Further Isoflavonoids from White Lupin Roots

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Further investigation of the isoflavonoids in white lupin roots has revealed a new coumaronochromone with a 2-hydroxy-3-methyl-3-butenyl side chain (lupinalbin G), three new pyrano-isoflavones (lupinisoflavones K and L, and allolicoisoflavone B) and two new dihydrofurano-isoflavones each with a 2,3-dihydroxy-3-methylbutyl substituent (lupinisoflavones M and N). The biogenetic pathways for these and other lupin isoflavones are briefly discussed.

Introduction

As reported in our earlier papers, the roots of white lupin (*Lupinus albus* L. *cv.* Kievskij Mutant; Leguminosae) contain numerous isoflavonoids including three simple isoflavones and a remarkable range of complex derivatives characterized by mono- or diprenylation, or the possession of pyrano, dihydropyrano or dihydrofurano side structures [1–4]. The roots of *L. albus* have also yielded a series of rare coumaronochromones [3, 4]. A further survey of the constitutive isoflavonoids in methanol extracts of white lupin roots has now revealed the presence of a new coumaronochromone (lipinalbin G) and an additional five complex isoflavones. Two of the latter (lupinisoflavones M and N) are the first plant-derived isoflavones to be recognized with a 2,3-dihydroxy-3-methylbutyl side structure although various naturally occurring coumarins with this substituent

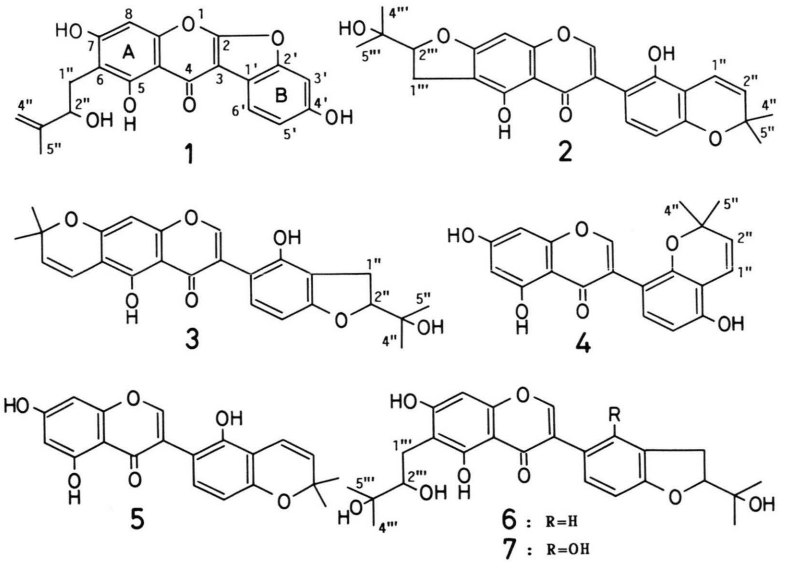

Fig. 1. New isoflavonoids (**1–4, 6,** and **7**) from white lupin roots and licoisoflavone B (**5**).

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have been reported [5]. Our previous studies [6–10] have also shown that 2,3-dihydroxy-3-methylbutyl-substituted isoflavones are occasionally produced as fungal metabolites when prenylated isoflavones are administered to cultures of *Botrytis cinerea* and *Aspergillus flavus*.

Results and Discussion

The first compound, lupinalbin G (**1**), proved to be a new coumaronochromone with 5,7,4′-trihydroxy- and 6-(2-hydroxy-3-methyl-3-butenyl) substituents. On silica gel TLC plates viewed under long wavelength (365 nm) UV light, **1** fluoresced yellow-orange in common with other lupin coumaronochromones [3]. In methanol, **1** had a major UV λ_{max} at 260 nm shifting bathochromically (± 10 nm) in the presence of $AlCl_3$ (C-5 OH) [11], and broadening upon addition of NaOAc. This latter feature is typical of 7-hydroxylated coumaronochromones, the methanol spectrum being quickly regenerated by the further addition of H_3BO_3. The relative intensities of the methanolic UV λ_{max} of **1** [260(100), 286(45) and 336(32)] effectively excluded the possibility that the compound **1** possessed an isomeric coumestan-type skeleton [*cf.* UV λ_{max}, nm (rel. int.) of glycyrol: EtOH 227(135), 244(100), 256(79), 347(115) and 356(105)] [12].

