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ISOFLAVONES FROM *LUPINUS ANGUSTIFOLIUS* ROOT

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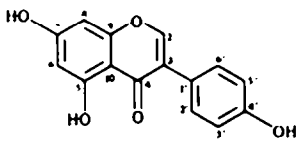
Key Word Index *Lupinus angustifolius*; Leguminosae; isoflavones; ^{13}C NMR; coumaronochromone.

Abstract - Two novel isoflavones, 5,7,2'-trihydroxy-6-(3,3-dimethylallyl)-[6",6"-dimethylpyrano(2",3":4',3')]-isoflavone and 5,2',4'-trihydroxy-3'-(3,3-dimethylallyl)-[6",6"-dimethylpyrano(2",3":7,6)]isoflavone, have been isolated from the roots of *Lupinus angustifolius* cv. Uniharvest. Structures were established by analysis of ^{13}C NMR and other spectral data, and by chemical conversion of one of the compounds to a coumaronochromone.

INTRODUCTION

In the course of an investigation of the insect feeding deterrent and antifungal activity of an ethanol extract of the roots of *Lupinus angustifolius* cv. Uniharvest nine isoflavones (**1**-**9**) were isolated and their insect feeding deterrent activity and antifungal activity determined [1]. Of these compounds, genistein (**1**), wighteone (**2**), 2'-hydroxygenistein (**3**) and luteone (**4**), were known constituents of the aerial parts [2-7] and roots of several *Lupinus* sp. including *L. angustifolius* cv. Beliak [7], and licoisoflavone A (**5**) and licoisoflavone B (**7**) have been isolated from the roots of *L. albus* together with other prenyl-, chromenyl- and dihydrofurano-isoflavones



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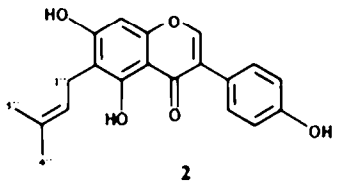

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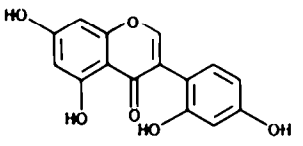

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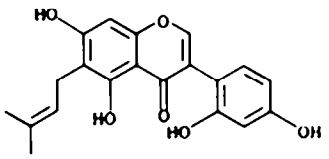

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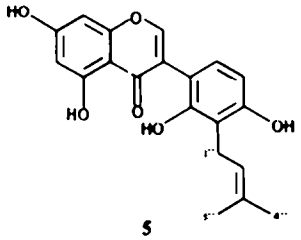

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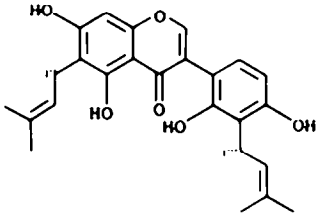

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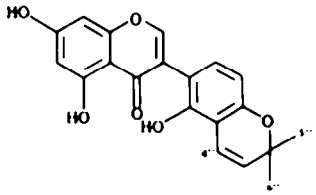

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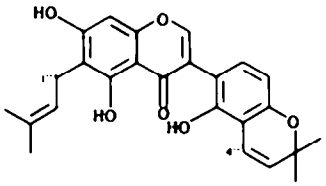

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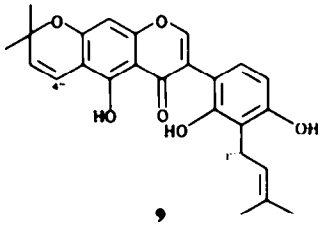

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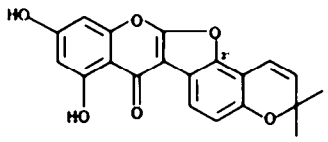

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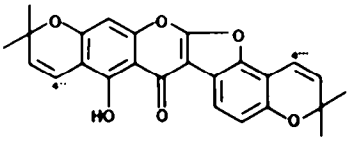

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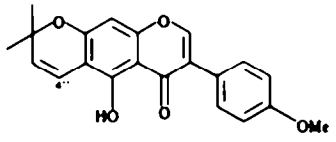

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[7, 8]. In the insect feeding deterrent study [1] three further components with the characteristic deep purple fluorescence and fast blue reaction on TLC of 5-hydroxyisoflavones were isolated for testing, and given the names angustone A (**6**), angustone B (**8**) and angustone C (**9**). The isolation of **6** as an insect feeding deterrent from *L. angustifolius* has been briefly reported in a preliminary form [9]. **6** has recently been reported from the roots of *L. albus* [8] (as 2'-hydroxylupalbigenin) and a compound from *Millettia pulchra* has been assigned the same structure [10]. The data on which the characterization of **6** was based is reported here together with the elucidation of structures **8** and **9**.

RESULTS AND DISCUSSION

The isolation of **1–9** from an extract of *Lupinus angustifolius* root by partitioning and repeated column chromatography on silica gel and Sephadex-LH20, and the identification of **1**, **5** and **7** has been described [1].

