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PRENYLATED FLAVONOIDS IN THE ROOTS OF YELLOW LUPIN

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IN HONOUR OF PROFESSOR RAGAI K. IBRAHIM'S SIXTY-FIFTH BIRTHDAY

Key Word Index—*Lupinus luteus*; Leguminosae; flavonoids; prenylated flavanones; isoflavone; chromone; coumaronochromone; antifungal activity.

Abstract—A further investigation of the methanol-soluble compounds in yellow lupin roots has revealed a new diprenylchromone, a new coumaronochromone (lupinalbin H), a new isoflavone 5,7,4'-trihydroxy-8,3'-di-(3,3-dimethylallyl)isoflavone (isolupalbigenin), and some complex flavanones. The latter compounds have been identified as two known diprenylated flavanones (lonchocarpol A and euchrestaflavanone A), two diasteroisomeric pairs of dihydrofuranoflavanones (lonchocarpols C_1 and C_2, and lonchocarpols D_1 and D_2; the structures formerly proposed for lonchocarpols C and D were also reinvestigated), a new furanoflavanone (lupinenol), and three 8-prenylflavanones with an additional (2*RS*)-hydroxy-3-methyl-3-butenyl side chain. The structures of the latter flavanones were unambiguously identified by spectroscopic (^{1}H NMR) comparison with 6-, 8- and 3'-prenylnaringenins chemically prepared from (2*S*)-naringenin. The antifungal activity of the prenylated naringenins, and of the various yellow lupin flavanones, was determined by TLC place bioassays using *Cladosporium herbarum* as the test fungus.

INTRODUCTION

We have previously reported that yellow lupin roots contain 6- or 8-prenylated isoflavones (e.g. luteone and 2,3-dehydrokievitone) and derivatives of these compounds in which the prenyl group has been variously cyclized and/or oxygenated [1–3], 5-*O*-methylisoflavones [4, 5], a coumaronochromone [3], and two 3-methoxy-6-prenylflavones (topazolin and topazolin hydrate) [6]. The present study, which was focused on the less polar constituents in yellow lupin roots, revealed that in addition to luteone (157 mg kg^{-1} fr. roots), methanol extracts also contained very substantial amounts of 6,8-diprenylnaringenin [= lonchocarpol A, **1**; 5,7,4'-trihydroxy-6,8-di-(3,3-dimethylallyl)flavanone, 647 mg kg^{-1} fr. roots]. The latter compound co-occurred with the known regioisomer 8,3'-diprenylnaringenin (euchrestaflavanone A, **2**) [7].

Lonchocarpol A and two dihydrofuranoflavanones (lonchocarpols C and D) were first obtained from the leaves of *Lonchocarpus minimiflorus* and characterized on the basis of their chemical and spectroscopic properties [8]. All three compounds were also found in yellow lupin roots. However, when compared with authentic 6- and 8-prenylnaringenins, both lonchocarpol C and lonchocarpol D exhibited certain ^{1}H NMR features which were inconsistent with the structures originally proposed [8]. Our conclusion from this ^{1}H NMR comparison is that the structures shown for lonchocarpols C and D in ref. [8] should be interchanged.

Lonchocarpols C and D, and the 2-hydroxy-3-methyl-3-butenyl substituted flavanones (lupiniols A and B) which occur in yellow lupin roots have been found to be a mixture of diastereoisomers, those of the first three compounds being successfully separated by HPLC to give lonchocarpols C_1 (**3**) and C_2 (**4**), lonchocarpols D_1 (**5**) and D_2 (**6**), and lupiniols A_1 (**7**) and A_2 (**8**). Because of the very small quantities available for investigation, the structure of lupiniol B (**9**) was determined using the diastereoisomeric mixture.

In addition to the above compounds, the present study also revealed another new flavanone (lupinenol, **10**) with the 2-(1-hydroxy-1-methylethyl)furano side attachment recently found in piscerisoflavone E from *Piscidia erythrina* [9]. Previous studies on yellow lupin roots [3, 4] failed to reveal any diprenylated isoflavones, although compounds of this type with 6,3'-diprenylation (e.g. lupalbigenin and 2'-hydroxylupalbigenin) occur in *L. albus* [10]. However, the present investigation has resulted in both the discovery of the first diprenylated (C-8/3') isoflavone (isolupalbigenin, **11**) in yellow lupin, and the identification of a novel diprenylated chromone (lupichromone, **13**). Finally, yellow lupin roots have also been found to contain a new coumaronochromone (lupinalbin H, **12**) structurally related to parvisoflavone B [3].

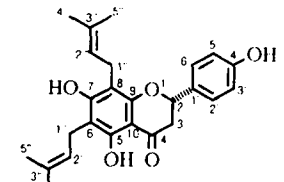
TLC plate bioassays carried out on the diastereoisomeric components of lonchocarpols C and D, and lupiniol A, revealed some evidence for stereochemical-related activity (e.g. lonchocarpol D_1 compared with D_2) [Table 3]. The regiospecific influence of a prenyl side chain on fungitoxicity was also evident from a comparison of 6-, 8- and 3'-prenylnaringenins. Interestingly, 8-prenylnaringen (**15**) was much more fungitoxic than the corresponding 6-prenylated isomer (**14**) [Table 3]. This observation is in direct contrast to the situation found in prenylated isoflavones where luteone and wighteone (prenyl at C-6) are stronger fungitoxins than 2,3-dehydrokievitone and lupiwighteone (prenyl at C-8) [10 and unpublished data].

RESULTS AND DISCUSSION

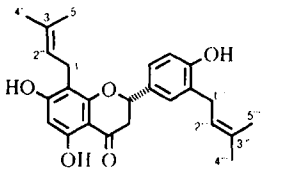
Methanolic extracts of yellow lupin roots were treated as described in the Experimental to yield, after column chromatography on silica gel, several fractions containing relatively non-polar constituents. After being concentrated and allowed to stand at -20°, one of the fractions (Fr-I) deposited a pale yellow solid (8.41 g from 13 kg fr. roots) which was subsequently identified as lonchocarpol A (6,8-diprenylnaringenin, **1**). The large quantity of **1** occurring in yellow lupin prompted us to carry out a detailed investigation of the less polar root components. As a result, we have now identified a second diprenylflavanone, various other prenylated flavanones with additional dihydrofurano or 2-hydroxy-3-methyl-3-butenyl substitution, and several minor compounds including an isoflavone and a chromone (both having diprenylation), and a new coumaronochromone.

Preparation of 6-, 8- and 3'-prenylnaringenins and their spectroscopic properties

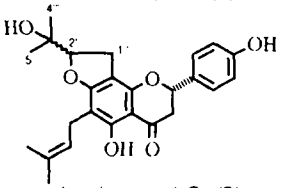
As a preliminary examination (UV, MS, Shinoda test) had shown that the major flavonoids in the less polar fractions of yellow lupin extracts were prenylflavanones, or closely related compounds, we chemically prepared three monoprenylnaringenins from (2*S*)-naringenin for use as model compounds in the identification process.



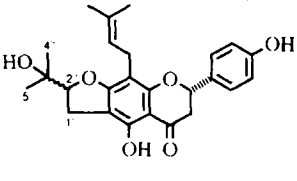
lonchocarpol A (1)



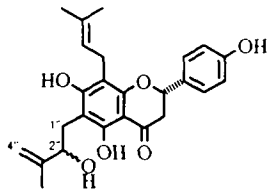
euchrestaflavanone A (2)



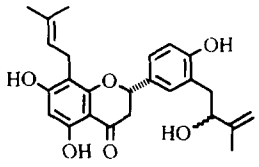
lonchocarpol C_1 (3)
lonchocarpol C_2 (4)



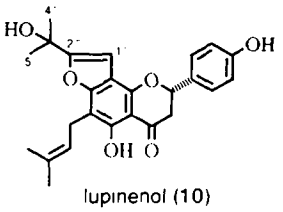
lonchocarpol D_1 (5)
lonchocarpol D_2 (6)



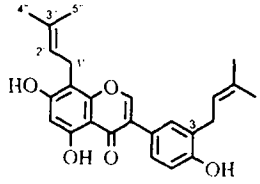
lupiniol A_1 (7)
lupiniol A_2 (8)



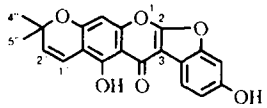
lupiniol B (9)



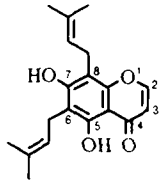
lupinenol (10)



isolupalbigenin (11)



lupinalbin H (12)



lupichromone (13)

Thus, authentic 6-, 8- and 3'-prenylnaringenins (**14**–**16**) were obtained as a mixture by non-selective prenylation of naringenin, the individual compounds then being separated and purified using silica gel column and thin layer chromatography [11, 12].

