

## Supplementary Materials:

# Tyrosinase Inhibitors Derived from Chemical Constituents of *Dianella ensifolia*

Yu-Chang Chen <sup>1</sup>, Sheng-Han Su <sup>2,†</sup>, Jheng-Cian Huang <sup>2,†</sup>, Che-Yi Chao <sup>3</sup>, Ping-Jyun Sung <sup>4</sup>, Yih-Fung Chen <sup>5,6</sup>, Horng-Huey Ko <sup>1,6,7,8,\*</sup> and Yueh-Hsiung Kuo <sup>9,10,11,\*</sup>

<sup>1</sup> School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>2</sup> Department of Chemistry, National Taiwan University, Taipei 106, Taiwan

<sup>3</sup> Department of Food Nutrition and Health Biotechnology, Asia University, Taichung 413, Taiwan

<sup>4</sup> National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan

<sup>5</sup> Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>6</sup> Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>7</sup> Department of Fragrance and Cosmetic Science, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>8</sup> Drug Development and Value Creation Center, Kaohsiung Medical University, Kaohsiung 807, Taiwan

<sup>9</sup> Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung 404, Taiwan

<sup>10</sup> Chinese Medicine Research Center, China Medical University, Taichung 404, Taiwan

<sup>11</sup> Department of Biotechnology, Asia University, Taichung 413, Taiwan

\* Correspondence: hhko@kmu.edu.tw (H.-H.K.); kuoyh@mail.cmu.edu.tw (Y.-H.K.); Tel.: +886-7-3121101 (ext. 2643) (H.-H.K.); +886-4-22053366 (ext. 5709) (Y.-H.K.)

† These authors contributed equally to this work.

**Figure S1.** The chemical structures of compounds **1–71**.

**Figure S2.** IR spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

**Figure S3.** EIMS spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

**Figure S4.** The <sup>1</sup>H-NMR spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**; 400 MHz, CDCl<sub>3</sub>)

**Figure S5.** The <sup>13</sup>C-NMR spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**; 100 MHz, CDCl<sub>3</sub>)

**Figure S6.** COSY spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

**Figure S7.** NOESY spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

**Figure S8.** HMQC spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

**Figure S9.** HMBC spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

**Figure S10.** IR spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

**Figure S11.** EIMS spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

**Figure S12.** The <sup>1</sup>H-NMR spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**; 500 MHz, CDCl<sub>3</sub>)

**Figure S13.** The <sup>13</sup>C-NMR spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**; 125 MHz, CDCl<sub>3</sub>)

**Figure S14.** COSY spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

**Figure S15.** NOESY spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

**Figure S16.** HMQC spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

**Figure S17.** HMBC spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

**Figure S18.** IR spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

**Figure S19.** EIMS spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

**Figure S20.** The <sup>1</sup>H-NMR spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**; 500 MHz, CDCl<sub>3</sub>)

**Figure S21.** The <sup>13</sup>C-NMR spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**; 125 MHz, CDCl<sub>3</sub>)

**Figure S22.** COSY spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

**Figure S23.** NOESY spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

**Figure S24.** HMQC spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

**Figure S25.** HMBC spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

**Figure S26.** IR spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

**Figure S27.** EIMS spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

**Figure S28.** The <sup>1</sup>H-NMR spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**; 400 MHz, CDCl<sub>3</sub>)

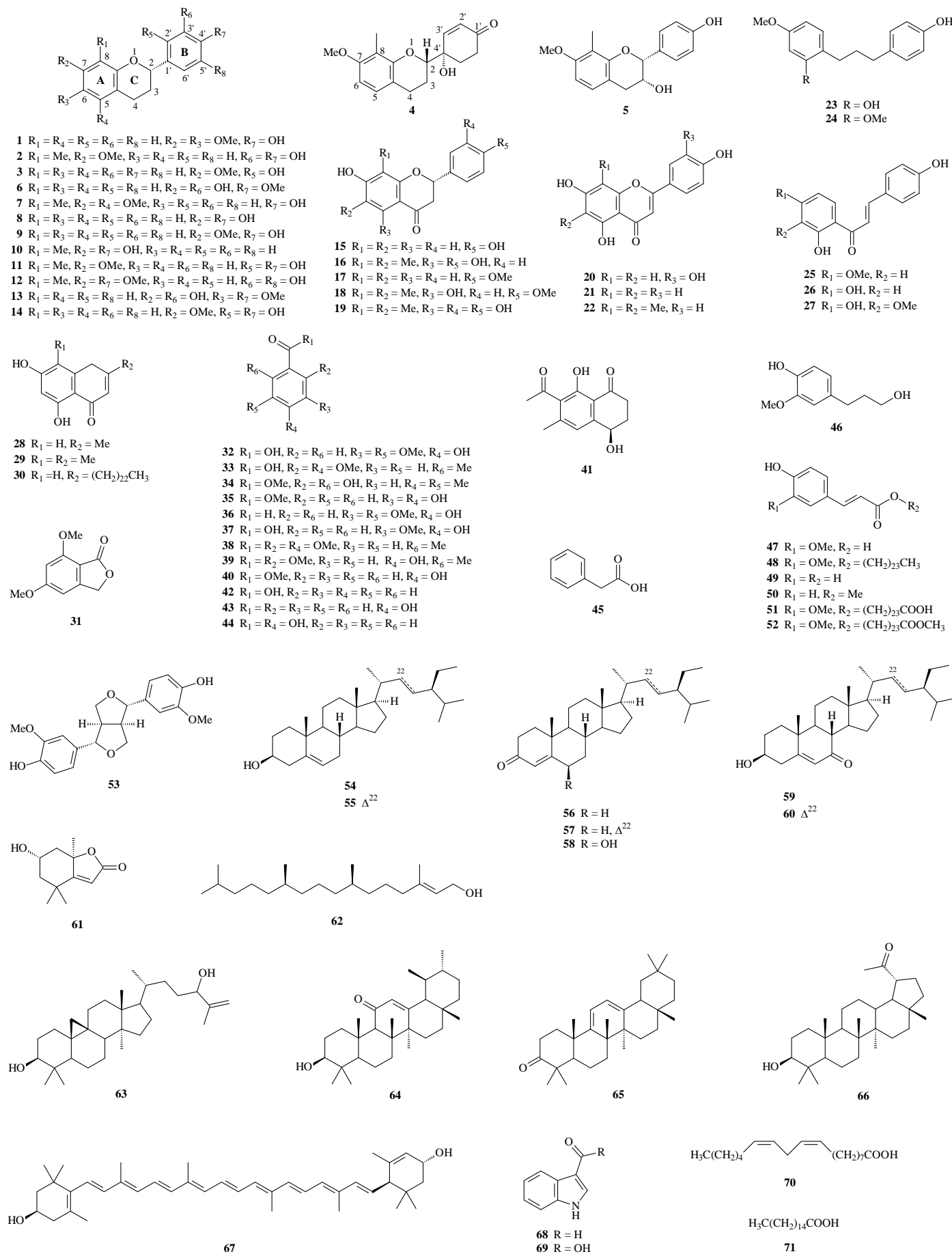
**Figure S29.** The <sup>13</sup>C-NMR spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**; 100 MHz, CDCl<sub>3</sub>)

**Figure S30.** COSY spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

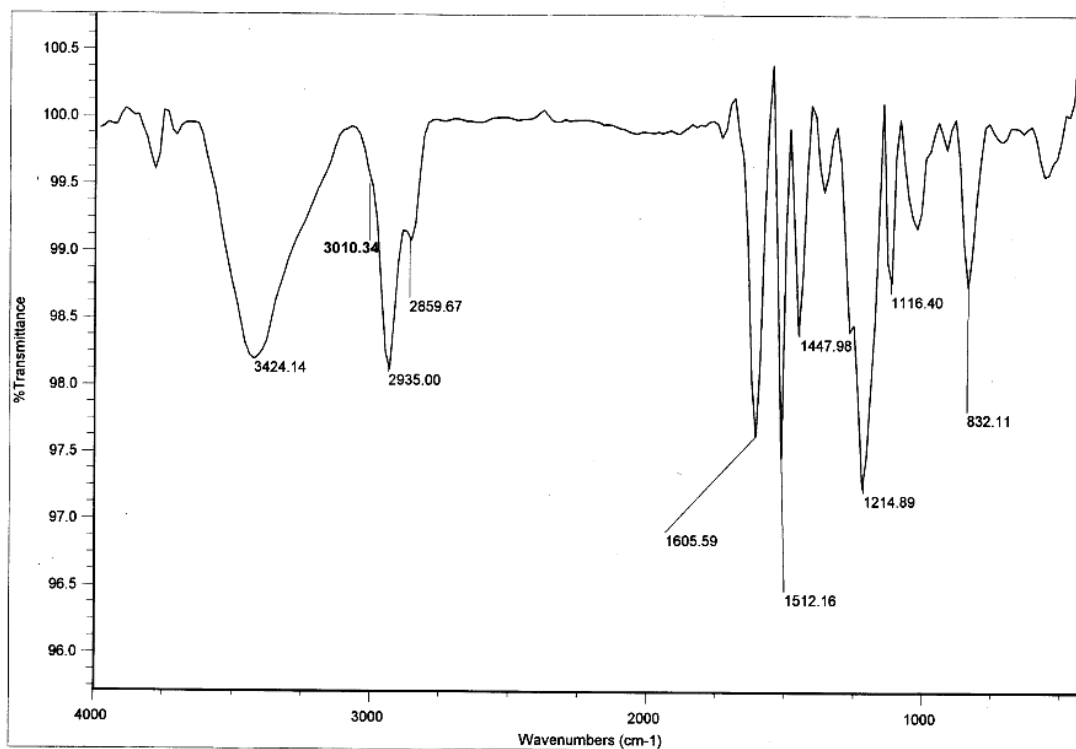
**Figure S31.** NOESY spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

**Figure S32.** HMQC spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

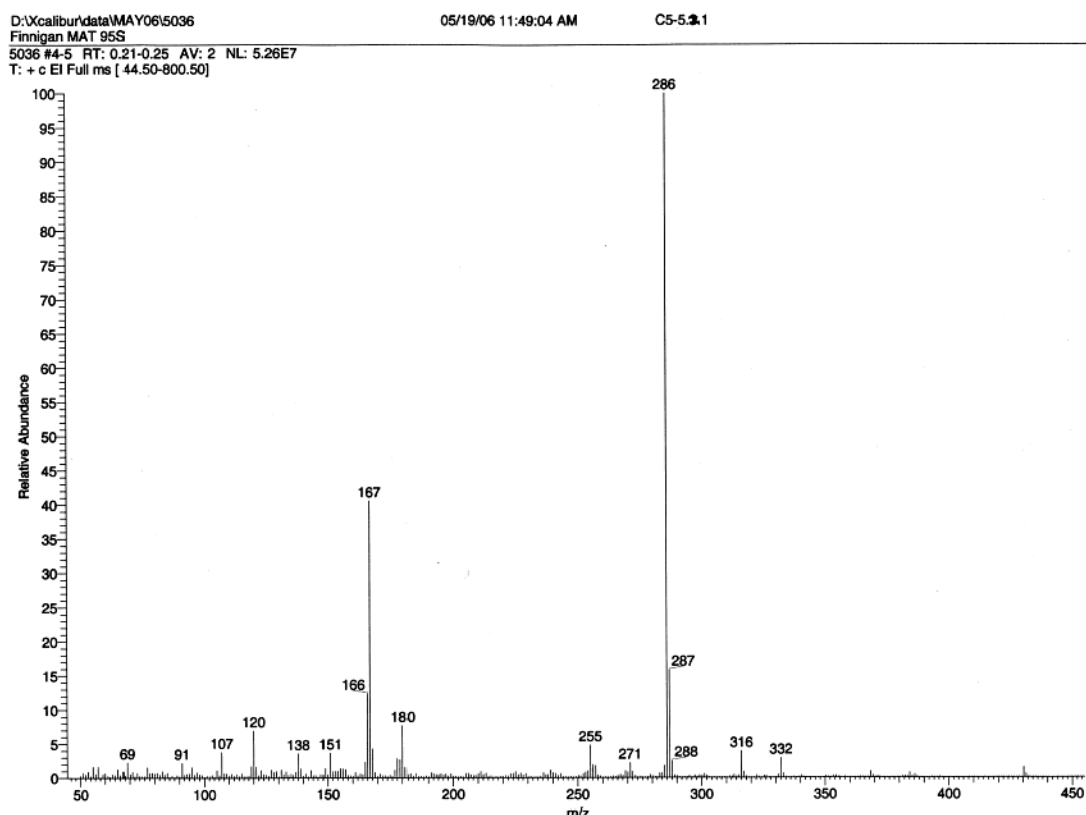
**Figure S33.** HMBC spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)



