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SEVENTEEN ISOFLAVONOIDS FROM *LUPINUS ALBUS* ROOTS

SATOSHI TAHARA, SUNAO ORIHARA, JOHN L. INGHAM* and JUNYA MIZUTANI

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan; *Department of Food Science, University of Reading, Whiteknights, P.O. Box 226, Reading RG6 2AP, U.K.

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Key Word Index—*Lupinus albus*; Leguminosae; isoflavonoids; prenyl-isoflavones; dihydrofurano-isoflavones; pyrano- and dihydrohydroxypyrano-isoflavones; 2-hydroxy-3-methyl-3-butenyl-substituted isoflavones; coumaronochromone.

Abstract—A further investigation of the methanol extractives from white lupin (cv. Kievskij Mutant) roots has revealed a new coumaronochromone (lupinalbin F) and 13 new isoflavones. The latter compounds have been identified as four dihydrofurano-isoflavones (lupinisoflavones G–J), three dihydrohydroxypyrano-isoflavones (lupinisolones A–C), three 2-hydroxy-3-methyl-3-butenyl-substituted isoflavones (lupinisols A–C), two pyrano-isoflavones (isoderrone and isochandalone), and a diprenyl-isoflavone (2′-hydroxyisolupalbigenin). The structures of all 14 isoflavonoids were principally deduced by spectroscopic comparison (MS, UV and ^{1}H NMR) with structurally similar compounds previously isolated from *L. albus* or obtained as fungal metabolites of prenylated isoflavones. Chemical transformation and/or the Gibbs test response was used to provide supporting structural evidence in some cases. The known isoflavones chandalone, angustone B and angustone C were also isolated from *L. albus* roots.

INTRODUCTION

The roots and leaves of white lupin (*Lupinus albus* L.) are known to contain numerous isoflavones [1–3], as well as a series of rare coumaronochromones (lupinalbins A–E) [4]. Apart from three simple isoflavones (genistein **1**, 2′-hydroxygenistein **2**, and 3′-*O*-methylorobol **3**) [1–3], white lupins have also yielded a remarkable range of complex derivatives, these being distinguished by mono or diprenylation (wighteone **4**, luteone **5**, licoisoflavone A **6**, lupisoflavone **7**, lupalbigenin **8**, and 2′-hydroxylupalbigenin **9** [= angustone A]), or by the presence of pyrano- (alpinumisoflavone **10**, parvisoflavone B **11**, and licoisoflavone B **14**) or dihydrofurano- (lupinisoflavones A–F **18**, **19**, **23**, **24**, **26**, **27**, and erythrinin C **20**) side structures attached to ring A and/or ring B. Our studies have also shown that three distinct oxygenation patterns occur within the white lupin isoflavones, these being 5,7,4′-trihydroxy, 5,7,2′,4′-tetrahydroxy and 5,7,4′-trihydroxy-3′-methoxy [1–3].

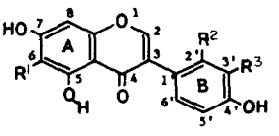
Re-examination of the methanol soluble components from *L. albus* roots has now revealed 17 minor isoflavonoids including a new coumaronochromone (**31**). Of the 16 identified isoflavones, only three, namely angustones B (**12**) and C (**15**) from *L. angustifolius* roots [5] and chandalone (**13**) from *Derris scandens* [6], have previously been recognized as natural products. The remainder are described for the first time in this report. In addition to compounds with prenyl, pyrano and dihydrofurano groups, several isoflavones with dihydrohydroxypyrano (**36**, **38**, **40**) and 2-hydroxy-3-methyl-3-butenyl (**42**–**44**) side structures have also been identified. Although the latter substituent is found within the naturally occurring coumarins [7], to our knowledge its appearance amongst the isoflavonoids has until now been confined to dolichins A and B, two isomeric pterocarpans obtained from the bacteria-infiltrated leaves of *Dolichos biflorus* [8].

RESULTS AND DISCUSSION

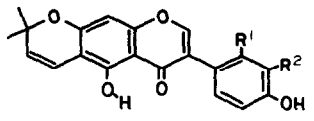
White lupin roots (8 kg of fresh material) were exhaustively extracted with methanol [3], and the neutral and phenolic constituents (*ca* 40 g) were then fractionated by column chromatography on silica gel and Florisil. When necessary, compounds of interest in the eluates were further purified by preparative silica gel TLC (PTLC). The identity of each pure compound was then established by means of the Gibbs test [9], and by detailed spectroscopic (UV, MS, ^{1}H NMR) comparison with isoflavonoids previously obtained from *L. albus*, or from other sources. Throughout this paper, oxygenation at C-7 (as in all known isoflavonoids) and at C-4′ (as in every reported *Lupinus* isoflavonoid) is assumed. All the compounds studied were found to be hydroxylated at C-5 (effect of $AlCl_3$ on the methanolic UV spectrum [10]; detection of an ^{1}H NMR singlet between *ca* 12.50 and 13.60 attributable to an H-bonded C-5 OH group [10, 11]).

Identification of Lupinus *isoflavonoids*

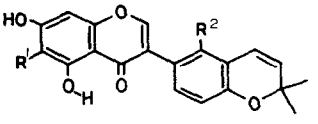
Diprenyl-substituted isoflavone (*2′-hydroxyisolupalbigenin*, **32**). Like 2′-hydroxylupalbigenin **9** (= angustone A) [3, 5], one of the isoflavones from column fractions Fr″-31/32 and Fr‴-3 (see Experimental for column details) was found to be dihydroxylated and monoprenylated in both ring A and ring B [M^+ 422, with partly alkylated RDA fragments at *m/z* 165 (63%, ring A) and *m/z* 147 (32%, ring B)]. Apart from an isoflavone 2-H singlet (δ 8.29) [11], the ^{1}H NMR spectrum consisted of



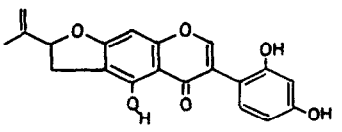
1 $R^1=R^2=R^3=H$, genistein
2 $R^1=R^3=H$, $R^2=OH$, 2'-hydroxygenistein
3 $R^1=R^2=H$, $R^3=OMe$, 3'-*O*-methylorobol
4 $R^1=CH_2CH{=}C(Me)_2$, $R^2=R^3=H$, wighteone
5 $R^1=CH_2CH{=}C(Me)_2$, $R^2=OH$, $R^3=H$, luteone
6 $R^1=H$, $R^2=OH$, $R^3=CH_2CH{=}C(Me)_2$, licoisoflavone A
7 $R^1=CH_2CH{=}C(Me)_2$, $R^2=H$, $R^3=OMe$, lupisoflavone
8 $R^1=R^3=CH_2CH{=}C(Me)_2$, $R^2=H$, lupalbigenin
9 $R^1=R^3=CH_2CH{=}C(Me)_2$, $R^2=OH$, 2'-hydroxylupalbigenin



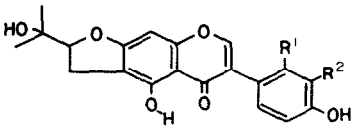
10 $R^1=R^2=H$, alpinumisoflavone
11 $R^1=OH$, $R^2=H$, parvisoflavone B
12 $R^1=OH$, $R^2=CH_2CH{=}C(Me)_2$, angustone C
13 $R^1=H$, $R^2=CH_2CH{=}C(Me)_2$, chandalone



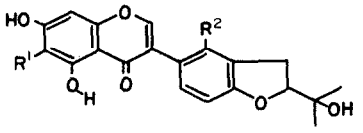
14 $R^1=H$, $R^2=OH$, licoisoflavone B
15 $R^1=CH_2CH{=}C(Me)_2$, $R^2=OH$, angustone B
***16** $R^1=R^2=H$, isoderrone
***17** $R^1=CH_2CH{=}C(Me)_2$, $R^2=H$, isochandalone



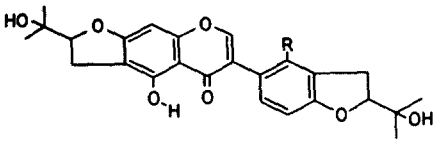
18 lupinisoflavone A



19 $R^1=OH$, $R^2=H$, lupinisoflavone B
20 $R^1=R^2=H$, erythrinin C
***21** $R^1=H$, $R^2=CH_2CH{=}C(Me)_2$, lupinisoflavone G
***22** $R^1=OH$, $R^2=CH_2CH{=}C(Me)_2$, lupinisoflavone H



23 $R^1=R^2=H$, lupinisoflavone C
24 $R^1=H$, $R^2=OH$, lupinisoflavone D
***25** $R^1=CH_2CH{=}C(Me)_2$, $R^2=OH$, lupinisoflavone I



26 R=H, lupinisoflavone E
27 R=OH, lupinisoflavone F

two sets of protons, one of which was assigned to a 2',4'-dihydroxy-3'-prenylated B-ring (Table 1), as in the known compound licoisoflavone A **6** (5'-H and 6'-H at δ6.51 *d* and 6.93 *d* respectively; prenyl protons at δ1.65 *s*, 1.78 *s*, 3.44 *br d* and 5.33 *br t* [3]). The remaining (A-ring) signals more closely resembled those of the 5,7-dihydroxy-8-prenyl isoflavone, 2,3-dehydrokievitone **33** (6-H at δ 6.39; prenyl protons at δ1.66 *s*, 1.81 *s*, 3.46 *br d* and 5.26 *br t* [12]) than its 6-prenylated isomer luteone **5** (8-H at δ6.53 *s*; prenyl protons at δ 1.65 *s*, 1.78 *s*, 3.37 *br d* and 5.28 *br t* [13]). On this basis, therefore, the new isoflavone (2'-hydroxyisolupalbigenin) can be considered to have structure **32**. Although isoflavones **32** and **9** (2'-hydroxylupalbigenin) are close isomers, they can be separated by silica gel TLC in CM (25 : 1) (**9**, R_f 0.54; **32**, R_f 0.66).