In the ^{1}H NMR spectrum of **1**, a set of *o*- (δ 7.83), *o*/*m*- (δ 7.03) and *m*- (δ 7.15) coupled signals were assigned respectively to 6′-, 5′- and 3′-H from a comparison with lupinalbin A (5,7,4′-trihydroxycoumaronochromone) of known constitution [δ and *J* values in acetone-d_6: 6′-H, 7.81 d (*J* = 8.3 Hz); 5′-H, 7.01 dd (*J* = 8.3, 2.2 Hz); and 3′-H, 7.13 d (*J* = 2.2 Hz)] [3]. Similarly, the side chain gave a ^{1}H NMR absorption pattern almost indistinguishable from that previously associated with lupinisols A, B and C [*e.g.* lupinisol B, δ 1.84 (3 H, s, 5″-H_3); 4.77 and 4.98 (both 1 H, two br s, 4″-Ha and 4″Hb); 4.46 (1 H, dd, *J* = 7.1, 4.2 Hz, 2″-H); and 2.90 and 2.97 (both 1 H, two dd, *J* = *ca.* 15.0, 7.1 Hz, and 15.0, 4.2 Hz, 1″-Ha and 1″-Hb)] [4]. Location of the side chain at C-6 was based on the chemical shift value of the single A-ring proton (δ 6.63 s, 8-H) which closely resembled that of the 6-prenylated coumaronochromone lupinalbin B (8-H at δ 6.66). In contrast, the 6-H signal (δ 6.42) of lupilutin, a coumaronochromone with a 2,3-dihydro-3-hydroxyprenyl side chain at C-8, appeared at a quite different position [13]. Thus the structure of lupinalbin G can be depicted as in **1.**

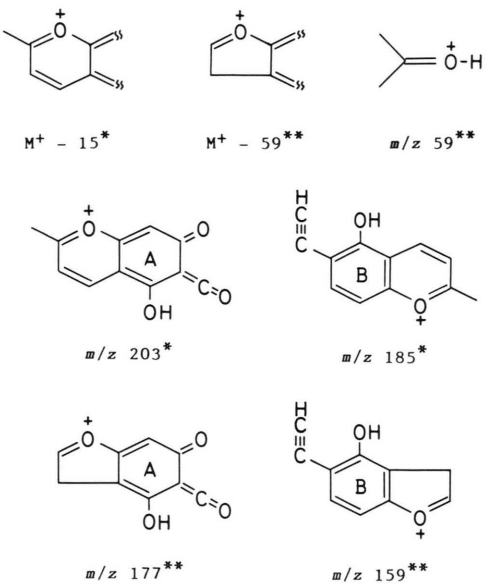

Fig. 2. Some mass fragments mentioned in the text: * from 2,2-dimethylpyrano-isoflavones; ** from 2,3-dihydro-2-(1-hydroxy-1-methylethyl)furano-isoflavones.

The second and third *Lupinus* compounds [lupinisoflavones K (**2**) and L (**3**)] were isomeric isoflavones (mol. wt. 436), each being found to contain, a 2,2-dimethylpyrano group and a 2,3-dihydro-2-(1-hydroxy-1-methylethyl)furano substituent. Support for the presence of these two cyclic ether ring systems was provided by the MS fragments at *m*/*z* 377 ($M^+ - 59$) and *m*/*z* 59 characteristic of the dihydrofurano attachment [9], and the significant loss of 15 mass units from the molecular ion to give the base peak at *m*/*z* 421, a feature typical of the 2,2-dimethylpyrano side structure (Fig. 2) [14]. Both compounds were found to be hydroxylated at C-5 (bathochromic UV shift of the methanolic λ_{max} induced by $AlCl_3$) [11] and C-2′ (immediate development of a clear blue color with Gibbs reagent) [2, 15]. Assuming oxygenation at C-7 and 4′ as in other *Lupinus* isoflavones, these two positions must be incorporated into the cyclic ether attachments.

