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Prenylated flavonoids from *Moghania philippinensis*

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Abstract

Five prenylated flavonoids, 8-(1,1-dimethylallyl)genistein (**1**), 5,7,3′,4′-tetrahydroxy-2′,5′-di(3-methylbut-2-enyl)isoflavone (**2**), 5,7,3′-trihydroxy-2′-(3-methylbut-2-enyl)-4′,5′-(3,3-dimethylpyrano)isoflavone (**3**), (2*R*)-5,2′,4′-trihydroxy-8,5′-di(3-methylbut-2-enyl)-6,7-(3,3-dimethylpyrano)flavanone (**4a**) and (2*S*)-5, 2′, 4′-trihydroxy-8,5′-di(3-methylbut-2-enyl)-6,7-(3,3-dimethylpyrano)-flavanone (**4b**), were isolated from the roots of *Moghania philippinensis*. The structures of these compounds were determined on the basis of spectroscopic and chemical means.

Keywords: Moghania philippinensis; Fabaceae; prenylated flavonoid

1. Introduction

In the course of developing phyto-estrogenic agents from crude drugs used in traditional medicine, an extract of the roots of *Moghania philippinensis* (Merr. et Rolfe) Li (Fabaceae) was found to have appreciable estrogenic activity when tested for its effect on the proliferation of MCF-7 human breast cancer cells. *M. philippinensis* is a shrubby herb growing in southwestern China, the roots of which have been used in folk medicine as treatment for osteoscopic pains of rheumatism, arthritis of parturition, leukorrhea and arthropathy, (Jiang Su New Medical College, 1977; Chen et al., 1990). The 70% ethanol extract of the roots was reported to have cytotoxic activity against P388 lymphocytic leukemia cells in culture (Chen et al., 1991). As regard chemical constituents, isoflavonoids, prenylated flavonoids, flemiphilippinins A, B, C and flemichin D have been reported from the plants belonging to this genus (Chen et al., 1990, 1991). In the present paper, we report isolation of five new prenylflavanones (**1**–**4a,4b**) and 13 known compounds (**5**–**17**) from the roots of *M. philippinensis,* in which the structure of 8-(1,1-dimethylallyl)-genistein (**1**) was confirmed by synthesis through Claisen (sigmatropic) rearrangement of 7-*O*-prenyl-genistein (Barron and Mariotte, 1994).

2. Results and discussion

A MeOH extract of the roots of *M. philippinensis* (Fabaceae) was further extracted with hexane followed by $CHCl_3$. The $CHCl_3$-soluble fraction was subjected to repeated column chromatography on ODS, Sephadex LH-20 and silica gel to give five new compounds (**1**–**4a,4b**) together with 13 known compounds [genistein (**5**), biochanin A (**6**), 2′-hydroxygenistein (**7**) (Lane and Newman, 1987), lupinalbin A (**8**) (Tahara et al., 1985), 3′-*O*-methylorobol (**9**) (de Almeida and Gottlieb, 1974), 5,7,3,4′-tetrahydroxy-6,8-diprenylisoflavone (**10**), auriculasin (**11**) (Nkengfack et al., 1989), flemiphilippinin A (**12**) (Chen et al., 1991), (2*S*)-liquiritigenin (**13**) (Yahara et al., 1989), (2*S*)-naringenin (**14**) (Osawa et al., 1992), (3*R*)-isoferreirin (**15**) (Adesanya et al., 1985), flemiphilippinin D (**16**) and flemichin D (**17**) (Chen et al., 1990)]. respectively. The known compounds were identified by comparing their spectral data with those reported in the

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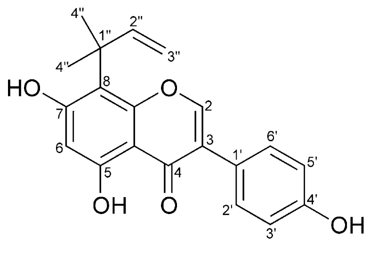

