Pergamon

0031-9422(94)00720-9

Phytochemistry, Vol. 38, No. 2, pp. 519–525, 1995

0031-9422/95 $9.50 + 0.00

SIX FLAVONOSTILBENES AND A FLAVANONE IN ROOTS OF *SOPHORA ALOPECUROIDES*

MUNEKAZU IINUMA,* MASAYOSHI OHYAMA and TOSHIYUKI TANAKA

Department of Pharmacognosy, Gifu Pharmaceutical University, 6-1 Mitahora-higashi 5 chome, Gifu 502, Japan

(*Received in revised form* 1 *September* 1994)

Key Word Index—*Sophora alopecuroides*; Leguminosae; roots; flavonostilbenes; flavanone; alopecurones A–G.

Abstract—Six novel flavonostilbenes, alopecurones A–F, were isolated from the roots of *Sophora alopecuroides*, in addition to a new 5-deoxyflavanone with a lavandulyl group, alopecurone G. The structures of alopecurones A–F, which are flavonostilbenes composed of a flavanone [5, 7, 2′,4′-tetrahydroxy-8-lavandulylfavanone (sophoraflavanone G) or its 2′-methyl ether (leachianone A)] condensed with a stilbene [3, 5, 4′-trihydroxystilbene (resveratrol)] through the A ring of the flavanone skeleton, were established by spectroscopic analysis. Chemical relationships between *S. alopecuroides*, *S. leachiana* and *S. moorcroftiana* are discussed on the basis of their phenolic components.

INTRODUCTION

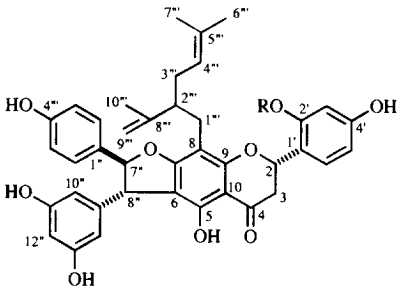
As to the chemical constituents of *Sophora alopecuroides* [1] (syn. *Vexibia alopecuroides*, *Goebelia alopecuroides*), distributed in west and middle Asia, the presence of matrine-type quinolizidine alkaloids [2], flavanones with a lavandulyl and/or an isoprenyl groups [3, 4], and several isoflavonoids has been reported. In the present paper, we describe the isolation and structural determination of six novel flavonostilbenes, alopecurones A (**1**) – F (**6**), and a new flavanone, alopecurone G (**7**) from the roots of this species. In addition, vexibidin (**8′**) and vexibinol (**9′**) [4, 5], the structures of which have been revised to leachianone A (**8**) [5] and sophoraflavanone G (**9**), respectively [16], were isolated and confirmed the preceding revision.

RESULTS AND DISCUSSION

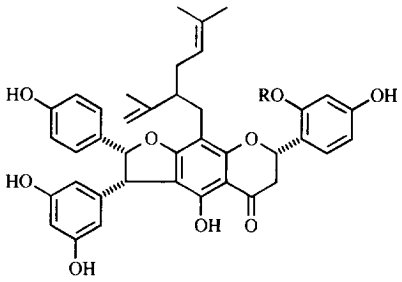
Purification of an acetone extract of the roots of *Sophora alopecuroides* by a combination of silica gel and Sephadex LH-20 column chromatography, and vacuum liquid chromatography, resulted in the isolation of 15 phenolics, including seven new compounds.

Alopecurone A (**1**), obtained as a yellow amorphous solid, showed a $[M - H]^-$ ion at m/z 649 in the negative ion FAB mass spectrum, which corresponds to the empirical formula $C_{39}H_{38}O_9$. In the ^1H NMR spectrum, typical one-proton double doublets at δ2.78 (*dd*, J = 17.1 and 2.7 Hz), 3.12 (*dd*, J = 17.1 and 13.2 Hz) and 5.73 (*dd*, J = 13.2 and 2.7 Hz) assigned to H-2 and H-3 of a 2′-oxygenated flavanone [7] were observed, in addition to proton signals for a lavandulyl group [δ1.50, 1.58, 1.66 (3H each, *br s*, vinylic Me), 2.05 (2H, *m*, CH_2), 2.58 (1H, *m*, CH), 2.65 (1H, *m*, CH_2) 4.57, 4.67 (1H each, *br s*, CH_2 =) and 5.01 (1H, t like *m*, CH =)], and six hydroxyl groups [δ8.15 (×2), 8.39, 8.49, 8.67 and 12.28 (chelated)]. Aromatic methine protons coupled in an ABX-system at δ6.48 (*dd*, J = 8.3 and 2.2 Hz), 6.51 (*d*, J = 2.2 Hz) and 7.41 (*d*, J = 8.3 Hz) indicated that **1** has a 2′,4′-dihydroxyphenyl moiety as its B ring. Furthermore, the ^1H NMR spectrum showed the presence of a 4-hydroxyphenyl [δ6.87 (2H, *d*, J = 8.8 Hz) and 7.23 (2H, *d*, J = 8.8 Hz)] and a 3,5-dihydroxyphenyl group [δ6.20 (2H, *d*, J = 2.4 Hz) and 6.27 (1H, *t*, J = 2.4 Hz)], as well as mutually coupled benzyl methine protons at δ5.50 (*d*, J = 5.4 Hz, Ph-C<u>H</u>-O) and 4.40 (*d*, J = 5.4 Hz, Ph-C<u>H</u>), indicating the presence of 3,5,4′-trihydroxystilbene (resveratrol). In the ^1H–^1H long range COSY spectrum, the benzyl methine protons at δ5.50 and 4.40 were correlated with the *ortho*-coupled doublet at δ7.23 and the *meta*-coupled doublet at δ6.20 through ^4J, respectively. The positions condensed with the resveratrol moiety and substituted with the lavandulyl group were determined as follows. In the COLOC spectrum (Fig. 1), the chelated hydroxyl group at C-5 was correlated with three carbons at δ104.0 (C-10), 108.1 (C-6) and 158.3 (C-5) through ^3J and ^2J. Moreover, the benzyl methine proton at δ4.40, assigned to H-8″ of resveratrol, caused a cross-peak with C-6 through ^2J. On the other hand, the methylene protons of the lavandulyl group at δ2.65 were coupled with three quaternary carbons at δ103.5 (C-8), 162.7 (C-9) and 167.9 (C-7). Consequently, the resveratrol moiety was condensed with an oxygen at C-7 and C-6; the lavandulyl group was at C-8. Thus, **1** was regarded as a new natural