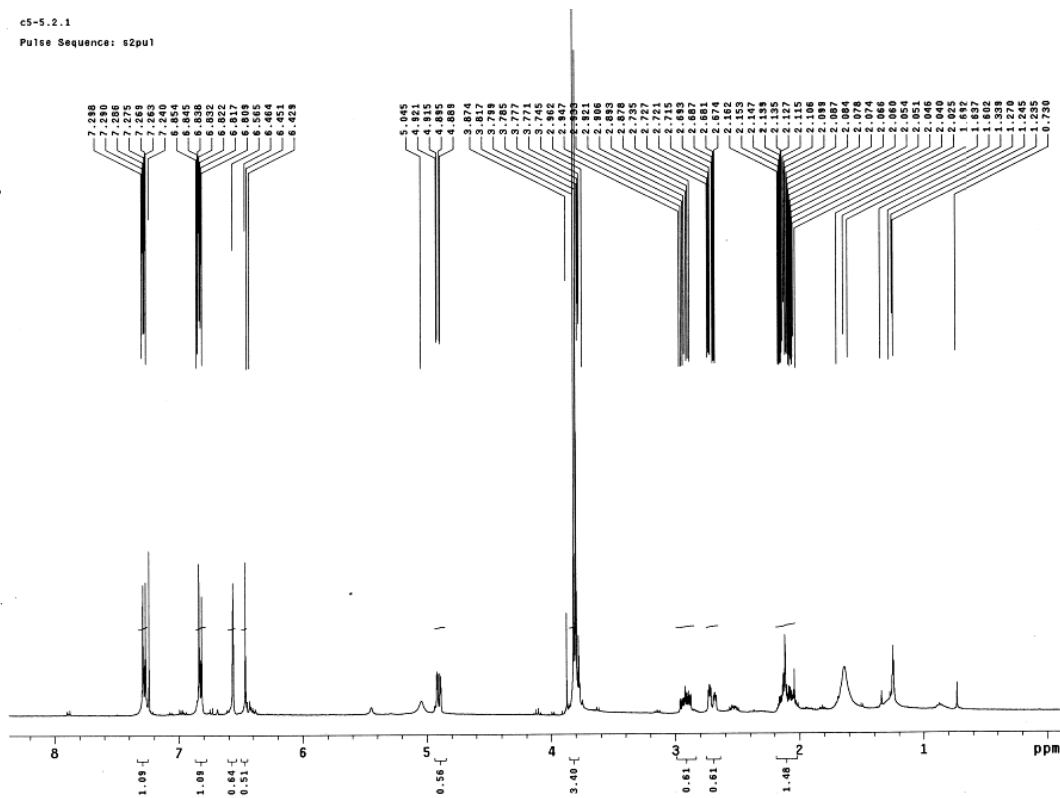
**Figure S1.** The chemical structures of compounds **1–71**.



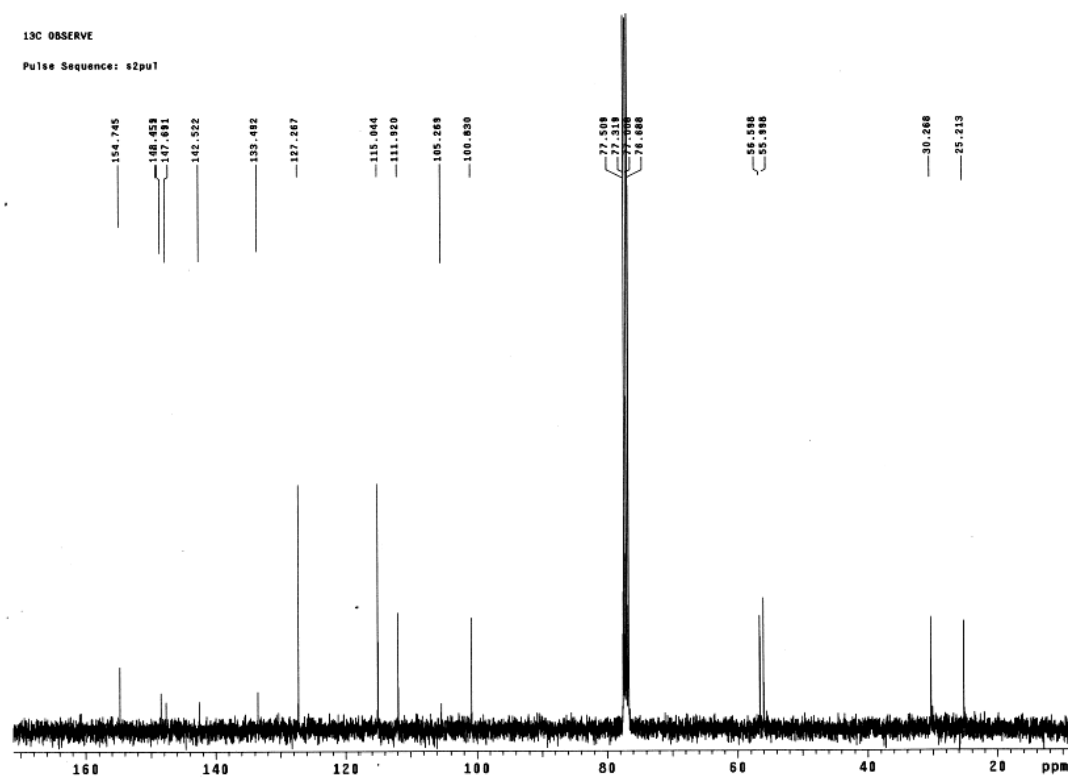
**Figure S2.** IR spectrum of (2S)-4'-hydroxy-6,7-dimethoxyflavan (**1**)



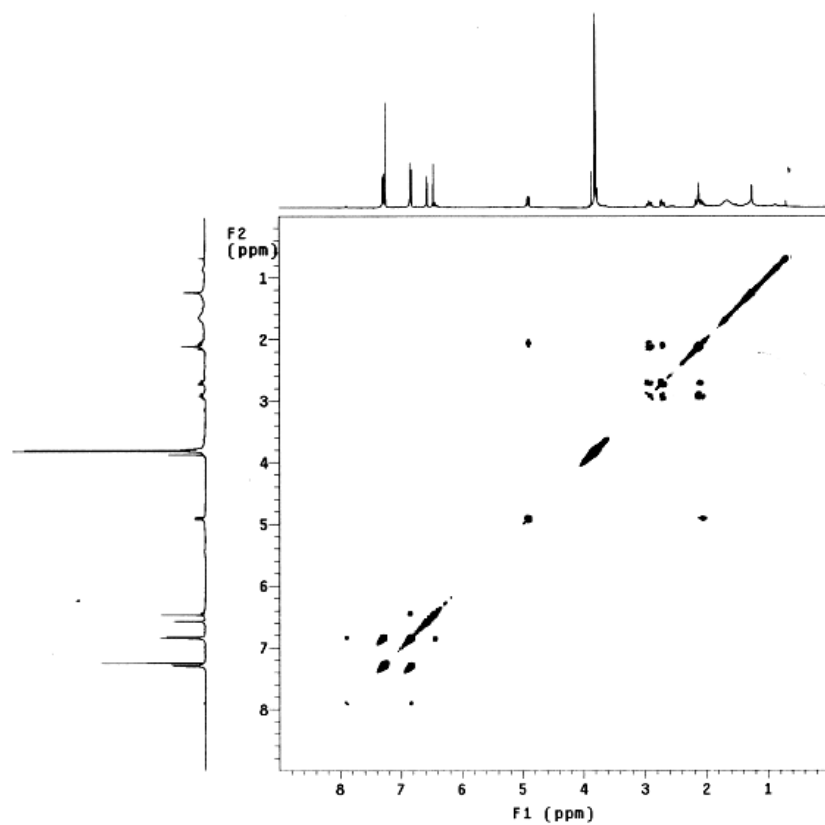
**Figure S3.** EIMS spectrum of (2S)-4'-hydroxy-6,7-dimethoxyflavan (**1**)



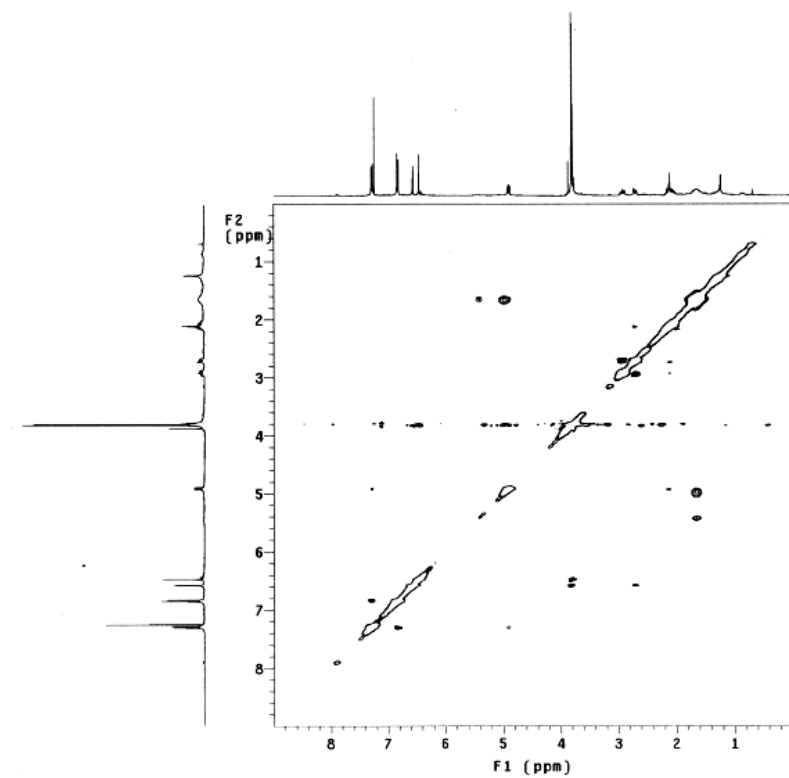
**Figure S4.** The  $^1\text{H}$ -NMR spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**; 400 MHz,  $\text{CDCl}_3$ )