^{1}H NMR detection of the protons in a 1,3,4-trisubstituted B-ring [13], together with the intense MS ion at *m/z* 153 (91%) attributable to an RDA fragment from the dihydroxylated A-ring [14], confirmed that **16** was 3'-prenylnaringenin. The products corresponding to 6- and 8-prenylnaringenins were distinguished using a combination of UV and ^{1}H NMR spectroscopy. Tsukayama *et al.* [15] have recently reported that 8-substituted 5-hydroxy isoflavones (angular position) chelated more easily with $AlCl_3$ than do compounds substituted at C-6 (linear position). Furthermore, we have previously shown that when directly compared in acetone-d_6, 8-substituted (e.g. prenyl) isoflavones and flavones give δ values for 5-OH which are consistently smaller than those of the 6-substituted analogues [16]. Thus, **15** (8-prenylnaringenin [17]) exhibited a bathochromic UV shift after addition of one drop of 5% $AlCl_3$ solution, with the 5-OH signal appearing at δ12.15. In contrast, **14** (6-prenylnaringenin [18]) gave a bathochromic shift only after addition of seven drops of $AlCl_3$ solution (unchanged after one drop), whilst the 5-OH resonated at δ12.47. Complete ^{1}H NMR data for 6-, 8- and 3'-prenylnaringenins (**14**–**16**) in acetone-d_6 are shown in Table 1. It should be noted that the chemical shift values for the prenyl protons of these three naringenin derivatives show significant differences: 6-prenyl, δ3.25 (CH_2), 5.24 (CH), 1.75 (Me) and 1.64 (Me); 8-prenyl, δ3.22 (CH_2), 5.19 (CH) and 1.60 (2 × Me); 3'-prenyl, δ3.36 (CH_2), 5.36 (CH) and 1.72 (2 × Me).

Diprenylated flavanones from yellow lupin

The major compound (**1**, $C_{25}H_{28}O_5$) from yellow lupin roots was easily recognized as a flavanone from its positive Shinoda test [19], and the detection of ^{1}H NMR signals characteristic of the C-2-H (δ5.43 *dd*) and C-3-H_2 (δ3.14 *dd* and 2.77 *dd*) protons of a flavanone C-ring (Table 1) [13]. Two sets of prenyl signals confirmed that the compound was also diprenylated (Table 1). The presence of aromatic protons attributable to a symmetric 1,4-disubstituted benzene part structure (H-2′ and H-6′ at δ7.40 *d*; H-3′ and H-5′ at δ6.90 *d*), and UV bathochromic shifts with $AlCl_3$ (slow, 5-OH) and NaOAc (7-OH) were indicative of a naringenin-type skeleton [20]. The two prenyl groups must therefore be attached at C-6 and C-8 allowing **1** to be identified as lonchocarpol A, previously isolated from the leaves of *Lonchocarpus minimiflorus* [8].

^{1}H NMR data for ring B of the second diprenylated flavanone (**2**, $C_{25}H_{28}O_5$) were in good agreement with those given by the model compound 3′-prenylnaringenin (**16**) [Table 1]. Similarly, the A-ring proton signals (including the prenyl group) were closer to those of 8-prenylnaringenin (**15**) than to 6-prenylnaringenin (**14**). As **2** is an isomer of **1**, and since OH groups can be placed at C-5 and C-7 from UV shift measurements, the third OH must be located at C-4′. Compound **2** is thus 8,3′-diprenylnaringenin (euchrestaflavanone A) which was first isolated from *Euchresta japonica* [7].

Dihydrofuranoflavanones from yellow lupin

We previously reported [10, 21] that isoflavonoids possessing a 2-(1-hydroxy-1-methylethyl)-2,3-dihydrofurano side attachment afford characteristic mass fragments at $[M-59]^+$ and/or *m/z* 59 $[(Me)_2C{=}OH]^+$, and give a ^{1}H NMR oxymethine signal at *ca* δ4.8. These features were also associated with two isomeric compounds ($C_{25}H_{28}O_6$) obtained from yellow lupin roots. ^{1}H NMR data revealed that these compounds had identical B-(4-hydroxyphenyl) and C-(flavanone) rings, that both were monoprenylated, and that each was hydroxylated at C-5 (*ca* δ12.7 and 12.3). From the above results it can be inferred that the compounds are regioisomers, one of which has a prenyl group at C-6 and the dihydrofurano substituent cyclized C-8→7[O] with the other having the reverse arrangement (prenyl at C-8, and dihydrofurano group cyclized C-6→7[O]) as shown in **3/4** and **5/6**, respectively. In fact, the lupin compounds had spectroscopic features (MS, ^{1}H NMR) almost identical with lonchocarpols C and D which have been isolated, together with lonchocarpol A (**1**), from *Lonchocarpus minimiflorus* [8].

However, from a ^{1}H NMR comparison with authentic 6-prenylnaringenin **14** (6-prenyl: δ3.25, CH_2; δ5.24, CH; δ1.64 and 1.75, 2 × Me) and 8-prenylnaringenin **15** (8-prenyl: δ3.22, CH_2; δ5.19, CH; δ1.60, 2 × Me), it was clear that the chemical shift values reported for the prenyl groups in lonchocarpol C (δ3.19, CH_2; δ5.17, CH; δ1.66 and 1.64, 2 × Me) and lonchocarpol D (δ3.24, CH_2; δ5.24, CH; δ1.69 and 1.77, 2 × Me) were inconsistent with the structures proposed in ref. [8] (6-prenyl in lonchocarpol C; 8-prenyl in lonchocarpol D). Moreover, as we reported previously [16], dihydrofurano cyclization of the 8-prenyl group (8→7[O]) in isoflavones shifts the 5-OH signal to lower field, whereas 6-prenyl cyclization (6→7[O]) shifts the 5-OH to higher field. In lonchocarpol A (uncyclized) the 5-OH is reported to occur at δ12.28 [8] with the corresponding signal in lonchocarpol C (cyclized, 8→7[O]) at δ12.10, and in lonchocarpol D (cyclized, 6→7[O]) at δ12.47, exactly the opposite of what would be expected based on our study of model isoflavones [16]. To avoid confusion, we have retained the names lonchocarpols C and D for the *Lupinus* compounds, with the structures shown in ref. [8] (C, 6-prenyl; D, 8-prenyl). However, the spectroscopic properties (^{1}H NMR) formerly attributed to lonchocarpols C and D [8] must be interchanged. That lonchocarpol C is prenylated at C-6 is confirmed by the significantly slower UV bathochromic shift given with $AlCl_3$ when compared with lonchocarpol D (see earlier discussion on shift data). Similarly, ^{1}H NMR data for the prenyl protons of lonchocarpol D are in close agreement with those of the 8-prenyl-6→7[O]-pyranoflavanone lupinifolin (δ3.21, CH_2; δ5.15, CH; δ1.65, 2 × Me) [22].

Although lonchocarpol C and lonchocarpol D were initially isolated from lupin roots as single compounds, detailed ^{1}H NMR analyses (500 MHz) indicated that both actually consisted of two diastereoisomers resulting from epimerization of the asymmetric centre in the cyclic side attachment. Separation of the two sets of diastereoisomers (lonchocarpols C_1 and C_2, **3/4** and lonchocarpols D_1 and D_2, **5/6**) was achieved by HPLC as described in the Experimental.

2-Hydroxy-3-methyl-3-butenyl substituted flavanones from yellow lupin

Lupiniols A and B both contained a prenyl group characterized by ^{1}H NMR chemical shift values (δ3.23–3.24, CH_2; δ5.19–5.21, CH; δ1.60–1.61, 2 × Me) similar to those given by the prenyl substituent of 8-prenylnaringenin (Tables 1 and 2). In addition, the lupiniols gave ^{1}H NMR signals attributable to a 2-hydroxy-3-methyl-3-butenyl side chain (benzyl methylene, Ha at *ca* δ2.8 *dd* or *m*, and Hb at δ2.9–3.05 *dd* or *m*; hydroxylated methine, δ4.35–4.42 *brd*-like; vinyl methylene, Ha at δ4.96–5.02 *br s*, and Hb at δ4.78–4.82 *br s*; allyl methyl, δ1.80–1.83 *s*). This type of side chain has previously been found in dolichins A and B, two diastereoisomeric pterocarpans from bacteria-infected leaves of *Dolichos biflorus* [23], and in three isoflavones (lupinisols A–C), and one coumaronochromone (lupinalbin G) from white lupin [24, 25].