The detection of a prominent fragment at *m*/*z* 185 (Fig. 2) in the MS of **2** can be explained if the 2,2-dimethylpyrano substituent is located (3′→4′[O]) on ring B. Thus, in **2** the dihydro-

furano group must be attached to ring A, a linear (6→7[O]) arrangement being favored as the single A-ring proton (δ 6.49, 8-H) resonates at much lower field than in an angular (8→7[O])-type dihydrofurano-isoflavone such as 2,3-dehydrokievitone metabolite DK-M 1 (6-H at δ 6.24) [10]. ^{1}H NMR data are also entirely consistent with the formulation of lupinisoflavone K as **2.**

Because only small quantities of **3** were available for investigation. ^{1}H NMR data could not be obtained for this lupin isoflavone. However, we have assigned structure **3** to lupinisoflavone L for the following reasons. The detection of a MS fragment at *m/z* 203 (Fig. 2) indicated that the 2,2-dimethylpyrano side structure was located on ring A. This was placed in the linear position (6→7[O]) from the methanolic UV λ_{max} at 282 nm (*cf.* alpinumisoflavone [16] and parvisoflavone B [2] each with a linear side attachment, λ_{max} MeOH 282 nm). In contrast, angular-type isoflavones (side structure 8→7[O]) such as derrone [16] and parvisoflavone A [17] have UV maxima at 270 nm). The dihydrofurano attachment of **3** must therefore be located on ring B in the position shown (**3,** 3′→4′[O]) to satisfactorily account for the rapid Gibbs test response (clear blue; 2′-OH group with 5′ unsubstituted) mentioned earlier [2, 15].

The fourth *Lupinus* compound (allolicoisoflavone B, **4**) was isomeric (mol. wt. 352) with the known licoisoflavone B (**5**) [1, 18]. Hydroxylation at C-5 and C-7 was evident from bathochromic shifts of the methanolic UV λ_{max} at 260 nm induced by $AlCl_3$ and NaOAc respectively, whilst loss of 15 mass units to give the MS base peak at *m/z* 337 indicated the presence of a 2,2-dimethylpyrano side attachment. This was assigned to ring B, as in **5,** from the prominent MS ion observed at *m/z* 185. However, **4** was readily distinguished from its isomer **5** by TLC [*e.g.* R_f values for **4** and **5** in PEAa were 0.41 and 0.60 respectively; in CAAm, 0.19 and 0.29; and in CM, 0.24 and 0.56]. Both compounds also differed in their Gibbs test response: **4** slowly gave a blue color similar to that of cyclolicoisoflavone A_2 (side structure 3′→2′[O]) [2] whereas **5** reacted rapidly to give a clear blue color comparable with that noted for cyclolicoisoflavone A_1 (side structure 3′→4′[O]) [2]. The above evidence supports structure **4** for allolicoisoflavone B, although it is unexpected to find in *Lupinus* an isoflavone with a 3′→2′[O] oriented side attachment when the 4′-OH is underivatized.