High resolution mass spectrometry showed angustone A (**6**) to have the molecular formula $C_{25}H_{26}O_6$. The IR spectrum showed the presence of a carbonyl group (ν_{max} 1642 cm^{-1}), and the presence of four hydroxy groups was indicated by the formation of a tetraacetate (*m/z* 590 $[M]^+$) on acetylation. The UV data [λ_{max} 269 nm; bathochromic shifts with NaOAc (+7 nm), $AlCl_3$ (+3.5 nm), and NaOMe (+15 nm)] suggested a 5,7-dihydroxyisoflavone [11]. Evidence that ring A and ring B each bore two hydroxy groups and a 3,3-dimethylallyl substituent was provided by the mass spectrum. Characteristic ions arising from the loss of C_3H_7· and C_4H_7· from the molecular ion, and subsequent loss of C_4H_8 [12] were observed (*m/z* 379, 367, 323, 311) together with the corresponding metastables. A major ion of *m/z* 165 $[C_8H_5O_4]^+$ (57%) corresponds to subsequent retro Diels Alder fragmentation [12] with charge retention on ring A as observed for the 6-prenyl-5,7-dihydroxyisoflavones **2** and **4** [2, 4]. Ions of *m/z* 351 $[M-C_5H_{11}]^+$ and 203 $[C_{11}H_7O_4]^+$ may arise by an alternative fragmentation pathway via a chroman [13].

The ^{1}H NMR spectrum (in DMSO-d_6) showed four broad phenolic hydroxyl singlets downfield (δ13.09, 10.78, 9.23 and 8.18), an isoflavone 2-H singlet (δ8.09) and characteristic signals for the methylene, olefinic methine and non-equivalent terminal methyl protons of two 3,3-dimethylallyl side-chains [δ5.19 (2H, m), 3.30 (4H, m), 1.74 (6H, *br s*), 1.65 (6H, *br s*)]. A 2',3',4',5,6,7-substitution pattern was indicated with signals for three aromatic protons, one isolated, the other two an *ortho* pair δ6.77 (1H, *d*, *J* = 8 Hz), 6.47 (1H, *s*), 6.38 (1H, *d*, *J* = 8 Hz). The chemical shift of the isolated proton signal is in the range for H-8 of a 5,7-dihydroxyisoflavone [11]. The *ortho* pair was assigned as H-6' and H-5' of a trisubstituted ring B, and the close similarity of the chemical shifts to those for **4** (δ6.74 (H-6'), 6.36 (H-5') [14]) suggested a homologous 3'-prenyl-2',4'-hydroxylated structure **6** for angustone A. These data are in accord with those reported by Tahara *et al.* [8] for the compound from *L. albus* root, and the data for the tetraacetate derivative (Experimental) are also in agreement apart from the higher melting point recorded. There appear to be significant differences in the mp and UV data reported for the *Millettia* compound [10], and the chemical shift reported for the ring A proton in the ^{1}H NMR spectrum of the isoflavone and its tetraacetate is at higher field.

Supporting evidence for structure **6** was provided by analysis of the ^{13}C NMR data (Table 1) for **1–6**. Assignments are based on chemical shift arguments, and determination of the number of directly bonded hydrogens by inversion recovery experiments, fully coupled or single-frequency off-response decoupled (sford) spectra. The spectrum of 2'-hydroxygenistein (**3**) was assigned by comparison with published assignments for genistein (**1**) [15–17], using substituent effects for the introduction of a 2'-hydroxy group into ring B of an isoflavone derived from lit. data [16, 17]. The resonances for C-4' and C-2' were assigned on the basis of relaxation measurements. Proton–carbon dipole–dipole interactions should make the spin-lattice relaxation time, T_1, shorter for C-4' with two adjacent C–H moieties than for C-2' with one adjacent C–H group [18, 19, R. H. Newman, unpublished]. The signals at δ158.7 (T_1 2.2 ± 0.3 sec) and δ156.6 (T_1 3.4 ± 0.4 sec) were accordingly assigned to C-4' and C-2', respectively.

The ring B and C carbon resonances of the 6-prenyl compounds **2** and **4** are comparable to those of the respective parent compounds **1** and **3**. The ring A and side-chain carbon resonances of **2** and **4** are essentially identical. The 3,3-dimethylallyl side-chain resonances were identified on the basis of characteristic chemical shifts [20] and the number of attached protons. C-6 and C-8 alkylated flavonoids are readily distinguished by ^{13}C NMR [21], and the pattern of ring A carbon resonances is consistent with the C-6 alkylated structure of **2** and **4**. The C-6 and C-8 signals are well resolved in **1** and **3** (*ca* 99 and 94 ppm), and the C-6 signal is shifted 12 ppm downfield and the *ortho* and *para* carbon resonances are shifted 2.1–3.1 ppm upfield in **2** and **4**, while the C-8 resonances are shifted only slightly.

The spectrum of the 3'-prenylisoflavone, **5**, was similarly assigned on the basis of comparisons with that of **3**, characteristic values of 3,3-dimethylallyl resonances [20] and alkylation shifts of ring B resonances similar to those observed for 6-prenylation of ring A. With these assignments to hand, the spectrum of **6** could be seen to consist of two sets of resonances, corresponding to those of the side-chain and ring A of **2** and **4**, and the side-chain and rings B and C of **5**, in keeping with the proposed structure.