Pyrano-substituted isoflavones (*angustone B* **15**, *angustone C* **12**, *chandalone* **13**, *isoderrone* **16**, and *isochandalone* **17**). The extractives in column fraction Fr-4 were identified as the known pyrano-isoflavones angustone B (**15**), angustone C (**12**), and chandalone (**13**) by comparison of their spectroscopic data (UV, MS, ^{1}H NMR) with previously reported literature values [5, 6].

Isoderrone (M^+ 336; from fraction Fr''-31) afforded an intense MS ion at $[M-15]^+$ (*m/z* 321, base peak) typical of a pyrano-isoflavone [14]. The location of two hydroxyl groups on ring A, and the alkyl substituent on ring B, was apparent from RDA fragments observed respectively at *m/z* 153 and 169. A 10 nm bathochromic shift of the neutral (MeOH) UV maximum (259 nm) was induced by sodium acetate, thus permitting the second OH group to be placed at C-7 [10]. The ^{1}H NMR spectrum of isoderrone revealed a low-field singlet (δ 8.21, isoflavone 2-H [11]), two *meta*-coupled A-ring protons (6-H and 8-H), and a 2,2-dimethylpyrano ring (δ1.43 *s*, 6H, 2 × Me; δ5.77 *d* and 6.45 *d*, two olefinic protons with $J=10.0$ Hz) associated with three aromatic protons having *o*-, *o/m*- and *m*-coupling. Since 4'-oxygenation is assumed, the above data allow isoderrone to be assigned structure **16**.

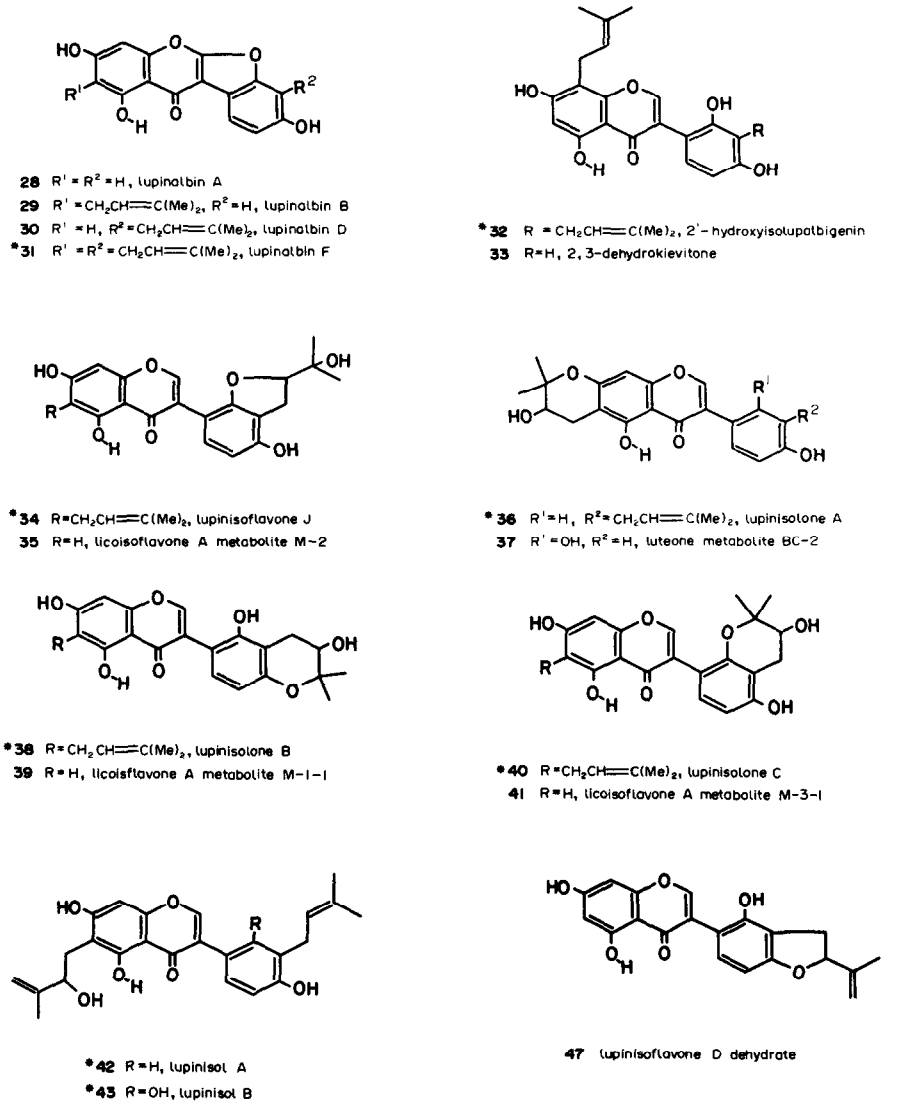


Fig. 1. Structures of the isoflavonoids mentioned in the text (compounds marked with an asterisk have been newly identified during the present study). Compounds ***44** (lupinisol C), **45** (LCF-1) and **46** (LCF-2) are shown in Scheme 1.

Apart from isoderrone, two other naturally occurring pyrano derivatives of 5,7,4′-trihydroxyisoflavone have been reported [15], these being derrone (A-ring alkylation; angular type) [16] and alpinumisoflavone **10** (A-ring alkylation; linear type) [14]. The latter isoflavone is known to occur in *L. albus* roots [2].

In addition to chandalone (**13**, see above), column fraction Fr-4 also contained approximately comparable amounts of an isomeric monoprenylated pyrano-isoflavone ($[M]^+$ 404, base peak at $[M-15]^+$) for which we propose the name isochandalone. The B-ring of isochandalone clearly resembled that of isoderrone **16** (Table 1) and thus only the A-ring substitution pattern remained to be established. Hydroxylation at C-5 and C-7, as in isoderrone, was evident from UV shift data ($AlCl_3$, NaOAc) whilst a blue Gibbs test colour [8] indicated that C-8 was unsubstituted. Ring A (prenyl at C-6) must therefore resemble that of wighteone **4** (8-H at δ6.49 *s*; prenyl protons at δ1.65 *s*, 1.78 *s*, 3.37 *br d* and 5.28 *br t* [17]) and luteone **5** [13] giving structure **17** for isochandalone.

Dihydrofurano-substituted isoflavones (lupinisoflavones G-J; **21**, **22**, **25** *and* **34**). As reported in our earlier papers [3, 4], seven dihydrofurano-isoflavones (erythrinine C **20**, and lupinisoflavones A-F, **16**, **19**, **23**, **24**, **26** and **27**) have already been isolated from *L. albus* roots. The present study has resulted in the discovery of four more compounds of this type differing from those previously described in having an intact prenyl substituent attached to either ring A (**25** and **34**) or ring B (**21** and **22**). The 2,3-dihydro-2-(1-hydroxy-1-methylethyl)furan attachment common to all these isoflavones can be unambiguously distinguished from the isomeric 2,3-dihydro-3-hydroxy-2,2-dimethylpyrano substituent by ^{1}H NMR spectroscopy [18]. In addition, compounds possessing the above dihydrofurano attachment characteristically afforded MS fragments at $[M-59]^+$ and *m/z* 59 [3].