The two remaining compounds, lupinisoflavones M (**6;** M^+ 456) and N (**7;** M^+ 472) were recognizable as isoflavones from their characteristic UV spectra (λ_{max}, 266 nm in MeOH), and from the low-field ^{1}H NMR singlet (2-H) at δ 8.17 (**6**) and δ 8.18 (**7**). Both isoflavones were found to be hydroxylated at C-5 and C-7 (UV shifts induced by $AlCl_3$ and NaOAc respectively [11]). Apart from 2-H and four aromatic protons, the ^{1}H NMR spectrum of **6** revealed the presence of a 2,3-dihydroxy-3-methylbutyl substituent [9] and a 2,3-dihydro-2-(1-hydroxy-1-methylethyl)furano side attachment [9], the former being assigned to C-6 (ring A) following a comparison with the glycol derivatives of wighteone, luteone (both with a C-6 side chain, and 8-H at δ 6.45 and δ 6.49 respectively [6, 8]) and 2,3-dehydrokievitone (C-8 side chain, 6-H at δ 6.34 [10]). Location of the cyclic furan side attachment on ring A was precluded by the detection of OH groups at C-5 and C-7 as discussed above. Only three aromatic protons could be assigned to ring B of lupinisoflavone M. Since oxygenation at C-4′ (*cf.* other *Lupinus* isoflavones) can be safely assumed, the furano side structure of **6** must be attached (3′→4′[O]) as in the known compound lupinisoflavone C [2]. Chemical shift values reported for 2′-H (δ 7.42), 5′-H (δ 6.75) and 6′-H (δ 7.30) of lupinisoflavone C [2] were identical with those given by the ring B protons of lupinisoflavone M which, from the foregoing discussion, can thus be represented by structure **6**.

Finally, lupinisoflavone N (**7;** M^+ 472 = 6 + [O]) was identified as the 2′-hydroxy derivative of lupinisoflavone M (**6**). All the ^{1}H NMR signals (A/C-ring, and side structure protons) evident in the spectrum of **6** were also given by **7,** the difference between these compounds being that only two *ortho*-coupled protons could be assigned to ring B of **7.** These must be located at C-5′ and C-6′ since no other B-ring arrangement is compatible with both the presence of a cyclic (3′→4′[O]) side structure and an additional OH group. In fact the chemical shift values recorded for the A- and B-ring protons, and their associated side structures, were identical with those respectively reported for luteone glycol [6] and lupinisoflavone D [2] thereby establishing **7** as the structure of lupinisoflavone M. In a previous study [9], **7** was identified

