, *d*, J = 2.2 Hz, 8-H), 6.51 (1H, *d*, J = 8.6 Hz, 5'-H), 6 93 (1H, *d*, J = 8.6 Hz, 6'-H), 8.18 (1H, *s*, 2-H), 12.5 (*s*, C-5-OH).

Cyclisation of licoisoflavone A (**7a**) [3]. *p*-Toluenesulphonic acid (10 mg) was added to **7a** (26 mg) in C_6H_6 (15 ml) and the mixture was then heated at 80° for 10 hr. After working up as previously described [1], the products were separated by PTLC

using solvent system CM, 50:3 (cyclolicoisoflavone A_1, **7b**, R_f 0.52; cyclolicoisoflavone A_2, **7c**, R_f 0.30). A marker of starting material (**7a**) was located at R_f 0.36. Yields: **7b**, 12 mg and **7c**, 10 mg. *Cyclolicoisoflavone* A_1 (**7b**). Gibbs test (+), rapid, clear blue. MS *m/z* (rel. int.): 335 $[M+1]^+$ (12), 354 $[M]^+$ (82), 311 $[M-43]^+$ (24), 300 (8.9), 299 $[M-55]^+$ (100), 298 (37), 153 (3.3). UV λ_{max}^{MeOH} nm: 223 sh, 252 sh, 263, 290 sh; +NaOMe, 272, 325; +$AlCl_3$, 228 sh, 255 sh, 270, 311, 371; +NaOAc, 275, 330. ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.32 (6H, *s*, 4″- and 5″-H_3), 1.82 (2H, *br t*, J = 6.8 Hz, 2″-H_2), 2.73 (2H, *br t*, J = 6.8 Hz, 1″-H_2), 6.34 (1H, *d*, J = 2.2 Hz, 6-H), 6.37 (1H, *d*, J = 8.2 Hz, 5′-H), 6.49 (1H, *d*, J = 2.2 Hz, 8-H), 6.99 (1H, *d*, J = 8.2 Hz, 6′-H), 8.21 (1H, *s*, 2-H), 12.5 (*s*, C-5-OH). *Cyclolicoisoflavone* A_2 (**7c**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 354 $[M]^+$ (72), 311 $[M-43]^+$ (13), 299 $[M-55]^+$ (100), 298 (25), 153 (4.2). UV λ_{max}^{MeOH} nm: 225 sh, 250 sh, 260, 290 sh; +NaOMe, 225 sh, 269, 321; +$AlCl_3$, 227 sh, 256 sh, 269, 308, 370; +NaOAc, 252, 268, 324. ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.27 (6H, *s*, 4″- and 5″-H_3), 1 79 (2H, *br t*, J = 6.8 Hz, 2″-H_2), 2.71 (2H, *br t*, J = 6 8 Hz, 1″-H_2), 6.27 (1H, *d*, J = 2 1 Hz, 6-H), 6.40 (1H, *d*, J = 2.1 Hz, 8-H), 6.44 (1H, *d*, J = 8.3 Hz, 5′-H), 7.00 (1H, *d*, J = 8.3 Hz, 6′-H), 7.99 (1H, *s*, 2-H), 13.2 (*s*, C-5-OH).