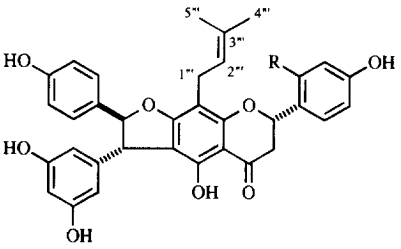
*Author to whom correspondence should be addressed.



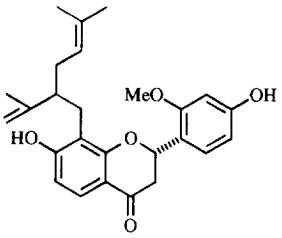
1: R = H, alopecurone A
4: R = Me, alopecurone D



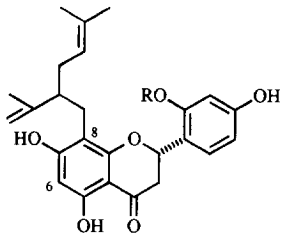
2: R = H, alopecurone B
5: R = Me, alopecurone E



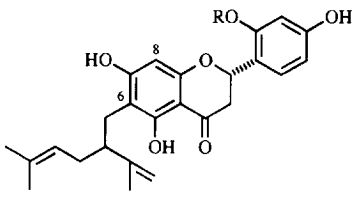
3: R = H, alopecurone C
6: R = OH, alopecurone F



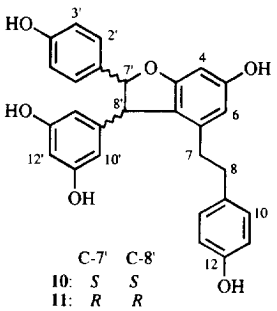
7: alopecurone G



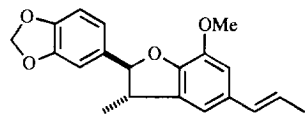
8: R = Me, leachianone A
9: R = H, sophoraflavanone G



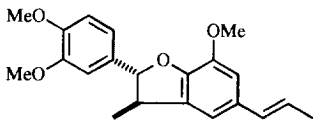
8': R = Me, vexibidin
9': R = H, vexibinol



	C-7'	C-8'
10:	*S*	*S*
11:	*R*	*R*
14:	*trans*	
15:	*cis*	



12



13

product formed by coupling of sophoraflavanone G and resveratrol to give a new framework, e.g. $(C_6)_2$-C_2-$^{A}C_6$-C_3-$^{B}C_6$ [A and B superscript refer to rings A and B in the flavonoid numbering system]. The CD data [($\Delta\varepsilon$: + 3.3 (336), − 11.2 (297)] suggested the configuration at C-2 to be *S* [8]. NOE enhancements I (Fig. 2) indicated that the aryl groups of the resveratrol molecule were *trans*-oriented [9]. The absolute stereochemistry at C-7″ was *S* for **1** (negative Cotton effect at 279 nm), which was established by CD evidence in comparison with the following compounds: gnetin F (**10**) [negative Cotton effect at 300 nm) [9], 7,8,7′,8′-tetrahydro derivative of (−)-ε-viniferin (**11**) [positive Cotton effect at 294 nm] [10], the neolignans **12** (negative Cotton effect at 280 nm) and **13** (positive Cotton effect at 280 nm) [11]. Consequently, these data confirmed the absolute configurations at C-7″ and C-8″ to be *S*.

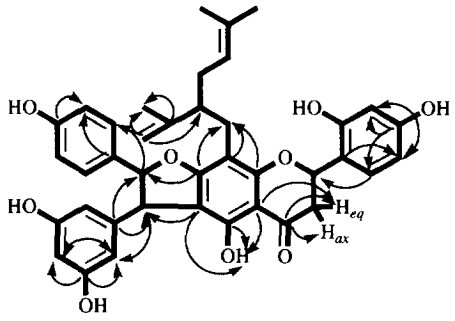


Fig. 1. Long-range correlations of alopecurone A (**1**) in COLOC spectrum (J = 5 Hz).

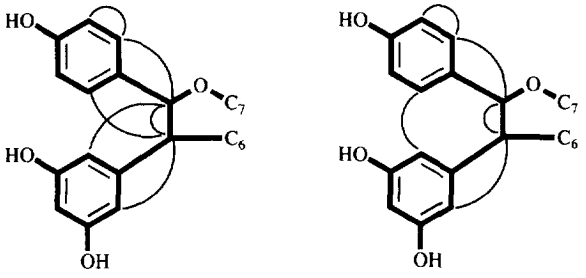


Fig. 2. NOE interactions in partial structures of alopecurones A (I) and B (II) in PSNOESY and DIFNOE spectra.

