Naturally Occurring Coumaranochroman-4-ones: a New Class of Isoflavonoids from Lupins and Jamaican Dogwood

Satoshi Tahara, Masaaki Moriyama, Sunao Orihara, John L. Ingham*, Jun Kawabata, and Junya Mizutani

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan
* Department of Food Science, University of Reading, P. O. Box 226, Whiteknights, Reading RG 6 2AP, England

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Leguminosae, *Lupinus*, *Piscidia erythrina*, Isoflavonoids, Coumaranochroman-4-ones, Coumaronochromones

A further investigation of the isoflavonoid components of *Piscidia erythrina*, *Lupinus albus* and *L. luteus* has revealed the presence of 3-hydroxy- and 3-methoxy-coumaranochroman-4-ones. These compounds are the first known representatives of a new type of naturally occurring isoflavonoid. Using a combination of chemical and spectroscopic methods, the compounds were identified as 3,5,7,4′-tetrahydroxy-5′-methoxy-3′-(3,3-dimethylallyl)coumaranochroman-4-one (piscerythrol, **1**), 3,5,7,4′-tetrahydroxy-6,3′-di(3,3-dimethylallyl)coumaranochroman-4-one (lupinol A, **3**), 5,7,4′-trihydroxy-3-methoxy-6,3′-di(3,3-dimethylallyl)-coumaranochroman-4-one (lupinol B, **6**) and 3,5,7,4′-tetrahydroxy-6-(3,3-dimethylallyl)-coumaranochroman-4-one (lupinol C, **7**). All four isoflavonoids were found to have the *cis* relative configuration at C-2/C-3. Possible biogenetic relationships between the coumaranochroman-4-ones and other isoflavonoids (isoflavones and coumaronochromones) found in *Piscidia* and *Lupinus* are briefly discussed.

Introduction

Our earlier studies on the isoflavonoids of *Lupinus albus* (white lupin) and *L. luteus* (yellow lupin) resulted in the identification of various simple isoflavones with 5,7,4′-, 5,7,2′,4′- or 5,7,3′,4′-oxygenation [1–3]. A range of complex isoflavones with prenyl groups at C-6, C-8 and/or C-3′ also occur in *Lupinus* [1–6]. In *L. albus* and *L. luteus*, these compounds are variously accompanied by isoflavones with prenyl-related side structures, and by the coumaronochromones lupilutin (*L. luteus* [4]) and lupinalbins A–G [5–7]. The Jamaican dogwood (*Piscidia erythrina*) has also proved to be a rich source of isoflavonoids, yielding simple and complex isoflavones [8–11], a variety of rotenoids [8, 12], and the coumaronochromones lisetin **8** [8], the first compound of this type to be recognized as a natural product and 8-prenyl-lisetin [11].

During a further chemical examination of *Lupinus* and *Piscidia*, we encountered four closely related acid-labile compounds which appeared to be examples of a new class of isoflavonoid. All four compounds gave a UV (MeOH) maximum between 295 and 310 nm, and a prominent MS fragment corresponding to either M^+-H_2O or M^+-CH_3OH. None of the compounds afforded ^1H NMR signals attributable either to the isoflavone 2-H (δ 7.8–8.1 s in $CDCl_3$ [13]; δ 7.7–8.3 s in acetone-d_6 [2, 5, 14]) or the rotenoid 6-Ha/Hb (δ 4.1–4.6 dd in $CDCl_3$ [15]; δ 4.3–4.6 dd in acetone-d_6 [12]). However, their close chemical relationship to coumaronochromones was demonstrated by acid-conversion of the *Piscidia*-derived compound (piscerythrol) to lisetin (**8**).

This paper presents evidence to show that the new isoflavonoids are 3-hydroxy(or 3-methoxy)-coumaranochroman-4-ones possessing structures **1** (piscerythrol), **3** (lupinol A), **6** (lupinol B) and **7** (lupinol C). In each case the relative stereochemistry (C-2/C-3) was deduced to be *cis*. Coumaranochroman-4-ones appear to represent a biogenetic link between the 2′-hydroxyisoflavones and coumaronochromones found in *L. albus*, *L. luteus* and *P. erythrina* (see Fig. 1 for general ring structures and numbering systems).

Reprint requests to Dr. S. Tahara or Dr. J. L. Ingham.

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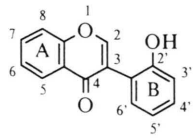
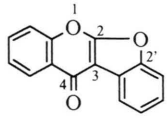
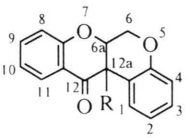


2'-Hydroxyisoflavone

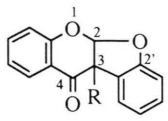


Coumaronochromone



Rotenoid (R=H)

12a-Hydroxyrotenoid (R=OH)



3-Hydroxycoumarano-chroman-4-one (R=OH)

3-Methoxycoumarano-chroman-4-one ($R=OCH_3$)

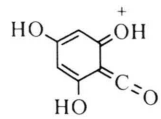
Fig. 1. Ring systems of 2'-hydroxyisoflavones, coumaronochromones, rotenoids and coumaranochroman-4-ones (coumaronochromones and coumaranochroman-4-ones are numbered as shown for 2'-hydroxyisoflavones).

Results and Discussion

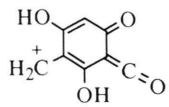
Piscerythrol (**1**) from *P. erythrina* was found by FD-MS to have a mol. wt. of 400 corresponding to $C_{21}H_{20}O_8$.

5,7-Dihydroxylation of ring A was evident from the bathochromic shifts of the UV (MeOH) maximum at 296 nm caused by addition of $AlCl_3$ (+ 20 nm) and NaOAc (+ 46 nm), respectively [16], and from the MS fragment at *m/z* 153 (**a**) [17]. In the ^1H NMR spectrum of piscerythrol, a pair of *meta*-coupled aromatic protons (δ 5.99 d and δ 6.00 d, $J = 2.2$ Hz) were assigned to 6-H/8-H (Table I) to give an A-ring part structure identical with that found in many other *Piscidia* isoflavonoids [8–11, 14]. Signals attributable to a prenyl (3,3-dimethylallyl) side-chain (Table I), a methoxyl substituent (δ 3.78 s), and a single aromatic proton (δ 6.84 s) were also observed, these being provisionally located on ring B together with an OH group.