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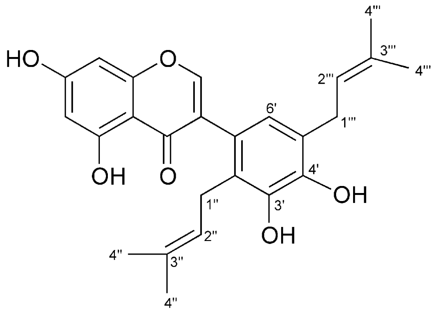

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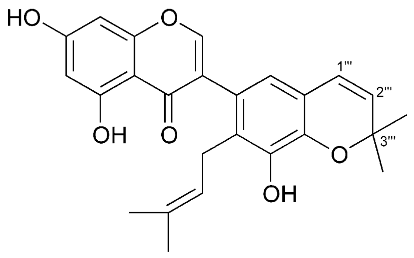

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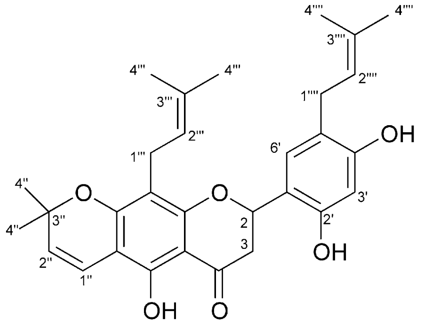

4 (a = 2*R*; b = 2*S*)

literature, and the structures of new compounds were determined as follows.

Compound **1** was shown to have the molecular formula $C_{20}H_{18}O_5$ by HREIMS. The IR spectrum showed the presence of a carbonyl group (1650 cm^{-1}) and the MS spectrum displayed characteristic fragment ion peaks at *m/z* 295 $[M-43]^+$ and *m/z* 283 $[M-55]^+$, indicating the presence of a dimethylallyl substituent (Hashidoko et al., 1986). The UV absorption at 265 nm suggested the presence of either an isoflavonoid or flavonoid skeleton. In the ^1H NMR spectrum, one singlet signal observed at δ_H 7.91 was characteristic of an isoflavone skeleton. Two sets of doublet proton signals at δ_H 6.89 (2H, *d*, $J = 8.8$ Hz, H-3′ and H-5′) and 7.43 (2H, *d*, $J = 8.8$ Hz, H-2′ and H-6′) were correlated with carbon-13 signals at δ_C 115.6 (C-3′, 5′) and 130.3 (C-2′, 6′), respectively, in the HMQC spectrum, indicating the presence of a 4′-monosubstituted B ring. A 1,1-dimethylallyl substituent was assigned on the basis of characteristic ^1H NMR signals [δ_H 1.64 (6H, *s*, H-4″×2), 5.40 (1H, *dd*, $J = 0.7$ and 10.8 Hz, H-3″ *cis*), 5.47 (1H, *br d*, $J = 18.1$ Hz, H-3″ *trans*), and 6.45 (1H, *dd*, $J = 10.8$ and 18.1 Hz, H-2″)], which were correlated with carbon-13 signals [δ_C 27.8 (C-4″×2), 113.7 (C-3″), and 148.9 (C-2″)] in the HMQC spectrum. In the HMBC experiment, a singlet proton signal at δ_H 13.10 (HO-5) was correlated with a carbon-13 signal at δ_C 101.6 (C-6), and a proton signal at δ_H 6.45 (H-2″) with carbon signals at 109.7 (C-8) and 27.8 (C-4″×2). Based on these findings, compound **1** was concluded to be 8-(1,1-dimethylallyl)-genistein. The structure was further confirmed via synthesis of **1** from genistein; 7-isoprenylgenistein (**1a**) was prepared by prenylation of genistein without any protective group. The structure of **1a** was determined by an HMBC experiment. Compound **1a** was subjected to thermal rearrangement by refluxing with acetic anhydride/ sodium acetate for 48 h, according to the method of Barron and Mariotte (1994). The resulting product was deacetylated and purified by column chromatography on silica gel to yield 8-(1,1-dimethylallyl)genistein, which was spectroscopically identical with compound **1**.