The ^{1}H NMR data also indicated that ring B of lupiniol A was a symmetric 1,4-disubstituted benzene, whilst that of lupiniol B was an asymmetric 1,3,4-trisubstituted one. Assuming hydroxylation at C-4′ (as in the other yellow lupin flavanones), and dihydroxylation of ring A at C-5 (from ^{1}H NMR and UV shift measurements) and C-7 (UV shift), the hydroxylated side chain

Table 1. ^{1}H NMR data (δ values) for prenylated flavanones*

	6-Prenylnaringenin	8-Prenylnaringenin	3′-Prenylnaringenin	Lonchocarpol A	Euchrestaflavanone A	Lupinenol (**10**)	
	14	**15**	**16**	**1**	**2**	in acetone-d_6	in benzene-d_6
2β	5.45 *dd* (12.8, 3.0)	5.45 *dd* (12.5, 3.3)	5.43 *dd* (12.8, 3.1)	5.43 *dd* (12.7, 3.0)	5.43 *dd* (12.5, 3.1)	5.51 *dd* (12.0, 3.4)	4.83 *dd* (12.6, 2.9)
3α	3.17 *dd* (17.2, 12.8)	3.15 *dd* (17.2, 12.5)	3.18 *dd* (17.2, 12.8)	3.14 *dd* (17.1, 12.7)	3.12 *dd* (17.0, 12.5)	3.26 *dd* (17.3, 12.0)	2.68 *dd* (17.1, 12.6)
3β	2.70 *dd* (17.2, 3.0)	2.76 *dd* (17.2, 3.3)	2.72 *dd* (17.2, 3.1)	2.77 *dd* (17.1, 3.0)	2.76 *dd* (17.0, 3.1)	2.87 *dd* (17.3, 3.4)	2.48 *dd* (17.1, 2.9)
5-OH	12.47 *s*	12.15 *s*	12.19 *s*	12.48 *s*	12.15 *s*	12.75 *s*	13.22 *s*
6	–	6.03 *s*	5.949 *s*	–	6.02 *s*	—	—
8	6.03 *s*	–	5.947 *s*	–	–	—	–
2′	7.39 *d*-like (8.6)	7.42 *d* (8.8)	7.29 *d* (2.2)	7.40 *d* (8.5)	7.32 *d* (2.2)	7.44 *br d* (8.5)	7.07 *d* (8.4)
6′	[2H]	[2H]	7.21 *dd* (8.2, 2.2)	[2H]	7.22 *dd* (8.2, 2.2)	[2H]	[2H]
3′	6.90 *d*-like (8.6)	6.91 *d* (8.8)	—	6.90 *d* (8.5)	—	6.91 *br d* (8.5)	6.69 *d* (8.4)
5′	[2H]	[2H]	6.89 *d* (8.2)	[2H]	6.89 *d* (8.2)	[2H]	[2H]
Ha-1″	3.25 *br d* (7.3)	3.22 *br d* (7.3)	3.36 *br d* (7.2)	3.33 *br d* (6.9)	3.22 *br d* (7.3)	3.45 *br d* (7.6)	3.59 *dd* (14.5, 7.5)
Hb-1″	[2H]	[2H]	[2H]	[2H]	[2H]	[2H]	3.60 *dd* (14.5, 7.2)
2″	5.24 *br t* (7.3)	5.19 *br t* (7.3)	5.36 *dt*-like (7.2)	5.18 *br t* (*ca* 7.0)	5.20 *br t* (7.3)	5.29 *br t* (7.6)	5.51 *br t* (*ca* 7.3)
H_3-4″	1.75 *br s*	1.60 *br s*	1.72 *s*	1.76 *s*	1.61 *s*	1.72 *s*	1.73 *s*
H_3-5″	1.64 *d* (1.0)	[6H]	[6H]	1.65 *d* (0.7)	[6H]	1.61 *s*	1.65 *s*
Ha-1‴	—	–		3.29 *br d* (7.1)	3.36 *br d* (7.2)	6.66 *s*	6.71 *s*
Hb-1‴	–	—	–	[2H]	[2H]	[1H]	[1H]
2‴	–	—	–	5.15 *br t* (*ca* 7.0)	5.37 *br t* (7.2)	—	—
H_3-4‴		–	–	1.62 *d* (0.7)	1.73 *s*	1.61 *s*	1.44 *s*
H_3-5‴	–		—	1.60 *s*	[6H]	[6H]	[6H]
7-OH	9.50 *br s*	9.53*br s*	*ca* 9.6 *br s*	*ca* 8.3 *br s*	9.53 *br s*	—	—
4′-OH	8.51 *br s*	8.46 *br s*	8.45 *br s*	*ca* 8.3 *br s*	8.42 *br s*	8.52 *s*	5.62 *br s*
alcoholic OH	–	—	–	–		4.44 *s*	2.06 *br s*

*Values were determined in acetone-d_6 at 100 MHz (**10**), 270 MHz (**14**–**16**) and 500 MHz (**1**, **2**), or in benzene-d_6 at 500 MHz (**10**). J in Hz.

Table. 2. ^{1}H NMR data (δ values) for diastereoisomeric flavanones*

	Lonchocarpol		Lonchocarpol		Lupiniol		Lupiniol B† (9)
	C_1 (3)	C_2 (4)	D_1 (5)	D_2 (6)	A_1 (7)	A_2 (8)	(A mixture of B_1 and B_2)
2β	5.47 *dd* (12.7, 3.0)	5.48 *dd* (12.6, 3.0)	5.45 *dd* (12.7, 3.1)	5.44 *dd* (12.8, 3.0)	5.42 *dd* (12.8, 3.0)	5.45 *dd* (12.5, 3.1)	5.42 *dd* (*ca* 13.3)
3α	3.16 *dd* (17.0, 12.7)	3.18 *dd* (17.2, 12.6)	3.13 *dd* (17.0, 12.7)	3.13 *dd* (17.1, 12.8)	3.14 *dd* (17.0, 12.8)	3.14 *dd* (17.1, 12.5)	3.13 *dd* (17.0, 12.8), 3.12 *dd* (17.0, 12.8)
3β	2.75 *dd* (17.0, 3.0)	2.76 *dd* (17.2, 3.0)	2.76 *dd* (17.0, 3.1)	2.75 *dd* (17.1, 3.0)	2.75 *dd* (17.0, 3.0)	2.78 *dd* (17.1, 3.1)	2.74 *dd* (17.0, 3.3), 2.74 *dd* (17.0, 3.3)
5-OH	12.70 *s*	12.71 *s*	12.26 *s*	12.28 *s*	12.68 *s*	12.65 *s*	12.16 *s*
2′	7.40 *d* (8.5)	7.40 *d* (8.5)	7.40 *d* (8.6)	7.41 *d* (8.6)	7.41 *d* (8.6)	7.41 *d* (8.5)	7.33 *d* (*ca* 2)
6′	[2H]	[2H]	[2H]	[2H]	[2H]	[2H]	7.28 *dd* (8.2, 2.0)
3′	6.90 *d* (8.5)	6.90 *d* (8.5)	6.90 *d* (8.6)	6.91 *d* (8.6)	6.91 *d* (8.6)	6.91 *d* (8.5)	–
5′	[2H]	[2H]	[2H]	[2H]	[2H]	[2H]	6.88 *d* (8.2)
Ha-1″	3.21 *br d* (7.4)	3.21 *br d* (7.4)	3.06 *dd* (15.4, 9.5)	3.05 *dd* (15.3, 9.6)	2.77 *dd* (14.8, 8.5)	2.83 *dd* (15.1, 7.0)	3.23 *br d* (7.2)
Hb-1″	[2H]	[2H]	3.11 *dd* (15.4, 7.6)	3.11 *dd* (15.3, 7.4)	3.05 *dd* (14.9, 2.1)	3.01 *dd* (15.1, 2.1)	[2H]
2″	5.25 *br t* (7.4)	5.25 *br t* (7.4)	4.78 *dd* (9.5, 7.6)	4.77 *dd* (9.6, 7.4)	4.35 *br d*-like (*ca* 8)	4.36 *br d*-like (*ca* 7)	5.21 *br t* (7.2)
$H_3(H_2)$-4″	1.74 *s*	1.75 *s*	1.25 *s*	1.249 *s*	4.82 *br s* [1H]	4.82 *br s* [1H]	1.61 *s*
					5.02 *br s* [1H]	5.01 *d* (0.8) [1H]	[6H]
H_3-5″	1.64 *s*	1.65 *s*	1.24 *s*	1.246 *s*	1.83 *s*	1.83 *s*	
Ha-1‴	3.02 *dd* (15.3, 9.4)	3.02 *dd* (15.4, 9.6)	3.17 *br d* (7.4)	3.17 *m*	3.24 *br d* (7.3)	3.24 *br d* (*ca* 7)	*ca* 2.9 *m*
Hb-1‴	3.08 *dd* (15.3, 7.8)	3.09 *dd* (15.4, 7.3)	[2H]	[2H]	[2H]	[2H]	[2H]
2‴	4.75 *dd* (9.4, 7.8)	4.77 *dd* (9.6, 7.3)	5.19 *br t* (7.4)	5.20 *br t* (7.4)	5.19 *t*-like (7.2)	5.21 *t*-like (7.2)	4.42 *br d*-like (8.2)
$H_3(H_2)$-4‴	1.25 *s*	1.25 *s*	1.62 *s*	1.62 *s*	1.60 *s*	1.61 *s*	4.78 *br s* [1H] 4.96 *br s* [1H]
H_3-5‴	1.22 *s*	1.23 *s*	[6H]	[6H]	[6H]	1.61 *s*	1.80 *s*
7-OH	—	—	—	—	not observed	10.3 *br s*	not observed
4′-OH	8.54 *br s*	8.53 *br s*	8.58 *br s*	8.56 *br s*	8.44 *br s*	8.53 *br s*	not observed
alcoholic OH	3.71 *br s*	3.73 *br s*	3.74 *br s*	3.74 *br s*	not observed	not observed	not observed