In addition to the prenyl and methoxyl carbons, the ^{13}C NMR spectrum (Table II) revealed only three non-aromatic carbons at δ 193.2 (C=O), 110.9 (acetal CH) and 79.8 (oxygenated C). All were assigned to the heterocyclic proton of the molecule along with the singlet proton at δ 6.23 (Table I). The significance of the oxygenated non-aromatic carbon (δ 79.8) was clear from the MS which contained a major dehydration signal (*m/z* 382; $M^+ - H_2O$, 63%) indicative of a tertiary alcohol (C–OH) arrangement as in the 6a-hydroxypterocarpans [*e.g.* 3,6a,9-trihydroxypterocarpan, M^+ 272 (100%), $M^+ - H_2O$ (*m/z* 254, 40%)] [18].



a (*m/z* 153)



b (*m/z* 165)

When treated with H_2SO_4 in toluene (see Experimental), piscerythrol was rapidly dehydrated to afford a product indistinguishable (UV, MS, ^1H NMR, TLC) from an authentic sample of the *Piscidia* coumaronochromone lisetin (**8**) [8]. Smaller quantities of cyclo-lisetin (**9**) were also isolated from the reaction mixture. Thus, formation of compounds **8** and **9** indicates that piscerythrol has the B-ring part structure shown in **1** with the tertiary alcohol group located (C-3) in a position analogous to that of 12a-hydroxyrotenoids [12]. Supporting evidence for **1** was provided by the COLOC NMR spectrum which showed cross peaks between 2-H and C-1' (*via* 3 bonds, $H-C_2-C_3-C_{1'}$), between 2-H and C-2' (*via* 3 bonds, $H-C_2-O-C_{2'}$), and between 2-H and C-9 (*via* 3 bonds, $H-C_2-O-C_9$). In contrast to lisetin (**8**), the 5-OH and 6'-H NMR signals of piscerythrol were detected at higher than expected field (δ 11.94 and 6.84 respectively, *cf.* lisetin, 5-OH at δ 13.00 and 6'-H at δ 7.38 [8]). This difference may be due to the fact that in piscerythrol the effect of the C=O group (C-4) on both 5-OH and 6'-H is modified by the 3-OH which could H-bond to the carbonyl oxygen.

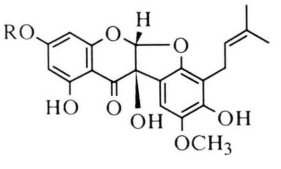
The relative stereochemistry at C-2/C-3 of piscerythrol was considered to be *cis* from the detection of an NOE between 2-H (δ 6.18 s) and 3-OH (δ 4.82 s) in the ^1H NMR spectrum of 7-O-methylpiscerythrol (**4**) determined in benzene-d_6. The absolute stereochemistry of piscerythrol remains to be firmly established.

Our studies on the isoflavonoids of *Lupinus albus* (white lupin) and *L. luteus* (yellow lupin) have shown that these species also contain coumaranochroman-4-ones. Lupinol A (**3;** mol. wt. 438, $C_{25}H_{26}O_7$) and lupinol B (**6;** mol. wt. 452,

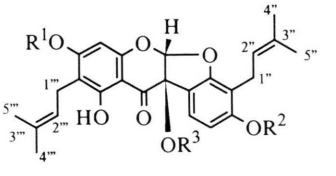
$C_{26}H_{28}O_7$) from white lupin roots were 5,7-dihydroxy (UV shifts with $AlCl_3$ and NaOAc respectively [16]) coumaranochroman-4-ones, each being found to possess two prenyl substituents. One of the prenyls was assigned to ring A from the ion at *m/z* 165 (**b**) in the MS of each compound. Lupinol B differed spectroscopically from lupinol A in two important respects. First, the ^1H NMR spectrum of lupinol B contained a high field 3H signal (δ 3.43 s, $-OC\underline{H}_3$) attributable to a non-aromatic methoxyl group. Secondly, the MS of lupinol B afforded a major fragment at M^+-CH_3OH (*m/z* 420) rather than at M^+-H_2O as in lupinol A. These observations suggested that lupinol B (mol. wt. = lupinol A+CH_2) was the 3-O-methyl derivative of lupinol A (*cf.* the previously recognized series of 12a-hydroxy- and 12a-methoxy-rotenoids [19]). Supporting evidence for 3-methoxylation was provided by the ^{13}C NMR data obtained for lupinol B which indicated that C-3 (δ c 84.4) was shifted to lower field by 5 ppm when compared with that of lupinol A (Table II). An OH group, the second prenyl substituent, and two aromatic protons (δ 6.50/6.55 and δ 7.06/7.12, $J = 8.2$ Hz) were all provisionally assigned to ring B of lupinols A and B.

Upon treatment with H_2SO_4/toluene at room temperature, lupinol A gave the cyclo-dehydration products **10, 11,** and **12** (all coumaronochromones). Similarly, when heated (60 °C) with *p*-TsOH, lupinol B yielded **10** and **13** by methanol elimination and cyclization. Structures **10–13** were deduced from UV and MS data, and from a detailed ^1H NMR comparison with the known *Lupinus* coumaronochromones lupinalbin F (**16**).

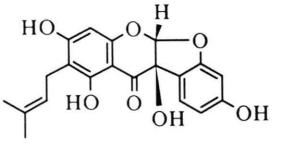
[5,7,4′-trihydroxy-6,3′-di(3,3-dimethylallyl)-coumaronochromone: 8-H, δ 6.67 s; 5′-H, δ 7.03 d $J = 8.3$ Hz; 6′-H, δ 7.66 d $J = 8.3$ Hz] [5] and lupilutin (**17**) [5,7,4′-trihydroxy-8-(3-hydroxy-3-methylbutyl)coumaronochromone: 6-H, δ 6.42 s] [4]. This allowed the signal at δ 6.68 s in the ^1H NMR spectrum of **12** to be readily assigned (8-H), and confirmed that ring B in all four compounds was underivatized at 5′- and 6′-H (*o*-coupled doublets be-



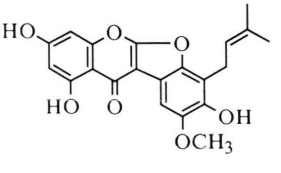
1: R=H, Piscerythrol
2: R=CH_3, 7-*O*-Methylpiscerythrol