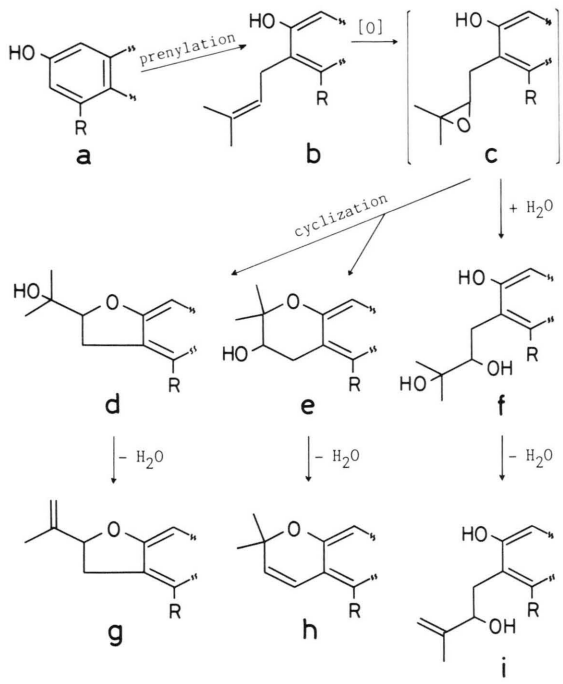

Fig. 3. Biogenetic pathways for complex isoflavone production in white lupin *(Lupinus albus)*. The above scheme is equally applicable to substituents on ring A (illustrated, R=OH) or ring B (R=H or OH) of isoflavones from *L. albus*. – *Key to part structures:* **a:** Simple isoflavones: genistein, 2′-hydroxygenistein and 3′-O-methylorobol; **b:** prenylation normally occurs at C-6 and/or C-3′ (only one *Lupinus* isoflavone with 8,3′-diprenylation has been reported [4]): wighteone, luteone, lupisoflavone, licoisoflavone A, lupalbigenin, 2′-hydroxylupalbigenin (= angustone A) and 2′-hydroxyisolupalbigenin (prenylated at C-8 and 3′). Other prenylated isoflavones with additional side structures *e.g.* lupinisoflavone I, lupinisolone A, angustone B, and lupinisol A [4] are included in categories **d, e, h,** or **i; c:** a possible metabolic intermediate (the formation of an epoxy intermediate was recently confirmed during an investigation of the metabolism of prenylated isoflavones by *Botrytis cinerea* [19]); **d:** lupinisoflavones B–J, and erythrinin C (excluding dihydrofurano-isoflavones with **f-** or **h-**type side structure); **e:** lupinisolones A–C; **f:** lupinisoflavones M and N; **g:** lupinisoflavone A; **h:** alpinumisoflavone, isoderrone, parvisoflavone B, licoisoflavone B, allolicoisoflavone B, chandalone, angustones B and C, isochandalone and lupinisoflavones K and L; **i:** lupinisols A–C. In white lupin roots, the above isoflavones are associated with coumaronochromones of types **a** (lupinalbin A), **b** (lupinalbins B, D and F), **d** (lupinalbins C and E) and **i** (lupinalbin G).

as a fungal metabolite (AF-d-6) of the diprenylated isoflavone 2′-hydroxylupalbigenin (= angustone A). A comparison of the physico-chemical data for **7** derived from *L. albus* and fungal cultures confirmed that the two compounds were identical. As expected, on silica gel TLC plates sprayed with Gibbs reagent [2, 15] compound **6** reacted slowly to afford a dark blue (genistein-type) color, whereas **7,** with a 2′-OH group and the *para* (C-5′) position free, immediately gave a clear blue color resembling that of cyclolicoisoflavone A_1 [2].

Fig. 3 shows the A-ring part structures of all the white lupin isoflavones isolated during the course of this and previous studies [1–4]. The compounds are arranged in a plausible biogenetic sequence which requires the involvement of a transitory epoxide intermediate (**c**). As yet we have not been able to detect such an epoxide in *L. albus* extracts although we have recently found that an epoxide is formed during the conversion of 7-*O*-methyl-2,3-dehydrokievitone to the corresponding glycol by cultures of *Botrytis cinerea* [19]. We believe that in white lupin a stereospecific epoxidation yields isoflavones with part structures **d–i** [2, 20], and that a comparable step is involved in the fungal metabolism of prenylated isoflavones to give **d-** and **e-**type metabolites as well as **f-**type glycols [6–10].

Crombie *et al.* [21] suggested the O_2-dependent enzymatic 2,2-dimethylpyrano-ring formation *via* quinone methide in their recent study on deguelin (one of rotenoids) biosynthesis by *Tephrosia vogellii*. More recently, Welle and Grisebach [22] have proposed another reaction scheme for oxidative cyclization of prenylated pterocarpans to glyceollin isomers in the elicitor-challenged soybean cell suspension cultures. Their NADPH- and O_2-dependent microsomal enzyme(s) seems to catalyze the cyclization reaction to yield both 2,2-dimethylpyrano- and 2,3-dihydro-2-isopropenylfurano-pterocarpans not *via* an epoxy-intermediate but directly.

Experimental

Except where indicated preparative TLC (PTLC) was carried out on silica gel using the following solvent systems: CAAm = $CHCl_3$–acetone–conc. aq. NH_3 (35:30:1), CM = $CHCl_3$–MeOH (25:1), PEAa = *n*-pentane–diethyl ether–glacial AcOH (75:25:4) and CAEM = $CHCl_3$–acetone–EtOAc–MeOH (40:5:5:2).

