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Synthesis and Structure of Flavan-4-ols and 4-Methoxyflavans as New Potential Anticancer Drugs

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Abstract—Reduction of a series of substituted flavanones afforded synthetic access to flavan-4-ols and was followed for some of them by an S_N2-type acid-catalysis in methanol to provide 4-methoxyflavans. The stereochemistry of these compounds was established by ^1H and ^{13}C NMR data. Flavan-4-ols and 4-methoxyflavans have been resolved into enantiomers which are being evaluated as anticancer drugs.

Introduction

There is now overwhelming evidence from epidemiological studies that a high consumption of fruits and vegetables is consistently associated with a low incidence of many types of cancer. This may be relevant in hormone-dependent cancers with considerable variations in incidence in different countries. Among compounds of known structure, flavonoids deserve special attention because they are present in practically all dietary plants, fruits and roots and are consumed daily in considerable amounts. Additionally, flavonoids have been shown to exert a wide variety of health-protective physiological and biological properties.[1,2] Support for a role of certain flavonoids in breast cancer prevention is derived from several observations: first, they were found to inhibit the growth of established cancer cell lines derived from human breast tumors;[3] then, earlier studies have shown that some flavones and flavanones (particularly 5 and 7-substituted)[4] are able to inhibit cytochrome P450 aromatase[4–6] and 17β-hydroxysteroid dehydrogenase,[4,7] two enzymes responsible for the synthesis of estrogens, resulting in a decrease in the level of estrogen in women.

Wähälä and coworkers[8] showed that the *cis*- and *trans*-4′,7-dihydroxyisoflavan-4-ols, identified as metabolites of daidzein, were potent inhibitors of the growth of prostate cancer cells in culture. However, the synthesis of flavan-4-ols and 4-methoxyflavans, which are of a rare occurrence in nature,[9] for anticancer properties, appears to be an unexplored field. The aim of the present investigation was to prepare a number of such prototypic molecules in order to evaluate their cytotoxic and inhibitory activities.

Results and Discussion

The route adopted to the synthesis of flavan-4-ols and 4-methoxyflavans is outlined in Scheme 1. Reduction of the flavanones **1**, **2**, **3** and **4** was performed with sodium borohydride to give the corresponding flavan-4-ols **5**, **6**, **7**, **8**, **9** and **10** and, except for 7-hydroxyflavanone **1**, weak amounts of the corresponding flav-3-enes **11**, **12**, **13**. Flav-3-enes are conveniently obtained in one step by $NaBH_4$ reduction of 2′-hydroxychalcones whose chemical equilibrium with flavanones is well known.[10] However, in our study, the formation of these flav-3-enes occurred after acidification of the reaction mixture and evaporation. Therefore, we interpreted this formation as arising from an intramolecular dehydration of the flavan-4-ols. This hypothesis was confirmed since acidification and heating of **6** in $CHCl_3$ led to the compound **11**.

We exploited the reactivity of flavan-4-ols to synthesize compounds which belong to a rare class of natural products[9]: thus, compounds **5** and **6** were subjected to the action of methanol and HCl and afforded the corresponding 4-methoxyflavans **14** and **15**.

The assignment of stereochemistry to these flavan-4-ols and 4-methoxyflavans is readily made on the basis of the vicinal coupling constants. The key signals and the associated coupling constants in the ^1H NMR spectra of the flavan-4-

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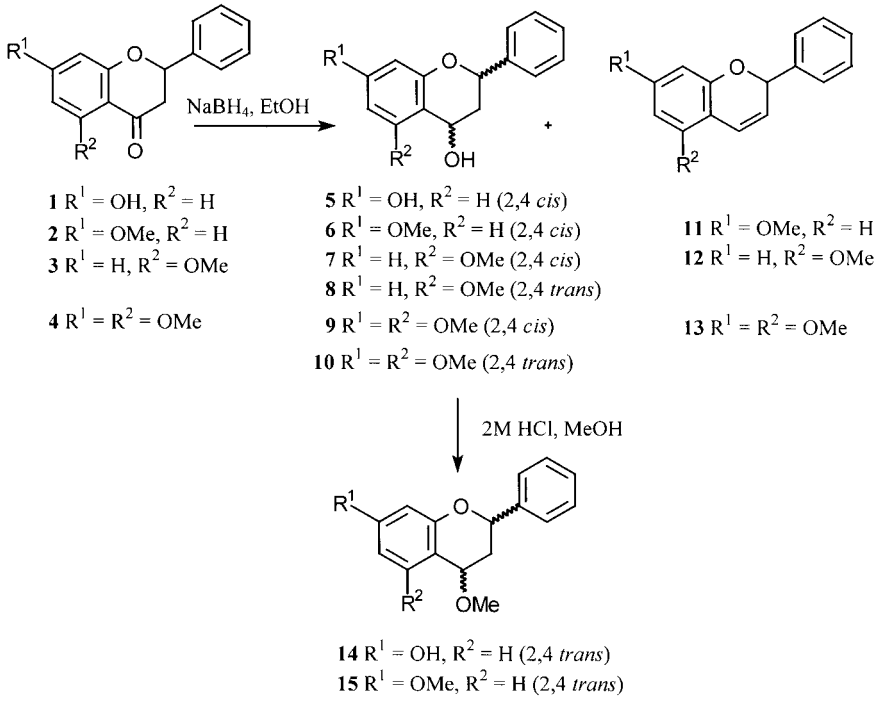


Scheme 1.

ols and 4-methoxyflavans are given in Table 1. The heterocyclic ring protons of these compounds have ABXY-type spectra in which J_{XY}=0 Hz (the 3_{eq}, 3_{ax}, 2 and 4 protons are labeled A, B, X and Y).