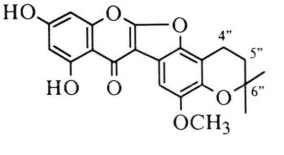
3: $R^1=R^2=R^3=H$, Lupinol A
4: $R^1=CH_3$; $R^2=R^3=H$, Monomethyl-lupinol A
5: $R^1=R^2=CH_3$; $R^3=H$, Dimethyl-lupinol A
6: $R^1=R^2=H$; $R^3=CH_3$, Lupinol B



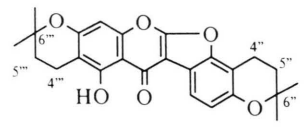
7: Lupinol C



8: Lisetin



9: Cyclo-lisetin



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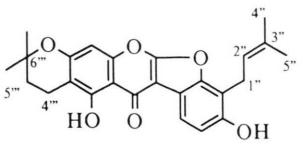

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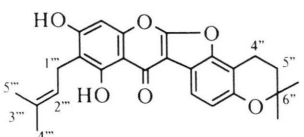

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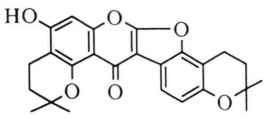

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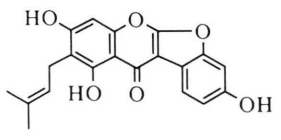
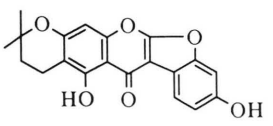
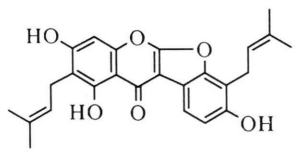


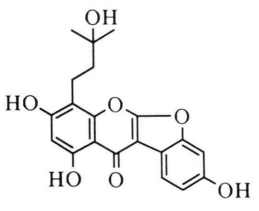
14: Lupinalbin B



15: Cyclo-lupinalbin B



16: Lupinalbin F



17: Lupilutin

tween δ 6.88 and 7.71). The prenyl side-chain on ring A of lupinols A and B must therefore be located at C-6, with prenylation on ring B occurring at C-3′ since oxygenation (OH) at C-4′ can be assumed. Lupinols A and B are thus represented by structures **3** and **6** respectively. The *Lupinus* isoflavones lupalbigenin and 2′-hydroxylupalbigenin (= angustone A) are also known to be prenylated at both C-6 and C-3′ [1, 2, 20].

The relative configuration at C-2/C-3 of **3** and **6** was established as *cis* using the NOE method applied to piscerythrol. Methylation of lupinol A yielded a mixture of the 7-O-methyl (**4**) and 7,4′-di-O-methyl (**5**) derivatives which were separated by PTLC. Irradiation of the 3-OH (δ 4.82) of **4** in benzene-d_6 resulted in a celar NOE on the 2-H indicative of the *cis* stereochemistry. The NOE between 2-H (δ 6.40) and 3-OCH_3 (δ 3.43) of lupinol B was detected by NOE difference spectroscopy and NOESY in acetone-d_6.

The third *Lupinus* 5,7-dihydroxycoumaranochroman-4-one (lupinol C, **7**) was isolated from leaf washings of *L. luteus*. MS analysis gave the mol. wt. as 370 ($C_{20}H_{18}O_7$; = lupinol A minus one prenyl group), and the expected dehydration peak at *m/z* 352. When allowed to stand for 3 h at room temperature with H_2SO_4/toluene, lupinol C was converted to the cyclo-derivative (**15**) of the known *Lupinus* coumaronochromone lupinalbin

Table I. ^1H NMR data (δ values) for the coumaranochroman-4-ones (**1, 3, 6** and **7**). Spectra were recorded in acetone-d_6 at either 270 MHz (lupinols A and C) or 500 MHz (piscerythrol and lupinol B). Coupling constants are given in Hz. TMS was the internal standard.

Proton	Piscerythrol (**1**)	Lupinol A (**3**)	Lupinol B (**6**)	Lupinol C (**7**)
2-H	6.23 s	6.25 s	6.40 s	6.24 s
5-OH	11.94 s	12.19 s	12.27 s	12.15 s
6-H	5.99 d, J = 2.2			
8-H	6.00 d, J = 2.2	6.08 s	6.10 s	6.08 s
3′-H				6.43 d, J = 2.2
5′-H		6.50 d, J = 8.2	6.55 d, J = 8.2	6.55 dd, J = 8.2, 2.2
6′-H	6.84 s	7.06 d, J = 8.2	7.12 d, J = 8.2	7.24 d, J = 8.2
1″-H_2	3.33 d, J = 7.1 (2H)	3.31 m (4H)	3.29 m (2H)	3.22 d, J = 7.2 (2H)
1‴-H_2			3.22 d, J = 7.2 (2H)	
2″-H	5.29 t-like, J = 7.1	5.21 m (2H)	5.26 t-like, J = 7.3	5.17 t-like, J = 7.2
2‴-H			5.17 t-like, J = 7.2	
4″-H_3	1.75 s (3H)	1.73 s (6H)	1.74 s (3H)	1.73 s (3H)
4‴-H_3			1.72 s (3H)	
5″-H_3	1.64 s (3H)	1.63 s (3H)	1.63 s (3H)	1.61 s (3H)
5‴-H_3		1.62 s (3H)	1.61 s (3H)	
3-OCH_3			3.43 s (3H)	
5′-OCH_3	3.78 s (3H)			

Table II. ^{13}C NMR data (δ values) for coumaranochroman-4-ones (**1, 3, 6** and **7**). Spectra (DEPT or INEPT) were recorded in acetone-d_6 at either 67.5 MHz (lupinol C) or 125 MHz (other compounds). Assignments for chemical shifts with the same note are interchangeable.