High resolution mass spectra showed both angustone B (**8**) and angustone C (**9**) to have the molecular formula $C_{25}H_{24}O_6$. Both compounds showed evidence for the presence of a carbonyl group in the IR spectrum [ν_{max} 1655 cm^{-1} (**8**); 1649 cm^{-1} (**9**)], and each formed a triacetate (*m/z* 546 $[M]^+$) on acetylation. These data suggested **8** and **9** to be cyclodehydrogenated analogues of angustone A (**6**).

The UV spectrum of **9** showed maxima at 226 and 288 nm, and bathochromic shifts with NaOH (+10 mm) and $AlCl_3$ (+5 nm) but not NaOAc, characteristics of a 5-hydroxyisoflavone with a ring A fused 2,2-dimethylpyranyl ring [22–24]. The mass spectrum provided evidence for the presence of a 3,3-dimethylallyl substituent in addition to the dimethylpyranyl ring. Characteristic ions corresponding to the loss of CH_3·, C_3H_7·, and C_4H_8 from the molecular ion (*m/z* 405, 377, 365), and a daughter ion and corresponding metastable for the loss of C_4H_8 from the $[M-CH_3]^+$ fragment (*m/z* 349) were observed [12, 13]. The base peak at *m/z* 203, reported for other ring-A fused 2,2-dimethylpyrano-5-hydroxyisoflavones [22–24], corresponds to the ring A retro Diels Alder fragment from the $[M-CH_3]^+$ and $[M-CH_3$

Table 1. ^{13}C NMR of isoflavones

Carbon	1*	2	3	4	5	6	7	8	9	12†
2	153.6	153.8	155.4	155.1	155.8	155.6	155.8	155.6	156.0	152.3
3	122.4[a]	122.3[a]	120.6	120.5	121.4	121.2	120.7	120.6	121.5	122.9[a]
4	180.2	180.4	180.6	180.7	181.2	181.2	180.8	180.8	181.5	180.7
5	162.1	159.0	162.0	158.9[a]	162.0	158.8	162.0	158.8	156.1[a]	156.8[b]
6	98.6	111.2	99.0	111.2	99.1	111.3	99.1	111.3[a]	104.9[b]	105.2[c]
7	164.3	162.1	164.3	161.9	164.4	162.2	164.3	162.1	158.9	159.6
8	93.7	93.0	93.8	93.0	93.8	93.1	93.8	93.1	94.8	94.7
9	157.6	155.5	157.8	155.5	158.0	155.7	157.9	155.5	157.0[a]	157.1[b]
10	104.6	104.4	104.6	104.5	104.9	104.6	104.8	104.6	105.8[b]	106.1[c]
1′	121.4[a]	121.5[a]	108.8	109.1	109.8	110.0	111.4[b] (110.3)‡	111.6[a]	109.5	123.3[a]
2′	130.0	130.3	156.6	156.6	154.2	154.2	151.3 (151.2)	151.4	154.2	129.9
3′	115.2	115.2	102.8	102.9	115.7	115.7	110.0[b] (109.4)	111.0[a]	115.7	114.0
4′	157.6	157.5	158.7	158.7[a]	156.6	156.6	153.8 (154.0)	153.8	156.7[a]	159.6
5′	115.2	115.2	106.4	106.5	107.0	106.9	107.6 (107.4)	107.7	107.0	114.0
6′	130.0	130.3	132.3	132.4	128.8	128.8	131.5[a] (131.8)	131.5[b]	128.9[c]	129.9
1″		21.2		21.2	22.6	21.2				
2″		122.3		122.4	123.7	122.4	75.5	75.5	78.1	77.8
3″		130.8		130.8	129.7	130.8	128.7[a]	129.0[b]	129.1[c]	128.0
4″		25.6		25.6	25.6	25.6	117.1	117.2	114.8	115.5
5″		17.8		17.8	17.9	17.8	27.6	27.6	27.9	28.2
6″							27.6	27.6	27.9	28.2
1‴						22.6		21.2	22.6	
2‴						123.7		122.4	123.7	
3‴						129.7		130.8	129.7	
4‴						25.6		25.6	25.7	
5‴						17.8		17.8	17.9	
CH_3O										55.2

*Data from [15] (DMSO-d_6 as solvent) with C-4′ and C-5 reassigned as [17].
†Data (in $CDCl_3$ as solvent) from Vilain and Jadot [26, 27].
‡Calculated values (see text).
[a,b,c]Interchangeable pairs.

$-C_4H_8]^+$ ions. From these data the presence of the dimethylallyl group and two hydroxy groups on ring B could be inferred. The ^{1}H NMR spectrum was consistent with this evidence with three downfield phenolic proton singlets (δ13.27, 9.32, 8.32), an isoflavone 2H singlet (δ8.13), and characteristic signals for a 2,2-dimethylpyranyl ring [AB system, δ6.62, 5.76, (J = 10 Hz); 6H singlet δ1.42], and a 3,3-dimethylallyl moiety [δ5.21 (1H, t, J = 7 Hz), 3.29 (2H, d, J = 7 Hz), 1.72 (3H, s), 1.62 (3H, s)]. The close similarity of the aromatic proton pattern [δ6.78 (1H, d, J = 8 Hz), 6.45 (1H, s), 6.40 (1H, d, J = 8 Hz)] to that for **6**, suggested a similar substitution pattern, and angustone C was deduced to be the linear fused pyranoisoflavone 5,2′,4′-trihydroxy-3′-(3,3-dimethylallyl)-[6″,6″-dimethylpyrano(2″,3″:7,6)]-isoflavone (**9**).