The coupling constants are fully compatible with either the half-chair (a) or sofa (b) conformation of the heterocyclic ring in which the 2-aryl group is in the *equatorial* position (Scheme 2).[11]

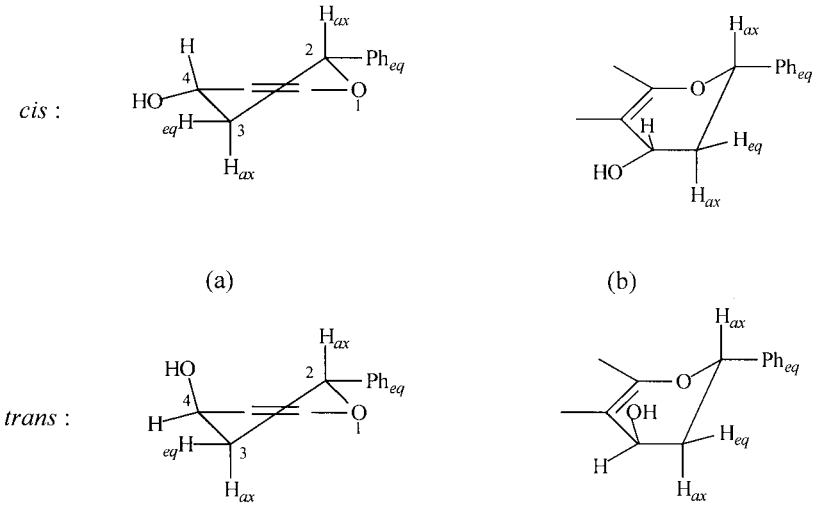
Assignment of the axial proton at C-3 as the high-field proton of the H-3eq, H-3ax pair agrees with results obtained in cyclohexanes.[12,13] Then, the value of $J_{2,3ax}$ for these compounds is so large that it can only arise from a *trans-diaxial* coupling: thus, H-2 is axial and the 2-phenyl group is equatorial. In the case of the compounds **5**, **6**, **7** and **9**, the large value $J_{4,3ax}$ requires H-4 to be *quasi-axial*. Therefore, these products have the 2,4-*cis* structure with both substituents occupying equatorial positions. In the case of the compounds **8** and **10**, the lower value of $J_{4,3ax}$ means that H-4 is in a *quasi-equatorial* position. Thus, these two products have the 2,4-*trans* structure with the 4-hydroxyl group in a *pseudo-axial* position. Confirmation of stereochemical analysis of 4-substituted flavans spectra is based on the sum $J_{4,3ax}+J_{4,3eq}$. The sum of the coupling constants of C(4) H to its two neighbors at C(3) for compounds **8** and **10** is 6.0 Hz which is characteristic for a *trans* arrangement while the sum for compounds **5**, **6**, **7** and **9** is between 16.5 and 17.3 Hz which is characteristic for a *cis* arrangement.[9] As to the 4-methoxyflavans **14** and **15**, the sum $J_{4,3ax}+J_{4,3eq}$ which is 5.5 Hz allows to determine a *trans* relative configuration of these compounds.

All ^{13}C NMR signals could be assigned by the C–H COSY and long-range C–H COSY techniques and are in agreement with values found in the literature (see Table 2).[14] The spectra of the *cis* and *trans* isomers are very similar, the only notable difference being in the chemical shifts of C-2. In the *trans*-flavan-4-ols, this carbon (at δ 73.0 ppm) is shifted upfield from the corresponding carbon (at δ 77.0 ppm) in the *cis* isomers. This upfield shift is evidently due to the γ-*gauche* effect of the axial hydroxyl in the *trans* isomers. The C-4 resonances for compounds **7**, **8**, **9** and **10** are shielded for the presence of the 5-methoxy group.

$NaBH_4$ reduction of 7-hydroxyflavanone **1** and 7-methoxyflavanone **2** is stereoselective since it leads respectively to the 2,4-*cis*-7-hydroxyflavan-4-ol **5** and the 2,4-*cis*-7-