The first new dihydrofurano-isoflavone (lupinisofla-

Table 1. ^{1}H NMR data for the minor isoflavonoids isolated from white lupin roots*

Compound Proton	Chandalone **13**	Isoderrone **16**	Isochandalone **17**	Lupinisoflavone G **21**	Lupinisoflavone H **22**	Lupinisoflavone I **25**	Lupinalbin F **31**	2′-Hydroxyiso-lupalbigenin **32**
2-H	8.15 *s*	8.21 *s*	8.19 *s*	8.14 *s*	8.21 *s*	8.14 *s*		8.29 *s*
5-OH	13.47 *s*	12.99 *s*	13.29 *s*	13.28 *s*	12.69 *s*	12.89 *s*	13.31 *s*	12.46 *s*
6-H		6.29 *d* (2.2)						6.43 *s*
8-H	6.36 *s*	6.43 *d* (2.2)	6.50 *s*	6.37 *s*	6.48 *s*	6.55 *s*	6.67 *s*	
2′-H	7.35 *d* (2.2)	7.30 *d* (2.2)	7.30 *d* (2.2)	7.33 *d* (2.2)				
5′-H	6.89 *d* (8.2)	6.79 *d* (8.0)	6.79 *d* (8.1)	6.89 *d* (8.1)	6.51 *d* (8.3)	6.33 *d* (8.3)	7.03 *d* (8.3)	6.51 *d* (8.3)
6′-H	7.28 *dd* (8.2, 2.2)	7.35 *dd* (8.0, 2.2)	7.36 *dd* (8.1, 2.2)	7.28 *dd* (8.1, 2.2)	6.94 *d* (8.3)	7.01 *d* (8.3)	7.66 *d* (8.3)	6.96 *d* (8.3)
B-ring side attachment‡								
1″-{H_a or H 1″-{H_b	3.37 *br d* (7.2) [2H]	6.45 *d* (10) [1H]	6.45 *d* (9.8) [1H]	3.37 *br d* (7.1) [2H]	3.44 *br d* (6.8) [2H]	3.17 *br d* (6.5) [2H]	3.60 *br d* (6.5) [2H]	3.44 *br d* (*ca* 7) [2H]
2″-H	5.38 *br t* (7.2)	5.77 *d* (10)	5.77 *d* (9.8)	5.38 *br t* (7.1)	5.32 *br t* (6.8)	4.69 *dd* (*ca* 9, 8)	5.39 *br t* (6.5)	5.32 *br t* (*ca* 7)
4″-H_3	1.37 *br s*	1.43 *s*	1.43 *s*	1.73 *br s*	1.77 *s* [3H]	1.27 *s* [3H]	1.85 *s* [3H]	1.78 *s* [3H]
5″-H_3	[6H]	[6H]	[6H]	[6H]	1.64 *s* [3H]	1.24 *s* [3H]	1.67 *s* [3H]	1.65 *s* [3H]
A-ring side attachment‡								
1‴-{H_a or H 1‴-{H_b	6.69 *d* (10) [1H]		3.37 *br d* (6.8) [2H]	3.17 *br d* (8.8) [2H]	3.20 *br d* (8.8) [2H]	3.38 *br d* (7.3) [2H]	3.40 *br d* (7.2) [2H]	3.46 *br d* (*ca* 7) [2H]
2‴-H	5.77 *d* (10)		5.28 *br t* (*ca* 7)	4.84 *dd* (9.8, 8.3)	4.88 *dd* (9.0, 8.1)	5.29 *br t* (*ca* 7)	5.30 *br t* (7.2)	5.40 *br t* (*ca* 7)
4‴-H_3	1.47 *s*		1.78 *s* [3H]	1.29 *s* [3H]	1.30 *s* [3H]	1.77 *s* [3H]	1.80 *s* [3H]	1.81 *s* [3H]
5‴-H_3	[6H]		1.65 *s* [3H]	1.25 *s* 13H]	1.26 *s* [3H]	1.65 *s* [3H]	1.67 *s* [3H]	1.65 *s* [3H]

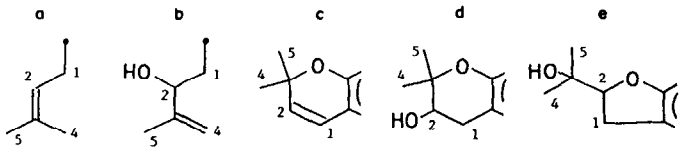
Table 1. *Continued*

Compound Proton	Lupinisoflavone J **34**	Lupinisolone A **36**†	Lupinisolone B **38**†	Lupinisolone C **40**	Lupinisol A **42**	Lupinisol A **42** in $CDCl_3$	Lupinisol B **43**	Lupinisol C **44**
2-H	8.30 *s*	8.14 *s*	8.21 *s*	7.99 *s*	8.13 *s*	7.82 *s*	8.18 *s*	8.14 *s*
5-OH	13.31 *s*	13.42 *s*	12.82 *s*	13.44 *s*	13.57 *s*	13.28 *s*	13.03 *s*	13.03 *s*
6-H								
8-H	6.50 *s*	6.36 *s*	6.58 *s*	6.48 *s*	6.44 *s*	6.47 *s*	6.52 *s*	6.55 *s*
2′-H		7.32 *d* (2.2)			7.34 *d*(i)	7.26 *d*(i)		
5′-H	6.41 *d* (8.5)	6.89 *d* (8.3)	6.39 *d* (8.5)	6.46 *d* (8.3)	6.89 *d* (8.1)	6.86 *d* (8.8)	6.51 *d* (8.3)	6.50 *d* (8.4)
6′-H	7.23 *d* (8.5)	7.28 *dd* (8.3, 2.2)	7.00 *d* (8.5)	7.01 *d* (8.3)	7.28 *dd* (8.1, 2.2)	7.26 *dd* (i)	6.93 *d* (8.3)	7.02 *d* (8.4)
B-ring side attachment‡								
1″-H_a	3.18 *m*	3.37 *br d* (6.8)	2.61 *dd* (17.3, 7.3)	2.57 *dd* (17.2, 7.8)	3.37 *br d* (*ca* 7)	3.40 *br d* (7.6)	3.44 *br d* (7.3)	*ca* 2.9 *dd* (*i*)
1″-H_b	[2H]	[2H]	3.03 *dd* (17.3, 5.4)	3.00 *dd* (17.2, 5.9)	[2H]	[2H]	[2H]	3.24 *dd* (14.5, 2.5)
2″-H	4.67 *dd* (9.2, 7.9)	5.38 *br t* (*ca* 7)	3.82 *m* (*br t*-like)	3.77 *br t* (*ca* 7)	5.37 *br t* (*ca* 7)	5.42 *br t* (7.6)	5.32 *br t* (*ca* 7)	4.35 *br d* (*ca* 8)
4″-H_2 or H_3	1.29 *s* [3H]		1.36 *s* [3H]	1.30 *s* [3H]			1.78 *s* [3H]	4.80 *br s* [1H]
		1.72 *br s*			1.73 *s*	1.79 *s*		5.01 *br s* [1H]
5″-H_3	1.20 *s* [3H]	[6H]	1.26 *s* [3H]	1.23 *s* [3H]	[6H]	[6H]	1.65 *s* [3H]	1.85 *s* [3H]
A-ring side attachment‡								
1‴-H_a	3.37 *br d* (6.6)	2.60 *dd* (*ca* 17, 6.8)	3.39 *br d* (7.1)	3.37 *br d* (7.1)	2.8–3.1 *m* (i)	2.91 *dd* (14.7, 7.6)	2.90 *dd* (*ca* 15, 7.1)	3.37 *br d* (7.3)
1‴-H_b	[2H]	2.97 *dd* (*ca* 17, 5.4)	[2H]	[2H]	[2H]	3.20 *dd* (14.7, 2.4)	2.97 *dd* (*ca* 15, 4.2)	[2H]
2‴-H	5.28 *br t* (6.6)	3.88 *m* (*ddd*-like)	5.28 *br t* (7.1)	5.28 *br t* (7.1)	4.35–4.50 *m*	4.43 *m* (*br d*-like)	4.46 *dd* (7.1, 4.2)	5.28 *br t* (7.3)
4‴-H_2 or H_3	1.78 *s* [3H]	1.39 *s* [3H]	1.79 *s* [3H]	1.78 *s* [3H]	4.77 *br s* [1H]	4.88 *br s* [1H]	4.77 *br s* [1H]	1.78 *s* [3H]
					4.93 *br s* [1H]	4.99 *br s* [1H]	4.94 *br s* [1H]	
5‴-H_3	1.65 *s* [3H]	1.33 *s* [3H]	1.66 *s* [3H]	1.65 *s* [3H]	1.84 *s* [3H]	1.87 *s* [3H]	1.84 *s* [3H]	1.65 *s* [3H]

*^{1}H NMR spectra were determined at 100 MHz in acetone-d_6 (TMS reference). *J* are in Hz. An i in parentheses indicates an incomplete signals for which a coupling constant (*J*) could not be determined. Unless stated otherwise, the signals integrated for one proton.