Confirmatory evidence for structure **9** was provided by the ^{13}C NMR data (Table 1). Resonances essentially identical to those of the ring B and C, and ring B side-chain carbons of **5** and **6** were observed. A 5,6-fused pyranoisoflavone structure can be excluded as the C-4 resonance is unshifted [25]. As with C-6 and C-8 prenylation (above) Vilain and Jadot [26, 27] have shown that linear 6,7-fused and angular 7,8-fused 5-hydroxypyranoisoflavones can be readily distinguished by ^{13}C NMR. In each case the remaining protonated ring A carbon resonance (C-8 or C-6, respectively) was distinctive, being shifted only slightly by comparison with the parent isoflavone **1**. While the C-5 and C-7 resonances were shifted upfield in both cases, the C-9 resonance was shifted 5 ppm further upfield in the angular isomer. The linear fused structure for **9** is thus confirmed by the close similarity of the ring A and 2,2-dimethylpyranyl carbon resonances to those reported for **12** (Table 1) [26, 27].

The UV spectrum of angustone B (**8**) showed a maximum at 271 nm, and bathochromic shifts were observed with NaOAc (+6 nm), $AlCl_3$, (+4.5 nm) and NaOH (λ_{max} 344 nm), indicative of a 5,7-dihydroxyisoflavone [11]. The presence of a 2,2-dimethylpyranyl ring fused to ring B and a 3,3-dimethylallyl substituent attached to ring A was suggested by the mass spectrum. Characteristic ions for the loss of CH_3 (m/z 405) and subsequent loss of C_4H_8 (m/z 349, confirmed by metastable) were observed. Ions of m/z 185 [$C_{12}H_9O_2$], as observed in the MS of the 3′,4′-dimethylpyranyl isoflavone **7** [7, 13], and m/z 165 [$C_8H_5O_4$]$^+$ as observed in the mass spectrum of the 6-prenyl compounds **2**, **4** [2, 4] and **6** (above) correspond to retro Diels–Alder fragments with charge retention in ring B and ring A, respectively [12]. The ^{1}H NMR was very similar to that for **9** with three phenolic proton singlets (δ13.07, 10.82, 8.81), an isoflavone 2-H singlet (δ8.11), and a 2,2-dimethylpyranyl ring [AB system, δ6.67, 5.64 (J = 10 Hz); 6H singlet δ1.36] and a 3,3-dimethylallyl moiety. A structure homologous with **6** and **9** was indicated by the similar pattern of aromatic proton signals [δ6.87 (1H, d, J = 8 Hz), 6.46

(1H, *s*), 6.31 (1H, *d*, *J* = 8 Hz)], and angustone B was deduced to be 5,7,2'-trihydroxy-6-(3,3-dimethylallyl)-[6",6"-dimethylpyrano(2",3": 4,3')]isoflavone (**8**).

The ^{13}C NMR data (Table 1) for **8** was analysed by comparison with the data for **2**, **4**, **6** and **7**. The ring A and C carbon resonances of **7** were readily assigned by comparison with those of **1**, **3** and **5**, and the 2,2-dimethylpyranyl ring carbon resonances were assigned by comparison with lit. values [20, 26, 27]. Assignment of the ring B carbon resonances was based on calculated values. Substituent chemical shifts for the effect of a ring B fused 2,2-dimethylpyranyl ring were derived from lit. data for the ring A carbon resonances of **1** [15] and **12** [26, 27], and these shifts were applied to the ring B resonances of **3**. The calculated values are in good agreement with the observations (Table 1). The ring A and 3,3-dimethylallyl carbon resonances of **8** are comparable with those of the 6-prenyl compounds **2**, **4** and **6**, while the ring B and C and 2,2-dimethylpyranyl ring carbon resonances are essentially identical to those of **7**, in accordance with the proposed structure.

Due to the symmetrical pattern of the 2,2-dimethylpyranyl substituent shifts, an alternative 2',3'-fused structure could not be eliminated on the basis of the ^{13}C NMR data. A distinctive property of 2'-hydroxyisoflavones is oxidative cyclization to the corresponding coumaronochromone structure on treatment with a range of oxidizing agents including $K_3Fe(CN)_6$ [28, 29], lead tetraacetate [30] and Ag_2CO_3 [31]. The formation of products identified as the coumaronochromones **10** and **11** on treatment of **7** and **8** with DDQ provided confirmation of the presence of a free 2'-hydroxy group and hence a 3',4'-fused structure in these isoflavones. The distinctive pattern of spectroscopic properties characteristic of a coumaronochromone structure was first identified by Ollis and coworkers in the natural product lisetin [28, 29], and several coumaronochromones related to the lupin isoflavones have been recently reported from the roots of *Lupinus albus* [33].