Table 1. Selected ^{1}H NMR data on flavan-4-ols and 4-methoxyflavans

	δ H-3_{ax}	δ H-3_{eq}	δ H-4	δ H-2	$J_{3ax-3eq}$	J_{2-3ax}	J_{2-3eq}	J_{4-3ax}	J_{4-3eq}	$J_{4-3ax}+J_{4-3eq}$
5	2.10	2.51	5.04	5.16	13.1	11.2	1.9	10.3	6.2	16.5
6	2.13	2.52	5.05	5.17	13.2	11.4	2.0	10.2	6.3	16.5
14	2.02	2.34	4.25	5.25	14.4	12.1	1.9	3.2	2.3	5.5
15	2.04	2.36	4.27	5.28	14.3	12.2	2.2	3.2	2.3	5.5
7	2.27	2.54	5.32	5.04	13.2	12.1	1.7	10.1	7.2	17.3
8	2.06	2.30	5.05	5.15	14.4	12.0	1.7	4.1	1.9	6.0
9	2.24	2.51	5.26	5.03	13.4	11.9	1.8	9.8	7.2	17.0
10	2.04	2.27	5.00	5.16	14.5	12.4	1.6	4.1	1.9	6.0



Scheme 2.

methoxyflavan-4-ol **6**. The hydride attack on the opposite side from the phenyl group is responsible for this stereochemistry.

Reduction of the 4-keto group of the 5-methoxyflavanone **3** afforded a mixture of 2,4-*cis*-5-methoxyflavan-4-ol **7** and 2,4-*trans*-5-methoxyflavan-4-ol **8** in a 70:30 ratio. In the same way, reduction of 5,7-dimethoxyflavanone **4** led to the 2,4-*cis*-5,7-dimethoxyflavan-4-ol **9** and 2,4-*trans*-5,7-dimethoxyflavan-4-ol **10** in a 75:25 ratio. We interpreted the formation of the two isomers *cis* and *trans* as arising from the presence of a 5-methoxy group which creates a steric hindrance decreasing the facial discrimination due to the phenyl group.

The 2,4-*cis*-7-hydroxyflavan-4-ol **5** and 2,4-*cis*-7-methoxyflavan-4-ol **6**, through the action of methanol and HCl led to the 2,4-*trans*-7-hydroxy-4-methoxyflavan **14** and 2,4-*trans*-4,7-dimethoxyflavan **15** respectively. Inversion of configuration involves an acidic catalyzed S_N2 reaction in which methanol acts as nucleophile.

The convenient synthesis described here uses moderately cheap reagents and affords two series of compounds, the flavan-4-ols and the 4-methoxyflavans, which are of a rare occurrence in nature. Flav-3-enes were also identified as by-products but they were not further investigated in view of their instability. Reduction, which was performed on racemic flavanones, was expected to afford flavan-4-ols as racemates. Direct enantiomeric separation of the flavan-4-ols and 4-methoxyflavans has been achieved by HPLC using an analytical column (CHIROSE C1®) as a chiral stationary phase. Chromatographic results indicate that these compounds exist as a (1:1) mixture of two optical isomers: 2*R*,4*R* and 2*S*,4*S* for *cis*-compounds, 2*R*,4*S* and 2*S*,4*R* for *trans*-compounds. Once preparative separation of the compounds **5**–**10** and **14**–**15** into enantiomers is carried out, they will be tested for their antiproliferative and aromatase inhibitory properties. Detailed biochemical results will be reported elsewhere.

Table 2. Selected ^{13}C NMR data on flavan-4-ols

	5	**6**	**7**	**9**	*cis*[a]	*trans*[a]	**8**	**10**
C-2	77.1	77.1	77.0	77.3	76.9	73.0	73.3	73.7
C-3	40.2	40.2	37.8	37.9	40.2	38.2	37.6	37.6
C-4	65.6	65.5	63.7	63.4	65.9	63.8	59.5	59.3

[a] From Ref. 13.

Experimental

NMR spectra were recorded on a Bruker 400 MHz spectrometer with Me_4Si as internal standard. High resolution mass spectra were obtained with a VG AUTOSPEC instrument using EI ionization at 70 eV and ESP-MS spectra were performed on a Waters Alliance system equipped with an API-MS interface. IR spectra were recorded in CH_2Cl_2 with a Satellite Mattson FT-IR spectrophotometer and UV with a 930 UVIKON spectrophotometer. Enantiomeric separation of flavan-4-ols was carried out using CHIROSE C1® no. 9604/3202, 5 μm, 250×4.6 mm^2 (CHIRALSEP) as a chiral stationary phase. The flow-rate of the mobile phase (hexane/*i*-PrOH) was 1 ml/min. 7-Hydroxyflavanone was obtained from Indofine Chemical Company; 7-methoxyflavanone and 5-methoxyflavanone were purchased from Sigma Aldrich while 5,7-dimethoxyflavanone was obtained from Lancaster Synthesis.