*δ Values were determined in acetone-d_6 at 500 MHz. J in Hz.

†The signal attributable to H-6 was observed at δ6.05 *s*.

can be placed at C-6 in lupiniol A and at C-3′ in lupiniol B, since C-8 in both compounds is occupied by the prenyl group.

As with the two diastereoisomers comprising lonchocarpols C (**3**/**4**) and D (**5**/**6**), it was possible to separate the components of lupiniol A by HPLC using a reversed phase column to give lupiniols A_1 (**7**) and A_2 (**8**). ^{1}H NMR data for the separated compounds are given in Table 2. Lupiniol B (**9**) was isolated from yellow lupin roots only as a very minor constituent. Although **9** would also be expected to exist as a diastereoisomeric mixture (lupiniols B_1 and B_2), it was not separated into its individual components.

Furanoflavanone from yellow lupin

^{1}H NMR analysis indicated that the B- and C-rings of the final yellow lupin flavanone (lupinenol $C_{25}H_{26}O_5$) were identical with those of lonchocarpols A, C_1/C_2 and D_1/D_2, and lupiniols A_1 and A_2 (Tables 1 and 2). In addition, lupinenol was found to contain a 5-OH (δ12.75 *s*; slow UV shift with $AlCl_3$) and a 6-prenyl substituent (cf. chemical shift data for prenyl protons with those of the same substituent in 6-prenylnaringenin and lonchocarpols C_1/C_2, Tables 1 and 2).

Based on data previously reported for glyceofuran from *Glycine max* [26], and the *Piscidia* isoflavones piscerisoflavone E and erythbigeneol [9], the remaining ^{1}H NMR signals were attributed to the presence of a 2-(1-hydroxy-1-methylethyl)furano side attachment (δ6.66 *s*, olefinic β-proton of an α-substituted furan ring; δ1.66 *s*, 6H, two methyls on a hydroxylated carbon; δ 4.44 *br s*, aliphatic OH). As the UV (MeOH) spectrum of lupinenol was unaffected by NaOAc (C-7-OH derivatized), the furano group was placed (8→7[O]) as shown in structure **10**. Supporting evidence for the β-proton (H-1‴) of an α-substituted benzofuran part structure was provided by long range C–H correlations (via 2 or 3 bonds) to C-7, C-8 and C-2‴ using HMBC spectroscopy. 6-Prenylation of **10** was also confirmed by HMBC correlations of the prenyl methylene protons (H_2-1″) to C-5, C-6 and C-7 (see Experimental for correlation data).

Isoflavone, coumaronochromone and chromone from yellow lupin

Isolupalbigenin (**11**) was initially identified as an isoflavone from the sharp, low-field ^{1}H NMR singlet (δ8.23) characteristic of the isoflavone H-2. The mass spectrum gave a molecular ion at *m/z* 406 ($C_{25}H_{26}O_5$) and prominent RDA fragments at *m/z* 165 (20%; A-ring with dihydroxylation and a CH_2 remnant from a prenyl group) and *m/z* 131 (9%; B-ring with OH and CH_2 remnant).

From a ^{1}H NMR comparison of isolupalbigenin with the known lupin isoflavone lupalbigenin (6,3′-diprenylgenistein) [1], it was clear that both compounds had identical B-rings (4′-OH, 3′-prenyl). However, whereas the A-ring aromatic proton of lupalbigenin (H-8) appeared at δ6.51 [1], that of isolupalbigenin occurred at δ6.36. This suggests that the prenyl side chain of ring A is located at C-8, rather than C-6, giving structure **11** (8,3-diprenylgenistein) for isolupalbigenin. Confirmation of the A-ring substitution pattern was provided by a ^{1}H NMR comparison with lupiwighteone (8-prenylgenistein) which gave signals for 5-OH (δ13.01), H-6 (6.37) and the prenyl protons almost identical with those of isolupalbigenin [3] (cf. 5-OH of 6-prenylgenistein at δ13.32 [16]). Although isolupalbigenin is reported here for the first time, its 2′-hydroxy derivative is already known to occur in white lupin roots [24].

The second lupin isoflavonoid (lupinalbin H, $C_{20}H_{14}O_6$) was identified as a coumaronochromone from its UV maxima (MeOH) at 274 nm (rel. int. 100%) and 338 nm (rel. int. 25%), fluorescence under $UV_{365\ nm}$ light (dull orange), and the absence of a low-field ^{1}H NMR signal characteristic of the isoflavone H-2 [25, 27]. A prominent mass fragment at *m/z* 335 ($[M-Me]^+$, 100%) suggested the presence of a 2,2-dimethylpyrano side structure. This was confirmed from the ^{1}H NMR spectrum which showed two equivalent methyls (δ1.49 *s*, 6H) and two olefinic protons (δ5.80 *d* and 6.71 *d*) with $J = 10.2$ Hz [1].

Various coumaronochromones are known to occur in white and yellow lupins [3, 24, 25, 27], and from a ^{1}H NMR comparison with these compounds, it was clear that the B- and C-rings of lupinalbin H (see data in Experimental) were identical with those of lupinalbin A (H-3′, δ7.13 *d*, $J = 2.2$ Hz; H-5′, δ7.01 *dd*, $J = 8.3$ and 2.2 Hz; H-6′, δ7.81 *d*, $J = 8.3$ Hz) [27]. As lupinalbin H has a 5-OH (δ13.39 *s*), only the position (angular or linear) of the pyrano group remained to be established.

The chemical shift value for the single A-ring proton (δ6.55) closely resembled that of lupinalbin C (H-8, δ6.57), a linear-type dihydrofurano-coumaronochromone [27]. In contrast, H-6 (δ6.42) of lupilutin, a coumaronochromone with a 2,3-dihydro-3-hydroxyprenyl side chain at C-8, appeared at quite a different position [3]. The effect on H-6 of coumaronochromone ring formation has been calculated to be 0.01 0.05 ppm [27] when parvisoflavone A (angular-type pyranoisoflavone, H-6 at δ6.22 [3]) is transformed to the corresponding coumaronochromone. In the latter compound, H-6 would be expected to resonate around δ6.23–6.27, a position rather different from that of the A-ring proton in lupinalbin H. Thus, lupinalbin H (**12**) is the coumaronochromone derivative of the linear-type pyranoisoflavone parvisoflavone B which also occurs in white and yellow lupin roots [3, 10].