The product of DDQ treatment of **7** was crystalline, and of molecular formula $C_{20}H_{14}O_6$ by MS, corresponding to the loss of H_2. The base peak in the mass spectrum at *m/z* 335 $[M-CH_3]^+$ suggested the 2,2-dimethylpyranyl ring remained intact [13]. Ions of *m/z* 198 $[C_{13}H_{10}O_2]^+$ and *m/z* 153 $[C_7H_5O_4]^+$ were observed, corresponding to retro Diels-Alder fragments with charge retention on ring B and ring A, respectively, and indicating dehydrogenation had taken place in the ring B/C region. The ^1H NMR spectrum showed the characteristic features of a coumaronochromone [28, 29] with no isoflavone H-2 signal, and the H-6' (isoflavone numbering) doublet shifted to low field (δ7.73, *d*, *J* = 8 Hz). The signals for the remaining aromatic protons [δ6.91 (*d*, *J* = 8 Hz), 6.53 (*d*, *J* = 2 Hz), 6.36 (*d*, *J* = 2 Hz)] and the dimethylpyranyl ring [AB system, δ6.79, 5.85 (*J* = 10 Hz); 6H singlet δ1.60] indicated the structure was otherwise unaltered. The coumaronochromone structure **10** was further supported by the IR data (ν_{max} cm^{-1}: 1655 C=O) and UV data (λ_{max} nm: 237 sh, 254, 283 sh, 338) which are similar to those reported for the naturally occurring coumaronochromone lisetin (ν_{max} cm^{-1}: 1653; λ_{max} nm: 258, 284, 338 [28, 29]) and allow an alternative formulation as a coumestan to be excluded.

Reaction of **8** with DDQ gave a bis-dehydrogenation product of molecular formula $C_{25}H_{20}O_6$ by MS accurate mass assigned the structure **11**. The MS showed major ions corresponding to the sequential loss of two CH_3 moieties (*m/z* 401 $[C_{24}H_{17}O_6]^+$ (base peak), 193 $[C_{23}H_{14}O_6]^{2+}$), suggesting the presence of two 2,2-dimethylpyranyl rings. The ^1H NMR spectrum showed signals for two 2,2-dimethylpyranyl rings [δ6.75 (2H, *d*, *J* = 10 Hz), 5.85 (1H, *d*, *J* = 10 Hz), 5.70 (1H, *d*, *J* = 10 Hz), 1.52 (12H, *s*)]. A coumaronochromone structure was indicated by the absence of an isoflavone H-2 singlet, the downfield shift of the H-6' signal [δ7.73 (*d*, *J* = 8 Hz)] and the IR (ν_{max} cm^{-1}: 1652) and UV data (λ_{max} nm: 230, 273, 280 sh, 336) [28 29]. The observation of bathochromic shifts with NaOMe and $AlCl_3$ but not NaOAc in the UV spectrum were in keeping with a 1-hydroxycoumaronochromone structure with a 2,3-fused-2,2-dimethylpyranyl ring similar to the naturally occurring compound milletin (λ_{max} nm: 280, 354) [32]. Cyclodehydrogenation of angustone A (**6**) with DDQ similarly gave **11**, confirming the structural homology of angustone A and angustone B (**8**).

EXPERIMENTAL

Mps are uncorr. TLC systems: I $CHCl_3$–MeOH (19:1), II pentane–Et_2O–HOAc (75:25:3), III petrol–EtOAc (1:1) on silica gel 60 F_{254} aluminium sheets (Merck); IV H_2O–HOAc (2:1) on cellulose (Macherey-Nagel); V Me_2CO on polyamide (Wang). Spots were visualized with UV light (254 nm and 366 nm) or by spraying with Fast Blue B salt soln. HPLC: H_2O–MeOH on Whatman Partisil PXS 10/25 ODS and Whatman Partisil M9 10/25 ODS-2 columns, with UV detection at 254 nm. MS: EI (probe), 70 eV. ^1H NMR at 60 MHz were run on a Varian T-60 spectrometer; ^1H NMR at 79.5 MHz and ^{13}C NMR at 20 MHz were run on a Varian FT-80A spectrometer; δ rel. to TMS as int. standard. Petrol refers to the fraction bp 60–80°C. All evaporation of volatile material was performed under red. press.

Extraction and isolation. Ground dried roots (3.13 kg) of field grown *Lupinus angustifolius* cv. Uniharvest were extracted with 90% EtOH in a Soxhlet for 48 hr. Evaporation of the solvent yielded a gum which was partitioned (3 ×) between petrol and MeOH–H_2O (4:1). The MeOH–H_2O phase was evapd and the residue partitioned between *n*-BuOH and H_2O. Evaporation of the BuOH phase yielded a gum (75 g) which was chromatographed on silica gel (2 kg).

Fractions were collected following elution with petrol–Et_2O (4:1) (1.85 g), petrol–Et_2O (1:1) (1.14 g), Et_2O (9.95 g), Et_2O–MeOH (9:1) (8.38 g), Et_2O–MeOH (1:1) (14.46 g) and MeOH (31.8 g). **6**, **8** and **9** were isolated from the Et_2O fraction by CC on silica gel and Sephadex LH-20 as described [1]. The estimated total yields by TLC and HPLC of mixed fractions (% dry wt) were: **6**, 0.06; **8**, 0.003; **9**, 0.001.