Alopecurone B (**2**), obtained as a yellow amorphous solid, also showed a $[M - H]^-$ ion at m/z 649 in the negative ion FAB mass spectrum, which is consistent with the molecular formula of **1**. The ^{1}H NMR spectrum was closely similar to that of **1**. In the COLOC spectrum, a proton signal of the C-5-OH was correlated with carbon signals of C-5 (δ158.2), C-6 (δ109.9) and C-10 (δ104.3). A benzyl methine proton at δ4.62 (H-8″) was correlated with a signal of C-6. Hence, the resveratrol in **2** formed a dihydrofuran ring between the C-7-OH and C-6, the same as in **1**. The position of a lavandulyl group was established to be at C-8 by correlations between a methylene proton at δ2.68 (H-1‴) and a carbon at δ103.9 (C-8). The structure of **2** is compatible with that of **1**, except for the absolute configuration. The orientation of C-2 was concluded to be *S* by a positive [$\Delta\varepsilon$: + 5.6 (336)] and a negative Cotton effect [$\Delta\varepsilon$: − 3.9 (293)] in the CD spectrum. In the phase-sensitive NOESY (PSNOESY) spectrum, NOE interactions were observed, II (Fig. 2). The enhancement between H-2″ (6″) and H-10″ (14″) was explainable if each aryl group was *cis*-oriented. The NOE results and the positive Cotton effect [$\Delta\varepsilon$: + 6.3 (315)] in the CD spectrum supported the absolute configurations of C-7″ and C-8″ top be *R* and *S*, respectively.

Comparison of the ^{1}H NMR spectral data of **1** and **2** revealed a significant difference in the chemical shifts of protons (H-2″, 3″, 5″, 6″ and H-10″, 12″, 14″) on the aryl groups of a 4-hydroxyphenyl group at C-7″ and of a 3,5-dihydroxyphenyl group at C-8″. In the case of **2**, the signals appeared at a relatively higher field than those of **1**; this was attributed to the shielding effect produced by the spatial proximity of the aryl groups. Furthermore, the orientations of C-7″ and C-8″ were reflected in the coupling constants between H-7″ and H-8″; the values of **1** (J = 5.4 Hz) and **2** (J = 8.3 Hz) are compatible with those of ε-viniferin derivatives [**14** (*trans*): J = 6.2 Hz and **15** (*cis*): J = 8.3 Hz] [10].

The chemical shifts of the chelated hydroxyl group at C-5 give valuable information on whether the alkyl side chain, e.g. an isoprenyl and a lavandulyl group is substituted at C-6 or C-8 [12]. The shift values (**1**: δ12.28 and **2**: 12.22), which are consistent with those of a flavanone lacking such an alkyl side chain at C-6, showed that they are not affected by the dihydrofuran moiety in the above flavonostilbenes, suggesting that they can be applied to the eludiation of the substitution of an A ring moiety in the flavonostilbenes described above.

Alopecurone C (**3**), isolated as a powder, gave a $[M - H]^-$ ion peak at m/z 565 in the negative ion FAB mass spectrum, which is consistent with the molecular formula $C_{34}H_{30}O_8$. On the basis of the ^{1}H NMR spectrum, the framework was thought to be a flavonostilbene, e.g. **1**. The presence of an isoprenyl group [δ1.59, 1.64 (3H each, *br s*, vinylic Me), 3.26 (2H, *d*, J = 7.3 Hz, CH_2) and 5.27 (1H, *t*, J = 7.3 Hz, CH =)] was supposed in **3**, instead of a lavandulyl group. In the COLOC spectrum, long-range couplings were observed between a proton signal of the C-5-OH and carbon signals of C-5 (δ158.5), C-10 (δ104.3) and C-6 (δ108.4); the last carbon signal was coupled with H-8″ (δ4.36). In addition, a methylene proton at δ3.26, assigned to H-1‴ in the isoprenyl group, was coupled with carbons at δ161.8 (C-9), 167.5 (C-7) and 103.9 (C-8) through ^3J and ^2J. The position of the isoprenyl group was thus at C-8, which was also supported by the chemical shift to the chelated hydroxyl group at δ12.25. The structure of **3** was therefore elucidated to be a flavonostilbene consisting of 5,7,4′-trihydroxy-8-isoprenylflavanone (sophoraflavanone B) [13, 14] and resveratrol. Considering the coupling constant (J = 4.9 Hz) between H-7″ and H-8″ in the resveratrol molecule, and the chemical shifts of protons on the 4-hydroxyphenyl and the 3,5-dihydroxyphenyl groups at C-7″ and C-8″, the aryl groups are suggested to be in a *trans*-configuration; this was supported by NOE correlation between H-7″/H-10″ (14″) and H-8″/H-2″ (6″) in the PSNOESY spectrum. The absolute stereochemistry was concluded from the CD spectrum as follows. As in the case of **1**, the CDs [$\Delta\varepsilon$: + 5.5 (338) and − 14.4 (297)] characterized the configuration at C-2 to be *S*, and that at C-7″ and C-8″ to be *S* [$\Delta\varepsilon$: − 11.0 (283)].

Alopecurone D (**4**), obtained as a yellow oil, showed a $[M - H]^-$ ion at m/z 663 in the negative ion FAB mass spectrum consistent with the formula $C_{40}H_{40}O_9$ which corresponds to a monomethyl ether of **1** or **2**. The ^{1}H NMR spectral signals of **4**, which showed the presence of a methoxyl group at δ 3.84 were similar to those of **1**. A 4-hydroxy-2′-methoxyphenyl substituent was proposed from the NOE enhancement observed between a *meta*-coupled aromatic proton at δ6.57 and the methoxyl

group in the PSNOESY spectrum. Substitution on the B ring was further confirmed by comparison of the ^{13}C NMR spectral data with those of leachianone A (**8**) [5]. The chemical shifts of the chelated hydroxyl and the aryl groups, including the resveratrol moiety, were similar to those of **1**, indicating that the lavandulyl group was substituted at C-8 and that the aryl groups at C-7″ and C-8″ were *trans*-oriented. The absolute configurations of C-2, C-7″ and C-8″ were shown to be *S*, by the Cotton effects at 335 (positive), 297 (negative) and 281 nm (negative in the CD spectrum. The structure of alopecurone D is therefore the 2′-methyl ether of alopecurone A.