Finally, the ^{1}H NMR data of **13** (lupichromone, $C_{19}H_{22}O_4$) showed the presence of two prenyl groups and two hydroxyl (phenolic) protons at δ8.30 and 13.07 (H-bonded). UV shifts with NaOAc and $AlCl_3$ confirmed hydroxylation at the positions corresponding, respectively, to 7-OH and 5-OH of flavonoids. The only other ^{1}H NMR signals (δ6.23 *d* and 8.15 *d*, both $J = 5.9$ Hz) were assigned to two olefinic protons. The above data can only be satisfactorily explained if **13** is 5,7-dihydroxy-6,8-di-(3,3-dimethylallyl)chromone, a compound which might arise from lonchocarpol A (**1**) by a post-mortem

degradative process [28, 29]. The chemical shift values (olefinic protons) and the coupling constant of **13**, and its UV spectrum in MeOH, closely resembled those reported by Fukushima *et al.* [30] for leptorumol (5,7-dihydroxy-6,8-dimethylchromone).

Fungitoxic activities of some prenylated flavanones

The three semi-synthetic prenylated (6-, 8-, 3'-) naringenins (**14–16**), and the various yellow lupin flavanones (**1–8, 10**), except for lupiniol B (**9**), were compared for fungitoxicity against *Cladosporium herbarum* using the TLC plate bioassay method [31, 32]. As shown in Table 3, the two diprenylated flavanones, lonchocarpol A (**1**) and euchrestaflavanone A (**2**), both lupiniols (A_1, **7**; A_2, **8**) and lupinenol (**10**) were either inactive, or only very weakly antifungal, even at the maximum level (200 μg) applied to TLC plates.

It is not clear why the diprenylflavanones (**1** and **2**) exhibit no significant fungitoxicity, whereas 8-monoprenyl and 3'-monoprenylnaringenin (**15, 16**) are both quite strongly antifungal. That the position of the prenyl group is important, at least in the monoprenylnaringenins, is clear from Table 3 since 6-prenylnaringenin (**14**) is inactive, unlike its 8- and 3'-prenyl analogues. Amongst the isoflavones a somewhat different situation has been observed with 6-prenyl (e.g. luteone and wighteone) and 3'-prenyl (licoisoflavone A) compounds being considerably more fungitoxic than the 8-prenyl analogue (2,3-dehydrokievitone) [10 and unpublished data]. Transformation of these isoflavones to the dihydrofurano or dihydropyrano derivatives has been found to greatly reduce or eliminate their fungitoxic effects [33, 34].

Several flavanones with cyclic (furano/dihydrofurano) or acyclic (2-hydroxy-3-methyl-3-butenyl) side structures were either inactive (**7, 8**) or only weakly fungitoxic (**3, 4, 10**). However, within the dihydrofurano group of compounds there is a distinct difference between lonchocarpol D_1 (**5**, strongly antifungal) and its diastereoisomer lonchocarpol D_2 (**6**, weakly antifungal). The two other dihydrofuranoflavanones, lonchocarpols C_1 (**3**) and C_2 (**4**) both exhibited slight fungitoxicity, with diastereoisomer C_1 (**3**) being marginally more active than C_2 (**4**). It is interesting that lonchocarpols C_1, C_2, D_1 (the most active compound tested) and D_2 all possess varying degrees of antifungal activity, whereas the corresponding diprenylflavanone, lonchocarpol A (**1**), is inactive.

EXPERIMENTAL

General. Analyt. and prep. TLC sepns, and detection of lupin compounds on TLC plates, was as described in our earlier papers [10, 24]. Solvents used for chromatography are abbreviated as follows: A = acetone; Am = conc. NH_3 water; B = benzene; C = $CHCl_3$; E = EtOAc; F = formic acid; H = *n*-hexane; M = MeOH; MeCN = acetonitrile; T = toluene; THF = tetrahydrofuran. Instrumental analyses were carried out using equipment and conditions reported elsewhere [9, 24]. The antifungal activity

Table 3. Fungitoxicity of lupin flavanones and synthetic monoprenylnaringenins*

Compound	Growth of *Cladosporium herbarum*† as affected by the amount of flavanone (μg) applied to each 14 mm diameter test zone				
	13	25	50	100	200
Monoprenylnaringenins					
6-Prenylnaringenin (**14**)	—	—	—	—	—
8-Prenylnaringenin (**15**)	+	+ +	+ + +	+ + +	+ + +
3'-Prenylnaringenin (**16**)	–	+	+ +	+ + +	+ + +
Diprenylnaringenins					
Lonchocarpol A (**1**)	—	–	–	—	– –
Euchrestaflavanone A (**2**)	—	–	–	—	+
Dihydrofuranoflavanones					
Lonchocarpol C_1 (**3**)	–	—	+	+	+
Lonchocarpol C_2 (**4**)	– –	—	–	+	+
Lonchocarpol D_1 (**5**)	+ +	+ + +	+ + +	+ + +	+ + +
Lonchocarpol D_2 (**6**)	·	—	+	+ +	+ + +
Other compounds					
Lupiniol A_1 (**7**)	—	– –	–	—	—
Lupiniol A_2 (**8**)	–	—	—	–	–
Lupinenol (**10**)	– –	—	—	– –	+

*Each test compound was applied in acetone to give a 14 mm diameter zone on a silica gel 60 TLC plate (Merck, F254; layer thickness; 0.25 mm). The plates were then sprayed with a spore suspension of *Cladosporium herbarum* and incubated under warm (25°) moist conditions until fungal growth was apparent (two–three days).

†+ + +: Complete inhibition of fungal growth, + + and +: decreasing level of inhibition, —: no inhibition of fungal growth.

of lupin flavanones and synthetic monoprenylnaringenins was determined by TLC plate bioassays against growth of *Cladosporium herbarum* using a modification [32] of the method described by Homans and Fuchs [31].

Plant material and isolation of constituents. Roots of yellow lupin (*Lupinus luteus* cv Topaz, fr. wt 13 kg), collected immediately after flowering, were extracted (×2) with 90% MeOH (*ca* 60 l). The combined extracts were concd to *ca* 1 l and diluted with acetone (4 l) followed by concn of the resulting liquid phase. H_2O (1 l) was then added, and the extracts were shaken with EtOAc (×3, 3 l). The EtOAc layers were collected, washed with 5% aq. $NaHCO_3$ and satd aq. NaCl, concd to *ca* 200 ml, and applied to a column of silica gel (200 g). Relatively less-polar root flavonoids/isoflavonoids and other constituents (lipids, etc.) were washed out of the column with EtOAc (1.5 l). After reducing the eluate to near dryness, the residue was taken up to 90% MeOH (800 ml) and shaken with hexane (×3, 500 ml each) to remove fatty material. The hexane layer was discarded and the aq. MeOH layer was concd (55 g) and applied to a silica gel column (550 g) settled in B. Elution with mixts of E in B (E/B) gave the following frs: Fr.-I (10% E/B, 1000 ml, followed by 15% E/B, 500 ml), Fr.-II (20% E/B, 500 ml) and Fr.-III (20% E/B, 750 ml).

After standing at −20° (freezer), concd Fr.-I deposited 1050 mg of lonchocarpol A (**1**). The amount of **1** in crude root extracts was estimated by UV spectroscopy to be *ca* 8.41 g. The mother liquor (13.55 g), containing more **1** and various other constituents, was rechromatographed over silica gel (180 g) to afford frs Fr.-I-1 (solvent C, 1000 ml), Fr.-I-2 to 4 (1% M in C, 200 ml each), Fr.-I-5 to 7 (3% M in C, 200 ml each) and Fr.-I-8 to 10 (6% M in C, 200 ml each). The major constituents in Fr.-I-1 and 2 were found by silica gel TLC to be mixts of the ferulates of higher alcohols (R_f 0.45 in HE = 4:1). A minor compound in Fr.-I-2 which ran above the ferulates (R_f 0.52 in HE = 4:1) was purified by prep. TLC in HE (4:1) to give fine needles of lupichromone **13** (8.4 mg). Fr.-I-3 yielded more lonchocarpol A (2.015 g). Frs Fr.-I-4 and 5 were combined (*ca* 7 g) and rechromatographed over silica gel (130 g; 2%M in C as eluting solvent) to give the following FFr.-I frs: 1 (198 ml), 2 (52 ml), 3 (39 ml), 4 (13 ml), 5 (39 ml), 6 (26 ml), 7 (39 ml), and 8 (39 ml).