Angustone A (**6**). Mp 159–160° (petrol–Et_2O) lit. 155–157° [8], 145–146° [10]. IR ν_{max}^{KBr} cm^{-1}: 1642 (C=O). UV λ_{max}^{MeOH} nm (log ε) 269 (4.43), 340 sh (3.3); $\lambda_{max}^{MeOH+NaOAc}$ nm 276, 340; $\lambda_{max}^{MeOH+NaOMe}$ nm: 284, 342; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 272.5, 310 sh, 364 sh. ^1H NMR (80 MHz, DMSO-d_6): δ13.09 (1H, *s*, OH), 10.78 (1H, *s*, OH) 9.23 (1H, *s*, OH), 8.18 (1H, *s*, OH), 8.09 (1H, *s*, H-2), 6.77 (1H, *d*, *J* = 8 Hz, H-6'), 6.47 (1H, *s*, H-8), 6.38 (1H, *d*, *J* = 8 Hz, H-5'), 5.19 (2H, *m*, H-2", H-2"'), 3.30 (4H, *m*, H-1", H-1"'), 1.74 (6H, *br s*, H-4", H-4"'), 1.65 (6H, *br s*, H-5", H-5"'). MS *m/z* (rel. int.): 422.1726 [M]$^+$ (70) (calc. for $C_{25}H_{26}O_6$ 422.1728), 379 $[C_{22}H_{19}O_6]^+$ (37), 367 $[C_{21}H_{19}O_6]^+$ (59), 351 $[C_{20}H_{13}O_6]^+$ (64), 323 $[C_{18}H_{11}O_6]^+$ (100), 311 $[C_{17}H_{11}O_6]^+$ (60), 203 $[C_{11}H_7O_4]^+$ (29), 165 $[C_8H_5O_4]^+$ (57).

Tetraacetate. (Py–Ac_2O) Mp 155–156° (EtOAc) lit. 127–129° [8]. UV λ_{max}^{MeOH} nm (log ε): 243.5 (4.44), 307 (3.80). ^1H NMR

(60 MHz, $CDCl_3$): δ 7.75 (1H, *s*, H-2), 7.19 (1, *s*, H-8), 7.12 (1H, *d*, J = 9 Hz, H-6'), 7.02 (1H, *d*, J = 9 Hz, H-5'), 5.04 (2H, *br m*, H-2'', H-2'''), 3.25 (4H, *br m*, H-1'', H-1'''), 2.40 (3H, *s*, AcO), 2.33 (3H, *s*, AcO), 2.26 (3H, *s*, AcO), 2.10 (3H, *s*, AcO), 1.69 (12H, *br s*, H-4'', H-4''', H-5'', H-5'''). MS *m/z* (rel. int.): 590 [M^+] (12), 549 (28), 548 (78), 507 (49), 506 (37), 464 (27), 463 (50), 451 (27), 447 (21), 421 (30), 409 (42), 407 (38), 367 (25), 366 (34), 365 (100), 351 (29), 349 (22), 323 (45), 311 (33), 219 (25), 203 (36), 165 (69).

Angustone B (**8**). Mp 160–161° (petrol–Et_2O). IR ν_{max}^{KBr} cm^{-1}: 1655 (C=O). UV λ_{max}^{MeOH} nm (log ε): 231 sh (4.36), 271 (4.51), 310 sh (3.98); $\lambda_{max}^{MeOH+NaOAc}$ nm: 277, 345 sh; $\lambda_{max}^{MeOH+NaOH}$ nm: 272, 344; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275.5, 315. ^{1}H NMR (80 MHz, DMSO-d_6): δ 13.07 (1H, *s*, OH), 10.82 (1H, *br s*, OH), 8.81 (1H, *br s*, OH), 8.11 (1H, *s*, H-2), 6.87 (1H, *d*, J = 8 Hz, H-6'), 6.67 (1H, *d*, J = 10 Hz, H-4''), 6.46 (1H, *s*, H-8), 6.31 (1H, *d*, J = 8 Hz, H-5'), 5.64 (1H, *d*, J = 10 Hz, H-3''), 5.17 (1H, *t*, J = 7 Hz, H-2'''), 3.24 (2H, *d*, J = 7 Hz, H-1'''), 1.71 (3H, *s*, H-4'''), 1.60 (3H, *s*, H-5'''), 1.36 (6H, *s*, H-5'', H-6''). MS *m/z* (rel. int.): 420.1565 [M]$^+$ (28) (calc. for $C_{25}H_{24}O_6$ 420.1571), 405 [$C_{24}H_{21}O_6$]$^+$ (100), 349 [$C_{20}H_{13}O_6$]$^+$ (23), 185 [$C_{12}H_9O_2$]$^+$ (29), 175 [$C_{20}H_{14}O_6$]$^+$ (13), 165 [$C_8H_5O_4$]$^+$ (15).

Triacetate. (Py Ac_2O) UV λ_{max}^{MeOH} nm (log ε): 250.5 (4.67), 309 (3.92). MS *m/z* (rel. int.): 546 [M]$^+$ (22), 504 (31), 489 (42), 448 (31), 447 (100), 405 (28), 348 (33), 185 (35).