Lupinisoflavone A (**8a**). Gibbs test (+), rapid, dark blue. Pale yellow needles, mp 189–191°. $[\alpha]_D^{23}$ +74° (*c* 0.108; MeOH). HRMS, MW 352.094 ($C_{20}H_{16}O_6$ requires 352.094). MS *m/z* (rel. int.): 353 $[M+1]^+$ (15), 352 $[M]^+$ (100), 338 (3.8), 337 $[M-15]^+$ (62), 219 (3.3), 203 (32), 134 (2.0). UV λ_{max}^{MeOH} nm (log ε): 214 (4.58), 263 (4.47), 287 sh (4.23); +NaOMe, 282, 290–300 sh; +$AlCl_3$, 235 sh, 271, 316, 365; +NaOAc, 229, 263, 287 sh. ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.79 (3H, *s*, 5″-H_3), 2.98 (1H, *dd*, J = 16 and 7.3 Hz, 1″-H_a), 3 42 (1H, *dd*, J = 16 and 9.4 Hz, 1″-H_b), 4.96 and 5.12 (each 1H, both *br s*, 4″-H_2), 5.47 (1H, *dd*, J = 9.4 and 7.3 Hz, 2″-H), 6 45 (1H, *dd*, J = 7.8 and 2.4 Hz, 5′-H), 6.49 (1H, incomplete *d*, 3′-H), 6.49 (1H, *s*, 8-H), 7.13 (1H, *br d*, J = 7.8 Hz, 6′-H), 8.19 (1H, *s*, 2-H), 13.0 (*s*, C-5-OH). *Dimethyl ether* (**8b**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 381 $[M+1]^+$ (9.6), 380 $[M]^+$ (100), 379 (6.4), 366 (7.5), 365 $[M-15]^+$ (82), 349 $[M-31]^+$ (8.9), 219 (8.2), 161 (7.1), 148 (4.2). UV λ_{max}^{MeOH} nm: 215, 253 sh, 262, 287; +$AlCl_3$, 237 sh, 272.5, 317, 366. The MeOH spectrum of **8b** was unaffected by NaOAc. *Trimethyl ether* (**8c**). Gibbs test (−). MS *m/z* (rel. int.): 395 $[M+1]^+$ (17), 394 $[M]^+$ (100), 393 (7 3), 363 $[M-31]^+$ (34), 161 (8.2). UV λ_{max}^{MeOH} nm: 216 sh, 247 sh, 253, 286, 310 sh. The MeOH spectrum of **8c** was unaffected by $AlCl_3$ and NaOAc. TLC R_f in CM (50:3): **8a**, 0.45; **8b**, 0.94; **8c**, 0.92. *Triacetate* (**8d**): MS *m/z* (rel. int.): 478 $[M]^+$ (2.8), 436 (30), 394 (29), 353 (7.1), 352 (100), 337 (17), 203 (5.4). UV λ_{max}^{MeOH} nm: 233 sh, 245, 252 sh, 299, 310 sh ^{1}H NMR (acetone-d_6, 100 MHz): δ 1 78 (3H, *s*, 5″-H_3), 3.03 (1H, *dd*, J = 16 and 7.6 Hz, 1″-H_a), 3.47 (1H, *dd*, J = 16 and 9.3 Hz, 1″-H_b), 4.97 and 5.14 (each 1H, both *br s*, 4″-H_2), 5.50 (1H, *dd*, J = 9.3 and 7.6 Hz, 2″-H), 6.84 (1H, *s*, 8-H), 7.06 (1H, *d*, J = 2.2 Hz, 3′-H), 7.08 (1H, *dd*, J = 8.9 and 2.2 Hz, 5′-H), 7.40 (1H, *d*, J = 8.9 Hz, 6′-H), 8.06 (1H, *s*, 2-H), acetyl protons at 2.10 (3H, *s*) and 2.28 (6H, *s*).