2,4-*cis*-7-Hydroxyflavan-4-ol 5. To a stirred solution of 7-hydroxyflavanone **1** (70 mg, 2.9×10^{-4} mol) in ethanol (30 ml) at room temperature, was added 210 mg of $NaBH_4$ (5.7×10^{-3} mol). The reaction was monitored by TLC on silica gel (hexane–ethyl acetate, 7:3) and after three days, the reaction mixture was diluted with H_2O (30 ml), acidified with aqueous HCl (pH 6) and extracted with Et_2O (3×30 ml). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via preparative TLC on silica gel (hexane–ethyl acetate, 7:3) afforded **5** (26.6 mg, 38%). R_f 0.13 (hexane–ethyl acetate, 7:3); λ_{max} (MeOH)/nm 219, 282; ν_{max} (CH_2Cl_2) cm^{-1}: 3221, 1619, 1596; δ_H (400 MHz; $CDCl_3$) 2.10 (1H, ddd, $J_{3ax,3eq}$=13.1 Hz, $J_{3ax,2}$=11.2 Hz,

$J_{3ax,4}$=10.3 Hz, 3_{ax}-H), 2.51 (1H, ddd, $J_{3eq,3ax}$=13.2 Hz, $J_{3eq,4}$=6.2 Hz, $J_{3eq,2}$=1.9 Hz, 3_{eq}-H), 5.04 (1H, dd, $J_{4,3ax}$=10.0 Hz, $J_{4,3eq}$=6.2 Hz, 4-H), 5.16 (1H, dd, $J_{2,3ax}$=11.2 Hz, $J_{2,3eq}$=1.7 Hz, 2-H), 6.38 (1H, d, $J_{8,6}$=2.4 Hz, 8-H), 6.49 (1H, dd, $J_{6,5}$=8.4 Hz, $J_{6,8}$=2.4 Hz, 6-H), 7.37 (1H, d, $J_{5,6}$=8.6 Hz, 5-H), 7.34–7.45 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 40.2 (CH_2, C-3), 65.6 (CH, C-4), 77.1 (CH, C-2), 103.2 (CH, C-8), 108.7 (CH, C-6), 118.5 (Cq, C-4a), 126.0 (2×CH, C-2′ and C-6′), 128.3 (2×CH, C-4′ and C-5), 128.6 (2×CH, C-3′ and C-5′), 140.4 (Cq, C-1′), 155.6 (Cq, C-8a), 156.3 (Cq, C-7); *m/z* (EI), M^+ 242, (Found: M^+, 242.0938. $C_{15}H_{14}O_3$ requires M, 242.0943).

2,4-*trans*-7-Hydroxy-4-methoxyflavan 14. To a stirred solution of 2,4-*cis*-7-hydroxyflavan-4-ol **5** (15 mg, 6.2×10^{-5} mol) in methanol (15 ml), was added 2 M HCl. The reaction mixture was heated at 40°C for 5 min, then diluted with H_2O (30 ml) and extracted with Et_2O (3×30 ml). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via preparative TLC on silica gel (hexane–ethyl acetate, 7:3) afforded **14** (12 mg, 76%). R_f 0.39 (hexane–ethyl acetate, 7:3); λ_{max} (MeOH)/nm 227, 280, 286; ν_{max} (CH_2Cl_2) cm^{-1}: 3324, 1619; δ_H (400 MHz; $CDCl_3$) 2.02 (1H, ddd, $J_{3ax,3eq}$=14.4 Hz, $J_{3ax,2}$=12.3 Hz, $J_{3ax,4}$=3.2 Hz, 3_{ax}-H), 2.34 (1H, br dt, $J_{3eq,3ax}$=14.3 Hz, J=2.3 Hz, 3_{eq}-H), 3.45 (3H, s, 4-OCH_3), 4.25 (1H, br t, J=2.8 Hz, 4-H), 5.25 (1H, dd, $J_{2,3ax}$=12.1 Hz, $J_{2,3eq}$=1.9 Hz, 2-H), 6.41 (1H, d, $J_{8,6}$=2.5 Hz, 8-H), 6.44 (1H, dd, $J_{6,5}$=8.2 Hz, $J_{6,8}$=2.5 Hz, 6-H), 7.13 (1H, d, $J_{5,6}$=8.2 Hz, 5-H), 7.34 (1H, m, 4′-H), 7.40 (2H, m, 3′-H and 5′-H), 7.46 (2H, m, 2′-H and 6′-H); δ_C (100 MHz; $CDCl_3$) 35.2 (CH_2, C-3), 55.8 (OCH_3), 72.3 (CH, C-4), 73.4 (CH, C-2), 103.5 (CH, C-8), 108.0 (CH, C-6), 113.5 (Cq, C-4a), 126.3 (2×CH, C-2′ and C-6′), 128.1 (CH, C-4′), 128.6 (2×CH, C-3′ and C-5′), 132.0 (CH, C-5), 141.1 (Cq, C-1′), 156.2 (Cq, C-8a), 157.1 (Cq, C-7); *m/z* (EI), M^+ 256, (Found: M^+, 256.1103. $C_{16}H_{16}O_3$ requires M, 256.1099).

2,4-*cis*-7-Methoxyflavan-4-ol 6 and 7-methoxyflav-3-ene 11. To a stirred solution of 7-methoxyflavanone **2** (100 mg, 4×10^{-4} mol) in ethanol (30 ml) at room temperature, was added 76 mg of $NaBH_4$ (2×10^{-3} mol). The reaction was monitored by TLC on silica gel (toluene–Et_2O, 8:2) and after 4 h, the reaction mixture was diluted with H_2O (50 ml), acidified with aqueous HCl (pH 4) and extracted with Et_2O (3×30 ml). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via preparative TLC on silica gel (toluene–Et_2O, 8:2) afforded **6** (60 mg, 59%) and **11** (6 mg, 6%).