Extraction and isolation of isoflavonoids

For details of the extraction procedure, and initial silica gel column fractionation of lupin isoflavonoids, see ref. [4]. Column fraction (Fr)-8 and a portion of subfractions Fr′-4 and Fr′-5 (from Fr-6 + Fr-7) were combined (2.3 g) and subjected to further column chromatography over Florisil (120 g moistened with 5% H_2O, w/w). The third and fourth fractions (each 75 ml) eluted with 50% EtOAc in benzene were combined and reduced to dryness. The residue (42 mg) was chromatographed (PTLC) in CAAm to give 8 bands. Elution (EtOAc) and re-PTLC of band 1 (R_f approx. 0.85) in CM yielded lupinisoflavone K (**2,** 3.1 mg, R_f 0.83), a minute amount of lupinisoflavone L (**3,** R_f 0.73) and the known lupinisoflavone H (2.6 mg, R_f 0.59) [4]. Bands 2, 3, and 7 from the CAAm chromatogram were respectively identified as parvisoflavone B (9.6 mg, R_f 0.62) [2], lupinisoflavone J (5.3 mg, R_f 0.50) [4] and lupinisol B (6.7 mg, R_f 0.19). Band 6 (R_f 0.27) contained a new compound which was purified by PTLC in CM to yield 1.4 mg of a colorless powder (lupinalbin G, **1,** R_f 0.34). The identity of material constituting bands 4 (R_f 0.38), 5 (R_f 0.33) and 8 (R_f 0.10) could not be established.

Another new isoflavone (**4**) was isolated from Fr-9 (2.2 g) [4] which was further fractionated by column chromatography over Florisil as described above. The third and fourth fractions (each 75 ml) eluted with 40% EtOAc in benzene were combined and concentrated (79.8 mg). Silica gel PTLC of this concentrate in CAAm gave bands fluorescing respectively dark purple (R_f 0.20–0.25) and dark brown (R_f 0.10–0.20) under UV_{365nm} light. After elution and re-PTLC in PEAa the upper band yielded luteone (1.9 mg, R_f 0.26) and the new pyrano-isoflavone allolicoisoflavone B (**4,** 8.2 mg, R_f 0.41). Further PTLC of the lower band in CAAm gave the known isoflavonoids, lupinalbin D (1.5 mg, R_f 0.28) [3] and licoisoflavone A (6.8 mg, R_f 0.17) [2].

Our earlier paper [4] dealt only with the constituents in the initial silica gel column fractions up to Fr-9 (eluted with 30% EtOAc in benzene). Following the collection of Fr-9, any remaining isoflavonoids were eluted from the column (all fractions 250 ml each) as follows: Fr-10, 11, 12 (40% EtOAc in benzene), Fr-13, 14, 15 (55% EtOAc in benzene), Fr-16, 17, 18 (70% EtOAc in benzene), and Fr-19, 20, 21 (EtOAc only). Examination of Fr-19 by PTLC in CAEM afforded two dark purple fluorescing (UV_{365nm}) bands at R_f 0.23 (lupinisoflavone M, **6**) and 0.15 (lupin isoflavone N, **7**). After elution with EtOAc, both compounds were finally purified by multiple development PTLC in CAAm (70:60:1, × 4). Column fraction Fr-19 yielded 3 mg of **6,** and 14.5 mg of **7.**

Physico-chemical properties of the new lupin isoflavonoids

For spectroscopic details (instrumentation *etc.*), see our earlier papers [2–4, 9].

Lupinalbin G (**1**): Colorless powder, UV_{365nm} fluorescence: orange. Gibbs test: (+), slow, blue-purple. MS (rel. int.): *m/z* 368 (M^+; 10), 351 (10), 350 (M^+-H_2O; 49), 349 (10), 336 (19), 335 (350–CH_3; 87), 298 (44), 297 (M^+-71; 100), 167 (10), 163 (10), 55 (11). UV: λ_{max}, nm (rel. int.): MeOH 244 sh, 260 (100), 286 (45), 336 (32); +NaOMe 258 sh, 275, 305, 360; +$AlCl_3$ 237.5, 270, 288 sh, 323, 370; +NaOAc 256 (br), 269 sh, 291, 344 (H_3BO_3 regenerated the MeOH spectrum). ^1H NMR (acetone-d_6, 100 MHz): δ 1.85 (3H, s, 5″-H_3), 2.65–2.75 and 2.97–3.09 (both 1H, two m, 1″-Ha and 1″-Hb), 4.45 (1H, m, 2″-H), 4.78 and 4.95 (both 1H, two br. s, 4″-Ha and 4″-Hb), 6.63 (1H, s, 8-H), 7.03 (1H, dd, J = 8.3, 2.4 Hz, 5′-H), 7.15 (1H, d, J = 2.4 Hz, 3′-H), 7.83 (1H, d, J = 8.3 Hz, 6′-H), 13.49 (s, 5-O<u>H</u>).