Compound **2** was assigned the molecular formula $C_{25}H_{26}O_6$ by HREIMS. The IR spectrum showed absorptions due to an α,β-unsaturated ketone group (1654 cm^{-1})), and a hydroxyl group (3691 and 3019 cm^{-1}). A hydrogen-bonded hydroxyl group was deduced from the presence of a signal at δ_H 12.80 (HO-5) in the ^1H NMR spectrum. The UV absorption (λ_{max} at 260 nm in MeOH) and ^1H NMR signal at δ_H 7.72 (H-2) were characteristic of an isoflavone. The EIMS showed a molecular ion peak at *m/z* 422 $[M]^+$ and fragment ion peaks at *m/z* 353, which could be accounted for by loss of a prenyl group $[M-69]^+$, and a peak at *m/z* 153, resulting from a retro-Diels–Alder (RDA) fragmentation, suggesting the presence of two hydroxyl groups on the A ring (Redaelli and Santaniello, 1984). The ^1H NMR spectrum showed *meta*-coupled signals at δ_H 6.27 ($J = 2.0$ Hz, H-6) and δ_H 6.34 ($J = 2.0$ Hz, H-8). The presence of prenyl groups were confirmed from the ^1H NMR signals [(δ_H 3.23 (2H, *br s*, H-1″), 3.31(2H, *d*, $J = 7.1$ Hz, H-1‴), 5.23 (1H, *t*, $J = 7.1$

Hz, H-2″), 5.31 (1H, *t*, $J = 7.1$ Hz, H-2″) 1.67 (3H, *s*, H-4″), 1.69 (3H, *s*, H-4″) and 1.74 (6H, *s*, H-4‴×2)], in which correlations were observed among allylic methylene signals [δ_H 3.23 (H-1″) and 3.31 (H-1‴)] and oxygenated carbon signals at [δ_C 142.5 (C-3′) and 143.1 (C-4′)], and carbon signals at [δ_C 121.5 (C-1′) and 123.4 (C-6′)], indicating the presence of prenyl moieties at positions C-2′ and C-5′. All ^{1}H and ^{13}C NMR assignments for **2** were performed by COSY, HMQC and HMBC experiments. Thus, compound **2** was determined to be 5,7,3′,4′-tetrahydroxy-2′,5′-di(3-methylbut-2-enyl)isoflavone.

Compound **3** was shown to have the molecular formula $C_{25}H_{24}O_6$ by HREIMS. The EIMS spectrum exhibited an $[M]^+$ ion peak at *m*/*z* 420, and fragment ion peaks at *m*/*z* 365 $[M-55]^+$ and 351 $[M-69]^+$ suggested the presence of a prenyl group. The ^{1}H NMR spectrum showed signals at δ_H 3.25 (2H, *br s*, H-1″) and δ_H 5.08 (1H, *t*, $J = 7.0$ Hz, H-2″) and two methyl signals at δ_H 1.49 and 1.59. The ^{1}H NMR spectral feature of **3** was similar to that of **2** except for lack of one isoprenyl group and the appearance of a characteristic signal for a 2,2-dimethylpyranyl ring [δ_H 5.57 (1H, *d*, $J = 10.0$ Hz, H-2‴), 6.26 (1H, *d*, $J = 10.0$ Hz, H-1‴)] and a singlet signal at [δ_H 1.47 (6H, *s*, H-4‴×2)]. A signal for one aromatic proton was observed at δ_H 6.43, which is assigned to H-6′ in the B ring. The HMBC spectrum showed a correlation of signals between C-6′ and H-1‴, indicating the position of chromene moiety. On the basis of these findings, compound **3** was determined to be 5,7,3′-trihydroxy-2′-(3-methylbut-2-enyl)-4′,5′-(3,3-dimethylpyrano)isoflavone.