2,4-*cis*-7-Methoxyflavan-4-ol 6. R_f 0.37 (toluene–Et_2O, 8:2); λ_{max} (MeOH)/nm 229, 282; ν_{max} (CH_2Cl_2) cm^{-1}: 3226, 1619, 1580; δ_H (400 MHz; $CDCl_3$) 2.13 (1H, ddd, $J_{3ax,3eq}$=13.2 Hz, $J_{3ax,2}$=11.4 Hz, $J_{3ax,4}$=10.2 Hz, 3_{ax}-H), 2.52 (1H, ddd, $J_{3ax,3eq}$=13.2 Hz, $J_{3eq,4}$=6.3 Hz, $J_{3eq,2}$=2.0 Hz, 3_{eq}-H), 3.78 (3H, s, 7-OCH_3), 5.05 (1H, br s, 4-H), 5.17 (1H, dd, $J_{2,3ax}$=11.4 Hz, $J_{2,3eq}$=2.0 Hz, 2-H), 6.46 (1H, d, $J_{8,6}$=2.5 Hz, 8-H), 6.59 (1H, dd, $J_{6,5}$=8.6 Hz, $J_{6,8}$=2.5 Hz, 6-H), 7.36 (1H, d, $J_{5,6}$=8.6 Hz, 5-H), 7.34–7.45 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 40.2 (CH_2, C-3), 55.3 (OCH_3), 65.5 (CH, C-4), 77.1 (CH, C-2), 101.1 (CH, C-8), 108.1 (CH, C-6), 118.1 (Cq, C-4a), 126.0 (2×CH, C-2′and C-6′), 127.7 (CH, C-4′), 128.2 (CH, C-5), 128.6 (2×CH, C-3′ and C-5′), 140.4 (Cq, C-1′), 155.5 (Cq, C-8a), 160.5 (Cq, C-7); *m/z* (EI), M^+ 256, (Found: M^+, 256.1104. $C_{16}H_{16}O_3$ requires M, 256.1099).

7-Methoxyflav-3-ene 11. R_f 0.88 (toluene–Et_2O, 8:2); λ_{max} (MeOH)/nm 280, 305; δ_H (400 MHz; $CDCl_3$) 3.75 (3H, s, 7-OCH_3), 5.66 (1H, dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.4 Hz, 3-H), 5.88 (1H, dd, $J_{2,3}$=3.1 Hz, $J_{2,4}$=2.0 Hz, 2-H), 6.38 (1H, d, $J_{8,6}$=2.4 Hz, 8-H), 6.43 (1H, dd, $J_{6,5}$=8.2 Hz, $J_{6,8}$=2.5 Hz, 6-H), 6.49 (1H, dd, $J_{4,3}$=9.8 Hz, $J_{4,2}$=1.9 Hz, 4-H), 6.92 (1H, d, $J_{5,6}$=8.2 Hz, 5-H), 7.34–7.45 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 55.3 (OCH3), 77.1 (CH, C-2), 101.8 (CH, C-8), 107.0 (CH, C-6), 114.7 (Cq, C-4a), 121,9 (CH, C-3), 123.7 (CH, C-4), 127.1 (2×CH, C-2′ and C-6′), 127.3 (CH, C-5), 128.4 (CH, C-4′), 128.6 (2×CH, C-3′ and C-5′), 140.9 (Cq, C-1′), 154.4 (Cq, C-8a), 160.9 (Cq, C-7).

2,4-*trans*-4,7-Dimethoxyflavan 15. To a stirred solution of 2,4-*cis*-7-methoxyflavan-4-ol **6** (20 mg, 7.9×10^{-5} mol) in methanol (10 ml), was added 2 M HCl. The reaction mixture was heated at 40°C for 5 min, then diluted with H_2O (30 ml) and extracted with Et_2O (3×30 ml). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via preparative TLC on silica gel (upper phase of toluene–Et_2O–H_2O 10% AcOH, 50:50:50) afforded **15** (17 mg, 80%). R_f 0.75 (upper phase of toluene–Et_2O–H_2O 10% AcOH, 50:50:50); λ_{max} (MeOH)/nm 233, 279; ν_{max} (CH_2Cl_2) cm^{-1}: 1617, 1581; δ_H (400 MHz; $CDCl_3$) 2.04 (1H, ddd, $J_{3ax,3eq}$=14.3 Hz, $J_{3ax,2}$=12.1 Hz, $J_{3ax,4}$=3.2 Hz, 3_{ax}-H), 2.36 (1H, br dt, $J_{3eq,3ax}$=14.2 Hz, J=2.3 Hz, 3_{eq}-H), 3.47 (3H, s, 4-OCH_3), 3.78 (3H, s, 7-OCH_3), 4.27 (1H, br t, J=2.8 Hz, 4-H), 5.28 (1H, dd, $J_{2,3ax}$=12.2 Hz, $J_{2,3eq}$=2.2 Hz, 2-H), 6.52 (1H, d, $J_{8,6}$=2.5 Hz, 8-H), 6.54 (1H, dd, $J_{6,5}$=8.0 Hz, $J_{6,8}$=2.5 Hz, 6-H), 7.19 (1H, d, $J_{5,6}$=8.0 Hz, 5-H), 7.34–7,50 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 35.2 (CH_2, C-3), 55.3 (OCH_3), 55.7 (OCH_3), 72.3 (CH, C-4), 73.5 (CH, C-2), 101.4 (CH, C-8), 107.5 (CH, C-6), 113.3 (Cq, C-4a), 126.3 (2×CH, C-2′ and C-6′), 128.0 (CH, C-4′), 128.5 (2×CH, C-3′ and C-5′), 131.6 (CH, C-5), 141.2 (Cq, C-1′), 156.1 (Cq, C-8a), 161.0 (Cq, C-7); *m/z* (EI), M^+ 270, (Found: M^+, 270.1256. $C_{17}H_{18}O_3$ requires M, 270.1256).