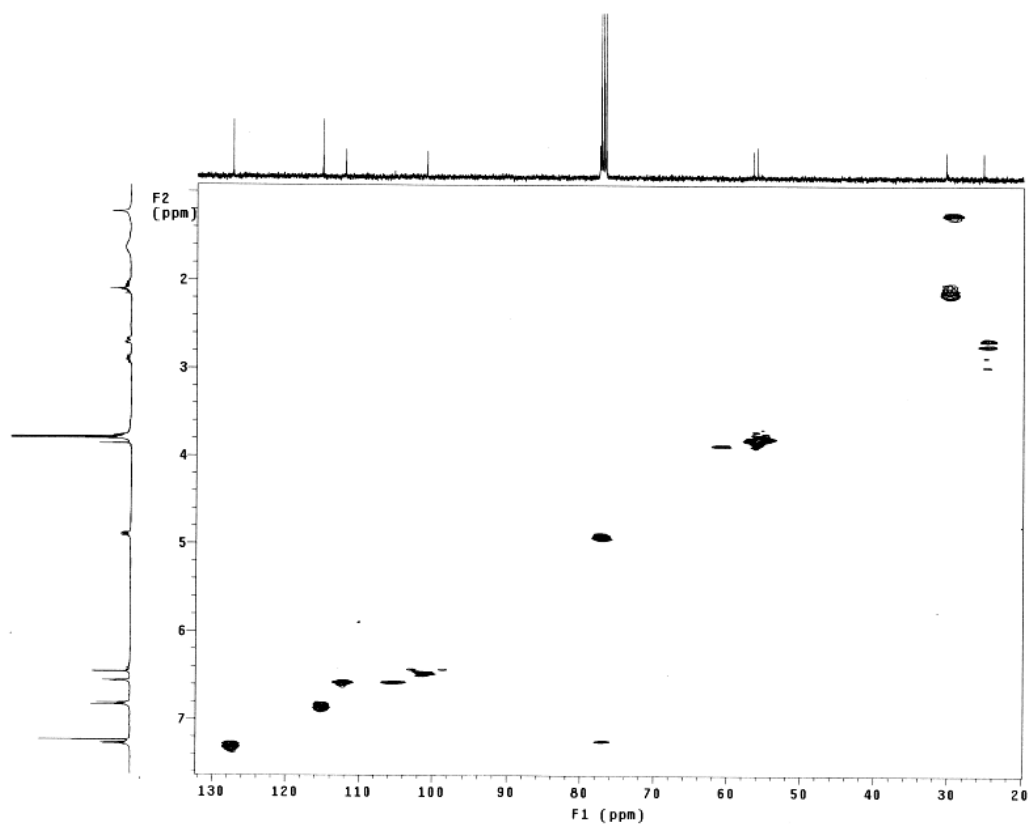
**Figure S5.** The  $^{13}\text{C}$ -NMR spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**; 100 MHz,  $\text{CDCl}_3$ )



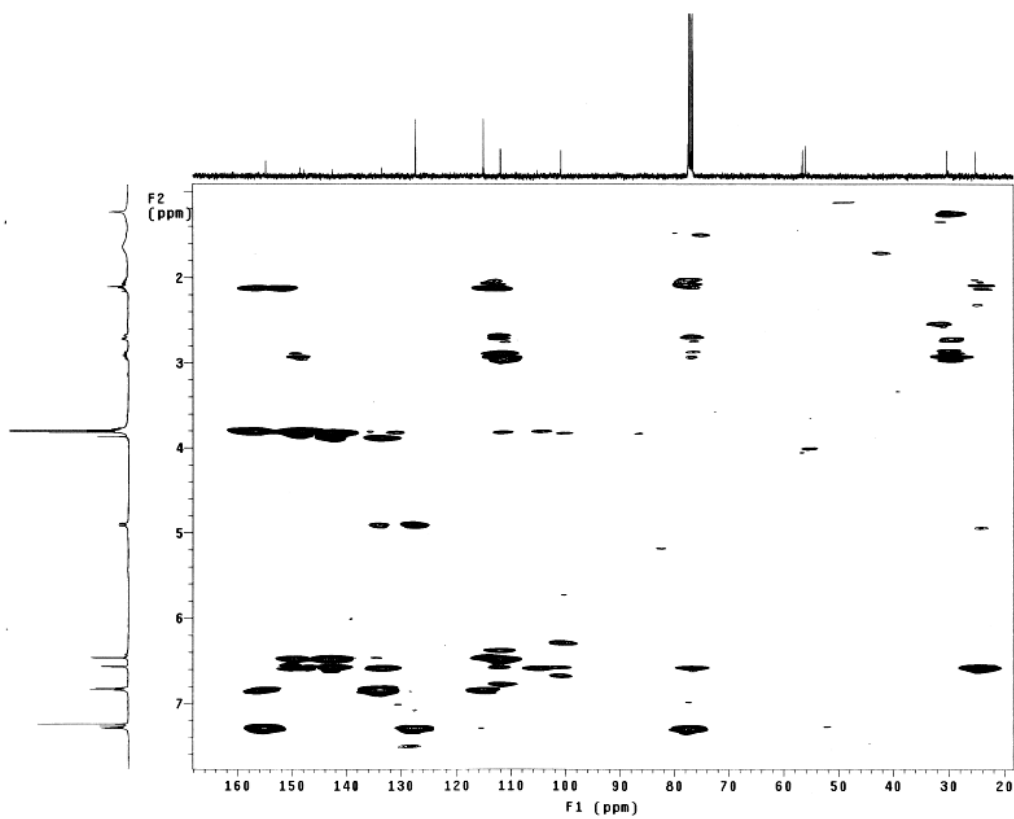
**Figure S6.** COSY spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)



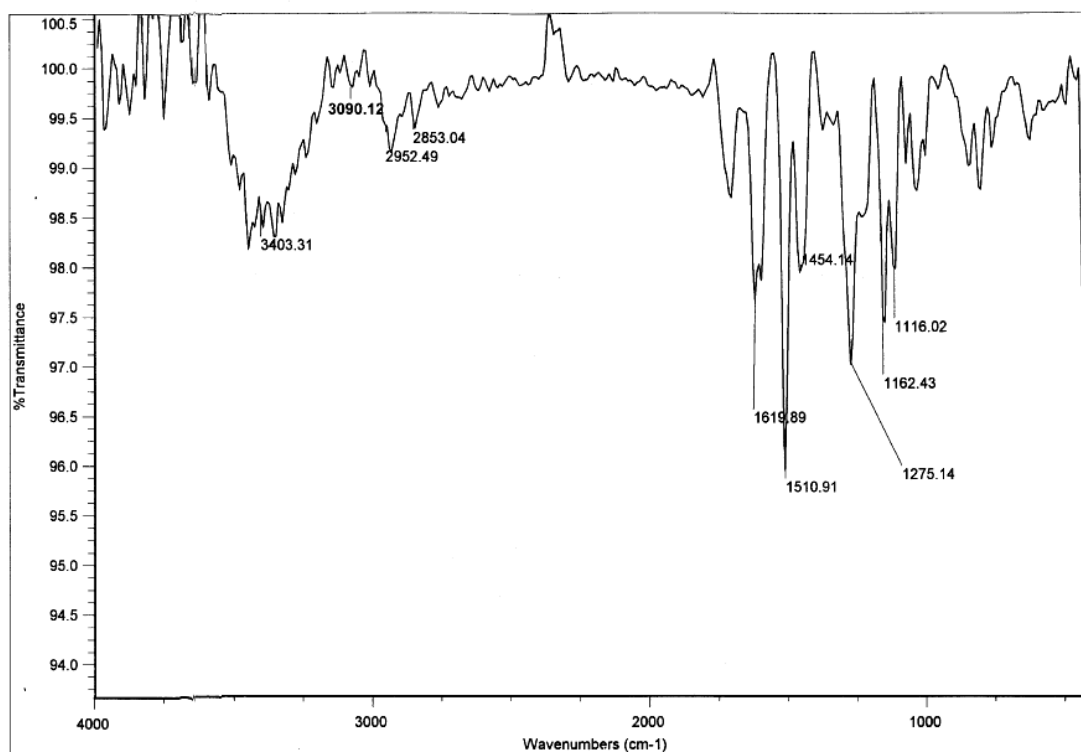
**Figure S7.** NOESY spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)



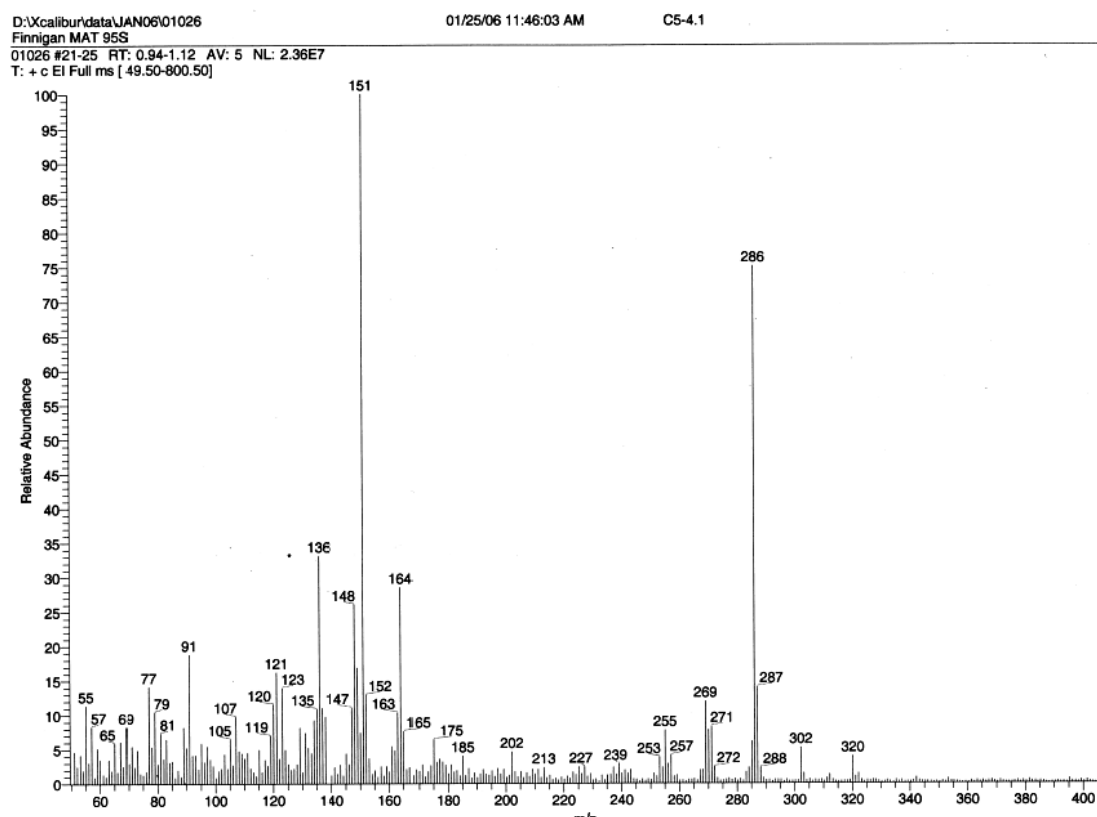
**Figure S8.** HMQC spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)