Alopecurone E (**5**), a yellow amorphous solid, showed a $[M-H]^-$ ion at *m/z* 663 in the negative ion FAB mass spectrum, the same empirical formula $C_{40}H_{40}O_9$ as **4**. In the ^1H NMR spectrum, differences of the coupling constants between H-7″ and H-8″, and of the chemical shifts of aromatic protons in a resveratrol moiety as found in **1** and **2** were observed in **4** and **5**, indicating that **5** is a stereoisomer of **4**. Among them, the higher shifts of proton signals attributable to the resveratrol moiety than those of **4** and the coupling constant (J = 8.3 Hz) between H-7″ and H-8″ characterized that the aryl groups at C-7″ and C-8″ are *cis*-oriented; this was further confirmed by NOE interactions between H-2″ (6″) and H-10″ (14″) in the PSNOESY spectrum. The similar Cotton effects to those of **2** confirmed that the absolute configuration at C-2, C-7″ and C-8″ to be *S*, *R* and *S*, respectively.

Alopecurone F (**6**), obtained as a yellow amorphous solid, showed a $[M-H]^-$ ion at *m/z* 581 for an empirical formula of $C_{34}H_{30}O_9$. In the ^1H NMR spectrum, the presence of an isoprenyl; [δ1.60, 1.64 (3H each, *br s*), 3.27 (2H, *d*, J = 7.3 Hz) and 5.29 (1H, *t* like *m*)] was observed. The chemical shift of the chelated hydroxyl group at δ12.28 showed the position of the isoprenyl group to be at C-8, not at C-6. The PSNOESY spectrum showed cross-peaks between H-8″ H-2″ (6″) and H-7″ H-10″ (14″), indicating that the aryl groups were *trans*-oriented. The absolute stereochemistry of C-2, C-7″ and C-8″ in **6** was concluded to be *S* by the CD spectrum; the structure was therefore the 2′-hydroxy derivative of alopecurone C.

Alopecurone G (**7**), a pale yellow oil, gave positive reactions to Gibbs and Mg–HCl tests, and a negative one to $FeCl_3$ reagent. The ^1H NMR spectrum showed three one-proton double doublets at δ2.67, 2.90 and 5.65 assigned to H-3 and H-2 of a 2′-oxygenated flavanone. In the spectrum, the presence of a methoxyl (δ3.82) and a lavandulyl group [δ1.48, 1.56, 1.67 (3H each, *br s*, vinylic Me), 2.05 (2H, *m*, CH_2), 2.61 (1H, *t*-like *m*, CH), 2.74 (2H, *d*, J = 6.8 Hz, CH_2), 4.55, 4.59 (1H each, *br s*, CH_2 =), 5.00 (1H, *t*-like *m*, CH =)] was observed. Aromatic methine protons coupled in an ABX-system at δ6.53 (*dd*, J = 7.8, 2.0 Hz), 6.55 (*d*, J = 2.0 Hz) and 7.46 (*d*, J = 7.8 Hz), and a set of *ortho*-coupled aromatic protons at δ6.61 (1H, *d*, J = 8.8 Hz) and 7.60 (1H, *d*, J = 8.8 Hz) were also observed. In the EI mass spectrum, prominent fragment ions at *m/z* 149 and 150 suggested that the lavandulyl group and a hydroxyl group were located on the A ring, and the methoxyl and another hydroxyl group on the B ring. In the difference NOE spectrum, enhancement of the *meta*-coupled aromatic proton at δ6.55 appeared after irradiation of the methoxyl group at δ3.82, which indicated the substitution pattern of the B ring to be 4′-hydroxy-2′-methoxyl. On the contrary, the deshielded *ortho*-coupled proton at δ7.60 showed the A ring to have a 7-hydroxy-8-lavandulyl substitution. In the CD spectrum [$\Delta\varepsilon$: + 6.0 (333), − 10.3 (302)], C-2 in **7** was shown to have *S*-configuration. The structure of alopecurone G was concluded to be (2*S*)-7,4′-dihydroxy-8-lavandulyl-2′-methoxyflavanone (**7**).

The other compounds isolated were characterized by spectral analysis as known flavanones of leachianone A (**8**) [5], sophoraflavanone G (**9**) [6], glabrol [15] and lehmannin [16], a pterocarpan of sophoracarpan B [17], a coumestanol of medicagol [18], an isoflavone of 2′-hydroxygenistein [19] and a resveratrol dimer of ε-viniferin [9], respectively.

Previously, 5,7,4′-trihydroxy-6-lavandulyl-2′-methoxyflavanone (**8′**) and 5,7,2′,4′-tetrahydroxy-6-lavandulylflavanone (**9′**) were obtained from roots of *Vexibia alopecuroides* and named vexibidin and vexibinol [4]. From the physical properties and the chemical shifts of the chelated hydroxyl group, the lavandulyl group was proposed to be substituted, not at C-6 but at C-8, and the tentative structures of vexibidin and vexibinol were revised to (2*S*)-5,7,4′-trihydroxy-8-lavandulyl-2′-methoxyflavanone (leachianone A, **8**) [5] and (2*S*)-5,7,2′,4′-tetrahydroxy-8-lavandulylflavanone (sophoraflavanone G, **9**) [5, 6].

Up to now, the occurrence of flavonostilbenes which have a resveratrol condensed with a flavone through its B ring have been reported in the roots of *S. leachiana* [20, 21] and *S. moorcroftiana* [22]; their framework is designated $^AC_6$-C_3-$^BC_6$-C_2-$(C_6)_2$. On the other hand, flavonostilbenes in *S. alopecuroides* have a resveratrol found with a flavanone through its A ring to form a different framework $(C_6)_2$-C_2-$^AC_6$-C_3-$^BC_6$. In our previous papers [23–25], the differences between *S. leachiana* and *S. moorcroftiana* were shown by the kind of oligostilbenes present, i.e. the oligostilbene in *S. leachiana* is characterized as a product oligomerized through a pallidol. In contrast, the oligostilbenes in *S. moorcroftiana* are through ε-viniferin. From the roots of *S. alopecuroides*, resveratrol oligomers involving leachianols A [23], F, G [25] and pallidol [23, 26] were isolated, which indicates the similarity between *S. alopecuroides* and *S. leachiana* from the standpoint of oligostilbene production, but the two species have different pathways for the formation of flavonostilbenes.