2,4-*cis* and 2,4-*trans*-5-Methoxyflavan-4-ols 7 and 8, 5-methoxyflav-3-ene 12. To a stirred solution of 5-methoxyflavanone **3** (50 mg, 2×10^{-4} mol) in ethanol (30 ml) at room temperature, was added 38 mg of $NaBH_4$ (10^{-3} mol). The reaction was monitored by TLC on silica gel (toluene–Et_2O, 9:1) and after 2 h, the reaction mixture was diluted with H_2O (30 ml), acidified with aqueous AcOH (pH 5) and extracted with Et_2O (3×30 ml). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via preparative TLC on silica gel (toluene–Et_2O, 9:1) afforded **7** (20 mg, 39%), **8** (8.2 mg, 16%) and **12** (1.5 mg, 3%).

2,4-*cis*-5-Methoxyflavan-4-ol 7. R_f 0.41 (toluene–Et_2O, 9:1); λ_{max} (MeOH)/nm 232, 277; ν_{max} (CH_2Cl_2) cm^{-1}: 3552, 1590, 1470; δ_H (400 MHz; $CDCl_3$) 2.27 (1H, ddd, $J_{3ax,3eq}$=13.2 Hz, $J_{3ax,2}$=12.1 Hz, $J_{3ax,4}$=10.1 Hz, 3_{ax}-H), 2.54 (1H, ddd, $J_{3eq,3ax}$=13.5 Hz, $J_{3eq,4}$=7.2 Hz,

$J_{3eq,2}$=1.7 Hz, 3_{eq}-H), 3.91 (3H, s, 5-OCH_3), 4.07 (1H, br s, 4-OH), 5.04 (1H, dd, $J_{2,3ax}$=11.9 Hz, $J_{2,3eq}$=1.1 Hz, 2-H), 5.32 (1H, br t, J=8.6 Hz, 4-H), 6.51 (1H, d, $J_{6,7}$=8.2 Hz, 6-H), 6.59 (1H, d, $J_{8,7}$=8.3 Hz, 8-H), 7.14 (1H, t, $J_{7,6}$ and $J_{7,8}$=8.3 Hz, 7-H), 7.34 (1H, m, 4′-H), 7.40 (2H, m, 3′-H and 5′-H), 7.46 (2H, m, 2′-H and 6′-H); δ_C (100 MHz; $CDCl_3$) 37.8 (CH_2, C-3), 55.7 (OCH_3), 63.7 (CH, C-4), 77.0 (CH, C-2), 102.8 (CH, C-6), 110.6 (CH, C-8), 114.4 (Cq, C-4a), 126.3 (2×CH, C-2′ and C-6′), 128.2 (CH, C-4′), 128.6 (2×CH, C-3′ and C-5′), 129.0 (CH, C-7), 140.4 (Cq, C-1′), 156.0 (Cq, C-8a), 158.6 (Cq, C-5); *m/z* (EI), M^+ 256, (Found: M^+, 256.1095. $C_{16}H_{16}O_3$ requires M, 256.1099).

2,4-*trans*-5-Methoxyflavan-4-ol 8. R_f 0.32 (toluene–Et_2O, 9:1); λ_{max} (MeOH)/nm 229, 277; ν_{max} (CH_2Cl_2) cm^{-1}: 3575, 1597, 1470; δ_H (400 MHz; $CDCl_3$) 2.06 (1H, ddd, $J_{3ax,3eq}$=14.4 Hz, $J_{3ax,2}$=12.0 Hz, $J_{3ax,4}$=4.1 Hz, 3_{ax}-H), 2.30 (1H, br dt, $J_{3eq,3ax}$=14.4 Hz, J=1.9 Hz, 3_{eq}-H), 2.66 (1H, br s, 4-OH), 3.91 (3H, s, 5-OCH_3), 5.05 (1H, m, 4-H), 5.15 (1H, dd, $J_{2,3ax}$ 12.0, $J_{2,3eq}$=1.7 Hz, 2-H), 6.51 (1H, d, $J_{6,7}$=8.2 Hz, 6-H), 6.63 (1H, d, $J_{8,7}$=8.4 Hz, 8-H), 7.20 (1H, t, $J_{7,6}$ and $J_{7,8}$=8.2 Hz, 7-H), 7.34 (1H, m, 4′-H), 7.40 (2H, m, 3′-H and 5′-H), 7.46 (2H, m, 2′-H and 6′-H); δ_C (100 MHz; $CDCl_3$) 37.6 (CH_2, C-3), 55.6 (OCH_3), 59.5 (CH, C-4), 73.3 (CH, C-2), 102.1 (CH, C-6), 110.4 (CH, C-8), 113.2 (Cq, C-4a), 126.3 (2×CH, C-2′ and C-6′), 128.0 (CH, C-4′), 128.5 (2×CH, C-3′ and C-5′), 129.6 (CH, C-7), 141.0 (Cq, C-1′), 155.7 (Cq, C-8a), 158.5 (Cq, C-5); *m/z* (EI), M^+ 256, (Found: M^+, 256.1091. $C_{16}H_{16}O_3$ requires M, 256.1099).