Compound **4** was obtained as a yellow oil, and the molecular formula was determined to be $C_{30}H_{34}O_6$ by HREIMS. The IR spectrum of **4** indicated the presence of hydroxyl (3442 cm^{-1}), conjugated carbonyl (1647 cm^{-1}), and aromatic (1610 and 1508 cm^{-1}) groups. Its UV absorptions at 225, 265, 273, 297, 312 and 363 nm suggested a pyranoflavanone chromophore (Smalberger et al., 1974). A fragment ion peak at *m*/*z* 475 $[M-Me]^+$ in the EI mass spectrum gave further evidence for the presence of methyl substituents on the pyran ring (Mahidol et al., 1997). Ion peaks at *m*/*z* 286 and 204 were considered to be derived by the RDA fragmentation. An ion peak at *m*/*z* 286 suggested the presence of γ,γ-dimethylallyl and 2,2-dimethylchromene moieties attached to the A ring. On the other hand, an ion peak at *m*/*z* 204 was considered to be derived from the B ring, which subsequently yields an ion peak at *m*/*z* 149 by loss of an C_4H_7 moiety. Therefore, the B ring also contained one 3,3-dimethylallyl group. From these findings, it was apparent that a 3,3-dimethylallyl group was attached to each of the A and B rings (Shirataki et al., 1982).

The ^{1}H NMR spectrum of **4** exhibited three protons at δ_H 2.82 (*dd*, $J = 3.0$, 17.3 Hz, H-3 *cis*), 3.15 (*dd*, $J = 13.2$, 17.3 Hz, H-3 *trans*) and 5.48 (*dd*, $J = 3.0$, 13.2 Hz, H-2) assignable to H_2-3 and H-2 of a flavanone skeleton. In addition, a hydrogen-bonded hydroxyl group was observed at δ_H 12.27 (*s*, HO-5). In the HMBC experiment, correlations were observed among signals of an allylic methylene at δ_H 3.21 (H-1‴) and two oxygenated carbons at δ_C 158.7 (C-9) and 159.7 (C-7), indicating the presence of a prenyl moiety at C-8. In the ^{1}H NMR spectrum, a *gem*-dimethylchromene ring was assigned by the presence of two singlet signals at δ_H 1.44 and 1.45 and two doublet signals due to olefinic protons at δ_H 5.51 (1H, d, $J = 10.0$ Hz, H-2″) and δ_H 6.64 (1H, *d*, $J = 10.0$ Hz, H-1″). In addition, aromatic protons were concluded to be located at H-3′ (*s*, 6.41) and H-6′ (*s*, 6.94). In the HMBC spectrum, correlations of a signal at δ_H 3.29 (H-1⁗) were observed with signals of δ_C 128.1 (C-6′) and δ_C 155.6 (C-4′), suggesting that the second prenyl group is attached at C-5′ (δ_C 118.9) On the basis of the above findings, compound **4** was determined to be 5,2′,4′-trihydroxy-8,5′-di(3-methylbut-2-enyl)-6,7-(3,3-dimethylpyrano)flavanone. Since compound **4** showed no appreciable Cotton effects (CE) in the circular dichroic (CD) spectrum, it is expected that **4** is a mixture of enantiomers. The relative ratio was determined by HPLC on a chiral column (Chiral CD-Ph). The two enantiomers **4a** and **4b** were eluted at retention times of 34.7 and 36.2 min, respectively, in a ratio of 37:64, when hexane/2-propanol (96:4) was used as a mobile phase. Furthermore, **4a** and **4b** were characterized by their CD spectral features (Gaffield, 1970). Since **4a** showed a positive CE at 300 nm ($\Delta\varepsilon$ +0.77), and negative CEs at 262 nm ($\Delta\varepsilon$ −0.052) and 360 nm ($\Delta\varepsilon$ −0.099), the absolute configuration was concluded to be *R* at C-2. Thus, the structure of **4a** was determined to be (2*R*)-5,2′,4′-trihydroxy-8,5′-di(3-methylbut-2-enyl)-6,7-(3,3-dimethylpyrano)flavanone. On the other hand, **4b** showed a negative CE at 300 nm ($\Delta\varepsilon$ −1.07) and positive CEs at 261 nm ($\Delta\varepsilon$ +0.51) and 366 nm ($\Delta\varepsilon$ +0.45), thus allowing the assignment of the *S*-configuration at C-2 in **4b** (Gaffield, 1970).