**Figure S9.** HMBC spectrum of (2*S*)-4'-hydroxy-6,7-dimethoxyflavan (**1**)

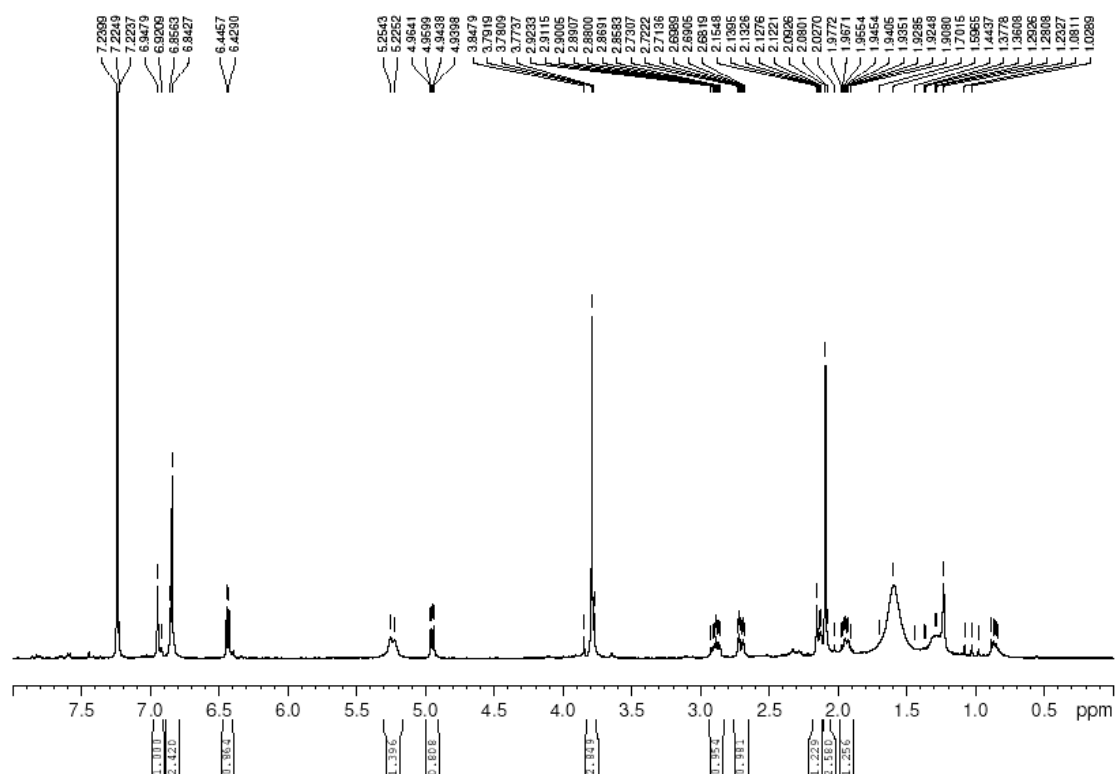


**Figure S10.** IR spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

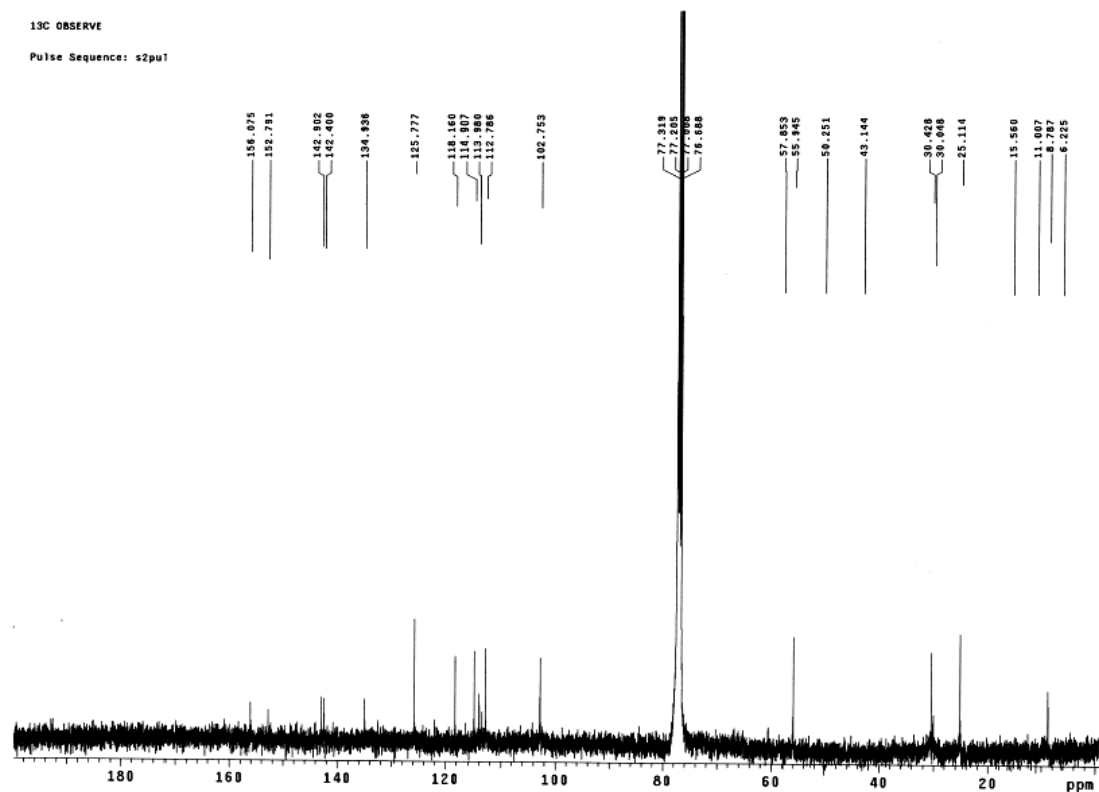


**Figure S11.** EIMS spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

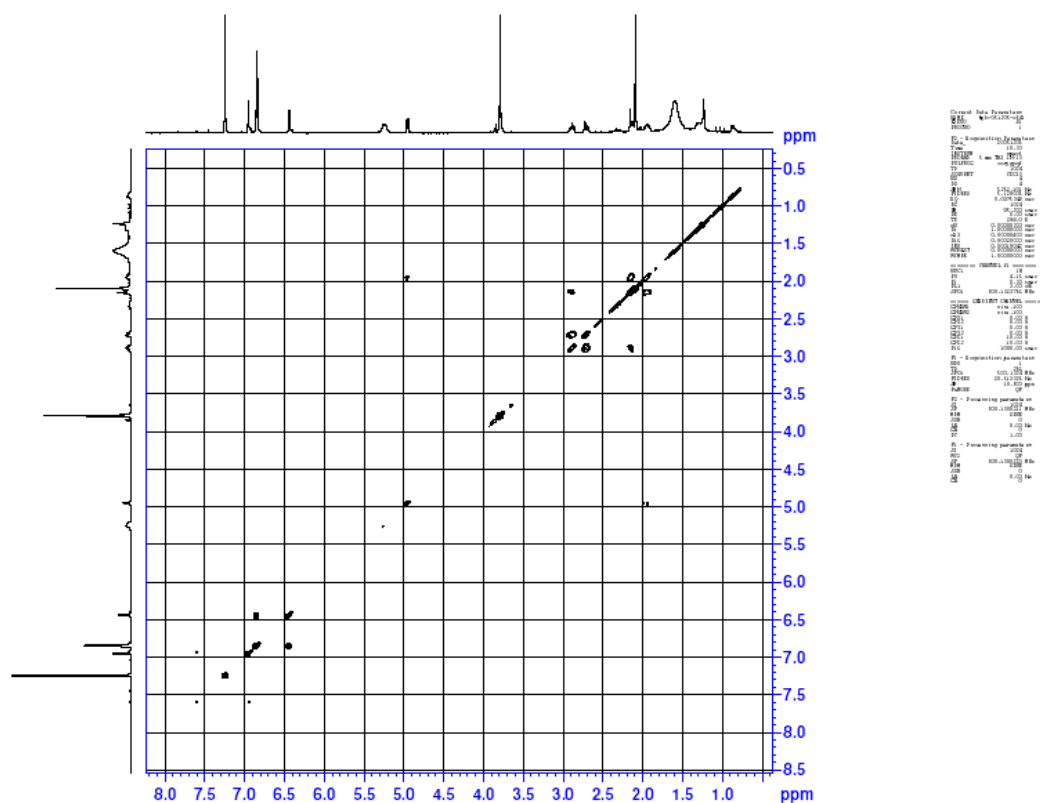




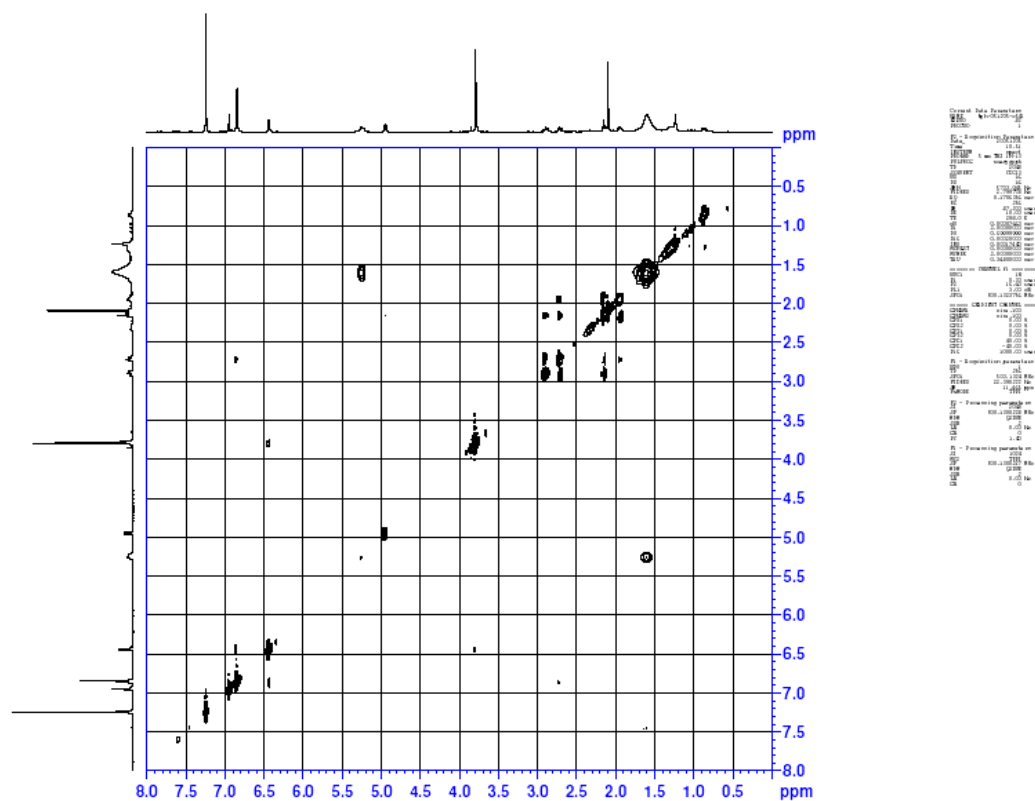
**Figure S12.** The  $^1\text{H}$ -NMR spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**; 500 MHz,  $\text{CDCl}_3$ )