5-Methoxyflav-3-ene 12. R_f 0.78 (toluene–Et_2O, 91); δ_H (400 MHz; $CDCl_3$) 3.83 (3H, s, 5-OCH_3), 5.76 (1H, dd, $J_{3,4}$=10.0 Hz, $J_{3,2}$=3.5 Hz, 3-H), 5.85 (1H, dd, $J_{2,3}$=3.2 Hz, $J_{2,4}$=2.0 Hz, 2-H), 6.43 (1H, d, $J_{6,7}$=8.8 Hz, 6-H), 6.46 (1H, d, $J_{8,7}$=9.1 Hz, 8-H), 6.89 (1H, dd, $J_{4,3}$=10.0 Hz, $J_{4,2}$=1.4 Hz, 4-H), 7.05 (1H, t, $J_{7,6}$ and $J_{7,8}$=8.2 Hz, 7-H), 7.30–7.50 (5H, m, Ph).

2,4-*cis* and 2,4-*trans*-5,7-Dimethoxyflavan-4-ols 9 and 10, 5,7-dimethoxyflav-3-ene 13. To a stirred solution of 5,7-dimethoxyflavanone **4** (50 mg, 1.7×10^{-4} mol) in ethanol (20 ml) at room temperature, was added 20 mg of $NaBH_4$ (5×10^{-4} mol). The reaction was monitored by TLC on silica gel (toluene–Et_2O, 9:1) and after 8 h, the reaction mixture was diluted with H_2O (20 ml), acidified with aqueous AcOH (pH 6) and extracted with Et_2O (3×30 ml). The combined ethereal extracts were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification via preparative TLC on silica gel (toluene–Et_2O, 9:1) afforded **9** (20 mg, 41%), **10** (6.5 mg, 13%) and **13** (2.5 mg, 5%).

2,4-*cis*-5,7-Dimethoxyflavan-4-ol 9. R_f 0.29 (toluene–Et_2O, 9:1); λ_{max} (MeOH)/nm 240, 264; ν_{max} (CH_2Cl_2) cm^{-1}: 3559, 1616, 1589; δ_H (400 MHz; $CDCl_3$) 2.24 (1H, ddd, $J_{3ax,3eq}$=13.4 Hz, $J_{3ax,2}$=11.9 Hz, $J_{3ax,4}$=9.8 Hz, 3_{ax}-H), 2.51 (1H, ddd, $J_{3eq,3ax}$=13.5 Hz, $J_{3eq,4}$=7.2 Hz, $J_{3eq,2}$=1.8 Hz, 3_{eq}-H), 3.75 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.88 (1H, s, 4-OH), 5.03 (1H, dd, $J_{2,3ax}$=11.9 Hz, $J_{2,3eq}$=1.5 Hz, 2-H), 5.26 (1H, br t, J=8.4 Hz, 4-H), 6.13 (2H, d, $J_{6,8}$=2.3 Hz, 6-H and 8-H), 7.34 (1H, m, 4′-H), 7.40 (2H, m, 3′-H and 5′-H), 7.46 (2H, m, 2′-H and 6′-H); δ_C (100 MHz; $CDCl_3$) 37.9 (CH_2, C-3), 55.4 (OCH_3), 55.7 (OCH_3), 63.4 (CH, C-4), 77.3 (CH, C-2), 92.4 (CH, C-6), 93.9 (CH, C-8), 107.2 (Cq, C-4a), 126.3 (2×CH, C-2′ and C-6′), 128.2 (CH, C-4′), 128.6 (2×CH, C-3′ and C-5′), 140.3 (Cq, C-1′), 156.6 (Cq, C-8a), 159.3 (Cq, C-5), 160.7 (Cq, C-7); ESP-(40V) *m/z*, $[M-H]^-$ 285 (Found $[M-H]^-$, 284.8227, $C_{17}H_{18}O_4$ requires $[M-H]^-$, 285.1132).