Angustone C (**9**). Mp 178–180° (petrol Et_2O). IR λ_{max}^{KBr} cm^{-1}: 1649 (C=O). UV λ_{max}^{EtOH} nm (log ε): 226 (4.36), 288 (4.99), 342 infl (3.56); $\lambda_{max}^{EtOH+NaOAc}$ nm: 288; $\lambda_{max}^{EtOH+NaOH}$ nm: 298; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 293. ^{1}H NMR (80 MHz, DMSO-d_6): δ 13.27 (1H, *s*, OH), 9.32 (1H, *s*, OH), 8.32 (1H, *s*, OH), 8.13 (1H, *s*, H-2), 6.78 (1H, *d*, J = 8 Hz, H-6'), 6.62 (1H, *d*, J = 10 Hz, H-4''), 6.45 (1H, *s*, H-8), 6.40 (1H, *d*, J = 8 Hz, H-5'), 5.76 (1H, *d*, J = 10 Hz, H-3''), 5.21 (1H, *t*, J = 7 Hz, H-2'''), 3.29 (2H, *d*, J = 7 Hz, H-1'''), 1.72 (3H, *s*, H-4'''), 1.62 (3H, *s*, H-5'''), 1.42 (6H, *s*, H-5'', H-6''). MS *m/z* (rel. int.): 420.1560 [M]$^+$ (34) (calc. for $C_{25}H_{24}O_6$ 420.1571), 405 (76), 377 (21), 365 (16), 349 (34), 203 (100).

Triacetate. (Py-Ac_2O) UV λ_{max}^{MeOH} nm (rel. int.): 264.5 (100), 327 (19). MS *m/z* (rel. int.): 546 [M]$^+$ (7), 505 (22), 504 (63), 489 (51), 462 (47),461 (33), 448 (28), 447 (100), 470 (24), 419 (43), 405 (33), 403 (22), 349 (21), 203 (70).

DDQ cyclization of licoisoflavone B. (**7**) (10 mg) and DDQ (8 mg) were heated to reflux in toluene (3 ml) for 30 min. The mixture was cooled and filtered, and the filtrate evapd: CC on Sephadex LH-20 with petrol–$CHCl_3$–EtOH (13:6:1) gave **10** (8.3 mg). Mp 254–256° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 1655 (C=O). UV λ_{max}^{EtOH} nm (log ε) 237 sh (4.33), 254 (4.51), 283 sh (3.88), 338 (4.01); $\lambda_{max}^{EtOH+NaOAc}$ nm: 251, 268 sh, 345; $\lambda_{max}^{EtOH+NaOH}$ nm: 247, 268, 314, 348; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 238, 266, 316, 376. ^{1}H NMR (60 MHz, $CDCl_3$–CD_3OD): δ 7.73 (1H, *d*, J = 8 Hz, H-6'), 6.91 (1H, *d*, J = 8 Hz, H-5'), 6.79 (1H, *d*, J = 10 Hz, H-4''), 6.53 (1H, *d*, J = 2 Hz, H-8), 6.36 (1H, *d*, J = 2 Hz, H-6), 5.85 (1H, *d*, J = 10 Hz, H-3''), 1.60 (6H, *s*, H-5'', H-6''). MS *m/z* (rel. int.): 350.0791 [M]$^+$ (30) (calc. for $C_{20}H_{14}O_6$ 350.0789), 335 [$C_{19}H_{11}O_6$]$^+$ (100), 198 [$C_{13}H_{10}O_2$]$^+$ (8), 153 [$C_8H_5O_4$]$^+$ (5).

DDQ cyclization of **8**. A mixture of **8** (25 mg) and DDQ (33 mg) in toluene (5 ml) was heated to reflux under N_2 for 30 min. The mixture was cooled and filtered, and the filtrate evapd. CC on silica gel with petrol Et_2O (19:1) afforded **11** (7.5 mg). Mp 222–223° (EtOH). IR ν_{max}^{KBr} cm^{-1}: 1652 (C=O). UV λ_{max}^{EtOH} nm (log ε): 230 (4.49), 273 (4.73), 280 sh (4.71), 336 (4.11); $\lambda_{max}^{EtOH+NaOAc}$ nm: 273, 281 sh, 336; $\lambda_{max}^{EtOH+NaOH}$ nm: 248 sh, 277, 376; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 275, 280, 335 sh. ^{1}H NMR (60 MHz, $CDCl_3$–CD_3OD): δ 7.73 (1H, *d*, J = 8 Hz, H-6'), 6.88 (1H, *d*, J = 8 Hz, H-5'), 6.75 (2H, *d*, J = 10 Hz, H-4'', H-4'''), 6.51 (1H, *s*, H-8), 5.85 (1H, *d*, J = 10 Hz, H-3''), 5.70 (1H, *d*, J = 10 Hz, H-3'''), 1.52 (12H, *s*, H-5'', H-6'', H-5''', H-6'''). MS *m/z* (rel. int.): 416.1249 [M]$^+$ (38) (calc. for $C_{25}H_{20}O_6$ 416.1259), 402 (29), 401 [$C_{24}H_{17}O_6$]$^+$ (100), 193 [$C_{23}H_{14}O_6$]$^{2+}$ (37).