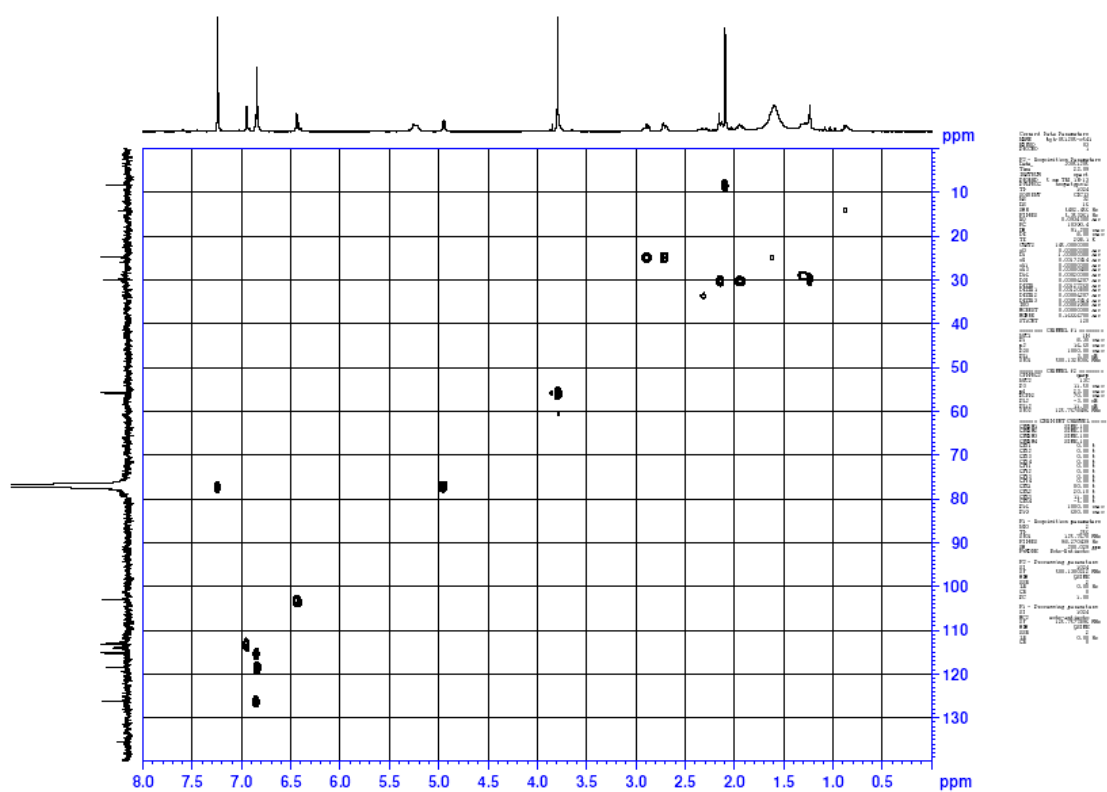
**Figure S13.** The  $^{13}\text{C}$ -NMR spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**; 125 MHz,  $\text{CDCl}_3$ )

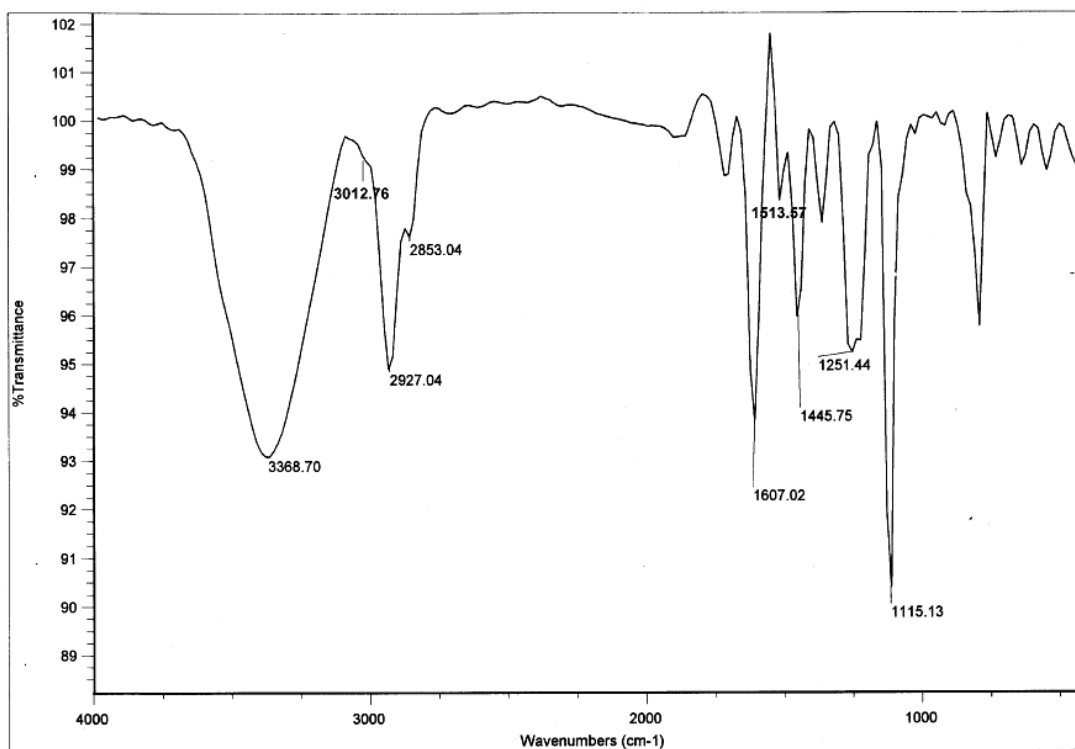


**Figure S14.** COSY spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

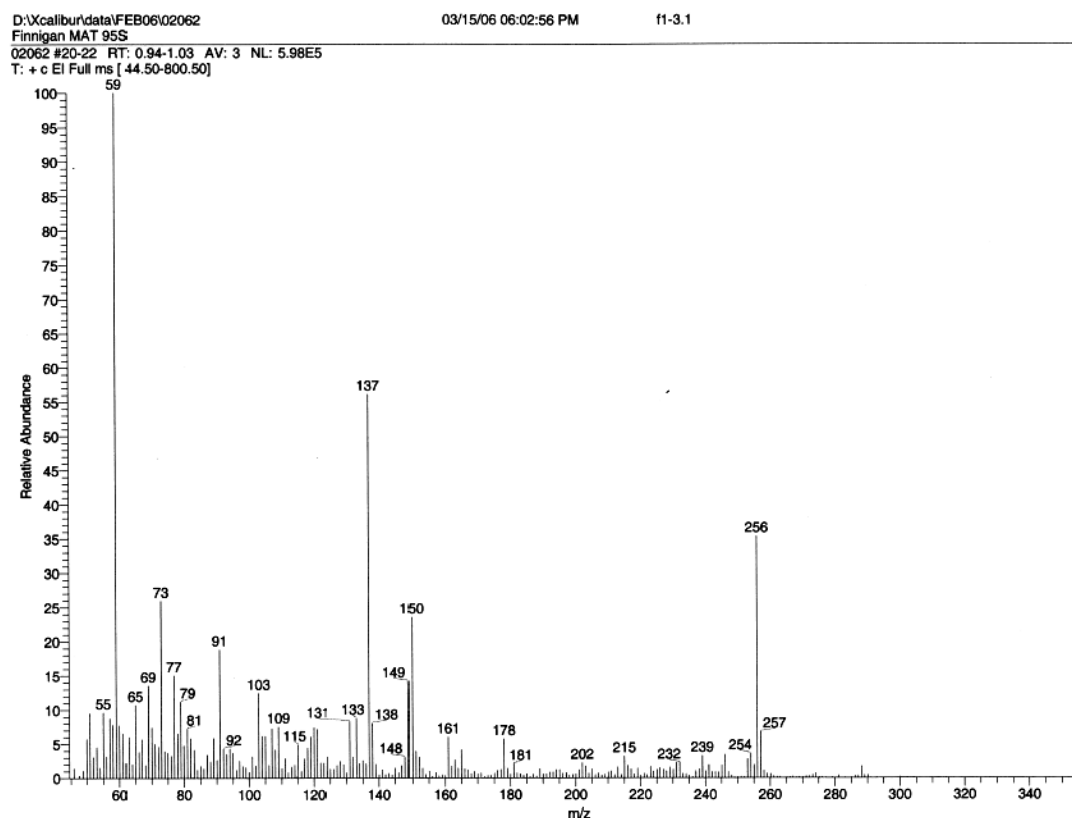


**Figure S15.** NOESY spectrum of (2*S*)-3',4'-dihydroxy-7-methoxy-8-methylflavan (**2**)

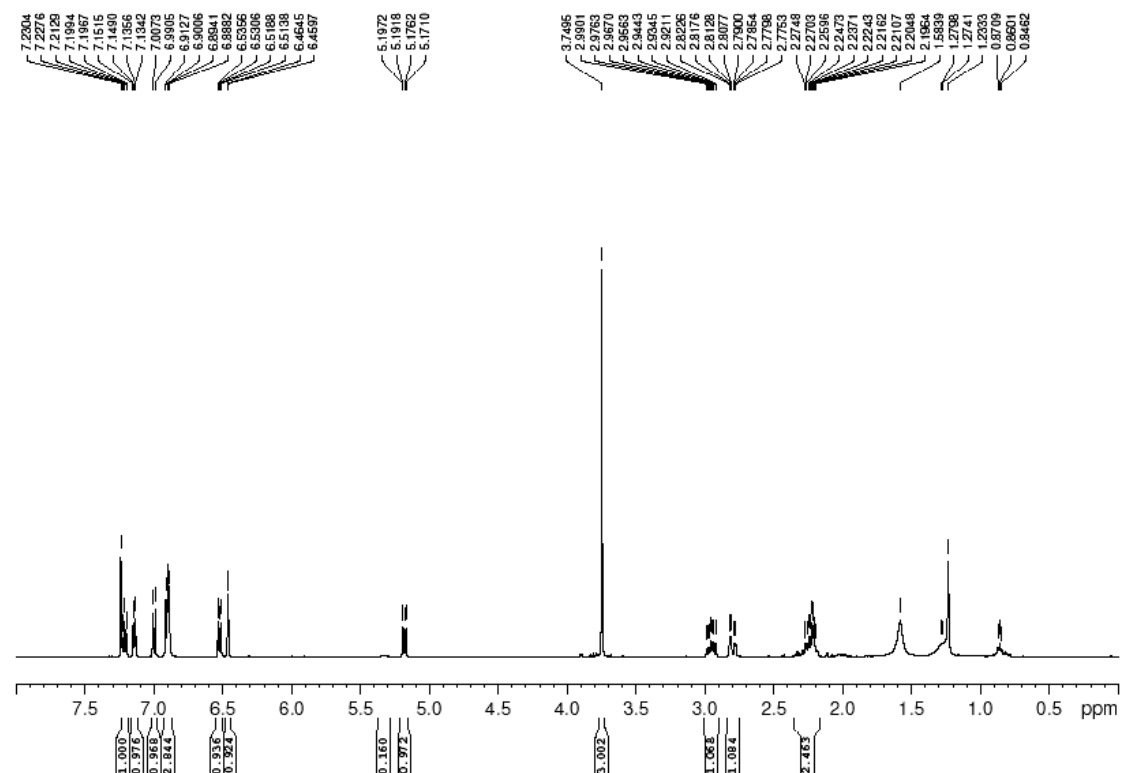




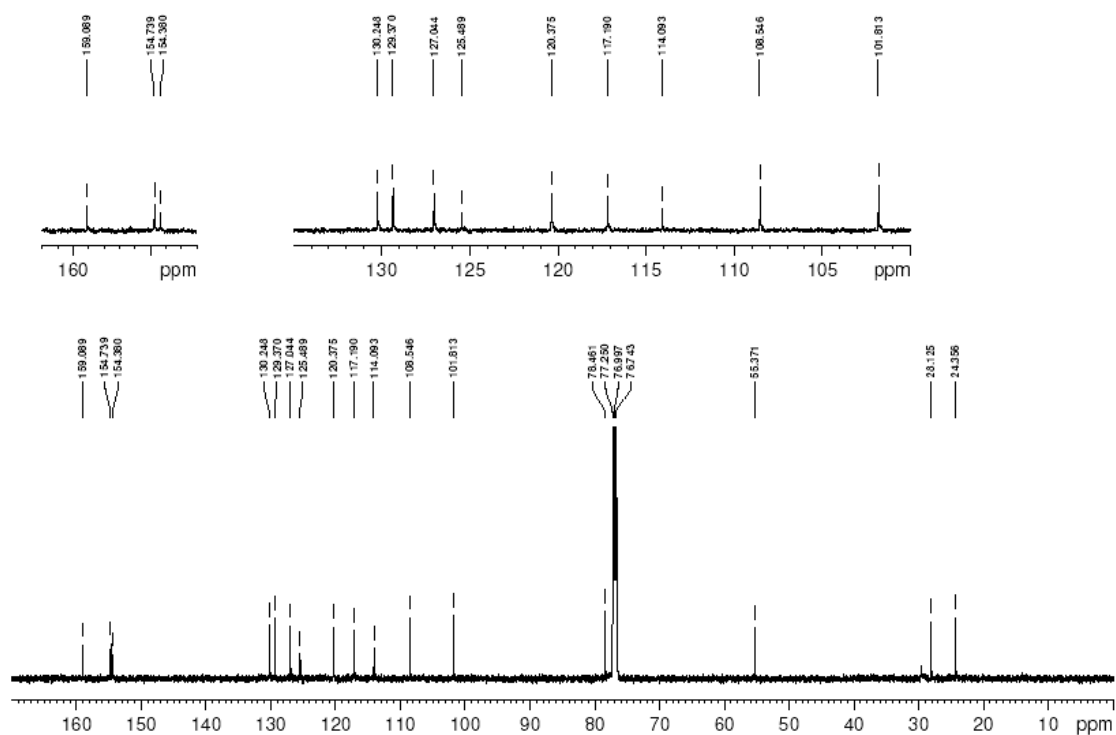
**Figure S18.** IR spectrum of (2S)-2'-hydroxy-7-methoxyflavan (**3**)