3. Experimental

3.1. General

Melting points are uncorrected. Optical rotations were measured with a DIP-360 automatic polarimeter. UV spectra were measured with a UV-2200 UV–vis recording spectrophotometer. CD spectra were obtained with a J-805 spectropolarimeter, and IR spectra were measured with an FT/IR-230 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were measured with a JHA-LAA 400 WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz; Jeol) or Varian UNITY Plus 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometers, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. HR-EIMS were acquired with a JMX-AX 505 HAD mass spectrometer, whereas EIMS were obtained with a JMS DX-300 mass spectrometer at an ionization voltage of 70 eV. CC was carried out on silica gel (BW-820MH), ODS DM 1020T (Fuji Silysia), and Sephadex LH-20 (Pharmacia Co.). Prep HPLC was performed on a Tosoh CCPM-CCPM-II system (Tosoh Co.) equipped with a UV 8020 detector and a TSK gel ODS-80Ts column (21.5×300 mm, Tosoh Co.) Chiral HPLC analysis was performed using a column CD-Ph (4.6×250 mm, 5 μm, Fine Chemicals, Shiseido Co., Yokohama, Japan), using a hexane/2-propanol (96:4) as eluent (1.0 ml/min). 4-Bromo-2-methyl-2-butene (prenyl bromide) was purchased from Aldrich (USA), and genistein from Tokyo Kasei (Japan).

3.2. *Plant material*

The roots of *M. philippinensis* were purchased from Nanning, Guangxi Province, People's Republic of China, in March 2002 and identified by Katsuko Komatsu. A voucher specimen (TMPU 19843) was deposited in the herbarium of the Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Japan.

3.3. *Extraction and isolation*

The roots of *M. philippinensis* (2.5 kg) were extracted with MeOH (3×8 l, reflux). The combined MeOH solutions were filtered and evaporated in vacuo to give a residue (190 g). The residue was suspended in MeOH–H_2O (9:1) and extracted with hexane. The residual MeOH solution was concentrated in vacuo to give a residue, which was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated and applied on a silica gel column eluted with hexane–EtOAc (9:1→5:1) and $CHCl_3$–MeOH (20:1→5:1) to yield ten fractions. Compounds **1** (4 mg), **2** (30 mg), **3** (10 mg), were obtained from fraction 6 **4** (10 mg), **6** (4 mg) and **12** (24 mg) after further CC on Sephadex-LH 20 with hexane–$CHCl_3$–MeOH (3:5:1). Compounds **5** (140 mg), **8** (4 mg), **10** (120 mg), **11** (130 mg), **14** (7 mg), **15** (3 mg), **16** (45 mg) and **17** (8 mg) were obtained from fractions 7 and 8 by repeated CC on ODS, Sephadex LH-20 and silica gel. Compounds **7** (24 mg), **9** (8 mg) and **13** (4 mg) from fraction 8 were obtained by prep HPLC [MeOH–0.1% TFA/H_2O, 5 ml/min, monitored at 256 nm].

3.3.1. *Separation of enantiomers **4a** and **4b***

Enantiomers **4a** and **4b** were separated by HPLC on a chiral CD-Ph column (4.6×250 mm, 5 μm, Fine Chemicals, Shiseido Co., Yokohama, Japan), using a mixed solvent of hexane/2-propanol (96:4) as an eluate (1.0 ml/min). Two peaks were observed at retention times of 34.7 and 36.2 min in an approximate ratio of 37:64. Repeated separation of **4** yielded optically pure **4a** (1.5 mg) and **4b** (2.5 mg).