Lupinisoflavone K (**2**): Pale yellow gum, UV_{365nm} fluorescence: dark purple. Gibbs test: (+), rapid, clear blue. MS (rel. int.): *m/z* 437 (M^++1; 7.1), 436 (M^+; 22), 423 (5.5), 422 (29), 421 (M^+-15; 100), 405 (8.2), 404 (6.7), 403 (22), 361 (7.4), 349 (5.4), 203 (4.4), 194 (5.7), 185 (18), 177 (4.4; Fig. 2), 59 (16). UV: λ_{max}, nm: MeOH 217, 227 sh, 268; +NaOMe 234 sh, 262, 276 sh, 283 sh, 340 sh (br); +$AlCl_3$ 223, 231, 273, 310 (br), 361; +NaOAc unchanged. ^1H NMR (acetone-d_6, 100 MHz): δ 1.26 and 1.30 (both 3H, two s, 4‴-H_3 and 5‴-H_3), 1.42 (6H, s, 4″- and 5″-H_3), 3.2–3.3 (2H, br. d-like m, 1‴-H_2), 4.88 (1H, m, 2‴-H), 5.70 (1H, d, J = 10.5 Hz, 2″-H), 6.40 (1H, d, J = 9.0 Hz, 5′-H), 6.49 (1H, s, 8-H), 6.79 (1H, d, J = 10.5 Hz, 1″-H), 7.05 (1H, d, J = 9.0 Hz, 6′-H), 8.28 (1H, s, 2-H), 12.66 (s, 5-O<u>H</u>).

Lupinisoflavone L (**3**): Pale yellow gum, UV_{365nm} fluorescence: dark purple. Gibbs test: (+), rapid,

clear blue. MS (rel. int.): *m/z* 437 (M^++1; 20), 436 (M^+; 64), 422 (29), 421 (M^+−15; 100), 403 (18), 377 (M^+−59; 31), 204 (11), 203 (83), 159 (3.2, Fig. 2), 59 (22). UV: λ_{max}, nm: MeOH 225.5, 282, 288 sh; +NaOMe 232 sh, 277−285 (br), 300 sh; +$AlCl_3$ 235, 292−302 (br), 361; +NaOAc unchanged.

Allolicoisoflavone B (**4**): Pale yellow gum, $UV_{365\,nm}$ fluorescence: dark purple. Gibbs test: (+), slow, blue-purple. MS (rel. int.): *m/z* 353 (M^++1; 5.7), 352 (M^+; 23), 338 (25), 337 (M^+−15; 100), 321 (11), 185 (10), 169 (10), 153 (5.9). UV: λ_{max}, nm: MeOH 220 sh, 260, 287 sh, 320 sh (br); +NaOMe 232, 270.5, 282 sh, 325; +$AlCl_3$ 227, 269, 307, 362; +NaOAc 269, 326 (br) (H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 100 MHz): δ 1.38 (6 H, s, 4″-H_3 and 5″-H_3), 5.66 (1 H, d, *J* = 10.3 Hz, 2″-H), 6.28 (1 H, d, *J* = 2.2 Hz, 6-H), 6.41 (1 H, d, *J* = 2.2 Hz, 8-H), 6.47 (1 H, d, *J* = 8.4 Hz, 5′-H), 6.73 (1 H, d, *J* = 10.3 Hz, 1″-H), 7.04 (1 H, d, *J* = 8.4 Hz, 6′-H), 8.03 (1 H, s, 2-H), 13.09 (s, 5-O<u>H</u>).