DDQ cyclization of **6**. A mixture of **6** (50 mg) and DDQ (100 mg) in toluene (5 ml) was heated to reflux under N_2 for 30 min. The mixture was cooled and filtered, and the filtrate evapd. CC on silica gel with petrol–Et_2O (19:1) afforded **12** (10 mg). Mp (EtOH) and mmp, IR, UV, ^{1}H NMR, and MS data and R_f on TLC in systems I, II and III identical to that for material prepared from **8**.

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REFERENCES

1. Sutherland, O. R. W., Lane, G. A. and Skipp, R. A. *J. Chem. Ecol.*, in press.
2. Fukui, H., Egawa, H., Koshimizu, K. and Mitsui, T. (1973) *Agric. Biol. Chem.* **37**, 417.
3. Harborne, J. B., Ingham, J. L., King, L. and Payne, M. (1976) *Phytochemistry* **15**, 1485.
4. Ingham, J. L., Keen, N. T. and Hymowitz, T. (1977) *Phytochemistry* **16**, 1943.
5. Schroder, G., Zahringer, U., Heller, W., Ebel, J. and Grisebach, H. (1979) *Arch. Biochem. Biophys.* **194**, 635.
6. Ingham, J. L. and Dewick, P. M. (1980) *Z. Naturforsch.* **35c**, 197.
7. Ingham, J. L., Tahara, S. and Harborne, J. B. (1983) *Z. Naturforsch.* **38c**, 194.
8. Tahara, S., Ingham, J. L., Nakahara, S., Mizutani, J. and Harborne, J. B. (1984) *Phytochemistry* **23**, 1889.
9. Russell, G. B., Sutherland, O. R. W., Lane, G. A. and Biggs, D. R. (1979) In *Proceedings of the 2nd Australasian Conference on Grassland Invertebrate Ecology* (Crosby, T. K. and Pottinger, R. P., eds) p. 95. Government Printer, Wellington.
10. Baruah, P., Barua, N. C., Sharma, R. P., Baruah, J. N., Kulanthaivel, P. and Herz, W. (1984) *Phytochemistry* **23**, 443.
11. Markham, K. R. and Mabry, T. J. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds) p. 45. Chapman and Hall, London.
12. Mabry, T. J. and Markham, K. R. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds) p. 78. Chapman and Hall, London.
13. Drewes, S. E. (1974) *Chroman and Related Compounds*, p. 15. Verlag Chemie, Weinheim.
14. Kinoshita, T., Saitoh, T. and Shibata, S. (1978) *Chem. Pharm. Bull.* **26**, 141.
15. Wagner, H., Chari, V. M. and Sonnenbichler, J. (1976) *Tetrahedron Letters* 1799.
16. Pelter, A., Ward, R. S. and Gray, T. I. (1976) *J. Chem. Soc. Perkin I* 2475.
17. Pelter, A., Ward, R. S. and Bass, R. J. (1978) *J. Chem. Soc. Perkin I* 666.
18. Wehrli, F. W. (1973) *J. Chem. Soc. Chem. Commun.* 379.
19. Norton, R. S. and Wells, R. J. (1982) *J. Am. Chem. Soc.* **104**, 3628 and references cited therein.
20. Wenkert, E. and Gottlieb, H. E. (1977) *Phytochemistry* **16**, 1811.
21. Chari, V. M. and Wagner, H. (1979) *Recent Advances in Phytochemistry*, Vol. 12 *Biochemistry of Plant Phenolics* (Swain, T., Harborne, J. B. and Van Sumere, C. F., eds) p. 29.

Plenum Press, New York.
22. Jackson, B., Owen, P. J. and Scheinmann, F. (1971) *J. Chem. Soc. C* 3389.
23. Chibber, S. S. and Sharma, R. P. (1980) *Phytochemistry* **19**, 1857.
24. Martinez Olivares, E., Lwande, W., Delle Monache, F. and Marini Bettolo, G. B. (1982) *Phytochemistry* **21**, 1763.
25. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
26. Vilain, C. and Jadot, J. (1977) *Bull. Soc. Chim. Belg.* **86**, 473.
27. Vilain, C. and Jadot, J. (1979) *Bull. Soc. Chim. Belg.* **88**, 273.
28. Falshaw, C. P., Ollis, W. D., Moore, J. A. and Magnus, K. (1966) *Tetrahedron* Suppl. No. 7, 333.
29. Falshaw, C. P. and Ollis, W. D. (1966) *Chem. Commun.* 305.
30. Kurosawa, K. and Araki, F. (1979) *Bull. Chem. Soc. Japan* **52**, 529.
31. Antus, S. and Nógrádi, M. (1979) *Acta Chim. Acad. Sci. Hung.* **100**, 179.
32. Subba Raju, K. V., Srimannarayana, G., Ternai, B., Stanley, R. and Markham, K. R. (1981) *Tetrahedron* **37**, 957.
33. Tahara, S., Ingham, J. L. and Mizutani, J. (1985) *Agric. Biol. Chem.* **49**, 1775.