2,4-*trans*-5,7-Dimethoxyflavan-4-ol 10. R_f 0.20 (toluene–Et_2O, 9:1); λ_{max} (MeOH)/nm 234, 277; ν_{max} (CH_2Cl_2) cm^{-1}: 3575, 1615, 1591; δ_H (400 MHz; $CDCl_3$) 2.04 (1H, ddd, $J_{3ax,3eq}$=14.6 Hz, $J_{3ax,2}$=12.4 Hz, $J_{3ax,4}$=4.1 Hz, 3_{ax}-H), 2.27 (1H, br dt, $J_{3eq,3ax}$=14.4 Hz, J=1.9 Hz, 3_{eq}-H), 2.50 (1H, br s, 4-OH), 3.77 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 5.00 (1H, m, 4-H), 5.16 (1H, dd, $J_{2,3ax}$=12.2 Hz, $J_{2,3eq}$=1.6 Hz, 2-H), 6.12 (1H, d, $J_{6,8}$=2.2 Hz, 6-H), 6.16 (1H, d, $J_{8,6}$=2.2 Hz, 8-H), 7.34 (1H, m, 4′-H), 7.40 (2H, m, 3′-H and 5′-H), 7.48 (2H, m, 2′-H and 6′-H); δ_C (100 MHz; $CDCl_3$) 37.6 (CH_2, C-3), 55.4 (OCH_3), 55.6 (OCH_3), 59.3 (CH, C-4), 73.7 (CH, C-2), 91.8 (CH, C-6), 93.4 (CH, C-8), 106.1 (Cq, C-4a), 126.3 (2×CH, C-2′ and C-6′), 128.0 (CH, C-4′), 128.6 (2×CH, C-3′ and C-5′), 140.9 (Cq, C-1′), 156.4 (Cq, C-8a), 159.3 (Cq, C-5), 161.2 (Cq, C-7); *m/z* (EI), M^+ 286, (Found: M^+, 286.1203. $C_{17}H_{18}O_4$ requires M, 286.1205).

5,7-Dimethoxyflav-3-ene 13. R_f 0.80 (toluene–Et_2O, 9:1); λ_{max} (MeOH)/nm 277; δ_H (400 MHz; $CDCl_3$) 3.74 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 5.61 (1H, dd, $J_{3,4}$=9.9 Hz, $J_{3,2}$=3.4 Hz, 3-H), 5.83 (1H, dd, $J_{2,3}$=3.3 Hz, $J_{2,4}$=1.9 Hz, 2-H), 6.03 (1H, d, $J_{6,8}$=2.2 Hz, 6-H), 6.05 (1H, d, $J_{8,6}$=2.2 Hz, 8-H), 6.80 (1H, dd, $J_{4,3}$=9.9 Hz, $J_{4,2}$=1.8 Hz, 4-H), 7.34–7.47 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 55.4 (OCH3), 55.6 (OCH3), 77.2 (CH, C-2), 91.9 (CH, C-6), 93.8 (CH, C-8), 104.4 (Cq, C-4a), 118,8 (CH, C-4), 119.8 (CH, C-3), 127.1 (2×CH, C-2′ and C-6′), 128.3 (CH, C-4′), 128.6 (2×CH, C-3′ and C-5′), 140.9 (Cq, C-1′), 154.9 (Cq, C-5), 156.3 (Cq, C-8a), 163.1 (Cq, C-7).

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References

1. Havsteen, B. *Biochem. Pharmacol.* **1993**, *32*, 1141.
2. Di Carlo, G.; Mascolo, N.; Izzo, A. A.; Capasso, F. *Life Sci.* **1999**, *65*, 337.
3. So, F. V.; Guthrie, N.; Chambers, A. F.; Carroll, K. K. *Cancer Lett.* **1997**, *112*, 127.
4. Le Bail, J. C.; Laroche, T.; Marre-Fournier, F.; Habrioux, G. *Cancer Lett.* **1998**, *133*, 101.
5. Kellis, J. T.; Vickery, L. E. *Science* **1984**, *225*, 1032.
6. Adlercreutz, H.; Bannwart, C.; Wähälä, K.; Mäkelä, T.; Brunow, G.; Hase, T.; Arosemena, P. J.; Kellis, J. T.; Vickery, L. E. *J. Steroid Biochem. Mol. Biol.* **1993**, *44*, 147.
7. Mäkelä, S.; Poutanen, M. J.; Kostian, M. L.; Lehtimaki, N.;

Strauss, L.; Santti, R.; Vihko, R. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 310.
8. Wähälä, K.; Koskimies, J. K.; Mesilaakso, M.; Salakka, A. K.; Leino, T. K.; Adlercreutz, H. *J. Org. Chem.* **1997**, *62*, 7690.
9. Dehmlow, E. V.; Van Ree, T.; Guntenhöner, M. *Phytochemistry* **1998**, *49*, 1805.
10. Clark-Lewis, J. W.; Jemison, R. W.; Skingle, D. C.; Williams, L. R. *Chem. Ind.* **1967**, 1455.
11. Clark-Lewis, J. W. *Aust. J. Chem.* **1968**, *21*, 2059.
12. Jackman, L. M. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, Pergamon: London, 1959 (pp 115–119).
13. Bolger, B. J.; Hirwe, A.; Marathe, K. G.; Philbin, E. M.; Vickars, M. A. *Tetrahedron* **1966**, *22*, 621.
14. Senda, Y.; Ishiyama, J.; Imaizumi, S.; Hanaya, K. *J. Chem. Soc., Perkin Trans. 1* **1977**, 217.