Carbon	Piscerythrol (**1**)	Lupinol A (**3**)	Lupinol B (**6**)	Lupinol C (**7**)
C-2	110.9	111.3	109.4	110.6[e]
C-3	79.8	79.3	84.4	78.8
C-4	193.2	193.5	192.1	193.4
C-5	168.4	165.8	166.2	165.8
C-6	97.8[a]	110.3[b]	110.5	110.5[e]
C-7	165.6	162.6	162.7	162.6
C-8	96.6[a]	96.0	96.0	96.1
C-9	161.0	159.8[c]	160.3[d]	161.8[f]
C-10	100.6	100.5	101.8	100.6
C-1′	115.8	118.4	114.9	118.6
C-2′	153.5	158.9[c]	159.3[d]	161.7[f]
C-3′	112.7	112.6	112.5	98.9
C-4′	148.1	158.5[c]	158.2[d]	158.4
C-5′	144.1	110.2[b]	110.3	111.6
C-6′	105.9	123.5	125.0	126.7
C-1″	23.5	23.2	23.2	21.6
C-1‴		21.6	21.6	
C-2″	122.4	122.8	122.7	123.1
C-2‴		123.1	123.0	
C-3″	132.4	132.1	132.2	131.7
C-3‴		131.6	132.2	
C-4″	17.9	17.9	17.9	17.8
C-4‴		17.8	17.8	
C-5″	25.8	25.8	25.8	25.8
C-5‴		25.8	25.8	
C-3−OCH_3			53.9	
C-4′−OCH_3	56.6			

B (**14**) [7]. Thus, lupinol C can be assigned structure **7.**

The relative stereochemistry of lupinol C was considered to be *cis* from the general similarity in chemical shift values of 2-H and 6′-H when compared with those of lupinol A (Table I). It has previously been shown that *cis*- and *trans*-12a-hydroxyrotenoids can be differentiated by the chemical shift value of 1-H (= 6′-H in coumaranochroman-4-ones), which in *cis* compounds appears at considerably higher field than in the *trans* isomers [21]. A comparable situation with respect to the 6′-H signal of *cis*/*trans*-coumaranochroman-4-ones can also be envisaged in view of their structural similarity to 12a-hydroxyrotenoids.

Our earlier studies have shown that the effect of *p*(3′)-prenylation on the 6′-H signal is about −0.16 to −0.20 ppm (*cf.* luteone, 6′-H at δ 7.12 [7] → 2′-hydroxylupalbigenin [*p*-prenyl], 6′-H at δ 6.92 [2], and lupinalbin B, 6′-H at δ 7.82 [7] → lupinalbin F [*p*-prenyl], 6′-H at δ 7.66 [5]). It is significant, therefore that the 6′-H signal of lupinol C appears at δ 7.24 (Table I), within the δ 7.22−7.26 range (δ 7.06 of lupinol A, plus a shift effect of +0.16 to +0.20 ppm due to the absence of *p*-prenylation) which would be expected if the configuration around C-2, C-3 and C-6′ was the same *(cis)* as in lupinol A.

Two classes of isoflavonoids (6a-hydroxypterocarpans and 12a-hydroxyrotenoids) with a tertiary alcohol group at the position corresponding to C-3 of coumaranochroman-4-ones have been described [22]. In both cases the hydroxyl group is thought to be inserted at a stage in biosynthesis following construction of the basic (tetracyclic) ring system [23]. Although coumaranochroman-4-ones may be 3-hydroxylated in a comparable manner, it is possible that compounds **1, 3** and **7** could arise *via* 2,3-epoxidation of an isoflavone (Fig. 2), subsequent interaction between C-2 and

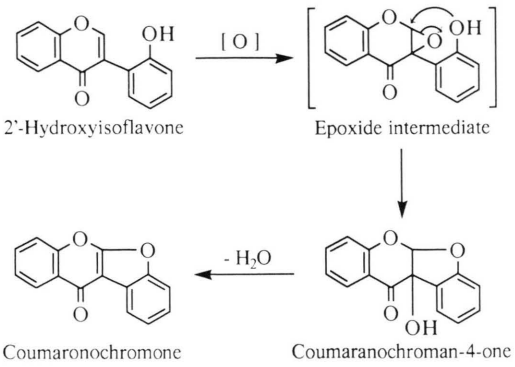


Fig. 2. Proposed pathway for the biosynthesis of coumaranochroman-4-ones and coumaronochromones.

the 2′-OH yielding the hydroxy-dihydrofuran ring (*e.g.* 2′-hydroxylupalbigenin [isoflavone] [2, 20] → lupinol A (**3**) in *L. albus,* and luteone [isoflavone] [24] → lupinol C (**7**) in *L. luteus*).

Because of their ease of dehydration, 3-hydroxy-coumaranochroman-4-ones may also represent a biosynthetic link between 2′-hydroxyisoflavones and correspondingly substituted coumaronochromones (*e.g.* in *P. erythrina:* piscerythrone [isoflavone] → piscerythrol (**1**) → lisetin (**8**) [coumaronochromone]). Similar sequences could account for the formation of *Lupinus* coumaronochromones which typically co-occur with their isoflavone analogues (*e.g.* 2′-hydroxygenistein/luteone/lupinisoflavone B/licoisoflavone A/lupinisoflavone D/2′-hydroxylupalbigenin [isoflavones] → lupinalbins A–F respectively [coumaronochromes]) [1, 2, 5, 7].

Experimental

General procedures

Analytical and preparative TLC (PTLC) separations were carried out using Merck pre-coated silica gel 60 plates (F-254, layers thickness, 0.25 or 0.50 mm). The developing solvent systems were as follows, (a) $CHCl_3$–MeOH (CM), (b) $CHCl_3$–acetone-conc. aqueous NH_3 (CAAm), (c) *n*-hexane–EtOAc (HE), (d) *n*-hexane–EtOAc–HCOOH (HEF), and (e) *n*-pentane–diethyl ether–AcOH (PEeAa). Details relating to the composition of solvent systems are given at the appropriate point(s) in the text. All compounds were eluted from chromatograms with EtOAc. Detection of isoflavonoids on developed thin-layer plates was by inspection under long (365 nm) and short (254 nm) wavelength UV light, and by the characteristic colours formed upon spraying with Gibbs reagent. FD-MS spectra were recorded on a JEOL JMS-OlSG-2. UV spectra were determined on a Hitachi Model U 3210 in methanol with or without a shift reagent [16]. NMR data (^1H, ^{13}C, NOE, NOESY and COLOC) were obtained using a Bruker AM 500 or a JEOL JNM-FX-270, in acetone-d_6 or benzene-d_6 with TMS as the internal standard.