3.4. *8-(1,1-Dimethylallyl)genistein (**1**)*

Yellow prisms ($CHCl_3$); mp 94–96°; UV (MeOH) nm λ_{max} (log ε): 265 (4.38), 375 (*sh* 3.44); IR ($CHCl_3$) cm^{-1}: 3407, 1650, 1614, 1583, 1515, 1455, 1363, 1313; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.64 (6H, *s*, H_3-4″×2), 5.40 (1H, *dd*, $J = 0.7$, 10.8 Hz, H-3″ *cis*), 5.47 (1H, *d*, $J = 18.1$ Hz, H-3″, *trans*), 6.32 (1H, *s*, H-6), 6.45 (1H, *dd*, $J = 10.8$, 18.1 Hz, H-2″), 6.89 (2H, *d*, $J = 8.8$ Hz, H-3′, 5′), 7.43 (2H, *d*, $J = 8.8$ Hz, H-2′, 6′), 7.91 (1H, *s*, H-2), 13.10 (1H, *s*, OH-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.8 (C-4″×2), 40.9 (C-1″), 101.6 (C-6), 106.8 (C-10), 109.7 (C-8), 113.7 (C-3″), 115.6 (C-3′, 5′), 122.8 (C-1′), 123.1 (C-3), 130.3 (C-2′, 6′), 148.9 (C-2″), 151.7 (C-2), 155.9 (C-4′), 156.2 (C-9), 161.0 (C-5), 161.7 (C-7), 181.3 (C-4); EIMS *m/z* (rel. int.): 338 $[M]^+$ (94), 323 (100), 295 (29), 283 (50), 270 (18), 141 (22), 69 (20); HREIMS *m/z*: 338.1145 (calc. for $C_{20}H_{18}O_5$, 338.1154).

3.5. *5,7,3′,4′-Tetrahydroxy-2′,5′-di(3-methylbut-2-enyl)isoflavone (**2**)*

Amorphous yellow solid; UV (MeOH) nm λ_{max} (log ε): 250 (*sh* 4.14), 260 (4.22), 295 (*sh* 3.76), 350 (*sh* 3.53); IR (MeOH) cm^{-1}: 3691, 3019, 2919, 1654, 1602, 1515, 1457, 1375, 1310, 1267; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.67 (3H, *s*, H_3-4″), 1.69 (3H, *s*, H_3-4″), 1.74 (6H, *s*, H_3-4‴×2), 3.23 (2H, *br s*, H-1″), 3.31 (2H, *d*, $J = 7.1$ Hz, H-1‴), 5.23 (1H, *t*, $J = 7.1$ Hz, H-2″), 5.31 (1H, *t*, $J = 7.1$ Hz, H-2‴), 6.27 (1H, *d*, $J = 2.0$ Hz, H-6), 6.34 (1H, *d*, $J = 2.0$ Hz, H-8), 6.52 (1H, *s*, H-6″), 7.72 (1H, *s*, H-2), 12.80 (1H, *s*, OH-5) ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.8 (C-4″), 17.8 (C-4‴), 25.7 (C-4″), 25.8 (C-4‴), 27.7 (C-1″), 28.7 (C-1‴), 94.1 (C-8), 99.5 (C-6), 105.9 (C-10), 121.5 (C-1′), 121.8 (C-2‴), 122.2 (C-2″), 123.4 (C-6′), 124.4 (C-3), 124.9 (C-2′), 125.4 (C-5′), 134.2 (C-3‴), 134.6 (C-3″), 142.5 (C-3′), 143.1 (C-4′), 153.9 (C-2), 158.2 (C-9), 162.3 (C-7), 162.7(C-5), 181.2 (C-4); EIMS *m/z* (rel. int.): 422 $[M]^+$ (23), 366 (16), 353 (12), 298 (11), 229 (5), 207 (7), 153 (100), 69 (38); HREIMS *m/z*: 422.1683 (calc. for $C_{25}H_{26}O_{6,}$, 422.1729).

3.6. *5,7,3′-Tri-hydroxy-2′-(3-methylbut-2-enyl)-4′,5′-(3,3-dimethyl-pyrano)isoflavone (**3**)*

Amorphous yellow solid; UV (MeOH) nm λ_{max} (log ε): 225(4.31), 250 (*sh* 4.23), 285 (*sh* 4.01); IR ($CHCl_3$) cm^{-1}: 3609, 3021, 2927, 1652, 1618, 1508, 1455, 1282;