EXPERIMENTAL

Plant material. Roots of *S. alopecuroides* L. were collected at Xinjiang, China in June 1993. A voucher specimen is deposited in the Herbarium of Gifu Pharmaceutical University.

Extraction and isolation of compounds. Dried and pulverized roots (600 g) were extracted with Me_2CO at room temp. After concn, the extract (40 g) was subjected to

Table 1. ^{1}H NMR data for alopecurones A (**1**) – F (**6**) in acetone-d_6

No.	**1**	**2**	**3**	**4**	**5**	**6**
2	5.73 (*dd*, 13.2, 2.7)	5.77 (*dd*, 13.7, 2.9)	5.51 (*dd*, 13.3, 2.9)	5.70 (*dd*, 13.7, 2.9)	5.74 (*dd*, 13.2, 2.9)	5.75 (*dd*, 13.2, 2.9)
3*eq*	2.78 (*dd*, 17.1, 2.7)	2.80 (*dd*, 17.1, 2.9)	2.77 (*dd*, 17.1, 2.9)	2.72 (*dd*, 17.1, 2.9)	2.74 (*dd*, 17.1, 2.9)	2.76 (*dd*, 17.1, 2.9)
3*ax*	3.12 (*dd*, 17.1, 13.2)	3.13 (*dd*, 17.1, 13.7)	3.16 (*dd*, 17.1, 13.3)	3.12 (*dd*, 17.1, 13.7)	3.13 (*dd*, 17.1, 13.2)	3.17 (*dd*, 17.1, 13.2)
2′			7.44 (*d*, 8.3)		–	—
3′	6.51 (*d*, 2.2)	6.52 (*d*, 2.0)	6.93 (*d*, 8.3)	6.57 (*d*, 2.4)	6.57 (*d*, 2.4)	6.50 (*d*, 2.4)
5′	6.48 (*dd*, 8.3, 2.2)	6.49 (*dd*, 8.3, 2.0)	6.93 (*d*, 8.3)	6.55 (*dd*, 8.3, 2.4)	6.56 (*dd*, 8.3, 2.4)	6.46 (*dd*, 8.6, 2.4)
6′	7.41 (*d*, 8.3)	7.42 (*d*, 8.3)	7.44 (*d*, 8.3)	7.45 (*d*, 8.3)	7.46 (*d*, 8.3)	7.38 (*d*, 8.6)
2″(6″)	7.23 (*d*, 8.8)	7.02 (*d*, 8.5)	7.20 (*d*,8.5)	7.23 (*d*, 8.8)	7.02 (*d*, 8.3)	7.20 (*d*, 8.5)
3″(5″)	6.87 (*d*, 8.8)	6.64 (*d*, 8.5)	6.86 (*d*, 8.5)	6.86 (*d*, 8.8)	6.63 (*d*, 8.3)	6.86 (*d*, 8.5)
7″	5.50 (*d*, 5.4)	5.96 (*d*, 8.3)	5.58 (*d*, 4.9)	5.49 (*d*, 5.4)	5.97 (*d*, 8.3)	5.56 (*d*, 4.9)
8″	4.40 (*d*, 5.4)	4.62 (*d*, 8.3)	4.36 (*d*, 4.9)	4.39 (*d*, 5.4)	4.62 (*d*, 8.3)	4.35 (*d*, 4.9)
10″(14″)	6.20 (*d*, 2.4)	5.82 (*d*, 2.0)	6.22 (*d*, 2.0)	6.19 (*d*, 2.2)	5.81 (*d*, 2.0)	6.20 (*d*, 2.0)
12″	6.27 (*t*, 2.4)	6.03 (*t*, 2.0)	6.28 (*t*, 2.0)	6.27 (*t*, 2.2)	6.03 (*t*, 2.0)	6.26 (*t*, 2.0)
1‴	2.65 (2H, *m*)	2.68 (2H, *m*)	3.26 (*d*, 7.3)	2.63 (2H, *m*)	2.68 (2H, *m*)	3.27 (*d*, 7.3)
2‴	2.58 (*m*)	2.62 (*m*)	5.27 (*t*, 7.3)	2.58 (*m*)	2.60 (*m*)	5.29 (t-like *m*)
3‴	2.05 (2H, *m*)	2.08 (2H, *m*)		2.05 (2H, *m*)	2.05 (2H, *m*)	
4‴	5.01 (*t*-like *m*)	5.05 (*t*-like *m*)	1.64 (3H, *br s*)	4.99 (*t*-like *m*)	5.02 (*t*-like *m*)	1.64 (3H, *br s*)
5‴	—	—	1.59 (3H, *br s*)	—	—	1.60 (3H, *br s*)
6‴	1.58 (3Hm *br s*)	1.58 (3H, *br s*)	—	1.58 (3H, *br s*)	1.58 (3H, *br s*)	—
7‴	1.50 (3H, *br s*)	1.52 (3H, *br s*)	—	1.50 (3H, *br s*)	1.53 (3H, *br s*)	—
9‴	4.57 (*br s*)	4.63 (*br s*)	—	4.56 (*br s*)	4.61 (*br s*)	—
	4.67 (*br s*)	4.74 (*br s*)	–	4.68 (*br s*)	4.76 (*br s*)	
10‴	1.66 (3H, *br s*)	1.69 (3H, *br s*)		1.65 (*br s*)	1.69 (3H, *br s*)	
OMe	—	—	—	3.84 (3H, *s*)	3.86 (3H, *s*)	—
OHs	8.15 (× 2), 8.38,	7.80 (× 2), 8.21,	8.13 (× 2)	8.15 (× 2), 8.50,	7.78 (× 2), 8.18,	8.14 (× 2), 8.38,
	8.49, 8.67 (*br s*)	8.39, 8.64 (*br s*)	8.44 (× 2, *br s*)	8.56 (*br s*)	8.55 (*br s*)	8.45, 8.65 (*br s*)
C_5-OH	12.28 (*s*)	12.22 (*s*)	12.25 (*s*)	12.26 (*s*)	12.21 (*s*)	12.28 (*s*)