Isolation of coumaranochroman-4-ones

a) Piscerythrol (**1**)

This coumaranochroman-4-one was initially detected in *Piscidia* column fractions FFr-9 to FFr-12 [11, 12] as a spot which fluoresced dull green on TLC plates viewed under $UV_{365\ nm}$ light. The combined column fractions were first chromatographed (PTLC) in CM (25:1), the material at R_f 0.20–0.30 then being eluted and successively re-chromatographed in $CHCl_3$-acetone (7:6) fumed with NH_3 vapour (R_f 0.00–0.35), and $CHCl_3$–EtOAc-acetone-MeOH (50:5:5:1, R_f 0.45) to yield 105 mg of pure piscerythrol as a pale yellow amorphous powder.

b) Lupinols A (**3**) *and B* (**6**)

Both compounds were isolated from column fractions obtained during the course of earlier work on *L. albus* (*cv.* Kievskij Mutant) [5]. Lupinol A, a major constituent of Fr″-27 [5], was detected on PTLC plates developed in CAAm (35:50:1) as a dark purple fluorescent band ($UV_{365\ nm}$ light) at R_f 0.70. Elution and re-PTLC in CM (25:1, R_f 0.49) gave 14 mg of lupinol A as a pale yellow gum.

Lupinol B was isolated from column fraction Fr′-3 [5], and ran at about R_f 0.60 (PTLC) in CAAm (70:100:3). Like **3,** lupinol B fluoresced dark purple under $UV_{365\ nm}$ light. Elution of the CAAm band and re-PTLC in CM (25:1) yielded the known isoflavone angustone B [5, 20] at approx. R_f 0.90, and lupinol B (2.1 mg) at approx. R_f 0.80. Further quantities of lupinol B (21.5 mg) were also isolated from column fraction Fr-5 [5] as a pale brown oil.

c) Lupinol C (**7**)

Fresh leaves of *L. luteus* (*cv.* Barpine; 12.6 kg) were washed with MeOH for 20–30 sec. The neutral and phenolic constituents (approx. 8 g) extractable with EtOAc from the concentrated methanol washings were column chromatographed over 300 g of silica gel using mixtures of EtOAc and benzene as the eluting solvent. Initial elution of the charged column was with benzene only (Fr-1, 200 ml), followed successively with 5% EtOAc/benzene (Fr-2 to -10, 200 ml each) and 30% EtOAc/benzene (Fr-11 to -15, 200 ml each). Fraction Fr-12 was found to contain alpinumisoflavone, wighteone and parvisoflavone B, whilst Fr-13 and Fr-14 contained wighteone and luteone as major components. The two latter fractions were combined and re-chromatographed over 50 g of silica gel (eluting mixture, 25% EtOAc in benzene; each fraction 50 ml). TLC monitoring of the fractions revealed that in FFr-11 the major component (luteone) was accompanied by small quantities of a compound which fluoresced dark purple ($UV_{365\ nm}$ light). This minor compound (lupinol C, **7**) was separated from luteone by successive PTLC in CM (20:1), CAAm (70:60:1) and PEeAa (25:20:1): luteone, R_f 0.33, 0.50 and 0.16; lupinol C, R_f 0.28, 0.45 and 0.12 respectively. Lupinol C (**7**) was eventually obtained as a pale yellow gum (12 mg).

Physico-chemical properties of coumaranochroman-4-ones (**1, 3, 6** and **7**)

^1H and ^{13}C NMR data are shown in Tables I and II respectively.

Piscerythrol [*3,5,7,4'-tetrahydroxy-5'-methoxy-3'-(3,3-dimethylallyl)coumaranochroman-4-one,* **1**]. Pale yellow amorphous powder, m.p. 85–92 °C. $UV_{365\ nm}$ fluorescence: dull green. Gibbs test: (+), slow, purple → green-purple. FD-MS *m/z* (rel. int.): 401 (M^++1, 26), 400 (M^+, 100), 398 (17), 382 (12). EI-MS *m/z* (rel. int.): 383 (16), 382 (M^+-H_2O, 55), 365 (10), 328 (11), 327 (38), 326 (100), 297 (15), 283 (13), 231 (20), 153 (32), 149 (11), 69 (18), 57 (17), 55 (11). UV λ_{max} (nm), MeOH; 296 (ε = 17,400), 336; + NaOMe 253 sh, 336; +$AlCl_3$ 316; +NaOAc 253 sh, 342 (H_3BO_3 regenerated the MeOH spectrum).

Lupinol A [*3,5,7,4'-tetrahydroxy-6,3-di(3,3-dimethylallyl)coumaranochroman-4-one,* **3**]. Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (+), green-blue → green. FD-MS *m/z* (rel. int.): 439 (M^++1, 33), 438 (M^+, 100). EI-MS *m/z* (rel. int.): 438 (M^+, 8), 421 (6), 420 (M^+-H_2O, 19), 378 (7), 377 (27), 366 (7), 365 (27), 222 (14), 221 (100), 218 (8), 166 (9), 165 (83), 163 (13), 162 (12), 69 (7), 55 (7). UV λ_{max} (nm), MeOH: 303 (ε = 15,700); +NaOMe 251, 347; +$AlCl_3$ 242 sh, 313; +NaOAc 250 sh, 343 (H_3BO_3 regenerated the MeOH spectrum).

Lupinol B [*5,7,4'-trihydroxy-3-methoxy-6,3'-di(3,3-dimethylallyl)coumaranochroman-4-one,* **6**]. Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (+), green-purple → green. FD-MS *m/z* (rel. int.): 454 (M^++2, 10), 453 (M^++1, 37), 452 (M^+, 100), 450 (12), 423 (12), 422 (15). EI-MS *m/z* (rel. int.): 421 (16), 420 (M^+–MeOH, 52), 419 (8), 418 (8), 405 (16), 403 (20), 401 (8), 378 (23), 377 (82), 366 (27), 365 (100), 364 (8), 363 (7), 321 (15), 309 (17), 165 (7), 155 (7), 69 (7). UV λ_{max} (nm), MeOH: 307 (ε = 16,700), 330 sh (br.); +NaOMe 251, 353; +$AlCl_3$ 240 sh, 308, 350 sh; +NaOAc 254 sh, 349 (H_3BO_3 regenerated the MeOH spectrum). NOEs between the two singlets assignable to 2-H (δ 6.40) and 3-OCH_3 (δ 3.43) were observed by NOE difference spectroscopy and NOESY in acetone-d_6.