Lupinisoflavone B (**9a**). Gibbs test (+), rapid, purple–blue. Pale yellow prisms, mp 245–247°. $[\alpha]_D^{23}$ 0° (*c* 0.101; MeOH) HRMS, MW 370.103 ($C_{20}H_{18}O_7$ requires 370.105). MS *m/z* (rel. int.): 370 $[M]^+$ (100), 337 $[M-33]^+$ (32), 312 (57), 311 $[M-59]^+$ (91), 177 (18), 134 (16), 59 (67). UV λ_{max}^{MeOH} nm (log ε): 215 (4.55), 263 (4.44), 289 sh (4.19); +NaOMe, 260 sh, 280, 295 sh; +$AlCl_3$, 236 sh, 271, 318, 366; +NaOAc, 227, 263, 289 sh. ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.25 and 1.30 (each 3H, both *s*, 4″- and 5″-H_3), 3.15 (1H, *dd*, J = 16 and 9.3 Hz, 1″-H_a), 3.21 (1H, *dd*, J = 16 and 7.8 Hz, 1″-H_b), 4.86 (1H, *dd*, J = 9.3 and 7.8 Hz, 2″-H), 6.43 (1H, *s*, 8-H), 6.45 (1H, *dd*, J = 8.3 and 2.4 Hz, 5′-H), 6.49 (1H, *d*, J = 2.4 Hz, 3′-H), 7.13 (1H, *d*, J = 8.3 Hz, 6′-H), 8.18 (1H, *s*, 2-H), 12.96 (*s*, C-5-OH). *Dimethyl ether* (**9b**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 398 $[M]^+$ (100), 365 $[M-33]^+$ (14), 340 (26), 339 $[M-59]^+$ (82), 161 (8.0), 59 (40). UV λ_{max}^{MeOH} nm: 214, 240 sh, 262, 286; +$AlCl_3$, 238 sh, 272, 319, 370 sh. The MeOH spectrum of **9b** was unaffected by NaOAc. *Trimethyl ether* (**9c**). Gibbs test (−). MS *m/z* (rel. int.): 413 $[M+1]^+$ (17), 412 $[M]^+$ (100), 395 (16), 394 $[M-18]^+$ (26), 381 $[M-31]^+$ (33), 353 $[M-59]^+$ (16), 335 (13), 161 (20), 59 (39). UV λ_{max}^{MeOH} nm: 215 sh, 246 sh, 253.5, 287, 310 sh. TLC R_f in CM (50:3): **9a**, 0.32; **9b**, 0.89; **9c**, 0.71.

Lupinisoflavone C (**10a**). Gibbs test (+), slow, blue–green. Pale yellow gum; $[\alpha]_D^{23}$ −60° (*c* 0.108; MeOH). HRMS, MW 354.107 ($C_{20}H_{18}O_6$ requires 354.110). MS *m/z* (rel. int.): 354 $[M]^+$ (35), 321 $[M-33]^+$ (1.6), 295 $[M-59]^+$ (100), 153 (2.3), 59 (11). UV λ_{max}^{MeOH} nm (log ε): 250 sh (4.33), 263 (4.52), 292 sh (4.12); +NaOMe, 250 sh, 272.5, 295 sh, 326; +$AlCl_3$, 230 sh, 273, 312 sh, 375; +NaOAc, 228 sh, 272, 296 sh, 326 (addition of solid boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.23 and 1.28 (each 3H, both *s*, 4″- and 5″-H_3), 3.22 (1H, *dd*, J = 16 and 9.8 Hz, 1″-H_a), 3.31 (1H, *dd*, J = 16 and 8.3 Hz, 1″-H_b), 4.67 (1H, *dd*, J = 9.8 and 8.3 Hz, 2″-H), 6.29 (1H, *d*, J = 2.2 Hz, 6-H), 6.42 (1H, *d*, J = 2.2 Hz, 8-H), 6.75 (1H, *d*, J = 8 1 Hz, 5′-H), 7.30 (1H, *dd*, J = 8.1 and 1.5 Hz, 6′-H), 7.42 (1H, *d*, J = 1.5 Hz, 2′-H), 8.17 (1H, *s*, 2-H), 13.04 (*s*, C-5-OH). *Monomethyl ether* (**10b**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 368 $[M]^+$ (41), 310 (40), 309 $[M-59]^+$ (100), 59 (13). UV λ_{max}^{MeOH} nm: 250 sh, 264, 290 sh, 330 sh, +$AlCl_3$, 230 sh, 274.5, 310 sh, 373. The MeOH spectrum of **10b** was unaffected by NaOAc. *Dimethyl ether* (**10c**). Gibbs test (−). MS *m/z* (rel. int.): 383 $[M+1]^+$ (4.2), 382 $[M]^+$ (18), 364 $[M-18]^+$ (5.4), 349 $[M-33]^+$ (4.5), 324 (21), 323 $[M-59]^+$ (100), 181 (3.3), 162 (4.6), 149 (13), 59 (9.7). UV λ_{max}^{MeOH} nm: 246 sh, 258, 290 sh, 315 sh (unaffected by $AlCl_3$ and NaOAc). TLC R_f in CM (50:3): **10a**, 0.48; **10b**, 0.89; **10c**, 0.73.