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.47 (6H, *s*, H_3-4‴×2), 1.49 (3H, *s*, H_3-4″), 1.59 (3H, *s*, H_3-4″), 3.25 (2H, *br s*, H-1″), 5.08 (1H, *t*, *J* = 7.0 Hz, H-2″), 5.57 (1H, *d*, *J* = 10.0 Hz, H-2‴), 6.26 (1H, *d*, *J* = 10.0 Hz, H-1‴), 6.30 (1H, *br s*, H-6), 6.37 (1H, *br s*, H-8), 6.43 (1H, *s*, H-6′), 7.67 (1H, *s*, H-2), 12.83 (1H, *s*, OH-5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ17.6 (C-4″×2), 25.6 (C-4″), 26.8 (C-1″), 28.3 (C-4‴×2), 77.6 (C-3‴), 94.0 (C-8), 99.5 (C-6), 105.9 (C-10), 118.7 (C-5′), 119.8 (C-6′), 121.4 (C-1‴), 122.4 (C-1′), 122.7 (C-2″), 123.9 (C-3), 127.9 (C-2′), 130.0 (C-2‴), 131.5 (C-3″), 139.6 (C-4′), 142.7 (C-3′), 153.9 (C-2). 158.2 (C-9), 162.4 (C-7), 162.8 (C-5), 181.2 (C-4); EIMS *m/z* (rel. int.): 420 $[M]^+$ (77), 405 (21), 377 (19), 365 (15), 351 (30), 267 (8), 153 (100), 73 (73), 69 (23); HREIMS *m/z*: 420.1597 (calc. for $C_{25}H_{24}O_6$, 420.1573).

*3.7. (2R)-5,2′,4′-Trihydroxy-8,5′-di(3-methylbut-2-enyl)-6,7-(3,3-di-methylpyrano)flavanone (**4a**)*

Yellow oil; UV (MeOH) nm λ_{max} (log ϵ): 225 (*sh*, 4.09), 265 (*sh*, 4.31), 273 (4.34), 297 (*sh*, 3.86), 312 (3.80), 363 (3.16), IR ($CHCl_3$) cm^{-1}: 3675, 3590, 3442, 3019, 2927, 2857, 1647, 1610, 1508, 1446, 1380, 1299, 1261, 1164, 1122, 1010; ^{1}H NMR (400 MHz, $CDCl_3$): δ1.44, (3H, *s*, H_3-4″), 1.45 (3H, *s*, H_3-4″), 1.665 (3H, *s*, H_3-4‴), 1.667 (3H, *s*, H_3-4‴), 1.77 (6H, *s*, H_3-4⁗), 2.82 (1H, *dd*, *J* = 3.0, 17.3 Hz, H-3, *cis*), 3.15 (1H, *dd*, *J* = 13.2, 17.3 Hz, H-3, *trans*), 3.21 (2H, *d*, *J* = 7.8 Hz, H-1‴), 3.29 (2H, *d*, *J* = 7.36 Hz, H-1⁗), 5.10 (1H, *t* = 7.3 Hz, H-2‴), 5.29 (1H, *dt*, *J* = 7.3, 1.2, H-2⁗), 5.48 (1H, *dd*, *J* = 3.0, 13.2 Hz, H-2), 5.51 (1H, *d*, *J* = 10.0 Hz, H-2″), 6.41 (1H, *br s*, H-3′), 6.64 (1H, *d*, *J* = 10.0 Hz, H-1″), 6.94 (1H, *s*, H-6′), 12.27 (1H, *s*, OH-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ17.8 (C-4‴), 17.85 (C-4⁗), 21.4 (C-1‴), 25.76 (C-4‴), 25.8 (C-4⁗), 28.28 (C-4″), 28.34 (C-4″), 29.1 (C-1⁗), 43.0 (C-3), 77.5 (C-2), 78.2 (C-3″), 102.6 (C-10), 103.2 (C-6), 104.6 (C-3′), 108.7 (C-8), 115.6 (C-1″), 116.4 (C-1′), 118.9 (C-5′), 121.7 (C-2⁗), 122.2 (C-2‴), 126.2 (C-2″), 128.1 (C-6′), 131.8 (C-3‴), 135.1 (C-3⁗), 153.6 (C-2′), 155.6 (C-4′), 156.7 (C-5), 158.7 (C-9), 159.7 (C-7), 196.7 (C-4); EIMS *m/z* (rel. int.): 490 $[M]^+$ (25.1), 475 (17.9), 472 (31.0), 429 (37.8), 417 (15.7), 286 (5.3), 271 (30.2), 243 (21.0), 215 (60.8), 204 (4.9), 189 (21.2), 149 (21.1); HREIMS *m/z*: 490.2310 (calc. for $C_{30}H_{34}O_6$ 490.2356); CD (in $CHCl_3$): $\Delta\varepsilon_{360}$ −0.099, $\Delta\varepsilon_{300}$ +0.77, $\Delta\varepsilon_{262}$ −0.052; $[\alpha]_D$: +73.3° (c = 0.15, $CHCl_3$).