The major compound in FFr.-I-4 was euchrestaflavanone A (**2**, 170 mg) which was purified by prep. TLC in CM (50:1, R_f 0.47). Upon prep. TLC in TAF (40:20:1), FFr.-I-6 afforded 3 main bands which were eluted and further purified by TLC in HAE (6:1:1) to give lupinalbin H (**12**, 3.5 mg from upper band R_f 0.53), lupiniol A (**7**+**8**, 58.4 mg from the middle band, R_f 0.32) and derrone (75.5 mg from lower band). Lupiniol A obtained as above was contaminated with a minute amount of lupiniol B (**9**). Prep. TLC of FFr.-I-7 in CAAm (70:60:1) gave 3 major bands. The top band yielded 5.4 mg of isolupalbigenin (**11**) after further TLC in HE (3:1, R_f 0.42). Lonchocarpol D ($D_1 + D_2$, **5**/**6**, 25.9 mg) was isolated from the middle band, whilst the main component of the lower band was found to be lonchocarpol C ($C_1 + C_2$, **3**/**4**, 7.5 mg). Lonchocarpol C was also obtained from FFr.-I-8 (7.6 mg).

Investigation of Fr.-II (*ca* 3 g) gave, upon concn, ppts (269 mg) which were divided into the isoflavonoids lupinisoflavone A (207 mg) [10] and lupinalbin B (29.1 mg) [27] by recrystallization.

Fr. Fr.-III (9.10 g) was concd, and applied to a column of silica gel (230 g) which was eluted with 10% E in B (500 ml), 15% E in B (450 ml), and 20% E in B (100 and 200 ml). The final 200 ml fr. contained 4 main compounds which were purified by prep. TLC in CAAm (70:60:1) to give lupinenol **10** (24 mg, R_f 0.64), topazolin (40 mg, R_f 0.30) [6], 2,3-dehydrokievitone (64 mg, R_f 0.23) [3] and luteone (137 mg, R_f 0.21) [2].

HPLC separation of diastereoisomeric dihydrofurano- and 2-hydroxy-3-methyl-3-butenyl-flavanones. ^{1}H NMR analysis revealed that lonchocarpols C and D, and lupiniol A, each consisted of 2 diastereoisomers which were sepd from each other by HPLC using either a JASCO 88-PU or TRI ROTER-V chromatograph equipped with a UV detector (UVIDEC-100-V) set at 294 nm. R_ts were determined by analyt. HPLC using 4.6 × 250 mm column of Fine-Pak-Sil or Deverosil-ODS with solvent systems shown below and flow rate 1 ml min^{-1}, and details of column and running conditions were as follows.

*Lonchocarpols C_1 (**3**) and C_2 (**4**).* Column: Fine Pak-Sil (15 i.d. × 250 mm, JASCO). Solvent: CH_2Cl_2–MeOH (50:1, v/v, flow rate 5 ml min^{-1}). The prep. TLC fr. containing lonchocarpol C gave 2 peaks at R_t 9.4 min (lonchocarpol C_1, **3**) and R_t 10.2 min (lonchocarpol C_2, **4**). The assignment of absolute stereochemistry in the side attachment has still to be determined.

*Lonchocarpols D_1 (**5**) and D_2 (**6**).* Column: Inertsil-ODS (20 i.d. × 250 mm, GL Science). Solvent: MeOH–THF–H_2O (37:1:12, v/v), flow rate 5 ml min^{-1}. The 2 components of lonchocarpol D eluted at R_t 7.3 min (lonchocarpol D_1, **5**) and R_t 7.7 min (lonchocarpol D_2, **6**).

*Lupiniols A_1 (**7**) and A_2 (**8**), and lupiniol B (**9**).* Column: Inertsil-ODS (20 i.d. × 250 mm). Solvent: MeOH–MeCN–H_2O (65:11:24, v/v), flow rate 5 ml min^{-1}. The prep. TLC fr. containing lupiniol A afforded 2 major peaks at R_t 11.4 min (lupiniol A_1, **7**) and R_t 11.9 min (lupiniol A_2, **8**), and a very minor peak at R_t 13.1 min. MS and ^{1}H NMR data for this 3rd compound indicated that it was a regioisomer of lupiniol A with structure **9** (lupiniol B). Although lupiniol B would be expected to occur as a mixt. of 2 diastereoisomers, the very small quantities available for examination precluded attempts to separate these components.

Physicochemical properties of the yellow lupin compounds. Lonchocarpol A (**1**). Yellow solid, mp 90–92°, $UV_{365\ nm}$ fluorescence: dark red. Gibbs test: (−). MS *m/z* (rel. int.): 409 ([M+1]$^+$, 31), 408 ([M]$^+$, 100), 393 (16), 365 (13), 353 (37), 352 (16), 340 (10), 338 (11), 337 (44), 309 (21), 297 (28), 273 (31), 260 (24), 245 (29), 233 (52), 232 (34), 231 (28), 217 (53), 215 (13), 205 (12), 204 (21), 203 (13), 190 (12), 189 (74), 177 (48), 176 (11), 147 (11), 135 (11), 121 (14), 120 (18), 119 (11), 109 (16), 107 (18), 91 (19), 81 (12), 79 (10), 77 (12), 69 (14), 55 (15). UV λ_{max}^{MeOH} nm: 216sh, 297, 347; + NaOMe, 207, 243, 342; + $AlCl_3$ (shifted slowly), 203, 224, 320, 400; + NaOAc, 342 (+ H_3BO_3 regenerated the MeOH spectrum). $[\alpha]_D^{23}$ −20.4° (MeOH; *c* 0.334), CD

$[\theta]_{max}^{MeOH}$: $[\theta]_{400}$ 0, $[\theta]_{340}$ +5900, $[\theta]_{323}$ +4000, $[\theta]_{317}$ +5400, $[\theta]_{311}$ 0, $[\theta]_{293}$ −37600, $[\theta]_{263}$ 0. ^{13}C NMR δ_{TMS} (acetone-d_6, 125 MHz): 79.7 (C-2), 43.6 (C-3), 197.9 (C-4), 159.0 (C-5 or C-9), 108.7 (C-6), 162.3 (C-7), 107.9 (C-8), 160.2 (C-9 or C-5), 103.4 (C-10), 131.1 (C-1'), 128.9 (C-2' and C-6'), 116.1 (C-3' and C-5'), 158.6 (C-4'), 21.8 and 22.5 (C-1'' and C-1'''), 123.3 and 123.5 (C-2'' and C-2'''), 132.0 and 132.2 (C-3'' and C-3'''), 25.9 and 25.9 (C-4'' and C-4'''), 17.9 (C-5'' and C-5'''). ^{1}H NMR: Table 1.

Euchrestaflavanone A (**2**). Rods. mp 150–152°. $UV_{365\ nm}$ fluorescence: dark brown. Gibbs test: (−). MS *m/z* (rel. int.): 409 ([M + 1]$^+$, 24), 408 ([M]$^+$, 71), 393 (11), 365 (16), 353 (26), 233 (12), 221 (26), 220 (31), 219 (21), 205 (72), 192 (44), 191 (11), 188 (16), 177 (43), 175 (33), 166 (11), 165 (100), 133 (33), 123 (13), 77 (12), 69 (23). UV λ_{max}^{MeOH} nm: 228sh, 293, 339; + NaOMe, 248, 285, 333; + $AlCl_3$, 221, 316, 392; + NaOAc, 287, 297, 334 (+ H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR: Table 1.