Lupinisoflavone D (**11a**). Gibbs test (+), rapid, clear blue. Pale yellow fine needles, mp 189–191°. $[\alpha]_D^{23}$ −47° (*c* 0.102; MeOH). HRMS, MW 370.108 ($C_{20}H_{18}O_7$ requires 370.105). MS *m/z* (rel. int.): 370 $[M]^+$ (38), 337 $[M-33]^+$ (12), 311 $[M-59]^+$ (100), 299 (4.2), 160 (7.1), 153 (8.3), 59 (14). IR ν_{max}^{KBr} cm^{-1}: 3420, 3170, 2960, 1650, 1600, 1260, 1160, 1045. UV λ_{max}^{MeOH} nm (log ε): 226 sh (4 33), 252 sh (4.46), 261 (4.50), 290 sh (4.06); +NaOMe, 257 sh, 269, 319; +$AlCl_3$, 226 sh, 269, 310, 371; +NaOAc, 272, 327 (addition of solid boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.24 and 1.28 (each 3H, both *s*, 4″- and 5″-H_3), 3.14 (1H, *dd*, J = 16 and 9.5 Hz, 1″-H_a), 3.21 (1H, *dd*, J = 16 and 8.3 Hz, 1″-H_b), 4.69 (1H, *dd*, J = 9.5 and 8.3 Hz, 2″-H), 6.33 (1H, *d*, J = 2.0 Hz, 6-H), 6.34 (1H, *d*, J = 8.5 Hz, 5′-H), 6.47 (1H, *d*, J = 2 0 Hz, 8-H), 7.01 (1H, *d*, J = 8.5 Hz, 6′-H), 8.18 (1H, *s*, 2-H), 12.7 (*s*, C-5-OH). *Dimethyl ether* (**11b**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 398 $[M]^+$ (41), 340 (26), 339 $[M-59]^+$ (100), 167 (2.8), 59 (6.6). UV λ_{max}^{MeOH} nm: 225 sh, 252 sh, 260, 287 sh, +$AlCl_3$, 227 sh, 270, 308, 372. The MeOH spectrum of **11b** was unaffected by NaOAc. *Trimethyl ether* (**11c**). Gibbs test (−). MS *m/z* (rel. int.): 413 $[M+1]^+$ (15), 412 $[M]^+$ (82), 381 $[M-31]^+$ (27), 354 (18), 353 $[M-59]^+$ (100), 181 (8.2), 59 (16). UV λ_{max}^{MeOH} nm: 225 sh, 248 sh, 255, 280 sh (unaffected by $AlCl_3$ and NaOAc). ^{1}H NMR (acetone-d_6, 100 MHz): 1.21 (6H, *s*, 4″- and 5″-H_3), 3 33 (2H, *br d*, J = *ca* 9 Hz, 1″-H_2), 4.66 (1H, *dd*, J = 9.4 and 8.4 Hz, 2″-H), 6.42 (1H, *d*, J = 8.1 Hz, 5′-H), 6.49 (1H, *d*, J = 1.8 Hz, 6-H), 6.57 (1H, *d*, J = 1.8 Hz, 8-H), 6.96 (1H, *d*, J = 8.1 Hz, 6′-H), 7.80 (1H, *s*, 2-H), methoxy protons at 3.75, 3.87 and 3.93 (each 3H, all *s*). TLC R_f in CM (50:3): **11a**, 0.39; **11b**, 0.85; **11c**, 0.60. *Triacetate* (**11d**). Small prisms, mp 187–189°. MS *m/z* (rel. int.): 496 $[M]^+$ (3.3), 454 (5.6), 438 (4.2), 412 (4 5), 396 (53), 354 (78), 353 (100), 337 (7.5), 312 (26), 311 (62), 59 (27). UV λ_{max}^{MeOH} nm: 225 sh, 251, 260 sh, 290 sh, 305 sh. ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.23 and 1.26 (each

3H, both *s*, 4″- and 5″-H_3), 3.02 (1H, *dd*, J = 16 and 9.5 Hz, 1″-H_a), 3.21 (1H, *dd*, J = 16 and 8.2 Hz, 1″-H_b), 4.70 (1H, *dd*, J = 9.5 and 8.2 Hz, 2″-H), 6.66 (1H, *d*, J = 8.2 Hz, 5′-H), 6.97 (1H, *d*, J = 2.3 Hz, 6-H), 7.09 (1H, *d*, J = 8.2 Hz, 6′-H), 7.34 (1H, *d*, J = 2.3 Hz, 8-H), 8.04 (1H, *s*, 2-H), acetyl protons at 2.10, 2.30 and 2.34 (each 3H, all *s*).