*3.8. (2S)-5,2′,4′-trihydroxy-8,5′-di(3-methylbut-2-enyl)-6,7-(3,3-dimethylpyrano)flavanone (**4b**)*

Yellow oil; The UV, HREIMS, ^{1}H NMR, and ^{13}C NMR spectral data were identical with those of **4a**; CD (in $CHCl_3$): $\Delta\varepsilon_{366}$ +0.45, $\Delta\varepsilon_{300}$ −1.07, $\Delta\varepsilon_{261}$ +0.51; $[\alpha]_D$: −84.0° (c = 0.25, $CHCl_3$).

3.9. Synthesis of 8-(1,1-dimethylallyl)genistein

3.9.1. Prenylation at position 7 of genistein

A solution of 100 mg of genistein (0.37 mmol) and 0.05 ml of prenyl bromide (0.43 mmol) in 20 ml of Me_2CO containing 100 mg of anhydrous K_2CO_3 (0.72 mmol) was heated until reflux began, this being maintained for 3 h. Solid K_2CO_3 was removed by filtration and the acetone solution was evaporated to dryness in vacuo. The crude products were applied to a column of silica gel using hexane/EtOAc (2.5:1) as eluant. 7-(3,3-Dimethylallyl)genistein (**1a**) was obtained in a yield of 78 mg (62.2%). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.76 (3H, s, H_3-4″), 1,81 (3H, *s*, H_3-5″), 4.57 (2H, *d*, *J* = 7.0 Hz, H-1″), 5.48 (1H, *t*, *J* = 7.0 Hz, H-2″), 6.39 (1H, *d*, *J* = 2.5 Hz, H-6), 6.41 (1H, *d*, *J* = 2.5 Hz, H-8), 6.85 (2H, *d*, *J* = 9.0 Hz, H-3′, 5′), 7.35 (2H, d, *J* = 9.0 Hz, H-2′, 6′), 7.85 (1H, s, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ18.3 (C-4″), 25.8 (C-5″), 65.4 (C-1″), 93.1 (C-8), 98.8 (C-6), 106.1 (C-10), 115.5 (C-3′, 5′), 118.4 (C-2″), 122.6 (C-1′), 123.7 (C-3), 130.3 (C-2′, 6′), 139.4 (C-3″), 152.8 (C-2), 156.2 (C-4′), 157.9 (C-9), 162.5 (C-5), 164.8 (C-7), 180.9 (C-4); EIMS *m/z* (rel. int.): 338 $[M]^+$ (92.2), 323 (4.0), 304 (5.4), 295 (6.7), 283 (6.2), 270 (100), 153 (29.6), 118 (15.8), 69 (64.1); HREIMS *m/z*: 338.2222 (calc. for: 338.2246).

3.9.2. Claisen rearrangement in the presence of fused NaOAc

7-(3,3-Dimethylallyl)genistein (50 mg, 0.17 mmol, **1a**) and fused anhydrous NaOAc (50 mg) in 5 ml of acetic anhydride were refluxed with stirring for 48 h. Excess Ac_2O was decomposed by addition of ice. After 1 h, the mixture was extracted with $CHCl_3$ and the $CHCl_3$ solution was concentrated in vacuo to give a residue, which was dissolved in 5% methanolic KOH. After 1 h, the reaction mixture was diluted with H_2O, acidified (ice bath, 3 N HCl) and extracted with EtOAc. The EtOAc extract was subjected to CC on silica gel using hexane/EtOAc (3:1) as an eluting solvent. Pure compound **1** was obtained as yellow prisms in a yield of 20 mg (43.5%).

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