†OH in the side structure of **36** (lupinisolone A) appeared at δ 4.40 *d* (*J* = 5.1 Hz; 2‴-OH), and in **40** (lupinisolone C) at δ 4.22 *br s* (2‴-OH).

‡Side attachments are numbered (1″ to 5″ on ring B; 1‴ to 5‴ on ring A) as shown below.



vone G from fraction Fr'''-3; $[M]^+$ 422) was found to have a derivatized OH group at C-7 (absence of a UV shift with NaOAc [10]). A prenyl sidechain and a 2,3-dihydro-2-(1-hydroxy-1-methylethyl)furan substituent were evident from the ^1H NMR spectrum (Table 1). The prenyl group was allocated to ring B (C-3') along with an OH substituent (C-4') following an ^1H NMR comparison with chandalone (**13**), allowing the dihydrofurano side attachment to be placed on ring A. A linear arrangement was favoured in view of the blue-green Gibbs test colour (C-8 unsubstituted) given by lupinisoflavone G, and this was supported by the relevant ^1H NMR signals which had chemical shift values very similar to those associated with ring A of erythrinin C **20** (8-H at δ 6.38 *s*; dihydrofurano protons at δ 1.25 *s*, 1.29 *s*, 3.0–3.3 *m* and 4.84 *dd* [4]). Structure **21** was thus established for lupinisoflavone G.

From the spectroscopic data, it was clear that lupinisoflavone H ($[M]^+$ 438, fraction Fr'''-3) differed from lupinisoflavone G (**21**) only by its possession of an additional OH group. The OH at C-7 was found to be derivatized, permitting the dihydrofurano group to be provisionally placed in a linear fashion on ring A, an arrangement supported by an ^1H NMR comparison with lupinisoflavone B **19** (8-H at δ6.43; dihydrofurano protons at δ1.25 *s*, 1.30 *s*, 3.15 *dd*, 3.21 *dd* and 4.86 *dd* (400 MHz) [3]). The 6-H on ring A alternatively substituted in an angular fashion resonated quite differently at δ 6.24 [18]. Since lupinisoflavone H reacted rapidly with Gibbs reagent to afford a purple-blue colour (cf. the slow, blue-green response of **21**) the extra OH group must occur on ring B. Significantly, the B-ring aromatic protons appeared as two *ortho*-coupled doublets making possible only the following substitution patterns: 2',4'-dihydroxy-3'-prenyl, or 3',4'-dihydroxy-2'-prenyl. Comparison of the sidechain ^1H NMR signals with those of kwakhurin [19] effectively excluded a 2' (=6') location for the prenyl substituent thereby allowing ring-B to be formulated as in licoisoflavone A **6** (5'- and 6'-H at δ6.51 *d* and 6.93 *d* respectively; prenyl protons at δ1.65 *s*, 1.78 *s*, 3.44 *br d* and 5.33 *br t* [3]). Lupinisoflavone H is therefore represented by structure **22**.

The two remaining dihydrofurano-isoflavones (lupinisoflavones I and J from column fractions Fr''-28 and Fr''-31/32 respectively) had $[M]^+$ 438, with both affording a major RDA fragment at *m/z* 165 (A-ring with dihydroxylation and a CH_2 prenyl remnant). UV shift measurements ($AlCl_3$ and NaOAc) combined with the ^1H NMR data indicated that each compound had an A-ring part structure (5,7-dihydroxy-6-prenyl) resembling that of luteone **5** [13]. Thus, lupinisoflavones I and J can be envisaged as structural isomers in which the dihydrofuran attachment on ring B is cyclised to the 4'-oxygen (cf. lupinisoflavone D = licoisoflavone A metabolite M-1-2, **24**, [3, 20]) in one case, and to the anticipated 2'-oxygen (cf. licoisoflavone A metabolite M-2, **35** [20]) in the other. The detection of two *ortho*-coupled B-ring aromatic protons provides support for these alternative substitution patterns.

We reported earlier [3, 20] that the Gibbs test can be used to successfully and unambiguously distinguish 5,7,2'-hydroxyisoflavones (rapid response, deep blue colour) from 5,7,4'-hydroxyisoflavones (slow response, week blue-green colour) on thin-layer plates. When lupinisoflavones I and J were tested, the former immediately gave an intensely blue derivative (2'-OH free, 4'-OH derivatized) whereas the latter reacted only slowly to afford a less strongly coloured blue-green product (2'-OH derivatized, 4'-OH free). Structures **25** (for lupinisoflavone I) and **34** (for lupinisoflavone J) were also supported by the ^1H NMR data. Thus, the B-ring signals of lupinisoflavones **I** and **J** respectively resembled those of lupinisoflavone D **24** (= licoisoflavone A metabolite M-1-2,5'- and 6'-H at δ6.34 *d* and 7.01 *d*: dihydrofuran protons at δ1.24 *s*, 1.28 *s*, 3.17 *m* and 4.69 *dd* [20]) and licoisoflavone A metabolite M-2 **25** (5'- and 6'-H at δ6.24 *d* and 7.22 *d*: dihydrofuran protons at δ1.20 *s*, 1.29 *s*, 3.18 *m* and 4.66 *dd* [20]). Although the spectroscopic and colour test data do not entirely exclude an alternative B-ring arrangement for **34** (4'-OH, side structure 2' → 3' [O]), this can be effectively discounted since no isoflavones from any lupin species are known to have 2'-alkylation, whereas compounds with 3'-alkylation are frequently encountered.

2,3-Dihydro-3-hydroxy-2,2-dimethylpyrano-substituted isoflavones (lupinisolones A–C; **36**, **38** *and* **40**). In contrast to pyrano-isoflavones, relatively few dihydrohydroxypyrano-isoflavones have been described [15], the best known examples probably being mundulone [21] and psoralenol [22] from *Mundulea sericea* and *Psoralea corylifolia* respectively. Recently, however, we have shown that dihydrohydroxypyrano-isoflavones accumulate as metabolic products when certain prenylated isoflavones are incubated in liquid culture, with either *Aspergillus flavus* or *Botrytis cinerea* [13, 18, 20, 23]. As mentioned above, these compounds can be distinguished from isomeric 2,3-dihydro-2-(1-hydroxy-1-methylethyl)-furano-isoflavones by ^1H NMR and mass spectroscopy [18]. Our investigation of white lupin roots has now resulted in the isolation of three additional dihydrohydroxypyrano-isoflavones which we propose to name lupinisolones A–C.

The ^1H NMR spectrum of lupinisolone A ($[M]^+$ 422), from column fraction Fr'''-3, indicated the presence of both a prenyl group and a 3-hydroxy-2,2-dimethyldihydropyrano substituent (Table 1), the latter also being evident from the prominent mass fragment observed at *m/z* 351 $[M-71]^+$ [23]. Although the UV (MeOH) maximum at 266.5 nm was shifted bathochromically by aluminium trichloride (C-5 OH), no movement was caused by sodium acetate, an observation consistent with incorporation of the C-7 oxygen into the dihydropyrano ring. This was considered to be linearly disposed, as in luteone metabolite BC-2 **37** [13], from the slow development of a blue-green Gibbs test colour which ruled out the possibility of alkylation at C-8. Ring B (prenyl at C-3', and OH at C'-4) was found to resemble that of chandalone by comparison of the appropriate ^1H NMR signals giving structure **36** for lupinisolone A.

Lupinisolones B and C (both $[M]^+$ 438; from column fractions F''-27 and 32 respectively) were isomers, with each containing a prenyl sidechain in addition to the dihydrohydroxypyran attachment. A prominent mass fragment at *m/z* (A-ring derived RDA ion with dihydroxylation, and a CH_2 alkyl remnant), and the detection of only two B-ring protons (*ortho*-coupled) permitted the pyrano substituent to be placed unequivocally on ring B. Since UV shift data indicated that C-5 and C-7 were hydroxylated, the prenyl group must be attached at either C-6 or C-8, the former position being preferred for both compounds because of the comparatively low-field resonance of the single (8-H) A-ring proton (lupinisolone B at δ6.58, and lupinisolone C at δ6.48; cf. 6-H of the C-8 prenyl isoflavone 2,3-dehydrokievitone **33** which appears at δ6.39 [12]). Moreover, the methylene (1''-CH_2) pro-

tons of prenyl groups located at C-6 on 5,7-dihydroxylated isoflavones typically resonate (acetone-d_6) at *ca* δ3.37 (e.g. wighteone **4** [17], luteone **5** [13], 2′-hydroxylupalbigenin **9** [23] and isochandalone **17**), as observed for lupinisolones B and C, whereas those of C-8 prenyl groups occur at lower field (e.g. δ3.45 in the case of lupiwighteone [12], and δ3.46 for 2,3-dehydrokievitone **33** [12] and 2′-hydroxyisolupalbigenin **32**).