Lupinol C [*3,5,7,4'-tetrahydroxy-6-(3,3-dimethylallyl)coumaranochroman-4-one,* **7**]. Pale yellow gum. $UV_{365\ nm}$ fluorescence: dark purple. Gibbs test: (+), blue → blue-green. FD-MS *m/z* (rel. int.): 372 (M^++2, 11), 371 M^++1, 42), 370 (M^+, 100). EI-MS *m/z* (rel. int.): 352 (M^+-H_2O, 18), 337 (13), 335 (34), 310 (9), 309 (45), 298 (11), 297 (100), 221 (18), 165 (32), 71 (8), 69 (8), 57 (16), 55 (12). UV λ_{max} (nm), MeOH: 227, 294 sh, 304 (ε = 15,300), 340 sh; +NaOMe 248, 350; +$AlCl_3$ 227, 306; +NaOAc 228, 250 sh, 291 sh, 344 (H_3BO_3 regenerated the MeOH spectrum).

Derivatization of coumaranochroman-4-ones and preparation of reference compounds

Dehydration of piscerythrol (**1**)

One drop of conc. H_2SO_4 was added to toluene (10 ml), and the mixture was then ultrasonicated to give an H_2SO_4/toluene suspension. Piscerythrol (5.5 mg) was dissolved in 0.5 ml of the H_2SO_4/toluene suspension and allowed to stand at room temperature for 4 h. The mixture was then diluted

with EtOAc and washed with brine. TLC analysis (HEF, 60:40:1) of the concentrated EtOAc fraction revealed two yellow fluorescent compounds (PDH-1 and PDH-2) at R_f 0.45 and R_f 0.41 respectively. Small quantities of unchanged piscerythrol (R_f 0.36) were also detected. The two yellow fluorescent compounds were isolated by PTLC in HEF (70:30:1) to give 2.1 mg of PDH-1 (40% yield) and 1.6 mg of PDH-2 (30% yield).

The former product (PDH-1) was indistinguishable from authentic lisetin (**8**) [8] in a ^{1}H NMR and TLC comparison. The chromatographic (TLC) and spectroscopic (EI-MS, ^{1}H NMR, UV) properties of PDH-2 were in good agreement with those of the acid-catalyzed cyclization product of lisetin prepared as described below.

Acid-catalyzed cyclization of lisetin (**8**)

A mixture of lisetin (1.5 mg) and 90% HCOOH (0.4 ml) was heated at 80 °C for 5.5 h. The reaction mixture was diluted with EtOAc, and was then washed successively with aqueous 5% Na_2CO_3 and brine. The EtOAc layer was removed, concentrated *in vacuo* and chromatographed (PTLC; HEF, 70:30:1) to yield 1 mg (67%) of cyclo-lisetin (**9** = PDH-2).

Cyclo-lisetin (**9**). Yellow amorphous powder. $UV_{365\ nm}$ fluorescence: orange-yellow. Gibbs test: (+), blue. EI-MS *m/z* (rel. int.): 383 (M^++1, 18), 382 (M^+, 73), 368 (13), 365 (17), 327 (41), 326 (100), 313 (10), 312 (41), 153 (12), 69 (12). UV λ_{max} (nm), MeOH: 260, 284, 303 sh, 337 (br.); +NaOMe 255 sh, 265, 293, 349 (br.); +$AlCl_3$ 273, 292, 384 (br.); +NaOAc 260 (br.), 289, 343 (br.) (H_3BO_3 regenerated the MeOH spectrum). ^{1}H NMR δ (acetone-d_6, 500 MHz): 13.01 (s, 5-OH), 7.32 (1 H, s, 6′-H), 6.60 (1 H, d, J = 2.1 Hz, 8-H), 6.36 (1 H, d, J = 2.1 Hz, 6-H), 3.89 (3 H, s, 5′-OC$\underline{H}_3$), 3.00 (2 H, t, J = 6.7 Hz, 4″-H_2), 1.93 (2 H, t, J = 6.7 Hz, 5″-H_2), 1.39 (6 H, s, 2 × 6″-C$\underline{H}_3$).

Methylation of piscerythrol (**1**)

Piscerythrol (10 mg) was methylated with ethereal diazomethane [2] to give 7-O-methylpiscerythrol (**2**; 1.8 mg, 17% yield) which was isolated by PTLC in HEF (70:30:1, R_f 0.32).

7-O-Methylpiscerythrol **2** [*3,5,4′-trihydroxy-7,5′-dimethoxy-3′-(3,3-dimethylallyl)coumarano-chroman-4-one*]. Pale yellow gum. $UV_{365\ nm}$ fluorescence: dull green. Gibbs test: (+), purple. FD-MS *m/z* (rel. int.): 415 (M^++1, 27), 414 (M^+, 100), 412 (10), 396 (6). UV λ_{max} (nm), MeOH: 294, 336 sh; +NaOMe 244 sh, 289, 365 (br.); +$AlCl_3$ 315, 386 (br.); +NaOAc unchanged. ^{1}H NMR δ (benzene-d_6, 500 MHz): 12.16 (s, 5-OH), 6.63 (1 H, s, 6′-H), 6.12 (1 H, s, 2-H), 6.02 and 5.89 (both 1 H, two d, J = 2.2 Hz, 6- and 8-H), 5.73 (s, 3-OH), 5.63 (1 H, t-like, J = 7.2 Hz, 2″-H), 3.62 (2 H, d, J = 7.2 Hz, 1″-H_2), 2.97 and 2.91 (both 3 H, two s, 7- and 5′-OC$\underline{H}_3$), 1.79 (3 H, s, 4″-H_3), 1.60 (3 H, s, 5″-H_3). Irradiation at δ 5.73 (3-OH) increased the intensity of the singlet at δ 6.12 assignable to 2-H (NOE).

Dehydration of lupinol A (**3**)

Lupinol A (5 mg) was dehydrated in H_2SO_4/toluene (0.5 ml) over a period of 3 h at room temperature. Work up as described above for piscerythrol dehydration afforded three yellow-orange fluorescent spots at R_f 0.52 (LADH-1), 0.24 (LADH-2), and 0.19 (LADH-3) on thin-layer plates developed in HE (4:1). All three compounds were isolated by PTLC in the same solvent system to give LADH-1 (0.8 mg, 17%), LADH-2 (0.6 mg, 13%) and LADH-3 (0.2 mg, 4%).