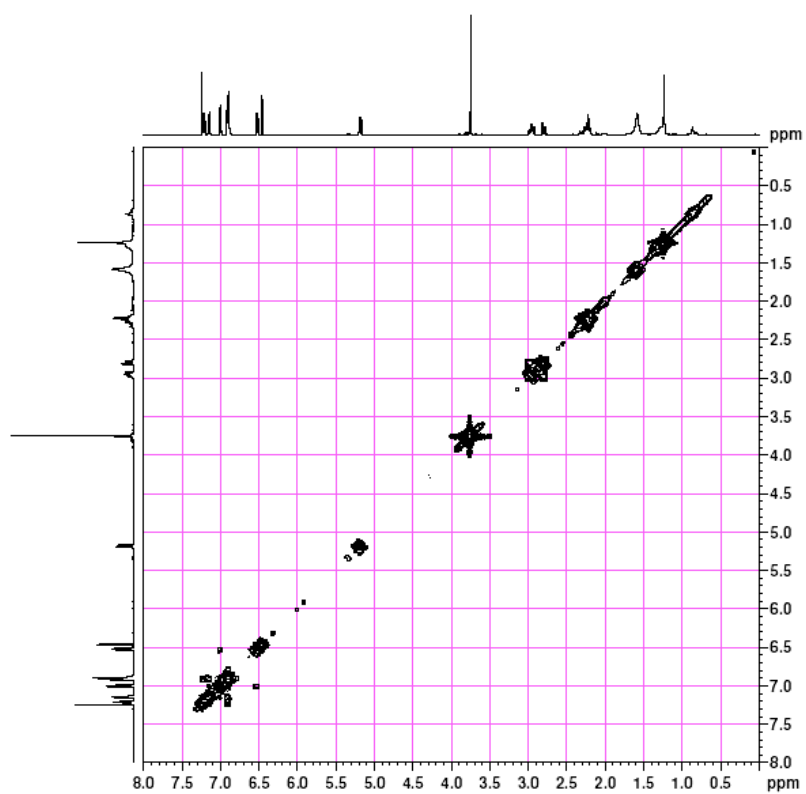
**Figure S19.** EIMS spectrum of (2S)-2'-hydroxy-7-methoxyflavan (**3**)



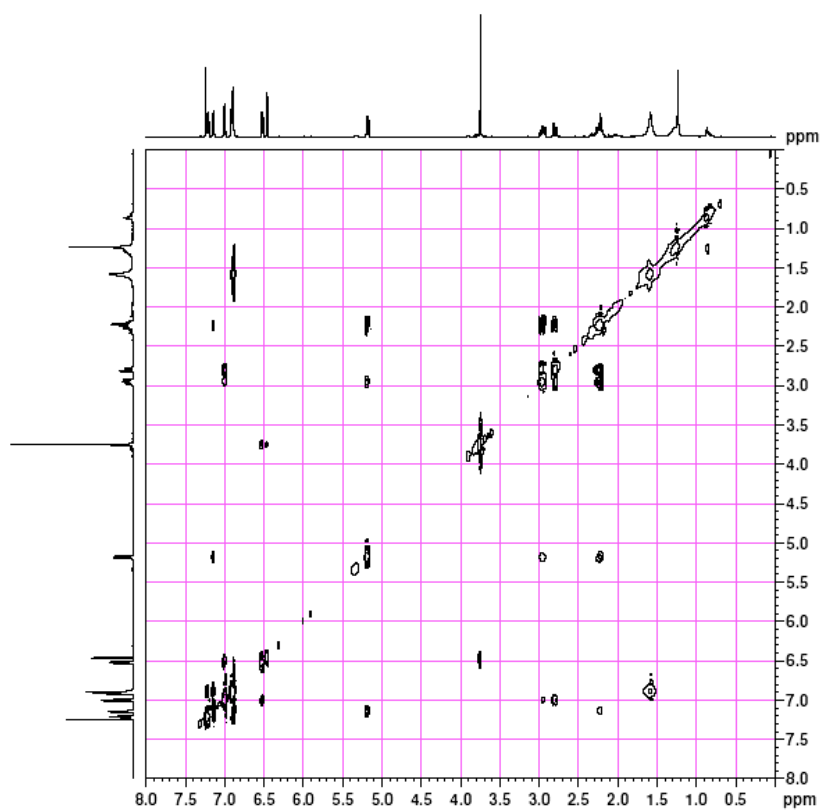
**Figure S20.** The <sup>1</sup>H-NMR spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**; 500 MHz, CDCl<sub>3</sub>)



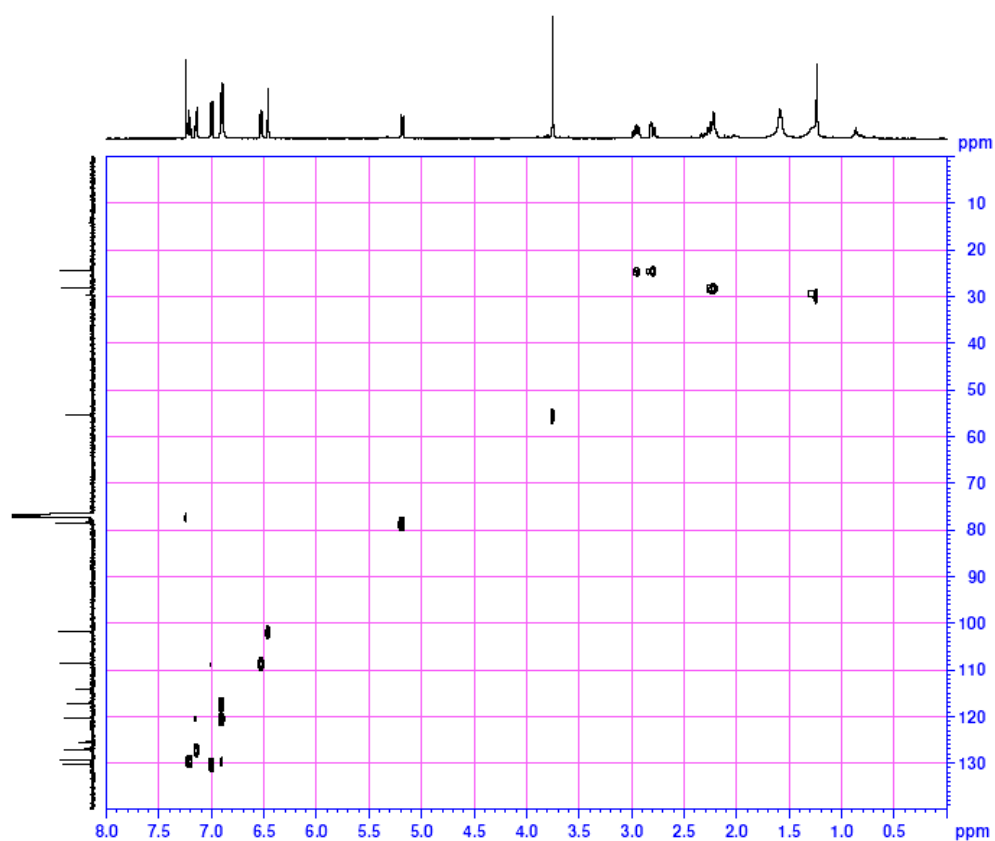
**Figure S21.** The <sup>13</sup>C-NMR spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**; 125 MHz, CDCl<sub>3</sub>)



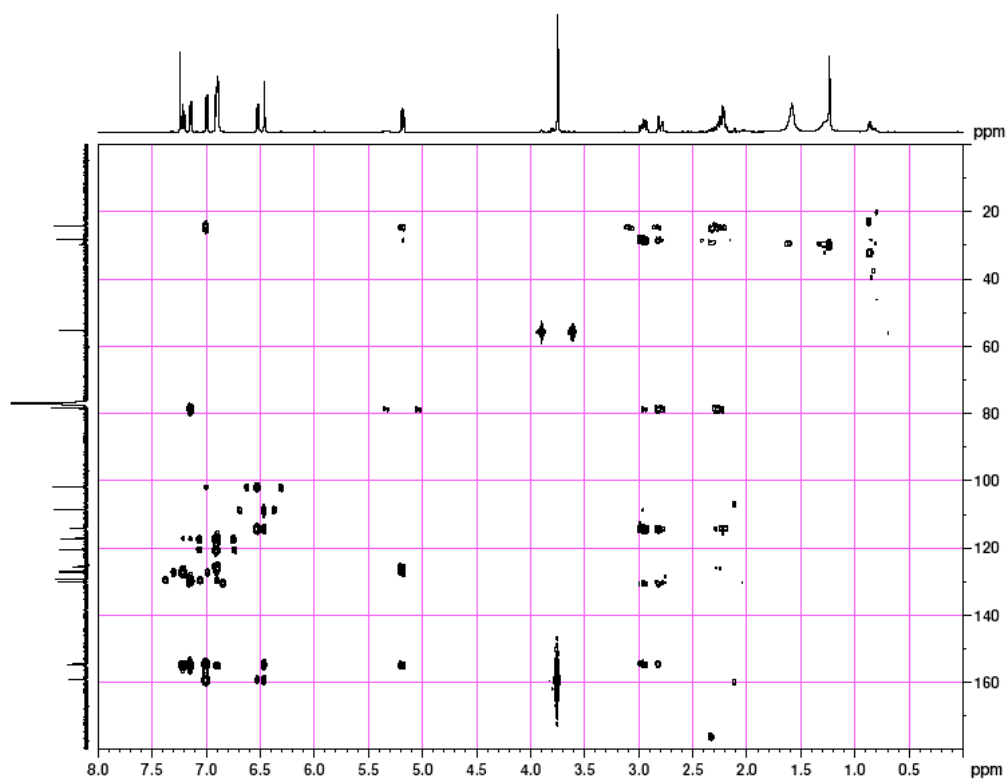
**Figure S22.** COSY spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)