When subjected to the Gibbs test, lupinisolone B rapidly afforded a deep blue colour whilst lupinisolone C reacted slowly to give a blue-green colour. If 4′-oxygenation is accepted, the only B-ring substitution pattern attributable to lupinisolone B which accounts for the Gibbs test response and the spectroscopic data (presence of a dihydrohydroxypyran substituent, and two *ortho*-coupled aromatic protons) is that proposed for licoisoflavone A metabolite M-1-1 (**39**; 2′-OH, side attachment 3′ → 4′[O]) [20]. Lupinisolone B must therefore have structure **38**. Since **38** and lupinisolone C are isomers with identical A-rings, it is logical to argue that the difference between these compounds reflects only the disposition of their side structures (cf. lupinisoflavones F **25**, and J **34**). Thus the B-ring of lupinisolone C can be drawn (4′-OH, side attachment, 3′ → 2′[O] as in licoisoflavone A metabolite M-3-1 (**41**) [20]. Structure **40** for lupinisolone C is supported by the spectroscopic data and by the blue-green Gibbs test colour (see further discussion relating to **39** and **40** in ref. 20) which is consistent with a derivatized 2′-hydroxyl group. As noted for lupinisoflavone J (**34**), however, the data relating to lupinisolone C do not entirely exclude a 4′-hydroxylated B-ring in which the side structure is cyclized from C-2′ → C-3′[O] although such an arrangement is very unlikely.

2-Hydroxy-3-methyl-3-butenyl-substituted isoflavones (lupinisols A–C, **42–44**). The final three isoflavones (lupinisols A–C) gave ^{1}H NMR signals indicative of the 2-hydroxy-3-methyl-3-butenyl sidechain (Table 1) previously found in certain coumarins (e.g. aurapten [7]), and in dolichins A and B, two isoflavonoids (pterocarpans) from the bacteria-stressed leaflets of *Dolichos biflorus* [8]. As yet, however, no isoflavones with a 2-hydroxy-3-methyl-3-butenyl substituent appear to have been reported within the isoflavone group of natural products. The alternative 1-hydroxy-3-methyl-3-butenyl sidechain was readily discounted since the methylene ($-CH_2-$) ^{1}H NMR signals would be expected to occur at a much higher field ($\sim\delta$ 2.1) than was actually observed (*ca* δ3.0).

The identification of lupinisol A ($[M]^+$ 422; major fragments at *m/z* 404 $[M-H_2O]^+$, 389 $[M-H_2O-Me]^+$ and 351 $[M-71]^+$, base peak), from column fraction Fr″-27, was uncomplicated as an ^{1}H NMR comparison with lupinisoflavone G **21** (Table 1) indicated that both compounds had identical B-rings (3′-prenyl, 4′-OH). 5,7-Dihydroxylation of ring A was confirmed by standard UV shift measurements, whilst the hydroxylated sidechain was placed at C-6 from the Gibbs test result (slow, blue-green) which confirmed that C-8 was unsubstituted. Lupinisol A can thus be represented by structure **42**.

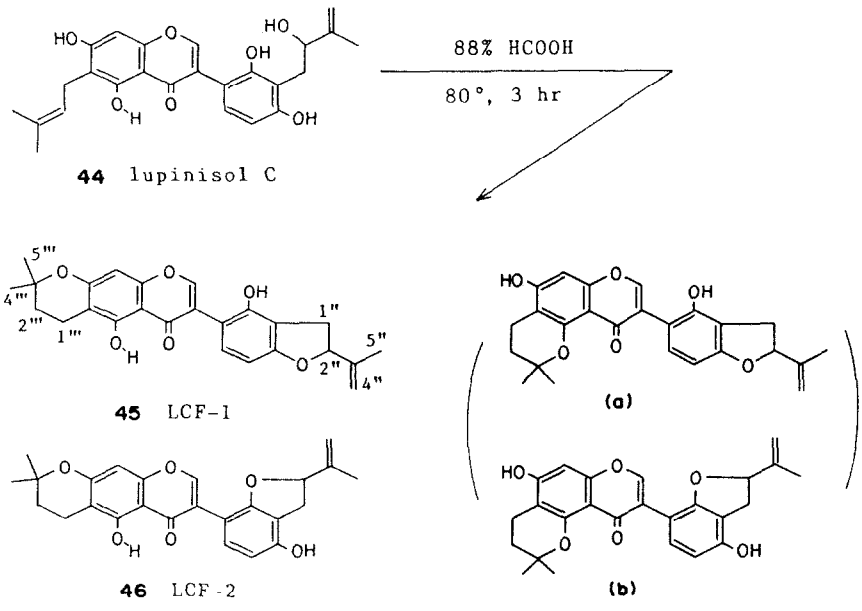
Lupinisols B (from column fraction Fr″-31) and C (from fractions Fr-8, Fr′-7/8 and Fr″-27) were isomeric, with each giving $[M]^+$ 438 and major fragments at *m/z* 420 $[M-H_2O]^+$, 405 $[M-H_2O-Me]^+$, 367 $[M-71]^+$, 311, 165 (RDA ion from a dihydroxylated A-ring $+CH_2$ alkyl remnant) and 147 (RDA fragment from a dihydroxylated B-ring$+CH_2$ alkyl remnant). UV shift measurements confirmed that both compounds were hydroxylated at C-5 and C-7. Apart from a 2-hydroxy-3-methyl-3-butenyl attachment, the ^{1}H NMR spectra of lupinisols B and C established the presence of a prenyl sidechain, a low-field aromatic singlet (8-H), and two *ortho*-coupled B-ring protons (5′- and 6′-H). Like luteone **5** each compound rapidly afforded a purple-blue Gibbs test colour suggesting B-ring hydroxylation at C-2′ and C-4′. Dialkylation in these isoflavones must therefore be at C-6 and C-3′, leaving only the precise location of the sidechains to be determined.

On the basis of a ^{1}H NMR comparison, the B-ring of lupinisol B (2′,4′-dihydroxy-3′-prenyl) was formulated as in licoisoflavone A **6** [3]. Likewise, the A-ring substitution pattern of lupinisol C (5,7-dihydroxy-6-prenyl) was supported by a ^{1}H NMR comparison with luteone **5** [13]. Since prenylation in lupinisols B and C occurs respectively at C-3′ and C-6, it follows from the preceding discussion that the hydroxylated sidechain must be at C-6 in the former compound and at C-3′ in the latter giving structures **43** (lupinisol B) and **44** (lupinisol C).

In order to obtain further proof for structure **44**, lupinisol C was treated with 88% HCOOH (80°; 3 hr) to afford two major products, LCF-1 (R_f 0.94; $[M]^+$ 420) and LCF-2 (R_f 0.84; $[M]^+$ 420). Both compounds were found to be hydroxylated at C-5 (UV shift with $AlCl_3$). As expected, ^{1}H NMR analysis (see Experimental section) indicated that the prenyl group of lupinisol C had undergone acid-catalysed cyclization (C-6 → C-7[O]) to yield a linear dihydropyran side structure, whilst dehydro-cyclization of the 2-hydroxy-3-methyl-3-butenyl substituent had similarly yielded a dihydrofuran attachment (Scheme 1). Significantly, the ^{1}H NMR spectrum of LCF-1 allowed the dihydrofuran ring, and hence also the hydroxy sidechain of lupinisol C, to be firmly located on ring B. Thus, the methine proton (2″-H) of LFC-1 had a chemical shift value (δ 5.29) identical with that of the corresponding proton in lupinisoflavone D dehydrate (**47**) [3]. In lupinisoflavone A **18**, with the same side structure linearly oriented on ring A, the methine proton has been shown to resonate up-field at δ 5.47 [3]. Lastly, LCF-1 rapidly gave a clear blue Gibbs test colour. This fact, coupled with the above discussion and the detection of two *ortho*-coupled B-ring aromatic protons, can only be explained in terms of the 2′-hydroxylated structure **45**. Consequently, LCF-2, with virtually identical spectroscopic characteristics, must be **44**, a suggestion in accord with its Gibbs test response (slow, blue-green; 2′-OH derivatized).

Apart from LCF-1 and LCF-2, the lupinisol C/HCOOH reaction mixture also contained two other products with $[M]^+$ 420. One compound (R_f 0.42) fluoresced greenish-white under long wavelength UV light, and the other (R_f 0.25) appeared purple-white (cf. LCF-1 and LCF-2, both of which exhibit the dark purple fluorescence typical of 5-hydroxyisoflavones). Although neither compound was obtained in quantities sufficient for chemical characterization, it is possible that they respectively resemble LCF-1 and LCF-2 except for cyclization of the A-ring (prenyl) sidechain to the alternative, but much less reactive, C-5 hydroxyl group (**a** and **b** in Scheme 1).