Dehydration product LADH-1 (**10**). EI-MS *m/z* (rel. int.): 421 (M^++1, 31), 420 (M^+, 100), 404 (6), 377 (16), 366 (17), 365 (74), 364 (51), 349 (8), 321 (10), 310 (10), 309 (39), 308 (22), 176 (6), 69 (6), 57 (7), 55 (8). UV λ_{max} (nm), MeOH: 262, 283 sh, 336 (br.). ^{1}H NMR δ (acetone-d_6, 500 MHz): 13.35 (s, 5-OH), 7.70 (1 H, d, J = 8.4 Hz, 6′-H), 6.88 (1 H, d, J = 8.4 Hz, 5′-H), 6.53 (1 H, s, 8-H), 3.02 (2 H, t, J = 6.8 Hz, 4″-H_2), 2.72 (2 H, t, J = 6.8 Hz, 4‴-H_2), 1.95 (2 H, t, J = 6.8 Hz, 5″-H_2), 1.90 (2 H, t, J = 6.8 Hz, 5‴-H_2), 1.39 and 1.38 (both 6 H, two s, 2 × 6″-C$\underline{H}_3$ and 2 × 6‴-C$\underline{H}_3$).

Dehydration product LADH-2 (**11**). EI-MS *m/z* (rel. int.): 421 (M^++1, 31), 420 (M^+, 98), 377 (22), 366 (24), 365 (100), 364 (60), 321 (14), 310 (11), 309 (45), 308 (33), 222 (11), 221 (78), 218 (13), 165 (37), 55 (12). UV λ_{max} (nm), MeOH: 261, 286 (br.), 336 (br.). ^{1}H NMR δ (acetone-d_6, 500 MHz): 13.37 (s, 5-OH), 7.66 (1 H, d, J = 8.4 Hz, 6′-H), 7.04 (1 H, d, J = 8.4 Hz, 5′-H), 6.54 (1 H, d, J = 2.0 Hz, 8-H), 5.39 (1 H, t-like, J = 7.4 Hz, 2″-H), 3.62 (2 H, br. d, J = 7.4 Hz, 1″-H_2), 2.72 (2 H, t, J = 6.9 Hz, 4‴-H_2), 1.90 (2 H, t, J = 6.9 Hz, 5‴-H_2), 1.85 (3 H, s, 4″-H_3), 1.68 (3 H, s, 5″-H_3), 1.39 (6 H, s, 2 × 6‴-C$\underline{H}_3$).

Dehydration product LADH-3 (**12**). EI-MS *m/z* (rel. int.): 421 ($M^+ + 1$, 18), 420 (M^+, 65), 405 (10), 378 (19), 377 (78), 366 (24), 365 (100), 321 (13), 309 (19), 221 (15), 69 (10), 57 (13). UV λ_{max} (nm), MeOH: 263, 298 (br.), 331 sh (br.). ^{1}H NMR δ (acetone-d_6, 500 MHz): 13.29 (s, 5-OH), 7.71 (1 H, d, J = 8.4 Hz, 6′-H), 6.88 (1 H, d, J = 8.4 Hz, 5′-H), 6.68 (1 H, d, J = 1.5 Hz, 8-H), 5.30 (1 H, t-like, J = 7.4 Hz, 2‴-H), 3.40 (2 H, br. d, J = 7.4 Hz, 1‴-H_2), 3.01 (2 H, t, J = 6.9 Hz, 4″-H_2), 1.94 (2 H, d, J = 6.9 Hz, 5″-H_2), 1.80 (3 H, s, 4‴-H_3), 1.66 (3 H, s, 5‴-H_3), 1.37 (6 H, s, 2 × 6″-CH_3).

Methylation of lupinol A (**3**)

Lupinol A (12.4 mg) was methylated as described for piscerythrol to give a mixture of 7-O-methyl-lupinol A (**4,** 5.5 mg, 44% yield), and 7.4′-di-O-methyl-lupinol A (**5,** 1.0 mg, yield 8%). Both compounds were isolated by PTLC in HE (1 : 1, **4** at R_f 0.60, and **5** at R_f 0.73).

7-O-Methyl-lupinol A (**4**). Pale yellow gum. $UV_{365\,nm}$ fluorescence: dark purple. Gibbs test: (+), red-purple. FD-MS *m/z* (rel. int.): 453 ($M^+ + 1$, 40), 452 (M^+, 100), 451 (17). EI-MS *m/z* (rel. int.): 434 ($M^+ - H_2O$, 5), 391 (16), 379 (14), 236 (11), 235 (71), 218 (5), 180 (11), 179 (100), 163 (15), 162 (11), 149 (7), 91 (10), 77 (10), 69 (8). UV λ_{max} (nm), MeOH: 300; +NaOMe 250 sh, 294, 350; +$AlCl_3$ 301; +NaOAc unchanged. ^{1}H NMR δ (acetone-d_6, 500 MHz): 12.00 (s, 5-OH), 8.83 (br. s, 4′-OH), 7.05 (1 H, d, J = 8.2 Hz, 6′-H), 6.52 (1 H, d, J = 8.3 Hz, 5′-H), 6.30 (1 H, s, 2-H), 6.22 (1 H, s, 8-H), 5.94 (br. s, 3-OH), 5.29 and 5.10 (both 1 H, two t-like, J = *ca.* 7 Hz, 2″- and 2‴-H), 3.92 (3 H, s, 7-OCH_3), 3.33 and 3.31 (both 1 H, two br. d, J = *ca.* 7 Hz, 1″- and 1‴-Ha), 3.19 (2 H, br. d, J = *ca.* 7 Hz, 1″- and 1‴-Hb), 1.76 and 1.72 (both 3 H, two s, 4″- and 4‴-H_3), 1.64 and 1.60 (both 3 H, two s, 5″- and 5‴-H_3). ^{1}H NMR δ (benzene-d_6, 500 MHz): 12.11 (s, 5-OH), 6.97 (1 H, d, J = 8.2 Hz, 6′-H), 6.18 (1 H, s, 2-H), 6.02 (1 H, d, J = 8.2 Hz, 5′-H), 5.64 (1 H, s, 8-H), 5.49 and 5.30 (both 1 H, two t-like, J = *ca.* 7 Hz, 2″- and 2‴-H), 4.82 (br. s, 3-OH), 3.55 (br. s, 4′-OH), 3.50 and 3.43 (both 2 H, two br. d, J = *ca.* 7 Hz, 1″- and 1‴-H_2), 2.96 (3 H, s, 7-OCH_3), 1.81 and 1.66 (both 3 H, two s, 4″- and 4‴-H_3), 1.59 and 1.49 (both 3 H, two s, 5″- and 5‴-H_3). The signal at δ 6.18 attributed to 2-H was intensified (NOE) by irradiation of the broad signal at δ 4.82 due to 3-OH.