Lonchocarpol C$_1$ (**3**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 425 ([M + 1]$^+$, 36), 424 ([M]$^+$, 100), 409 (34), 369 (50), 289 (57), 261 (20), 249 (27), 231 (23), 215 (23), 190 (25), 189 (39), 177 (26), 120 (22), 107 (14), 91 (17), 77 (10), 59 (51), 43 (24). UV λ_{max}^{MeOH} nm: 233sh, 301; + NaOMe, 240, 299; + $AlCl_3$ (shifted slowly), 304; + NaOAc, unchanged, ^{1}H NMR δ_{TMS} ($CDCl_3$, 500 MHz): 5.32 (1H, *dd*, *J* = 12.9, 2.9 Hz, H-2), 3.00 (1H, *dd*, *J* = 17.0, 12.9 Hz, Hα-3), 2.76 (1H, *dd*, *J* = 17.0, 2.9 Hz, Hβ-3), 12.47 (1H, *s*, 5-OH), 7.31 (2H, *d*, *J* = 8.5 Hz, H-2' and H-6'), 6.87 (2H, *d*, *J* = 8.5 Hz, H-3' and H-5'), 3.26 (1H, *dd*, *J* = 14.5, 7.0 Hz, Ha-1''), 3.21 (1H, *dd*, *J* = 14.5, 7.7 Hz, Hb-1''), 5.17 (1H, *t*-like, *J* = *ca* 7 Hz, H-2''), 1.77 (3H, *s*, H_3-4''), 1.69 (3H, *s*, H_3-5''), 3.06 (1H, *dd*, *J* = 15.2, 9.5 Hz, Ha-1'''), 2.90 (1H, *dd*, *J* = 15.2, 7.5 Hz, Hb-1'''), 5.24 (1H, *t*-like, *J* = *ca* 7 Hz, H-2'''), 1.33 and 1.19 (both 3H, two *s*, H_3-4''' and H_3-5'''). ^{1}H NMR data in acetone-d_6: Table 2.

Lonchocarpol C$_2$ (**4**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 425 ([M + 1]$^+$, 40), 424 ([M]$^+$, 100), 409 (31), 369 (39), 289 (54), 261 (20), 249 (25), 231 (25), 215 (17), 190 (20), 189 (39), 177 (24), 120 (21), 107 (14), 91 (17), 77 (10), 59 (40), 43 (23). UV λ_{max}^{MeOH} nm: 236sh, 302; + NaOMe, 237, 299; + $AlCl_3$ (shifted slowly), 308; + NaOAc, unchanged. ^{1}H NMR data in acetone-d_6: Table 2.

Lonchocarpol D$_1$ (**5**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 425 ([M + 1]$^+$, 33), 424 ([M]$^+$, 100), 409 (30), 369 (37), 353 (21), 289 (50), 249 (23), 231 (24), 189 (37), 177 (32), 120 (20), 91 (16), 77 (10), 59 (33), 43 (20). UV λ_{max}^{MeOH} nm: 235, 300; + NaOMe, 243, 298; + $AlCl_3$ (shifted slowly), 323, 367; + NaOAc, unchanged. ^{1}H NMR δ_{TMS} ($CDCl_3$, 500 MHz): 5.34 (1H, *dd*, *J* = 12.8, 3.0 Hz, H-2), 3.03 (1H, *dd*, *J* = 17.0, 12.8 Hz, Hα-3), 2.79 (1H, *dd*, *J* = 17.0, 3.0 Hz, Hβ-3), 12.12 (1H, *s*, 5-OH), 7.33 (2H, *d*, *J* = 8.4 Hz, H-2' and H-6'), 6.88 (2H, *d*, *J* = 8.4 Hz, H-3' and H-5'), 3.11 (1H, *dd*, *J* = 15.3, 9.6 Hz, Ha-1''), 3.03 (1H, *dd*, *J* = 15.3, 7.9 Hz, Hb-1''), 4.73 (1H, *dd*, *J* = 9.5, 7.9 Hz, H-2''), 1.32 and 1.21 (both 3H, two *s*, H_3-4'' and H_3-5''), 3.19 (2H, *br d*, *J* = 7.4 Hz, H_2-1'''), 5.17 (1H, *br t*, *J* = *ca* 7 Hz, H-2'''), 1.66 and 1.64 (both 3H, two *s*, H_3-4''' and H_3-5'''). ^{1}H NMR data in acetone-d_6: Table 2.

Lonchocarpol D$_2$ (**6**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 425 ([M + 1]$^+$, 35), 424 ([M]$^+$, 100), 409 (32), 369 (39), 353 (11), 289 (53), 249 (25), 231 (25), 190 (20), 189 (39), 177 (24), 120 (21), 91 (17), 77 (10), 59 (40), 43 (23). UV λ_{max}^{MeOH} nm: 233, 301; + NaOMe, 240, 300; + $AlCl_3$ (shifted slowly), 323, 390 *br.*; + NaOAc, unchanged. ^{1}H NMR data in acetone-d_6: Table 2.

Lupiniol A$_1$ (**7**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 425 ([M + 1]$^+$, 8), 424 ([M]$^+$, 3), 407 (20), 406 (9), 354 (22), 353 (81), 351 (16), 297 (59), 233 (57), 177 (100), 121 (23), 120 (20), 109 (24), 107 (16), 91 (21), 69 (17), 55 (21), 43 (21). UV λ_{max}^{MeOH} nm: 269, 297, 346; + NaOMe, 243, 337; + $AlCl_3$ (shifted slowly), 269, 310; + NaOAc, 299, 340 (+ H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR: Table 2.

Lupiniol A$_2$ (**8**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 425 ([M + 1]$^+$, 5), 424 ([M]$^+$, 3), 407 (15), 406 (9), 354 (22), 353 (76), 351 (10), 297 (60), 233 (56), 177 (100), 121 (21), 120 (16), 109 (19), 107 (13), 91 (16), 69 (15), 55 (20), 43 (17). UV λ_{max}^{MeOH} nm: 269, 297, 346; + NaOMe, 243, 337; + $AlCl_3$ (shifted slowly), 269, 310; + NaOAc, 299, 340 (+ H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR: Table 2.

Lupiniol B (**9**). MS *m/z* (rel. int.): 424 ([M]$^+$, 1), 408 (64), 353 (24), 221 (23), 220 (27), 205 (71), 192 (43), 177 (44), 175 (28), 165 (100), 133 (35). ^{1}H NMR: Table 2.

Lupinenol (**10**). Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark, and brownish yellow after fuming with NH_3. Gibbs test: brown→dull green→dull purple. Shinoda test: pink-purple. FD-MS *m/z* (rel. int.): 423 ([M + 1]$^+$, 29), 422 ([M]$^+$, 100). EI-MS *m/z* (rel. int.): 422 ([M]$^+$, 38), 405 (27), 404 ([M − H_2O]$^+$, 100), 349 (14), 302 (RDA fragment from ring A, 19), 287 (18), 284 (RDA fragment from dehydrated ring A, 50), 269 (30), 247 (13), 241 (36), 231 (30), 229 (49), 228 ([284 − C_4H_8]$^+$, 97), 91 (13). CD $[\theta]_{max}^{MeOH}$ nm: $[\theta]_{362}$ +3300, $[\theta]_{328}$ 0, $[\theta]_{291}$ −15600, $[\theta]_{253}$ 0, $[\theta]_{230}$ +15100. UV λ_{max}^{MeOH} nm: 251sh, 259, 270sh, 290, 368 *br.*; + NaOMe, 245, 262, 272, 285sh, 388 *br.*; + $AlCl_3$ (shifted slowly), 227, 270, 313, 362 *br.*; + NaOAc, unchanged. ^{1}H NMR in acetone-d_6: Table 1. ^{13}C NMR δ_{TMS} (benzene-d_6, 125 MHz): 79.2 (C-2), 43.6 (C-3), 199.1 (C-4), 155.9 (C-5), 105.4 (C-6), 160.6 (C-7), 111.8 (C-8), 156.1 (C-9), 105.1 (C-10), 131.0 (C-1'), 128.0 (C-2' and C-6'), 115.9 (C-3' and C-5'), 157.0 (C-4'), 22.5 (C-1''), 122.7 (C-2''), 131.9 (C-3''), 25.6 (C-4''), 17.8 (C-5''), 99.3 (C-1'''), 162.3 (C-2'''), 69.3 (C-3'''), 28.2 (C-4''' and C-5'''). HMBC correlations (benzene-d_6, 125 MHz): H-2→C-4, C-1', C-2' and C-6'; Hα-3→C-2, C-4 and C-1'; Hβ-3→C-4; 5-OH→C-5 and C-10; H-2' (6')→C-4'; H-3' (5')→C-1' and C-4'; H_2-1''→C-5, C-6, C-7, C-2'' and C-3''; H-2''→C-6, C-1'', C-3'', C-4'' and C-5''; H_3-4''→C-2'', C-3'' and C-5''; H_3-5''→C-2'', C-3'' and C-4''; H-1'''→C-7, C-8 and C-2'''; H_3-4''' (5''')→C-2''', C-3''' and C-5''' (4''').