Diprenylated coumaronochromone (lupinalbin F, **31**). *Lupinus albus* roots are known to contain five 5-hydroxycoumaronochromones (lupinalbins A–E), all of which fluoresce yellow-orange on silica gel thin-layer plates



Scheme 1. Acid-catalysed cyclization of lupinisol C (products in parentheses were not fully identified).

viewed under long wavelength UV light [4] (cf. the dark purple fluorescence of lupin 5-hydroxyisoflavones [3]). A further example of this rare type of isoflavonoid (lupinalbin F from column fraction Fr'-3) has now been isolated and assigned the diprenyl structure **31**. Initial recognition of the compound ([M]$^+$ 420) as a coumaronochromone rather than as an isoflavone was based on the absence of a 2-H signal (^{1}H NMR spectrum, Table 1), the failure to observe either A- or B-ring derived RDA fragments in the mass spectrum, and the unusual series of UV (MeOH) maxima at 261.5 nm (100%), 284 nm (44%) and 338 nm (32%) [4]. Hydroxylation at C-5 and C-7 was evident from UV shift ($AlCl_3$, NaOAc) measurements. As well as the expected 5-OH singlet (δ13.31), the remaining ^{1}H NMR signals of lupinalbin F were confidently attributed to a 5,7-dihydroxy-6-prenylated A-ring, and a 4'-hydroxy-3'-prenylated B-ring, by comparison of the former with those of lupinalbin B **29** (8-H at δ6.66 *s*; prenyl protons at δ 1.66 *s*, 1.80 *s*, 3.39 *br d* and 5.29 *br t* [4]), and those of the latter with lupinalbin D **30** (5'- and 6'-H at δ7.02 *d* and 7.64 *d* respectively; prenyl protons at δ1.68 *d* [J=0.73 Hz], 1.85 *s*, 3.60 *br d* and 5.39 *br t* [4]). The possibility of an isomeric coumestan-type structure for lupinalbin F was excluded by the detection of an IR absorption band at 1610–1640 cm^{-1} (α,β-unsaturated ketone) [24]. Lupinalbin F is thus 5,7,4'-trihydroxy-6,3'-di(3,3-dimethylallyl) coumaronochromone (**31**).

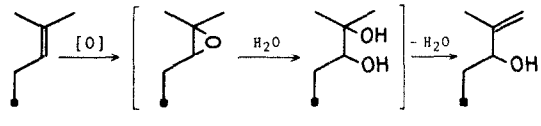
Biogenetic relationships

In an earlier paper [3] we described in detail the probable biogenetic relationships of white lupin isoflavones, and the significant position, on the route to furano- and pyrano-isoflavones, thought to be occupied by epoxide intermediates. The discovery of lupin isoflavones with a 2-hydroxy-3-methyl-3-butenyl sidechain is also consistent with the involvement of appropriate epoxide compounds which, by addition of water (to afford an intermediate diol with a sidechain as in luteone metabolite AF-2 [13]) and subsequent dehydration, could afford lupinisols A-C (Scheme 2). Interestingly, the present study has also revealed the occurrence, in white lupin roots, of dihydrohydroxypyrano-isoflavones which were previously proposed [3] as intermediates in the formation of pyrano-isoflavones (e.g. alpinumisoflavone **10**, and parvisoflavone B **11**). If this suggestion is correct, one of the compounds identified (lupinisolone B, **38**) could undergo dehydration to directly give the known angustone B **15** [5], and another (lupinisolone A, **36**) could afford chandalone **13**.

EXPERIMENTAL

General. Mps: uncorr. Analytical and preparative thin-layer separation were carried out on Merck pre-coated silica gel plates (F-254; layer thickness, 0.25 or 0.5 mm) using the following solvent systems: (a) CAAm = $CHCl_3$–Me_2CO–conc. NH_4OH, (b) HEA = *n*-hexane–EtOAc–Me_2CO, and (c) CM = $CHCl_3$–MeOH. Details relating to the composition of solvent systems are given at the appropriate point(s) in the text. All compounds were eluted from chromatograms with EtOAc. Detection of isoflavonoids on developed thin-layer plates was by inspection under long (365 nm) and short (254 nm) wavelength UV light, and by the characteristic colours formed upon spraying with Gibbs reagent [3, 9].

Extraction and purification of isoflavonoids. White lupin (*Lupinus albus* L. cv. Kievskij Mutant) roots (8 kg; washed and air-dried overnight) were extracted with 90% MeOH as previously described [3]. The neutral phenolic constituents were partitioned (×2) between *n*-hexane and 90% MeOH. The



Scheme 2. Possible pathway from the prenyl sidechain to 2-hydroxy-3-methyl-3-butenyl sidechain.

MeOH–H_2O phase was evapd to dryness to give *ca* 40 g of a yellow gum which was chromatographed on a Wako-gel C-200 column (400 g). The various root constituents were initially eluted with C_6H_6 (750 ml), and then with C_6H_6 plus increasing amounts of EtOAc. Eluates (250 ml per fraction) were collected as follows: Fractions (Fr)-1, 2 and 3, 5% EtOAc in C_6H_6; Fr-4, 5 and 6, 15% EtOAc in C_6H_6, and Fr-7, 8 and 9, 30% EtOAc in C_6H_6. The isoflavones in Fr-4 were separated by silica gel prep. TLC (PTLC) in CAAm (30:50:1) to give three broad bands at R_f 0.92, 0.86 and 0.66. Upon elution with EtOAc, the bottom band (R_f 0.66) was found to consist of pure angustone B (**15**, 10 mg) [5]. Further PTLC of the top band (R_f 0.92) in HEA (5:1:1) gave chandalone (**13**, R_f 0.46, 3.3 mg) [6] and two very minor, lower running compounds which were not identified. PTLC of the middle band (R_f 0.86) in $CHCl_3$–EtOAc–conc. NH_4OH = 30:6:1 (lower phase) afforded angustone C (**12**, R_f 0.57, 3.2 mg) [5], and isochandalone (**17**, R_f 0.35, 2.8 mg).

Column fractions Fr-6 and Fr-7 were combined and evaporated to give a solid (*ca* 5 g). This material was then re-chromatographed on a column of Wako-gel C-200 (120 g). After being washed initially with C_6H_6 (250 ml), the column was eluted with C_6H_6–EtOAc mixtures (100 ml per fraction) as follows: Fractions (Fr')-1, 2 and 3, 5% EtOAc in C_6H_6, and Fr'-4 to 9, 10% EtOAc in C_6H_6. Silica gel PTLC (CAAm, 70:100:3) of Fr'-3 afforded four Gibbs test-positive constituents at *ca* R_f 0.80, 0.74, 0.57 and 0.50. Elution and re-PTLC of the first band (HEA, 5:1:2) gave lupinalbin F (**31**, R_f 0.49, 3.1 mg) and lupalbigenin (**8**, R_f 0.39, 5.9 mg). Lupalbigenin was additionally isolated as a major component of the second band (PTLC in CM, 25:1, R_f 0.73, 29.8 mg), whilst the third consisted mainly of angustone B (**15**) which also occurred in Fr-4 and 5 (total yield, 25.3 mg). The fourth band was eluted to give 2'-hydroxylupalbigenin (**9**, 5.6 mg).

Fractions Fr'-4 and 5 were combined, and after evaporation the residue (*ca* 3 g) was applied to a column of Florisil (150 g, pretreated with 5% H_2O, w/w). The column was washed with C_6H_6 (300 ml), after which the isoflavonoid components were eluted as follows with C_6H_6–EtOAc mixtures (data in parentheses refer to fraction volumes, and % EtOAc in C_6H_6 respectively: Fractions (Fr'')-1 to 4 (75 ml, 3%), Fr''-5 to 8 (75 ml, 6%), Fr''-9 to 12 (75 ml, 10%), Fr''-13 to 16 (75 ml, 15%), Fr''-17 to 20 (75 ml, 20%), Fr''-21 to 24 (75 ml, 30%), Fr''-25 to 28 (75 ml, 40%), Fr''-29 and F4''-30 (150 ml, 50%), Fr''-31 (500 ml, EtOAc only, and Fr''-32 (1000 ml, EtOAc–MeOH, 1:1). PTLC of the residue (*ca* 60 mg) from Fr''-27 in CAAm (35:70:1) gave bands at R_f 0.70, 0.63 and 0.41 (lupinisol A, **42**, 3.3 mg). The composition of the upper band was not determined, but PTLC of the middle band (CM, 25:1) afforded lupinisolone B (**38**, R_f 0.34, 1.3 mg) and lupinisol C (**44**, R_f 0.29, 5.1 mg). The latter isoflavone (20.4 mg) was also obtained from combined column fractions Fr-8 + Fr'-7 and 8. Lupinisoflavone I (**25**, 1 mg) was isolated from Fr''-28 by PTLC in CM (25:1, R_f 0.33) with further quantities being obtained from combined fractions Fr-8 + Fr'-7 and 8. Separation of the constituents in Fr''-31 (PTLC, CAAm, 35:50:1) yielded 2'-hydroxyisolupalbigenin (**32**, R_f 0.77, 0.6 mg), lupinisoflavone J (**34**, R_f 0.63, 1.1 mg), isoderrone (**16**, R_f 0.40, 2.4 mg) and impure lupinisol B (**43**, R_f 0.22). Purification of lupinisol B by PTLC in CM (25:1) yielded small amounts of a colourless oil (R_f 0.52, 2.8 mg). 2'-Hydroxyisolupalbigenin **32** and lupinisoflavone J (**34**) were additionally found in fraction Fr''-32.