7,4′-Di-O-methyl-lupinol A (**5**). Pale yellow gum. $UV_{365\,nm}$ fluorescence: dark purple. Gibbs test: (+), red-purple. FD-MS *m/z* (rel. int.): 467 ($M^+ + 1$, 32), 466 (M^+, 100). EI–MS *m/z* (rel. int.): 448 ($M^+ - H_2O$, 2), 236 (17), 235 (100), 233 (6), 232 (13), 217 (7), 180 (11), 139 (87). UV λ_{max}, MeOH: 301 nm. ^{1}H NMR δ (acetone-d_6, 500 MHz): 11.97 (s, 5-OH), 7.20 (1 H, d, J = 8.4 Hz, 6′-H), 6.63 (1 H, d, J = 8.4 Hz, 5′-H), 6.32 (1 H, s, 2-H), 6.22 (1 H, s, 8-H), 5.22 and 5.10 (both 1 H, two t-like, J = *ca.* 7 Hz, 2″- and 2‴-H), 3.92 and 3.82 (both 3 H, two s, 7- and 4′-OCH_3), 3.30 and 3.19 (both 2 H, two d, J = *ca.* 7 Hz, 1″- and 1‴-H_2), 1.76 and 1.72 (both 3 H, two s, 4″- and 4‴-H_3), 1.63 and 1.60 (both 3 H, two s, 5″- and 5‴-H_3). ^{1}H NMR δ (benzene-d_6, 500 MHz): 12.15 (s, 5-OH), 7.08 (1 H, d, J = 8.4 Hz, 6′-H), 6.18 (1 H, s, 2-H), 6.05 (1 H, d, J = 8.4 Hz, 5′-H), 5.64 (1 H, s, 8-H), 5.57 and 5.49 (both 1 H, two t-like, J = *ca.* 7 Hz, 2″- and 2‴-H), 3.62 and 3.51 (both 2 H, two d, J = *ca.* 7 Hz, 1″- and 1‴-H_2), 3.19 and 2.95 (both 3 H, two s, 7- and 4′-OCH_3), 1.66 and 1.61 (both 3 H, two s, 4″- and 4‴-H_3), 1.59 and 1.49 (both 3 H, two s, 5″- and 5‴-H_3).

Acid-catalyzed methanol elimination from lupinol B (**6**)

Lupinol B (6.1 mg) was heated (60 °C) in toluene with approx. 0.1 mg of *p*-TsOH for 2 h. PTLC of the reaction mixture in HE (3 : 1) gave the products LBEM-1 (1.7 mg, 30% yield) at R_f 0.56, and LBEM-2 (0.9 mg, 16% yield) at R_f 0.09. LBEM-1 was indistinguishable from LADH-1 (**10**) obtained by dehydration of lupinol A.

Methanol elimination product LBEM-2 (**13**). Colourless powder. $UV_{365\,nm}$ fluorescence: light blue. Gibbs test: (−). EI-MS *m/z* (rel. int.): 421 ($M^+ + 1$, 29), 420 (M^+, 93), 403 (20), 377 (25), 366 (26), 365 (100), 364 (37), 321 (15), 310 (16), 309 (50), 97 (18), 83 (24), 81 (17), 73 (21), 71 (24), 69 (34), 67 (15), 60 (17), 57 (50), 56 (20), 55 (48). UV λ_{max} (nm), MeOH: 258, 289–297(br.), 331(br.). ^{1}H NMR (acetone-d_6, 500 MHz), 7.75 (1 H, d, J = 8.3 Hz, 6′-H), 6.82 (1 H, d, J = 8.3 Hz, 5′-H), 6.63 (1 H, s, 8-H), 2.98 (2 H, t, J = *ca.* 7 Hz, 4″-H_2), 2.74 (2 H, t, J = 6.9 Hz, 4‴-H_2), 2.28 (2 H, t, J = *ca.* 7 Hz, 5″-H_2), 1.86 (2 H, t, J = 6.9 Hz, 5‴-H_2), 1.41 and 1.36 (both 6 H, two s, 2 × 6″-CH_3 and 2 × 6‴-CH_3).

Dehydration of lupinol C (**7**)

Lupinol C (1.3 mg) was dehydrated in H_2SO_4/toluene (0.3 ml) over a period of 3 h. Work up (see piscerythrol dehydration), and PTLC (HE, 7:3) gave 0.6 mg (49% yield) of a product (R_f 0.45) identical by EI-MS, ^{1}H NMR and TLC comparison with cyclo-lupinalbin B (**15**) prepared as described below.

Acid-catalyzed cyclization of lupinalbin B (**14**)

Lupinalbin B (2 mg) [7] was treated with H_2SO_4/toluene (0.3 ml) for 3 h at room temperature. Work up and PTLC in HE (3:1) gave cyclo-lupinalbin B (**15,** 1.2 mg, 60% yield) at R_f 0.28.

Cyclo-lupinalbin B (**15**). Colourless semi-solid. UV_{365nm} fluorescence: orange. Gibbs test: (+), blue. EI-MS *m/z* (rel. int.): 353 (M^+1, 13), 352 (M^+, 61), 337 (8), 310 (6), 309 (32), 298 (18), 297 (100), 296 (46), 268 (5), 176 (16), 55 (7). UV λ_{max} (nm), MeOH: 259, 286, 336(br.); +NaOMe 270, 314sh, 363(br.); +$AlCl_3$ 235, 270, 288, 325(br.), 387(br.); +NaOAc unchanged. ^{1}H NMR (acetone-d_6, 500 MHz): 13.33 (s, 5-OH), 7.82 (1H, d, J = 8.4 Hz, 6′-H), 7.14 (1H, d, J = 2.1 Hz, 3′-H), 7.02 (1H, dd, J = 8.4, 2.1 Hz, 5′-H), 6.52 (1H, s, 8-H), 2.72 (2H, t, J = 6.8 Hz, 4″-H_2), 1.90 (2H, t, J = 6.8 Hz, 5″-H_2), 1.39 (6H, s, 2 × 6″-CH_3).

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