Values in ppm (δ_H) at 400 MHz. All protons were assigned with the aid of ^{13}C–^{1}H COSY and COLOC. Figures in parentheses are coupling constants (J) in Hz.

silica gel CC (*n*-hexane–Me_2CO_3 10:1 to 1:1, finally MeOH) to afford 22 frs. Frs 4–7 were further purified by repeated vacuum liquid chromatography (VLC) eluting with a *n*-hexane–EtOH system to obtain caffeic acid alkyl ester (44 mg), maackiain (2 mg), sophoracarpan B (32 mg), glabrol (62 mg), leachianone A (**8**, 977 mg), sophoraflavanone G (**9**, 733 mg) and alopecurone G (**7**, 56 mg). From frs 10–11, lehmanin (2 mg) and 2′-hydroxygenistein (2 mg) were isolated by prep. TLC ($CHCl_3$–MeOH, 20:1); further VLC ($CHCl_3$–MeOH system, gradient) and Sephadex LH-20 CC (MeOH, or Me_2CO–H_2O, 4:1) yielded alopecurones A (**1**, 804 mg), B (**2**, 349 mg), C (**3**, 151 mg), D (**4**, 677 mg), E (**5**, 3 mg) and F (**6**, 3 mg). Frs 17–18 gave leachianol A (10 mg) and a mixt. of leachianols F and G (16 mg), in addition to ε-viniferin (111 mg), using similar separating procedures.

Alopecurone A (**1**). Yellow amorphous solid. Negative ion FABMS: *m/z* 649. $[\alpha]_D^{27}$ − 13.5° (MeOH; *c* 0.13). CD (MeOH; *c* 3.69×10^{-5}): $\Delta\varepsilon^{20}$ + 3.3 (336), − 11.2 (297), − 2.3 (279), + 1.0 (255), − 1.5 (248). UV λ_{max}^{MeOH} nm): 228, 287, 300, 333sh. IR ν_{max}^{KBr} cm^{-1}: 3320, 1640, 1600, 1510. ^1H and ^{13}C NMR: Tables 1 and 2.

Alopecurone B (**2**). Yellow amorphous solid. Negative ion FABMS: *m/z* 649. $[\alpha]_D^{27}$ − 1.5° (MeOH; *c* 0.13). CD (MeOH; *c* 3.38×10^{-5}): $\Delta\varepsilon^{20}$ + 5.6 (336), + 6.3 (315), − 3.9 (293), − 3.2 (245), + 6.1 (238). UV λ_{max}^{MeOH} nm): 228, 287, 299, 355sh. IR ν_{max}^{KBr} cm^{-1}: 3350, 1650, 1610, 1520. ^1H and ^{13}C NMR: Tables 1 and 2.

Alopecurone C (**3**). Powder. Negative ion FABMS: *m/z* 565. $[\alpha]_D^{27}$ 50.7° (MeOH; *c* 0.10). CD (MeOH; *c* 4.59×10^{-5}): $\Delta\varepsilon^{20}$ + 5.5 (338), − 14.4 (297), − 11.0 (283), + 1.7 (253), − 0.6 (248). UV λ_{max}^{MeOH} nm: 228, 285, 300, 333sh. IR ν_{max}^{KBr} cm^{-1}: 3300, 1660, 1610, 1520. ^1H and ^{13}C NMR: Tables 1 and 2.

Alopecurone D (**4**). Yellow oil. Negative ion FABMS: *m/z* 663. $[\alpha]_D^{26}$ − 10.1° (MeOH; *c* 0.12). CD (MeOH;