A portion of Fr''-32 (*ca* one-fifth) was chromatographed (PTLC in CAAm, 35:40:1) to give two major bands at R_f 0.57 and 0.19. After elution, the components of these bands were re-chromatographed in CM (25:1) to respectively afford lupinisolone C (**40**, R_f 0.07, 1.6 mg) and licoisoflavone B (**14**, R_f 0.51, 7.7 mg). The remainder of Fr''-32 was reduced to dryness, and the resulting solid (172 mg) was then column chromatographed over silica gel (16 g). After washing the column with $CHCl_3$ (30 ml), isoflavones were eluted with $CHCl_3$–EtOAc (3:1). Four fractions (Fr''', each 12 ml) were collected, but attention was focused on Fr'''-3 which contained at least five components. Fr'''-3 was initially separated into three bands by PTLC in CAAm (35:50:1). The highest running band (R_f 0.86) was then re-chromatographed in CM (25:1) to yield lupinisoflavone H (**22**, R_f 0.64, 1.3 mg), lupinisoflavone G (**21**, R_f 0.62, 1.4 mg), and lupinisolone A (**36**, R_f 0.54, 1 mg). The second band (R_f 0.68) and the third one (R_f 0.18) respectively gave 2'-hydroxyisolupalbigenin (**32**) and licoisoflavone B (**14**). Lupinisoflavone G (**21**), lupinisoflavone H (**22**) and lupinisolone A (**36**) were also isolated from Fr'''-4.

Properties of lupin isoflavonoids. The known isoflavones, angustones B (**15**) and C (**12**), and chandalone (**13**), were identified by comparison of their spectroscopic data (UV, MS and ^{1}H NMR) with those previously reported [5, 6]. ^{1}H NMR data for the newly isolated *Lupinus* isoflavonoids are shown in Table 1.

2'-Hydroxyisolupalbigenin (**32**). Partly solidified gum. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. MS *m/z* (rel. int.): 423 $[M+1]^+$ (29), 422 $[M]^+$ ($C_{25}H_{26}O_6$, 100), 405 (14), 380 (19), 379 (69), 368 (20), 367 (78), 366 (30), 365 (14), 352 (11), 351 (47), 349 (10), 335 (10), 324 (19), 323 (81), 312 (14), 311 (59), 310 (23), 219 (18), 205 (10), 203 (30), 177 (20), 176 (14), 175 (14), 168 (14), 165 (63), 156 (11), 148 (15), 147 (32), 123 (14). UV λ_{max}^{MeOH} nm: 224sh, 267.5, 335sh (*br*); +NaOMe, 286, 339 (*br*); +$AlCl_3$, 225sh, 275, 313 (*br*), 364; +NaOAc, 283, 338 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Isoderrone (**16**). Colourless amorphous solid. $UV_{(365nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, dark blue. MS *m/z* (rel. int.): 337 $[M+1]^+$ (6.2), 336 $[M]^+$ ($C_{20}H_{16}O_5$, 27), 322 (22), 321 $[M-Me]^+$ (100), 279 (11), 207 (6.8), 169 (7.1), 167 (19), 161 (14), 153 (6.3), 152 (11), 151 (8.0), 149 (39), 129 (8.5), 125 (6.9), 123 (7.1), 115 (7.8), 113 (11), 112 (9.2), 111 (13), 109 (8.8). UV λ_{max}^{MeOH} nm: 220sh, 259, 300sh; +NaOMe, 240sh, 272, 331 (*br*); +$AlCl_3$, 234, 269, 312, 364; +NaOAc, 271, 329 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Isochandalone (**17**). Colourless amorphous powder. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, dark blue. MS *m/z* (rel. int.): 405 $[M+1]^+$ (12), 404 $[M]^+$ ($C_{25}H_{24}O_5$, 39), 390 (28), 389 $[M-Me]^+$ (100), 361 (12), 349 (13), 333 (10), 187 (8.1), 167 (25). UV λ_{max}^{MeOH} nm: 217, 269, 290sh; +NaOMe, 216sh, 240sh, 276, 337 (*br*); +$AlCl_3$, 235, 271, 302sh, 320sh, 360sh; +NaOAc, 274, 327 (*br*) (H_3BO_3 regenerated the MeOH spectrum). IR ν_{max}^{KBr} cm^{-1}: 3400, 2890, 1630, 1450, 1280, 1250, 1205, 1150, 1110, 1040, 800.

Lupinisoflavone G (**21**). Colourless gum. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, blue-green. MS *m/z* (rel. int.): 423 $[M+1]^+$ (32), 422 $[M]^+$ ($C_{25}H_{26}O_6$, 100), 404 (14), 395 (26), 389 (43), 383 (27), 367 (19), 365 (14), 364 (40), 363 $[M-59]^+$ (54), 349 (17), 309 (26), 308 (25), 307 (26), 295 (19), 179 (13), 165 (16), 59 (85). UV λ_{max}^{MeOH} nm: 213.5, 266, 290sh; +NaOMe, 282; +$AlCl_3$, 230sh, 275.5, 315 (*br*), 363; +NaOAc, unchanged.

Lupinisoflavone H (**22**). Colourless amorphous powder. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. MS *m/z* (rel. int.): 439 $[M+1]^+$ (28), 438 $[M]^+$ ($C_{25}H_{26}O_7$, 97), 423 (15), 396 (25), 395 (100), 384 (22), 383 (91), 382 (33), 379 $[M-59]^+$ (8.5), 377 (13), 365 (13), 337 (20), 324 (19), 323 (37), 321 (14), 311 (11), 237 (14), 203 (11), 177 (18), 165 (17), 147 (19), 91 (11), 59 (37). UV λ_{max}^{MeOH} nm: 266.5, 290sh; +NaOMe, 285; +$AlCl_3$, 272.5, 318, 362; +NaOAc, unchanged.

Lupinisoflavone I (**25**). Colourless amorphous powder. $UV_{(365\,nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, clear-blue. MS *m/z* (rel. int.): 439 $[M+1]^+$ (29), 438 $[M]^+$ ($C_{25}H_{26}O_7$, 100), 405 (17), 395 (37), 384 (12), 383 (48), 382 (13),

380 (21), 379 $[M-59]^+$ (58), 377 (26), 367 (11), 365 (14), 337 (21), 335 (13), 325 (19), 324 (27), 323 (65), 311 (14), 219 (11), 165 (71), 123 (14), 69 (11), 59 (39). UV λ_{max}^{MeOH} nm: 267, 292sh; + NaOMe, 271.5, 336 (*br*); + $AlCl_3$, 216sh, 231sh, 274, 314 (*br*), 363; + NaOAc, 274, 345 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Lupinisoflavone J (**34**). Colourless amorphous powder. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, blue-green. MS *m/z* (rel. int.): 438 $[M]^+$ ($C_{25}H_{26}O_7$, 3.0), 420 (7.8), 405 (10), 381 (16), 380 (67), 379 $[M-59]^+$ (58), 378 (14), 377 (31), 365 (19), 325 (17), 324 (47), 323 (100), 203 (16), 165 (22), 119 (10), 105 (12), 97 (11), 91 (16), 85 (17), 83 (15), 81 (12), 77 (12), 71 (35), 70 (12), 69 (27), 59 (13). UV λ_{max}^{MeOH} nm: 211, 266, 290sh, 330sh (*br*); + NaOMe, 276, 335; + $AlCl_3$, 271.5, 312sh, 364; + NaOAc, 272, 340 (H_3BO_3 regenerated the MeOH spectrum).

Lupinisolone A (**36**). Partly solidified gum. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, blue-green. MS *m/z* (rel. int.): 423 $[M+1]^+$ (29), 422 $[M]^+$ ($C_{25}H_{26}O_6$, 100), 407 (9.4), 405 (13), 389 (12), 367 (29), 366 (9.5), 352 (13), 351 $[M-71]^+$ (51), 350 (24), 296 (12), 295 (34), 294 (12), 165 (11), 131 (9.6), 69 (17). UV λ_{max}^{MeOH} nm: 212sh, 266.5, 292sh, 335sh (*br*); + NaOMe, 281.5; + $AlCl_3$, 230sh, 274.5, 310sh (*br*), 364; + NaOAc, unchanged.