Lupinisoflavone M (**6**): Pale yellow needles, m.p. 117−179 °C, $UV_{365\,nm}$ fluorescence: dark purple. Gibbs test: (+), slow, green-blue → dark blue. MS (rel. int.): *m/z* 456 (M^+; 10), 439 (11), 438 ($M^+ - H_2O$; 20), 398 (18), 397 (M^+ − 59; 47), 380 (12), 379 (34), 368 (29), 367 (87), 349 (15), 339 (14), 337 (39), 321 (11), 310 (17), 309 (38), 308 (26), 307 (69), 295 (12), 165 (17), 123 (13), 115 (17), 69 (12), 59 (100), 55 (22), 43 (37). UV: λ_{max}, nm: MeOH 201, 209 sh, 266, 289 sh, 327 sh; +NaOMe 205, 273, 337 (br); +$AlCl_3$ 211 sh, 229 sh, 272, 310 sh, 360 sh (br); +NaOAc 273, 337 (br) (H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 500 MHz): δ 1.23, 1.26, 1.28 and 1.29 (all 3 H, four s, 4″-, 4‴, 5″- and 5‴-H_3), 2.61 (1 H, dd, *J* = 14.5, 9.9 Hz, 1‴-Ha), 3.21 (1 H, dd, *J* = 15.9, 9.5 Hz, 1″-Ha), 3.25 (1 H, dd, *J* = 14.5, 1.9 Hz, 1‴-Hb), 3.31 (1 H, dd, *J* = 15.9, 8.4 Hz, 1″-Hb), 3.65 (1 H, dd, *J* = 9.9, 1.9 Hz, 2‴-H), 4.67 (1 H, dd, *J* = 9.5, 8.4 Hz, 2″-H), 6.45 (1 H, s, 8-H), 6.75 (1 H, d, *J* = 8.2 Hz, 5′-H), 7.30 (1 H, dd, *J* = 8.2, 1.6 Hz, 6′-H), 7.42 (1 H, incomplete d, 2′-H), 8.17 (1 H, s, 2-H), 13.51 (1 H, s, 5-O<u>H</u>).

Lupinisoflavone N (**7**): Colorless fine needles, m.p. 225−226 °C. $UV_{365\,nm}$ fluorescence: dark purple. Gibbs test: (+), rapid, clear blue. $[\alpha]_D^{21}$ −33° (*c* = 0.11, MeOH). MS (rel. int.): *m/z* 472 (M^+; 12), 455 (12), 454 ($M^+ - H_2O$; 40), 436 (20), 421 (20), 413 (M^+−59; 19), 403 (12), 396 (23), 395 (64), 384 (28), 383 (89), 377 (28), 365 (25), 353 (20), 325 (31), 324 (15), 323 (34), 311 (12), 203 (16), 166 (13), 165 (100), 160 (18), 147 (12), 123 (18), 69 (12), 59 (82), 55 (19), 43 (37). UV: λ_{max}, nm: MeOH 208, 266, 291 sh, 330 sh (br); +NaOMe 205, 222 sh, 271, 330 (br); +$AlCl_3$ 205, 274, 314, 369 (br); +NaOAc 274, 339 (br) (H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR (acetone-d_6, 500 MHz): δ 1.24, 1.26, 1.28 and 1.29 (all 3 H, four s, 4″-, 4‴-, 5″- and 5‴-H_3), 2.64 (1 H, dd, *J* = 14.4, 9.8 Hz, 1‴-Ha), 3.14 (1 H, dd, *J* = 15.8, 9.6 Hz, 1″-Ha), 3.21 (1 H, dd, *J* = 15.8, 8.3 Hz, 1″-Hb), 3.25 (1 H, dd, *J* = 14.4, 1.9 Hz, 1‴-Hb), 3.66 (1 H, dd, *J* = 9.8, 1.9 Hz, 2‴-H), 4.69 (1 H, dd, *J* = 9.6, 8.3 Hz, 2″-H), 6.34 (1 H, d, *J* = 8.2 Hz, 5′-H), 6.50 (1 H, s, 8-H), 7.01 (1 H, d, *J* = 8.2 Hz, 6′-H), 8.18 (1 H, s, 2-H), 13.13 (1 H, s, 5-O<u>H</u>).

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