Dehydration of lupinisoflavone D (**11a**). $SOCl_2$ (0.1 ml) was added under N_2 atmosphere to a chilled (ice-bath) soln of **11a** (15 mg) in pyridine (1 ml). The reaction mixture was stirred at 0 to −5° for 30 min, and then at room temp for a further period of 30 min. After diluting to 15 ml with chilled water, the mixture was extracted with EtOAc. The extract was washed successively with dil. HCl, 5% aq. $NaHCO_3$ and brine, and the organic layer was then recovered, dried (Na_2SO_4) and concd to near dryness *in vacuo*. This concentrate contained about 7 mg of two reaction products (DHY-1 and DHY-2) which were separated by PTLC in PEAa (starting material, **11a**, R_f 0.04; DHY-1, R_f 0.30; DHY-2, R_f 0.35). A mixture of DHY-1 and DHY-2 could not be resolved into its individual components by TLC in CM (single spot, R_f 0.52) or CAAm (single spot, R_f 0.04). *DHY*-1 (*major product*, **14a**): Colourless prisms, mp 197–199°. MS *m/z* (rel. int.): 352 $[M]^+$ (42), 337 $[M-15]^+$ (100), small fragments at *m/z* 185 and 153, no fragment at *m/z* $[M-59]^+$ or *m/z* 59. UV λ_{max}^{MeOH} nm: 225 sh, 252 sh, 261, 290 sh, 330 sh; +NaOMe, 232 sh, 257 sh, 268, 320; +$AlCl_3$, 228 sh, 257 sh, 269, 311, 369; +NaOAc, 228 sh, 258 sh, 271.5, 280 sh, 328 (addition of boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.79 (3H, *s*, 5″-H_3), 2.99 (1H, *dd*, J = 16 and 7.8 Hz, 1″-H_a), 3.39 (1H, *dd*, J = 16 and 9.8 Hz, 1″-H_b), 4.91 and 5 10 (each 1H, both *br s*, 4″-H_2), 5.29 (1H, *br t*, J = *ca* 9 Hz, 2″-H), 6.34 (1H, *d*, J = 2.0 Hz, 6-H), 6 41 (1H, *d*, J = 8.1 Hz, 5′-H), 6.48 (1H, *d*, J = 2.0 Hz, 8-H), 7.06 (1H, *d*, J = 8.1 Hz, 6′-H), 8.20 (1H, *s*, 2-H), 12.7 (*s*, C-5-OH). *DHY*-2 (*minor product*, **15a**) Colourless fine needles, mp 158–160°. MS *m/z* (rel. int.): 352 $[M]^+$ (93), 337 $[M-15]^+$ (100), 200 (4 9), 185 (7.5), 153 (1.9). UV λ_{max}^{MeOH} nm. 212 sh, 238 sh, 252 sh, 259, 292 sh, 330 sh; +NaOMe, 228 sh, 260 sh, 265, 315; +$AlCl_3$, 228 sh, 262–265 br, 311, 366; +NaOAc, 229 sh, 261 sh, 271, 330 (addition of boric acid regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.35 (6H, *d*, J = 6.8 Hz, 4″- and 5″-H_3), 3.10 (1H, *d sep*, J = 6.8 and 1.0 Hz, 3″-H), 6.34 (1H, *d*, J = 2.4 Hz, 6-H), 6.49 (1H, *d*, J = 2.4 Hz, 8-H), 6.61 (1H, *s*, 1″-H), 7.06 (1H, *dd*, J = 8.3 and 1.0 Hz, 5′-H), 7.15 (1H, *d*, J = 8.3 Hz, 6′-H), 8.24 (1H, *s*, 2-H), 12.7 (*s*, C-5-OH).