Isolupalbigenin (**11**). Pale yellow needles, mp 165–167°. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (−). MS *m/z* (rel. int.): 407 ([M + 1]$^+$, 43), 406 ([M]$^+$, 100), 392 (14), 391 (48), 363 (7), 352 (9), 351 (34), 350 (7), 338 (14), 335 (15), 307 (9), 295 (14), 283 (11), 165 (20), 148 (8), 131 (9), 79

(8), 77 (9), 69 (12). UVλ_{max}^{MeOH} nm: 203, 267, 330sh *br.*; +NaOMe, 282, 325sh; +$AlCl_3$, 278, 310sh, 369 *br.*; +NaOAc, 277, 332 *br.* (+H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz): 1.66 and 1.81 (both 3H, *d*, J = 0.8 Hz and *s*, H_3-5'' and H_3-4''), 1.71 and 1.74 (both 3H, *d*, J = 0.8 Hz, and *s*, H_3-5''' and H_3-4'''), 3.37 (2H, *br d*, J = 7.2 Hz, H_2-1'''), 3.45 (2H, *br d*, J = 7.2 Hz, H_2-1''), 5.25 (1H, *br t*, J = 7.2 Hz, H-2''), 5.39 (1H, *br t*, J = 7.2 Hz, H-2'''), 6.36 (1H, *s*, H-6), 6.90 (1H, *d*, J = 8.3 Hz, H-5'), 7.29 (1H, *dd*, J = 8.3, 2.2 Hz, H-6'), 7.36 (1H, *d*, J = 2.2 Hz, H-2'), 8.23 (1H, *s*, H-2), 8.43 (1H, *br s*, 4'-OH), 9.62 (1H, *br s*, 7-OH), 13.01 (1H, *s*, 5-OH).

Lupinalbin H (**12**). Pale yellow needles, mp 248–250°. $UV_{365\ nm}$ fluorescence: dull orange. Gibbs test: dark blue. MS *m/z* (rel. int.): 351 ([M + 1]$^+$, 6), 350 ([M]$^+$, 24), 349 (4), 336 (22), 335 ([M − Me]$^+$, 100), 168 (4), 167 (19), 77 (4). UVλ_{max}^{MeOH} nm (rel. int.): 210sh, 227sh, 274 (100), 282sh (88), 338 (25); + NaOMe, 274, 295sh, 368 *br.*; +$AlCl_3$, 245, 253, 282sh, 292, 300sh, 355 *br.*; +NaOAc, unchanged. ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz): 1.49 (6H, *s*, H_3-4'' and H_3-5''), 5.80 (1H, *d*, J = 10.2 Hz, H-2''), 6.55 (1H, *s*, H-8), 6.71 (1H, *d*, J = 10.2 Hz, H-1''), 7.03 (1H, *dd*, J = 8.4, 2.2 Hz, H-5'), 7.15 (1H, *d*, J = 2.2 Hz, H-3'), 7.82 (1H, *d*, J = 8.4 Hz, H-6'), 8.91 (1H, *br s*, 4'-OH), 13.39 (1H *s*, 5-OH).

Lupichromone (**13**). Fine needles, mp 144–146°. $UV_{365\ nm}$ fluorescence: dark red. Gibbs test: (−). MS *m/z* (rel. int.): 315 ([M + 1]$^+$, 8), 314 ([M]$^+$, 35), 271 (26), 259 (26), 244 (10), 243 (59), 215 (55), 204 (13), 203 (100), 77 (9), 69 (8). UVλ_{max}^{MeOH} nm: 210, 265, 303 *br.*, 330sh; +NaOMe, 225sh, 277, 351 *br.*; +$AlCl_3$, 276, 319, 364; +NaOAc, 274, 349 *br.* (+H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR δ_{TMS} (acetone-d_6, 500 MHz): 1.65 (6H, *s*, H_3-5' and H_3-5''), 1.77 and 1.78 (both 3H, two *s*, H_3-4' and H_3-4''), 3.42 (2H, *br d*, J = *ca* 7.0 Hz, H_2-1''), 3.50 (2H, *br d*, J = 7.0 Hz, H_2-1'), 5.17 (1H, *br t*, J = 7.0 Hz, H-2''), 5.21 (1H, *br t*, J = 7.0 Hz, H-2'), 6.23 (1H, *d*, J = 5.9 Hz, H-3), 8.15 (1H, *d*, J = 5.9 Hz, H-2), 8.30 (1H, *br s*, 7-OH), 13.07 (1H, *s*, 5-OH).

Preparation and physicochemical properties of the monoprenylated naringenins (**14–16**). Boron trifluoride/etherate (1.2 ml) was added with stirring to a suspension of (2*S*)-naringenin (5.00 g) in anhydrous dioxane (12 ml). The mixt. was then heated to 50°, and a soln of 2-methyl-3-buten-2-ol (1.84 g) in dioxane (5 ml) was added with stirring over a period of 30 min. Stirring was continued for 40 min (50°) after addition of the prenylating agent. The reaction products were worked-up as previously reported [11, 12], and then fractionated by silica gel CC using CM (39:1) as the eluting solvent. Eluates were monitored (TLC) for monoprenylated naringenins (mol. wt 340) which appeared on TLC plates developed in CM (20:1) as 2 major spots (R_f 0.41 and 0.31) running between lonchocarpol A (**1**, R_f 0.60) and naringenin (R_f 0.24). Prep. TLC in CM (20:1) yielded a mixt. of 8- and 3'-prenylnaringenins from the upper band, and 6-prenylnaringenin (**14**, 484 mg) from the lower band. 8-Prenylnaringenin (**15**, 800 mg) and 3'-prenylnaringenin (**16**, 85 mg) were sepd by prep. TLC in CAAm (70:60:1; upper band **15**, R_f 0.53, lower band **16**, R_f 0.21).

6-Prenylnaringenin (**14**). Fine needles, mp 211–213°. $UV_{365\ nm}$ fluorescence: reddish purple. Gibbs test: (+), very slow, dark blue-purple. MS *m/z* (rel. int.): 341 ([M + 1]$^+$, 19), 340 ([M]$^+$, 77), 325 (17), 297 (24), 285 (31), 220 (24), 219 (16), 205 (54), 203 (14), 192 (37), 177 (27), 166 (15), 165 (RDA fragment from ring A, with methylene remnant of a prenyl side chain, 100), 123 (23), 120 (RDA fragment from 4'-hydroxylated B-ring, 35), 119 (14), 107 (15), 91 (22), 77 (12), 69 (39), 65 (17). UVλ_{max}^{MeOH} nm: 215sh, 225, 294, 366; +NaOMe, 241, 331; +$AlCl_3$, one drop: unchanged, 7 drops: 227, 316, 366; +NaOAc, 300sh, 331 (+H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR in acetone-d_6: Table 1.

8-Prenylnaringenin (**15**). Powder, mp 194–196°. $UV_{365\ nm}$ fluorescence: dull brown. Gibbs test: (−), yellow brown. MS *m/z* (rel. int.): 341 ([M + 1]$^+$, 33), 340 ([M]$^+$, 99), 325 (22), 297 (28), 285 (38), 221 (12), 220 (29), 219 (21), 206 (11), 205 (79), 192 (52), 191 (12), 178 (11), 177 (64), 166 (17), 165 (100), 147 (10), 123 (11), 121 (12), 120 (39), 119 (14), 107 (16), 91 (23), 77 (14), 69 (37), 65 (17). UVλ_{max}^{MeOH} nm: 210sh, 225sh, 294, 338; +NaOMe, 245, 333; +$AlCl_3$, one drop: 223, 316, 385 *br.*; +NaOAc, 303sh, 333 (+H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR in acetone-d_6: Table 1.

3'-Prenylnaringenin (**16**). Plates from EtOAc–hexane, mp 129–131° (complex with EtOAc?). $UV_{365\ nm}$ fluorescence: dark yellow. Gibbs test: (+), dark blue-purple. MS *m/z* (rel. int.): 340 ([M]$^+$, 33), 339 (21), 188 (29), 179 (26), 176 (15), 175 (100), 173 (16), 171 (12), 153 (RDA fragment from the 5,7-dihydroxylated A-ring, 91), 152 (10), 133 (RDA fragment from ring B, with methylene remnant of a prenyl group, 65), 132 (11), 131 (10), 124 (13), 115 (12), 103 (10), 91 (12), 77 (17), 69 (26), 55 (13). UVλ_{max}^{MeOH} nm: 225sh, 287, 320sh *br.*; +NaOMe, 247, 324; +$AlCl_3$, 222, 311, 370 *br.*; +NaOAc, 325 (+H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR in acetone-d_6: Table 1.

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