Lupinisolone B (**38**). Colourless gum. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, clear-blue. MS *m/z* (rel. int.): 439 $[M+1]^+$ (29), 438 $[M]^+$ ($C_{25}H_{26}O_7$, 100), 420 (8.7), 395 (32), 384 (10), 383 (40), 382 (14), 377 (22), 368 (12), 367 $[M-71]^+$ (28), 366 (20), 365 (17), 323 (16), 312 (21), 311 (46), 310 (33), 219 (13), 203 (12), 165 (51), 147 (19), 123 (10), 91 (10), 69 (12). UV λ_{max}^{MeOH} nm: 211sh, 268.5, 290sh; + NaOMe, 273, 338 (*br*); + $AlCl_3$, 233sh, 274, 315, 363; + NaOAc, 277, 345 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Lupinisolone C (**40**). Glassy solid. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, blue-green. MS *m/z* (rel. int.): 439 $[M+1]^+$ (28), 438 $[M]^+$ ($C_{25}H_{26}O_7$, 100), 405 (22), 395 (37), 383 (77), 378 (20), 377 (76), 367 $[M-71]^-$ (20), 366 (29), 365 (41), 351 (50), 335 (22), 323 (65), 311 (66), 207 (25), 203 (69), 165 (40), 147 (27), 55 (28). UV λ_{max}^{MeOH} nm: 229sh, 264.5, 287sh; + NaOMe, 273, 336; + $AlCl_3$, 272 (*br*), 312, 363; + NaOAc, 269 (*br*), 338 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Lupinisol A (**42**). Colourless gum. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), slow, blue-green. MS *m/z* (rel. int.): 422 $[M]^+$ ($C_{25}H_{26}O_6$, 11), 405 (9.4), 404 $[M-18]^+$ (30), 390 (12), 389 $[M-18-15]^+$ (43), 353 (8.9), 352 (42), 351 $[M-71]^+$ (100), 349 (9.6), 296 (6.9), 295 (19), 283 (8.9), 167 (9.7), 165 (6.7), 131 (9.2), 69 (9.5). UV λ_{max}^{MeOH} nm: 212sh, 267; + NaOMe, 277, 330 (*br*); + $AlCl_3$, 215sh, 230sh, 241sh, 277.5, 314, 366; + NaOAc, 274, 336 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Lupinisol B (**43**). Colourless gum. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. MS *m/z* (rel. int.): 439 $[M+1]^+$ (8.2), 438 $[M]^+$ ($C_{25}H_{26}O_7$, 26), 420 $[M-18]^+$ (15), 405 $[M-18-15]^+$ (7.2), 377 (16), 368 (36), 367 $[M-71]^+$ (100), 365 (17), 313 (8.7), 312 (27), 311 (70), 165 (36), 147 (15), 123 (9.0). UV λ_{max}^{MeOH} nm: 212sh, 225sh, 268, 295sh; + NaOMe, 281 (*br*), 336 (*br*); + $AlCl_3$, 216sh, 232sh, 274, 315 (*br*), 364; + NaOAc, 277, 341 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Lupinisol C (**44**). Colourless gum. $UV_{(365\ nm)}$ fluorescence, dark purple. Gibbs test: (+), rapid, purple-blue. MS *m/z* (rel. int.): 439 $[M+1]^+$ (11), 438 $[M]^+$ ($C_{25}H_{26}O_7$, 36), 421 (10), 420 $[M-18]^+$ (33), 405 $[M-18-15]^+$ (34), 377 (26), 369 (20), 368 (83), 367 $[M-71]^+$ (63), 365 (26), 349 (12), 325 (20), 313 (34), 312 (69), 311 (100), 203 (12), 175 (12), 165 (49), 148 (11), 147 (16), 91 (15). UV λ_{max}^{MeOH} nm: 212sh, 266, 291sh, 340sh; + NaOMe, 283, 340 (*br*); + $AlCl_3$, 274, 316, 366; + NaOAc, 273, 344 (*br*) (H_3BO_3 regenerated the MeOH spectrum).

Lupinalbin F (**31**). Pale brown plates, mp 234–236°. $UV_{(365\ nm)}$ fluorescence, orange-brown. Gibbs test: (+), slow, dark blue. HRMS: $[M]^+$ 420.153 ($C_{25}H_{24}O_6$ requires 420.157). MS *m/z* (rel. int.): 421 $[M+1]^+$ (20), 420 $[M]^+$ (63), 405 (19), 403 (16), 378 (25), 377 (78), 366 (25), 365 (100), 364 (8.8), 347 (7.7), 323 (8.1), 321 (15), 309 (18), 203 (8.9), 130 (8.8), 83 (9.9), 81 (12), 71 (9.5), 69 (24). UV λ_{max}^{MeOH} nm (rel. int.): 215 (87), 245sh (59), 261.5 (100), 282 (44), 338 (32); + NaOMe, 224sh, 262sh, 276.5, 303 (*br*), 364; + $AlCl_3$, 213.5, 235sh, 271, 284sh, 328 (*br*), 378; + NaOAc, 259.5 (*br*), 285sh, 349 (*br*) (H_3BO_3 regenerated the MeOH spectrum). IR ν_{max}^{KBr} cm^{-1}: 3130, 2920, 1640, 1610, 1490, 1440, 1370, 1305, 1270, 1180, 1110, 1065, 1025, 780.

Acid-catalysed cyclization of lupinisol C (**44**). Formic acid (88%, 1 ml) was added to lupinisol C (*ca* 10 mg) in EtOAc (2 drops), and the mixture was then heated at 80° for 3 hr. After diluting with C_6H_6, the reaction mixture was concd under red. pres. to give an oily residue. This was chromatographed (silica gel PTLC) in CAAm (35:30:1) to yield two major dark purple fluorescing (365 nm UV light) compounds at R_f 0.94 (LCF-1) and 0.84 (LCF-2).

LCF-1 (**45**). Colourless gum (1.9 mg). Gibbs test: (+), rapid, clear-blue. MS *m/z* (rel. int.): 421 $[M+1]^+$ (24), 420 $[M]^+$ (85), 406 (27), 405 (100), 365 (13), 349 (22), 185 (10), 175 (16), 165 (29). UV λ_{max}^{MeOH} nm: 212 sh, 264.5; + NaOMe, 253, 261, 285.5; + $AlCl_3$, 232sh, 273, 315.5, 364; + NaOAc, unchanged. ^1H NMR δ_{TMS} (Me_2CO-d_6, 100 MHz): 8.18 (1H, *s*, 2-H), 7.06 (1H, *d*, $J=8.2$ Hz, 6′-H), 6.40 (1H, *s*, 8-H), 6.40 (1H, *d*, $J=8.2$ Hz, 5′-H), 5.29 (1H, *dd*, $J=ca\ 9$ and 8 Hz, 2″-H), 5.11 (1H, *br s*, 4‴-H_a), 4.91 (1H, *br s*, 4‴-H_b), 3.6–2.8 (2H, *m*, 1″-H_2), 2.71 2H, *t*, $J=6.6$ Hz, 1‴-H_2), 1.90 (2H, *t*, $J=6.6$ Hz, 2″-H_2), 1.79 (3H, *s*, 5‴-H_3), 1.38 (6H, *s*, 4″- and 5″-H_3).

LCF-2 (**46**). Colourless gum (2.9 mg). Gibbs test: (+), slow, blue-green. MS *m/z* (rel. int.): 421 $[M+1]^+$ (29), 420 $[M]^+$ (100), 406 (16), 405 (58), 379 (19), 377 (12), 366 (12), 365 (38), 364 (13), 349 (25), 323 (10), 175 (19), 165 (28). UV λ_{max}^{MeOH} nm: 213sh, 265, 300sh; + NaOMe, 279; + $AlCl_3$, 274, 318sh; + NaOAc, unchanged. ^1H NMR δ_{TMS} (Me_2CO-d_6, 100 MHz): 13.42 (1H, *s*, 5-OH), 8.21 (1H, *s*, 2-H), 7.26 (1H, *d*, $J=8.4$ Hz, 6′-H), 6.44 (1H, *d*, $J=8.4$ Hz, 5′-H), 6.32 (1H, *s*, 8-H), 5.26 (1H, *dd*, $J=9.3$ and 8.1 Hz, 2″-H), 5.11 (1H, *br s*, 4‴-H_a), 4.88 (1H, *br s*, 4‴-H_b), 3.5–3.0 (2H, *m*, 1‴-H_2), 2.69 (2H, *t*, $J=6.6$ Hz, 1‴-H_2), 1.88 (2H, *t*, $J=6.6$ Hz, 2″-H_2), 1.79 (3H, *s*, 5‴-H_3), 1.37 (6H, *s*, 4″- and 5″-H_3).

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