Dehydration of lupinisoflavone D trimethyl ether (**11c**). Compound **11c** (12 mg) was dehydrated using the basic method applied to **11a**. In the case of **11c**, however, the reaction mixture was stirred at 0° for 1 hr, and then for 2.5 hr at room temperature before being worked up as described for **11a**. The products (MDHY-1 and MDHY-2, R_f in $CHCl_3$: 0.19 and 0.23, respectively) were isolated and separated by PTLC in CM, 50:1. *MDHY*-1 (*major product*, **14b**). Colourless gum, 5.6 mg. MS *m/z* (rel. int.): 395 $[M+1]^+$ (17), 394 $[M]^+$ (100), 379 $[M-15]^+$ (16), 364 (9.4), 363 $[M-31]^+$ (78), 181 (8.9). UV λ_{max}^{MeOH} nm: 225 sh, 248 sh, 255, 285 sh (unaffected by NaOMe, $AlCl_3$ or NaOAc). ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1.80 (3H, *br s*, 5″-H_3), 3.12 (1H, *dd*, J = 16 and 9.1 Hz, 1″-H_a), 3.47 (1H, *dd*, J = 16 and 8.1 Hz, 1″-H_b), 4.93 and 5.12 (each 1H, both *br s*, 4″-H), 5.21 (1H, *br t*, J = *ca* 8.8 Hz, 2″-H), 6.36 (1H, *d*, J = 2.4 Hz, 6-H), 6.45 (1H, *d*, J = 2.4 Hz, 8-H), 6.57 (1H, *d*, J = 8.2 Hz, 5′-H), 7.07 (1H, *d*, J = 8.2 Hz, 6′-H), 7.71 (1H, *s*, 2-H), methoxy protons at 3.73, 3.89 and 3.91 (each 3H, all *s*). *MDHY*-2 (*minor product*, **15b**). Colourless gum, 1.6 mg. MS *m/z* (rel. int.): 395 $[M+1]^+$ (9.2), 394 $[M]^+$ (70), 379 $[M-15]^+$ (2.6), 364 (17), 363 $[M-31]^+$ (100), 181 (4.5). UV λ_{max}^{MeOH} nm: 248 sh, 254, 287 sh, 310 sh (unaffected by NaOMe or $AlCl_3$). ^{1}H NMR ($CDCl_3$, 100 MHz): δ 1 35 (6H, *d*, J = 6.8 Hz, 4″- and 5″-H_3), 3.08 (1H, *br sep*, J = 6.8 Hz, 3″-H), 6.37 (1H, *d*, J = 2.4 Hz, 6-H), 6.47 (1H, *d*, J = 2.4 Hz, 8-H), 6.48 (1H, *s*, 1″-H), 7.14 (2H, *br s*, 5′- and 6′-H), 7.76 (1H, *s*, 2-H), methoxy protons at 3.90, 3.91 and 3.92 (each 3H, all *s*).