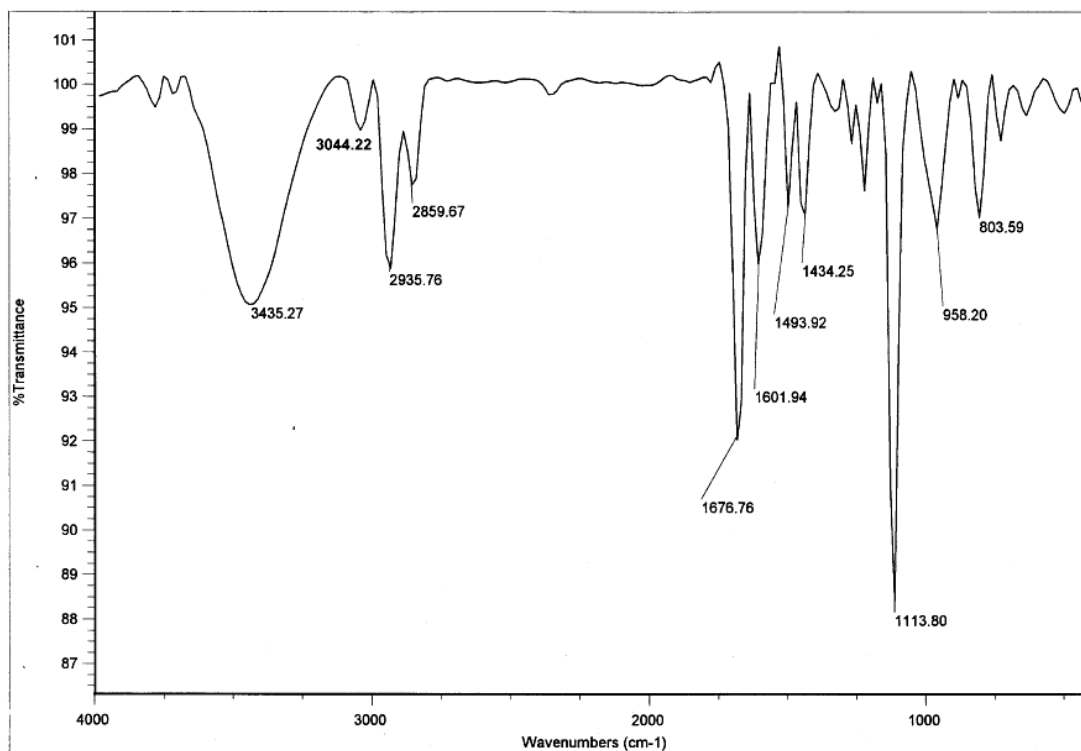
**Figure S23.** NOESY spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)



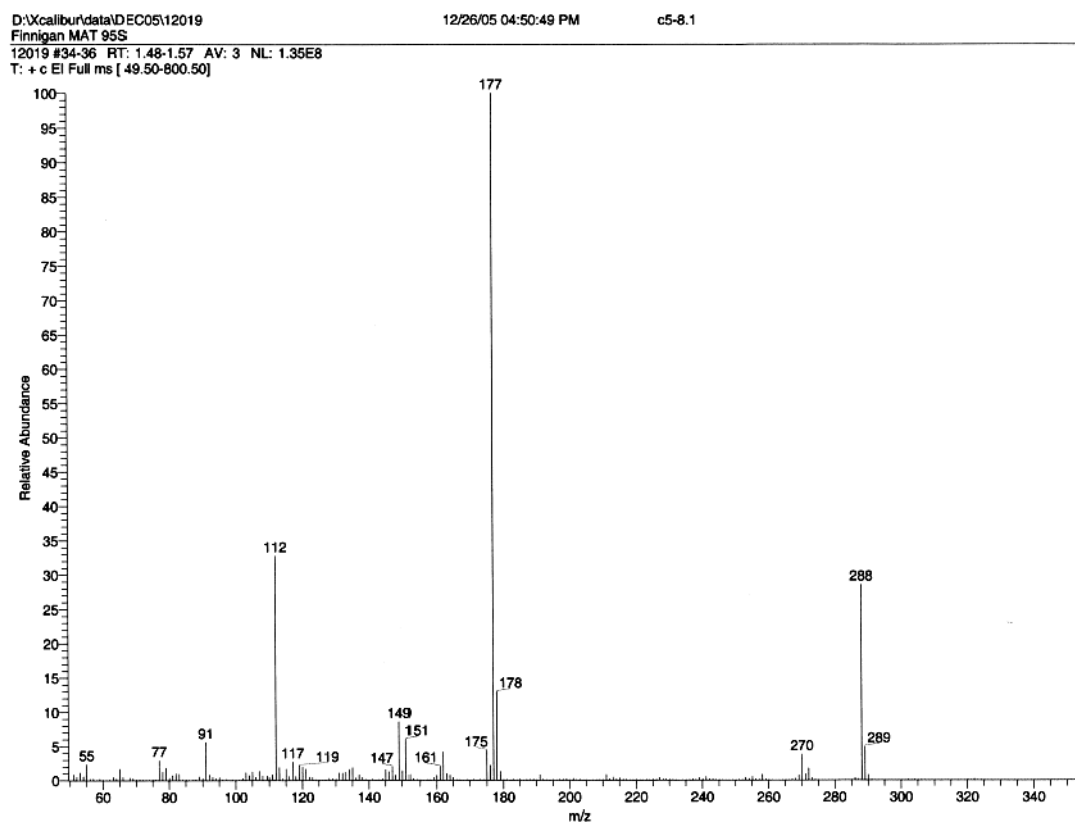
**Figure S24.** HMQC spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)



**Figure S25.** HMBC spectrum of (2*S*)-2'-hydroxy-7-methoxyflavan (**3**)

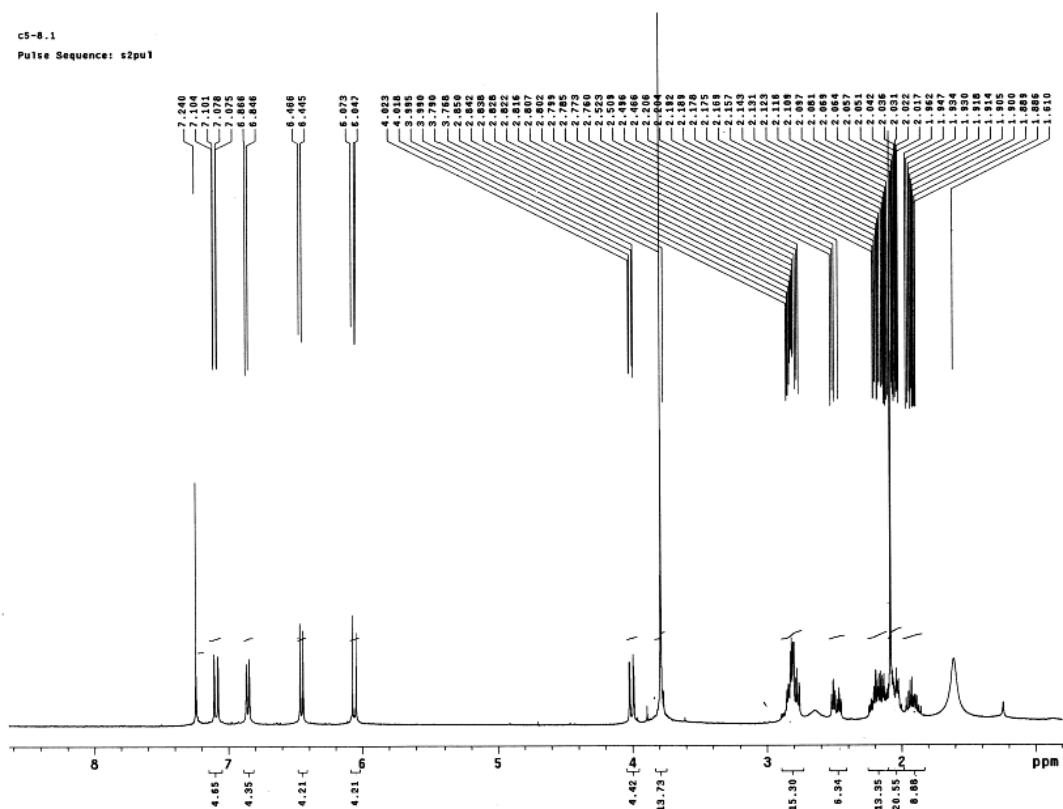


**Figure S26.** IR spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

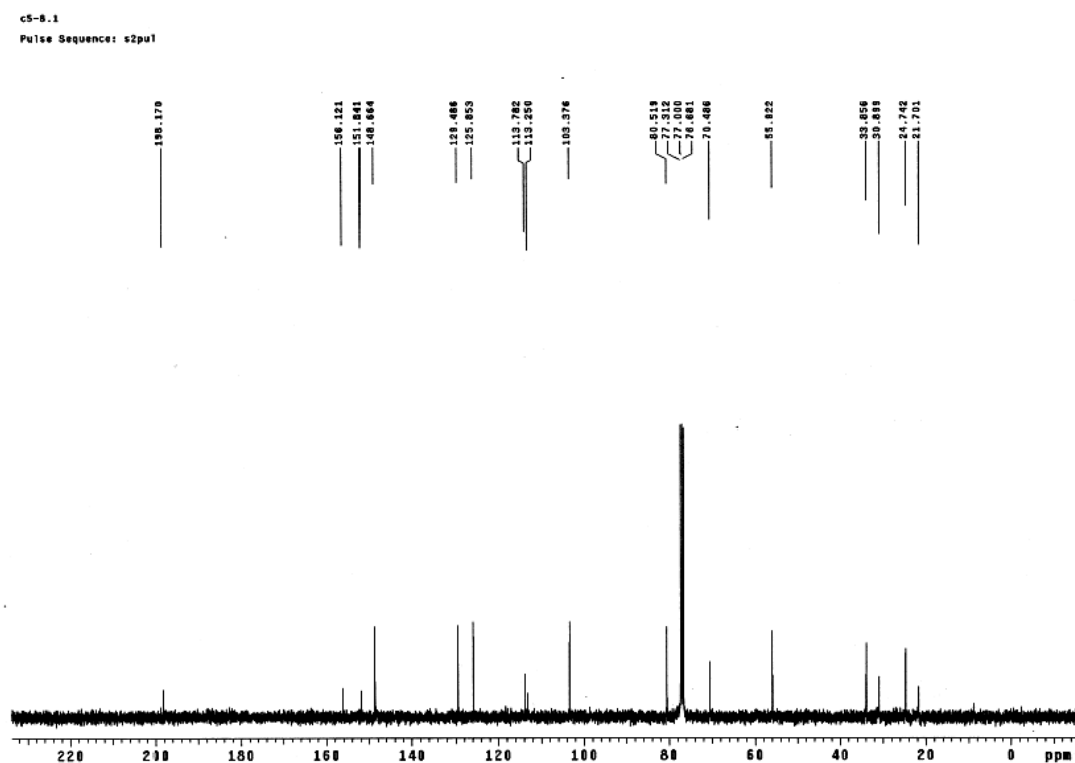


**Figure S27.** EIMS spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)





**Figure S28.** The  $^1\text{H}$ -NMR spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**; 400 MHz,  $\text{CDCl}_3$ )