Table 2. ^{13}C NMR data of alopecurones A (**1**) – F (**6**) in acetone-d_6

No.	1	2	3	4	5	6
1	75.5 (*d*)	75.6 (*d*)	80.0 (*d*)	75.2 (*d*)	75.2 (*d*)	75.5 (*d*)
3	42.5 (*t*)	42.6 (*t*)	43.3 (*t*)	42.5 (*t*)	42.5 (*t*)	42.5 (*t*)
4	198.4 (*s*)	198.5 (*s*)	197.8 (*s*)	198.4 (*s*)	198.5 (*s*)	198.4 (*s*)
5	158.3 (*s*)[a]	158.2 (*s*)	158.5 (*s*)	158.5 (*s*)	158.0 (*s*)	158.6 (*s*)
6	108.1 (*s*)	109.9 (*s*)	108.4 (*s*)	108.2 (*s*)	110.0 (*s*)	108.2 (*s*)
7	167.9 (*s*)	168.1 (*s*)	167.5 (*s*)	168.0 (*s*)	168.0 (*s*)	166.8 (*s*)
8	103.5 (*s*)	103.9 (*s*)	103.9 (*s*)	103.5 (*s*)	103.9 (*s*)	103.8 (*s*)
9	162.7 (*s*)	162.5 (*s*)	161.8 (*s*)	162.7 (*s*)	162.5 (*s*)	162.3 (*s*)
10	104.0 (*s*)	104.3 (*s*)	104.3 (*s*)	104.1 (*s*)	104.3 (*s*)	104.1 (*s*)
1′	117.5 (*s*)	117.6 (*s*)	130.9 (*s*)	118.9 (*s*)	119.0 (*s*)	117.7 (*s*)
2′	156.1 (*s*)	156.2 (*s*)	128.8 (*d*)	158.7 (*s*)	158.8 (*s*)	156.4 (*s*)
3′	103.4 (*d*)	103.5 (*d*)	116.2 (*d*)	99.8 (*d*)	99.8 (*d*)	103.5 (*d*)
4′	159.3 (*s*)	159.4 (*s*)	158.6 (*s*)	159.9 (*s*)	160.0 (*s*)	159.5 (*s*)
5′	107.8 (*d*)	107.8 (*d*)	116.2 (*d*)	107.9 (*d*)	107.9 (*d*)	107.9 (*d*)
6′	128.5 (*d*)	128.6 (*d*)	128.8 (*d*)	128.4 (*d*)	128.5 (*d*)	128.8 (*d*)
1″	132.7 (*s*)	132.0 (*s*)[b]	133.0 (*s*)	132.8 (*s*)	132.0 (*s*)[c]	133.1 (*s*)
2″(6″)	128.0 (*d*)	129.1 (*d*)	127.7 (*d*)	128.1 (*d*)	129.1 (*d*)	127.7 (*d*)
3″(5″)	116.2 (*d*)	115.1 (*d*)	116.3 (*d*)	116.3 (*d*)	115.1 (*d*)	116.3 (*d*)
4″	158.3 (*s*)[a]	157.5 (*s*)	158.4 (*s*)	158.4 (*s*)	157.6 (*s*)	158.4 (*s*)
7″	95.2 (*d*)	91.6 (*d*)	95.2 (*d*)	95.3 (*d*)	91.7 (*d*)	95.2 (*s*)
8″	54.7 (*d*)	50.6 (*d*)	55.0 (*d*)	54.8 (*d*)	50.7 (*d*)	55.0 (*d*)
9″	145.5 (*s*)	141.9 (*s*)	145.6 (*s*)	145.6 (*s*)	142.0 (*s*)	145.6 (*s*)
10″(14″)	106.6 (*d*)	108.6 (*d*)	106.6 (*d*)	106.6 (*d*)	108.6 (*d*)	106.6 (*d*)
11″(13″)	159.4 (*s*)	158.5 (*s*)	159.6 (*s*)	159.5 (*s*)	158.6 (*s*)	159.6 (*s*)
12″	102.1 (*d*)	101.7 (*d*)	102.2 (*d*)	102.1 (*d*)	101.8 (*d*)	102.1 (*d*)
1‴	28.0 (*t*)	28.0 (*t*)	22.7 (*t*)	28.1 (*t*)	28.0 (*t*)	22.8 (*t*)
2‴	47.8 (*d*)	48.0 (*d*)	123.0 (*d*)	47.8 (*d*)	47.9 (*d*)	123.1 (*d*)
3‴	32.1 (*t*)	32.5 (*t*)	132.0 (*s*)	32.2 (*t*)	32.5 (*t*)	131.9 (*s*)
4‴	124.0 (*d*)	124.2 (*d*)	25.0 (*q*)	124.1 (*d*)	124.2 (*d*)	25.9 (*q*)
5‴	131.9 (*s*)	132.0 (*s*)[b]	17.9 (*q*)	132.0 (*s*)	132.0 (*s*)[c]	17.9 (*q*)
6‴	25.8 (*q*)	25.8 (*q*)	—	25.8 (*q*)	25.8 (*q*)	—
7‴	17.9 (*q*)	17.9 (*q*)	—	17.9 (*q*)	17.9 (*q*)	—
8‴	148.4 (*s*)	148.4 (*s*)	—	148.5 (*s*)	148.4 (*s*)	—
9‴	111.7 (*t*)	112.1 (*t*)	—	111.8 (*t*)	112.2 (*t*)	—
10‴	18.8 (*q*)	19.0 (*q*)	—	18.8 (*q*)	19.0 (*q*)	—
OMe	—	—	—	55.8 (*q*)	55.8 (*q*)	—

Values in ppm (δ_C) at 100 MHz. All carbons were assigned with the aid of ^{13}C–^1H COSY and COLOC.

[a–c]Overlapping signals.

$c\,5.09\times10^{-5}$): $\Delta\varepsilon^{21}$ + 3.6 (335), − 12.5 (297), − 3.9 (281), + 0.9 (256), − 0.7 (248). UV λ_{max}^{MeOH} nm: 206, 224sh, 280, 299, 333sh. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Alopecurone E (**5**). Yellow amorphous solid. Negative ion FABMS: *m/z* 663. CD (MeOH; $c\,3.25\times10^{-5}$): $\Delta\varepsilon^{21}$ + 4.5 (334), + 6.7 (313), − 1.0 (290), − 3.0 (245), + 3.3 (238). UV λ_{max}^{MeOH} nm: 205, 225sh, 280, 299, 335sh. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Alopecurone F (**6**). Yellow amorphous solid. Negative ion FABMS: *m/z* 581. CD (MeOH; $c\,3.92\times10^{-5}$): $\Delta\varepsilon^{21}$ + 1.9 (340), − 4.3 (297), − 2.3 (280), + 0.8 (255), − 1.0 (248). UV λ_{max}^{MeOH} nm: 205, 220sh, 285, 296, 335sh. ^{1}H and ^{13}C NMR: Tables 1 and 2.