Lupinisoflavone E (**12a**). Gibbs test (+), slow, blue–green. Pale yellow prisms, mp 199–201°. $[\alpha]_D^{23}$ −111° (*c*0.085; MeOH). HRMS, MW 438.165 ($C_{25}H_{26}O_7$ requires 438.167). MS *m/z* (rel. int.): 439 $[M+1]^+$ (8.7), 438 $[M]^+$ (64), 380 (27), 379 $[M-59]^+$ (100), 321 (7.8), 307 (7.2), 59 (44). UV λ_{max}^{MeOH} nm (log ε): 215 5 (4.49), 267 (4.43), 296 sh (4.20); +NaOMe, 269, 295 sh; +$AlCl_3$, 233 sh, 277, 319, 371; +NaOAc, 215, 267, 295 sh. ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.23, 1.25, 1.28 and 1.30 (each 3H, all *s*, 4″-, 5″-, 4‴- and 5‴-H_3), 3.11–3.24 (2H, *m*, 1‴-H_2), 3.26–3.34 (2H, *m*, 1″-H_2), 4.67 (1H, *dd*, J = 9.5 and 8.1 Hz, 2″-H), 4.85 (1H, *dd*, J = 9 5 and 8.1 Hz, 2‴-H), 6.38 (1H, *s*, 8-H), 6.75 (1H, *d*, J = 8.1 Hz, 5′-H), 7.30 (1H, *dd*, J = 8.1 and 1.5 Hz, 6′-H), 7.42 (1H, *d*, J = 1.5 Hz, 2′-H), 8.18 (1H, *s*, 2-H), 13.2 (*s*, C-5-OH). *Monomethyl ether* (**12b**). Gibbs test (−). MS *m/z* (rel. int.): 453 $[M+1]^+$ (20), 452 $[M]^+$ (100), 434 $[M-18]^+$ (22), 394 (20), 393 $[M-59]^+$ (97), 375 (41), 59 (85). UV λ_{max}^{MeOH} nm: 216 sh, 245 sh, 261, 293 sh, 312 sh (unaffected by $AlCl_3$ or NaOAc). TLC R_f in CM (50:3): **12a**, 0.71; **12b**, 0 51.

Lupinisoflavone F (**13a**). Gibbs test (+), rapid, clear blue. Pale yellow needles, mp 215–216°. $[\alpha]_D^{23}$ −124° (*c*0.103; MeOH). HRMS, MW 454.162 ($C_{25}H_{26}O_8$ requires 454.162). MS *m/z* (rel. int.): 455 $[M+1]^+$ (9.4), 454 $[M]^+$ (64), 421 (4.5), 396 (32), 395 $[M-59]^+$ (100), 59 (29). UV λ_{max}^{MeOH} nm (log ε): 210 (4.62), 252 sh, (4.33), 264 (4.48), 263 sh (4.20); +NaOMe, 235 sh, 251, 260, 273 sh, 296; +$AlCl_3$, 272.5, 319, 369; +NaOAc, 252 sh, 264, 293 sh. ^{1}H NMR (acetone-d_6, 400 MHz): δ 1.24, 1.25, 1.27 and 1.30 (each 3H, all *s*, 4″-, 5″-, 4‴- and 5‴-H_3), 3.14–3.21 (2H × 2, *m*, 1″- and 1‴-H_2), 4.68 (1H, *dd*, J = 9.5 and 8.8 Hz, 2″-H), 4.87 (1H, *dd*, J = 9.5 and 8.1 Hz, 2‴-H), 6.34 (1H, *d*, J = 8.8 Hz, 5′-H), 6.45 (1H, *s*, 8-H), 7.02 (1H, *d*, J = 8.8 Hz, 6′-H), 8.20 (1H, *s*, 2-H), 12.8 (*s*, C-5-OH). *Monomethyl ether* (**13b**). Gibbs test (+), slow, blue–green. MS *m/z* (rel. int.): 469 $[M+1]^+$ (9.7), 468 $[M]^+$ (69), 410 (29), 409 $[M-59]^+$ (100), 59 (43). UV λ_{max}^{MeOH} nm: 212, 254 sh, 262, 293 sh; +$AlCl_3$, 238 sh, 273 5, 319, 366. The MeOH spectrum of **13b** was unaffected by NaOAc. *Dimethyl ether* (**13c**). Gibbs test (−). MS *m/z* (rel. int.): 483 $[M+1]^+$ (20), 482 $[M]^+$ (100), 464 $[M-18]^+$ (11), 451 $[M-31]^+$ (40), 423 $[M-59]^+$ (53), 405 (18), 59 (51). UV λ_{max}^{MeOH} nm: 247 sh, 253, 280 sh, 304 sh (unaffected by $AlCl_3$ or NaOAc). TLC R_f in CM (50·3): **13a**, 0.54; **13b**, 0.63; **13c**, 0.40.

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