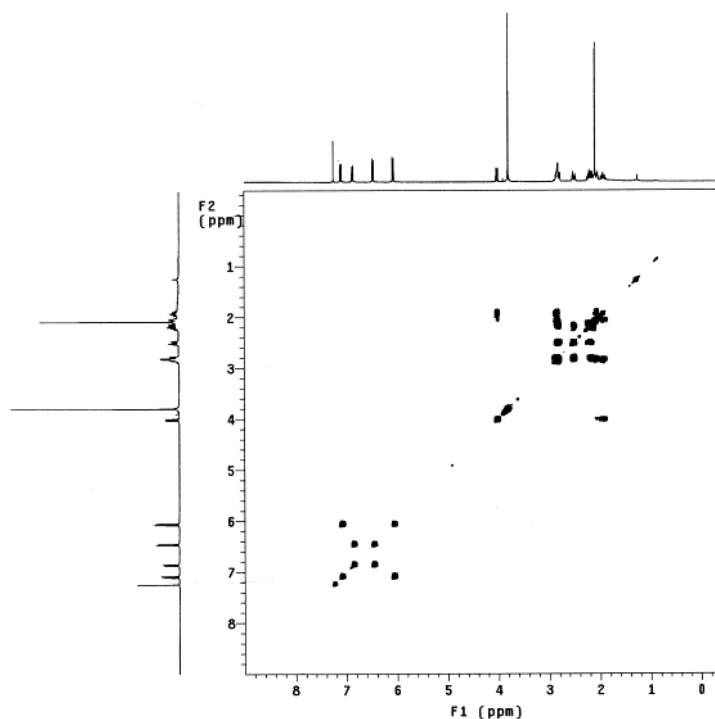


**Figure S29.** The  $^{13}\text{C}$ -NMR spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**; 100 MHz,  $\text{CDCl}_3$ )

## STANDARD 1H OBSERVE

exp2 relayh

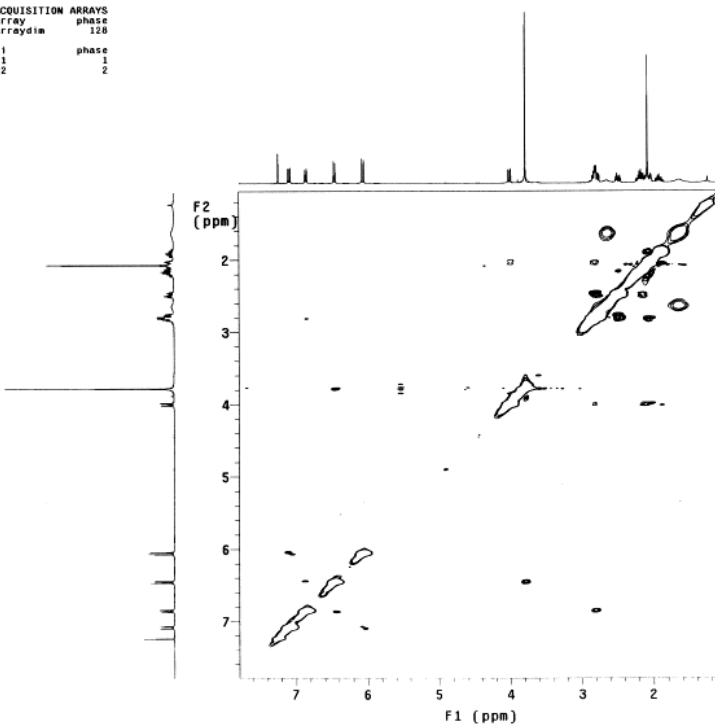
SAMPLE DEC. & VT  
 date Mar 7 2006 dfrq 399.877  
 solvent CDC13 dn M1  
 file exp dpr 44  
 ACQUISITION exp dof 0  
 sfrq 399.877 da nmh  
 tn H1 dm c  
 at 0.132 dmf 9700  
 np 1000  
 sw 3787.9 lb -38.55  
 fb not used gf 0.033  
 tpr 58 gfs not used  
 pw 17.0 wtfll 1  
 pl 17.0 proc ft  
 d1 1.000 fn 4096  
 tof -329.4  
 nt 8 werr  
 ct 8 wexp svf(n2)  
 tau 0 wbs wtt  
 relay 0 wnt  
 alock n 2D PROCESSING  
 gain 32 lb1 -75.284  
 FLAGS n gf1 0.017  
 in n gfs1 not used  
 dp y procl ft  
 2D ACQUISITION fnl 4096  
 sw1 3787.9  
 nl 256  
 phase 0  
 DISPLAY  
 sp -180.1  
 wp 3787.9  
 vs 800  
 sc 10  
 wc 116  
 hzmm 32.72  
 ls 500.00  
 rf1 190.1  
 rfp 0  
 th 11  
 ins 10.000  
 a1 2D DISPLAY  
 sp1 -180.1  
 wp1 3787.9  
 sc2 0  
 wc2 116  
 rf11 3085.2  
 rfp1 2895.1

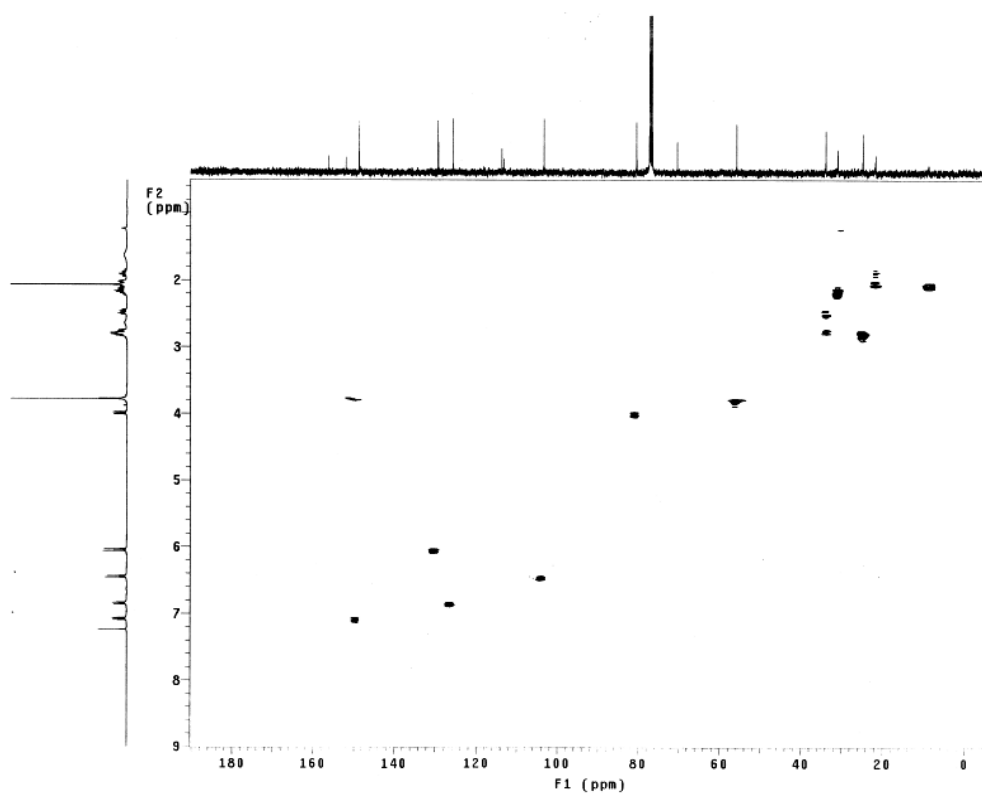
Figure S30. COSY spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)

## STANDARD 1H OBSERVE

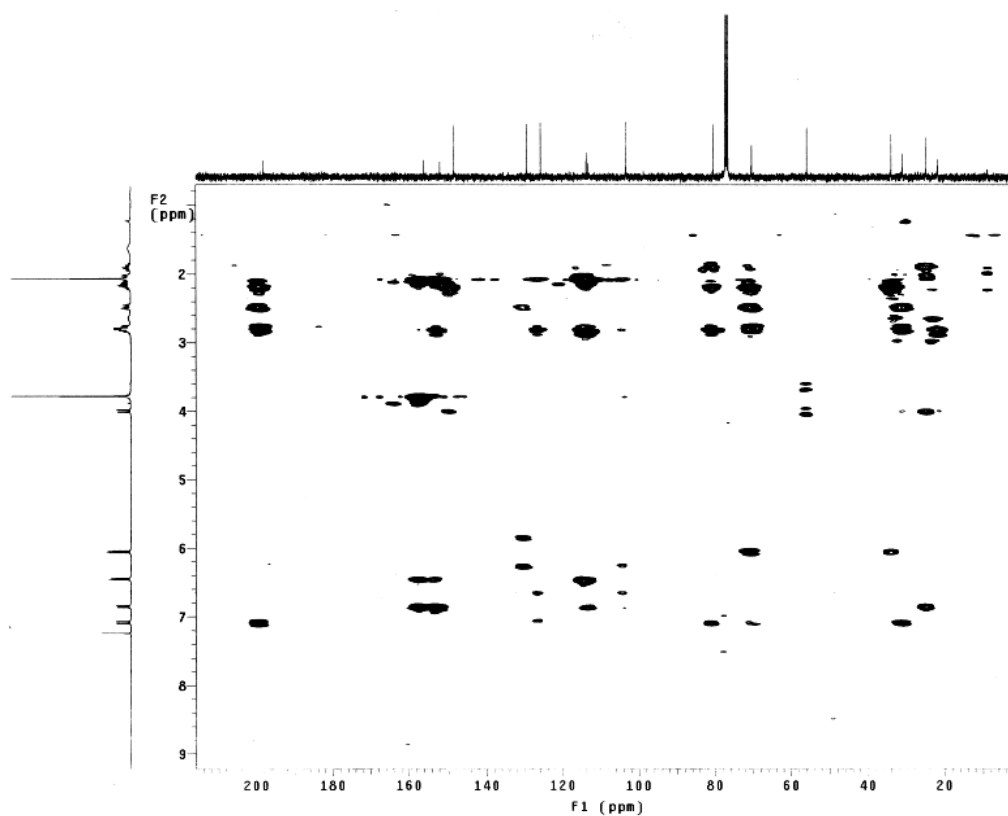
exp5 noesy

SAMPLE DEC. & VT ACQUISITION ARRAYS  
 date Mar 3 2006 dfrq 399.954 array phase  
 solvent CDC13 dn M1 arraydia 128  
 file exp dpr 30  
 ACQUISITION exp dof 0 1 phase  
 sfrq 399.954 da n 1  
 tn H1 dm c 2  
 at 0.122 dmf 200  
 np 1024 dseq 1.0  
 sw 4192.4 dres n  
 fb 2400 homo 24.0  
 tpr 57 temp  
 pw 10.0  
 d1 1.000 g PROCESSING  
 preset 0 gfs not used  
 mix 0.800 wtfll 1  
 tof -95.8 proc ft  
 nt 32 fn 4096  
 ct 32 meth f  
 alock n  
 gain 40 werr  
 FLAGS n wexp svf(n2)  
 in n wnt  
 dp y 2D PROCESSING  
 hs yn lb1 0.318  
 sspul n gf1 0.015  
 2D ACQUISITION n gfs1 not used  
 sw1 4192.4 wtfll 1p  
 nl 64 procl  
 phase arrayed fnl 4096  
 DISPLAY  
 sp 423.2  
 wp 2693.2  
 vs 1000  
 sc 10  
 wc 116  
 hzmm 23.27  
 ls 279.56  
 rf1 193.2  
 rfp 0  
 th 2  
 ins 100.000  
 a1 cdc ph  
 2D DISPLAY  
 sp1 423.2  
 wp1 2693.2  
 sc2 0  
 wc2 116  
 rf11 193.2  
 rfp1 0

Figure S31. NOESY spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)



**Figure S32.** HMBC spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)



**Figure S33.** HMBC spectrum of 4-hydroxy-4-(7-methoxy-8-methylchroman-2-yl)-cyclohex-2-enone (**4**)