Alopecurone G (**7**). Pale yellow oil. EIMS *m/z* (rel. int.): 422 (13), 299 (21), 269 (11), 203 (45), 150 (24), 149 (100), 123 (18). $[\alpha]_D^{26}$ − 69.7° (MeOH; *c* 0.10). CD (MeOH; $c\,5.59\times10^{-5}$): $\Delta\varepsilon^{21}$ + 6.0 (333), − 10.3 (302). UV λ_{max}^{MeOH} nm: 219, 231sh, 285, 307sh, 341sh. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.48, 1.56, 1.67 (3H, each *br s*, Me at C-6″, 7″, 10″), 2.05 (2H, *m*, H-3″), 2.61 (1H, *m*, H-2″), 2.67 (1H, *dd*, J = 16.6, 2.4 Hz, H-3*eq*), 2.74 (2H, *br d*, J = 6.8 Hz, H-1″), 2.90 (1H, *dd*, J = 16.6, 13.2 Hz, H-3*ax*), 3.82 (3H, *s*, OMe), 4.55, 4.59 (1H each, *br s*, H-9″), 5.00 (1H, *t* like *m*, H-4″), 5.65 (1H, *dd*, J = 13.2, 2.4 Hz, H-2), 6.53 (1H, *dd*, J = 7.8, 2.0 Hz, H-5′), 6.55 (1H, *d*, J = 2.0 Hz, H-3′), 6.61 (1H, *d*, J = 8.8 Hz, H-6), 7.46 (1H, *d*, J = 7.8 Hz, H-6′), 7.60 (1H, *d*, J = 8.8 Hz, H-5), 8.80 (*br s*, OH). ^{13}C NMR (100 MHz, acetone-d_6): δ 17.8 (C-7″), 19.1 (C-10‴), 25.8 (C-6″), 28.2 (C-1″), 32.0 (C-3″), 43.9 (C-3), 47.5 (C-2″), 55.8 (OMe), 75.5 (C-2), 99.8 (C-3′), 107.9 (C-5′), 110.3 (C-6), 111.2 (C-9″), 115.2 (C-10), 116.1 (C-8), 119.7 (C-1′), 124.4 (C-4‴), 126.4 (C-5), 128.4 (C-6′), 131.7 (C-5″), 149.1 (C-8″), 158.5 (C-2′), 159.7 (C-4′), 162.7 (C-9), 163.0 (C-7), 191.6 (C-4).

REFERENCES

1. Tsoong, P. C. and Ma, C. Y. (1981) *Acta Phytotaxonom, Sin.* **19**, 1.
2. Talkachev, O. N., Monakhova, T. E., Sheichemnko, V. I., Kabanov, V. S., Fesenko,. O. G. and Proskurnina, N. F. (1975) *Khim, Prir. Soedin.* 30.
3. Yusupova, S. S., Batirov, E. K., Abdullaev, Sh. V. and Malikov, V. M. (1984) *Khim. Prir. Soedin.* 250.
4. Batirov, E. K., Yusupova, S. S., Abdullaev, Sh. V., Vdovin, A. D., Malikov, V. M. and Yagudaev, M. R. (1985) *Khim. Prir, Soedin.* 35.
5. Iinuma, M., Tanaka, T., Mizuno, M., Shirataki, Y., Yokoe, I., Komatsu, M. and Lang, F. A. (1990) *Phytochemistry* **29**, 2667.
6. Shirataki, Y., Yokoe, I., Noguchji, M., Tomimori, T. and Komatsu, M. (1988) *Chem. Pharm. Bull.* **36**, 2220.
7. Iinuma, M., Ohyama, M., Tanaka, T., Mizuno, M. and Hong, S. K. (1992) *Phytochemistry* **31**, 2855.
8. Rao, K. N. and Srimannarayana, G. (1983) *Phytochemistry* **22**, 2287.
9. Lins, A. P., Yoshida, M., Gottlieb, O. R., Gottlieb, H. E. and Kubitzki, K. (1986) *Bull. Soc. Chim. Belg.* **95**, 737.
10. Kurihara, H., Kawabata, J., Ichikawa, S. and Mizutani, J. (1990) *Agric. Biol. Chem.* **54**, 1097.
11. Gottlieb, O. R., Mourao, J. C., Yoshida, M., Mascarenhas, Y. P., Rodrigues, M., Rorsenstein, R. D. and Tomita, K. (1977) *Phytochemistry* **16**, 1003.
12. Fukai, T. and Nomura, T. (1990) *Heterocycles* **31**, 1861.
13. Komatsu, M., Yokoe, I. and Shirataki, Y. (1978) *Chem. Pharm. Bull.* **26**, 3863.
14. Shirataki, Y., Yokoe, I., Endo, M. and Komatsu, M. (1985) *Chem. Pharm. Bull.* **33**, 444.
15. Mizuno, M., Tanaka, T., Tamura, K., Matsuura, N., Iinuma, M. and Cheih, C. (1990) *Phytochemistry* **29**, 2738.
16. Batirov, E. Kh., Yusupova, S. S., Sattikulov, A., Vdovin, A. D., Malikov, V. M. and Yagudaev, M. R. (1987) *Khim. Prir. Soedin.* 516.
17. Mizuno, M., Matsuura, N., Iinuma, M., Tanaka, T. and Phengklai, C. (1990) *Phytochemistry* **29**, 2675.
18. Mizuno, M., Tanaka, T., Katsuragawa, M., Saito, H. and Iinuma, M. (1990) *J. Nat. Prod* **53**, 498.
19. Ingham,. J. L. (1990) *Biochem. Syst. Ecol.* **18**, 329.
20. Iinuma, M., Tanaka, T., Kawai, M., Mizuno, M. and Lang, F. A. (1991) *Phytochemsitry* **30**, 3773.
21. Iinuma, M., Ohyama, M., Tanaka, T. and Lang, F. A. (1994) *Phytochemistry* **37**, 1157.
22. Shirataki, Y., Noguchi, M., Yokoe, I., Tomimori, T. and Komatsu, M. (1991) *Chem. Pharm. Bull.* **39**, 1568.
23. Ohyama, M., Tanaka, T. and Iinuma, M. (1994) *Chem. Pharm. Bull.* **42**, 2117.
24. Ohyama, M., Tanaka, T. and Iinuma, M. (1994) *Tetrahedron Letters* **35**, 7817.
25. Ohyama, M., Tanaka, T. and Iinuma, M. (1994) *Phytochemistry* (in press).
26. Khan, M. A., Nabi, S. G., Prakash, S. and Zaman, A. (1986) *Phytochemistry* **25**, 1945.